Stefano Goffredo · Zvy Dubinsky *Editors*

The Cnidaria, Past, Present and Future

The world of Medusa and her sisters

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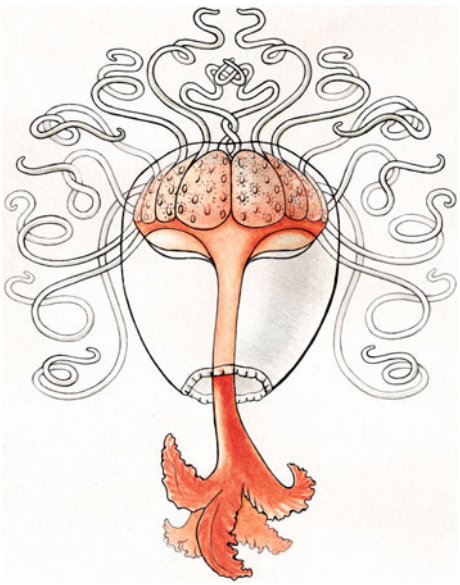

Medusa at the interface between science and arts (Illustration by: Anya Lauri, Lauri Projects, Italy; 2016)

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Preface

In the present volume, we exhibit a broad panorama of the current status of cnidarian research. On the timescale, the cnidarians are traced from the earliest geological records of their place at the very cradle of the evolution of multicellular organisms in the Earth's primordial oceans, to their response to present climate change effects in the warming and acidifying oceans. The latter aspects are described on the molecular and physiological responses followed by resulting population level effects. Their evolution is accompanied by their taxonomic diversification and geographic expansion, including their present role among invasive species. Their acquisition of neural networks and development of skeletal elements are described on the background of the evolutionary history of these systems covering both evolutionary and ontogenetic analyses.

The uniquely widespread and intimate mutualistic symbiotic associations with algae is described in taxa, such as jellyfishes and corals, revealing the roots of the dependence of so many cnidarian species on photosynthesis. That allows their symbiotic algae – the zooxanthellae – to provide most of the metabolic energy needs of the animal algae holobiont. Due to the dominant role of corals in the biogeochemical carbon and calcium cycles, the various aspects of cnidarian calcification are discussed in terms of accepted theories while exposing their difficulties.

The relation of the Cnidaria with mankind is approached on several levels, in accordance with the editors' philosophy of bridging the artificial schism between science, arts and humanities. On the direct level, the various encounters with humans result in a broad spectrum of medical emergencies of varying seriousness are reviewed. On a more detached level, humans from the very emergence of our species were fascinated by corals, and various coralline ornamental objects are found in burial sites in coastal regions in all continents. Chapters are dedicated to the history of coral harvesting down to current sustainable culture practices.

To the role of "Hydra" and "Medusa" in mythology, art and culture is devoted in the final section of the volume.

That section closes the unique, topical, multi-level, interdisciplinary coverage of the widespread, predominantly marine taxon, the Cnidaria. The present volume takes the reader through the earliest evolution of the taxon and the ramification of its diversity. It proceeds to the group's molecular and genomic make-up, through its ecology and physiology, all the way to its use in jewellery and invasion of human imagination in art and myth.

Bologna, Italy — Stefano Goffredo
Ramat-Gan, Israel — Zvy Dubinsky

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Part I

Genesis, Evolution, and Systematics

Emergence and Evolution of Early Life in the Geological Environment

1

Barbara Cavalazzi and Roberto Barbieri

Abstract

Based on our current assumptions, life on Earth started long before 3.5 billion years ago, the age of the oldest (accepted) terrestrial fossils. The issue on how life emerged from a non-living world, however, is still waiting for a solution. Aside from the many ideas generated by theoretical speculations, however, the issue on origin and evolution of the primordial life can be addressed through some of the oldest environmental conditions still preserved on the terrestrial sedimentary record (and their alleged modern analogues), and other planetary bodies, especially Mars, where rovers, orbiters and spectrometers can deal with unaltered rocks much older than the oldest one preserved on our planet and, perhaps, finally able to reveal some evidence of life.

Keywords

Hadean habitability • Archaean biosphere • Earliest habitat

1.1 Introduction

Knowing how, where and when life first emerged on Earth is one of the most fascinating, fundamental and yet elusive questions in science. Yet the answer to these questions remains enigmatic, as it combines highly controversial, unconstrained and/or unconfirmed assumptions.

The origin of life is still matter of different and often-divergent hypotheses, some of them providing interesting explanation for life's beginnings. Life potentially has a terrestrial origin as suggested by the attempts of synthetic biology and prebiotic chemistry to reconstruct the molecular bricks of living organisms, and the conditions of the primitive Earth since Urey-Miller's experiments (Bada and Lazcano 2003; Brack 2009). Two main hypotheses have been followed to reconstruct such a primitive life in a test tube: the heterotrophic origin of life (the primordial soup) versus an autotrophic origin (metabolism first). Life could have been originated on Earth and, in the same way it could have been or will take place (at least in its early steps) in similar planets (Zahnle et al. 2007; McKay 2010). Recent research for testing the survival of life forms to the re-entry into the Earth's atmosphere, do not bode well on the possible arrival of life from space, especially photosynthetic microorganisms (Cockell et al. 2007). However, a possible non terrestrial origin cannot be underestimated (e.g., Levin 2007), especially when considering all the possible ways of the transfer of life through the universe (e.g., the STONE experiment series, Brack et al. 2002), and the ubiquitous distribution of bio-elements, such as carbon and oxygen, and most of the life-based molecules and compounds, especially H_2O, in the cosmos (Ehrenfreund et al. 2011).

From our perspective, we agreed that life definitively appeared on a prebiotic early Earth at some point in its *ca.* 4.56 billion years long history, and there developed, evolved

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and diversified making as consequence our home planet truly unique if compared to the other rocky planets and hypothetically habitable moons, asteroids and comets known in the universe (Orò 1990; Kasting et al. 1993; Sleep 2010; Hazen 2012). Up to now, Earth is still the only known oasis of life in our solar system, and perhaps in the universe.

The emergence and progressive increase of life's complexity required a habitable planet with specific physical and chemical conditions able to sustain chemical systems capable of Darwinian-type evolution. From simple organic molecules dispersed in liquid water, these systems can then have evolved towards entities capable of transferring their molecular information via self-replication and also capable of evolving, i.e. primitive life. During the first steps of the evolutionary events prokaryotic microorganisms adapted to a variety of the different conditions of extreme life. Environments similar to those characterized by extreme conditions on the early Earth are still present in certain terrestrial regions, and provide a powerful tool for a direct investigation of key aspects of the dawn of life.

Life is a chemically complex system (e.g., Forterre and Gribaldo 2007) that opportunistically adapts and develops suitable strategies to take advantage of the energy and nutrient sources available in a variety of environments that in turn continually modify themselves. Therefore, the origin of life can in some way be considered the origin of the evolution (e.g., Hazen 2012). Billions of years ago life must have begun (originated on Earth or adapted to terrestrial primitive conditions) in a "prebiotic soup" scenario of a cooling, ocean-flooded prebiotic Earth in which geological context characterized by (geo)thermal and other energy gradients, as well as metabolically suitable carbon and other nutrient sources, were established (e.g., Zahnle et al. 2007; Abramov and Mojzsis 2009; Arndt and Nisbet 2012; Sleep et al. 2012). A generalized volcanic context seems the most geologically convenient one for such a beginning with its corollary of chemical disequilibria, as are those that characterize the hydrothermal systems (Russell et al. 1993; Schulte et al. 2006; Lane et al. 2010; Sleep 2010). From its origin to 3.8 billion years ago, the age of the rock that retains the disputed oldest geochemical evidence of life, Earth underwent irreversible changes, either gradual or instantaneous in terms of geological time. From that time, co-evolution of geological and biological events are still operating and continuously shaping our planetary system. Therefore, the building blocks of cells and the first simplest life forms should be appeared on Earth only at a certain point of this evolution. In an oxygenated environment such as on Earth soon after the *Great Oxidation Event*, *ca*. 2.4 billion years ago (Kopp et al. 2005), life would probably never been formed since all organic and inorganic ingredients of life would have been destroyed or driven in a different (evolutionary) direction (e.g., Hazen and Sverjensky 2010; Sleep et al. 2012) by reactive oxygen.

From the pioneering experiments by Miller (1953) in prebiotic chemistry, and the discovery by Tyler and Barghoorn (1954), who first reported microfossils of a few billion years old, there are still important gaps in our knowledge in the research on the origin of life that must be overcome. Researchers interested in origin of life are now seeking to understand via prebiotic chemistry and chemical evolution when autocatalysis became life (e.g., Forterre and Gribaldo 2007; Brack et al. 2010), and are striving to dig deep in the paleobiological and geological records in order to expand the understanding of the mechanisms of early life, its co-evolution with the changing early environment, and ultimately, the issue of environmental requirements for habitability (e.g., Schopf and Bottjer 2009). Paleobiologists have to deal with the rareness of rocks and minerals survived from the early Earth, the only record that provides direct evidences of the deep time of our planet. They are also developing the capacity to solve at a very fine scale biosignatures and other traces of life – left long after the disappearance of the living things that made them – (e.g., Cavalazzi 2007; Cavalazzi 2013; Oehler and Cady 2014) to detect cause-effect relationships in the interactions between biosphere and physical environments in the geological record (e.g., Kelley et al. 2013).

Based on the oldest evidences of life – including fossil remains and hints of biological activity, such as geochemical and molecular signals, that are only known from rocks aged 3.8–3.5 billion years – and on few other geological evidences – including the oldest rock and mineral records dating back up to 4.4 billion years – our planet is suspected to have been always a habitable one, with the only exception, perhaps, of the its first 150 million years. This chapter includes an updated state of the art of the current hypotheses (and available data) on the earliest phases of the Earth history, and on the early terrestrial habitats that made possible to our planet to became a suitable place for life.

1.2 Early Earth and the Geological Time Scale

Early Earth informally indicates an undefined broad interval of the geological time that spans from the formation of the Earth to the age of the oldest evidence of life *ca*. 3.5 billion years ago, and up to age of the rise of the oxygenated atmosphere, *ca*. 2.4 billion years ago (Orò 1990; Zahnle et al. 2007; Van Kranendonk 2012). For non-specialists, however, it can also represent the first 4 billion years of the history of the planet. A formal division in Eons and sub-division in Eras of the geological time scale of the early Earth has recently been proposed (Van Kranendonk 2012) on the basis of plausible hypotheses of the main geological and biological changes derived from the most ancient terrestrial rock record (on Earth, rocks older than *ca*. 4.0 billion years are not available) (Fig. 1.1).

The major events that have modified the early Earth, with a definitive change of its prebiotic conditions in favour of

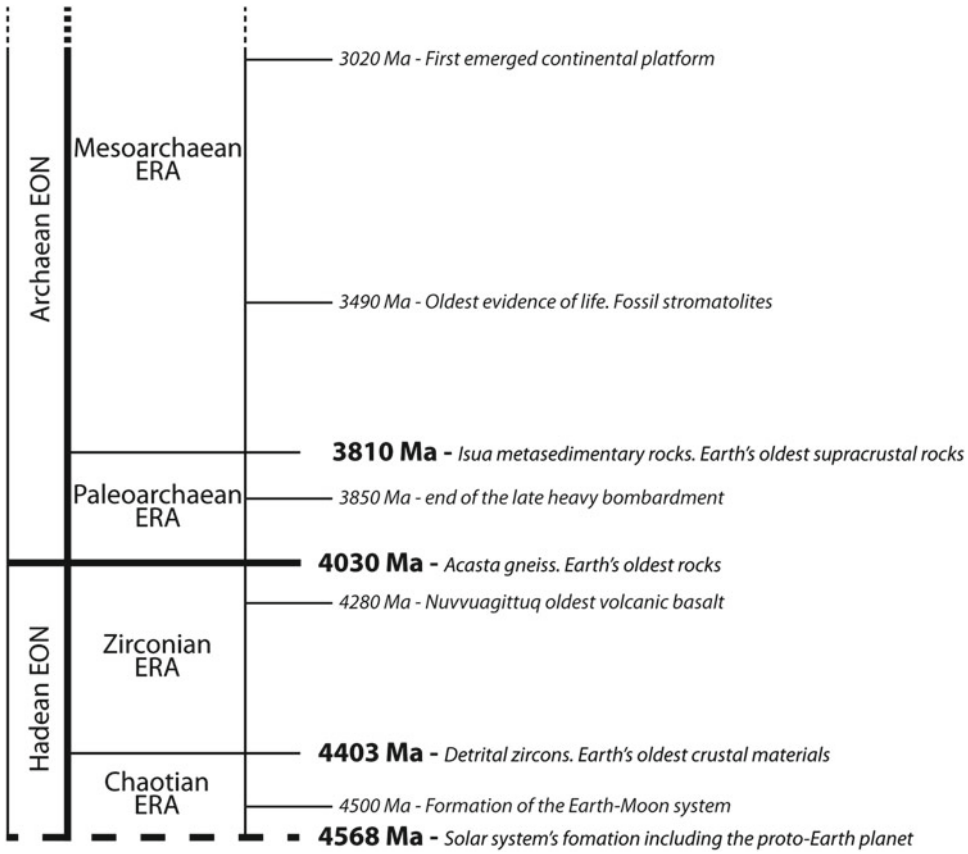

Fig. 1.1 Simplified scheme resuming the recently proposed Hadean and Archaean time-scale division (modified from Van Kranendonk 2012), and the major events in earliest Earth's history. *Ma* million years

more suitable conditions to support life probably occurred during the first 2 billion years history of the planet, that is to say during the Hadean and Archaean Eons. The Eon Hadean spans from *ca*. 4.5 to *ca*. 4.03 billion years ago, and the time interval between the end of Hadean and *ca*. 3.0 billion years ago is the early part of the Archaean Eon (ending ca. 2.4 billion years ago). During this time span, Earth has been literally shaped from a molten surface to a planet dominated by oxygen breathing organisms largely organized in the organo-sedimentary structures termed stromatolites (Fig. 1.2).

Although it is still unknown when exactly (during the Hadean or early Achaean) life appeared on Earth because of the incomplete geological record, the Hadean Earth might have been habitable relatively soon, and the rocks of Archaean age host traces of life from at least 3.5 billion years ago (the age of the oldest stromatolites) to now. The following sections will be particularly focused on the earliest geological conditions that made the Hadean and Archaean Earth a suitable place for life.

1.3 Early Habitable Earth

Based on our current knowledge on the requirements needed to support life forms, the conditions making a world habitable include a number of factors such as (i) suitable surface temperature ranges, (ii) liquid water availability, (iii) a suitable atmosphere to retain heat and protect from harmful radiation, (iv) availability of sources of energy and nutrients (e.g., Lunine 2013). Considering the solar system, only the Earth's surface presently combines all the above factors. Recent studies, such as new analyses on Martian meteorites (e.g., Shaheen et al. 2013) and the results being published through the geological discoveries of the rover Curiosity (e.g., Grotzinger et al. 2014), indicate that the conditions of habitability of Mars some 4 billion years ago may have been similar to the Earth today and probably to the primitive Earth. So Mars had chance to harbour primitive life. This is a strong argument in favour of those who consider plausible the discovery of evidences of fossil life (perhaps even older than those found on Earth) on that planet. For the moment, however, we should focus on the only known place in which were found clear traces of primordial life: the Earth.

1.3.1 Habitable Hadean (4.567–4.030 Billion Years) Earth

Earth-Moon system forms about 4.56 billion years ago in a *ca*. 50 million years old Solar System as a consequence of a giant impact (for a recent review see Hazen 2012; Geiss and Rossi 2013). The Early Hadean (post-impact) newly formed planet (and Moon) was an incandescent and chemically dynamic planet with an unusually large natural satellite, the Moon, if compared to other moons of the Solar System's planets.

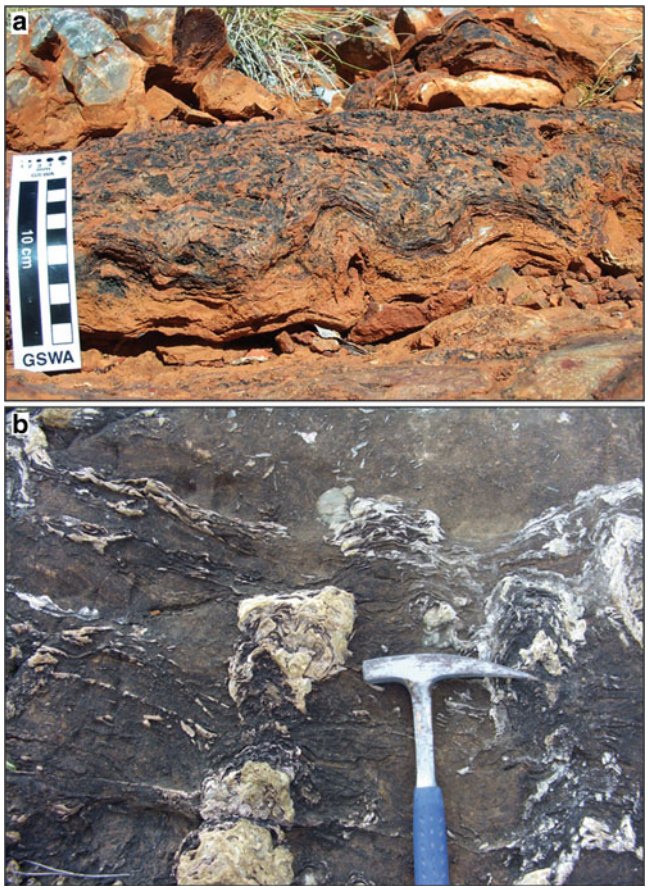

Fig. 1.2 The oldest evidence for life may be *ca*. 3.5 billion years old organo-sedimentary stromatolites. (**a**) Stromatolites from the *ca*. 3.49 billion years old Dresser Formation, North Pole Dome, Western Australia. Note the wrinkly- and finely-laminated mats forming small conical domes. (**b**) Stromatolites in the *ca*. 3 billion years old Pongola Supergroup, Barberton greenstone belt, South Africa. Note silicified columnar stromatolites with fine and irregularly wavy internal lamination. Hammer for scale

Immediately after its formation, the Earth began to cool and self-organize hardening and solidifying a thin layer of magma to the surface. Geological and cosmological activities, such as geothermal turbulence, heat-driven fracturing and cataclysmic bombardments period, were continuously interfering with the cooling-solidifying processes expected from a simple heat release, thus delaying the achievement of habitability conditions (Sleep et al. 2001; Sleep 2010; Arndt and Nisbet 2012). Considering the elevated temperature of the Earth 4.5 billion years ago, the sun should not have significantly influenced the thermal balance of our planet. It is, however, possible that a super-greenhouse effect would have occurred as a consequence of the formation of an atmosphere and oceans in the early Hadean. The relative molten state that the Earth's surface maintained during that time was also a consequence of the intense gravity-induced tides of the Moon.

Although the presence of the Moon has complicated the Earth's cooling history, it has ultimately made possible both life and its evolutionary direction. The Moon is now slowly moving away from Earth that gradually slows down by 15 s. per million years, and in a geological future it will eventually leave the Earth's orbit. Because the Moon has always been a stabilizing factor for the axis of rotation of the Earth, it had (and still has) a significant effect on the evolution of our planet. In the same way, its removal from the Earth's orbit is expected to have a catastrophic impact on the planet, and especially on the biosphere. Looking at Mars, for instance, it has dramatically wobbled on its axis over time due to the gravitational influence of all the other planets in the solar system. Because of this obliquity change, the ice that is now at the poles on Mars might sometimes drift up to the equator. Differently, through the stabilizing capacity of the Moon, the Earth's axis of rotation maintains a constant direction. This is the reason why the Earth has experienced, much less (dramatic) climate change than if had been alone. And this has, ultimately, changed the way how life evolved on Earth, allowing for the emergence of more complex multi-cellular organisms compared to a planet where recurrent, drastic climate change would have only permitted survival of small extremophiles. So without the stabilizing effects of the Moon, life on Earth would not have been started or, at most, it would be limited only to extremely protected niches to survive the environmental extremes.

According to the Earth-Moon model formation, 4.56 billion years ago our satellite was much closer to the Earth than it is today, with the result of more rapid rotation of the Earth around its axis (1 day = 5 h; 1750 days = 1 year), and of the Moon around the Earth. By driving the tides, the Moon could have determined or contributed to create on Earth a suitable place for life, or at least accelerated and driven its progression. This suggests that, without the Moon, the biological evolution on Earth would have taken a different path. Although no direct evidence on the type of tidal cycles during the Hadean is presently known, evidence supporting past different conditions do exist, it is the case, for example, of the growth lines of corals (Hazen 2012). Because the minimal growth lines of certain corals can indicate daily and annual growth cycles, it can be a useful tool to measure the number of days of the year. This ability, therefore, allows modern corals to produce about 365 growth lines per year, whereas Devonian fossil corals display more than 400 daily lines per year, suggesting that 400 million years ago, when the Moon was presumably closer to the Earth, the planet had a faster rotation rate (day length: about 22 h).

Because of the scanty geological evidences of the Hadean Earth, events such as the cooling of the crust and the formation of oceans and atmosphere can be hardly constrained. Zircons ($ZrSiO_4$) from the Jack Hills of Western Australia,

that represent the oldest (4.4 billion years) known fragments of the Earth's crust, indicate that their host rock was a granitic crust formed at a very early stage, probably in a differentiation process started before 4.4 billion years ago (Wilde et al. 2001; Valley et al. 2002). It is therefore likely that the Earth of the early Hadean had a prevailing molten surface and that soon after the formation of the planet this surface cooled enough to form the first basalts organized in solid, floating surfaces. These early fragments of crust may have developed a fast crustal recycling, and together with certain topographic features, such as volcanic cones and islands, erupting geysers and sulphur hot springs, may have contributed to develop an ocean-dominated potentially habitable planet (Valley et al. 2002; Arndt and Nisbet 2012; Sleep et al. 2012). The presence of liquid water is one of the essential ingredients for life (especially when coupled with the suitable environment such as hydrothermal systems), and one of the most important requirements for planetary habitability. A number of factors, such as the release of the Earth's internal heat, an intense greenhouse effect expected from the high atmospheric concentrations of CO_2 and/or organic molecules produced by ultraviolet radiation reacting with the large amount of the CH_4, the higher percentage of the Sun's energy absorbed by the iron-rich earliest oceans, or the combination of all these causes are in favour of a liquid Hadean global ocean (e.g., Kasting et al. 1993; Rosing et al. 2010). Otherwise, solely on the basis of the weak solar energy received by the early Earth (Sagan and Chyba 1997), these early oceans would be expected frozen (e.g., Zahnle et al. 2007; Arndt and Nisbet 2012).

Recent hypotheses favour a Haden planet able to release high thermal energy in 4.4 billion years old surface covered by highly saline, liquid oceans and shrouded by a dusty and dense, reducing atmosphere. This scenario depicts a potentially habitable planet where the most resistant extremophiles (hyperthermophiles) might have had the chance to originate and adapt in hydrothermal and/or deep, protected subsurface ecological niches (e.g., Russell and Arndt 2005; Zahnle and Sleep 2006; Abramov and Mojzsis 2009). The lunar radiometric dating and stratigraphy, however, have revealed that a dramatic rain of impacts, the so called Late Heavy Bombardment (LHB), between 4.1 and 3.85 billion years ago (Cohen et al. 2000), would have also invested our planet and, perhaps, evaporated the early Hadean oceans (Ryder 2002). Therefore, although the Hadean Earth could have been a habitable planet relatively soon after its formation, the LHB would have seriously compromised its habitability (Zahnle and Sleep 2006; Forterre and Gribaldo 2007; Abramov and Mojzsis 2009). In this case, life might have (re)started its story after 3.85 billion years ago, following a very first mass extinction. Therefore, Hadean Earth remained a desolate planet and life (in case) would have no really contributed to its changes or altered its surface.

1.3.2 Inhabited Archaean (4.030–2.40 Billion Years) Earth

The Archaean Earth at the end of LHB, although ready to host life, was a still cooling place. During the Paleoarchaean (Fig. 1.1), basaltic surfaces – with spots of isolated volcanic islands and continental crust largely flooded by oceans – have probably controlled the topography of the Earth (Arndt and Nisbet 2012).

The formation of the first granitic continental crust was an important step in the planetary differentiation of Earth, and a presumably important contributor to its habitability. The Earth had also a suitable combination of factors (e.g., size, temperature, timing of compositional differentiation) to become habitable, unlike bodies such as Mars, Mercury, and Moon, which were too small and cold to produce granitic crust. The oldest known terrestrial rocks (Fig. 1.1) are highly metamorphosed sediments of about 4 billion years ago from the Acasta Gneiss Complex, Canada (Bowring and Housh 1995). However, metamorphosed basaltic rocks of 4.3–4.4 billion years ago have been recently reported from the Nuvvuagittuq Greenstone Belt, Canada (O'Neil et al. 2012). Of primary interest are the rocks dating back 3.8 billion years ago of the Isua Supracrustal Belt, Greenland. These altered sedimentary rocks have been claimed to contain the controversial oldest evidence of life in the form of geochemical signals of highly fractionated and isotopically depleted ^{13}C (Mojzsis et al. 1996; Rosing 1999). Because they experienced tectonism and high-grade metamorphism, however, these rocks might have modified their geochemistry and/or destroyed any biogenic-related microstructural features (e.g., Fedo et al. 2006; Papineau et al. 2011). Therefore, this primacy makes the Isua Belt a place of unique significance to search for the origin of life on Earth despite still frustrating results.

Between 3.5 and 3.0 billion year ago the abundance and variety of the paleobiological records in the silicified sedimentary sequenced of the Pilbara Craton (Western Australia) and Barberton greenstone belt (South Africa) as well as the variety of their reconstructed paleoenvironments reveal how life was flourishing on the Earth during the Mesoarchaean (e.g., Furnes et al. 2004; Schopf 2006; Schopf et al. 2007).

Fossil stromatolites of *ca.* 3.5 billion years ago are reported from the Pilbara Craton in Western Australia. Here, in the *ca.* 3.49 billion years old Dresser Formation, hydrothermal-related stromatolites (Fig. 1.2a) are the likely result of the activity of hyperthermophilic microbes (Van Kranendonk 2006). For the stromatolites of the *ca.* 3.43 billion years old Strelley Pool Formation a shallow marine environment under the influence of hydrothermal activity has been hypothesized (Allwood et al. 2006). Similarly, marginal marine environments (tidal channels) have been proposed for the formation of columnar stromatolites by

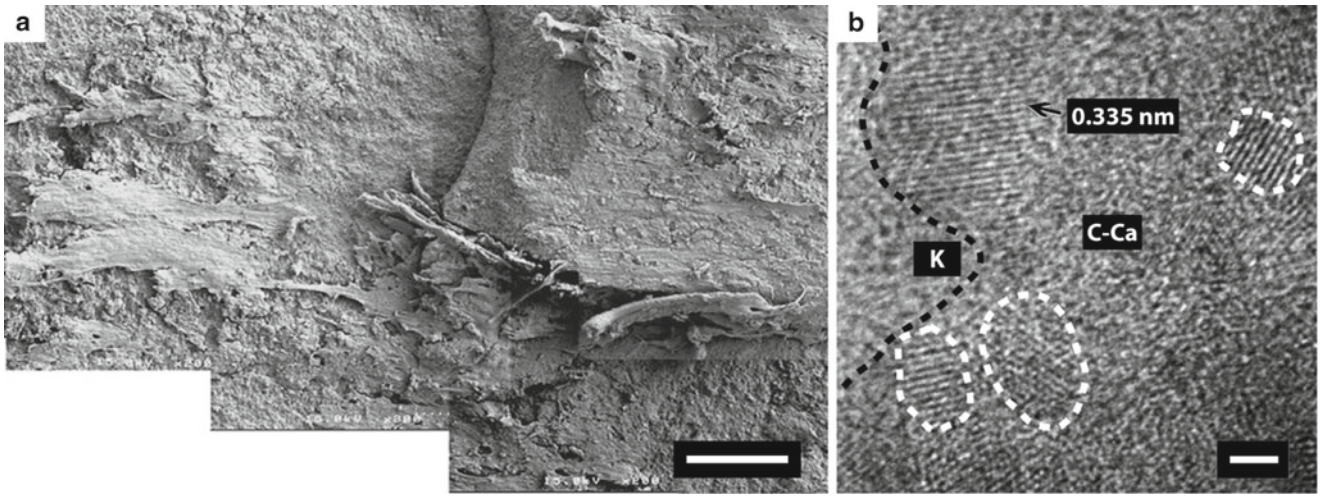

Fig. 1.3 Anoxygenic microbial mat from *ca*. 3.3 billion years old Josefsdal Chert, South Africa. (**a**) Scanning electron microscope micrograph of a silicified microbial mat. (**b**) High resolution transmission electron microscope micrograph of aragonite nanocrystals with 0.335 nm interlayer spacing and the d_{111} plane, observed within the carbonaceous calcified (C-Ca) area of the mat in **a**. The presence of aragonite coupled with other geochemical signatures, indicates anoxygenic behaviour of this ancient microbial mat. *K* kerogene. Scale bars are 200 μm and 2 nm in **a** and **b** images, respectively (Modified from Westall et al. 2011)

Fig. 1.4 Wrinkly laminated sandstones with desiccation cracked mud drapes, with the wrinkly laminae probably shaped by the presence of biomats from the *ca*. 3.2 billion years old Clutha Formation of the Moodies Group in Dycedale Syncline, Barberton greenstone belt, South Africa. Coin for scale (Image courtesy of A. Hofmann, University of Johannesburg)

oxygenic photoautotrophic cyanobacteria (Beukes and Lowe 1989) in the 3 billion years old Pongola Supergroup, South Africa (Fig. 1.2b).

Well preserved microbial mats and filamentous microfossils derived from presumable photosynthetic processes have been described in the Barberton greenstone belt (South Africa) from carbonaceous cherts belonging to the Swaziland Supergroup: (i) in the *ca*. 3.4 billion years old Buck Reef Chert (Tice and Lowe 2004); (ii) in the Onverwacht Group from *ca*. 3.4 to 3.3 billion years old deposits (Walsh and Lowe 1985); (iii) in the *ca*. 3.3 billion years old Josefsdal Chert, where fossil microbial mats of anoxygenic microorganisms (Fig. 1.3) have been interpreted as dominated by photosynthesizers and sulfur reducing bacteria (Westall et al. 2011). Tubular morphologies attributed to biotic alteration, have also been described in *ca*. 3.4–3.3 billion years old pillow lavas of South Africa and Western Australia (Furnes et al. 2004; Banerjee et al. 2007). Again from the Barberton greenstone belt, in tidal deposits of the Moodies Group, *ca*. 3.2 billion years old, microbially induced sedimentary structures (Fig. 1.4) have been described (e.g., Noffke 2010).

The oldest fossil evidences of life suggest that the early Archaean was inhabited by relatively diverse forms of life. Much of these evidences, however, seem ambiguous (e.g., Brasier et al. 2006; Grosch and McLoughlin 2014). The geochemical proxies, especially the mass fractionation of sulphur isotopes by atmospheric processes (Farquhar et al. 2000), suggest that (i) the early environmental conditions were

anoxic and evolved to oxic around 2.4–2.32 billion years ago (Kopp et al. 2005), or even earlier (Ohmoto et al. 2006), and (ii) the earliest microbial ecosystems were sulphur-based (Shen et al. 2001). Although we still do not know when exactly microorganisms developed photosynthetic oxygen production, their contribution to the oxygenation of the environment was a necessary prerequisite for the evolution of a more complex life (e.g., Catling et al. 2005).

1.4 The Early Life and Habitat on Earth and Their Modern Analogues

In the depicted Hadean-early Archaean scenario, it can be assumed that life took its first steps in a turbulent, chemically active, oxic and magmatic-dominated environment with common release of water vapour, methane, carbon dioxide and sulphur exhalations.

Despite the limiting factors that might have compromised the real chances of habitability of the Earth during the Hadean (at least until the end of the LHB), and the planetary recycling processes (active since the planet's formation), with consequent destruction of any record of the early Earth's history, it seems likely that impacts and hydrothermalism have played a key role on the prebiotic precursors and the origin of life. Although it is not possible to know whether any life that appeared in the Hadean was then destroyed during the LHB, and re-started later, the evolutionary process of the first forms of life should have been significantly affected by the energy released during the LHB (e.g., Zahnle et al. 2007). During and before the LHB, for example, impact-induced hydrothermal systems would also have favoured the establishment of suitable habitats for life in the subsurface (Abramov and Kring 2005; Russell and Arndt 2005; Abramov and Mojzsis 2009).

Hydrothermal vent systems, which were abundant in the primordial Earth, with their availability of geothermal energy, ions abundance, and availability of minerals with high reactive surface could have played a primary role in synthesizing and stabilizing the complex organic molecules that are the basis of life (e.g., Holm 1992; Deamer 2007; Martin et al. 2008; Hazen and Sverjensky 2010; Golding and Glikson 2011). The chemical equilibrium of the hydrothermal-derived H_2 and the CO_2 dissolved in the early ocean may have ensured the synthesis of reduced carbon compounds, the essential constituents for life (for review see Schrenk et al. 2013). The cycling of hydrogen, methane, sulphur and fermentative processes appear to be important metabolic activities implemented by microbial ecosystems in the carbonate chimneys of Lost City (Kelly et al. 2005), an outstanding hydrothermal system associated with the mid-Atlantic Ridge. Here, the serpentinization processes may have provided energy, raw materials, and supplied hydrogen, during the abiogenic synthesis of organic matter that would have supported chemosynthetic/chemotrophic microbial communities. Similar vent systems could also have existed in the Hadean oceans. Serpentinization processes, that were probably active in early Earth's hydrothermal environments, are currently considered a plausible scenario in which to place the origin and early evolution of life (e.g., Russell et al. 2010).

The phylogenetic relationships of living organisms based on 16S rRNA molecules support the assumption that the earliest terrestrial organisms and their metabolisms were rooted in the Archaea branch and emerged from a prokaryote-like, thermophilic, last common ancestor (for reviews see Forterre and Gribaldo 2007; Brack et al. 2010). The first life forms may, therefore, have been thermophilic, anaerobic chemoautotrophs, similar to those that today inhabit high-temperature habitats, notably the marine and continental hydrothermal springs (e.g., Stetter 2006). Some of the oldest evidences for life have been observed in hydrothermally controlled paleoenvironments, such as in the Pilbara (Western Australia) and the Kaapvaal (South Africa) Cratons (e.g., Schopf et al. 2002; Van Kranendonk et al. 2007; Westall et al. 2011; Hofmann 2011), that appear compatible with a thermophilic and non-photosynthetic metabolic pathway (e.g., Stetter 2006; Abramov and Mojzsis 2009; Sleep 2010; Golding and Glikson 2011).

Modern hydrothermal vent systems are regulated by thermodynamic and geochemical environments able to sustain diverse microbial ecosystems (Fig. 1.5). Because of suitable conditions to favour the emergence of life (e.g., Russell and Hall 2006; Sephton and Hazen 2013; Barbieri and Cavalazzi 2014), these systems – especially those that occur in continental (sub-aerial) areas, with shallow magma plumes generated by surface volcanic hot spots, such as at Yellowstone, El Tatio, and Dallol (Fig. 1.6) – are now intensively studied to understand the chemical and geobiological processes that underlie them.

1.4.1 From Primordial Thermophiles to Modern Cnidaria: A Red (Hot) Thread?

Whether the last common ancestor was a thermophile or not, hydrothermal environments host heat-loving, life forms from the beginnings to the present day. Moving on from the world of prokaryotes to that of animals, the discovery of microorganisms growing in the boiling hot springs of Yellowstone National Park (Brock and Brock 1966) nearly 50 years ago, and that of the deep sea hydrothermal communities 10 years later (Corliss and Ballard 1977), has allowed the recognition of previously unthinkable relationships between animals and hot habitats, such

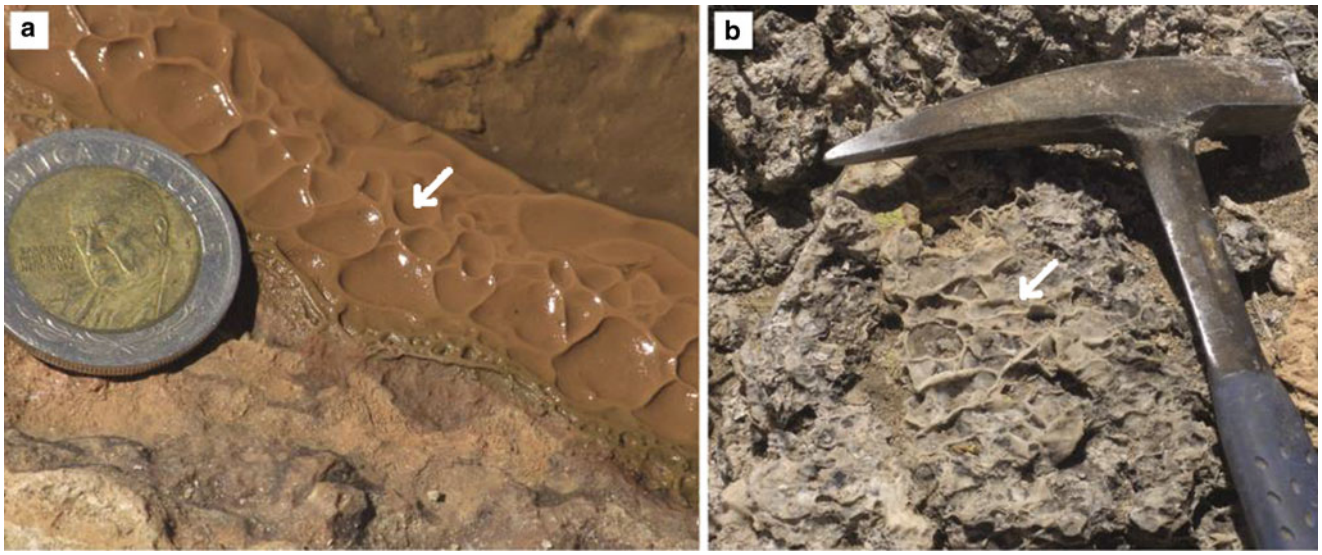

Fig. 1.5 El Tatio Geyser Field (Chilean Andes): alveolar network (*white arrows*) produced by mucilaginous sheaths of filamentous bacteria along the edge of an output channel from a pool of hot water. In (**a**) the network is fresh (coin for scale), in (**b**) it is permineralized by silica and delivered to the rock record (hammer for scale) (Modified from Barbieri and Cavalazzi 2014)

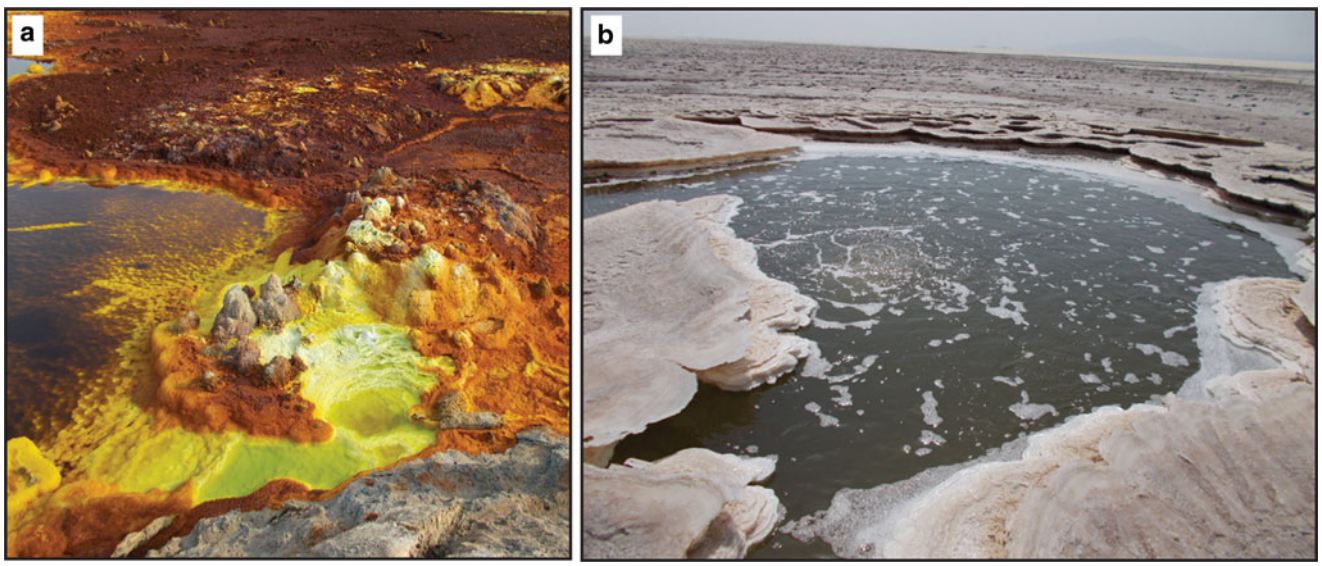

Fig. 1.6 The most extreme environment on Earth is the Dallol geothermal field in the Danakil depression, Afar region, Ethiopia. Here, the strongly salty and acidic water can reach pH well below 1 and temperatures exceeding 100 °C. Hydrothermal spring field (**a**) and perpetual boiling pool (**b**) sourced by deep subsurface volcanic activity

as new demands on metabolisms and symbiosis that are only partially known. Cnidaria also contribute to the composition (and endemism) of hydrothermal vent faunas (e.g., Wolff 2005). Similarly to modern ones, also the thermophilic fossil corals – that go back to the Devonian (Berkowski 2004; Bełka and Berkowski 2005) – belong to Anthozoa, the largest class of cnidarians. How these corals (living and fossil) interact with other components of the thermophilic communities is a subject of speculation that could also preclude real relationships with the current descendants of primitive prokaryotes. Recent results that would indicate the absence of symbiosis between chemoautotrophs and cnidarians (Childress and Girguis 2011), for example, seem to go in this direction.

1.4.2 Primordial Thermophiles in the Light of Panspermia Hypothesis

On Mars examples of hydrothermalism, especially impact-induced hydrothermalism and venting have been detected in a number of regions (e.g., Schulze-Makuch et al. 2007).

These sites have a great importance in an astrobiological perspective and in the current debate on the (past) Martian habitability. Because of the presumably Noachian age (a geologic period roughly equivalent to the Earth's Hadean and early Archaean, i.e. when life probably appeared) of a number of these hydrothermal-interpreted environments and rocks, a relationship with the hypothesis of panspermia is evident. Microbial life exchanges between Mars and Earth, and vice versa can therefore be taken into account when considering the great ability to survival of hyperthermophilic microorganisms (Stetter 2006; Lunine 2013). This sheds new light on interplanetary travel of living matter and allowing realistically considering life and its emergence as an event not necessarily confined to our planet.

1.5 Summary

Long history of Earth changes have strongly influenced and driven the life evolution since autocatalysis became life and mutating population of microbes continuously and efficiently adapted to these transformative planetary forces. At whatever time life emerged in the Hadean or Archaean Eons, it changed forever the fate of our planet.

Crucial questions or details about the origin of life are probably still waiting to be discovered, or are loosed forever with plate tectonics or impacts. However, all life forms evolved from earlier species of organisms, all living species continued and continue to evolve by natural selection and to co-evolve with the geosphere. Earth will host life and life will continue to occupy all suitable niches for billions of years, and they will adapt and adjust each other's in unique co-driving forces.

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Integrated Evolution of Cnidarians and Oceanic Geochemistry Before and During the Cambrian Explosion

2

Jian Han, Xingliang Zhang, and Tsuyoshi Komiya

Abstract

Molecular phylogenetic analysis reveals that the splitting of the phylum Cnidaria into Anthozoa and Medusozoa probably took place in deep time as early as Cryogenian–Ediacaran (720–635 Ma), a long period with low dissolved oxygen concentration in the ocean. However, the pelagic life-style of medusae and the skeleton construction of anthozoans are expensive oxygen-consuming behaviours. Thus the advent of these evolutionary events, including the medusoid phase with statoliths necessary for medusa swimming, should be constrained by Neoproterozoic–Cambrian ocean geochemical environments, particularly the progressive oxygenation of Neoproterozoic–Cambrian ocean. This is well consistent with the dominance of Ediacaran–Cambrian cnidarian fossils by polypoid forms and later rise of medusae.

Keywords

Cnidaria • Oxygen • Cambrian explosion • Ediacaran • Statolith

2.1 Introduction

The Cnidaria is a diverse group of relatively simple diploblastic animals characterized by a highly complex cellular product, the cnida. Extant forms consist of five classes, namely the class Anthozoa (Tabulata, Rugosa, Hexactinaria and Octocoral) and four medusozoan classes, i.e. Cubozoa, Hydrozoa, Scyphozoa, and Staurozoa. Anthozoans include sea anemones, corals with calcareous exoskeletons, and sea pens with endoskeletons. Medusozoans are represented in general by animals with a three-phase life cycle, i.e. planula, sessile polypoid and pelagic medusoid stages. As the Cnidaria is generally accepted as a sister group of the Bilateria, a better understanding of all aspects of cnidarians is of critical importance to investigate the evolution of metazoans. The origin of the Cnidaria, including the biomineralization of corals and the multi-phase life cycle of medusae, has been widely approached from developmental biology (Kraus et al. 2015), molecular and morphological phylogenetic analysis (Bridge et al. 1995; Marques and Collins 2004), as well as paleontology (Van Iten et al. 2014; Young and Hagadorn 2010). According to the molecular clock estimate, cnidarians, together with sponge, ctenophores, and bilaterians, emerged in the Cryogenian Period (Erwin et al. 2011; Park et al. 2012; dos Reis et al. 2015). Combination of molecular and paleontological analyses suggests that the splitting of medusozoans and anthozoans took place about 531.5–641.8 Ma ago (Fig. 2.1) (dos Reis et al. 2015) and the medusa phase might have been independently derived from polyps in different classes of medusozoans (Collins 2002; Collins et al. 2006; Marques and Collins 2004). However, a better understanding of cnidarian evolution additionally requires an integrated knowledge of the physical and chemical condition of the ancient ocean.

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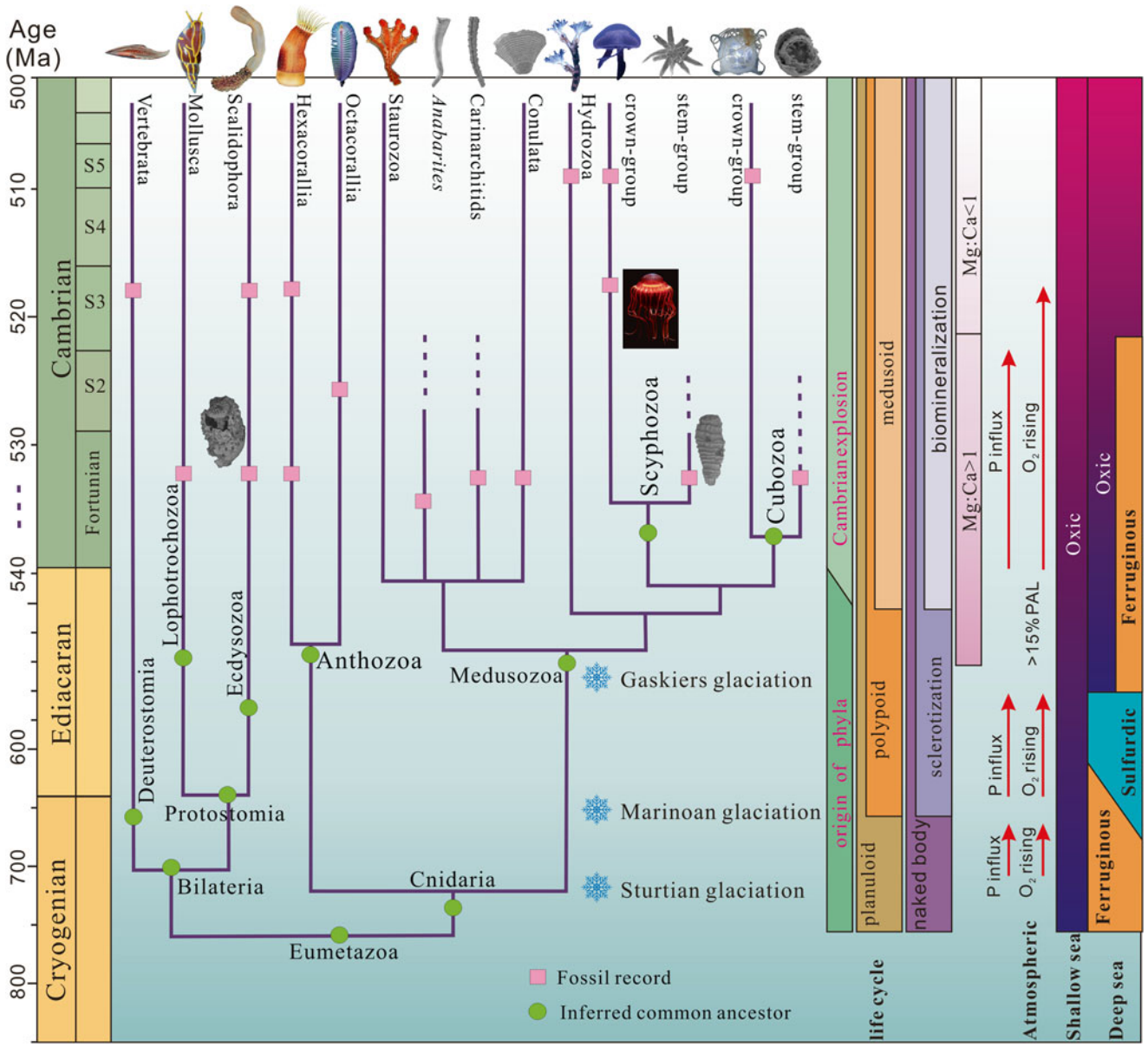

Fig. 2.1 Neoproterozoic–Cambrian geological events and the inferred phylogeny of fossil cnidarians (Modified from Zhang et al. 2014 and Han et al. 2016a)

In the past 20 years, increasing geochemical data of Neoproterozoic–Cambrian ocean redox suggested the composition of seawater had changed dramatically. Particularly the concentration of oxygen, although lacking of precise value, were probably rather low in Cryogenian but reach near-modern value for first time perhaps during Ediacaran–Cambrian (635–542 Ma) (Canfield et al. 2007, 2008; Chen et al. 2015). Oxygen is absolutely essential for metazoans maintaining many metabolic and physiological processes, which allows for effective aerobic respiration, mobility, body size increase, skeletonization, and synthesis of collagen and cholesterin (Zhang et al. 2014). Therefore, it strengthens previous hypothesis that the explosive radiation of animals were kick-started by the rise of O_2 level in the atmosphere and ocean (Nursall 1959; Berkner and Marshall 1965; Cloud 1968; Xiao 2014). Generally, it was further proposed that Cryogenian animals were probably dominated by osmotrophic and microphagous feeders that demand relatively low levels of oxygen; whereas motile and actively feeding animals became more prominent toward the late Ediacaran and early Cambrian periods (Fedonkin and Rich 2007; Xiao 2014). However, up to date, little attention has been specifically focused on the relationships between the rise of oxygen and the evolution of cnidarians. Here, we attempt to provide a preliminary link between the theoretical cnidarian ancestors and their fossils records at the background of

Neoproterozoic–Cambrian ocean geochemistry, especially the oxygen level.

2.2 Neoproterozoic–Cambrian Ocean Geochemistry and Oxidation Events

Seasonally or permanently hypoxic environments in modern ocean have significant impact on distribution and mortality of organisms (Purcell et al. 2001). However, the oceanic environments remain anoxic during the first 2.5 billion years of Earth history. Overwhelming geochemical data suggested a stratified ocean throughout the most time of the Proterozoic Eon (Kendall et al. 2012; Lyons et al. 2012). The surface oceans were mildly oxygenated after the initial rise of atmospheric O_2 at the beginning of the Proterozoic, i.e. the Great Oxidation Event (GOE) of 2.4–2.1 Ga, but the deep ocean was still anoxic, ferruginous or euxinic (Anbar and Knoll 2002; Shen et al. 2003; Komiya et al. 2008a). When ferruginous condition prevailed in the open ocean, euxinic water bodies rimed the global ocean as "dynamic wedges" at mid-water depths (Li et al. 2010) or were limited to restricted, marginal marine basins. These possibilities of the marine redox structure likely existed at different times and also at the same time in different places of the ocean (Lyons et al. 2012).

It is evident that the surface oceans were oxygenated long before the Cambrian radiation of metazoans. The problem is the lack of precise values of oxygen level before and during initial appearances of diverse metazoan fossils. Therefore, it is difficult to determine when oxygen concentrations in shallow water rose above the critical requirement capable of supporting the metazoan diversity. The atmospheric Po_2 was estimated to be approximately 0.01–10 % of the present atmosphere level (PAL) after the Paleoproterozoic GOE, which may be not sufficient for maintaining animal diversity (Zhang and Cui 2016) because the critical level of Po_2 are *ca.* 1 % PAL for biosynthesis of collagen hydroxyproline (Towe 1970), *ca.* 1–10 % PAL for 0.2 to a few mm in size or in tissue thickness without a circulatory system (Catling and Claire 2005; Raff and Raff 1970; Runnegar 1991), and *ca.* 1 % (1 mm in size) to 100 % PAL (10 cm in size) for motility of animals without a circulatory system (e.g. Runnegar 1991).

Geological and geochemical evidence suggested a second rise of atmospheric oxygen level during Neoproterozoic–Cambrian transition, which is crucially important for metazoan diversification (Zhang et al. 2014). The ocean redox models suggest the oxygen level varying between 15 and 40 % of PAL during the Ediacaran–Cambrian period (Canfield 2005; Canfield et al. 2007; Kump 2008). It is worth mentioning that cerium anomaly recognized in South China suggested a stepwise rise of oxygen levels in the shallow marine environments during the Ediacaran Period (Komiya et al. 2008a; Ling et al. 2013). These studies also implied that shallow oceans remained anoxic or suboxic before 551 Ma, and became well oxygenated afterward. The record of U contents of shales also implied that the atmospheric oxygen content was constantly rising during the Ediacaran–Cambrian transition (Partin et al. 2013; Kendall et al. 2015). Uranium and Molybdenum isotope ratios of black shales in the Member IV (M4 in Fig. 2.2) of the Doushantuo Formation indicate that seawater became oxic during the late Ediacaran (Kendall et al. 2015). Molybdenum isotope data from South China suggest the areal extent of oxygenated bottom waters increased in the early Cambrian and modern-like oxygen levels characterized the ocean at ~521 Ma for the first time in Earth history (Fig. 2.2) (Chen et al. 2015). Although the geochemical proxies of atmospheric O_2 and redox state of seawater are not sensitive to highly oxic condition because most are changeable under the transition from anoxic through suboxic to oxic conditions; these results suggest that seawater became oxic in the terminal Ediacaran to the early Cambrian, fairly compatible with the fossil record that metazoans suddenly occurred in abundance in the early Cambrian. The emergence of cnidarians across the Cryogenian–Cambrian transition must have been intercalated with most part of Neoproterozoic–Cambrian Oxidation events.

In addition, recent comprehensive geochemical studies of drill core samples in South China give more detailed view of secular changes of redox condition and some nutrient contents of seawater from the Ediacaran to Early Cambrian. Figure 2.2 shows a summary of the chemostratigraphies of C, O, radiogenic and stable Sr, and Ca isotopes and Fe, Mn, REE (Ce anomaly) and P contents of carbonates or carbonate rocks, Mo isotope values of black shales and nitrogen isotopes of organic matter (Ishikawa et al. 2008; Komiya et al. 2008a, b; Tahata et al. 2013; Sawaki et al. 2008, 2010, 2014; Shimura et al. 2014; Kikumoto et al. 2014; Kendall et al. 2015). The C, radiogenic and stable Sr, and Ca isotopes are considered as proxies for primary productivity and biological activity, continental influx, and strontium and calcium contents of seawater, respectively. Phosphorus, Fe and Mn contents and Ce anomaly of carbonates provide phosphate, Fe and Mn contents and redox condition of seawater, respectively. Nitrogen isotopes of organic matter and Mo isotope values of black shales give estimates of nitrate and Mo contents of seawater as well as the redox condition (Fig. 2.2). Because the detailed description and interpretation of each proxy are beyond topics of this paper and also mentioned elsewhere, this paper presents the summary of proxies related with redox state and nutrient contents of ancient seawater.

Fe, Mn and phosphorus contents, Ce anomalies and Fe speciation indicate that the seawater was relatively less oxic until the Gaskiers glaciation and became oxic after then (Fig. 2.1, see also Canfield et al. 2008; Shen et al. 2008). The

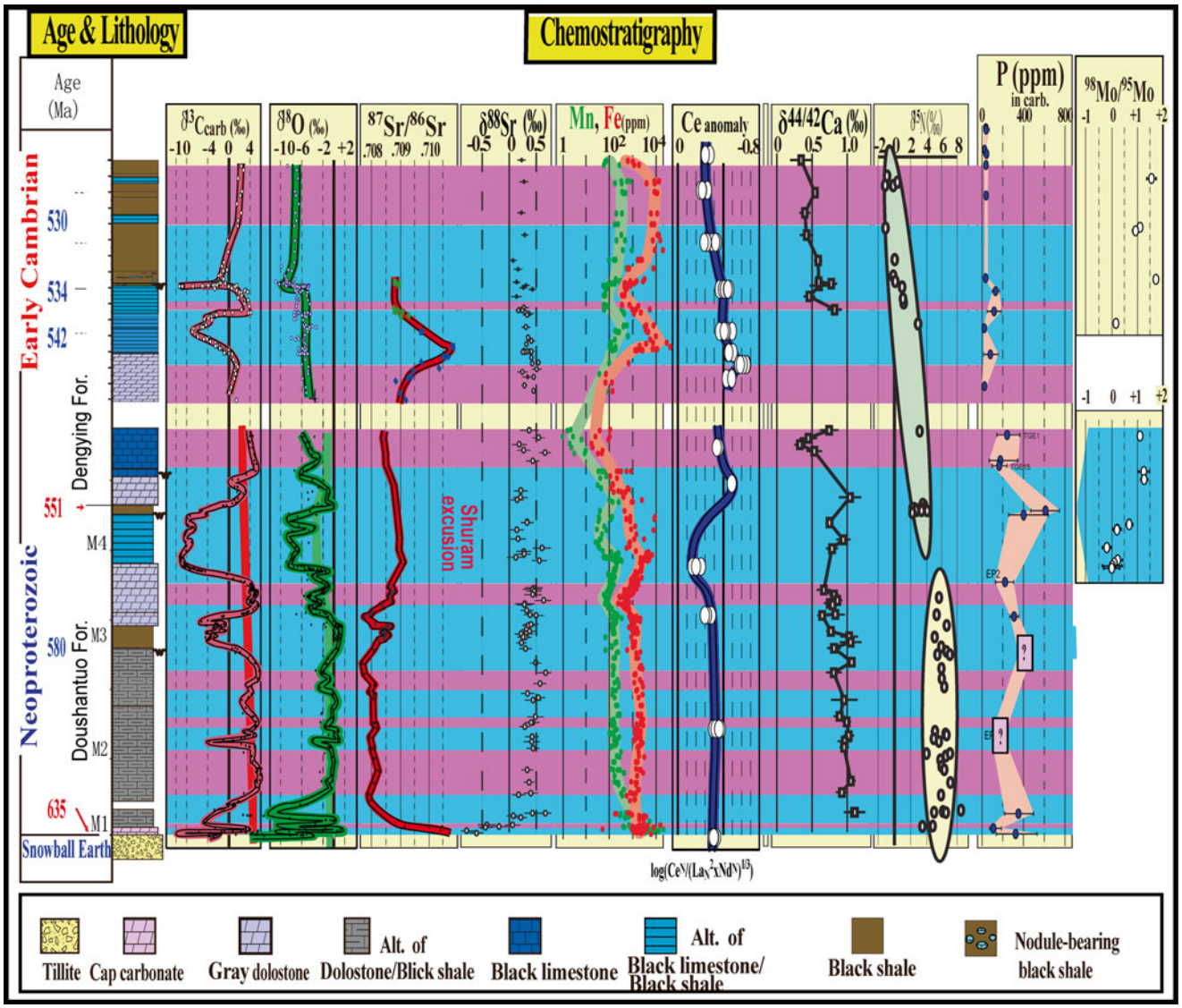

Fig. 2.2 Evolution of the seawater composition through Cryogenian–Cambrian illustrated by an integrated chemostratigraphy of drilling sections in the Three Gorge area, South China

oxidation in the terminal Ediacaran is accompanied with increase of nitrate and calcium contents of seawater as well as the Mo contents (Fig. 2.2). The oxidation of seawater and increase of nitrate contents favored emergence of motile animals whereas the increase of Ca contents resulted in calcium biomineralization such as *Cloudina*, *Sinotubulites* and probably sponges with spicules.

2.3 Physiological Response of Cnidarians to Oxygen

Laboratorial physiological experiments (Condon et al. 2001; Ishii et al. 2008) and field observations (Purcell et al. 2001) reveal that cnidarians require oxygen to maintain their life cycle, including planula, polypoid, and medusoid phases. In addition, the skeletal formation of polyps, especially corals, is also affected by oxygen depletion.

2.3.1 Planulae

The ciliated planulae, approximately 80–2000 μm in length, are microphagous feeders capable of settling, crawling or swimming. Although different cnidarian species can tolerant different level of dissolved oxygen; but generally, benthic planulae need less oxygen than medusae. Laboratory settlement experiment reveals that most planulae of *Aurelia aurita* can survive at least 48 h with dissolved oxygen concentration as low as 0.2 mlO^2L^{-1}; but those of the scyphomedusa

Cnyaneac apillata, showed abnormal attachment orientation in reduced dissolved oxygen concentration and high mortality at 0% oxygen (Brewer 1976). Different oxygen level affect the settlement ratio of planulae and a decrease in dissolved oxygen concentration promote planula settlement; it well consists with the decrease of their respiration rate after settlement (Okubo et al. 2008) from 3.5 to 1.05 $\mu lO_2DW^{-1}h^{-1}$ (Ishii et al. 2008). According to the Blastea-Planuloid theory, the sexually reproducing, bottom-pelagic planulae were the proxy of primitive cnidarians for the pre-polyp ancestral condition (Salvini-Plawen 1978). Taken together with the long hypoxic environment of Neoproterozoic ocean, it is reasonable to perceive tiny settling or crawling planulae, possibly including minute polyps, as the best candidates of cnidarians adaptive for extreme low oxygen during Cryogenian–Ediacaran. Probably the rise of dissolved oxygen concentration can promote the transition from settling or slowly-crawling planulae to swimming planulae although the dispersal distance of the latter are rather limited.

2.3.2 Polyps

Solitary polyps, which have at least four modes of locomotion (Robson 1971), develop organs such as mouth, extensible tentacles and a stomach with or without mesenteries, thus demanding a vascular system for oxygen transportation. The metagenesis from planulae to predatory polyps most probably represents the escalation of mobility, body size as well as improved efficient of reproduction.

The minimum requirement of dissolved oxygen concentrations for polyps is poorly known. But oxygen level apparently affects the size and asexual reproduction of scyphozoan polyps (Condon et al. 2001; Ishii et al. 2008). With a dissolved oxygen concentration above 2 mlO^2L^{-1}, the polyps of *Aurelia aurita* can grow well, but they can't survive for 7 days with oxygen concentration below 0.2 mlO^2L^{-1}. Some species of sea anemones under low oxygen conditions were measured with an oxygen consumption rate ranging from 30 to 100 $\mu l\ g^{-1}h^{-1}$ (Sassaman and Mangum 1972). These studies suggest that the meiofaunas appear to be more broadly tolerant to oxygen depletion than macrofaunas (Levin 2003), and simplicity of internal organization is adaptive for low or unstable oxygen environments.

As above mentioned, the appearance of cnidarians, either in planula or polypoid forms, must have been intercalated with early part of Neoproterozoic–Cambrian ocean oxidation events. As the stepwise rise of dissolved oxygen concentration, the evolutionary transition of ancestral polyps from minute planulae might have undergone a long period of time as it will involve a series of evolutionary innovations, including the increase of body size, the invention of gastric cavity, the mouth and further partition of gastric cavity by mesenteries, formation of tentacles, and the development of several types of nematocysts (see David et al. 2008) followed by the invention of predatory habit and the development of different reproduction strategies. As the polyps differ so remarkably from their planulae, a diversity of intermediate forms between polyp and planulae might have been invented during this transition.

2.3.3 Skeletonization of Polyps

Except for protective function, skeletonization (biomineralization and sclerotization) among extant five classes of cnidarians, although following markedly different plans of skeletogenesis (Sorauf 1980), may also be related to the oxygenation of environments. Extant hexacorallians, octocorals, and some hydrozoans, secret calcite skeletons such as septa, epitheca, tabulae, spicules. These skeletons are invariably mediated by organic matrix (Sorauf 1980; Lowenstam and Weiner 1989).

The advent of widespread biomineralization among metazoan lineages at the Neoproterozoic–Cambrian transition has been proposed as a result of either paleoceanographic changes or an ecological process due to competitive predator-prey interactions (Bengtson 2002; Knoll 2003). Generally, the formation of biomineralized hard parts consumes more energy for organisms transporting ions across the cell membrane. Similarly, the locomotion loaded with heavy skeletons invariably demands much energy. Thus, the advent of biomineralization may have also been constrained by the raised dissolved oxygen concentration at the Neoproterozoic–Cambrian transition. This coincides with the modern oceanographic observations that marine invertebrates with biomineralized skeletons require more oxygen, and those calcified invertebrates are absent where oxygen levels fall below 0.3 mlO^2L^{-1} (Levin 2003). The oxygen minimum zone (OMZ) (<0.5 mlO^2L^{-1}) in the extant ocean are dominated by soft-bodied forms among the metazoans (Levin 2003).

Modern ocean acidification has been well known as a threat for organisms with calcareous skeletons, such as reef-building corals (Hoegh-Guldberg et al. 2007). Nonetheless, the hypothetical stratified Ediacaran ocean (Li et al. 2010) will produce a faintly acidic water body with a PH (Komiya et al. 2008a; Ohnemueller et al. 2014) lower than current 7.9 of modern alkalescent ocean (Winans and Purcell 2010), thus disfavoring the precipitate of calcite that is the major mineral for biomineralization.

The sclerotization of extant cnidarians is mainly represented by mesoglea of body wall, the periderm of polyps, cyst of planulae, and perisarc of colonial hydrozoan polyps. As inorganic minerals generally precipitate on an organic matrix, the evolution of organic matrix, or sclerotization within a phylum, may have preceded the appearance of

biomineralization (Bengtson et al. 1990). The most primitive forms can be further inferred being nonskeletonized members, but may not be successful in competition with those of skeletonized forms under oxic conditions. This case is well illustrated by octocorals. Large solitary octocorals are rather rare in modern ocean (Bayer and Muzik 1976) in comparison with those of colonies with calcite spicules (i.e. Gorgonian) that are more success in ecology. However, octocorals with organic matrix within the spicules of gorgonian were also reported. Octacoral, and other cnidarian classes, might have experienced enlargement, sclerotization and biomineralization in response to Ediacaran–Cambrian oceanic oxygenation.

2.3.4 Medusae

The metamorphosis from minute sessile polyps to large pelagic medusae apparently demands relatively more dissolved oxygen in the ocean. Extant medusae differ from their polyps in many aspects, for instance: (1) larger body size without a stalk; (2) thicker mesoglea; (3) more types of nematocysts; (4) longitudinal muscular cords in polyps versus coronal muscles in medusa; (5) up-side down orientation in life position; (6) sense organs (statocyst and ocelli) leading to periodical contraction of the bell for respiration and swimming, that finally leading to positive predation and wider dispersal; (7) devoid (or loss) of exoskeletons in medusoid form; (8) hypostome in polyps versus extensible manubrium with oral lips in medusae; (9) development of a canal system; (10) specialized tentacles. Thus, medusae are not merely up-side down polyps as previously suggested (Thiel 1966), but its rise may have involved a suite of morph-anatomical and functional innovations. Some of these innovations, such as first, sixth, and ninth items, were apparently related to the rise of oxygen. For instance, the respiration rate of medusae increases with increased body size or mass (Arai 1997). In another words, meiofaunas appear to be more broadly tolerant to oxygen depletion environment than macrofaunas (Levin 2003). Items of first to fourth, seventh, eighth, and tenth are adaptive for a pelagic and predatory life, thus their appearances indirectly reflect a higher dissolved oxygen level in the ocean.

In summary, the advent of planulae, polyps, skeletonization of polyps and medusae, was not only a suite of pure evolutionary innovations in mirror with life cycles, but also a process potentially remotely related to the course of Neoproterozoic–Cambrian oceanic oxygenation. Thus it deserves a fresh look at the fossil record of early cnidarians.

2.4 Fossil Record of Cnidarians

2.4.1 Fossils Records of Planulae

The fossil record of planulae in the geohistory is lacking. However, early prehatched stage of *Olivooides* (Fig. 2.3b, c) from early Cambrian Kuanchuanpu Formation (Fortunian Stage, ca. 535 Ma) in South China, was proposed potentially equivalent to the planula stage enveloped within a cyst (Bengtson and Yue 1997; Han et al. 2016b) because: (1) embryonic *Olivooides* has a size close to extant planulae; (2) the completely closed globular stellate cyst of *Olivooides* in early prehatched stage (Fig. 2.3b, c; see also Bengtson and Yue 1997; Dong 2009; Yue and Bengtson 1999) was most likely secreted by the entire surface of epithelium of planulae or earlier embryonic stage; the stellate spines potentially correspond to the evenly-distributed planula cilia, each of which is surrounded by a circle of microvilli (Fig. 2.3c; see Hirose et al. 2008); (3) additionally embryonic cyst occasionally exhibiting a small area with centripetal-directed stellae indicates the position of the blastopore and animal pole, as previously suggested (Hua et al. 2004); (4) the later serial addition of dense longitudinal striations on the juvenile and adult tube wall was likely secreted by the epithelium of the oral end. The change of cyst ornament (Fig. 2.3d, e) probably reflects a metamorphosis from planulae to polyps associated by reduced cilia on the oral epithelium. Nevertheless, the above arguments need fossil evidence.

2.4.2 Fossils Records of Polyps

2.4.2.1 Soft-Bodied Cnidarian Polyps

A number of forms have been reported from the Ediacaran Period. Their phylogenic positions, however, remain problematic. A suite of tubular microfossils, i.e. *Sinocyclocyclicus*, *Ramitubus*, *Crassitubus* and *Quadratitubus* from the Ediacaran phosphorites (580 Ma) of South China, were previously interpreted as soft-bodied animals comparable to tabulate corals (Chen et al. 2002; Xiao et al. 2000). But recent analysis by X-ray tomographic microscopy supported that the features of their internal cross walls, originally interpreted as tabulae, are incompatible with a cnidarians interpretation (Cunningham et al. 2015). *Eolympia* (Han et al. 2010) and an unnamed phosphatized microfossil (Steiner et al. 2004) from the lowest Cambrian of South China exhibit a whorl of tentacles, a central mouth, radial septa, and a basal pedicles (Fig. 2.3h), and thus can be more confidently inter-

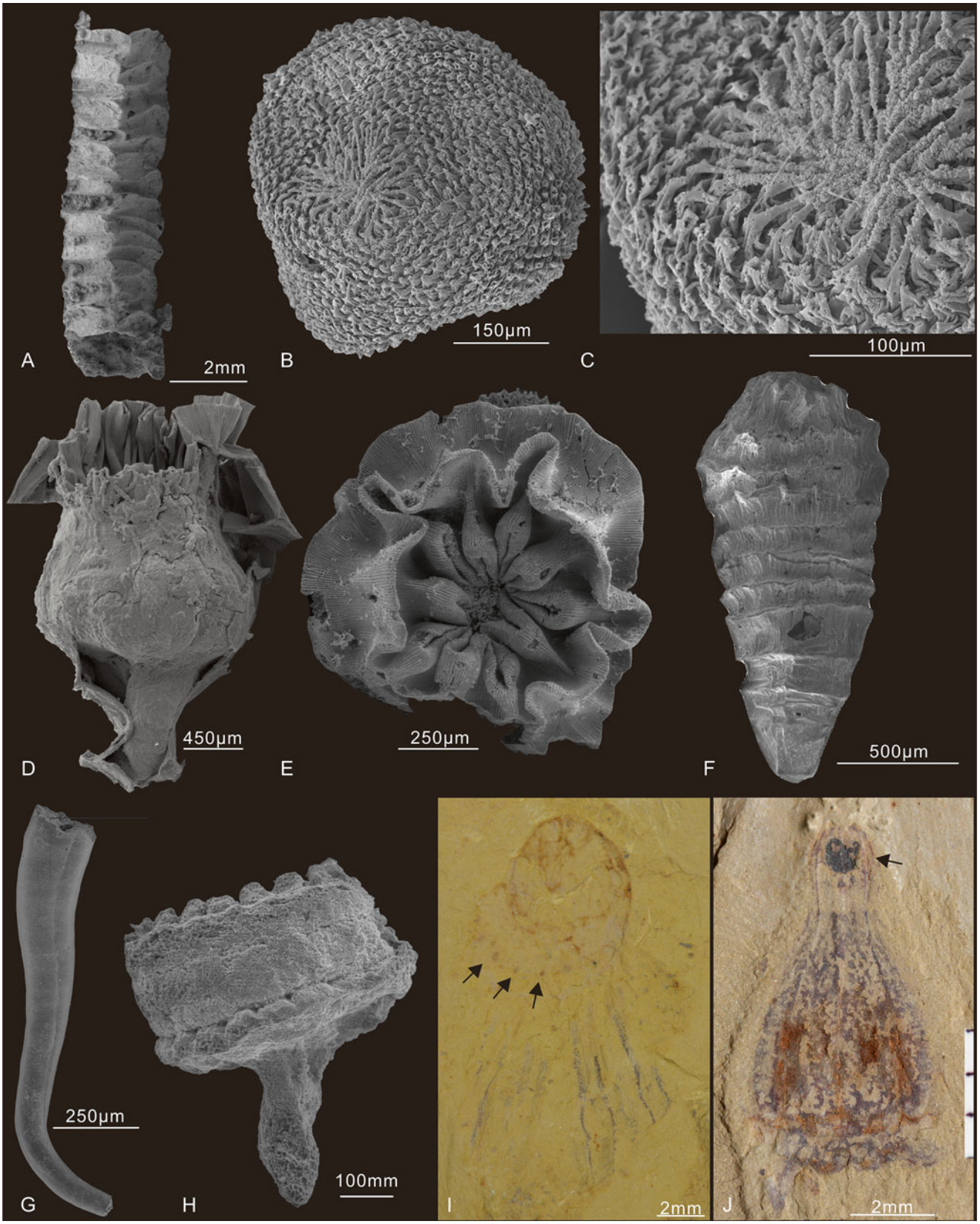

Fig. 2.3 Representatives of fossil cnidarians during Ediacaran–Cambrian transition. (**a**) *Sinotubulites triangularis* Cai et al. 2015 (Courtesy of YP Cai). (**b**–**g**), small shelly fossils from early Cambrian Kuanchuanpu Formation in south China. (**b**–**e**). *Olivooides mirabilis* (Steiner et al. 2014). (**b**, **c**), Prehatched stage with embryos enclosed by a stellate cyst (**b**); a small area with centripetal-directed stellae. (**d**) A young individual showing the internal polyp with a stalk inside the peridermal tube (courtesy of M. Steiner). (**e**) The oral disc of the young tube. (**f**) tetraradial *Quadrapyrgites ningqiangensis*; (**g**) Triradial *Anabarites trisulcatus*. (**h**) *Eolympia pediculata* Han et al. 2010. (**i**) *Yunnanoascus haikouensis* Hu et al. 2007 (Courtesy of SX, Hu), a pelagic medusa with rhopalia from Chengjiang biota in south China. (**j**) Undetermined ctenophore with apical statocyst from Chengjiang biota. Statoliths in (**i**–**j**) are indicated by *arrows*

preted as cnidarian polyps. *Archisaccophyllia*, a centimeter-sized polyp from the Chengjiang fauna, shows a whorl of 12 tentacles, 12 mesenteries, and probably a pedal disc, was believed to be an ancient sea anemone (Van Iten et al. 2014; Hou et al. 2005). Notably *Archisaccophyllia* resembles *Xianguangia* in several aspects, but the 'tentacles' of *Xianguangia* are found with stiff setae (Hou et al. 2005) highly reminiscent of lophophore tentacles of co-occurring brachiopods (see Zhang et al. 2009). Thus, the zoological affinities of *Archisaccophyllia* and *Xianguangia* remain unresolved.

2.4.2.2 Skeletonized Polyps

Some macrofossils with possible "tentacles" and a cone-shape "theca" from the Liantian biota (600 Ma) in South China were suggested as cnidarian-like organisms (Yuan et al. 2011) or conulariid cnidarians (Van Iten et al. 2013), thus indicating a greater amount of free oxygen in the Ediacaran ocean (Yuan et al. 2011). However, narrow strip-like bands extending through the 'theca' of these organisms are more or less similar to those of co-occurring algae.

The earliest fossil record of biomineralization of metazoans occurs at the late Ediacaran (ca. 550 Ma), as represented by tubular fossil *Cloudina* (Hua et al. 2005; Wood and Curtis 2015) which is characterized by a calcareous composite tube with "funnel in funnel" structure. The appearance of calcified forms in the late Ediacaran is consistent with the geochemical data that suggest an oxygen level close to modern oceans (see also Fig. 2.3). However, the affinity of *Cloudina* remain controversial, which has been interpreted as either annelids (Hua et al. 2005), stem-group metazoans, or mostly accepted as cnidarians capable of asexual reproduction (Knoll 2003; Van Iten et al. 2014; Vinn and Zaton 2012). Co-occurring *Sinotubulites* with a similar "funnel in funnel" structure from Late Ediacaran Gaojiashan biota in South China (Fig. 2.3a) exhibits flexible organic dominated tube walls with weak mineralization (Chen et al. 2008). In terms of ecological tiering essential for sessile suspenders, the cone-shaped, "funnel in funnel" tube construction of *Conotubus* (Cai et al. 2011) is more efficient than that of "cylinder in cylinder" as illustrated by *Sinotubulites*. The multiple symmetric patterns (0, 3, 5, and 6) (Cai et al. 2015), the constantly oriented longitudinal ridges (Fig. 2.2b in Chen et al. 2008); Figs. 2.2 and 2.3 in Sun et al. 2012; Cai et al. 2015), and the irregular transverse crests on a single layered tube wall of *Sinotubulites* are highly reminiscent of extant scyphopolyp periderm (Werner 1973), early Cambrian tubular fossils (i.e. *Anabarites*, Fig. 2.3g), and conulariids (Cai et al. 2015), as well as peridermal theca secreted by sessile polyp-shaped *Olivooides* and *Quadrapyrgites* (Liu et al. 2014b; Steiner et al. 2014). Such coordinate-oriented tubes were more likely secreted by an inactive sedentary soft-tissue with only circular and longitudinal muscles, instead of a free, twistable worms with complex muscles. In addition, the lacking of fossil evidence for soft tissues inside *Sinotubulites* tube is incompatible with the cuticularized integuments and setae of extant annelids resistant to decay. Therefore, *Sinotubulites* and other relatives are more acceptable as sessile polypoid cnidarians with organic or weak biomineralized skeletons.

Palaeozoic corals or coralomorphs with calcified skeletons were rather diverse and abundant (Hicks 2006; Oliver 1983). They have been systematically reviewed in many publications (Debrenne and Reitner 2001; Oliver 1996; Scrutton 1997). Here we will focus on three aspects of these fossils related to the oxygen level. (**1**) **Tabulae**, or tabularium, is a horizontal calcified plate serving as a supporting floor for the soft parts (Chen and Dong 2008). It is common in calcitic corallite skeletons of anthozoans (i.e. Tabulata) (Oliver 1983) and some stem-group cnidarians (Park et al. 2011). Most probably it represents an adaptive strategy of sessile polyps for completion of ecological tiering. Notably organic tabulae are also present in early Cambrian medusozoan *Olivooides*. It agrees with the implication of biomineralization that the ancestral condition of calcified tubulae should be organic. (**2**) **The septa of polyps**. Some polyps of extant corals such as octacorals and Edwarsiids of hexactinarians (Daly et al. 2002), and fossil polyps i.e. Cambrian Tabulae (Oliver 1983), *Eolympia* (Han et al. 2010) *Cambroctoconus* (Park et al. 2011) as well as *Lipopora* (Cambrian Stage 4) and *Tretocylichne* (Cambrian Stage 5) from Australia exclusively exhibit a few of fixed-numbered septa (Hicks 2006). Three septa of *Anabarites* (Fig. 2.3g) are indicated by three longitudinal tube furrows. The fixed-numbered septa in extant cnidarian are not always indicative of primitive condition and of phylogenetic significance (Daly et al. 2002). Nevertheless, it can be alternatively interpreted as a reflection of Ediacaran–Cambrian oceanic oxygenation and an increase of Ca contents of seawater from the terminal Ediacaran (Sawaki et al. 2014). The skeletonized Ediacaran cnidarians, restricted by the oxygen level, have limited body size supported by a few of septa. As the rise of oxygen level during Ediacaran–Cambrian transition, their body size increases thus requiring more septa to partition and support the body for better respiration. In comparison with fixed numbered septa, both serial and cycle insertions of septa in anthozoans probably represent derived features. (**3**) **The colony**. Octocoral and Tabulata are colonial forms undoubtedly developed from solitary forms. The earliest fossil reminiscent of octocoral spicules, *Microcoryne*, comes from Lower Cambrian of South Australia (Bengtson et al. 1990). A feather-like frondose taxon, *Chengjianggopenna wangii*, from early Cambrian Chengjiang fauna was interpreted as sea pen of octocorals on the basis of separate branches with regularly-arranged microscopic nodes (Shu et al. 2006). Tabulata, which were probably originated in the Cambrian

but prosperous in Ordovician, developed massive calcified skeletons even reefs; thus they require an oxygen level equivalent to modern corals. The presence of colonized octocorals and tabulates can also be regarded as a biological indicator for the rise of oxygen level.

Although the lack of convincing fossils of planulae and medusae may have been ascribed partly to taphonomic bias, the dominance of sedentary polypoid forms which are more tolerant in oxygen-deficient condition than medusae is an indisputable fact that was most likely related to the Neoproterozoic–Cambrian oceanic oxygenation.

2.4.3 The Rise of Medusae and Their Fossil Records

Fossil medusae have been the subject of recent reviews (Van Iten et al. 2014; Young and Hagadorn 2010). Here we would like to concentrate on the rise of medusae based on the evolutionary, morphological and geochemical constrains. The disc-shaped fossils from Ediacaran biota (ca. 580–541 Ma) (Glaessner 1984) have concentric rings and radial lines, which were respectively interpreted as ring canals and radial canals. However, these disc-like objected were alternatively regarded as holdfasts of frond-like organisms unrelated to cnidarians (Debrenne and Reitner 2001; Young and Hagadorn 2010). Here we provide four additional arguments against cnidarian affinity: (1) A medusa-interpretation does not merit as the ring canals and radial canals are result of endodermic fusion of subumbrella and exumbrella (Han et al. 2016a) that have never been seen in polyps and basal lineage of medusozoans, i.e. stauromedusans (See Collins and Daly 2005). (2) It is unlikely to compare them with pelagic colony forms of chondrophoran hydromedusae, likewise a highly derived feature of evolution. (3) Additionally such decimeter-scaled medusae are not congruent with low oxygen level in Ediacaran oceans although we lack precise data of oxygen concentration. (4) Finally, the statocyst with biomineralized statoliths used for keeping balance in extant swimming medusa has not been found in these fossils. Thus presently, no fossil record of pelagic medusae can be confirmed in Ediacaran (Van Iten et al. 2014; Young and Hagadorn 2010). Representatives of major lineages of nektonic medusozoans with umbrellar shape, gonads, strong coronal muscles, and elongate retractile tentacles possibly with nematocyst batteries were present in Cambrian Stage 5 in Utah (Cartwright et al. 2007). Late Ediacaran medusozoans that have survived in recent evaluations, i.e. *Crumbella*, *Vendoconularia* and *Haootia quadriformis* (Liu et al. 2014b), were proposed as polypoid forms (Van Iten et al. 2014), although we know little of their soft-tissues and internal anatomy.

Yunnanoascus haikouensis (Hu et al. 2007) (Fig. 2.3i) from the Chengjiang biota were originally interpreted as a centimeter-scaled ctenophore. Recently we reinterpreted it as a tetraradial pelagic crown-group medusozoan on the basis of diagnostic features of pelagic medusozoans, including 16 pairs of thick-based marginal tentacles, 16 rhopalia, 16 marginal lappets, and a manubrium (Han et al., 2016c). Thus, the onset of medusoid forms should precede Cambrian Stage 3.

Fossil records of millimeter-sized tubular polypoid medusozoans, such as *Anabarites*, olivooids (Fig. 2.3b–f), and carinarchitids, conulariids (Van Iten et al. 2006; Kouchinsky et al. 2009; Kouchinsky et al. 1999; Yue and Bengtson 1999) are abundant and divergent in the Cambrian Fortunian Age (541–529 Ma) (see Fig. 2.4). These minute polypoid medusozoans most likely represent an ecological success and a large-scale radiation of cnidarians prior to the prosperous of calcified Paleozoic corals. As those polypoid medusozoans with a flexible tube are relatively rarer than those with calcite skeletons, their ecological and evolutionary successes were most likely ascribed to the first widespread skeletonization and biomineralization of medusozoans. However, the soft bodied *Olivooides* and their co-occurring relatives, although possessing a sessile aboral stalk in the juvenile stage (Fig. 2.3d) (Steiner et al. 2014), exhibit an internal anatomy more complex than that of extant medusozoan polyps (Dong et al. 2013; Han et al. 2013, 2016a). Some features, such as coronal muscles, frenulae, apertural lappets and a large manubrium (Han et al. 2013, 2016b) are analogous to those of extant adult medusae. Therefore, they can be alternatively termed as polyp-shaped medusae akin to extant stauromedusans (see Collins and Daly 2005), thus highly promising as a suite of intermediate forms between sessile polypoid and pelagic medusoid phases. As sessile forms generally consume less oxygen than pelagic forms, sessile polyp-shaped medusa, as indicated by *Haootia* and stauromedusans, is highly expected in the Neoproterozoic–Cambrian ocean. If correct, the evolutionary transitions from sessile polyp-shaped medusa into pelagic medusae in applicable oxygen level, although varying in different lineages of medusozoans, could be a course of uniformitarianism.

Considering the respiration of these tubular microfossils could give hints for the rise of medusa. As the epidermis of extant polyps directly contacts with seawater, the dissolved oxygen can reach body tissue by both osmotic diffusion and ciliary current. However, early Cambrian *Olivooides* and their relatives need invent a circulatory system for oxygen consumption as the external tube (Fig. 2.3d–f) is a barrier for osmotic diffusion of oxygen. One solution is frequent contraction/relaxation of the soft body by circular muscles that will bring/expel water (also food) to/from the oral side of body surface through tube aperture. The following evolutionary innovations can promote the respiration efficiency. For instance, (i) the invention of a deep subumbrellar cavity can increase the volume of water in one contraction; (ii) the development of coronal circular muscles is helpful in captur-

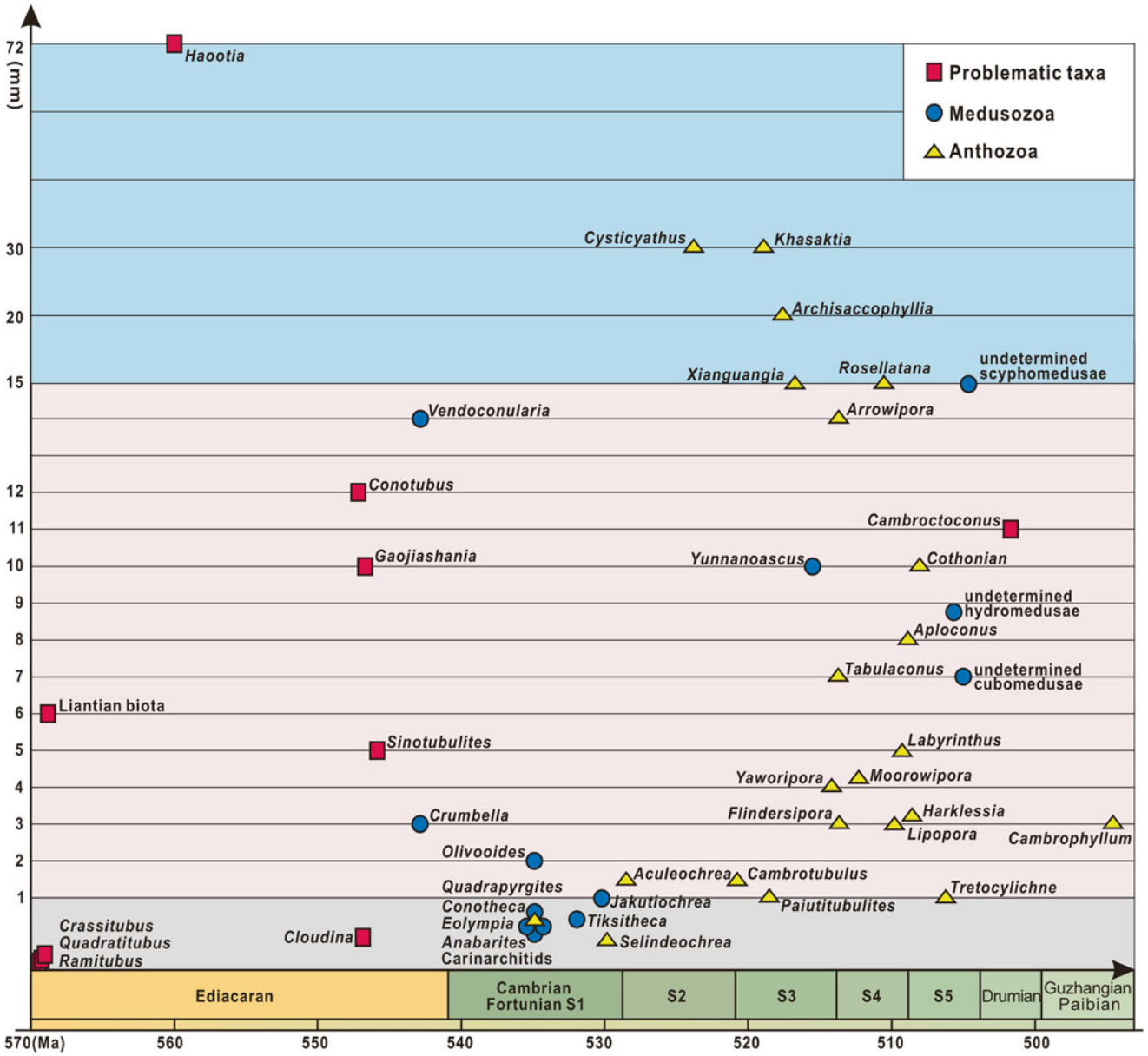

Fig. 2.4 An incomplete statistic of maximal body width of Ediacaran–Cambrian cnidarians. S2–5, Cambrian stages 2–5. The absolute age data and body size of the fossils come from references: (Cai et al. 2011, 2015; Chen et al. 2002; Cartwright et al. 2007; Han et al. 2010; Hicks 2006; Hou et al. 2004; Hu et al. 2007; Hua et al. 2005; Kouchinsky et al. 2009; Liu et al. 2014a, Liu 2011; Park et al. 2011; Van Iten et al. 2013; Yuan et al. 2011)

ing preys; (iii) the frenulae connecting to the apertural lappets seen in the soft body of *Olivooides* (Han et al. 2016b) are also useful for controlling bell and tube aperture.

Swimming of most medusae depends on rhythmic contraction of the coronal muscles to produce a jet of water, and elastic recoil of the mesoglea to restore the resting shape (Arai 1997). Thus, the locomotion mode of extant swimming medusae quite resembles the inferred respiration manner of Cambrian tube-dwelling polyps. One difference between them is that rhythmic simultaneous contraction of circular muscles in the pelagic medusa requires the evolution of nerve control from marginal sense organs. Thus, we propose that the rise of swimming behavior of medusae might be a by-product of respiration of sessile polyps.

2.5 The Geochemical Constraints on the Formation of Medusa Statoliths

Extant medusozoans except for staurozoans have statoliths for gravity sensing. Up to date, when did statoliths firstly appear in the geohistory remains unknown. As currently understood, the statoliths of hydrozoans and ctenophores are

composed of calcium magnesium phosphate, whereas statoliths of scyphozoans and cubozoans are composed of bassanite (calcium sulfate hemihydrate) (Chapman 1985; Singla 1975; Sötje et al. 2011; Tiemann et al. 2006; Tiemann et al. 2002; Becker et al. 2005). Experiments on *Aurelia aurita* (Scyphozoa) revealed that the environmental sulfate for statolith synthesis is incorporated at a very early stage of strobilation (Spangenberg 1968). Statolith formation in the laboratory experiments is almost completely inhibited in sulfate-deficient water; and ephyrae without statoliths usually sink to the bottom or appear as weak swimmers (Spangenberg 1968). Therefore, the statolith formation is limited by the sulfate concentration in seawater.

Sulfate is the second most abundant anion in modern seawater with a concentration of 28 mM (Mills and Vogt 1984). However, the ancient ocean is stratified, sulfidic with extremely low sulfate concentrations (<0.2 mM) before 2.4 billion years ago (Habicht et al. 2002), and the first evidence for elevated sulfate concentrations (*ca*. 20 mM) was near the latest Precambrian and earliest Cambrian (Brennan et al. 2004; Shen et al. 2003). The deposition of sulfate minerals from the ocean became a major sulfur removal pathway until Phanerozoic (Canfield and Farquhar 2009; Li et al. 2010). These phenomena were believed to be results of efficient oxidative weathering of ocean sulfides in response to increased atmospheric oxygen (Canfield and Farquhar 2009). Although sulfate statolith formation in the presumed ancestral medusae might have happened several times at the interglacial periods of three great Neoproterozoic glaciations, severe stratification of water column during the glaciations will sustain permanent oxygen depletion (Canfield and Farquhar 2009; Li et al. 2010) that will be unfavorable for the survival of statoliths; thus the statolith formation might be correlated to the prominent increase of ocean oxygen in the aftermath of the Gaskiers glaciation (ca. 580 Ma) (Canfield et al. 2007, 2008; Komiya et al. 2008a; Li et al. 2010). In this sense, a termination of stratified ocean by the stirring of swimming medusa, is unlikely.

The phosphorus in apatite skeletons of modern metazoans is derived from metabolic P accumulated from foods (Bengtson 2002). However, the primary source of P for phosphate statoliths is still not clear. If it is derived directly from nutrient-enriched bottom waters, the three major phosphogenic events worldwide across the Neoproterozoic–Cambrian (Cook and Shergold 1986) including in south China (Figs. 2.1 and 2.2), which formed as an upwelling ocean current during transgression thus representing the break of stratified ocean, was probably related to the formation of insoluble phosphatic statoliths in both hydrozoans and ctenophores (Chapman 1985; Singla 1975). Additionally, as mentioned above, a faintly acid condition in a stratification Neoproterozoic ocean (Li et al. 2010; Ohnemueller et al. 2014) disfavors the precipitation of calcite; in contrast, insoluble calcium magnesium phosphate and bassanite were more suitable as statoliths.

The fossil record of pelagic medusa provides direct evidence for statoliths. *Yunnanoascus haikouensis*, as mentioned above, is supported to be a crown-group, predatory scyphozoan bearing 16 rhopalia possibly with prominent statocyst (Fig. 2.3i, Han et al. 2016c). Diverse ctenophores with statoliths (Fig. 2.3j) were recently documented from the early Cambrian Chengjiang biota (Hu et al. 2007; Ou et al. 2015). Thus both sulfate and apatite statoliths might have been invented at the Cambrian Stage 3.

Notably, although both seawater sulfate and phosphorus concentrations during Cambrian period were lower than that of the earlier period of the Cambrian (Shen et al. 2003; Shimura et al. 2014), and being fluctuated in the later geohistory (Brennan et al. 2004), ctenophores and cnidarians were proposed rarely switching their mineralogy of statoliths after initial acquirement independently in different lineages (Spangenberg 1968; Porter 2007). Medusae and ctenophores might have independently developed a mechanism to regulate the concentration of sulfate, magnesium, and calcium ions (Mills and Vogt 1984). If correct, high Mg/Ca ratio of statoliths in Leptomedusae, Hyromedusa and tentaculate ctenophores (Chapman 1985) could be further correlated with oscillations of seawater Mg/Ca ratio through Neoproterozoic–Cambrian; and these two clades might be originated at Late Ediacaran–Cambrian Stage II (ca. 550–521 Ma) when seawater Mg/Ca ratios >2 (see Porter 2007; Zhuravlev and Wood 2008; Hardie 1996, 2003).

2.6 Conclusion

We have attempted to integrate fossil records and limited physiological data of extant cnidarians with imprecise geochemical data of Neoproterozoic–Cambrian ocean for a better understanding of the environmental constraints on the evolution of cnidarians. The progressive oxidation of Ediacaran–Cambrian oceans is proposed most likely as an important role in shaping modern-type cnidarians and a critical prerequisite for the evolution of three-phase life cycle of medusae or the skeletons of both medusozoans and anthozoans during the late Ediacaran and Cambrian. However, our preliminary approach is far from conclusive in dealing with such complicated evolutionary event rooted in deep earth history.

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Author Contributions X. Z designed the research. J.H, X.Z, and T.K. wrote the manuscript. J.H. and T.K prepared figures. All authors participated in discussion of conclusions.

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3 Origin and Early Diversification of Phylum Cnidaria: Key Macrofossils from the Ediacaran System of North and South America

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Abstract

Recent molecular clock studies place the origin of phylum Cnidaria within the Cryogenian Period (ca. 850–635 Ma), with the split between the two subphyla (Anthozoaria and Medusozoa) likewise occurring during this time interval. However, the oldest cnidarian macrofossils, all medusozoans, occur in rocks of the late Ediacaran Period (ca. 560–541 Ma). Lightly skeletonized *Corumbella werneri*, currently known from late Ediacaran strata of Brazil, Paraguay and Nevada (USA), has been allied with coronate and conulariid scyphozoans, but it also shares gross morphological similarities with *Carinachites spinatus*, a possible conulariid from Cambrian Stage 1 (China), and it may be compared with *Sinotubulites* and *Wutubus annularis* from the late Ediacaran Dengying Formation (China). The strongest evidence of affinity with coronate scyphozoans is exhibited by *Paraconularia* sp. from a *Corumbella*-bearing shale interval in the latest Ediacaran Tamengo Formation of central Brazil. Furthermore, *Paraconularia* sp. from this rock unit establishes conulariids as a cnidarian clade that crossed the Proterozoic-Phanerozoic boundary. Finally, *Haootia quadriformis* from the late Ediacaran lower Fermeuse and Trepassy formations (southeastern Newfoundland, Canada) exhibits intriguing gross morphological similarities to extant

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staurozoans and may represent the earliest record of metazoan musculature. Together, *C. werneri* and latest Ediacaran *Paraconularia* sp. fix the split between the medusozoan classes Cubozoa and Scyphozoa at no later than ca. 543 Ma. If *H. quadriformis* was indeed a staurozoan or stem staurozoan, then this fossil taxon fixes the split between the class Staurozoa and all other medusozoan cnidarians at no later than ca. 560 Ma.

Keywords
Cnidaria • Medusozoa • Conulariids • Cryogeniam • Ediacaran

3.1 Introduction

Students of phylum Cnidaria have long agreed that the origin of this monophyletic group of diploblastic eumetazoans probably lies well within the Neoproterozoic Era (see for example Van Iten et al. 2014a and references cited therein). The phylogenetic relationships of cnidarians to other metazoans, as well as relationships among the major groups within Cnidaria, have been subject to divergent interpretations, but at present the most widely accepted phylogenetic hypothesis for the five cnidarian classes appears to be ((((Cubozoa, Scyphozoa) Hydrozoa) Staurozoa) Anthozoa). Nonetheless, even classical hypotheses (e.g., the traditional concept of Scyphozoa) come to light from time to time, based on new data (e.g., Zapata et al. 2015). Similarly, molecular clock studies have yielded a broad range of age estimates for the origins of major cnidarian groups, yet all agree that at least some cnidarian clades probably are older than suggested by their fossil records, with, for example, Park et al. (2012) estimating that Cnidaria arose 819–686 Ma, Medusozoa 670–671 Ma and Hexacorallia 684–544 Ma.

Until fairly recently it was widely thought that the hypothesis of a Cryogenian-Ediacaran origin for Cnidaria and its major subclades received direct support from the fossil record, in particular from certain iconic, polyp- or medusa-like soft-bodied taxa (e.g., Glaessner and Wade 1966; Wade 1972) of the three classical Ediacaran assemblages (Avalon, ca. 575–560 Ma; White Sea, ca. 560–550 Ma; Nama, ca. 550–541 Ma; sensu Waggoner 2003). However, more recent investigations (e.g., Antcliffe and Brasier 2007; Young and Hagadorn 2010) have demonstrated that similarities between extant cnidarians and cnidarian-like, soft-bodied Ediacaran fossils are not mutually equivalent. Moreover, there are no cnidarian body fossils of Cryogenian age, and no trace fossils from this period can be attributed to this phylum. It should be noted, however, that certain phosphatic microfossils from the middle Ediacaran (ca. 585 Ma) Doushantuo Formation of South China have collectively been interpreted as embryos, larvae and minute adult specimens of anthozoans and hydrozoans (Chen et al. 2002).

In general, estimates of the timing of phylogenetic branching events based on molecular clocks vary substantially, and of course molecular clocks are frequently calibrated using the fossil record. Partly for this reason, we focus in this contribution on the anatomy and phylogenetic affinities of macroscopic Ediacaran body fossils showing the strongest evidence of homology with skeletons or soft bodies of extant cnidarians. To be sure, these fossils continue to present important problems of interpretation, and some of these are reviewed here. Be that as it may, we argue that these fossils can legitimately be used as calibration limits for molecular phylogenetic studies of the early diversification of cnidarians and other metazoan clades.

3.2 *Corumbella werneri*

3.2.1 Background

Conspicuously annulated, possibly weakly mineralized skeletons of *Corumbella werneri* have been described from latest Ediacaran strata in central Brazil, southern Nevada and Paraguay (Hahn et al. 1982; Hagadorn and Waggoner 2000; Babcock et al. 2005; Pacheco et al. 2011, 2015; Fairchild et al. 2012; Warren et al. 2012). Formations hosting *Corumbella* also contain calcareous skeletons of the cosmopolitan genus *Cloudina*, which may have been a cnidarian polyp (Vinn and Zatoń 2012; Cortijo et al. 2015). Examination of specimens showing three-dimensional preservation (Fig. 3.1a–d) revealed that complete skeletons of *Corumbella werneri* consisted of two regions or portions, with the probable aboral portion, approximately circular in transverse cross section, grading into a longer, polyhedral (quadrate) portion having four sub-rounded corners lacking a longitudinal sulcus (Pacheco et al. 2015). No evidence of a terminal holdfast structure has been detected, and indeed the aboral end may have been closed (Fig. 3.1e). The four faces of the quadrate portion are nearly parallel, and at the widest or oral end the skeleton is open, with each face having a straight to gently arcuate apertural margin. The largest skeletons may originally have measured about 100 mm long and up to 5 mm wide. The entire skeleton is transversely corrugated, and in longitudinal cross-sections the corrugations are trochoidal in form, with the crests of the trochoid pointing away

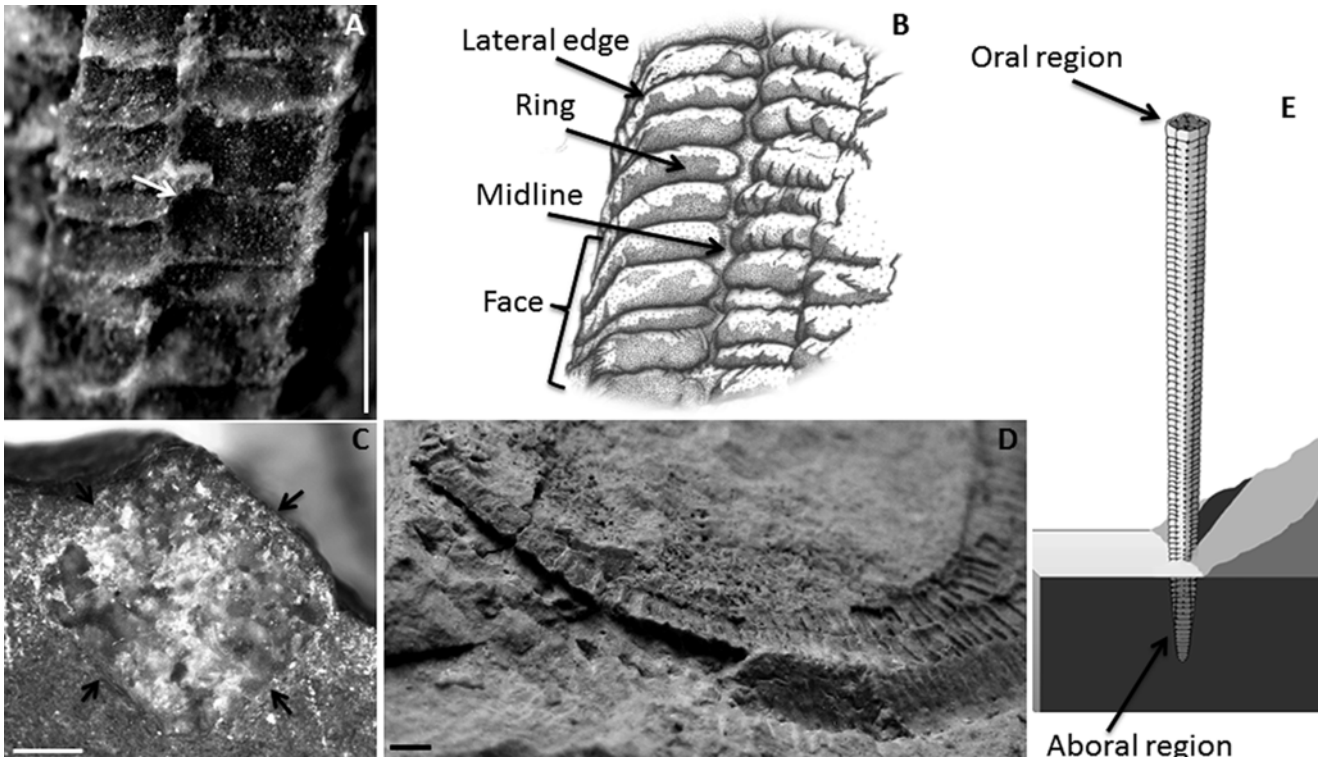

Fig. 3.1 *Corumbella werneri* (latest Ediacaran, Tamengo Formation, Mato Grosso do Sul, Brazil and Wood Canyon Formation, Nevada, USA) (**a**) part of the inner surface of a single face, showing the zigzagged midline sulcus and narrow angular interspaces (specimen number GP1E-4210, Geosciences Institute, University of São Paulo, Brazil) (**b**) drawing of part of GP1E-4210, showing details of a corner, sulcate midline and broadly rounded transverse ridges (rings) (**c**) transverse cross-section through the quadrate portion of another specimen, showing the four faces (*arrows*; specimen number 12802, Los Angeles County Museum of Natural History, California, USA) (**d**) specimen showing the aboral portion of the skeleton (*left*) grading into the polyhedral (quadrate) portion of the skeleton (specimen number DGM-5601-I, Earth Sciences Museum, DNPM, Brazil) (**e**) reconstruction of the complete periderm of a solitary polyp in its original life orientation and with its closed apical region embedded in bottom sediment (All scale bars represent 1 mm)

from the external surface of the skeleton (Fig. 3.1a, b), the thickness of which appears to be less than 0.1 mm. Viewed externally, the skeleton exhibits relatively broad, rounded, ring-like transverse ridges ("rings") that number about 4 per mm (long.) and alternate with narrow angular troughs corresponding to the crests of the aforementioned trochoid. In the short circular portion, the transverse ridges and troughs completely encircle the skeleton, and there is no other ornament. In the quadrate portion, the transverse ridges and troughs are continuous across the corners but are interrupted along the midline of the faces by a narrow, zigzagged angular groove that is expressed on the inner surface of the faces as a corresponding low ridge or carina (Fig. 3.1a). Also along the midline the transverse ridges and troughs are straight to recurved and exhibit slight offset (hence the zigzagged course of the midline sulcus). Results of SEM imaging and elemental analysis (Warren et al. 2012; Pacheco et al. 2015) indicate that the skeleton is finely lamellar, and further that individual lamellae consist of a mosaic of numerous, tightly interlocking polygonal plates measuring approximately 10–120 μm wide and 5 μm thick. The plates exhibit minute (~1 μm in diameter) pores and pillar-like features (papillae) arranged in regular rows. Finally, the trough between adjacent ridges appears to be the site of a single circumferential suture, hence the tendency of *Corumbella* fossils to be incomplete and broken along lines parallel to the annulations.

The hypothesis of a scyphozoan affinity for *Corumbella* is based on a suite of anatomical similarities uniquely shared by this genus with thecate scyphopolyps of the extant order Coronata and the extinct order Conulariida (Van Iten et al. 1996, 2006a, 2014a, b). Like coronates and most conulariids, the quadrate portion of *Corumbella* exhibits tetrameral symmetry, accentuated in all three taxa by the presence of four or eight inwardly projecting, seriated or continuous longitudinal sulci (*Corumbella* and many conulariids) or carinae (many conulariids and some coronates). The four sulcate midlines of *Corumbella* can be interpreted as being homologous to the scyphozoan interradii, which in many living scyphozoans are sites of an endodermal gastric septum, while the four corners can be homologized with the scyphozoan perradii. In many conulariids both the midlines (interradial)

and the corners (perradial) likewise are sulcate, but in several species the corners are rounded, as in *Corumbella*. The transverse ridges and troughs of this genus can be homologized with the transverse ribs and interspaces, respectively, of rib-bearing conulariids and coronates. In sum, then, the skeleton of *Corumbella* can be interpreted as an ectodermally derived, originally non- or weakly mineralized periderm, the open wide end of which was oral (Fig. 3.1e). At this point no detailed alternative hypotheses of homology between *Corumbella* and non-scyphozoan groups such as annelids have been offered.

3.2.2 Additional Comparisons

Corumbella may also be compared with *Carinachites*, a possible conulariid genus from basal Cambrian (ca. 540 Ma) phosphorites of the Yangtze Platform (South China; Conway Morris and Chen 1992; Van Iten et al. 2005a), and with certain annulated body fossils from the late Ediacaran Dengying Formation, also on the Yangtze Platform. Specimens of *Carinachites*, which measure less than 5 mm long, generally are broken at both ends, though recently paleontologists in China have discovered specimens preserving a conulariid-like apertural margin (J. Han, written communication, 2015). Both *Carinachites* and the polyhedral portion of *Corumbella* are square in transverse cross section, and the angle of taper of the skeleton is extremely low (<5° as opposed to ~10–30° in most conulariids; e.g., Babcock and Feldmann 1986). Additionally, the thickness of the skeleton appears to have been less than 0.1 mm. The annulations of *Corumbella* are similar to the rounded, gently arcuate transverse ridges of *Carinachites spinatus* (see Conway Morris and Chen 1992, Fig. 3.5), and these in turn are similar to the smooth, sinusoidal transverse ribs of the conulariid genus *Conularina* (see for example illustrations in Sinclair 1942). Also in these three taxa, the midline of each face is marked by a sulcus (*Corumbella* and *Conularina*) or by a low ridge (*Carinachites spinatus*), and many or all of the transverse ridges are reduced in amplitude and recurved along the midline. Like the rounded transverse ridges of *Corumbella*, the transverse ridges of *Carinachites spinatus* are smooth and more or less rounded, arch gently toward the wide end of the skeleton, and appear to represent corrugations of the thin skeletal wall. The troughs (interspaces) between the transverse ridges likewise are smooth. Unlike *Corumbella*, though, the corners of all species of *Carinachites* are sulcate, in some cases (*C. spinatus*) bearing a transverse ornament that is finer than that crossing the faces (a characteristic shared with conulariids such as *Climacoconus*; see for example Van Iten et al. 1996).

Parsimony-based cladistic analysis of phylogenetic relationships among these taxa lies beyond the scope of the present study. However, two possible hypotheses suggested by the foregoing discussion are (1) that *Corumbella* was more closely related to *Carinachites* than it was to conulariids (based in part on the shared presence of an extremely low rate of skeletal taper; Fig. 3.2a) and (2) that *Corumbella* was more closely related to conulariids than it was to *Carinachites* (based in part on the shared presence of a corner sulcus; Fig. 3.2b).

Finally, the Dengying Formation hosts several taxa, including *Wutubus annularis* and the genus *Sinotubulites*, which consist of an elongate body or tubular skeleton showing conspicuous annulation (Chen et al. 2014; Cai et al. 2015). Like *Corumbella*, the most complete specimens of *W. annularis* (up to 180 mm long) exhibit two regions, one short and narrow and the other long and wide, and its annulations may be ruga-like or broadly rounded (Chen et al. 2014). However, it is not clear whether *W. annularis*, which was more or less cylindrical (transv.) as opposed to quadrate and lacked longitudinal sulci, had a skeleton or even a mouth opening. Skeletons of *Sinotubulites*, broken at both ends and thought to have originally been calcareous (Cai et al. 2015), are laminated and composed of two main layers. In some species, the outer layer is polyhedral, having three, five or six faces, and it exhibits coarse transverse corrugation. However, unlike *Corumbella*, the external ridges of *Sinotubulites* are sharp-crested rather than broadly rounded (being also offset along the corners of the skeleton), and the midline of the faces of *Sinotubulites* appears to lack a sulcus (see Cai et al. 2015, figs. 5, 6b and 8). For these reasons, then, hypotheses of homology between *Corumbella* and other Ediacaran tubular genera appear to be relatively weak, and we do not offer any preliminary phylogenetic hypotheses for these taxa here.

3.3 First Definite Precambrian Conulariid (*Paraconularia* sp.)

3.3.1 Comparisons with Phanerozoic Conulariids

In addition to *Cloudina* and *Corumbella*, the latest Ediacaran Tamengo Formation of central Brazil has also yielded a single specimen of a conulariid (Leme et al. 2013; Van Iten et al. 2014a) (Figs. 3.3 and 3.4). Collected by one of us (TRF) near Corumbá in Mato Grosso do Sul State (Fig. 3.4a), the specimen (Fig. 3.3) is most similar to species in the genus *Paraconularia* and has been assigned to this taxon (Van Iten et al. 2014a), the monophyly of which however has been questioned (Van Iten et al. 2008). Other Ediacaran fossils, including *Conomedusites* (Fedonkin et al. 2007), *Vendoconularia triradiata* (Ivantsov and Fedonkin 2002; Van Iten et al. 2005b) and certain fossils from the Lantian Formation of South China (Van Iten et al. 2013), have been allied or compared with conulariids, but the Tamengo

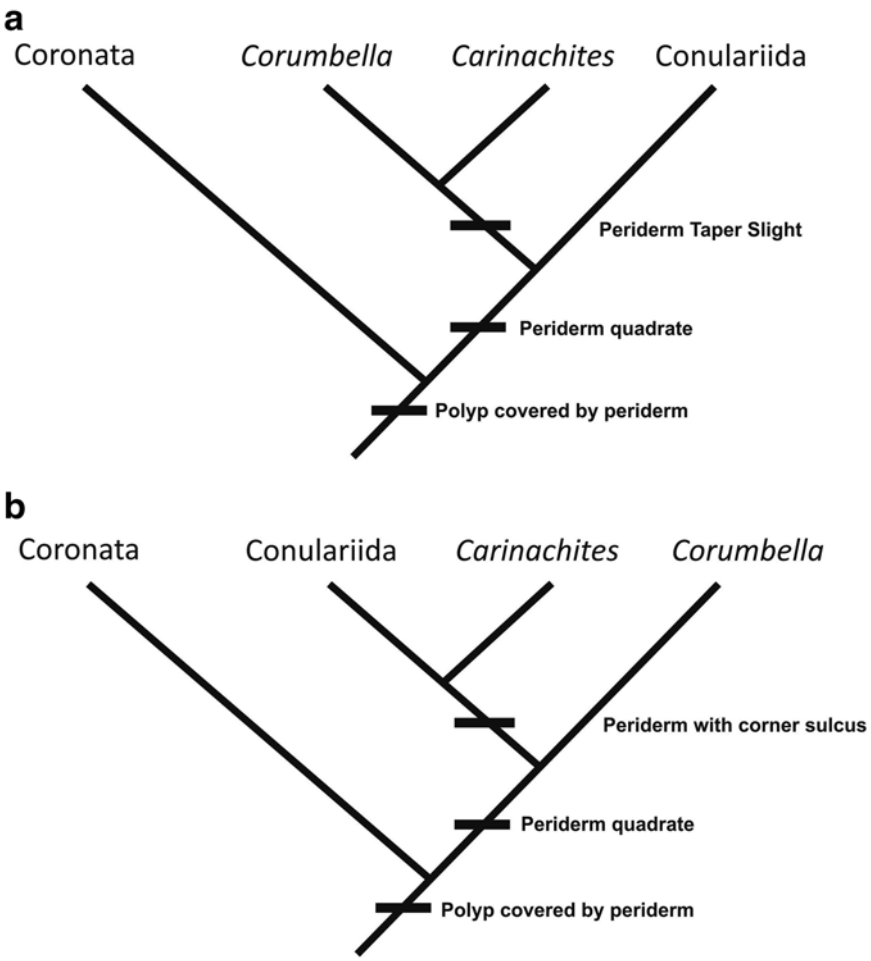

Fig. 3.2 Cladograms showing two alternative interpretations of the phylogenetic relationships among coronate scyphozoans, *Carinachites*, conulariids (order Conulariida) and *Corumbella* (**a**) *Carinachites* and *Corumbella* as sister taxa united by possession of a thin-walled quadrate periderm that tapers very gently (**b**) *Carinachites* and conulariids as sister taxa united by possession of an external sulcus at the corners of the periderm

Formation specimen (Fig. 3.3) is the first such fossil showing compelling evidence of affinity with a particular conulariid genus. The specimen, which has been flattened and is missing the aboral region and apertural margin, measures approximately 26 mm long and 15 mm wide and is oriented parallel to bedding. We estimate that the complete periderm originally measured at least 100 mm long. The conulariid was found in a 1.5-m-thick interval of variegated, *Corumbella*-bearing laminated shale in the middle part of the Tamengo Formation, approximately 35 m below the topmost occurrence of *Cloudina*, a latest Ediacaran index fossil (Gaucher et al. 2003; Cortijo et al. 2015), and approximately 40 m below the topmost occurrence of *Corumbella* (Fig. 3.4b). Zircons from altered volcanic tuffs situated approximately 40 m above the level at which the conulariid was found have yielded a radiometric (U-Pb) age date of 543 ± 3 Ma (see Gaucher et al. 2003 and references cited therein).

Comparisons of this fossil with taxa other than conulariids, including previously described, soft-bodied Ediacaran genera, failed to yield any similarities as detailed as those uniquely shared with *Paraconularia*. Conulariids in general consist of a four-sided (rarely three-, five- or six-sided), gently to strongly tapered lamellar periderm having (in most genera) sulcate corners and a well-defined transverse ornament consisting of transverse ridges (ribs) and/or nodes. The thickness of the periderm ranges from <0.1 mm to several mm, and the corners and/or midlines may be sites of an internal carina (midlines and corners) or pair of carinae (midlines). All *Paraconularia* possess trochoidal (long.), adaperturally arching transverse ribs, thickest beneath their crest, which in most species bears a single row of minute, more or less closely spaced nodes (see Babcock and Feldmann 1986, figs. 23.5, 28.6 and 33.8). Some of the transverse ribs may be offset along the facial midline, and all of the transverse ribs terminate within the corner sulcus, bending towards the aperture on the shoulders of the sulcus and (within the sulcus) alternating with the terminal portion of the transverse ribs of the adjoining face (see Babcock and Feldmann 1986, figs. 22.2, 25.3, 26.1 and 27.5). In most *Paraconularia* possessing nodes, the adapertural half of each node is developed into a short, spine-like ridge (adapertural spine) that extends part way across the interspace (the trough

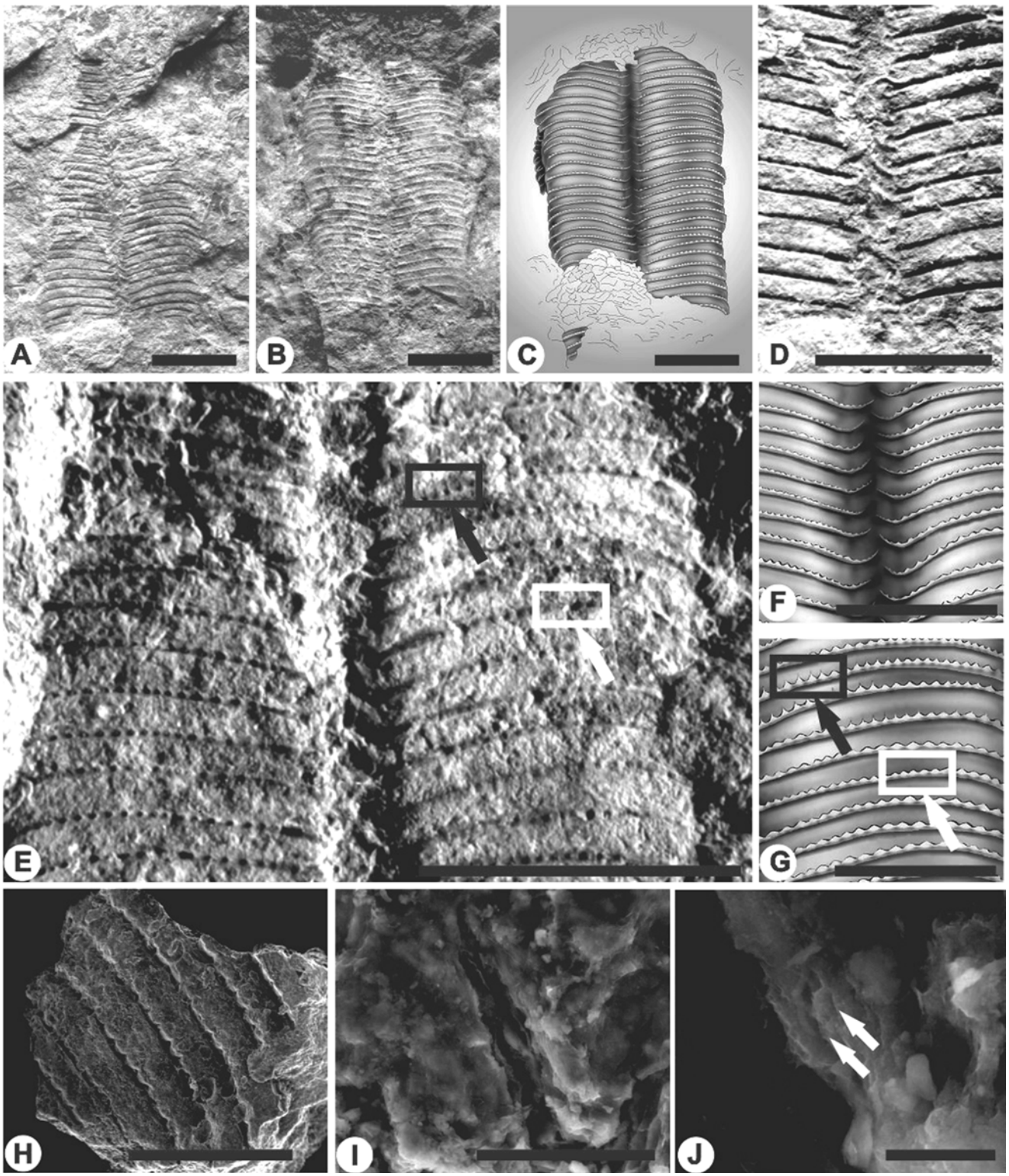

Fig. 3.3 *Paraconularia* sp. (latest Ediacaran, middle Tamengo Formation, Corumbá Group, Mato Grosso do Sul, Brazil; specimen number GP-1T 2301, Geosciences Institute, University of São Paulo, Brazil) (**a**) the counterpart of the specimen (**b**) the part of the specimen, showing the morphology of two faces and the intervening corner sulcus (**c**) schematic drawing of **b**, (**d**) detail of the corner sulcus of the part (**e**) detail of several nodes (*white square* and *arrow*) and adapertural spines (*black square* and *arrow*) (**f**) schematic drawing of the corner sulcus region shown in **e**, (**g**) schematic drawing of the highlighted nodes (*white square* and *arrow*) and adapertural spines (*black square* and *arrow*) shown in **g**, (**h**) scanning electron photomicrograph of a periderm fragment showing transverse ribs and nodes (**i**, **j**) scanning electron photomicrographs of broken and exfoliated periderm showing microscopic lamellae (*white arrows* in **j**) Scale bars: **a**–**c** and **e** = 10 mm; **d** = 7 mm; **f, g** = 5 mm; **h** = 3 mm; **i** = 40 μm; **j** = 5 μm

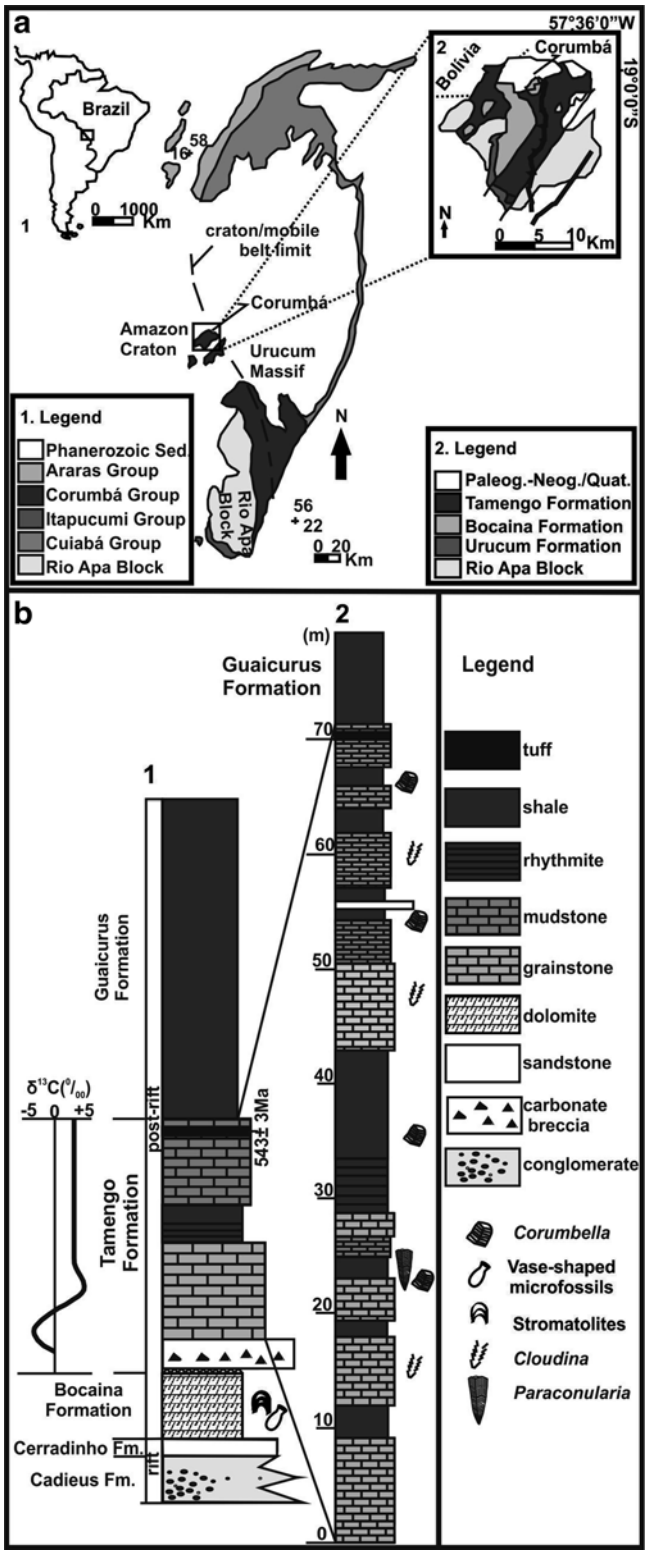

Fig. 3.4 (**a**) Geological map of the Paraguay Belt in South America, showing in (*1*) the location of the Corumbá (Brazil) area and in (*2*) the geology of the Corumbá Group near Corumbá (**b**) measured stratigraphical columns for (*1*) the Corumbá Group and (*2*) the Tamengo Formation, showing in (*2*) the levels of occurrence of *Paraconularia* sp., *Cloudina* and *Corumbella*

between adjacent transverse ribs) (see Babcock and Feldmann 1986, figs. 28.6 and 33.8). Finally, the pattern of arrangement of nodes on the faces is such that the nodes of every other transverse rib are collinear, forming longitudinal series that are approximately parallel to the facial midline or corners.

The relatively thin-walled (~0.2–0.5 mm), gently tapered Tamengo Formation *Paraconularia* exhibits the full suite of gross morphological features uniquely exhibited by conulariids in general, and it is most similar to node-bearing species of *Paraconularia* having adapertural spines. It consists of large portions of all four faces, though because it is flattened only two of the faces and the corner sulcus between them are readily visible (the other two faces, though mostly covered, project slightly beyond the apical edge of the two exposed faces of the part). The two exposed faces (Fig. 3.3a–g) are well-preserved, exhibiting 32 trochoidal, adapertural arching transverse ribs bearing relatively large, widely spaced nodes with adapertural spines (Fig. 3.3e, h). The midlines and corners lack an internal carina. Next to and within the corner sulcus (Fig. 3.3d–f), the transverse ribs bend and alternate in a manner similar to that of all other *Paraconularia*, and the arrangement of the nodes and adapertural spines on the faces likewise is similar.

Essentially the only difference between the Tamengo Formation *Paraconularia* and Phanerozoic conulariids in general is that whereas the periderm of the latter fossils consists of alternating organic and phosphatic "microlamellae" (Ford et al., in press), the finely lamellar periderm of the Tamengo Formation conulariid (Fig. 3.3i, j) appears to be non-mineralized. Analysis of the periderm using Raman spectrometry yielded multiple bands characteristic of unaltered organic material, but none in the range (900–1100 cm^{-1}) for phosphate (Fig. 3.5). Similarly, phosphate was not detected in the host rock matrix. At present it is unclear whether the Tamengo Formation specimen originally was non-mineralized or whether original skeletal phosphate was removed secondarily, perhaps during diagenesis or weathering (see Muscente and Xiao 2015 and references cited therein). Be that as it may, the detailed gross morphological similarities between the Tamengo Formation specimen and Phanerozoic *Paraconularia*, many shared only by these fossils, constitute compelling evidence of homology.

3.3.2 Implications

The discovery of *Paraconularia* sp. in strata of latest Ediacaran age has important implications for our understanding of the evolution of both conulariids and cnidarians prior to their expansion during the Early Paleozoic. Because soft-bodied Ediacaran fossils originally interpreted as cni-

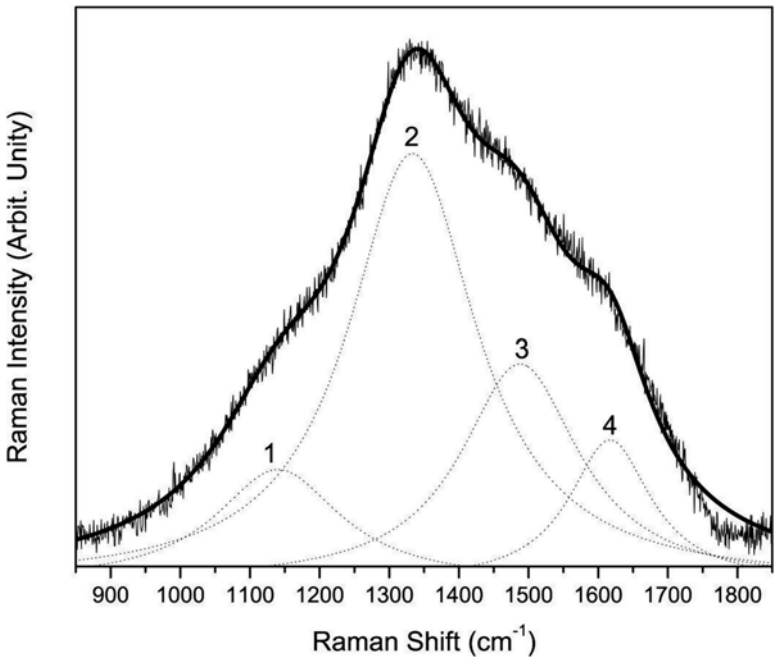

Fig. 3.5 Raman spectra of the periderm of *Paraconularia* sp. (middle Tamengo Formation, Mato Gross do Sul, Brazil)

darians have since been shown to be unrelated to this phylum (see Van Iten et al. 2014a and references cited therein), the Tamengo Formation *Paraconularia* sp. now constitutes the strongest paleontological evidence of the presence of conulariids, scyphozoans or any cnidarians during Neoproterozoic times. Moreover, conulariids survived the end-Proterozoic extinction event (Laflamme et al. 2013), a status currently shared with the probable latest Ediacaran-early Cambrian annelid, *Sabellidites cambriensis* (Moczyłowska et al. 2014). Cladistic analyses of phylogenetic relationships among conulariids (Leme et al. 2008; Van Iten et al. 2014b) show *Paraconularia* to be a relatively apical branch in the conulariid tree. This result implies that conulariids originated even earlier in the Neoproterozoic Era, and it suggests that additional conulariid taxa may occur in rocks of this age (possibly as sub-microscopic fragments; e.g., Van Iten et al. 2006b). Conulariids originated after the medusozoan sister classes Scyphozoa and Cubozoa split from each other, and after these two clades together split from their most recent common ancestor with the class Hydrozoa and, even earlier, after the subphylum Medusozoa split from its most recent common ancestor with the subphylum Anthozoaria (Van Iten et al. 2014a). Therefore the origin of conulariids was preceded by at least three major branching events in the history of phylum Cnidaria. Finally, together with *Corumbella werneri*, the Tamengo Formation *Paraconularia* constitutes a new, internal calibration point for molecular clock studies of early cnidarian evolution, dating the split between Scyphozoa and Cubozoa at no later than ca. 543 Ma.

3.4 *Haootia quadriformis:* The Oldest Fossil Cnidarian?

A new genus and species of (originally) soft-bodied fossil, *Haootia quadriformis*, has recently been described from the late Ediacaran (ca. 560 Ma) lower Fermeuse and Trepassy formations of the Bonavista Peninsula, southeastern Newfoundland (Liu et al. 2014, 2015; Miranda et al. 2015). At present *H. quadriformis* is represented by two specimens, namely the holotype from the Fermeuse Formation and a single incomplete paratype from the slightly older Trepassy Formation. Liu et al. (2014) interpreted *H. quadriformis* as a muscular cnidarian or a muscular metazoan of cnidarian grade. Additionally, these authors proposed that the new species was a sessile benthic animal, originally about 100 mm long, having a circular basal attachment disc connected to a narrow peduncle passing adorally into a broad quadrate calyx bearing four distally bifurcating branches. One of the most salient features of both the holotype and paratype is their unique system of narrow, closely spaced low ridges, interpreted by Liu et al. (2014) as sediment molds of bundled collagenous muscle fibers. These authors stated further that *H. quadriformis* may represent the earliest record of metazoan musculature. More specifically, Liu et al. (2014) posited that ridges in the stalk-like peduncle correspond to longitudinal (retractor) muscles, and that ridges paralleling the upper margin of the calyx correspond to circular coronal muscles. Based in part on these interpretations, Liu et al. (2014) proposed that *H. quadriformis* may have been most

closely related to extant staurozoans, a group of sessile medusae originally placed in the class Scyphozoa.

Still more recently, Miranda et al. (2015) published an alternative interpretation of *H. quadriformis*, supporting the hypothesis of a medusozoan affinity for this taxon but also calling into question certain aspects of Liu et al.'s (2014) reconstruction of the living animal. Specifically, Miranda et al. (2015) noted that the putative muscle impressions appear to overlap each other, possibly owing to post-mortem deformation including superposition of soft-part structures, and thus that it is difficult to interpret how the muscles might have been organized in the living body. Concerning Liu et al.'s (2014) interpretation of narrow ridges paralleling the putative oral margin of the calyx, Miranda et al. (2015) argued that they are less extensive than shown in Liu et al.'s reconstruction (2014, Fig. 3.3b). In the alternative reconstruction offered by Miranda et al. (2015, Fig. 3.3), the muscles are predominantly longitudinal in alignment, extending much closer to the putative oral margin of the calyx than posited by Liu et al. (2014), an interpretation that agrees more closely with the predominantly longitudinal musculature of extant staurozoans. However, Miranda et al. (2015) also stated that if the reconstruction of Liu et al. (2014) is in fact accurate, then perhaps *H. quadriformis* was a benthic form exhibiting vigorous pulsation, possibly to facilitate feeding. This hypothesis has important implications for the evolution of medusozoan life histories, as such a condition could have given rise to a free-living nektonic descendant.

Finally, the possibility that *H. quadriformis* specimens underwent significant taphonomic modification is an important problem meriting further investigation. Assuming that the original host sediment(s) and pore-water chemistry can be duplicated in the laboratory, it might prove worthwhile to conduct controlled taphonomic experiments to determine whether muscles of extant staurozoans or other cnidarians can be replicated under conditions similar to those that existed during final burial of *H. quadriformis*. A positive result would corroborate the hypothesis that the two currently available fossil specimens preserve relic muscle fibers.

3.5 Summary

Crucial to understanding the origin and early diversification of phylum Cnidaria is a thorough understanding of the nature and temporal range of its fossil record. Although recent molecular clock studies place the origins of this clade and its major subgroups within the Neoproterozoic Era, there are no cnidarian body fossils from rocks of Cryogenian age, and no Cryogenian trace fossils can be attributed to cnidarians. The Ediacaran System has yielded numerous genera of macrofossils, but only three of these, *Corumbella*, *Haootia* and *Paraconularia*, are likely to have been cnidarians (medusozoans). *Paraconularia* sp. from the latest Ediacaran Tamengo Formation (Brazil) constitutes the strongest paleontological evidence yet documented of the presence both of cnidarians and scyphozoans below the Proterozoic-Phanerozoic boundary, and it establishes conulariids as a cnidarian clade that survived the terminal Neoproterozoic extinction event. Together with *Corumbella werneri*, a possible close relative of conulariids, *Paraconularia* sp. from the Tamengo Formation serves as a new internal calibration point for molecular clock estimates, fixing the split between the classes Cubozoa and Scyphozoa at no later than ca. 543 Ma. Finally, if *Haootia quadriformis* was indeed a muscular, crown- or stem-group staurozoan, then it fixes the split between the class Staurozoa and all other medusozoans at no later than ca. 560 Ma.

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The New Systematics of Scleractinia: Integrating Molecular and Morphological Evidence

4

Marcelo V. Kitahara, Hironobu Fukami, Francesca Benzoni, and Danwei Huang

Abstract

The taxonomy of scleractinian corals has traditionally been established based on morphology at the "macro" scale since the time of Carl Linnaeus. Taxa described using macromorphology are useful for classifying the myriad of growth forms, yet new molecular and small-scale morphological data have challenged the natural historicity of many familiar groups, motivating multiple revisions at every taxonomic level. In this synthesis of scleractinian phylogenetics and systematics, we present the most current state of affairs in the field covering both zooxanthellate and azooxanthellate taxa, focusing on the progress of our phylogenetic understanding of this ecologically-significant clade, which today is supported by rich sets of molecular and morphological data. It is worth noting that when DNA sequence data was first used to investigate coral evolution in the 1990s, there was no concerted effort to use phylogenetic information to delineate problematic taxa. In the last decade, however, the incompatibility of coral taxonomy with their evolutionary history has become much clearer, as molecular analyses for corals have been improved upon technically and expanded to all major scleractinian clades, shallow and deep. We describe these methodological developments and summarise new taxonomic revisions based on robust inferences of the coral tree of life. Despite these efforts, there are still unresolved sections of the scleractinian phylogeny, resulting in uncertain taxonomy for several taxa. We highlight these and propose a way forward for the taxonomy of corals.

Keywords

Azooxanthellate • Cnidaria • Coral • Integrative taxonomy • Phylogenetics • Reef • Species boundaries • Zooxanthellae

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4.1 Introduction

Stony corals belonging to the order Scleractinia (Anthozoa: Hexacorallia) are a clade of cnidarians that build a calcium carbonate skeleton in the form of aragonite, and are sister group to the non-stony corallimorpharians (Daly et al. 2003; Fukami et al. 2008; Kitahara et al. 2014; Lin et al. 2014). At present, Scleractinia contains 31 families, about 240 genera, and over 1,500 species (Cairns 1999, 2009; Appeltans et al. 2012; Huang and Roy 2015), including both zooxanthellate—hosting the symbiotic dinoflagallate *Symbiodinium*—and azooxanthellate corals. Zooxanthellate species typically inhabit shallow waters surrounding warm-subtropical and tropical seas and comprise the main coral reef framework builders with about 800 valid species. Azooxanthellate species are widely distributed in the world's oceans from shallow to deep waters and consist of about 700 valid species. Neither zooxanthellate and azooxanthellate nor shallow and deep species are distinguished phylogenetically and only partially separated at the family level taxonomically. Due to the ecological and economic importance of tropical coral reefs—e.g., high species diversity and mass fisheries production—zooxanthellate taxa have been the subject of a greater volume of research relative to azooxanthellate species. However, both groups have comparable richness, having diversified successfully over hundreds of millions of years. Therefore in this chapter on coral systematics, they deserve equal attention, limited only by the amount of published data available.

The coral skeleton has been and continues to be the main source of morphological characters used in scleractinian classification. Most coral species are colonial, but solitary corals have evolved in at least six lineages independently (Barbeitos et al. 2010). Among colonial species, each corallite (skeletal unit formed by an individual polyp) within a colony or species may have varying characteristics depending on growth rate, position in the colony and other environmentally-influenced traits. Consequently, morphological boundaries between species are generally obscure, and the task of identifying corals falling within and outside the limited pool of systematists has remained challenging at every taxonomic level since Linnaeus (1758) established *Madrepora*.

Fortunately, molecular phylogenetic analyses in the last two decades have undoubtedly advanced coral taxonomy by making large amounts of data available and inspiring the next generation of systematists. Understandably, the numerous name changes across the entire coral phylogeny that have ensued can cause considerable confusion for coral researchers outside the limited circle of systematists. To address this apparent disarray, we track the history of molecular data used for phylogenetic reconstruction, summarise the most recent phylogenetic understanding of corals, and describe recent taxonomic research at family, genus and species levels. Finally we conclude by highlighting taxonomic issues that remain unresolved in the hope that research efforts will be refocused to stabilise all of the problematic taxa.

4.2 The Rise of Molecular Phylogenetic Methods

Genetic data have been collected from scleractinian corals since the early 1980s, but these were first based on allozyme allelic frequencies obtained using gel electrophoresis (Ridgway 2005). Stoddart (1983, 1984) examined the genetic diversity of *Pocillopora damicornis* using up to ten enzymes, and found that populations from Western Australia and Hawaii were maintained predominantly via asexual reproduction. Willis and Ayre (1985) analysed eight enzyme loci from Great Barrier Reef *Pavona cactus* to show that genetically similar colonies tended to show the same growth form, and overall the species comprised highly clonal populations (Ayre and Willis 1988). Allozyme electrophoresis was also employed to clarify genetic boundaries of closely-related morphotypes, such as between *Montipora* species (Heyward and Stoddart 1985), *M. digitata* populations (Stobart and Benzie 1994), within the *Orbicella* (previously "*Montastraea*") *annularis* species complex (Knowlton et al. 1992; van Veghel and Bak 1993), and among *Platygyra* morphospecies (Miller and Benzie 1997).

Another early genotyping method was restriction fragment length polymorphism (RFLP), which hybridised digested DNA fragments to probes for determining their lengths, or to genomic DNA of known species to establish identity. McMillan and Miller (1988) used RFLP to distinguish the morphologically confusing corals, *Acropora formosa* (= *A. muricata*) and *A. "nobilis"* (= *A. intermedia*; see Veron and Wallace 1984).

The first set of scleractinian DNA sequence data to be published comprised highly repetitive sequences of 118 bp each, otherwise known as minisatellites, cloned from *Acropora muricata* and *A. latistella* (McMillan and Miller 1989). Five more species were sequenced for these repeats in a follow-up study, in which a maximum parsimony analysis did not support most of the morphological subgroups (McMillan et al. 1991).

The use of polymerase chain reaction (PCR), an essential technique of today, began for corals with the amplification of nuclear 28S ribosomal DNA (rDNA) that was then sequenced for reconstructing the phylogeny of Anthozoa (Chen et al. 1995). This analysis included nine species of scleractinian corals, and two families tested with more than one species each were recovered as clades. In a subsequent analysis that focused on Scleractinia, Veron et al. (1996) added six species

with improved representation from Fungiidae and Poritidae, which were found to be monophyletic.

At about the same time, the mitochondrial 16S rDNA was sequenced from 34 species to reconstruct a larger scleractinian phylogeny (Romano and Palumbi 1996, 1997). This analysis showed that all five genera and nine of ten families for which more than one taxon were tested formed monophyletic groups. However, of the seven suborders examined, only three—Meandriina, Poritiina and Dendrophylliina—were recovered unambiguously as clades, although only Dendrophylliina remains the only monophyletic suborder to emerge from recent studies (e.g., Fukami et al. 2008; Arrigoni et al. 2014a).

Other PCR-based methods were adopted earlier on but these contributed little to phylogenetic reconstruction and have largely been discontinued owing to the fall in DNA sequencing costs in recent years. For instance, random amplified polymorphic DNA (RAPD) detected by four 10-mer primers showed that *Favia fragum* and *Porites astreoides* underwent high levels of self-fertilisation (Brazeau et al. 1998). Five RAPD primers were also used to assess differentiation among populations of *Acropora surculosa* (= *A. hyacinthus*) in Guam (Romano and Richmond 2000). The four populations studied were not significantly distinct from one another, but the eastern and western coasts of Guam were found to be genetically distinct when the respective populations were pooled. Amplified fragment length polymorphism (AFLP) is another PCR-based tool related to the RFLP technique that amplifies the restriction fragments which are subsequently separated by gel electrophoresis. This method aided in the discrimination of *Orbicella faveolata* from the other two species of the *O. annularis* complex (Lopez and Knowlton 1997; Lopez et al. 1999). Interestingly, AFLP was able to detect a much greater proportion of distinct *Pavona cactus* genotypes at Eclipse Island compared to allozyme genotypes (Smith et al. 1997), which indicated highly clonal populations instead (Ayre and Willis 1988).

Microsatellites, short tandem sequence repeats of between two and five bp, are typically used in population genetic studies and in tests of species boundaries among closely-related species. The first coral microsatellite to be utilised was detected in *Orbicella franksi* and used to distinguish among members of the *O. annularis* complex (Lopez et al. 1999). Many taxon-specific sets of microsatellite markers were published at the turn of the century (Maier et al. 2001; Le Goff and Rogers 2002; Magalon et al. 2004; Miller and Howard 2004; Severance et al. 2004a; Shearer and Coffroth 2004), and continue to be developed in recent years (Davies et al. 2013; Torda et al. 2013c; Boulay et al. 2014; Serrano et al. 2014; Zilberberg et al. 2014; Addamo et al. 2015; Tay et al. 2015). Unfortunately, the extreme polymorphism exhibited by these markers even among sibling species diminishes their utility for inferring phylogenies, but they continue to be the main workhorse for population genetic studies.

The first multi-species evolutionary trees of Scleractinia were reconstructed on the basis of the mitochondrial 16S rDNA (Romano and Palumbi 1996) and nuclear 28S rDNA (Veron et al. 1996). Shortly after, the nuclear internal transcribed spacers 1 and 2 (ITS), which include the 5.8S rDNA between them (White et al. 1990), were amplified and sequenced from the *Orbicella annularis* complex (Lopez and Knowlton 1997), as well as species from *Acropora* (Odorico and Miller 1997) and *Porites* (Hunter et al. 1997). Lopez and Knowlton (1997) also obtained sequence data from the β-tubulin coding and intron regions, but found that ITS and these loci showed no diagnosable variability among the three *Orbicella* species. The *Acropora* species exhibited varying degrees of molecular separation, with only *A. longicyathus* clearly distinguished from the other four studied species (Odorico and Miller 1997). However, ITS from five species of *Porites* analysed under maximum parsimony appeared to resolve evolutionary relationships among them (Hunter et al. 1997).

These taxon-specific patterns of genetic resolution prompted researchers to expand on the repertoire of loci from both the nuclear and mitochondrial genomes for phylogenetic purposes (Severance et al. 2004b; Concepcion et al. 2006, 2010; Flot et al. 2008; Chen et al. 2009). These markers, along with the primers used to amplify them, are often clade specific. Among the nuclear loci that are still in use today, some of the earliest to be developed include the intron region of the mini-collagen gene (Wang et al. 1995), used almost exclusively to investigate the evolutionary history of *Acropora* (Hatta et al. 1999; Vollmer and Palumbi 2002; Fukami et al. 2003; Palumbi et al. 2012; Suzuki and Fukami 2012). The Pax-C 46/47 intron, introduced by van Oppen et al. (2000, 2001), continues to be used for *Acropora* phylogenetics (Richards et al. 2008, 2013) and taxonomically broader reconstructions (Fig. 4.1). The divergence of Pax-C intron is low among sibling species (van Oppen et al. 2000) but is much higher for more inclusive clades (van Oppen et al. 2001).

The awareness that gene duplication (Lopez and Knowlton 1997; Odorico and Miller 1997) and heterozygosity (van Oppen et al. 2000) are common in nuclear loci led many to clone their PCR products and sequence multiple clones in hopes of capturing intragenomic variability. These include amplifications of the Pax-C intron (van Oppen et al. 2001, 2004; Márquez et al. 2002; Richards et al. 2008, 2013), β-tubulin (Fukami et al. 2004b; Stefani et al. 2008a), ITS (Medina et al. 1999; van Oppen et al. 2000, 2002; Diekmann et al. 2001; Rodriguez-Lanetty and Hoegh-Guldberg 2002; Márquez et al. 2003; Chen et al. 2004; Vollmer and Palumbi 2004; Forsman et al. 2005, 2006, 2009, 2010, 2015; Wei

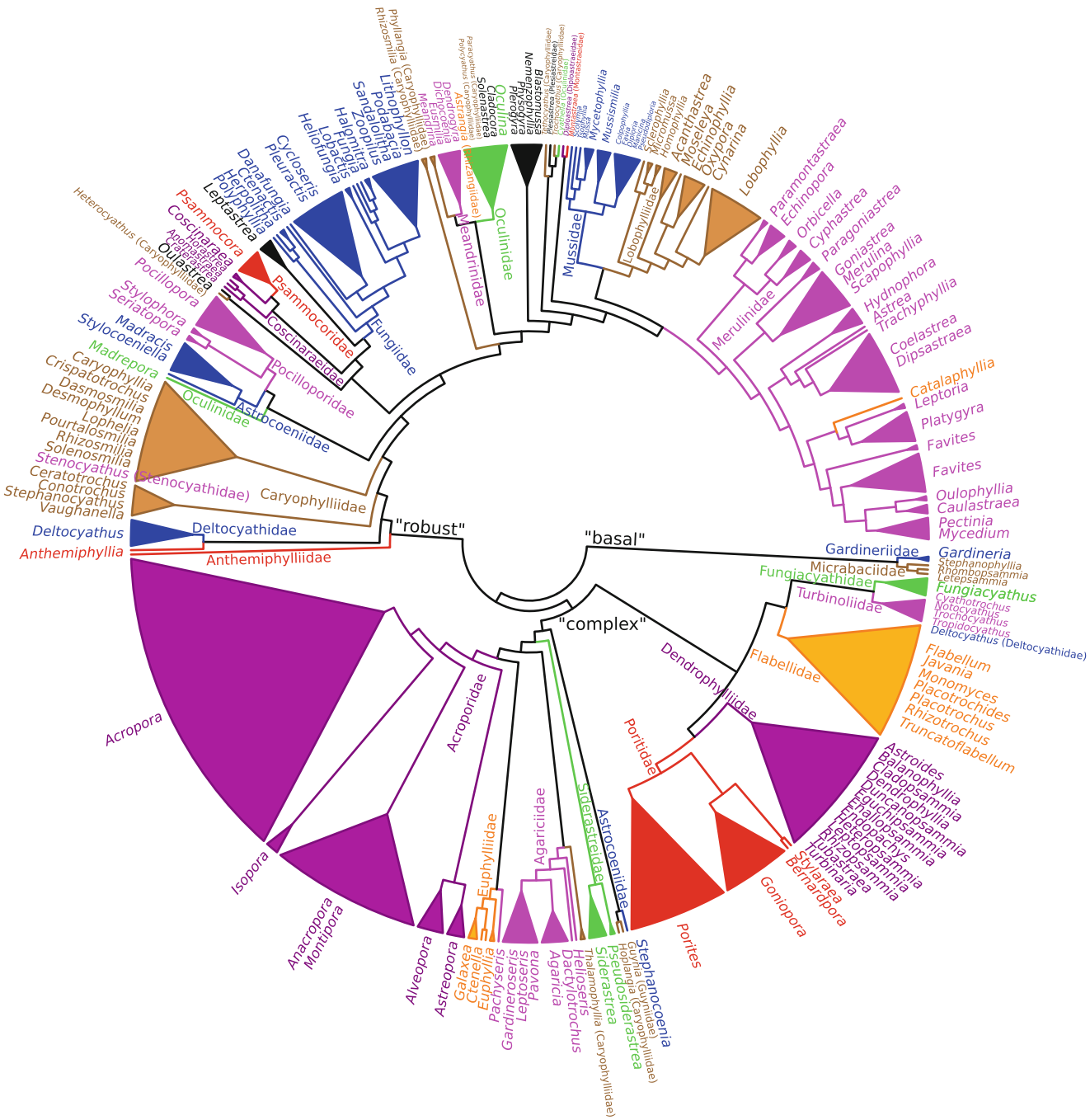

Fig. 4.1 Maximum likelihood genus-level phylogeny (576 species) of Scleractinia based on 12 DNA markers: mitochondrial 12S rDNA, 16S rDNA, ATP synthase subunit 6, cytochrome c oxidase subunit I, control region, cytochrome b and NADH dehydrogenase subunit 5; nuclear 18S rDNA, 28S rDNA, histone H3, internal transcribed spacers and Pax-C 46/47 intron. Data unavailable for Schizocyathidae, the only valid extant family not represented here. Branch supports not assessed in detail. *Colours* differentiate adjacent families and are not unique for any taxa, except for genera assigned *incertae sedis* that are shown in *black*

et al. 2006; Stefani et al. 2011; Kitano et al. 2013, 2014), and 28S rDNA (Chen et al. 2000; Cuif et al. 2003; Wolstenholme et al. 2003). For regions that have not diverged considerably between paralogues, such as the ITS, mixed PCR products can be split into two dominant sequences based on phase reconstruction of forward and reverse chromatograms of distinct lengths (Flot and Tillier 2006; Flot et al. 2006). The software Champuru was developed (Flot 2007) and used for processing direct sequencing data from *Pocillopora* (Flot et al. 2008, 2010; Schmidt-Roach et al. 2013; Adjeroud et al. 2014) and *Stylophora* (Flot et al. 2011). Variable amplicons with no intra-individual length variation can also be resolved

statistically using SeqPHASE (Flot 2010). Furthermore, direct sequencing of ITS has been carried out following PCR with primers demonstrating high fidelity for a single copy (Takabayashi et al. 1998a, b, 2003; Lam and Morton 2003; Benzoni et al. 2007, 2010, 2011, 2012a, b, 2014; Mangubhai et al. 2007; Stefani et al. 2008b; Knittweis et al. 2009; Huang et al. 2011; Benzoni and Stefani 2012). Nevertheless, since the intra-individual variability of these nuclear markers is not fully understood (Chen et al. 2004; Vollmer and Palumbi 2004), caution should be exercised even when using these primer sets.

Mitochondrial loci have also been popular markers in phylogenetic analyses. These are haploid, and thus unambiguous sequences can be obtained generally without cloning. While mitochondrial genes typically evolve faster than nuclear genes in metazoans, anthozoans show an opposite pattern (van Oppen et al. 1999; Shearer et al. 2002; Fukami and Knowlton 2005; Tseng et al. 2005; Hellberg 2006; Huang et al. 2008; Chen et al. 2009). Therefore, these genes are more informative for reconstructing deep coral phylogenies. Other than the 16S rDNA that established widespread subordinal non-monophyly (Romano and Palumbi 1996, 1997; Le Goff-Vitry et al. 2004), 12S rDNA, cytochrome b and cytochrome c oxidase subunit I (COI) were purposed for corals relatively early (Medina et al. 1999; van Oppen et al. 1999; Chen and Yu 2000; Fukami et al. 2000) and have been used for inferring large scleractinian trees effectively (Chen et al. 2002; Fukami et al. 2004b, 2008; Barbeitos et al. 2010; Kitahara et al. 2010b, 2013; Stolarski et al. 2011; Arrigoni et al. 2012, 2014c; Huang 2012; Huang and Roy 2013, 2015; Marcelino et al. 2013; Curnick et al. 2015; Fig. 4.1). The gene encoding ATP synthase subunit 6 is also commonly used, but primarily for Acroporidae (Fukami et al. 2000; Forsman et al. 2010).

Different taxa contain various intergenic regions within their mitochondrial genomes, but these may not be orthologous across species or are not amenable for alignment across distant clades. The noncoding intergenic region identified by Fukami et al. (2004a), for instance, was too variable to be aligned across all of Merulinidae (Huang et al. 2011) and is not orthologous with the intergenic region (or the putative control region) in *Acropora* (van Oppen et al. 2001; Wolstenholme 2004; Richards et al. 2008, 2013), *Montipora* (van Oppen et al. 2004; Forsman et al. 2010), *Porites* (Forsman et al. 2009) or Agariciidae (Luck et al. 2013; Pochon et al. 2015). These fast-evolving mitochondrial markers remain useful for phylogenetic studies among closely-related species.

Whole mitochondrial genomes have also been extremely important sources of data for large coral phylogenies (Medina et al. 2006; Emblem et al. 2011; Kayal et al. 2013; Lin et al. 2011, 2014). Nevertheless, we note that major clades appear to exhibit distinct patterns of mtDNA sequence evolution that could be responsible for various topological inconsistencies, such as the paraphyly of Scleractinia with respect to Corallimorpharia (Kitahara et al. 2014), i.e., the "naked coral" hypothesis (Medina et al. 2006).

On the one hand, single-gene analyses were the rule among the earliest studies because of the high cost of DNA sequencing and the paucity of suitable markers, primers and publicly available data. On the other hand, there were studies drawing phylogenetic inference based on more than one loci, including Lopez and Knowlton's (1997) analyses of two nuclear genes and AFLP. Early researchers also acknowledged that nuclear and mitochondrial genes evolve at different rates and thus both should be examined, albeit as separate datasets (Medina et al. 1999; Romano and Cairns 2000; van Oppen et al. 2001). Sequence data were combined beginning with the seminal study by Fukami et al. (2004b), which concatenated the cytochrome b and COI genes after passing the incongruence length difference test (Farris et al. 1995). The use of more than one marker for inferring species relationships has become the norm in more recent studies, aided by a variety of nucleotide substitution models (Posada and Crandall 2001) and the ability to use mixed models in a multilocus partitioned-by-gene analysis (Ronquist and Huelsenbeck 2003; Stamatakis 2006).

Authors remain split between concatenating markers to obtain hidden support (Huang et al. 2011; Addamo et al. 2012; Arrigoni et al. 2012, 2014a, b, c; Benzoni et al. 2012b) and making separate estimations of gene trees (Benzoni et al. 2011, 2012a, 2014; Gittenberger et al. 2011; Bongaerts et al. 2013; Kitano et al. 2013, 2014; Huang et al. 2014a; Arrigoni et al. 2015). With more markers available for inferring phylogenies, combined analyses of multilocus data may be the way forward. Recent large-scale studies (>450 species) have sought to concatenate data from seven or more loci (Huang 2012; Huang and Roy 2013, 2015; Curnick et al. 2015; Fig. 4.1). However, different genes cannot be assumed to share the same evolutionary history, and the phylogeny reconstructed for every gene may differ from the actual species history (Maddison and Knowles 2006). Thus, for species classifications, methods that use coalescent theory to jointly estimate gene trees and the species tree would be more appropriate (Liu and Pearl 2007; Liu 2008; Liu et al. 2008; Heled and Drummond 2010). A recent study of *Porites* corals based on the multilocus coalescence showed that the three branching forms found in the Caribbean are probably not distinct species (Prada et al. 2014).

These species tree methods have become especially relevant with the development of high-throughput sequencing technologies because it is now possible to generate orthologous sequence data in great abundance (McCormack et al. 2013). Such data can be obtained through the sequencing of expressed sequence tags (Philippe and Telford 2006), restriction site associated DNA (Rubin et al. 2012), and probe-based target enrichment of nuclear ultraconserved elements

(Faircloth et al. 2012; Lemmon et al. 2012), among several others. The assembly of the complete *Acropora digitifera* genome (Shinzato et al. 2011) has provided a much-needed reference to identify and utilise orthologous regions for phylogenetic analyses. Indeed, we expect these new methods to be applied on scleractinians extensively in the next decade, sustaining the "molecular revolution" (Stolarski and Roniewicz 2001: 1101) of coral systematics.

4.3 The Phylogeny of Scleractinia: Integrating Molecular and Morphological Evidence

The origin of modern Scleractinia is not well understood. Fossils appeared abruptly in the Middle Triassic (ca. 240 Ma ago) already represented by a wide variety of solitary and colonial forms (Roniewicz and Morycowa 1993; Veron 1995; Stanley Jr 2003). From colony integration, e.g., phaceloid, meandroid and thamnasteroid (Wells 1956; Stanley Jr 2003), to the structural organisation within individual corallites, e.g., septal ornamentation and axial structures (Roniewicz 1989; Roniewicz and Stanley Jr 1998; Roniewicz and Stolarski 1999, 2001), the range of morphological diversity observed among Triassic fossils is comparable to that in modern scleractinians. Moreover, the recent proposal that *Kilbuchophyllia* (Ordovician, ca. 450 Ma ago; Scrutton and Clarkson 1991; Scrutton 1993), *Numidiaphyllum* and *Houchangocyathus* (Permian, ca. 265–255 Ma ago; Ezaki 1997, 2000) were true scleractinian corals, in addition to molecular clock estimates (Stolarski et al. 2011), suggest an extensive Palaeozoic evolutionary history for Scleractinia.

The foundational studies of evolutionary relationships in the late nineteenth and early twentieth centuries relied exclusively on macromorphological skeletal characteristics of extant and extinct scleractinians. As they are sessile or have restricted capacity for movement (e.g., free-living and/or solitary), corals are subjected to the environmental conditions at their place of settlement. Consequently, they exhibit considerable morphological plasticity, driven in part by various ecological factors (Foster 1979a, b, 1980; Best et al. 1984; Hoeksema 1991; Budd 1993; Todd 2008). According to Lowenstein (1985), taxonomic research based exclusively on morphology is plagued by two major limitations. The first arises from convergence, in which unrelated taxa resemble one another as a result of having adapted to living in similar environments, so morphological similarities are not indicative of close evolutionary relationships. The second limitation concerns traits that may evolve at distinct rates in different lineages. Not surprisingly, the small number of "reliable" macromorphological characters, as indicated by Cairns (2001), and the uncertain impact of environmental variables on skeletal morphology have severely hampered attempts to infer relationships among scleractinian suborders and families (Romano and Cairns 2000; Stolarski and Roniewicz 2001; Le Goff-Vitry et al. 2004; Fukami et al. 2008). As such, evolutionary hypotheses based on morphological characters have resulted in several different taxonomic schemes (e.g., Vaughan and Wells 1943; Alloiteau 1952; Wells 1956; Chevalier and Beauvais 1987; Veron 1995; for a review of the first four schemes, see Stolarski and Roniewicz 2001). Despite the long history of the subject (e.g., Linnaeus 1758; Pallas 1766; Forskål 1775; Esper 1795; Lamarck 1801), taxonomic and evolutionary relationships within this important habitat-forming anthozoan order remain largely uncertain to date.

In their first comprehensive and consistent scheme that was heavily influenced by the skeletal macromorphological research of Milne Edwards and Haime (e.g., 1848a, b, c, d, e, 1850, 1851a, b, 1857), Vaughan and Wells (1943) hierarchically ordered several characters and devised keys to genera centered around an evolutionary hypothesis of Scleractinia. Although more recent analyses have included additional and more detailed subcorallite morphology, the revised version of this scheme published in the *Treatise on Invertebrate Paleontology* (Wells 1956) is still widely applied (Wood 1983; Veron 1986, 2000). The essence of Wells' (1956) scheme is that five scleractinian suborders can be distinguished based on characteristics of septal trabeculae and septal structure, with 33 families differentiated by wall type, occurrence of endotheca and type of budding.

The incorporation of subcorallite data into scleractinian classification was pioneered by Alloiteau (1952, 1957), who recognised a total of 65 families (30 with extant representatives) belonging to eight suborders. These groupings were later revised with greater emphasis on microstructural characters by Chevalier and Beauvais (1987), who proposed 11 suborders embracing 55 families. However, according to Stolarski and Roniewicz (2001: 1095), the microstructural criteria applied "to distinguish suborders containing only extinct taxa (i.e., Pachythecaliina, Distichophylliina, Archaeofungiina) are unclear or have not been supported by further research".

The most recent Scleractinia-wide classification divided the order into 13 suborders (7 with extant representatives) and 61 families (24 extant) (Veron 1995). However, as explicitly stated by the author, it had many points of uncertainty at subordinal and family levels. According to Budd et al. (2010), this evolutionary scheme had even lower resolution among families and suborders than the classification of Wells (1956), and by that time cladistic analyses had yet to contribute significantly to our understanding of scleractinian evolution. Indeed, the use of morphological characters to establish phylogenetic relationships within coral families have proved challenging and, as a consequence, applied to only a small number of extant families—Fungiidae (Cairns

1984; Hoeksema 1989, 1991, 1993), Mussidae and Siderastreidae (Pandolfi 1992), Turbinoliidae (Cairns 1997), Faviidae (Johnson 1998), Acroporidae (Wallace 1999), Dendrophylliidae (Cairns 2001), Atlantic Faviidae and Mussidae (Budd and Smith 2005), and Pacific Faviidae (Huang et al. 2009).

The recent recognition that the scleractinian skeleton is biologically controlled and not easily perturbed by environmental factors at the microstructural level (Janiszewska et al. 2011, 2013) has led to more detailed subcorallite observations (Cuif et al. 2003; Budd et al. 2012; Kitahara et al. 2012, 2013; Arrigoni et al. 2014a; Huang et al. 2014b; Janiszewska et al. 2015). Indeed, greater attention has been given to previously overlooked micromorphological and microstructural characters. Specifically, micromorphology considers the shapes of teeth along the wall, septa, columella, and septal face granulations, while microstructure is concerned with the cross-sectional wall structure, arrangements of rapid accretion centres and thickening deposits within the wall, septa, and columella (Cuif and Perrin 1999; Budd and Stolarski 2009, 2011). Together with improvements in our understanding of skeletal ontogeny, new studies of subcorallite morphology are shedding light on evolutionary relationships within the order. Indeed, the finding that intra-fibrous organic matrices containing complex macromolecular assemblages actually control nucleation, spatial delineation and organisation of basic microstructural skeletal units (Lowenstam and Weiner 1989) has provided support for several molecular clades (e.g., Cuif et al. 2003; Benzoni et al. 2007; Budd and Stolarski 2009, 2011; Janiszewska et al. 2011, 2015; Kitahara et al. 2012, 2013).

DNA sequences provide large numbers of phylogenetically informative characters that are independent of the high morphological variability of the coral skeleton. Various degrees of incongruence between morphological and molecular phylogenies are seen at all taxonomic levels, but the most striking is found at the subordinal level. While five suborders are recognised in the most widely-accepted morphological scheme (Wells 1956), only three main clades at the deepest nodes—"basal", "complex" and "robust"—have been recovered based on molecular analyses (Romano and Palumbi 1996; Kitahara et al. 2010b; Stolarski et al. 2011; Huang 2012). Nearly every genetic locus tested to date supports these latter groupings. The 28S rDNA (Chen et al. 1995; Cuif et al. 2003), 16S rDNA (Romano and Palumbi 1996, 1997; Le Goff-Vitry et al. 2004; Kitahara et al. 2010a), 12S rDNA (Chen et al. 2002), combined 16S rDNA and 28S rDNA (Romano and Cairns 2000), combined cytochrome b and COI, as well as β-tubulin (Fukami et al. 2008) all support the split between the "complex" and "robust" clades. The sister relationship between the "basal" clade and the rest of Scleractinia has been recovered by 12S rDNA, COI, 28S rDNA (Kitahara et al. 2010b; Stolarski et al. 2011), and most other mitochondrial loci (Huang 2012; Huang and Roy 2013, 2015; Kitahara et al. 2014; Lin et al. 2014). To date, no morphological characters associated with the hard skeleton have been found to correlate directly with the molecular splits. Interestingly, an examination of four "complex" and seven "robust" corals revealed that the two clades differ in embryonic developmental morphology ("prawn chip" in "complex" corals), with the notable exception of the "complex" *Pavona decussata*, which is more similar to "robust" clade representatives in this respect (Okubo et al. 2013). Expectedly, without any trace of soft tissue preserved, it would be even more challenging to position the extinct suborders on the coral phylogeny.

At the family level, the picture is not very different. Most families composed exclusively of zooxanthellate species have been shown by molecular data to be polyphyletic (Fukami et al. 2004b, 2008; Arrigoni et al. 2012). Among these, the most poorly understood families were Faviidae, Merulinidae, Pectiniidae and Trachyphylliidae (sensu Veron 2000). The Indo-Pacific representatives of these taxa had been called the "Bigmessidae" for their extremely chaotic and unnatural classification (Budd 2009; Huang et al. 2011). In contrast, the molecular evolutionary hypothesis posits that most families composed exclusively or predominantly of azooxanthellate corals are monophyletic. Therefore, apart from Caryophylliidae and Oculinidae, molecular groupings of azooxanthellate taxa are broadly consistent with classical taxonomy (Kitahara et al. 2010b; Stolarski et al. 2011).

According to our present understanding, the order Scleractinia comprises at least 30 clades that correspond to family-level groups. Among them, Gardineriidae and Micrabaciidae belong to the "basal" clade; Acroporidae, Agariciidae, Astrocoeniidae, Dendrophylliidae, Euphylliidae, Flabellidae, Fungiacyathidae, Guyniidae, Poritidae, Siderastreidae and Turbinoliidae from the "complex" clade; and Anthemiphylliidae, Caryophylliidae, Coscinaraeidae, Deltocyathiidae, Diploastraeidae, Fungiidae, Lobophylliidae, Meandrinidae, Merulinidae, Montastraeidae, Mussidae, Oculinidae, Plesiastreidae, Pocilloporidae and Psammocoridae represent the "robust" clade (Fig. 4.1). Genetic sampling for three families is limited or nonexistent. Rhizangiidae is represented only by the mitochondrial genome of an *Astrangia* species (Medina et al. 2006), which is closely related to *Oculina* (Huang 2012; Huang and Roy 2013, 2015). Stenocyathidae consists of three monotypic genera, of which only *Stenocyathus* has been sequenced and found nested within Caryophylliidae (Cuif et al. 2003; Kitahara et al. 2010b; Stolarski et al. 2011). Schizocyathidae contains three monotypic genera that have never been sampled for genetic data. Among the "robust" corals, *Madrepora* and *Heterocyathus* + *Oulastrea* appear to be two phylogenetically distinct lineages that cannot be placed in any of the above families.

4.4 New Taxonomic Revisions of Families and Genera

The abundance of taxonomic revisionary studies is increasing in recent years, but the resolution of all scleractinian families and genera is far from complete. A large amount of data and comprehensive taxonomic coverage are necessary to justify formal name changes following the International Code of Zoological Nomenclature, which have taken considerable time and effort by numerous coral taxonomists. Consequently, the first revision to jointly consider DNA sequence data and traditional forms of evidence such as morphology and reproduction in a phylogenetic context only emerged more than a decade after the first scleractinian molecular phylogenies by Romano and Palumbi (1996) and Veron et al. (1996).

The pioneering study by Wallace et al. (2007) used one mitochondrial (cytochome b) and one nuclear (histone 2a and 2b) gene to show that subgenus *Isopora*, previously placed within *Acropora*, was sufficiently distinct to be elevated to genus within family Acroporidae. *Isopora* tends to form more than one axial corallite per branch, while *Acropora* contains only a single axial corallite (Wallace et al. 2012). Reproductively, *Isopora* broods planula larvae and its oocytes are attached via a stalk to the mesenteries, in contrast to *Acropora* spp. which are broadcast spawners and have unstalked gonads.

Acroporidae expanded further when, following the comprehensive reconstruction of Fukami et al. (2008), Dai and Horng (2009a) transferred *Alveopora* from Poritidae to Acroporidae (see also Licuanan 2009). Like its new confamilials, *Alveopora* possesses synapticulothecal walls (Wallace 2012). Its exact phylogenetic placement is unstable to date, although evidence has pointed to a close relationship with *Astreopora* (Fukami et al. 2008; Kitahara et al. 2010b, 2014; Huang and Roy 2015; Kitano et al. 2014; Fig. 4.1).

Another group that underwent taxonomic changes relatively early was Siderastreidae. Fukami et al. (2008) first showed that the family was polyphyletic, with *Siderastrea* placed in the "complex" clade while the rest of the family was deep within the "robust" clade. Furthermore, Benzoni et al. (2007, 2010) found strong support to distinguish *Psammocora* from other "robust" siderastreids and resurrected Psammocoridae to accommodate the genus. The most recent analyses indicated that *Coscinaraea*, *Craterastrea*, *Horastrea* and *Anomastraea* constituted a monophyletic group that is sister to Psammocoridae, so the family Coscinaraeidae was proposed to contain these genera (Benzoni et al. 2012b; see also Huang 2012; Huang and Roy 2013, 2015).

These revisions implicated the closely-related Fungiidae as two former polystomatous and attached siderastreids, *Coscinaraea wellsi* and *Psammocora explanulata*, were genetically nested within the predominantly monostomatous and free-living Fungiidae and possessed the fungiid synapomorphy of compound synapticulae or fulturae, continuous buttress-like structures connecting the septa (Benzoni et al. 2007). The two rogue species were eventually transferred into *Cycloseris* (Benzoni et al. 2012a). Siderastreidae has thus been split into Siderastreidae, Psammocoridae and Coscinaraeidae, with two species transferred into Fungiidae. The latter also underwent a major reclassification based primarily on COI and ITS data, which supported the elevation of several subgenera previously in *Fungia* to genus, including *Cycloseris*, *Danafungia*, *Lobactis* and *Pleuractis* (Gittenberger et al. 2011). Several movements between genera were also proposed, such as the transfer of members of *Fungia (Verrillofungia)* into *Lithophyllon*, *Lithophyllon mokai* into *Cycloseris*, *Fungia (Danafungia) fralinae* into *Heliofungia*, and *Fungia (Wellsofungia) granulosa* into *Pleuractis*. Transformations of life history traits onto the molecular phylogeny further showed that the ability to be free living was lost four times and the evolution of multiple mouths occurred ten times, all independently throughout the evolutionary history of Fungiidae (Gittenberger et al. 2011).

The extreme polyphyly of the "robust" families Faviidae, Merulinidae, Mussidae and Pectiniidae revealed by Fukami et al. (2004b, 2008), coupled with the large number of species and genera in these taxa, posed severe challenges for taxonomic definitions of these corals. There was widespread acknowledgement that reclassification was necessary (Fukami 2008; Budd 2009; Budd et al. 2010), but the convergence of most macromorphological characters conventionally used to define genera and families hindered revisionary work. Many molecular (Huang et al. 2009, 2011; Benzoni et al. 2011; Arrigoni et al. 2012; Schwartz et al. 2012) and morphological (Budd and Smith 2005; Budd and Stolarski 2009, 2011) studies identified problematic taxa and highlighted phylogenetically informative characters—including molecular markers, macromorphology, micromorphology and microstructure—before the first taxonomic monograph was published.

In a massive undertaking, Budd et al. (2012) expanded Merulinidae to include all members of the "Bigmessidae" clade (XVII sensu Fukami et al. 2008), made up of mostly Indo-Pacific species from Faviidae, Merulinidae, Pectiniidae and Trachyphylliidae as defined by Veron (2000). They also relegated Faviidae to subfamily Faviinae as a group restricted to the Atlantic, and synonymised Pectiniidae and Trachyphylliidae as Merulinidae. Mussidae was redefined to include Mussinae (Atlantic mussids) and Faviinae. Finally, Pacific "mussids", the three remaining pectiniid genera, *Echinomorpha*, *Echinophyllia* and *Oxypora*, as well as

Moseleya were placed in the new family Lobophylliidae (Dai and Horng 2009b).

Budd et al. (2012) also proposed several modifications at the genus level. The highly polyphyletic *Favia* and *Montastraea* were trimmed of their Indo-Pacific and "Bigmessidae" members, which were accommodated by *Dipsastraea* and *Phymastrea* respectively, so that *Favia* now contains *F. fragum* and *F. gravida*, while *Montastraea* only includes *M. cavernosa. Montastraea* and *Diploastrea* were also placed in their own respective families Montastraeidae and Diploastraeidae as appropriate for their distinctiveness. *Scolymia* became an Atlantic genus, so its Indo-Pacific constituents became *Homophyllia australis* and *Parascolymia vitiensis*.

More recently, Huang et al. (2014a, b) examined Merulinidae more closely and found that more revisions at the genus level were necessary. In particular, *Astrea* was resurrected and a new genus *Paramontastraea* established to contain some species from *Phymastrea*, which was synonymised as *Favites. Coelastrea* was revived and a new genus *Paragoniastrea* described to accommodate distinct species previously classed in *Goniastrea*. *Barabattoia* and *Paraclavarina* were neither genetically nor morphologically separated from *Dipsastraea* and *Merulina* respectively, and were thus synonymised.

Major changes to the recently-established Lobophylliidae are ongoing, as Arrigoni et al. (2014b) considered *Australomussa* as a junior synonym of *Parascolymia*, and also resurrected *Sclerophyllia* to accommodate *S. margaritaricola* and its sister species *S.* (previously *Acanthastrea*) *maxima* that are endemic to waters surrounding the Arabian peninsula (Arrigoni et al. 2015).

Several taxa thought to be closely affiliated with Pacific "faviids" and "mussids"—Merulinidae and Lobophylliidae respectively—are now in distant "robust" taxa. Dai and Horng (2009b) transferred *Plesiastrea* into Plesiastreidae (clade XIV sensu Fukami et al. 2008), although only the move of the type species *P. versipora* has been validated since *P. devantieri* is in *Astrea*, Merulinidae (Huang et al. 2014b). *Blastomussa* was also transferred into Plesiastreidae (Dai and Horng 2009b), but it has been considered *incertae sedis* more recently (Budd et al. 2012; Benzoni et al. 2014) as *Plesiastrea* is more closely related to the azooxanthellate species *Cyathelia axillaris*, *Trochocyathus efateensis* and *Tethocyathus virgatus* (Kitahara et al. 2010b; Benzoni et al. 2011; Huang 2012; Huang and Roy 2013, 2015). Furthermore, the closest relatives of *Blastomussa* are *Physogyra*, *Plerogyra* and *Nemenzophyllia*, all previously in the "complex" Euphylliidae and now *incertae sedis* (Fukami et al. 2008; Kitahara et al. 2010b; Benzoni et al. 2014). *Oulastrea* is part of a deep-branching clade sister to Fungiidae, Psammocoridae and Coscinaraeidae (Huang 2012; Huang and Roy 2013, 2015), and may revert to the family Oulastreidae (Veron 2013). Perhaps the most enigmatic and still unresolved case of a former Pacific "faviid" is that of *Leptastrea*, which has been consistently shown as closely related to Fungiidae based on different markers (Romano and Palumbi 1996; Romano and Cairns 2000; Fukami et al. 2008; Kitahara et al. 2010b) despite striking differences in morphology between this genus and any of the known mushroom coral genera.

While the first integrative taxonomic revision was performed for Acroporidae in the "complex" clade, progress on other "complex" groups has been limited compared to the "robust" corals. Only recently was the first comprehensive revision of Poritidae published. Kitano et al. (2014) analysed samples from all five poritid genera using COI and ITS to show that *Porites* was monophyletic, but *Machadoporites* and *Poritipora* cannot be distinguished from *Goniopora* and were thus synonymised. The authors also found that *Goniopora stutchburyi* was genetically isolated from its congenerics but was the only sister species of *Stylaraea*, and thus moved it into a new genus, *Bernardpora*.

The azooxanthellate corals have lagged far behind in terms of revisionary work, due to much fewer taxonomists working on the numerous scleractinian lineages that contain them. Nevertheless, problematic taxa have been identified through broad-scale phylogenetic analyses (Kitahara et al. 2010b; Stolarski et al. 2011), and revisions have commenced. For instance, *Dactylotrochus cervicornis* was genetically nested among Agariciidae species, so Kitahara et al. (2012) moved it from Caryophylliidae into Agariciidae, making it the first extant agariciid that is solitary and azooxanthellate. An azooxanthellate shallow-water agariciid, *Leptoseris troglodyta*, was described shortly after (Hoeksema 2012). Finally, a new family Deltocyathiidae that included nearly all the species of *Deltocyathus* was established for an early-diverging clade traditionally placed in Caryophylliidae (Kitahara et al. 2013).

4.5 Detection of Species Boundaries

Identification of coral species has always been problematic. The overlap of morphological variation between and within colonies (i.e., between corallites) obscures species boundaries. Although species delimitation among scleractinian corals has been studied for many corals, data are still limited. The most studied coral in this respect is the *Orbicella annularis* complex. This group of three species, *O. annularis*, *O. franksi* and *O. faveolata*, is amongst the dominant corals of many Caribbean reefs. Historically, they have been considered as one species, *O. annularis*, with several morphs distributed along various environmental gradients, including different depths and reef zones (Graus and Macintyre 1976, 1989). However, a tremendous number of studies on morphology, reproduction, ecology, growth rates and genetics

have been carried out (Knowlton et al. 1992, 1997; van Veghel and Bak 1993, 1994; van Veghel 1994; van Veghel and Kahmann 1994; Weil and Knowlton 1994; van Veghel and Bosscher 1995; van Veghel et al. 1996; Lopez and Knowlton 1997; Szmant et al. 1997; Lopez et al. 1999; Medina et al. 1999; Manica and Carter 2000; Knowlton and Budd 2001; Fukami et al. 2004a; Levitan et al. 2004, 2011), nearly all of which showing that the complex is not a single species with high morphological variation but comprises three separate species.

The research effort devoted to resolving the *Orbicella annularis* complex was unprecedented for corals, and remains unmatched for other taxa that are seemingly as complex. Nevertheless, there have been several cases whereby species complexes showed varied levels of separation and no taxonomic action was taken. We describe some of these examples as follows.

In order to investigate species boundaries, crossing experiments and spawning observations are the most precise approaches to test for reproductive isolation between species (Lang 1984). However, data from such studies are limited in terms of taxonomic and geographic coverage. Crosses have been tested for a variety of *Acropora* species and interspecific fertilisation observed in several combinations (Willis et al. 1997; Hatta et al. 1999; van Oppen et al. 2002; Fukami et al. 2003; Isomura et al. 2013). Nevertheless, interspecific fertilisation rates tend to be lower than intraspecific ones (Wei et al. 2012), allowing species boundaries to be defined (Willis et al. 2006). *Acropora* colonies with intermediate morphologies between species are generally not used for such experiments and remain challenging subjects for taxonomic research. Species boundaries of such difficult morphologies have been explored in two instances. First, five species and seven morphs from the *A. humilis* species group were examined by Wolstenholme (2004) for their reproductive patterns and molecular phylogeny. The data indicated that the five species were valid and the morphs at different stages of divergence from the valid species. Second, Suzuki and Fukami (2012) analysed the fertilisation rates and molecular phylogenetic relationships of three morphs of *A. solitaryensis* and found that two morphs were actual variants of the species while the last one was an undescribed species.

The merulinid genus *Platygyra* has also been used in multiple experimental crosses due to its abundance in the Indo-Pacific and problematic species identities. Miller and Babcock (1997) performed crossing experiments and recorded spawning times to show that reproductive isolation was severely limited among seven species in the Great Barrier Reef. Moreover, Miller and Benzie (1997) found that three species, *P. daedalea*, *P. sinensis* and *P. pini* contained no fixed differences in allozyme frequencies. However, contrary to these results, molecular phylogenetic analysis using ITS sequences revealed clear genetic differences between *P. sinensis* and *P. pini* in Hong Kong (Lam and Morton 2003). To date, species boundaries among *Platygyra* species remain unresolved, although results have so far suggested that geographic variation in the degree of species separation is apparent.

Cryptic diversity within species exists in several other corals. For example, comparisons of *Mycedium elephantotus* colonies between different localities in Taiwan revealed the existence of at least two reproductive groups based on timings of gametogenesis and spawning, supported by allozyme electrophoretic data (Dai et al. 2000). In fact, intraspecific differentiation was detected between co-occurring populations of *Cycloseris costulata* in Indonesia (Gittenberger and Hoeksema 2006), *P. daedalea* in Kenya (Mangubhai et al. 2007), and *Favites valenciennesi* in Japan (Fukami and Nomura 2009). Larger geographic contrasts such as between Red Sea and Pacific Ocean populations of *Dipsastraea* and *Stylophora* have also revealed molecular separation between regions (Stefani et al. 2011; Arrigoni et al. 2012; Keshavmurthy et al. 2013). However, to reach a stage where taxonomic revisions can be attempted, broad geographic sampling across the Indo-Pacific and detailed studies of closely-related species are necessary, such as in the case of species in *Astreopora* (Suzuki and Nomura 2013), *Pocillopora* (Pinzón and LaJeunesse 2010; Pinzón et al. 2012, 2013; Torda et al. 2013a, b; Marti-Puig et al. 2014; Schmidt-Roach et al. 2013, 2014) and *Psammocora* (Benzoni et al. 2010; Stefani et al. 2008a). In particular, boundaries among *Psammocora* species were clarified through a series of rigorous molecular and morphological analyses (Stefani et al. 2008a, b; Benzoni et al. 2007, 2010), which saw 24 nominal species reorganised as seven valid species—*P. albopicta*, *P. contigua*, *P. digitata*, *P. haimiana*, *P. nierstraszi*, *P. profundacella* and *P. stellata*.

Crossing experiments are usually performed for broadcast-spawning corals because it is relatively easy to collect eggs and sperm, but are difficult to apply on species that brood, are gonochoric or release daughter colonies asexually. Temporal reproductive isolation has been examined in some fungiids (Loya et al. 2009), but for other taxa, detailed morphological analyses with type material combined with molecular methods have been used to define species boundaries, such as in *Pocillopora damicornis* (Schmidt-Roach et al. 2014) and *Goniopora stokesi* (Kitano et al. 2013). Considering that coral spawning usually occurs once a year, it may be prudent to use these approaches on top of crossing experiments. Unfortunately, the latter may be the only way to tell species apart as some closely-related corals may be indistinguishable with morphological and molecular methods (e.g., Forsman et al. 2009).

An important goal of species delimitation is to characterise intraspecific morphological variation, but cryptic species that are still undergoing introgression may occur without

fixed morphological differences throughout their distribution, such as in *Acropora cytherea* and *A. hyacinthus* (Ladner and Palumbi 2012). We expect more corals to possess such a signature, but an unambiguous procedure to deal with them taxonomically remains to be established.

4.6 Unresolved Taxa and the Future of Coral Systematics

Much of biology depends critically on a reliable taxonomic framework (Wheeler 2004). In modern times, such a framework has been built with molecular data on top of traditional and updated morphological evidence that has been the mainstay of taxonomy. Often, developmental, reproductive, and other ecological data are also gleaned for such research. Within the last two decades, coral biologists have developed a systematic phylogenetic approach that integrates these lines of evidence. Indeed, molecular data have been the major driving force in modern coral taxonomy, and together with the application of new techniques to explore subcorallite morphology, new light is still being shed on scleractinian phylogeny.

Although morphological evidence to support the three deep molecular clades is still scarce, microstructural characters such as the structure and arrangement of rapid accretion deposits and thickening deposits have proven to be phylogenetically informative at the family level (Budd and Stolarski 2011; Kitahara et al. 2013; Arrigoni et al. 2014a). Micromorphological traits such as shape of septal teeth, the development of secondary calcification axes and corresponding granulation on septal teeth and faces, the shape of the area between teeth, fulturae (Gill 1980), and others, are also useful for the differentiation of some genera within zooxanthellate coral families and genera (Benzoni et al. 2007; Budd and Stolarski 2009). In the same way, the delineation of primarily azooxanthellate coral families has largely been resolved, with few notable exceptions including Caryophylliidae (Kitahara et al. 2010b, 2012, 2013; Stolarski et al. 2011) and Oculinidae (Kitahara et al. 2010b; Huang and Roy 2015).

While rapid improvements have been achieved in scleractinian systematics, there are still unresolved taxa. In reality, the evolutionary positions of some families and genera, especially those still based solely on macromorphological characters (e.g., Wells 1956), remain tentative. Furthermore, only about one-third of all scleractinian species have been examined phylogenetically (Fig. 4.1; Huang and Roy 2015), and for most of these species, few genetic markers have been used. Families that are still showing considerable uncertainties in their evolutionary positions include Anthemiphylliidae, Astrocoeniidae, Caryophylliidae, Oculinidae and Siderastreidae (Benzoni et al. 2007; Fukami et al. 2008; Kitahara et al. 2010b, 2012; Huang 2012; Huang and Roy 2013, 2015). In the case of genera, the emerging picture is even more concerning, as we are still unable to place many of them precisely on the phylogeny. They include *Anthemiphyllia*, *Astrangia*, *Catalaphyllia*, *Cladocora*, *Culicia, Gyrosmilia, Indophyllia*, *Leptastrea*, *Montigyra*, *Paracyathus*, *Polycyathus*, *Simplastrea*, *Solenastrea* and *Stephanocyathus*. Representatives of some of these genera are rare or restricted to remote localities and sampling them for molecular analyses poses a practical challenge. Nevertheless, some genera with sufficient numbers of representatives tested have been shown to be para- or polyphyletic. Among them, some of the most problematic genera are within the families Agariciidae (*Leptoseris* and *Pavona*), Dendrophylliidae (*Balanophyllia*, *Cladopsammia*, *Dendrophyllia* and *Rhizopsammia*), Caryophylliidae (*Phyllangia* and *Rhizosmilia*), Euphylliidae (*Euphyllia* and *Galaxea*), Flabellidae (*Flabellum* and *Truncatoflabellum*) and Oculinidae (*Oculina*). Unfortunately, only a few genetic markers have been sequenced from these genera, and most are only informative at higher taxonomic levels.

Endeavouring to improve our understanding of scleractinian evolution as a lineage and as a system, we recognise and consider some important future research directions. Amongst these, the most obvious is "the species problem in corals" as foreshadowed by Hoffmeister (1926: 151) and nowadays made increasingly clear by the application of molecular techniques; establishing a clear and unambiguous phylogenetic framework must be one of the first challenges to be addressed. Since reliable taxonomic information is essential for the interpretation of molecular phylogenies, institutional and financial investments should be made toward building strong specimen collections and spurring rigorous taxonomic research. In particular, the inclusion of more material with broader taxon coverage and multiple sampling localities in future phylogenetic studies should be supported consistently by *in situ* images, collection of voucher specimens and fixed tissue samples for deposition in accessible repositories. This will allow re-examination of evidence as new molecular and morphological techniques become available. Moreover, importance should also be accorded to existing historical reference collections, including type material of extant and extinct coral taxa for which only a morphological approach can be used.

Although coral molecular phylogenetic studies generally focus heavily on few mitochondrial or ribosomal markers, and whilst these have greatly improved our understanding of scleractinian phylogenetic relationships, it is now clear that to achieve higher resolution and to be able to investigate all taxonomic levels, multiple genetic markers are essential (e.g., Dunn et al. 2008; Philippe et al. 2009; Regier et al. 2010). In the case of corals, a stumbling block to applying such multilocus phylogenetics is the paucity of single copy

nuclear markers that have been tested. To cross this hurdle, we must turn to high-throughput sequencing technologies for obtaining genomic or transcriptomic data for a range of corals. These methods could be used to collect near-exhaustive molecular data possibly containing phylogenetic signal at all levels. However, notwithstanding the progress expected with phylogenomics, we stress that improvement of techniques and better understanding of the taxonomic signals and environment-induced variability of morphological characters are essential for advancing the field.

As we go forth in this new age of coral systematics, the gap between the state-of-the-art classification and practical needs of the broader scientific community appears to be widening. Indeed, while taxonomic changes resulting from integrative analyses are increasingly being published, the outdated but understandably more widely-accepted scheme is still being applied in some recent work on corals and their associates (e.g., Ho and Dai 2014; Tsang et al. 2014; Work and Aeby 2014). A lag is to be expected before the new framework is embraced outside the restricted circle better informed of the ongoing revisions. To bridge this gap more rapidly, we urge more active collaborations between taxonomists and ecologists, as well as more user-friendly literature such as field illustrations of corals under the revised classification (e.g., Dai and Horng 2009a, b; Licuanan 2009).

Thus, apart from encouraging a new generation of taxonomists, molecular biologists, and paleontologists, the foment of multi- and interdisciplinary studies including taxonomy, ecology, morphology, molecular biology, palaeontology and oceanography, will shape future studies positively to help improve our understanding of scleractinian evolution. This is indeed a welcome development in a time of major scientific interest and intense public concern due to the uncertain fate of coral reefs in the face of anthropogenic challenges.

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Ceriantharia in Current Systematics: Life Cycles, Morphology and Genetics

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Sérgio N. Stampar, Maximiliano M. Maronna, Marcelo V. Kitahara, James D. Reimer, Julia S. Beneti, and André C. Morandini

Abstract

The systematics of the class Anthozoa has been built-up by a number of authors with different approaches. However, despite all efforts the evolutionary position of the tube-dwelling anemones (subclass/order Ceriantharia) remains unclear. This dilemma has arisen especially due to lack of understanding of the homologies that date back to the early twentieth century. Thus, this review focuses on discussing the morphological and genetic patterns and inferences of Ceriantharia in relation to other groups of Anthozoa. Initially we discuss the basic aspects of Ceriantharia, followed by a comparison of ceriantharian characters to other anthozoan groups and also provide a discussion on the uncertain systematic and evolutionary position of the order bringing up some perspectives on future studies of Ceriantharia.

Keywords

Tube dwelling anemones • Cnidaria • Anthozoa • Integrative taxonomy • Phylogenetics • Morphology

5.1 Introduction

The class Anthozoa is currently recognized as a natural group (monophyletic clade) formed by two main clades accepted as subclass ranks: Hexacorallia and Octocorallia (Daly et al. 2007). Fossils of Anthozoa, those representing hexacorallians, have been the basis for the hypothesis that the group had its onset between the Lower Cambrian in the Paleozoic (~540 million years ago) (Han et al. 2010) and the Ediacaran in the Pre-Cambrian (~600 million years ago) (Scrutton 1999).

The first studies of Anthozoa date back to Aristotle's manuscripts (384–322 BC) where two octocorals (*pneumōn* – later *Alcyonium palmatum* Pallas 1766 and holothourion – later *Veretillum cynomorium* (Pallas 1766) and two sea anemones (*acalēphē sklērē* – later *Actinia equina* (Linnaeus 1758) and *acalēphē edōdimos* – later *Anemonia viridis* (Forsskål 1775) were described (McMurrich 1910a). Modern taxonomic studies started with the publications of Linnaeus (1758) and Pallas (1766), and nowadays have resulted in the formal descriptions of approximately 7,500 extant species (Daly et al. 2007) and

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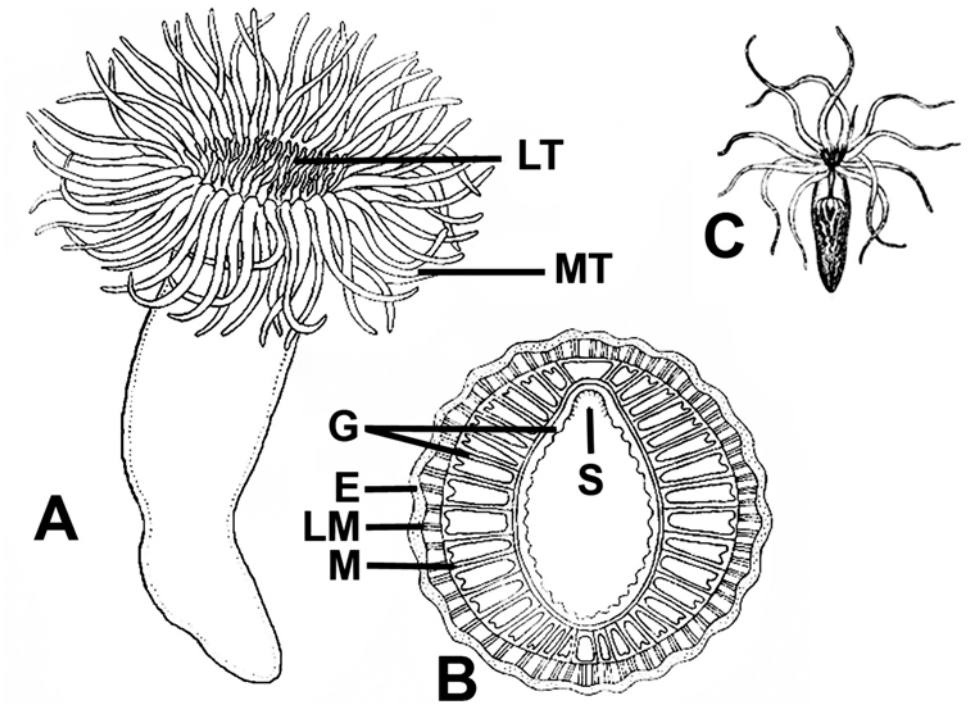


Fig. 5.1 Graphical representation of two main stages of the life cycle of Ceriantharia. (**a**) Benthic specimen; (**b**) Cross-section of a Ceriantharia at pharynx; **(c)** Planktonic specimen. *LT* Labial tentacles, *ML* Marginal tentacles, *G* Gastrodermis, (*E*) Epidermis, *LM* Longitudinal musculature, *M* Mesentery, *S* Siphonoglyph (Adapted from Nyholm 1943)

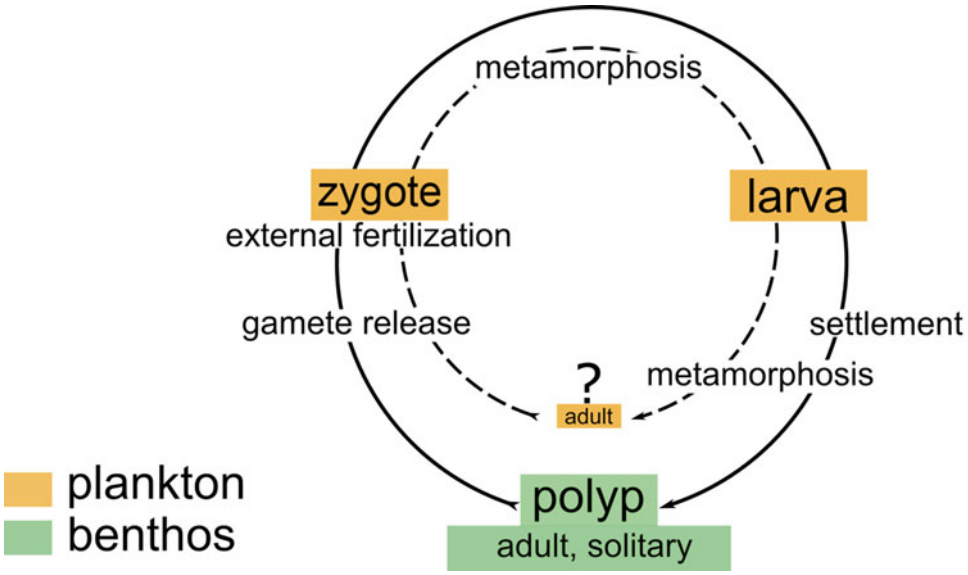
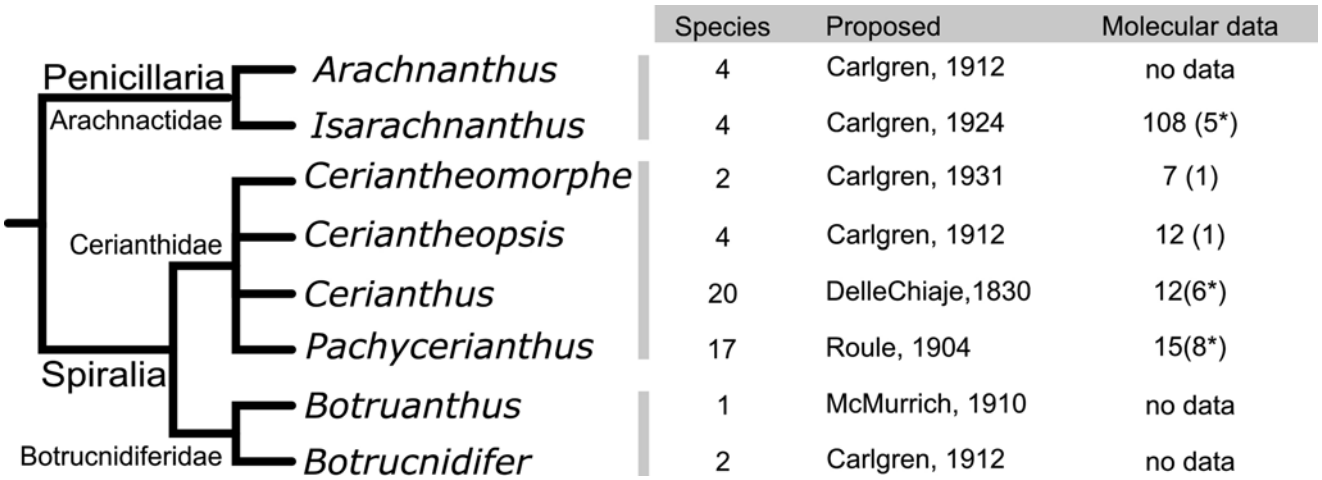


Fig. 5.2 Basic cerianthid life cycles, considering major stages (larva and polyp), events (e.g. gamete release, fertilization, settlement) and development mechanism (metamorphosis). Inner life cycle (*dash line*) represent the deduced life cycle for holoplanktonic species

6,572 extinct (fossil) species (Data from Paleobiology Database on 08 December, 2015 using "Taxon Count" search).

Although in depth anatomical studies remain scarce for some orders, three morphological/anatomical characters define the Anthozoa clade: the presence of an actinopharynx, a siphonoglyph region, and mesenteries with trilobed filaments (Won et al. 2001) (Figs. 5.1 and 5.2). The actinopharynx is a region formed by epidermal tissues which are usually curled at the region exposed to the gastrovascular cavity (Daly et al. 2007). The siphonoglyph is a highly ciliated area within the 'corner' of the actinopharynx known to have several forms within Anthozoa (Hyman 1940; Manuel 1981), and mesenteries that are sheet-like partitions of the body wall that extends to the gastrovascular cavity and, in anthozoans, have specialized filaments on their border (Won et al. 2001).

Unlike the general recognition of the monophyly of Anthozoa, the debate related to the validity of the Hexacorallia is very controversial (Chen et al. 1995) (Figs. 5.3 and 5.4). Currently 3,100–4,300 extant hexacorallian species are known (Grassé 1987; Appeltans et al. 2012), of which, conservatively speaking, are divided into six orders (Actiniaria, Antipatharia, Ceriantharia, Corallimorpharia, Scleractinia, Zoantharia) (Daly et al. 2007). Although attempts to define synapomorphies for this clade have been proposed (e.g. ratio of perfect mesenteries, position and formation of the mesen-



Species	Proposed	Molecular data
4	Carlgren, 1912	no data
4	Carlgren, 1924	108 (5*)
2	Carlgren, 1931	7 (1)
4	Carlgren, 1912	12 (1)
20	DelleChiaje,1830	12(6*)
17	Roule, 1904	15(8*)
1	McMurrich, 1910	no data
2	Carlgren, 1912	no data

Fig. 5.3 Current classification for Ceriantharia showing major groups (orders, families and genera) and detailing number of valid species, year of original proposals and available molecular data. Symbol * indicates presence of non definitive species identifications ("sp" in GenBank records)

teries, shape of mesenteric filaments, variation of cnidae types) (Won et al. 2001), the quest has not yet been successful. Polyp symmetry was also discussed as a possible synapomorphy for the clade, however the wide variation observed mainly in relation to the Ceriantharia restricts its usefulness (Manuel 1981; Daly et al. 2007; Stampar et al. 2014a). Authors suggesting use of this character as a synapomorphy are based on larval aspects of Ceriantharia Unfortunately, the usage of this character as a synapomorphy was based on larval aspects of Ceriantharia (van Beneden 1897), which, in some cases (e.g. Stampar et al. 2015c) clearly resemble adult polyps of some groups of Hexacorallia, in particular Antipatharia (Nyholm 1943; Brugler and France 2007; Stampar et al. 2015c). Based on this, van Beneden (1897) proposed the clade Cerianthipatharia combining Ceriantharia with Antipatharia. This relationship lasted for decades until molecular data (Chen et al. 1995) demonstrated that Ceriantharia and Antipatharia were not grouped within a monophyletic clade (Fig. 5.5).

5.2 Overview of Studies on Ceriantharia

Meaning "wax flower" and deriving from the most popular genus among Ceriantharia, *Cerianthus* Delle Chiaje, 1830, the generic name "cerianthid" is broadly used as a reference to all representatives of the Ceriantharia (also known as tube-dwelling anemones). Cerianthids have a cylindrical body that is usually worm-like (Tiffon 1987). The aboral side of the polyp is hollow and dull, opened by a pore. The column, oral disc and tentacles are smooth and their tissues are simple. The tentacles are divided into marginal tentacles on the edge of the oral disc, and labial tentacles, which are around the mouth (van Beneden 1924; Tiffon 1987) (Fig. 5.1). Many larval specimens were obtained from plankton samples and these were described as new species because some of them have reproductive tissues in this stage. Probably these specimens are just more advanced stages in the larval development, since to date no species with "recognizable complete" holoplanktonic life cycle has been described for the group (Molodtsova 2004; Stampar et al. 2015c) (Fig. 5.1).

Scientific knowledge of Ceriantharia dates back from the late 1700s, with the description of an aberrant and tubular hydroid with a membranous tube around its body; *Tubularia membranosa* (Spallanzani 1784). Renier (1807) described the sea anemone, *Actinia cylindrica*, also with a membranous tube, and latter repositioned it as *Moschata rhododattila*, another sea anemone (Reiner in de Blainville 1830). This classification was maintained and Ceriantharia species were kept mixed within Actiniaria until Delle Chiaje (1841) deduced that the *T. membranosa* was not a hydrozoan and indicated that it also not even to be grouped with other anemones. However, although a proposal to establish a group embracing only tube-dwelling anemones was not presented, Delle Chiaje (1841) defined the genus *Cerianthus* as sea anemones with two cycles of tentacles – "…*Corpo cilindraceo con apertura conica anteriore*, *cinta da duplice serie di tentacoli lunghi gli esterni o marginali*…".

In 1851, Milne-Edwards and Haime (pg. 13) indicated that cerianthids were distinct in relation to Actiniaria and thus proposed the family, Cerianthidae, implying that it would probably represent a new order. A few years later Hertwig (1882) started the discussion of the "phylogenetic" position of this group in relation to other Anthozoa. At that time, the definition of the characters for comparison between cerianthid species, in addition to the development of comparative proposals for higher levels had already begun. Therefore, improvements on the studies of tube-dwelling anemones occurred, mainly determined by van Beneden (1897) and McMurrich (1910a) that started to describe larval morphotypes as different species. These studies were based on Sars (1846), who had described a "swimming polyp" called *Arachnactis*. These pulses of studies have

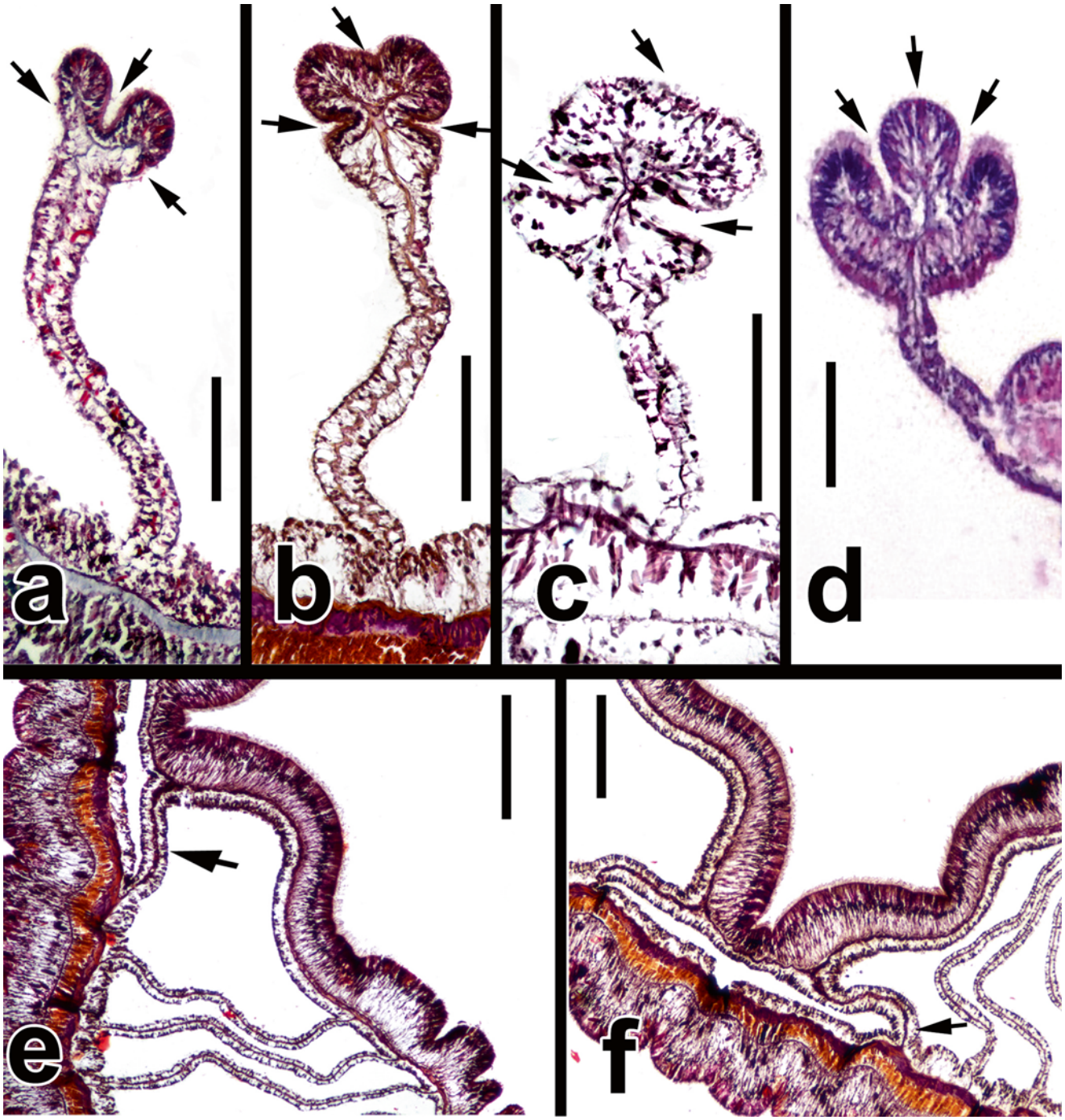


Fig. 5.4 Mesentery and mesenteric filament (*arrows*) in histological sections of different members of Ceriantharia (**a**) *Isarachnanthus nocturnus* (Arachnactidae), (**b**) *Ceriantheomorphe brasiliensis* (Cerianthidae) and (**c**) *Botrucnidifer novergicus* (Botrucnidiferidae), Actiniaria (**d** *Diadumene paranaensis*). Mesentery attached to siphonogryph (*arrows*) in Ceriantharia (**e** *Ceriantheomorphe brasiliensis*; **f** *Ceriantheomorphe* sp.) Scale 100 μm

resulted in the description of 85 species belonging to 32 genera until now (Molodtsova 2004; Stampar et al. 2015c). Furthermore, McMurrich (1910a) also undertook observations on the "benthic" species of Ceriantharia and erected the first systematic classification of the group, dividing Ceriantharia into two sub-orders: Acontiferae (including families Cerianthidae and Arachnactidae) and Botrucnidiferae (only Botrucnidiferidae). This classification was based on the relative position of the longer mesentery pair and was simply an adaptation of the proposition made by van Beneden (1897). However, this first systematic delineation was shortly discredited by Carlgren (1912),

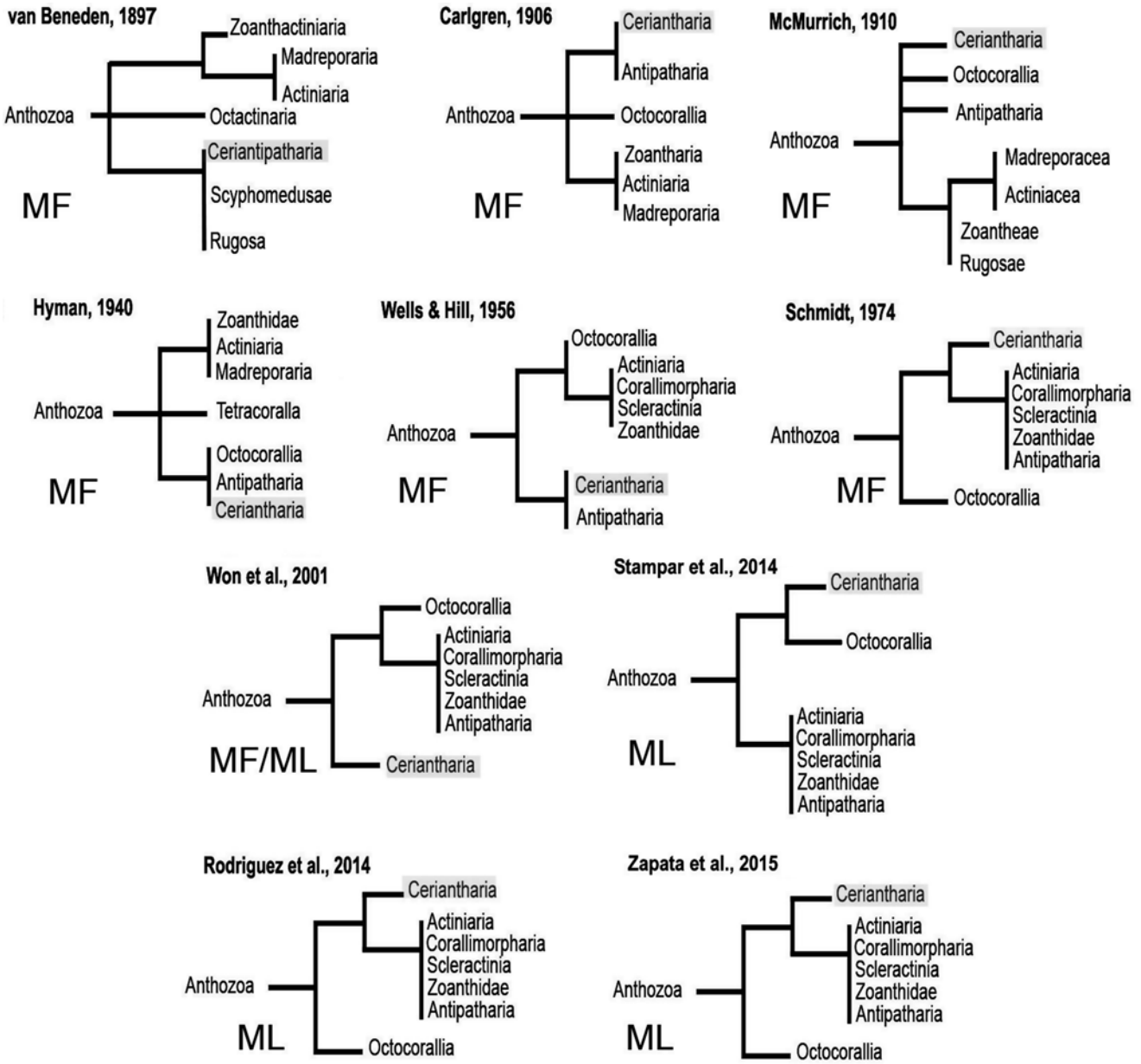


Fig. 5.5 Examples of classifications dealing with Ceriantharia in relation to other Anthozoa (*MF* Morphology, *MF/ML* Morphology/Molecular, *ML* Molecular)

which is one of the most influent studies on tube-dwelling anemones to the present day.

Although the most important aspects of tube-dwelling anemone taxonomy continued to be related to the shape and arrangement of the mesenteries and cnidome (compilation of cnidae types in each region of the polyp), Carlgren (1912) assembled all useful characters for Ceriantharia taxonomy, compiled the nomenclature of all genera of "benthic" Ceriantharia known (i.e.: *Arachnanthus*, *Botrucnidifer*, *Botruanthus*, *Ceriantheopsis*, *Cerianthus*, and *Pachycerianthus*), and attempted to synonymize some larval morphotypes (*Calpanthula*, *Cerianthula* and *Hesenanthula*) with the benthic genus *Botruanthus*. However, such synonymies were not readily adopted by subsequent authors (e.g. Leloup 1964), leading to likely synonymous names that are still valid by the absence of studies on the life cycle of many Ceriantharia species. Finally, Carlgren (1912) redescribed three families of Ceriantharia: Acontiferidae (later replaced by Arachnactidae den Hartog 1977), Botrucnidiferidae, and Cerianthidae.

Since then, few studies have been conducted with a systematic focus on Ceriantharia, being the majority related to taxonomy of benthic (few) and planktonic species (many) (e.g. Arai 1965; Carter 1995). Among these we should

mention the studies by Calabresi (1927, 1928) and especially Leloup (1931, 1932, 1942, 1960, 1964), which recorded a number of larval forms of Ceriantharia occurring in the plankton, describing many of these as "larval species". These descriptions are based only on larval morphotypes, and were often based only a single specimen (Molodtsova 2004; Stampar et al. 2015c).

Schmidt (1972, 1974) corroborated the families based on the cnidome as previously proposed by Carlgren (1912), a fact also verified by den Hartog (1977) on the studies of the taxonomy of Caribbean specimens. Nonetheless, beyond the cnidome, den Hartog also noted differences in the structure/shape of mesenteries and relative oral disk sizes, concluding that the Ceriantharia was represented by two main groups (suborders) – Spirularia (Cerianthidae and Botrucnidiferidae, 99 species (benthic/planktonic) and Penicilaria (Arachnactidae, 38 species (benthic/planktonic)) – that are still in use (Fig. 5.3).

5.2.1 Brief Historical Summary on Ceriantharia Taxonomic Proposals

Studies on benthic Ceriantharia have been limited by the difficulty of collecting specimens and therefore to define full life cycles and diagnostic characters in Ceriantharia. In shallow areas, it is often necessary to withdraw much sediment in order to collect a specimen (Rosa 1973). In this way, the amount of studies performed on benthic Ceriantharia is relatively few compared to other anthozoan groups.

The diagnostic characters for the taxonomy of benthic tube-dwelling anemones have only briefly been systematically addressed, and publications by van Beneden (1897) and McMurrich (1910a) form the basis of Ceriantharia studies. However, in both cases only descriptions of materials without comparisons of characters were made. The only study that has addressed characters in a concise and comparative way was carried by Carlgren (1912), who detailed all known characteristics, discussing them based on the description of several species. However, only a few of the analyzed species were represented by more than one specimen, and discussion was restricted to interspecific variation. Arai (1965) also presented some morphological characters that were used in Cerianthidae taxonomy, although the real utility of these characters was not discussed. In addition, although no new terms were introduced, Arai (1965) also compiled a list of Ceriantharia taxonomic and morphological terms, and den Hartog (1977) presented a comprehensive discussion on the cnidome variation in Ceriantharia, but did not comparatively address any other characteristics.

5.3 Diagnostic Characters and Classification of Main Ceriantharia Groups

Based on the study of larval forms, van Beneden (1897) divided the Ceriantharia into two groups: Acontiferae (species with acontia) and Botrucnidiferae (species with botrucnidae). Later, examining adults and larvae, McMurrich (1910a, b) proposed a subdivision of Acontiferae based on the position of the longest pair of mesenteries – family Cerianthidae (protomesenteries) and family Arachnactidae (metamesenteries). However, McMurrich (1910a) maintained the family Botrucnidiferidae as the sister group of other Ceriantharia. Modifications on only reorganization or addition of some genera have occurred (Carlgren 1931; Arai 1965). The most recent reorganization of families and genera was performed by den Hartog (1977), when he offered many reasons for differentiating Cerianthidae from Arachnactidae. At the same time, it was shown that Cerianthidae and Botrucnidiferidae have many similarities, and the proposition of two suborders based primarily on cnidome as well as anatomical aspects was put forward – Spirularia (Cerianthidae and Botrucnidiferidae) and Penicillaria (Arachnactidae) – based on primarily cnidome as well as anatomical aspects, nowadays with a total of eight genus (Fig. 5.3).

5.4 Review of the Systematic Position of Ceriantharia Within Cnidaria

The first reconstructions of the systematic position of Ceriantharia were obviously based on morphological aspects (e.g. van Beneden 1897; McMurrich 1910a). However, in the past two decades the tendency has been completely reversed, with more than a dozen studies being based on molecular data and only few studies conducted with morphological data. Both of these approaches deserve explanation and review.

5.4.1 Morphological and Biological Aspects

Unlike the profusion of phylogenetic reconstructions based on somewhat limited molecular data, the few existing morphological evaluations have utilized more comprehensive and differentiated approaches. Currently, however, there are still many inconsistencies in these results as well, particularly in the confirmation and also interpretation of homology within characters. As previously mentioned, since Andres

(1884) there have been attempts to associate Ceriantharia with other groups of Hexacorallia, a consequence of the "miscorrelation" of the external similarity between Actiniaria with Ceriantharia-like body shape (particularly the column) and tentacles. However, more detailed observations on the morphology (especially anatomy) and data on the ontogeny of ceriantharian species enabled the discrimination between tube-dwelling anemones and all other groups of Anthozoa (van Beneden 1924; Stampar et al. 2014b). There has still been no consistent discussion on characters and the manner that these can be discerned in the different Anthozoa clades. In this regard, the other large group of Cnidaria, Medusozoa, is much better defined as such patterns have been extensively studied in a comparative manner (see Marques and Collins 2004; Collins et al. 2006).

5.4.1.1 Morphological and Anatomical Characters

One of the most easily observable Ceriantharia characters are the tentacles, which have a distinct pattern from other anthozoans (Tiffon 1987). They are always organized in two isolated cycles, external and internal, also called marginal and labial (Carlgren 1912). The marginal tentacles are arranged in the peripheral region of the peristomium and delimit the oral disc (van Beneden 1897). These tentacles are typically much longer (Tiffon 1987), sometimes being longer than the total length of the polyp (e.g. *Ceriantheomorphe brasiliensis*) (Carlgren 1931; Spier et al. 2012).

Compared to other anthozoan groups, it is possible to highlight two important aspects related to the tentacles. The first aspect is the arrangement of the tentacles in two isolated regions, which is unique in tube-dwelling anemone. In Octocorallia the organization of the tentacles is assumed to be invariable, with a series of eight pinnate tentacles (McFadden et al. 2010). Nevertheless, hexacorallians display a more diverse organization. In Actiniaria the tentacles are always organized into cycles (typically 1–8), but always in only one region on the margin of oral disk or scattered around the innermost part of the disk (Manuel 1981; Beneti et al. 2015). Zoantharians have tentacles arranged in two rows in the margin of the oral disc (e.g. Fujii and Reimer 2013) and antipatharians normally display 6–12 tentacles organized in one or two rows in only one region (Manuel 1981). Scleractinia tentacles are arranged in one usually on the marginal region of the oral disk and often display a knob at the end, while corallimorpharians tentacles are organized in radial lines and can be branched or singles tentacles with knobs. The organization of these tentacles is usually in cycles, but they are also organized in radial lines. An additional aspect related to tentacles and which differs greatly in Ceriantharia compared to the other Anthozoa is the presence of large pores on the tentacle surfaces. They are exclusive to Ceriantharia (Fig. 5.6) and are purported to be an adaptation for expelling internal water – retreating the polyp faster into the tube (van Beneden 1924).

Another widely used character in cerianthid taxonomy is the types of cnidae. The first study on cnidae of tube-dwelling anemones was performed by Haime (1854), who made a detailed description of a cerianthid species highlighting the cnidae composition. However, the inclusion of cnidome data of tube-dwelling anemone species was not a rule in the 19th century, the period in which the highest number of species were formally described. The inclusion of such data as a matter of policy began in 1940 when Carlgren published a review focusing on the comparison of cnidae in all taxa within Anthozoa. In Carlgren (1940) it is evident that Ceriantharia has a highly "remarkable" and divergent type of cnida. This differentiated type of cnidae, the pytchocyst, was only formally described by Mariscal et al. (1977), and is considered as definitive character of Ceriantharia. In a recent publication, Zapata et al. (2015) discussed the importance of cnidae composition in relation to the systematics of Octocorallia, Hexacorallia, and Ceriantharia. Moreover, concluding that the similarity of cnidome composition of Ceriantharia and Hexacorallia is an extremely important character. However, the cnidomes states/characters is still incipient, particularly in Ceriantharia.

Among Ceriantharia subgroups the variation of form and use of cnidae, mainly pytchocysts, is quite interesting. Differing forms of tube construction reconciliate with the families Arachnactidae and Botrucnidiferidae. At the same time, members of the family Cerianthidae display a different manner to construct the tube using the pytchocysts (Stampar et al. 2015a). This is important because apparently some adaptations to the environment compared well with observed phylogenetic relationships.

Another important aspect that distinguishes Ceriantharia when compared to other Anthozoa is the shape and organization of the mesenteries. The first noticeable feature is the unique general layout of mesenteries at the pharynx level, as every Ceriantharia species has only complete mesenteries (=no incomplete mesenteries), a feature resembling more like Octocorallia than Hexacorallia (Tiffon 1987). Another point that is quite distinctive is the manner in which new mesenteries appear. In Ceriantharia new mesenteries always arise from a single area called the multiplication chamber. This structure is unique among anthozoan groups (Tiffon 1987). Also related to the mesenteries, the mesenteric filaments can be compared between anthozoan groups. In Fig. 5.4, it is possible to observe an example of such a difference (a-c

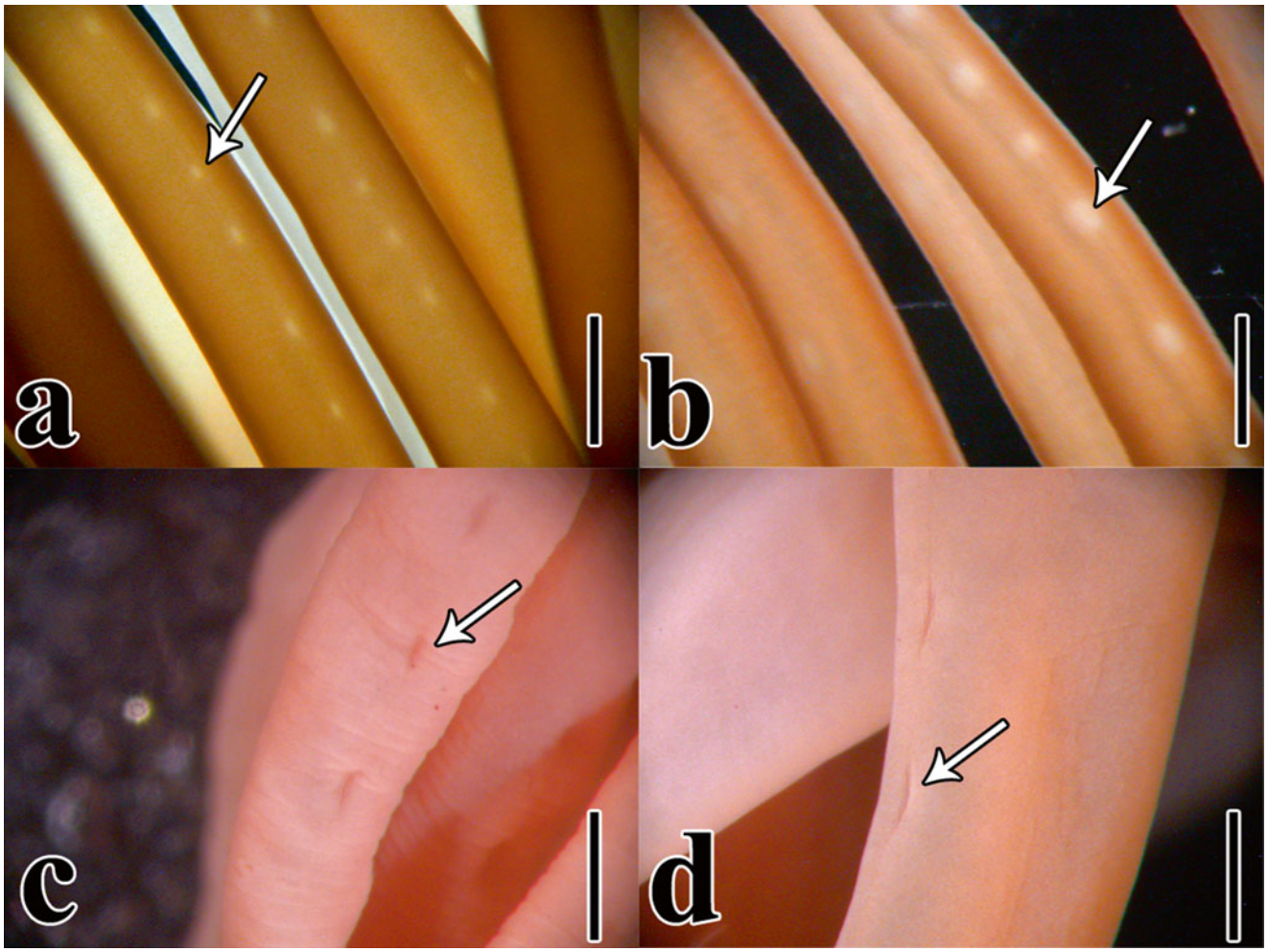


Fig. 5.6 Details of the tentacular pores in Ceriantharia tentacles (**a**) *Ceriantheomorphe brasiliensis*; (**b**) *Ceriantheopsis* sp.; (**c**) *Ceriantheopsis lineata*; (**d**) *Isarachnanthus nocturnus*. Scale 3 mm

Ceriantharian mesenteric filaments and d – Actiniarian mesenteric filament). The organization of mesenteric filaments is quite different in Ceriantharia. The division into three regions is not very clear and can be interpreted as only two. Anyway, deeper comparative studies are needed.

5.4.1.2 Life Cycle and Biological Characters

The life cycle and larval development of majority of Ceriantharia representatives is still unknown (around 98 % – Stampar et al. 2015c). However, for species that information exists, we can deduce there are two different patterns: (1) life cycle without long-term larvae – a pattern similar to other Anthozoa that have regular planula larvae (Nyholm 1943; Uchida 1979); and (2) life cycle with long-term larvae with adaptations for planktonic life (see Nyholm 1943; Uchida 1979; Molodtsova 2004; Stampar et al. 2015c) – a fact that can confuse ceriantharian larvae with other planktonic organisms (e.g. jellyfish) (Rodriguez et al. 2011). Apparently the second pattern is predominant in Ceriantharia, mainly due to the huge number of larval forms described (see Stampar et al. 2015c).

Many Ceriantharia genera and species have been named solely from pelagic larvae with the adults and their life cycle being unknown (Molodtsova 2004). Some authors (e.g. Leloup 1964) described these planktonic ceriantharians as valid species while others have believed they are larval or young stages of benthic species (Tiffon 1987; Molodtsova 2004). This type of larvae with specific adaptations for planktonic life is unique among Anthozoa and apparently represents an evolutionary lineage entirely different from other groups.

Other biological aspects such as behavior, reproductive cycle and ecological niches and that are used in various groups are almost absent in tube-dwelling anemones (see some data in Child 1908; Torelli 1938; Shepard et al. 1986; Stampar et al. 2010). Additionally, information on patterns of behavior, regeneration, and nutrition are still scarce.

5.4.2 Molecular Aspects

Molecular studies that have included sequences of tube-dwelling anemones started with the seminal work by Chen and collaborators (1995) (Fig. 5.5). In this study the authors described the "unusual" outcome of analyses based on partial sequences of the large nuclear gene 28S of *Cerianthus* sp. (later identified as *Pachycerianthus magnus*). In their reconstruction, the Ceriantharia terminal was allocated between the external outgroup (a cubozoan – *Chironex fleckeri*) and all other anthozoan groups. The authors argued that the tube-dwelling anemone's position was confusing and therefore withdrew this terminal for other discussions. Still, the authors argued that the former "Ceriantipatharia" proposition was not valid based on these results. Soon after, France et al. (1996) published the first study on the group based on mitochondrial data (partial 16S rDNA gene). In this reconstruction the Ceriantharia clade was recovered as a sister group of Hexacorallia. However, the sequences utilized in this study (*Ceriantheopsis americana*, *Cerianthus* (*Pachycerianthus*) *borealis*) have regions that are quite incongruous with other, newer sequences of the same species for the same regions, leading to the that the topology recovered (Ceriantharia + Hexacorallia) might be an artifact. Subsequently, Song and Won (1997) presented an analysis based on nuclear ribosomal 18S gene marker (*Cerianthus filiformis*), concluding that the Ceriantharia clade is a sister group of the clade Octocorallia + Hexacorallia. Berntson et al. (1999) also performed analyses based on 18S sequences from two species of tube-dwelling anemones (*Ceriantheopsis americana*, *Cerianthus* (*Pachycerianthus*) *borealis*) and once again the Ceriantharia sequences were not consistent with other tube-dwelling anemone sequences obtained in several other studies (see Stampar et al. 2014a). The authors argued that the tube-dwelling anemones' position was not consistent (low support values). Two subsequent studies, Won et al. (2001) and Daly et al. (2003), addressed the class Anthozoa by integrating morphology and molecular data from different markers. Nonetheless, both studies recovered the Ceriantharia in distinct positions, most probably due to bioinformatic approaches employed in phylogenetic reconstructions. Another study that included Ceriantharia was conducted by Collins et al. (2006; partial 18S and 28S molecular markers), in which Maximum Likelihood analyses recovered Ceriantharia as an independent subclass of Anthozoa. The data presented in Collins et al. (2006) indicate congruence with the results of Chen et al. (1995) and Song and Won (1997). Based on these results (Collins et al. 2006) the group Ceriantharia + Hexacorallia exists only with the addition of Octocorallia sequences into analyses with low non-parametric support.

Compared to these studies, more recent research shows two distinct patterns: (1) Ceriantharia as a sister lineage of the members of Hexacorallia (Kayal et al. 2013; Rodriguez et al. 2014) – both studies, based on mitochondrial data the sister relationship between Ceriantharia and Hexacorallia was only observed by a certain handling of sequences (e.g. exclusion/weighing sectors, setting a root a priori); and (2) Ceriantharia as sister group of Hexacorallia + Octocorallia or even as sister group of all other cnidarians (see discussion in Stampar et al. 2014a; the latter results are consistent with those originally presented by Chen et al. (1995) and Song and Won (1997)). Based on mitochondrial protein coding genes, Park et al. (2012) theorized that Anthozoa is paraphyletic with Octocorallia being the sister group of Medusozoa, but Ceriantharia was not included in the analysis. Kayal and collaborators (2013) also showed similar results; however there was no discussion of the retrieved pattern. Recently, Zapata et al. (2015) published an extensive phylogeny based on transcriptome and nuclear genes. However, although the inclusion of Ceriantharia (only one species) within Hexacorallia was justified by the similarity of their cnidae, bootstrap and posterior probability did not recover high support values for such a relationship and the dataset was very restricted.

Among the "orders" that are traditionally grouped into Hexacorallia, only Ceriantharia has no species with mitochondrial genome described to date. Phylogenetic studies of Ceriantharia based on mitochondrial and nuclear markers have recovered differing results, and are also distinctive from those based on morphology (see discussion in Stampar et al. 2014a). This may be reflection of a lack of extensive discussion of the relation between the traditional anthozoan "orders", once nearly all studies until now have reported only the most preferred topologies or focused the discussion only on the nodes with highest support values.

Broader taxon and data sampling would define a more appropriate analysis, because ceranthids are always recovered as an anthozoan "earlier lineage" possibly with a rapid irradiation. This historical scenario would be a combination of several difficulties like methodological issues (e.g., taxon sampling alter modeling of genetic evolution, rooting the trees) (Such et al. 2015); as well as biological ones (e.g., outgroups and incomplete lineage sorting) (Pisani et al. 2015; Such et al. 2015). The systematic relationship between Ceriantharia, Hexacorallia, Octocorallia has to be evaluated in a more intensive approach, because there is no robust result about these relationships even coming from the biggest dataset (Zapata et al. 2015) or from the analysis with the broadest taxon sampling and methodological analyses (Stampar et al. 2014a). Thus, taking into account, we would prefer to maintain the Ceriantharia clade as an "*incertae sedis*" group among Anthozoa (Stampar et al. 2014b).

5.5 Perspectives on Studies of Ceriantharia

Discussions on the phylogenetic position of Ceriantharia are still far from reaching a consensus, especially due to the scarce molecular and comparative morphological data. The inclusion of Ceriantharia among hexacorallians weakens this group from both taxonomic and phylogenetic points of view, and Ceriantharia's systematic position has retrieved low support on any scenario comprehensively evaluated this far. Thus, the accumulation of basic information on Ceriantharia is still needed.

The low number of species for which the life cycle has been described, as well as the scarce molecular data available to date may represent the most notorious "fragility" in understanding the group (Stampar et al. 2012, 2014b, 2015c). Thus, rectifying this problem should be one of the main aims of future studies on the group. For example, a recent study on the magnitude of Ceriantharia species diversity listed a possible increase of only a few species (Appeltans et al. 2012), but recent studies shows that this estimate is not realistic, and the number of species of tube-dwelling anemones is likely to increase considerably in coming years (Stampar et al. 2012, 2015b).

Another very important focus for future studies should also address robust evolutionary reconstruction and patterns considering evolutionary reconstructions based on molecular data and also investigate the intriguing evolutionary rates observable so far for Ceriantharia (Stampar et al. 2014a). Molecular evolution of the mitochondrial DNA of Ceriantharia appears to be completely different from all other Anthozoa (Stampar et al. 2014a). Thus, the study of the causes, organization, and possible ramifications of this distinctive pattern should be examined, particularly on a comparative basis. In addition, Ceriantharia has not yet been utilized as a model in almost any population ecology or biogeography research. To date, there has been only one study that has addressed aspects of biogeography in Ceriantharia (Stampar et al. 2012). In Stampar et al. (2012) the closure of Isthmus of Panama was proposed as the cause in the formation of current East Pacific and Caribbean species. From this example it is clear that tube-dwelling anemones can be used as models of great utility for the study of evolutionary processes in marine environments.

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Origin and Evolution of the Nervous System Considered from the Diffuse Nervous System of Cnidarians

6

Osamu Koizumi

Abstract

Cnidarians have the most primitive nervous system in animal kingdom. Diffuse nerve net (called "diffuse nervous system") covers the whole body. Cnidarians are considered the earliest metazoans to have evolved a nervous system and therefore offer possible insight into some of the fundamental properties of early nervous system. Recent studies show the cnidarian nervous system is much more complex than it was expected before. Genome projects and molecular developmental biological studies clarified indistinctness between Radiata and Bilateria. As to the nervous system, the situation is the same, namely there are many similarities between two animal groups. The cnidarian nervous system has all fundamental components with the molecular, morphological, and functional aspects. This might be also the case for the central nervous system, namely cnidarians are suggested to have primitive central nervous system.

I will make comprehensive description of the cnidarian nervous system based on morphological, functional, developmental studies in many biological levels from gene and molecule to individual, and compare with the concentrated nervous system of bilaterians. Subsequently, I will discuss the origin and evolution of the nervous system.

Keywords

Nervous system • Evolution • Cnidarian • Nerve ring • Neuropeptide

6.1 Introduction

6.1.1 Diversity of Nervous Systems (Fig. 6.1)

Nervous systems in animal kingdom are very diverse. Figure 6.1 shows the phylogenetic tree of animals' nervous systems. Chordata including vertebrates have main neuronal components in the dorsal side, which is called notoneuralia. The evolution of this nervous system, tubular nervous system has started due to the emergence of a neural tube. Acadian in Urochordata and amphioxus in Cephalochordata has dorsal neural tube. Other Deuterostomia, Ambulacaria include Echinodermata and Hemichordata, have simpler nervous systems. For example starfish in Echinodermata has a nervous system composed of a nerve ring and radial nerves, called radial nervous system.

Protostomia among invertebrates have main neural components in the ventral side, which is called gastroneuralia. Insects in Ecdyzoa have micro-brain, which is a masterpiece of information processing equipment with small size, light weight, and low cost. This nervous system is called ladder-like nervous system, which is typical nervous system in gastroneuralia. Mollusca in Lophotrochozoa have modified type of ladder-like nervous system, called tetra neural nervous system. Cephalopoda including octopus and squid have large brain, which is called "vertebrate-like mega brain". An earthworm in Annelida has typical ladder-like nervous system, and planarian *Dugesia japonica* has orthogonal nervous system, which is primitive type of ladder-like nervous system. Cnidaria have simple nervous system, diffuse nervous system, and Porifera have no neurons.

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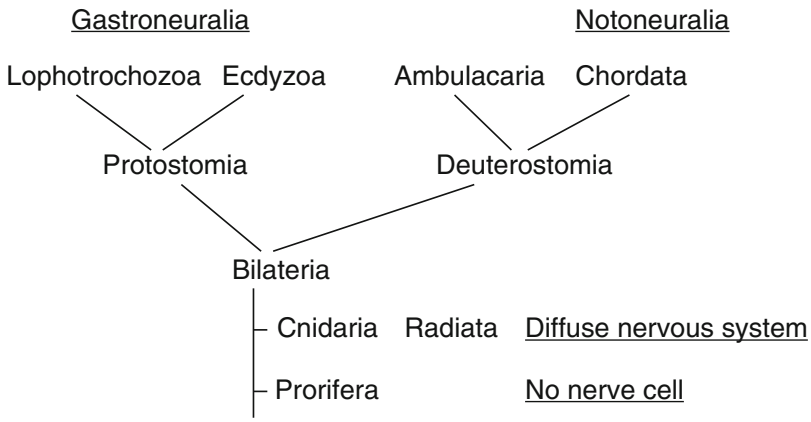

Fig. 6.1 Phylogenetic tree of the nervous systems of animals

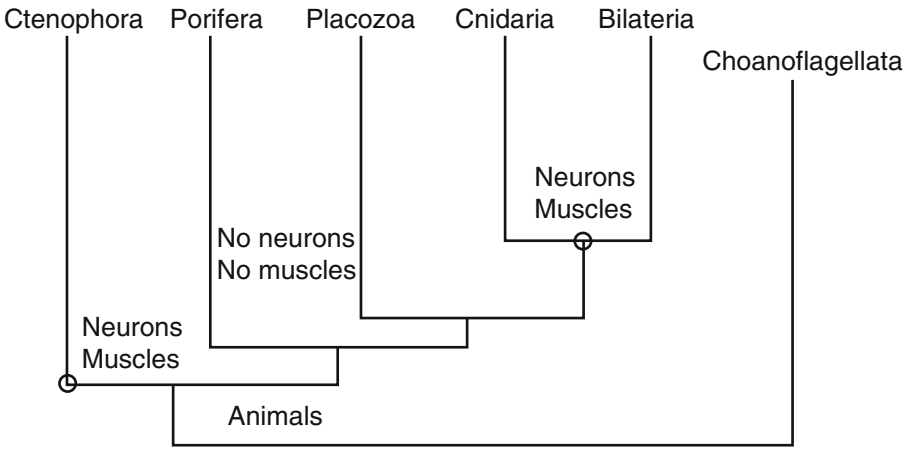

Fig. 6.2 Unique phylogenetic position of ctenophores and hypothesis of independent appearance of nervous systems in ctenophores and cnidarians. *Two circles* indicate emergences of the nervous system (Modified from Moroz et al. 2014)

6.1.2 Ctenophore's Hypothesis (Fig. 6.2)

Cnidarians and ctenophores (comb jellies) used to belong to one animal group, coelenterate, among basal metazoans traditionally, because they both have jelly-shaped forms and neurons. However, Cnidaria and Ctenophora are now divided into different phyla after many differences were recognized between the two animal groups. Moreover, very recently Moroz et al. (2014) showed that ctenophores are remarkably distinct from other animals in their content of neurogenic, immune and developmental genes. Their integrative analyses place Ctenophora as the earliest lineage within Metazoa. This hypothesis is supported by comparative analysis of multiple gene families, including the apparent absence of HOX genes, canonical microRNA machinery, and reduced immune complement in ctenophores.

Many bilaterian neuron-specific genes and genes of 'classical' neurotransmitter pathways either are absent or, if present, are not expressed in neurons in ctenophores. Hence it has been proposed that the nervous systems originated independently in Ctenophora and Cnidaria (Moroz et al. 2014). The nervous system of ctenophore is different from bilaterian nervous system, but those of cnidarian and bilaterian are similar (Watanabe et al. 2009).

6.1.3 Characters of Cnidarians

Recent molecular biological studies of cnidarian have given rise to doubt the clear distinguished between cnidarian and bilaterian. Cnidarian were considered to be a different animal group from the other higher animals, bilaterian. Cnidarian shows radial symmetry, and is diploblastic without the mesoderm, which are called radiate. On the other hand, all higher animals are bilaterian, and show bilateral symmetry with a dorsal-ventral axis, and are triploblastic with a mesoderm.

Genome projects of *Nematostella* and *Hydra* show genome sizes of these cnidarians are much larger than of nematode (*Caenorhabditis elegance*) or a fruit fly (*Drosophila melanogaster*) and comparable to vertebrates. Properties of genome structure of cnidarians were more similar to mammalians than nematode and fruit fly (Putnam et al. 2007; Chapman et al. 2010).

Although cnidarians lack obvious mesoderm, many jellyfish have musculature with similarities to striated muscle in triploblastic animals at both ultrastructural and molecular levels. The presence and expression patterns of genes related to those involved in mesoderm and muscle specification in triploblastic animals were observed during jellyfish (hydrozoan medusa; *Podocoryne carnea*) muscle development. Similar results were observed in a sea anemone, *Nematostella vectensis* and coral, *Acropora millepora* (Ball et al. 2004; Martindale et al. 2004).

Expression of genes involved in patterning the dorsal-ventral (DV) axis were observed in cnidarians. The expression pattern of a gene clearly related to *dpp/Bmp4* – the key determinant of the DV axis in bilateral animals – has been asymmetrical during the embryonic development of *A. millepora* (Ball et al. 2004) and *N. vectensis* (Matus et al. 2006).

Many properties of bilateral symmetry could be observed in cnidarians. For example, formation and the structure of septa or mesenteries in the coelenteron of sea anemone, and rhopalial nervous system of box jellyfish *Tripedalia cyctophora*, show clear bilateral symmetry (Berking 2007; Skogh et al. 2006).

6.2 Cnidarian Nervous System

6.2.1 Cnidarian Nervous System (Fig. 6.3)

Immunohistochemistry using neuropeptide antisera and monoclonal antibodies specific to hydra neurons on whole mounts has made it feasible to study the nerve net of hydra (Grimmelikhuijzen 1985; Dunne et al. 1985). These studies have shown that the hydra nerve net contains numerous subsets of neurons and that the spatial distributions are highly position-specific (Fig. 6.3). Numerous subsets of neurons containing different neuropeptides and several subsets of neurons defined by monoclonal antibodies were noted (Grimmelikhuijzen et al. 1982, 1995; Grimmelikhuijzen 1985; Dunne et al. 1985; Koizumi and Bode 1986, 1991; Koizumi et al. 1988; Yaross et al. 1986). The regional distribution of each subset tends to be constant (Koizumi and Bode 1986; Koizumi et al. 1988; Bode et al. 1988).

6.2.2 Neuron's Morphology: Sensory Cell and Ganglion Cell (Fig. 6.4)

The nerve net contains two types of nerve cells, ganglion cells and sensory cells (Mackie and Passano 1968; Davis et al. 1968; Koizumi and Bode 1991; Grimmelikhuijzen and Westfall 1995). Ganglion cells lie close to the muscle processes at the basal ends of the epithelial cells. Sensory cells have elongated cell bodies that extend from the level of the muscle processes in an apical direction and an elaborate ciliary cone at the apical end of the cell body (Fig. 6.4) (Westfall 1973; Westfall and Kinnamon 1978; Bode et al. 1988; Koizumi and Bode 1991; Grimmelikhuijzen and Westfall 1995).

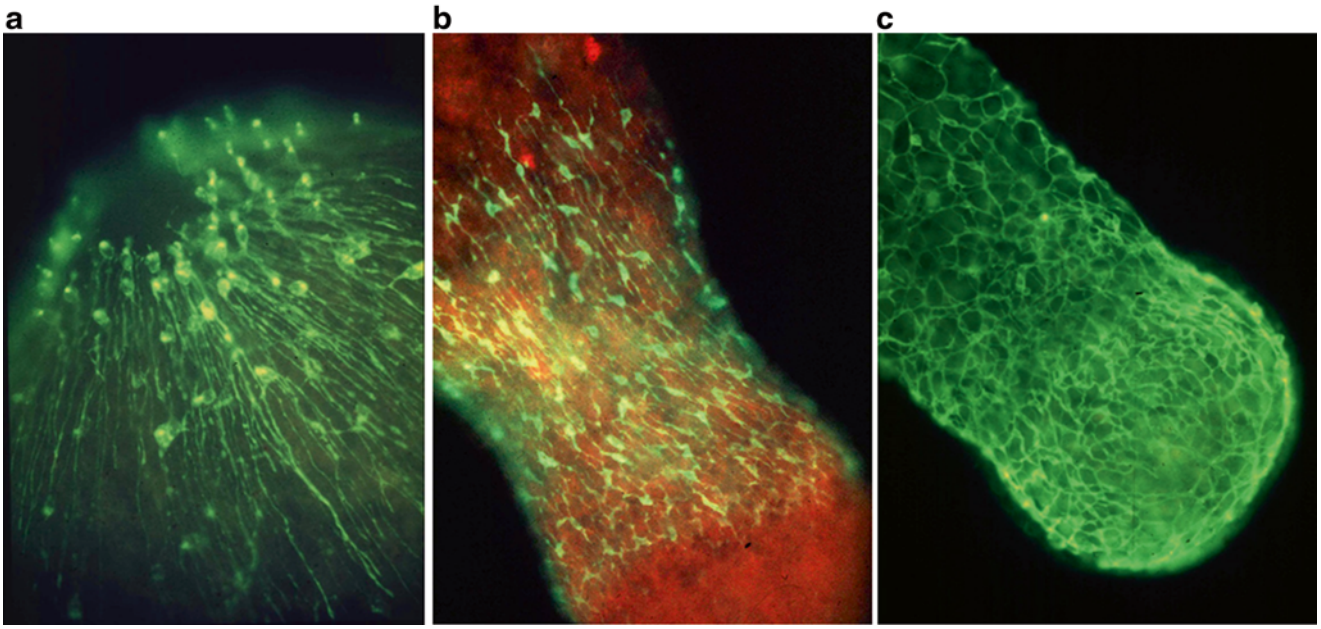

Fig. 6.3 Cnidarians nervous system visualized with different antibodies: a monoclonal antibody, JD1 (**a**), antiserum against neuropeptides, RFamide (**b**), antiserum against neuropeptides, GLWamide (**c**). **a** is hypostome of hydra, and (**b**, **c**) are foot part of hydra

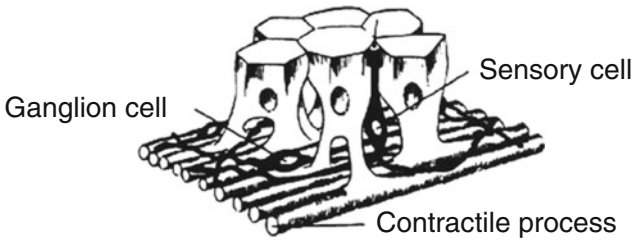

Fig. 6.4 Organization of a cnidarian epithelium and two types of nerve cells, a sensory cell and a ganglion cell (Modified from Mackie and Passano 1968)

6.2.3 Electrical Conduction

Most unique feature of the nerve cell is rapid conduction of electrical signals called electrical conduction along a nerve fiber. In cnidarians, differentiation of axon and dendrites is not observed. All nerve fibers are called neurites. However, action potentials and electrical impulses have been observed extracellularly and intracellularly from neurons and neurites (Horridge 1954; Bullock and Hrridge 1965; Josephson 1974; Mackie and Meech 1985; Anderson and Schwab 1983; Mackie and Meech 1995). Voltage dependent sodium channel was also reported, although some characters are different from other animals, such as TTX (tetrodotoxin, toxin of puffer fish)-insensitivity or very slow processes of repolarization of action potential (Anderson and Schwab 1983; Anderson 1987, 1989; Spencer et al. 1989; Anderson et al. 1993; Spafford et al. 1999; Barzilai et al. 2012). Voltage dependent potassium channel has also been demonstrated at electrophysiological and gene levels (Meech and Mackie 1993; Grigoriev et al. 1997; Klassen et al. 2008).

6.2.4 Chemical Transmission

Presence of the chemical directional synapse in cnidarian nerve nets are currently recognized (Anderson and Spencer 1989). Successive studies at ultrastructural level by Westfall's group contributed a lot to establish the presence of the chemical synapse in cnidarians (Westfall et al. 1971; Westfall 1973; Westfall and Kinnamon 1978; Kinnamon and Westfall 1982; Westfall 1987). Together with Westfall and Grimmelikhuijzen I also demonstrated that a neuropeptide is present only in neuronal dense-cored vesicles in hydra. Using immunogold electron microscopy, RFamide-peptide was present only in vesicles in nerve cells (Koizumi et al. 1989). Moreover, evidences for the presence of chemical synapse are accumulated at physiological and molecular levels today (see Grimmelikhuijzen and Westfall 1995 for review).

En passant synapses are general rather than synapse of the nerve terminal in cnidarian nerve net, and neurosecretory nonsynaptic release of neurotransmitters was also observed in cnidarians. Bidirectional synapses and electrical synapses have also been demonstrated in addition to directional chemical synapse in cnidarians (Anderson 1985; Anderson and Spencer 1989; Spencer 1989).

Various neuropeptides were reported as neurotransmitters in cnidarian nervous system. Grimmelikhuijzen and his collaborators contributed to establish the idea that peptides are major neurotransmitters in cnidarian nervous system (see Grimmelikhuijzen et al. 1989; McFarlane et al. 1989; Grimmelikhuijzen and Westfall 1995 for review). Peptide-gated ion channel was also identified in hydra (Golubovic et al. 2007), which suggests Hydra-RFamide neuropeptides work as first neurotransmitters in hydra.

Hydra peptide project also demonstrated that a huge number of peptide works as neurotransmitters in hydra. The project involves a systematic, large-scale screening of the peptide signal molecules in hydra, by adopting a novel comprehensive approach. Individual peptides were purified systematically to homogeneity without any biological assays using HPLC. The isolated peptides were subjected to structural analysis and then synthesized chemically. The synthetic peptides were then subjected to a series of biological tests to examine their functions in hydra. During the process, antibodies against each peptide were produced, and their locations were clarified. Half of peptides have been found to be neuropeptides, while half were epitheliopeptides. We estimated 200 neuropeptides in hydra (Takahashi et al. 1997, 2000, 2003; Yum et al. 1998; Morishita et al. 2003, see Koizumi 2002; Takahashi et al. 2008 for review).

Classical transmitters, such as acetylcholine and monoamines have been demonstrated in some species, but with many exceptions (see Anctil 1989; Grimmelikhuijzen et al. 1989; Scemes 1989 for reviews). However, analysis of neurotransmitter-related genes has shown cnidarians have all transmitters present in mammals (Watanabe et al. 2009).

6.2.5 Directional Conduction of Excitation (Fig. 6.5)

It is widely accepted that in a diffuse nervous system of hydra an external stimulus is conducted in all directions over the net. However, Shimizu (2002) reported that hydra tentacles respond to feeding and wounding stimuli in a unidirectional manner. Upon contact of a tentacle with a brine shrimp larva during feeding, tissue on the proximal side of the point of contact contracted strongly, whereas tissue on the distal side contracted only very weakly. Unidirectional conduction was obtained also by mechanically pinching the tissue. The response of tentacles devoid of neurons examined was bidirectional, demonstrating that the nervous system is responsible

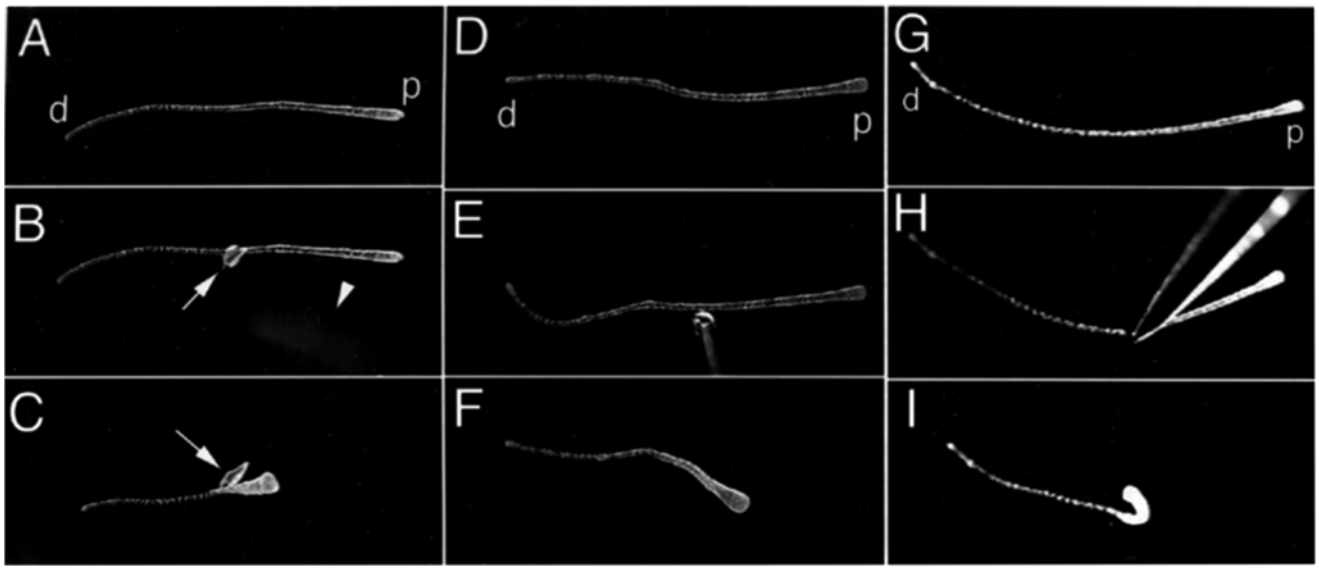

Fig. 6.5 One directional conduction of nerve response in a tentacle of hydra. Stimuli of *Artemia* (**a**–**c**), or glutathione (**d**–**f**), mechanical pinching (**g**–**i**) were applied on the middle of excised tentacle. **a**, **d**, and **g**: before stimulation, **b**, **e**, and **h**: on stimulation, and **c**, **f**, **i**: responses after stimulation. Responses conduct one way only into proximal (p) direction, but not into distal direction (d). Tentacle excised from nerve free hydra showed no responses by application of these stimuli (Shimizu 2002)

for the unidirectional responses. These observations suggest that polarized property of the nerve net in hydra tentacles is responsible for the unidirectional tentacle contraction.

6.2.6 Non-directional Epithelial Conduction: Gap Junction Between Epithelia-Muscular Cells

One of the remarkable features of cnidarians nervous system is non-nervous epithelial conduction. Cnidarian epithelial cells are also muscular cells, called epitheliomuscular cells. Epithelial cells are connected with gap junction. Propagation in nonnervous epithelia is typically all-or-none, nondecremental, and unpolarized (Josephson 1974; Josephson and Schwab 1979). The spread of excitation in conducting epithelia is associated with effector responses. The nervous system interacts with the conduction system. Spontaneous activity appears to originate in the nervous system (Mackie 1965; Mackie and Passano 1968; Satterlie and Spencer 1987).

6.2.7 Multifunctionality of Neurons in Hydra (Fig. 6.6)

The nervous system of hydra has several unique features. The most remarkable of these is the multifunction of neurons. Each neuron in hydra possesses the entire repertory of nerve-cell functions (Westfall 1973; Westfall and Kinnamon 1978), i.e., the neurons are all sensory-motor-interneurons with neurosecretory granules. For example, a sensory cell has sensory cilia as a sensory neuron, synaptic connections to the muscle layer as a motor neuron, synaptic connections to neurites or the cell body of a ganglion cell as an interneuron, and aggregations of granules in non-synaptic regions of proximal sites of the cell body as a neurosecretory cell (Fig. 6.6) (Westfall and Kinnamon 1978). Ganglion cells have the same features (Westfall 1973). Moreover, as is shown in Fig. 6.6, sometimes a single neuron innervates two different types of effectors: muscle fibers and a nematocyte (Westfall et al. 1971; Grimmelikhuijzen and Westfall 1995).

6.2.8 Distinction Between Endodermal and Ectodermal Nervous System

The presence of nerve cells of hydra has been demonstrated in the endoderm as well as in the ectodermal nerve net (Lentz 1968; Davis 1972; Westfall and Townsend 1977; Epp and Tardent 1978; Westfall and Rogers 1990; Westfall et al. 1991; Murate et al. 1996). However, the nerve nets visualized so far by neuropeptide antisera are all ectodermal nerve nets (Fig. 6.3). To examine the endodermal nerve net, we made inside-out hydras and visualized the endodermal nerve net using immunocytochemistry. Using a monoclonal antibody to α-tubulin, we could visualize endodermal nerve net. However, we never detected endodermal nerve cells using neuropeptide antibodies. Neuropeptide antibodies show only an ectodermal nerve net. Hence, these experiments reveal a remarkable difference between the ectodermal and endoder-

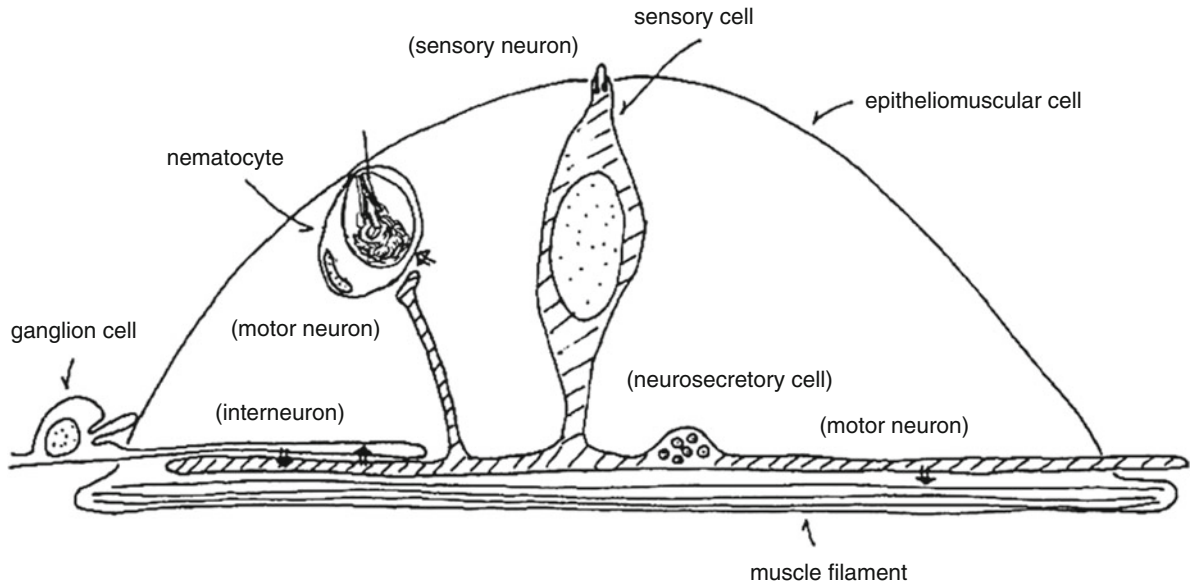

Fig. 6.6 Multifunctional nerve cells in hydra. A single sensory cell has synaptic connections to the muscle sheet of an epithliomuscular cell, a nematocyte, and a ganglion cell. Moreover, it has sensory cilia and neurosecretory granules. The *arrows* show synapses and their polarities (Koizumi 2002)

mal nervous system in regard to neuropeptide expression (Koizumi et al. 2004).

6.2.9 Sensory System (Fig. 6.12)

Cubozoan have elaborate sensory systems, rhopallia, at the margin of the bell. The system has camera eyes like mammalian eyes and a statocyst shown in Fig. 6.12. This jellyfish species, *Tripedalia cystophora* have four rhopallia connected with the nerve ring (Garm et al. 2007). The neuronal organization in rhopallium is like ganglion and is used for integration of plural sensory information containing light and gravity stimulus (Garm et al. 2006, 2007; Nilsson et al. 2006; Skogh et al. 2006).

All cnidarian medusae have eye-spots, and even cnidarian polyps have sensory cells. There is evidence that all cnidarian including polyps have sensitivity to light and gravity (see Rushforth 1973b; Josephson 1974; Satterlie 2011 for reviews).

6.2.10 Motor System

Many physiological studies show the evidence of neuromuscular system in cnidarians (Bullock 1943; Hoyle 1960; Singla 1978; Spencer 1979; Weber et al. 1982; Spencer 1982; Kerfoot et al. 1985).

In hydra, ectodermal epitheliomuscular cells have longitudinal muscles and endodermal digestive cells have circular muscles in the proximal part of the cell. Contraction of longitudinal muscle causes contraction of the polyp, and contraction of circular muscle causes elongation of the polyp. Both are antagonistic to each other. When we want to separate two layers of hydra, procaine, an activator of muscle contraction, is applied to the body column. Simultaneous contraction of both layers give us separated layer: a contracted ectodermal ring and an elongated endodermal bar.

Micro-connections between ectodermal muscle sheet and endodermal muscle sheet crossing through mesoglea were observed, which suggest the structure to carry out the antagonistic interactions between both muscles (Takahashi-Iwanaga et al. 1994).

6.3 Functional Ability of the Diffuse Nervous System

6.3.1 Hydra's Behavior

Hydra has many abilities to perform neuronal functions that might be comparable to those performed by the central nervous system of bilaterians (Rushforth 1973a, b). For example, inhibitions and modifications of feeding behavior in satiated hydra were observed (Koizumi and Maeda 1981; Blanquet and Lenhoff 1968). Figure 6.4 shows the rise of feeding threshold in satiated hydra. Habituation was also observed in hydra by Rushforth (1973a, b). Habituation (inhibition of response by repeated neutral stimuli) is not due to adaptation of sensory receptor or fatigue of motor muscle, but due to function of central nervous system.

6.3.1.1 Locomotion

Hydra shows a various modes of locomotion although it usually has sessile life as other polyps. Surprisingly, as many as seven different forms of locomotion have been reported. They are inchworm or caterpillar movements, walking on substratum using tentacles, somersaulting, positive gliding downward from water surface, floating by means of gas bubble, active gliding along substratum on base with the aid of tentacles and body movements, active gliding along substratum on base without aid of tentacles and body movements, walking along water surface film (for review, Rushforth 1973a). During this locomotion hydra selectively uses a specific one type of four nematocysts.

6.3.1.2 Feeding Behavior (Figs. 6.7 and 6.8)

Feeding activities in hydra, like several modes of locomotion, consist of a series of complex behavioral sequences. These sequences of the capture and engulfment of *Artemia salina* nauplia by hydra are enumerated as (1) nematocyst discharge; (2) tentacular movement; (3) mouth opening, creeping over the prey and closure; (4) inhibition of endogenous tentacle and body contractions (Fig. 6.7a). Hydra use two types of nematocyst in the first step of feeding behavior; desmoneme to capture preys and stenotele to kill preys by injection of toxin from the tip of discharged thread.

Discharge of nematocysts is evoked by mechanical stimulus to a cnidcile, but chemical stimulus modifies the discharge. A nematocyte is mechano- and chemo-sensory cell in addition to effector cell. The nematocyte is elaborate masterpiece, and is found only in cnidarians, and is not found in other animal groups.

Second and third steps of feeding behavior, tentacular movements and mouth opening, are evoked by feeding stimulus, glutathione released from the injured hole of prey (Fig. 6.7b).

Central nervous system is involved in the control of feeding responses in bilaterians, e.g., fly or mouse. Various modifications of feeding responses are observed in hydra in a similar manner to fly or mouse. Various types of inhibitions of feeding responses were observed in hydra. Rise of threshold of feeding stimulus in satiated hydra (Fig. 6.8), elongation of time to ingest a brine shrimp in satiated hydra, inhibition of nematocysts discharge, and so on (Koizumi and Maeda 1981; Smith et al. 1974).

Neck formation is also modification of feeding behavior appeared in satiated hydra. Hydra that still contains partially digested food in their gastrovascular cavity undergoes a

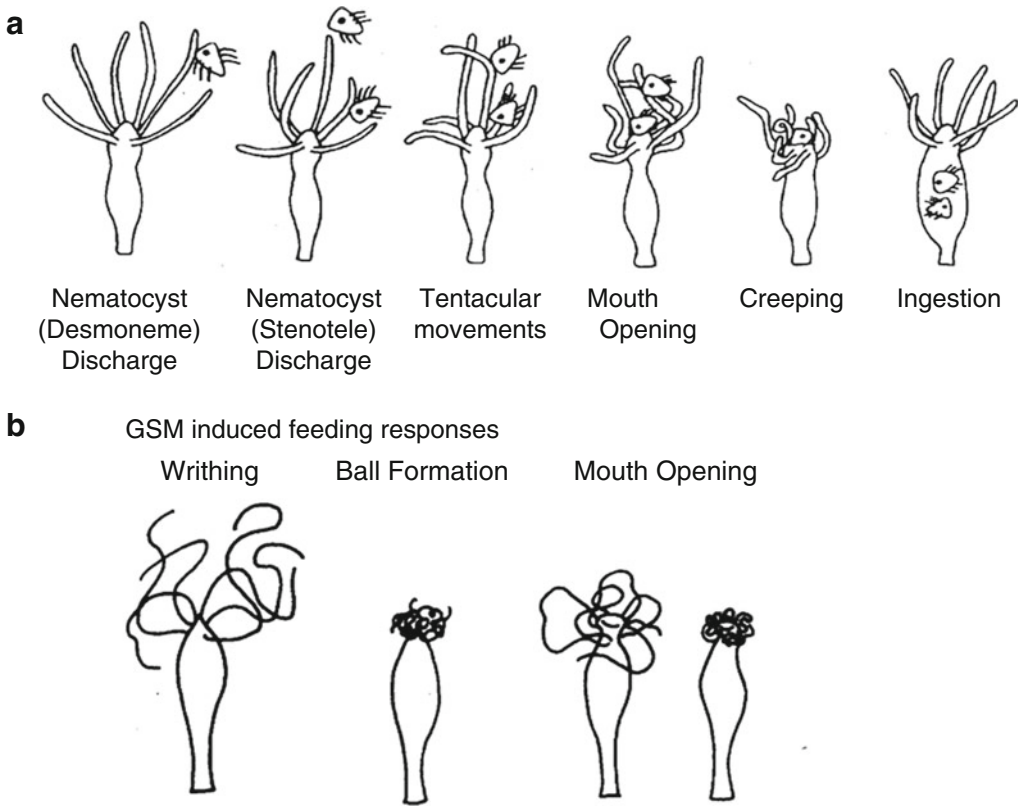

Fig. 6.7 Feeding behavior of hydra. (**a**) Feeding behavior of ingestion of *Artemia* brine shrimps. (**b**) Feeding responses induced by chemical feeding stimulus, S-methyl-glutathione (GSM)

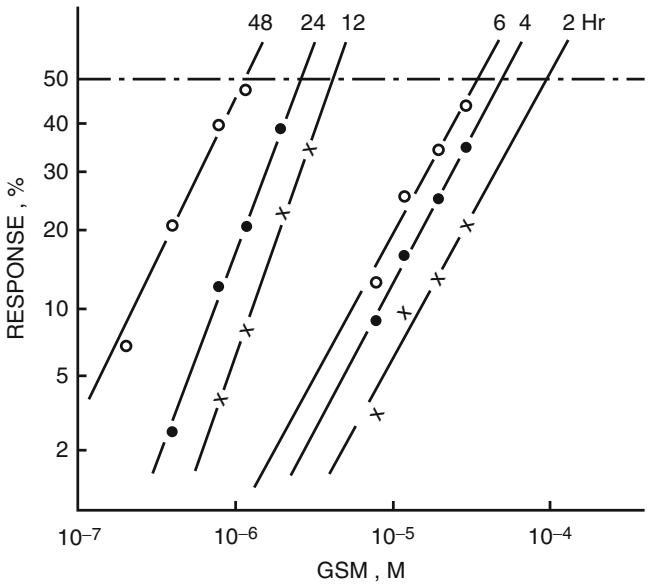

Fig. 6.8 Inhibition of feeding response in satiated hydra. Shift of regression lines of feeding response along the abscissa by shortening the starvation periods. Vertical axis indicates fractions of feeding response (feeding stimulus-induced mouth opening response), and abscissa indicates log concentrations of feeding stimulus, S-methylglutathione (GSM). Number attached with each *line* indicates the starvation periods (hours). Concentration where 50 % response is obtained is the mean value of the threshold (Koizumi and Maeda 1981)

modified feeding response when it ingests additional prey. A localized constriction is formed just below the sites of tentacle attachment, preventing the loss of gastrovascular contents. This response involves the interaction of an ectodermal receptor system for glutathione and an endodermal receptor system for tyrosine (Blanquet and Lenhoff 1968).

6.3.1.3 Habituation (Fig. 6.9)

Rushforth (1965, 1967, 1973a, b) reported hydra, *Hydra pirardi* and *H.virivis* have the ability of habituation, a primitive type of learning as shown in Fig. 6.9. The contraction response of the animals to intermittent mechanical stimulation was represented by a response curve: a plot of the proportions of a group of hydra contracting at various rotation speeds (Fig. 6.9a, response curve). If hydras are exposed to repeated stimulation at fixed rotation speed, they become less responsive to continued exposure (Fig. 6.9b, habituation curve). After habituation, a lowering of the response curve is observed (Fig. 6.9c). Recovery from the habituation needs 4 h (Fig. 6.9d).

Increasing the strength of the stimulus produces a slower rate of habituation. Contractions are readily evoked in hydras habituated to mechanical stimulation by a different stimulus, that of light, which is similar to dishabituation. These observations show this process is not considered to be one of muscular fatigue. Rushforth (1973a, b) argues the lowering the sensitivity to mechanical agitation fit to criteria of habituation. Habituation is recognized as a function of brain in higher animals, bilaterians, and as a primitive learning.

6.3.1.4 No Classical Conditioning

We have no evidences of associative learning of cnidarian's nervous system. Classical conditioning was also not demonstrated. This nervous system might not reach the level of complex learning although it has abilities of integration of plural sensory information and of memory (see Rushforth 1973a, b; Mackie 2004 for review).

6.3.1.5 Autonomic Nervous Functions

The mammalian digestive tract undergoes various digestive movements such as peristalsis and segmentation movement. A widely accepted view has been that, early in evolution, the digestive process was static based upon diffusion, and later it became dynamic involving digestive movements. Here, we report digestive movements which occur in hydra. We find that the body column of hydra undergoes a series of movements when fed with *Artemia*. Comparison of the movements to those in mammals showed similarities in appearance to esophageal reflex, segmentation movement, and defecation reflex. When nerve cells were eliminated, polyps did not show these movements, demonstrating that the diffuse nerve net in the body column of hydra primarily regulates the movements just as the netlike enteric nervous system does in mammals (Shimizu et al. 2004).

Moreover, antagonistic interactions between sympathetic nervous system and parasympathetic nervous system were demonstrated in hydra (Shimizu and Okabe 2007). Autonomic regulation is most developed in mammals, in which a part of peripheral nervous system, termed the autonomic nervous system plays the dominant role. Circulatory activity and digestive activity in vertebrates change in opposite phases to each other. The stage where circulatory activity is high and digestive activity is low is termed the "fight or flight stage" while the stage where circulatory activity is low and digestive activity is high is termed the "rest and digest stage". It has been thought that the autonomic nervous system originated in early vertebrate phyla and developed to its greatest extent in mammals. They compared the pattern of change of circulatory and digestive activities in several invertebrates and found that the two stages seen in mammals are also present in a wide variety of animals, including evolutionarily early-diverging invertebrate cnidarian taxa. They proposed a novel possibility that the basic properties of the autonomic nervous system were established very early in metazoan evolution (Shimizu and Okabe 2007).

6.3.2 Cnidarian's Behavior (Fig. 6.10)

Other groups of cnidarians show wide variety of more complex behaviors. For example, a sea anemone, *Stomphia coccinea*, shows complex swimming behavior when it contact with a starfish as shown in Fig. 6.10 (Robson 1961). The behavior after stimulus, the contact with a starfish, is com-

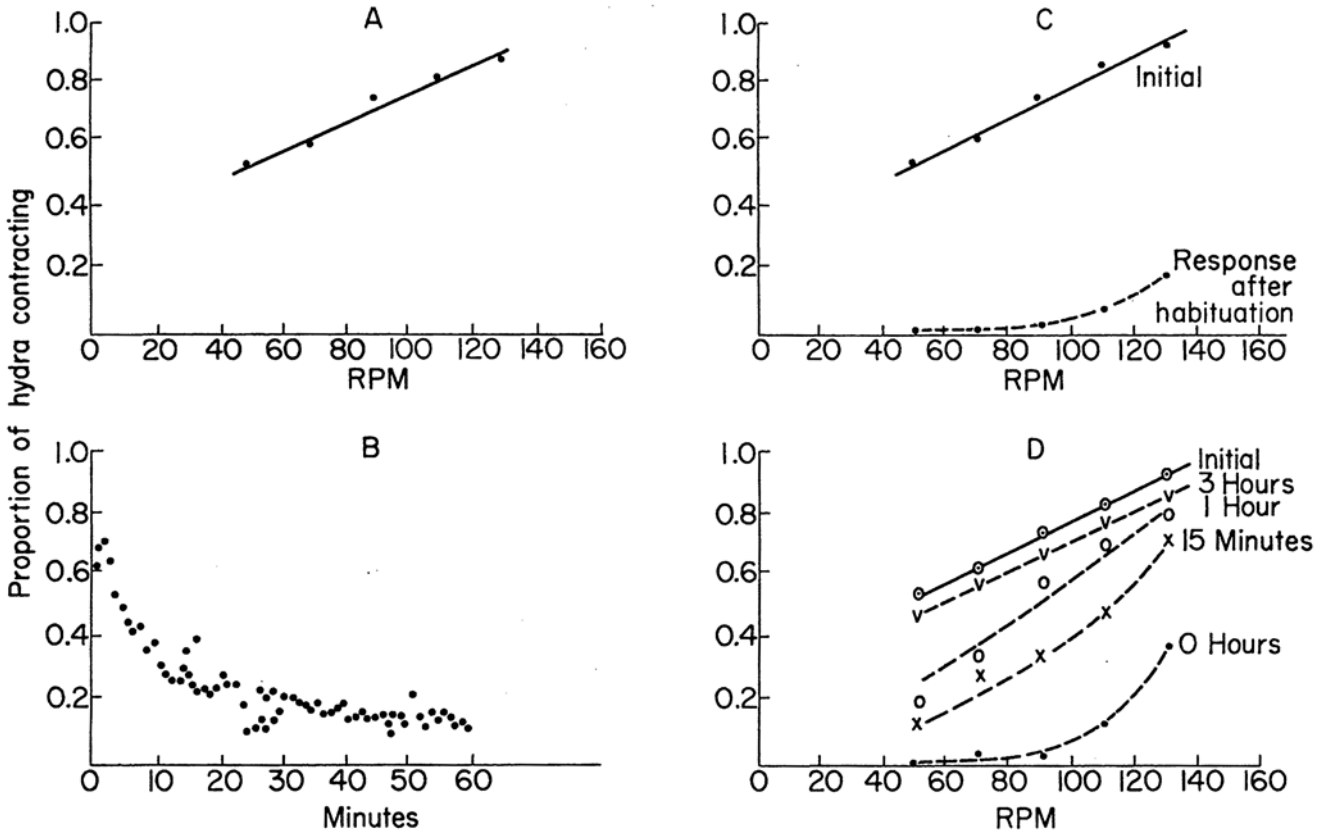

Fig. 6.9 Habituation of contraction response by intermitted mechanical stimulations. (**a**) Response curve, (**b**) Habituation curve, (**c**) Response curve after habituation, (**d**) Recovery curve after habituation. See text in detail (Rushforth 1973a)

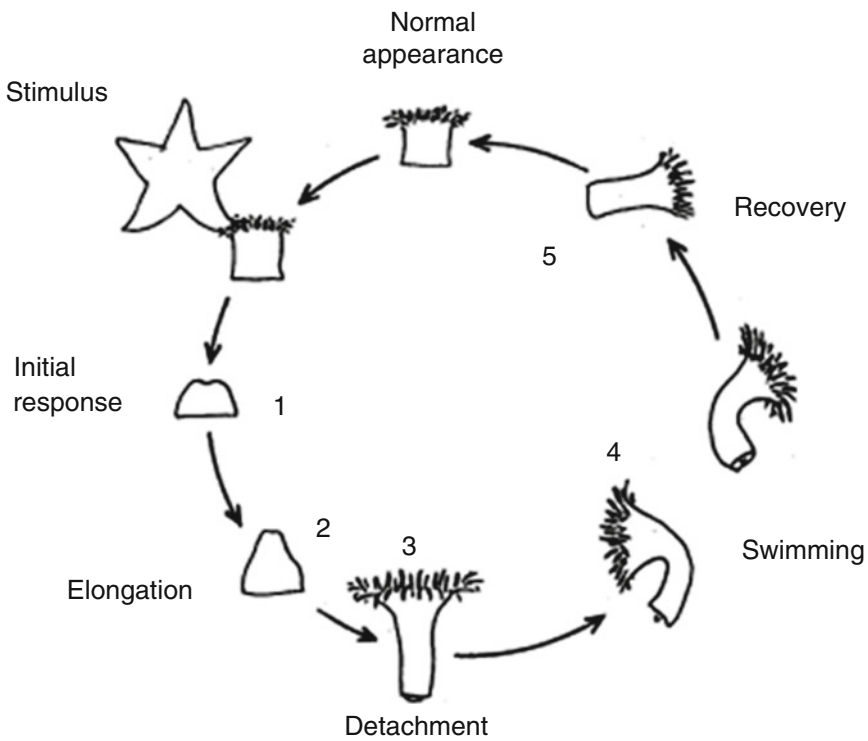

Fig. 6.10 Sea anemone behavior after contact with a starfish (Robson 1961)

posed of the following responses: initial response, elongation, detachment, swimming, and recovery. The behavior is initiated by chemoreception from starfish.

Another example is various actions of hydrozoan medusa, *Stomotoca*. They are swimming, crumpling (protective involution), tentacle posture, pointing (unilateral reciprocal flexing of the manubrium and margin), and visceral movements (Mackie and Singla 1975). There are now many examples of series of complex behaviors of cnidarians, as bilaterians show (see Rushforth 1973a, b; Spencer and Schwab 1982; Passano 1982; Satterlie 1985; Arkett and Spencer 1986; Shelton 1982 for review).

6.4 Developmental Characters of Cnidarian's Nervous System (Fig. 6.11)

6.4.1 Hydra's Developmental Dynamics of Diffuse Nervous System

Hydra has three types of cell lineages: an ectodermal epithelial cell lineage, an endodermal epithelial cell lineage, and a interstitial cell lineage. The interstitial cell lineage is composed of interstitial cells, nerve cells, nematocytes, gland cells, and gametes. Interstitial cells are multipotent stem cells, committed precursors, and differentiating intermediates (David and Gierer 1974; Bode and David 1978; Bode 1996).

In an adult hydra, nerve cells are produced continuously by constant differentiation from interstitial cells (Bode et al. 1988; David and Hager 1994). Nerve-cell production in the nerve net is balanced by a loss of neurons at the extremities and by the supply of neurons to young buds. Therefore, neurons are continuously changing their axial location by moving with epithelial cells either towards the apical end (the apex of the hypostome or the tip of a tentac1e) or towards the basal end (the basal disk) (Campbell 1976 1973; Bode et al. 1988; Bode 1992). However, the distribution of each subset of neurons expressing a certain neural phenotype is maintained (Bode et al. 1988; Bode 1992).

How is the constant nerve net maintained in spite of the active growth dynamics in hydra described above? In experiments related to this question, it was demonstrated that neurons can change the expression of FMRF amide-like peptide and vasopressin-like peptide depending upon their position in hydra (Koizumi and Bode 1986, 1991). Moreover, it was demonstrated that ganglion cells were converted to sensory cells when the neurons were moved from the body column to the hypostome (Koizumi et al. 1988). These dynamic features of neurons in the adult hydra correspond to properties of developing nerve cells in embryos of higher animals.

Hydra has high capacity to regenerate: if it is decapitated, new head appears within a few days, and head-specific nervous

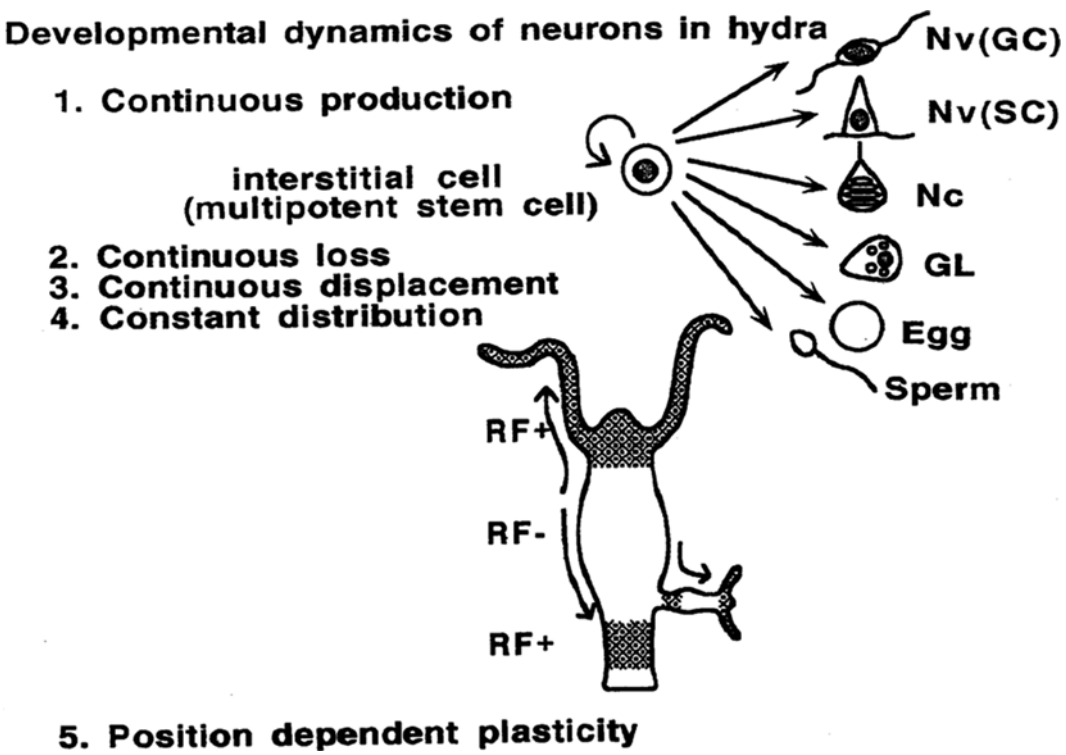

Fig. 6.11 Developmental dynamics of neurons in hydra. See text in detail

system reappears at the same time, which can perform the normal feeding behavior (Koizumi 2002). These active features of reorganization and plasticity of neurons are remarkable in cnidarians as described as 'Hydra is steady state embryo'.

6.4.2 *Nematostella*: Model Animal of Developmental Neurobiology

A sea anemone, *Nematostella* is a new model animal in cnidarians, because it has an advantages to carry out developmental biology of embryo very easily. Molecular developmental biology of this animal now is successful and productive. As to developmental neurobiology, importance of this animal has begun to be recognized (Technau and Steele 2011; Nakanishi et al. 2011; Layden et al. 2012; Watanabe et al. 2014).

6.5 Central Nervous System of Cnidarians

6.5.1 Cubozoa Rhopalium-Ring Nerve Complex (Fig. 6.12)

A nerve ring connecting the rhopalia has been reported in cubozoan medusae (Garm et al. 2006; Nilsson et al. 2006; Skogh et al. 2006; Satterlie 2011). Garm et al. (2007) wrote: "What are the distinctions, if any, between the peripheral and the central nervous system in cnidarians? This is by no means a trivial question, especially since cnidarians possess multifunctional neurons. Here, we define the central nervous system as the part of the nervous system in which several information channels are integrated and processed, with an output signal being produced to control other parts of the body, e.g., the motor neurons. The receptor cells and motor neurons therefore make up the bulk of the peripheral nervous system. The rhopalial nervous system situated within the

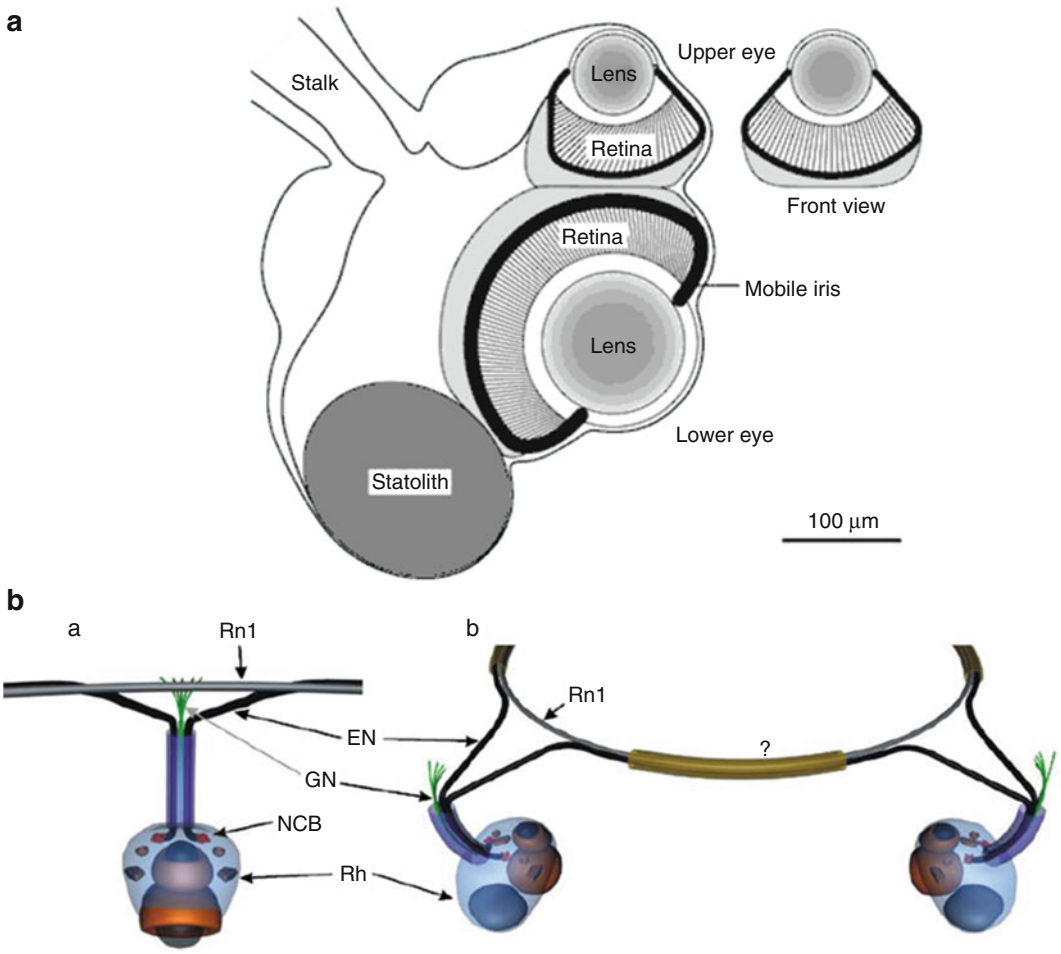

Fig. 6.12 Rhopalia and nerve ring in a cubozoan medusa, *Tripedalia cystophora*. (**a**) Complex eyes in a rhopalium. (**b**) Nerve ring connecting rhopalia (Nilsson et al. 2005; Garm et al. 2006)

eye-bearing rhopalia probably fulfils the requirements for being a central nervous system, since it receives input from several eyes and part of its output is a pacemaker signal that directly controls swim pulses. The cubozoan nerve ring is structurally similar to the hydrozoan ring nerve and, on the assumption that this indicates similar functions, the ring nerve also constitutes a part of the central nervous system."

6.5.2 Hydrozoan Nerve Ring (Figs. 6.13 and 6.14)

As to the hydrozoan medusae, two types of nerve ring are observed: the inner nerve ring and the outer nerve ring running through the marginal zone of the bell (Figs. 6.13 and 6.14) (Mackie et al. 2003; Koizumi et al. 2014). Hydrozoan jellyfish, such as *Polyorchis penicillatus* and *Aglantha digitale*, have inner and outer nerve rings. The inner nerve ring is considered to be a fused single giant axon, in which neurons show dye and electrical coupling with gap junctions (Spencer and Satterlie 1980). This morphological feature of the inner nerve ring enables rapid conduction of electrical signals and simultaneous movements of tentacles called crumpling (Roberts and Mackie 1980; Satterlie and Spencer 1983; Mackie 2004). The outer nerve ring of hydrozoan jellyfish is known to integrate pleural sensory information (Mackie and Meech 2000; Mackie 2004).

Mackie (2004) stated: "The term 'central nervous system' can legitimately be applied to hydromedusan nervous systems as these animals have concentrations of hundreds of axons running in parallel forming 'nerve rings' in the margin. There are two such rings, an inner and an outer, but axonal processes cross between them at many points and the two rings essentially function as single unit. In cross sections of the nerve rings of *Aglantha*, a total of about 800 axon profiles are seen. The nerve rings include several functionally distinct nerve pathways, and they often interact in complex ways. The fact that the central nervous system takes the form of an annulus rather than a single, compact ganglion does not make it any less 'central' in terms of the functions carried on within it. The annular configuration is simply an adaptation to radial symmetry. It does mean, however, that pacemakers and synaptic interactions are replicated at numerous points around the ring, rather than being localized to specific zones as in the neuropil of a conventional ganglion."

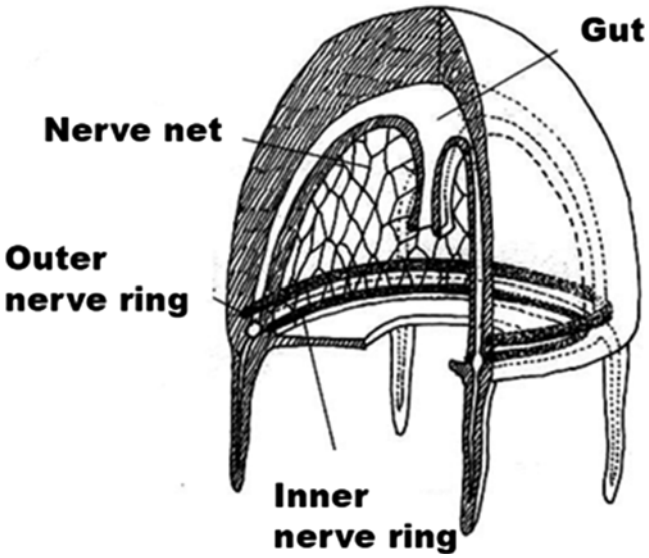

Fig. 6.13 Illustration of hydrozoan jellyfish containing two nerve rings: inner nerve ring and outer nerve ring around the margin of the bell

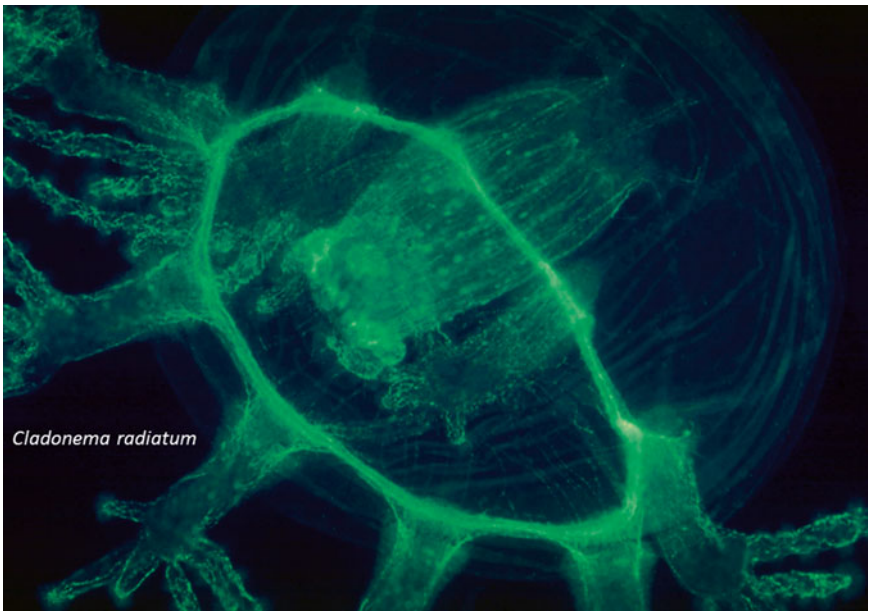

Fig. 6.14 Picture of the nerve ring observed in the margin of the bell of a hydrozoan medusa, *Cladonema radiatum*. Nervous system is visualized immunohistochemically using RFamide antibody

6.5.3 Hydra's Nerve Ring (Fig. 6.15)

In addition to diffuse nerve nets, a nerve ring is observed in the lower hypostome of hydra. It is a ring composed of ganglion cells, whose neurites make a bundle and run circumferentially around a mouth (Koizumi et al. 1992). Ultrastructural studies have shown around 30 neurites make a bundle (Yuura 2008). Hence the nerve ring is a sole neural structure showing a tight association of neurons in contrast to the diffuse nerve net seen in other regions.

This nervous structure has unique features in the hydra nervous system (Koizumi et al. 1992; Koizumi 2007). It shows static developmental characters in contrast to the dynamic features of hydra nerve net present in other regions. This character is similar to higher animal's nervous system. Moreover, its structure and location are similar to the well-known central nervous system of other animals without a complex central nervous system such as nematodes and starfishes. As to functions of the hydra nerve ring, the identified function is a crumpling of the tentacles, corresponding to the function of the inner nerve ring of hydrozoan jellyfish. The jellyfish nerve ring is considered to be a primitive central nervous system of radiates.

We identified "crumpling" as a function of nerve ring of *Hydra oligactis* so far. Destruction experiment also supports the results (Koizumi 2007). Hydrozoan jellyfish, *Polyorchis penicillatus* and *Aglantha digitale*, have inner and outer nerve rings (Fig. 6.3). The inner nerve ring is considered to be a fused single giant axon, in which neurons show dye- and electrical-couplings with gap junctions (Spencer and Satterlie 1980). This morphological feature of the inner nerve ring enables rapid conduction of electrical signals and simultaneous movements of tentacles, which is called as "crumpling" (Roberts and Mackie 1980; Satterlie and Spencer 1983; Mackie 2004). The outer nerve ring of hydrozoan jellyfish is known to integrate pleural sensory information. Crumpling of hydra corresponds to the function of the inner nerve ring of hydrozoan jellyfish (Roberts and Mackie 1980; Spencer and Satterlie 1980; Satterlie and Spencer 1983; Koizumi 2007).

Hydra has many abilities to perform neuronal functions that might be comparable to those performed by the central nervous system of bilaterians as described in Sect. 6.3.1. Considering all the information available, we proposed a hypothesis, 'The nerve ring in the head of hydra is a central nervous system -like neural structure" (Koizumi 2007).

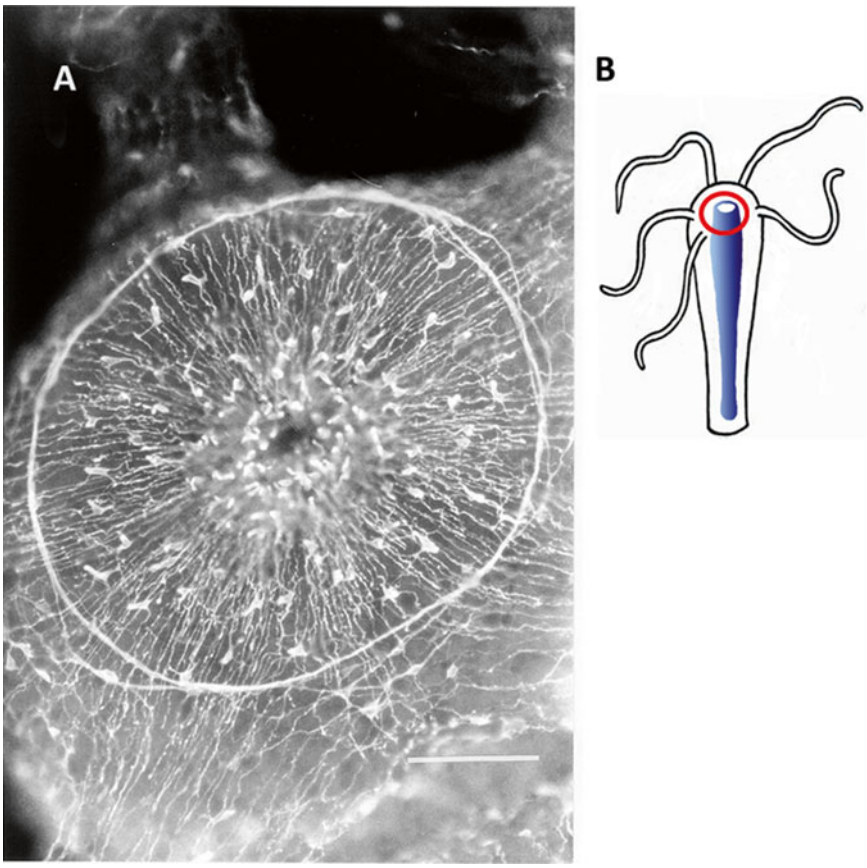

Fig. 6.15 Nerve net observed in the hypostome of *Hydra oligactis* by means of immunohistochemistry using antiserum against RFamide neuropeptides. (**a**) Picture of the nerve ring, (**b**) Schematic illustration showing the position of the nerve ring in hydra. Scale indicates 100 μm in (**a**)

6.5.4 Anthozoan Nerve Ring

Recent molecular phylogenetic studies show that anthozoans are the most primitive group among cnidarians (Bridge et al. 1992). Especially as anthozoans have well-developed nerve ring, which was the fundamental neural structure since the beginning of cnidarians.

The nerve ring can be visualized in the hypostome of a sea anemone, *Aiptasia sp*. visualized with RFamide antiserum. A clear circle of nerve ring in the hypostome, running circumferentially around the mouth was observed. This is the first observation of nerve ring in anthozoans including cell bodies and neurites. About 70 neurons are present in the nerve ring. Similar nerve ring were observed in the hypostome of other sea anemone species, *Aiptasiomorpha minuta*, and *Actinia equina* (Koizumi et al. 2011).

A new model cnidarian, sea anemone, *Nematostella vectensis* was reported to have two nerve rings around a pharynx although the circular bundle was not visualized because it was examined by in situ hybridization not by immunohistochemistry (Marlow et al. 2009).

As to coral, fluorescent immunolabeling is not easy because of the intensive endogenous fluorescence of both green and red wavelength and hard skeleton. But these difficulties can be managed using various techniques such as enzyme-immunostaining and decalcification. Unexpectedly, a coral polyp of *Pocillopora damicornis* shows nerve ring in the hypostome around the mouth. Using antibodies against RFamide, GLWamide, and vasopressin, nerve ring was visualized in addition to dense nerve net in tentacles. GLWamide and vasopressin antibodies visualized two nerve rings, inner nerve ring and outer nerve ring. The other coral, *Galaxea fascicularis* also showed the nerve ring (Koizumi et al. 2011).

6.5.5 Scyphozoan Nerve Ring (Fig. 6.16)

The nerve ring around the mouth in the head has first time observed in a polyp of scyphozoan, *Aurelia auria* (Fig. 6.16). The nerve ring runs circumferentially around the mouth and shows thicker bundle like other polyp of cnidarians. More than 70 neurons were counted in the nerve ring (Koizumi, unpublished data).

6.5.6 A Hypothesis That Cnidarian Nerve Ring Is a Central Nervous System-Like Neuronal Structure

Satterlie (2011) has proposed the presence of a central nervous system in cnidarian medusae. The rhopalium-nerve ring complex of cubomedusae, the rhopalia in the scyphomedusae, and the two nerve rings in hydromedusae have all been proposed to be primitive central nervous systems.

In our preliminary experiments, polyps of the scyphozoan *Aurelia auria* showed well-developed nerve rings in the

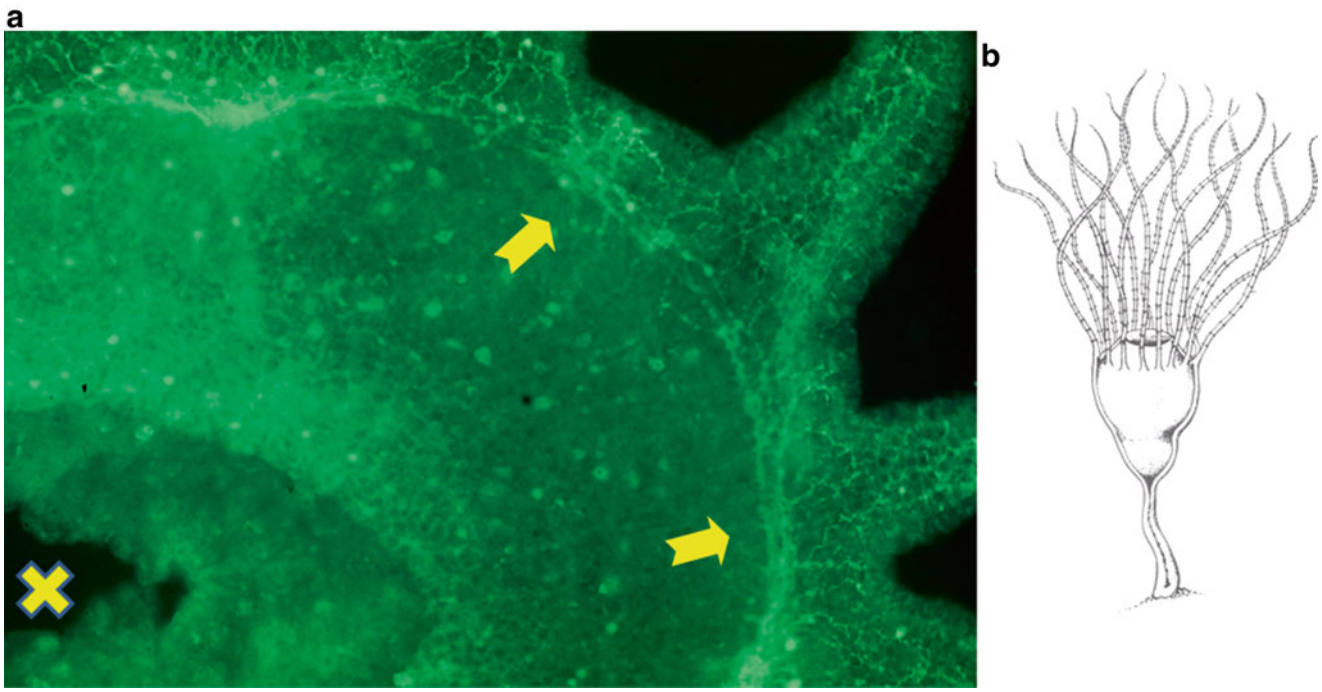

Fig. 6.16 Nerve ring in a polyp of scyphozoan, *Aurelia aurita*. (**a**) Nerve ring visualized with RFamide antiserum. (**b**) Sketch of a polyp. *Arrows* show nerve ring, and cross shows mouth opening

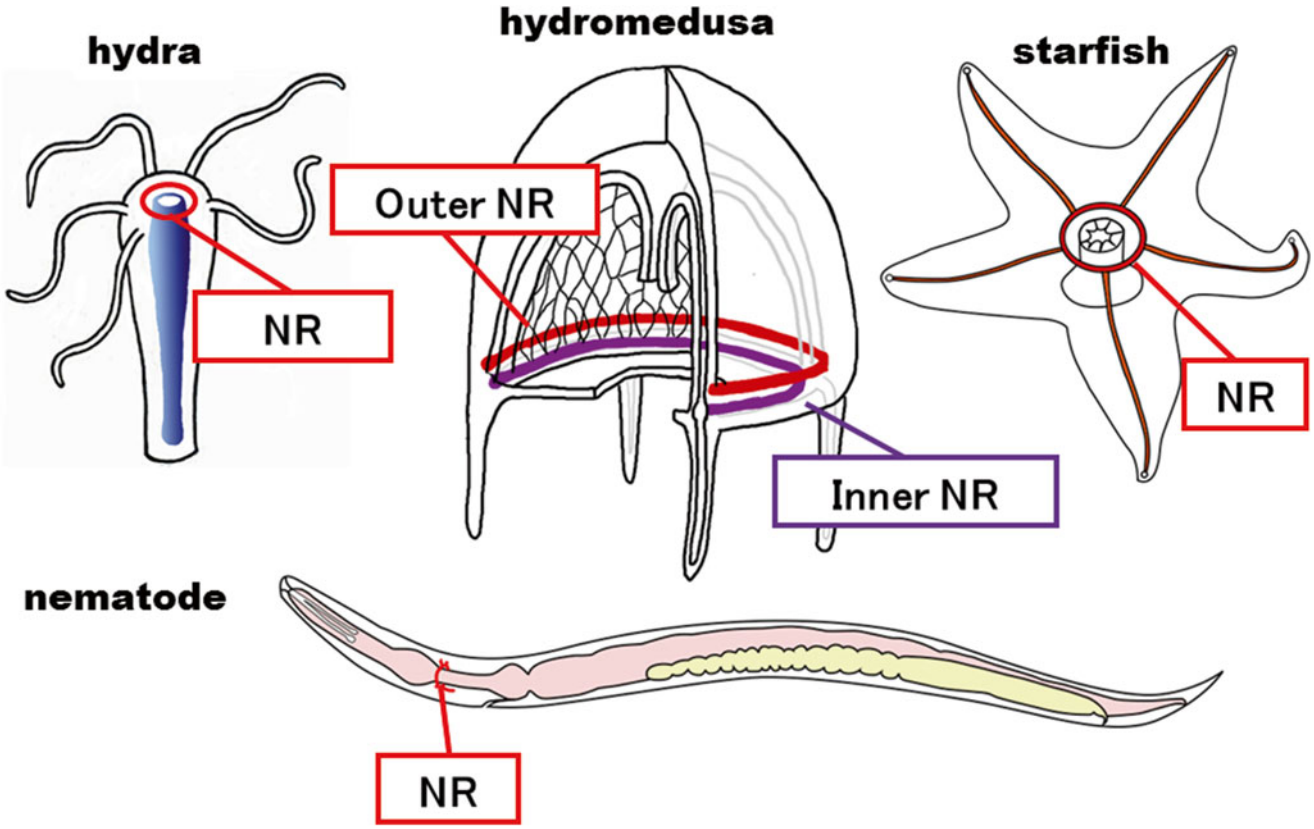

Fig. 6.17 Illustration of nerve rings observed in two cnidarians, hydra and hydromedusae, starfish, and nematode. *NR* nerve ring

hypostome (Figs. 6.16 and 6.17), and anthozoan polyps, the sea anemone *Aiptasia sp*., and the coral, *Pocillopora damicornis* also showed nerve rings around the mouth (Koizumi et al. 2011). A new model cnidarian, the sea anemone, *Nematostella vectensis* has been reported to have two nerve rings around pharynx (Marlow et al. 2009).

Recent molecular phylogeny shows that Anthozoa is an animal group first evolved in the cnidarians, and Medusozoa including Hydrozoa and Scyphozoa is a latecomer (Kayal et al. 2013). Hence, it is suggested that the nerve ring is an ancestral feature of Cnidaria.

We have already demonstrated the presence of a nerve ring in the polyp of hydra, which runs circumferentially around the mouth in the hypostome (Koizumi et al. 1992). All these results strongly suggest that there are two kinds of the neural structure of the nerve ring, one running around the mouth in the polyp head (type 1) and the other running around the margin of the medusa bell (type 2). In my opinion they are general neuronal structures in cnidarians (Koizumi et al. 2014).

As to their morphological properties, both nerve rings are nerve associations with bundles of neurites and cell bodies. As to their physiological properties, the nerve rings in the medusa (type 1) have been proposed as a central nervous system by Mackie (2004) and Satterlie (2011). The nerve ring in the hydrozoan polyp head (type 1) has also been proposed as a central nervous system-like system by Koizumi (2007). Here, I propose a working hypothesis that the neural association called the nerve ring is a primitive central nervous system-like neural structure in cnidarians.

6.6 Origin and Evolution of the Nervous System Considered from the Diffuse Nervous System of Cnidarians

Three epoch-making incidents can be expected when we think about the history of the nervous system, a long time when huge various nervous systems established after the first nervous system appeared in the earth. First event is the emergence of nerve cells and the nervous system. Second event is the emergence of brain, which causes prosperity of gastroneuralia. Third event is the emergence of a neural tube, which causes prosperity of notoneuralia. In this section, origin and evolution of the nervous system will be discussed, based on the studies on cnidarian nervous system.

6.6.1 Centralization and Functional Specialization

By viewing nervous systems from the bottom of phylogenetic tree to top of gastroneuralia or notoneuralia, we can easily imagine their evolutional process. Centralization and specialization are considered the direction of the nerve evolution. In fact, cnidarians have not remarkable large brain although they have its primitive form.

A cnidarian neuron is multifunctional, each of which has a set of all nerve functions. Muscle cells are epithelial cells or digestive cells at the same time. In contrast, neurons in higher animal work as either a sensory neuron, or a interneuron, or a motor neuron. Moreover, each motor neuron controls a few specific muscular cells.

6.6.2 Specialization of Nerve Cells at Developmental and Morphological Level

Specialization of nerve cells in higher animals appears clearly by comparison of cnidarians and mammals. Neurons are produced from interstitial cells, multipotent stem cells in an adult cnidarians continuously appear and die. Hence, neurons show active turn-over in cnidarians like various cells except neurons of higher animals. Developmental strategies of the nervous system in higher animals are long-term survival of differentiated nerve cells and consequential loss of ability of division and differentiation.

Hydra's neurons show active dynamics such as neurotransmitter phenotypic plasticity in an adult (Bode 1992). These characters are observed only in developing processes in bilaterians.

Specialization of nerve cells is also related to the polarity of neurons. Neurons have precise polarity: side of input of neural information, dendrite, and side of output of neural information, axon. Cnidarian neuron does not have such differences, but all have only neurites. Hence, during evolution of the nervous system, neurons might become specialized in many aspects from unspecialized nerve cells like as observed in cnidarian.

6.6.3 From Peptides to Classical Transmitter

Neuropeptides are more remarkable than classical transmitter in cnidarian nervous system. We thought the origin of the neurotransmitter might be peptides, but not choline or monoamine (Grimmelikhuijzen et al. 1989; Scemes 1989). Peptides are produced from precursor protein via protein synthesis from DNA. As to monoamines, specific enzymes for sysnthesis from amino acids (tyrosine or phenylalanine) are essential. I expect this system emerged later than peptide biosynthesis. However this hypothesis needs to be verified in more detail.

Genomic analysis of ctenophore's nervous system suggests that an amino acid, glutamate is used as neurotransmitter and probably neuropeptide but not classical transmitters, such as acetylcholine and any monoamines (Moroz et al. 2014).

6.6.4 All Fundamental Components are Present in Cnidarian Nervous System

If cnidarian nervous systems were compared with those of mammals, insects or octopus, there are huge differences in complexities and functional levels. However, we can see all fundamental components of this nervous system. Rapid electrical conduction along neurites, chemical transmission in synapses, information processing in nerve nets, sensory systems, and motor systems. It is also in the case of central nervous system at morphological, physiological, and molecular levels as described in the present review. Functional abilities of integration of plural sensory information, and primitive learning are remarkable examples observed in cnidarian.

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Part II

Zoogeography

Zoogeography of Hydrozoa: Past, Present and a Look to the Future

7

Cinzia Gravili

Abstract

The Hydrozoa, an inconspicuous taxon of invertebrates, contributes significantly to the bulk of biodiversity but, usually, is noticed only by specialised taxonomists. The taxon Hydrozoa of the phylum Cnidaria comprises about 3800 nominal species, about which knowledge has greatly progressed in recent decades due to the scientific research of some specialists, particularly in the Mediterranean area. Past and present-day information on the global geographic distribution of hydrozoan species was analysed and compared to select those species that have shown fluctuations and geographic shifts in relation to warming of oceanic and marine waters and that can play a powerful and functional 'sentinel-role' of global warming. The growth of knowledge of the occurrence and distribution of hydrozoan species is attributable to the labours of numerous zoologists and naturalists, from the beginning of the eighteenth century onwards through many investigations at sea by geographical and oceanographic expeditions. Moreover, the expedition collections were frequently sent to different researchers who tended to specialise either in medusae or in hydroids. The defects of this dual system and large gaps in knowledge of relationships between medusae and hydroids have remained for a long time. Biogeography of the Hydrozoa is a broad field of inquiry combining different approaches, such as the evolutionary (biogeographical) and contemporary (ecological) ones. Nonetheless, numerous efforts have been made to bridge this gap and we are closer to a biogeographical synthesis than ever before. Therefore, initiatives to promote integration among disciplines should be encouraged to achieve a synthetic and comprehensive science of zoogeography.

Keywords

Hydrozoa • Zoogeography • Taxonomy • Biodiversity • Global warming

7.1 Introduction

The taxon Hydrozoa is broad (currently comprising about 3800 nominal species), heterogenous and 'inconspicuous', being the most diverse group of cnidarians (Schuchert 2014). The *status* of many nominal species is currently unclear and, often, their identification has been considered difficult because of the presence of many synonyms and similar species can be noticed only by specialised taxonomists (Boero 2001). Hydrozoans are a group of medusozoan cnidarians found in all marine environments, displaying a wide array of life-cycle strategies (Bouillon et al. 2006) and, although marine species far outweigh freshwater ones, the genus *Hydra* may certainly be considered the best known hydrozoan. It appears in most textbooks of invertebrate zoology, but this simple animal is not representative of the Hydrozoa as a whole and thus gives a misleading impression of the complexity of this taxon. Hydrozoans are characterised by their way of producing the pelagic stage (hydromedusae),

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which are budded laterally from the polyps rather than by complete transformation of the polyp (Cubozoa) or arising by strobilation (Scyphozoa).

Currently, there is still a lack of consensus about classification of the Hydrozoa and alternative names remain in use for suprafamily taxa (see Bouillon et al. 2006; Cartwright and Collins 2007; Maronna et al. 2016; Schuchert 2014). Bouillon and Boero (2000), on the basis of embryological, developmental and morphological features, proposed a classification with Hydrozoa as a superclass including three classes (Automedusa, Hydroidomedusa, Polypodiozoa). Other classifications take into account cnidarian phylogenetic relationships as revealed by mitochondrial and nuclear genomic data (Cartwright et al. 2008; Collins et al. 2008; Daly et al. 2007; Kayal et al. 2013, 2015). Daly et al. (2007) subdivided Hydrozoa species into subclass: Trachylina including four orders (Actinulida, Limnomedusae, Narcomedusae, Trachymedusae) with 15 families, and Hydroidolina including three orders (Anthoathecata, Leptothecata, Siphonophorae) with 98 families. The Hydroidolina is the largest clade, without the ecto-endodermal statocysts characteristic of other cnidarian taxa (Collins et al. 2006), including all the athecate and thecate hydroids (most of which have free-living planktonic medusa stages in their life cycles) and Siphonophora. Trachylina is a small clade of four lineages, of which three (Limnomedusae, Narcomedusae and Trachymedusae) contribute to the planktonic animal life. A characteristic feature of the taxon Hydrozoa, forming complex life cycles, is the strong tendency towards formation of polymorphic colonies. This taxon is characterized by three distinct morphs: the planula (usually considered as the larva) metamorphoses into the polyp stage that, in turn, will produce medusae by budding, a strategy displayed by many Hydroidomedusae. It is often the case, however, that either the hydroid or the medusa stage are absent from the life cycles of these species (Boero et al. 1997). Siphonophores (currently comprising about 191 valid species), in this respect, are exceptional, because most species have both polyps and medusae combined into polymorphic colonial planktonic organisms (Mapstone 2014).

Two early classification systems considered polyps and medusae as separate entities (Hincks 1868; Mayer 1910). Since Brooks (1885) highlighted the need for a classification of hydroidomedusae including both stages in a single system and, through subsequent studies of life-cycles by several scientists (Brinckmann-Voss, Naumov, Russell, Rees, and others), the basis of a natural classification has been posed. Further research (Piraino et al. 1996; Carlà et al. 2003) have shown that even phenomena such as the inversion of the ontogenetic sequence can occur in hydroidomedusae; the adult medusae of *Turritopsis dohrnii* (Weismann 1883) transform into hydroids, offering new perspectives in evolutionary and developmental biology (Miglietta et al. 2007).

Hydrozoans are some of the most important planktonic (medusa stage) and benthic (polyp stage) predators consuming fish larvae, crustaceans, and other benthic and planktonic organisms (Purcell and Mills 1988). Some species may feed on phytoplankton, bacteria, protozoans, and dissolved organic matter (Gili et al. 1998); other species harbour symbiotic intracellular algae (Bossert and Dunn 1986). These strongly seasonal gelatinous organisms (Coma et al. 2000) can reach very high abundance under favourable environmental conditions, causing seasonal blooms in planktonic communities (Boero et al. 2008a; Boero 2013; Gibbons and Richardson 2013 and references therein), and many specimens perform seasonal and daily vertical migrations between epipelagic and mesopelagic waters (Andersen et al. 1992). Hydromedusae are known as 'indicators of upwelling systems' (Buecher and Gibbons 2000; Pagès and Gili 1992a, b), and as bio-indicators of environmental conditions as omnipresent components of the benthic rocky-shore fauna (González-Duarte et al. 2014). Moreover, their distribution and disappearance are known to be influenced by a variety of environmental factors (abundance of food resources for pelagic and benthic communities; biotic factors, such as epibiosis, and those associated with human activities) (Arai 1992; Bourget et al. 2003). Temperature is often considered a key factor in determining the distribution of benthic (Boltovskoy and Wright 1976) and planktonic (Beaugrand et al. 2013) organisms.

In this chapter, zoogeography of the taxon Hydrozoa is summarized from the past to the present with a look to the future characterized by most recent developments and conceptual progress that include new research programs accompanied by increasing data availability and advances in analytical techniques that show a further step towards the integration of ecological and historical biogeography.

7.2 Past

The first investigations of hydroids and the study of their nature were carried from the second half of the seventeenth century and the first half of the eighteenth. The freshwater *Hydra* was discovered by van Leeuwenhoek through the development of the compound microscope. At the beginning of the nineteenth century, Péron and Lesueur (1810a, b), reporting the description of species collected along the Normandy and the Mediterranean coasts, suggested a new classification of the medusae, but without distinguishing the differences between hydro- and scyphomedusae. Cuvier (1817) proposed the distinction between Acalephae (medusae, siphonophores, anemones and ctenophores) and Polypes (sponges, hydroids, anthozoans and polyzoans) recognizing the structural differences between anthozoan and hydrozoan polyps, even if hydroids as 'Polypi' continued to be associated together with corals and polyzoans. The relationship

between polyp and medusa stages was demonstrated by several scientists (e.g. Sars, Loven, Dujardin) through hydrozoan and scyphozoan biological cycle description. Later, Leuckart (1848, 1853), recognizing the heterogeneity within the Radiata, separated this taxon into Echinodermata and a new phylum (Coelenterata) including the current Cnidaria, Ctenophora and Porifera. In Vogt (1851) created the term Hydromedusae. The name Hydrozoa was proposed by Huxley (1856) and Hatschek (1888) separated the Coelenterata of Leuckart into three phyla: Porifera, Cnidaria and Ctenophora.

During the eighteenth and early nineteenth centuries several expeditions of geographical exploration were launched by France, England, and Russia. Russian expeditions (1815–1826) involved the naturalists Chamisso and Eschscholtz: the Eschscholtz's researches (1829) have allowed recognition of the fundamental differences between hydromedusae and scyphomedusae, and subsequently establishing the distinct taxa Ctenophorae and Siphonophorae. Geographical explorations, therefore, were fundamental steps to achieve the most important progress in Hydrozoa taxonomy, specifically, understanding their life cycles and recognizing the relationships between the stages of hydroid and medusa (Edwards 1972). Many zoologists (Agassiz, Allman, Bigelow, Browne, Haeckel, Hartlaub, Maas, Mayer, Nutting, Vanhoffen and others) were engaged in the study and description of the hydroids and medusae collected during these expeditions. The middle decades of the nineteenth century witnessed an extraordinary growth of interest that extended to several biotic realms (benthic, planktonic, and pelagic). The British, European and eastern North American coastal and shallow-sea marine faunas became well known through intensive collecting in coastal waters and shelf seas. In particular, description of British hydrozoan species was pursued by Alder, Allman, Gosse, Hincks, Wright and others. The monographic works on hydroids by Hincks (1868) and Allman (1871–1872) are still consulted today.

Haeckel described the medusae of the world in a monograph (1879–1880), in which different stages of the same medusa are often described under different taxonomic arrangements. About 30 years later, Mayer's (1910) monograph included the world's medusae with the known hydroids, but omitted descriptions of any hydroids that do not produce medusae.

A few life history data were collected by several zoologists during the nineteenth century despite the significant gaps in knowledge about relationships between hydroids and medusae. For example, through rearing hydroids at the Plymouth Laboratory, Rees and Russell (1937) were able to fill these several of those gaps.

Kramp (1959, 1968) provided faunistic descriptions and also an account of the Atlantic and Indo-Pacific hydromedusae distribution with an attempted zoogeographical explanation. For this purpose, Kramp (1968) divided the species into the three ecological groups (neritic, slope and oceanic species, with the latter subdivided into epipelagic and bathypelagic groups), and for the division into zoogeographical regions he mainly followed Ekman (1953). Kramp (1968) found that greatest percentage of species common among the oceans was within the Arctic and Antarctic regions characterized by their uninterrupted ranges of coastal areas.

The best-studied areas certainly include the Mediterranean Sea. The zoogeography of the area 'Red Sea-Mediterranean' and the biological consequences of the migration of Lessepsian species have been well documented by Spanier and Galil (1991 and references therein).

Interesting studies about Hydrozoa have been carried out in Italy, due to the geographical position of the peninsula in the Mediterranean that has been regarded as the most representative of the basin (Bouillon et al. 2004). In the past, many visitors to the Stazione Zoologica of Naples have helped to extend the studies of hydrozoan biodiversity (Du Plessis, Günther, Yamada, Vannucci, Soares Moreira, Brinckmann-Voss). Other areas of the Italian waters were studied due to their peculiar geographical position, such as the Strait of Messina (Gegenbaur and Metschnikoff), and the Gulf of Trieste (Heller, Graeffe, Schulze, Schneider, Neppi and Stiasny).

Analysis of historical faunal inventories at our disposal revealed a large percentage of endemic species localized mainly in the following world areas: Pacific (Calder 2010; Calder et al. 2003), Indian (Hamond 1974; Mammen 1963, 1965a, b; Millard and Bouillon 1973, 1975; Navas-Pereira and Vannucci 1991) and Antarctic archipelagos (Marques and Peña Cantero 2010; Miranda et al. 2013; Peña Cantero et al. 2010, 2013; Peña Cantero and Manjon-Cabeza 2014; Soto Angel and Peña Cantero 2015). Endemisms often disappear gradually due to numerous oceanographic expeditions and advancement of scientific knowledge. For example, the species *Rhabdoon singulare* was recorded in the Mediterranean Sea by Goy (1983), but also is widespread along the Australian coast (Hamond 1974). There are a few cosmopolitan species that 'characterize' an area with their presence, such as species of *Aglaura*, *Liriope*, *Rhopalonema* and *Solmundella* in all temperate oceanic areas, *Aglantha digitale* along the northern part of the Atlantic Ocean, and the association between *Persa incolorata* and *Solmissus albescens* in the Mediterranean basin during the winter homothermy (Goy 1993). A final group consists of a few non-indigenous species with Indo-Pacific distributions that recently entered into the eastern side of the Mediterranean basin (Gravili et al. 2013 and references therein). Other cases are so striking, for example *Arctapodema australis*, known from the Southern Ocean, but then recorded in the Planier canyon (Gili et al. 1998), that transport by unnatural ways, such as by ballast waters, is probable (Gravili et al. 2013; Spanier and Galil 1991). Moreover, collection techniques have remarkable effects on the ecological study of the

Table 7.1 Five hydrozoa genera with vicariant species

Arctic Ocean	Antarctic Ocean
Halitholus cirratus	*H. intermedius*
Margelopsis haeckelii	*M. australis*
Ptychogena lactea	*P. antarctica*
Calycopsis nematomorpha	*C. borchgrevinki*
Bougainvillia superciliaris	*B. macloviana*

Modified by Goy (1993)

Table 7.2 Hydrozoa collected over 3000 m deep

Species	Depth	Ocean
Abietinaria variabilis	4000	Pacific
Acryptolaria conferta australis	4400	Pacific
Acryptolaria flabellum	3330	Pacific
Aglaophenia galatheae	7000	Indian
Branchioceriathus imperator	5307	Pacific
Bythotiara depressa	6000	Pacific
Cladocarpus millardae	5020	Indian
Cryptolarella abyssicola	4970	Pacific, Atlantic
Filellum contortum	3570	Pacific
Halisiphonia megalotheca	4755	Pacific, Indian
Hydractinia ingolfi	3200	Atlantic
Lafoea benthophila	3246	Antarctic, Indian
Lytocarpia tenuissima	6770	Pacific
Sertularella gigantea	4820	Pacific, Atlantic

Modified by Vervoort (1966)

taxonomic group; inventories of hydrozoan fauna are totally different if they are established by dredging the benthos or caught in the plankton. After the studies of Hickson and Gravely (1907) and Browne (1910), the high proportion (about 45 %) of endemic species (such as *Arctapodema antarctica*, *Vallentinia falklandica*, *Zanclonia weldoni*), within populations of Antarctic Hydrozoa, were explained by the extreme climatic conditions of Antarctic waters. In cases of vicarious species, within the same genus, one species may live in the Arctic Ocean and the other in the Antarctic Ocean (see Table 7.1).

Deep waters also deserve special attention; fauna of this environment is little known and it is often difficult to distinguish the influence of temperature and depth. For that purpose, Ekman (1953) proposed to divide the fauna of the abyss into four major groups: (1) Atlantic, (2) Pacific, (3) Arctic, and (4) Antarctic. Later, a more detailed biogeography of the deep ocean floor was proposed by Watling et al. (2013). Vervoort (1966) reported about 50 species, characterized by hydranths of considerable size, collected in bathyal and abyssal waters (in Table 7.2 some Hydrozoa collected over 3000 m deep). In particular, the species *Branchiocerianthus imperator* (Allman 1888) is present up to a depth of 5000 m. Some species, such as *Botrynema brucei*, *Crossota brunnea*, *Halicreas minimum*, and *Pantachogon haeckeli* are present both in polar and bathyal waters. This phenomenon is known as 'equatorial submergence'; it is not a 'true' bipolarity due to continuity in the distributions in waters with temperatures not exceeding 4 °C.

Despite numerous studies and monographs about inventories of species and their distribution in several oceans, the information is still fragmentary (Goy 1993). Some species are present both in the Atlantic and the Indian oceans: *Botrynema brucei*, *Calycopsis papillata*, *Cirrholovenia tetranema*, *Ectopleura minerva*, *Koellikerina elegans*, *Persa incolorata*, *Rhabdoon singulare*, *Sminthea eurygaster*, *Tiaropsidium roseum*. Other species are common in the Atlantic and Pacific oceans: *Cladonema radiatum*, *Cunina globosa*, *Haliscera bigelowi*, *Hydractinia ocellata*, *Solmissus incisa*, *Zanclea prolifera*. The Hydrozoa species common to the three oceans are approximately 10 % of tropical Atlantic fauna (among them, *Diphasia digitalis*, *Dynamena crisioides*, *D. quadridentata*, *Pennaria disticha*, *Halopteris diaphana*, *Hebella scandens*, *Hincksella cylindrica*, *Idiellana pristis*, *Macrorhynchia philippina*, *Obelia bidentata*, *Obelia dichotoma*, *Plumularia setacea*, *Sertularia marginata*, *Sertularia tongensis*, *Sertularia turbinata*, and *Synthecium tubithecum*). With a few exceptions, all the species present along the western coast of Panama are found in the Atlantic Ocean, proving that the populations have settled before the closure of the isthmus (Deevey 1954).

In temperate areas, as in the Netherlands and the Mediterranean Sea, thermic characteristics evolved where surface water temperatures differed by more than 10 °C during the year. In these areas, few species, such as *Coryne eximia*, indicate the extension of the temperate zone (Hutchins 1947). By considering the life cycle of some species, Goy (1974) and Mills (1981) drew up a faunistic inventory associated with a 'calendar of release' of medusae related to the characteristics of the medusa stage. As suggested by Werner (1963), some species (mostly Trachymedusae) are found in the period of thermal stability, others when temperatures increase, as for most Anthomedusae, while most Leptomedusae are released when temperatures decrease.

The hydrozoan species of continental waters deserve a separate discussion. Among those, the family Hydridae is characterized by solitary fresh-water hydroids with asexual reproduction by lateral buds and without a medusa phase. In current taxonomy the family comprises only the widespread genus *Hydra* (Schuchert 2010). Finally, two species (stenothermal thermophiles) that live in continental waters steadily warm (*Limnocnida tanganyicae* and *Craspedacusta sowerbyi*). The species *Limnocnida tanganyicae* Günther, 1893 is responsible for spectacular blooms throughout the African hydrographic network (Salonen et al. 2012 and references therein) and lives in very constant conditions, with its

medusae constantly present in all stages of development (Bouillon 1957). *Craspedacusta sowerbii* Lankester, 1880 has colonized, since the end of the nineteenth century, water bodies in subtropical to temperate areas worldwide (Gasith et al. 2011 and references therein).

7.3 Present

Currently, an accurate number of described hydrozoan species is not known due to a large number of dubious and invalid species. Indeed, May and Nee (1995) estimated that about 20 % of all described species names are actually synonyms. The *status* of many hydrozoan nominal species (about 3800) is currently unclear (a considerable number of names represent synonyms); therefore, the taxonomy of the group still needs revision. The study of Hydrozoa biodiversity and zoogeography requires knowledge of the species lists and distribution records that are the starting point for research and marine biodiversity management (Costello et al. 2001; Gravili et al. 2013; Mora et al. 2011). Appeltans et al. (2012) used WoRMS (the World Register of Marine Species with about 226,000 eukaryotic marine species) as a starting point to estimate how many more species may still be discovered assessing that, at present, between one-third and two-thirds of marine species may be undescribed. Costello et al. (2013) described how WoRMS, born through the co-operation amongst over 240 collaborating scientists from 133 institutions in 31 countries, published online quality information on marine species despite the scale of the problems related to their synonyms and classification. The check-list includes about 460,000 taxonomic names and Regional Species Databases covering less than half of the oceans, with more than 17,000 cnidarian taxa (infra-species and above), of which about 5000 records are Hydrozoa species (Costello et al. 2013). As argued by Appeltans et al. (2012), this database fosters a continuous decline of the introduction rate of synonyms through better access to type specimens and related publications, in addition to greater availability of systematic revisions and communication among taxonomists.

Today, biogeography of the taxon Hydrozoa is a broad field of inquiry combining different approaches, such as evolutionary (biogeographical) and contemporary (ecological). As Wallace (1876) noted, "nothing like a perfect zoological division of the earth is possible." Therefore, the use of biogeographic data in a global classification is inevitably influenced by relatively rapid changes in community structure and dominant habitats; for example, climate change may significantly increase the instability of many marine boundaries with shifting oceanographic conditions (Beaugrand et al. 2013; Hiscock et al. 2004). With regard to this, Spalding et al. (2007) proposed the Marine Ecoregions of the World (MEOW), a new classification system for planning coastal and shelf areas, which provides considerably better spatial resolution than earlier global systems for the world's areas. This nested system consists of 12 realms, 62 provinces, and 232 ecoregions preserving many common elements that can be cross-referenced to many regional biogeographic classifications.

A few recent papers attempt to trace the biogeography of selected hydrozoan taxa (e. g. family Bougainvilliidae (Mendoza-Becerril and Marques 2013) and genus *Halecium* (Gravili and Boero 2014)), focusing on the information of little-known zoogeographical areas (Peña Cantero 2014; Soto Angel and Cantero 2015) from an historical perspective through analysis of their potential distribution based on their ecology. In particular, Gravili and Boero (2014) compared past and present-day information on the geographic distribution of *Halecium* spp. as a 'sentinel taxon' of the global warming to identify the species that have shown fluctuations and geographic shifts. They pointed out that large species, such as *Halecium halecinum* and *H. labrosum*, are regressing in the temperate areas and are restricted to temperate-cold ecoregions; while only inconspicuous species such as *H. pusillum* and *H. tenellum* are mostly recorded in warm-temperate ecoregions.

The Mediterranean basin is well studied and particularly rich in biodiversity, with an estimated 13,200 species (Coll et al. 2010; Gravili et al. 2013; Templado 2014). This is an impressive number considering that the Mediterranean represents less than 0.8 % of the total world ocean area, while the number of species that inhabit it represents about 5 % of the total known recent marine species. A monograph of the Hydrozoa from this area covers 457 species (Bouillon et al. 2004) and was recently updated (Gravili et al. 2008a, b, 2010, 2013; Mastrototaro et al. 2010; Morri et al. 2009; Schuchert 2010, 2014); the total represents about 10 % of the 3702 currently valid species reported in a recent world assessment of hydrozoan diversity (Bouillon et al. 2006). The number of species recorded in the Mediterranean almost doubled in the last 30 years and the number of new records is still increasing. The Mediterranean hydromedusan fauna is composed of about 20 % endemic species whose origin is debated. Most of the remaining Mediterranean species are present in the Atlantic and could have entered the Mediterranean from Gibraltar after the Messinian crisis. Only about 8.0 % of the hydrozoan fauna have Indo-Pacific affinities and are mainly restricted to the eastern basin, presumably having migrated from the Red Sea via the Suez Canal (Lessepsian migrants).

One of the most remarkable biogeographic phenomena today is the influx of thousands of tropical species into the Mediterranean (Galil et al. 2014). Changes due to climate change involve not only nonindigenous species, subjected to a process called "tropicalization" (increasing number, abundance and success of sub-tropical species in the warm-

temperate Mediterranean Sea) (Bianchi 2007; Bianchi and Morri 2004). Lejeusne et al. (2010) considered the use of this term to be exaggerated and suggested "meridionalization" as a more realistic term to indicate the increase of thermophilic species that is causing a progressive homogenization of Mediterranean marine biota (Philippart et al. 2011), as well as shifts in the geographical distribution of species to keep them within their thermal regime through local extinction and contraction/expansion of their geographical ranges (Boero et al. 2008b; Templado 2014).

On the other hand, high percentages of endemic Mediterranean marine species characterize the central part of the basin (Boudouresque 2004; Gravili et al. 2013), where they have colonized several unique habitats such as meadows of the sea grass *Posidonia oceanica*, vermetid reefs, and coralligenous assemblages (Ballesteros 2006; Goren and Galil 2001; Green and Short 2003; Sardà et al. 2004). The western Mediterranean basin, characterized by the continued penetration of Atlantic species with the incoming flux of water (Harmelin and d'Hont 1993), exhibits strong Atlantic affinities, while the Levant Sea, after the opening of the Suez Canal, disclosed the influence of Red Sea species (Galil 2008). Acclimated Lessepsian species within the Mediterranean (Golani 1998) now are so abundant that Por (1999) reserved a separate biogeographic province for this portion of the basin.

Boero (2014) suggested the possibility of identifying some trends and future scenarios about the Mediterranean basin. The identification of cells of ecosystem functioning is proposed by Boero (2014) as a challenge for future studies, stressing the importance of both large and small scale circulation patterns in the Mediterranean Sea. The Gulf of Lion, the Northern Adriatic Sea and the Northern Aegean are areas of deep-water formation and renewal of the basin deep water through a cascade that flows down through canyons. In turn, the Mediterranean canyon system along the coast can give rise to upwelling currents and generating benthic-pelagic coupling (Boero 2014 and references therein). The influence of global warming on these 'cold engines', with a consequent reduction of Mediterranean deep-water formation, might have a catastrophic impact on biodiversity, producing a permanent stratification (Boero 2014). Furthermore, many endemic hydrozoan species, such as species in the families Paracorynidae and Tricyclusidae, have been described from 'cold engine' areas suggesting the possibility that some of them might become extinct (Boero et al. 2013).

Gravili et al. (2013), moreover, showed that species lists are dynamic, requiring continual updating that considers both additions (introduced species) and subtractions of species with historical records, but not contemporary ones (Boero and Gravili 2013). The later authors estimated that the Mediterranean hydrozoan fauna (excluding Siphonophora) comprises 69 nonindigenous species (NIS), mainly introduced by ship traffic through the Suez Canal. The number of NIS in the Mediterranean Sea is probably underestimated (Gravili et al. 2013) and, hopefully, molecular approaches will improve our understanding of the origins of NIS and their modes of dispersal (Miglietta and Lessios 2009). Of the almost 400 non-siphonophoran-Hydrozoa species known from the Mediterranean Sea, records in the last 10 years are available only for 156 species (39%), while there are no positive records of 67 species for at least the last 40 years (Gravili et al. 2013). Some observed changes are sometimes interpreted as natural ones with cyclical or episodic frequency or catastrophic events, but most of them are of anthropic origin and their impacts interact synergistically (Templado 2014) and, therefore, their interaction may be greater than the sum of individual impacts. Although episodic or cyclical jellyfish blooms are a natural feature of pelagic ecosystems (Piraino et al. 2002), a pattern is now emerging of more frequent and intense outbreaks in many areas.

Nowadays, the combined pressure of both human impacts and global change are causing a rapid alteration of species distribution and biodiversity, and great changes in food webs are accelerating loss of populations and species (Boero and Gravili 2013; Templado 2014). Many biologists, after studies on marine communities over a period of time, have reported significant declines of populations or local disappearance of some species (Templado 2014) involving also the taxon Hydrozoa (Benović and Lučić 2003; Benović et al. 2000; Boero and Gravili 2013; Gravili and Boero 2013). On the other hand, it is very difficult to confirm the disappearance of a species in the marine environment; this is the case, for example, of the hydroid species *Tricyclusa singularis* (Schulze 1876) (Fig. 7.1), that has never been recorded after its original description (see Boero and Bonsdorff 2007 for more details).

The history of Hydrozoa records showed that, as reported for the Mediterranean Sea (Bouillon et al. 2004), the world hydrozoan contingent is currently undergoing profound changes and, therefore, species records can be divided in six categories: (a) 'evident' common species known for a long time (such as *Aglaophenia* spp., *Dynamena disticha*, *Obelia dichotoma* and *O. geniculata*), (b) 'cryptic' species now collected more frequently due to the increased efficiency of the sampling, such as *Halocoryne epizoica* (see Piraino et al. 1992) and *Perarella schneideri* (see Bavestrello et al. 2000) that are strictly symbiotic with a few bryozoan species, (c) species that are alternately rare and common, such as *Paracoryne huvei* and *Ectopleura crocea*, frequent only for relatively narrow time windows, (d) species that newly entered the considered area (non-indigenous species in several areas of the oceans and seas, such as *Clytia hummelincki*, a Lessepsian immigrant in the Mediterranean basin (see

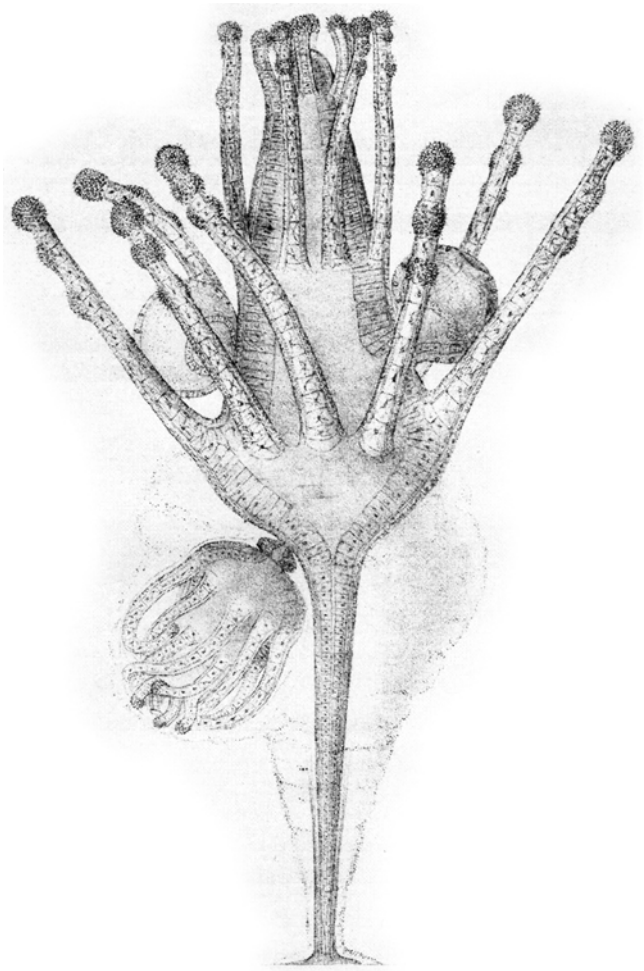

Fig. 7.1 *Tricyclusa singularis* polyp (After Schulze 1876)

Gravili et al. 2008b, 2013)), (e) species that are rare (geographically and/or ecologically) since their discovery (in the Mediterranean Sea, an example of the first type of rarity is *Errina aspera* that is restricted to the Strait of Messina (Salvati et al. 2010; Zibrowius and Cairns 1992), and the second type of rarity includes *Rhysia autumnalis* present only in autumn (Brinckmann 1965)), (f) declining species not found for several years, such as the siphonophore *Apolemia uvaria* (see Carré and Carrè 1995).

Boero and Bouillon (1993) reported contradictory evidence regarding the influence of life cycle patterns on species distribution in the Mediterranean Sea; their opinion was that the distribution of hydromedusae does not depend on their strategies of dispersal, but on their limits of environmental tolerance. Therefore, the absence of a given species from a certain area is probably due to its inability to adapt to local conditions and may not depend on its ability to reach it. In fact, over short periods, the presence of a long-lived pelagic stage seems to be a successful strategy of dispersal, as indicated by the predominance of species with medusae in the Mediterranean Indo-Pacific contingent (Boero and Bouillon 1993; Gravili et al. 2013). Boero and Bouillon (1993) emphasized how efficiency of dispersal is important during the first stages of colonization (e.g., the prevalence of species with a medusa stage among those that entered via Suez Canal), but which seems unimportant over geological time (e.g., the group that entered via Gibraltar Strait appears 'balanced'). As remarked by Sarà (1985), understanding the causes of the distributions of marine animals will be possible by taking into account historical aspects (theories of vicariance and dispersal) that cannot be separated from the knowledge of present-day environmental features, such as climatic factors, that play an important role in 'shaping' a given fauna. In recent decades, the great originality of the Mediterranean fauna has been highlighted by Boero and Bouillon (1993); their research showed that the endemic Mediterranean hydromedusan fauna is second only to the circumtropical group. The Mediterranean environmental conditions vary during the year, favouring hydromedusae characterized by a marked tendency towards seasonality, and the confinement of hydrozoan species that evolved in the Mediterranean could be explained by differences in salinity and features of circulation within the basin (Boero and Bouillon 1993; Gravili et al. 2013). Species with fixed gonophores prevail over those with medusae among cosmopolitan species, while among the circumtropical contingent, species with a medusa stage prevail; thus, the whole endemic hydromedusan fauna shows no significant difference between the two general types of life cycle, despite those living on *Posidonia* leaves showing a strong tendency towards medusa suppression (Boero and Bouillon 1993). Therefore, the presence of a medusa stage with a high degree of vagility seems unimportant in the patterns of distribution of the Mediterranean hydromedusae (see Picard 1958; Boero and Bouillon 1993). Furthermore, the tendency to medusa reduction is evident also on Mediterranean species of boreal affinity, as also observed by Cornelius (1981) who, analysing the distribution of boreal hydroids, found that two-thirds of the species lacked a medusa stage.

The majority of Siphonophorae species, characterized by polymorphic colonial planktonic organisms, exhibit instead a cosmopolitan distribution thoughout their geographical ranges, surrounding the globe within latitudinal bands depending on both ocean currents and water temperature (Mapstone 2014). They are not linked to shallow continental shelf waters in any way, being deep-sea pelagic organisms present in the Mediterranean and all three great oceans (Grossman and Lindsay 2013; Mapstone 2014). Gibbons and Thibault Botha (2002) examined the match between prevailing patterns of surface ocean circulation and zoogeography of epipelagic siphonophores around southern Africa, noting that species richness is greater in oceanic than coastal waters, peaking in subtropical waters. Some warm water siphonophores (e. g., *Forskalia contorta* and *Hippopodius hippopus*)

inhabit shallow epipelagic layers at tropical latitudes. Other species occupy a broader latitudinal range in either deeper mesopelagic layers at lower latitudes or epipelagic layers at higher latitudes (e. g., *Agalma elegans*, *Physophora hydrostatica*, *Vogtia serrata*), others are bipolar (e.g., *Crystallophyes amygdalina* and *Muggiaea bargmannae*) or restricted to just one polar region (e.g., *Marrus orthocanna* and *M. antarcticus*). Finally, a few species have restricted ranges (e.g., *Bargmannia lata* and *Nectopyramis thetis*).

7.4 Future

Future frontiers of zoogeography in the taxon Hydrozoa are progressive conceptual advances through innovative theories and research programmes, such as macroecology, which deals with the relationships between organisms and their environment at large spatial scales, and phylogeography, the historical processes that may be responsible for the contemporary geographic distributions, accompanied by advances in analytical techniques (e.g., digital distribution maps, species distribution modelling, molecular sequencing), and increasing data availability (Schuchert 2014; Villalobos et al. 2012). Moreover, phylogenetic diversity, the historical component of biodiversity important at local/ecological scales, shows how applied biogeography can contribute to disciplines such as conservation biology and community ecology (Villalobos et al. 2012 and references therein).

We need to act effectively with due haste; the extinction rate of species is increasing both on land and in the oceans (Carlton et al. 1999; Dulvy et al. 2003) and many species, mostly smaller invertebrates, are going extinct even before a formal description (Boero and Gravili 2013; Costello and Wilson 2011; Costello et al. 2013; Gravili and Boero 2013). Boero and Bonsdorff (2007) asserted that the loss of biodiversity could lead to profound changes of ecosystem functionality. Biogeographic and ecological theories, supported by long-term data, predict that half of all present species may be extinct within the next 100–300 years due to climate change, pollution, over-harvesting, and habitat fragmentation and loss (Boero and Bonsdorff 2007; Chapin et al. 2000; Costello and Wilson 2011; Jackson 2008).

Clearly, the most important problems of zoogeography are to trace the origin of the species and determine their 'time distribution'. Several areas are insufficiently explored: most deep-sea habitats, and marine areas such as the southeast Pacific, the Indian subcontinent, the Arabian and Persian gulfs, tropical West Africa, Southeast Asia and the Pacific islands (Spalding et al. 2007; Van Soest et al. 2012). The knowledge of the worldwide biogeography of the taxon Hydrozoa is, however, far from complete. This gap is mainly due to two reasons: continuous arrival of new species in the several marine ecoregions of the world, and insufficient or geographically focussed research efforts in some coastal and shelf areas. The biogeography of the Hydrozoa revealed that we are still very far from a complete evaluation of species presence and distribution (structure of biodiversity) and the understanding of biodiversity function is even less developed (Boero et al. 2003). Recent analyses characterized by an extensive monitoring over time (González-Duarte et al. 2014 and references therein) show that the anomalous climate of the past few decades is already affecting the physiology and distribution of some species. Long-term monitoring, indeed, may confirm the stability and consistency of the changes in the hydroid assemblages over time, excluding the influence of other factors such as the natural fluctuation of the marine benthic populations (González-Duarte et al. 2014). Past and present information about the global geographic distribution of hydrozoan species allows selection of taxa that have shown fluctuations and geographic shifts in relation to warming of the oceanic and marine waters and that, therefore, can play a powerful and functional 'sentinel-role' of the global warming (Gravili and Boero 2014).

Change is a natural feature of all systems (Jackson and Johnson 2001), but the rate of change can vary with different modalities and, sometimes, it may be alarmingly fast (Boero and Bonsdorff 2007). Therefore, experimental research is required within the field of functional marine biodiversity to identify the interactions between species-assemblages and any failure of nodes of food webs (Bulling et al. 2006; Ieno et al. 2006; Solan et al. 2006), as well as for the disappearance of some species (Gravili and Boero 2013), arrival of others (Gravili et al. 2013), or changes in their relative abundances (Reise et al. 2006).

Mapping the habitat distribution must be followed by the compilation of species lists, derived from literature-based and original research, for each habitat and communities using multiple approaches from local taxonomy to remote sensing (Nagendra and Gadgil 1999). It is clear that compiling a list of hydrozoan species requires the collaboration of ecologists and taxonomists to merge biodiversity measurements at both the habitat and species level. The importance of the relationship biodiversity-ecosystem functioning was stressed by Boero and Bonsdorff (2007) as an approach to obtain information about the evolution of biodiversity (Zenetos et al. 2012). Therefore, only one integrated approach would recognize the resilience of the marine ecosystem after disappearance of species and resulting 'catastrophic' shifts (Lotze et al. 2006; Scheffer et al. 2001). A comprehensive approach considers the importance of taxonomic knowledge as the basis for biology, zoogeography and ecology, but the integrative nature of the discipline is not yet fully realized (Wiens and Donoghue 2004).

The present incomplete taxonomic knowledge of most taxa complicates the matter further. It is possible that the increasing number of recognized species consequently

narrows the ecological niches of the species and, thus, increases the number of ecological guilds, as reported for the coral reefs (Knowlton and Jackson 1994). Within the present-day biogeography, niche and species modelling represent an active research area integrated by historical biogeographers (Escalante et al. 2007a, b). Past biogeographical studies about the taxon Hydrozoa have commonly taken a historical, rather than an ecological approach. General patterns have been investigated in the species' distribution maps and explained in terms of past or present distribution barriers. Any biogeographical hypotheses attempt to explain the observed species distribution patterns, specifying which factors are considered to be explanatory variables (e.g., latitude, altitude, seasonality, and so on). In general, because those variables are strongly correlated with each other, it may prove impossible to determine which have causal relationships with the observed biogeographical phenomena. Therefore, the interconnections among available dispersal vectors, local currents, and life-history strategies cannot be overlooked in the interpretation of marine biogeographic patterns.

Over recent decades, the essential link between biogeography and ecology has been clearly acknowledged through the recognition of historical-contingency and scale-dependency (Ricklefs 2004; Whittaker et al. 2001). If zoogeographic hypotheses attempt to explain the origin of species, in most cases the exact place, time and duration of this event are unknown. Cladistic analyses, vicariance biogeography and DNA data can be used to obtain a relative time scale and extricate the order of speciation events, but cannot easily solve this problem, because they do not directly indicate the reasons for speciation and the absolute time scale remains ambiguous.

In the absence of direct knowledge of the past, more complete ecological and environmental understanding of the present marine zoogeographical areas are needed for evaluating the relative roles of historical and ecological factors in hydrozoan biogeography and biodiversity.

Lyell's well-known slogan "The present is the key to the past" in Principles of Geology' (1830) should be remembered because the present can be observed in so much more detail and compared to the past. The past can only be reconstructed on the basis of what is known about the present. Therefore, both biodiversity and biogeographical studies would benefit from a more rigorous documentation of present ecological conditions and their influence on the biota.

At present, most of the international projects exploring biodiversity are dedicated to the informatization of biodiversity information (i.e., the European Network of Biodiversity Information, Lifewatch, the Census of Marine Life, the European Register of Marine Species, Tree of Life) (for more details see Boero 2010). Unfortunately, the current policies in funding scientific research discourage the maintenance of long-term studies on the different marine taxonomic groups. With regard to this, Boero et al. (2014) warns that dynamics of the historical systems can be understood only if opportunely described in the course of time. Only long time series, indeed, ensure measurement not only of simple abiotic features, but also complex biological variables (e.g., species diversity, abundances). The few long time series in European waters and in other seas and oceans must, therefore, be interlinked and networked to facilitate the formation of nodes (series of global observatories) that together should promote a better understanding of the causes and effects of change in ecosystems. I believe that a synthetic approach, developed by biogeographers and ecologists, will become the standard for biogeographic studies in coming years. Therefore, initiatives to promote integration among disciplines should be encouraged to achieve a synthetic and comprehensive science of zoogeography. Nonetheless, numerous efforts have been made to bridge this gap and we are closer to a biogeographical synthesis more than ever before.

Hydrozoa records were, and continue to be, strongly influenced by the distribution of the taxonomists, so that some areas appear to be particularly rich. Therefore, a priority in future studies on hydrozoan biogeography is the merging of zoogeographical gaps. On the other hand, Appeltans et al. (2012) confirmed a high rate of marine species description, driven by the increasing number of taxonomists and the current opportunity to explore previously unknown geographic areas, as well as the challenge enabled through molecular studies to estimate the diversity of cryptic species.

The current trend is to implement the European policies to increase networking and partnerships (Katsanevakis et al. 2013). A witness that follows this trend is the Rome Declaration presented at the EurOCEAN 2014 Conference (October 2014, Rome), an official event of the Italian EU Presidency. The Rome Declaration sets out high level goals and associated actions for delivering a vision for seas and ocean science with the aim of realizing an ecosystem approach to the management of Europe's marine resources. Moreover, the European Marine Strategy Framework Directive changed the approach regarding the *status* of the environment through 11 descriptors of GES (Good Environmental Status) and, instead of considering the putative causes of impact, considers their effects on the biotic components (Boero 2014).

Multidisciplinary programmes become a favoured research approach; to overcome this difficulty, an effective solution is to involve citizen-scientists. An experiment in citizen science called 'Watch for Jellies', endorsed by the popular science magazine FOCUS (http://www.focus.meduse.it/meduse/), asked citizens to report sightings of gelatinous macro-zooplankton, a few of which are hydrozoan species, i. e., *Physalia physalis*, *Velella velella*, *Porpita*

porpita, *Aequorea aequorea*, and *Olindias phosphorica*). This initiative, conceived by the University of Salento, led to the creation of a dataset (METEOMEDUSE project), for the first time allowing the presence of such organisms to be monitored, over vast spatial (Italian coasts) and temporal (2009–2015) scales.

Finally, I believe that we need to increase our ethical wisdom within ecology (Minteer and Collins 2005) and, therefore, this goal might be achieved only if scientists in different fields of biodiversity (from phenotypes to genotypes, ecological niches, life cycles, populations, communities) combine their efforts.

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Diversity and Distribution of Octocorallia

8

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Abstract

Octocorals are a group of striking presence in marine benthic communities. With approximately 3400 valid species the taxonomy of the group is still not resolved, mainly because of the variability of morphological characters and lack of optimization of molecular markers. Octocorals are distributed in all seas and oceans of the world, from shallow waters up to 6400 m deep, but with few cosmopolitan species (principally pennatulaceans). The Indo-Pacific is the region that holds the greatest diversity of octocorals, showing the highest level of endemism. However, endemism in octocorals as we know might be the reflex of a biased sampling effort, as several punctual sites present high level of endemism (e.g. Alaska, Antarctica, South Africa, Brazil, Gulf of Mexico). Since around 75 % of described octocoral species are found in waters deeper than 50 m, increasing knowledge on octocoral diversity and distribution is still limited by the availability of resources and technologies to access these environments. Furthermore, the limited number of octocoral taxonomists limits progress in this field. Integrative taxonomy (i.e. morphology, molecular biology, ecology and biogeography) appears to be the best way to try to better understand the taxonomy of such a diverse and important group in marine benthic communities.

Keywords

Octocoral • Soft coral • Anthozoa • Worldwide distribution • Deep sea

8.1 Diversity in Octocorals of the World: Number of Valid Species, New Trends in the Classification of Octocorals

Cnidarians in the subclass Octocorallia constitute a faunistic group with significant presence in benthic communities, made remarkable by their beauty, diversity, abundance and interspecific relationships. This group is represented by a number of growth forms, varying from encrusting, filiform and membranous, to complex and elaborate arborescent architectures. Octocorals are distributed in all marine environments, but are generally conspicuous and diverse in shal-

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low tropical reefs and in deep-sea habitats (e.g. seamounts), where they are important structural components of the community (Williams and Cairns 2013).

To this day there are over 3400 valid species of octocorals (Williams and Cairns 2015), whose taxonomy is far from being resolved. As an example, 3490 octocoral nominal species in >370 genera are listed in the World Register of Marine Species, but from these only 3103 species are considered valid names (van Ofwegen 2015).

Attempts to understand the taxonomy and phylogenetic relationships in this group have been hampered by a shortage of useful morphological characters, widespread homoplasy (parallelisms, convergences, and reversals) and pronounced intraspecific variation in characters such as colony growth form and morphology of sclerites (Williams 1997). Recent molecular phylogenies have shown how branching patterns can evolve independently several times from unbranched hypothetical ancestors involving different axial materials (Sánchez et al. 2003a, b; Etnoyer et al. 2006). Added to this, as pointed out by Daly et al. (2007), numerous species remain either undescribed or simply unidentifiable; lost type specimens and the poor quality of most nineteenth and early twentieth centuries species descriptions preclude species-level identifications in many groups.

To estimate the precise number of described species in Octocorallia is a very difficult task, as most genera have never been revised, and several regions of the world are still to be explored. Furthermore, the number of octocoral taxonomists is very limited, and the literature is quite dispersed (Bayer 1981a; Cairns 2007a). Efforts to condense and compile information on Octocorallia have started with a series of studies by Dr. Frederick M. Bayer (Bayer 1981a and Bayer et al. 1983).

Until the 1990s, octocoral systematics was mainly based on alpha-taxonomy (Kölliker 1880; Hickson 1916; Bayer 1955, 1956; Williams 1992a, b, 1997). However, the limitation of octocorals in the fossil record made it more difficult to polarize taxonomic characters in phylogenetic reconstructions (Bayer 1956).

The current classification of octocoral orders and families is mainly based on the studies by Kükenthal (1906, 1915, 1919, 1921, 1924, 1925), Hickson (1906, 1916, 1930), and Bayer (1956, 1981b) who created a classification system that is still in use, with few modifications (Tables 8.1 and 8.2).

Among the orders, Helioporacea (blue corals) and Pennatulacea (sea pens) are the only ones with well-defined synapormorphies. The blue corals because of their massive aragonite skeleton, and the sea pens because of their proximal peduncle used for anchorage in the soft substrate. Although the other orders also have well-defined stereotypes, they also have intermediate forms.

Bayer (1981b) suggested that increased research on octocorals slowly approximated the orders originally established by Hickson, Kükenthal, and others. According to Bayer, based on colonial organization and skeletal structure, the only distinguishable taxa were Pennatulacea, Helioporacea and the "restricted" Holaxonia that lack a chambered axis medulla (families Ellisellidae, Ifalukellidae, Chrysogorgiidae, Primnoidae, and Isididae). On the other hand, Stolonifera, Telestacea, Gastraxonacea, Alcyonacea, Scleraxonia, and the "medullate" Holaxonia (with chambered axial medulla) should be united by intermediate forms, and be included in the order Alcyonacea. Therefore, Bayer suggested that the traditional subdivisions (suborders) should be kept in a quasi-subordinal grade level, only as convenient levels, not taxa (Table 8.3).

The system proposed by Bayer (1981b) was well accepted by the scientific community, and it is still in use. Based on skeletal apomorphies, Grasshoff (1999) subdivided the order Alcyonacea in: Holaxonia sensu stricto (the medullate Holaxonia of Bayer) and Calcaxonia (the "restricted" Holaxonia of Bayer). Calcaxonia was created to include gorgonians with an axis lacking a cross-chambered hollow core, but having large amounts of calcareous material, either calcite or aragonite. The remnant four groups in the order (Alcyoniina, Protoalcyonaria, Scleraxonia, Stolonifera) can be maintained for convenience, as suggest by Bayer (1981b),

Table 8.1 Octocoral classification by Kükenthal (1925)

Orders	Suborders	Families (n)	Genera (n)
Alcyonaria		9	37
Gorgonaria	Scleraxonia	4	24
	Holaxonia	8	85
Pennatularia	Sessiliflorae	11	18
	Subselliflorae	3	10
Total		35	174

Table 8.2 Octocoral classification by Hickson (1930)

Orders	Suborders	Families (n)	Genera (n)
Stolonifera		3	16
Telestacea		1	4
Alcyonacea		5	29+
Gorgonacea	Scleraxonia	4	11+
	Holaxonia	8	53+
Coenothecalia		1	1
Pennatulacea		13	31
Total		35	145+

Table 8.3 Octocoral classification by Bayer (1981b)

Orders	Sub-ordinal groups	Families (n)	Genera (n)
Helioporacea		2	2
Alcyonacea	Protoalcyonaria	1	1
	Stolonifera	5	19
	Alcyoniina	6	48
	Scleraxonia	7	23
	Medullate Holaxonia	4	40
	Restricted Holaxonia	5	67
Pennatulacea		14	28
Total		44	228

Table 8.4 Current octocoral classification system (Bayer 1981b; Grasshoff 1999; Daly et al. 2007). Records of valid names based on World Register of Marine Species (van Ofwegen 2015)

Orders	Sub-ordinal groups	Families (n)	Genera (n)	Species (n)
Helioporacea		2	2	4
Alcyonacea	Protoalcyonaria	1	1	1
	Stolonifera	7	27	165
	Alcyoniina	6	86	1235
	Scleraxonia	7	28	249
	Holaxonia	4	74	541
	Calcaxonia	6	111	685
Pennatulacea		14	37	223
Total		47	364	3103

however they do not represent actual clades and should be seen only as grades of colony architecture (Fabricius and Alderslade 2001) (Table 8.4).

In the 1990s the use of molecular techniques started to be employed in the study of octocorals. Molecular phylogenetic studies support the monophyly of Octocorallia, but there is no consensus yet regarding the relationships among groups below the subclass level (McFadden et al. 2010). Apparently, the major problem is in the optimization of molecular markers, as well as the lack of available material for molecular use, since many studies have analyzed the molecular phylogeny using a scarce number of taxa samples.

The rapid development of new molecular markers has helped to improve the resolution of molecular techniques in the taxonomy of octocorals. For instance, the first molecular markers used in the phylogeny of octocorals (mt 16 s and nuclear 18 s) were only able to resolve individuals at the genus or family level (Berntson et al. 2001; France 2007), whereas more recently developed mt markers such as *msh1* and *ND2* have a better resolution than these original markers (McFadden et al. 2006a, b, 2010).

The most complete phylogeny published for Octocorallia is that by McFadden et al. (2006a, b) who sequenced two mitochondrial protein-coding regions, *msh1* and *ND2*, for 115 genera representing 29 of 47 families. The phylogenetic analysis resulted in two major clades: one clade (Pennatulacea-Calcaxonia) comprised Calcaxonia plus Pennatulacea, with Helioporacea included in some analyses; another clade (Holaxonia-Alcyoniina) included all members of Holaxonia, a majority of Alcyoniina, and representatives of Scleraxonia and Stolonifera; and a third small clade (*Anthomastus-Corallium*) included deep-water Scleraxonia and several taxa of Alcyoniina. Although this study agreed with the previous findings using 16 s and 18 s genes (France et al. 1996; Berntson et al. 2001), none agreed with the traditional taxonomic divisions placed within the Octocorallia (McFadden et al. 2006a, b). The addition of cytochrome oxidase I (COI) and complete 28S rDNA sequences to existing 16S, 18S, and mtDNA datasets continues to support the two major clades of Octocorallia (Holaxonia–Alcyoniina and Calcaxonia–Pennatulacea), but provides no further insights into relationships within the unresolved Holaxonia–Alcyoniina clade (McFadden et al. 2010). In addition, incongruence between mitochondrial and nuclear gene trees suggests that hybrid speciation and reticulate evolution may be an important mechanism of diversification in some genera (McFadden et al. 2010).

The proposed molecular phylogenetic resolutions for the order Alcyonacea are consistent with those proposed by Bayer (1981b), and they support the idea that the previous orders created by Kükenthal and Hickson cannot be supported by traditional taxonomy or molecular phylogenetics. The molecular phylogenetic studies published to date support the monophyly of Pennatulacea (McFadden et al. 2010) although the phylogeny of this order is not yet resolved. Molecular analyses do not support its traditional classification, as the suborder Sessiliflorae is paraphyletic, and Subselliflorae is polyphyletic. Apparently, the high frequency of morphological homoplasy in pennatulaceans has led to many misinterpretations in the systematics of the group (Dolan et al. 2013).

The solution for the taxonomy of Octocorallia seems to be in the reconciliation of morphology and molecular data, as suggested by McFadden et al. (2010). The current octocoral classification is based on morphological characters, some of which are highly plastic or present a high convergence, often influenced by the environment. In these cases, molecular phylogenies do not agree with the groups suggested by the traditional classification. For instance, certain characters including polyp sclerites, sclerite ornaments, and mineralization of the axis in Plexauridae and Gorgoniidae are consistent with molecular phylogenies. On the other hand, mineralization, surface sclerites and length of sclerites are highly homoplasious (Sánchez et al. 2003b). The opposite can also be observed, as molecular analyses have revealed the taxonomic importance of certain morphological characters not frequently used to classify certain groups. For instance, in the soft corals *Sinularia*, *Sarcophyton*, and *Lobophytum* the presence, arrangement, and form of sclerites in the polyps are not frequently considered in the taxonomy of these genera. However, molecular analyses have

shown that these characters are actually congruent with the major molecular clades (McFadden et al. 2006b, 2009). Although the use of character mapping has been shown to be useful at differentiating octocoral genera and families, additional molecular studies are necessary before this approach can be successfully applied at subordinal levels (McFadden et al. 2010).

The main issue in the current taxonomy of octocorals is to understand the taxonomic relevance of the intraspecific variation, since the lack of understanding of morphological variation and the absence of variation in the molecular markers at the species level make difficult the establishment of limits between taxa, and consequently of a suitable classification. As pointed out by McFadden et al. (2010), the development of molecular markers, new tools and approaches to the study of morphological variations are needed to deepen the knowledge of species and speciation processes in octocorals.

8.2 Current Patterns of World Distribution of Octocorals: Regions of Endemism and Hotspots

Octocorals can be found from the intertidal zone to depths up to 6400 m, inhabiting both reef environments and soft bottoms (Williams 2011). The deepest octocoral record is 6400 m *Thouarella vityaz*, by Zapata-Guardiola and Lopez-Gonzalez (2012). Although common in all oceans, they have peculiarities on their local distribution, which is directly influenced by factors such as presence/absence of strong currents, suspended organic matter, and distance from coast (Fabricius and Alderslade 2001). Globally, they can be found from tropical to polar environments. Few species are cosmopolitan, with most of them being found in the order Pennatulacea (Williams 2011). Species with wide depth ranges – such as pennatulaceans – tend to occupy more areas, which is the case of several abyssal species that are cosmopolitan, while species usually inhabiting continental margins are more geographically restricted (Williams 2011).

The actual diversity in the deep-sea is not always evident. The distinction of limits between species and morphologic plasticity sometimes can only be detected with the use of molecular techniques (Baco and Cairns 2012; McFadden and Ofwegen 2013; Quattrini et al. 2013). Furthermore, biogeographic barriers can be subtle and identification complex.

Examples of deep-water octocoral endemism include *Callogorgia delta* Cairns and Bayer, 2002, which is only known from the Gulf of Mexico (Quattrini et al. 2013); and *Primnoella delicatissima* Kükenthal, 1908 and *Primnoella polita* Deichmann, 1936, which are restricted to tropical latitudes in South America (Cairns 2006). Endemism in the deep-sea is a complex subject, and several studies have been trying to better understand it. Some of these have discussed the role of seamounts as hotspots, refuges or centers of endemism (Thoma et al. 2009; Rowden et al. 2010). However, because knowledge on the organisms living in the deep sea is still scarce and fragmented, what we consider now an endemic species might also be the result of a biased sampling effort. Reproduction mechanisms and recruitment events are also not well known, limiting information on dispersion and colonization (Rogers et al. 2007).

Understanding the distribution of deep-water octocorals is very challenging, considering the important logistics associated with accessing the deep sea. In this context, the field of marine habitat mapping has come to fill an important gap. For instance, habitat suitability models have been increasingly used to map the distribution of cold-water octocorals at both large (e.g. Yesson et al. 2012) and local scales (e.g. Bryan and Metaxas 2007). Based on known information on environmental requirements of different species, these studies allow one to further predict species distribution. In a global suitability model on cold-water octocorals, Yesson et al. (2012) estimated that salinity, temperature, broad scale slope, productivity, and oxygen and calcite saturation states are important factors influencing the distribution of cold-water octocorals. According to this study, the suborder Sessiliflorae (Pennatulacea) has the widest potential habitat range, whereas all octocoral suborders suggest a habitat preference for continental shelves and margins, particularly in the Northwest Atlantic and Western Pacific Rim.

As for scleractinian corals (Cairns 2007a), the world region that supports the highest diversity of octocorals is certainly the Indo-Pacific (Fig. 8.1), where a great number of endemic species is found. As an example, in Australia 457 deep-water octocoral species have already been documented (~21 % or 94 of the identified species are Australian endemics) (Alderslade et al. 2014). Some sub-regions considered as possible centers of origin and potentially highly diverse include the Philippines, Southeast Australia, New Caledonia, and New Zealand (McCoy and Heck 1976).

In 1981, Bayer (1981a) indicated several areas in the Indo-Pacific and Pacific coast of the Americas south of Panama as "poorly known"(Fig. 8.1 – areas 13 and 18), the latter with only two studies (Philippi 1892; Hickson 1928). More than 20 years later, Bayer (2002) reaffirmed the paucity of knowledge on gorgonians from the Indo-West Pacific areas. In the same document, he emphasized that knowledge on octocorals of the eastern Pacific, Indo-west Pacific and Southern Ocean presented a "greater problem" (Bayer 2002) when compared with the Atlantic, for example. The knowledge of octocorals from the Indo-Pacific region has greatly improved since then. To date, more than 100 genera of shallow-water octocorals from the Indo-Pacific have been described (Fabricius and Alderslade 2001; Paulay et al. 2003; Fabricius and McCorry 2006). However, there are still major

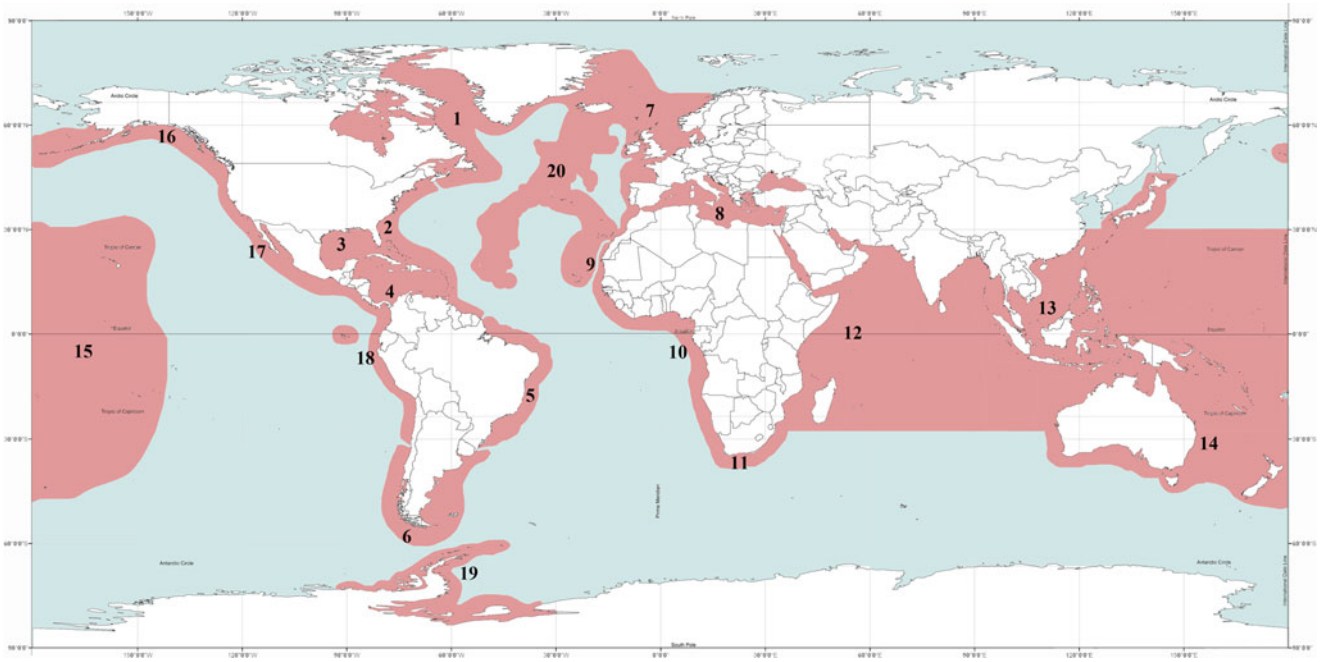

Fig. 8.1 Map of geographical areas and richness estimates of known species by region, based on local surveys. *1* Atlantic Canada (~40 spp.), *2* Atlantic USA (~140 spp.), *3* Gulf of Mexico (~170 spp.), *4* Caribbean (~156 spp.), *5* Brazil (~100 spp.), *6* Subantarctic (~55 spp.), *7* Northeast Atlantic (>80 spp.), *8* Mediterranean (~54 spp.), *9* Macaronesia (~60 spp.), *10* Western Africa (~24 Pennatulacea), *11* Southern Africa (~170 spp.), *12* Red sea and Western Indian (~208), *13* Western Pacific (~890), *14* Australia (~460 spp.), *15* Central Pacific (~70), *16* Alaska (~98 spp.), *17* Pacific coast of North America (~76 spp.), *18* Pacific coast of Central and South America (~69 spp.), *19* Antarctic (~30 spp.), *20* Northern mid-Atlantic ridge (~30 spp.)

gaps in the knowledge of octocoral distribution in this region, mainly because past inventories described the octocoral fauna at the family level or in terms of colony shape patterns, such as "soft coral" or "sea fans", which can make identification inaccurate (Fabricius et al. 2007).

A series of studies has been published since the 1980s, including descriptions of species (Alderslade 1983, 1986, 1991, 2003; Bayer 1990; Williams 1990; Bayer and Stefani 1988; Ofwegen and Benayahu 2006; Alderslade and McFadden 2007; Ofwegen and Alderslade 2007), reviews of several taxa (Alderslade 1985; Williams 1992; Cairns 2010; Namin and Ofwegen 2010; Ofwegen et al. 2013) and regional surveys (Grasshoff 2007; Benayahu et al. 2004; Benayahu and Chou 2010; Benayahu and Fabricius 2010; Benayahu and Ofwegen 2012; Benayahu 2013; Alderslade et al. 2014). And yet, it is unlikely that we are getting closer of knowing the total octocoral diversity in these regions.

The Indo-Pacific is a very large region that covers a tropical range of Indian and Pacific oceans, including the Red Sea, Oceania, and the islands of the Pacific (Melanesia, Micronesia and Polynesia). This area is very heterogeneous and it is treated uniformly in several works of taxonomic inventories, which makes it difficult to study. Local surveys are the best way to improve the understanding of the octocoral diversity in the Indo-Pacific (Fig. 8.1 – areas 11–15) and in many other areas in the world. Thus, here we present a quick overview of some sub-areas of this region.

A series of studies performed by Williams since 1980s revealed a richness of ~170 species in the southern Africa region (Fig. 8.1 – area 11). South Africa is one of the most important centers of endemism for Octocorallia. From the described species, ~50 % are endemic with at least seven endemic genera (Williams 1989a, b, 1992, 2000; 2003; Williams and Little 2001). From the north-western region of Madagascar, Tixier-Durivault (1966) and Verseveldt (1969, 1971) listed 63 species. The octocoral fauna of the Red Sea is composed of approximately 60 shallow-water species (Verseveldt and Benayahu 1978; Benayahu 1990; Benayahu et al. 2002; Halász et al. 2014) and around 85 shallow-water species for India (Kumar et al. 2015).

In the Western Pacific the reports are generally for islands or archipelagos (Fig. 8.1 – area 13). For instance, the region of Taiwan has around 70 species listed, mostly from shallow waters (Benayahu et al. 2004). Fabricius et al. (2007) made an octocoral inventory of the archipelago of Palau and they found a great diversity containing at least 76 genera in 24 families (~150 spp.), with the family Nephtheidae being the dominant taxon with tall colonies and high abundance; alcyoniid species of the genera *Sinularia*, *Sarcophyton* and *Lobophytum* were also conspicuous at many places. Also in Micronesia, Paulay et al. (2003) inventoried the octocoral

fauna of Guam (Mariana Islands) and recorded 79 species (32 genera) with a great predominance of the soft coral *Sinularia*. They pointed out that the diversity and abundance of gorgonians (*Acabaria*, *Suberogorgia*, *Junceella*) increases strikingly with depth. The available knowledge on octocorals from China's Exclusive Economic Zone is the result of a few studies, mainly focusing on gorgonians (Stiasny 1938; Ren-Lin et al. 1991) and alcyonaceans (Li 1982a, b, 1984, 1986, 1993; Zou et al. 1991; Malyutin 1993) from the southern China. These studies, which have been published since the 1980s, indicate the presence of about 40 shallow-water species in the region. There are no recent reviews in addition to species descriptions given by Benayahu and Ofwegen (2009) and Benayahu and Fabricius (2010). The region of New Caledonia has an estimated richness of 266 species, but the accuracy of this number is questionable. Ofwegen (2007) and Grasshoff (2007) cited respectively 173 and 93 species for this area. Ofwegen (2007) pointed out, however, that the list was based mainly on data from Tixier-Durivault (1970) and as many of her identifications are frequently challenged, an intense working revision is necessary. Of the 280 species known from the New Zealand EEZ (Cairns et al. 2009; Cairns in press), only 12 occur in shallow waters (Grange and Brook 2010). In Japan 260 species of octocorals are known (144 gorgonians and 36 pennatulaceans), of which 120 can be found in the upper bathyal to abyssal depths (Matsumoto et al. 2007). The shallow water epicentre of marine diversity, the Coral Triangle (tropical marine waters of Indonesia, Malaysia, Papua New Guinea, Philippines, Solomon Islands and Timor-Leste), is poorly known but the deep waters of this region might house a great octocoral diversity. For example, the Malay Archipelago contains about 25 species of *Chrysogorgia* (Chrysogorgiidae), which is more than 40 % of the current species richness of this genus (Watling et al. 2011).

The region of Australia (Fig. 8.1 – area 14) is probably the largest, both in terms of diversity and number of published studies on octocorals. In 2005, an expedition on the shelf and slope of Western Australia (100–1000 m) detected 141 species of soft corals, 80 % of which have been estimated to be new to science (Butler et al. 2010). Then, Alderslade et al. (2014) published the most comprehensive study on octocorals in the region, listing, as stated previously, 457 species, belonging to 131 genera and 28 families. Of this total, more than 45 % occur at depths >500 m, and 69 are probably new species.

The Hawaiian Archipelago (Fig. 8.1 – area 15) has a pattern different from other shallow-water reefs of the world, with few octocoral species described (only four). However, the diversity of deep-water octocoral is very high, with at least 70 confirmed species (Grigg and Bayer 1976; Cairns and Bayer 2008; Cairns 2009, 2010).

The cost of North Pacific of the Aleutian Archipelago and Alaska (Fig. 8.1 – area 16) has around 300 seamounts, representing a vast area of hard substrate for populations of octocorals that forming dense gardens with big colonies (Stone and Shotwell 2007). Heifetz et al. 2005 suggested that this region may be a possible center of origin for some octocoral families. Gorgonians are the most diverse group with more than 60 species that making dense assemblages of *Primnoa* spp and big colonies of *Paragorgia arborea* and bamboo corals. True soft corals and pennatulaceans are not a rich group (nine and ten species respectively) but they are very abundant, as species of *Clavularia* with densities of 1.7 colonies m^2 and with giant colonies as the sea pen *Halipteris willemoesi* which can reach up to 3 m high (Stone 2006; Stone and Shotwell 2007).

In the Pacific coast of North America (Fig. 8.1 – area 17) seems to repeat the same pattern of octocoral diversity of Alaska. More richness of gorgonians (36 species from 10 families) with dense "forest" of the bubblegum coral *Paragorgia arborea* and isidids, few species of true soft corals (8) and a high abundance of sea pens (28 species) principally of *Stylatula* spp., *Anthoptilum grandiflorum* and *Umbellula* spp. which are found coast wide (Stone and Shotwell 2007).

In the region that includes the Pacific coast of Central and South America (south Panama) (Fig. 8.1 – area 18) an increasing number of species has been revealed since the 1980s (Bayer 1986; Williams and Breedy 2004; Breedy and Guzman 2005, 2012; Guzman and Breedy 2012; Breedy and Cortés 2011; Breedy and Williams 2013; Cairns 2007b; Ofwegen et al. 2009). The octocoral fauna in the Galapagos Islands was virtually unknown until a few years ago, when Breedy et al. (2009) recorded 15 gorgonian (mainly *Pacifigorgia* and *Muricea*) and 3 pennatulacean species (*Virgularia galapagensis*, *Ptilosarcus undulatus*, and *Cavernulina darwini*), most of which were considered endemic to the archipelago.

The region between Antarctica and surroundings (Subantartic) – including the southern South American continent – (Fig. 8.1 – area 6 and 19) has received special attention in the recent years. Octocorals living in this area are limited to cold and dark environments (as deep-water octocorals), where so far species richness has been considered low. However, it seems to be a potential center of endemism, with some genera and over a dozen species being described only in the last decade (Zamponi and Pérez 1995; López-González and Gili 2000; Pérez and Zamponi 2000; López-González and Williams 2002; López-González et al. 2002; Williams and López-González 2005; López-González 2006; Häussermann and Försterra 2007; Ofwegen et al. 2006, 2007; Zapata-Guardiola and López-González 2010; McFadden and Ofwegen 2013).

Most of the known species in the Arctic were studied in more than 30 manuscripts written by Dr. Hjalmar Broch between the years 1910 and 1965 (e.g. Broch 1929), and it is difficult to assume a number of species, since no further reviews or checklists of octocoral species have been developed for this region. Neptheids and sea pens such as *Umbellula* spp. are some of the most common octocorals reported in Arctic waters (e.g. Broch 1956; Bluhm et al. 2005). Broch (1956) also reported *Acanella arbuscula* and *Virgularia*. Bamboo corals (e.g. *A. arbuscula*, *Keratoisis* sp.) and sea pens have been frequently caught as fishing bycatch in the Baffin Bay area (Eastern Arctic) (e.g. Neves et al. 2014), and a bamboo coral identified as *Isidella lofotensis* has also been found in high densities in East Greenland (Mayer and Piepenburg 1996).

In contrast to the Indo-Pacific, where communities of reef octocorals are mainly composed of soft corals (Xeniidae, Nephtheidae, Alcyoniidae) (Benayahu and Loya 1981) (Fig. 8.2a), in the Western Atlantic (Fig. 8.1 – areas 2, 3, 4 and 5) Gorgoniidae and Plexauriidae (Fig. 8.2b) are the most representative families (Bayer 1953, 1961).

In the Western Atlantic, the highest diversity is concentrated in the Caribbean and Gulf of Mexico. Around 10 % of the octocoral species found in the Gulf of Mexico are endemic (Cairns and Bayer 2009). This region is considered the probable center of origin for tropical species in the Southwestern Atlantic (Brazil) (Rocha 2003), where diversity is lower but endemism is higher (~24 % of endemism and at least two endemic genera) (Castro et al. 2010). Cairns and Bayer have published a series of eight manuscripts on the 59 deep-water calcaxonian species found in this region (Cairns 2001, 2006, 2007a, c; Cairns and Bayer 2002, 2003, 2004a, b).

In the Mediterranean (Fig. 8.1 – area 8) only 54 octocoral species have been recorded (Vafidis et al. 1994; Pastor 2006; Sartoretto 2012), although it presents an important endemism (18.5 %). In the Azores (Fig. 8.1 – area 9), about 41 species have been recorded, with most of these being known to live near continental margins, near islands and seamounts (Tixier-Durivault and d'Hondt 1973; Braga-Henriques et al. 2013), its octocoral fauna, however has been little studied. Further south, according to Raddatz et al. (2011), cold-water corals occur in abundance in the archipelago of Cape Verde, but few publications include descriptions of specimens in the region. In the Canary Islands, the situation is not much different, however, at least two species were described in the 1990s (Ocaña et al. 1992; López-González et al. 1995) and one genus and species in the 2000s (Ocaña and Ofwegen 2003). The African Atlantic coast is much less studied. Lopéz-Gonzaléz et al. (2001) revealed that – at the time of their study – only 15 studies had reported on Pennatulacea for that region. These authors recorded 24 species of pennatulaceans for the African Atlantic coast, from depths ranging 0–6200 m (Fig. 8.1 – area 10).

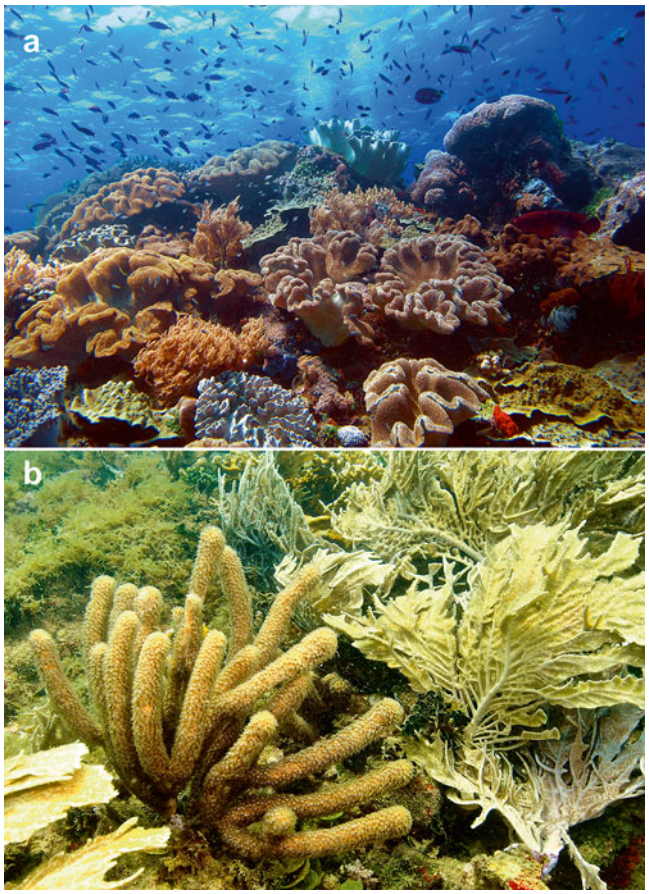

Fig. 8.2 Comparison between octocoral reef communities in the Indo-Pacific and Western Atlantic. (**a**) Alcyoniids from Komodo Island (Photo: Henrique Maranhão). (**b**) Gorgonids and plexaurids in a Brazilian reef (Bahia State) (Photo: Clovis B. Castro)

Finally, the deep-sea fauna of the mid-Atlantic ridge is particularly important in terms of understanding dispersion and connectivity patterns between eastern and western Atlantic populations. In the recent years there was an increase in the knowledge of this particular octocoral fauna, mainly in the northern mid-Atlantic ridge, where recent reports indicate an approximate number of 30 species (Watling 2007; López-González and Gili 2008; Mortensen and Buhl-Mortensen 2008; Watling et al. 2011; Molodtsova 2013). The octocoral fauna of the southern mid-Atlantic remains largely unknown, although recent efforts MAR-ECO (Patterns and processes of the ecosystems of the northern mid-Atlantic) have started to be made in order to explore the region (Pérez et al. 2012).

8.2.1 Richness According to Depth

It is a common perception that due to a dependence on light, shallow-water zooxanthellate octocorals have a more limited distribution than their azooxanthellate counterparts. Shallow-water species living in coastal environments are exposed to

river runoff and large variations in temperature and salinity. These factors can expose organisms living in such environments to speciation and a higher endemism, by creating geographic barriers. In the deep sea, speciation is as frequent as in shallow water environments, as evidenced by the high diversity found in the deep-sea (>75 % of octocoral species are found in waters >50 m; Cairns 2007a, b). However, the endemism observed in shallow waters is more easily detected (see Thoma et al. 2009; Quattrini et al. 2013).

The Gulf of Mexico (Fig. 8.1 – area 3) is a good example of how octocoral richness varies with depth. In this region, about 80 species are known from environments up to 50 m, while there is an increase in the number of species at >50 m (Cairns and Bayer 2009) (Fig. 8.3).

Even though the octocoral fauna in the Gulf of Mexico region has been classified as "poorly known", richness in waters >50 m is almost 40 % higher than in shallower waters. Richness, however, is not homogeneously distributed and tends to decrease with depth (Cairns and Bayer 2009). Another example is the New Zealand EEZ, from which of its 281 known species (Cairns et al. 2009; Cairns 2012), only 12 (3.6 %) occur in shallow water (Grange and Brook 2010). A higher octocoral diversification in deeper environments is also notable in the continental shelf and seamounts, where an increasing slope gradient with depth leads to more diverse habitats, despite geographic proximity (Quattrini et al. 2013). These habitats are influenced by different ranges of temperature, pressure, dissolved oxygen, which promote speciation. Towards the abyssal plain, richness tends to decrease and species are more adapted to more extreme life conditions.

Similarly to how certain families are more common in shallow/tropical environments (e.g. Gorgoniidae and Clavulariidae), certain taxa also tend to occupy deep rather than shallow-water environments (see section on deep-sea octocorals).

8.3 Deep-Sea Octocorals

8.3.1 History, Families and More Representative Genera

The deep sea is generally defined as the zone that begins at the end of the shelf edge (Gage and Tyler 1991). By this definition, deep-water octocorals are those species usually found at depths below 200 m; although corals living at depths >50 m have also been considered deep-water corals (Cairns 2007a; Roberts et al. 2009). The terms deep-water and cold-water corals have commonly been used interchangeably, since most findings and observations of these organisms come from cold and deep-water environments. However, certain primarily deep-water species can eventually be observed in much shallower environments, a phenomenon known as deep-water emergence. In Tracey Arm – a fjord in Alaska – the red-tree coral *Primnoa pacifica* (a primarily deep-water octocoral) can be found at depths as shallow as 10 m (Cairns 2010; Waller et al. 2014). The opposite pattern can also occur, with cases of primarily shallow-water species being eventually found in deeper waters. In the Red Sea, shallow-water soft corals have been observed at depths of

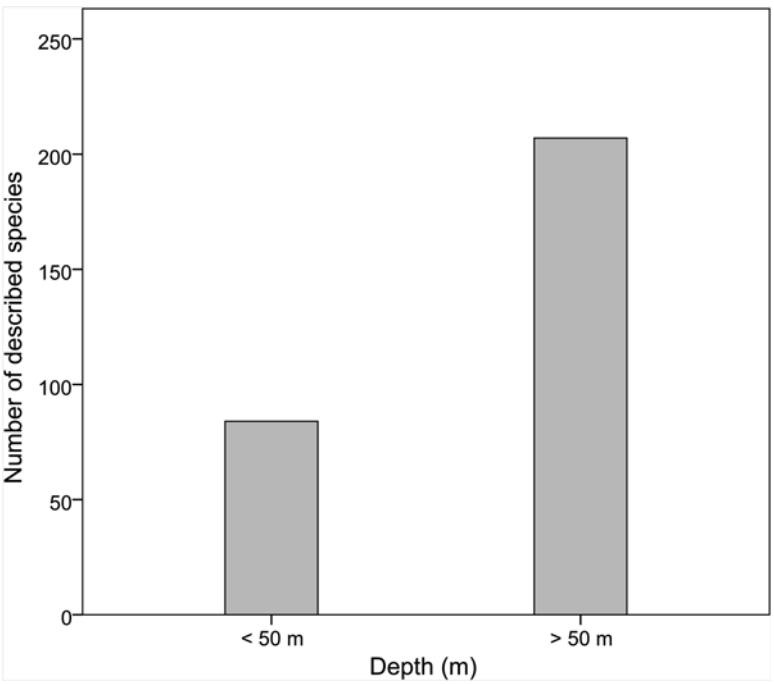

Fig. 8.3 Richness of octocorals in the Gulf of Mexico in two bathymetric classes: 0–50 m and ≥50 m (Data from Cairns and Bayer 2009)

360–720 m, where the temperature can be as high as 20 °C (Qurban et al. 2014). Both cases indicate that factors other than depth alone influence the distribution and growth of these octocorals (Freiwald et al. 2004; Roberts et al. 2009).

Deep-water octocorals – as other corals – have been known for centuries. At least since the eighteenth century, these organisms have been caught in fishing gear, as bycatch. They were particularly familiar to fishermen, who did not know the nature of these structures and fragments that came in their nets and lines (Roberts et al. 2006). The first known mention of the sea pen *Umbellula* in the literature came from the description of a colony caught in a fishing line, during a whale fishery near Greenland (Ellis 1753).

Knowledge of deep-water octocorals started to increase with results from early oceanographic expeditions, such as the *HMS Challenger* (1872–1876) (Wright and Studer 1889). In the nineteenth and twentieth centuries, important octocoral taxonomic and anatomical studies were published, based on descriptions of a number of deep-water specimens collected during such expeditions (e.g. Kölliker 1880; Versluys 1906; Kükenthal and Broch 1911; Kükenthal 1915, 1919, 1924; Jungersen 1904; Hickson 1916; Verrill 1922; Deichmann 1936). It was with the advent of novel underwater technologies – including remotely operated vehicles (ROVs), automated underwater vehicles (AUVs), sonar technologies, etc. – that the understanding of the ecology and distribution of deep-sea corals has considerably improved (Roberts et al. 2009). These technologies allow the direct sampling, in situ observation and monitoring in a way not possible before.

All three Octocorallia orders (i.e. Alcyonacea, Pennatulacea and Helioporacea) have representatives in the deep-sea. As already mentioned, around 75 % of the described octocoral species are known to be found in waters deeper than 50 m (Cairns 2007a), with around 67 % of octocoral families being found deeper than 200 m (Watling et al. 2011). Although most species are found in the continental shelf and shelf break (Watling et al. 2011), many species extend their ranges to much deeper waters. The primnoid *Convexella krampi* and the sea pen *Umbellula* sp. for example, are some of the deepest known octocorals, with records from 5850 m to >6000 m, respectively (Williams 1995, 2011; Madsen 1956).

Primnoidae, Chrysogorgiidae and Isididae are considered the three major deep-sea octocoral families. Primnoidae is a primarily deep-water family (Cairns and Bayer 2009), as is Chrysogorgiidae (Pante et al. 2012). In Isididae, some taxa are exclusive of deep-water environments, such as species in the subfamily Keratoisidinae (Alderslade 1998). Paragorgiidae and Parisididae are examples of families exclusively found in waters >200 m (Sánchez 2005; Watling et al. 2011).

Families of soft corals such as Nephtheidae and Alcyoniidae also have some genera exclusive to cold-water (but not necessarily deep) environments. *Gersemia* is an example of temperate-polar nephtheid with an eurybathic distribution (Williams and Lundsten 2009). These soft corals are commonly reported in North Atlantic waters (Mortensen and Buhl-Mortensen 2005a, b; Wareham and Edinger 2007). The mushroom soft corals *Anthomastus* and *Heteropolypus* are some of the common alcyoniids found in deep-water environments (Molodtsova 2013). Colonies of the recently described octocoral family Aquaumbridae are also found in deep-waters (Breedy et al. 2012).

In Pennatulacea most families can be found in both shallow and deep waters. The three deepest pennatulacean genera are *Umbellula*, *Kophobelemnon*, and *Porcupinella* (Williams 2011). Although *Umbellula* and *Kophobelemnon* can also be found at much shallower depths (<200 m), *Porcupinella* is only known from its type location and depth (5300 m) (López-González and Williams 2011). In Helioporacea only the genus *Epiphaxum* is found in deep-water environments (Bayer 1992).

8.3.2 Adaptations and Environmental Requirements

Some of the features that characterize deep-water octocorals and differentiate them from their shallow-water counterparts include the absence of association with zooxanthellae, high longevities, slow growth rates, tolerance to low water temperatures and limited food availability. Cold-water corals are usually found in environments where temperature ranges 4–12 °C, although there are species that support even colder temperatures (Roberts et al. 2009; Baker et al. 2012; Neves et al. 2014).

Many deep-water octocorals have a suspension feeding habit and depend upon currents to have access to food. The flabellate colonies of some gorgonians such as *Primnoa* and *Paragorgia* have a concave shape in response to strong and unidirectional currents, characteristic of deep-water environments (Grigg 1972; Mortensen and Buhl-Mortensen 2005b; Tong et al. 2012). This shape is usually associated to adjusting position in relation to currents to maximize prey capture (Grigg 1972).

In Pennatulacea, morphological adaptations to deep-water environments can be identified in taxa living at great depths (e.g. >3000 m), and include reduction in the number of feeding polyps (with an increase in their size), and absence of polyp leaves (Williams 2011). The alcyoniid genus *Anthomastus* also shows a reduction in the number but increase in size of polyps, which are much larger than polyps of other soft coral species (Bayer 1993; Molodtsova 2013); this character has not been explicitly referred to as an adaptation to deep-water environments in this taxon.

8.3.3 Substrate Requirements

The distribution and growth of deep-water octocorals depends on substrate availability. Most deep-water sea pens (Pennatulacea) are found inhabiting soft bottoms, with only two genera (*Anthoptilum* and *Calibelemnon*) known to have species adapted to colonize hard substrates (Williams and Alderslade 2011). Conversely, most soft corals and gorgonians require hard substrates for larval attachment and subsequent growth. Large gorgonians such as *Primnoa*, *Paragorgia*, *Paramuricea* and *Keratoisis* are frequently observed attached to bedrock, boulders and cobbles (Edinger et al. 2011). However, certain bamboo corals (Isididae) and chrysogorgiids can also be found in soft bottoms, as many species have root-like branches (holdfasts) to anchor in the soft substrate (Deichmann 1936). For instance, the bamboo coral *Keratoisis* sp. was observed forming dense forests on a soft bottom environment in West Greenland (Neves et al. 2014).

8.3.4 Longevity and Growth Rates

Most of the studies on growth rates and longevity of deep-water octocorals have focused on gorgonians, whereas growth in soft corals and sea pens has rarely been addressed. Several species of deep-water gorgonians lay down growth rings in their skeletons (Cairns 2002; Sherwood et al. 2005; Roark et al. 2009; Sherwood and Edinger 2009), and in certain taxa (e.g. *Primnoa*, *Acanella* and *Keratoisis*) ring formation periodicity has been shown to be annual (Sherwood and Edinger 2009). Longevity in these octocorals can reach hundreds of years, with linear growth rates being as slow as <1 cm.year^{-1} (Sherwood and Edinger 2009). The high longevities presented by some deep-water octocorals suggest they can be archives of environmental change, being of particularly interest in palaeoceanography (e.g. Thresher et al. 2004; Roark et al. 2005; Hill et al. 2012). The analysis of the internal axis of deep-water sea pens suggest they are also slow growing organisms (2–5 cm.year^{-1}), but with a decadal longevity (Wilson et al. 2002; Neves et al. 2015).

8.3.5 Vulnerability and Conservation

Deep-water octocorals are important components of Vulnerable Marine Ecosystems (VME) (Roberts et al. 2009) for several reasons. They are highly vulnerable to anthropogenic activities such as bottom fisheries, as contact with fishing gear can damage, dislodge, and kill colonies (Jones 1992; Watling and Norse 1998; Pauly et al. 2003; Troffe et al. 2005; Heifetz et al. 2009; Althaus et al. 2009). They have slow growth rates and high longevities (as outlined above), which suggest that full recovery from damage can take from decades to centuries, depending on the species. They also make structure and habitat for a number of other species (e.g. Auster 2005; Buhl-Mortensen and Mortensen 2004; Buhl-Mortensen et al. 2010).

In some locations, deep-water octocorals can form large assemblages commonly referred to as forests or gardens (Auster et al. 2013; Bullimore et al. 2013; Neves et al. 2014). These gardens are usually reported as being dense aggregations of gorgonians and other arborescent corals. Deep-water sea pens too, can be found forming dense fields up to 1 km in length (Baker et al. 2012; Kenchington et al. 2014), and possess a rich invertebrate associated fauna (Baillon et al. 2014). The finding of redfish larvae associated with different species of deep-water sea pens in the Northwest Atlantic highlighted their importance as habitat for these commercially important fish species, in an otherwise flat environment (Baillon et al. 2012).

Deep-water octocorals are diverse, abundant and widespread. New taxa are often being described and new hotspots of octocoral diversity being discovered. Concomitantly, threats, such as commercial fisheries, tend to expand to deeper environments (e.g. Paulay et al. 2003), with pristine areas becoming rarer. A better understanding of deep-water octocoral distribution and the taking of measures to protect and better know them is therefore essential for the maintenance of these populations. On the bright side, as underwater technologies become more accessible and logistics less constrained, an increase in the knowledge of deep-water octocorals will hopefully be witnessed in the next years, helping to increase protection and conservation of these organisms.

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Diversity and Distribution of Actiniaria

9

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Abstract

Sea anemones (Order Actiniaria) are among the most well successful groups of animals on the planet. They are distributed along the entire planet in several environments, from pole to pole, and so far, there are more than 1000 species known by scientists. Despite the small number of species when compared to other groups, the knowledge of sea anemones phylogenetic relationships is still obscure. Some of the reasons for this obscurity are the challenges imposed by the phenotypic variability and the lack of more strong morphological characters. Such difficulty faced by taxonomists also affects other areas of the study such as biogeography and ecology, therefore, the knowledge of these fields continues to be scant when compared to other groups of cnidarians. In this chapter, information about the distribution of species around the globe and also suggestions that could be used in studies focusing on the relationship among sea anemones are presented. In conclusion, despite the fact that there has been an increase in the number of researches regarding Actiniarians' biogeography, phylogeny and ecology, these areas still suffer the consequences of the low number of specialists and proper methods to study the group.

Keywords

Sea anemone • Hexacorallia • Biogeography • Taxonomy • Actinofauna

9.1 Species Diversity in Actiniaria: Taxonomy and Phylogeny

Actiniaria is one of the most successful and diverse taxa of Anthozoa. Sea anemones can be found in all marine habitats, depths and latitudes despite its body simplicity (Daly et al. 2008). The group comprises 48 families, 269 valid genera and more than 1000 species (Daly et al. 2007; Fautin 2013). Until recently, the taxonomy of sea anemones was based solely on a limited number of morphological characters, but the significance of them is debatable, and the lack of fossil records (soft body animals) hinders further assessments of these relations (McCommas 1991; Berntson et al. 2003).

Most of characters in sea anemone taxonomy are well studied regarding their function and tissue origin. Some features are linked to external morphology: protuberances that can function to adhere material to the column, carry nematocysts or other functions are observed by its arrangement at

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the column; Acrorhagi (a defensive organ) evaluated in taxonomy by its presence or absence; Cinclids are openings in the column and are estimated by its position and number; Tentacles, or more specialized tentacles such as catch-tentacles, are observed by morphology and arrangement. In general these structures are related to defense, ornamentation or regulation of hydrostatic pressure (Stephenson 1920). From internal morphology, taxonomists separate taxa based on presence or absence of basilar muscles, acontia and by morphology of sphincter, retractors and mesenteries. Acontia are thread-like organs full of nematocysts existent at the edge of mesenteries that can be exposed through the cinclids or mouth, and are appraised by its presence or absence (Stephenson 1920). The sphincter is a muscle responsible to close the oral disc in a transversal direction and retractors are the muscles that contract the column in a longitudinal way. Development and origin of musculature (mesogleal, endodermal or ectodermal) and its structure (weak/fragile, strong) can characterize suborders and families within Actiniaria.

It is common in taxonomic studies of Actiniaria to also have its set of nematocysts estimated, in acontiarian members they are used to distinguish families (Fautin et al. 1988). But, the use of nematocysts as valid characters to separate groups within sea anemones is still controversial and generates debates between scientists (Fautin 1988), due to its variability, excepting for acontiarian anemones, nematocysts are not a useful character for taxonomic and phylogenetic studies (Acuña et al. 2003). Furthermore, the numerous nematocyst classifications found in the literature (e.g. Carlgren 1940; Schmidt 1972; Mariscal 1974; England 1991; Östman 2000) often leads to conflicting diagnoses of the cnidom. Most of these classifications are grounded on the morphology of discharged capsules, but in most of the groups the research is conducted with undischarged capsules from preserved specimens, making difficult to distinguish different types of nematocysts. Some measurements of nematocysts (length × width) are also taken into consideration on diagnosis, but the variation in these measures is common even for individuals of the same species because they can vary according to the geographical and bathymetrical distribution (Zamponi and Acuña 1991), thus, these are not good characters to be used in diagnosis of species (Acuña et al. 2003). In addition, the high diversity of nematocyst could be explained by different mechanisms to construct cnidom in each species of sea anemones (Karalis and Chintiroglou 1997). Acuña et al. (2003) concluded that a set of other characteristics should also be used to define each taxon, not exclusively variation of cnidae, but it can be used as an additional piece of information.

There is a crescent urge to find more features in Actiniaria that could elucidate the phylogenetic relationships between its members (McCommas 1991). Schmidt (1974) was the first to introduce the use of cladistics in Anthoza, later, Bridge et al. (1995) analyzed Cnidaria using 29 morphological characters. However, these early studies were insufficient to show higher relationships in Anthozoa (Won et al. 2001). Although in some instances genetic molecular and morphological studies reflects the ambiguity of morphological features to distinguish groups, in other cases it supports some divisions. For example, the presence of acrorhagi in the Family Actiniidae according to Francis (1973) might be a monophyletic character, in opposition to what is concluded by Daly et al. (2007).

Currently, the most used classification is the one proposed by Carlgren (1949), which divides the order into three suborders: Protantheae Carlgren (1899), Endocoelantheae Carlgren (1925), and Nynantheae Carlgren (1899) (Table 9.1). The suborders Protantheae and Endocoelantheae are small, up to two families, and hold fewer than 20 species of the total and their members are hardly collected in shallow waters. The suborder Nynantheae contains more than 30 families. It is divided into three infraorders and three superfamilies. Since Nynantheae holds approximately 98 % of sea anemone species, it is the most diverse group, and its members are abundant at all depths and latitudes. Consequently, it is the most utilized group in phylogenetic studies and has been regarded as monophyletic despite its size and complexity (Daly et al. 2010). Although Carlgren's (1949) division

Table 9.1 Proposed classifications comparison, note that Schmidt joins Acontiaria in Late/Early Mesomyaria; Carlgren joins all infraorders proposed by Stephenson in Suborder Nynantheae but Athenaria and Boloceroidaria hold their status when Endomyaria, Mesomyaria and Acontiaria are ranked as superfamilies

Stephenson (1935)	Schmidt (1974)	Carlgren (1949)		
Infraorder		Suborder	Infraorder	Superfamily
Athenaria		Nynantheae	Athenaria	
Boloceroidaria			Boloceroidaria	
Endomyaria			Thenaria	Endomyaria
Mesomyaria	Late/Early Mesomyaria			Mesomyaria
Acontiaria				Acontiaria
		Protantheae		
		Endocoelantheae		

grouped taxa by the presence of some features, these same characteristics can be present in external groups (e.g. the basilar muscles is absent in Protantheae, Endocoelantheae and also in some members in Nynantheae). This kind of situation results in a difficult distinction of taxa, once the infraorders in Nynantheae are differentiated by the absence or presence of basilar muscles (Fautin 2013; Rodriguez et al. 2014a). Indeed, only a few taxa within Actiniaria possess an exclusive status of a certain attribute.

The suborder Nynantheae is branched into three Infraorders: Boloceroidaria, Athenaria and Thenaria. The infraorder Boloceroidaria has just two families (Boloceroididae Carlgren 1924; Nevadneidae Carlgren 1925) with four valid genera in total. Boloceriodarian sea anemones lack marginal sphincter and basilar muscles, possess longitudinal muscles at the column with deciduous tentacles, and are mainly recognized by its ecology, swimming behaviour and small size (Carlgren 1924). As a consequence, scientists do not know the members relationship clearly. One good example that illustrates the uncertainty within Boloceroidaria is the paper published by Rodriguez et al. (2014a) in which the authors propose a new genus and family for *Boloceroides daphneae* (a big boloceriodarian) and ranked it as *incerti ordinis*. The infraorder Athenaria also shows some taxonomic inconsistencies; its members are grouped together by the lack of basilar muscles, overall morphology and life style. It contains burrowing sea anemones with elongated bodies, but this characteristic is too simplistic and seems to have been lost or gained multiple times within the suborder (Rodríguez et al. 2012). The infraorder Thenaria is defined and delimited by the presence of basilar muscle (Carlgren 1949). However, as usual in sea anemones, there is an exception. The members of family Actinodendronidae Haddon 1898 lack basilar muscles, thus they go on the inverse direction of the definition of Thenaria, the group they belong. The infraorder Thenaria comprehend most of sea anemone's species, so it has been divided into three superfamilies: Endomyaria Stephenson (1921), Mesomyaria Stephenson (1921), and Acontiaria Stephenson (1935).

Endomyaria (Actinioidea Rafinesque 1815) contains 14 valid families of sea anemones with (or without) endodermal sphincter and no acontia (Carlgren 1949; Fautin 2013). Mesomyaria (Actinostoloidea Carlgren 1932) is characterized by mesogleal sphincter, no acontia and comprises four valid families (Carlgren 1949; Fautin 2013). Acontiaria (Metridioidea Carlgren 1893) members are among the most complex sea anemones presenting, in general, a composite cnidom in acontia. It holds 14 valid families (Carlgren 1949; Fautin 2013). The relationship between these superfamilies in some phylogenetic analysis could shift regarding methods used. In a few combined analysis, Acontiaria and Endomyaria have basilar muscles as a synapomorphy but if more taxa and less type of data are used it shows a parallelism (Daly et al. 2002). Synapomorphies inside Acontiaria (mesogleal marginal sphincter and acontia) are clearly observed, but in some analyzes it forms a clade with other mesomyarians who lack acontia and present mesogleal sphincters (Daly et al. 2003).

There are 48 valid families (Table 9.2) in total distributed within the sub and infraorders and superfamilies in Actiniaria (following Carlgren's Classification). Some of them are highlighted here due to its abundance, number of species, representativeness in some environment (deep sea or shallow waters) or even importance in evolutional discussions inside the order.

Actiniidae Rafinesque (1815), a family within Thenaria and superfamily Endomyaria, is the biggest in number of species and the most varied family of sea anemones, being found from shallow water to abyssal depths (McCommas 1991; Song and Cha 2002). It comprises more than 50 genera and approximately 300 valid species (Carlgren 1949; Song and Cha 2002; Fautin 2013). Its members can be characterized by absence of acontia and presence of endodermal sphincter. Opinions are divergent about the monophyly of Actiniidae. The diagnosis of the family is simple and contains several features that are present in other families (Daly et al. 2003). Francis (1973) affirms that acrorhagi is only seen within the family but not in all members. Acrorhagi is probably an ancestral feature and it presence is a synapomorphy (McCommas 1991). Nevertheless, recent molecular studies have not supported its members as monophyletic taxa (Daly et al. 2003). The confusion and lack of knowledge could be due the difficulties of having a minimum taxa assessed because the quantity of genera in the family.

Aiptasiidae Carlgren (1924), an acontiarian family, holds 6 genera and 24 valid species (Carlgren 1949; Fautin 2013; Grajales and Rodriguez 2014). The family is not monophyletic (Rodriguez et al. 2014a) and it is not apparently distinguished by any exclusive feature. But, Grajales and Rodriguez (2014) proposed an amend in the diagnosis (e.g. new type of nematocyst in the acontia) to better feat the relatively homogeneous members of the family.

Hormathiidae Carlgren (1932), an acontiarian family containing 15 valid genera and approximately 120 valid species, it is the second most representative family in deep seas (Fautin and Barber 1999; Fautin 2013). Although Hormathiidae is characterized by presence of acontia, there are several genera and species with dubious diagnosis, for instance some *Amphianthus* and *Actinauge* species may lack acontia (Carlgren 1949; Rodriguez et al. 2012). According to Gusmão and Daly (2010) Hormathiidae is probably paraphyletic. There is also a hypothesis that Actinoscyphiidae are hormathids that have lost acontia (Rodríguez et al. 2008; Daly et al. 2008).

Isophelliidae Stephenson (1935), an acontiarian family that comprehends 7 genera and approximately 40 valid species (Daly et al. 2007; Fautin 2013). Isophelliidae

Table 9.2 All valid families of Actiniaria in their respects Sub and infraorders with brief comments for each. Based on Hexacorallians of the World (Fautin 2013) and WoRMS (2015). Endomyaria = Actinoidea; Mesomyaria = Actinostoloidea; Acontiaria = Metridioidea. Genera/species column shows in total how many valid species and genera are within the respective family; Species with *nomen dubium*, *nomen nudum* or incorrect spelling were not counted. Number of current genera/species with *incertae sedis* is 45/47. Total of valid genera and species (plus *incertae sedis*) is 269 and 1089 respectively

Suborder infraorder	(Superfamily) family	Genera/species	Short remarks
Prontantheae	Gonactiniidae	2/2	Northern distribution, lacks most of actiniarian taxonomic features
Endocoelantheae	Halcuriidae	2/10	Ample latitudinal and depth distribution. Unique syphonoglyph
Endocoelantheae	Actinernidae	4/7	Unique arrangement of mesenteries. Deep sea
Nynantheae Boloceroidaria	Boloceroididae	3/8	Swimming sea anemones. Usually small
	Nevadneidae	1/1	Monospecific. Without sphincter at the base of tentacles
Nynantheae Athenaria	Andresiidae	1/1	Monospecific. From sublittoral of Mediterranean Sea
	Andvakiidae	3/11	Sand-encrusted, burrowing anemones
	Edwardsiidae	7/92	Burrowing anemones with eight pairs of perfect mesenteries
	Galatheanthemidae	1/2	Restrict to abyssal depths. Strong chitinous tube
	Halcampidae	8/24	Elongated body with cuticle
	Halcampoididae	8/14	Soft-bottom anemones. Boundaries with Halcampidae hard to distinguish
	Haliactinidae	6/9	Elongated body. Most members with acontia
	Haloclavidae	10/30	Soft-bottom anemone, burrowing anemones
	Limnactiniidae	1/2	No tentacles. Bipolar distribution
	Octineonidae	1/3	With acontia. Flattened body
	Polyopidae	2/2	Deep sea
Nynantheae Thenaria	(Endomyaria) Actiniidae	55/308	Most diverse family. Cosmopolitan
	Actinodendridae	3/9	Dendritic tentacles Shallow-water anemones
	Aliciidae	4/10	Symbiosis with zooxanthellae
	Capneidae	2/6	Lobed tentacles with unusual arrangement
	Condylanthidae	6/8	Macro- and micro-cnemes. Sometimes disc-shaped
	Homostichanthidae	1/1	Monospecific. Shallow waters
	Iosactinidae	1/2	Monogeneric. Deep sea. Bipolar distribution
	Liponematidae	2/4	Deep sea and polar waters
	Minyadidae	3/11	Neritic. Pedal disc used for buoyancy
	Phymanthidae	2/13	Distinct verrucae and knoblike tentacles.
	Preactiniidae	2/2	Partially fused mesenteries. Southern distribution
	Ptychodactinidae	1/2	Monogeneric. Northern distribution
	Stichodactylidae	2/9	Unique arrangement of tentacles. Clownfish sea anemones
	Thalassianthidae	4/8	Oral disc lobed, dendritic tentacles. Shallow waters
	(Mesomyaria) Actinoscyphiidae	8/14	Flytrap sea anemones. Deep sea
	Actinostolidae	19/71	Unique arrangement of mesenteries. Most species from deep waters
	Exocoelactinidae	1/2	Irregular arrangement of mesenteries
	Isanthidae	6/8	Elongated body, macro- and micro-cnemes
	(Acontiaria) Acontiophoridae	3/5	Mostly in shallow waters. Lacks a unique feature
	Aiptasiidae	6/24	Shallow water. Cosmopolitan. Dinoflagellate symbiosis

(continued)

Table 9.2 (continued)

Suborder infraorder	(Superfamily) family	Genera/species	Short remarks
	Aiptasiomorphidae	1/4	Monogeneric. Shallow waters. Lacks a unique feature
	Antipodactinidae	1/2	Monogeneric. Deep sea, bipolar distribution
	Bathyphelliidae	4/8	Deep sea. With acontia reduced
	Diadumenidae	1/9	Monogeneric. Shallow waters, catch-tentacles
	Haliplanellidae	1/1	Monospecific. Shallow waters
	Hormathiidae	18/127	Deep sea anemones, cosmopolitan. Sometimes shows symbiosis with crabs
	Isophelliidae	7/44	Similar to Andvakiidae but has basilar muscles
	Kadosactinidae	2/6	Deep sea and hydrothermal vents, sometimes symbiont with sponges
	Metridiidae	2/8	Ample depth distribution. Lacks exclusive features
	Nemanthidae	1/3	Sometimes symbiont with crabs. Tropical and subtropical distribution
	Sagartiidae	17/94	Cosmopolitan, shallow waters
	Sagartiomorphidae	1/1	Monospecific. Shallow waters

distinguishing features are very similar to Andvakiidae, even though they are in different superfamilies (Daly et al. 2008). In a recent study (Rodríguez et al. 2012), Isophelliidae was synonymized with Andvakiidae because their only difference is that the later lacks basilar muscles, the history of the groups (Andvakiidae traditionally belongs to Athenaria) probably had prevented them from being considered synonyms (Daly and Goodwill 2009; Rodríguez et al. 2012).

Sagartiidae Gosse (1858) acontiarian family with 17 genera and approximately 85 valid species (Carlgren 1949; Daly et al. 2007; Fautin 2013) is often found in shallow waters and SCUBA dive reaching depths. Acontia with a set of nematocysts and the presence of mesogleal sphincter are the only differential features and they are not exclusive of the family, therefore it is probably not monophyletic (Daly et al. 2007).

Actinostolidae Carlgren (1893) family placed in Mesomyaria is one of the most representative families from deep sea, polar waters and chemosynthetic environment (Rodríguez and Daly 2010; Fautin 2013). It contains 19 genera and approximately 70 valid species (Daly et al. 2003). The family lacks unique features; it is characterized only by mesogleal sphincter and no acontia. Recent phylogenetic studies (Rodríguez et al. 2008) strongly suggest that Actinostolidae is not monophyletic. Some genera actually belong to Actinoscyphiidae.

Haloclavidae Verrill (1899) an athenarian family that holds 10 genera and approximately 25 valid species (Rodríguez and López-González 2003; Fautin 2013). The majority of characters in the diagnosis not just overlaps with the family Halcampoididae but also shows a high variability, unique features are: length of tentacles and a possible absence of siphonoglyph in Halcampoididae (Rodriguez et al. 2003). The problem is that the form of tentacles in preserved specimens depends on the circumstances of the death (Stephenson 1920). Some phylogenetic analyses, puts together the type genus *Haloclava* with some members of Actiniidae (Berntson et al. 2003; Daly et al. 2003).

Edwardsiidae Andres (1881) within Athenaria, has 7 genera and more than 90 valid species (Daly 2002; Fautin 2013). The genus *Edwardsia* de Quatrefages (1842) actually is the richest in terms of species in Actiniaria with approximately 56 valid species (Daly et al. 2008; Fautin 2013). Specialists gave more importance to the study of this family probably due to the vermiform body of the adults, resembling the developmental stage shared by other sea anemones (Daly 2002). Other features of this family includes a naked physa and eight pairs of mesenteries, the latter is a derived state supporting monophyly of the family (Daly 2002; Daly et al. 2002). Several authors consider Edwardsiidae a higher rank than family (Bourne 1916; Stephenson 1921; Daly et al. 2002; Rodríguez et al. 2014a).

Prior to Carlgren's (1949) classification, Stephenson (1935) proposed the extinction of Thenaria, putting Athenaria, Acontiaria, Boloceroidaria, Endomyaria and Mesomyaria as infraorders (Table 9.1). Later, Schmidt (1974) distinguished Mesomyaria into Late Mesomyaria and Early Mesomyaria, containing the acontiarians and treats Athenaria as polyphyletic. Therefore, one can conclude that the characteristics used on the several proposed classifications lead to "unnatural" groups (Daly et al. 2007). It is clear that Carlgren's classification of 1949 it is not based on phylogenetic analysis. Although the studies cited on this text have shown some strong monophyly, for example in Nynantheae, they also showed that some infraorders and

Table 9.3 Classification proposed by Rodriguez et al. (2014a), with the included families and thus correspondent taxa and status of species in Carlgren 1949

Rodriguez et al. (2014a)			Carlgren (1949)
Suborder	Superfamily	Included families	Members taxa
Anenthemonae	Edwardsioidea	Edwardsiidae	Family of Athenaria
	Actinernoidea	Actinernidae, Halcuriidae	Suborder Endocoelantheae
Enthemonae	Actinostoloidea	Actinostolidae, Exocoelactinidae	Superfamily Mesomyaria
	Actinioidea	Actiniidae, Actinodendridae, Andresiidae, Capneidae, Condylanthidae, Haloclavidae, Homostichanthidae, Iosactinidae, Limnactiniidae, Liponematidae, Minyadidae, Oractinidae, Phymanthidae, Preactiniidae, Ptychodactinidae, Stichodactylidae, Thalassianthidae	Superfamily Endomyaria and some families of Athenaria
	Metridioidea	Acontiophoridae, Actinoscyphiidae, Aiptasiidae, Aiptasiomorphidae, Aliciidae, Amphianthidae[a], Andvakiidae, Antipodactinidae, Bathyphelliidae, Boloceroididae, Diadumenidae, Gonactiniidae[a], Halcampidae, Haliactinidae, Haliplanellidae, Hormathiidae, Isanthidae[a], Kadosactinidae, Metridiidae, Mimetridiidae[a], Nemanthidae, Nevadneidae, Octineonidae, Ostiactinidae[a], Phelliidae[a], Ramireziidae[a], Sagartiidae, Sagartiomorphidae	Most Acontiara but also members of Mesomyaria, Endomyaria, Boloceroidaria, Athenaria

[a]Families not in Carlgren (1949)

superfamilies are paraphyletic. Because of the grouping using higher and broader taxonomic levels it is hard to identify and spot errors. Some works could detect divergences at superfamilies and infraorders, especially in Acontiaria and Mesomyaria (Daly et al. 2007). Just a few families have been studied by a phylogenetic approach; hence, monophyly is not well established (Rodriguez et al. 2014a).

This grouping could reflect a lack of sensible observations on relationships and traits based on synapomorphies, leading to a poor correspondence between the taxa since its diagnosis is mostly based on possession or not of a unique structure. Strong relationships are rare among the suborders (Rodriguez et al. 2014a). The addition of more morphological features could be difficult because of the simplicity of these animals in what concerns body structure, and its high level of phenotypic plasticity that is affected by development, nutrition stages and clonal reproduction (McCommas 1991). Some polyp structures (acrorhagi, sphincter musculature, etc.) in some occasions are difficult to distinguish in preserved specimens or by several different definitions (Fautin 1984; Cappola and Fautin 2000; Daly and den Hartog 2004). Phylogenetic relationships of some families and Actiniaria in general have being explored since late 1920s, but just in the last few decades they have become more clear. Although monophyly is unclear for most groups, several new proposals are relighting the discussions on Actinian' systematic (Daly et al. 2008; Rodríguez et al. 2012, 2014a).

Rodríguez et al. (2014a) conducted a phylogenetic analyzes with members of all three suborders and several molecular markers and proposed a new arrangement in the classification, regrouping all actiniarians in only two suborders Anenthemonae and Enthemonae (Table 9.3). Anenthemonae includes two superfamilies, Edwardsioidea (eight pairs of perfect mesenteries actiniarians) and Actinernoidea (members of Endocoelantheae). Enthemonae includes sea anemones with the common mesenterial display and contains three superfamilies: Actinostoloidea (former family Actinostolidae), Actinioidea (Endomyaria and Ptychodacteae plus some former Athenaria) and Metridioidea (acontiate members plus some members without acontia as Boloceroidaria and Protantheae). These proposed orders divide (on a high-level) sea anemones based on arrangements of mesenteries. Rodriguez et al. (2014a) also provided a diagnosis for each proposed clade. However, some of taxa among those proposed groups still show a mosaic pattern and challenge these new relationships. The precise position of *Boloceroides daphneae*, is uncertain. It was allocated in the genus *Relicanthus* and a new family was created to support the genus, Relicanthidae, but the work also gives *incerti ordinis* to this family, because they do not show a close relation with Actiniaria not belonging to it, excluding them from the classification proposed. Although both molecular and morphological results support this new higher-level classification it also puts under a magnifying glass the significance of several morphological features.

9.1.1 Conclusion

Advancements from cladistics techniques and technology are creating a tendency for more complex and profound studies regarding sea anemones taxonomy. Morphology-only based classifications are being challenged by recent phylogenetic studies. However even phylogenetic studies are not yet sufficient to demystify gaps on knowledge about the relationships of the group. Probably the lack of a unifying methodology aggregated with several confusing morphological features, due high phenotypic plasticity, is one of the causes that difficulties in a more assertive congruence of diagnostics approaches for the actiniarians. Specific approaches, such as: finding strong taxonomic features and establish solid relations among the species are needed to highlight from deep the relations among Actinarian members. In addition, there is only few researchers currently investigating the concerns of such broad taxa.

Phylogenetic tools should be an ally and probably not the only approach to solve the doubts on actiniarian taxonomy. Knowledge from diverse but completing fields (e.g. ecology, biogeography, molecular) when aggregated is more probable to solve the questions about species diversity and relationship. Integrative taxonomy is most likely the key to enlighten most problems within Actiniaria, since taxonomy or phylogeny alone cannot explain the confusion between the taxa.

9.2 Biogeography of Actiniarians

9.2.1 Patterns of World Distribution of Actiniaria

Sea anemones form a well represented group of marine invertebrates that are present in all regions of the globe. This distribution is reflected and confirmed by the existence of data from the different oceans of the planet, at several depths – from intertidal zone up to 3500 m – and at almost all latitudinal zones (Sanamyan and Sanamyan 2007; Fautin 2013; Fautin et al. 2013). Such tolerance to different environmental conditions exposes the great plasticity of sea anemones' species and may be one of the main reasons why Actiniarians have survived global extinction events and can be considered as one of the most successful groups of this Era.

Very conspicuous in the intertidal zone, sea anemones are also an important component of coral reefs communities; see grass beds; mangroves; mud flats, and other marine ecosystems around the world. Furthermore, some actiniarians are also present in the Antarctic continent, which represents one of the most inhospitable environments on earth. During the beginning of the last decade several species of sea anemones were described to the Antarctic continent and its surroundings (Rodríguez and López-González 2001, 2002, 2003; Rodríguez 2012). More recently the discovery of a new species has amazed the scientific community. Daly et al. (2013) presented the description of *Edwardsiella andrillae*, apparently just another new species of sea anemone. However, what drew the attention of the scientists was not any morphological or anatomical trait. The interesting fact about those individuals was the environment where they were sampled. Using a remote operated vehicle, Daly and colleagues found *E. andrillae* burrowed on the ice in the lower side of the Ross Ice Shelf, in a quite dense population. This represents the first record of an animal community dominated by sea anemones in the underside of the frozen continent.

Despite the fact that most of the known species of sea anemones occur in the depth zone between 0 and 500 m, there are some species present in very deep parts of the oceans, even with this region of the ocean being poorly explored (Fautin and Barber 1999; Sanamyan and Sanamyan 2007; Ammons and Daly 2008). At great depths, actiniarians are commonly found on thermal vents, which may be considered small, warm oasis in the bottom of the ocean. The best explored vent-systems are located at the Atlantic and Pacific Oceans (Van Dover et al. 2002). In some the Atlantic vents, the density of sea anemones can reach as many as 100 individuals per square meter, with the individuals generally distributed 15–60 m around the center of the vent and the highest density in the farther zone (Fautin and Barber 1999; Van Dover 1995). Unfortunately, little is known about the biogeography and the connections between organisms of different thermal vents and different cold seeps. It is believed that, in cases where two separated thermal vents present the same community structure, such similarity is a consequence of the great ability that larval stages have to disperse; it has been demonstrated that some larvae may stay as part of the planktonic community for as much as 13 months, and thus being able to reach long distances (Van Dover et al. 2002; Arellano and Young 2009). According to Rodríguez and Daly (2010), it is possible that the species inhabiting the vents have been originated from at least two different lineages inside the actiniarian's tree. By the moment, there is no scientific paper in the literature about the biogeography of sea anemones from neither thermal vents, cold seeps or any other deep-ocean habitat, probably due to the difficulties imposed by the study of sea anemones from those environments.

Another point that draws the attention in the geographical disposition of actiniarians around the planet is their latitudinal pattern of distribution. Sea anemones' latitudinal distribution seems to follow a different pattern then the one that is considered common to other animal groups such as terrestrial vertebrates and the close related group of hexacorallians, the Scleractinia. In those groups, there is an increase in diversity of species as we move from the pole to the equator,

with the peak at, or close to, 0° (Willig et al. 2003; Harriott and Banks 2002). It is now known that the great majority of sea anemones' species occurs in temperate regions (30–40°N and 30–40°S), and in a general perspective, the group presents a tendency to reduce its diversity as we move towards the equator and the poles, with the least diversity of species found in the poles (Fautin et al. 2013). At first glance, it seems counterintuitive and one could think that these results may be biased by the reduced knowledge of the species occurring in tropical zones, but, as discussed by the authors, this is not the case. They also affirm that this tendency encountered for sea anemones is common to other marine organisms, and consequently, such distribution may not be the exception, but the rule (see Fautin et al. 2013 for more details).

9.2.2 The Current Status on Biogeographic Studies

Currently, in biogeographic studies, one of the main challenges faced by the scientists is the difficulty to determine areas of endemism, which are considered by some authors as the fundamental units of this subject and a very important tool to be used when inferences about the processes that have shaped the distribution of organisms are intended to be made (Morrone 1994). Therefore, there has been an increase in the number of publications proposing new methods to determine those units (for a general view of some of those methods, see DaSilva 2011 and Posadas et al. 2006). Even though areas of endemism have been used in most biogeographic studies, the practical and philosophical definitions of it and the consequences of assuming one or another existing definition still generates debates among the scientific community (Harold and Mooi 1994; Hausdorf 2002; Crother and Murray 2011). Such uncertainty about the definition of what in fact is an area of endemism did not reduce the use of it in other areas of biology and they are now considered an important part of studies that focus on species conservation (Crother and Murray 2011).

In what concerns the use of actiniarians as the model organism to biogeographic studies, we are still in an initial phase, where most of the papers published only provide basic and descriptive information. Historically, the great majority of papers about sea anemones' biogeography have as the main goal to determine which species occur in a certain area and what the spatial arrangement of those species is. Hence, the main questions of such investigations continuous to be "*how* the organisms are distributed?", with almost no research targeting "*why*" such pattern has arisen (some examples are: Zamponi et al. 1998; Acuña and Griffiths 2004; Fautin et al. 2005, 2013; Häussermann 2006).

Currently, some researchers are conducting investigations to find out which forces are responsible for molding the existing distribution of sea anemones and how two different areas are related or had been related to each other in the past. Rodríguez et al. (2007) presented data about the composition of the Antarctic fauna of sea anemones that shows its similarity with the communities of the surrounding regions (e.g. Sub-Antarctic). From that they have inferred the connections between the surrounding areas and concluded that the existing actinofauna of the frozen continent is not a consequence of only one origin event. Another work that deserves attention is the one Daly (2004) developed about the biogeographic origins of sea anemones of the genus *Anthopleura* applying systematic methods. As a result, some of the deductions she could make were that the direction from which the genus has spread is from north to south and that the species of *Anthopleura* have, probably, a pan-tropical origin; besides, the North Atlantic species are a result of more than one radiation.

Miranda and Marques (2011) suggest some reasons that could explain the low number of studies conducted in marine environments that apply the cutting-edge methods in biogeography. For instance, most of those methodologies are proposed by biologists that have their research interest in terrestrial animals, therefore, the vast majority of them do not take into account the fact that marine environments have a third dimension (depth). One possible explanation for the lack of more complex biogeography studies using actiniarians is the very complicated identification process and the still unsolved phylogenetic relationship of the main groups of sea anemones (Stephenson 1921, 1922, 1923; Carlgren 1949; Daly et al. 2008; Rodríguez et al. 2012, 2014a). Another point that is worth to highlight is that there are only few studies addressed to understand the historical biogeography of sea anemones, what may be a consequence of the reduced number of sea anemones' fossil samples found in the geological register. Such absence of information could impose difficulties in determining the connections between two different areas that were once in contact but now have no apparent affinity.

9.2.3 Hot-Spots of Diversity and Geographical Gaps in Knowledge

According to Reid (1998), biodiversity hotspots are defined as areas that present at least one or more of the following characteristics: High levels of endemism, elevated richness of species, rare species, and high levels of threatening to those rare species. Usually hotspots became a synonym of the first and the second, in other words, they are assumed by most of the scientists as areas with high levels of endemism

and species richness. Hotspot has become a very common term among scientists that are concerned about the current status of the biodiversity on earth and has been employed to determine areas that deserve more attention from conservation programs (Myers et al. 2000). In marine environments most of the hotspots are related to coral reef systems, mainly because they possess great diversity and high levels of endemism. Furthermore, they are among the most threatened environments on earth and in the recent decades have been suffering impacts and losing area as consequence of man-related actions (Edinger et al. 1998; Carpenter et al. 2008; Reaka et al. 2008). In such context, it is important to conduct researches that are more extensive in order to look for other hotspots that comprise different marine ecosystems as important to the biodiversity of the oceans as coral reefs.

When it comes to anemones, there is no literature devoted to determine hotspots of biodiversity or endemism. However, it does not mean that these areas are inexistent or difficult to be established. In its work, Häussermann and Forstera (2005) determined that the fjord region of the Chilean coast must be treated as a hotspot for species of sea anemones once this coastal habitat presents a high level of endemism and great species richness. Around the globe, other regions could also be considered actiniarian's hotspots as well, but it seems that there is no apparent interest among specialists in determining such areas. Accessing the data available at the web site Hexacoralians of the World (the most complete online database for sea anemones species; developed by Dra. Daphne G. Fautin of Kansas University) and conducting a simple research, we easily find the number of sea anemones species from any location and can use this data as a fairly reliable estimation of the sea anemones' richness of that area (Fig. 9.1). The resultant data may be used as one of the parameters to determine where are located the Actiniarian's hotspots. For example, if we type in "Australia", the result shown is that there are 99 valid species (almost 10 % of all known species in the world) recorded for the country; with such high number of species it is reasonable to consider Australia (or at least one specific region of its coast) as a candidate to become a hotspot. As one can imagine, determining hotspots requires much more research and time and it is beyond the scope of this chapter. But it is important to understand that the use of such tools is very valuable and that determining hotspots is an important step towards more complete plans of conservation of species.

Even though more sea anemones' species have been discovered and described in the recent decades – according to Fautin (2013) there are more than 1000 species known by the science currently – the world map of sea anemones distribution still presents some gaps (Fig. 9.1). The North of Canada

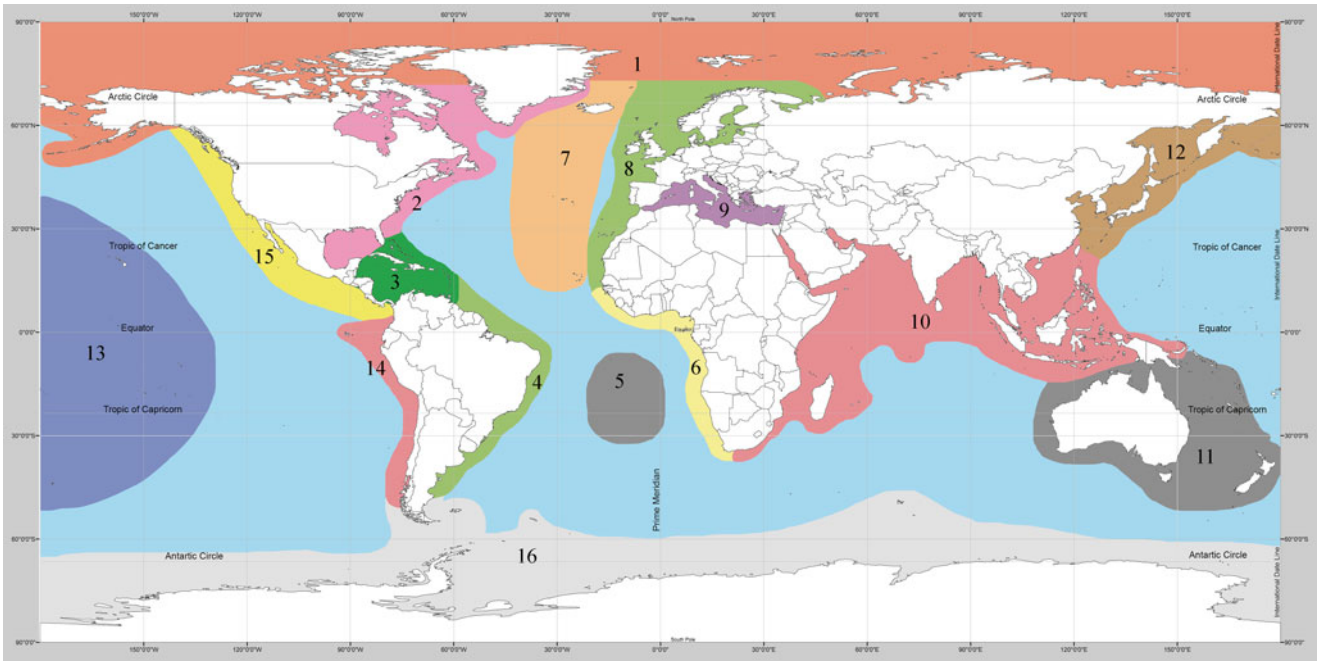

Fig. 9.1 Map of geographical areas and richness estimates of known species by region, based on Hexacorallians of the World (Fautin 2013; WoRMS 2015). *1* Arctic Region (~37 spp.), *2* Northwest Atlantic Ocean (~93 spp.), *3* Caribbean (~74 spp.), *4* Southwest Atlantic Ocean (~76 spp.), *5* Central South Atlantic Ocean (~19 spp.), *6* Southeast Atlantic Ocean (~51 spp.), *7* Central North Atlantic Ocean (~16 spp.), *8* Northeast Atlantic Ocean (~215 spp.), *9* Mediterranean Sea (~71 spp.), *10* Indian Ocean and part of Indo-Pacific region (~155spp.), *11* Australian Region (~171 spp.), *12* Northwest Pacific Ocean (~110 spp.), *13* Central Pacific Ocean (~21 spp.), *14* Southeast Pacific Ocean (~75 spp.), *15* Northeast Pacific Ocean (~100 spp.), *16* Antarctic and Subantarctic Region (~63 spp.)

and Russia, the Coast of Oman and Yemen, the west coast of Africa (mainly Angola and Namibia) and the Amazon Costal Zone (which comprises the north of Brazil, French Guyana, Suriname, Guyana and part of Venezuela) are among some important regions where more robust data about the fauna of sea anemones is still missing (Fautin 2013). Many of those areas are located in regions that represent transitional zones to other larger biogeographic provinces, as is the case of the Amazon Costal Zone, located in the north of South America in between the Caribbean and Brazil's provinces. This zone is believed to represent a very important area for studies of the biogeographic relationships between South and Central Americas. It was included in the results of the study of Spalding et al. (2007) as representing a separate biogeographical province; but studies about the fauna of sea anemones of this region are still very scarce and imprecise (Zamponi et al. 1998).

9.2.4 Conclusion

In order to better understand the processes that have lead the sea anemones to acquire the distribution pattern that they present today it is important that some areas of the globe – specially those where the fauna of actiniarians is still poorly known – receive more attention. Furthermore, it is important the phylogenetic relationships of actiniarians species become better understood. Along with that, with the advance of biogeography techniques, methods will be better suited for the application in marine environments. As we notice, there is a vast number of possibilities opened in what concerns the study of biogeography using sea anemones as model organisms, hence, more investments must be done in order to achieve a higher understating of the natural history of Actiniarians.

In addition, it is important to give some attention to the number of specialists in sea anemones taxonomy currently in activity around the world. There are only a few groups of researches that are dedicated to identification and classification of Actiniarians. Thus, in order to advance more rapidly in the area of biogeography, it is mandatory that the number of scientists specialized in sea anemones' taxonomy and systematic increase, once both are the base for the currently methodologies in this field.

9.3 Functional Diversity in Actiniaria: Ecological Aspects and New Approaches

Sea anemone's simple body structure contrasts with their enormous ecological diversity. In addition to their distribution in a wide variety of environments (e.g. coral reefs, rocky shores, shallow water mud flats, deep sea, artificial structures and other), they can also establish important ecological relationships with different organisms, such as photosynthetic microorganisms, other invertebrates and fishes. Symbiosis (e.g. sea anemones of the genus *Calliactis* and hermit crabs; see Gusmão and Daly 2010) and parasitism (e.g. the case of larvae of the species *Peachia quinquecapitata*; see McMurrich 1913; Spaulding 1972). Such diversity is also reflected in the chemical's production where compounds have been studied and isolated from sea anemones (Rodriguez et al. 2014b). Therefore, the order Actiniaria has an important part in biotechnology and great value for functional ecology studies, considering its role on the ecosystems' dynamics where they are present.

In general, the abundance of sea anemones is relatively small in tropical regions, but in the temperate parts of the globe, they can form big aggregates that take over the landscape and represent the base of these local communities (Fig. 9.2). They could also be part of the fauna associated to ship hulls and port structures, becoming a potential invasive organism, which is the case of the starlet sea anemome *Nematostella vectensis* (Reitzel et al. 2008; Silva et al. 2010). Especially in regions where they are abundant, sea anemones can play an important role in population size control through the predation of larvae from other organisms such as those of mollusks and Fishes (Minchin 1983; Castellanos and Lopretto 1990). Usually, sea anemones are considered opportunistic polyphagous predators, feeding from whatever is available in the system at that moment, and their diet may vary according to the season, location, or even depth (Quesada et al. 2014; Acuña et al. 2001; Davenport et al. 2011; Chintiroglou and Koukouras 1992). Another source of energy to Actiniarians is the organic matter dissolved in the water, which they absorb through the body surface (Schlichter et al. 1986). However, sea anemones are also a source of food themselves. They are predated by animals of several groups such as mollusks, starfishes and some vertebrates (Stephenson 1928; Mauzey et al. 1968; Edmunds et al. 1976; Ates 1989).

Sea anemones' association with photosynthetic microorganism (zooxanthellae) provides them another nutrition source, in this association they are beneficiated by the product of the photosynthesis (Trench 1993). This relationship has revealed some important aspects of the group's diversity. Photosynthetic dinoflagellates (genus *Symbiodinium*) are common symbionts of other anthozoans, and in general, each group presents a certain diversity of genetically different subgroups that present a distinctive biogeographical, ecological and host-specific pattern (Casado-Amezúa et al. 2014). However, recent researches using zooxanthellae associated to scleractinians corals have revealed that some symbionts taxa have a wide geographical distribution and associated with many hosts, while others present a certain endemism and host-specificity (Loh et al. 2001; Rodriguez-Lanetty and Hoegh-Guldberg 2003; Santos et al. 2002;

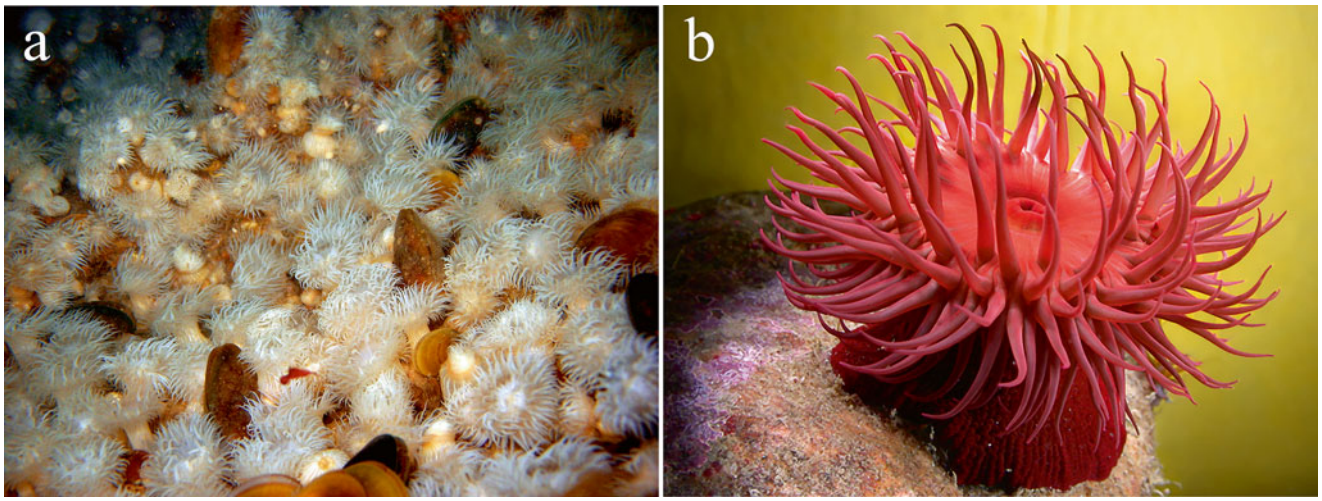

Fig. 9.2 Abundance of sea anemones. (**a**) The temperate sea anemone *Anthothoe chilensis* (Lesson 1830) forming a large aggregation in a shipwreck in Mar del Plata, Argentina (Photo: Gabriel N. Genzano. Universidad Nacional de Mar del Plata, Argentina) **(b)** the solitary tropical sea anemone *Bunodosoma caissarum* Corrêa in Belém 1987 from São Sebastião, São Paulo, Brazil (Photo: Alvaro E. Migotto. **Anêmona-do-mar)**. Image Bank: *Cifonauta*. Available at: http://cifonauta.cebimar.usp.br/photo/3812/ Accessed 08 April 2015

LaJeunesse et al. 2004, 2010). These patterns may represent a piece of information that could help on the understanding of the phylogenetic relationships of actiniarians and their biogeographical pattern.

Sea anemones produce a vast number of chemical compounds, including bioactive proteins and peptides like cytolysins, phospholipases, ion channel toxins and protease inhibitors (Oliveira et al. 2012). Recent studies have demonstrated that the complexity of those compounds is even higher than thought before (Zaharenko et al. 2008). Some toxins have already been isolated and studied from species of ten distinct families, revealing compounds that apparently are shared with other animal groups such as Arachnids and Mollusks (Rodriguez et al. 2014b). Despite this chemical diversity and their specificity to certain taxa these data is still not applied on studies that have as objective to clarify the phylogentic diversity of the group.

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Cnidarian Alien Species in Expansion

10

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Abstract

The study of the diversity and composition of marine communities is the first step in understanding the development of marine ecosystems. While some cnidarian populations are in decline, others invade new regions and habitats. The pressure of the human actions on the marine ecosystems has increased in the past decades. The artificial structures in marine environments, shipping, aquaculture, global warming and interoceanic canals have contributed to the dispersion, establishment and invasion of many places by alien species around the world, in some cases, with major ecological and socio-economic impacts. Some cnidarian species may serve as examples of the truly widespread reach of invasive species. Benthic phase of hydroids and anthozoans are common components of harbours and fouling communities and, probably, they have been transported on ship hulls. Ephyrae and hydromedusae are also frequently found in ballast water. Additionally, the global climatic change allows cnidarian of tropical affinity to extend their range into the temperate zones. The biological invasions and range extensions may cause local native biodiversity and economic losses. Only recently have these biological invasions attracted the attention of the scientific community. Though invasive alien cnidarians are recognized now as a global threat to biodiversity, and monitoring their presence and impacts is considered a prerequisite for marine environmental management and sustainable development, it seldom takes place even in the coastal regions most vulnerable to introductions

Keywords

Alien species • Invasive species • Vectors of dispersion • Cryptogenic species • Pseudoindigenous species • Hydrozoa • Anthozoa • Scyphozoa • Comb jellies

10.1 Alien Species: Problematic and Causes

The recently adopted regulation of the European Parliament and of the Council on the prevention and management of the introduction and spread of invasive alien species (European Commission 2013), defines 'alien species' as "species that are transported as a result of human action outside of their natural range across ecological barriers", and 'Invasive Alien Species' (IAS) as those with "a *significant* negative impact on biodiversity as well as *serious* economic and social consequences", but declined to clarify the levels of disturbance to be considered *significant* and *serious*. A companion

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document, Commission Staff Working Document, Impact Assessment (European Commission SWD 2013, Annex VIII, p. 80), defines IAS as "alien species whose introduction or spread has been found, *through risk assessment*, to *threaten biodiversity and ecosystem services*, or to have a negative impact on the environment, society and the economy", again, without delineation of threat levels. Since quantitative, or even qualitative, levels of impact of alien species on biodiversity, economy and society have not been delineated, let alone discussed and agreed upon, it is best at the present time to adhere to plain and clearly defined terms. Therefore, we use the term 'invasive' for clearly quantifiable 'wide spread', defined in the regulations as "an invasive alien species whose population has gone beyond the naturalization stage, in which a population maintains a self-sustaining population, and has spread to colonize a large part of the potential range where it can survive and reproduce" (there, Article 3, p. 16).

Alien species are considered by many researchers as constituting one of the major threats to biodiversity in marine environments (Bax et al. 2001). The study and control of these species is crucial for the maintenance of biodiversity and functioning of an ecosystem in a given area (Byers et al. 2002). Alien species can dramatically alter the diversity and productivity of ecosystems (Carlton 2009), the biotic relationships among native species (Byers et al. 2002), and may eventually lead to their extinction (Haugom et al. 2002). Furthermore, the alteration of an ecosystem by the alien species can facilitate subsequently the introduction of additional new exotic species (Simberloff and Holle 1999). Thus, the effect of alien species on ecosystems and their native species is one of the most important goals of conservation biology (Bax et al. 2003).

The first step in the study of alien species is to understand their dispersion, ecologic effects and possible control strategies (Byers et al. 2002). Unfortunately, the historical records and distribution of many alien species are poorly known (Byers et al. 2002; Carlton 2009), even in well studied zones (Carlton 1999; Galil 2000). Furthermore, many alien species are not detected because they are inconspicuous, very small (few millimetres) and/or little known (Bax et al. 2001). Therefore, the number of alien species in a given zone is usually underestimated (Carlton 2009).

In the sea, unlike the terrestrial environment, the only sure way to prevent the potential damage, both ecological and economical, caused by the introduction of alien species is prevention (Floerl et al. 2005). This is mainly due to the vectors of dispersion and the difficult detection of these species.

10.1.1 Vectors and "Hubs" of Introduction

Maritime traffic is the main vector of introduction of alien species in marine environment (Ruiz et al. 2000; Flagella and Abdulla 2005), by the hull fouling and ballast water (Ruiz et al. 2000). Some authors pointed out the earliest transport of species as possibly in Vikings' vessels (Petersen et al. 1992; Carlton 2009). Nevertheless, this phenomenon became global with the opening of the interoceanic maritime routes in the sixteenth century (Galil 2000; Carlton 2009). Harbours and marinas are artificial habitats associated with maritime traffic, and nowadays are qualified as vector hubs and hot spots for invasions (Ruiz et al. 2000; Flagella and Abdulla 2005; Forrest et al. 2009). Commercial harbours are usually located in estuary zones (Ruiz et al. 2009), where pontoons, pilings, marinas, etc. can also be found. The alien species seem to be more frequent in artificial habitats (Bulleri and Airoldi 2005; Tyrrell and Byers 2007; Glasby et al. 2007; Ruiz et al. 2009; Megina et al. 2013). The environmental conditions within harbours (for instance, higher turbidity, pollution, more frequent impacts), could give a better chance for opportunistic species, including new invaders transported by shipping (Occhipinti-Ambrogi and Savini 2003). Alien species, translocated by shipping and established within commercial harbours, can be later spread at local and regional levels by recreational boats (Ashton et al. 2006; Minchin et al. 2006).

Some authors pointed out that floating structures associated with marinas, like pontoons and docks, are habitats where alien species are significantly abundant (Glasby et al. 2007; Megina et al. 2016). These types of habitats increasingly occur on coasts worldwide (Minchin et al. 2006) and they are novel habitats for marine benthic organisms, forming different communities from those developing on natural rocky habitats (Connell 2001; Glasby et al. 2007). It has been suggested that hydrological conditions (conditions that affect the transport of nutrients) affect especially filter-feeders, which are the dominant organisms in artificial floating structures (Perkol-Finkel et al. 2006).

Artificial habitats are becoming very profuse, and in some regions, they are more abundant than natural rocks (Reise 2005; Pister 2009). Therefore, they must be considered as key features of coastal management (Megina et al. 2013).

The worldwide phenomenon of aquaculture is the other important vector of introduction of alien species (Streftaris et al. 2005; Ruesink et al. 2005). This comprises both intentional release of species of economic interest and unintentional introduction of associated species (Minchin et al. 2009). These associated species are more numerous than intentional introductions (Streftaris et al. 2005), and they are usually less known and less controlled (Minchin et al. 2009). Cultured species tolerate a wide range of physical conditions (therefore, they could be potential invaders) and culture conditions (high density of individuals and confinement) can favour the spread of parasites and pests (Minchin 2007).

10.1.2 Global Warming and Alien Species

The climate is changing and the average surface temperature of the sea is increasing (Brierley and Kingsford 2009). The clime is an important determinant in the range of distribution of species (Thomas 2010), and rapid climatic changes influence both the distributional range of the species and the composition of the communities (Walther et al. 2002; Harley et al. 2006). Indeed, global warming and the increase of the warm season are changing the distributional boundaries of species and cold water species are being replaced by warm water ones (Occhipinti-Ambrogi 2007). This process is known as "tropicalization" (Bianchi 2007).

The climatic change and the global warming constitute a complex scenario, which influences the alien species-phenomenon increasing the number of introductions and the dominance of alien on native species (Stachowicz et al. 2002). Global warming can affect the competitive interactions between native species (CIESM 2008) and the physiological stress originated by abnormally high temperatures can cause mass mortalities in benthic organisms (Coma et al. 2009; Lejeusne et al. 2010), leaving empty niches for new colonisers (Occhipinti-Ambrogi 2007). In this scenario, many alien species can replace native species with similar ecological roles (Galil 2007). Stachowicz et al. (2002) showed how alien species had higher recruitment than native species in warm winters and their growth was faster at high temperatures. In artificial habitats like harbours, hot spots of introduction of species (as we mentioned above), the increase of temperature facilitates the survival and growth of alien species of tropical affinity in the fouling communities (Sorte et al. 2010).

Global warming can also change the distribution of these alien species already introduced. Species better adapted to higher temperatures will be favoured by a changing environment (with progressively higher temperatures), facilitating their dispersion once introduced (Lewis 1999; Occhipinti-Ambrogi and Galil 2010; González-Duarte et al. 2013a, b). The impact on native species is also increased by global warming, due to alterations in the abundance of the populations of alien species or the available resources (Occhipinti-Ambrogi and Galil 2010).

The phylum Cnidaria Verrill, 1865 include around 11,000 spp., present in all aquatic habitats (Daly et al. 2007). This group is wide and heterogeneous, with solitary and colonial species, species with polyp stage only, medusa stage only or both (Daly et al. 2007). These features make them very diverse and plastic. In this chapter we discuss why many cnidarian species have spread further from their original distribution and the characteristics as invaders of this group.

10.2 Hydroids as Alien and Invasive Species

The Class Hydrozoa Owen, 1843 comprises around 4000 spp. (Bouillon et al. 2006), present in all aquatic ecosystems (Boero and Bouillon 1993; Gili and Hughes 1995; Bouillon et al. 2006). They are particularly abundant in coastal rocky shores (Boero 1984; Gili and Hughes 1995; González-Duarte et al. 2013a, b, 2016), where hydroids are usually present with a large number of colonies and species, including a high variety of forms that play diverse roles within their assemblages (Boero 1984; Boero and Fresi 1986; Gili and Hughes 1995; Piraino et al. 2002). They exhibit a generalised behaviour with regard to substrate selection (Cornelius 1982; Genzano and Rodriguez 1998), and they also adapt well to a diversity of artificial structures including ship hulls of commercial vessels and recreational crafts (Floerl and Inglis 2005; Minchin et al. 2006; Farrapeira et al. 2011), buoys (Calder 1997; Kirkendale and Calder 2003), pilings (Migotto 1996; Galea 2008), fish cage nets (Guenther et al. 2010; Bloecher et al. 2013) or harbours and marinas (Floerl and Inglis 2005; Cangussu and Altvater 2010; Megina et al. 2013, 2016). As previously mentioned, shipping and harbours are nowadays considered main actors in the artificial translocation of alien species. In fact, Megina et al. (2013) found a notably high diversity of hydroids in sea-walls within commercial harbours, comparable with that found in well preserved natural habitats, and some of the typical components of harbour assemblages were found to be alien species.

The essential life cycle of hydroids comprises several phases, with a polypoid stage, typically benthic and sessile (although this can be modified), a pelagic medusa stage and swimming planula larvae (Boero and Bouillon 1993; Bouillon et al. 2006). This can also be secondarily modified and almost half of the hydroid species have lost a true medusa phase, having a short-living, non-feeding medusoid or fixed gonophores within the mother colony (Cornelius 1990; Bouillon et al. 2004). Hydroids can be transported either during their benthic stages, as fouling organisms on ship hulls (Floerl and Inglis 2005; Minchin et al. 2006; Farrapeira et al. 2011) or during their pelagic stages, within cargoes' ballast water, as medusa or as different types of propagules (including pieces of benthic forms and diverse forms of resistance) (Carlton 1985; Carlton and Hodder 1995; see below).

Hydroids frequently present encysted stages in their life cycle, which allows them to pass less favourable periods, but it also helps them to survive the adverse travelling conditions in the ballast water or on the ship hulls (Boero 2002). The most common form of resistance is probably the dormant hydrorhizae (Boero et al. 2002), but also podocyst, a simple

fragment of living tissue enveloped by chitinous perisarc, which can act as resting stages or as propagules (Bouillon et al. 2004), or embryonic or planula encystments (Boero et al. 2002; Bouillon et al. 2004). This, together with a high regenerative and asexual-reproduction potential (Boero et al. 2002) and, unless in some species, the ability for reverse development (Piraino et al. 2004), allows them to adapt well to instable environments and makes them very efficient invaders.

The presence or the absence of a well-developed medusa stage is associated with the life history traits of hydroids, where two different evolutionarily stable strategies have been identified in this regard (Cornelius 1990; Leclère et al. 2009): a group of species are characterised by colonies of small size, relatively short lifespan and the retention of the ancestral life cycle within hydrozoan, with a free-swimming medusa stage. These species exhibit a "pioneering" life style, and are thought to depend on continuously locating and colonising new spatial refuge (Cornelius 1990; Leclère et al. 2009). On the other hand, species that have lost a true medusa phase and, theoretically, have a reduced dispersion potential, generally tend to present larger or more elaborated colonies (Cornelius 1990; Bouillon et al. 2004). In comparison with the previous strategy, these species exhibit a "competitor's" life style (Megina et al. 2013; Fernandez et al. 2014), with a higher investment in growth and somatic tissues, and an enhanced competition capacity for space and the long term maintenance of local patches (Cornelius 1990; Leclère et al. 2009; Megina et al. 2013).

When examining the hydroid assemblages in harbours, Megina et al. (2013) found clear differences with assemblages developing on well preserved rocky coast: "pioneering" species were significantly better represented in harbours, where human activity generates a higher frequency of disturbances and other associated alterations (e.g. pollution, modified water circulation, etc.) (Piola and Johnston 2008; Megina et al. 2013). Similar observations were made by researchers studying floating structures in harbours (i.e. ship hulls and experimental plates) (Millard 1959). However, "competitor style" species with large colonies are also well represented in fouling and harbours assemblages and, indeed, some of them are well-known alien or invasive species (Millard 1959; Morri and Boero 1986; Megina et al. 2013, 2016). In fact, "competitor" species are those that can potentially be a more serious threat for benthic native biodiversity. Small, short living and pioneering, they have a limited competitive capacity during their benthic stage, and a more limited possibility to exclude a native species, as they are reliant on temporal spatial refuges. Still, they can have a significant ecological impact on bentho-pelagic trophic nets, mainly through their medusa stage (see below).

An additional complexity exists to determine the status of alien species for hydroids, as many of them are small, inconspicuous and easily overlooked by non-specialists (Gravili et al. 2008). This is particularly true for the pioneering species. The history of their human-mediated geographical movements is not known for most of them and sometimes their native distributional range is just determined by the history of their records (Carlton 2009; Haydar 2012). As we discussed previously, the ship-mediated introductions of marine species started several centuries before and, therefore, some (or many) hydroid could be in fact alien species mistaken as native (Pseudoindigenous species). As an indication, they have one of the highest percentages of cryptogenic species (species that are not reliably demonstrated to be alien or native) and species with a disjunct distribution (also considered as an indication of a possible artificial translocation) (Haydar 2012). Indeed, only a few hydroid are usually included on lists of alien species (Streftaris et al. 2005; Zenetos et al. 2010), but it is thought that the number of species artificially translocated is significantly underestimated (Haydar 2012).

An example of this problematic is *Pennaria disticha* Goldfuss, 1820, a widespread circumtropical species, common in harbours and other fouling communities (Carlton and Eldredge 2009; Mead et al. 2011). *P. disticha* was described in the Mediterranean in 1820 (Goldfuss 1820) and for this reason it is sometimes cited as a European species (Coles et al. 1999; Carlton and Eldredge 2009) and not considered as an alien species in the Mediterranean (Zenetos et al. 2005). However, in areas where comparative studies exist between artificial and natural habitats (i.e. the Western Mediterranean and Strait of Gibraltar), this species was only recorded in harbours (Megina et al. 2013). This may suggest that it was a past and undocumented introduction in this region and this species could be considered as cryptogenic.

10.2.1 Some Examples of Alien and Invasive Hydroids

As stated, only a small number of potential alien and invasive hydroids are regularly listed as such. Here, we provide examples of some whose negative effects have been studied or have a high invasive potential.

Blackfordia virginica Mayer, 1910 is an example of Pseudoindigenous species sensu Carlton (2009): a species considered as native and described as new after its introduction. *B. virginica* is a Leptothecata species with small and rarely ramified benthic colonies (Bouillon et al. 2006). Usually, only the medusa stage is recorded. This species, native in the Black Sea, was described by Mayer (1910) from the estuarine zone of the Chesapeake Bay in Virginia (Western Atlantic Ocean), and later was found in several bays and estuarine zones in USA (Wintzer et al. 2013;

Harrison et al. 2013). Harrison et al. (2013) showed a low genetic difference among the population of *B. viginica*, consistent with a founder introduction effect and the subsequent spread, pointing to the alien-character of this species in the USA.

Nowadays, *Blackfordia virginica* has been introduced in estuarine zones of all oceans by shipping (Genzano et al. 2006; Haydar 2012), it is frequently found in ballast water and hull fouling (WoRMS 2014) and is usually recorded as invasive (Mills and Sommer 1995; Genzano et al. 2006; Bardi and Marques 2009). A characteristic of *B. viginica* (in common with other marine invasive species) is its high tolerance to a range of environmental conditions (particularly salinity: 3–35 %) (Moore 1987; Mills and Sommer 1995). This species has been recorded in the eastern Mediterranean (Bouillon et al. 2004), western and eastern Atlantic Ocean (Moore 1987; Genzano et al. 2006; Nogueira and de Oliveira 2006; Bardi and Marques 2009; Chícharo et al. 2009), western and eastern Pacific Ocean (Mills and Sommer 1995; Silva et al. 2003; Hayes and Sliwa 2003; Wintzer et al. 2011) and Indian Ocean (Mao and Jinbiao 1990; Santhakumari et al. 1999; Quasim 2003). *B. viginica* is a non-selective zooplankton predator (Wintzer et al. 2013) and place high predation pressure on planktonic crustaceans and fish eggs (Mills and Sommer 1995; Chícharo et al. 2009). It is a threat to the native species, and some authors described its effect also on commercial fish populations (Chícharo et al. 2009; Wintzer et al. 2013), with economic consequent.

Clytia hummelincki (Leloup 1935) is a recent invader in the Mediterranean Sea, first recorded in 1996 on the coast of Calabria (Ionian Sea, Italy) (Boero et al. 1997). There are records from the Balearic Islands to South Adriatic Sea (Gravili et al. 2008), however, this species was not collected in the Strait of Gibraltar or the Alboran Sea (the Atlantic entrance to the Mediterranean Sea) (Gravili et al. 2008; González-Duarte et al. 2013a, b, 2016), or the Levant Sea, (the first entrance for a Lessepsian immigrant) (Gravili et al. 2008; Morri et al. 2009). Thus, this species most probably arrived by maritime traffic, as suggested by several authors (Boero 2002; Gravili et al. 2013).

Clytia hummelincki is a Caribbean species described by Leloup (1935) in Bonaire Island, the Netherlands Antilles. There are several later records of this species in the Caribbean Sea (Vervoort 1967; Calder 1993, 1998; Galea 2008; Calder and Cairns 2009). However, its known distributional range has been expanded to the western and eastern Atlantic: Brazil (Migotto 1996; Kelmo and Attrill 2003), Ghana (Buchanan 1975), South Africa (Millard 1975); Indo-Pacific Ocean: Galapagos Islands (Calder et al. 2003), Papua New Guinea (Boero and Bouillon 1986, unpublished data see Gravili et al. 2008), Indonesia (Di Camillo et al. 2008) and Pakistan (Moazzam and Moazzam 2006); and Mediterranean Sea (See Gravili et al. 2008 for a revision of the Mediterranean Records). *C. hummelincki* can be considered as a cosmopolitan species, recorded in both natural and artificial substrates in a wide range of habitats (Cornelius 1982).

The benthic phase of this species is stolonal and produces a high number of relatively large medusa (Bouillon et al. 2004) which are able to live for several days (Gravili et al. 2008) and can have an important impact by predating on eggs and larvae (Bouillon et al. 2004).

Cordylophora caspia (Pallas 1771) is an athecate hydroid species (Family Cordylophoridae; previously included in Oceaniidae or in Clavidae) (Schuchert 2004). The type locality of this species is the Caspian Sea, however, the original description of *Tubularia caspia* (Pallas 1771) is short and imprecise. Therefore, this species has been re-described at least six times in different parts of the world: Indo-Pacific Ocean (New Zealand and Australia), Atlantic European Coast (Germany and Ireland) and Atlantic American coast (Carlton 2009). Several authors considered *C. capia* today as a "cryptic complex species" (Folino-Rorem et al. 2009).

Cordylophora caspia occurs both in freshwater and brackish habitats (like coastal lagoons and estuaries) in temperate and sub-tropical zones globally (Folino 1999; Folino-Rorem et al. 2009), although there are also records of its existence in the North Sea (Buschbaum et al. 2012) or the fjord region of southern coasts of Chile (Galea 2007). This species tolerates a wide range of temperatures and salinities (Smith et al. 2002; Folino-Rorem and Indelicato 2005) and can survive for long periods without food (Schuchert 2004). *C. caspia* is able to develop spheres of coenosarc in the perisarc as resistance structure during unfavourable conditions and regenerate itself when the favourable conditions return (Roos 1979). Furthermore, it is a typical fouling-species on artificial habitats like harbours, marinas, pilings, etc. (Morri and Boero 1986; Mineur et al. 2012; Karlson and Osman 2012). This species has been translocated by shipping into estuaries at global level (Carlton and Hodder 1995; Folino-Rorem and Indelicato 2005; Carlton 2009). *C. caspia* can also invade rivers and fresh water habitats (CIESM 2002; Gravili et al. 2010). Several authors also pointed out the negative economic effects for industry (this species grows in the pipes of industrial installations) and boating (common component of the fouling on boat hulls) caused by this species (Leppäkoski 2002).

Eudendrium carneum Clarke, 1882 is a well-known alien species around the world (Bavestrello and Piraino 1991; Boero and Bouillon 1993; Oliveira et al. 2000; Occhipinti-Ambrogi et al. 2011), and a "potential pest" in several seas (Marino

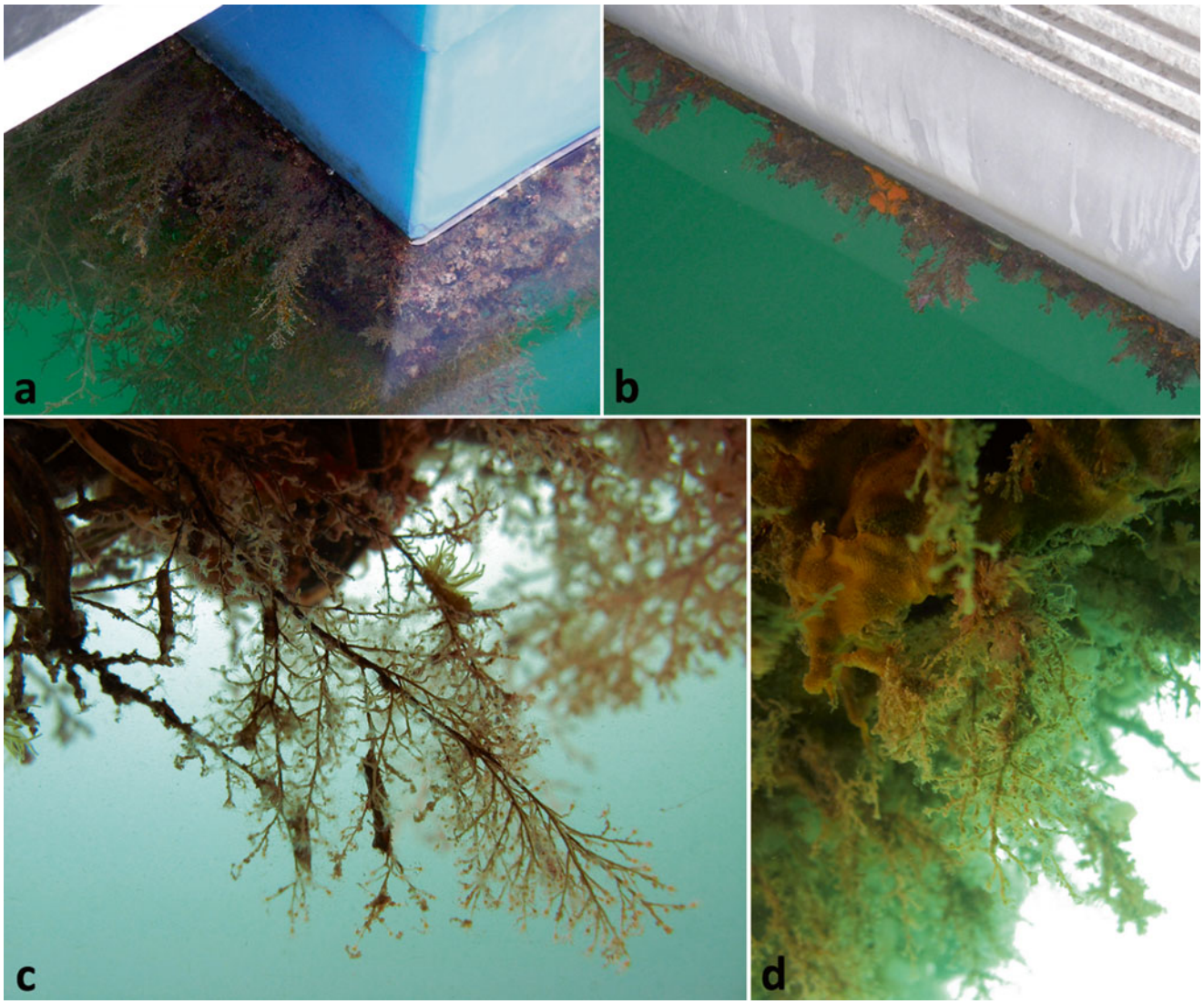

Fig. 10.1 Colonies of the hydroid *Eudendrium carneum* on floating pontoons within recreational marinas in Cádiz (Southern Iberian Peninsula). (**a**, **b**) General view, (**c**, **d**) details of the colonies

et al. 2007). *E. carneum* is a conspicuous species with large polysiphonic colonies, up to 105 mm high. A main characteristic of this species is its abundance in harbours and marinas (Fig. 10.1) (Schuchert 2008; Megina et al. 2013, 2016) and it is one of the most frequently recorded hydroid species in fouling communities (Millard 1959; Karlson and Osman 2012). The type locality of this species was Virginia (USA, Atlantic Ocean). Today, it is a cosmopolitan species (Marques et al. 2000; Schuchert 2008) listed as invasive in several seas like the Mediterranean (Zenetos et al. 2010; Occhipinti-Ambrogi et al. 2011). This species is present in all oceans and seas around the world: [e.g. Australia (Huisman et al. 2008; Sliwa et al. 2009), South Africa (Mead et al. 2011), Florida, USA (Karlson and Osman 2012), Palau (Marino et al. 2007), Caribbean Sea (Calder and Kirkendale 2005), Brazil (Marques et al. 2013), California, USA, Ecuador and Mexico (Fraser 1948)]. Its global distribution and high abundance in harbours and marinas make it a potential invader worldwide.

Garveia franciscana (Torrey 1902) is another Pseudoindigenous species. Although some authors considered the origin of this species in the Indian Ocean (Carlton 2009), the type locality of *G. franciscana* is unclear and it has been described in diverse zones as different species: San Francisco Bay (Torrey 1902), Eastern coast of USA (Fraser 1943), European waters (Kinne 1956), Western coast of Africa (Billard 1927), etc. Vervoort (1964) considers *G. franciscana* as a marine species that has colonised brackish habitats, unlike *Cordylophora caspia*, which would be a fresh water species with tolerance to high salinities. At any rate, *G. franciscana* is a common species in fouling communities in artificial habitat (Morri and Boero 1986; Neves et al. 2007; Mineur et al. 2012) and it can tolerate high levels of aquatic pollution, like high copper concentrations (Crooks et al. 2011). These opportunistic characters have favoured its dispersion by ship-hulls and ballast waters in brackish habitats around the world (Fofonoff et al. 2009). Nowadays, this

species is considered as an alien species in San Francisco Bay (Ruiz et al. 2000; Carlton 2009), North Eastern and Western Atlantic (Streftaris et al. 2005; Karlson and Osman 2012) and Mediterranean (Boero 2002; Zenetos et al. 2010), where it is identified as invasive (Gravili et al. 2013). There are also records of this species in Western Coast of Africa (Billard 1927; Buchanan 1956) and Eastern and Western coasts of India (Leloup 1932).

Macrorhynchia philippina (Kirchenpauer 1872) is a distinctive species of large polysiphonic colonies (up to 30 cm) of the family Aglaopheniidae (Calder 1997; Bouillon et al. 2004; Morri et al. 2009). This species has been known in the eastern Mediterranean since the 1990s (Bitar and Bitar-Kouli 1995; Morri et al. 2009). *M. philippina* is now one of the alien hydroid species most widely spread in the Levant Sea (Çinar et al. 2006; Morri et al. 2009), having arrived on the Turkish coasts (Çinar et al. 2006). Zenetos et al. (2010) pointed out its high invasive potential and, indeed, this species has widely spread in the eastern Mediterranean Sea, on coastal habitats from the surface to 40 m in depth and is recurrently fertile (Morri et al. 2009).

The records for the Gulf of Aden (the connection between Indian Pacific and Red Sea), the Red Sea and Suez Canal (see Gravilli et al. 2013) indicate that it is a Lessepsian invader which arrived in the Mediterranean through the Suez Canal (CIESM 2002). The type locality of *Macrorhynchia phylippina* is Manile (Philippines), in the western Pacific Ocean (Kirchenpauer 1872), and it is now a circumglobal species in all tropical and sub-tropical oceans in continental coasts (Migotto 1996; Calder 1997; Watson 2002; Bouillon et al. 2004) and often oceanic islands (Guam, Fiji, Hawaii, etc.) (Kirkendale and Calder 2003). There are also some records for temperate zones in the eastern Atlantic Ocean (Maderia and Azores) (Wirtz 2007; Moura et al. 2012). *M. phylippina* is a stinging species and could be harmful to human health due to its large nematocyst (100 μm in length) (Marques et al. 2002). The increase of the density and abundance of this species could reduce tourist activities (Çinar et al. 2006), and it could potentially have a negative impact on local economies.

10.3 Anthozoans as Alien and Invasive Species

The Class Anthozoa Ehrenberg, 1834 are around 6300 spp., although some estimations increase to ca. 10,000 the total species number in this group (Appeltans et al. 2012). They are present in all oceans in a wide range of bathymetric levels and environmental variables, from intertidal zones to >6100 m depth (Williams 2011), from hydrothermal to deep-sea, tropical reefs, soft and rocky bottoms. Anthozoans are often considered passive suspension feeders, but their prey ranges from macromolecules to fishes (Fautin and Mariscal 1991, among others). Some of them are associated with endosymbiont unicellular algae as an additional source of nutrients (Furla et al. 2005). From an ecological point of view, anthozoans are an essential link in the transference of matter and energy from the pelagic to the benthic subsystems (Gili and Coma 1998).

Octocoral anthozoans are characterised as colonial forms (a few solitary species exist, see Bayer and Muzik 1976) with a high structural variety, from stolonate to massive, branching, whip-like or feather-like colony forms (Bayer et al. 1983; Fabricius and Alderslade 2001). Hexacoral anthozoan includes solitary and colonial forms, structurally diverse in shape, from the solitary sea anemones to colonial whip- or bushy-like antipatharians, and massive or tree-like branched scleractinians. Anthozoans are probably the main marine bioconstructors: they are a very important structural component of marine benthic communities. Although usually recognised as contributing to the tropical reefs diversity (Roberts et al. 2002; among many others), they are also present in moderate deep sea bottoms as cold coral reef, as well as associated with soft deep sea bottoms, thus increasing local and regional diversity (Mortensen et al. 1995; Idjadi and Edmunds 2006). Anthozoans reproduce in sexual and asexual varieties (Bocharova and Kozevich 2011; Kahng et al. 2011, among others), and can be present in the environments as minute, sometimes ephemeral cloned individuals, or large, permanent and long-live components of the benthic ecosystem landscape (Jackson and Coates 1986; Andrews et al. 2002; Betti et al. 2011).

When arrival occurred in the past it was not always easy to distinguish what is alien and what is not. Some biological interactions such as aggressive behaviours can be of help, especially when they are accompanied by other biological capabilities such as a wide range of prey captures, salinity and thermal tolerances, sexual and asexual reproduction (fragmentation, basal laceration, transversal or longitudinal division, polyp expulsion) (e.g. Kramarsky-Winter et al. 1997; Fautin 2002; Kružić 2007), photosynthetic symbionts, among others (see Kahng et al. 2008; Lira et al. 2009; among many others). The introduction of an alien invasive species can produce important changes in the indigenous fauna, not only in the composition but also in the general structure and functioning of the communities (Galil 2000; Bax et al. 2003; Bianchi 2007), especially in spatially restricted environments such as islands or reduced communicated seas (Zibrowius 1992; Galil and Zenetos 2002; Davis 2003; Lejeusne et al. 2010). Furthermore, the impact of invasive species can alter the evolutionary pathway of native species

by competitive exclusion, niche displacement, hybridisation, introgression, predation and, ultimately, extinction (Mooney and Cleland 2001).

Another important question concerns the geographic scale we use for detecting alien species and their importance for management of native fauna reservoirs. Local and regional scientific policies, as well as faunistic originality of some areas are clearly affected by the geographic scale used. For the redaction of environmental policies and conservation strategies, the record of *Astroides calycularis* in the Northern Adriatic Sea (endemic from the South-Western Mediterranean) (Kružić et al. 2002) and, on the other hand, the Lessepsian introduction of the gorgonian *Melithaea erytraea* native of the Red Sea (Fine et al. 2005 as *Acabaria erytraea*), should be considered as different cases. The first case seems a natural enlargement of distribution of an already geographically restricted species, as a result of recent warming of sea water, while the second, is the unintentional but clearly human facilitated introduction of a new gorgonian family in the Mediterranean Sea.

As in the case of *Astroides calycularis* recently detected in the Adriatic Sea, the existence of previous faunistic inventories as well as historical collections in this area (Hoeksema et al. 2011; Rocha et al. 2014) facilitates recognition of the first detections of this species. Sometimes, the original localities where a species is described as new to Science do not necessarily correspond to the true centre of diversity and distribution of a species. Among many other possible examples, the scleractinian coral *Dendrophyllia laboreli* Zibrowius and Brito, 1986 was reported as new for European waters (López-González et al. 2010) as it was not previously collected during extensive sampling surveys from the late 1980s to the mid 1990s, along both sides of the Strait of Gibraltar in the same localities where it was recently found. The reduction of colour variability (exclusively orange-yellow) of this species in the Canary Islands and those localities northward suggests that the origin of this species is around the Gulf of Guinea, although a molecular approach is desirable to support this hypothesis. *D. laboreli* is apparently expanding its northern distributional limits. The consequence of including Canary Islands specimens in the original description of this species and further surveys was the inclusion of *D. laboreli* in the regional red list (as "species of interest for Canarian marine ecosystems"). A thousand kilometres to the north, in the Gulf of Cádiz in continental Europe, *D. laboreli* is under study as a recent Southern immigrant, awaiting more biological information about its reproduction and interactions with native fauna (López-González et al. 2008). A number of additional records of this and other cnidarian species are still probably hidden in grey literature as well as submarine or scuba divers' guides.

Aspects whose importance has not always been correctly valued (or has simply been neglected) in the last decades are those related to the current taxonomic knowledge of the alien species reported to date (in all its varieties), as well as (and not less important) the certitude of the taxonomic identifications provided. An example of insufficient taxonomic knowledge of an alien anthozoan species can be illustrated by the octocoral *Carijoa*. This octocoral species has been largely considered an alien invasive species in Pacific areas after colonies were transported as fouling of commercial ships from Caribbean localities (Grigg 2004; Reaser et al. 2007). Recent molecular studies (Concepcion et al. 2010) showed that *Carijoa* is native to the Indo-Pacific. All published reports of geographically wide ranging invasions of *Carijoa* throughout the Pacific are unfounded because the higher molecular diversity throughout the Pacific relative to the Caribbean-Atlantic indicates a longer evolutionary presence of *Carijoa* in the Pacific. These data clearly refute a Caribbean origin for *Carijoa* in the Hawaiian Archipelago.

10.3.1 Some Examples of Alien and Invasive Anthozoans

In the following paragraphs we compile comments and selected references to the most common anthozoan species considered as alien and invasive species, at different geographical scales and introduction pathways. They are grouped by Order taxonomic groups.

Alcyonacea Octocorallia At least three species have been discussed as alien species: *Melithaea erythraea* (Ehrenberg, 1834), *Carijoa riseii* (Duchassaing and Michelotti 1860), and a xeniid soft-coral species genetically close to *Xenia mebranacea* Schenk, 1896. *M. erythraea* (often as *Acabaria erythraea* in the literature) is a scleraxonian gorgonian from the Red Sea recently reported for the eastern Mediterranean waters (Fine et al. 2005) as a result of Lessepsian immigration. *C. riseii* is a clavulariid octocoral species apparently widespread in tropical Indo-Pacific and Atlantic areas. However, after the findings of Conception et al. (2010), the taxonomic status of *Carijjoa* species should be revised on a comparative molecular and morphological study. The supposition of a native Western Atlantic *C. rissei* invading Indo-Pacific areas could completely change in the light of new evidence from modern taxonomy. If it is a single variable species or a species complex, it is true that the aggressive behaviour of this species overgrowing on native benthic communities has an important ecological and economical impact (Kahng and Grigg 2005; Kahng 2006; Sánchez and Ballesteros 2014). Biocontrol experiments using specialised natural predators from a different population did not provide successful results (Wagner et al. 2009). The records of a xeniid species in Venezuelan Caribbean waters (a soft-coral family from Indo-Pacific coral reefs) are highly worrying as

it is supposed to have been intentionally introduced for propagation and farming purposes by commercial aquarists (Ruiz Allais et al. 2014). On a different geographical scale within the Pacific, monitoring studies of faunistic displacements of *Carijoa* and *Sarcothellia* species will be of special interest (O'Connor et al. 2008) for the elaboration of further management policies on islands like Hawaii.

Actiniaria Hexacorallia At least 12 sea anemone species have been reported as alien. The tolerance to different thermal and salinity conditions in combination with efficient asexual reproductive modes, and, on occasions, association with unicellular algal symbionts, seems to be responsible for the success of sea anemones in the colonisation of new areas. Fouling on ship hulls or with aquiculture species are apparently the main ways of dispersion. A number of families and genera can be briefly commented on here, as the literature and reports for this group are relatively abundant to be treated in this chapter. The family Diadumenidae (including the previous Haliplanellidae) includes four species now in the genus *Diadumene* [*D. cincta* Stephenson, 1920; *D. franciscana* Hand, 1956; *D. leucolena* (Verrill, 1866); and *D. lineata* (Verrill, 1869)] and one species in the genus *Aiptasiomorpha* [*A. minima* (Sthephenson, 1978)] reported as alien species. All four *Diadumene* species have been reported by Lee and Reusser (2012) as alien species for Hawaii North Pacific, although the native localities of some are not known for certain. The orange-striped green anemone (*D. lineata* = *Haliplanella luciae* Verrill, 1898) reported as native to the coast of Japan has been registered worldwide (see Molina et al. 2009, among others). *D. cincta* and *D. leucolena* supposedly native to North-Western Atlantic, have also been reported for European waters (Preda et al. 2012; among others). Some of these species can probably be considered cryptogenic. Cohen et al. (2005) reported as exotic four *Diadumene* species for San Francisco Bay, including *D. franciscana*, whose origin is suggested to be western or southwestern Pacific Ocean, Indian Ocean, or Australasia (Carlton and Eldredge 2009). *Aiptasiomorpha minima* was reported for Japan and as native to South Pacific (New Zealand) (see Mito and Uesugi 2004). Previous records of *Aiptasia* spp. as alien species (see Gruet et al. 1976 as *A. pulchella* Carlgren, 1943; and Mito and Uesugi 2004 as *A.* cf. *insignis*, among others) can probably be attributed to the recently re-described *A. pallida* (Agassiz in Verrill, 1864), considered as a new genus: *Exaiptasia* by Grajales and Rodríguez (2013). Additional molecular evidence supports a single, widely distributed species (Grajales 2014). This sea anemone is also proposed as a species for experimental research in laboratory (Howe et al. 2012 as *A. pulchella*), as well as a known plague in public and private tropical aquariums (often as *Aiptasia* sp.).

Although the edwardsiid sea anemone *Nematostella vectensis* Stephenson 1935 was originally described for North Europe, it is distributed on both sides of the North Atlantic, on Western American coasts, and recently reported for Brazilian coasts (Silva et al. 2010). However, Hand and Uhlinger (1995) only found both sexes in some populations of the Atlantic and Pacific North America; those populations from England are composed of only female individuals, suggesting that this is the result of asexual reproduction. More recent evidence on genetic diversity of UK populations suggests that this species is native to the east coast of North America (Pearson et al. 2002). Paradoxically, although this sea anemone is considered rare and threatened in the UK (destruction of lagoonal habitat, see Wells et al. 1983; Manuel 1988), its European populations are apparently the result of an old introduction from very few individuals from the United States. Thus, *N. vectensis* could be finally considered an old alien species of North Eastern Atlantic, which grows fast and prolifically and its identification as an invasive or non invasive species should be made with caution as it is extremely tolerant (temperature −1 to 28 °C, salinity 2–52 p.p.t, see Stefanik et al. 2013), perhaps currently retained by its preferential habitat in lagoonal environments. The relatively easy sexual and asexual reproduction of this species under laboratory conditions resulted in the use of this sea anemone as a cnidarian model for evolutionary research (see Hand and Uhlinger 1992; Martindale et al. 2004; Darling et al. 2005; Marlow et al. 2009; Fritz et al. 2013, among many others).

Other species belonging to the families Sagartiidae [*Sagartia ornata* (Holdsworth, 1855) (misspelled as *S. ornate*), *Cereus pedunculatus* (Pennant, 1777)], Actiniidae (as *Bunodeopsis* sp.), or Andvakiidae [*Synandwakia hozawai* (Uchida, 1932)] have also been reported as alien species at different geographical scales (Gruet et al. 1976; Engle and Richards 2001; Haupt et al. 2010; Lee and Reusser 2012, among others).

Scleractinia Hexacorallia At least two main areas of introduction and seven scleractinian coral species have been reported as alien species. The first case includes species now present in the Mediterranean Sea, but originally reported (or considered) as from other water masses. This is the case of *Oulastrea crispata* (Lamarck, 1816), a zooxanthellate scleractinian found in shallow waters of Corsica and native of central Indo-Pacific (see Hoeksema and Ocaña 2014). For the other largely considered alien scleractinian in the Mediterranean Sea, *Oculina patagonica* de Angelis, 1908, there is an important body of studies dating back to 1974, describing its spread throughout the Mediterranean (see Zibrowius 1974; Fine et al. 2001; Serrano et al. 2013, among others), and according to its behaviour it is considered the

only invasive scleractinian species in this sea (Zenetos et al. 2010). Other scleractinian species, like *Dendrophyllia laboreli*, are at the threshold of the Alboran Sea, and stable populations have already been reported for the Algeciras Bay, Spain (López-González et al. 2010), and Cabo Negro, Morocco (Ocaña et al. 2011), both localities on the Mediterranean side of the Strait of Gibraltar. A second affected area includes the introduction of Indo-Pacific species in Western Atlantic. This is the case of the non-zooxanthellate species *Tubastraea coccinea* Lesson, 1829 and *Tubastraea micranthus* (Ehrenberg, 1834), being this last the single, most abundant coral on artificial substrata in the Gulf of Mexico (see additional details of these two species in Sammarco et al. 2004, 2010). The presence of the introduced Indo-Pacific coral *Lobactis scutaria* (Lamarck, 1810) in the Atlantic (Discovery Bay, Jamaica) clearly demonstrates the remarkable resilience of a coral species despite negative environmental events (hurricanes and bleaching by thermal stress), and collapsing native coral populations. It is even able to retain its original symbiotic algal lineage by occupying a niche poorly exploited by native Atlantic coral species (as *Fungia scutaria* in LaJeunesse et al. 2005). Other scleractinian corals, such as *Culicia rachelfitzhardingeae* Cairn, 2006, evidently involved in translocations in the Indo-Pacific areas, will be surely reported in the next decades (Lee and Reusser 2012), as well as those translocations in the Mediterranean Sea (Kružić et al. 2002; Pećarević et al. 2013).

10.4 Jellyfish and Comb Jellies as Alien and Invasive Species

Intentional and unintentional transport of marine species goes back millennia, yet there was little recognition of the phenomenon until the expansion of maritime trade in the nineteenth century and the much-publicised plans of the Saint-Simonians for a canal at the Isthmus of Suez. Even before the Suez Canal was fully excavated, the French zoologist Vaillant (1865) argued that the breaching of the isthmus would bring about migration of species and mixing of faunas. It fell to Keller (1888) to note the incursion of the upside-down jellyfish *Cassiopea andromeda* (Forsskål, 1775) from the Red Sea into the canal itself and the adjoining lagoons – an augury of the Erythraean invasion to come. A single sentence testifies to the first ever invasion of an alien scyphozoan jellyfish: "Ich habe an den Riffen der Küste von Cypren *Cassiopeia andromeda* selbst gesammelt" (Maas 1903: 42). Half a century passed before the species was recorded for the Mediterranean again: Schäfer (1955) reported the occurrence of specimens on Neokameni, a small volcanic island near Thira, in the southern Aegean Sea, where the medusae flourished in rocky pools with water temperatures reaching 36 °C due to volcanic activity. Presciently Schäfer concluded "Sicherlich aber sind sie im Mittelmeer seltene und doch neue Gaste, denn noch keine 100 jahre besteht zwischen Indischen Ozean und Mittelmeer durch den Suezkanal eine dauernde und breite Verbindung, die sie fur ihre Ausbreitung im Mittelmeer haben benutzen können". Yet, although records of alien jellyfish kept appearing in the scientific literature, their number and impact were considered negligible and they failed to elicit scientific or public interest. As long as their impacts were inconspicuous, induced no direct economic cost or impinged on human welfare, jellyfish and comb jellies have been ignored by scientists, conservationists, policy makers and managers. However, a sequence of catastrophic events in the Black Sea beginning in the early 1980s has changed that. The rapid spread and conspicuous impacts of a pair of invasive comb jellies, *Mnemiopsis leidyi* A. Agassiz, 1865 and *Beroe ovata* sensu Mayer, 1912 (Pereladov 1988; Kideys 1994; Shiganova et al. 2004), helped raise awareness of the impacts of alien jellyfish. It is now widely recognised that jellyfish and comb jellies are invaders of global concern, with some invasive populations so abundant as to endanger the environmental and economic viability of their recipient ecosystem.

A dearth of information on jellyfish and comb jellies due to high temporal and spatial population variability, poor sampling and preservation methodologies, limited search effort and erosion of taxonomic expertise, hampered efforts to assess the historical timeline of the expansion of their populations and to understand invasion patterns and processes. The magnitude of the gap is difficult to assess as data are rarely gathered through region-wide standardised surveys specifically targeting jellyfish and comb jellies. Data are presumably most accurate for large and conspicuous species, but increasingly used molecular tools have revealed cryptic species and erroneous identifications (Holland et al. 2004; Dawson et al. 2005).

Jellyfish and comb jellies are considered synanthropic, benefiting from human actions, i.e. overfishing, eutrophication, habitat modification, aquaculture, global warming and human-mediated dispersal (Purcell et al. 2007; Purcell 2012; Brotz et al. 2012; Boero 2013). In this chapter we identify which populations of marine alien jellyfish and comb jellies have been recently on the rise and where.

10.4.1 Spread of Invasive Alien Jellyfish and Comb Jellies

Recent large-scale analyses have purported to show that pullulations of jellyfish and comb jellies populations are linked to large-scale inter-annual climatic regime shifts (Purcell 2005; Brotz et al. 2012; Condon et al. 2012, 2013). However,

sparsity of long-term replicate series of population-level data of jellyfish and comb jellies precludes a categorical statement that their populations are increasing (Purcell 2005; Dawson et al. 2015). The extant data on alien jellyfish and comb jellies indicate a rapid spread of certain populations over extensive areas in a remarkably short time. These spatial and temporal differences between sites are important determinants of population dynamics. Graham and Bayha (2007) listed only four well known invasive alien scyphozoans (*Phyllorhiza punctata, Cassiopea andromeda, Rhopilema nomadica, Aurelia* spp.), in addition to the pair of comb jellies *M. leidyi* and *B. ovata*; later adding *Mastigias* spp. (Bayha and Graham 2014). Purcell's (2007: 166) listing of *Sanderia malayensis* among "Species…known to have been inadvertently introduced to non-native habitats" is clearly erroneous (identified as *Cyanea* sp., Dong et al. 2010). Yet, the past decade alone saw two scyphozoan jellyfish, new to science (*Marivagia stellata* Galil and Gershwin, 2010, *Pelagia benovici* Piraino et al. 2014), described as alien introductions, and a momentous increase in the secondary spread of invasive alien jellyfish and comb jellies.

10.4.2 Newly Described Alien Jellyfish

The earliest record of *Marivagia stellata* in the Mediterranean Sea concerned a single specimen collected in January 2006 on the southern rim of Haifa Bay, Israel. Another specimen was photographed in June 2008, and in the summer of 2010 additional specimens were collected and photographed in several locations along the Israeli coast. In October 2010, a swarm of *M. stellata* was reported and photographed off the southern coast of Lebanon (The Daily Star – Lebanon News – Odd marine changes strike coast of Sidon, 7 October 2010). Galil et al. (2010) argued that *M. stellata* was introduced to the Mediterranean through the Suez Canal, as it is highly unlikely that a large native littoral species, markedly different from known Mediterranean scyphozoans, would escape attention until the twenty first century. Yet, the question of its origin remained unanswered at the time. Specimens of *M. stellata* recorded in 2010 and 2013 off the mouth of the River Indus, Pakistan, and Vizhinjam, Kerala, India, respectively, establish the species' origin in the Arabian Sea (Galil et al. 2013; Gull et al. 2014).

A prolonged bloom of a previously unknown semaestome jellyfish near the Po River Delta and the Gulf of Trieste, North Adriatic Sea, lasting from September 2013 to March 2014, attracted the attention of scientists, who identified it as a species new to science: *Pelagia benovici* (Piraino et al. 2014). The discovery of sexually mature specimens at sites hundreds of kilometres apart suggests the existence of an already established population, and the bloom that alerted scientists to the species' presence may be an augury of its outbreak potential. The storied long-term gelatinous zooplankton studies in the North Adriatic, and in particular, the attention paid to jellyfish blooms in recent decades, suggest that *P. benovici* is a recent introduction. The Gulf of Venice and the North Adriatic have been inundated by shipping and mariculture mediated alien species (Occhipinti-Ambrogi et al. 2011; Galil 2012), and it is likely *P. benovici* was transported in ballast waters from as yet unknown native region.

While unusual, the fact that *Marivagia stellata* and *Pelagia benovici* were first described in their introduced rather than native region can be ascribed to serendipity, availability of taxonomical expertise and unequal research efforts. Indeed, they follow a now familiar pattern: when swarms of a previously unknown rhizostomid jellyfish appeared off the Israeli coast in the 1980s, it was compared with Red Sea rhizostomids (Stiasny 1938, 1939), since the Suez Canal is the principal pathway of Levantine invasive aliens, and subsequently described as a species new to science: *Rhopilema nomadica* (Galil et al. 1990).

10.4.3 Recent Secondary Spread of Invasive Alien Jellyfish

Phyllorhiza punctata Lendenfeld, 1884 is indigenous to the tropical western Pacific (Graham et al. 2003), and was not recorded outside the Indo-Pacific Ocean until the mid twentieth century. In the 1950s a large but short-lived population was described off southern Brazil (Mianzan and Cornelius 1999; Migotto et al. 2002; Haddad and Nogueira 2006), reappearing off Bahia in 1991 (Silveira and Cornelius 2000) and spreading northwards to Ceará and southwards to Santa Catarina (Haddad and Nogueira 2006). In the Gulf of Mexico it was first recorded in 1993, and a massive population explosion occurred in the summer of 2000 off Alabama, Mississippi, and Louisiana, spreading in the following summer to the Indian River Lagoon, Florida (Graham et al. 2003). Small numbers were recorded along the Georgia coast in 2007 and 2008 (Verity et al. 2011), but no irruption has been detected since. The first record in the Mediterranean Sea dates back to 1965, collected off Israel; additional specimens were collected in 2005, 2006, 2009, and the presence of sexually mature specimens throughout the year indicates the presence of an established population (Galil et al. 1990, 2009). In 2005 and 2006, ephyrae and medusae were collected off Lefkada Island, on the Ionian coast of Greece, but the population has evidently existed there for a number of years (Abed-Navandi and Kikinger 2007). In the past 5 years a sudden spate of records attest to the growing presence of the species in the Mediterranean: in Syria (Durghan 2011), the Mediterranean and Aegean coasts of Turkey (Çevik et al. 2011; Gülşahin and Tarkan 2012), Tunisia, where 1428 individuals were recorded along a 4 km transect in Bizerte

Lagoon (Gueroun et al. 2015), Sardinia (Boero et al. 2009) and the Ebro Delta, Spain (http://www.elperiodico.com/es/noticias/sociedad/llegada-masiva-una-segunda-medusa-invasora-delta-del-ebro-2185742).

Cassiopea andromeda (Forsskål, 1775) is a semi-sessile rhizostomid jellyfish introduced from the Red Sea to the Mediterranean through the Suez Canal over a century ago (see before). It is well established in the Levantine and Aegean Seas, but was hitherto unknown west of the south-central Aegean (Galil et al. 1990; Çevik et al. 2006; Özgür and Öztürk 2008). However, recent records evince local outbreaks as well as spread to the central Mediterranean. In July 2011, a rapid assessment survey in the Kyklades, south Aegean Sea, uncovered a dense population on Paros Island, attaining in places densities over 20 individuals/m^2 (Katsanevakis 2011). In 2009, an aggregation of approximately 50 individuals was observed at the mouth of Marsamxett Harbour, Malta (Schembri et al. 2010). Whether these individuals were introduced from the Red Sea in shipping, spread with current from Levantine populations, or from elsewhere is yet to be determined by molecular tools (Holland et al. 2004).

Rhopilema nomadica Galil 1990, first recorded in the Mediterranean in the early 1970s, is notorious for the swarms it has formed each summer since the early 1980s along the Levant coast (Galil et al. 1990; Kideys and Gücü 1995). The swarms adversely affect tourism: a socio-economic survey, carried out in Israel in 2013, estimated that swarming reduces the number of seaside visits by 3–10.5 %, with an annual monetary loss of €1.8–6.2 million (Ghermandi et al. 2015). The annual swarming results each year in envenomation victims suffering adverse effects that may last weeks and even months after the event (Benmeir et al. 1990; Silfen et al. 2003; Yoffe and Baruchin 2004; Sendovski et al. 2005; Öztürk and İşinibilir 2010). Coastal trawling and purse-seine fishing are disrupted for the duration of the swarming due to net clogging and inability to sort yield. Jellyfish-blocked water intake pipes pose a threat to desalination plants and seawater cooling systems of coastal power plants: in the summer of 2011, Israel Electric removed tonnes of jellyfish from its seawater intake pipes at its largest power plants (www.bbc.co.uk/news/world-middle-east-14038729). In 2004, two individuals were sighted off the Maltese Islands. Though the species has yet to establish a reproducing population there, this record forecasted its spread to the central Mediterranean (Deidun et al. 2011). Indeed, in 2008, *R. nomadica* was recorded in the Gulf of Gabes, Tunisia. Since 2010, it occurs regularly in the summer months in the Gulf of Tunis, where, in 2013, densities as high as 10–100 individuals/1000 m^3 were observed, and the beach was strewn with stranded specimens (1 individual/m^2) (Daly Yahia et al. 2013).

10.4.4 Secondary Spread of Invasive Alien Comb Jellies

Mnemiopsis leidyi A. Agassiz, 1865 is one of the best studied gelatinous species due to its invasion success and harmful consequences (reviewed in Kideys 2002; Costello et al. 2012). Indigenous to western Atlantic coastal waters, from New England to Argentina, *M. leidyi* has spread in the past three decades to the Black, Caspian, Mediterranean, Baltic and North seas (Mianzan 1999; Shiganova et al. 2001a; Javidpour et al. 2006; Faasse and Bayha 2006). Genetic analysis confirmed the origin of the populations in the Black and Caspian Seas in the vicinity of the Gulf of Mexico, whereas the North and Baltic Seas populations were traced to New England (Ghabooli et al. 2011, 2013; Reusch et al. 2010).

Mnemiopsis leidyi was first observed in the Mediterranean Sea in 1990, in the Gulfs of Saronikos and Elefsis, western Aegean Sea (Shiganova et al. 2001b). In 1992, it appeared in Mersin, on the Mediterranean coast of Turkey, and in 1993 in Kusadasi, on the Aegean coast of Turkey (Uysal and Mutlu 1993; Kideys and Niermann 1994), and off the Syrian coast (Shiganova 1997). As all locales were in the vicinity of ports, and no population persisted for long, these were considered ballast mediated introductions (Shiganova et al. 2001a). Between 1991 and 1996, and again in 1998, *M. leidyi* swarms were observed in the summer months in the northern Aegean Sea; these were considered to have been swept with the outflow of the Black Sea water masses (Shiganova et al. 2001a, 2004). It was observed in the Berre Lagoon and other coastal lagoons along the Gulf of Lion, and in the Bay of Piran, in the northern Adriatic Sea (Shiganova and Malej 2009). Suddenly and concurrently, large swarms appeared along the Ligurian, Tyrrhenian and Ionian shores of Italy, the Mediterranean coast of Spain, including the Balearic Islands, and the SE Levant, in 2009, where it has been intermittently observed on the coast ever since (Boero et al. 2009; Fuentes et al. 2009; Galil et al. 2009, 2011; Marambio et al. 2013). Genetic analysis confirmed the Mediterranean populations result from a secondary introduction originating in the Black Sea (Bolte et al. 2013).

Recent near-simultaneous records considered *Mnemiopsis leidyi* in Norwegian fjords (Oliveira 2007; Hosia et al. 2011), the Baltic Sea (Hansson 2006; Javidpour et al. 2006; Kube et al. 2007), Danish territorial waters (Tendal et al. 2007), the German Bight (Boersma et al. 2007), the Netherlands (Faasse and Bayha 2006), Belgium (Dumoulin 2007; Van Ginderdeuren et al. 2012) and France (earliest record 2005 *fide* Antajan et al. 2014), to have resulted from multiple ballast-mediated introductions to European harbours, followed by secondary spread by advection (Antajan et al. 2014).

Beroe ovata Bruguière, 1789, a specialised predator of *Mnemiopsis leidyi*, is indigenous to western Atlantic coastal waters, from the USA to Argentina (Mayer 1912; Mianzan 1999). In 1997, it was first sighted in the Black Sea (Konsulov and Kamburska 1998; Seravin et al. 2002). Observations conducted over 25 years in the Black Sea confirm the predator-prey interactions of *B. ovata* and *M. leidyi*: their observed annual succession of peaks (maximum annual values) suggested that the maximum annual abundance of *B. ovata* is related to that of its prey (Shiganova et al. 2014).

In the Mediterranean *Beroe ovata* was first observed in 2004, in Evvoikos Gulf, Greece (Shiganova et al. 2007), followed in 2005, by sighting in the Bay of Piran, northern Adriatic Sea (Shiganova and Malej 2009). In both instances it was found together with its prey. The occurrence of *B. ovata* in the Evvoikos Gulf was attributed to the outflow of the Black Sea water masses (Shiganova et al. 2007), though its presence in the nearly landlocked Gulf, but not in the localities in the northern Aegean where masses of *Mnemiopsis leidyi* swarms had been observed, is puzzling. We suggest it is likelier that *B. ovata* arrived with ballast offloaded at the port of Chalkis, as it arrived in the Bay of Piran with ballast originating from the Black Sea to the nearby Port of Koper (Shiganova and Malej 2009). In 2011, it was first recorded off Israel near the port of Ashdod, suggesting that like *M. leidyi*, it is a ballast-mediated introduction from the Black Sea (Galil et al. 2011). Its arrival soon after its prey follows the pattern of spread elsewhere, yet its presence in the warm and saline waters of the SE Levant is a surprise. In the winter of 2011–2012, *B. ovata* was first recorded in Kerteminde Harbour, Great Belt, Denmark (Shiganova et al. 2014).

10.4.5 Fit for the Anthropocene

Recently described fossils of late-stage embryo of a comb jelly from the Early Cambrian (540 million years old) Meishucun fossil assemblage of Ningqiang County, China (Chen et al. 2007), and scyphozoan jellyfish fossils in the Middle Cambrian (505 million years old) Marjum Formation of Utah (Cartwright et al. 2007), leave no doubt these organisms are well equipped for survival come what may. Indeed, both Jellyfish and comb jellies have evolved life history traits enabling rapid population growth – ingenious reproductive strategies (asexual reproduction, hermaphroditism), high fecundity, rapid growth, short generation time, broad physiological tolerance and dietary flexibility (Costello et al. 2012; Bayha and Graham 2014). These traits, which clearly served them well over the past tumultuous half billion years on earth, are the "opportunistic" traits of "invasiveness", regarded as determinants of invasion success (Ehrlich 1989; Catford et al. 2012). Bayha and Graham (2014: 45), who sought to "analyze organismal attributes of marine jellyfishes that promote their success as bioinvaders (*invasiveness*) and characteristics of recipient ecosystems that increase likelihood of successful invasions by marine jellies (*invasibility*)", failed to elucidate which traits, or combination thereof, distinguish the invasive alien species from the hundreds of species that have not (yet?) made use of the various anthropogenic pathways and vectors to spread to new regions. Their attempt "to examine the characteristics of heavily invaded ecosystems in order to determine which characteristics might make one ecosystem more susceptible to invasion than another" (there, p. 61) is similarly inconclusive. Some "sheltered estuarine and inland sea ecosystems" especially "degraded systems" (there, p. 62) have their share of alien jellyfish, but similar sheltered inland ecosystems and degraded locales escaped their fate so far. The authors (there, p. 63) then posit "the invasion of *Mnemiopsis* … in the North and Baltic Seas followed years of increasing North Sea temperatures, indicating that temperature may have reached a minimum threshold" – that for a species notorious for its "capacity to tolerate temperatures between 0 and 32 °C" (Costello et al. 2012). Be that as it may, temperature could not have held off the establishment of *Mnemiopsis* in the Mediterranean Sea prior to the 2009 sudden irruption.

The Mediterranean Sea is clearly the locale where populations of marine alien jellyfish and comb jellies have been recently on the rise. The largest number of alien jellyfish and comb jelly species have been recorded there, in addition to more than 700 other multicellular alien species (Galil et al. 2014). Bayha and Graham (2014: 58, 59) postulate that "medusozoans are more likely to be transported as hull-fouling organisms (as polyps or cysts) on the exposed surfaces of ocean-going vessels", whereas "Ballast water transfer is likely the main vector only in holoplanktonic organisms such as ctenophores". If this were the case, one would find congruence among the "network of global cargo ship movements with port environmental conditions and biogeography" and hot spots of bioinvasion, but the distribution pattern of alien jellyfish and comb jellies fails to fit the predictions (Seebens et al. 2013: 782). The important determinant of alien population dynamics in the Mediterranean is the Suez Canal – one of the most potent pathways for invasions by marine species in the world, that has served for the introduction of more than half the total number of alien species in that sea, and three (possibly four) of the five human-mediated introductions of scyphozoan species. Propagule pressure – a composite measure of the number of viable alien individuals, genotypes and taxa, the number of discrete introduction events, their frequency and duration – is recognised as the primary determinant of invasion success even overwhelming disturbance and biodiversity (Wonham et al.

2001; Verling et al. 2005; Von Holle and Simberloff 2005; Colautti et al. 2006; Simberloff 2009). Of the three major propagule conveyances – shipping, culture and Suez Canal – the latter supplies the largest number of successfully established aliens in the Mediterranean by virtue of the magnitude, frequency and duration of the trans-isthmian corridor invasion and the common evolutionary history of its components. In 2014, the Egyptian government announced its plans to enlarge further the Suez Canal in order to double its shipping capacity (Galil et al. 2015). The expansion is sure to have effects, at local and regional scales, on both the biological diversity and the ecosystem goods and services of the Mediterranean Sea. It is quite likely jellyfish will be among the next cohort to enter the Mediterranean Sea.

10.5 Concluding Remarks

The alien species are one of the major threats to biodiversity in marine environments. Cnidarian species are frequently translocated and spread far from their native areas. Many cnidarians have been successfully transported as fouling on ship hulls and in ballast waters (Carlton and Hodder 1995; Gollasch and Riemann-Zürneck 1996; Boero et al. 2002), and as associated fauna with species of mariculture interest (Minchin et al. 2009). Some Cnidarians haver enlarged their distribution by rafting on natural or man-made flotsam (Jokiel 1990; Thiel and Gutow 2005; Hoeksema et al. 2012), or with currents in transoceanic canals (Galil et al. 1990; Fine et al. 2005). They have established populations in artificial and human-modified habitats as well as, into natural habitats, frequently as results of a secondary spread (Leppäkoski 2002; Sammarco et al. 2004, 2010; Megina et al. 2013; Serrano et al. 2013). Furthermore, their wide range of sexual and asexual reproductive strategies making them efficient invaders.

The loss of biodiversity by the introduction of alien and invasive species could not simply be considered in terms of displacements of some native species in favour of their equivalent newcomers. All associated fauna and relationships can be finally altered (Byers et al. 2002). The ecological, socio-economical and evolutionary implications of the alien species phenomenon are far from correctly understood (Grosholz 2002; McFadden and Hutchinson 2004; Hoover et al. 2007). Their interplay with anthropogenic changes, e.g., euthophication, overfishing, habitat alteration, global warming and other components in the foodweb, is unknown and it is an important research field to contribute to maintain the global biodiversity. In view of all the above, cnidarians are a group of special interest for monitoring studies on alien and invasive species.

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Part III

Calcification

Calcification in the Cnidaria Through Time: An Overview of Their Skeletal Patterns from Individual to Evolutionary Viewpoints

11

Jean-Pierre Cuif

Abstract

Compared to the several hundreds of Families recognized among the Cnidaria phylum, the number of taxa in which a biomineralization process has been developed appears rather restricted. However an in-depth understanding of the resulting hard-parts is of major importance with respect to both evolutionary history of some major components of the phylum and contribution to a general model of calcium carbonate mineralization among living organisms. Results of a top-down approach involving recent physical characterization methods are summarized, emphasizing the remarkable and somewhat paradoxical aspects of this biochemically driven crystallization process.

Keywords

Biomineralization • Microstructure • Classification

11.1 Calcification in the Cnidaria Compared to Their Overall Evolutionary Tree

Cnidocytes, these sophisticated cellular weapons common to the organisms gathered in the phylum Cnidaria were characterized by cytologists since the nineteenth century (Hatschek coined *Cnidaria* in 1888) but it is only recent molecular investigations that revealed the huge time-gap existing between creation of these specialized cells (i.e. the potential origin of the phylum) and the Ordovician period, the lowest part of the geological column in which sediments contain a noticeable amount of well recognizable fossil coral skeletons (−485 to −443 million years). Actually, notwithstanding the remaining discrepancies among molecular phylogenies and uncertainty regarding calibration of the molecular clock, results converge to indicate that origins of the phylum Cnidaria (as well as occurrence of its main branching phases) are deeply rooted in Precambrian times, i.e. largely older than the 540 My time-line, the basal Cambrian limit that corresponds to the first development of mineralized skeletons in a wide array of Invertebrate animals. Erwin et al. (2011) suggested that origin of the Cnidaria took place during the Cryogenian, this enigmatic period of time during which most -if not all- of the Earth surface was covered by ice and snow (−850 to −635 million years). Waggoner and Collins (2004), then later Cartwright and Collins (2007) suggested an ancestry of more than 1 billion years. More recent investigations led van Iten et al. (2014) to consider that placing the major diversification of the Cnidaria (sub-phylum and class levels) « in the Ediacarian or even the Cryogenian period » is a « very plausible » working hypothesis. Origin of octocorals for instance, considered either as sister group of Hexacorallia following the Collins' hypothesis (2009) or sister group of the Medusozoa (according to Park et al. 2011) occurred well before the Ediacarian time (formerly Vendian) 635–542 million years ago (Taylor et al. 2013).

Evidence that major evolutionary divergence had already occurred long before the lower limit of the Cambrian is established by the recently discovered middle Cambrian (−505 My) jellyfish fauna from Utah (Cartwright et al. 2007), comprising specimens that exhibit diagnostic characters at the family level. In addition to the surprising fact that such a

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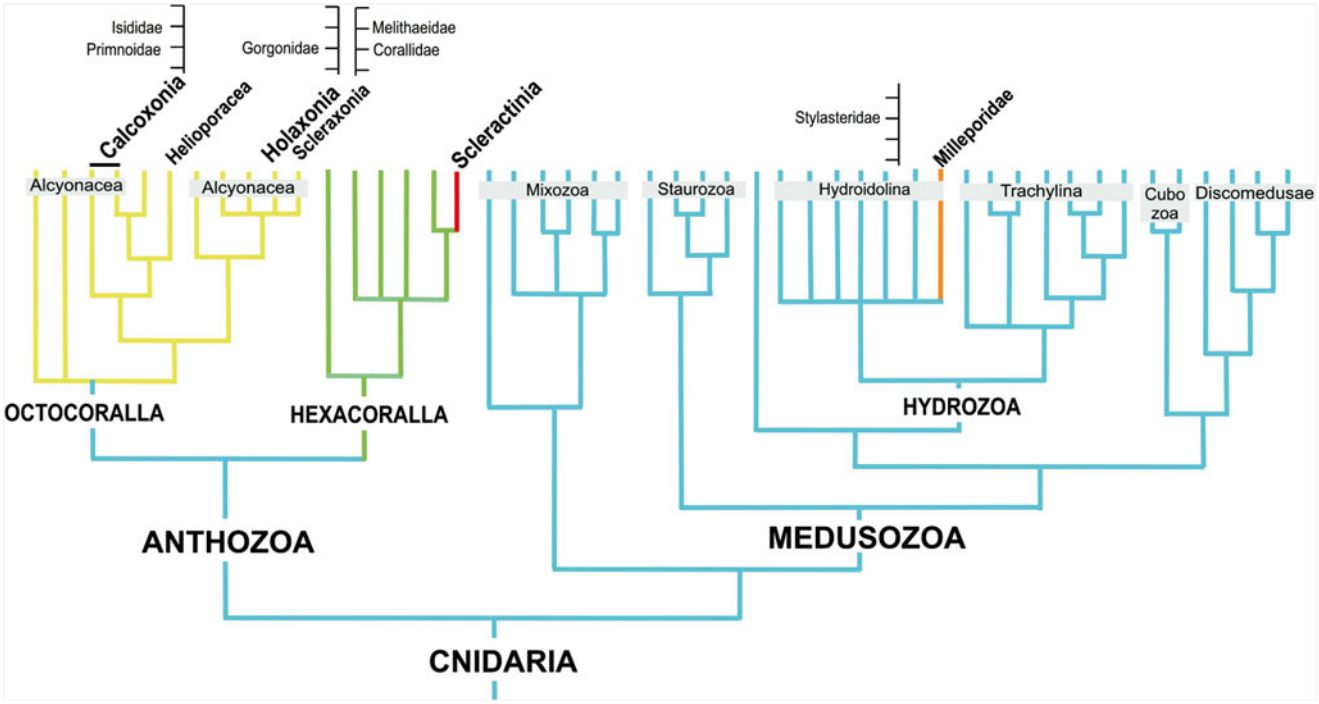

Fig. 11.1 A recent phylogenic arborescence based on molecular data (Collins 2009) on which mention is made of the major calcifying groups among living Cnidaria

significant set of data is provided by non-calcified animals, this also provides a milestone example allowing assessment of a quite advanced status of the evolutionary branching process for the phylum Medusozoa as early as Middle Cambrian times. According to the Marques and Collins' remark (2004) that "Medusozoa is just as basal within Cnidaria as is Anthozoa" an equivalent taxonomical complexity may exist in both groups. Supporting such a statement, the long-term investigation of Jenkins studying the world-wide distributed Ediacarian frond-like fossils (1978–1984) led him to conclude that a "likely presence of spicules" may suggest attribution of some of them to "highly evolved octocorals" (Jenkins 1984, p 100).

When considering calcification, an overview of the distribution of the calcareous skeletons among extant Cnidaria lineages makes obvious that a majority of them do not develop a biomineralization mechanism (Fig. 11.1).

It is remarkable that the prominent role recognized to Cnidaria in marine geology relies essentially on a single lineage, the Scleractinia, due to the unique ability of this group to construct solid calcareous structures distributed from the highly dynamic sea waters of the tropical reef barriers to the dark and cold deep-sea waters. This feature reflects the contrasted morphologies of the mineralized structures that living Cnidaria are able to produce. Animals belonging to the Cnidaria are extremely diverse from a morphological viewpoint and in their large majority have developed a colonial mode of life. What makes specimens of calcifying Hexacorallia so easily recognized and recorded even in ancient sedimentary rocks is their remarkable ability to produce skeletons in which the radial organization of the polyp body is generally well recognizable (Fig. 11.2a). Considering the whole phylum this is a quite rare property. Although a radial anatomical symmetry of the individual polyp is a general pattern in the phylum Cnidaria, the calcified structures produced by the octocoral colonies are solid ramified constructions whose organization does not reflect the anatomy of the individual polyps (Fig. 11.2b). Even more distant from polyp anatomy is the third form of calcification that is used in the Cnidaria: the calcareous sclerites of octocorals (Figs. 11.2c and 11.3). These are essentially single cell products (or sometimes more or less syncytial), with sometimes a remarkable taxonomic specificities (e.g. Primnoidae).

These images explain why geological investigators are facing major difficulties with respect to taxonomical identification of fossilized remains. Sclerites are dispersed after decay of the animal tissues, forming multispecies post-mortem assemblages whereas the calcified axes of colonies are generally unable to provide valuable criteria at the lower taxonomic levels (Bengtson 1981). This also explains the origin of the large under-evaluation of the non-Hexacorallia skeletons as sedimentary contributors.

With respect to animal anatomy the mineralized hard-parts of the Cnidaria are always ectodermal in origin but can be produced through two modes of biomineralization:

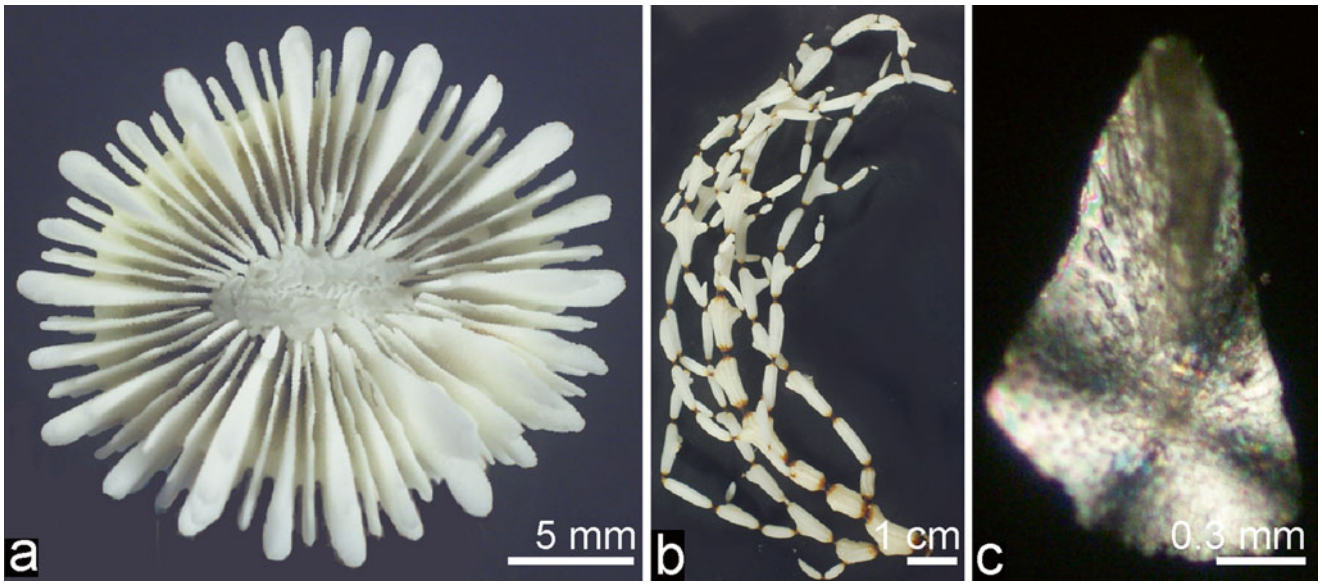

Fig. 11.2 Contrasting morphologies of calcified structures produced by Cnidaria. (**a**) A perfectly radial skeleton built by a Scleractinia (*Cynarina*); (**b**) An arborescent support built by *Isidella* (Octocorallia, Gorgonacea); (**c**) One of the numerous types of sclerites surrounding the animal body from a Primnoidae colony (Octocorallia, Gorgonacea; polarized light, crossed nicols)

intracellular and extracellular (i.e. epithelial) calcification. The former results in small and discrete mineral units (called sclerites or spicules) dispersed into the ectodermal tissues and mesoglea (the gel-like substance located between the ectodermal and endodermal cell layers). Conversely the epithelial mode of mineralization results in macroscopic individual specimens the dimension of which may be up to the meter range in colonial species (e.g. *Porites* colonies).

Regarding skeleton mineralogy extant Cnidaria exclusively use calcium carbonate although Bayer and McIntyre (2001) have detected carbonate hydroxy-apatite as minor component of the axis of many gorgonians. In addition, a strong biological control is exerted on selection of the Ca-carbonate polymorph: calcite or aragonite. Scleractinian corals for instance produce aragonite skeletons even when living on deep-sea floors (e.g. *Lophelia*) where at such temperatures, depths and pCO_2 aragonite particles descending from sea surface are dissolved before reaching sea floor. However calcite was used by the Paleozoic predecessors of the present reef builders. Contrastingly the Octocorallia use calcite with the remarkable exception of *Heliopora* (the Blue Coral) whose skeletal fibers are composed of aragonite. Such exceptions raise questions about the mode of selection of the skeletal Ca-carbonate polymorph and the continuity of this mineralogical character through time (see also below Tabulate corals). It is noteworthy that no example has been found of simultaneous production of calcite and aragonite in distinct areas of the skeleton, as occurs regularly in some Pelecypods for instance (e.g. blue mussel or pearl oysters).

11.2 Skeletal Calcification: Evidence of a Stepping Biological Process Acting at the Micrometer Scale

Interestingly intracellular and epithelial modes of calcification can be employed simultaneously in Cnidaria skeletogenesis, as exemplified by the *Corallium rubrum* whose intensely red colored solid axes contributed to the myth of Medusa as they were interpreted as "the blood which continued to drip from Medusa's severed head » (Ovid, Metamorphoses). These branching axes which were used since antiquity as source of handcrafted jewels are only a part of the mineralized structures produced by this species. At the periphery of the axis a cortical structure consisting of sclerites densely distributed into the mesoglea (but always individually distinct) contributes to protection of the polyps (Figs. 11.3 and 11.4).

Each species produces a set of morphologically specific sclerites, providing evidence of the biological control exerted during the biomineralization process. Overall, each sclerite epitomizes the still unsolved paradox of Ca-carbonate skeletal units. It has long been known from optical observations that whatever their morphological complexity each sclerite exhibits a crystal-like behavior, i.e. displaying optical extinction when rotated between crossed-nicols (Fig. 11.3g, h). On the other hand Floquet and Vielzeuf (2012) have established by careful back-scattered electron diffraction measurements that the mineral components of the sclerites were arranged in conformity with axial orientations of a calcite lattice. In contrast to these crystallographic patterns, back scattered electron

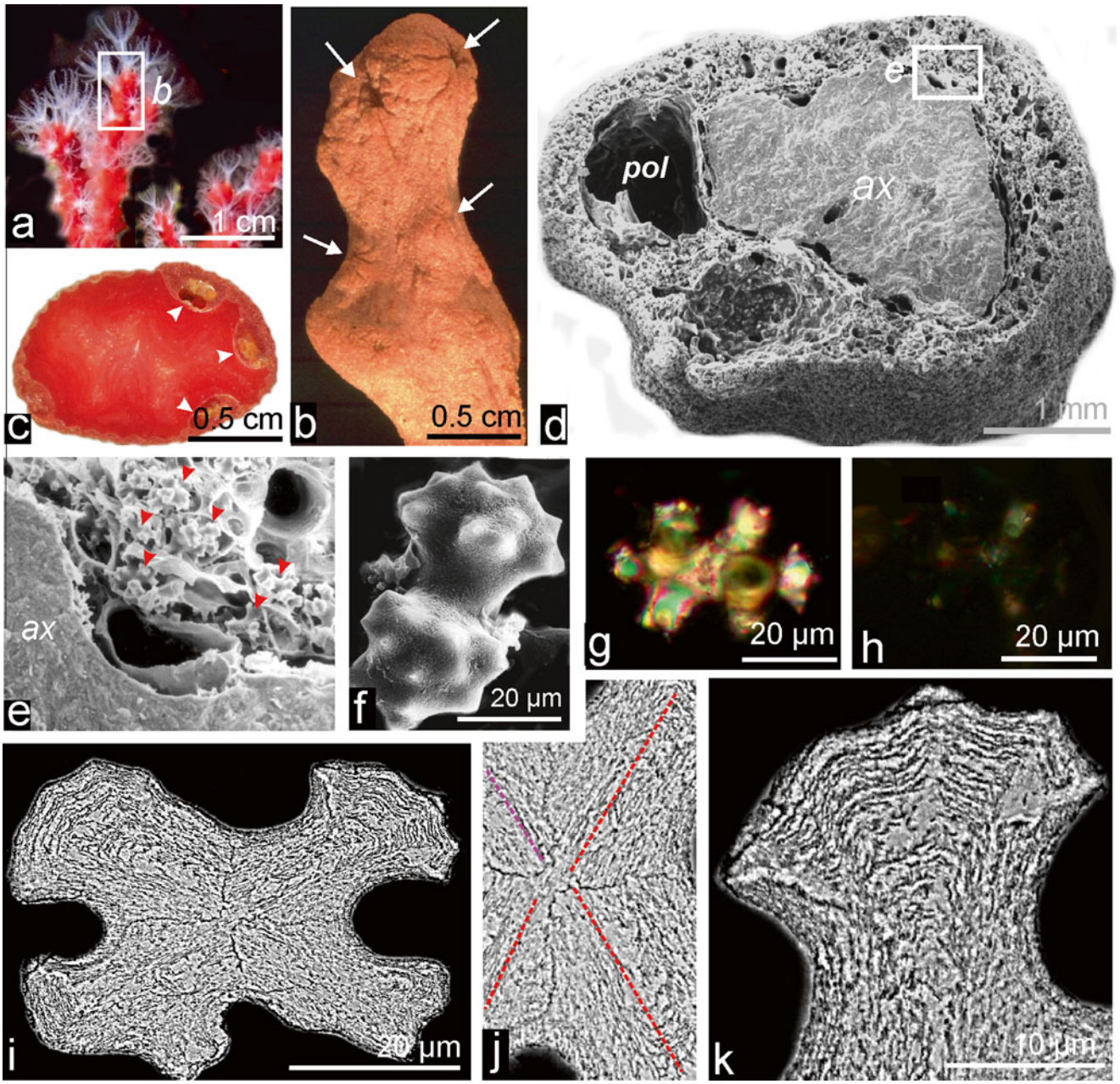

Fig. 11.3 Cortical skeleton made of sclerites in *Corallium rubrum*. (**a**–**c**) The extended polyps (**a**) can be retracted within chambers covered by the peripheral cortex (**b**: *arrows* external view) and section (**c**); (**d**, **e**) SEM view of a section perpendicular to growth direction of the skeletal axis (*ax*), *red arrows*: sclerites. (**f**) Typical view of an isolated sclerite; (**g**, **h**) Crystal-like behavior of a sclerite observed in polarized light (crossed nicols): rotation changes from brightly colored (**g**) to extinct appearance (**h**) of the whole sclerite; **i**–**k** Evidence of the layered growth mode of the sclerite obtained on a polished surface observed with electron microscope in the back-scattered mode. With this observational mode the contrast is due to the proportion of back-scattered electrons that depends on the physical properties of substrates: typically organic and mineral areas are delineated. Note the distinctly growing branches (**j**), each of them with its own growth direction. Detailed observation of a branch (**k**) shows the progressive shape modification of the superposed growth layers resulting in the species-specific morphology of the sclerite

imaging of *C. rubrum* spicules reveals that morphology of the superposed growth layers which progressively built the sclerites (Fig. 11.3i–k) is completely independent of the growth surfaces of the virtual crystal that can be reconstructed through the series of back-scattered electron diffractions. Varying during growth, the curved profiles of the micrometer-thick growth layers of the sclerites (Fig. 11.3k) clearly show that, if viewed as crystal-like skeletal units, the sclerites do not grow in agreement with the classical rules of crystal growth (formation of plane surfaces forming regular angles between them).

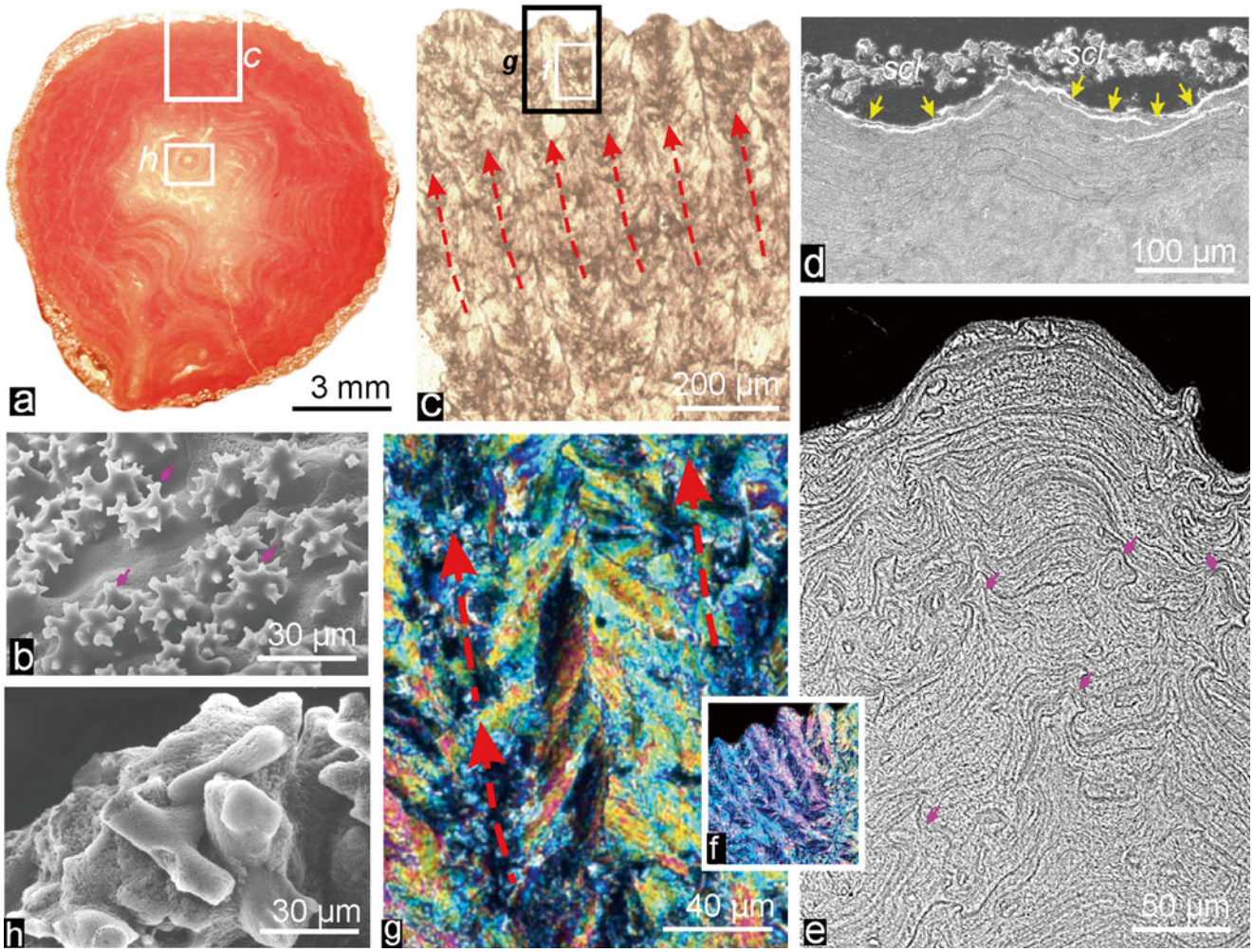

Fig. 11.4 Microstructure of the skeletal axis of *Corallium rubrum*. (**a**) Overall section; (**b**) Spinose expansions on axis surface; (**c**) Microscopic thin section (natural light) showing the arrangement of fibers into radial fascicles (*arrows*); (**d**) Thin cortex of sclerites covering the axis surface marked by longitudinal groves (*arrows*); (**e**) Enlarged view of a fibrous fascicle showing the distorted profile of the micrometer thick growth layers (*arrows*); (**f**, **g**) Microscopic ultra-thin section (approximately 4–5 µm thick) showing the diverging arrangement of fibers; (**h**) Young immature sclerites visible at the distal ends of the axes, in which they are eventually incorporated

Of importance is that each sclerite grows inside the biological envelope formed by one or several associated cells. Formation of species-specific morphologies for this few tens of micrometers sized unit is obtained through repeated deposition of mineralized growth layers whose thickness and shape locally vary through time, providing typical illustration of a layered growth and crystallization process (Cuif et al. 2011). Formation of species-specific morphologies implies that the involved cell (or group of cells) develops a sequential mineralization process in which the layered deposition of calcium carbonate is controlled not only with respect to selection of the used polymorph (e.g. calcite in octocoral sclerites) but, much more fundamentally, through a genetically driven crystallization program which precisely modulates the secretion activity of the mineralizing areas.

The microstructure of the solid skeletal axis, which is by far the most familiar structure of the *C. rubrum*, provides additional evidence of this layered biomineralization process. A detailed description of the skeletal axis of *C. rubrum* was made by Lacaze-Duthiers (1864). Taking into account the spinose expansions covering the axis surface (Fig. 11.4a, b) his interpretation was that the whole axis was constructed of cemented sclerites. However, observation of polished surface and microscopic thin sections disproves but also explains this interpretation, by establishing the layered growth mode of the whole axis. Figure 11.4e–g provide a new example of the paradoxical crystallization mode previously observed in the sclerites. Here at the surface of the branching axis the ectodermal epithelium produces continuous layers of mineralized materials. Instead of a regular

concentric arrangement these micrometer thick layers display strong distortions (Fig. 11.4e), producing the spinose expansions visible on the outer surface of the axis (Fig. 11.4b), that were long considered as spicules partly emerging from the bulk.

At the very top of the axis (Fig. 11.4h) corresponding to the central part of axis section (4a) young and not fully developed sclerites may be incorporated into the axis (note their morphological difference with fully developed sclerites (Fig. 11.3f). However below the axis tip the whole structure is constructed through a layered mineralization process (Fig. 11.4e). From a petrographic view point the *Corallium* axes are built by calcite crystals (high magnesium content calcite) arranged into fascicular units (Fig. 11.4f, g). However, as in the sclerite case, traces of the growth layering observed within the axis (Fig. 11.4e) reveal the stepping growth mode of the crystal-like fibers, formed by superposition of variously contorted mineralization layers. Once again microstructure and crystallography of the axis offer contradictory patterns, as previously observed in the sclerites, allowing assessment of a biological control exerted on mineral deposition by the ectodermal cell layer.

From an historical view-point these superposed growth lines establishing the existence of a stepping growth mode for the *Corallium rubrum* skeleton components (both sclerites and axes) were first reported by microscopic observation made by Kölliker (1864) not on the *C. rubrum* but on a Scleractinia corallite identified as *Astrea*. The first diagrams of such growth lines were given by Ogilvie (1896) in her extensive structural analysis of the coral skeletons. Since these milestone studies the concept of a layered growth mode of skeletons fell into disfavor and prevalence was given to the more apparent crystalline patterns. The formal statement marking this conceptual change is found in the Bryan and Hill's paper (1941) in which the coral fiber is described as "a single crystal of orthorhombic aragonite". Over several decades this concept of a crystalline coral fiber was considered definitive, thereby providing the foundation for the interpretation of isotopic or chemical measurements in the geochemical investigations using corals as environmental archives. A series of microstructural observations completed by physical characterizations (see below Figs. 11.7 and 11.8) have re-established the primary importance of the layered growth and crystallization as the basic calcification mechanism. Figures 11.3 and 11.4 have shown that both the sclerites and axes of the *Corallium rubrum* were also built by such a mode of growth.

11.3 Skeletal Diversity Among Octocorallia

Corallium rubrum, whose skeleton comprises both sclerites and solid axes, exemplifies a type of dual calcification largely represented among corals belonging to the subclass Alcyonaria (=Octocorallia). Apart from species in which sclerites remain essentially isolated as in the "soft corals" (e.g. *Dendronephthya*, Fig. 11.5a), an important difference exists among octocoral species producing axes regarding the origin and structure of this supporting organ. In contrast to the *Corallium rubrum*, in which the massive but layered structure of the axes has been recognized (once disproved the initial interpretation), three-dimensional associations of sclerites have been developed as for example in *Melithaea* resulting in complex axial architectures (Fig. 11.5b–g). In this species, the large and densely packed sclerites form thick laminae associated into a reticulated structure, very solid although not massive. Calcified sclerites exhibit a readily visible fibrous structure that is actually the microstructural organization of the whole colony.

Thus, the entire supporting skeleton is built by densely packed sclerites. Such a fundamentally fibrous organization with its variously oriented building units explains the remarkable mechanical properties of this type of supporting structure. It allows *Melithaea* and comparable species to create large colonies, erect but remaining flexible (contrasting with the massive axis of the red coral) and able to accommodate relatively intense water movements.

Other types of dual supporting structure are exploited by members of the Gorgonids, with two distinct organizations of the axes. In the typical form the axial structure is essentially constructed by superposed horny layers. In aging individuals, calcification of the horny layers may occur (Fig. 11.6b, arrows) mostly at the basis of the axis (Esford and Lewis 1990; Bayer and MacIntyre 2001). In addition, as in *Eunicella* for instance, the horny axis is covered by a cortex composed of closely joined balloon-club sclerites supported by a more internal layer of spindle sclerites (Fig. 11.6c, d).

In contrast a strongly calcified axial skeleton is observed in many other species in which flexibility is maintained by regularly spaced non-mineralized nodes (Fig. 11.1b: *Isidella*). These are the "bamboo corals" that are currently intensively studied owing to their potential as environmental archives for the reconstruction of chemical or physical changes that may have occurred in deep sea-waters. Slow growing, heavily calcified and widely distributed with respect to depths of living areas, these bamboo corals complement scleractinian deep-sea corals in the study of oceanic circulations. Calcified structures among these species are weakly diversified: many of them display regular deposition of calcite layers associated to organic components, the latter made visible by Orange acridine staining (Fig. 11.6f–h). As a result, skeletal microstructures consist of superposed simple calcite layers (Fig. 11.5i, j) whose detailed mineralization patterns do not enable formation of detailed taxonomic criteria (Noe and Dullo 2006). Conversely such a simple growth layering is advantageous with respect to chemical or isotopic measurements: a simple radial sampling provides a reliable access to time.

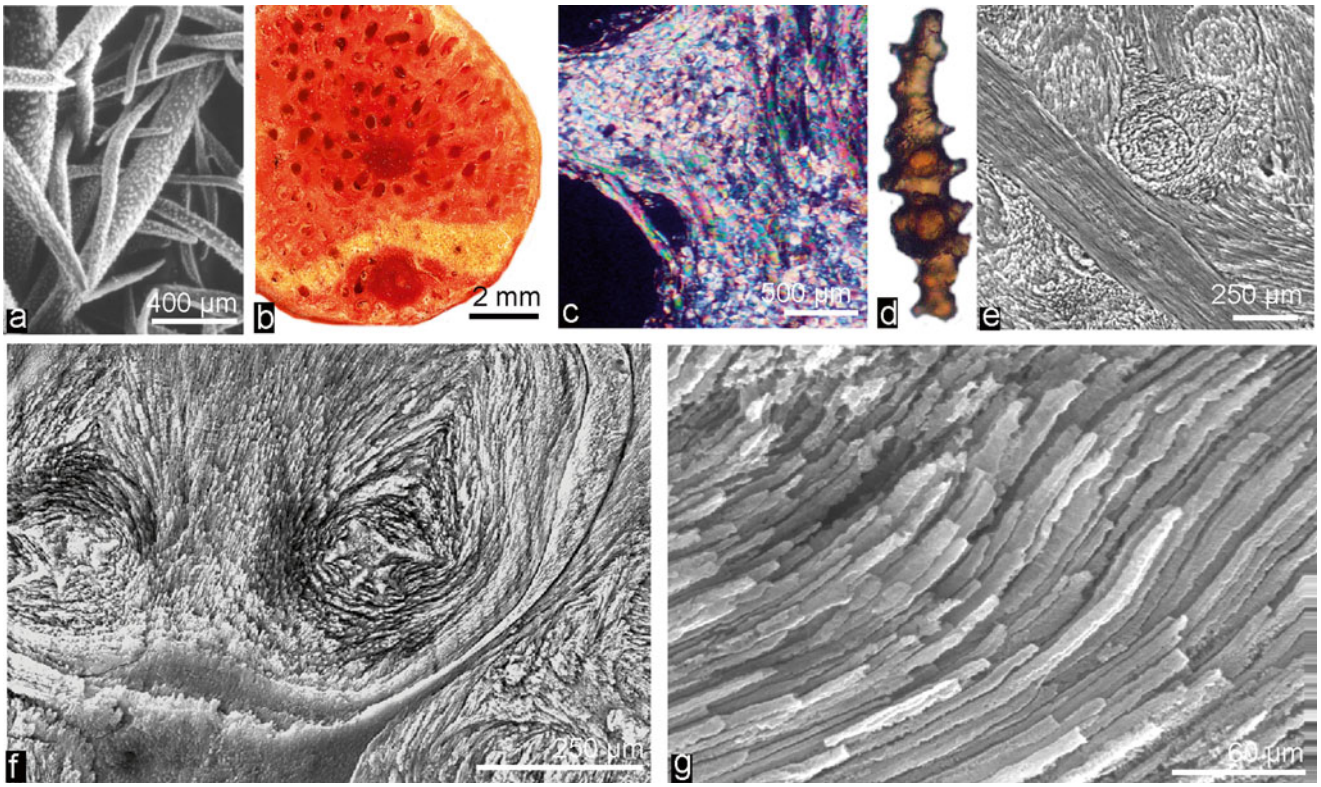

Fig. 11.5 Two spatial arrangements of sclerites. (**a**) Variously sized isolated sclerites (*Dendronephthya*); (**b**–**g**) Complex axial supporting architectures built by reticulated thick laminae made of densely packed elongated sclerites in *Melithea*: (**b**) section of the supporting axis; (**c**) optical view (polarized light); (**d**) isolated sclerite; (**e**) SEM image (medium enlargement) of the axial spicules; (**f**, **g**) fibrous structure of the densely packed sclerites

However, some members of the group Isididae (e.g. *Isidella*, Fig. 11.6k–o) exhibit structural indications of a more diversified control on calcification. Microstructural units result from deposition of areas enriched in organic components whose regular variation during growth (Fig. 11.6k, l) creates apparently incurved structures. Within the resulting sectors, however, the layered growth mode (Fig. 11.6m) and orientation of fiber fascicles conform throughout to a radial mode of growth as shown by polarized light (Fig. 11.6n, o).

11.4 Innovative Calcification in the Hexacorallia: Access to Long Term Evolution Through Microstructural Analysis

In spite of obvious morphological differences the calcareous structures of Octocorallia examined so far (sclerites or axes) have in common a low degree of microstructural differentiation. In the mineralizing Hexacorallia, in addition to the fact that the skeleton secreting epithelium includes the basal ectodermal layer of the polyps themselves (explaining why radial organization is visible in the Scleractinia skeletons; see Fig. 11.1), this calicoblastic ectoderm exhibits a remarkable histological specificity regarding organization of the microstructural units, a property whose elucidation was achieved through a century long controversy.

It is noteworthy that up to the last decades of the nineteenth century (including the influential Milne-Edwards and Haime's studies from 1848 up to 1860) only morphological approaches were used to classify the Scleractinia skeletons. The transition to structural analysis at the micrometer scale is due to Ogilvie who established the taxonomic value of the microstructural patterns (three dimensional arrangements of fibers) and coined the term "center of calcification" as a key point in formation of the Scleractinia septa (1896). However at that time the 10-year old controversy between von Heider (1886) and von Koch (1886) (based on their opposing views regarding the role of the ectodermal cell layer in the mineralization process) was still continuing. It is only after Bournes' careful histological studies of the basal cell layer of the polyp (1899) that the extracellular calcification of the skeleton was conceded, disproving the von Heider's opinion that coral skeletons were constructed by calcification of the epithelial cells themselves. Unfortunately this later view was supported by Ogilvie, thereby casting doubt on her own results. This paved the way to the chemical concept of a spherulitic

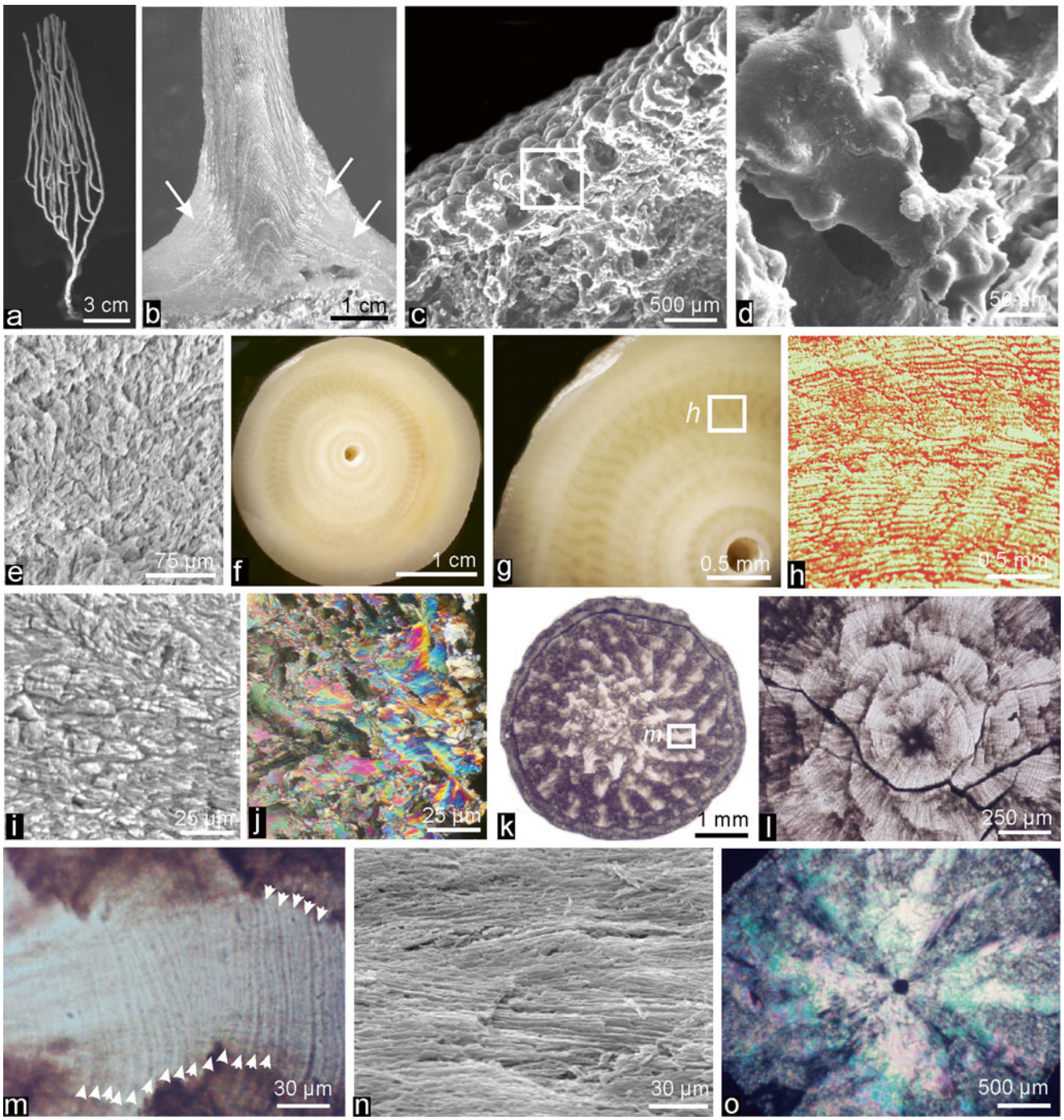

Fig. 11.6 Calcification in gorgonian corals. (**a**–**c**) In *Eunicella* the closely assembled sclerites form a protective cortex; (**d**, **e**) Calcification limited to the basal layers basis of the axis (**d**) exhibits weakly organized calcareous units (**e**); (**f**–**j**) A remarkably regular layering of calcification in the axis of a Isididae coral. Orange acridine staining (**h**), SEM view of the mineral phase (**i**) and microscopic thin section (**j**, crossed-nicols) show that no long range crystallinity can be observed; (**k**–**o**) A weak coordination between mineralizing areas (**k**–**m**) results in some consistency between crystallization of successive layers (**m**, **n**) and an overall structural organization of the axis (**o**)

crystallization formulated by Bryan and Hill (1941): "the coral fiber is a single crystal of orthorhombic aragonite" and the long standing view of a "biologically induced" origin of the coral fibers (up to Veis 2005). Simultaneously nature and role of the "centers of calcification" were questioned. This was still the case in the two main treatises on Scleractinia published in the middle of the twentieth century. The concept of centers of calcification was used "for practical purposes" by Vaughan and Wells (1943, p. 32), in association with the neighboring fiber fascicle to form the sclerodermites viewed as the actual skeletal units whereas "center of calcification" was always written with quotation marks in the Wells' chapter "Scleractinia" (1956: R.C. Moore, Treatise on Invertebrate Paleontology part F, p. 337, Fig. 231). At that time, and subsequently for a long period, no evidence was available to support the concept of a biological control of crystallization by the secreted mineralizing matrices outside the ectodermal cell layer.

A key result towards a better understanding of corallite ontogenesis was obtained by Vandermeulen and Watabe (1973) who established that formation of septa and walls was a two-step process. The early mineralization stage consists in deposition of small granular crystals forming the very first wall and radial septa, later reinforced (step 2) by deposition of fibrous layers on both sides of the initial micro-granular structures. Remarkably, all along corallite or colony life, the superposed micrometer thick growth layers produced by the basal cell layer of the polyps replicate the same dual secretion process.

The controversial points that, over several decades, had queried the existence of the "centers of calcification" and the biochemical control of mineral deposition were explained by converging data from scanning electron microscopy, atomic force imaging and synchrotron-based characterizations (Fig. 11.7). These observations showed that distinct areas of the calicoblastic ectoderm were dedicated to production of specific organic compounds generating the microgranular structures typical for early (=distal) calcification whereas lateral areas are producing the fiber growth layers (Cuif and Dauphin 1998, 2005; Cuif et al. 2003a, 2012).

Here lies the structural innovation that makes the calcification process of Hexacorallia lineages distinct from those of Octocorallia. Not only is the radial organization of the polyp reflected in the calcareous skeleton but also the spatial arrangement of the distal mineralization area have demonstrated an exceptional evolutionary potential, as established by fossil data distributed from Ordovician to recent. Surprisingly this major influential role was first highlighted by Volz (1896) using fossil specimens, taking advantage of the exceptional preservation status of the coral fauna in the Triassic Saint-Cassian strata from Süd-Tyrol (then Dolomites). In most of these fossils a specific spatial arrangement of the initial calcification stages was emphasized by differential fossilization. Volz coined the term "urseptum" (= primitive septum, 1896, p. 9 Fig. 4) hypothesizing that these figures could reflect the initial step in formation of the septa. It is noteworthy that the biological and physical characterizations that fully supported this view were only obtained about a century later. Origin of this full biological control on formation of the granular "urseptum" and fibrous skeletal structures was made more understandable through atomic force microscopy in the phase-contrast mode, showing that both skeletal areas (distal and lateral) forming a growth layer were built by mineral granules of 75–100 nm in mean diameter surrounded by an irregular organic cortex (Fig. 11.7j). Obviously, far from a simple spherulitic crystallization, the cyclic mechanism forming the corallite growth-layers involves precisely distributed organic compounds in a controlled crystallization process which is not fully explained yet at molecular scale.

Importance of the Volz concept cannot be overestimated. Not only can diversity of septal structures between genera and families be accurately established (e.g. Figs. 11.8 and 11.9 in this paper) but also through a careful reconstruction of changes in the microstructural patterns during coral ontogenesis does a detailed knowledge of the species specific septal developments become accessible. By so doing a comprehensive understanding of the skeletal ontogenic program that drives mineral deposition by the calicoblastic ectoderm can be obtained. Well preserved fossils (e.g. the Triassic *Distichophyllia norica* Fig. 11.9a–f) exemplify the possibility of reconstructing skeletal ontogenesis from very ancient samples. That microstructural ontogenesis has a strong evolutionary potential was assessed by a comparative study involving molecular based phylogeny from polyp tissues and in parallel, analysis of septal microstructures in the corresponding corallites (Cuif et al. 2003a, b). By this method inadequacy of the classical and long authoritative supra-generic taxonomy and phylogenetic arborescence of families from the Triassic to recent Scleractinia (as suggested by Wells 1956, p. F363) was established long before occurrence of molecular studies.

Creating a common evolutionary scheme encompassing skeleton-producing Hexacorallia is a long standing project, first attempted by investigators at the end of the nineteenth century (e.g. Frech 1890; Ogilvie 1896) who admitted continuity between corals distributed from Paleozoic to recent. In contrast, the mid-twentieth century Treatises (Vaughan and Wells 1943; Wells 1956 and recent revisions) integrated the Haeckel suggestion (1896) that Paleozoic and post-Paleozoic corals were fully distinct lineages, the later having been produced by "newly calcifying anemones".

An in-depth reexamination of this concept (authoritative throughout the whole twentieth century) is currently ongoing mostly through molecular investigations. Several studies have converged to consider that scleractinian lineages (the "modern corals") are more or less deeply rooted within the Paleozoic period (e.g. Medina et al. 2006), a result that clearly disproves the Haeckel concept that was based

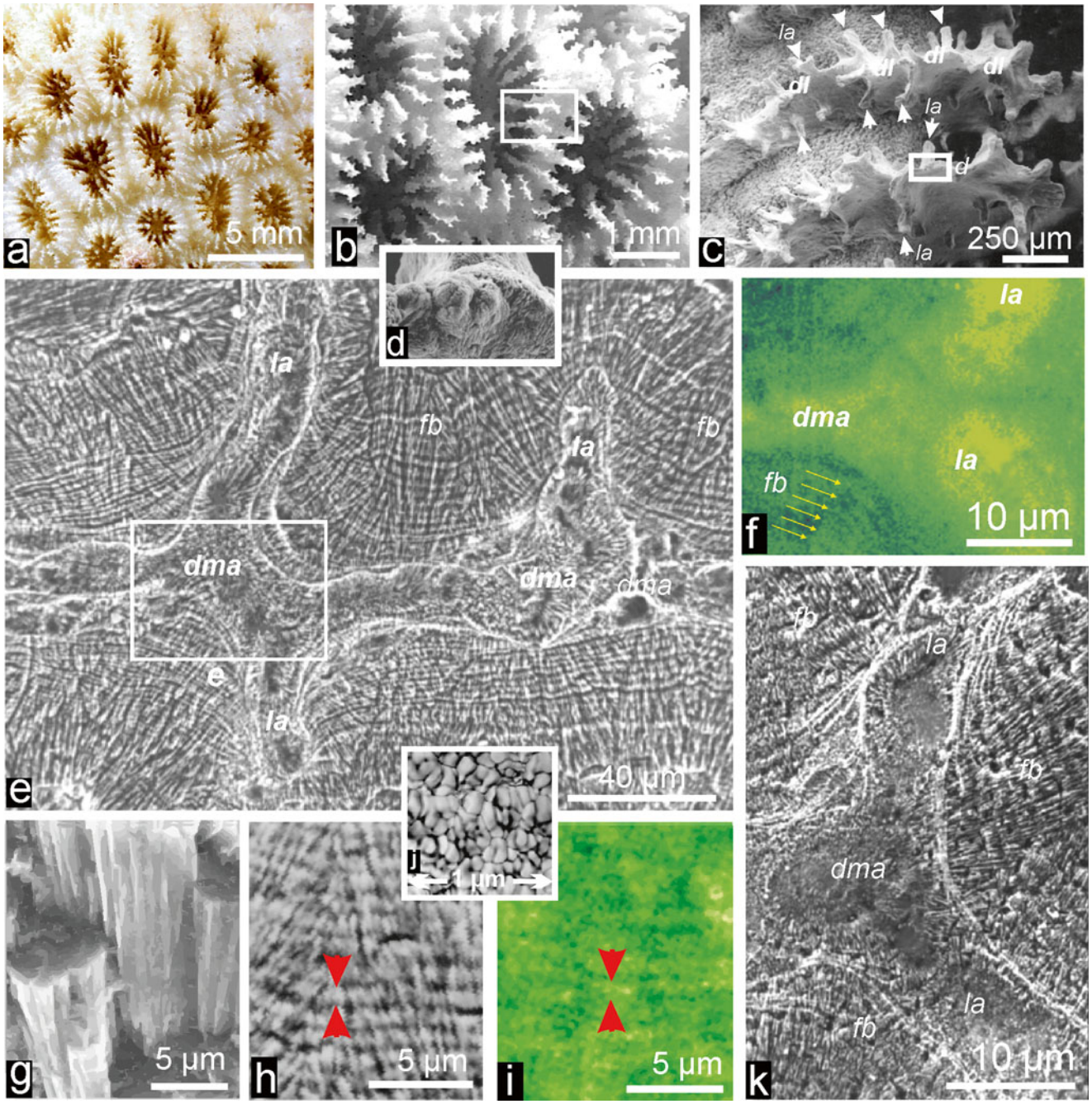

Fig. 11.7 Distal (or early) mineralization areas followed by stepping deposition of fibers exemplified by *Favia stelligera* skeleton. (**a**–**d**) Close examination of septa reveals the distal mineralization area (*dma* = Volz "Urseptum") with its distinctive mode of microcrystalline mineralization (**d**). This area comprises the distal line (*dl*), variously designed depending upon species) and the lateral axes (*la*) whose spatial arrangement will generate the genus/species specific septal microstructure; (**e**) Distal line and lateral axes can be made visible through appropriate sections of the septa (polished and etched surfaces), opening possibility of reconstructing septal development through time; (**f**) Synchrotron-based mapping of sulfated polysaccharides emphasizes the biochemical contribution to both initial and secondary steps of septum construction: distal line made of more or less fused spots with higher polysaccharide concentration than in the calcified layers forming fibers; (**g**–**i**) The usual SEM view of fibers as elongated compact aragonite crystals strongly contrasts with pictures obtained from polished sections (**i**) (mapping of polysaccharides) and (**h**) (stepping growth visible after etching); (**j**) Atomic Force Microscopy (phase contrast imaging) reveals the granular organo-mineral structure forming the skeletal growth layer. Note the 1 µm wide field view; (**k**) Superimposed deposition of fibrous layers units onto the initial septal framework the Volz' Urseptum)

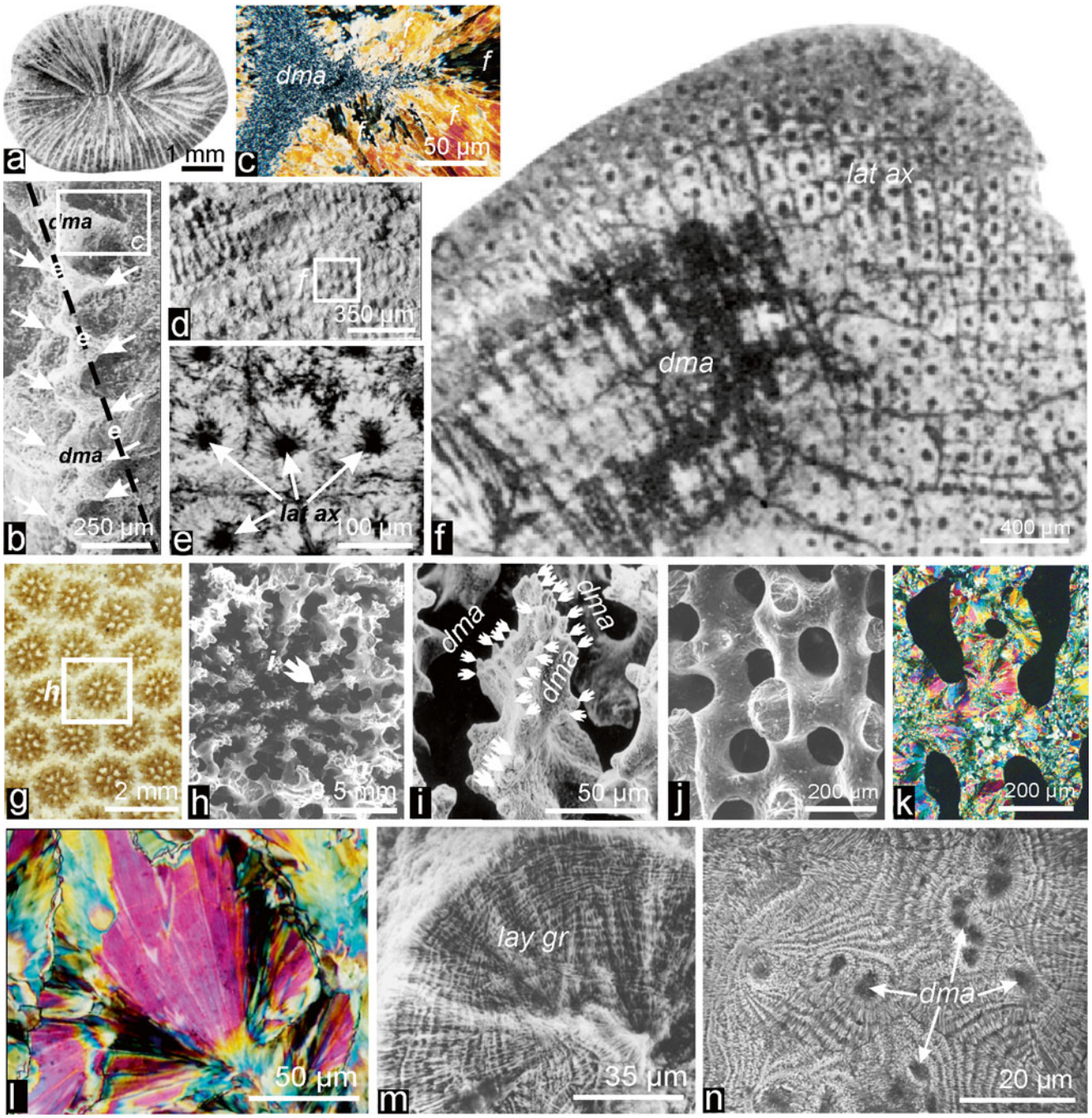

Fig. 11.8 Two distinct types of spatial distribution of the distal (or early) mineralizing areas (*dma* = "centers of calcification" = Volz' "urseptum") viewed from upper surface of the septa (the distal growth line) and from inside septal structures. (**a**) Overall morphology of *Distichophyllia*, Triassic species from the Austrian Zlambach schichten; (**b**) Zig-zag morphology of the distal mineralization upper line; (**c**) fibrous fascicles inserted onto one of the lateral axes expanding from the distal-zig-zag line (note the granular microstructure of the axis); (**d**, **e**) Granules visible on the lateral surfaces of the septa (**d**) are caused by growth of the lateral axes surrounded by fibers as shown by microscopic thin sections (**e**); (**f**) Overall view of a microscopic thin section in a septum of *Distichophyllia* slightly oblique to the median plan of the septum: growth patterns of the two distinct microstructural areas can be observed. (**g**, **h**) Morphology of a *Porites* corallite; (**i**) morphology of the upper part of a skeletal rod: note the distribution of the distal mineralizing areas as small and distinct spherical units; (**j**–**l**) Morphology and microstructure of the skeletal rods in SEM view (**j**), crossed nicols microscopic thins sections (**k**, **l**); (**m**, **n**) Layered growth (lay gr) of the skeletal rods (**m**) and dispersed distribution of the distal mineralizing areas surrounded by the layered growth of fibers (**n**)

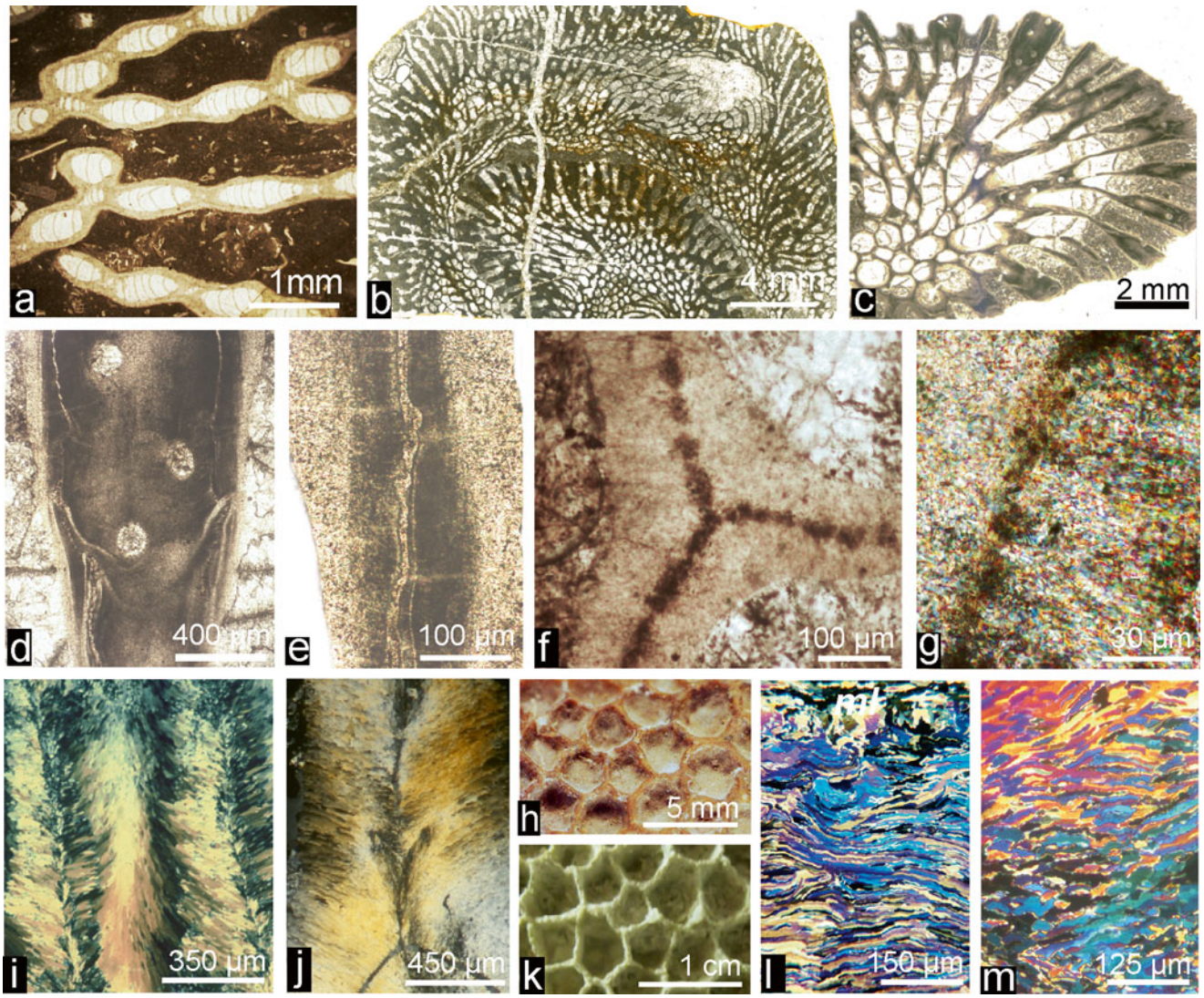

Fig. 11.9 Morphological and histological patterns of some Tabulate corals. (**a**–**c**) Section in colonies of *Halysites* (**a**), *Alveolites* (**b**), *Thamnopora* (**c**); (**d**, **e**) Pores in the wall of *Thamnopora* and section perpendicular to the wall; (**f**–**h**) Fibrous microstructure of the wall of *Favosites* (**f**, **g**), and morphology of the corallites (**h**). Note the distinct spots forming the median line; (**i**, **j**) Other examples of fibrous microstructures: *Thamnopora* (**i**), *Trachypsammia* (**j**) both from Plusquellec coll. (University of Brest); (**k**–**m**) Morphology of the calices (**k**) and wall microstructures (**l**, **m**) in *Michelinia*, examples of lamellar microstructure (Lafuste coll., MNHN Paris) *ml* median line between calices

essentially on skeleton morphology. During the two most recent decades the exclusive use of molecular data as phylogenic tools produced a number of diverging phylogenetic arborescences (e.g. Chen et al. 1995; Veron et al. 1996; Romano and Palumbi 1996; Romano and Cairns 2000) up to present (Fukami et al. 2008; Budd et al. 2010; Stolarski et al. 2011). As pointed out by Budd et al. (2010) confusion as a "hall-mark of scleractinian classification… reemerged in the late 20th century when molecular techniques began to be applied to scleractinian systematics".

From a methodological view-point microstructural investigation based on the concept of evolving microstructures through both individual skeletal ontogenesis and long-term evolution offers a unique opportunity to take advantage of the large fossil data-base collected during two centuries of paleontological studies. As the permanent biological control of the corallite calcareous structures is now well established, collaborating investigations involving gene expression and molecular mechanisms of skeletogenesis coupled to microstructural analysis of the resulting structures could contribute to creating a more consistent evolutionary framework.

It is remarkable that among the many research studies that aim at deciphering the biomineralization mechanisms, a growing number is studying influence of natural organic components extracted from calcified biominerals (e.g. Reggi et al. 2014) in place of organic chemicals as it was initially done (e.g. Kitano and Hood 1965). Further steps consist in using mineralizing molecules produced by molecular clon-

ing and gene expression (Mass et al. 2013). Deciphering the sequence of genomic events (the mineralization program) that control the spatial organization of the mineral particles successively generating the initial septal framework (the Volz' "urseptum") followed by stepping deposition of the layered fibrous tissue may reduce the gap between morphological and molecular phylogenies.

11.5 How Do Extinct Lineages May Contribute to Our Representation of Long Term Evolution of the Calcifying Cnidaria

Molecular phylogeny (e.g. Fig. 11.1) yields models in which the distances between groups (i.e. potential evolutionary affinities) result in series of generally bifurcating branches. Such overall schemes obviously suggest that evolution of the phylum consists in a progressive differentiation through time, more or less similar to the classical Darwinian scheme. Taking into account the fossil data leads to a completely different view.

An overall survey of fossils identified as Cnidaria in the first part of the fossiliferous period (Paleozoic) shows that during about 250 million years Cnidaria diversity is higher than in any later period. In addition to the presence of a complex series of genera and families having produced undoubtedly Hexacorallia coral looking samples (comprising septa, walls and endothecal structures), a number of various colonial forms exhibit such distinct patterns that they were identified as a major taxon as early as 1850: the Tabulata Milne-Edwards and Haime. Tabulata (Tabulate corals) formed colonies whose tubular units, freely growing or associated into massive constructions lack radial internal subdivision. In contrast, the tube internal space is subdivided by calcified layers (tabulae) typically perpendicular to growth axis of the corallites (Fig. 11.9). Septa are lacking, but regularly distributed spines may exist, as well as communication pores between neighbor corallites.

The order Tabulata encompasses at least six distinct taxonomic units whose consistency was recently challenged by the biological evidence that some Demosponges (Sponges with siliceous spicules) were able to built additional calcareous skeletons made of densely packed tubes whose internal space was transversally subdivided by typical tabulae. *Acanthochaetetes* for instance, admittedly related to the Paleozoic Chatetetid group was accepted as a fossil coral up to the discovery of a living species *A. wellsi* (Hartmann and Goreau 1975) whose soft tissues demonstrated that it belongs to Sponges. Among the remaining Tabulate corals important microstructural differences are observable. Many of them are built by fibrous tissues, and in some cases skeletal patterns suggest possible "centers of calcification" (Fig. 11.9f, g).

A remarkable contrast exists between these fibers, more or less perpendicular to the common wall between neighbor corallites and the skeletal lamellae that are basically parallel to the corallite walls. Figures of the fibrous walls in the Tabulate corals *Thamnopora* and *Trachypsammia* (Fig. 11.9i, j) and the lamellar *Michelinia* (Fig. 11.9l, m) epitomize this difference in the biomineralization processes: both taxa gather colonies that are composed of polygonal units (Fig. 11.9h–k). Some criticisms have been presented against the use of microstructural patterns in Tabulate studies due to potential influence of diagenetic processes (e.g. Oekentorp 2007). Interestingly, a series of recent isotopic measurements (Zapalski 2014) suggests that in some cases (as it may also occur for skeletons of Scleractinia) the influence of these post-mortem changes might have been less than suspected. Additionally, remarkable improvements have been brought to histological studies during last decades. Ultra-thin sections (thickness 4–5 μm) observed between crossed nicols (Lafuste 1963) enable examination of individual skeleton units as exemplified by Fig. 11.9h, i, l, m.

Recent publications (e.g. Zaika 2010) have supported the use of investigations based on the concept of biomineralization. Considering what has occurred to the morphology-based evolutionary scheme of the Scleractinia (e.g. Wells 1956) when it was confronted to microstructural analysis and then molecular studies, working hypothesis can reasonably be made that precise histological characterizations of Tabulate corals may determine an in-depth modification of the classical figure of lineages diverging from a single ancestor (e.g. Scrutton 1983, p. 113).

11.6 Genomic Control Versus Environmental Influences on Cnidaria Calcification

In addition to evolutionary processes, another type of influence may affect, and indeed may be recorded within Cnidaria skeletons. From early Ordovician times to present the mechanisms of calcification in the Cnidaria have been submitted to the continuous changes that have occurred in sea-water composition. In addition to these slow running changes much more abrupt variations may have been influential on the biomineralization process. Since the landmark paper by Raup and Sepkowski (1982) the origin of the "big five" evolutionary crises has triggered a number of investigations in which magma floods linked to plate tectonics and asteroid collisions with Earth are associated. Variations in chemical composition of sedimentary carbonates through the geological column led Sandberg (1983) to construct an oscillating curve summarizing the dominance of calcite or aragonite precipitation in the Paleozoic and post-Paleozoic times respectively. This result has been tentatively correlated to the

catalogue of Ca-carbonate polymorphs used by living organisms through time (Stanley and Hardie 1998; Ries et al. 2006). Distribution of calcite versus aragonite in the Cnidaria skeletons that have been examined through the whole geological column can be summarized as a general agreement marked by noticeable exceptions. Most of the Paleozoic Cnidaria were using calcite as skeletal mineral but in the Tabulate family Tetradiidae skeletons were recognized aragonitic (Wendt 1989). Post-Paleozoic Cnidaria offer a contrasted view. Among the numerous and diversified Octocorallia calcite is quite dominant (in contrast to the Sandberg's aragonite sea) with the exception of the Blue Coral (*Heliopora coerulea*) that constructs its skeleton with aragonitic fibers. Scleractinia have produced aragonite skeletons since the Triassic period but during Cretaceous some examples have convincingly established evidence of shift to calcitic skeletons (Stolarski et al. 2007). Interestingly some aragonite corals were found in the upper Permian (Wendt 1990) whereas other representatives of the Paleozoic corals still have their calcite skeletons. Some remarkable Triassic corals produced aragonite skeletons although having preserved the typical "bauplan" (or "skeletogenesis program") of a typical Permian calcitic family (Cuif 2013). This complex result suggests that instead of being submitted to a simple chemical control, determination of the used polymorph and its possible variation through time should be examined from a biomineralization viewpoint.

It is now established that the biological calcification process involves several tens (or hundreds) molecules whose genomic approaches have revealed the diversity and taxonomy-linked specificity. Secreted outside the mineralizing organ (e.g. the basal ectoderm of the Cnidaria) these complex molecular assemblages control every aspect of skeletal deposition. Which part of these secreted compounds determines the used calcium carbonate polymorph is still unknown, as is the sensitivity of this mineralogy driving molecular assemblage to environmental conditions. What underlines the paleontological data is that a mineralogical shift may occur without changing the microstructure of the skeletons. These recent data have important taxonomic implications as they clearly suggest that skeletal mineralogy as a taxonomic character must be subordinated to the much more complex genomic mechanism that drives the three-dimensional assemblage of the skeletal units. Among the present Cnidaria the Stylasteridae (skeleton producing Hydrozoa) offers the most demonstrative example that mineralogy cannot be considered as major criteria because if the majority of them produce aragonite skeletons a significant part use the calcite polymorph without significant correlation to taxonomy (Cairns and Macintyre 1992).

What has been revealed about histological diversity of the Tabulate corals during the last decades suggests that distinct branches are deeply rooted in the Precambrian, and should be considered as illustrations of the huge gap between formation of the phylum and separated emergences of calcification.

Undoubtedly overall evolutionary process of the Cnidaria during the Paleozoic (as seen from calcifying forms) appears more comparable to what can be seen in other phyla. The 510 million year old fossils of Burgess (middle Cambrian) bring punctual evidence for a proliferating branching pattern inherited from early stages of Arthropod diversification. This is also well demonstrated by the phylum Echinodermata in which about 15 major subdivisions in the Class range were recognized during the Paleozoic era (and sometimes more e.g. Paul and Smith 1988). Of these 15 Classes, only five survived the mass extinction that marks the end of the Paleozoic era. Diversity of the Tabulata corals through Paleozoic offers a strikingly comparable panorama, the last family becoming extinct during the Permian. From a methodological viewpoint evaluating biological diversity by taking into account low level taxa obviously favors the recent periods of time, neglecting the more deeply original lineages which deserve higher taxonomic ranking but have become extinct during geological history.

11.7 Enigmatic Specimens from the Cambrian Period and Earliest Indication of a Cnidarian Type Calcification

Below the Lower Ordovician, fossil records do not provide unambiguous information about Cnidaria. Data illustrating at best this uncertainty are the numerous imprints first found by Reginald Sprigg (1947) in the Ediacara site (an about 635–542 million years old outcrop of the Flinders Range, South Australia) and subsequently discovered in many other places always older than the Cambrian period (i.e. the occurrence of animals bearing mineralized structures). This led to the concept of an Ediacarian age predating the "Cambrian biological explosion". There is a wealth of literature (Pratt et al. 2001) dedicated to these fossils since the Glaessner's paper "Precambrian animals" (1961). Exemplifying how uncertain interpretation of these documents can be, the long standing attribution to marine jellyfishes of many of the samples known from the Ediacara outcrops have been challenged during recent years by a series of new observations. The familiar figures of *Dickinsonia*, *Cyclomedusa* etc. long viewed as primitive Cnidaria were more probably fossil remains of "lichens or microbial consortia developed in paleosols formed onto emerged sandy sea shores" (Retallack 2013).

Many small and morphologically diverse calcareous structures have been discovered in sediments of the Cambrian period. Reviewing these fossils, Jell (1984) made a rather skeptical statement regarding our ability to decide on morphological basis only whether they actually belong to the phylum

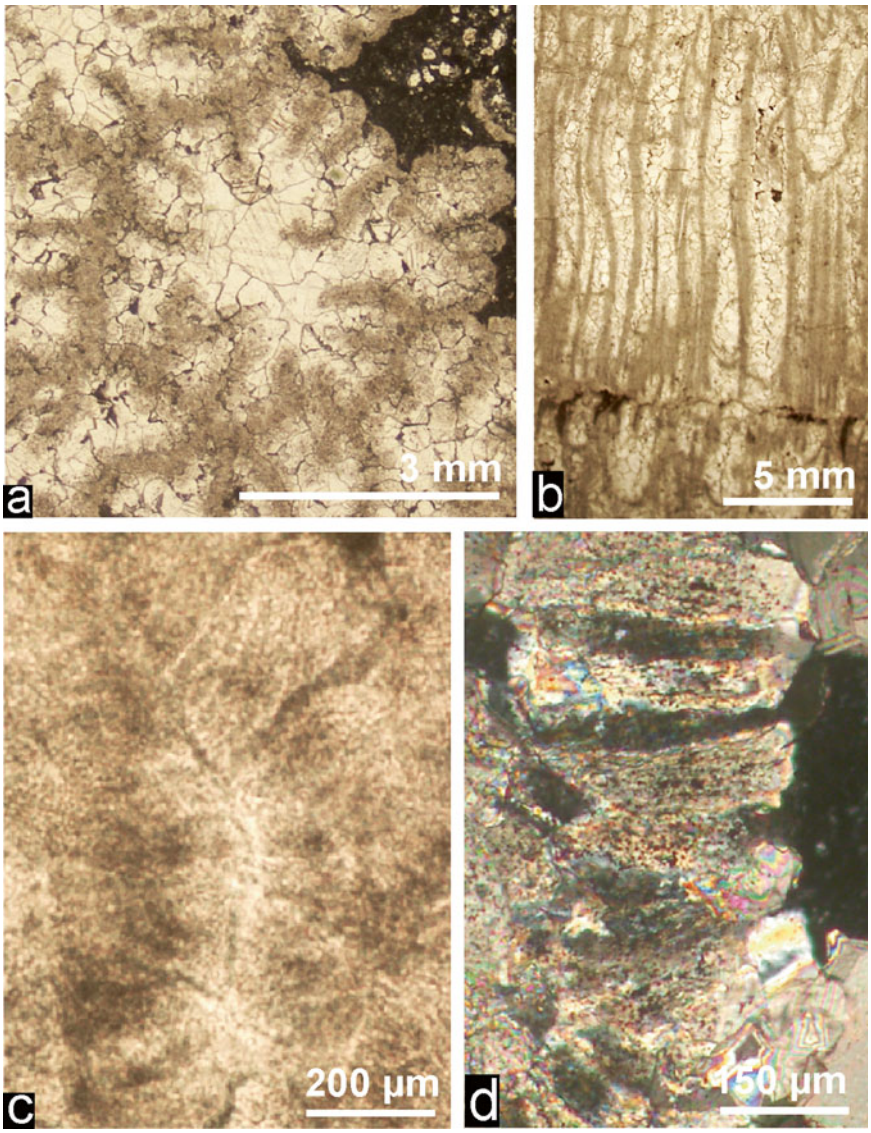

Fig. 11.10 *Flindersipora* morphological and microstructural patterns. (**a**, **b**) Section perpendicular to growth axis: possible wall and radial components of the skeleton; (**c**, **d**) Fibrous microstructure viewed on natural light (**c**); crossed-nicols polarized light show that fibers have undergone a slight diagenetic process restricted to crystallographic coalescence of the neighbor fibrous units (**d**)

Cnidaria or not. A recent example is given by some Middle Cambrian (−509 to −493 My) conically shaped calcareous structures possessing height symmetrically arranged internal radial lamellae (*Cambroctoconus* Park et al. 2011). Compared to these small and localized fossils the enigmatic Conularids appear much more important. However, as a recent episode of the 150 year old controversy regarding their biological placement, the Babcock (1991) versus Van Iten (1991) confrontation could not permit a firmly based opinion to be established. Ranging from middle Cambrian to Triassic (Moore and Harrington 1956) these organisms were producing phosphatic pyramidal shells reminding that Bayer and McIntyre (2001) have detected carbonate hydroxy-apatite in skeleton of some Gorgonians. Fourfold symmetry (a pattern known from some Medusozoa) and exceptional soft-part preservation revealing soft protruding tentacles support Cnidarian interpretation as a definitely marginal sub-phylum. Recent re-interpretation of the Cambrian bryozoa *Pywackia* as an octocoral (Taylor et al. 2013) provides an example of these calcareous structures reinforcing the evidence that not only were the major branches of the Cnidaria well established far below the basal Cambrian but also indicate that the complex metabolic pathway leading to production of mineralized structures has been undergone by a variety of lineages.

Among them *Flindersipora* (Lafuste et al. 1988, 1991) appears as a remarkable exception. This colonial skeleton is dated from the Lower Cambrian of Australia through its association with Botomian Archaeocyathids. The thick skeletal layers are made of vertical laminae frequently radially arranged (Fig. 11.10a), a structure suggesting a colonial organization laterally expanding. Noticeably, each component of the calcified structure exhibits a double-sided fibrous microstructure with a continuous median line between the fibrous layers (Fig. 11.10c, d).

What makes *Flindersipora* an interesting case-study in the search for earliest corallian organization is the similarity

between the microstructural patterns observable in these basal Cambrian specimens (Fig. 11.10) and equivalent pattern in more recent specimens. Architecture of the *Flindersipora* is presently unique among the morphological diversity of the numerous Cambrian enigma (Babcock 1991) and cannot be definitely placed in any of the present higher taxa. But its histological features indicate that the sequential coordination of gene activity required to produce a bilateral fibrous biocalcification was well established at the lowest Cambrian times, providing the earliest support to prevalence of microstructural characteristics over morphological and architectural patterns.

Consistency of the structural and biochemical data obtained by applying physical methods to recent and fossil Cnidaria skeletons offers a renewed perspective in the project of an integrated taxonomic approach enabling a more reliable evolutionary scheme to be progressively constructed.

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A Non-traditional Stable Isotope Perspective on Coral Calcification

12

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Abstract

Corals are unique marine organisms whose skeletons are a primary component of both tropical and cold water reefs. The ability of corals to convert aqueous seawater ions into the calcium carbonate ($CaCO_3$) mineral aragonite at a tremendous rate in the tropics, and at all in the inhospitable deep ocean, is an impressive feat that has long been a topic of intrigue. The isotope and trace element composition of annually-banded coral skeletons are often used to generate multi-century reconstructions of past environmental variability with a near-absolute chronology. However, geochemical signals preserved in coral skeleton that cannot be attributed to environmental variables also provide insight into the biomineralization process. The fractionations of carbon (C) and oxygen (O) isotopes between coral skeleton and seawater have been particularly valuable in this regard due to the ease with which they can be compared with abiogenic aragonite precipitation experiments that hope to isolate the effects of specific processes. Such studies have prompted the development of a number of semi-quantitative coral biomineralization models, some of which indicate that bulk transport of seawater to the calcifying space, and subsequent biologically mediated modification of alkalinity and dissolved inorganic carbon, is a likely explanation for observed variability. A number of "non-traditional" isotope systems can now be measured with unprecedented precision due to recent advances in mass spectrometry, including boron (B), calcium (Ca), magnesium (Mg), and strontium (Sr), as well as the "clumping" of heavy carbon and oxygen isotopes in multiply substituted isotopologues. This chapter reviews the fractionation of these isotopes between coral and seawater, and interprets their trends in the context of biomineralization models based on C and O isotopes. Based on consistent differences between corals and abiogenic experiments, it can be generalized that coral skeletogenesis is an extremely rapid, biologically induced process that does not favor equilibrium isotope fractionation. Processes such as pH, precipitation rate and Rayleigh fractionation can explain much of the variability in coral non-traditional stable isotopes, although their relative importance varies among elements and coral species. For example, pH is the primary control on coral B isotope composition, but only influences clumped isotopes indirectly by affecting the rate of isotopic equilibration. Rayleigh fractionation may be of importance to Ca isotope fractionation, and perhaps that of Sr isotopes, but cannot explain

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Mg isotope fractionation, which may be more strongly impacted by precipitation rate. Despite many remaining uncertainties, this review demonstrates that geochemical processes influenced by, but not completely controlled by, physiology can explain the non-traditional stable isotope composition of corals.

Keywords
Coral • Stable isotopes • Boron isotopes • Clumped isotopes • Calcium isotopes • Strontium isotopes • Magnesium isotopes • Biomineralization • Vital effects

12.1 Introduction

Coral reefs are among Earth's largest and most impressive bioconstructions. Hermatypic tropical reefs are common in low latitudes covering about 617,000 km^2 and 15 % of the ocean's shallow seabed (Smith 1978), while ahermatypic corals occur throughout the high latitudes and deep oceans (Cairns and Stanley 1982). Both environments host a rich diversity of life and even conservative estimates suggest that there are hundreds of thousands of reef species (McIntyre 2010). Habitat for many of these organisms is provided by a framework of coral skeletons cemented together by crustose coralline algae to form dense, erosion-resistant reefs (Goreau 1963). Individual coral colonies combine calcium (Ca^{2+}) and carbonate (CO_3^{2-}) ions to precipitate the calcium carbonate ($CaCO_3$) polymorph aragonite, and in most shallow species this occurs in tight symbiosis with photosynthetic zooxanthallae. The existence of reefs therefore relies on $CaCO_3$ being deposited more rapidly than physical, chemical and biological erosion can break them down. Historically, this has been the case, as evidenced by their evolutionary success over hundreds of millions of years (Hofmann 1963) and ability to outpace rapid sea level changes due to volcanic subsidence and glacial-interglacial changes in global ice volume. However, ongoing anthropogenic activity will present new and uncertain challenges for coral existence and calcification (e.g. ocean warming and acidification) in the coming century and beyond.

Coral calcification is sensitive to a number of environmental conditions including light, temperature, nutrient supply, ocean pH and turbidity (Goreau 1959; Grottoli 2002; Krief et al. 2010; McCulloch et al. 2012a). In addition to making corals susceptible to future change, this sensitivity makes them attractive targets for reconstructing past environmental variability. With life spans approaching half a millennium, massive coral colonies are some of the longest-lived organisms in the shallow ocean and ahermatypic deep sea species may live even longer (Roark et al. 2009). Physical and geochemical variations in the skeletons of corals are commonly interpreted as proxies for past environmental change allowing paleoceanographic conditions to be estimated prior to significant anthropogenic influence.

Many of the paleoceanographic proxies measured in corals are based on the isotopic composition of the coral skeleton. The stable isotopes of carbon (C) and oxygen (O) are likely the most common, although extensive work has also been conducted on other isotope systems such as the nitrogen stable isotopes and radiocarbon (Druffel 1981; Heikoop et al. 1998; Yamazaki et al. 2011). As described in this chapter, variations in coral isotopes are not only controlled by environmental variables, but can also be strongly affected by coral physiology. Often referred to as "vital effects", these biological impacts on coral geochemistry can sometimes overwhelm environmental signals leading to considerable frustration among paleoceanographers. The silver lining of this complication is that vital effects on the isotopic composition of corals can be used to probe their calcification processes, giving unique insight that complements observational studies. Advances in mass spectrometry over the past two decades have allowed new isotope systems to be measured with far greater precision. These so called "non-traditional" stable isotopes include Ca^{2+}, magnesium (Mg^{2+}), strontium (Sr^{2+}), boron (B) and others (Li, Fe, Cu, Zn, Ba etc.). Recent work has also started to explore the controls on the bonding of heavy C isotopes to heavy O isotopes in $CaCO_3$ referred to as carbonate clumped isotopes (Δ_{47}). Although investigations of these isotopes in corals are in their infancy, available data already provides considerable insight into coral calcification. The field is evolving quickly however, and new data will undoubtedly affect the conclusions presented in this chapter.

Following a brief introduction to isotope notation, this chapter first reviews what has been inferred about coral calcification from stable C and O isotopes, including conceptual models based on these data. We then discuss non-traditional isotopes relevant to the carbonate ion (CO_3^{2-}) component of coral aragonite, specifically B and Δ_{47} and how they may be used to refine, edit and build upon calcification models. Finally, we examine the stable isotopes of calcium and other divalent cations, including Mg^{2+} and Sr^{2+} that may substitute for Ca^{2+} in the aragonite lattice.

12.2 Basics of Isotope Fractionation in Carbonates

12.2.1 Isotope Notation

This review will focus primarily on the difference in the isotopic composition of corals relative to the seawater from which they precipitate their skeleton. The isotopic composition of a substance is reported in delta notation with units of permil (‰) deviation from an international standard:

$$\delta^H X = \left(\frac{\left(R_X\right)_{sample}}{\left(R_X\right)_{standard}} - 1 \right) \times 1000$$

where X is the element of interest, the superscript H refers to the mass of the heavy isotope and R_X is the ratio of the heavy to light isotopes in either the sample or standard:

$$R_X = \frac{^H X}{^L X}$$

For example, the oxygen isotope composition of a coral might be reported relative to a common belemnite standard from the PeeDee formation (PDB):

$$\delta^{18}O_{coral} = \left(\frac{\left(\frac{^{18}O}{^{16}O}\right)_{coral}}{\left(\frac{^{18}O}{^{16}O}\right)_{PDB}} - 1 \right) \times 1000$$

When the isotopic composition of two substances is known, the isotopic fractionation between them is given by the fractionation factor:

$$a_{A-B} = \frac{R_{X,A}}{R_{X,B}}$$

where A and B are the two substances of interest and R_X is as defined above. For the fractionation of oxygen isotopes between coral and seawater the fractionation factor is:

$$a_{coral-sw} = \frac{\left(\frac{^{18}O}{^{16}O}\right)_{coral}}{\left(\frac{^{18}O}{^{16}O}\right)_{sw}}$$

Because this value is so close to 1, it is very common for fraction to be reported as 1000lnα, which can be related to delta values, provided they are reported relative to the same standard:

$$1000 \ln a_{A-B} = \frac{1000 + \delta^H X_A}{1000 + \delta^H X_B}$$

It is also increasingly common, particularly with non-traditional stable isotopes, for fractionations to be reported using capital delta notation:

$$\Delta^H X_{A-B} = \delta^H X_A - \delta^H X_B$$

This is roughly equivalent to the 1000lnα notation, though the later is more rigorous. For the case of coral-seawater O isotope fractionation then:

$$1000 \ln a_{coral-sw} = \frac{1000 + \delta^{18}O_{coral}}{1000 + \delta^{18}O_{sw}} \approx \Delta^{18}O_{coral-sw} = \delta^{18}O_{coral} - \delta^{18}O_{sw}$$

It must be noted that the Δ value above is distinct from that used in clumped isotope notation (Δ_{47}) and discussed in Sect. 6.

12.2.2 Processes Governing Isotope Fractionation

Isotopic fractionations between materials reflect differences in the energy of their molecules. These energy differences derive from variations in how individual atoms within a molecule vibrate, which in turn depends on the mass of those atoms and temperature (Urey 1947). At equilibrium it is expected that the isotope composition of a carbonate mineral will depend only on temperature and the isotopic composition of the fluid from which it precipitates (Epstein et al. 1953; O'Neil 1986; Rustad et al. 2010; Urey 1947). Whether or not a particular fractionation reflects equilibrium is difficult to prove, but these conditions are likely to be approached by natural carbonate minerals precipitated very slowly over thousands of years at stable conditions (Coplen 2007; Fantle and DePaolo 2007). Alternatively, equilibrium isotope fractionations can also be estimated using theoretical calculations that are based on the principles of thermodynamics and quantum mechanics, although the results of such calculations are usually sensitive to the assumptions used to solve the Schrödinger equations (e.g. O'Neil et al. 1969; Rustad et al. 2010; Schauble 2004). Finally, carbonate minerals precipitated slowly in laboratory experiments may approach equilibrium isotope fractionation (Böhm et al. 2012; Kim et al. 2007; Kim and O'Neil 1997; Sanyal et al. 2000; Tang et al. 2008; Wang et al. 2013b; Zaarur et al. 2013), and this method is most convincing when the same fractionation factor is achieved under various experimental conditions (e.g. O'Neil et al. 1969). However, the methods above are rarely, if ever, entirely consistent, leading to some uncertainty in the true carbonate-water isotopic equilibrium signature for most elements.

When carbonate precipitates out of isotopic equilibrium, a number of factors other than temperature and the isotopic composition of the fluid may be important. These include diffusion, desolvation, mineral growth rate, pH and the chemical

composition of the fluid. In general, the diffusion coefficient of light isotopes is greater than that of heavier isotopes of the same element (Bourg et al. 2010), and diffusion of dissolved cations through a fluid or membrane results in carbonate that is enriched in the light isotope. Similarly, the desolvation of light isotopes at the surface of a growing mineral is thought to be faster for many aqueous cations (Hofmann et al. 2012), also leading to light isotope enrichment in the mineral. Both processes may help explain carbonate-water isotope fractionations that show a dependence on growth rate (Böhm et al. 2012; DePaolo 2011; Gussone et al. 2003; Tang et al. 2008; Watkins et al. 2013), but other factors, such as the entrapment of distinct boundary layers at the fluid-mineral interface could also be important (e.g. Gabitov et al. 2012; Watson 2004).

The pH of the solution from which carbonate minerals precipitate is also a potential source of non-equilibrium isotope fractionation, particularly for C, O and Δ_{47}. Dissolved inorganic carbon (DIC) exists predominately as aqueous carbon dioxide ($CO_{2(aq)}$) at low pH, bicarbonate ion (HCO_3^-) at intermediate pH and carbonate ion (CO_3^{2-}) at high pH (Zeebe and Wolf-Gladrow 2001). The oxygen isotope fractionation factor between each of these DIC species and water decreases such that $1000\ln\alpha_{CO2(aq)\text{-}H2O} > 1000\ln\alpha_{HCO3\text{-}H2O} > 1000\ln\alpha_{CO3\text{-}H2O}$ (Beck et al. 2005) and variations in the contribution of each DIC species to the mineral can affect the overall mineral-water isotopic fractionation (Hill et al. 2014; Wang et al. 2013a). Furthermore, solution pH affects the rate of isotopic equilibration among DIC species, potentially leading to non-equilibrium fractionation if mineral growth rate is faster than the time necessary for isotopic equilibration (McConnaughey 1989a).

12.3 Basics of Coral Calcification Relevant to Stable Isotopes

The topic of coral calcification is discussed in detail in Cohen and McConnaughey (2003), Allemand et al. (2011), and Tambutté et al. (2011). Although considerable uncertainty exists on this subject, it is generally agreed that a coral's tissue layer is made up of four cellular layers: the oral ecto- and endoderm, which are separated from the aboral endoderm and the calicoblastic ectoderm by the coelenteron (Tambutté et al. 2011, Fig. 12.1). The coelenteron is open to ambient seawater through the mouths of coral polyps. Coral calcification takes place in a semi-isolated extracellular calcifying compartment below the calicoblastic ectoderm and the fluid (or gel) occupying this thin sub-calicoblastic space likely contains the ions necessary for calcification.

Descriptions of coral calcification can generally be divided into biologically induced and biologically controlled models (Tambutté et al. 2011). Biologically induced models suggest that corals actively manipulate the chemical composition of the calcifying fluid to promote aragonite precipitation, but do not control the actual mineral growth, which is largely an abiotic process (e.g. Holcomb et al. 2009). In contrast, biologically controlled models suggest that the coral

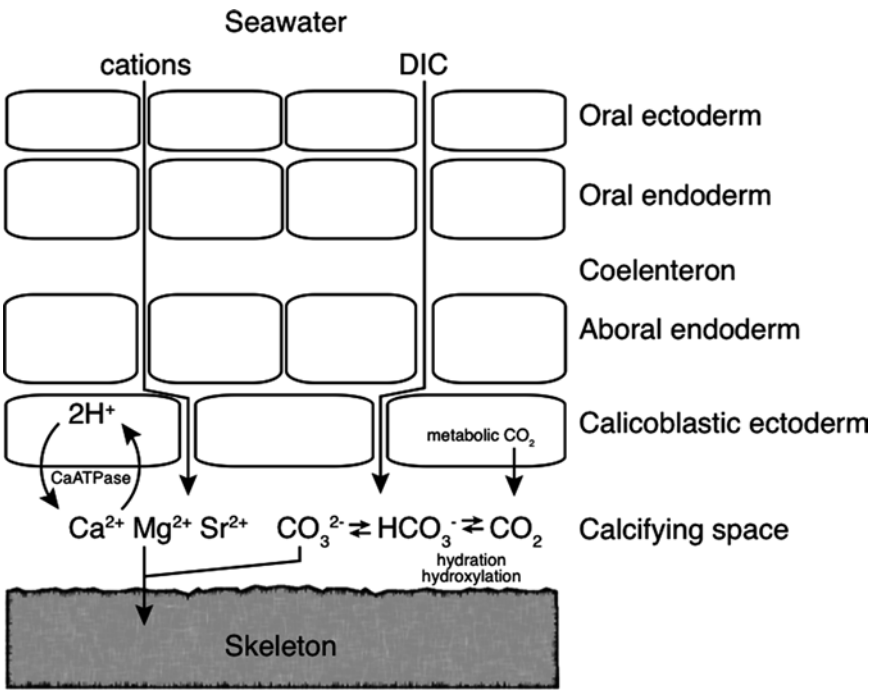


Fig. 12.1 Schematic cross section of a coral tissue layer illustrating the major processes of the simple calcification model assumed in this review. Cations are supplied primarily from bulk seawater with a small additional Ca^{2+} contribution from CaATPase pumping. This and possibly other ion transporters elevate pH_{cf}. Carbon and oxygen are supplied from seawater DIC, metabolic CO_2 and an inorganic internal carbon pool. Seawater DIC is assumed to be in isotopic equilibrium while C and O derived from inorganic and metabolic CO_2 may be prone to kinetic effects associated with the slow rate of hydration and or hydroxylation

closely regulates mineral formation by, for example, actively transporting ions to the calcifying space or constructing an organic template to guide aragonite growth (e.g. Allemand et al. 1998). As elaborated on below, we adopt a biologically induced view of calcification in this chapter, in part because the processes known to affect isotopic fractionation in abiogenic experiments are capable of explaining the majority of the variability observed in corals, but also because the isotopic fractionations expected from various biologically controlled processes are poorly known.

As a starting point for understanding many isotope systems, we assume seawater is transported directly to the calcifying fluid either through paracellular pathways or between polyps, thereby supplying a high concentration of ions without a significant energetic cost compared to active transport through the cell (e.g. Böhm et al. 2006). This view is supported by experiments in which cell impermeable fluorescent dyes and beads were incorporated into the skeleton of multiple coral species, likely by transport from the ambient water to the calcifying space via intercellular junctions (Tambutte et al. 2012). Similarly, NanoSIMS profiles of corals cultured in seawater enriched in Ca, Sr, Ba and the rare earth element terbium (Tb) demonstrate that all four elements are taken up at the same rate, consistent with the direct transport of seawater to the extracellular calcifying space (Gagnon et al. 2012).

If seawater enters the calcifying space without modification, the concentrations of Ca^{2+} and DIC would be approximately 10 mM and 2 mM, respectively. However, microelectrodes (Al-Horani et al. 2003) and steady state geochemical models (Gagnon et al. 2012) suggest that the concentration of Ca^{2+} in the calcifying space can be elevated by ~5 % above ambient values. This increase in Ca^{2+} is widely attributed to the activity of a Ca-ATPase pump, which transports Ca^{2+} to the calcifying space. Support for this mechanism comes from experiments that show that Ca^{2+} concentration is not elevated when Ca-ATPase activity is inhibited (Al-Horani et al. 2003), and from the localization of the enzymatic pump within coral cells (Zoccola et al. 2004). It is generally believed that charge balance is maintained during Ca-ATPase activity by removing two hydrogen ions (H^+) from the calcifying space for each Ca^{2+} that enters (Carafoli 1991; Fig. 12.1), thereby elevating calcifying fluid pH (pH_{cf}). However, similar elevation of pH_{cf} could also be achieved through other proton transporters such as Na^+/H^+ exchangers (Laurent et al. 2014). Regardless of the mechanism by which pH_{cf} is raised, microelectrode and fluorescent dye studies support its occurrence and suggest that pH at the site of calcification is elevated by ~0.2–1.0 units above ambient (Al-Horani et al. 2003; Venn et al. 2011).

The relationship between Ca^{2+}, pH_{cf} and calcification can be understood in terms of the aragonite saturation state of the calcifying fluid (Ω_{cf}):

$$\Omega_{cf} = \frac{\left[Ca^{2+}\right]_{cf}\left[CO_3^{2-}\right]_{cf}}{K_{ar}}$$

where $[Ca^{2+}]$ and $[CO_3^{2-}]$ are the calcium and carbonate ion concentrations in the calcifying fluid and K_{ar} is the solubility constant of aragonite. When the product of $[Ca^{2+}]$ and $[CO_3^{2-}]$ is greater than K_{ar}, Ω_{cf} is greater than 1, the calcifying fluid is supersaturated and precipitation is favored. When the product of $[Ca^{2+}]$ and $[CO_3^{2-}]$ is less than K_{ar}, Ω_{cf} is less than 1, the calcifying fluid is undersaturated and dissolution is favored. Re-writing the relationship above in terms of $[DIC]_{cf}$ and pH_{cf} gives:

$$\Omega_{cf} = \frac{\left[Ca^{2+}\right]_{cf}\left[DIC\right]_{cf}}{K_{ar} \times \left\{1 + \frac{\left[H^+\right]}{K_2^*} + \frac{\left[H^+\right]^2}{K_1^* K_2^*}\right\}}$$

where $pH_{cf} = -\log[H^+]_{cf}$ and K_1^* and K_2^* are the first and second equilibrium constants of the seawater/carbonate system ($K_1^* = [HCO_3^-][H^+]/[CO_2]$ and $K_2^* = [CO_3^{2-}][H^+]/[HCO_3^-]$). Thus, it can be seen that elevating $[Ca^{2+}]$, $[DIC]_{cf}$ and pH_{cf} all have the potential to promote coral growth by elevating Ω_{cf}.

Given the demonstrated ability of corals to manipulate their calcifying fluid composition, it seems logical that they would also concentrate carbon to the point that DIC concentration equaled that of Ca^{2+}. Such carbon concentration is likely linked to elevated pH_{cf}, which promotes CO_2 diffusion into the calcifying space, thereby increasing DIC (Allison et al. 2014). Experiments in which corals were grown in ^{45}Ca and ^{14}C enriched solutions suggest that a portion of this CO_2 may derive from metabolism (Erez 1978; Furla et al. 2000; Goreau 1959), consistent with evidence that skeletal carbon acquires the isotopic signature of radioactively labeled food (Pearse 1970). Although dual ^{45}Ca and ^{14}C studies suggest that the majority (~70 %) of carbon may derive from respired CO_2 (e.g. Furla et al. 2000), carbon isotope balance indicates that this may be an overestimate. For example, assuming the $\delta^{13}C$ of seawater DIC is ~1‰ and that of respired CO_2 is similar to coral tissue (~−15‰; Swart et al. 1996), typical skeletal $\delta^{13}C$ values of −2 to −4‰ imply that only 20–30 % of carbon comes from respired CO_2. If this is the case, the additional CO_2 needed to balance DIC and Ca^{2+} concentrations likely derives from an internal carbon pool such as acidified seawater in the coelenteron or calicoblastic space in much the same way as it is supplied by acidic vesicles within foraminifera (Bentov et al. 2009). A similar mechanism may operate in deep sea corals, which incorporate only ~5–10 % of respired CO_2 into their skeletons (Adkins et al. 2003).

In shallow species, calcification is intimately linked to photosynthesis, and light enhanced calcification has long been known to occur in corals (Goreau 1959). Although

much remains unknown about this relationship, it is often argued that calcification supplies aqueous CO_2 for photosynthesis while photosynthesis supplies nutrients and carbonate ions for calcification (Cohen and McConnaughey 2003; Schneider and Erez 2006). However, photosynthesis is clearly not required for calcification as evidenced by dark calcification in shallow corals and the shear existence of deep-sea corals that thrive far below the photic zone.

We use these observations as the basis for a simple calcification model that will serve as a starting point for interpreting coral stable isotope data (Fig. 12.1). In this model, Ca^{2+} is supplied to the calcifying fluid primarily through the direct transport of seawater through either paracellular pathways or between polyps, with the possibility that small amounts of additional Ca^{2+} comes from Ca-ATPase pumping. Other cations that may substitute for Ca^{2+} in the aragonite lattice (i.e. Mg^{2+}, Sr^{2+}) are supplied by seawater, but we assume these elements are not transported by the Ca-ATPase pump. Seawater DIC also brings C and O to the calcifying space with their speciation dependent on pH_{cf}. We assume seawater DIC is chemically and isotopically equilibrated. In contrast, additional C and O derived from metabolic or acidified seawater CO_2 must react with water or hydroxyl ion (OH^-) through hydration and hydroxylation, respectively, to produce HCO_3^- prior to being used for skeletal construction, and may not be in isotopic equilibrium. Furthermore, the proportion of HCO_3^- formed through hydration or hydroxylation will depend on pH_{cf}, with hydroxylation favored when pH is greater than ~8.7. The enzyme carbonic anhydrase (CA), which promotes isotopic equilibrium by catalyzing CO_2 hydration, may also be important particularly at low pH where hydration dominates.

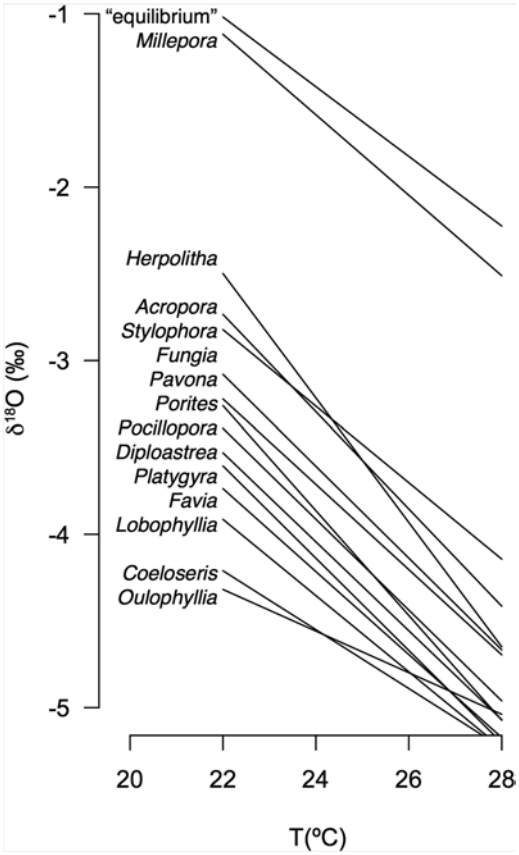


Fig. 12.2 Temperature dependent oxygen isotope variability in various coral genera (Data from Weber and Woodhead 1972b)

12.4 Stable Carbon and Oxygen Isotopes

To inform the discussion of non-traditional stable isotopes in corals, it is helpful to discuss briefly the rich literature on the stable C and O isotopic composition of coral skeleton. In this section we first describe the patterns these isotopes exhibit in corals followed by conceptual and quantitative models that have been proposed to explain them. Our review is far from complete, and the reader is directed elsewhere for a more comprehensive discussion of the topic (Erez 1978; Goreau 1977; Grottoli 2000; Grottoli and Eakin 2007; Leder et al. 1996; Swart 1983; Swart et al. 1996).

Corals were excluded from early paleoclimate reconstructions because their $\delta^{18}O$ differed from the value expected based on both laboratory experiments and the temperature dependence of other marine calcifyers (Lowenstam and Epstein 1954). Drs. Jon Weber and Peter Woodhead resumed research on coral isotopes in the early 1970s by conducting large surveys of hundreds of coral specimens from dozens of genera (Weber and Woodhead 1970, 1972a, b). An investigation of Heron Island, Australia corals found $\delta^{18}O$ and $\delta^{13}C$ to vary by 4‰ and 13‰, respectively, among different genera with linearly-correlated offsets toward values lower than expected at apparent equilibrium (Weber and Woodhead 1970). This was attributed to varying proportions of skeletal $CaCO_3$ derived from respired CO_2 and seawater DIC, with additional enrichments in ^{13}C, but not ^{18}O, due to the photosynthetic activity of zooxanthallae (c.f. Swart 1983). Expansion of this work to other sites showed that most corals also exhibit a $\delta^{18}O$ temperature dependence similar to apparent isotopic equilibrium, but with an offset that varies among genera (Weber and Woodhead 1972b; Fig. 12.2). No temperature dependence was observed in $\delta^{13}C$, and Weber and Woodhead (1972b) attributed the offset from apparent equilibrium to constant, genera-specific, effects of respired CO_2, seawater DIC and photosynthesis on coral-seawater isotope fractionation that was overprinted by a temperature signal.

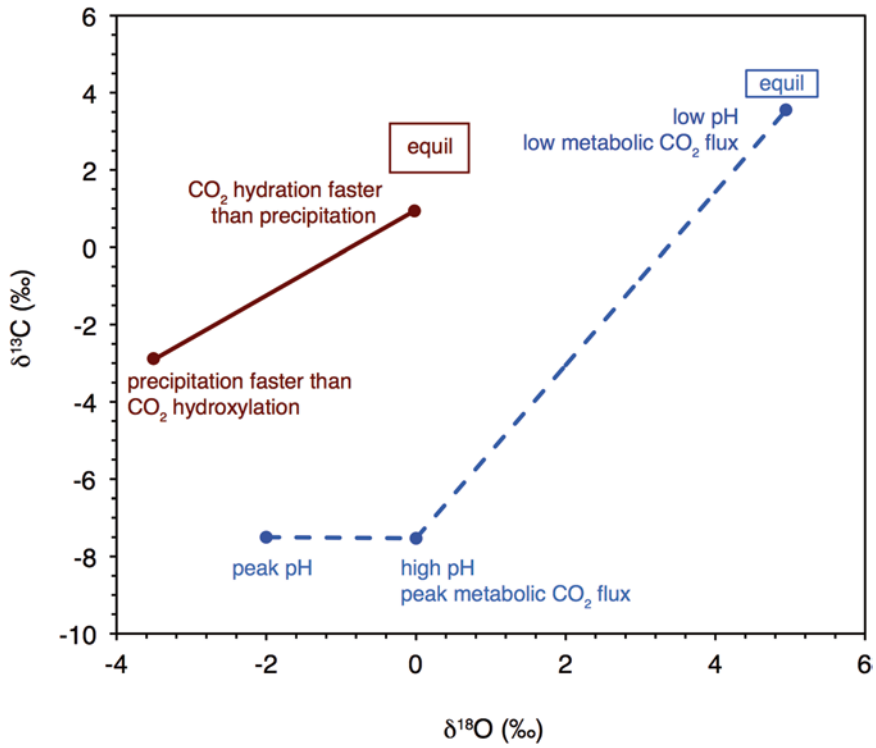


Fig. 12.3 Schematic illustration McConnaughey's (1989b; *red*) and Adkins et al.'s (2003) biomineralization models. The box labeled "equil" approximates isotopic equilibrium

Subsequently, Goreau (1977) presented a physiologically-based box model that explained both $\delta^{13}C$ and $\delta^{18}O$ in hermatypic corals, and suggested ~40 % of skeletal carbon derived from seawater and ~60 % from respired CO_2. However, this model included many degrees of freedom that added considerable uncertainty to these conclusions. Work by Erez (1978) combined physiological measurements with $\delta^{13}C$ and $\delta^{18}O$ data to demonstrate discrepancies between the models of Weber and Woodhead (1972b) and Goreau (1977). He showed for the first time that hermatypic corals likely have internal carbon pools that may contain an appreciable amount of respiratory CO_2 capable of inverting $\delta^{13}C$-photosynthesis trends and affecting $\delta^{18}O$. Additional work has supported many of these ideas (Leder et al. 1996; Swart et al. 1996).

Previous models were elaborated on by McConnaughey (1989a) who suggested that correlated offsets in $\delta^{13}C$ and $\delta^{18}O$ from apparent equilibrium could be explained by a kinetic mechanism associated with hydration and hydroxylation if the majority of skeletal C and O derived from respired CO_2. Specifically, he argued that the rate at which isotopes equilibrated during hydration and hydroxylation was relatively sluggish compared to typical calcification, leading to preferential incorporation of light isotopes during rapid coral growth (Fig. 12.3). This idea was supported by evidence that both C and O isotopes approached the expected equilibrium value in slow growing regions of azooxanthallate *Tubastrea sp*. corals (McConnaughey 1989b). Zooxanthallate *Pavona sp*. corals showed similar trends, with $\delta^{13}C$ and $\delta^{18}O$ approaching apparent equilibrium in regions of slow growth, but had shallower slopes that were attributed to photosynthetically-driven enrichments in skeleton ^{13}C (Fig. 12.3). However, not all data is consistent with this model. For example, Erez (1978) observed that both $\delta^{13}C$ and $\delta^{18}O$ decrease with increasing photosynthesis, while Tambutté et al. (2007) showed that carbonic anhydrase (CA) affects *Tubastrea sp*. calcification and is present in both the tissue and organic matrix of the skeleton, making disequilibrium less likely (see below).

Similar linear trends in the $\delta^{13}C$ and $\delta^{18}O$ of deep-sea corals were difficult to explain by the same mechanism since these corals incorporate very little respired CO_2 into their skeleton and exhibited offsets toward lower $\delta^{18}O$ in rapidly precipitated trabecular centers that were not accompanied by coordinated offsets in $\delta^{13}C$ (Adkins et al. 2003). Instead, Adkins et al. (2003) proposed that slowly precipitated skeleton was formed near ambient pH_{cf}, mostly from HCO_3^- derived from seawater DIC, leading to $\delta^{13}C$ and $\delta^{18}O$ values near apparent equilibrium (Fig. 12.3). They suggested that rapidly precipitated skeleton was deposited when very active Ca-ATPase pumping significantly elevated pH_{cf}, forcing DIC speciation toward ^{18}O depleted CO_3^{2-} and driving more diffusion of isotopically light respired CO_2 into the calcifying fluid, consistent with the internal carbon pool suggested by Erez (1978). When diffusion of respired CO_2 reached a maximum, Adkins et al. (2003) suggested $\delta^{13}C$ would plateau, but additional proton pumping could still explain lower $\delta^{18}O$ values if pH_{cf} exceeded values of 10 (c.f. McConnaughey 2003).

CA catalyzes the hydration of CO_2, and is a critical variable in evaluating these and other models of coral $\delta^{13}C$ and $\delta^{18}O$ variability. CA accelerates the rate at which isotopes equilibrate among DIC species during CO_2 hydration, and concentrations of ~3.7×10^{-9} M can halve the time needed to reach equilibrium (Uchikawa and Zeebe 2012). Although its concentration is not known, CA has been isolated near the calcifying space in the calicoblastic ectoderm (Tambutté et al. 2007; Moya et al. 2008). If CA actively catalyzes CO_2 hydration, coral calcification would have to be rapid (although perhaps not unreasonably so) for the model proposed by McConnaughey (1989a, b) to be realistic. The presence of CA also makes it more realistic for isotopic equilibrium to be achieved at the extremely high pH values proposed in Adkins et al.'s (2003) model. Although CA does not catalyze the hydroxylation reaction, which would dominate at high pH values, the Baertchi effect (Lazar and Erez 1991, 1992) could still lower $\delta^{13}C$.

12.5 Boron Isotopes

12.5.1 A Proxy for Calcifying Fluid pH

The boron isotope ($\delta^{11}B$) proxy is based on the pH-dependent speciation of boron between borate ($B(OH)_4^-$) and boric acid ($B(OH)_3$) and the ~27‰ fractionation between them (Hemming and Hanson 1992; Klochko et al. 2006). It is generally believed that only the charged borate species is incorporated in aragonite, allowing pH_{cf} to be calculated from $\delta^{11}B$ under the assumption that solution $\delta^{11}B$ is known and constant (Hemming et al. 1998; Hemming and Hanson 1992; Hönisch et al. 2004) although there is some debate on this topic (Klochko et al. 2009). Early work suggested the $\delta^{11}B$ of coral skeleton was primarily controlled by the $\delta^{11}B$ of seawater (Vengosh et al. 1991), but this view has evolved, and today the proxy is primarily thought to reflect calcifying fluid pH (pH_{cf}) (Allison and Finch 2010a; Hönisch et al. 2004; Krief et al. 2010; McCulloch et al. 2012a; Reynaud et al. 2004; Rollion-Bard et al. 2003; Trotter et al. 2011).

The interpretation of $\delta^{11}B$-based pH depends on which equilibrium isotope fractionation factor is used. Studies based on a theoretical value (Kakihana et al. 1977) estimate that pH_{cf} is negligibly different from seawater (e.g. Reynaud et al. 2004), which is not consistent with the elevated pH that would result from proton pumping (Fig. 12.1). In contrast, an experimentally derived value (Klochko et al. 2006) calculates pH_{cf} values above ambient (e.g. Krief et al. 2010; McCulloch et al. 2012b) that are consistent with microelectrode studies (Al-Horani et al. 2003) and proton pumping, suggesting it may be the more accurate fractionation factor. Furthermore, recent work using confocal microscopy in combination with pH-sensitive fluorescent dyes suggests that *Stylophora sp*. pH_{cf} is elevated to about the degree suggested by $\delta^{11}B$ in the same species when the experimental value of Klochko et al. (2006) is assumed (Venn et al. 2011, 2013). This provides strong evidence for the up-regulation of pH_{cf} as well as the use of the experimentally derived boron isotope fractionation factor (Klochko et al. 2006).

Using a consistent fractionation factor, $\delta^{11}B$ in bulk sampled corals suggests that most zooxanthallate corals calcify at pH_{cf} of approximately 8.4–8.6, while azooxanthallate species precipitate their skeletons at modestly higher pH_{cf} values close to 8.8 (Fig. 12.4). Secondary ion mass spectrometry (SIMS) data, which can achieve high spatial resolutions of tens of microns, show similar results, but suggest that portions of azooxanthallate *Lophelia sp*. skeleton may occur at exceptionally high pH values of ~10 (Blamart et al. 2007, Fig. 12.4c). These results are generally consistent with the calcification schemes described in Sect. 4, in particular the high pH required by Adkins et al.'s (2003) model.

12.5.2 Relationship to $\delta^{13}C$ and $\delta^{18}O$

The likelihood that coral $\delta^{13}C$ and $\delta^{18}O$ are at least partially influenced by the pH at which skeleton is formed makes it logical to incorporate $\delta^{11}B$ analyses into investigations of stable C and O isotope variability. Variations in $\delta^{11}B$, $\delta^{13}C$ and $\delta^{18}O$ at approximately monthly resolution can show higher $\delta^{11}B$-based pH values coincident with higher $\delta^{13}C$ values, suggesting that periods of enhanced photosynthesis (which consume ^{12}C, leaving an isotopically-heavy residual C pool) may fuel greater ion pumping that raises pH_{cf} (Hemming et al. 1998). However, studies that reared corals under various pH, light, depth and feeding regimes (Grottoli 2002; Hönisch et al. 2004) showed that the difference between ambient water pH and pH_{cf} (ΔpH) was relatively constant, indicating that seasonal $\delta^{11}B$ variations could not be due to photosynthetic activity, and were more likely caused by seasonal variations in local seawater. Further evidence against a strong link between pH_{cf}, $\delta^{13}C$ and the extent of proton pumping comes from SIMS data, which shows little correlation between $\delta^{11}B$ and $\delta^{13}C$ at fine spatial scales that may reflect variations in the $\delta^{13}C$ of respired CO_2 over time (Allison and Finch 2012).

Studies investigating $\delta^{11}B$, $\delta^{13}C$ and $\delta^{18}O$ often indicate that the relationship between C and O isotope fractionation is not as closely coupled as suggested by many of the models in Sect. 4. For example, Hemming et al. (1998) found that variations in $\delta^{11}B$ and $\delta^{13}C$ were not accompanied by synchronous changes in $\delta^{18}O$. Seasonal variations in seawater $\delta^{18}O$ could have masked a relationship between $\delta^{13}C$ and $\delta^{18}O$, but this is not supported by SIMS analyses made on temporal scales too short for appreciable seawater $\delta^{18}O$ variability that also do not show a clear covariation between $\delta^{11}B$, $\delta^{13}C$ and

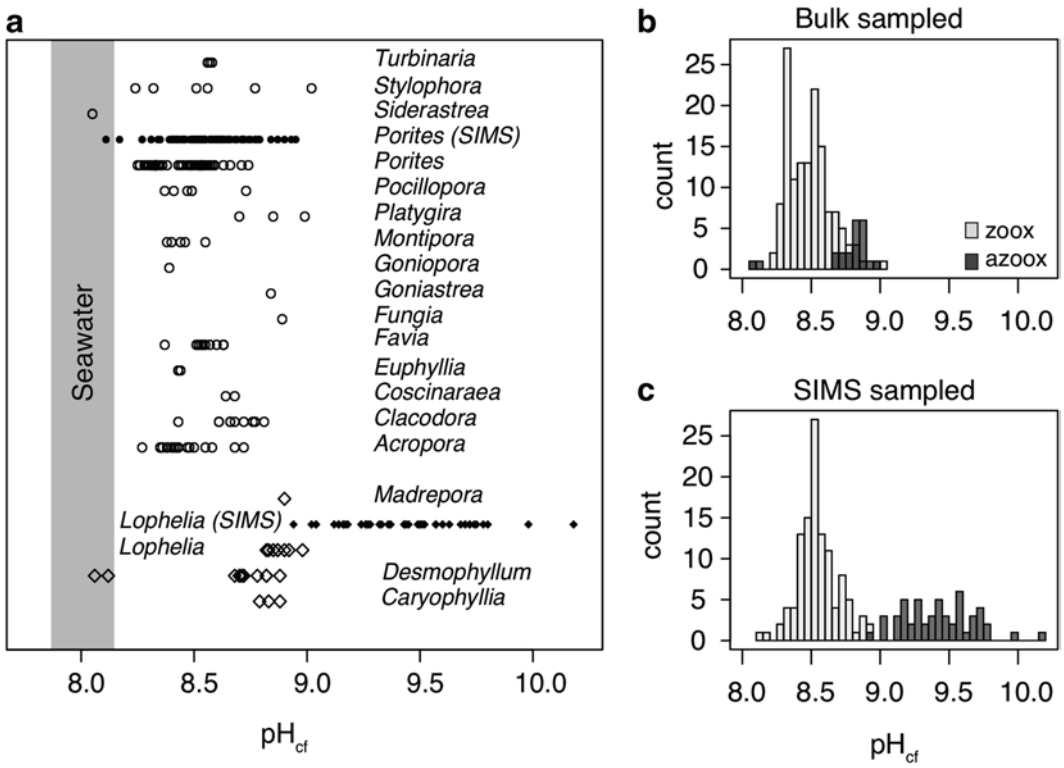


Fig. 12.4 **a** δ^{11}B-based pH_{cf} in various coral genera relative to typical seawater values. **b** histogram of δ^{11}B-based pH_{cf} in bulk sampled zooxanthallae (*light grey*) and azooxanthallae (*dark grey*) corals. **c** as in **b** for corals measured using SIMS (Data from Allison and Finch (2010a), Blamart et al. (2007), Calvo et al. (2007), Douville et al. (2010), Gaillardet and Allègre (1995), Hemming et al. (1998), Holcomb et al. (2014), Hönisch et al. (2004), Kasemann et al. (2009), Krief et al. (2010), McCulloch et al. (2012b), Pelejero et al. (2005), Reynaud et al. (2004), Rollion-Bard et al. (2003, 2011), Trotter et al. (2011), Vengosh et al. (1991), Wei et al. (2009), and Xiao et al. (2006, 2013))

δ^{18}O (Allison and Finch 2010a, b, 2012; Rollion-Bard et al. 2011, 2003). Although it is very likely that some kinetic and/or biologic processes affect both C and O isotope fractionation, the data above suggests that attributing δ^{13}C and δ^{18}O offsets from equilibrium to a single mutual source is an oversimplification.

Despite the equivocal relationship between δ^{11}B and δ^{13}C, the covariability of δ^{11}B and δ^{18}O can be satisfactorily explained using the principles described in Sect. 4. For example, cultured *Porites* and *Stylophora sp*. corals show higher δ^{18}O values at lower pH that parallel the shape expected from a pH-dependent shift in DIC speciation toward isotopically heavy HCO_3^- (Krief et al. 2010). However the trend is also consistent with kinetic effects that favor ^{18}O enrichment during slower calcification at lower pH, and is therefore consistent with both the models of McConnaughey et al. (1989a, b) and Adkins et al. (2003). Rollion-Bard et al. (2003, 2011) combined elements from these models to explain large δ^{18}O variations in SIMS data that spanned from near equilibrium to significantly ^{18}O depleted and could not be explained by temperature (Fig. 12.5). They proposed that ^{18}O depleted skeleton approached a kinetic endmember that inherited the δ^{18}O of the H_2O, CO_2 and OH from which it formed with little isotopic equilibration. The value of this kinetic endmember depended on the proportion of CO_2 that was hydrated or hydroxylated and therefore was pH dependent (Fig. 12.5). Furthermore, it was argued that ^{18}O enriched skeleton approached an equilibrium endmember whose value was set by DIC speciation and was therefore also pH dependent (Rollion-Bard et al. 2003, 2011). Most δ^{18}O data fell between these extremes reflecting partial equilibration of DIC species that depended on their residence time in the calcifying fluid prior to precipitation (Fig. 12.5). Allison and Finch (2010b) derived a similar model, but assumed only carbonate ions were incorporated into the coral skeleton. Although this is likely the case, modeling of abiogenic experiments strongly suggest that aragonite can acquire the isotopic signature of other DIC species (Wang et al. 2013a).

12.5.3 Biomineralization Response to Ocean Acidification

The rise in anthropogenic atmospheric CO_2 concentrations will further lower ocean pH and aragonite saturation state (Ω_{ar}) in the coming century (Broadgate et al. 2013). Understanding how corals will respond to this challenge

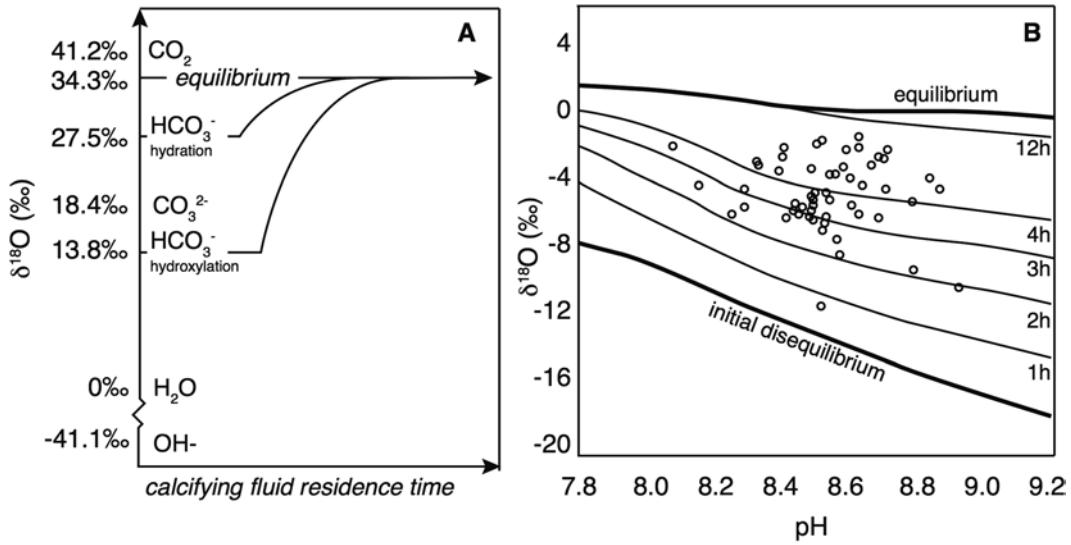


Fig. 12.5 **a** Theoretical evolution of calcifying fluid oxygen isotope composition due to the relative proportions of oxygen derived from hydration and hydroxylation, as well as their degree of isotopic equilibration (From Rollion-Bard et al. (2003)). **b** Coral δ^{11}B-based pH_{cf} and δ^{18}O overlaid on modeled δ^{18}O at various pH values and calcifying fluid residence times (From Rollion-Bard et al. (2011))

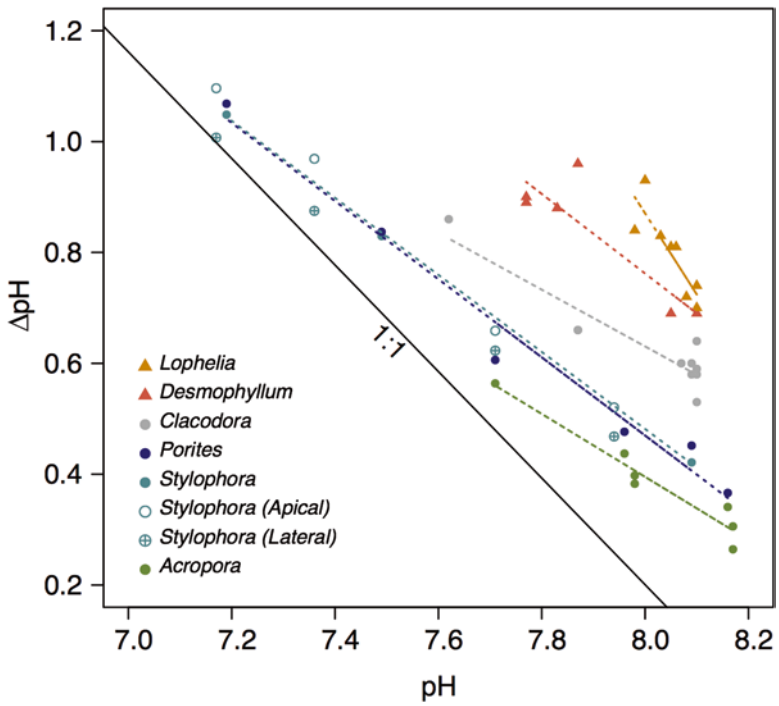


Fig. 12.6 The difference between δ^{11}B-based pH_{cf} and ambient water pH (ΔpH) at various ambient water pH values for various coral genera. The 1:1 *line* indicates the slope required for pH_{cf} to remain constant at lower ambient pH (Data from Holcomb et al. (2014), Hönisch et al. (2004), Krief et al. (2010), McCulloch et al. (2012b), Reynaud et al. (2004), and Trotter et al. (2011))

depends, in part, on how well they are able to elevate pH_{cf} under these conditions (Cohen and Holcomb 2009; Erez et al. 2011). Results from δ^{11}B studies have been valuable in this regard, and have also stimulated new theories on fundamental calcification processes.

Corals grown at elevated CO_2 concentrations (and lower ambient pH) demonstrate that calcification continues, albeit at a reduced rate, even when seawater becomes undersaturated ($\Omega_{cf} < 1$, see Sect. 3) (Hönisch et al. 2004; Krief et al. 2010; Reynaud et al. 2004). Boron isotope data indicate that this is possible in part because elevated pH_{cf} increases $[CO_3^{2-}]$ and allows the calcifying fluid to remain supersaturated. The magnitude of pH_{cf} elevation varies with ambient pH such that ΔpH increases by about 0.05–0.07 units for each 0.1 unit decrease in ambient pH (Holcomb et al. 2014; Hönisch et al. 2004; Krief et al. 2010; McCulloch et al.

2012b; Reynaud et al. 2004; Trotter et al. 2011; Fig. 12.6). Although this up-regulation will cause pH_{cf} to decline less rapidly than ambient pH (McCulloch et al. 2012a), it does not keep pace, suggesting that, all else being equal, coral calcifying fluid will become undersaturated with sufficient ocean acidification (Fig. 12.6).

Assuming the up-regulation of pH_{cf} inferred from $\delta^{11}B$ is related to proton pumping, larger ΔpH values likely come at an energetic cost. McCulloch et al. (2012b) estimate elevating pH_{cf} by 0.87 units requires ~10 % more energy than elevating it by 0.80 units. In symbiotic colonies, the extra energy necessary for up-regulation may be relatively small (<1 %) compared to that produced by photosynthesis (McCulloch et al. 2012a), but even these modest excess energy requirements may require adaptation. Some data suggests that enhanced photosynthesis may supply the extra energy, and that increases in ΔpH at more acidic ambient conditions may be achieved by fewer zooxanthallae with higher chlorophyll concentrations (Krief et al. 2010) working with greater efficiency to maintain the same carbon supply to the host coral (Tremblay et al. 2013). However, other studies found that lowering CO_3^{2-} concentrations (either by decreasing pH or lowering DIC at constant pH) did not affect the symbiont photosynthesis (Schneider and Erez 2006). In either case, more negative $\delta^{13}C$ values in the tissue of corals that exhibit large ΔpH suggest that aqueous CO_2 may replace HCO_3^- derived from calcification as the primary carbon source for photosynthesis (Krief et al. 2010; Mook et al. 1974; Schneider and Erez 2006). Since it has been suggested that the primary reason for coral calcification is to keep coelenteron pH down and CO_2 concentrations high (Cohen and McConnaughey 2003), it is conceivable that decreased coral calcification in high CO_2 experiments may occur not only because aragonite saturation states are lower, but also because calcification is less necessary to support photosynthesis under lower pH and higher CO_2 ambient conditions (Schneider and Erez 2006).

Photosynthesis clearly cannot supply the extra energy required for pH up-regulation in azooxanthallate corals, suggesting a metabolic mechanism may be more appropriate. Cold water corals exhibit a mixed responses to lower ambient pH with some specimens showing relatively normal growth and metabolism (Form and Riebesell 2012), others lower respiration rates without a change in calcification (Hennige et al. 2014) and still others increased calcification at higher pH without change at ambient or lower values (Maier et al. 2012). These studies are restricted to only a few genera however, and none employed $\delta^{11}B$ as a proxy for pH_{cf}, making even broad generalizations about the mechanism(s) driving ΔpH variability in cold water corals difficult. We look forward to what boron isotope variability is able to tell us about this intriguing question in the near future.

12.6 Carbonate Clumped Isotopes

12.6.1 A Temperature Proxy Without Vital Effects?

Carbonate clumped isotope thermometry (Δ_{47}) is a relatively new tool for estimating temperatures from the abundance of ^{13}C-^{18}O bonds in a $CaCO_3$ mineral (see Eiler 2007, 2011 for recent reviews). Unlike all other isotope systems in this chapter, which depend on the fractionation of isotopes between water and coral, Δ_{47} is independent of solution composition at equilibrium and is derived entirely from the mineral phase. Low Δ_{47} values reflect a stochastic distribution at high temperature with relatively few ^{13}C-^{18}O bonds, while high Δ_{47} values reflect preferential ^{13}C-^{18}O bonding at lower temperatures. Similar Δ_{47}-temperature relationships in abiogenic experiments and many biogenic carbonates (including mollusks, foraminifera, brachiopods, otoliths, snails and egg shells; Came et al. 2007; Dennis and Schrag 2010; Eagle et al. 2010; Ghosh et al. 2007, 2006; Henkes et al. 2013; Tripati et al. 2010; Wacker et al. 2014; Zaarur et al. 2011, 2013) have been used to suggest the proxy is relatively uninfluenced by biological vital effects and may reflect temperature without the need for empirical calibration (Fig. 12.7). However, it should be noted that the degree to which such arguments are convincing is dependent on the abiogenic temperature relationship to which natural carbonates are referenced.

The first investigation of coral Δ_{47} found that both shallow and deep sea specimens with known $\delta^{13}C$ and $\delta^{18}O$ vital effects generally agreed with abiogenic data (Ghosh et al. 2006). However, higher than expected Δ_{47} values in portions of *Porites sp*. skeleton deposited in winter yielded unrealistic temperature estimates near freezing that were attributed to a surprising, but unknown vital effect (Ghosh et al. 2006). Results from more comprehensive studies of Δ_{47} variability in corals generally confirm that deep sea species behave similarly to abiogenic experiments (Thiagarajan et al. 2011) while shallow water species exhibit clear offsets toward higher Δ_{47} that estimate temperatures ~8 °C cooler than observed (Saenger et al. 2012). However, very recent work suggests that vital or kinetic effects may also affect deep sea corals (Spooner et al. 2016)

Models for coral vital effects in other isotope systems make kinetic and/or pH effects logical candidates for the observed Δ_{47} offsets in shallow water species. Similar to the variations in oxygen and carbon isotope fractionation observed among DIC species and water, theoretical calculations and laboratory experiments suggest that the Δ_{47} of $CO_3^{2-} < HCO_3^- < CO_{2(aq)}$ (Hill et al. 2014; Tripati et al. 2015), raising the possibility that Δ_{47} could be sensitive to pH. However, higher pH_{cf} would increase the concentration

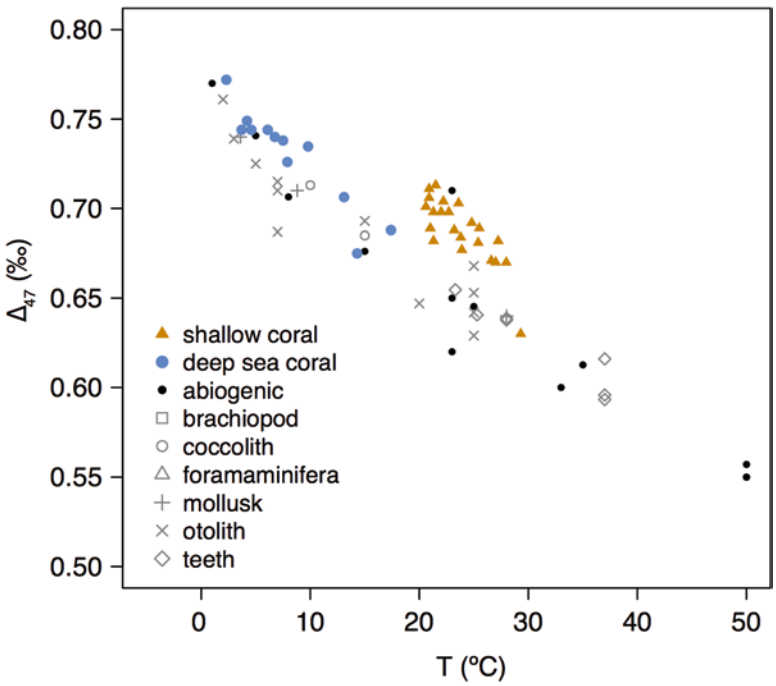


Fig. 12.7 Temperature dependent carbonate clumped isotope composition of various biogenic carbonates, including deep sea corals (*blue*) and shallow *Porites* species (*orange*), compared with abiogenic data (*black*) (Data compilation from Zaarur et al. (2013) and references therein)

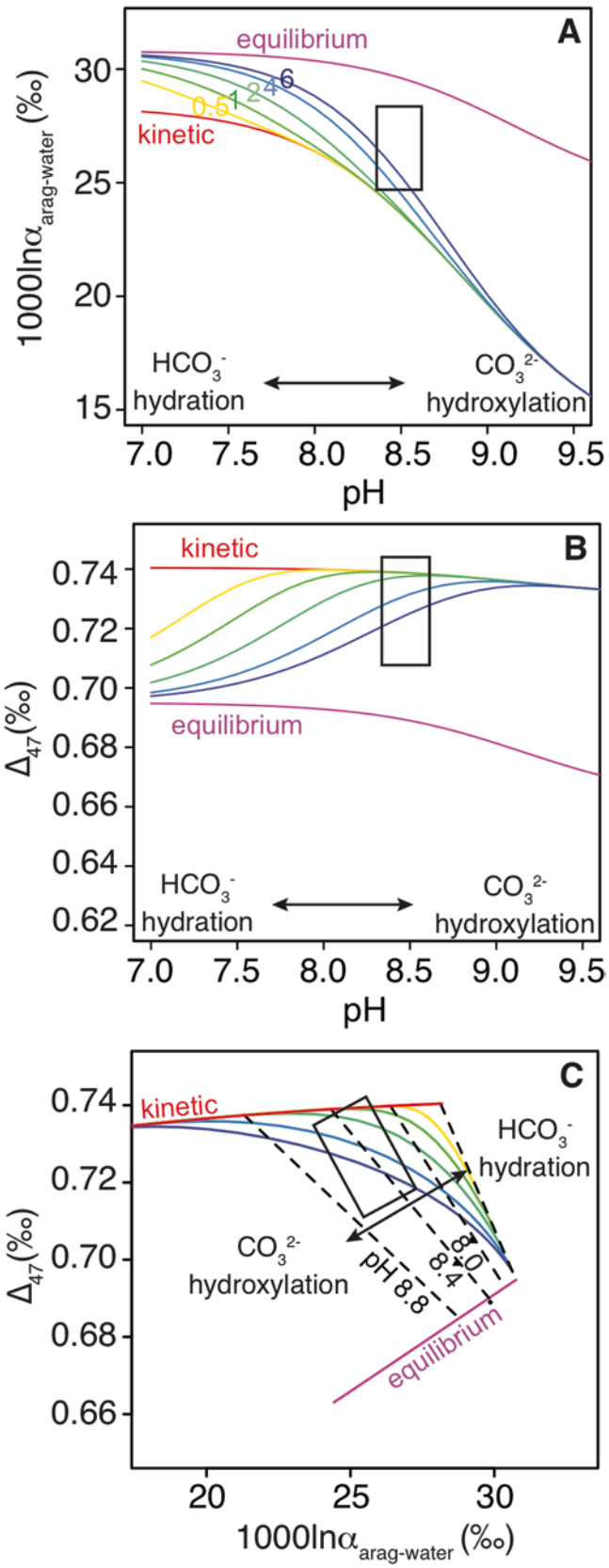
of CO_3^{2-} relative to HCO_3^- and favor lower Δ_{47} values opposite the trend observed. Furthermore, deep sea corals typically exhibit higher pH_{cf} than shallow water species (Fig. 12.4), but show no obvious Δ_{47} vital effect.

Recent work demonstrates that the rate at which clumped isotopes equilibrate during CO_2 hydration is identical to that for oxygen isotopes (Affek 2013), raising the possibility that hydration and/or hydroxylation of metabolic CO_2 may impart vital effects in coral skeleton if calcification is faster than the time required for isotopic equilibration. Kinetic effects associated with dehydration and dehydroxylation reactions during rapid precipitation of cave calcite have been invoked to explain offsets toward lower Δ_{47} and higher $\delta^{18}O$ in speleothems (Daëron et al. 2011; Kluge et al. 2013), and suggest that the reverse reaction of hydration/hydroxylation could explain higher Δ_{47} and lower $\delta^{18}O$ in corals (Saenger et al. 2012). Since the H_2O or OH that react with CO_2 in these reactions have only a single oxygen atom, they cannot exhibit "clumping" implying that kinetic effects cause coral skeleton to inherit a disequilibrium Δ_{47} value, which we suggest to be that of CO_2 from metabolism or an internal carbon pool. Laboratory experiments support this possibility and show that when $CaCO_3$ is grown quickly at elevated pH, the mineral acquires the Δ_{47} signature of the CO_2 from which it precipitates (Falk and Guo 2014).

12.6.2 Relationship to $\delta^{13}C$, $\delta^{18}O$ and $\delta^{11}B$

With additional experimental constraint on the Δ_{47} values of CO_3^{2-}, HCO_3^- and H_2CO_3, it may be possible to incorporate clumped isotope data into existing biomineralization models based on $\delta^{18}O$, $\delta^{13}C$ and $\delta^{11}B$. Figure 12.8 illustrates one potential approach using Rollion-Bard's (2003) model as a starting point. At equilibrium, the $\delta^{18}O$ and Δ_{47} of coral skeleton are both expected to exhibit a pH-dependence that leads to higher values at low pH (HCO_3^- dominated) and lower values at high pH (CO_3^{2-} dominated) (Fig. 12.8a, b). The kinetic end member with respect to $\delta^{18}O$ would be set by the proportion of HCO_3^- generated via the hydration or hydroxylation pathway, with hydration dominating at low pH (~95 % at pH = 7) and hydroxylation dominating at high pH (~75 % at pH = 9) (Fig. 12.8a). As argued above, the kinetic end member with respect to Δ_{47} may be set by the Δ_{47} of CO_2 from metabolism and/or an internal carbon pool (Fig. 12.8b), which we hypothesize to be ~0.05 ‰ lower than equilibrium CO_2, as is true for human breath (Affek and Eiler 2006). These kinetic effects would only impact carbon or oxygen derived from CO_2 (Fig. 12.1), and the absolute value of $\delta^{18}O$ and Δ_{47} kinetic end members would therefore also depend on the proportion of skeleton derived from metabolic CO_2 and/or an internal carbon pool.

Values in between the equilibrium and kinetic end members may reflect the residence time of ions in the calcifying fluid prior to mineral formation. When fluid residence time is short, we anticipate that the coral calcification rate will exceed the rate of isotope equilibration between HCO_3^- and CO_2 in hydration and/or hydroxylation reactions, leading to $\delta^{18}O$ and Δ_{47} values near the kinetic end member. At sufficiently long residence times, the rate of isotopic equilibration should out pace the rate of calcification causing $\delta^{18}O$ and Δ_{47} to approach the equilibrium end member. Since $\delta^{18}O$



and Δ_{47} appear to equilibrate at the same rate (Affek 2013) we expect $\delta^{18}O$ vital effects to be proportional to those in Δ_{47}. Furthermore, since both isotope systems share a pH dependence, we anticipate kinetic and equilibrium end members to be linearly correlated in a plot of $\delta^{18}O$ versus Δ_{47} (Fig. 12.8c). Typical coral data support this model and suggest that observed $\delta^{18}O$ and Δ_{47} values can be explained at a pH_{cf} consistent with $\delta^{11}B$ data if the residence time of calcifying fluid is on the order of 4 h. If CA is present and active, the model could likely still explain observed $\delta^{18}O$, Δ_{47} and $\delta^{11}B$ variability with a significantly shorter residence time. However, testing the potential influence of CA is not possible without data on its concentration and activity in the calcifying space.

12.7 Calcium Isotopes

The critical role of Ca^{2+} in calcification makes understanding the processes controlling its isotopic composition ($\delta^{44}Ca$) in coral skeleton a logical complement to proxies associated with the CO_3^{2-} ion. For example, it may be reasonable to expect $\delta^{44}Ca$ and $\delta^{11}B$ to partially covary because of their common relationship to Ca-ATPase pumping, although this would be less true if other proton pumps are responsible for elevating pH_{cf}. However, the variables that may impact coral $\delta^{44}Ca$ are diverse and in addition to Ca-ATPase pumping, may include temperature, calcification rate, Rayleigh fractionation, membrane transport and interaction with organic matrices. The laborious high precision analyses required to resolve subtle Ca isotope fractionations (Russell et al. 1978) have historically limited its regular application, providing little data to distinguish among these mechanisms. However, recent advances in mass spectrometry, most notably the advent of instruments with multiple detectors (Heuser et al. 2002), has made the measurement of $\delta^{44}Ca$ increasingly routine, allowing some insight into the processes above.

Fig. 12.8 **a** Modeled coral oxygen isotope fractionation at various pH values and calcifying fluid residence times. The version shown assumes 70 % of coral carbon derives from inorganic and/or metabolic CO_2 at a growth temperature of 25 °C. At low pH, DIC is predominately HCO_3^- and metabolic CO_2 follows a hydration pathway. At high pH, DIC is predominately CO_3^{2-} and inorganic/metabolic CO_2 follows a hydroxylation pathway. At short residence times, the coral acquires the kinetic disequilibrium signature, while longer residence times favor equilibrium. The *black box* bounds typical *Porites sp.* coral oxygen isotope fractionation factors, and $\delta^{11}B$-based calcifying fluid pH values. **b** As in panel **a**, but for Δ_{47}. The hydration and hydroxylation rate constants are assumed to be the same for $\delta^{18}O$ and Δ_{47} (Affek 2013). The Δ_{47} of inorganic/metabolic CO_2 (assumed to be 0.05‰ lower than equilibrium CO_2) and the relative proportion of hydration/hydroxylation determine the kinetic endmember. **c** Cross plot of oxygen isotope fractionation and Δ_{47}. *Dashed lines* indicate lines of constant pH, but variable residence times

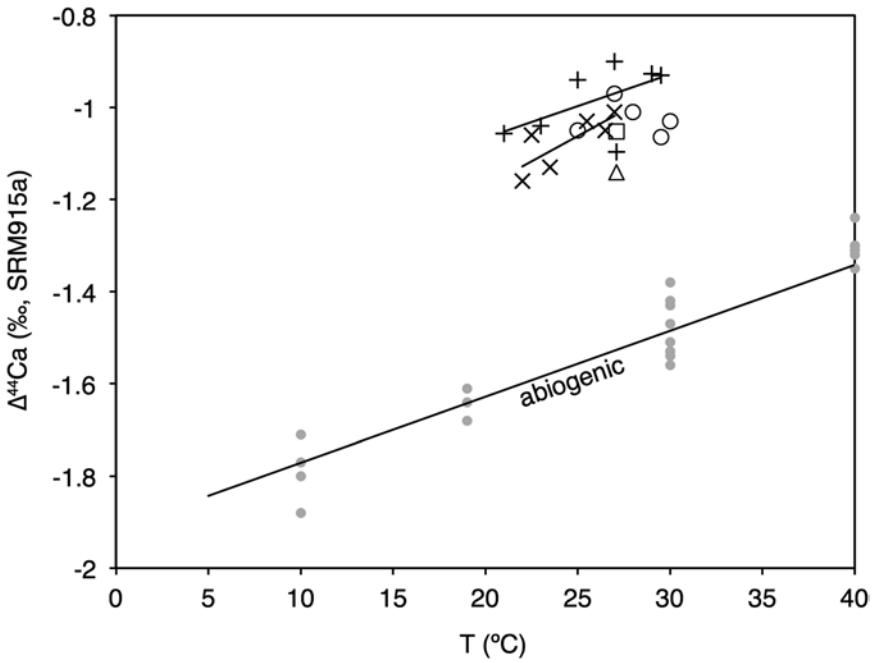


Fig. 12.9 Temperature dependent coral Ca isotope fractionation in *Pavona sp*. (*exes*), *Acropora sp*. (*crosses*), *Montipora sp*. (*squares*), *Stylophora sp*. (*triangles*) and *Porites sp*. (*circles*) compared with that of abiogenic experiments Gussone et al. (2003) (Data from Böhm et al. (2006), and Pretet et al. (2013))

Both corals and abiogenic aragonites exhibit $\delta^{44}Ca$ values lower than the fluid from which they precipitate (i.e. $\Delta^{44}Ca$ is negative) as well as a weak temperature dependence of ~0.02‰/°C (Böhm et al. 2006; Gussone et al. 2003; Inoue et al. 2015 Fig. 12.9). However, monthly-resolved data from a *Porites sp*. coral did not show clear annual cycles as would be expected if temperature were the primary driver of $\Delta^{44}Ca$ variability, although it must be noted that this was a fossil specimen (Pretet et al. 2013). Instead, evidence that coral $\Delta^{44}Ca$ is offset from the abiogenic trend by ~0.5‰ regardless of genus (Böhm et al. 2006; Pretet et al. 2013; Inoue et al. 2015) suggests that a common biomineralization strategy among most species leads to biological and/or kinetic effects that incorporate more heavy Ca into the skeleton than expected. A number of the biologically induced effects discussed above (i.e. rapid calcification at high pH and Ω_{ar}) may contribute to the offset between coral and abiogenic aragonite. Furthermore, ^{44}Ca and other cation isotopes may be sensitive to processes not discussed above, such as Rayleigh fractionation in a semi-closed system (see below).

12.7.1 Precipitation Rate

As for isotopes associated with the CO_3^{2-} portion of the skeleton, coral $\Delta^{44}Ca$ may deviate from the abiogenic trend if calcification proceeds faster than the time necessary to achieve isotopic equilibrium. Contrary to some calcites (e.g. Tang et al. 2008), Ca isotope fractionation appears to decrease at faster calcification rates in aragonite leading to less negative $\Delta^{44}Ca$ (Gussone et al. 2003; Niedermayr et al. 2013). The high saturation states in the calcifying fluid may result in coral calcification rates that are 1–2 orders of magnitude faster than Gussone et al.'s (2003) experiments (Lough and Barnes 1997), suggesting that their more positive $\Delta^{44}Ca$ may be a kinetic effect. Böhm et al. (2006) explored this possibility by extrapolating the precipitation rate dependence of abiogenic experiments (Gussone et al. 2003) to typical coral growth rates, but found that coral $\Delta^{44}Ca$ was ~0.5–0.9‰ more negative than predicted, and argued against a significant precipitation rate dependence. Similarly, Inoue et al. (2015) found growth rate was not significantly correlated with Ca isotope fractionation in cultured corals. However, recent abiogenic aragonite experiments conducted over a wider range of precipitation rates that overlap with coral growth produce $\Delta^{44}Ca$ of −0.91 to −1.55‰ that reasonably bracket the range from apparent equilibrium to corals (Niedermayr et al. 2013). This suggests that calcification rate cannot be ruled out as an important control on coral Ca isotope fractionation.

Kinetic effects in calcite $\Delta^{44}Ca$ have been attributed to the faster rate of light isotope attachment/detachment at the mineral surface, leading to equilibrium $\Delta^{44}Ca$ of ~0‰ at extremely slow growth and more negative $\Delta^{44}Ca$ as growth rates increase (DePaolo 2011; Tang et al. 2008). However, this mechanism can only explain aragonite data if $\Delta^{44}Ca$ is negative at equilibrium and increases toward ~0‰ at faster growth rates, which is not consistent with faster light isotope attachment/detachment. Alternatively, $\Delta^{44}Ca$ kinetic effects may reflect "unfractionated" Ca with an isotope composition similar to seawater being incorporated into the skeleton as a distinct boundary layer (Gussone et al. 2005; Watson 2004)

or as fluid inclusions, which are known to exist in biogenic and abiogenic carbonates (Fleitmann et al. 2003; Lécuyer and O'Neil 1994). However, a simple mixing calculation between apparent equilibrium fractionation and seawater suggests that fluid inclusions are an unreasonable explanation, as they would have to account for more than third of Ca compared to the 1–2 % typical of fluid inclusions.

It is also likely that at least a portion of coral skeleton forms through a non-classical pathway in which non-crystalline amorphous calcium carbonate (ACC) may be important. Coral skeletons exhibit small bundles of granular centers of calcification from which elongate aragonite crystals radiate (Cohen et al. 2001; Wells 1956). Morphological similarities between corals and abiogenic experiments suggest that centers of calcification form at very high pH values and aragonite saturation states (Ω_{ar}), while surrounding fibrous crystals are precipitated at lower values (Holcomb et al. 2009). In combination with the high Mg concentration of seawater (which inhibits calcite nucleation) and fast precipitation rates (which outpace aragonite nucleation), elevated Ω_{ar} during center of calcification formation would favor an ACC precursor that subsequently transforms to very high Mg calcite (Wang et al. 2012). Consistent with this possibility, calcite may occur in centers of calcification (Constantz and Meike 1989; Meibom et al. 2004), but these findings have been challenged (Cuif and Dauphin 1998). Both ACC and high Mg calcite are expected to have higher Δ^{44}Ca values with smaller fractionations from seawater than carbonate formed at apparent equilibrium (Tang et al. 2008), consistent with the offsets observed (Fig. 12.9). Furthermore, inter-species Δ^{44}Ca variations may be partially explained by different patterns of calcification center formation in which some species show small discrete spots while others exhibit larger continuous bands (Cohen and McConnaughey 2003; Gagnon et al. 2007).

As outlined above, kinetic explanations for coral Δ^{44}Ca vital effects often depend on nucleation and growth rates at the very fine scales of individual ions or crystals, which are not necessarily expressed in the annual or whole-colony averages typically measured (e.g. Lough and Barnes 1997). That is, Δ^{44}Ca and other geochemical proxies in "slow growing" deep sea corals should not necessarily be expected to be closer to equilibrium than "fast growing" tropical species simply because they deposit less aragonite in a given year. In fact, the higher calcifying fluid pH and Ω_{ar} of deep sea corals (Fig. 12.4) may favor faster nucleation and crystal growth that leads to larger vital effects. Although little growth rate or Δ^{44}Ca data is available at the scale of individual crystals for most species, emerging coral culturing methods and micro-analytical techniques (e.g. Gagnon et al. 2012; Venn et al. 2013) are helping to fill that void and will hopefully provide new insight into the impact of crystal-scale precipitation rate on geochemical proxies in the near future.

12.7.2 Rayleigh Fractionation

The negative Δ^{44}Ca values measured in abiogenic aragonites (Fig. 12.9) indicate that light ^{40}Ca is preferentially incorporated into the crystal lattice. When skeleton is formed from a "batch" of calcifying fluid, preferential removal of ^{40}Ca leaves the remaining calcifying fluid with slightly more ^{44}Ca, thereby raising the Δ^{44}Ca of skeleton formed from the next "batch" of fluid. As the fraction (*f*) of calcifying fluid consumed to precipitate skeleton increases, so too does its Δ^{44}Ca, until the calcifying fluid is replenished with ambient seawater and the processes starts over. Referred to as Rayleigh fractionation (Rayleigh 1896), this process has been a popular explanation for variations in the concentrations of cations in coral skeleton (Gaetani et al. 2011; Gagnon et al. 2007) suggesting it may also explain their isotopes.

An estimate of how Rayleigh fractionation would affect coral Δ^{44}Ca suggests that approximately half of the Ca in the calcifying fluid would have to be consumed to explain the observed ~0.5 ‰ enrichment relative to abiogenic data (Fig. 12.10). If this is the case, it would require carbon concentrations to be significantly elevated above typical seawater concentrations. For example, assuming an initial calcifying fluid Ca concentration of ~10.5 mM (Al-Horani et al. 2003), of which half is used for calcification, would require the carbon concentration to increase by ~2.5 times. This may be reasonable if an appreciable amount of the carbon used for calcification comes from metabolic CO_2 and/or an internal carbon pool (Erez 1978; Furla et al. 2000). If it is assumed that seawater DIC with a concentration of 2 mM represents 30 % of the calcifying fluid carbon pool, the total carbon pool may be estimated as 2 mM/0.3, or 6.67 mM, which would be sufficiently high for more than half of the Ca in the calcifying fluid to be consumed. Consuming such a large fractionation of the Ca in each "batch" of calcifying fluid would likely lead to periods of significant decreases in its Ca concentration. However, Al-Horani et al.'s (2003) measurement of calcifying fluid Ca concentration show variations of only ~5 % that never drop significantly below ambient values, potentially making a Rayleigh-based mechanism less realistic. Furthermore, Inoue et al. (2015) found that Ca isotope and Sr/Ca data in cultured corals could not be explained by a common proportion of calcifying fluid consumed during calcification.

A check on the importance of Rayleigh fractionation to Δ^{44}Ca variability may come from deep sea corals. Most of these species use very little metabolic CO_2 for skeletogenesis (Adkins et al. 2003, Sect. 4), suggesting that they may be less capable of concentrating carbon in their calcifying fluid, although the presence of a significant internal carbon pool cannot be ruled out. Without any carbon concentration, the amount of Ca consumed from any given "batch" of calcifying fluid could not exceed the ~2 mM carbon concentration,

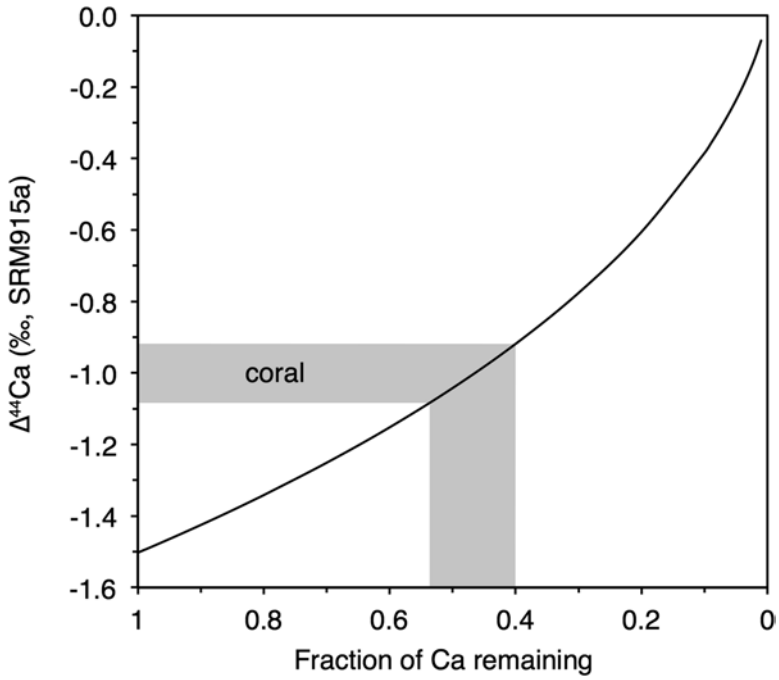


Fig. 12.10 Evolution of coral $\Delta^{44}Ca$ due to Rayleigh fractionation during calcification from a closed calcifying fluid. Typical coral $\Delta^{44}Ca$ values can be explained if ~50 % of each "batch" of calcifying fluid is consumed for skeletogenesis (See Saenger et al. (2013) for Rayleigh fractionation calculations)

limiting *f* in any Rayleigh-based model of deep sea coral $\Delta^{44}Ca$ variability to about 20 %, which is consistent with independent estimates (Gagnon et al. 2007). Thus, arguments that require an appreciably larger proportion of a deep sea coral's Ca to be consumed without a mechanism for carbon concentration may overestimate the importance of Rayleigh fractionation at the expense of other mechanisms described in this section.

12.8 Magnesium and Strontium Isotopes

Many of the processes that affect $\Delta^{44}Ca$ during skeletogeneis are also expected to influence the fractionation of Mg and Sr isotopes, which readily substitute for Ca in the aragonite crystal lattice. Therefore, evaluation of $\Delta^{26}Mg$ and $\Delta^{88}Sr$ may provide new insight and important checks on the mechanisms discussed in Sect. 7. However, despite advances in multi-collector mass spectrometry (Galy et al. 2001) that have made $\Delta^{26}Mg$ and $\Delta^{88}Sr$ analyses increasingly routine, relatively few studies exist on these isotopes systems, making their interpretation less certain.

Comparing abiogenic and coral data for both $\Delta^{26}Mg$ and $\Delta^{88}Sr$ show some common trends to $\Delta^{44}Ca$, supporting the possibility that similar processes may control their fractionation. Like $\Delta^{44}Ca$, light Mg and Sr isotopes are preferentially incorporated into coral skeleton (Fietzke and Eisenhauer 2006; Raddatz et al. 2013; Saenger et al. 2013; Wombacher et al. 2011), but not to the degree observed in abiogenic aragonites (Fig. 12.11). Thus, corals typically exhibit negative $\Delta^{26}Mg$ and $\Delta^{88}Sr$ values that are positively offset from abiogenic data. Furthermore, abiogenic $\Delta^{26}Mg$ and $\Delta^{88}Sr$ may both exhibit a subtle temperature dependence, with smaller fractionations at warmer temperatures (Fietzke and Eisenhauer 2006; Wang et al. 2013b). Annual cycles in the $\Delta^{26}Mg$ of *Porites sp*. corals have been shown to be in phase with $\delta^{18}O$ and the coral's strontium to calcium (Sr/Ca) ratio (Saenger et al. 2013) pointing to some degree of temperature control in corals as well. Similarly, a strong correlation between $\Delta^{88}Sr$ and temperature in *Pavona clavus* (Fietzke and Eisenhauer 2006) and *Lophelia pertusa* (Rüggeberg et al. 2008) also support a temperature dependence. However, the apparent temperature sensitivity of both $\Delta^{26}Mg$ and $\Delta^{88}Sr$ in corals is significantly steeper than in abiogenic experiments (Fig. 12.11). Although this may reflect a seasonal vital effect (Saenger et al. 2013), it may also be an analytical issue. Recent work using an extremely precise technique found *Lophelia pertusa* and a *Porites sp*. spanning a 6–25 °C temperature range to have a constant $\Delta^{88}Sr$ of ~0.19‰ (Raddatz et al. 2013), suggesting little or no temperature dependent fractionation. Intriguingly, very recent abiogenic aragonite and coral data also find a constant $\Delta^{88}Sr$ of ~0.19‰ (Fruchter et al. 2016) that is inconsistent with published experiments (Fietzke and Eisenhauer 2006). This makes it unclear if coral $\Delta^{88}Sr$ exhibits a vital effect or is identical to apparent equilibrium. With the significant caveat that close examination of Fruchter et al.'s (2016) work and similar studies will revise the arguments below, we proceed under the assumption that coral $\Delta^{88}Sr$ is positively offset from the abiogenic trend due to biologically induced mechanisms such as precipitation rate or Rayleigh fractionation as may be the case for $\Delta^{40}Ca$ and $\Delta^{26}Mg$.

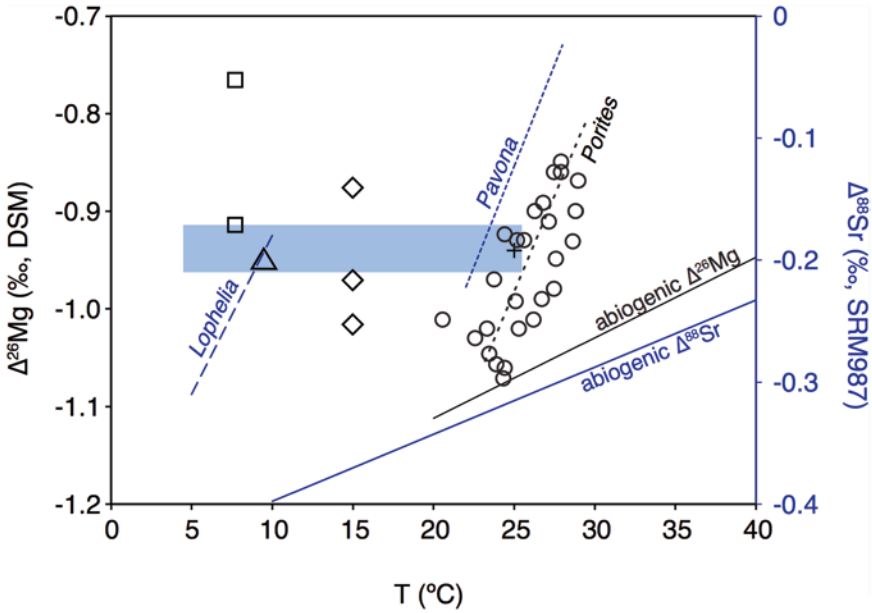


Fig. 12.11 Temperature dependent coral Mg isotope fractionation (*black*, left y-axis) in *Acropora/Pocillopora sp.* (*crosses*), *Porites sp.* (*circles*), *Astrangia* (*diamonds*), *Lophelia sp.* (*squares*), and *Desmophyllum sp.* (*triangles*) compared with that of abiogenic aragonites (Wang et al. 2013b) (Data from Chang et al. (2004), Saenger et al. (2013), Wombacher et al. (2011), and Yoshimura et al. (2011)). Also shown are the temperature dependent coral Sr isotope fractionation trends (*blue*, right y-axis) for *Lophelia sp.* (Rüggeberg et al. 2008), *Pavona sp.* and abiogenic aragonites (Fietzke and Eisenhauer 2006). The blue box bounds the constant Δ^{88}Sr for *Lophelia sp.* and *Porites sp.* coral standard JCp-1 measured by high precision double spike TIMS (Raddatz et al. 2013), which generally agree with abiogenic and coral data in Fruchter et al. (2016)

12.8.1 Precipitation Rate

Precipitation rate does not appear to affect Δ^{88}Sr strongly (Fruchter et al. 2016) and seems unlikely to cause any offset between abiogenic and coral data. If it is assumed that a similar mechanism controls Δ^{88}Sr and Δ^{44}Ca vital effects, the absence of a Δ^{88} Sr precipitation rate dependence argues against some of the kinetic mechanisms that may affect Ca isotope fractionation (Sect. 7.1) and in favor of other processes such as Rayleigh fractionation (Sect. 7.2 and below).

The effect of precipitation rate on Δ^{26}Mg has not yet been studied, although abiogenic data suggest that it is not sensitive to the degassing rate of experiments (Wang et al. 2013b). However, calcite shows smaller Mg isotope fractionation at faster precipitation rates (Immenhauser et al. 2010; Mavromatis et al. 2013) that are consistent with the offset of coral Δ^{26}Mg from the abiogenic trend. Mg is strongly excluded from aragonite, with approximately a 1000-fold decrease in Mg/Ca ratio between seawater and coral. This suggests Δ^{26}Mg may be particularly sensitive to kinetic effects as even a small concentration of unequilibrated Mg may have a large influence on the coral's bulk isotopic composition. As for Δ^{44}Ca, unequilibrated Mg may derive from entrapment of a distinct boundary layer, an ACC precursor or fluid inclusions (Saenger et al. 2013; Saenger and Wang 2014). The later may be particularly important given the sluggish dehydration of Mg at the mineral surface and evidence for water and less negative Δ^{26}Mg values in rapidly precipitated carbonates (Mavromatis et al. 2013). Unlike Δ^{44}Ca (Sect. 7.1), a simple mixing calculation reproduces coral Δ^{26}Mg and Mg/Ca trends, suggesting that small increases in the incorporation of hydrated Mg with a seawater-like isotopic composition at rapid precipitation rates may explain the apparent offset from abiogenic data (Fig. 12.12). If this is the case, it suggests that the source of vital effects in coral Δ^{26}Mg may differ from those for Δ^{44}Ca and Δ^{88}Sr.

12.8.2 Rayleigh Fractionation

The absence of a strong precipitation rate effect on Δ^{88}Sr suggests that coral vital effects may be better explained by Rayleigh fractionation (c.f. Fruchter et al. 2016). Repeating the calculations in Sect. 7.2 for the Δ^{88}Sr of *Lophelia* and *Porites sp.* (Raddatz et al. 2013) shows that the offset of coral data from the abiogenic trend of Fietzke and Eisenhauer (2006) could be explained if ~50 % of each "batch" of calcifying fluid was consumed for skeletogenesis (Fig. 12.13a). This agrees well with the *f* value required to explain Δ^{44}Ca (Fig. 12.13b) suggesting that the Sr and Ca isotope composition of corals could share a mutual dependence on Rayleigh fractionation. Furthermore, this similarity provides additional support for a biomineralization model in which skeletal precipitation occurs from modified seawater with a significant contribution of inorganic and metabolically-derived carbon (see Sect. 7.2). Following this logic, comparable Δ^{88}Sr offsets from the

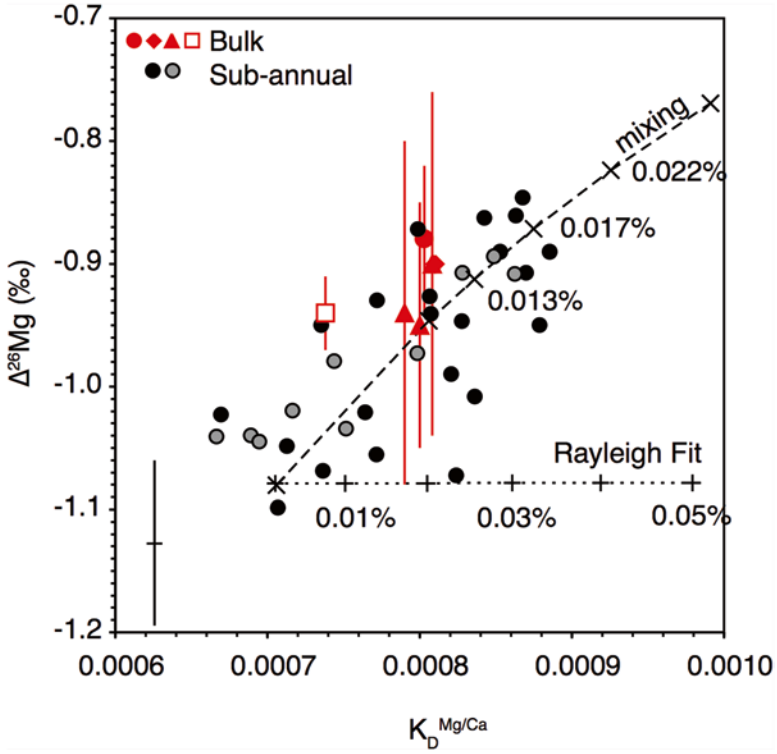


Fig. 12.12 Mg isotope fractionation and $K_D^{Mg/Ca}$ ($K_D^{Mg/Ca} = (Mg/Ca_{coral})/(Mg/Ca_{seawater})$) in bulk sampled (*red*) and sub-annually sampled (*black*/*grey*) *Porites sp*. corals overlain with mixing and Rayleigh fractionation calculations. Values for mixing indicate the percent of Mg with an isotopic value of seawater. Values for Rayleigh fractionation are percent of calcifying fluid Mg consumed (Adapted from Saenger et al. (2013). Data from Saenger et al. (2013), Wombacher et al. (2011), and Yoshimura et al. (2011))

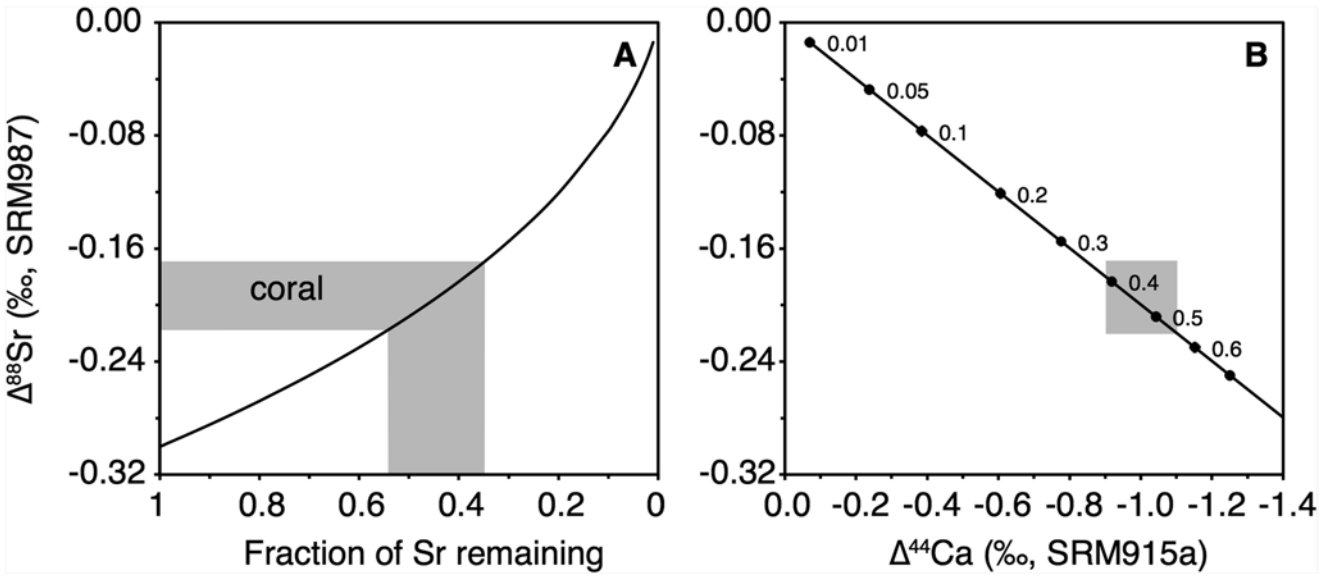


Fig. 12.13 **a** Evolution of coral $\Delta^{88}Sr$ due to Rayleigh fractionation during calcification from a closed calcifying fluid using *Lophelia sp*. and *Porites sp*. coral standard JCp-1 values (Raddatz et al. 2013). **b** Comparison with Rayleigh calculations for $\Delta^{44}Ca$ in Fig. 12.9. Marked values indicate the fraction of calcifying fluid consumed. Coral $\Delta^{44}Ca$ and $\Delta^{88}Sr$ values overlap when ~50 % of each "batch" of calcifying fluid is consumed for skeletogenesis (See Saenger et al. (2013) for Rayleigh fractionation calculations)

abiogenic trend in *Lophelia* and *Porites sp*. imply both genera consume approximately equal fractions of calcifying fluid and a similar amount of inorganic and metabolic carbon. Consistent with this possibility, *Lophelia sp*. are known to incorporate far more metabolic carbon into their skeleton than other azooxanthallae cold water genera (Adkins et al. 2003).

Although Rayleigh fractionation may strongly impact coral Mg/Ca (Gaetani et al. 2011; Gagnon et al. 2007), it is not consistent with Mg isotope fractionation (Saenger et al. 2013). As shown in Fig. 12.12, Rayleigh fractionation can reproduce the changes in coral Mg/Ca when only small amounts of calcifying fluid are consumed, but $\Delta^{26}Mg$ is essentially invariant over

this range, inconsistent with coral data. This supports the possibility that coral Δ^{26}Mg may be primarily controlled by precipitation rate or other variables, while Δ^{44}Ca, and possibly Δ^{88}Sr, are more strongly influence by Rayleigh fractionation.

12.9 Biologically Controlled Effects

Although the discussion above suggests that the range of Δ^{44}Ca, Δ^{26}Mg and Δ^{88}Sr values in corals can be adequately explained by abiogenic processes following biological preconditioning, a brief discussion of how active biological control of calcification may impact non-traditional stable isotope fractionation is warranted. Some calcification models suggest that the calcifying space is permanently isolated from seawater and that Ca^{2+} and other ions are supplied to the calcifying fluid through a two step process (Allemand et al. 2011). First, the large gradient in ion concentration between seawater and the cytoplasm of calicoblastic ectoderm cells (Fig. 12.1) causes cations to enter into the cell through a voltage-gated channel (Marshall 1996). Second, ions are dehydrated and transported by cation-binding proteins through calicoblastic ectoderm cells before being actively pumped against a strong concentration gradient into the calcifying space (Allemand et al. 2011). Isotope fractionations seems likely at a number of steps in this scenario including during transport across cell membranes, during dehydration and during pumping. Indeed, Böhm et al. (2006) argued that most Ca used for skeletogenesis followed this path, and attributed the difference between coral and abiogenic Δ^{44}Ca to biologically controlled processes.

However, Böhm et al. (2006) did not provide a clear hypothesis for why the processes above would discriminate against ^{40}Ca. Given that ^{40}Ca is expected to diffuse and be dehydrated more quickly than ^{44}Ca (Bourg et al. 2010; DePaolo 2011; Richter et al. 2006) active transport of Ca seems likely to enrich coral skeleton in ^{40}Ca relative to abiogenic experiments (Nielsen et al. 2012). Indeed, Inoue et al. (2015) suggest a isotope fractionation of –1.2‰ for transcellular Ca transport. The same would likely be true for other cations, resulting in coral Δ^{44}Ca, Δ^{26}Mg and Δ^{88}Sr values lower than the abiogenic relationship that are not consistent with observations (Figs. 12.9 and 12.11). Inoue et al. (2015) explained this apparent discrepancy by suggesting that after the initial –1.2‰, no additional fractionation associated with transport or pumping occured, and that all Ca was consumed during calcification, resulting in no isotope fractionation between calcifying fluid and coral skeleton. Although this Ca pathway cannot be ruled out, it seems inconsistent with relative high and stable Ca concentrations in the calcifying fluid (Al-Horani et al. 2003), and would be energy intensive compared to one in which the majority of ions were supplied by seawater (e.g. Gagnon et al. 2012), suggesting that the latter may be more realistic.

Strong biological control on calcification and isotope composition may still occur even if ions are supplied directly to the calcifying fluid from seawater. For example, if an organic matrix at the site of calcification helps to isolate the calcifying fluid, regulate aragonite deposition or provide structural stability (e.g. Clode and Marshall 2002), cation binding proteins in that matrix may concentrate, and subsequently deliver, ions for calcification (Puverel et al. 2005), affecting Δ^{44}Ca, Δ^{26}Mg and Δ^{88}Sr in the process if particular isotopes were favored. Unfortunately, such biologically controlled mechanisms are difficult to evaluate with laboratory experiments, making the direction of any such fractionation poorly constrained. However, recent work has observed in vitro precipitation of $CaCO_3$ by proteins in the organic matrix, which can form highly ordered crystalline structures (Mass et al. 2013, 2014), and future analyses of these carbonates may provide important constraint on how biologically controlled calcification associated with the organic matrix affects the isotope composition of coral skeleton.

12.10 Summary and Conclusions

Although much remains uncertain regarding the mechanisms by which corals transform seawater ions into aragonite skeleton, a number of inferences about the process emerge from this review. Perhaps most important is that the non-traditional stable isotope signature of both symbiotic, hermatypic shallow corals and asymbiotic deep sea species can be satisfactorily explained by a biologically mediated calcification model in which physiological processes create conditions that promote aragonite precipitation, but do not directly control mineral growth. Arguments for direct seawater transport to the calcifying space are generally consistent with non-traditional stable isotope data if ion pumping and transport of inorganic and metabolic CO_2 at high calcifying fluid pH (pH_{cf}) increases DIC concentration to promote rapid precipitation from a closed reservoir. Boron isotope data support this and suggest that pH_{cf} is typically 8.4–8.6 for shallow, symbiotic species and 8.8 or higher in cold water, asymbiotic corals. Stable calcium, and perhaps strontium, isotope fractionation may be strongly impacted by Rayleigh fractionation, with about half of each "batch" of calcifying fluid consumed. The magnitude of carbon concentration required for this amount of precipitation is broadly consistent with independent estimates of the amount of inorganic and metabolic CO_2 transported to the calcifying fluid. Adapting semi-quantitative biomineralization models to include carbonate clumped isotope data suggest that each "batch" of calcifying fluid is consumed within less than 6 h at typical pH_{cf}, and potentially even more quickly if the influence of carbonic anhydrase is considered. Rapid calcification is supported by apparent kinetic effects on Mg isotope fractionation that may reflect the unique isotope

composition of a mineral boundary layer, fluid inclusions or an amorphous precursor.

Our argument for a biologically mediated calcification model based on non-traditional stable isotope signatures has a number of caveats, and does not rule out other models that rely on more active biological control. Determining which, if either, of these scenarios is more consistent with coral non-traditional stable isotopes will require considerable additional research using abiogenic precipitation experiments, biologic culturing, micro-analytical techniques and geochemical models. From a geochemical standpoint, a number of avenues seem likely to significantly improve our understanding of the processes controlling isotopic variability in corals. Among these are a better understanding of the formation and isotopic signature of amorphous calcium carbonate in abiogenic aragonite and corals, the development of ion-by-ion models of aragonite construction consistent with those for calcite and the measurement of isotopic variability in carbonates precipitated by organic matrix proteins under controlled conditions. Analytical precision presents an overarching challenge to all aspects of future work, and efforts to standardize laboratory protocols, standards and instrumental parameters will be critical to using non-traditional stable isotopes to understand coral calcification.

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Influences of Coral Intra-skeletal Organic Matrix on Calcium Carbonate Precipitation

13

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Abstract

Corals are among the most important calcium carbonate mineralizers and form the main structures of the reefs, which provide an important socio-economical support. Despite this, and the fact the is quite generally accepted that coral mineralization is a biological controlled process, few studied have so far addressed the role of the intra-skeletal organic matrix in the calcification process. This chapter makes a scientific path on what is known on the biological control of coral mineralization describing the more relevant studies. The sections are sequenced with the aim to guide the readers to be conscious of the importance of the organic matrix in the mineralization process that is finally illustrated through a series of experiments in vivo and in vitro. Accordingly the chapter presents an overview on coral biomineralization, anatomy and physiology, skeleton microsctructure, tissue-skeleton, minor element distribution, organic matrix, biomineralization proteins and finally calcium carbonate precipitation in the presence of coral organic matrix.

Keywords

Corals • Organic matrix • Calcium carbonate • Crystallization • Biomineralization

13.1 Overview on Coral Biomineralization

Biomineralization is the science that studies the formation, structure and properties of minerals deposited by organisms, usually referred as biominerals (Lowenstam and Weiner 1989; Mann 2001).

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Among mineralizing organisms corals play an important role. Indeed, they lead to a calcium carbonate ($CaCO_3$) production of about 10^{12} kg year^{-1} (Milliman and Droxler 1996). Beside this, coral calcification is a globally important biological and geochemical process as it allows the tiny polyps of coral colonies to build the most important bioconstruction of the world, coral reefs (Spalding et al. 2001). Coral skeletons not only serve as the 3D-framework for reef building but may also play indirect physiological roles such as light scattering to enhance light absorption by symbionts (Enriquez et al. 2005). In addition, coral skeletons are used for paleoclimate reconstruction (Adkins et al. 2003) and as bone implants (Petite et al. 2000).

The "reef building" hard corals, the Scleractinian order, which accrete exoskeletons are distinguished from soft corals (*Octocorallia* and *Antipatheria*), which permeate their tissue with supportive $CaCO_3$ spicules.

Scleractinian corals are catalogued according to their capability to build reef. Those reef-building are mainly colonial and hermatypic, meaning that they host symbiotic algae,

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or zooxanthellae, in the polyp tissue endoderm. The ahermatypes, the other Scleractinia group, do not have zooxanthellae. In several reviews details of the structure of scleractinian corals are available (Cohen and McConnaughey 2003; Benayahu et al. 2004; Cuif and Dauphin 2005a).

Although the study of coral calcification started with the study of coral biology more than 150 years ago (Dana 1846), the knowledge still remains patchy, with few species used in multiple studies. Skeleton formation was initially considered as a purely mineralogic process where the basic element of the skeleton – that is, the fiber – was described as a single orthorhombic crystal of aragonite formed without biological control (Bryan and Hill 1941). Since then, many geologists and geochemists have widely applied this concept of a chemically dominated process. Conversely, biologists have based their research on a biological concept, such that today it is admitted that coral skeleton formation is a process of "extracellular biologically controlled biomineralization" (Weiner and Dove 2003), and as such involves both mineral and organic fractions.

13.2 Coral Anatomy and Histology

The scleractinian corals are organisms composed of polyps covering the skeleton (Fig. 13.1). In colonial corals the polyps are linked together by a tissue, the coenosarc that is missing in solitary corals. The exoskeleton (extracellular) is located at the base of the coral soft tissues. The oral tissues are in contact with seawater, whereas the aboral tissues are facing the skeleton. Tissues consist of two epithelial layers, an ectoderm and an endoderm, separated by a connective layer of mesoglea. The oral and aboral endoderms delimit the coelenteric cavity. Desmocytes mechanically anchor the tissues to the skeleton (Muscatine et al. 1997; Goldberg 2001a). The aboral ectoderm in contact with the skeleton, the calicoblastic ectoderm, has a topography that exactly complements the growth surface of the skeleton (Johnston 1980; Brown et al. 1983). Calicoblastic cells are long (10–100 mm), highly interdigitated, and overlap each other. Their high mitochondrial content suggests that they possess an active metabolic role (Allemand et al. 2004), while the presence of calcium channels and calcium ATPases in calicoblastic cells suggests that they play an important role in calcification (Zoccola et al. 1999). The space between the skeleton and the calicoblastic cells has a species-specific size and does not exceed a few nanometers (Zoccola et al. 2004; Goldberg 2001b).

13.3 Coral Skeleton Microstructure

The main aspects of scleractinian architecture are the centers of calcification (COCs) and the fibrous aragonite crystals that radiate from around these centers (Fig. 13.2). These basic building blocks are structurally similar in hermatypic and ahermatypic corals (Wainwright 1964). The spatial arrangement of the centers and the incremental zonation of the fibers vary between taxa (Perrin 2003). The coral growth occurs via a two-step matrix-mediated process has been proposed. The initial mineralization is characterized by randomly oriented microgranular components and the second step is characterized by consistent crystallographic alignment that determines the crystallography of the aragonite fibers (Cuif and Dauphin 2005b). The COCs have a high organic content in which $CaCO_3$ grains are embedded (Cuif and Dauphin 1998). The crystallinity of the $CaCO_3$ within the centers is lower than in the aragonitic fibers. The fibrous aragonite crystals comprise the bulk of the coral composition and, in contrast to the centers, have a low organic concentration of about 1 % by weight (Cohen and McConnaughey 2003) while the entire skeleton contains at least 3 % by weight organic material (Dauphin et al. 2006).

Corals with symbionts build skeleton both day and night (dark calcification) (Barnes 1985; Chalker et al. 1985) as do corals without symbionts (Jacques and Pilson 1980; Jacques et al. 1983). Annual density banding in corals (Dodge and Vaisnys 1975; Fang and Chou 1992; Matthews et al. 1996) enables dating and thus the recording of temporal resolution environmental information, which make corals archives exploitable as climate proxies.

The biological control over coral aragonite deposition influences composition and morphology (Enmar et al. 2000). The diversity of skeletal morphology has been historically used as a basis for classification of scleractinian corals (Wells 1956; Vaughan and Wells 1943; Milne-Edwards and Haime 1857), and has been generally confirmed by modern molecular methods (Benzoni et al. 2007; Cuif et al. 2003b) though in some cases there is a discrepancy between the two methods (van Oppen et al. 2001). Skeletal morphology is thus a characteristic of species and skeleton morphogenesis is therefore under genetic control. However, despite this genetic control, coral morphology may also greatly vary with environmental parameters, a phenomenon known as phenotypic plasticity (Garland and Kelly 2006). However, not all coral species (for example *Pavona cactus*) respond to environmental parameters (Willis 1985), nor genotypes within plastic species (Foster 1979) are plastic.

13.4 Tissue: Skeleton Interface in Corals

One of the first studies on the tissue-skeleton interface was carried out in the site of calcification of the branching coral *Pocillopora damicornis* (Vandermeulen 1975). Microscopic analysis were performed on the calicoblastic ectoderm of newly settled larval stages and of adult coral. During settling, the heterogeneous cell composition of the planktonic larva

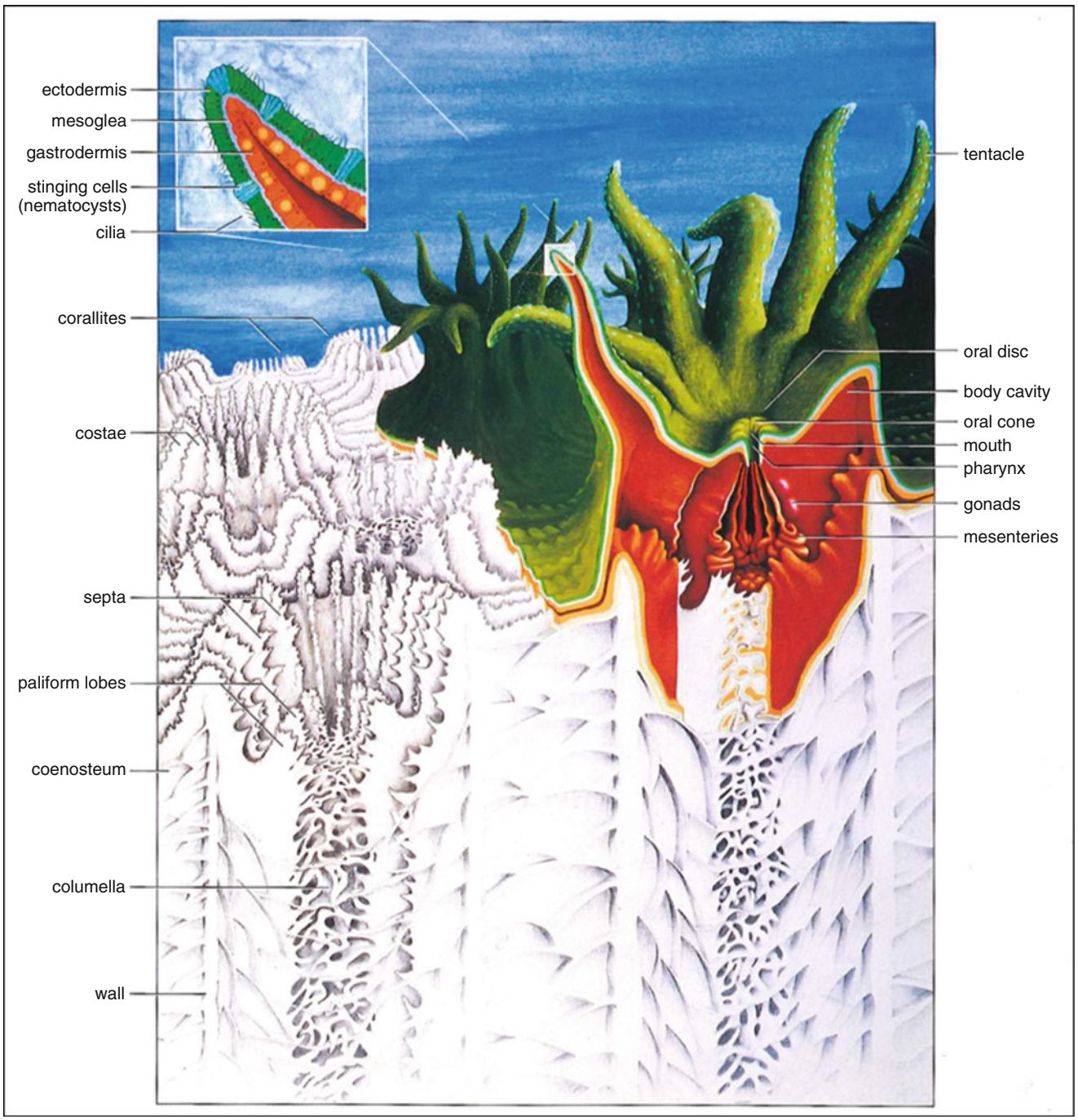

Fig. 13.1 The general structure of a polyp and underlying skeleton. The corallite is a tube enclosed by a wall, which is intercepted by flattened plates, the septa radiating out from the tube center. The paliform lobes are outgrowths of the septa. Extensions of the paliforms lobes meet in the center to form the columella (Reproduced from Veron 1986, with permission)

epidermis was replaced by a epithelium consisting calicoblast cell. This metamorphosis was linked to settling and was marked by the appearance of a new secretory cell. The calicoblast cell of the adult coral was extremely flattened, and interdigitates extensively with adjacent calicoblast cells. This cell contained large membrane-bound vesicles with homogeneously fine granular contents. A higher calcium content in these vesicles than in surrounding tissue was present. The calicoblast epithelium was separated from the underlying coral skeleton by a narrow gap. This gap appeared devoid of substructure, either organic or inorganic. The coral soft tissues were attached to the skeleton by mesogleal

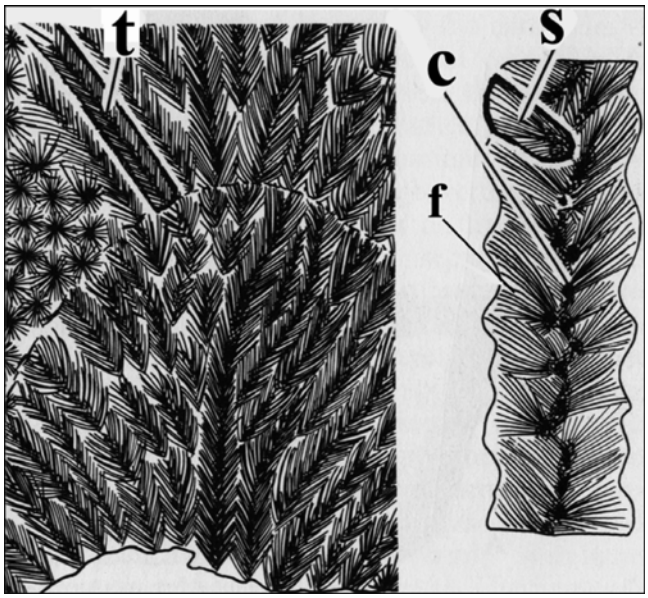

Fig. 13.2 Detail of the scleractinian septal structure. At *right*, aragonite fiber bundles (*f*) emerge from centers of calcification (*c*) and grouped into sclerodermites (*s*). Groups of sclerodermites growing upward together form the trabecula (*t*). This septum of *Galaxea sp.* is a palisade of trabeculae, shown at *left* (Reproduced from Wells 1956)

attachment processes. These consisted of a complex fibrous network originating in the mesoglea, and inserting onto the skeleton via specialized attachment regions consisting of electron-opaque membranous plaques.

The scleractinian coral *Galaxea fascicularis* revealed the presence of organic fibrils located between calicoblastic ectodermal cells and the underlying $CaCO_3$ skeleton and having a diameter of 26 nm (Clode and Marshall 2003). Small (37 nm in diameter) nodular structures observed upon this fibrillar organic material were associated to localised Ca-rich regions. Those Ca-rich regions appeared associated with the organic material are nascent crystals of $CaCO_3$. Significant amounts of S were also detected throughout the calcifying interfacial region, further verifying the likely presence of organic material. The ultrastructural nature of the calcifying interface in the scleractinian coral *G. fascicularis* has been further investigated (Clode and Marshall 2002). Two distinct types of vesicles (380 and 70 nm in diameter, respectively), were predominant throughout the calicoblastic ectodermal cells of frozen-hydrated preparations, but these were never seen to be entering, or to be contained within, sub-skeletal spaces, nor did they contain any crystalline material. A network of organic filaments (26 nm in diameter) extended from the apical membranes of calicoblast cells into these small sub-skeletal cavities. A thin sheath was also frequently observed adjacent to the apical membrane of calicoblast cells.

Histological observations of the coral tissue at the interface with the skeleton (Fig. 13.3), using hermatypic coral *Stilophora pistillata* as a model, showed that: (*i*) a morphological correspondence between the tissues and the skeleton was present and that the calicoblast cell layer was in direct physical contact with the skeletal surface; (*ii*) the distribution and density of desmocyte cells, which anchor the calicoblastic ectoderm to the skeletal surface, vary spatially and temporally during skeletal growth; (*iii*) the tissue above the coenosteal spines lack endoderm and consists only of ectodermal cell-layers separated by mesoglea (Tambutte et al. 2007a).

Skeletogenesis in *S. pistillata* was also studied by a coral nubbin attached to a glass coverslip glued to the bottom of a Petri dish (Raz-Bahat et al. 2006). The horizontal distal tissue edges developed thin transparent extensions of ectodermal and calicoblastic layers only. Four stages of skeletogenesis were observed: (*i*) a thin clear layer of coral tissue advanced beyond the existing peripheral zone; (*ii*) primary fusiform crystals (1 μm each) were deposited, forming a primary discontinuous skeletal front; (*iii*) needle-like crystals appeared, covering the primary fusiform crystals; (*iv*) lengthening of the needle-like crystals, a process that resulted in occlusion of the spaces between adjacent crystals. Two basic skeletal structures, "scattered" and "laminar" skeletons, were formed, integrating the growth patterns of the needle-like crystals.

A crucial aspect of coral biomineralization is the mechanisms behind the transfer of ions from the surrounding sea water to the site of calcification. An important research was carried out using the fluorescent dye calcein (Tambutte et al. 2012). The results showed that (*i*) calcein passes through coral tissues by a paracellular pathway, (*ii*) intercellular junctions control and restrict the diffusion of molecules, (*iii*) intercellular junctions should have pores of defined sizes, and (*iv*) a specific resistance of the tissues owing to paracellular junctions is present.

13.5 Corals Minor Element Distribution

Detailed knowledge of the distribution of trace and minor elements as well as stable isotopes allows to enhance the understand of the biological control and also contribute to the accurate interpretation of climate proxies such as Sr/Ca and Mg/Ca ratios, which, in corals, fluctuate systematically yet inversely with $\delta^{18}O$ (Cohen et al. 2006).

Distribution of trace and minor elements is not uniform throughout the coral skeleton (Allison 1996). The COCs contain higher concentrations of magnesium (Meibom et al. 2004), strontium, and barium than the fibers (Meibom et al. 2006). Heterogeneity in strontium concentration occurs both within and between the COCs and the fascicule (Allison et al. 2005). Despite this, since strontium replaces calcium by isomorphic substitution in scleractinian corals, this simplifies the relationship between the sea surface temperature and the Sr/Ca ratio, since the free energies of two differ-

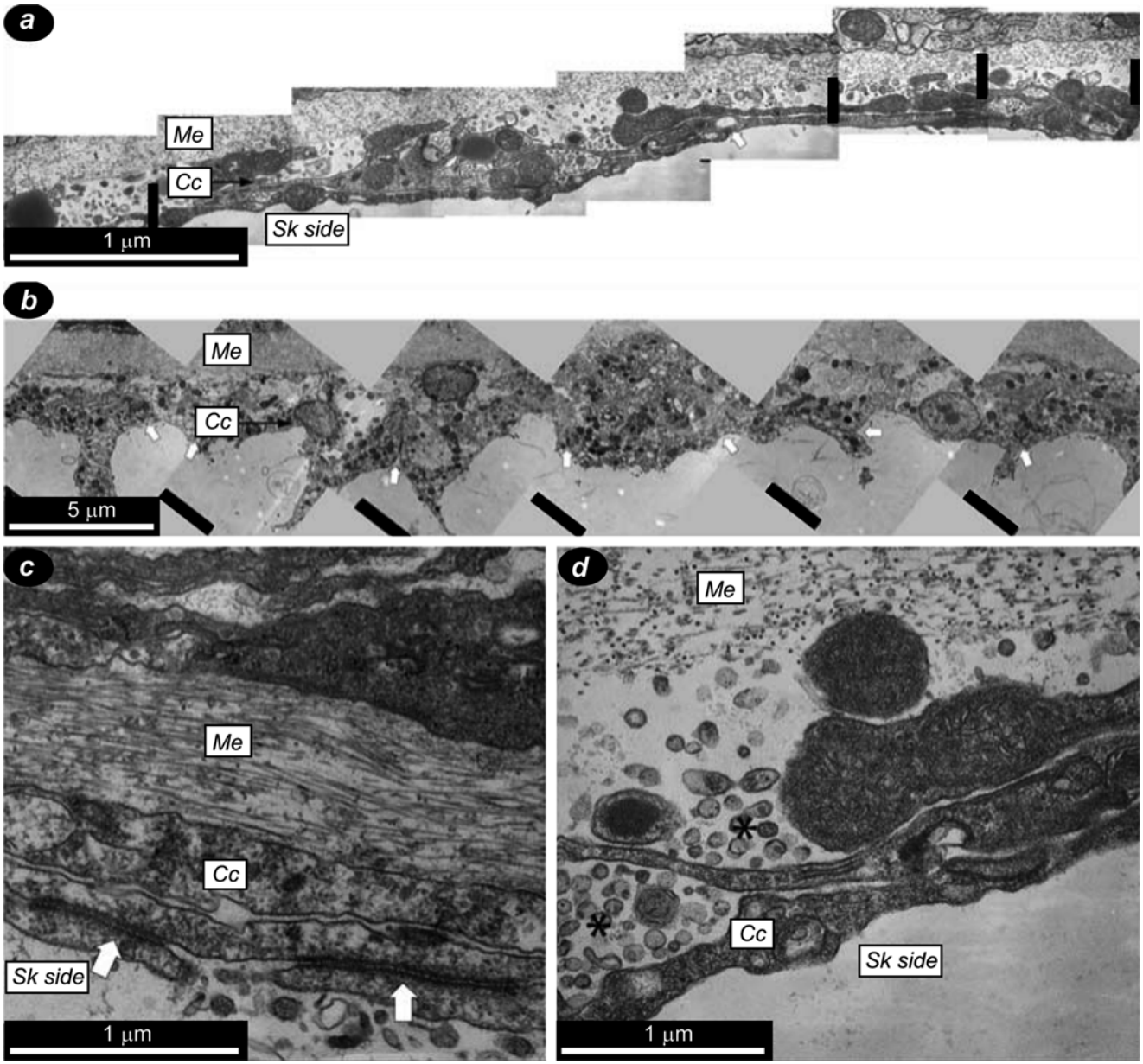

Fig. 13.3 Calicoblastic ectoderm and junctions in sections of decalcified samples processed for transmission electron microscopy. (**a**) Calicoblastic ectoderm showing long, thin and flat calicoblastic cells. (**b**) Calicoblastic ectoderm showing cup-like calicoblastic cells. (**c**) Magnification of septate junctions (*broad white arrows*) between calicoblastic cells. (**d**) Magnification of spherical extracellular material (marked with *asterisk*) between mesoglea and calicoblastic cells and between calicoblastic cells. *Broad white arrows* in **a**–**c** indicate septate junctions. *Cc* calicoblastic cell, *Me* mesoglea, *Sk* skeleton (Reproduced from Tambutte et al. 2007b with permission)

ent phases would result in a more complicated relationship (Cohen et al. 2002). Other factors that may influence strontium concentration and distribution are the activity of symbiont zooxanthellae and tidal modulation (Cohen and Sohn 2004). In *Porites lobata*, Mg/Ca ratios are broadly consistent with annual cycles in sea surface temperature, while inter annual fluctuations are not associated with temperature (Fallon et al. 1999). Magnesium distribution is different from that of strontium, with changes in magnesium concentration corresponding to the layering in the aragonite fibers. The fact that magnesium concentration in the COC is >10 times that of the fibers suggests that magnesium concentration is under biological control and that seasonal or subseasonal relationships between water temperature and coral trace element composition may be secondary (Blamart et al. 2007). Ranges of trace element concentration in deep sea corals (Hart and Cohen 1996; Gnagnon et al. 2007), suggested that biology may be the driving force for the changes in trace elements in coral skeletons. Synthetic growth experiments indicate that growth rate influences the incorporation of magnesium but

not strontium into aragonite, and this is relevant to coral biogenic aragonite structures (Gabitov et al. 2006). This is, however, not in agreement with suggestions that strontium incorporation is influenced by growth rate (Cohen et al. 2001). A study with cultured Porites species grown at two different temperatures suggests that it is strontium incorporation that is influenced by water temperature while magnesium incorporation is influenced by growth rate (Inoue et al. 2007). Studies on the massive brain coral, *Diploria labyrinthiformis*, suggested that changes in partitioning of specific elements with temperature, "surface entrapment" (Watson 1996; Watson and Liang 1995), and seasonal fluctuations in the amount of aragonite precipitated from the calcifying fluid form the basis of the temperature information recorded in scleractinian corals (Gaetani and Cohen 2006).

13.6 The Organic Matrix

In a wide definition the organic matrix (OM) should include molecules both into the skeleton and at the interface between the tissue and the skeleton. Those found into the skeleton, though initially present at the interface, can be not necessarily representative of those at the interface. However, the majority of knowledge on the OM comes from the molecules extracted from the skeleton, which could have a composition and a role diverse from those at the interface. On the other hand it has to be considered that only the molecules able to interact with the growing $CaCO_3$ can be likely entrapped into the skeleton, giving strength to the use of the intra-skeletal OM. Only a deeper knowledge of the skeleton-tissue interface (see Sect. 13.1.4) will help to the understanding of the role and distribution of the OM molecules.

In pioneering experiments the production of three extra cellular matrices by using an in vitro culturing system for coral cells was reported (Helman et al. 2008). There the excretion of extra cellular matrices by primary tissue cultures of both soft (*Xenia elongata*) and hard (*Montipora digitata*) corals was demonstrated. Structural differences between the extra cellular matrices produced by *X. elongata* cell cultures and that of *M. digitata* were observed. In cell cultures of *M. digitata*, together with the production of OM, extracellular mineralized particles were also observed.

Antibodies raised against OM demonstrated that even if other cell types, including zooxanthellae, can supply precursors for OM synthesis, only calicoblast cells facing the skeleton are directly responsible for the synthesis and secretion of the OM components (Puvarel et al. 2005a).

The OM components are usually arbitrarily divided as a function of their solubility in water upon extraction from the skeleton. The majority of the studies concern the soluble organic matrix (SOM), while so far much less attention has been dedicated to the insoluble organic matrix (IOM).

The SOM of *S. pistillata* and *P. cactus* were investigated (Puvarel et al. 2005b). Both SOM showed high amounts of acidic amino acids and glycine, while content of glycosaminoglycans and SDS-PAGE analyses were different. Three proteins of *S. pistillata* and at least five proteins of *P. cactus* were detected, some of them being able to bind calcium.

Newly synthesized SOM components were studied in microcolonies from *S. pistillata* (Puvarel et al. 2007). The presence of low molecular mass matrix components (<3.5 kDa), but no free amino acids in the skeletal SOM was detected. These molecules formed the bulk of newly synthesized SOM components.

The combination of several nucleic acid resources to a recent proteomic analysis of the *Acropora millepora* coral SOM enabled the identification of several skeletal matrix proteins (Ramos-Silva et al. 2014). The skeletal matrix proteins show a large range of isoelectric points, compositional patterns and signatures. Besides secreted proteins, there are a significant number of proteins with membrane attachment sites with strong adhesion properties. In terms of sugar moieties, there is an enrichment of the SOM in arabinose, and the monosaccharide composition exhibits the same signature as that of mucus of acroporid corals.

Soluble OMs extracted from aragonitic skeletons produced by recent zooxanthellate and nonzooxanthellate scleractinian corals were studied to investigate possible effects of symbionts in the mineralization process (Gautret et al. 1997). The characterization of their amino acid and monasaccharide compositions showed compositional differences correlated with the symbiotic or non-symbiotic character in both proteic (via Asp, Glu, Ala and Ser) and glucidic phases of SOM (via GAIN, GIcN and Gal).

The occurrence of a mucopolysaccharide layer at the outer surface of the mineralizing ectoderm of the polyp suggested its role in coral mineralization (Goldberg 2001b; Goreau 1959). A high content of probable proteoglycan-originated sulfur has been found in the SOM and within the COCs of corals (Cuif et al. 2003b). Additional evidence comes from the reported adsorption ability of GAGs on coral surfaces which depends on the charge density due to sulfate groups (Volpi 2002).

In the skeletal OM associated to the epithelium of *Mycetophyllia reesi* the calicoblast cells appeared to be the source of extracellular material that also appears to contribute to the composition of the mineralizing matrix (Goldberg 2001b). Moreover, a hyaluronan-like substance appeared to play a significant role in matrix structure.

Lipids were extracted from the aragonitic skeletons of seven modern coral species (Farre et al. 2010). The lipids differed in quantity and composition between the species. Higher proportions of sterols and sterol esters in skeleton extracts as compared to a much higher abundance of waxes and triglycerides in previously studied extracts from scleractinian soft tissues suggested a role in the mineralization process.

13.7 Coral Biomineralization Proteins

The role of proteins in the control coral biomineralization is central. For this reason many studies has been focused on these mineral-enzymes.

The most complete study of protein from corals is that of the 53 kDa protein named Galaxin extracted from the exoskeleton of the reef coral *G. fascicularis* (Fukuda et al. 2003; Watanabe et al. 2003). A cDNA encoding this protein was cloned, and the primary structure of 298 amino acids was deduced. It has a tandem 30 residue repeat and is glycosylated, but with no apparent calcium-binding activity. Interestingly, there are two conserved dicysteines (Cys-Cys) in each of the nine repeats that may invoke cross-linking to form a polyprotein network. Two less abundant proteins of approximate masses 45 and 20 kDa, were simultaneously isolated from *G. fascicularis*: neither showed calcium-binding activity.

Scleritin has been the first protein isolated and characterized from the OM of the sclerites of the alcyonarian, *Corallium rubrum* (Debreuil et al. 2012). Scleritin is a secreted basic phosphorylated protein which exhibits a short amino acid sequence of 135 amino acids and a signal peptide of 20 amino acids.

In a similar fashion, four proteins have been isolated from *Tubastrea aurea* of approximate masses 70, 53, 46, and 32 kDa, of which the 46 kDa band was the most abundant and likely to be a glycoprotein but not a calcium-binding protein. However, the 70 and 53 kDa proteins did show calcium binding activity and may be involved in the calcification process, perhaps inducing nucleation of $CaCO_3$ crystals. The major protein of 46 kDa was not homologous to galaxin (Fukuda et al. 2003).

A study on the carbonic anhydrase from *T. aurea* showed that the inhibition its activity affects the calcification rate (Tambutte et al. 2007b).

A number of proteins have been isolated from the SOM of *S. pistillata* and *P. cactus* corals (Puvarel et al. 2005b). *S. pistillata* has yielded three acidic proteins of apparent molecular weights 55, 47, and 37 kDa, of which only the 55 kDa entity stained positive for calcium-binding. In the coral *P. cactus* 5 acidic proteins were defined from SDS-PAGE analysis of the SOM with apparent MWs 68, 50, 47, 37, and 33 kDa. The proteins of 68, 50, and 47 kDa stained as calcium binding proteins. The amino acid content of the proteins found in the SOM for these two species is consistent with that obtained for other biomineral species (Cuif et al. 1999; Albeck et al. 1993; Addadi and Weiner 1985; Bedouet et al. 2001).

Of particular interest in this study is the long polyaspartate (36 Asp) domain of the 55 kDa protein from *S. pistillata*. It has long been proposed (Moradian-Oldak et al. 1992; Furedimilhofer et al. 1994; Falini et al. 1996) that this particular sequence denotes control of polymorph outcome.

High-performance liquid chromatography (HPLC) investigation of the SOM from *Monsastrea curta* and *Porites australiensis* showed only one main protein at 160 kDa for *M. curta* and one main protein at 200 kDa and a less abundant protein at 25 kDa for *P. australientis* (Dauphin et al. 2006). In both instances, high molecular weight acidic sulfated polysaccharides were found as well.

The identification of 36 soluble OM proteins in the staghorn coral *A. millepora* was carried out using a combination of proteomics and transcriptomics (Ramos-Silva et al. 2013). Besides secreted proteins, extracellular regions of transmembrane proteins were also present, suggesting a close control of aragonite deposition by the calicoblastic epithelium. In addition to the expected soluble OM proteins (Asp/Glu-rich, galaxins), several proteins containing known extracellular matrix domains were present. The number of coral-specific proteins was low, many soluble OM proteins having counterparts in the non calcifying cnidarians. The comparison with the skeletal OM proteomes of other metazoans allowed the identification of a pool of functional domains shared between phyla.

Four highly acidic proteins, derived from expression of genes obtained from the coral *S. pistillata* was carried out (Mass et al. 2013). Each of these four proteins catalyzed the precipitation of $CaCO_3$ in vitro. The results demonstrated that coral acid-rich proteins not only bind Ca^{2+} stoichiometrically, but also precipitate aragonite in vitro in seawater.

13.8 Calcium Carbonate Precipitation in the Presence of Coral's Organic Matrix

The influence of OM macromolecules on the precipitation of calcium carbonate has been investigated using diverse experimental assays. One experimental strategy has exploited the in vivo mineralization of coral recruits of other simpler biological systems. On the other hand the in vitro approach as used the extracted OM as additive able to work as $CaCO_3$ crystallization modifiers.

The $CaCO_3$ precipitation was achieved using *S. pistillata* coral tissue cultures that aggregate to form "*proto-polyps*" (Mass et al. 2012). In this experimental system the calcification was facilitated at the cellular level and simultaneously the in vitro manipulations of the calcifying fluid is allowed. Using an enriched seawater medium the primary cell cultures assembled into "*proto-polyps*" which formed an extracellular OM and precipitated aragonite crystals (Fig. 13.4). The precipitation of aragonite was independent of photosynthesis by the zooxanthellae, and did not occur in control experiments lacking coral cells.

The study of biomineralization of $CaCO_3$ in scleractinian coral recruits allowed to establish a baseline for how coral initiate skeletal formation (Gilis et al. 2014). A multiscale,

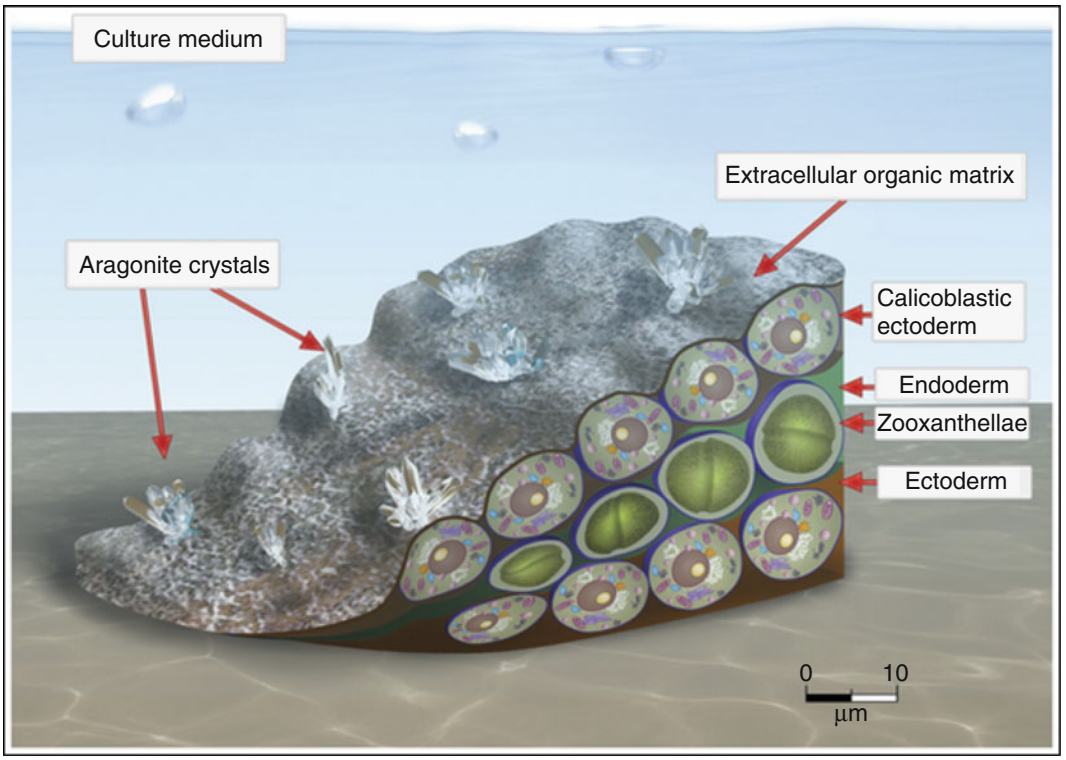

Fig. 13.4 Drawing of a proto-polyp showing the three cell layers organization. The lower layer, the ectoderm that is attached to the plate, is covered by the endoderm containing *Symbiodinium* sp. cells, which in turn is covered by the upper layer of calicoblastic cells. The calicoblastic layer secretes extra cellular matrix. Aragonite crystals form on top of the extra cellular matrix (Reproduced from Mass et al. 2012)

microscopic and spectroscopic, investigation of skeletal elements deposited by *P. damicornis* recruits was carried out based on appearance and morphology of skeletal structures The presence of calcite among the earliest components of the basal plate, which consist of micrometer-sized, rod-shaped crystals with rhomboidal habit was detected. All later $CaCO_3$ skeletal structures are composed exclusively of aragonite. High-resolution scanning electron microscopy reveals that, externally, all $CaCO_3$ deposits consist of <100 nm granular units. Fusiform, dumbbell-like, and semispherulitic structures, 25–35 mm in longest dimension, occurred during the earliest stages, with morphologies similar to structures formed abiotically or induced by organics in in vitro $CaCO_3$ crystallization experiments. All other skeletal structures of the basal plate are composed of vertically extending lamellar bundles of granules. In the latter stage, straight fibrils, 40–45 nm in width and presumably of organic composition, form bridges between these aragonitic bundles emerging from the growing front of fusing skeletal structures. These results show a clear evolution in the coral polyp biomineralization process as the carbonate structures develop toward those characterizing the adult skeleton.

The capability of intra-crystalline OM molecules to influence the in vitro precipitation of $CaCO_3$ has been studied both by $CaCO_3$ overgrowth on the coral skeleton sections and by $CaCO_3$ precipitation from solutions.

Overgrowth experiments allow to study specific crystal-macromolecules interactions under conditions that minimally affect the macromolecules native state (Aizenberg et al. 1994). They are certainly more conducive to preserving their structure that after isolation and separation procedures. Moreover, not all the intraskeletal macromolecules could be released by the skeletal elements.

Calcium carbonate overgrowth experiments were carried out on a surface section, normal to the oral-aboral axis, close to the growing tips of *Lophelia pertusa*, *Montipora caliculata*, *Acropora digitifera*, *Balanophyllia europaea*, *Leptopsammia pruvoti*, *Cladocora caespitosa* and *Astroides calycularis*. Aragonite formed on the surface of all coral skeletons (Fig. 13.5). This effect, which could be due to secondary nucleation events, brought to the growth of crystals having species specific size and texture. These crystals appeared as hexagonal prisms in different orientations, probably reflecting the orientation of the underlying fibers of ara-

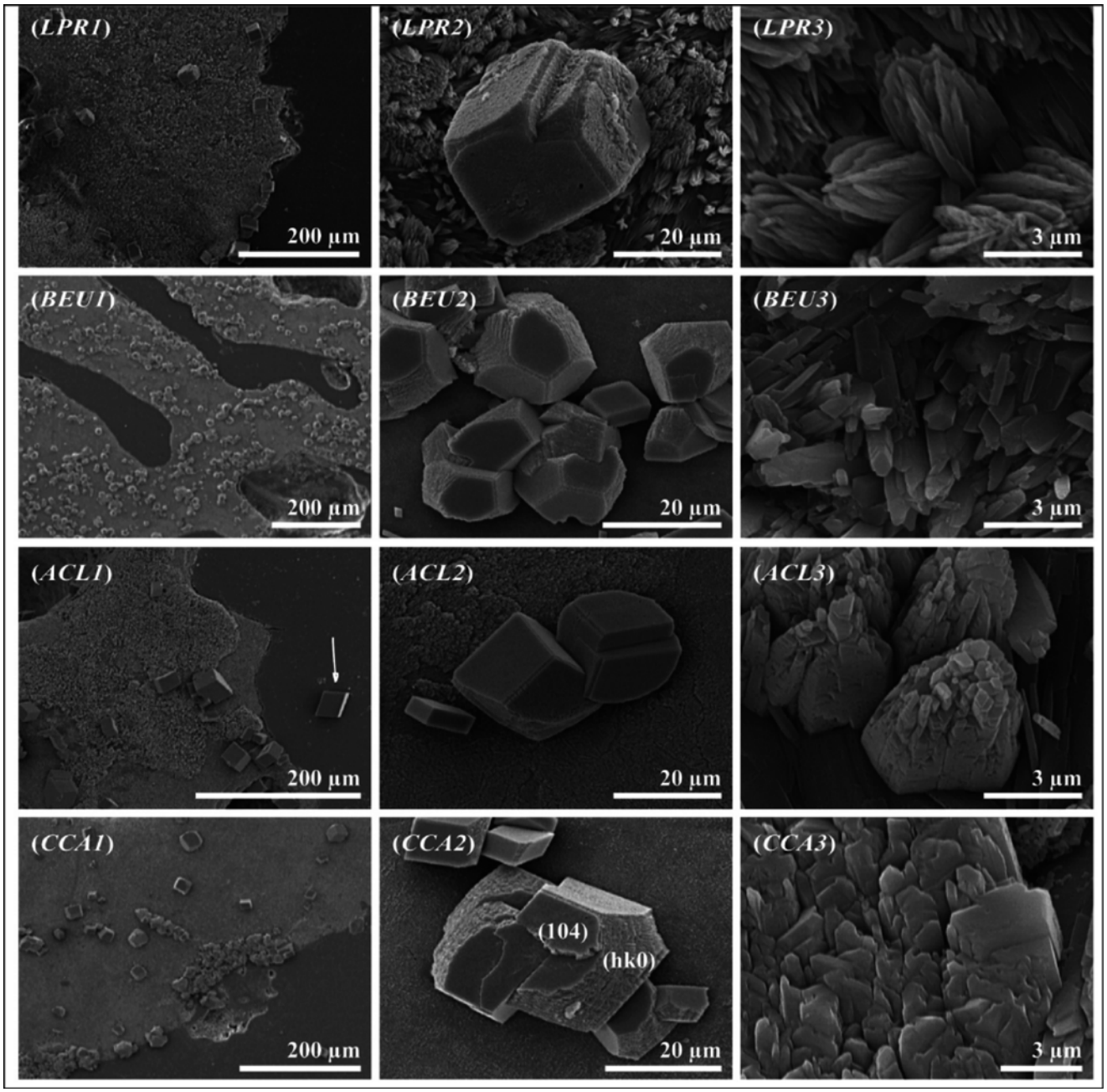

Fig. 13.5 SEM pictures at increasing magnifications (*1–3*) of sections of coral skeleton of *L. pruvoti* (*LPR*), *B. europaea* (*BEU*), *A. calycularis* (*ACL*), and *C. caespitosa* (*CCA*) after calcium carbonate overgrowth experiments. A new layer of crystals with aragonite typical morphology grew on the skeleton surfaces in all the species (magn. *3*). On these new layers more or less smoothed calcite crystals were observed (magn. *2*) that in some cases they showed additional *{hk0}* faces to the rhombohedral *{10.4}*. Outside the skeletons sections only rhombohedra of calcite precipitated (*ACL1*, indicated by the *arrow*) (Reproduced from Reggi et al. 2014 with permission)

gonite. The average cross section of these prisms was about 2 µm for *L. pertusa* and *M. caliculata* and less than 1 µm for *A. digitifera*. Onto the coral skeleton of *L. pertusa* and *M. caliculata* calcite crystals were also observed. The prisms appeared as bundles of needle-like crystals in *L. pruvoti* and as single hexagonal prisms in *B. europaea*, the bundles were about 3 µm in diameter, while the needle-like crystals and the single prisms had an average diameter smaller than 1 µm.

In *A. calycularis* and *C. caespitosa* the hexagonal prisms observed on *B. europaea* were coherently fused in big prisms having a diameter usually above 4 μm (Falini et al. 2013; Reggi et al. 2014). On the coral skeleton calcite crystals were also observed, and for *B. europaea* and *C. caespitosa* exhibited additional {hk0} faces to ones.

The precipitation of $CaCO_3$ has been carried in the presence of intra-skeletal OM components. This in vitro assay is far from the real biological environment, but is able to simulate the mineralization process by increasing concentration of carbonate ions (Addadi et al. 1987). That assay has been widely used for the study of the OM role in the precipitation of $CaCO_3$ for mollusk shells (Falini and Fermani 2013), but only recently has been exploited for intra-skeletal OM from corals.

The first study has been carried out using the OM from the skeleton of the *B. europaea*, a solitary scleractinian coral endemic to the Mediterranean Sea. The results showed that both soluble and insoluble OM components influence calcium carbonate precipitation and that the effect is enhanced by their co-presence. The role of magnesium ions was also affected by the presence of the OM components (Goffredo et al. 2011).

The OM from the Mediterranean coral *C. caespitosa* was also investigated (Sondi et al. 2011). The results obtained confirmed and complemented the hypotheses relating to the significant role of the soluble acidic protein matrix and the biologically induced colloid-chemical processes in the phase formation and growth of scleractinian submicrometer fibrous aragonite units. In that research was shown that the general strategy for the morphogenesis of fibrous structured aragonite lies in the nanoscale aggregation and subsequent coalescence processes that occur simultaneously. The subsequent morphological conversion of the initially formed submicrometer fibrous aragonite units into well-defined, micrometer-sized, prismatic facets in the skeletal structures of the corals has been demonstrated.

After this researches using Mediterranean corals, additional data were obtained using the intra-skeletal OM from tropical corals (Reggi et al. 2014). In the absence of magnesium ions, and in the presence of the OM fractions, the precipitation of calcite was always observed. The presence of SOM stabilized also the precipitation of amorphous calcium carbonate (ACC) and vaterite in *M. caliculata* and *L. pertusa*, respectively. The SOM from *A. digitifera* acted diversely having no effect on the polymorphism of $CaCO_3$. The main effect of OM fractions was on the precipitate's morphology. Aggregates of calcite particles having the shape of dumbbells, rods and peanuts were observed in the presence of the SOM from all the species. These aggregates always appeared to be formed by the assembly of sub micron sized particles, and the AFM observations showed that they were covered by an organic shell. The granular structure suggests that their precipitation occurred by a non-classical mechanism in which the precipitation took place by the assembly of preformed units (Song et al. 2009). Indeed, the coral skeleton is composed of nanosized grains (Motai et al. 2012). Interestingly, the presence of SOM from *M. caliculata* stabilized ACC. The presence of magnesium ions in the extra-cytoplasmic calcifying fluid, at the nucleation site of corals, has been reported (Holcomb et al. 2009). The analysis of the results of the $CaCO_3$ precipitation in the presence of magnesium ions and OM fractions showed, differently from what observed in their absence, a strong effect on the polymorphism and a lower effect on the morphology of the precipitated particles. While in the control experiments the co-precipitation of magnesium calcite and aragonite was observed in the presence of SOM from all the species the precipitation of aragonite was inhibited and that of ACC favored. As a seemingly opposite effect, magnesium ions favor the precipitation of aragonite (Falini et al. 2009). This apparent dichotomy could be explained by considering the role of OM components. Some of these components having strong affinity for aragonite (they are in an aragonitic skeleton) could interact with the aragonite nuclei inhibiting their growth, favoring the precipitation of other phases, and changing the final crystal's shape.

The precipitation of $CaCO_3$ in artificial sea water was carried out in the presence of OM from several Mediterranean corals (Fig. 13.6). They were *B. europaea* and *L. pruvoti, C. caespitosa* and *A. calyculari*. The results showed that the OM from *B. europaea*, as well the one from *A. calycularis*, precipitated mainly a form of ACC different from that obtained with OM from *L. pruvoti* or *C. caespitosa*. The ACC from *B. europaea* was the most stable upon heating up to 100 °C and was the one that mainly converted into aragonite instead of magnesium calcite after heating at 300 °C (Fig. 13.7). All this indicated a higher control of *B. europaea* OM over the calcium carbonate polymorphism than the other species.

To explore the coral biomineralization process, a series of $CaCO_3$ crystallization experiments were carried out in a counter-diffusion system containing a viscous agarose sol with dissolved SOM extracted from *B. europaea* or *L. pruvoti* (Sancho-Tomas et al. 2014). Indeed, the crystallization of skeletal aragonite by corals takes place in sites whose physical characteristics resemble those of a highly viscous sol or a gel. In these sites, biomolecules are secreted by calicoblast cells of the coral and some of them become entrapped in the skeleton. The influence of the viscosity of the media and the presence of

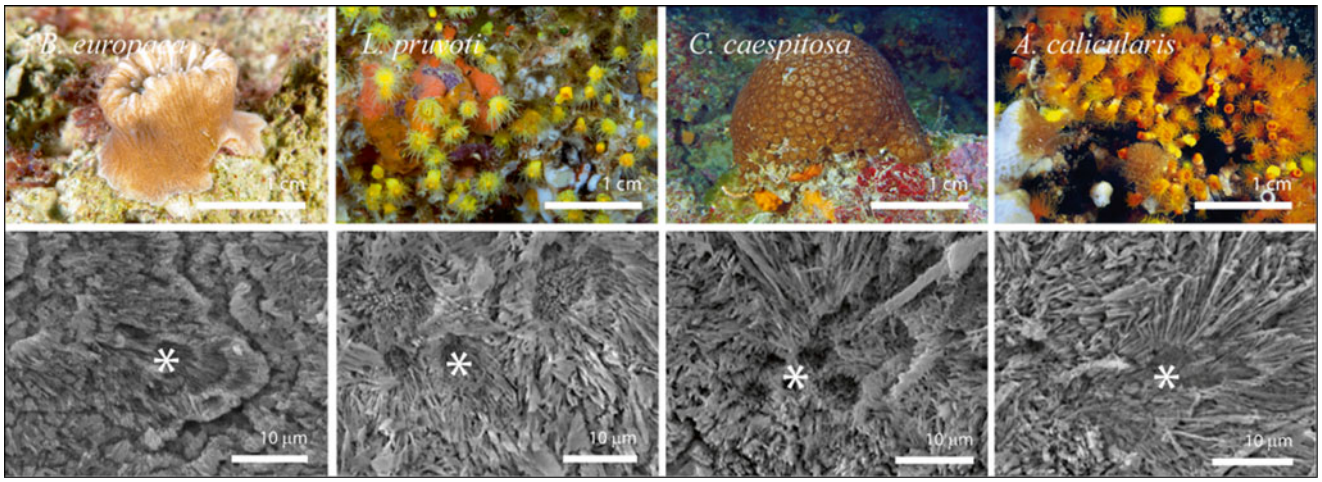

Fig. 13.6 Underwater in situ camera pictures of *B. europaea*, *L. pruvoti*, *A. caliculata* and *C. caespitosa* and SEM cross section images of their skeletons. In them the different microscale organization of the aragonitic fibers and of the centres of calcification is shown. The *asterisks* indicate the centre of calcifications surrounded by fibers of aragonite

magnesium ions were investigated in two additional sets of experiments, one using an agarose gel of variable viscosity, and another allowing magnesium ions to diffuse from the cationic reservoir (Fig. 13.8). The main findings are the following: (*i*) the species-specific molecular composition of the two SOMs has a different impact on the crystallization parameters and morphology of calcium carbonate; (*ii*) the viscosity of the gelling media, and thus its porosity, is important in regulating the SOM action; (*iii*) magnesium ion is important in defining specific and sharp limits of supersaturation under which crystallization occurs; (*iv*) the polymorph distribution is determined by SOM concentration.

13.9 Conclusions

All the studies on the deposition of aragonite in coral skeletons converge on the concept that it is a biological controlled process, although this process can influenced by environmental parameters.

The recent studies on the influence of the intraskeletal OM on the precipitation of $CaCO_3$ confirm and support this. Moreover, they add some important knowledge. (i) Despite the diverse growth and trophic form all the scleractinian corals show the same structural organization (i.e. radiating aragonitic fibers from a COC), however the composition of the OM differ from one species to another. When some compositional common features are observed, or analogies in controlling in vitro the precipitation of $CaCO_3$, these are not related at all with the growth and trophic form. In each species the OM has specific peculiarities in controlling in vitro the deposition of $CaCO_3$. The composition of the OM changes among species mainly in the relative content of proteins, polysaccharides and lipids. The proteins, which show all a high content of acidic residues, represent only a minor component of the OM, thus more investigations are necessary to address, and eventually to define, the role of lipids and polysaccharides. The recent studies showed that in vitro lipids are directly involved in the stabilization of ACC. (iii) Magnesium ions have an important role in controlling the deposition of $CaCO_3$. This observation opens important issues on the mechanism of ions transport, on the composition of the calcifying fluid at the mineral growing sites and on the discharge of side products of the $CaCO_3$ precipitation reactions.

From the above points it can be stated, with a reasonable degree of confidence, that the precipitation of aragonite in corals is controlled by the OM molecules, thus is a biologically controlled process. This even if coral skeleton do not show an apparent elegant and accurate architectural assembly of the aragonitic building units as it is in echinoderm and mollusk skeletons.

In conclusion corals, due to their simple structure when compared to other calcified organisms, represent almost an ideal gym for the study of the biomineralization processes. A biomineralization addressed study of the skeleton formation has been for a long time almost completely ignored, only recent studied shed new light from this prospective point a view and collected the attention of many scientists. Thus, there are good chances that in the near future more and more researchers will address their activities on these studies.

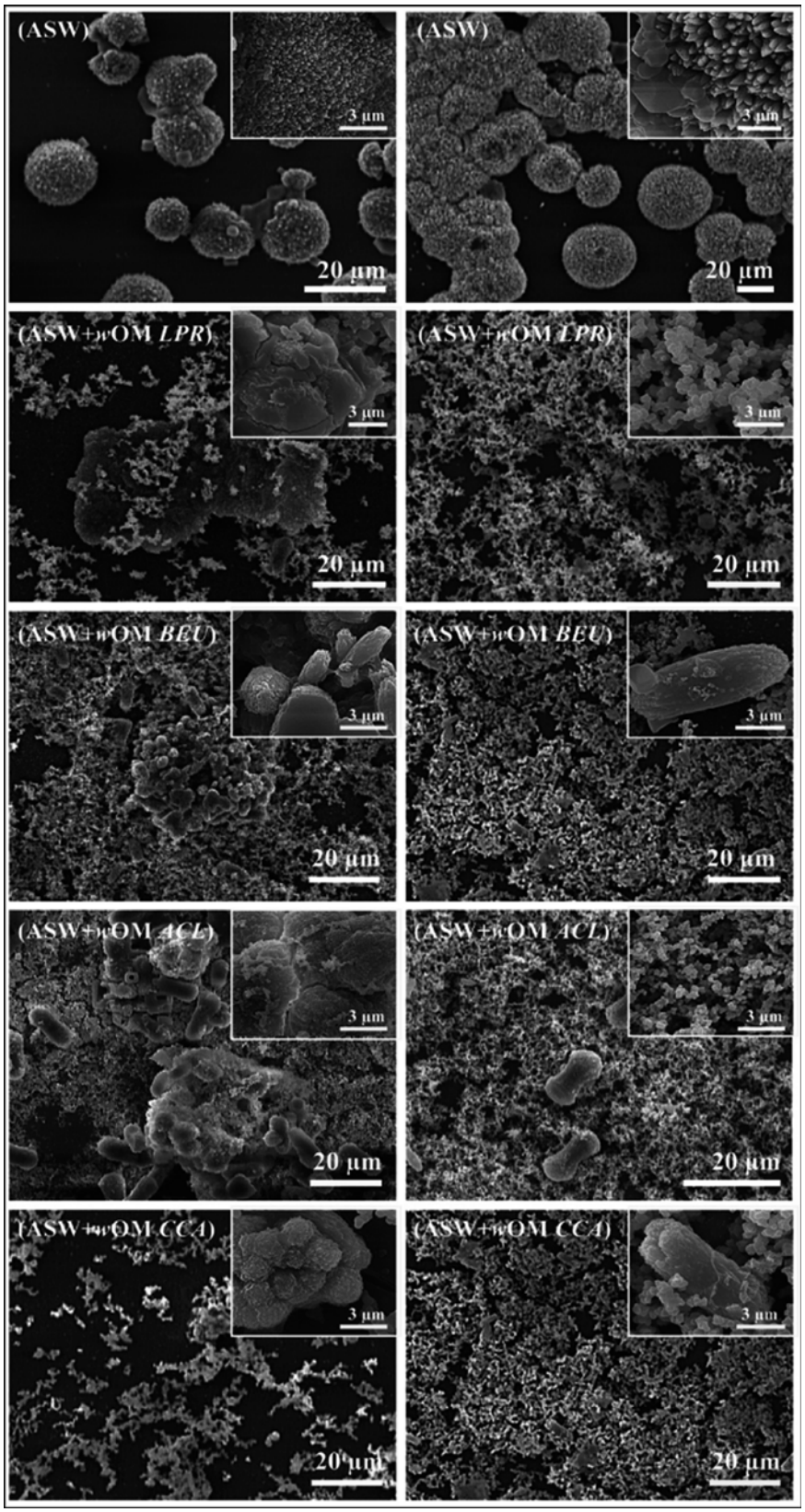

Fig. 13.7 SEM pictures of particles obtained from precipitation experiments of $CaCO_3$ from artificial seawater (*ASW*) in the presence of the whole organic matrix (*wOM*), after the synthesis (*left*), and after the thermal treatment at 300 °C for 12 h (*right*). The spherical nanoparticles (*second to fourth row*) were made of ACC before the thermal treatment and of magnesium calcite after the thermal treatment. In the presence of the *wOM* from *B. europaea*, aragonite co-formed with magnesium calcite. The *wOM* was extracted from *L. pruvoti* (*LPR*), *B. europaea* (*BEU*), *A. calycularis* (*ACL*), and *C. caespitosa* (*CCA*). These pictures are the most representative of the populations of observed particles (Reproduced from ref. Reggi et al. 2014 with permission)

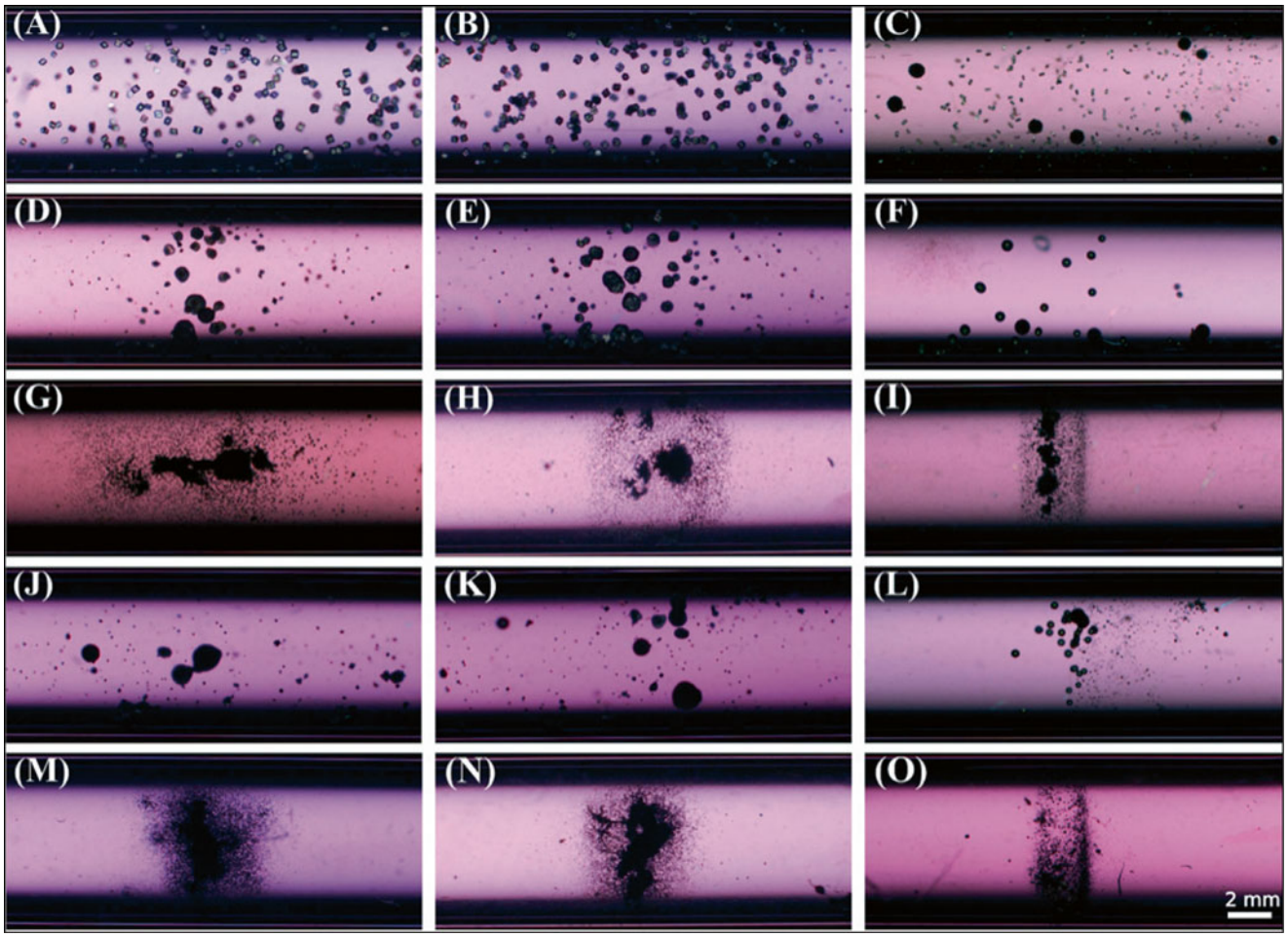

Fig. 13.8 Optical microscope images of crystal growing spaces (Δ) after 14 days, in the absence (**a–c**) and in the presence of SOMs *from B. europaea*, at concentration *c* (**d–f**) and *5c* (**g–i**), and from *L. pruvoti*, at concentration *c* (**j–l**) and *5c* (**m–o**). *The left column* refers to the highly viscous sol experiments, *the middle column* to the gel experiments and *the right column* to the highly viscous sol experiments, adding Mg^{2+} into the cation reservoir (Reproduced from ref. Sancho-Tomas et al. 2014)

There are many clues that the multidisciplinary approaches, the exploitation of several new experimental techniques and the growing interest of the response of coral calcification to environmental stresses, are going to put corals among the Olympus of biomineralizing organisms.

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From Molecules to Morphologies, a Multiscale Modeling Approach to Unravel the Complex System of Coral Calcification

14

Eva S. Deutekom, Pirom Konglerd, Paula Ramos-Silva, and Jaap A. Kaandorp

Abstract

Calcification can be viewed as an emergent behavior resulting from a complex system in which several sub-processes on very different temporal and spatial scales (ranging from nanometer and nanoseconds to meters and years) are connected in a multiscale system. Sub-processes like gene regulation, organic molecules influencing the mineral deposition process, cellular and physiological processes and environment are all linked. Although different calcification mechanisms and pathways have been proposed, there is still no conclusive and comprehensive model of the process. In recent years, many efforts have been made to individually elucidate the regulatory subprocesses. It is important to integrate this knowledge into a multiscale model and analysis that will increase fundamental understanding, guide coral preservation efforts and find potentially important applications in material sciences. Computational models can effectively aid in this integration, by providing structure and serving as a tool for inferring and interpreting interactions and hypotheses. The multiple data that have been collected and the computational models that have been and could be developed will be reviewed, starting with the coral transcriptome, followed by the skeletal proteome, tissue physiology, and skeletal micro- and macromorphology.

Keywords

Scleractinian coral • Calcification • Multiscale model • Biomineralization • Computational models

14.1 Introduction

Biomineralization is the selective uptake of chemical elements from a local environment and their incorporation into a functional structure, which happens under strict biological control (Mann 2001). This functional structure provides a range of properties to an organism, such as structural support and mechanical strength. In scleractinian corals biomineralization, also called calcification, consists of the precipitation of calcium (Ca^{2+}) and carbonate (CO_3^{2-}) ions into an exoskeleton of the mineral form aragonite.

Calcification is a joint effort of the coral colony (Fig. 14.1a), consisting of polyps (Fig. 14.1b), which are connected through a tubular system called the coenosarc (Fig. 14.1d). The coral coenosarc (Figs. 14.1d and 14.2) is organized in two tissue layers, the oral (water oriented) (Fig. 14.2a, b) and aboral (skeleton oriented) (Fig. 14.2d, e) layer, each containing an endo- and ectodermal cell layer. Scleractinian corals can also live in a mutualistic symbiotic relationship with algae of the genus *Symbiodinium*, also collectively called zooxanthellae, which reside in the oral

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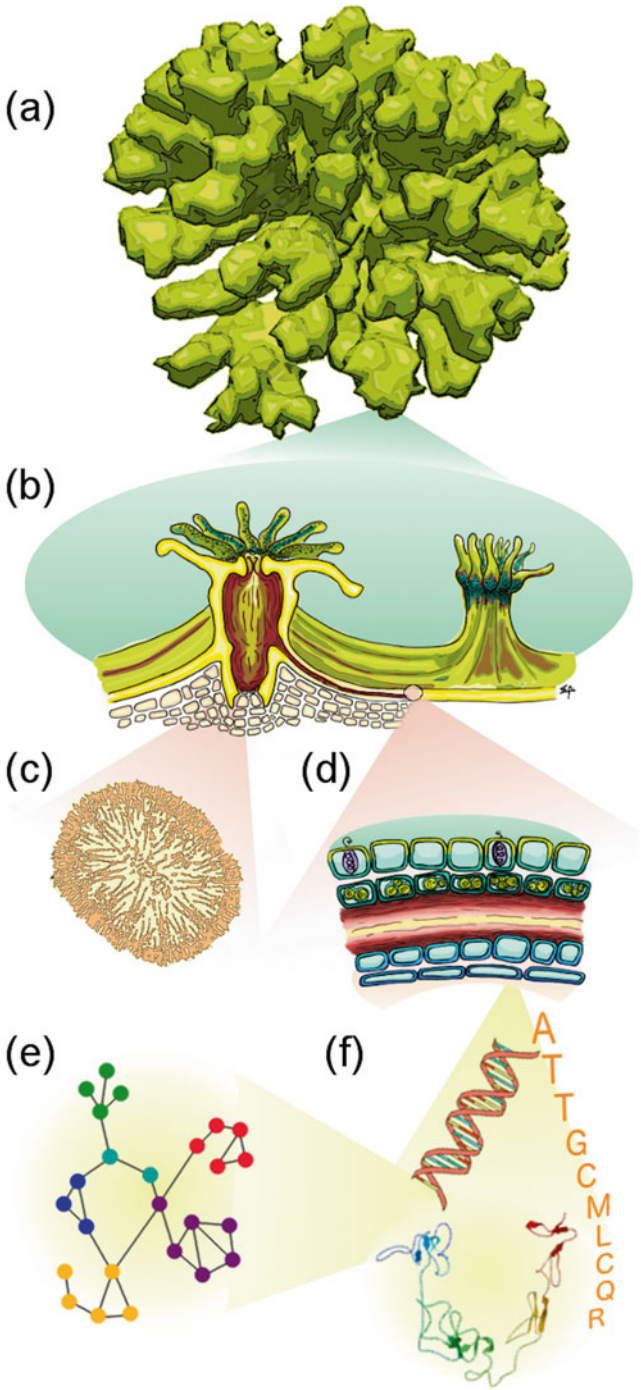

Fig. 14.1 Multiscale scheme of coral calcification research. Shown are the different scale levels of coral calcification research and modeling and their spatial and temporal scale given in parenthesis: (**a**) macromorphology (cm–m, months–years), (**b**) polyps, (**c**) micromorphology (µm–mm, days–weeks), (**d**) tissue physiology (1–100 µm, ns–µs), (**f**) molecular physiology (Å–nm, s–min), and (**e**) networks (regulatory, interaction or other)

endoderm (Fig. 14.2b). Due to photosynthesis, the algae influence calcification positively through the process of light enhanced calcification (LEC), even though the two processes are spatially separated. However, the exact mechanisms or combinations thereof are still debated (Allemand et al. 2011). Additionally, the paradoxal observation that zooxanthellate coral species have higher calcification rates where the algae concentration is the lowest shows it is difficult to assess the exact role of the zooxanthellae. At the macroscale level it is likely that zooxanthellae enhance calcification, at the microscale level it is still unclear.

The aboral ectoderm (Fig. 14.2e), also called the calicoblastic layer, is thought to be mainly responsible for skeleton formation. Yet, many different theories exist about the calcification process and how this cellular layer regulates it. At the fundamental level calcification is governed by genetics (Wirshing and Baker 2014), gene regulation (Moya et al. 2012, 2015) and physiology (Allemand et al. 2004, 2011), and also strongly influenced by the environment (Todd 2008). However, specifics on genes and their regulation, types of physiological processes and their interactions, and how multiple environmental perturbations influence calcification, remain to be fully understood. Two processes of control that are generally agreed upon for coral calcification (using Mann's (2001) definitions) are spatial and structural control through the process of boundary-organized and organic matrix-mediated biomineralization, respectively.

Boundary organization provides a means for regulating the chemistry of a solution near the mineralization site by spatial delineation, i.e. enclosing an area. This can for instance be done with physical boundaries and/or diffusion limited sites, and ion accumulation and transport (Mann 2001). For scleractinian corals, the control of ion transport and delivery to the site of calcification is done mainly using ion transport proteins and ion accumulation through enzymatic regulation by carbonic anhydrases (Allemand et al. 2011; Moya et al. 2008). The calicoblastic layer (Fig. 14.2e) is thought to be the delineating boundary.

In organic matrix-mediated biomineralization, the organic matrix (OM) acts as a macromolecular framework, possibly mediating different processes, such as mineral nucleation, surface stabilization of mineral from dissolution, mineral strength and spatial arrangement (Mann 2001). Different molecular components have been found in the coral OM, each ascribed a different function in the skeleton formation process (Dauphin and Cuif 1997; Goldberg 2001; Farre et al. 2010; Dauphin et al. 2008; Drake et al. 2013; Ramos-Silva et al. 2013). However, the exact role of the OM in coral calcification remains to be fully explored.

The control mechanisms described above, continue to be a highly debated subject and others have been proposed, such as: vesicles transporting preformed minerals (Weiner and Addadi 2011). Even though these other mechanisms should not be put aside, very little strong evidence has yet been found for them. A more elaborate discussion on this topic can be found in Allemand et al. (2011).

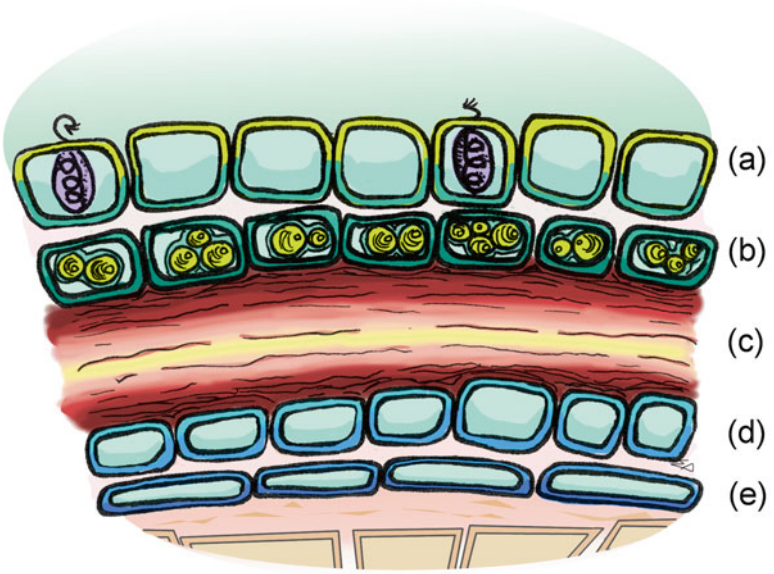

Fig. 14.2 Schematic coral coenosarc. (**a**) Oral ectoderm containing nematocysts oriented towards water (**b**) oral endoderm containing zooxanthellae, (**c**) coenorsarc's tubular cavity, (**d**) aboral endoderm, (**e**) aboral ectoderm, also called the calicoblastic cellular layer. The calicoblastic layer is located on top of the skeleton with the extracellular calcifying medium in between cells and skeleton

The study of biomineralization encompasses a large variety of concepts and methods in multiple applied and academic research fields (Allemand et al. 2011). Calcification, and biomineralization in general, can be studied on the physical, chemical, biochemical and biological level, since all levels dictate the function, composition and form of the coral skeleton and coral health. Each of these levels can also be divided into sublevels, such as water flow, crystal formation/nucleation, physiology and genetics. Researching these processes and their interactions experimentally can be costly, time consuming and hard to quantify.

When it comes to complex systems, it might be useful to use models and computational methods to aid in their understanding, function as a prediction tool, be used as a depository and organization of knowledge, gain insight on knowledge gaps, and test and generate new hypotheses. Additionally, quantitative inferences and mathematical rules can be obtained that capture the essence of different processes and their interactions.

The following sections will describe the latest computational models that have been and are being developed on different spatial and temporal scales (Fig. 14.1), from coral colony macromorphology (Fig. 14.1a) to tissue physiology (Fig. 14.1d), in an effort to understand coral calcification. In recent years, many data has been produced in the omics fields and the following sections will also suggest how to utilize most of these data to develop computational models for further analysis. The ultimate goal would be to connect all these components to form a multiscale model and analysis encompassing all spatial and temporal scales (Fig. 14.1a–f).

14.2 Calcification Genes and Gene Regulatory Networks

All species-specific characteristics must be explicable at the level of inherited genetic information and given the common protein families in the animal kingdom we must assume that differences in, for instance morphology, between animal species arise largely due to differential regulation of genes and their products (Bolouri and Davidson 2002). This regulation must also explain the different responses and susceptibilities of animals to their environment. Most of the research done on (environmental) perturbations influencing coral calcification has relied on measurements of coral growth, development and/or survival rates (Moya et al. 2015; Albright 2011), in other words by measuring a change in behavior of the system. To understand the responses of corals to environmental stressors, we must also focus on the regulation of these responses. It often happens that in a particular biological pathway, or network, environmental changes do not lead to the expected changes in behavior. This discrepancy can usually be explained by genetic regulation compensating for key components in some way.

Although it is very useful to analyze a system through its individual components, the ultimate goal would be to understand how these components work together to form a functioning system (Zvelebil and Baum 2008). Describing the network topology, i.e. arrangement and connectivity of components, of the calcification regulatory pathway will greatly enhance our understanding of the underlying mechanisms, the coral's response to external influences and the robustness

or sensitivity to environmental changes. These networks can yield an abundance of information, such as co-expression and molecular interactions, and inferring such a network is the first step in modeling a complex biological system.

With the latest advances in the transcripomics field, it has become relatively easy to measure gene expression levels of thousands of genes simultaneously, mainly using microarray or RNA-seq experiments. Relevant information and resources regarding microarray studies with corals have already been summarized by Forêt et al. (2007). Multiple studies on gene expression in corals (Grasso et al. 2008, 2011; DeSalvo et al. 2008; Reyes-Bermudez et al. 2009a; Voolstra et al. 2009; Moya et al. 2012, 2015; Vidal-Dupiol et al. 2013; Rosic et al. 2014) have made a significant contribution to understanding coral biology and providing a wealth of transcript data.

Moya et al. (2012) did the first comprehensive whole transcriptome analysis of the species *Acropora millepora*, studying the response to CO_2 driven acidification on the initiation of calcification. They found that acidification had little effect on ion transport protein expression, lowered secretory and membrane-associated carbonic anhydrase expression and had a dramatic effect on skeletal organic matrix protein expression. They later also studied the effect of short and prolonged exposure to acidified conditions, showing that juvenile *A. millepora* had the capacity to rapidly acclimate to these conditions by expressing specific heat shock and anti-apoptotic proteins (Moya et al. 2015). These intricate regulatory responses of *A. millepora* to the acidified conditions underline the importance of investigating this regulatory pathway and its interactions further.

No studies have yet inferred any regulatory networks to explain the mechanisms controlling calcification. The above studies used traditional techniques to find differentially expressed genes in different environmental conditions, for instance by cluster analysis. However, this traditional methodology cannot efficiently explain how thousands of RNAs interact in networks and pathways. Inferring regulatory networks from measured expression data is a very laborious task and computational tools and methods are needed to aid the process. Multiple methodologies, experimental technologies and data processing techniques to infer gene regulatory networks have been discussed previously (Bolouri and Davidson 2002; van Someren et al. 2002; Le Novère 2015). Most methods to identify networks from large data assume that the amount of variables in a system (e.g. gene transcripts) is substantially less than the amount of observations (e.g. replicates). In reality, this is most of the time not feasible due to experimental and financial constraints of a research group.

RNA-seq has become the method of choice for obtaining expression data since, unlike microarrays, RNA-seq can also detect novel transcripts and splice variants (Gibson and Muse 2009). This is especially useful when sequencing complex organisms like corals. Inferring gene regulatory networks from RNA-seq is still at its infancy. Nonetheless, some protocols have recently been developed (Zhu et al. 2013; Angelini and Costa 2014; Li et al. 2015; Schulze et al. 2015). In the case of microarrays, this is less of a problem since multiple tools are available commercially (Ingenuity (http://www.ingenuity.com) and Pathway Studio (http://www.elsevier.com/solutions/pathway-studio)) and noncommercially (FunRich (http://funrich.org), GenMAPP (http://www.genmapp.org) and Moksiskaan (http://csbi.ltdk.helsinki.fi/moksiskaan/)). Some of these tools are already adding features to implement RNAseq data.

RNA imaging techniques, such as in situ hybridization, have the advantage of adding spatiality to the data. Different mechanisms influencing coral calcification are spatially separated and therefore this technique can provide very useful additional information. There are some gene expression images produced from different coral larval stages, for instance gene expression during development (Grasso et al. 2008, 2011; Hayward et al. 2011) and organization and gastrulation (Hayward et al. 2015). A recently developed computational approach (Botman et al. 2014, 2015) has shown to be able to infer a basic gene regulatory network using expression images of developing *Nematostella vectensis* larvae. It is possible that this approach can also be used on coral larvae expression images (Hayward et al. 2011, 2015) in the near future. Both *N. vectensis* and scleractinian corals are anthozoans. Since *N. vectensis* is a noncalcifying anthozoan, comparing its regulatory network to that of a calcifying anthozoan might also prove to give valuable information regarding the regulation of calcification.

Building gene regulatory networks is the first stage in modeling regulatory interactions and calcification control. However, building these networks will be dependent on the data and knowledge that is available and if the data can be measured. Although this section has been on inferring regulatory networks, other types of networks can be inferred as well, such as: metabolic, protein, and signaling pathway networks. Eventually, these can be integrated to form the complete interaction system explaining function and behavior of the coral organism.

14.3 The Skeletal Organic Matrix: Protein, Molecular, Structural and Functional Characterization

Mann (2001) described a general model for the biomineral organic matrix (OM), consisting of a structural framework of hydrophobic macromolecules associated with acidic macromolecules that act as a nucleation surface for biomineralization. The coral skeletal OM consists of many different macromolecules, such as polysaccharides (Goldberg 2001),

glycoproteins (Dauphin and Cuif 1997), lipids (Farre et al. 2010), sugars (Dauphin et al. 2008) and proteins (Drake et al. 2013; Ramos-Silva et al. 2013). The exact role of the OM in coral calcification and the skeleton has not yet been fully elucidated. Multiple functions have been suggested (reviewed by Allemand et al. 2011; Tambutté et al. 2011a), ranging from strengthening the mineral to catalyzing skeleton deposition (Mass et al. 2014) or by providing a structural framework (Watanabe et al. 2003).

Many data are available describing the different components and amino acid composition of the OM in multiple coral species (Tambutté et al. 2007a). The way all these components work in vivo is still unknown due to the technical challenges in working at the tissue and cellular level in corals (Weis et al. 2008). Current developments in applying tandem mass spectrometry techniques to the purified skeletal organic matrices, in combination with genomic and transcripomic data, have allowed the first characterizations of OM proteomes. Drake et al. (2013) characterized 36 skeletal organic matrix proteins (SOMPs) in the coral *Stylophora pistillata* and extensively compared them to the genomes and transcriptomes of three other corals, invertebrates, single celled organisms and human. Their results suggest an OM proteome composed of cadherins, integrins, contactin and similar adhesion proteins that play a dual role in forming an extracellular matrix that adheres to newly formed skeleton and attach calicoblastic cells to this matrix; actins and tubulins providing flexibility of the calicoblastic space size and shape, i.e. between cells and skeleton, during crystal growth; carbonic anhydrases mediating favorable carbonate chemistry changes; collagens providing structural support within the calicoblastic space, where coral acid-rich proteins (CARPs) and analogous proteins can bind to create sites of mineral nucleation and growth. The CARPs have been shown to catalyze the precipitation of new aragonite crystals in vitro, possibly driven by an electrostatic interaction of Ca^{2+} ions with carboxylate groups on the proteins, followed by a dehydration step and precipitation of carbonate (Mass et al. 2013).

Ramos-Silva et al. (2013) identified 36 SOMPs in *Acropora millepora*, such as skeletal aspartic acid-rich proteins (SAARPs, homologous to CARPs in *S. pistillata*), extracellular matrix proteins, enzymes, a toxin, galaxins and other uncharacterized proteins (Fig. 14.3). These proteins contain signal peptides and transmembrane domains suggesting that they can be targeted to the extracellular (calcifying) space but may remain attached to the cell membranes. In the latter case the extracellular region of the protein facing the calcification site, is cleaved by proteins with proteolytic functions (peptidases) and enclosed within the skeleton. Figure 14.3 shows an example of a peptidase, a protein containing proteolytic domains. The presence of peptidases suggests that membrane-bound proteins may contribute to the calcification process by the cleaved extracellular domains. This evidence suggests that cell surfaces may exert a more effective control on the calcification site through the presence of transmembrane proteins that are incorporated into the OM.

Another recent study (Adamiano et al. 2014) of the OM in four Mediterranean species detected higher contents of lipids and saccharides than proteins. As discussed in Tambutté et al. (2011a) lipids and saccharides are thought to be involved in the nucleation and stabilization of calcium carbonate, suggesting that proteins are not the only controllers of calcification. Ramos-Silva et al. (2014) also found a high content of sugar moieties in *Acropora millepora* OM, supporting older studies with other *Acropora* species showing high sugar concentrations (Cuif et al. 1999).

The knowledge gained from these proteomic studies deals with the protein composition in terms of primary structure (i.e. sequence data) and do not provide quantitative or spatial information at the time of skeleton formation. Immunolocalization of seven proteins in the tissue and mineral of *S. pistillata* revealed that at the nanoscale, SOMPs are embedded within the aragonite crystals in a highly ordered arrangement consistent with a diel calcification pattern (Mass et al. 2014). The SOMPs are suggested to be involved in skeletal linear extension at night and skeletal thickening during the day. The crystalline units are composed of submicron particles in a coat of organic matter. The same proteins might also have other roles in coral metabolic pathways, since their presence is not restricted to the calicoblastic ectoderm.

The information obtained from these studies shows how intricate this aspect of biomineralization is. Many questions are still open regarding the coral OM. What exactly are the functions of the non-protein components, i.e. lipids and polysaccharides, in the OM? How do all the proteins and molecules interact with each other and the biomineral? An elaborate list of OM components is just the small first step to a more comprehensive analysis and a way towards a more complete model of the OM and its control on calcification. The next step would be to have a more elaborate description of functions and understand the different interactions of proteins and other molecules with the mineral in the OM framework.

For obtaining preliminary information regarding a protein function, a fast and relatively easy method is based on the protein primary structure, which can be used to query databases of protein domains and families (Apweiler et al. 2001; Finn et al. 2014). As shown in Fig. 14.3, it is possible to assign protein domains in this way and thus predict functions. The protocadherin protein, for example, is organized in many cadherin domains, which have calcium-binding functions, and laminin G domains, involved in cell adhesion (Sigrist et al. 2005). Moreover, the sequence contains several cysteine residues that are positioned to form disulfide bonds

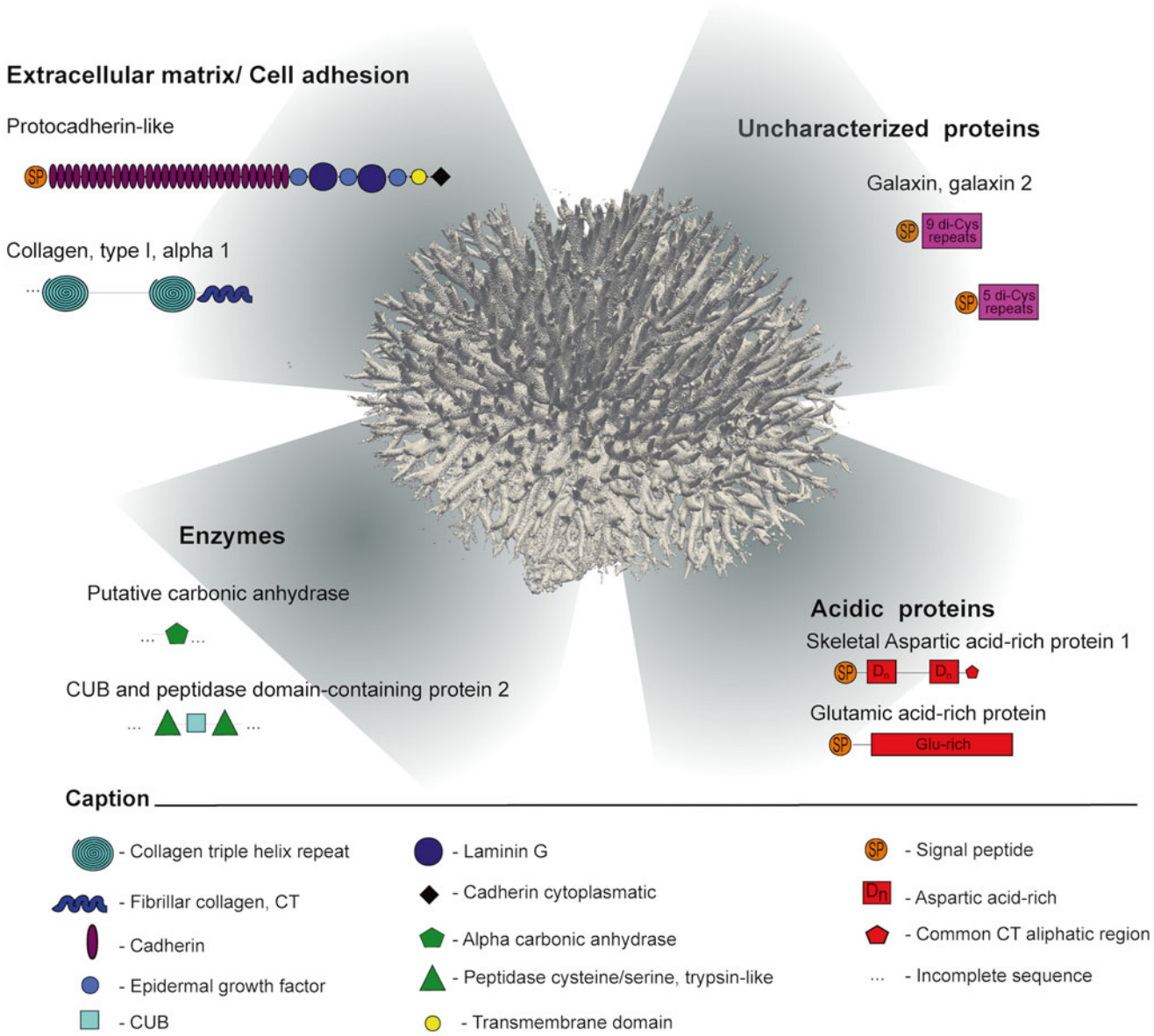

Fig. 14.3 Examples of skeletal OM matrix proteins identified in *Acropora millepora* (Ramos-Silva et al. 2013). Schematic representation of the domain architecture

that may produce a more stable protein or even linkage to other proteins forming dimers or trimmers. The same feature is observable in galaxins, a family of proteins that has been associated to skeletal formation in many corals and possesses several di-Cys repeats (Watanabe et al. 2003; Reyes-Bermudez et al. 2009b; Moya et al. 2012; Ramos-Silva et al. 2013). Besides the possible link to biomineralization and their identification in skeletal tissues, the functions of galaxins have not yet been determined. They do not show homology with other protein families at the domain level and they are therefore joining the large group of uncharacterized sequences in biomineralization that do not refer to any known protein family and/or function.

To answer the question of how OM proteins and other components cooperate in the biomineralization process, it is important to look at their possible interactions that can provide additional information on their function. There are multiple experimental methods to infer protein-protein (reviewed by Phizicky and Fields 1995) and protein-molecule interactions (reviewed by McFedries et al. 2013) that have not yet been used in corals. However, not all methods might work due to experimental constraints. There are also computational methods for the prediction of functional linkages between proteins (reviewed by Raman 2010).

Protein function or activity is not fully determined without knowing its three-dimensional structure (Zvelebil and

Baum 2008). Additionally, the structure can give the finer details of the protein surface that enables protein-protein or protein-molecule interactions. Obtaining a protein's shape experimentally, for instance by X-ray crystallography or nuclear magnetic resonance, can be laborious and costly. It is also not feasible to obtain all possible protein structures of all existent proteins this way. Ab initio methods that can predict a tertiary protein structure from its sequence are still unreliable, since the underlying protein folding principles are not fully understood, and impractical due to vast computer resources that are needed (Zvelebil and Baum 2008). This approach can only accurately predict the structure of small single domain proteins.

There are two other available methods for tertiary protein structure prediction. The first is by homology modeling and it is the modeling of the unknown structure with respect to a known structure of a homologous protein. When homologous protein structures are absent a second method, known as threading, can be applied to model the sequence of unknown structure onto all known protein folds to see which one fits best. This can then also be used as a more realistic structural model for the query protein in homology modeling. Once a good protein model is obtained, it can be analyzed like a real structure such as incorporating mutations to explore effects on function and searching for interactions using the surface properties.

However, coral protein research in both organism cells and skeleton has a long way to go before structural models or interaction networks can be fully determined. Nevertheless, modeling approaches to understand protein functions and interactions will become more important as more sequencing projects are completed for both corals and other Cnidaria, and as more representative structures are experimentally resolved to use as templates.

14.4 Coral Calcification Physiology Models

Additional to organic matrix-mediated biomineralization (Sect. 14.3), boundary organization is thought to be important for calcification control in scleractinians. This control mechanism is the partitioning of areas either within or outside of the cells by organic structures (Mann 2001). These enclosed areas are spatially defined and chemically separated and have multiple important functions such as controlling solution compositions and saturation state through ion pumping, enzymatic activity and water content, and moving mineralized structures to new construction sites. However, the exact organic structure used in boundary organization in scleractinian corals remains to be definitively proven and is still highly debated (reviewed by Allemand et al. 2011).

Vesicles with ions or preformed minerals have been suggested (Weiner and Addadi 2011). Intracellular vesicles have been observed in some scleractinian species and in others they have not (Tambutté et al. 2007b). Another possibility is space delineation using a group of cells in association with an existing mineralized structure (Mann 2001). The calicoblastic cellular layer located on top of the coral skeleton (Fig. 14.1d) is attached to the skeleton via specialized cells called desmocytes. It has been shown that there is a space between skeleton and the cells, possibly filled with a hydrogel, called the extracellular calcifying medium (ECM).

Hydrogels have lowered water content, favoring crystal formation (Weiner and Addadi 2011; Mann 2001). Additionally, the chemical composition of the ECM seems to be controlled, since the concentration of Ca^{2+} ions and pH are higher than the surrounding seawater (Al-Horani et al. 2003a, b). Carbonic anhydrases (CAs) have also been found at multiple locations in the coral tissue where they play major roles in different physiological processes (Bertucci et al. 2013). CAs are also secreted into the ECM, where they influence the carbonate chemistry locally and thus the calcification process.

Skeleton precursor ions are theorized to be transported to the ECM either with transporter proteins (transcellular pathway) or might pass the calicoblastic layer by diffusing between the cells (paracellular pathway). A possibility is also a combination of both. Multiple transporter proteins have been found (reviewed by Tambutté et al. 2011a; Allemand et al. 2011) to indicate a transcellular pathway, such as: a Ca^{2+}/H^{+}-exchanger (Zoccola et al. 2004), a Ca^{2+}-channel (Zoccola et al. 1999) and a HCO_3^- exchanger (Zoccola et al. 2015). Experiments using the membrane impermeable dye calcein suggest that there is a passage between cells to indicate the paracellular pathway, since this dye was incorporated into the skeleton. In the paracellular pathway there are also septate junctions present, which contain proteins indicating permselective properties (Ganot et al. 2014).

Hohn and Merico (2012, 2015) developed a mathematical box model implementing a detailed description of the carbonate chemistry significant for coral calcification and incorporating a simplified mechanism of photosynthesis and respiration. With this model they quantified the relative importance of both the paracellular and transcellular transport pathways, also in relation to ocean acidification. They tested four hypotheses describing alternate possibilities of how calcification is regulated by ion transport pathways through the tissue layer, which were: (p1) only the transcellular pathway; (p2) only the paracellular pathway; (p3) protons actively removed via the transcellular pathway and calcium passively diffusing between cells; (p4) a combination of both pathways. Additionally, energy costs of coral calcification were calculated for each of these pathways.

Their results showed that both the transcellular pathway (p1) and the combination of the transcellular and paracellular

pathway (p4) agreed best with experimental data (Al-Horani et al. 2003a). Increased CO_2 (acidification) caused a decrease in calcification rate for both p1 and p4 pathways. However, they argued that even though when implementing only the transcellular pathway (p1) gave good results, this mechanism couldn't explain the experimental evidence of calcein being incorporated into the growing skeleton (Tambutté et al. 2011b). Moreover, the results showed that the combination of both paracellular and transcellular pathway (p4) required the least energy for calcification compared to all other pathways.

The fact that the difference in results of the simulated transcellular pathway and a combination of both the transcellular and paracellular pathway was minimal, suggests that the paracellular pathway is of minor importance. Moreover, the simulations showed that the paracellular pathway actually causes a passive leakage of ions out of the ECM (Hohn and Merico 2015). These observations and others led them to conclude that calcification resilience in corals to ocean acidification is the consequence of active control of transcellular ion transport and paracellular leakage the reason for its vulnerability.

The model described above implements respiration and photosynthesis as a constant source and sink of CO_2, respectively. Additionally, active ion transport (requiring metabolic energy) through the transcellular pathway was modeled as an on- and off-switch during day and night conditions, respectively, to test the LEC hypothesis.

Nakamura et al. (2013) focused more on modeling one of the proposed mechanisms for LEC and did so by incorporating photosynthesis not only as a sink for CO_2, but additionally a source of oxygen and photosynthate that is later stored. Both the transcellular (only the Ca^{2+}/H^{+}-exchanger) and paracellular pathway were incorporated. Photosynthate can be consumed by respiration, which provides energy to drive the Ca^{2+}/H^{+}-exchanger.

The model was able to simulate the dark and night dynamics of calcification very well and from the results it could be concluded that the LEC mechanism can be explained by increased oxygen inside the coral polyp due to photosynthesis, which stimulates coral respiration in light conditions (or oxygen depletion inhibits respiration during dark conditions). This is followed by a calcification increases due to higher Ca^{2+}/H^{+}-exchanger activity, driven by higher respiratory energy availability.

The models described above do not explain all physiological mechanisms deemed important for the calcification process and each model has its own set of characteristics different from the other. Many questions still remain to be answered with these models, such as how spatiality affects calcification and how the results will change when other biological and abiotic components are added. Nevertheless, these models show that they are ideal tools for quantifying and analyzing coupled physiological processes in coral calcification that would otherwise be hard to research in biological experiments. Additionally, they helped answer fundamental questions in coral biology. Together with other physiological models (Ries 2011; Gagnon et al. 2012) there is now a comprehensive collection of modeling tools that has contributed to the understanding of coral calcification and physiology.

14.5 Microstructural Characterization of the Coral Skeleton

Since the coral skeleton is the final physical product of the calcification process, quantifying the properties of the skeleton by inspecting its external and, in particular, internal structure may possibly reflect the capacity of corals to calcify. There are a number of studies inferring the relationship between the calcification process and the quality or condition of the skeleton, including its changes due to environmental factors (Goffredo et al. 2007; Caroselli et al. 2011; Fantazzini et al. 2013, 2015). For example, the skeletal density and the skeletal extension rate are parameters used to calculate the coral calcification rate. A decrease of calcification causes an increase in skeletal porosity (Caroselli et al. 2011) with larger sized pores (Fantazzini et al. 2013), which decreases skeletal density (Goffredo et al. 2007) and may also result in lowering of the mechanical resistance (Fantazzini et al. 2013). To investigate the relationship more precisely, quantification at a very fine scale of the internal structures of the skeleton in order to obtain in-depth information is inevitable.

Microstructure describing the appearance of an object at micrometer length scale is of great interest to studying the quality of the material inside the object. There are several studies concerning the microstructural characterization in various fields such as medical (Massai et al. 2014), material science (Kastner et al. 2011), geoscience (Mahabadi et al. 2014), and biology (Popov 2014). With regard to corals, the characterization of microstructures and the skeletal properties such as density, porosity, pore distributions, shape complexity, and surface characteristics indicate skeletal quality. These properties are used in the environmental condition records of seawater (Nothdurft and Webb 2007), the effects of climate change (Tambutté et al. 2015), and coral graft as bone substitution studies (Puvaneswary et al. 2013).

With novel imaging techniques, microstructures can be obtained using a range of microscopy techniques such as Scanning Electron Microscopy (SEM). However, these techniques mainly analyze surface structures and are unable to analyze internal structures in 3D. Micro computed tomography scanning (μCT) is a noninvasive and nondestructive 3D imaging technique, and can be used to obtain and characterize

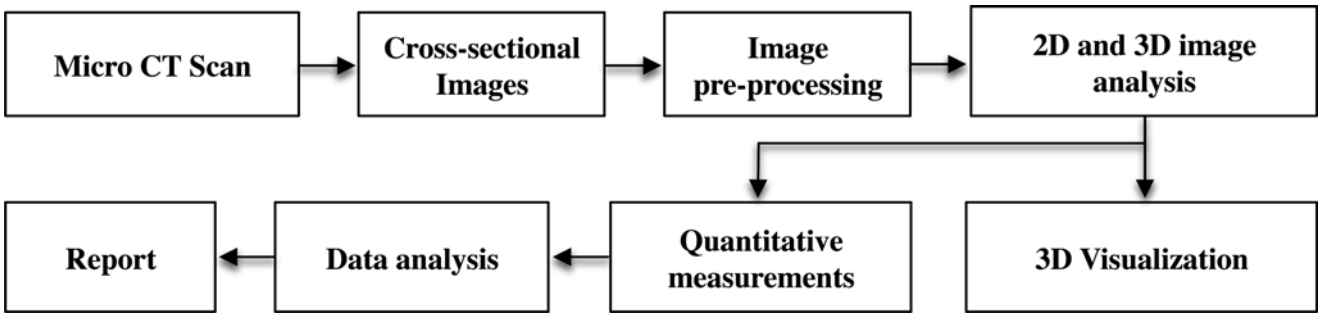

Fig. 14.4 Micro CT image processing and procedures

the microstructure of the skeleton in 3D, in particular the internal structures. Due to the success of applying μCT in various fields (Cnudde and Boone 2013; Wise et al. 2013; Peyrin et al. 2014; Hammel et al. 2012), it is not surprising that in recent years the focus has been on using this technology on corals to aid in identifying skeletal microstructure to provide in-depth information. To obtain accurate values of skeletal properties, 3D imaging techniques like μCT scanning at high resolution and their analysis are needed to reveal the finer detail of the porous structure inside the skeleton in 3D.

For μCT image processing and analysis procedures (Fig. 14.4), all cross-sectional images produced from a μCT scan need to be pre-processed to improve the quality of the images. The image pre-processing includes noise reduction by selecting the appropriate noise filter, image segmentation for better image handling, and binary operations such as closing (filling in) image pixels to join disconnected object parts that in reality are connected. The next step is image analysis is applying labeling to be able to extract each connected component in the image, or in this case the image of the skeleton. 3D rendering techniques are then used to visualize the skeletal structures in 3D and finally the skeletal properties are quantified for further data analysis.

Quantitative results from 3D analysis can include density, porosity, pore characterization, skeleton thickness, and surface characteristics such as surface area and surface roughness. Porosity and pore characterization are the most widely used properties to assess the condition of many porous materials. Porosity indicates how porous a material is and pore characterization reveals in-depth detail of pore structures. Figure 14.5 is an example of pore characterization that can be obtained using 3D analysis of 2D cross sections from a μCT image.

Additional to porosity, sub-porosity can say something about the condition of a particular area inside the skeleton. For example, the porosity value of an annual band in the skeleton can provide information about the effects of surroundings on calcification in a particular year. Similar to sub-porosity, the location of pores correlated with pore size, pore shape, and pore surface profiles can be used to keep track of changes of the skeleton in a certain period and can therefore be used as a historical record of environmental change. Besides porosity and pore characterization, skeleton thickness is also important property to indicate the strength of skeletal structures.

Advancing techniques in μCT combined with 2D and 3D image processing create the opportunity to directly visualize and quantify the internal architecture of the coral skeleton in 3D. The quantitative properties including skeleton porosity, pore characterization, and thickness are able to more accurately indicate the condition of the skeleton under study and the effects of environmental changes on coral calcification. Additionally, the quantitative measurements can be used in several fields of study such as paleoclimatology and coral graft studies.

14.6 Modeling Coral Macromorphology: Skeleton Growth and Form

Corals show a high degree of phenotypic variation, displaying diverse growth forms along a gradient of environmental conditions (Hennige et al. 2010; Todd 2008). Due to the heterogeneous reef environment it is difficult to identify the exact parameters affecting coral morphology in the field and there are many problems conducting suitable lab experiments (Todd 2008). However, evidence from multiple studies suggests that the most important parameters are water movement and light (reviewed by Todd 2008).

Branching coral colonies naturally show radially symmetrical growth forms. Presuming the shape of a coral colony is determined mainly by environmental factors, the symmetry of the colony will reflect the symmetry of the environment (Mass and Genin 2008). Mass and Genin (2008) researched this hypothesis by conducting an in situ flow experiment, where the coral *Pocillopora verrucosa* was exposed to unidirectional flow. The corals developed a more compact morphology and longer linear extension of the branches in the upstream direction of the flow. Additionally,

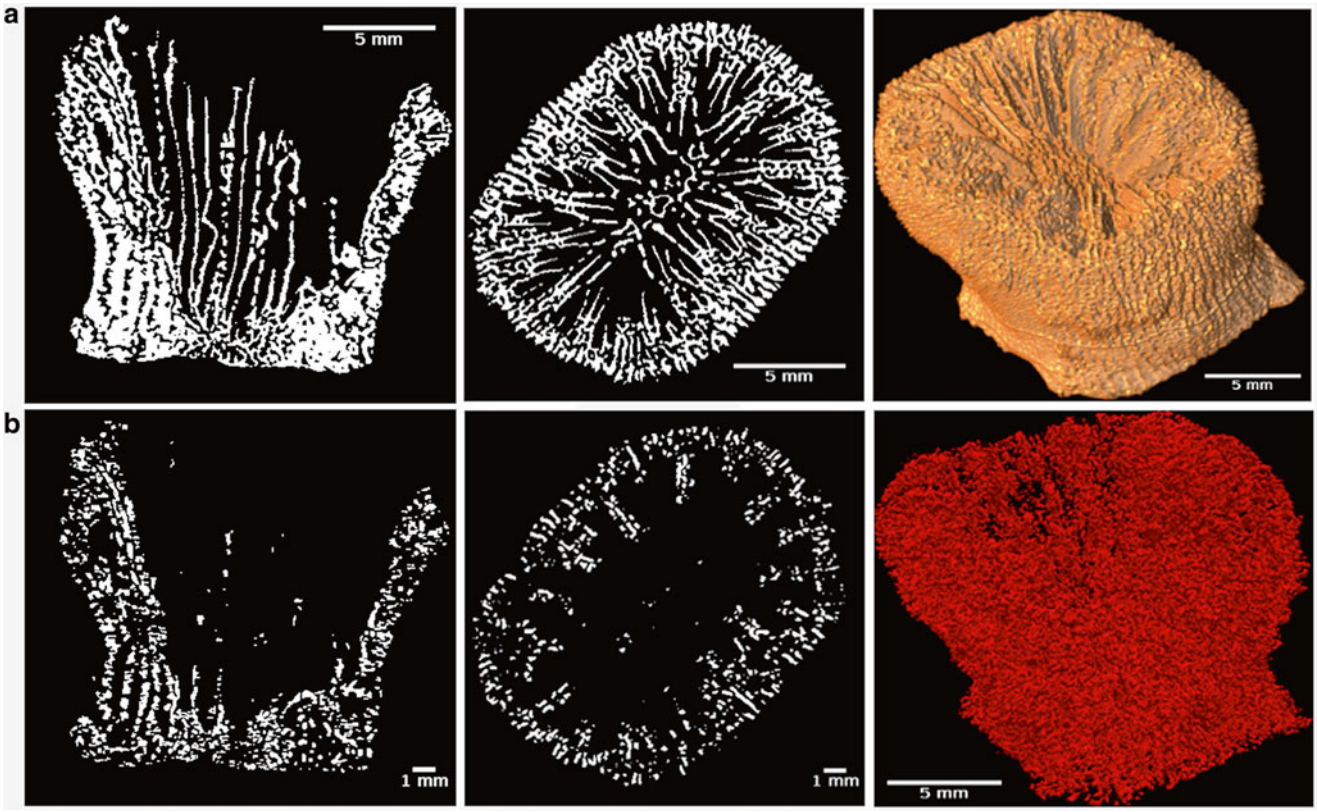

Fig. 14.5 Porous structures and pore distributions of *Balanophyllia europaea* skeleton. (**a**) Porous structures: side view (*left*) and top view (*middle*) of 2D cross sectional image produced from a micro CT scan and 3D rendered volume of the skeleton (*right*). (**b**) Pore distributions: side view (*left*) and top view (*middle*) of 2D cross sectional image and 3D visualization of pore distributions (*right*)

the corals had higher chlorophyll and protein concentrations, and a greater zooxanthellae density in the same direction, which could be explained by an enhanced nutrient uptake.

Coral growth forms have a high level of geometric complexity and simulation models can be an important option to complement experimental studies of growth in different environmental conditions (Chindapol et al. 2013). Multiple simulation methods modeling interactions of growth, branching, flow and nutrient distributions have been developed (Kaandorp et al. 1996, 2005; Kaandorp 1999; Kaandorp and Sloot 2001; Filatov et al. 2010) and are shortly reviewed by Kaandorp et al. (2011). The most recent computational simulation method was developed by Chindapol et al. (2013) in order to study the affects of nutrient uptake and unidirectional flow on coral symmetry by coupling a previously published polyp-based accretive growth model (Merks et al. 2004) to a nutrient transport flow model. The results could then be compared to computer tomography (CT) scans of *P. verrucosa* (Fig. 14.6) in different flow regimes (Mass and Genin 2008).

Simulations under no flow conditions (diffusion limited), showed symmetrical coral branch growth. Branch asymmetry increased with increasing unidirectional flow, with branches forming mainly towards the upstream side and downstream branches having a reduced growth (Fig. 14.6). This was similar to the corals from the flow experiments by Mass and Genin (2008). Additionally, the symmetry angles of the branches to the substratum were quantified, and maintained an angle of 45° in low flow conditions and changed drastically in high flow conditions. The surface/volume ratio could also be computed to quantify the degree of compactness of the simulated corals under different flow conditions. The results showed that an increase in flow brought about an increase of compactness, i.e. a decreased surface/volume ratio. The flow patterns around and inside, i.e. between branches, simulated corals were also considered. Flow inside the colony was greatly reduced due to an increase of compactness with most of the flow circumventing the coral and the along-stream flow becoming steeper at higher flow rates.

With both the study from Mass and Genin (2008) and the modeling approach by Chindapol et al. (2013) it could be concluded that the different colony symmetries of *P. verrucosa* found on reefs are likely due to different flow intensities. Chindapol et al. (2013) also showed that a biomechanical model could explain the formation of symmetrical and asymmetrical branching forms in a coral. Moreover, the computational model is the first example of an accretive growth model coupled to nutrient diffusion and flow model

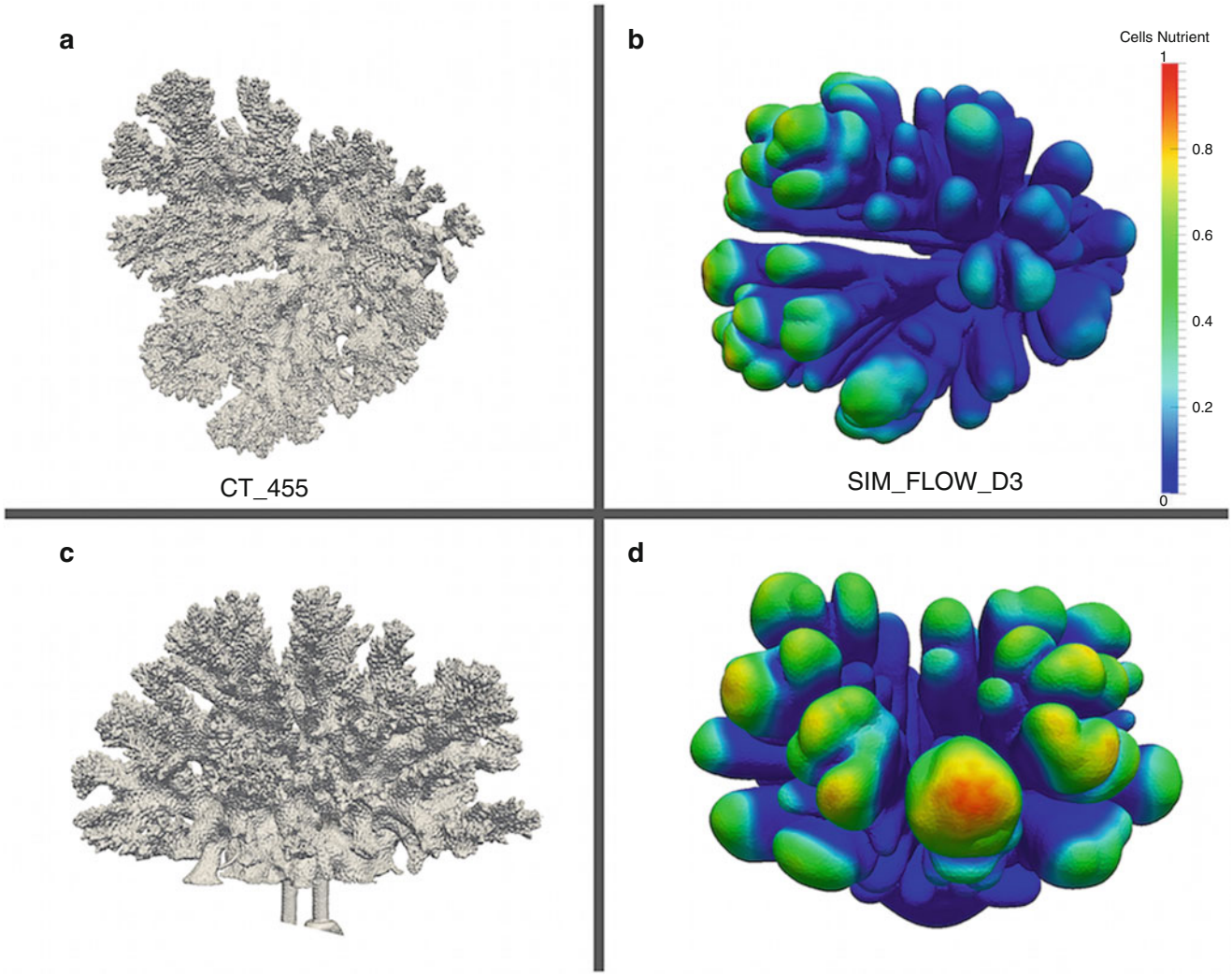

Fig. 14.6 Qualitative comparison between real (**a**, **c**) and simulated coral (**b**, **d**) in unidirectional flow conditions. The real coral was scanned with the CT scanning technique. Top view of coral (**a**, **b**) and side view (**c**, **d**) (Taken from Chindapol et al. (2013)). doi:10.1371/journal.pcbi.1002849.g007

and is able to simulate objects similar to biological growth forms under different hydrodynamic conditions. Nevertheless, it is still a challenge to include the full level of detail, i.e. branch roughness, of *P. verrucosa*. For this, the role of individual polyps will have to be included.

The model simulated coral growth under unidirectional flow, which is normally not found on the reef. Corals on reefs will naturally be exposed to bidirectional current due to tidal movements. The next question that could be answered with this growth model is if the radially symmetric coral colonies are caused by this bi-directional flow. Additionally, other physical processes influencing growth can be added, such as light and oxygen removal by flow. Moreover, the methods provided by Chindapol et al. (2013) can also be applied to analyze a large class of sessile marine organisms with complex morphological plasticity.

14.7 Going Multiscale: Coupling Different Models

The previous sections showed that using computational models to analyze different coral calcification topics could greatly increase our knowledge and quantify processes important in coral calcification. Subjects such as the coral transcriptome (Sect. 14.2) and proteome (Sect. 14.3) have only recently started being comprehensively researched and as such not many models have been developed that can aid in the understanding of complex interactions and regulatory networks. In contrast, relatively many computational models have been used to study coral physiology, each investigating a different physiological aspect in more detail (Sect. 14.4).

Section 14.5 showed that computational methods for image processing and analysis make it possible to obtain

microstructural skeleton properties, which can be used to infer skeleton health and changes due to environmental perturbations. Combining this quantitative microstructural analysis, for instance to an OM proteomic study under different environmental conditions, might provide interesting inferences regarding the way coral tune calcification.

Section 14.6 described the latest morphological modeling approach to study the affects of flow on coral growth and form, and compliments a comprehensive collection of morphological models. The accretive growth and nutrient flow model described in Sect. 14.6 is a good example of how different spatial and temporal scales can be coupled. However, this model is still a simple representation of the factors influencing coral morphology. Hennige et al. (2010) found changes in coral morphology according to an environmental gradient, but also changes in algae communities, respirations and photosynthesis rates. It has additionally been shown that water motion affects coral physiology mechanisms, with increasing photosynthesis and respiration following an increase in water motion (Patterson et al. 1991). Mass et al. (2010) subsequently reported that water flow enhances photosynthesis by increasing the efflux of oxygen from the coral to water. At the polyp level, water motion influences for instance particulate food capture (Sebens and Johnson 1991; Todd 2008). At the tissue level, increased water motion is believed to enhance diffusion of nutrients, ions and gases, and removal of waste products (Todd 2008). The above-mentioned observations could readily be implemented in both the physiology (Sect. 14.4) and growth (Sect. 14.6) models. For instance: by adding flow to the physiology model it can be examined how diffusion alters the carbonate chemistry in and around the coral; by adding light enhanced growth to the growth model it can be inspected how morphology changes.

Another example of a coupled computational method is the preliminary model done by Kaandorp et al. (2008). In this study, gene regulation controlling skeletogenesis in a branching sponge is coupled to an accretive growth model. The regulatory network controls skeletogenesis in time and space, and is closely linked to the environmental influences. For skeletogenesis, silicate must be absorbed from the environment. The model could predict the sponge morphology and the location of exhalant pores found on the sponge' surface. This methodology could also be conveyed onto a model with still to infer regulatory networks in coral calcification and coral growth and form. In this way, it might be possible to analyze and quantify (species specific) phenotypic plasticity of corals.

This chapter briefly explained the computational methods that can be and are being developed for understanding coral calcification on different spatial and temporal scales. In the last section several combinations of possible model couplings were summarized. A challenge would be to develop simulation models that not only ingrate a couple of processes, but rather multiple processes to form a multiscale model of calcification.

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Part IV

Reproduction and Population Ecology

Reproduction of Sea Anemones and Other Hexacorals

15

Ekaterina Bocharova

Abstract

The data on different modes of reproduction in sea anemones and other hexacorals are reviewed. There are colonial and solitary individuals, gonochoric and hermaphroditic organisms among them. Hexacorals can reproduce sexually in an ordinary way or by parthenogenesis. Asexual reproduction occurs in various forms. Specific features of different variants of sexual and asexual reproduction and their combinations in Hexacorallia populations from different habitats of the World Ocean are discussed.

Keywords

Sea anemone • Hexacoral • Sexual reproduction • Asexual reproduction • Parthenogenesis

15.1 Introduction

Reproduction is considered to be a crucial life history process for most of Metazoa. Patterns of mating and investment in offspring are expected to reflect strategies to maintaining populations. According to Fautin (2002), the border between sexual and asexual reproductions appears to be faint in Cnidaria Hatschek, 1888. Representatives of Anthozoa Ehrenberg, 1831 lack medusoid offspring, thus sexual and asexual reproductions are realized by polyps (Ivanova-Kazas 1975). Sea anemones and other hexacorals are known for their variety of sexual and asexual reproduction and their combinations (Bocharova and Kozevich 2011). Sexual reproduction occurs between individuals of the both sexes or via parthenogenesis (i.e. the development of oocytes without fertilization). During asexual reproduction new organism is formed from a fragment of parental body (pedal disk or tentacle) or from special formations such as buds (Ivanova-Kazas 1975). Some species have only sexual reproduction, others only use asexual ones and a great number of species prefers to combine different mechanisms of these reproductive modes, adopting to the changing habitat conditions. This suggestion was confirmed by other authors (Stephenson 1929; Ayre 1984; Billingham and Ayre 1996).

Besides the various reproductive modes, sea anemones possess some kind of parental care by brooding their offspring in the gastric cavity (Loseva 1974b; Lubbock and Allbut 1981; Monteiro et al. 1998; Mercier et al. 2011) or in the external body wall of adults (Dunn 1975a). Among other hexacorals internal brooding is known for Scleractinia Bourne, 1900 species (Goffredo and Telo 1998). There is some evidence of the juveniles that can appear as a result of cloning (Stoddart 1983; Monteiro et al. 1998; Sherman et al. 2007; Bocharova and Mugue 2012; Bocharova 2015). Brooding of clonal offspring can be associated with ameiotic parthenogenesis (Gashout and Ormond 1979) or parthenogenesis with restoring of oocyte diploidy (Grebelnyi 2005). However, a definite mechanism of such juvenile development in the gastric cavity of sea anemones still remains unclear.

15.2 Asexual Reproduction

15.2.1 Sea Anemones

Asexual reproduction was described for different species of sea anemones in detail. Modes of asexual reproduction vary greatly in Actiniaria, and blastogenesis is mainly based on a

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high ability of these animals to regeneration (Ivanova-Kazas 1975). Sea anemones are known for their pronounced reparative processes, which can proceed as "epimorphic regeneration" (i.e. reconstruction of absent organs) (Tokin 1959) or as somatic embryogenesis (Polteva 1967, 1970). In the latter case the development of new organism with all organs newly formed was observed. The order of mesenteries, tentacle rudiments development and pharynx formation in such case are comparable with those that occur in polyps during the sexual reproduction organogenesis (Polteva 1970). The common variants of asexual reproduction in sea anemones are transversal or longitudinal fission, laceration and autotomy of tentacles. Although, according to Ivanova-Kazas (1977) opinion, budding is not immanent for sea anemones, some authors noted an internal budding in *Actinia tenebrosa* Farquhar, 1898 (Ayre 1988) and *Actinia equina* Linnaeus, 1858 (Orr et al. 1982). In spite the fact that the anatomy of mentioned above species and other widespread brooding anemones was described in detail, the separation of multicellular compartments into the gastric cavity (an internal budding) was not found (Grebelnyi 1988).

Transversal fission was described for five sea anemone species belonging to two primitive families (Gonactiniidae and Aiptasiidae): *Anthopleura stellula* Ehrenberg, 1834 (Schmidt 1970), *Aiptasia mutabilis* Gosse, 1858 (syn. *Aiptasia couchii* (Stephenson 1935)), *Gonactinia prolifera* (Sars, 1835) (Chia et al. 1989), *Fagesia lineata* (Verrill, 1874) (Crowell and Oates 1980), *Nematostella vectensis* Stephenson, 1935 (Hand and Unlinger 1992).

Young individuals of *G. prolifera* start their fission by the appearance of tentacle rudiment band in the middle of the polyp body. Then the overstretching originates under the tentacle rudiments and separates the body into two parts. After the separation the upper body half starts regeneration of the pedal disk, while the lower side forms the oral disk. Sometimes an evidence of the next fission appears before the previous one has finished. Fission of *G. prolifera* has some features of paratomy (Ivanova-Kazas 1977).

Anthopleura stellula reproduces by transversal fission using architomy type (Fig. 15.1). Initially, the fission takes place, and then the lost body parts are regenerated. Schmidt (1970) suggested that sea anemones extract specific substances, which can be stimulus for fission initiation. Transversal fission of *A. stellula* and *N. vectensis* becomes more frequent, when water salinity decreases (Schmidt 1970; Hand and Unlinger 1992).

Longitudinal fission is the most widespread type of sea anemone asexual reproduction and occurs in *Aiptasia*, *Sagartia*, *Metridium*, *Anemonia*, *Haliplanella* and other genera representatives. The fission can initiate from both the apical (e.g. *Metridium senile* (Linnaeus 1761)) and the basal ends. In the last case the base stretches as long as the sea anemone body is broken (Fig. 15.1).

In *M. senile* the plane of longitudinal fission coincides with the plane of its pharynx, while in *Sagartia luciae* Verrill, 1898 and *Anemonia sulcata* Forskål, 1775 these planes are perpendicular to each other. Sometimes several longitudinal fissions start from lateral body sides, whereby several new individuals can be formed (Hyman 1940). After the fission edges of "sore" close and confluence, and mesenteries, tentacles and syphonoglyphs of newly formed individuals begin to form (Atoda 1976; Minasian and Mariscal 1979).

Detailed study of the longitudinal fission mechanism in *Haliplanella luciae* (Verrill, 1898) showed that induction of cell apoptosis is located in the sites of determined mesentery attachment to the body wall (Mire and Venable 1999). Firstly, the axis of the process is parallel to the axis of the animal stretching that is necessary for tissue thinning, and then it becomes perpendicular to the last one that is used for final separation of two individuals. Frequently fission occurs irregularly. Environmental changes can be stimulus for fission initiation. Under the laboratory conditions these are changing of aquarium water, extreme fluctuations of temperature and lightning, temporary desiccation, etc. (Ivanova-Kazas 1977).

Laceration is distributed among representatives of *Metridium*, *Aiptasia*, *Sagartia*, *Diadumene*, *Phellia*, *Heliactis* and other genera. During the process, small fragments of irregular shape being attached to substratum dissociate from the parental pedal disk, while an adult sea anemone crawls or remains stationary (Fig. 15.1). Each separated fragment contains parts of the pedal disk, mesenteries and pieces of lateral body walls. After the isolation the edges of "sore" fuse, and new tentacles and mesenteries are formed along the "sore". As a result, new individuals often have an irregular number of syphonoglyphs and mesenteries (Hyman 1940).

Sea anemone unequal longitudinal fission, pedal laceration and a variety of gradients between them are attended greatly (Polteva 1972). Current phenomenon was observed in *Metridium* (Hammat 1906; Bucklin 1979; Fautin et al. 1989; Anthony and Svane 1995), *Sagartia* (Stephenson 1929), *Haliplanella* (Dunn 1982; Johnson and Shick 1977), *Anthopleura* (Dunn 1982; Sebens 1983; Geller and Walton 2001; Geller et al. 2005), *Aiptasia* (Hunter 1984). To study the morphogenetic processes, taking place during the polyp regeneration, the natural laceration was imitated as cutting of fragments by application of longitudinal cuts (Polteva 1970).

Asexual reproduction of *Metridium senile* by pedal laceration can be compared with somatic embryogenesis type, which is characteristic for current species. In the last case young polyps are developed from the pedal disk fragments of an adult sea anemone (Polteva 1967). During the somatic embryogenesis main morphogenetic processes include changes of orientation and polarity, symmetrization (pharynx and pair mesenteries development), body growth and oral disk formation (Polteva 1972).

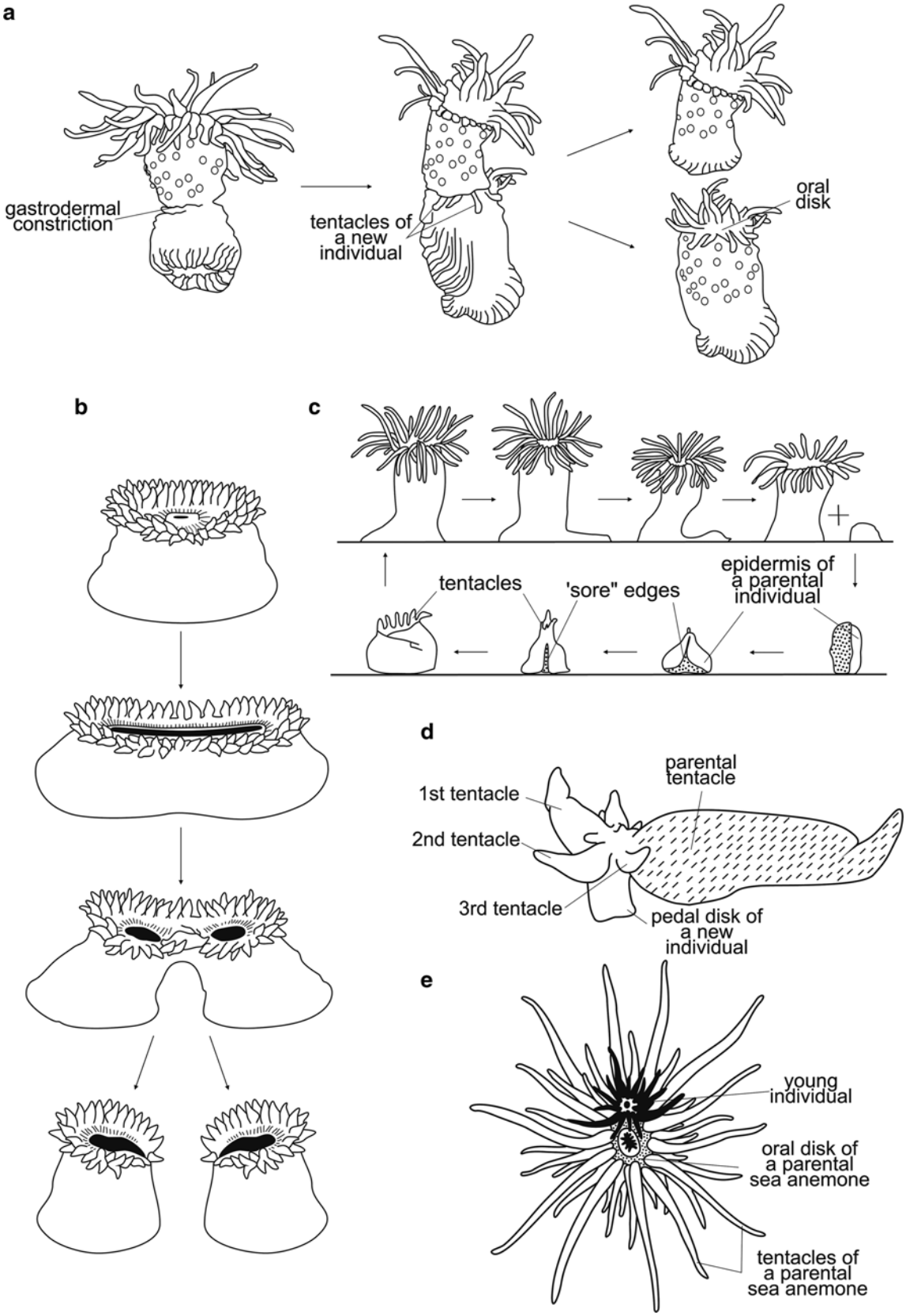

Fig. 15.1 Asexual reproduction modes of sea anemones: *A* scheme of transversal fission in *Anthopleura stellula* (According to Schmidt 1970), *B* scheme of longitudinal fission in *Anthopleura elegantissima* (According to Sebens 1983), *C* scheme of pedal laceration in sea anemones (According to Polteva 1970), *D* and *E* reproduction of *Boloceroides* sp. by autotomy of tentacles (According to Ivanova-Kazas 1977): *D* development of the young sea anemone from the isolated tentacle, *E* development of the young individual from the tentacle connected to the parental sea anemone

The representatives of *Boloceroides* and *Boloceractis* are described as using reproduction by autotomy of tentacles (Ivanova-Kazas 1977). Current species have sphincter in the base of each tentacle, thus the tentacle separation occurs using the circular muscle contraction. In the basal end of the isolated tentacle there is an aperture, which usually is closed by tissue "plug". Later, when active cell divisions begin, the "plug" is rejected that leads to the formation of a new individual (Fig. 15.1). In some species, a new polyp development occurs before the tentacle separation and young sea anemones can be observed on the oral disk of the parental adult (Fig. 15.1). Sometimes such kind of reproduction is called budding. When the "tentacle buds" are formed in situ, their oral ends are oriented to the parental organism (Hyman 1940).

15.2.2 Other Hexacorals

Fission is a primary mode of asexual reproduction in numerous anthozoans such as zoanthids and scleractinian corals (Hughes and Jackson 1980; Tanner 1999) and play an important role in population size (Tanner 1999), dynamics (Acosta et al. 2005), and structure in several species (Karlson 1991; McFadden 1991). Some Scleractinia species were described as having brooded larvae, which are produced asexually (Ayre and Miller 2004; Cairns 1988; Stoddart 1983), however, the precise nature of this process is unknown. Zoantharian *Palythoa caribaeorum* (Duchassaing et Michelotti, 1860) populations reproduce by fission at small colony size in Colombia and Brazil (Acosta and Gonzalez 2007). Asexual reproduction was also reported in such zoanthids as *Palythoa caesia* Dana, 1846 (Ryland 1997) and *Zoanthus* spp. (Karlson 1988).

Several authors reported on different modes of asexual reproduction within antipatharians, including the breaking away of fragments that can subsequently reattach to the substrate (Grigg 1984, 1993; Miller and Grange 1995; Bo et al. 2009), and asexual production of larvae (Miller and Grange 1995; Parker et al. 1997). In aquaria under stressful conditions, parts of *Antipathes fiordensis* Grange, 1990 (presumably tentacles) separate from colonies and then form highly mobile and ciliated planulae (Miller and Grange 1995; Parker et al. 1997)—a strategy also known for other hexacorals (Sammarco 1982; Harrison and Wallace 1990; Richmond and Hunter 1990). This phenomenon has never been observed in the field and it is not known whether it occurs naturally (Wagner et al. 2011). However, molecular studies on the population genetic structure of *A. fiordensis* performed in New Zealand suggest that asexual reproduction is common and geographically widespread within this species, because genetic clones have been reported to occur frequently and over long geographic distances (Miller and Grange 1995; Miller 1997, 1998).

Temperate as well as tropical corallimorpharians typically form large aggregations by asexual reproduction and can be the major components of some shallow-water benthic faunas (Foster 1958; Fishelson 1970; den Hartog 1980; Chadwick and Adams 1991). The diverse range of asexual reproductive strategies is displayed by various species, including pedal laceration, longitudinal fission and inverse or marginal budding (den Hartog 1980; Chen et al. 1995; Chadwick-Furman and Spiegel 2000). Marginal budding, a process whereby one or more sections of the margin, including part of the oral disk with a mouth and tentacles, column, and pedal disk, detach from the mother body, was only described in the Red Sea corallimorpharian—*Rhodactis rhodostoma* (Ehrenberg, 1934) (Chen et al. 1995). Chadwick-Furman and Spiegel (2000) speculated that it may occur in other common species.

There is some evidence that representatives of Ceriantharia are capable of asexual reproduction by budding, but it was not observed neither in the field nor under laboratory conditions.

15.3 Sexual Reproduction

15.3.1 Sea Anemones

Gamete origin and development in Cnidaria possess some special or characteristic features (Fig. 15.2). Coral polyps have gametes that originate from mesentery gastroderm. When differentiation begins, the entodermal cells migrate into mesoglea for the following maturation (Dunn 1975a, b; Larkman 1983; 1984; Wedi and Dunn 1983).

Reproductive system of sea anemones is organized very simply. In this case the term 'gonad' means gamete aggregation in the determined part of mesentery as real gonads are not formed in Actiniaria. Special reproductive channels are absent and gametes come to the gastric cavity through mesentery epithelium disruptions and then get inside through the mouth as usual (Kostina 1989). Sea anemones are classified as having 'diffuse type' of gamete development (Loseva 1970). Undifferentiated gonad of sea anemones looks like a swollen, longitudinal cord between gastric filament and retractor muscle in mesentery. Location of gonad development varies in different species: gonads can occur in all the mesenteries or only in the primary mesenteries, etc. For example, in *Metridium* sp. and *Bunodosoma cavernata* (Bosc, 1802) male gonads are located in the secondary mesenteries (Clark and Dewel 1974).

During early stages of the gamete differentiation in sea anemones the sex is not clearly determined (Fig. 15.2). Egg cells are normally not aggregated as it is observed during spermatogenesis. In addition, premeiotic modifications start early in future oocyte nuclei and neighbor egg cell sizes vary

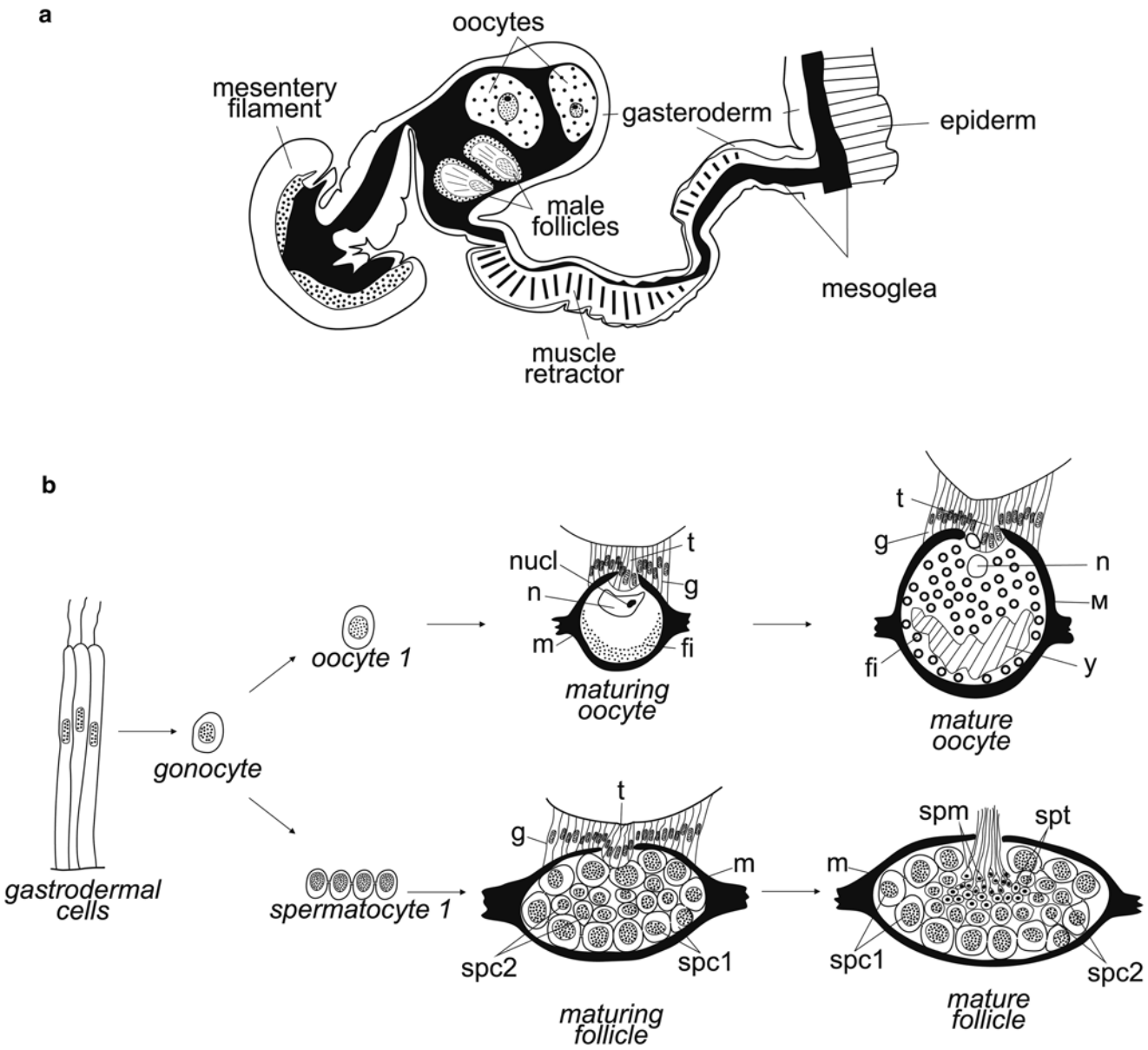

Fig. 15.2 Gametogenesis in sea anemones: *A* scheme of gonad location in the mesentery of *Aulactinia stella* (According to Bocharova and Kozevich 2011), *B* total scheme of spermatogenesis and oogenesis in sea anemones (According to Loseva 1972): *g* gastroderm, *fi* fat inclusions, *m* mesoglea, *n* nucleus, *nucl* nucleolus, *spc1* spermatocyte 1, *spc2* spermatocyte 2, *spm* sperm, *spt* spermatids, *t* trophonema, *y* yolk agglomerate

severely. Male cells of the same sizes have bright nuclei with weak chromatin grain (Loseva 1972).

Sea anemones are known by external (Chia 1976) and external-internal (Carter and Thorp 1979; Chia 1976; Gashout and Ormond 1979) fertilization. The first one is usual among dioecious sea anemone species after males and females spawn into the water. External-internal fertilization occurs in female gastric cavity, though it was noted that sperm can also penetrate into mesentery containing oocytes (Ivanova-Kazas 1975).

In *Metridium senile* and *Protanthea simplex* Carlgren, 1891 a great number of egg cells matures simultaneously. The sizes of current oocytes are not large (the average diameters are 170 μm vs 80 μm, respectively) with a low amount of nutrients, which are evenly distributed in the cytoplasm (Loseva 1971). When spawning occurs in both sexes, fertilization and larval development take place outside the maternal organism (Hyman 1940).

Fertilization of egg cells in some sea anemone (e.g. *Metridium* sp.) is characterized by cortical reaction (Clark and Dewel 1974; Dewel and Clark 1974). Within other species the cortical reaction is absent but the egg cells contain cortical granules, which function is unknown. Current granules are preserved during larva stages (Larkman and Carter 1984).

During long reproductive period brooding *Urticina crassicornis* (O.F. Müller, 1776) contain significantly more simultaneously maturing oocytes than *Aulactinia stella* (Verrill, 1864) (Loseva 1971). Apparently, the reproductive period of *U. crassicornis* is shorter than that of in *A. stella*. The oocytes of the first species are larger (average diameters are 600 μm vs 250 μm, respectively) and contain a lot of nutritive substances (Loseva 1970). Connection between mode of reproduction and size and number of simultaneously maturing egg cells is known for many sea animals (Giese 1959).

Some species spawn immature gametes. During oocyte development the meiosis stops at the certain stage and will be finished only using egg cell activation by sperm (Grebelnyi 2005). For instance, females of *Peachia quinquecapitata* McMurrich, 1913 spawn oocytes at the stage before the division of maturation (Spaulding 1974). *Urticina crassicornis* spawns spermatozoon containing cytoplasm abundance (Chia and Spaulding 1972).

Brooding is very widespread among sea anemones with external-internal reproduction and is described as offspring development in the parental organism prior to metamorphosis finish when it becomes to live separately (Loseva 1974a; Carter and Thorp 1979; Chia 1976; Gashout and Ormond 1979). Internal brooding of juveniles was found within *Actinia equina*, *Aulactinia stella*, *Hormathia digitata* (O.F. Müller, 1776), etc. (Kostina 1989).

Brooding is often used in sea invertebrates for the protection of juveniles at the early developmental stages, which are usually found in plankton (Dunn 1975a). Resettlement of brooding sea anemones is limited, because swimming larva stages are reduced or absent. Nevertheless, parental care increases the possibility of offspring survival within the equal productivity of the animals. For example, *Cribrinopsis fernaldi* Siebert et Spaulding, 1976 possesses external-internal fertilization and its larva forms in the gastric cavity of maternal organism (Siebert and Spaulding 1976). The pelagic stage lasts only several days. In evolutionary terms current reproductive mode can be considered as one of the most successful (Chia 1976).

Some northern species like *Epiactis prolifera* Verrill, 1869, *Epiactis lewisi* Carlgren, 1940, *Epiactis arctica* (Verrill, 1868), *Cnidopus japonicus* (Verrill, 1870) have no pelagic stages so their juveniles are developed in the brood chambers on the external body wall of the maternal organism. According to Carlgren (1949), there are 16 sea anemone species with external brooding of approximately 800 species worldwide distributed. Current species are limited by the polar and the temperate seas and at least five species inhabit the sublittoral zone exclusively (Dunn 1975a).

According to Dunn (1975a) the embryo of *Epiactis prolifera* leaves the gastric cavity of the maternal organism through the mouth and attaches to the lower part of the column under the pedal disk. Wall tissues of the parental column form the brood chambers where juveniles are developed prior to young polyp stages. Each individual of *E. prolifera* protects only its own offspring. In the lower part of adult *E. arctica* there is an annular bulb, which contains developing offspring, while *E. lewisi* possesses similar structure in the upper part of the polyp body (Kostina 1989).

In addition to the aforementioned variants of sexual reproduction, there are some data that do not allow to determine the mode of reproduction. Thus, *Aulactinia stella* described earlier has internal brooding. Juveniles of different ages occur in gastric cavity of parental organism simultaneously (Loseva 1970). Loseva (1974b) noted that not only females but also males, hermaphrodites and individuals without gonads brood offspring in the sea anemone population in Yarnyshnaya Bay of the Barents Sea. The similar situation was observed in *Anthopleura handi* Dunn, 1978 and *Haliplanella luciae* (Dunn 1982). In Californian population of *Epiactis prolifera* there were only females, hermaphrodites and individuals without gonads (Dunn 1975b). Such observation can be an evidence of sex change (sequential hermaphroditism) or parthenogenesis (asexual reproduction, cloning) existence within the sea anemones (Grebelnyi 2008).

Parthenogenetic populations of sea invertebrates occur rather frequently among Cnidaria (especially among brooding species) and Mollusca (Lively and Johnson 1994). According to author's opinion, appearance of the parthenogenetic populations at the edge of the range or under extreme conditions gives some advantages to the current species versus to its bisexual populations with external fertilization since animals do not have to spend a lot of energy for maturation of great amount of gametes to increase the fertilization success. Representatives of *Alcyonium*, *Tubastrea*, *Pocillopora* can form both bisexual and parthenogenetic populations. For instance, some planulaes of *Pocillopora damicornis* (Linnaeus, 1758) were found to be genetically identical to the parental organisms (Stoddart 1983).

Hypotheses of possible parthenogenesis in sea anemones have been proposed a long time ago. *Cereus pedunculatus* (Pennant, 1777) (Rossi 1975) and *Sagartia troglodytes* (Price in Johnston, 1847) (Shaw et al. 1987) are known to reproduce sexually and without brooding. Also there are parthenogenetic populations of current species, where individuals brood offspring (Lively and Johnson 1994).

Generally, if an initial stage of parthenogenesis is a matured egg cell, the organism, which develops from it, will be haploid. However, in the most cases there are cytological mechanisms, which facilitate preserving or restoring the offspring diploidy (Ivanova-Kazas 1995). Divisions of maturation start only after oocyte activation by sperm within the majority of animals, so incomplete of meiosis during parthenogenesis can be considered as a direct consequence of

fertilization failure. It is remaining unclear what exact factors stimulate the beginning of oocyte development. Grebelnyi (1989) suggested that parthenogenesis of sea anemones has appeared comparatively recently due meiosis disturbance at the last stages.

15.3.2 Other Hexacorals

Modularity of Scleractinian corals can potentially lead to a diverse array of sexual systems (Weiblen et al. 2000). However, there are essentially only two sexual systems in scleractinians. Colonies are either predominately outcrossing, consisting of simultaneous hermaphrodites with each polyp having both male and female functions, or colonial polyps of single sex only throughout their life; thus colonies are either male or female and species are gonochoric (Harrison and Wallace 1990).

Most hermaphroditic corals are simultaneous producing both eggs and sperm within single complete breeding cycle. Some corals, for example *Stylophora pistillata* Esper, 1797 (Rinkevich and Loya 1979), are protandrous simultaneous hermaphrodites. Such colonies display solely male function while small-sized, becoming simultaneous hermaphrodites once colonies exceed a species-specific characteristic size. Protandrous hermaphroditism is readily explained by models that predict delayed allocation to more energetically costly female function until larger sizes of colonies are attained (Charnov 1982). At least four solitary fungiid species were detected as sequential protandrous hermaphrodites, i.e. polyps display solely male function when small and solely female function when become larger (Kramarsky-Winter and Loya 1998; Loya and Sakai 2008) in accordance with sex-allocation theory (Charnov 1982). In addition, the fungiid *Ctenactis echinata* (Pallas 1766) can change sex in both directions, possibly in response to energetic and/or environmental constraints (Loya and Sakai 2008).

Mixed breeding systems are rarely found in scleractinian corals. In a few colonial species, some polyps are male and others are females. At least one scleractinian, *Galaxea fascicularis* (Linnaeus, 1767), is pseudo-gynodioecious. Current species populations consist of female colonies that release eggs and male colonies that release sperm packaged with nonviable eggs, which potentially provide buoyancy for sperm (Harrison 1988). In addition, a low proportion of hermaphrodites is often found in otherwise gonochoric populations, including species in Agariciidae (Delvoye 1988; Glynn et al. 1996) and Poritidae (Glynn et al. 1994; Soong 1991) families; such species are described by Giese and Pearse (1974) as stable gonochores. Although these are important exceptions, the vast majority of scleractinian species can be classified as either hermaphroditic or gonochoric.

Similarly, there are essentially only two modes of larval development in scleractinian corals (Baird and Guest 2009). Fertilization is either internal, when the embryo develops within the polyp and is released as a motile planulae larva (brooding), or external, when the embryo develops in the water column (broadcast spawning).

The order Zoantharia is divided into two suborders: Macrocnemina and Brachycnemina. In Macrocnemina, with few exceptions, colonies are gonochoric while in Brachycnemina colonies are commonly hermaphroditic (Hirose et al. 2011). Shallow-water zoanthids often have annual cycles of gametogenesis (Ryland 1997, 2000) while deep-water *Epizoanthus* species breed continuously (Muirhead et al. 1986). There are a few reports on the spawning behavior of zoanthids in the laboratory (Kimura et al. 1972) and in the field (Ryland and Babcock 1991) and internal brooding has been confirmed only in *Isozoanthus giganteus* Carlgren in Chun, 1903 (Ryland 1997). Babcock and Ryland (1990) raised the fertilized eggs of *Protopalythoa* sp. to zoanthella larvae and to date this is the only description of zoanthid development from spawning to settlement.

Except for a few studies of shallow-water (<50 m depth) species (Miller 1996; Parker et al. 1997; Bo 2008; Gaino and Scoccia 2008, 2009; Gaino et al. 2008; Scoccia and Gaino 2010), most information on the sexual reproduction of antipatharians derives from scarce notes on the anatomy of reproductive tissues accompanying taxonomic descriptions (Wagner et al. 2011). All species of black corals, which have been studied to date, were described as gonochoric, with no appreciable morphological difference between male and female colonies (Parker et al. 1997; Gaino et al. 2008; Gaino and Scoccia 2008). As in all modular organisms, which grow through asexual reproduction, gamete differentiation of antipatharians is an important event, as it ensures the high genetic diversity that enables species to adapt to environmental changes. Nowadays, gamete release and ensuing fertilization is the subject of speculation, owing to the almost total lack of fieldworks (Gaino and Scoccia 2010).

Patterns of sexual reproduction have been investigated in few species of Corallimorpharia, both of which form large aggregations via clonal replication (Chadwick-Furman and Spiegel 2000). In the temperate *Corynactis californica* Carlgren, 1936, all polyps in each clonal group are of the same sex and gametogenesis leads to annual synchronous spawning of gametes during winter (Holts and Beauchamp 1993). In contrast, in the tropical *Rhodactis indosinensis* Carlgren, 1943 polyp size and sex are influenced by position within each clonal aggregation, with edge polyps smaller being a male, and central polyps larger being a female (Chen et al. 1995). This species follows an annual gametogenetic cycle in both sexes, with gamete spawning in midsummer during the period of peak seawater temperature and day length (Chen et al. 1995).

All representatives of Ceriantharia are hermaphrodites, however, oocytes and sperm mature in different periods (Hinsch and Moore 1992). As a rule, protandric hermaphroditism is appropriate to these animals. Features of sexual reproduction in Ceriantharia have not been studied yet.

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Sexual Reproduction in Stony Corals and Insight into the Evolution of Oogenesis in Cnidaria

16

Shinya Shikina and Ching-Fong Chang

Abstract

The sexual reproduction of stony corals has long captivated the attention of researchers and has been studied in many species and locations worldwide. Although fundamental findings and research interests were revealed during the past three decades, the intrinsic mechanisms of sexual reproduction remain almost unknown. In this chapter, we review recent progress in molecular and cellular aspects of the sexual reproduction of scleractinians, with a particular focus on oogenesis. We describe some common characteristics in the molecular and cellular mechanisms of oogenesis between scleractinians and oviparous bilaterian animals to provide insight into the evolution of cnidarian oogenesis. The conclusions are presented as a list of important issues to be addressed in the future to improve understanding of the intimate mechanisms of sexual reproduction of stony corals.

Keywords

Sexual reproduction • Scleractinian corals • Coral reefs • Gametogenesis • Germ cell marker • Vasa • Piwi • Germline stem cells • Oogenesis • Vitellogenin • Egg protein • Evolution • Hormones

16.1 Introduction

Scleractinians, the stony corals, are an essential group in building the framework of coral reefs. Scleractinians are the largest order of anthozoans with over 1500 species. The sexual reproduction of scleractinians has long captivated the attention of researchers and has been studied in many species and locations worldwide. In the past three decades, primarily from an ecological perspective, various aspects of sexual reproduction, such as spawning, sexuality, and gametogenesis, were extensively investigated in more than 444 species, approximately one-third of the existing scleractinians (Harrison 2011).

Although fundamental findings are accumulating and research into the sexual reproduction of scleractinians has begun, we must admit that our understanding of the intrinsic mechanisms of sexual reproduction remains weak. For example, little information is available on the molecular and cellular mechanisms that operate in sexual reproduction or on the types of hormones that induce germ cell development and maturation.

Currently, interest is continuing to grow in scleractinian coral biology and ecology because of the increasing human concerns about reef degradation and the importance of the conservation of coral reefs (Tarrant 2005). A deeper understanding of both environmental and intrinsic factors that control sexual reproduction in scleractinians would allow us to examine the restoration of corals from a new perspective, such as the propagation of target scleractinians by using sperm and eggs obtained in aquaculture. Furthermore, by

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combining propagation with a technique for cryopreservation of the sperm and eggs of corals, we would establish an integrated approach to conserve genetic resources of corals for the future.

Additionally, the study of the intrinsic mechanisms of scleractinian reproduction is so interesting because of another feature; research on scleractinians, and more generally cnidarians, has the potential to provide important insights into the early evolution of metazoans. The Cnidaria are the sister group to the Bilateria (Technau and Steele 2011) and exhibit intermediate complexity between the Porifera (sponges) and Bilateria. Cnidarian bodies are diploblastic and possess functionally defined tissue but have no well-organized organs and/or systems as found in vertebrates. Thus, cnidarians are evolutionarily primitive metazoans. One of the surprise findings revealed by recent cnidarian expression sequence tags (EST) and genomic analyses is that, despite the morphologic simplicity, cnidarians share most of their genes with advanced vertebrates including humans and exhibit a high level of genetic complexity (Technau et al. 2005; Hemmrich and Bosch 2008). Their protein sequences often more similar to mammalian sequences than to those of the fruit fly *Drosophila* or the nematode *Caenorhabditis elegans* (Kortschak et al. 2003; Technau et al. 2005). Hence, cnidarians are useful and informative animals for speculation on the biological characteristics of the eumetazoan ancestor and to trace the evolutionary origin of various biological mechanisms that exist in present-day metazoans. Currently, several model systems are contributing to evolutionary developmental biology, i.e., the freshwater hydra *Hydra magnipapillata* (Bosch 2009), the sea anemone *Nematostella vectensis* (Darling et al. 2005), the hydrozoan jellyfish *Clytia hemisphaerica* (Houliston et al. 2010), and the stony coral *Acropora millepora* (Miller and Ball 2000).

In the present chapter, we first concentrate on the sexual reproduction of scleractinians, particularly of hermatypic species, and then briefly summarize our current state of knowledge on various aspects of scleractinian sexual reproduction for the general reader. We do not intend to review this topic in detail because there are many excellent reviews that summarize the essential and integrated knowledge of coral reproductive biology over the past three decades in great detail. The interested reader is referred to these previous reviews for further general and detailed information (Fadlallah 1983; Harrison and Wallace 1990; Richmond and Hunter 1990; Richmond 1997; Harrison and Jamieson 1999; Kolinski and Cox 2003; Harrison and Booth 2007, Baird et al. 2009; Harrison 2011). Second, as the primary topics of the present chapter, we focus on the molecular and cellular aspects of sexual reproduction of scleractinians and review recent progress in specific research areas. The subjects that will be addressed include (i) germ cell marker genes, (ii) origin of germline cells, (iii) yolk protein formation in oogenesis, and (iv) hormones in scleractinian tissues and possible relationships with sexual reproduction. We describe the shared characteristics in the molecular and cellular mechanisms of sexual reproduction between scleractinians and advanced animals. We then provide new insight into the evolution of cnidarian oogenesis, with the aim to promote discussion in this field and to open new doors for future research.

16.2 Summary of Reproductive Biology of Scleractinians

Scleractinians are among the most familiar marine invertebrates in the tropics and subtropics, and their diverse reproductive strategies have supported the successful propagation of scleractinians on the planet to date. Scleractinians reproduce both asexually and sexually. In asexual reproduction, scleractinians create genetically identical clones by budding, fragmentation and/or polyp bail out, which allows scleractinians to amplify the numbers of individual polyps and to establish colonies in a relatively short period. However, in sexual reproduction, scleractinians release gametes for external fertilization, which facilitates expansion into divergent habitats and creation of new populations, providing genetic links between one reef region and another. Sexual reproduction also allows scleractinians to foster genetic diversity, an advantage in adaptation to environmental changes.

Sexuality. Based on the recent review by Harrison (2011), information is available now on at least 444 out of the more than 1500 recognized scleractinian species. The body of data revealed that scleractinians have complex sexual patterns. Most corals investigated are either hermaphroditic or gonochoric (create male or female gametes in separate colonies). Hermaphrodism is the dominant sexual pattern among scleractinians (approximately 70.9 %; 294 out of 416 species for which data are available), whereas the gonochoric species are found less frequently (approximately 26.2 %; 109 out of 416 species). The remainder of the colonies (2.9 %; 13 species) exhibit a mixed pattern and are either hermaphroditic or gonochoric. Additionally, a change in sex (from male to female or bidirectional) was reported in some mushroom corals (Loya and Sakai 2008; Loya et al. 2009).

Gonad formation and allocation. For sexual reproduction, scleractinians generate gametes in a specific site of the endodermal mesentery within the polyp. The site of gametogenesis is often referred to as a "gonad", although corals possess no true organs. The gonad is generally observed as a small swelling, and the swelling becomes more distinct as corals approach the spawning period because of the development of oocytes and/or sperm within the gonad. Mature ovaries and testes are distinguished by their size, color, shape and location (Harrison and Wallace 1990). Typically, ovaries

have color (i.e., pink or pale green, among other colors) that is different from other mesentery tissues or testes. In gonochoric scleractinians, the polyps in a colony only have one type of gonad, ovary or testis. By contrast, in hermaphroditic species, the polyps in a colony have both ovary and testis, with three types of gonad arrangement within a polyp (Harrison and Wallace 1990): (1) ovaries and testes are intermingled within an identical mesentery, (2) both ovaries and testes are present in an identical mesentery, but they develop separately at different sites, and (3) ovaries and testes separately develop on different mesentery within a polyp. The genetic or epigenetic mechanisms of sex determination in gonochoric species are still unknown, and little information is available on the mechanisms of gonadal sex determination in polyps of hermaphroditic species.

Gametogenesis. Oogenesis begins with oogonial proliferation adjacent to the mesenterial mesoglea layer, which is a gelatinous extracellular matrix in the gonad. After the oogonia begin meiosis, the early oocytes infiltrate into the mesoglea layer with deformation of their morphology. The oocytes are subsequently enveloped by a thin mesoglea layer in which they develop with an increase in size by accumulating yolk proteins until maturation. As maturation is approached, in several species, the oocyte nucleus moves from a central location to the peripheral membrane where its morphology is changed into a semicircular or U-shape caused by an indentation of the plasma membrane (Vargas-Angel et al. 2006; Shikina et al. 2012). Spermatogenesis begins with the formation of spermaries, which are dozens of spermatogonia surrounded by a thin layer of mesenterial mesoglea in the gonad. The proliferation of spermatogonia, differentiation into spermatocytes, and spermiogenesis occur within each spermary, and sperm are typically visible a few weeks before spawning.

Spawning species. The two reproductive strategies of scleractinians are spawning and brooding. The spawning species, approximately three-quarters of scleractinians, release gametes through the mouth into the ocean for external fertilization; the larvae settle on suitable substrates, metamorphose, and become new polyps. In colonial species, the polyps undergo asexual reproduction and form new colonies. The timing of spawnings of scleractinians, in most cases, is highly synchronized within a species and even within closely related species that reside in the same area. In Australia's Great Barrier Reef, more than 130 scleractinian species participate in a simultaneous spawning event over eight nights (Harrison et al. 1984; Willis et al. 1985; Babcock et al. 1986; Guest et al. 2005). This "mass-spawning event" is the best-known phenomenon of scleractinian reproduction. Currently, synchronized spawning of multiple species is found in a variety locations, including a different location in Australia (Simpson 1985), the Gulf of Mexico (Gittings et al. 1992), Okinawa (Hayashibara et al. 1993), Taiwan (Dai et al. 1992), Singapore (Guest et al. 2002), the Solomon islands (Baird et al. 2001), Palau (Penland et al. 2004), and French Polynesia (Carroll et al. 2006). Because synchronized spawning results in large numbers of gametes and larvae, it maximizes the chances of fertilization and increases the survival rate of larvae. Moreover, because closely related species also release gametes within a similar time window, synchronized spawning among multiple species also has the potential for hybridization. Indeed, molecular evidence for hybridization was found between two sympatric staghorn coral species that spawned simultaneously in the Caribbean (Van Oppen et al. 2000; Vollmer and Palumbi 2002). The majority of spawning species generally release gametes once a year and have a single annual gametogenic cycle. The gametogenesis in these corals is completed within the 12 months of each year. Soon after spawning in these corals, developing germline cells are typically not observed in the gonads, which indicate that the annual gametogenic cycles do not overlap. In most corals, oogenesis precedes spermatogenesis by a couple of months. In a few species, oogenesis requires more than 1 year, and immature oocytes begin to develop before the matured oocytes are spawned (Fadlallah 1982; Fadlallah and Pearse 1982).

Brooding species. The remaining approximately one-quarter of scleractinians are brooding species that release planula larvae but not gametes. The planula larvae are generally produced by internal fertilization within the polyps. The brooding species typically have multiple gametogenic cycles per year, but the number of gametogenic cycles each year differs among species. The gametogenic cycles in some species are correlated with lunar cycles (Fan et al. 2002), with some exhibiting a bimonthly gametogenic cycle (Kojis 1986). The planulae from brooding species are usually larger than the larvae created by external fertilization and are released at a further stage of development (Harrison and Wallace 1990). These planulae also have a tendency to settle soon after release. Therefore, brooding species readily concentrate planulae close to the parent colony (Twan et al. 2006b). In a cauliflower coral, *Pocillopora damicornis* molecular evidence identified planulae that were sexually produced and planulae that were asexually produced (Yeoh and Dai 2010; Combosch and Vollmer 2013) through parthenogenesis, which is a mode of asexual reproduction in other anthozoans such as sea anemones (Gashout and Ormond 1979).

Environmental factors affecting sexual reproduction. The timings of sexual reproduction and spawning are controlled by various environmental factors (Babcock et al. 1986; Richmond and Hunter 1990; Harrison and Wallace 1990; Kolinski and Cox 2003; Harrison and Booth 2007, Baird et al. 2009; Harrison 2011). In spawning species, maturation and the annual gametogenic cycle are influenced by seasonal changes in sea surface temperature, day length, moonbeams in the lunar cycle, and solar insolation. The length of daily

light/dark periods and the night irradiance of the lunar cycle may influence the control of events, such as date of spawning, at a finer scale. The timing of gamete release on the day of spawning is strongly influenced by the change in light intensity during sunset. However, the environmental factors that affect sexual reproduction vary among species and even within regions, and the exact cues that trigger the dramatic annual spawning remain unclear.

16.3 Recent Progress in Molecular and Cellular Aspects of Sexual Reproduction in Scleractinians

For scleractinians, and more generally for cnidarians, knowledge of the intrinsic mechanisms underlying sexual reproduction is limited. For example, little is known about how scleractinians integrate and transduce the environmental signals to the physiological processes in reproduction (i.e., germ cell development or spawning). Additionally, little is known about the types of molecular and cellular regulations that operate in the creation of gametes or the types of hormones that are involved. Because Cnidaria is an early branch of the metazoan lineage, information on the shared molecular, cellular and endocrine characteristics of sexual reproduction between cnidarians and advanced vertebrates would provide important insights into the evolutionarily conserved mechanisms of metazoan sexual reproduction. After a decade of studies on the molecular and cellular aspects of sexual reproduction in scleractinians, particularly on gametogenesis, (1) the germ cell marker genes, (2) the possible origin of germline cells, and (3) the identity of yolk proteins and the synthetic site were determined. In the following section, aspects of the advances achieved from such recent studies will be outlined.

16.3.1 Germ Cell Markers

The precise assignment of cell types is required to study the intrinsic mechanisms by which cell-cell interactions, hormones, and growth factors mediate intercellular communication. The use of molecular markers to identify germ cells is generally accepted as one of the most reliable methods. For example, the use of evolutionarily conserved genes expressed in germ cells as molecular markers was used to identify germ cells and to investigate their behaviors in vertebrates and invertebrates (Kobayashi et al. 1996; Mochizuki et al. 2001; Sato et al. 2006; Gustafson and Wessel 2010). Particularly for scleractinians, which possess no true organs, molecular markers for germ cells can be used as a valuable tool to unambiguously distinguish germ cells from somatic cells in the body. Recently, two genes, *vasa* and *piwi*, were identified as markers for scleractinian germline cells (Shikina et al. 2012, 2015).

16.3.1.1 Vasa and Piwi Genes

The genes *vasa* and *piwi* are evolutionarily conserved genes that are expressed in germline cells in a variety of metazoans. Vasa is a member of an ATP-dependent RNA helicase family of DEAD-box proteins that unwind the double-stranded RNA loops to translate germline-specific target mRNAs (Hay et al. 1988; Johnstone and Lasko 2001; Raz 2000). Piwi is a member of the Argonaute (AGO) protein family, characterized by two major motifs, the Piwi/Argonaute/Zwille (PAZ) domain and the PIWI domain. Piwi proteins associate with Piwi-interacting RNAs (piRNAs) to modulate epigenetic programming and posttranscriptional regulation, and may be implicated in transposon silencing, genome integrity, germline specification, and germ cell maintenance (Houwing et al. 2007; O'Donnell and Boeke 2007; Juliano et al. 2011). In cnidarians, the expression of these genes in germline cells was reported in the freshwater hydra *H. magnipapillata* (Mochizuki et al. 2001, Nishimiya-Fujisawa and Kobayashi 2012), the hydrozoan jellyfish *Podocoryne carnea* (Seipel et al. 2004) and *C. hemisphaerica* (Leclère et al. 2012), the colonial marine hydroid *Hydractinia echinata* (Rebscher et al. 2008), and the sea anemone *N. vectensis* (Extavour et al. 2005).

In scleractinians, though a partial sequence of the *vasa* gene was cloned in *Acropora digitifera* (Mochizuki et al. 2001), molecular characterization remains incomplete. Recently, the full-lengths of *vasa*- and *piwi*-like cDNA sequences were determined in the stony coral *Euphyllia ancora* (Shikina et al. 2012, 2015). The deduced amino acid sequence of the coral vasa contained nine conserved domains (i.e., ATPase motifs, RNA unwinding motifs, and a helicase C-domain), which typify the Vasa-like proteins (Shikina et al. 2012). The deduced amino acid sequence of the coral piwi contained a PAZ domain and a PIWI domain at the C-terminal end and displayed a high degree of homology with other metazoan piwi protein family members (Shikina et al. 2015).

16.3.1.2 Vasa and Piwi Are Expressed in Early Stage Germline Cells in Scleractinians

The expression patterns of Vasa and Piwi in germline cells in corals were identified by immunohistochemistry with specific antibodies against the coral piwi or vasa in *E. ancora* (Shikina et al. 2012, 2015). Vasa and Piwi exhibited nearly identical expression patterns in the coral. In males, strong immunoreactivity was detected in unclustered spermatogonia alongside the mesoglea (Fig. 16.1a), in clustered spermatogonia (Fig. 16.1b), and in primary spermatocytes (Fig. 16.1c). The immunoreactive signals were faint or almost undetectable in the secondary spermatocytes, spermatids,

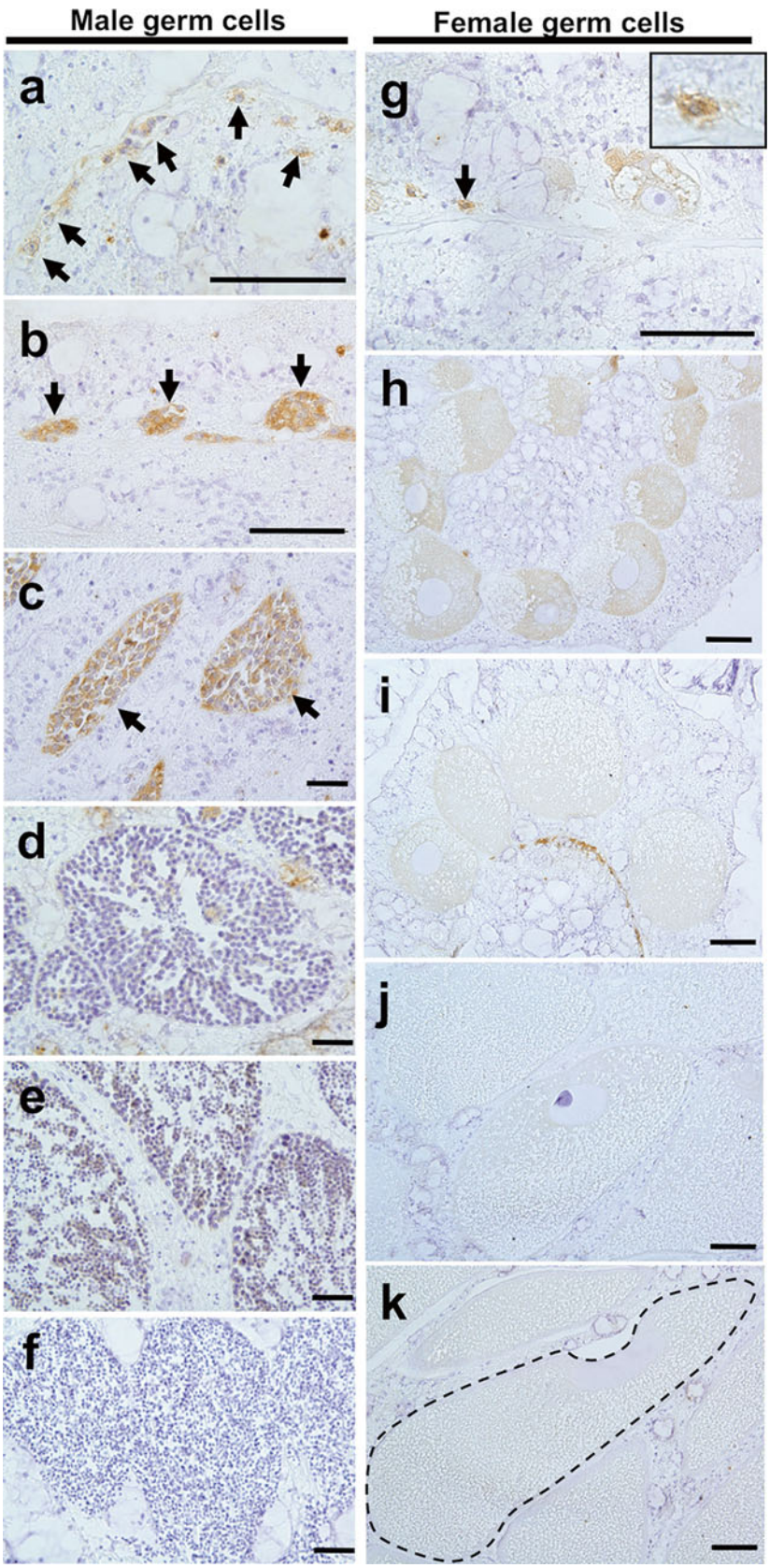

Fig. 16.1 Expression pattern of Piwi (a germ cell marker) during gametogenesis in the stony coral *E. ancora*, as assessed by immunohistochemistry. **a** Spermatogonia before forming clusters. *Arrows* indicate spermatogonia. **b** Spermatogonia that formed clusters. *Arrows* indicate spermatogonial clusters. **c** Primary spermatocytes. *Arrows* indicate clusters of spermatocytes. **d** Secondary spermatocytes. **e** Spermatids. **f** Mature sperm found during the month of spawning. **g** Oogonia and early oocytes (stage I, 20–50 µm diameters). The *arrow* indicates the oogonia visible in *inset*. **h** Developing oocytes (stage II, 51–125 µm diameters). **i** Developing oocytes (stage III, 126–200 µm diameters). **j** Developing oocytes (stage IV, 201–275 µm diameters). **k** Mature oocytes (stage V, >276 µm diameters, with 'U'-like germinal vesicles). *Broken line* indicates the mature oocytes. *Dark blue color*, the nuclei stained with hematoxylin. *Brown color*, piwi immunoreactivity. All bars, 50 µm (Reproduced from Shikina et al. 2015, modified)

and sperm (Fig. 16.1d–f). In females, strong signals were detected in oogonia and early oocytes (Fig. 16.1g). The signals became weaker as oocyte development progressed (Fig. 16.1h–j) and were faint or almost undetectable in the cytoplasm of mature oocytes (Fig. 16.1k). These results indicate that Vasa and Piwi are useful markers for detecting the early stages of germ cells in scleractinians.

16.3.1.3 Expression of Germ Cell Marker Genes in Extra-gonadal Sites

In most advanced animals, the *vasa* and *piwi* genes are exclusively expressed in germline cells (Gustafson and Wessel 2010; Lin and Spradling 1997; Cox et al. 1998; Deng and Lin 2002; Qiao et al. 2002; Houwing et al. 2007; Wang and Reinke 2008; Juliano et al. 2011). However, in some animals, the *vasa* and/or *piwi* genes are expressed in both multipotent (or somatic) stem cells and germline cells, including the planaria *Dugesia japonica* (Shibata et al. 1999; Reddien et al. 2005), the colonial tunicate *Botryllus primigenus* (Sunanaga et al. 2010), the freshwater hydra *H. magnipapillata* (Mochizuki et al. 2001; Nishimiya-Fujisawa and Kobayashi 2012), the colonial marine hydroid *H. echinata* (Rebscher et al. 2008; Seipel et al. 2004), the hydrozoan jellyfish *C. hemisphaerica* (Leclère et al. 2012), and the cydippid ctenophore *Pleurobrachia pileus* (Alié et al. 2011). Notably, in *E. ancora*, *vasa* transcripts were detected not only in mesentery tissues with gonads but also in isolated tentacles assessed by RT-PCR (Shikina et al. 2012). Vasa immunoreactivity was also confirmed in the small number of cells in tentacle endoderm and mesenterial filament (Shikina et al. 2012). Piwi immunoreactivity was also detected in a very small number of cells in the mesenterial filament (Shikina et al. 2015). The detailed characteristics and cell types with Vasa and Piwi immunoreactivity in *E. ancora* extra-gonadal tissues remain undetermined.

16.3.2 Origin of Germline Cells in Scleractinians

Most scleractinians have annual or multiple spawning cycles, which implies that scleractinians possess fundamental mechanisms to maintain undifferentiated germline cells as the "reservoir population" or the "origin" at steady state levels by providing a constant supply of cells destined to become gametes. A large number of previous studies primarily focused on the processes of oocyte development in females and sperm formation in males, whereas only a few described the origins of germline cells.

16.3.2.1 Germline Stem Cells

The continual production of eggs and sperm throughout adulthood requires some form of stem cell system (White-Cooper et al. 2009). Germline stem cells (GSCs), which both self-renew and differentiate, are the key cells responsible for this system in various metazoans. The processes in the system, such as the maintenance of stem cell potency or differentiation ability, are regulated typically by the interactions between the germline and the somatic components of the gonad (Li and Xie 2005). Moreover, the role of the GSC system is widely demonstrated in the gametogenesis of both vertebrates and invertebrates (Spradling et al. 2011).

16.3.2.2 Origin of Germline Cells in Hydrozoans: Interstitial Stem Cells

In cnidarians, the origin of germline cells was intensively studied in hydrozoans. In gonochoric hydra, such as *H. magnipapillata* and *H. oligactis*, germ cells originate from interstitial cells, called i-cells, which are found in the interstitial space of the ectodermal epithelium of the body column (Nishimiya-Fujisawa and Kobayashi 2012). The population of i-cells primarily consists of two types of stem cells, multipoint stem cells (MPSCs) and germline stem cells. Although they cannot be distinguished histologically, these cells differ in function. The MPSCs both self-renew and differentiate into multiple somatic cell types, such as nematocysts, nerve cells, gland cells, and GSCs; the MPSCs do not differentiate into ectodermal and endodermal epithelial cells. By contrast, the GSCs self-renew but only generate germline cells and do not generate somatic cells (Nishimiya-Fujisawa and Kobayashi 2012). Thus, the GSCs are unipotent stem cells that specifically produce gametes in the adult hydra. During the budding or regeneration process of asexual reproduction of hydra, the GSCs are typically transmitted to newly generated buds or body parts. However, when the GSCs are lost in the body during asexual reproduction events, the MPSCs generate new GSCs (Nishimiya-Fujisawa and Kobayashi 2012). Thus, the sexual reproduction of hydra is supported by a "dual" stem cell system. In the colonial marine hydroid *H. echinata*, i-cells are also present in the body, similar to that of the freshwater hydra (Müller et al. 2004). The *H. echinata* i-cells, by contrast, give rise to all cell types including ectodermal and endodermal epithelial cells and germline cells (Müller et al. 2004; Künzel et al. 2010; Plickert et al. 2012).

16.3.2.3 Origin of Germline Cells in Scleractinians: A Classical View

Classically, scleractinian germ cells are assumed to originate from interstitial cells, such as occurs in hydrozoans; however, the information was from a limited number studies (Szmant-Froelich et al. 1980, 1985; Harrison and Wallace 1990). Because the scleractinians reproduce asexually with budding and fragmentation, interstitial cells are likely present in the body. However, it remains unclear whether scleractinians possess true interstitial cells because the chemotherapy,

cloning, transplantation, and transgenic techniques that were applied to characterize interstitial cells in hydrozoans have not provided direct evidence (Bode et al. 1976; Nishimiya-Fujisawa and Sugiyama 1993, 1995; Khalturin et al. 2007). Basic information on these cells, such as morphology and spatial and temporal distribution patterns, remains largely missing. Indeed, interstitial cells have so far been demonstrated experimentally only in hydrozoans and not in anthozoans (Technau and Steele 2011; Gold and Jacobs 2013).

16.3.2.4 Implication of the Presence of GSCs in Scleractinian Gametogenesis

Most recently, a GSC system in scleractinian gametogenesis was proposed for *E. ancora* (Shikina et al. 2015). The authors hypothesized that because scleractinian gametogenesis exhibits annual or multiple cycles and gametes are produced at specific sites in endodermal mesentery tissues (gonads) but not in tentacles or in ectodermal epithelium of the body column, GSCs may reside in endodermal mesentery tissues for continual and stable gamete production in every cycle. To address this possibility, the spatial and temporal distribution patterns of early germ cells (oogonia) were investigated with immunohistochemical analysis using an anti-*E. ancora* piwi antibody. The early germ cells were present throughout the year within/or near the gonad of the mesentery tissues, regardless of the sexual reproductive cycle (Fig. 16.2a–i). Notably, even in the corals with fully matured gonads on the month of spawning, small but stable numbers of early germ cells (approximately 1600–1800 cells/mesentery) were present (Fig. 16.3). During spawnings, these early germ cells were not released with the sperm or eggs and remained in the mesentery tissues. These findings suggest that these early germ cells most likely serve as a reservoir of germline cells and that some of these cells produce the cells destined to become gametes for the next period of sexual reproduction; hence, these cells would function as GSCs.

Notably, in *E. ancora*, the early germ cells tended to be in two specific areas of mesentery tissues: (1) the area between the gonad and the mesenterial filament and (2) the area between the body wall and the gonad (Fig. 16.4a, b). Only small proportions of early germ cells were observed within the gonads with mature sperm or oocytes. Additionally, uneven distributions of early germ cells were also observed in the vertical direction of the mesentery tissue, with relatively larger proportions of early germ cells distributed in the mesentery tissues within the upper area of gonads in both sexes (Fig. 16.4c, d). The GSCs in some metazoans, such as *Drosophila* and *C. elegans*, reside in a specific site with a special microenvironment termed the "niche", which supports stem cell abilities to self-renew and differentiate (Li and Xie 2005). The mesenterial sites with early germ cells in *E. ancora* may be niche-like microenvironments in which early germ cells maintain their capacities and/or survival, though the key intrinsic factors that maintain the early germ cells within these sites remain unknown.

In Fig. 16.5, the schematic diagram of the proposed GSC system underlying oogenesis in *E. ancora* is presented. During the early stage of oogenesis, early germ cells are distributed throughout the gonad alongside the mesoglea (i). Then, a majority of oogonia differentiate into oocytes, which develop with increased diameters until maturation (ii and iii); however, a few germline cells remain in an undifferentiated state (undifferentiated germline cells, iii) and remain in the specific site of the mesenteries. These germline cells, which most likely have stem cell potency, remain at the mesentery site even after spawning (iv). For the production of differentiated germ cells for the next cycle of sexual reproduction, the germline cells migrate into the site of gametogenesis and proliferate (v). At present, the evidence for this process (v) is missing (Shikina et al. 2015).

16.3.3 Yolk Protein Formation and Accumulation During Oogenesis

In oogenesis within the female reproductive organ, diploid oogonia are transformed into mature haploid oocytes that contain the materials required for the initiation and maintenance of the metabolism and development of an embryo (Song and Wessel 2005). The formation and accumulation of yolk proteins, which are a source of energy and nutrients that support embryonic development, are among the most conspicuous aspects of oogenesis and are commonly observed biological phenomena in oviparous vertebrate and invertebrate animals. In cnidarians, the appearance and accumulation of yolk granules during oogenesis were confirmed with histological and/or ultrastructural analysis in many species, i.e., a hydrozoan jellyfish (Kessel 1968), a scyphozoan jellyfish (Eckelbarger and Larson 1988, 1992), sea anemones (Wedi and Dunn 1983; Eckelbarger et al. 2008), a sea pen (Eckelbarger et al. 1998), a sea fan (Tsai et al. 2014), and scleractinian corals (Szmant-Froelich et al. 1980). Recently, first shown in the scleractinians of the cnidarians, the identities of major yolk proteins, the site of yolk synthesis, and the timing of accumulation were determined by molecular evidence. Several aspects of the advanced achievements from these recent studies will be outlined in the following section.

16.3.3.1 Yolk Proteins in Cnidarians: Vitellogenin (Vg) and Egg Protein (Ep)

Vitellogenin (Vg), a member of the large lipid transfer protein (LLTP) superfamily (Babin et al. 1999; Smolenaars et al. 2007), is a major yolk protein precursor and is involved in oogenesis in numerous oviparous bilaterian animals (Prowse and Byrne 2012; Shyu et al. 1986). Imagawa et al.

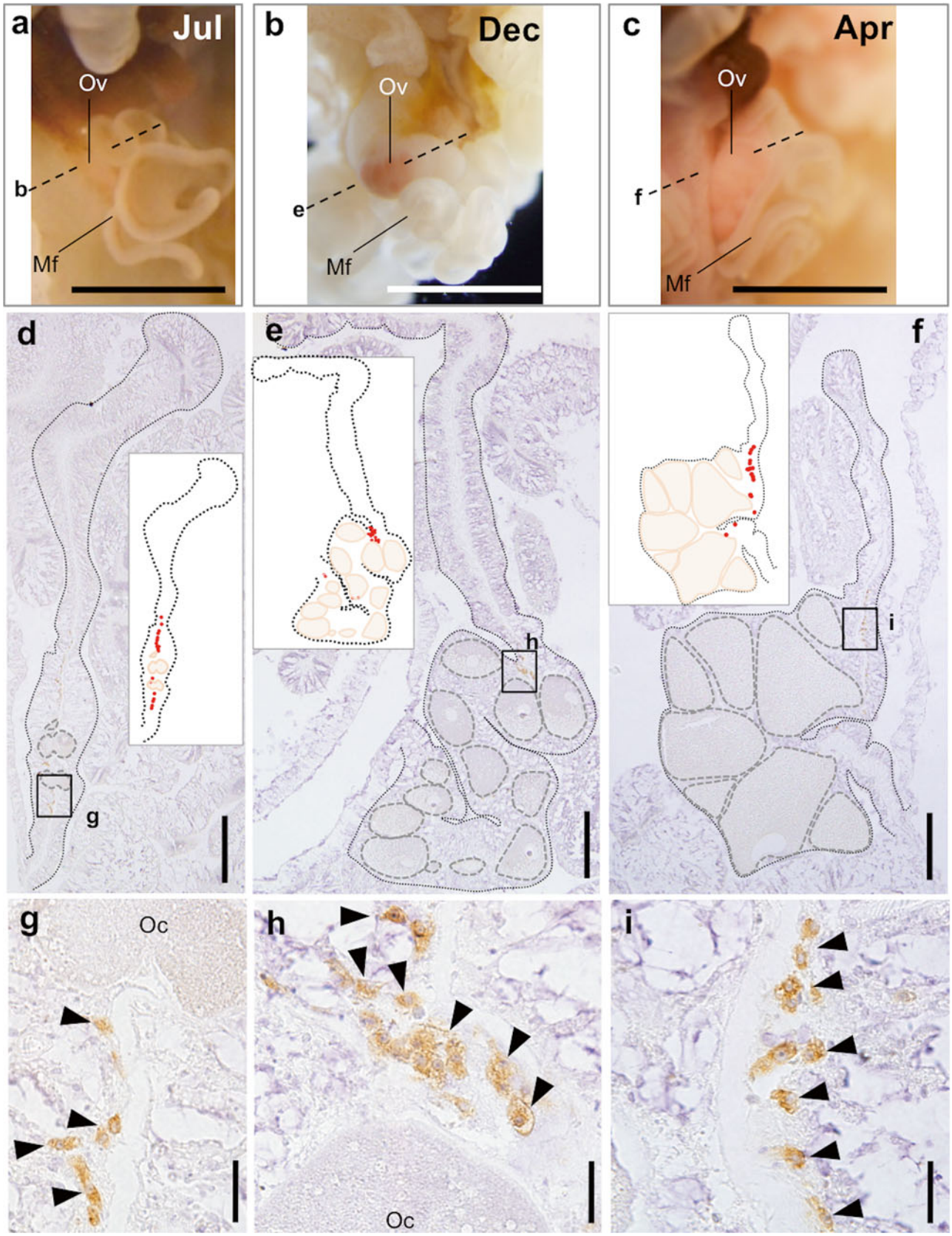

Fig. 16.2 The distribution of oogonia with Piwi immunohistochemical staining. **a–c** Micrograph of a portion of *E. ancora* mesentery tissue with putative ovarian tissue and a mesenterial filament in a female sample collected in Jul **a**, in Dec **b**, and in Apr (month of spawning, **c**). **d–f** Mesentery tissue horizontally sectioned at the approximate middle line shown in **a**, **b**, and **c** with anti-Piwi immunoreactivity. The *broken lines* indicate the shape of the mesentery tissues and the oocytes, and the *insets* are the schematic illustrations of the distribution of the oogonia with Piwi immunoreactivity (*red dot*). **g–i** The *insets* shown in **d–f**, respectively. *Flesh color*, the oocytes; *Dark blue color*, the nuclei stained with hematoxylin; and *Brown color*, oogonia with Piwi immunoreactivity. *Ov* ovary, *Mf* mesenterial filament; and *Oc* oocyte. *Bar* is **a–c** 2 mm; **d–f** 200 μm; and **g–i** 20 μm (Reproduced from Shikina et al. 2015)

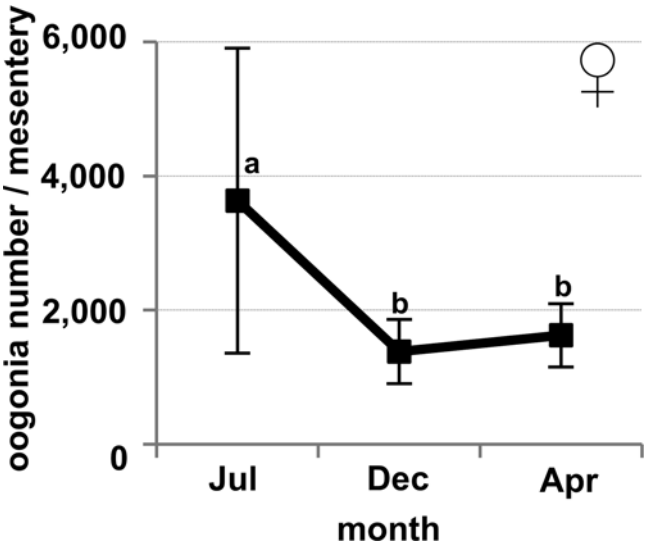

Fig. 16.3 The changes in the numbers of *E. ancora* oogonia with Piwi immunoreactivity per mesentery tissue at different periods of oogenesis in females, as assessed by immunohistochemistry. The month of *E. ancora* spawning was Apr. The data are shown as the means ± SD (n = 9 mesenteries, three colonies, at each period) (Reproduced from Shikina et al. 2015)

(2004) first demonstrated that Vg was in the eggs of the hermaphroditic coral *Favites chinensis* with SDS-PAGE analysis on soluble proteins extracted from released eggs. The N-terminus sequences of the major yolk protein present in *F. chinensis* eggs were determined, and the partial cDNA sequence encoding the protein was elucidated. The deduced amino acid sequences exhibited some similarities to the Vg of oviparous vertebrates and invertebrates. Thereafter, the full-length of *vg* cDNA was determined in two other stony corals *Galaxea fascicularis* (Hayakawa et al. 2005, 2006) and *E. ancora* (Shikina et al. 2013). In addition to scleractinians, Vg was also identified as a major oocyte yolk protein in the starlet sea anemone *N. vectensis* by recent proteome analysis on released eggs (Lotan et al. 2014). Presently, in cnidarians, *vg* cDNA was determined in the four species, but molecular identification and characterization of Vg have not been reported in the Meduazoa.

Although small variations in the Vg sequence occurred between these species, the deduced amino acid sequence of the Vg of those cnidarians generally contains a signal peptide known to release proteins and Vg domain at the N-terminus (Vitellogenin-N), a domain of unknown function (DUF1943), and a von Willebrand factor type-D domain (VWD) at the C-terminal end. The sequence KTLGNAG, which is highly conserved in the Vg of invertebrates and vertebrates, was also identified (Shikina et al. 2013), in addition to a no-polyserine domain. Moreover, based on the data from N-terminus peptide sequencing of major polypeptides isolated from unfertilized eggs and the full-length of *vg* cDNA, the entire primary structure of the Vg precursor and its processing pattern were proposed for *G. fascicularis* (Hayakawa et al. 2006). The Vg precursor are most likely processed in at least one or two sites and would generate two or three polypeptides, as was observed in the processing of Vg in other oviparous animals (Retzek et al. 1992; Havukainen et al. 2012), though the cleavage enzymes have yet to be determined.

The expression of *vg* transcripts was detected almost specifically in female colonies (Hayakawa et al. 2005; Shikina et al. 2013), and increased expression of *vg* transcripts during periods of oogenesis was observed as the coral approached the spawning season (Imagawa et al. 2004; Shikina et al. 2013).

A novel major yolk protein, egg protein (Ep), of approximately 30 kDa was identified with analysis of soluble proteins extracted from the eggs of *G. fascicularis* (GfEp-4, *G. fascicularis* Egg protein-4; Hayakawa et al. 2007). The deduced amino acid sequence contains a signal peptide known to release proteins and no putative conserved domains. In *E. ancora*, Ep products (both mRNA and protein) are highly expressed in female colonies, and the mRNA expression levels are highly correlated with reproductive cycles of female colonies (Shikina et al. 2013). The Ep sequence is not similar to Vg or the major echinoderm yolk protein, which are similar to transferrin (Unuma et al. 2001; Yokota et al. 2003), and the sequence is also not similar to insect yolk protein, which is similar to lipases (Bownes 1992; Hens et al. 2004). These results suggest that Ep is a novel yolk protein of corals with an evolutionary history that is independent from yolk proteins in other oviparous animals.

16.3.3.2 The Site of Vg and Ep Synthesis and the Timing of Accumulation

In various oviparous animals, Vg is produced in extraovarian sites, secreted into the circulatory system, and subsequently incorporated into developing oocytes through receptor-mediated endocytosis (Byrne et al. 1989; Raikhel and Dhadialla 1992). The primary extraovarian site of Vg synthesis differs among taxa; for example, the site is in the liver of vertebrate species (Wangh and Knowland 1975; Green and Tata 1976; Gruber et al. 1976; Lubzens et al. 2010), the fat body of insects (Raikhel and Dhadialla 1992), the intestine of nematodes (Kimble and Sharrock 1983), and the coelomocytes of annelids (Porchet et al. 1989). In some invertebrate species, Vg synthesis occurs in extraovarian tissues and/or within ovaries, e.g., in the hepatopancreas and ovarian somatic cells of marine shrimp (Tsang et al. 2003; Kung et al. 2004) and in the ovarian somatic cells (follicle cells) of oyster, scallop, and abalone (Osada et al. 2004; Kung et al. 2004; Matsumoto et al. 2008). A somatic lineage generally produces Vg proteins and not a germline, although the sites for Vg synthesis differ among animals. As an exception, an isoform of Vg produced by oocytes themselves was identified recently in ascidian eggs (Akasaka et al. 2013).

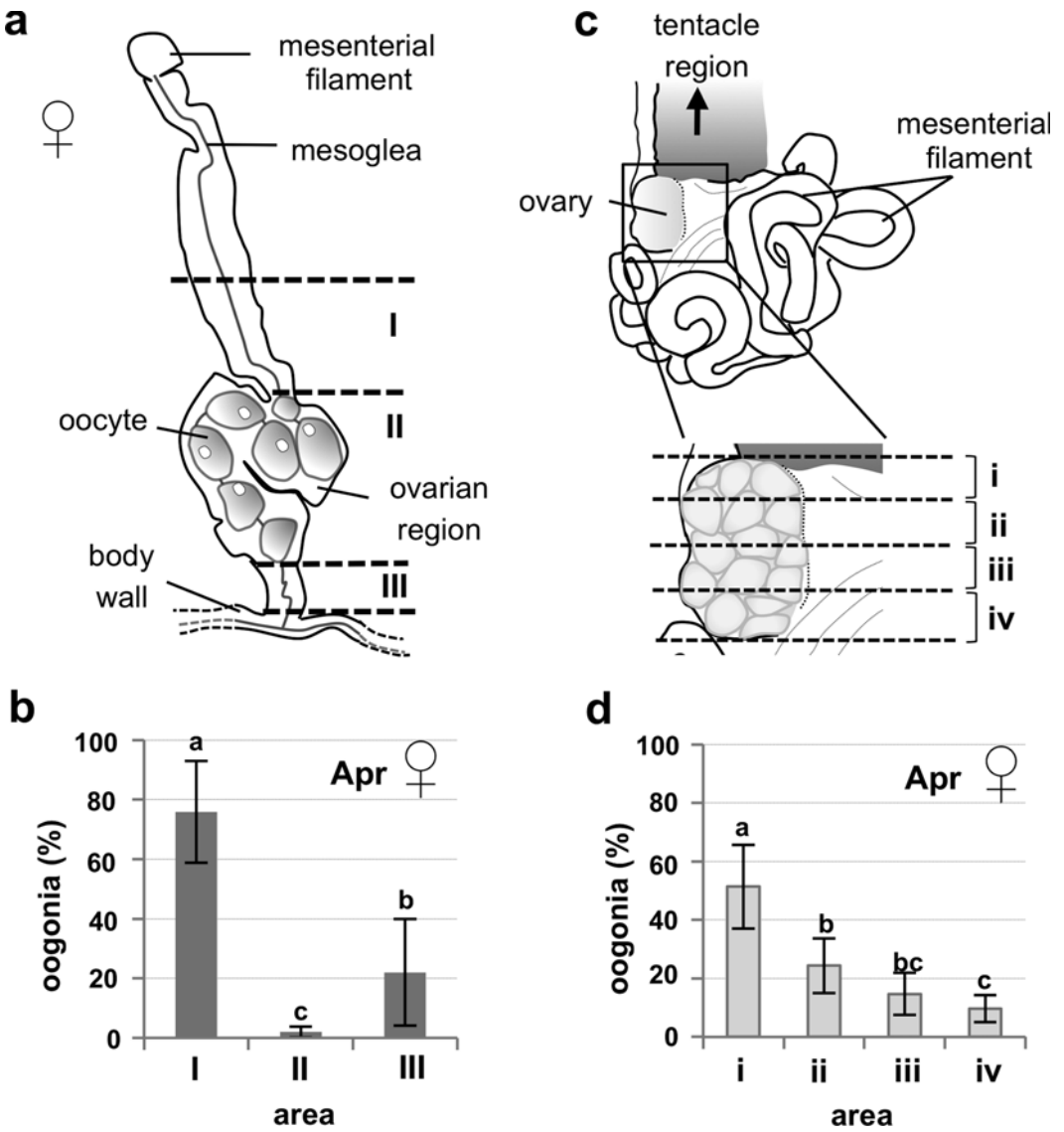

Fig. 16.4 **a** Schematic of a horizontally dissected *E. ancora* mesentery tissue with an ovary. The mesentery was artificially divided into three areas: (I) Between the ovarian region and the mesenterial filament; (II) The ovarian region with oocytes; and (III) Between the body wall and the ovarian region. **b** Distribution percentage of oogonia in the each area (I–III) in female corals collected in April (month of spawning). **c** Schematic diagram of a portion of mesentery tissue containing an ovary. A portion of mesentery tissue with an ovary was vertically divided into four areas (i–iv). **d** Vertical distribution frequency of oogonia in each area (i–iv) in female corals collected in April (month of spawning). Oogonia were identified by immunohistochemistry with the anti-*E. ancora* piwi antibody. The data are shown as the means ± SD of the frequency distribution percentages of oogonia that were given as the sum of the total percentages (n = 9 mesenteries, three colonies) (Reproduced from Shikina et al. 2015)

In cnidarians to date, the site of yolk synthesis was investigated in various species primarily by ultrastructural analysis. The mode of yolk synthesis was suggested to be either or both oocyte autosynthesis and somatic synthesis (heterosynthesis) (Eckelbarger and Larson 1998, 1992; Eckelbarger et al. 1998, 2008). Recently, the site of yolk protein synthesis and the timing of yolk accumulation were determined in *E. ancora* (Shikina et al. 2013). An analysis of tissue distributions demonstrated that *vg* transcripts were highly expressed in isolated ovarian tissues, as assessed by semiquantitative RT-PCR analysis (Fig. 16.6a–j). mRNA in situ hybridization demonstrated that *vg* mRNAs were present in ovarian (mesenterial) somatic cells, also called follicle cells (Fig. 16.6l–o). The *ep* transcripts exhibited similar distribution patterns to those of *vg* (Shikina et al. 2013). Subsequent immunohistochemical analyses with anti-*E. ancora* Vg or Ep antibodies indicated immunoreactivity in both oocytes and ovarian somatic cells (Fig. 16.7a–d). The localization of these transcripts and proteins demonstrated that ovarian somatic cells adjacent to oocytes, but not oocytes themselves, synthesize the Vg and Ep in the coral ovarian tissues (Shikina et al. 2013). Thus, yolk proteins produced in the ovarian somatic

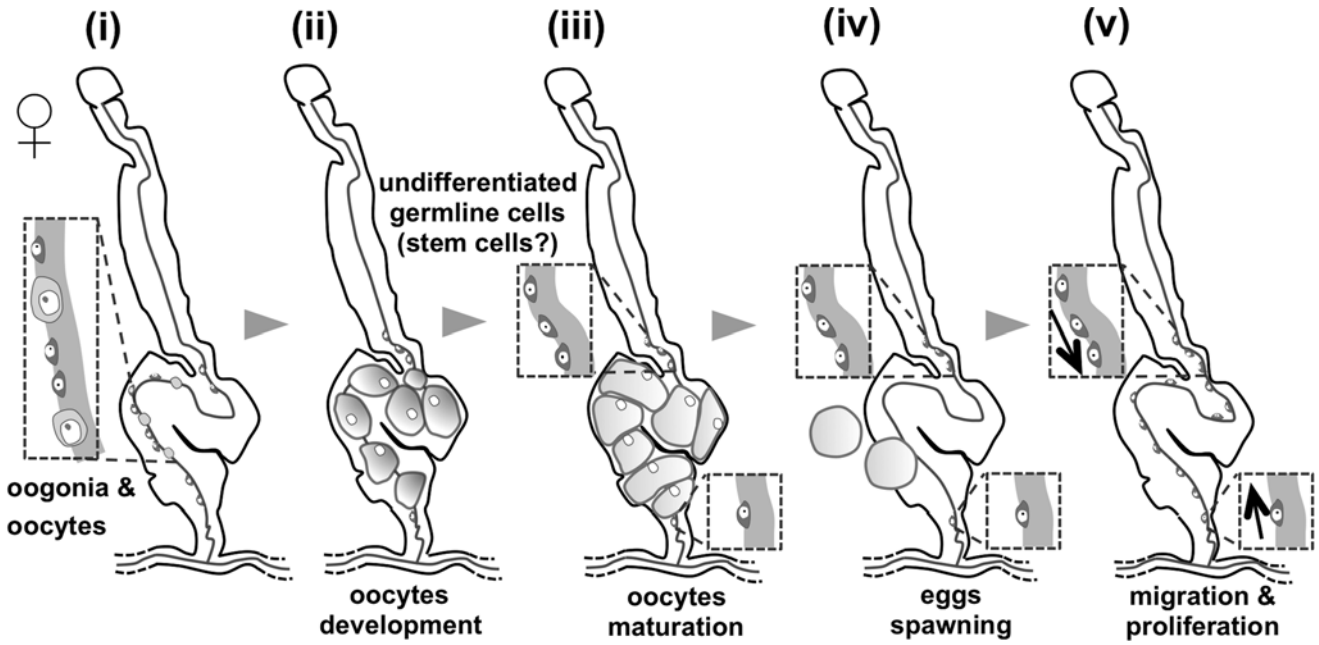

Fig. 16.5 Schematic diagram of the proposed germ line stem cell system underlying coral oogenesis in females of *E. ancora* (Reproduced from Shikina et al. 2015)

cells would be transported to the oocytes via either transmesoglea pores or somatic cell-oocyte contact through mesoglea with subsequent uptake by oocytes through receptor-mediated endocytosis (Fig. 16.8). In *N. vectensis*, by contrast, based on the low amounts of *vg* mRNA (Helm et al. 2013) and vigilin, a protein that acts as a stabilizer for *vg* mRNA, in fertilized eggs (Lotan et al. 2014), the hypothesis was that Vg would be synthesized within the oocytes. An ultrastructural observation suggested that both heterosynthesis and autosynthesis of yolk occurred during *N. vectensis* oogenesis (Eckelbarger et al. 2008). In corals, it is possible that other isoforms of Vg or undetermined yolk proteins are synthesized by oocytes themselves.

The timing of Vg and Ep accumulation in oocytes was also revealed in *E. ancora* by immunohistochemical analysis (Shikina et al. 2013). The accumulation of Vg begins in early stage oocytes (20–50 μm in diameter) 2–3 months after spawning, whereas Ep accumulation begins in developing (middle) stage oocytes (150 μm in diameter) approximately 6 months after spawning (Shikina et al. 2013). These results suggest that mechanisms control the timing of each type of yolk formation according to the developmental process of the stage of oocyte. Currently, however, little is known about the intrinsic mechanisms that regulate the formation of yolk in corals.

16.3.3.3 The Possible Functions of Yolk Proteins in Scleractinians

The possible functions of yolk protein were addressed in *E. ancora* (Shikina et al. 2013). During *E. ancora* embryogenesis, the amount of Vg polypeptides (160, 82, 77, and 60 kDa) significantly decreased 64 h after fertilization, as assessed by western blot analysis. Because *E. ancora* embryos start to swim actively 64 h after fertilization (pear stage), the suggestion is that embryos consumed the polypeptides as a source of energy or nutrients. The protein profiles of *Fungia scutaria* (mushroom coral) embryogenesis assessed by SDS page also showed two major bands at 84 and 79 kDa that were depleted with the onset of settlement and metamorphosis. Although the identities of these two major bands were not confirmed, based on the molecular weight, these two major bands were most likely polypeptides derived from *F. scutaria* Vg (Schwarz et al. 1999). By contrast, Ep levels did not dramatically decrease during *E. ancora* embryogenesis. The functions of Ep in coral bodies are currently unknown, although *ep* transcripts were detected in extraovarian sites such as tentacles in both sexes of *E. ancora* colonies by RT-PCR analyses. Additionally, the presence of Ep was demonstrated in pseudo-eggs of *G. fascicularis* by immunoblotting (Hayakawa et al. 2006). Thus, Ep may have multiple functions in coral that are different from those of Vg.

16.3.3.4 Evolutionarily Conserved Characteristics of Scleractinian Oogenesis: Insight into the Evolution of Oogenesis in Cnidaria

The Vg is present in cnidarian eggs, is involved in oogenesis, and its expression profiles are strongly correlated with the reproductive cycles of female colonies. Furthermore, somewhat similar to most oviparous bilaterians, coral Vg is produced by somatic cell lineages, not by oocytes and is

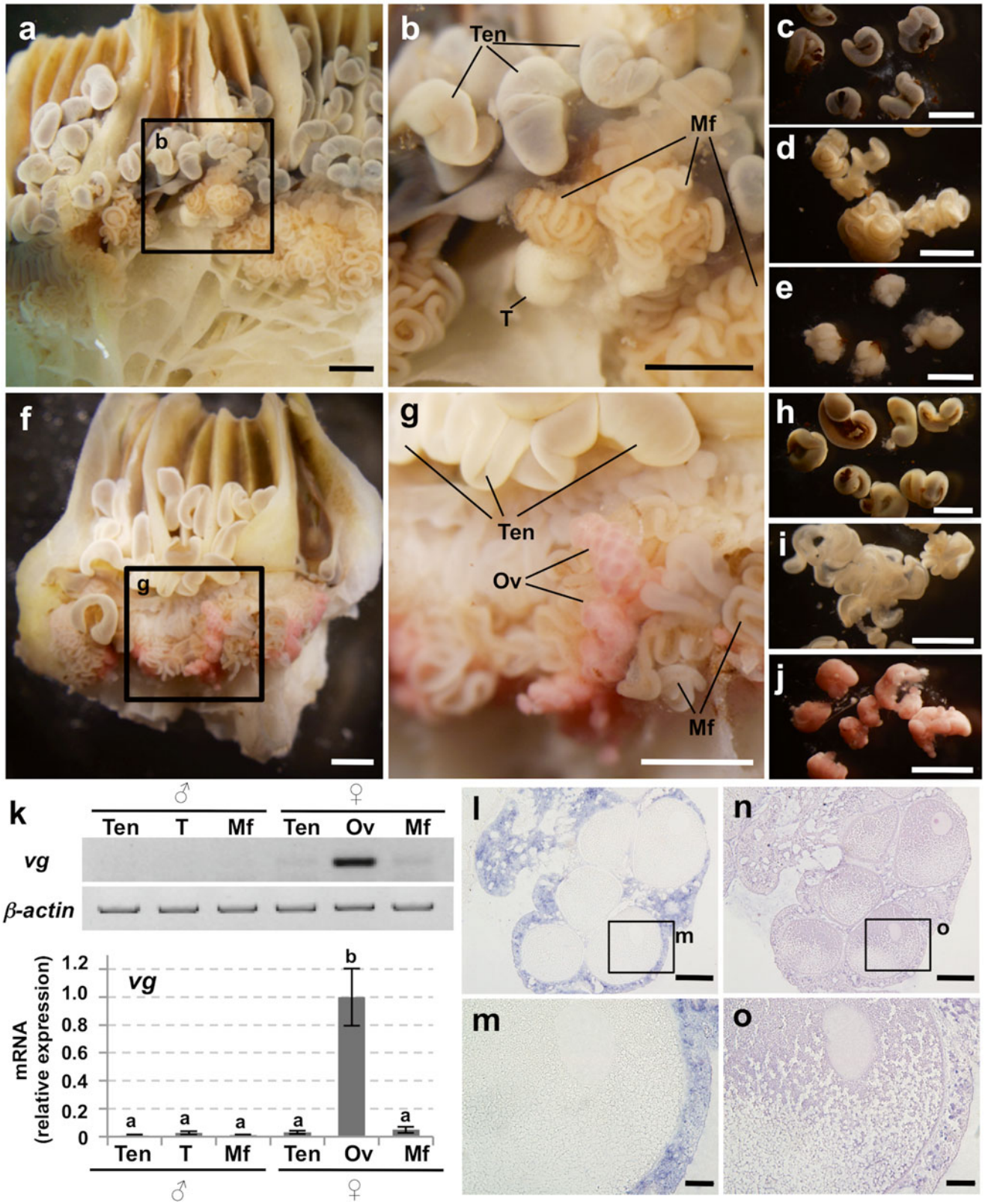

Fig. 16.6 The dissection of coral tissues and expression of *vitellogenin* (*vg*). **a** A portion of *E. ancora* male dissected skeleton and polyps collected 2 months before spawning. **b** A magnified view of the inset shown in **a. c** Isolated male tentacles. **d** Isolated male mesenterial filaments. **e** Isolated testicular tissues. **f** Dissected female polyps collected 2 month before spawning. **g** A magnified view of the *inset* shown in **f. h** Isolated female tentacles. **i** Isolated female mesenterial filaments. **j** Isolated putative ovarian tissues. **k** *Upper*: Distribution of *vg* transcripts in the different tissues of male and female samples as determined by semiquantitative RT-PCR analysis. *β-actin* was the internal control gene. *Lower*: Quantified expression levels of *Vg*. **l** and **m** Localization of *vg* mRNA-positive cells in an isolated putative ovarian tissue. **n**, **o** Sequential sections were stained with hematoxylin-eosin. **m**, **o** Higher-magnification views of the *insets* shown in **l**, **n**. *Ten* tentacles, *Mf* mesenterial filaments, *T* testicular tissues; and *Ov* ovarian tissues. *Bar* **a**–**j**, 2 mm; **l**, **n**, 200 μm; and **m**, **o**, 20 μm (Reproduced from Shikina et al. 2013, modified)

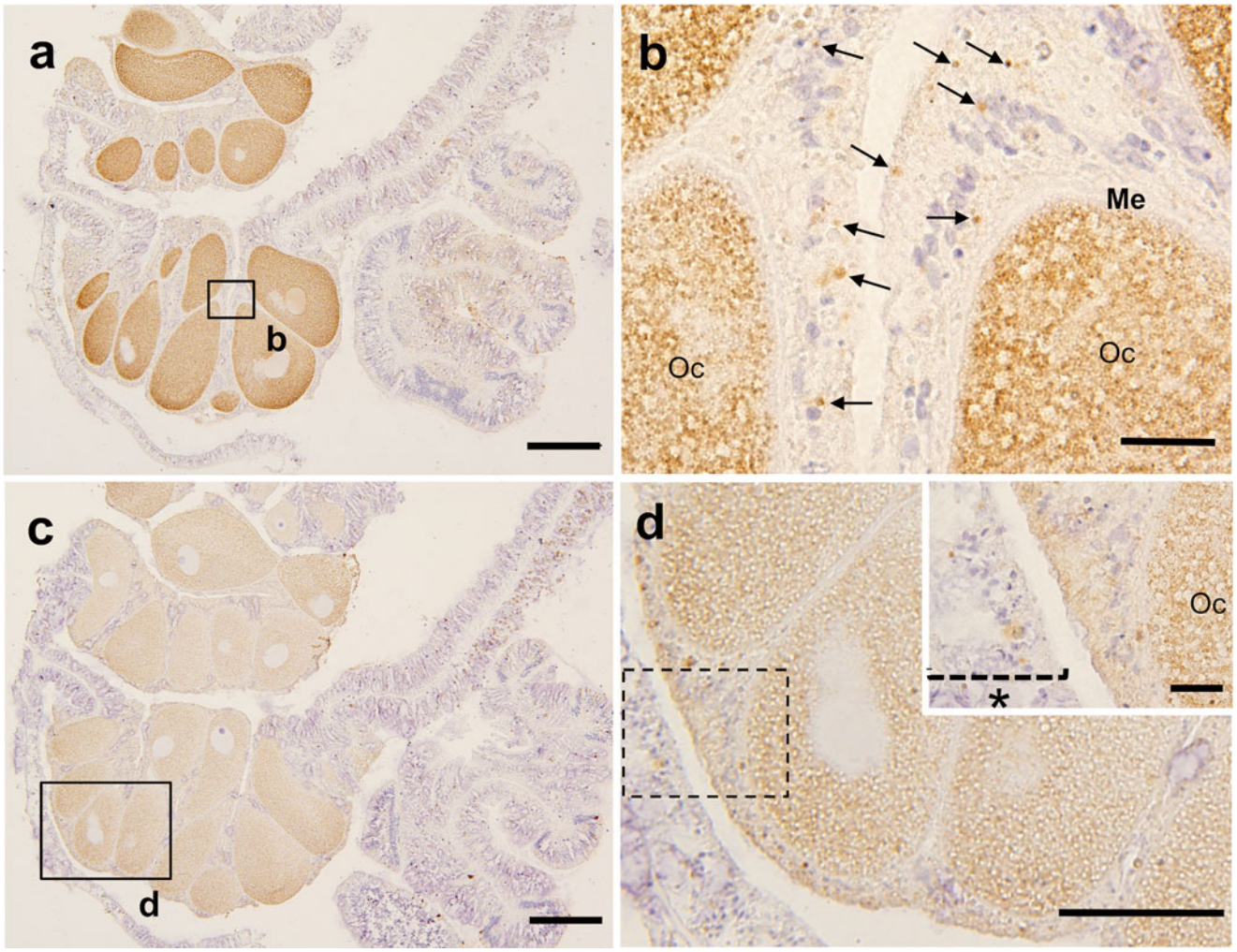

Fig. 16.7 Localization of Vg (vitellogenin) and Ep (egg protein) in *E. ancora* as assessed by immunohistochemistry. **a** Localization of Vg. **b** Higher-magnification view of the inset shown in **a**. Vg immunoreactivity was also detected in the somatic cells adjacent to the oocytes (*arrows*). **c** Localization of Ep. **d** Higher-magnification view of the inset shown in **c**. Ep immunoreactivity was detected in oocytes and somatic cells adjacent to oocytes but was faint or almost undetectable in cells of body wall (*inset*; *). *Oc* oocytes, *Me* mesoglea. **a**, **c** Bars = 200 μm; **d** Bar = 50 μm; and **b** and inset in **d** Bars = 20 μm (Reproduced from Shikina et al. 2013)

accumulated in oocytes; the Vg is likely used as a source of nutrition and/or energy during embryogenesis. These results raise the hypothesis that these features of Vg are most likely present in the oogenesis of some common ancestor before the divergence of the cnidarian and the bilaterian lineages.

How does the soma influence oogenesis in cnidarians? In advanced animals, the development of the oocytes is supported by somatic cells within the ovary, i.e., follicle cells in vertebrates. The somatic cells, called supporting cells or accessory cells, play essential roles in regulating the survival and growth of oocytes by supplying a favorable microenvironment with adhesion molecule-mediated intercellular communication, growth factors, and other substances (Richards 2001; Griswold 1995; Xie and Spradling 2000; Kiger et al. 2000; Matova and Cooley 2001). Currently, for cnidarians, a number of oocyte-somatic cell associations have been reported. In anthozoans (Eckelbarger et al. 1998), scyphozoans (Eckelbarger and Larson 1993), and hydrozoans (Thomas and Edwards 1991; Eckelbarger et al. 2008), somatic cells (follicle cells) adjacent to germ cells are speculated to be involved in nutrition production and transportation based on the cellular position and morphological characteristics and as assessed by histological and ultrastructural observation. In some anthozoans (order Actinaria) (Larkman and Carter 1982; Wedi and Dunn 1983; Eckelbarger et al. 2008) and scyphozoans (orders Semaeostome and Rhizostome) (Eckelbarger and Larson 1988, 1992; Eckelbarger 1993), oocytes maintain contact with specialized gastrodermis cells "trophocytes", which may serve a nutritive function to oocytes (Larkman and Carter 1982). Trophocytes are unique accessory cells and are not observed outside the Cnidaria (Wourms 1987; Eckelbarger et al. 2008).

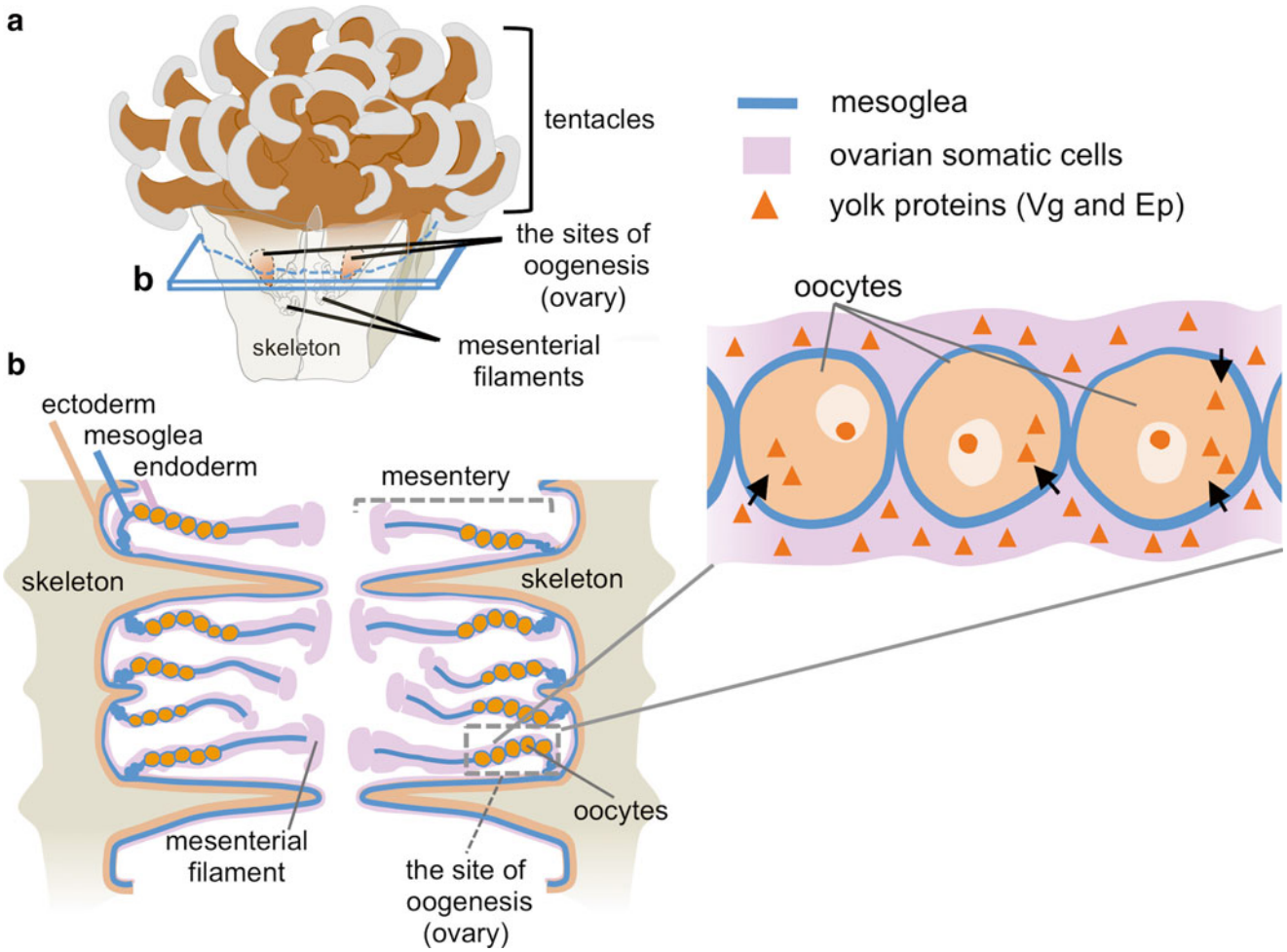

Fig. 16.8 Schematic representation of proposed major sites of yolk protein synthesis in *E. ancora*. Schematic of *E. ancora* body structure **a** and horizontally dissected **b** at the point indicated in **a**. Yolk proteins (Vg and Ep) synthesized in somatic cells adjacent to oocytes may be transported to oocytes (Reproduced from Shikina et al. 2013)

According to our best information, trophocytes have yet to be observed in scleractinian corals. Notably, however, not all cnidarians require somatic cell support for oocyte development. For example, in hydra, the development of oocytes is primarily supported by the cell fusion of germ cell-derived nurse cells with oocytes, but not by somatic cells. The nurse cells undergo apoptosis after the cytoplasmic transfer and are eventually phagocytosed by the oocytes (Miller et al. 2000; Technau et al. 2003; Alexandrova et al. 2005).

The recent findings for the coral *E. ancora* provide the first molecular evidence in cnidarians that the ovarian somatic cells (follicle cells) act as a type of supporting cell and supply yolk proteins to the oocytes, similar to some oviparous invertebrates (i.e., mollusks, Kung et al. 2004; Osada et al. 2004; Matsumoto et al. 2008). Compared with triploblastic metazoan animals, corals have a simple diploblastic body and no distinct organs, and the borders among the different tissues of polyps are also vague. Notably, even in such primitive animals, the somatic cells in ovary are functionally well specified to support germ cell development.

What is the ancestral prototype of cnidarian oogenesis? To address this issue, further analysis of oogenesis in various species across different cnidarian classes is essential not only at cellular levels but also at molecular levels, including the presence or absence of yolk proteins (i.e., Vg and Ep).

16.4 What Are the Hormones Regulating Oogenesis in Cnidarians?

In a variety of animals, hormones (i.e., steroids, growth factors, peptides, and other substances) play important roles in various aspects of sexual reproduction. Cnidarians, in contrast to advanced animals, do not have a vascular system or secretory organs and only have a passive transport system that depends on diffusion and a simple nerve net (Tarrant 2005). Advanced endocrine systems, such as those found in vertebrates, are unlikely in cnidarians. At present, limited data are available on the hormones that regulate oogenesis. During last two decades, however, several vertebrate-type

hormones were identified in cnidarian tissues and associations with sexual reproduction were proposed (Tarrant 2005).

16.4.1 Gonadotropin-Releasing Hormone (GnRH)

The gonadotropin-releasing hormone (GnRH) is a neuropeptide produced by the hypothalamus region in the brain of vertebrates. The GnRH acts on pituitary cells and induces the production of two gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH). Gonadotropins released to the vascular system are transported to the gonads to stimulate germ cell development and synthesis of several growth factors and steroid hormones. The sex steroids including progestin, androgens, and estrogens are essential in regulating ovarian functions, yolk formation, and oocyte maturation (Pierantoni et al. 2002). This pathway, the brain-pituitary-gonadal axis, is evolutionarily conserved in vertebrates (Licht et al. 1985; Stamper and Licht 1990; Hirschenhauser et al. 2000; Schulz et al. 2010).

In cnidarians, a GnRH-like peptide was reported for a few anthozoans in the last two decades. The GnRH-positive neuron was first identified in the sea anemone *N. vectensis* and the sea pansy *Renilla koellikeri* by whole-mount immunofluorescent analysis using antisera against mammalian GnRH (Anctil 2000). In *N. vectensis*, GnRH-positive neurons were detected in the endodermal region of various tissues in autozooid polyps, including in mesenterial tissue that encompassed the oocytes (Anctil 2000). Some of the neurites reached to the cortex of oocytes, suggesting that a GnRH-like peptide might have some specific function at that location. Furthermore, in *R. koellikeri*, the rhythmic peristaltic contractions, which are strongly enhanced during spawning, were inhibited by treatment with a GnRH-like peptide extracted from *R. koellikeri* and vertebrate GnRHs, which suggested that GnRHs were not involved or were negatively involved in the spawning of *R. koellikeri*. In the stony coral *E. ancora*, the presence of a GnRH-like peptide was also demonstrated by radioimmunoassay. Notably, the levels of the GnRH-like peptide increased approximately tenfold in the tissues in the month of spawning compared with those of nonspawning periods. This observation suggests that a GnRH-like peptide may play important roles in the regulation of oocyte maturation and/or spawning in the coral (Twan et al. 2003, 2006a, b), although the exact functions of the GnRH-like peptide and the amino acid sequence remain undetermined.

16.4.2 Sex Steroids

Sex steroids were also detected by immunoassay in several anthozoans. Estrogen was first detected in spawned eggs of stony corals (Atkinson and Atkinson 1992). Thereafter, other types of sex steroids such as testosterone, estron, and progesterone were detected in the tissues of several anthozoans, and some correlations between sex steroids levels and gametogenic cycles were observed (i.e., soft coral *Sinularia polydactyla*, Slattery et al. 1999; stony coral *Montipora verrucosa*, Tarrant et al. 1999; sea pansy *R. koellikeri*, Pernet and Anctil 2002; stony coral *E. ancora*, Twan et al. 2003; and sea anemone *Aiptasia diaphana*, Armoza-Zvuloni et al. 2014). Correlations between elevated estrogen levels and the timing of gonad maturation and spawning were also reported in the sea pansy *R. koellikeri* (Pernet and Anctil 2002) and the stony coral *E. ancora* (Twan et al. 2003, 2006a, b). These findings suggest that sex steroids may be involved in the regulation of gametogenesis, oocyte maturation, and/or spawning in anthozoans.

Steroid biosynthesis is catalyzed by the activities of various steroidgenic enzymes, including the members of the cytochrome P450 (CYP), short-chain dehydrogenase (SDR), and aldo-keto reductase (AKR) families (Miller and Auchus 2011). With assessment of the ability to metabolize labeled steroids, which suggested that coral tissues possess steroidgenic enzymes, the metabolic activities of steroids in cnidarians were confirmed in tissue extracts of several anthozoans (Gassman and Kennedy 1992; Slattery et al. 1997; Tarrant et al. 2003; Blomquist et al. 2006; Twan et al. 2006a). Additionally, the sequences of various steroidgenic enzyme genes were identified in the genome of the sea anemone *N. vectensis* (Tarrant et al. 2009). These findings suggest that cnidarians, at least anthozoans, at present, have the ability to metabolize and synthesize sex steroids.

The effects of exogenous steroids on stony coral physiology were investigated in a few species. Colonies of the stony coral *Montipora capitata* treated with estradiol-17β (E_2) for 3 weeks before spawning released fewer egg-sperm bundles (29 % decrease) (Tarrant et al. 2004). Fragments of the stony coral *Porites compressa* that were exposed continuously to E_2 for 2–8 weeks had lower (13–24 %) skeletal growth rates than those of nontreated corals (Tarrant et al. 2004). Although the inhibitory effects on spawning and skeletal growth were observed, nevertheless, these studies demonstrated that estrogen acts on the physiology of stony corals. To date, because no steroid receptors have been identified in cnidarians, the mechanisms of steroid action remain largely unknown.

The reports of sex steroids in cnidarians have so far been restricted to anthozoans. As the research accumulates, the possibility that sex steroids exist in cnidarians increases, and as in advanced animals, the steroids may be involved in the regulation of sexual reproduction. However, the sources, the sites of synthesis, and the true functions of sex steroids in cnidarians are not determined yet. More data are necessary to fully assess the physiological functions (Lafont and Mathieu 2007). In vertebrates, sex steroids also play

fundamental roles in various biological processes such as embryonic development (Hsu et al. 2006), metabolism (Faulds et al. 2012), brain function (McEwen 1992), and immune system function (Garcia-Gomez et al. 2013; Shiau et al. 2014). Thus, sex steroids in cnidarians may also be involved in functions not related to sexual reproduction.

16.4.3 Growth Factors, Other Neuropeptides, and Other Substances

In the sea pansy *R. koellikeri*, serotonin induced the spawning of both egg follicles and sperm (Tremblay et al. 2004). In the hydrozoan jellyfish *Cytaeis uchidae*, the neuropeptides, originally identified in the freshwater hydra *H. magnipapillata*, Hym-53 (NPYPGLW-NH_2) and CGLWamide (CGLW-NH_2) induced oocyte maturation and spawning events (Takeda et al. 2013). In the stony coral *Acropora tenuis*, dopamine had an inhibitory effect on the spawning phenomenon (Isomura et al. 2013). Furthermore, our recent transcriptome analysis of *E. ancora* gonads revealed that the receptors for various growth factors such as insulin-like growth factor, epidermal growth factor, basic fibroblast growth factor, serotonin, melatonin, and activin are expressed in the ovary (Shikina and Chang unpublished data). Some of these factors are involved in sexual reproduction of vertebrates (Pang and Ge 1999; Lonergan et al. 1996; Armstrong et al. 2002; Sheng et al. 2005). These findings favor the view that some of these factors, including other undetermined molecules, participate in the pathways of germ cell development, maturation and spawning in scleractinian oogenesis.

16.5 Conclusions and Challenges

Although oogenesis in cnidarians shares common molecular characteristics with that of oviparous bilaterians, it also possesses characteristics that developed in the unique way during the evolution of the process. The cellular processes, in particular, in cnidarian oogenesis are highly diverse among the classes of cnidarians. The studies on scleractinian oogenesis at molecular and cellular levels have only begun recently, and many of the intrinsic mechanisms of the stem cell system and oocyte development and maturation remain unknown. This review demonstrates that numerous questions remain to be answered.

- What are the basic genetic, cytological, and physiological mechanisms of sex determination of the individual polyps in gonochoric species?
- Do corals have interstitial stem cells (i-cells) or multipotent stem cells?
- Are there other types of major yolk protein in cnidarians?
- How do scleractinian oocytes incorporate yolk proteins produced by somatic cells surrounding the oocytes? Are receptors for these molecules present on the surface of oocytes?
- What is the ancestral prototype of cnidarian oogenesis?
- Are Vg and Ep present in the oogenesis of other cnidarians?
- What are the intrinsic factors that regulate oocyte development, including yolk formation, in scleractinians?
- What are the intrinsic factors that regulate the final maturation of oocytes and spawning? How do scleractinians synchronize the timing of spawning?
- What are the functions of the GnRH-like peptide and steroid hormones found in corals?

To address these questions, the use and integration of several approaches such as genomics, transcriptomics, and proteomics to investigate both embryo and adult germ cell development will be necessary. The development of functional assays, such as foreign gene transfer and gene inactivation (knock down), will also be important to demonstrate the roles of specific gene-encoding proteins and to discover the particular genes responsible for oogenesis. Furthermore, in vitro organ culture systems could be a key technique to identify intrinsic factors that regulate yolk formation in scleractinians. The two yolk proteins, Vg and Ep, could be useful indicators to identify molecules that promote oocyte development in scleractinians.

The hope is that the studies on oogenesis in cnidarians will promote discussion of the evolution of metazoan oogenesis and open new doors for future research. Moreover, the hope is that development of applied biological techniques such as induction of oocyte growth and maturation in aquaculture will be stimulated.

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Sexual Reproduction of Mediterranean Scleractinian Corals

17

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Abstract

The reproduction of scleractinian corals is a fundamental process for maintaining their populations and is essential for understanding the corals' ecology. However, it has been described in less than 30 % of known species. The majority of these studies were carried out in the tropics. In the Mediterranean, data are limited to historical observation by Lacaze-Duthier and more recent in-deep research on reproductive biology of *Balanophyllia europaea*, *Leptopsammia pruvoti*, *Cladocora caespitosa, Astroides calycularis*, *Caryophyllia inornata* and the invasive species *Oculina patagonica*. The Mediterranean Sea is characterized by marked seasonal patterns of seawater temperature that is driven by photoperiod and solar radiation, distinctive of temperate latitudes. Temperature and photoperiod are some of the environmental factors that influence organisms at all structural levels by controlling their physiological and reproductive processes. Climate change is leading to variations of environmental factors such as temperature, which could affect the population biology of corals by reducing their reproductive efficiency, as confirmed by several studies. This chapter focuses on analyzing the studies on sexual reproduction of Mediterranean scleractinians, integrating current understanding of the potential impacts of environmental changes on corals' reproductive output. Investigating how reproductive processes are affected by changing environmental conditions under different future scenarios of stress levels (in mesocosm or in field experiments), could help to identify important processes that may have been overlooked in the past.

Keywords

Temperate corals • Reproductive cycle • Sexuality • Reproductive mode • Environmental parameters

17.1 Introduction

Corals have a range of reproductive traits, such as sexuality (gonochorism *vs* hermaphrodism) and reproductive mode (brooding *vs* spawning), that promote their evolution. This includes the common occurrence of asexual reproduction, e.g. fragmentation and fission process or asexual production of larvae, in addition to sexual reproduction (Harrison 2011), with its high levels of genetic diversity and the interspecific hybridization in some taxa (van Oppen et al. 2011). The simultaneous occurrence of these reproductive traits are believed to be an evolutionary response to their sessile conditions, which require constant adaptation to environment, since the organisms are unable to move in a more suitable environment (van Oppen et al. 2015). This feature provides corals with incredible ability to grow, regress and regenerate their bodies as needed, resulting in a high potential for transgenerational

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acclimatization (Petralia et al. 2014). Such traits provide high environmentally induced epigenetic changes and permit the transmission of somatic mutations from one generation to the next one. Conversely, organisms with strictly sexual reproduction have segregated germ and somatic cell lines during gamete/embryo development (van Oppen et al. 2015). Consequently, transgenerational plasticity may be a powerful mechanism by which populations of some species will be able to adjust to projected environmental changes. Therefore, corals possess a variety of traits that make them suitable candidate organisms for understanding future dynamics and developing targeted management actions.

Improving knowledge on coral reproduction is necessary for the management and preservation of coral reefs. For example, successful reproduction may allow the addition of new individuals to existing populations and increase the dispersal potential (Kinlan et al. 2005), the colonization of new areas (Fiorillo et al. 2013), and the recovery of populations damaged by natural or human disturbances (Kersting et al. 2013).

Despite the great importance of coral reproduction, to date the reproductive biology has been described for only five Mediterranean scleractinian species (Table 17.1 and Fig. 17.1): *Balanophyllia europaea* (Family: Dendrophylliidae), *Leptopsammia pruvoti* (Family: Dendrophylliidae), *Cladocora caespitosa* (Family: Oculinidae), *Astroides calycularis* (Family: Dendrophylliidae) and *Caryophyllia inornata* (Family: Caryophylliidae). Sexual reproduction is also described for *Oculina patagonica* (Family: Oculinidae), which is an invasive species in the Mediterranean Sea (Table 17.1). This review aims to summarize the reproductive traits described for the above mentioned Mediterranean species, highlighting possible future perspectives in coral reproduction research.

17.2 Sexual Reproduction

17.2.1 Sexuality

Scleractinians can display two different sexual patterns: hermaphroditism and gonochorism. Hermaphroditic corals develop both male and female gametes within polyps or colonies during their lifetime, whereas gonochoric corals have separate sexes. Hermaphroditism is usually simultaneous, but there are some forms of hermaphroditism which are more complex to detect, such as the cyclic sequential, characterized by oocytes and spermaries that mature at different times in the same breeding season (Waller et al. 2005), and the protandrous or protogynous sequential during the lifetime (Loya and Sakai 2008).

The zooxanthellate solitary coral *B. europaea* (Fig. 17.1A) was observed to be simultaneous hermaphrodite at the Calafuria study site (Fig. 17.2; Goffredo and Telò 1998; Goffredo et al. 2000, 2002; Goffredo and Zaccanti 2004) and reaches sexual maturity at 6–10 mm in length, which corre-

Table 17.1 Reproductive traits in Mediterranean corals

Species	Sexual pattern	Mode of development	Gametes maturation period (month)		Fertilization period	Sites	References
			Oocytes	Spermaries			
Balanophyllia europaea (Risso, 1826)	H	B	24	12	March–June	1	Goffredo and Telò (1998) and Goffredo et al. (2000, 2002)
	H	B	24	12	March–June	1, 2, 3, 4, 5, 6	Airi et al. (2014)
Leptopsammia pruvoti Lacaze-Duthiers, 1897	G	B	24	12	January–April	1	Goffredo et al. (2005, 2006)
	G	B	24	12	January–April	1, 2, 3, 4, 5, 6	Airi et al. *personal observation*
	G	B	–	–	–	7	Lacaze-Duthiers (1897)
Astroides calycularis (Pallas, 1766)	G	B	24	12	February–May	4	Goffredo et al. (2010b, 2011)
	G	B	–	–	April–June	16	Casado-Amezúa et al. (2013)
	H	B	–	–	–	14	Lacaze-Duthiers (1897)
	H	–	–	–	–	15	Cirino et al. (1993)
Cladocora caespitosa (Linnaeus, 1767)	–	BS	–	–	June	23	Schiller (1993)
	H	BS	–	–	June	8	Kružić (2005) and Kružić et al. (2008)
	G	BS	7–8	7–8	August	8, 9, 10, 11, 12, 13	Kersting et al. (2013)
Caryophyllia inornata (Duncan, 1878)	G	B	5–6	12	April–July	3	Goffredo et al. (2012) and Marchini et al. (2015)
Oculina patagonica De Angelis D'Ossat, 1908	G	BS	5	3	September	17, 18, 19, 20, 21, 22	Fine et al. (2001)

H hermaphroditic, *G* gonochoric, – unknown, *B* brooder, *BS* broadcast spawner

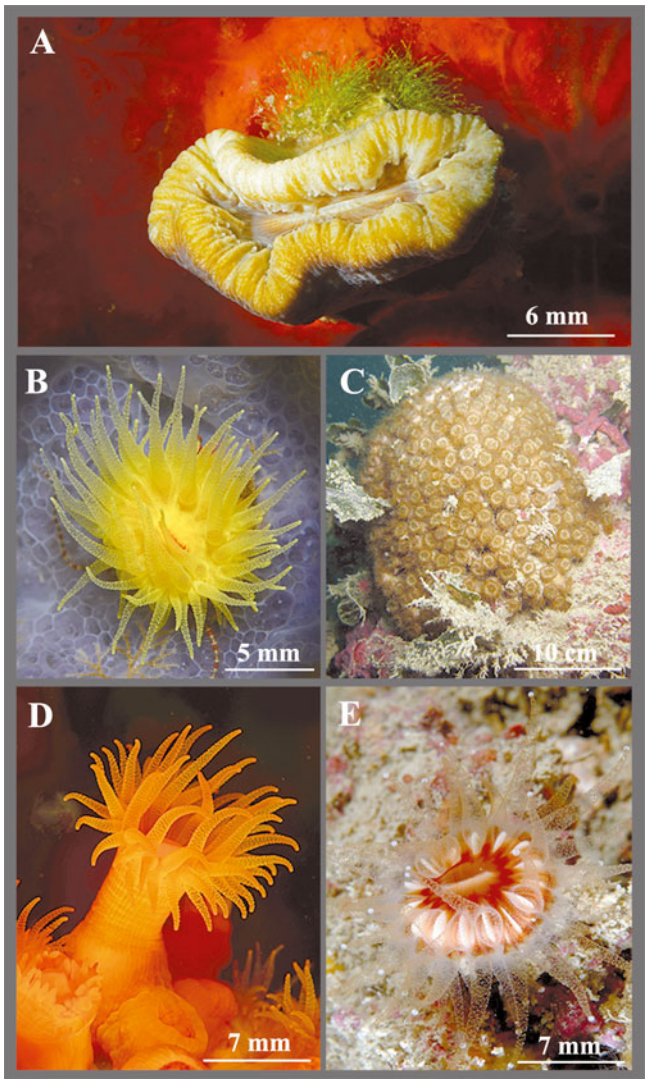

Fig. 17.1 Living specimens of the five Mediterranean scleractinians whose reproduction is known (**A**) *Balanophyllia europaea*; (**B**) *Leptopsammia pruvoti*; (**C**) *Cladocora caespitosa*; (**D**) *Astroides calycularis*; (**E**) *Caryophyllia inornata* (Photos **A**, **B**, **D**: Francesco Sesso; Photo **C**: Erik Caroselli; Photo **E**: Gianni Neto)

sponds to 3–4 years of age (Goffredo et al. 2004). The same sexual pattern was found in several populations of this species along the Western Italian coast (Genova, Elba Island, Palinuro, Scilla and Pantelleria; Fig. 17.2; Airi et al. 2014). Despite sexuality (gonochorism *vs* hermaphroditism) of scleractinians tends to be phylogenetically correlated and therefore constant within genera and families (Harrison 1985; Harrison and Wallace 1990; Richmond and Hunter 1990; Ward 1992; Shlesinger et al. 1998; Fautin 2002), *B. europaea* is an exception, since in dendrophylliids the prevalent sexuality is gonochorism (Fadlallah 1983; Baird et al. 2009).

The non-zooxanthellate solitary coral *L. pruvoti* (Fig. 17.1B) is gonochoric at the Calafuria study site (Fig. 17.2; Goffredo et al. 2005) and along the Western Italian coast (Fig. 17.2; Airi et al. *personal observation*). The same sexuality was observed by Lacaze-Duthiers (1897) at the Gulf of Lion, near Marseille (Fig. 17.2). In *L. pruvoti* sexual maturity occurs at 3 mm length, which corresponds to 2 years of age (Goffredo et al. 2010a), suggesting that reproductive activity begins early in this species.

Sexuality can vary not only at a genus and family level but also within species. Indeed, the zooxanthellate colonial *C. caespitosa* (Fig. 17.1C) was described as hermaphroditic in the Adriatic Sea (Fig. 17.2), since polyps within one colony develop both oocytes and spermaries on separate mesenteries (digonic; Kružić 2005). Conversely, this species is gonochoric in the Western Mediterranean (Fig. 17.2), since all polyps examined within the same colony are of the same sex (Kersting et al. 2013).

The non-zooxanthellate colonial *A. calycularis* (Fig. 17.1D) has the same mixed sexuality. This species is described as hermaphroditic in Algeria by Lacaze-Duthiers (1873; Fig. 17.2), with colonies mainly formed by separate sex polyps (monoic hermaphroditism) and rarely by simultaneous hermaphroditic polyps. The same condition was observed in aquarium by Cirino et al. (1993) in Naples (Fig. 17.2). Conversely, *A. calycularis* has been found to be gonochoric, both at the polyp and the colony level, in the Southern Tyrrhenian Sea (Palinuro; Fig. 17.2; Goffredo et al. 2010b) and in the Northern Alboran Sea (Punta de la Mona; Fig. 17.2; Casado-Amezua et al. 2013). The different sexuality expressed in these populations of *A. calycularis* might be an adaptation to different environments. For example, the high population density of *A. calycularis* in Palinuro (up to 90 % coverage of the rocky substrate) increases the probability of fertile encounters and gonochorism would be advantageous since it would ensure cross-fertilization and maintain the genetic variability of the population (Goffredo et al. 2010b). Colonies of this species are sexually mature at 3–4 cm^2 in area, with mature polyps at 3–4 mm in length, indicating that reproductive activity begins at an intermediate polyp size with reference to the range for this family (Goffredo et al. 2011).

A recent study of the non-zooxathellate solitary coral *C. inornata* (Fig. 17.1E) performed at Elba Island (Fig. 17.2) reveals that this species is gonochoric and reaches sexual maturity from 6 to 8 mm in length (Marchini et al. 2015), corresponding to ~ 7–10 years of age (Caroselli et al. 2016), which is later than the other Mediterranean species studied. This delay may be due to the peculiar reproductive strategy adopted by *C. inornata*, probably producing both sexual and asexual embryos during its lifetime. In fact, it seems that up to a certain size (between 4 and 6 mm) almost 50 % of fertile polyps brood embryos, thus, they could start to produce embryos before sexual reproduction occurs. Moreover, the population is characterized by females, males and sexually inactive polyps with three times more male polyps than female (Marchini

Fig. 17.2 General map of study sites on reproduction in Mediterranean scleractinians. *1* Calafuria, Ligurian Sea, Italy, *2* Genova, Ligurian Sea, Italy, *3* Elba Island, Northern Tyrrhenian Sea, Italy, *4* Palinuro, Central Tyrrhenian Sea, Italy, *5* Scilla, Southern Tyrrhenian Sea, Italy, *6* Pantelleria Island, Strait of Sicily, Italy, *7* Gulf of Lion, France, *8* Mljet Island, Adriatic Sea, Croatia, *9* Columbretes Islands Marine Reserve, Balearic Sea, Spain, *10* Eivissa Island, Western Mediterranean Sea, Spain, *11* Medas Island Marine Reserve, Northwestern Mediterranean Sea, Spain, *12* Natural Reserve of Scandola, Northwestern Mediterranean Sea, France, *13* Cap de Creus, Northwestern Mediterranean Sea, Spain, *14* Algeria, Southwestern Mediterranean Sea, *15* Naples, Southern Tyrrhenian Sea, Italy, *16* Punta de la Mona, Northern Alboran Sea, Spain, ***17*** Tel Aviv, Southeastern Mediterranean Sea, Israel, *18* Beirut, Southeastern Mediterranean Sea, Lebanon, *19* Alexandria, Southeastern Mediterranean Sea, Egypt, *20* Marseille, Gulf of Lion, France, *21* Savona, Ligurian Sea, Italy, *22* Alicante, Western Mediterranean Sea, Spain, *23* Bay of Piran, Adriatic Sea, Croatia

et al. 2015). A male biased sex ratio could be due to a clonal propagation where male clones are more likely to reproduce asexually than females, as has been reported in some solitary scleractinians of the Fungidae family (Kramarsky-Winter and Loya 1998; Colley et al. 2000; Gilmour 2002).

The zooxanthellate colonies of the invasive *O. patagonica* could have been accidentally transported by a transoceanic ship to the Mediterranean Sea from the temperate Southwestern Atlantic (Zibrowius 1974) and have been described using Pleistocene fossil samples collected in northern Argentina. This species is gonochoric in some localities of the Mediterranean Sea (Israel, Lebanon, Egypt, France, Italy and Spain; Fig. 17.2) and is sexually mature above a diameter of 2 cm, corresponding to 1–2 years of age (Fine et al. 2001). It is believed that this early reproductive age is distinctive of an opportunistic, short-lived organism colonizing new habitats (Horn and Mac Arthur 1972; Parsons 1982).

17.2.2 Reproductive Mode

Scleractinians with the above-mentioned sexual patterns can be either broadcast spawners, with external fertilization of their gametes and successive embryo and larval development, or have internal fertilization, brooding embryos within the coelenteron of the polyp (Harrison 2011).

The last reproductive mode is the most frequent among the studied Mediterranean corals. Indeed, the three dendrophylliids (*B. europaea, L. pruvoti* and *A. calycularis*) are brooders, as the majority of the species in this family (Fadlallah 1983; Harrison 1985; Goffredo and Telò 1998; Goffredo et al. 2005, 2010b; Casado-Amezúa et al. 2013). *C. inornata* not only develops embryos in the gastrovascular cavity, but also in the mesenterial septa of females, males and sexually inactive individuals. Early embryos have been observed in close morphological continuity with mesenterial septa or detached from it and sometimes are located inside the body of embryos in a more advanced stage of development (Fig. 17.3; Goffredo et al. 2012).

Colonies of *C. caespitosa* spawn their gametes in the water column. Spawning was observed in the Northern Adriatic Sea (Bay of Piran; Fig. 17.2; Schiller 1993), in the Southern Adriatic Sea (Mljet Island; Fig. 17.2; Kružić et al. 2008) and in the Western Mediterranean Sea (Columbretes Islands Marine Reserve, Eivissa, Medas Island Marine Reserve, Natural Reserve of Scandola, Cap de Creus; Fig. 17.2). The same reproductive mode was observed in the invasive species *O. patagonica* (Fine et al. 2001).

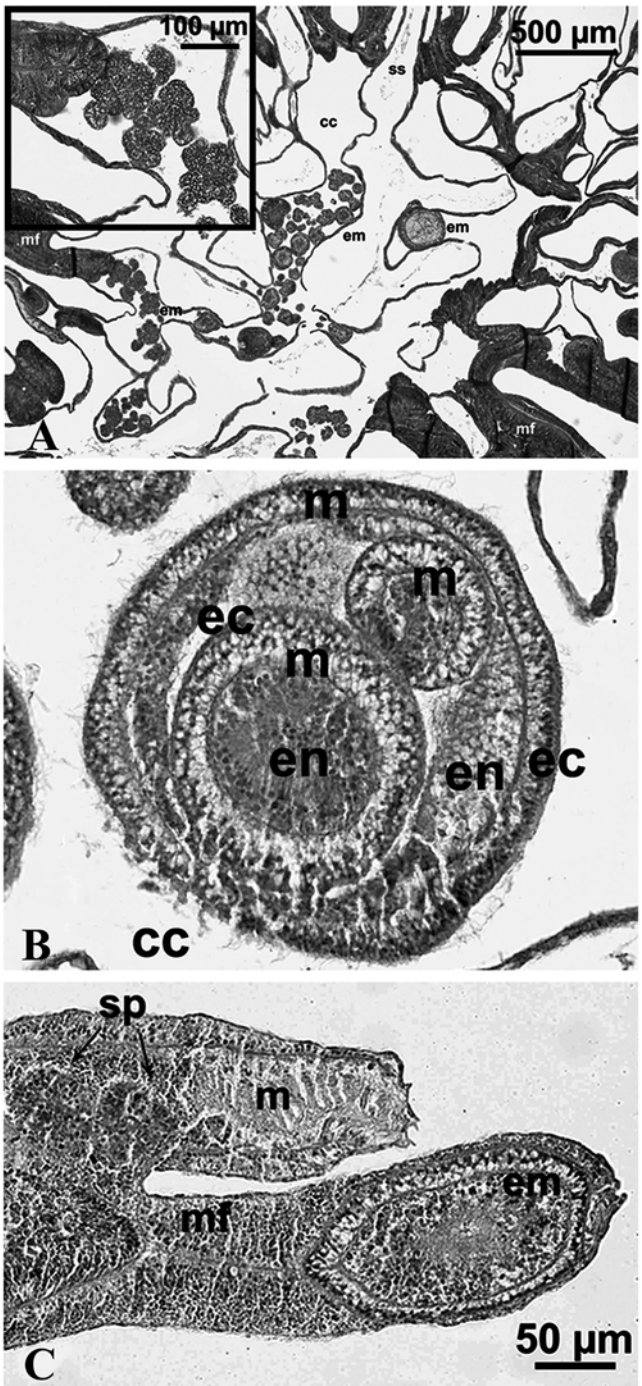

Fig. 17.3 *Caryophyllia inornata*. (**A**) Embryos within the gastrovascular cavity and in morphological continuity with mesenterial septa (detail in the *square*). (**B**) Early embryos located inside an embryo in a more advanced stage of development. (**C**) Embryo (late stage) placed in the coelentric cavity of a male polyp. Note the presence of spermaries in the same section. *cc* coelentric cavity, *ss* skeletal septum, *mf* mesenterial filament, *em* embryo, *ec* ectoderm, *en* endoderm, *m* mesoglea, *sp* spermary

17.3 A Few Insights on Asexual Reproduction

Colonial corals such as *A. calycularis* and *C. caespitosa* are modular organisms that form their colonies through asexual budding, producing genetically identical polyps within each colony. In particular, asexual reproduction in *C. caespitosa* can occur by fragmentation with small fragments that grow to form a new colony (Schiller 1993; Kružić 2005) and by budding that is both extratentacular (on the lateral side of the old polyp) and intratentacular (longitudinal fission of the disc of the original polyp; Zibrowius 1980; Kružić 2005). A new mode of asexual reproduction was observed in *O. patagonica*, which produces the new polyp by expulsion. At first, this polyp grows on a lengthened calcareous stalk and then it settles, spreading its tissues on the substrate and beginning to form the colony by budding (Fine et al. 2001).

Concerning solitary corals, asexual reproduction was suggested for *C. inornata*. Embryos are found not only in females, as expected, but also in males (Fig. 17.3C) and in sexually inactive individuals throughout the entire year without a clear seasonal trend. Therefore, it is possible that this species produces agamic embryos by some form of internal budding (Marchini et al. 2015). However, further studies are necessary to determine the origin of these embryos.

17.4 Coral Life Cycle

Understanding environmental and biological cycles is fundamental in defining which factors affect the reproductive output and timing and to comprehend the factors that regulate population dynamics, such as rates of recruitment, patterns of connectivity (Bouwmeester et al. 2014) and population recovery (Kinlan et al. 2005).

In *B. europaea* spermatogenesis follows an annual cycle with male germ cells taking 12 months to mature. In contrast, the oogenesis is characterized by a 2-year cycle, defined by the presence of two stocks of oocytes. The smaller stock consists of immature oocytes and the second stock of larger germ cells, ready to be fertilized (Goffredo et al. 2002). The same timing for gamete development was assessed in another congeneric species, *B. elegans*, in the temperate waters off the coast of California (Fadlallah and Pearse 1982; Beauchamp 1993) and in the other two Mediterranean species belonging to the same family: *L. pruvoti* (Goffredo et al. 2006) and *A. calycularis* (Goffredo et al. 2011).

The alien species *O. patagonica* is characterized by an annual reproductive cycle, showing a faster oogenesis and spermatogenesis (5 and 3 months respectively; Fine et al. 2001). A longer maturation period for oocyte development compared to the spermaries is typical in anthozoans gametogenesis (Schleyer et al. 2004; Guest et al. 2005, 2012; van Woesik et al. 2006; Ribes and Atkinson 2007; Hellstrom et al. 2010; van Woesik 2010; Madsen et al. 2014), since energetic investment in sperm development is considerably less than for oocytes (Goffredo et al. 2002; Baillon et al. 2011). Nevertheless, there are some exceptions, such as the Mediterranean colonial coral *C. caespitosa*, characterized by both reproductive elements taking 7–8 months to reach maturity (Kersting et al. 2013). In the solitary coral *C. inornata* male germ cells require about 12 months to mature (Marchini et al. 2015), as shown for the Mediterranean dendrophyllids. A similar spermatogenesis has been documented, within the Caryophylliidae family, for the deep coral *L. pertusa* in Norway (Brooke and Järnegren 2013). On the other hand, the oocytes of *C. inornata* take only 5–6 months to mature, showing a shorter oogenesis than spermatogenesis and differing from *L. pertusa* oogenesis (13–14 months in duration, with 1 or 2 months overlapping between cycles) and the other Mediterranean scleractinians.

17.5 Environmental Patterns of Coral Reproduction

17.5.1 Past Perspectives on Coral Reproduction

Reproductive cycles in these species are related to seasonal variations in water temperature and photoperiod, which could be major factors controlling the reproductive activities of temperate corals (Glynn et al. 2000; Penland et al. 2004). In Mediterranean corals, the decrease in photoperiod and water temperature in autumn and winter could be a cue for gamete development. The increase in photoperiod and water temperature, during winter and spring, coincides with sperm release and oocyte fertilization. Planulation takes place during spring and summer, when water temperature and photoperiod start to increase, reaching the annual maximum.

B. europaea showed a significant increase in "gonadal" development toward January-February, fertilization takes place from March to June and planulae are released between August and September (Goffredo et al. 2002). "Gonadal" size of *L. pruvoti* increases sixfold in males and threefold in females from November to January, fertilization takes place from January to April, and planulation during May and June (Goffredo et al. 2006). *A. calycularis* gamete size increases from November to March, more rapidly in males than in females. Subsequent fertilization occurs from February to May and planulae are released between June and July (Goffredo et al. 2011). The germ cell size of *C. inornata* increases significantly from March to May, when both photoperiod and water temperature rise after the minimum of the year. Fertilization takes place from April to July, when photoperiod is the longest of the year (Marchini et al. 2015). The alien broadcast spawner *O. patagonica* was studied in different localities spread over several countries (Fig. 17.2) and with two different trophic strategies: zooxanthellate and non-zooxanthellate. In all localities (Western and Eastern Mediterranean), the maximum "gonadal" development corresponds to the highest water temperature in August. Spawning occurs in September with the beginning of temperature decrease (Fine et al. 2001). Non-zooxanthellate colonies inhabiting small dark caves spawn simultaneously to the zooxanthellate colonies exposed to light (Fine et al. 2001). The broadcast spawner *C. caespicosa*, was studied in two localities of the Mediterranean Sea (Adriatic Sea; Fig. 17.2; Kružić et al. 2008) and Columbretes Island (Western Mediterranean; Fig. 17.2; Kersting et al. 2013). Reproductive cycles have been showing a strong correlation with seawater temperature in both of the populations studied, but differences in seawater temperature between the two sites result in a shifted spawning period (Kersting et al. 2013). While in the Columbretes Islands the spawning process occurs at the end of the summer (Kersting et al. 2013), in the Adriatic Sea it happens at the beginning (Schiller 1993; Kružić et al. 2008).

17.5.2 Recent Advances in Coral Reproduction Research

Sea water temperature is an important environmental factor, controlling gametogenetic cycles and planulae release in scleractinian corals (Richmond and Hunter 1990; Soong 1991; Clayton and Collins 1992; Beauchamp 1993; Babcock et al. 1994; Fan and Dai 1995; Steiner 1995; Fadlallah 1996; Tanner 1996; Gilmour 2002; Penland et al. 2004; Baird et al. 2009; Harrison 2011). This correlation could demonstrate that some corals display a reproductive plasticity or alterations in the timing of gamete release to adapt to environmental changes, representing an important mechanism for larval survival and fitness with rising ocean temperatures associated with climate change (Crowder et al. 2014).

Climatic models predict that the Mediterranean Sea will be one of the most impacted regions by the ongoing warming trend (Field 2012). Several studies have assessed the negative impacts of environmental changes on reproductive output of many tropical corals (Michalek-Wagner and Willis 2001; Baird and Marshall 2002; Mendes and Woodley 2002; Coles and Brown 2003; Cox 2007; Hoegh-Guldberg et al. 2007; Donner 2009; Randall and Szmant 2009; Albright et al. 2010; Armoza-Zvuloni et al. 2011; Albright and Mason

2013; Baums et al 2013; Crowder et al. 2014; Levitan et al. 2014) and Mediterranean gorgonian colonies (Gori et al. 2007; Linares et al. 2008; de Putron and Ryland 2009; Bauman et al. 2011; Torrents and Garrabou 2011; Kipson et al. 2012), but little information about temperate scleractinians is available.

The effects of temperature on Mediterranean corals were tested on *B. europaea* and *L. pruvoti* from six sites along a wide latitudinal gradient (Genova, Calafuria, Elba Island, Palinuro, Scilla and Pantelleria; Fig. 17.2). Oocyte abundance in the zooxanthellate *B. europaea* increases with increasing temperature during the earliest stages of gamete development, but was constant among sites just before the fertilization process. The spermaries size decreased with increasing temperature, while their abundance was not different among sites (Airi et al. 2014). On the contrary, polyps of *L. pruvoti* showed a reproductive output not influenced by increasing temperatures (Airi et al. *personal observation*). Oocyte abundance was constant along the gradient in *L. pruvoti*, whereas warm populations of *B. europaea* lost a great amount of earlier oocytes (Airi et al. 2014). *B. europaea* in warmer sites could resorb smaller oocytes, adjusting its energetic budget by reallocating the resources destined for oocyte maturity into other vital functions depleted by the negative effect of temperature (Airi et al. 2014). Resorption of oocytes after stress was detected in many corals, balancing the energy between reproduction and survival processes (Michalek-Wagner and Willis 2001; Okubo et al. 2007; Lueg et al. 2012). The initial effort of *B. europaea* in warmer populations was not enough to guarantee the same performance of colder populations (Airi et al. 2014). In fact, warmer populations are less abundant, less stable, and contain fewer young individuals (Goffredo et al. 2007, 2008), probably due to the negative effect of increasing temperature on post-fertilization life stages, such as larval survival and settlement (Airi et al. 2014). In *L. pruvoti*, the absence of an evident correlation with environmental parameters confirmed the previous findings on population density, growth and structure stability of this species along the same gradient (Goffredo et al. 2007; Caroselli et al. 2012a, b). The different response to environmental parameters of the two species could be due to their different trophic strategies. When temperature are above the optimal, several zooxanthellate species show a reduction in photosynthetic efficiency (Nakamura et al. 2004; Al-Horani 2005), followed by a decline in energy available for biological processes, such as gametogenesis (Rinkevich 1989; Baird and Marshall 2002), growth (Goffredo et al. 2008), skeletal density (Goffredo et al. 2007) and calcification rate (Goffredo et al. 2009). This could lead non-zooxanthellate and zooxanthellate species to a different performance and adaptability to environmental change, suggesting that heterotrophic species seem to be less sensitive to temperature change than zooxanthellate ones.

17.5.3 Future Perspectives in Coral Reproduction Research

Key findings on biological processes highlighted the effect of ocean warming and acidification on the geographical distribution of organisms, suggesting that they affect the timing of life history events such as reproduction and migration (Pörtner et al. 2014; Poloczanska et al. 2014). The composition and functions of many ecosystems are changing, giving rise to the concept of "novel ecosystems" (Doney et al. 2012). These impacts on coral ecosystems may act as a driving force to improve our understanding on coral species vulnerability, leading to new targeted management actions and assessments of ecosystem changes (Graham et al. 2014). Further researches are necessary to investigate coral ecosystems under different stress level scenarios and in different biophysical and geographic locations. Therefore, to understand how ecosystem processes will be influenced by changing environmental conditions in the near future, it will be useful to focus on different processes that may have been largely overlooked in the past, performing new mesocosm experiments, manipulative field experiments and simulation modeling.

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Brooding Corals: Planulation Patterns, Larval Behavior, and Recruitment Dynamics in the Face of Environmental Change

18

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Abstract

The brooding reproductive mode in scleractinian corals is often associated with high recruitment success facilitating replenishment of populations following disturbance events. Thus, if conditions continue to deteriorate on coral reefs from anthropogenic impacts and global climate change, a clear understanding of patterns of planulation, larval behavior and recruitment by brooding species is needed to accurately predict future population dynamics and the overall resilience of coral reefs. Here, we review the current knowledge of these topics with specific emphasis on the effects of environmental factors and discuss implications for reproductive success and population stability. Brooding corals typically release mature larvae during planulation events that vary in synchrony on a seasonal, monthly and daily basis linked to various environmental conditions, such as seasonal sea surface temperature, the lunar and diel cycles. Release time dictates the environmental conditions that larvae will experience, such as light availability and wave action, and thus affects dispersal potential and recruitment success. Differences in larval size exist among species, as well as within broods released during the planulation events of a single species; a possible strategy to maximize the chance of fitness in unpredictable habitats. Upon release, brooded larvae are typically competent to settle within hours to days and respond to a variety of environmental cues, such as type of benthic cover and irradiance, to facilitate settlement choice. Species-specific larval photosensitivity aids in depth and substrate selection promoting survival and can ultimately influence adult distribution patterns. Studies indicate that shifts in benthic cover from environmental changes, such as algal abundance and sedimentation, may inhibit or change patterns of larval settlement, which will affect species composition and reef resilience. Research on the effects of ocean acidification and rising sea surface temperatures on the physiology, settlement and early calcification of larvae of brooding corals show mixed results, but patterns suggest that if global climate change continues at or beyond projected scenarios over the next century, recruitment may be compromised. Thus, an increased understanding of planulation patterns, larval behavior, and recruitment dynamics of brooding species will be essential for conservation and management in the face of environmental change.

Keywords

Coral • Reproduction • Larvae • Settlement • Recruitment • Climate change • Ocean acidification

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18.1 Introduction

The process of sexual reproduction by corals is fundamental to population maintenance, resilience and adaptive evolution, as recruitment of new sexually produced individuals to a population will ultimately shape community dynamics. Shallow water scleractinian corals have two general strategies of sexual reproduction, broadcast spawning and brooding. Among coral species worldwide, broadcasting is the dominant reproductive mode, exhibited by roughly 83 % of species (reviewed by Harrison 2011). In broadcasting corals, individual polyps release gametes into the water column and thus fertilization and development occur externally. Subsequent development of zygotes into planula larvae takes place over the course of several days to weeks and once competent the larvae seek a suitable substrate for settlement and metamorphosis (Fadlallah 1983; Harrison and Wallace 1990; Gleason and Hofmann 2011; Harrison 2011).

In brooding corals, fertilization is internal with spermatozoa released through the mouth of one polyp and drawn into the gastrovascular cavity of another where eggs are fertilized and mature planula larvae are released several days to weeks later (Fig. 18.1; Smith 1971; Fadlallah 1983; Harrison and Wallace 1990; McGuire 1998; Harrison 2011). Variations are the "quick-releasing" strategy of internal fertilisation followed by a short or no brooding period seen in some scleractinian corals (Hagman et al. 1998; de Graaf et al. 1999; de Putron 2003; Vermeij et al. 2004) and surface brooding of embryos on the colony surface after fertilisation either internally or at the colony surface, so far only reported in the gorgonian corals *Paramuricea clavata* (Coma et al. 1995) and *Briareum asbestinum* (Brazeau and Lasker 1990), two alcyonacean and two stoloniferan species (see review in Benayahu et al. 1990). A possible selective advantage to these variations of the 'typical' brooding pattern could be to increase brood size, which is space restrictive when brooding to late stage larvae, often resulting in a trade-off between size and the number of larvae produced (Harrison and Wallace 1990). The release of premature larvae has also been reported as a stress response in *Agraicia humilis* (Peterson and Van Moorsel 2005). For the majority of corals with the more 'typical' brooding reproductive mode, mature larvae are released that commonly are competent to metamorphose into primary polyps within minutes to hours of planulation (Carlon and Olson 1993; Ayre and Hughes 2000; Goodbody-Gringley 2010). However, some larvae retain their competency for extended periods, such as larvae of *Pocillopora damicornis* that are able to spend up to 100 days in the plankton, likely accounting for the wide distribution of this species across the entire Pacific, Red Sea and Indian Ocean (Richmond 1988). The more usual short dispersal time associated with brooding has been suggested as a mechanism for retaining propagules (Szmant 1986) and promoting recruitment to replenish the population (Richmond 1987) if the parent habitat is unfavorable or unstable.

Fig. 18.1 *Porites astreoides* larvae, 24 h post release. Scale bar = 0.5 mm (Photo by Dr. Samantha de Putron)

Brooding corals display hermaphroditic, gonochoristic, and mixed sexual states, with hermaphroditism being slightly more common than gonochorism (Harrison 2011). In the former state, male and female gametes are often produced within the same polyp. While simultaneous hermaphroditism ensures that gametes ripen concurrently increasing the rate of successful fertilization (Policansky 1982), it also allows for the possibility of self-fertilization (Carlon 1999). In fact, an increasing number of studies have found evidence of inbreeding through self-fertilization in a variety of brooding coral species, reaching rates up to 50 % for *Favia fragum*, *Agaricia agaricites*, and *Porites astreoides* on some reefs of the Florida Keys (Brazeau et al. 1998; Bassim et al. 2002; Sherman 2008). Likewise, evidence of mixed modes of reproduction by *P. damicornis*, including sexually and asexually produced larvae, indicates that clonal reproduction might also significantly contribute to coral recruitment (Yeoh and Dai 2010). Despite potential decreased fitness due to inbreeding depression, self-fertilization and clonal reproduction may be beneficial by purging deleterious recessive alleles and enhancing the abundance of locally adapted genotypes, which may be selectively advantageous in populations with low adult densities and in isolated populations. Gonochorism, on the other hand, minimizes the chance of self-fertilization but imposes increased risks to successful gamete encounter (Carlon 1999). A variation of sexual

pattern is the occurrence of gynodioecy in a population, which is the absence of male colonies and the presence of hermaphroditic and female colonies, as has been recorded for *P. astreoides* in Jamaica and may allow for both cross and self-fertilization (Chornesky and Peters 1987). The variety of sexual states used by brooding corals and production of propogules both sexually and asexually may further facilitate increased recruitment following disturbance events and in unstable conditions (Knowlton and Jackson 1993; Sherman 2008; Yeoh and Dai 2010). In fact, brooding species are documented to experience higher recruitment efficiency to space created by disturbances compared to species that broadcast gametes (Rylaarsdam 1983; Smith 1992). Thus areas with high disturbance histories, such as those affected by thermally induced bleaching events, may experience a shift in community composition towards dominance by brooding species.

18.2 Reproductive Synchronization

Sessile or sedentary marine species such as corals rely on movement of sperm between individuals, and rapid gamete dilution coupled with a relatively short gametic lifespan means that fertilization is proximity dependent (Carlon 1999; Sherman 2008). Likewise, successful fertilization depends on reproductive synchronization to ensure gamete encounter (Harrison 2011). Reproductive timing varies widely among species; however, within a given species reproductive events are often highly synchronized, cued to a wide range of environmental factors (Yonge 1931; Abe 1937; Richmond and Jokiel 1984; Szmant-Froelich et al. 1985; Tanner 1996; Mendes and Woodley 2002; Neves and da Silveira 2003; Guest et al. 2005; Fan et al. 2006; Zakai et al. 2006). This is particularly evident in broadcasting species, which must synchronize gamete release among conspecifics to ensure gamete encounter and successful fertilization (Harrison et al. 1984). The most elaborate case occurs on the Great Barrier Reef, where more than 20–30 coral species from a range of scleractinian families are recorded to synchronize nocturnal release of gametes after the full moon periods of the austral late spring and early summer (Willis et al. 1985; Babcock and Heyward 1986).

In contrast to broadcasting coral species that typically have one or two gametogenic cycles a year producing many gametes, brood size after internal fertilization is space constrained and brooding coral species that allocate energy to a small number of offspring typically increase overall fecundity by having many reproductive cycles per year. Although reproductive patterns of brooding species do not usually display the same degree of tight synchronization as broadcasting species, planulation events are often documented to occur in accordance with the same proximate controls leading to varying degrees of synchrony on a seasonal, monthly, and daily basis (Wilson 1888; Duerden 1902; Vaughan 1919; Lewis 1974; Van Moorsel 1983; Szmant-Froelich et al. 1985; Soong 1991; Johnson 1992; McGuire 1998; Fan et al. 2002; Vermeij et al. 2004; Goodbody-Gringley and de Putron 2009).

In low latitude regions, many brooding species are documented to reproduce year round with monthly cycles of planulation; however, several studies document seasonally restricted reproduction in brooding coral species at high latitudes (Tanner 1996; McGuire 1998; Ben-David-Zaslow et al. 1999; Villanueva et al. 2008; Goodbody-Gringley and de Putron 2009). For example, reproduction by *P. astreoides* is restricted to 2–3 months in Bermuda (de Putron and Smith 2011), extends 5–6 months in the northern Florida Keys, and is year round in the lower Caribbean (McGuire 1998). Seawater temperature is often suggested as a controlling factor in seasonal reproductive patterns for brooding corals, where wider ranges in seawater temperature at high latitudes are thought to limit the reproductive season to the shorter period of warmer seawater temperatures (Tanner 1996; McGuire 1998; Villanueva et al. 2008; Goodbody-Gringley and de Putron 2009; de Putron and Smith 2011). Other environmental factors that correlate with reproductive patterns and vary with latitude such as solar insolation (Penland et al. 2004; van Woesik et al. 2006), absence of heavy rainfall (Mendes and Woodley 2002), photoperiod (Kruger and Schleyer 1998), and the duration of regional calm periods that may enhance fertilization and larval retention (van Woesik et al. 2006) are also proposed to serve as alternate proximate or evolutionary controls of reproductive seasonality (Harrison 2011).

Within the reproductive season, larval release often occurs in accordance with monthly lunar cycles for many brooding species (Szmant-Froelich et al. 1985; Fan et al. 2006; Goodbody-Gringley and de Putron 2009). Brooding corals show varying degrees of linkage between lunar periodicity and planular release, however, from no observed pattern (Harriott 1983), to a weak periodicity of a monthly peak among continuous low levels of planular release each month (Fan et al. 2002), to a strict synchrony over just a few days each month (Johnson 1992). One possible benefit of synchronizing release with the lunar cycle is predator avoidance, such as by satiation and/or by release near the new moon reducing light availability for visual predators (Morgan 1995). Oceanic tides are also correlated with lunar cycles, and planulation at various tidal stages (spring vs. neap) could facilitate either long-range dispersal or local retention (Morgan 1995).

Synchronization can also occur on an hourly basis within a given night in which planulation occurs. For example, Fan et al. (2002) found varying degrees of diel periodicity in planulation, ranging from highly coordinated with one daily

peak by *Seriatopora hystrix* and *Stylophora pistillata*, to two daily peaks by *P. damicornis* and *Euphyllia glabrescens*, to continuous release with no peak by *Tubastraea aurea*. Likewise, Goodbody-Gringley (2010) found planulation by *F. fragum* occurred in a single peak near dawn. This diel synchronization likely occurs in response to the light/dark cycle, as many corals display photosensitive behavior (Harrison and Wallace 1990). In particular, sensitivity to blue light is documented in corals (Gorbunov and Falkowski 2002), which is known to affect the circadian clocks of other animals. Lin et al. (2013) found the pattern of diel planulation by *S. hystrix* follows the Hourglass model, where the onset of the light phase is the trigger that initiates the planulation process, thus cyclical sunrise results in cyclical planulation. Moreover, Levy et al. (2007) found cryptochromes (CRYs), which are DNA photolyase-like photoreceptor proteins in corals, were expressed in response to daylight and moonlight, further indicating that light may be mediating diel reproductive synchronization in corals (Levy et al. 2007). The evolutionary reasons for periodicity of diel release differ from those of broadcasting corals, however, as fertilization has already occurred (Szmant-Froelich et al. 1985; Fan et al. 2006). It is suggested that synchronized diel release occurs in response to environmental conditions at the time of release, such as light availability, tidal cycles, and predator abundance, promoting larval fitness, dispersal, and successful recruitment (Goodbody-Gringley 2010), while reducing predation losses (Harrison and Wallace 1990). The effects of any future environmental change, such as those associated with global climate change scenarios, on the synchronization of larval release by brooding species are currently not well understood. However, any alteration to reproductive synchronization will likely affect larval fitness and recruitment success and therefore warrants further examination.

18.3 Inter- and Intra-species Variation in Larval Characteristics

Recent research has highlighted that larvae produced by many brooding corals are not functionally equivalent, as is also the case for many other marine invertebrates (Strathmann 1986; Marshall and Keough 2008). Differences in larval size have been shown to exist among species (Isomura and Nishihira 2001), among closely related species of the same genus (Van Moorsel 1983; Vermeij et al. 2003), and even inter-site variation within the same species (de Putron et al. in review). Some of these observed size variations in brooded larvae have simply been related to inter-specific differences in corallite size (Van Moorsel 1983; Isomura and Nishihira 2001). However, larval size variation also occurs among larvae released over several lunar days from the same population for several species, as documented for three *Acropora* species (Nozawa and Okubo 2011), three Pocilloporid corals (Isomura and Nishihira 2001), and the larvae of *Goniastrea aspera* (Nozawa and Harrison 2005). Interestingly, functional differences have also been documented to occur with larval phenotypic variation in some species. For example, *P. astreoides* larvae released from colonies across several lunar days exhibited differences in algal symbiont (zooxanthellae) density, productivity and mortality rate (Edmunds et al. 2001). Similarly, *P. damicornis* larvae varied in size, zooxanthellae density and photosynthetic ability dependent on lunar day of release (Putman et al. 2010). In addition, larger larvae and/or those released later in the reproductive cycle or during peak periods of diel periodicity had better settlement success (Isomura and Nishihira 2001; Nozawa and Harrison 2005; Goodbody-Gringley 2010; Cumbo et al. 2012). The occurrence of phenotypic variation in larvae within the reproductive cycle of brooding coral species may be a 'bet-hedging' strategy to increase the chance of a phenotypic match to changing environmental conditions in the unpredictable coral reef environment (Cumbo et al. 2012).

18.4 Settlement Behavior and Patterns

Coral larval dispersal, settlement and subsequent survival and recruitment of juvenile corals play key roles in shaping coral community structure (Connell 1985; Glassom et al. 2006) and can contribute to connectivity between reefs (Ayre and Hughes 2000; Goodbody-Gringley et al. 2010, 2012). Coral larvae do not settle randomly (Babcock and Mundy 1996), with settlement studies demonstrating a high variability in settlement rates between reef areas and even within the same reef (Glassom et al. 2004). Such variability results from a wide array of complex behaviors that exist in response to environmental conditions at the time of settlement. Factors such as irradiance, substrate type and orientation, algal type and abundance, sedimentation, and grazing pressure all have significant effects on larval settlement and subsequent survival (Ritson-Williams et al. 2009).

The phototactic responses of coral larvae have been well documented (Harrison and Wallace 1990). Lewis (1974) found *F. fragum* larvae to be positively phototaxic at the time of release but later become negatively phototaxic. Mundy and Babcock (1998) found larvae of the coral *Goniastrea favulus* had high settlement rates under light levels with shallow water spectral composition, but low rates under low intensity blue light. Mason and Cohen (2012), document coral larval sensitivity to both red and blue/green light for the brooding species *A. agaricites* and *P. astreoides*, as well as the broadcasting species *Acropora cervicornis, Acropora palmata*, and *Orbicella faveolata*. These authors, and others, suggest that photosensitivity is involved in substrate selection (Mason et al. 2011) and depth orientation (Gleason et al.

2006) at the time of settlement. For example, settlement frequency of *P. astreoides* larvae is highest on orange and red substrata, which may be facilitated by long-wavelength photosensitivity (Mason et al. 2011; Mason and Cohen 2012). In *A. agaricites*, heightened sensitivity to red wavelengths is thought to aid in avoidance of shallow zones with high irradiance, resulting in adult distributions concentrated at depths greater than 10 m or at shaded reef habitats (Mason and Cohen 2012). Yet despite all this evidence of light-mediated larval behavior, very little is known regarding the mechanisms of photoreception in corals. Rhodopsin-based photoreception is suggested to be involved in tentacle retraction/expansion (Gorbunov and Falkowski 2002) and spawning synchronization by adult corals (Sweeney et al. 2011), and opsin-like proteins were found in larvae from several coral species, with blue-light-sensing cryptochromes described from larvae of the broadcasting species *Acropora millepora* (Levy et al. 2007). Thus, further work examining the cellular and molecular mechanisms of photoreceptivity in coral larvae is necessary.

Properties of the benthic substrata also highly influence coral larval behavior, where various substratum types may result in either induction or deterrence of larval settlement (Ritson-Williams et al. 2009). For many marine invertebrates, larval settlement is induced by chemical cues released by conspecifics and/or other organisms that indicate appropriate habitat for survival and growth (Pawlik 1992; Hadfield and Paul 2001). Seminal research on the Caribbean brooding coral *A. humilis* found settlement and metamorphosis was induced by the presence of the coralline red alga *Hydrolithon boergesenii*, and it was suggested that an algal cue might serve as a common chemosensory mechanism for coral larvae (Morse and Morse 1991; Morse et al. 1994). More recently, however, studies have revealed wide degrees of variation in responses across coral species to crustose coralline algae (Baird and Morse 2004; Golbuu and Richmond 2007; Ritson-Williams et al. 2010, 2014). In Australia, Baird and Morse (2004) found that settlement and metamorphosis by larvae of the brooding coral *Acropora palifera* only occurred in the presence of coralline red algae, whereas larvae from the brooding coral *S. pistillata* metamorphosed onto glass surfaces in the absence of an algal chemical cue. In the Caribbean, the presence of a specific coralline algal species, *Titanoderma prototypum*, was documented to increase coral larval recruitment, but only for a few coral species in the genus *Agaricia* (Arnold et al. 2010; Arnold and Steneck 2011). However, in Guam, larvae of the brooding coral *Stylaraea punctaca* showed preference for metamorphosis on biofilmed rubble (Golbuu and Richmond 2007). Furthermore, it is suggested that depth-related differences in bacterial communities of biofilms may allow coral larvae to distinguish suitable depths for settlement (Webster et al. 2004). It is now understood, therefore, that a variety of induction cues exist for coral larval settlement, including coralline algae and their associated biofilms, which may be species specific.

While benthic cover such as coralline algae induce coral larval settlement, other biological inhabitants, such as algal turfs, macroalgae, and cyanobacteria may deter settlement (Kuffner and Paul 2004; Birrell et al. 2005, 2008; Kuffner et al. 2006). For example, settlement success of *P. astreoides* in the Caribbean is reduced when exposed to the brown algae, *Dictyota pulchella* and *Lobophora variegata*. Likewise, the algae *Sargassum polysystum* and *Laurencia papillosa* negatively impact settlement of *P. damicornis* larvae in the Phillippines (Birrell et al. 2008). Settlement and metamorphosis of *P. damicornis* is also negatively impacted by the cyanobacterium *Lyngbya majuscula* (Kuffner and Paul 2004), while settlement of *P. astreoides* larvae is deterred in the proximity of the cyanobacterium *Lyngbya polychroa* (Kuffner et al. 2006). Contrastingly, larvae of *F. fragum*, were found to settle and metamorphose directly onto the surface of the calcareous green alga, *Halimeda opuntia* (Nugues and Szmant 2006). However, the general trend of coral larval response to macroalgae is towards settlement inhibition. This response likely results from direct negative interactions of larvae with algae such as shading, abrasion, overgrowth, increased exposure to bacteria, increased exposure to dissolved organic matter, and natural products (Ritson-Williams et al. 2009; Gleason and Hofmann 2011). Therefore, the documented shifts from coral to macroalgal dominance on many reefs that occur as a result of anthropogenic impacts such as overfishing, added nutrients, and climate change, may act as a feedback mechanism to further reduce coral recruitment and reef resilience (Hughes et al. 2010).

18.5 Effects of Climate Change on Reproduction, Larval Physiology, Settlement and Recruitment

There is mounting concern about the potential impacts of global climate change induced ocean acidification (OA), notably the increase in pCO_2 and lowering of the carbonate ion concentration and calcium carbonate (aragonite) saturation state, on the ability of tropical reef corals to form their skeletons (Gattuso et al. 1999; Kleypas et al. 1999). Global climate change models also predict sea surface temperatures will rise by 2–3 °C by the end of the century (Bindoff et al. 2007), with expected diverse detrimental effects on corals (McClanahan et al. 2009), especially in combination with OA (Erez et al. 2011; Fabricius et al. 2011). Variation in environmental factors, both natural and anthropogenic, outside tolerable ranges can disrupt the timing of reproductive processes and can impair reproductive success and reduce

fecundity (Harrison 2011). Elevated temperatures can cause expulsion of the symbiontic zooxanthellae (i.e. coral bleaching) and sub-lethal bleaching can have implications on reproductive success (Levitan et al. 2014). Indeed, natural temperature fluctuations of just 1 °C negatively affected larval production of *P. damicornis* (Jokiel and Guinther 1978) and *P. astreoides* (de Putron and Smith 2011), providing an insight as to possible effects of future ocean warming, dependent on the capacity for corals to acclimatize. The limited results on the effects of OA on reproduction are encouraging with no effect on gametogenesis for the brooding coral species *Madracis pharensis* (Fine and Tchernov 2007), which is the same result seen for broadcasting species (Fine and Tchernov 2007; Jokiel et al. 2008). There were also no effects on planulation (number of larvae released) of the temperate coral *Balanophyllia elegans*, although female colonies were only exposed to elevated pCO_2 a few days before planula release began (Crook et al. 2013). Elevated pCO_2 is thought to affect fertilization efficiency in corals, as has been shown for other marine invertebrates (Albright 2011) and so for brooding corals, incubations of maternal corals under conditions of OA well before brooding begins are likely needed to assess any possible impact. Elevated temperature has also been shown to decrease fertilization success and slow embryogenic development in broadcasting coral species (Bassim et al. 2002; Negri et al. 2007). Further study is needed on whether temperature and OA effect internal fertilization of brooding corals, such as from decreased sperm motility.

There has been a recent focus on the effects of temperature and OA on larval physiology, survival, settlement and new recruit growth of both broadcasting and brooding coral species. For the purpose of this review on brooding corals, we provide a summary on these effects on the well-studied Atlantic species *P. astreoides* (Fig. 18.2) and *F. fragum* and the Pacific species *P. damicornis*. Elevated pCO_2 significantly depressed larval metabolic rates of *P. astreoides* (Albright and Langdon 2011) but did not affect metabolic rates of *P. damicornis* (Cumbo et al. 2013a). In contrast, elevated temperature increased respiration, oxidative damage, and antioxidant enzyme activity of *P. astreoides* (Olsen et al. 2013). Respiration rates of *P. damicornis* larvae showed a parabolic response to temperature variation with a positive threshold around 28 °C that coincided with the natural temperature of peak larval release suggesting high metabolic rates have selective value (Edmunds et al. 2011). Elevated temperature, however, was detrimental to photosynthesis of *P. astreoides* (Edmunds et al. 2001) and *P. damicornis* (Putman et al. 2010). Decreases in metabolic rate and productivity may hold implication for larval fitness and motility, thereby affecting survival or dispersal. Indeed, elevated pCO_2 reduced survivorship of *P. damicornis* larvae, especially when in combination with increased seawater temperature (Cumbo et al. 2013a). *P. astreoides* larval survival was similarly reduced under high temperature (5 °C above ambient; Edmunds et al. 2001) but there was no effect under elevated temperature (3 °C above ambient; Olsen et al. 2013). Elevated temperature also reduced survivorship of *F. fragum* larvae (Randall and Szmant 2009). Interestingly, brooded larvae of the coral *P. damicornis* differed in their response (metabolic rate and mortality) to high temperature and elevated pCO_2 depending on lunar day of release (Cumbo et al. 2013b). Future research is needed to further evaluate species specific and especially within species responses of larval physiology and survival to global climate change.

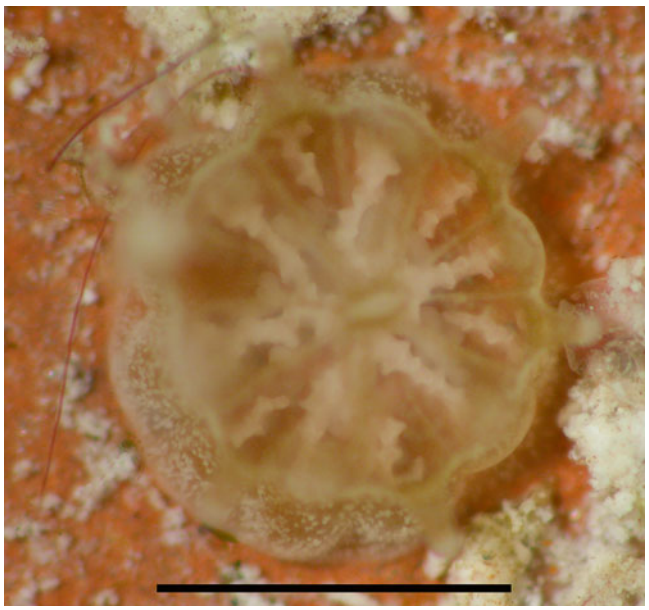

Fig. 18.2 One week old *Favia fragum* polyp showing the skeleton formation. Scale bar = 1 mm (Photo by Dr. James Wood)

The impact of OA on coral larval settlement has been mixed and negative effects seen for some species appear to be an indirect effect of OA induced changes of substrate community composition, such as decreased crustose coralline algae (CCA) cover altering settlement cues (Albright 2011). Elevated temperature reduced settlement of *F. fragum* (Randall and Szmant 2009) but actually increased metamorphosis in *P. astreoides* larvae (Edmunds et al. 2001). The success of coral recruits after settlement is believed to be determined by the same combination of biotic (e.g., competition and predation) and abiotic (e.g., temperature, light, carbonate chemistry conditions, water flow) factors as adult corals, although there has been little investigation into directly comparing the influence of these factors on adults and larvae of the same species (Putman et al. 2008, 2010). The majority of studies evaluating the effects of OA on new recruits have indicated decreased calcification (Albright 2011), including studies on *P. damicornis* (but the response was dependent on light level; Dufault et al. 2013), *P. astre-*

Fig. 18.3 Skeleton of *Favia fragum* polyps after 1 week post-settlement growth in ambient pH conditions (*bottom*) and showing reduced skeleton formation under reduced pH (ambient −0.4, *top*) (Photo by Dr. Anne Cohen (Cohen and Holcomb 2009; Cohen et al. 2009))

oides (Albright and Langdon 2011; de Putron et al. 2011) and *F. fragum* (Fig. 18.3; Cohen et al. 2009; de Putron et al. 2011). Feeding new recruits significantly increased their size and alleviated some of the stress response to OA; however, fed corals still showed sensitivity to elevated pCO2 (Crook et al. 2013; Drenkard et al. 2013). In contrast to the recent surge in research on the effects of OA on calcification of new recruits, there is little experimental work on the effects of temperature on new recruit survival and growth. However, field studies indicated that declining growth of juvenile Caribbean corals over several years tracked rising seawater temperatures leading to changes in species abundance (Edmunds 2004, 2007).

These patterns suggest that if global climate change continues at or beyond projected scenarios over the next century, the recruitment of *P. astreoides*, *F. fragum*, and *P. damicornis* as well as several other brooding and broadcasting coral species (Bassim et al. 2002; Bassim and Sammarco 2003; Putman et al. 2008; Table 1 in Edmunds et al. 2011; review by Albright 2011; Chua et al. 2013; Crook et al. 2013; Doropoulos and Diaz-Pulido 2013) may be compromised by either OA or elevated seawater temperature or a combination of these factors. However, it is important to note that many results may be species specific and variable, especially when in combination with other environmental factors, or when there is variation in experimental design. Interestingly, a recent study reported a null or stimulatory effect of elevated pCO_2 on calcification and survival of new recruits of the brooding coral *Seriatopora caliendrum* when pCO_2 was delivered in an diurnally oscillating range (Dufault et al. 2012). The usual methodology used in studies of employing a steady regime mimicking OA induced seawater chemistry changes rather than a natural oscillatory cycle may therefore be overestimating the detrimental effects of OA. Further

studies concerning the interactive effects of other environmental parameters, such light and food, are also needed, as well as the potential differential response of brooded coral larvae relating to the phenotypic variation observed in many species dependent on the lunar day of release.

18.6 Conclusion

Although brooding corals represent the minority worldwide, they are relatively more abundant in the western Atlantic, representing a high proportion of coral fauna in this region (Szmant 1986). In fact, on many Caribbean islands, reefs are experiencing functional shifts in species composition that correlate with reproductive mode, where reefs formerly dominated by species that broadcast gametes are moving towards those that brood planula larvae (Aronson and Precht 2001; Green et al. 2008). Indeed, recruitment efficiency to space created by disturbances is often higher in species with a brooding reproductive mode (Rylaarsdam 1983; Smith 1992) since plankton mortality is reduced in the typically short dispersal stage and larvae usually contain algal symbionts (zooxanthellae) when they are released, likely providing energetic advantages to the new recruits (Richmond 1987). Likewise, the ability to incorporate mixed modes of larval production may enable rapid recruitment and recovery following disturbance events (Knowlton and Jackson 1993; Sherman 2008; Yeoh and Dai 2010). Thus as conditions continue to deteriorate on coral reefs due to the effects of Global Climate Change and other anthropogenic impacts, a clear understanding of patterns of planulation, larval behavior and recruitment by brooding species will be essential for conservation and management.

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Larval Dispersal and Population Connectivity in Anthozoans

19

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Abstract

Concern about the future of coral reef communities, and the recognition that larval dispersal plays a key role in the resilience of populations has spurred research to determine whether populations are connected by larval exchange or instead maintained by local supply of larvae. In the process of these endeavors, the generalized belief that marine populations were well connected via larval dispersal gave way to the notion that local retention of larvae is common among many benthic species, and that patterns of population connectivity are very complex owing to the multitude of factors and scales involved in larval transport. This review synthesizes the progress in our understanding of the processes affecting dispersal and connectivity in anthozoans (primarily corals), and examines the caveats inherent to the genetic and modeling approaches used in most connectivity studies. Conclusions about the scales of larval dispersal in anthozoans are often species-specific and almost always location-specific. However, some generalities can be recognized. Extreme cases of larval philopatry are more common in brooders. Conversely, large-scale dispersal is seemingly more common in broadcast spawners where a pre-competency period and a potentially long-lived dispersal phase provides a template for more distant dispersal. In between these extremes the divide is less clear and complex patterns of connectivity are widespread between the two developmental modes. Resolving connectivity at scales relevant to demographics remains a major challenge and considerable methodological improvements are needed. Nevertheless, both genetic analyses and biophysical modeling are rapidly maturing and provide increasingly sophisticated analyses of connectivity.

Keywords

Coral reefs • Life history • Larval ecology • Pelagic larval duration • Dispersal kernel • Biophysical models • Gene flow • F_{ST}

19.1 Introduction

Most coastal marine organisms have complex life cycles that involve at least two habitat-distinct life stages, a free-swimming larval phase and a sedentary (or sessile in many invertebrates) adult phase (Thorson 1950). The bipartite nature of the life cycle implies the larval phase is effectively the only time dispersal between populations occurs. This has important implications for the ecology and evolution of these species, and as might be expected, larval biology and the ecological significance of the larval phase have been studied

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for well over 50 years (reviewed in Young 1990). However, interest in the subject grew dramatically in the 1980s and 1990s with the development of supply-side ecology and recruitment limitation theory (e.g., Doherty 1981; Gaines and Roughgarden 1985; Roughgarden et al. 1985; Underwood and Fairweather 1989; Caley et al. 1996). Those studies effectively shifted our attention to the processes occurring during larval dispersal and the importance of recruitment to the dynamics and structure of marine populations (Grosberg and Levitan 1992). It has become clear that dispersal has a key role to our understanding of the population dynamics, population genetic structure and biogeography of many coastal species (Gaines and Roughgarden 1985; Warner 1997; Shulman 1998; Strathmann et al. 2002; among others).

In concert with these schools of thought, the collapse of many fishing stocks (Jackson et al. 2001; Hutchings and Reynolds 2004), and the unprecedented decline and degradation of coastal habitats (Hughes 1994; Jackson 1997; Jackson et al. 2001), has reinforced the need to understand the full extent of larval dispersal in space and time. Knowledge about the magnitude of larval exchange among subpopulations (coined "population connectivity" in contemporary literature), and identification of which populations act as sinks and sources of larvae, have become crucial components of strategies aiming to conserve and/or restore marine populations, especially those based on the establishment of marine protected areas (reviewed in Botsford et al. 2009 and McCook et al. 2009).

As a result, the number of publications studying dispersal and connectivity of marine species has increased rapidly over the past two decades (Levin 2006; Jones et al. 2009), and that progress is underscored by the publication of several compilations of review articles, *Bulletin of Marine Science* [vol. 70, no. 1 (supplement), 2002]; *Oceanography* (vol. 20, no. 3, 2007); and *Coral Reefs* (vol. 28, issue 2, 2009). Cnidarians, especially reef corals, have been included in many of these reviews (van Oppen and Gates 2006; Jones et al. 2009), but have received relatively less attention compared to other groups despite the crucial role of cnidarians as ecosystem engineers in many marine environments. A Web of Science search for articles published between 1986 and 2013 using keywords that referred to *larval dispersal* or *connectivity* in marine species showed that only 7 % of all published articles were on cnidarians (Fig. 19.1). Studies of fishes, on the other hand, accounted for almost 35 % of all studies (Fig. 19.1). This disparity in representation results (at least to some extent) from the lack of appropriate methods to directly estimate larval dispersal in cnidarians (Jones et al. 2009), and it underlines how poorly we understand connectivity and dispersal in this group, particularly at ecologically relevant time scales.

The context for this review starts with the paradigm in place prior to the 1990s, that marine populations were well connected (i.e., demographically open) via the dispersive larval phase (Roughgarden et al. 1985; Caley et al. 1996; Roberts 1997). Several factors contributed to the prevalence of that view, most notably the poor understanding of the abiotic and biotic processes controlling the movement of larvae between populations, and the lack of direct evidence about the scale of larval dispersal at sea. Dispersal was viewed as a function of water circulation, i.e., passive transport, and duration of the pelagic larval phase (days to months depending on the species), and consequently the potential for transport by currents was considered to be high (Roberts 1997). With the exception of unusual species whose larvae settle immediately after release (e.g., Olson 1985), most larvae recruiting to a local population were assumed to come from distant populations, and self-recruitment was presumed unimportant (Gaines and Roughgarden 1985; Underwood

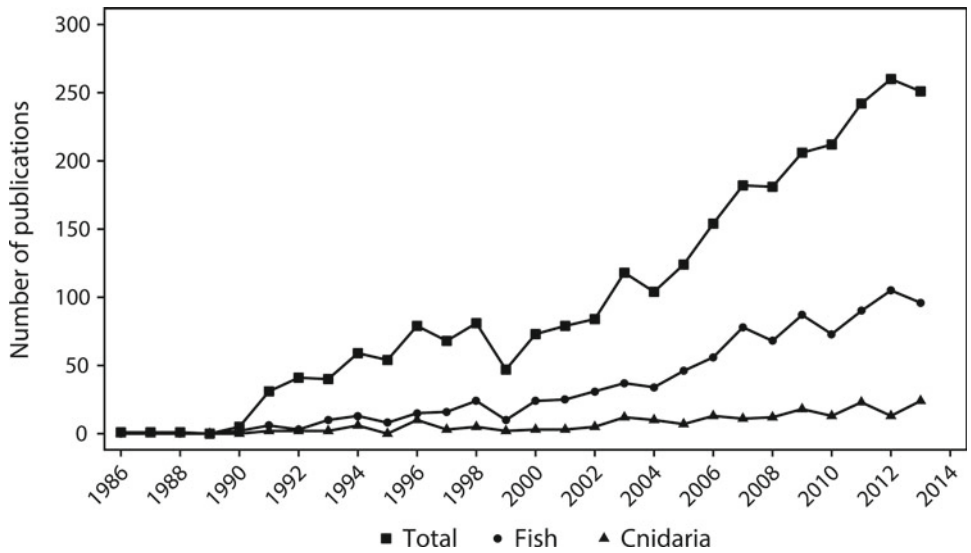

Fig. 19.1 Number of articles published between 1986 and 2013 using keywords that referred to *larval dispersal* or *connectivity* as obtained from a Web of Science search using the following criteria: larva* AND (ocean* OR sea OR seas OR marine) AND (dispers* OR connectivity). The results from this basic search were further refined by a number of filters to exclude obvious outliers (e.g., *Not* ^ freshwater OR lake), as well as to focus on each taxonomic group of interest (i.e., fish and cnidaria)

and Fairweather 1989; Caley et al. 1996). However, a continuously growing body of research has provided compelling evidence that self-recruitment is common in many species (Black et al. 1991; Miller 1997; Jones et al. 2004; reviewed in Levin 2006), thus challenging the assumption of broad larval dispersal and high population connectivity. These findings are a consequence of increasing understanding of the complexity of near-shore water circulation and the recognition that larval transport is not entirely passive. In most marine species, the larvae display a wide range of swimming behaviors in response to various external cues (reviewed in Kingsford et al. 2002) that have the potential of promoting dispersal (or retention) to areas with suitable habitat for settlement (Kingsford et al. 2002; Cowen et al. 2006).

Despite its well recognized importance and the growing body of research on larval dispersal, the extent to which marine populations are open or closed systems remains unanswered for most species and locations. The biophysical integration of a stochastic, temporally variable physical environment with the diverse assortment of life-history traits exhibited by marine organisms is extremely complex. This makes identifying useful predictors of connectivity in marine systems a non-trivial task (Cowen et al. 2007; Weersing and Toonen 2009). Population genetic structure has commonly been used as a proxy of connectivity, and findings of high to moderate degrees of population structure have been used as evidence of variation in dispersal across a variety of taxonomic groups and spatial scales, as well as between species with different dispersal potentials (e.g., Doherty et al. 1995; Murray-Jones and Ayre 1997; Barber et al. 2002; Addison and Hart 2004; Whalan et al. 2004; Jones et al. 2005; Miller and Ayre 2008a). Overall, it is becoming increasingly recognized that patterns of larval dispersal and population connectivity are quite diverse, and that the "openness" of populations will likely fall along a continuum depending on the life-history traits of species, the seascape, and the spatial and temporal scales of interest (Cowen et al. 2000, 2007; Mora and Sale 2002; Jones et al. 2009).

The aims of this chapter are to synthesize the available research on larval dispersal in anthozoan taxa, and identify the directions and challenges that lie ahead. We start by reviewing the literature available for the group while centering our discussion on the differences (or similarities) of patterns observed among taxa and across life histories. We then explore how readily those patterns can be attributed to differences in life-history traits. The various methodologies currently used to study dispersal and connectivity in marine systems have been comprehensively covered elsewhere (Hellberg et al. 2002; Thorrold et al. 2002; Levin 2006; Hellberg 2007; Cowen and Sponaugle 2009), and will not be discussed in detail here. However, we do address the mismatches between some of these approaches, particularly for those species that do not appear to disperse far. Finally, we close with some considerations about the challenges and directions faced by connectivity research on cnidarians.

19.2 State of Affairs: Evidence, Scales and Patterns of Dispersal

Research about the extent of dispersal in cnidarian taxa is available for only a limited number of groups, with most work focused on corals of the order Scleractinia. The Web of Science search (Fig. 19.1) indicates that ~74 % of the literature investigating larval dispersal or connectivity in Cnidaria is focused on scleractinian corals, whereas studies of octocorals (Octocorallia) accounted for about 17 % of all studies. Other anthozoan groups that lack the medusa stage, including the orders Actiniaria (sea anemones), Antipatharia (black corals), Ceriantharia (tube-dwelling anemones), Corallimorpharia (solitary, soft-bodied hexacorallians) and Zoanthidea (zoanthids), remain underrepresented in the literature and little is known about their larval dispersal or population connectivity. Taxa with a medusa, i.e., jellyfishes and hydroids, represent an inherently different system with regard to dispersal owing to the nature of their life cycle. Few data are available for medusoid species; thus our treatment will center exclusively on anthozoans, especially scleractinians and octocorals, which are the best-studied groups.

19.2.1 What Is Our Perception About the Scales of Dispersal?

The scales of larval dispersal in anthozoans have been inferred to vary extensively, and dispersal distances ranging from tens of meters to hundreds (thousands in some cases) of kilometers are seemingly widespread across species and seascapes (reviewed in Jones et al. 2009). Below we summarize the progress in our understanding of the extent of larval dispersal in anthozoans, primarily corals and reef species. For simplicity, our discussion is divided in four categories of increasing dispersal distance (Table 19.1): (1) *restricted* (<1 km), within reefs and between patch reefs; (2) *local* (<10 km), between reefs; (3) *moderate to regional* (<250 km), between sites and islands; and (4) *broad-scale* (100s km–1000s km), between reef systems and across basins. These categories capture common patterns of larval dispersal in a great many cases, but the dispersal categories are a simplified categorization of the dispersal continuum. As in any classification system, the data for some species do not neatly fit into these categories due to either the biology of species in question; the sampling scheme used in the studies; or the geographic setting or seascape.

Table 19.1 Scales of coral larval dispersal discussed in this review with examples of representative taxa for each category defined

Scale	Distance range	Geographic range	Representative species and studies
Restricted	<1 km	Within reefs and between patch reefs	*Corallium rubrum*: Costantini et al. (2007b) and Ledoux et al. (2010a)
			Balanophyllia elegans: Gerrodette (1981) and Hellberg (1994, 1996)
			Seriatopora hystrix: Underwood et al. (2007)
Local	<10 km	Between reefs	*Paramuricea clavata*: Mokhtar-Jamaï et al. (2011, 2013)
			Clavularia koellikeri: Bastidas et al. (2002)
			Platygyra daedalea: Miller and Ayre (2008a)
Moderate to regional	<250 km	Between sites and islands	*Orbicella faveolata*: Baums et al. (2010)
			Favia fragum: Goodbody-Gringley et al. (2010)
			Montipora capitata: Concepcion et al. (2014)
Broad-scale	100s km–1000s km	Between reef systems and across ocean basins	*Acropora palmata*: Baums et al. (2005, 2006); *Orbicella annularis*: Foster et al. (2012)
			Pocillopora spp.: Pinzón and LaJeunesse (2011), Pinzón et al. (2013) and Torda et al. (2013b)

For further detail about the spatial extent of dispersal in coral taxa please refer to the text, and reviews by van Oppen and Gates (2006) and Jones et al. (2009)

19.2.1.1 Restricted Dispersal

Consistent with theoretical expectations, the lower end of the dispersal continuum (i.e., <1 km) is in general occupied by brooding species. Because the larvae of most brooders are fully developed at the time of release (thus close to or already competent to settle) these species have long been assumed to be poor dispersers, and populations are assumed to be mostly self-seeded. These premises have been generally supported by a number of studies that have included direct observations of larval behavior, as well as genetic data. One of the earliest reports of limited dispersal is the solitary cup coral *Balanophyllia elegans*. The crawling larvae (i.e., non-pelagic) of this species have been observed to settle on average <0.5 m away from the maternal colonies (Gerrodette 1981; Fadlallah and Pearse 1982), which concurs with the low levels of gene flow among populations separated by as few as 4–30 m, and a pattern of isolation by distance (IBD) along most of the Pacific coast of North America (Hellberg 1994, 1996).

In another remarkable example of philopatric recruitment, Ledoux et al. (2010a) uncovered a complex pattern of kinship structuring within a small patch (<0.5 m^2) of colonies of the Mediterranean precious red coral *Corallium rubrum* (Octocorallia). IBD and kinship analyses suggested mean dispersal distances of less than 0.5 m, and identified a considerable proportion of half-sib relationships (9.4–10.5 %) among some of the size-classes sampled, which suggests small breeding units may be common in this species. These results were in agreement with previous studies that found high levels of genetic structuring at scales ranging from a few meters to 100 s of kilometers, as it would be expected in a species with limited effective dispersal (Costantini et al. 2007a, b; Ledoux et al. 2010b).

Underwood et al. (2007) analyzed the spatial extent of larval dispersal and population connectivity of the brooding scleractinian *Seriatopora hystrix* at an isolated reef system off northwestern Australia. Spatial autocorrelation analyses of genetic distance showed that recruitment is extremely philopatric at this location with most larvae settling within 100 m of the maternal colonies. Consistent with those data, most populations were significantly differentiated and self-seeded at the spatial scales analyzed (2–60 km; Underwood et al. 2007), and similar patterns of differentiation among *S. hystrix* populations have been reported in several other studies (Ayre and Dufty 1994; van Oppen et al. 2008; Noreen et al. 2009).

Among other anthozoan taxa, restricted dispersal and larval philopatry has been inferred in the black coral *Antipathes fiordensis*. Miller's (1998) study of the fine-scale (<50 m) genetic relationships between colonies of *A. fiordensis* at Doubtful Sound, Fiordland (New Zealand) revealed that at one of three sites larvae were likely to settle in close proximity to their parents. The population showed a marked pattern of IBD, which in conjunction with the strong spatial association of colony genotypes separated by as little as 5 m suggests larval dispersal may be extremely localized (Miller 1998). Interestingly, *A. fiordensis* has been inferred to be a broadcast spawner (Parker et al. 1997). The larvae are negatively buoyant and weak swimmers (Miller 1996), which in concert with restricted water flow within fjords may explain the species' restricted dispersal (Miller 1997, 1998).

19.2.1.2 Local Dispersal

At a slightly larger spatial scale, dispersal that is restricted to 1–10 km also appears to be more common among brooders than in broadcast spawners, albeit exceptions clearly exist

(see below). The presence of population genetic structure at inter-reef and larger spatial scales (Hellberg 1996; Ayre and Hughes 2000; Underwood et al. 2009) suggests the commonness of localized recruitment over ecological time scales among brooding corals. This pattern appears to hold for both octocorals and scleractinians despite brooding being a more common (and hence better documented) reproductive strategy among octocorals (Harrison 2010; Kahng et al. 2011). For example, estimates of genetic differentiation suggest that gene flow is extremely limited among populations of the brooding soft corals *Discophyton rudyi* (northeastern Pacific) and *Clavularia koellikeri* (Great Barrier Reef, GBR) separated by more than a few kilometers, which is consistent with the prevalence of local recruitment (McFadden 1997; Bastidas et al. 2002). Similarly, the Mediterranean endemic coral *Astroides calycularis* (Scleractinia), which broods negatively buoyant demersal larvae, exhibits a pattern of IBD within the Alboran Sea. The relatively low numbers of first-generation migrants originating from distant localities, indicates most larval exchange occurs between adjacent populations (Casado-Amezúa et al. 2012).

Among octocorals, external surface brooding, a seemingly intermediate strategy to broadcast spawning and internal brooding that is absent in scleractinian corals (Kahng et al. 2011), is often associated with the lower end of the dispersal continuum. In surface brooding species the eggs or zygotes are released by the polyps but retained on the surface of the maternal colonies (or in mucous coatings) where embryogenesis occurs. Once released, it has been observed that most larvae stay in the water column for only a short period of time (e.g., Coma et al. 1995; Gutiérrez-Rodríguez and Lasker 2004a), which suggests most larvae are retained locally. The Mediterranean red gorgonian *Paramuricea clavata* is one of such cases. The species exhibits strong population genetic differentiation and a pattern of IBD across its distribution (Mokhtar-Jamaï et al. 2011), which in conjunction with the occurrence of high self-recruitment (25 %) and parentage relationships at small spatial scales at one site (Mokhtar-Jamaï et al. 2013) indicate dispersal distances are effectively short. Similarly, Lasker and Porto-Hannes (2015) found that most recruits of the Caribbean gorgonian *Antillogorgia elisabethae* were likely of local or adjacent origin along the Little Bahamas Bank (LBB). They found a strong IBD signal among a group of populations along the LBB, as well as regional clustering (*sensu* Selkoe et al. 2014) when more distant sites were considered, a pattern also reported in previous studies (Gutiérrez-Rodríguez and Lasker 2004b; Gutiérrez-Rodríguez et al. 2005).

Local retention of larvae at reef scales has also been inferred to occur in some species of corals that broadcast spawn, as suggested by the significant, but generally small, levels of population genetic subdivision at these scales (Ayre and Hughes 2000; Whitaker 2004; Miller and Ayre 2008a; Underwood et al. 2009). For example, Miller and Ayre (2008a) found significant levels of genetic subdivision among populations of two species of broadcast spawning scleractinians separated by less than 4 km at One Tree Island in the southern GBR, which is indicative of some local retention and hence short dispersal distances. In the soft coral *Sinularia flexibilis*, evidence of limited gene flow between populations separated by less than 2 km in some areas of the GBR also suggests a potential for the local retention of larvae, despite the absence of significant genetic subdivision among other sites where populations were much farther apart (Bastidas et al. 2001). In general, the magnitude of self-seeding among broadcast spawning species at spatial scales of 1–10 km remains unclear, but seemingly is rarer than that presumed to occur in brooding species.

19.2.1.3 Moderate to Regional Dispersal

While it may still be premature to pinpoint the dominant scale of larval dispersal in coral taxa, moderate to regional dispersal distances (10–250 km) are commonplace among broadcast spawning corals, and generally less so among brooding species (Mackenzie et al. 2004; Underwood et al. 2009; Hemond and Vollmer 2010; Macdonald et al. 2011). Much of the support for this claim also comes from indirect genetic methods (e.g., F_{ST}'s), which estimate gene flow (and hence dispersal and connectivity) integrated over multiple generations. For instance, along the Florida Keys, the reef-building broadcast spawners *Acropora cervicornis* and *Orbicella faveolata* show no significant population subdivision, indicating that gene flow between locations separated by up to ~250 km is sufficient to maintain homogeneous populations. At broader spatial scales (i.e., across the Caribbean), however, considerable population genetic structure exists (Baums et al. 2010; Hemond and Vollmer 2010). It is plausible that similar patterns occur at ecological or demographic temporal scales, although many factors (see sections below) can distort this pattern and alter the scale of larval dispersal in both brooding and broadcast spawning species.

One brooding coral species that appears to disperse on a regional scale (i.e., >10 km) is the Caribbean scleractinian *Favia fragum*. The larvae of *F. fragum* have been observed to actively swim for a very short time (≈4 min) before dropping and attaching to the substrate (Carlon and Olson 1993), which has been regarded as evidence that dispersal distances may be small. Paradoxically, the lack of strong population genetic structure at scales <35 km suggests considerable larval exchange occurs between sites in some areas (Goodbody-Gringley et al. 2010), and restricted gene flow is only evident on a Caribbean-wide scale (i.e., 100s–1000s km).

A number of studies have provided evidence of coral larval dispersal at regional spatial scales over a few or single generations. Along the Hawaiian Archipelago, populations

of the scleractinian *Montipora capitata* (broadcast spawner) have been inferred to cluster into four regional groups, two at opposite ends of the archipelago between which little larval exchange is observed, and two additional groups of mixed ancestry at the central part of the archipelago (Concepcion et al. 2014). However, the authors found significant genetic structuring among most locations (i.e., islands), as well as evidence of high self-recruitment at the island level (>67 %) with most contemporary migration occurring between geographically proximate islands (10's to a few 100's km apart). In a study using multi-locus genotype assignment tests of *Pocillopora damicornis* recruits, Torda et al. (2013a) found that for one of the most common molecular operational taxonomic units (MOTUs, type ß *sensu* Schmidt-Roach et al. 2013), a large fraction of the larvae recruiting to two sites in the Northern and Central GBR were likely migrants from locations >10 km away.

19.2.1.4 Broad-Scale Dispersal

At large spatial scales (i.e., 100s–1000s of km) the great majority of coral species show marked signs of restricted larval exchange. This is particularly true for brooding species but also for many broadcast spawners (Ayre and Hughes 2000; Bastidas et al. 2001; Gutiérrez-Rodríguez and Lasker 2004b; Polato et al. 2010; among others). Nevertheless, a number of species have broad distribution ranges over which moderate to high levels of gene flow (and thus dispersal) have been inferred to occur (Severance and Karl 2006; Ridgway et al. 2008; Miller and Ayre 2008b; Porto-Hannes et al. 2014). For example, in the Caribbean basin, populations of the elkhorn coral *Acropora palmata* form two genetically distinct clusters west and east of the Mona Passage (located between the Dominican Republic and Puerto Rico), which acts as a bio-oceanographic filter to larval dispersal between the two regions (Baums et al. 2005, 2006). With the exception of an admixing/transition zone at Puerto Rico, western and eastern populations appear to be mostly self-seeded at the regional level, with limited gene flow between them in the recent past (Baums et al. 2005, 2006; Mège et al. 2014). Within each of the two provinces, however, migration between populations separated by as much as 1800 km (western region) occurs frequently enough to reduce population genetic differentiation (Baums et al. 2005; Mège et al. 2014), even when rates of local self-recruitment appear to be relatively high (Baums et al. 2005). Likewise, results from several studies indicate that gene flow among populations of the reef-building *Orbicella annularis* (Foster et al. 2012) and *Montastraea cavernosa* (Nunes et al. 2009; Goodbody-Gringley et al. 2011; Serrano et al. 2014) within major regions of the Caribbean and western Atlantic are moderate to high, although the exact spatial scales involved vary among regions, species and studies. For instance, levels of gene flow in *O. annularis* were found to be surprisingly low at relatively small spatial scales in Mesoamerica (Belize and Honduras; 150 km apart), but high along 100 s of km between areas like Tobago and Curaçao (Foster et al. 2012).

Broad-scale dispersal also occurs among corals in other regions and communities. Peripheral populations of the broadcast spawner *Acropora digitifera* spanning over 1000 km in the Nansei Islands (Japan) show little evidence of population genetic differentiation consistent with high genetic connectivity along the island chain (Nakajima et al. 2010). In deep sea communities in the North Atlantic Ocean, populations of the cosmopolitan deep-water coral *Lophelia pertusa* appear moderately connected by larval dispersal over broad geographic distances within some regions, albeit genetically distinct across ocean basins (e.g., Gulf of Mexico *vs.* Northwestern Atlantic) and in some cases at smaller spatial scales (e.g., offshore *vs.* fjord populations) (Le Goff-Vitry et al. 2004; Morrison et al. 2011).

One of the most studied species in the coral literature, and perhaps the most controversial, is the widespread Indo-Pacific scleractinian *P. damicornis*. A mix of reproductive strategies have been reported throughout its distribution range, encompassing broadcast spawning in the Tropical Eastern Pacific (TEP) (Glynn et al. 1991), and brooding of asexually (and to a lesser extent sexually) produced larvae across the Indo-West Pacific, although indirect evidence suggests broadcast spawning also occurs (Stoddart and Black 1985; Ward 1992; Yeoh and Dai 2010). Recent studies show that *P. damicornis*, and *Pocillopora* in general, comprise several genetically distinct lineages (sympatric in many cases) that do not in most cases relate to traditional morphospecies taxonomy (Souter 2010; Pinzón and LaJeunesse 2011; Pinzón et al. 2013; Schmidt-Roach et al. 2013; but see Flot et al. 2008). The combination of the genetic data, the mixed reproductive strategies, and the presence of highly clonal populations on some reefs (e.g., Adjeroud et al. 2014; Baums 2014) has confounded (and possibly undermined) many of the previous inferences about patterns of gene flow in *Pocillopora* (Stoddart 1984; Ayre et al. 1997; Magalon et al. 2005; Whitaker 2006). However, recent endeavors have provided evidence of large-scale dispersal among populations of the same genetic lineage. For example, Pinzón and LaJeunesse (2011) found no evidence of population genetic structure among populations of their "type 1" *Pocillopora* lineage across several locations in the TEP (100s–1000s km apart), which suggests long-distance larval dispersal may occur frequently. A subsequent study including samples of *Pocillopora* from the entire Indo-Pacific showed that additional genetic lineages exhibit moderately homogeneous populations across thousands of kilometers (Pinzón et al. 2013). In another study in the GBR, Torda et al. (2013b) found that genetic similarity and divergence was evident at

multiple spatial scales in two MOTUs of *P. damicornis* (types α and ß *sensu* Schmidt-Roach et al. 2013). They argued that local genetic divergence (<10 km) was likely the result of stochastic recruitment events from multiple sources, whereas genetic similarity at large spatial scales (over 1000 km) likely reflects a synergy between the long competency period of the larvae, year-round larval release and strong along-shore larval transport.

In summary, the spectrum of dispersal distances inferred to occur in coral species is diverse and has produced complex patterns of population connectivity. In general, the very ends of the spectrum, i.e., extremely restricted *vs.* broad-scale dispersal, appear to be mostly occupied by those species where life-history traits restrain or promote dispersal (i.e., brooding *vs.* broadcast spawning, respectively), particularly at ecological time scales. However, as described above and further discussed below important exceptions exist. In between these extremes the divide is less clear, if it exists at all, and likely the result of other factors working at various spatial and temporal scales.

19.2.2 Are All Broadcast Spawners Good Dispersers and All Brooders Poor Dispersers?

In a nutshell, the answer is "not necessarily". While the most extreme known cases of self-seeded coral populations are in fact brooding species, there is mounting evidence suggesting pelagic larval duration (PLD) and reproductive mode (i.e., brooding *vs.* broadcast spawning) do not always explain patterns of population connectivity and/or realized dispersal distances. For example, Ayre and Hughes (2000) measured genetic variation in five species of brooding and four of broadcast spawning corals at local and regional scales along the GBR, and found no clear association between reproductive strategy and levels of gene flow. They noted that while multi-generational, long-distance gene flow (and thus dispersal) was enough to maintain population genetic homogeneity at regional scales in many of the brooders (three species) and all the broadcast spawners studied, individual reefs appear to rely primarily on local recruitment as substantial genetic subdivision was observed among populations separated by only a few kilometers.

Among broadcast spawning corals, in particular, a surprisingly number of studies have noted the lack of correlation between dispersal potential suggested by life-history traits, and inferred levels of gene flow and population genetic structure (Ayre and Hughes 2000; Whitaker 2004; Severance and Karl 2006; Miller and Ayre 2008a; Reyes and Schizas 2010; van Oppen et al. 2011b; Andras et al. 2012; Concepcion et al. 2014). For example, Miller and Ayre (2008a) found similar levels of small but significant fine- to meso-scale (<4 km) population genetic subdivision in *Goniastrea favulus* and *Platygyra daedalea*, two broadcast spawners with contrasting modes of larval development (benthic *vs.* planktonic, respectively). This suggests larval dispersal may be equivalent in both species despite what was perceived to be a high potential for localized settlement in *G. favulus*. In contrast, the sympatric species *Orbicella annularis* and *O. faveolata* exhibit seemingly similar life-history traits, yet they have strikingly different patterns of population genetic differentiation along the northern Caribbean (significant inter-reef subdivision in the former but not the later) (Severance and Karl 2006).

More recently, van Oppen et al. (2011b) noted that some populations of the widely distributed *Acropora millepora* in the GBR can be largely self-seeded even when moderate levels of population connectivity occur at larger spatial scales, i.e., among populations within regional clusters, similar to that observed for *M. capitata* along the Hawaiian Archipelago (Concepcion et al. 2014). In one of the few studies describing population genetic structure in a broadcast spawning octocoral, Andras et al. (2012) found that populations of the Caribbean sea fan *Gorgonia ventalina* appear to be well admixed across broad distances via gene flow, but are probably ecologically independent as the estimated rates of migration (over a few generations) were in most cases very low, even between sites separated by <2 km.

Similar to broadcast spawners, the conjecture that brooders do not disperse far as the result of life-history traits restraints does not necessarily hold for all species. For example, using a comparative analysis of ribosomal DNA sequences in adults and juveniles of the brooder *Stylophora pistillata* in the Gulf of Aqaba (Red Sea), Zvuloni et al. (2008) found that while adults from populations ~10 km apart exhibited genetic differentiation, the juveniles were genetically similar and more diverse, which suggests high larval connectivity (or a common source) between the studied sites, and implicates post-settlement processes in explaining the pattern observed among adult populations. Several recent studies have shown that populations of the predominantly self-seeded coral *S. hystrix* are occasionally supplemented with recent migrants (2–6 % of sampled colonies) from adjacent and/or distant reefs (Underwood et al. 2007, 2009; van Oppen et al. 2008; Noreen et al. 2009). Likewise, larval exchange between distant populations (160 km apart on average) of the surface brooding octocoral *P. clavata* in the Mediterranean have been inferred to occur at least sporadically (Mokhtar-Jamaï et al. 2011). The extent to which long-distance dispersal in brooding corals results from single migration events or rather stepping-stone dispersal over a few generations is not entirely clear at this point. However, it suggests larval exchange over broad spatial scales (i.e., 10s

to 100s of km) also occurs in species with extremely philopatric recruitment.

Taken together, levels of population genetic differentiation are in general markedly higher, and thus inferred dispersal distances smaller, in brooding corals than in broadcast spawning species (Hellberg 1996; Ayre and Hughes 2000, 2004; Underwood et al. 2009). However, an increasing number of studies show that "exceptions" to those patterns are probably more common than previously appreciated. More importantly, these studies reinforce the notion that larval dispersal and population connectivity in marine species is more complex than developmental pattern alone.

19.2.3 What Are the Major Determinants of Dispersal and Population Connectivity in Corals?

As noted in the preceding sections larval dispersal in the marine environment is not a function of physical or biological processes alone, but rather the net result of the interplay between the two and hence biophysical in nature (Gawarkiewicz et al. 2007; Werner et al. 2007; Cowen and Sponaugle 2009). As in other benthic species, the high variability in coral larval dispersal (and hence in patterns of connectivity) is inherently tied to several factors, including but not exclusively the biology of the species (Ayre and Hughes 2004; Severance and Karl 2006; Miller and Ayre 2008b); environmental stochasticity affecting dispersal and recruitment (van Oppen et al. 2008; Nakamura and Sakai 2009); the spatial and temporal scales studied (Underwood et al. 2007; Mokhtar-Jamaï et al. 2011; Smilansky and Lasker 2014); the approach used to infer dispersal and connectivity (Ayre and Hughes 2000; van Oppen et al. 2008; Foster et al. 2012); the structural complexity and isolation of the reef system (van Oppen et al. 2008; Noreen et al. 2009); as well as habitat patchiness and seascape (Pinsky et al. 2012; Riginos and Liggins 2013). Table 19.2 summarizes the major processes controlling larval dispersal and connectivity in anthozoans. We divide those into biological, ecological and physical effects, as a way of contrasting those traits/processes controlled by the phenotype, interactions with other organisms and those features of the physical environment that control dispersal. The full consideration of all of the traits in Table 19.2 goes beyond the scope of our review, and we focus on a subset of processes that have been commonly considered and for which there are substantial data.

19.2.3.1 Hydrodynamics

Certainly, the many hydrodynamic processes that are involved in larval transport and retention play a substantial role in determining the spatial extent of dispersal and connectivity (reviewed in Sponaugle et al. 2002, Largier 2003, and Gawarkiewicz et al. 2007). These processes are scale-dependent both spatially and temporally (e.g., cross-shelf transport/retention by tides *vs.* along-shore ocean currents), and are particularly numerous and complex around shallow nearshore or reef regions where water flow interacts with features such as shoreline and bottom topography (Gawarkiewicz et al. 2007; Pineda et al. 2007). Consequently, how larvae are transported varies considerably across seascapes and time, making it difficult to predict larval dispersal and discern overarching patterns (Gawarkiewicz et al. 2007; Pineda et al. 2007). However, considerable advances have been achieved over the last decade or so (Cowen and Sponaugle 2009), and the continuing development of biophysical models should improve our understanding of how hydrodynamic processes interact with the various biological traits mediating larval dispersal.

19.2.3.2 Reefscape

In many respects, the extent to which coral populations are self-seeded or receive immigrants from other (sometimes distant) populations is linked to geographic isolation and habitat patchiness. Many marine habitats, especially coral reefs, are patchy and often separated by large stretches of deep water (e.g., Pacific reefs), which by itself makes larval subsidy from distant populations unlikely. A well-known example in the coral literature is the case of Lord Howe Island (LHI) located offshore of southeast Australia and separated from other reef areas by >600 km of open-ocean (Ayre and Hughes 2004; Miller and Ayre 2004). Ayre and Hughes (2004) showed that for several species of coral, encompassing different reproductive modes, the absence of stepping stone reefs connecting populations in the GBR with LHI was a far more important factor restricting dispersal than distance itself, as inferred levels of gene flow at similar spatial scales within the GBR (relatively contiguous reef system) were markedly higher than between GBR and LHI.

In such cases, population connectivity is likely maintained by episodic events of larval transport. For instance, hydrodynamic models suggest that the occurrence of extreme, periodic climatic conditions like those generated by cyclones during coral spawning season can substantially (and rapidly) increase larval transport distances well within the competency period of most species (Radford et al. 2014). Similarly, it has been suggested that during El Niño Southern Oscillation (ENSO) events, the increase in eastward flow of the North Equatorial Counter Current can facilitate larval dispersal (and thus population connectivity) across the Eastern Pacific Barrier, the most effective biogeographic barrier for marine species (Richmond 1990). Recent molecular data for the scleractinian *Porites lobata*, however, showed that with the exception of one marginal population in the Eastern Tropical Pacific (Clipperton Atoll), the changes in oceanographic conditions resulting from ENSO events

Table 19.2 Processes controlling larval dispersal and connectivity of anthozoans in marine systems. Adapted from Sponaugle et al. (2002), Pineda et al. (2007), Werner et al. (2007), and Cowen and Sponaugle (2009)

Biological	Physical (the seascape)
• Gametogenic cycle	• Reefscape
◦ Frequency and timing of spawning	◦ Distribution of suitable habitat
◦ Degree of synchrony in spawning	▪ Across basins
▪ Over time	▪ Along island chains
▪ Across individuals	▪ Along reef tracts
◦ Mode of spawning	▪ Around islands
▪ Brood, surface brood or broadcast	◦ Isolation of the system
• Larval traits	• Bathymetry
◦ Pre-competency period	• Hydrodynamics
◦ Competency period	◦ Monthly and daily variation (e.g., tides)
▪ PLD	◦ Seasonal variation
◦ Physical traits	◦ Long-term fluctuations (e.g., El Niño)
▪ Buoyancy	• External forcing
▪ Motility	◦ Wind-forcing
◦ Behavioral traits	◦ Episodic events (e.g., hurricanes)
▪ Sensory capabilities	
▪ Behavioral responses	
• Plasticity	
◦ Temperature	
◦ Productivity	
◦ Water quality	
Ecological	
• Larval mortality	
◦ Predation	
◦ Physical stress	
◦ Availability of habitat at settlement	
• Post-settlement mortality[a]	

[a]Note that population connectivity in the traditional sense refers to dispersal until the time of settlement (Cowen and Sponaugle 2009), and generally does not take into account the effects of post-settlement mortality and survival until reproduction, i.e., reproductive population connectivity (*sensu* Pineda et al. 2007)

appear insufficient to maintain connectivity between populations of the Central Pacific and East Tropical Pacific (Baums et al. 2012).

In addition to geographic isolation, coral reef patchiness can limit larval exchange among populations at smaller spatial scales (10–100 s of km). For example, using a simple model of the degree of population openness, Pinsky et al. (2012) argued that largely closed populations can be expected in coral reef seascapes where patch (i.e., habitat) spacing is more than twice the standard deviation of a species' dispersal kernel, the probability distribution of larvae as a function of distance dispersed. Certainly, the various life-history traits described above, as well as the existence of both hydrodynamic barriers and "corridors" affecting the degree of connectivity between coral reef patches, will greatly influence the overall role of habitat patchiness in the extent to which populations are demographically open or closed.

19.2.3.3 Timing of Spawning

Timing and frequency of coral spawning or larvae release set the initial environmental conditions for larval transport, and are therefore also relevant for dispersal. Reproductive patterns in coral taxa are fairly diverse both at the species and population levels across regions, forming a continuum that ranges from highly synchronized mass or multispecies spawning events over one or a few nights in many broadcast spawners, to year-round release of larvae in many brooders (reviewed in Harrison and Wallace 1990, Harrison 2010, and Kahng et al. 2011). Thus, when and where gamete or larval release occur will greatly affect whether dispersal or retention is favored, particularly in broadcast spawners where early stages (i.e., pelagic eggs) are at the mercy of hydrodynamic transport. It has been suggested that seasonal mass spawning events (timing and duration) are coupled with regionally calm periods as to ensure fertilization and facili-

tate larval retention, thereby increasing local recruitment (van Woesik 2010). However, extended breeding periods with multiple spawning episodes (i.e., split-spawning) or year-round larvae release as observed in several species of broadcast spawning (gonochoric species mostly) and brooding corals (Harrison and Wallace 1990; Bastidas et al. 2005) means the environmental conditions experienced by larvae during dispersal are likely to differ. That introduces variation to the shape of dispersal kernels, particularly at the tails of the distribution (i.e., extremes of retention or dispersal).

19.2.3.4 Pelagic Larval Duration and Pre-competency to Settle

The duration of the pelagic larval phase (i.e., PLD) has been regarded as the chief biological trait determining dispersal potential of marine benthic species. In the fluid marine environment, the potential for larval transport is considered to be high, and for this reason the time propagules (i.e., eggs and larvae) spend in the water column is expected to correlate well with dispersal distances and population connectivity (e.g., Shanks et al. 2003; Shanks 2009). Species with longer PLDs should in general disperse farther than species with shorter PLDs (Scheltema 1968). While PLD is most certainly an important determinant of dispersal in anthozoans, the extent to which it is the primary biological parameter influencing the shape of a species' dispersal kernel is unclear. In general, the upper competency limit on which most studies tend to focus may be more relevant in dictating how far larvae can go (i.e., tail of the dispersal kernel) rather than in explaining the dispersal distances of the majority of larvae. Indeed, biophysical models of larval dispersal indicate PLD is much more important in determining broad-scale patterns of connectivity than local to meso-scale patterns (Treml et al. 2012; Wood et al. 2013), which suggests it may be a better predictor of evolutionary connectivity (Treml et al. 2012).

In this respect, the minimum time to settlement competency may have a great effect on dispersal distances and the resultant patterns of connectivity, particularly at ecological time scales and among taxa with weakly swimming larvae such as corals (Miller and Mundy 2003; Underwood et al. 2009; Treml et al. 2012; Figueiredo et al. 2013). For instance, in many species of corals, including species with pelagic larval development, onset of competency to settle occurs rapidly enough to allow high local retention prior to the larvae being advected from the natal reefs (Miller and Mundy 2003; Connolly and Baird 2010; Figueiredo et al. 2013). This effect can be especially important in complex reef mosaics like some sections of the GBR where numerical modeling has shown flushing of water and larvae can be very poor (Willis and Oliver 1988; Black et al. 1991; Wolanski and Spagnol 2000; Andutta et al. 2012). Transport of pre-competent larvae away from natal reefs can, however, occur rapidly in areas exposed to strong currents, as well as in reefs characterized by long residence time of water masses, but that are subject to temporal variation in hydrodynamic conditions (e.g., spring tide *vs.* neap tide) (Black et al. 1991; Wolanski and Spagnol 2000; Andutta et al. 2012). In such cases, the proportion of long-distance dispersal will be higher provided larvae survive and remain competent to settle until being advected over suitable habitat.

19.2.3.5 Larval Behavior

In the last two decades, the role of larval behavior in mediating the spatial extent of dispersal has also been emphasized in the literature. Larvae of virtually all marine benthic species are sensitive to a myriad of environmental cues that may be used to detect suitable settlement habitat and therefore influence the outcome of larval transport (reviewed in Kingsford et al. 2002). Unlike other taxa such as fishes and decapods, which have strong orientation and swimming abilities (e.g., Leis et al. 2005; Kough et al. 2014), behavior of coral larvae in nature is thought to be limited to partial navigation (*sensu* Kingsford et al. 2002) involving vertical "migration" between stratified current flows that may favor larval transport (or retention) to a settlement location. The influence of *in situ* coral larval vertical swimming behavior on dispersal distances is, to date, unknown. However, mounting evidence suggests larvae respond to many of the same cues used by marine larvae of other groups for navigation, including sound, light, ultraviolet radiation, hydrostatic pressure, temperature and waterborne biotic chemicals (Morse et al. 1996; Raimondi and Morse 2000; Edmunds et al. 2001; Stake and Sammarco 2003; Gleason et al. 2005, 2009; Vermeij et al. 2006, 2010). The fact that coral larvae respond to acoustic cues, in particular, suggests their sensory capabilities may include at least one cue (sound) that could be used for orientation on much larger spatial scales (Vermeij et al. 2010). The scales over which acoustic cues can be used remains largely unknown. Overall, active vertical swimming behavior adds to the complexity of coral larval transport in the pelagic environment, and reinforces the importance of incorporating behavior in models of coral larval dispersal as it may greatly affect the extent of dispersal and connectivity (Werner et al. 2007). Indeed, it has been shown that parameterization of biophysical models with active coral larval behavior (changes in buoyancy) has a large impact on the resulting patterns of population connectivity relative to passive scenarios (Holstein 2013).

19.2.3.6 Phenotypic Plasticity

Plasticity and the temporal dynamics of larval traits are also important biological components of coral larval dispersal. Most empirical data (if not all) indicate there is substantial within-cohort variation in the onset and duration of larval competency to settle (e.g., Harii et al. 2002; Nishikawa et al. 2003; Connolly and Baird 2010), which as shown by dynamic models of larval dispersal potential in broadcast spawning scleractinians has the potential to increase the extent of both

self-recruitment and long-distance dispersal (Connolly and Baird 2010). This variation is likely magnified by the stochastic nature of the marine environment and the heterogeneous conditions experienced by larvae in the field. That further alters the overall shape of the dispersal kernel (Connolly and Baird 2010). Similarly, larval survivorship is not uniform throughout the duration of the larval phase but rather temporally dynamic (Harii et al. 2002; Graham et al. 2008; Connolly and Baird 2010; Nozawa and Okubo 2011). In several species of broadcast spawning corals, survivorship is typically characterized by high initial mortality rates, followed by a period of relatively low/constant mortality rate before progressively increasing again late in larval life (Nozawa and Harrison 2008; Graham et al. 2014). In general, high early larval mortality means most larvae are unlikely to survive long enough to disperse very far, thereby increasing the relative importance self-recruitment (Graham et al. 2008). Thus, the timing and magnitude of the high mortality phase early in larval life contribute to defining the spatial extent of dispersal. For instance, Connolly and Baird (2010) showed that the inclusion of temporally varying mortality rates (as opposed to a constant rate throughout larval life) in theoretical models of dispersal in corals qualitatively changes the shape of the dispersal kernel by increasing the magnitude of both self-recruitment and long-distance dispersal.

In summary, as in other benthic taxa, patterns of larval dispersal and connectivity in coral species are the product of many physical and biological factors that interact at multiple temporal and spatial scales. Certainly, some are more important than others, but all contribute to defining the shape of dispersal kernels, and all are crucial to make realistic inferences about population connectivity. A PLD of 100 day may mean little if most larvae settle and metamorphose within 20 day, or alternatively do not survive past 20 day due to high larval mortality in nature. Likewise, a pre-competency period of 3 days may not be the most relevant parameter in cases where larvae get immediately washed off the reef and remain in open water for a long period of time. Consideration of all these parameters (and others discussed elsewhere; see Kingsford et al. 2002, Sponaugle et al. 2002, Gawarkiewicz et al. 2007, and Pineda et al. 2007) is critical to advance our ability to understand and explain the patterns of larval dispersal and connectivity observed in the marine systems.

19.2.4 The Special Case of Vertical Connectivity: Mesophotic Populations and the "Deep Reef Refugia" Hypothesis

Interest in deep coral reef communities, particularly mesophotic coral ecosystems (i.e., 30 m to ~80–100 m depending on the location) has increased dramatically in recent years (reviewed in Lesser et al. 2009, Bongaerts et al. 2010a, and Kahng et al. 2010, 2014). With the unparalleled decline of shallow water coral reef ecosystems over the past decades, deep water communities, which may be less susceptible to environmental disturbances such as temperature stress and storm-induced waves relative to their shallower counterparts (see Bongaerts et al. 2010a and references therein), have been hypothesized to provide refugia that may be crucial in the recovery of shallow water populations (Bongaerts et al. 2010a). However, the extent to which MCE may act as refugia for coral reef organisms will largely depend on the degree of connectivity between shallow and deep populations, which remains unknown for many coral taxa (Kahng et al. 2010; Slattery et al. 2011).

To date, few studies have looked at the extent of larval exchange between MCE and shallow populations in coral taxa. However, similar to what has been observed among shallow populations, patterns of vertical connectivity appear to vary among species, and most importantly across locations. For example, populations of the brooding scleractinian *S. hystrix* and its associated algal symbionts sampled along a depth range of 30 m at two reefs in northeast Great Barrier Reef (20 km apart) showed strong genetic segregation across depths within reefs (but not among sites within habitats), suggesting local adaptation to distinct habitats (Bongaerts et al. 2010b). At one of the sites there was no evidence of migration between deep and shallow populations further supporting the occurrence of habitat segregated gene pools (van Oppen et al. 2011a). In contrast, at the isolated Scott Reef system (NW Australia), substantial larval exchange between the deeper and shallower populations of *S. hystrix* was inferred to have occurred recently despite the high genetic differentiation among populations (van Oppen et al. 2011a). This led the authors to suggest mesophotic populations at Scott Reef can aid in the recovery of shallow populations following localized mortality events.

Similar to *S. hystrix*, the degree of vertical connectivity between mesophotic and shallow populations of the broadcast spawner *M. cavernosa* has been inferred to be extremely low at some locations in The Caribbean (e.g., Florida Keys and Little Cayman Island), but high enough to maintain genetic homogeneity in others (e.g., Barbados and Lee Stocking Island) (Brazeau et al. 2013; Serrano et al. 2014) in spite of seemingly moderate to high levels of horizontal connectivity among shallow populations separated by 100–1000 s of kilometers (Nunes et al. 2009; Goodbody-Gringley et al. 2011; Serrano et al. 2014). Interestingly, migration from shallow to intermediate/deep populations appeared to be the most likely scenario of larval exchange in *M. cavernosa* (Serrano et al. 2014). The Caribbean octocoral *Eunicea flexuosa* also has a broad depth range but genetically (and morphologically) distinct shallow and deep water populations across several locations (Prada et al. 2008; Prada and Hellberg 2013). *E. flexuosa* broadcast spawns and Prada and

Hellberg (2013) suggested that the seemingly low level of connectivity between the depth segregated morphotypes was the result of habitat-specific post-settlement selection rather than the result of restricted dispersal or selective settlement. The patterns of depth related differences may, however, be more complex as Kim et al. (2004) found a similar pattern in the Florida Keys, but the morphotypes that they examined do not fit the description of those studied by Prada and Hellberg (Lasker pers. obs.).

Recent work using an individual-based biophysical model of larval dispersal has explored among-species differences in patterns of vertical connectivity in the U.S. Virgin Islands (Holstein et al. 2016). Model simulations, which focused on the broadcast spawner *O. faveolata* and brooder *Porites astreoides*, coupled several aspects of the reproductive biology of each species (e.g., onset of larval competency, PLD and egg/larval buoyancy) with a hierarchy of nested oceanographic models (max. horizontal resolution of 300 m). For both species, the model predicted demographically significant larval contribution of mesophotic populations to their shallower counterparts, but the magnitude of these contributions was higher in *O. faveolata*. Early onset of larval competency in *P. astreoides* ultimately increased local larval retention, thereby reducing vertical connectivity. Furthermore, estimates of mesophotic larval subsidy to shallow reefs were sensitive to depth-specific larval productivity, fertilization success and post-settlement survivorship, which further underline the importance of incorporating these factors in modeling approaches and to the general understanding of dispersal and connectivity in marine systems.

While the notion of MCE refers to warm-water, light-dependent coral ecosystems (Bongaerts et al. 2010a; Kahng et al. 2010), understanding the degree of vertical connectivity among deep and shallow populations of temperate and cold-water coral taxa is equally important. The threats faced by coral reef ecosystems are not exclusive to tropical and sub-tropical regions (although they are the most well studied areas), and therefore warrant better geographic and taxonomic coverage in the literature. Two studies have explicitly addressed the implications of vertical connectivity in non-tropical coral species. Costantini et al. (2011) focused on the azooxanthellate octocoral *C. rubrum*, which has been historically overharvested for use in jewelry (Tsounis et al. 2007) and whose populations more recently have suffered mass-mortality events in the NW Mediterranean (Cerrano et al. 2000; Garrabou et al. 2001). The authors found evidence of a vertical gradient in genetic structuring and diversity at two distinct locations of the western Mediterranean, and identified a critical boundary for larval exchange between "deep" and "shallow" populations at 40–50 m depth. Given the shift in commercial harvesting to deeper populations that followed the ban of shallow water fishing (<50 m) (Tsounis et al. 2013), the implicit low levels of vertical connectivity has major implications for management in this endangered species (Costantini et al. 2011, 2013). In another study in the Mediterranean, Mokhtar-Jamaï et al. (2011) found different patterns of vertical connectivity in the gorgonian *P. clavata* depending on location and depth range. While in some sites restrictions to gene flow were evident, the same was not true in others, which led the authors to suggest that local hydrodynamics and variation in the position of the summer thermocline may strongly influence the patterns of gene flow across depths in *P. clavata*.

19.3 Approaches Used in Connectivity Studies: Mismatches and Caveats

Despite major developments in the methods used to study larval dispersal and population connectivity in marine systems, much of what is known about the scale of dispersal and population connectivity in anthozoans has been inferred from indirect data. Unlike other taxa with a pelagic larval phase (e.g., fish: Jones et al. 1999, 2005; decapod crustaceans: DiBacco and Levin 2000; gastropods: Moran 2000), cnidarians lack the morphological structures that enable the application of most tagging approaches currently used (Jones et al. 2009), thus making it extremely challenging to obtain direct evidence of the full extent of larval dispersal. Genetic approaches, including both indirect and more recently direct (i.e., assignment and parentage tests) methods, as well as coupled biophysical models of larval dispersal, have been the best-available alternatives to study connectivity, but are not without their own shortcomings. Below we briefly discuss some of the limitations inherent to each of those two methods, as well as approaches that have been proposed to improve the accuracy of the data obtained.

19.3.1 Genetic Methods

The effects of isolation, and conversely dispersal, on population genetic structure is one of the fundamental predictions of population genetic theory (Wright 1943). As such, the use of genetic structure as a proxy for dispersal and connectivity has long standing in the literature, and has proved to be highly informative. However, as recently discussed by Selkoe and Toonen (2011), the relationship between life history traits and estimates of connectivity based on population genetic structure have not been as strong as most researchers have expected. PLD, in particular, has been found to exhibit poor correlation with measures of genetic structure, especially F_{ST} (Bradbury et al. 2008; Weersing and Toonen 2009). Population structure, while related to dispersal is not perfectly correlated with dispersal. Thus, the correspondence between population genetic structure and life-history param-

eters also incorporates the imperfect relationship between population structure and dispersal.

F_{ST} and similar metrics have been the most commonly used indices of population structure. F_{ST} is a function of dispersal to populations, survival of those migrants and genetic drift, which is a function of population size and mutation rate. The number of migrants successfully recruiting to a population is proportionate to Nm where N is the effective population size and m the proportion of migrants dispersing to and surviving in that population. When adults are surveyed it provides an estimate that is averaged over time (Hedgecock et al. 2007). However, use of F_{ST} as a simple index of dispersal assumes equilibrium between gene flow (m) and genetic drift, as well as equal and unchanging effective population sizes (Ne). In simulation studies, Faurby and Barber (2012) found that violations of these assumptions, especially unequal Ne, affected F_{ST} and the ability to discern the relationship between F_{ST} and PLD.

Assignment approaches do not necessarily rely on equilibrium assumptions and are particularly attractive when used with individuals known to have recruited within a specific time frame. This approach has been successfully applied to fishes (Hogan et al. 2011; Buston et al. 2012), but has been less successful when applied to anthozoans (Lasker and Porto-Hannes 2015). Critical to the success of such techniques is a genetic landscape that facilitates assignments for which there is a high level of confidence. Lasker and Porto-Hannes (2015) collected octocoral adults from six sites and recruits from four sites along 220 km of the southern edge of the Little Bahama Bank in The Bahamas. Analysis of the adults identified a strong pattern of IBD. However, it was difficult to unambiguously assign recruits to specific source populations because the adult populations were so similar that the probabilities of assignment were similar for multiple source reefs. The lack of certainty in the assignments makes it impossible to determine whether the populations at the sites are demographically open, i.e. receive substantial numbers of recruits from other sources. Such data are critical for developing management plans for species. Assignment approaches also require sampling all potential source populations. Among species exhibiting patterns of IBD or spatial clustering, sampling a relatively small number of locations scattered over great distances could lead to conclusions of high levels of self-recruitment when recruitment from sites 10s or 100s of km distant might be an important component of the species' demography. Distinguishing such patterns is a necessary precursor to designing marine protected areas.

In all of the genetic approaches, the growing availability of massive amounts of genotypic data from next generation sequencing holds the promise of increasing the resolving power of analyses. Seeb et al. (2011) have suggested a trajectory for molecular analyses of population genetics in which the currently used fragment length based techniques will give way first to the use of single nucleotide polymorphisms (SNPs), which are themselves developed using next generation sequencing, and ultimately to next generation sequence based approaches. At this time most studies of cnidarian connectivity are based on microsatellite data (i.e., the many studies already cited). Microsatellite development has been aided tremendously by next generation sequencing (cf., Porto-Hannes and Lasker 2013), but SNPs and next generation sequencing approaches have not been widely used in studies of cnidarian population structure.

Reitzel et al. (2013) used RAD Seq, a so called genotype by sequencing approach, to identify SNPs in populations of the anemone *Nematostella vectensis*. They found patterns of genotypic diversity that were similar to those reported from microsatellite studies, but were able to develop a more highly resolved phylogeography than had been achieved with other markers. A number of authors have identified SNPs in scleractinian species (Meyer et al. 2009; Wang et al. 2009a; Lundgren et al. 2013), and Wang et al. (2009b) demonstrated that SNPs developed from colonies at one site on the GBR could be amplified and often were polymorphic at sites 80 and 570 km distant. Reviews have highlighted the greater precision and power that should be achieved in the detection and estimation of connectivity using the volume of data that can be derived from SNPs and next generation sequencing (Seeb et al. 2011; Willette et al. 2014).

While SNPs and sequence-based analyses are not a panacea, they should increase the power of analyses focused on identifying population structure. Morin et al. (2009) simulated differentiation in two populations with Ne of 1000 and per generation rates of dispersal (m) of 0.05 between two populations. They found that 40 SNPs in a sample of 50 individuals had over 90 % success in recognizing the presence of two populations with F_{ST} of 0.01. Increasing the sample size to 100 individuals and 75 SNPs identified significant differences between populations with an F_{ST} of 0.0025 in 88 % of the simulations. The F_{ST} of 0.0025 was chosen as the level of differentiation expected in demographically independent populations, whereas the higher F_{ST} of 0.01 was the expected for evolutionarily significant units (i.e., one migrant per generation). Heylar et al. (2011) summarize the results of a large number of such analyses and in general report that SNPs can equal or improve the performance of microsatellites for population scale comparisons, i.e. F_{ST} based techniques. Independent of the strengths and weaknesses of the genetic markers and the analyses used, the scale of sampling may also limit, and in some cases bias, our understanding of dispersal. Meirmans (2012) has noted that patterns that appear to be IBD can be created in studies of structured populations in which samples from within clusters that are geographically close, have high genetic similarity, whereas pairwise comparisons of samples from different clusters will exhibit low genetic similarity associated with greater pairwise dis-

tance. Similarly, incomplete sampling over the range of a population can mistakenly portray populations as structured when instead they are part of a continuum linked by stepping stone populations that were not sampled.

The scale of sampling relative to the oceanographic processes that affect dispersal also plays an important role in our understanding of dispersal and connectivity. In all systems, dispersal of propagules is controlled by the time required for a propagule to move/be moved between two locations and the life-history traits controlling the longevity of the dispersive phase. The time required for a propagule to move between two points can be affected by traits of the propagule, but in general a correlation with distance is expected. That of course forms the basis for IBD models, i.e., the expectation that dispersal should be related to the longevity of the dispersive phase. In marine systems, and in particular in coral reef systems, the effects of hydrodynamic processes and the complex geographic settings of reefs distort the relationship between time and dispersal distance. These more complex relationships have been characterized as the seascape and in these systems a form of isolation by distance characterized as isolation by oceanographic distance has been identified in some systems (White et al. 2010; Alberto et al. 2011). Sampling over such dispersal ranges is an even greater challenge, and some patterns characterized as chaotic (*sensu* Selkoe et al. 2014) may reflect these more complex relationships between distance and dispersal time (see Sect. 19.4).

19.3.2 Models of Larval Dispersal

An alternative approach to genetic methods that has been increasingly applied to marine species is the use of biophysical models. The precursors of present-day biophysical models used simple advection-diffusion models that incorporated some element of the species biology (e.g., larval mortality; Cowen et al. 2000). In most modern approaches, however, numerical models of hydrodynamic circulation are coupled with Lagrangian stochastic particle-tracking subroutines (i.e., Individual-Based Models, IBMs) to move and track virtual eggs/larvae over space and time within the model domain (e.g., Heath and Gallego 1998; Baums et al. 2006; Cowen et al. 2006; Guizien et al. 2006; Paris et al. 2007). The IBM framework of these models allows the introduction of individual biological attributes and hence variation in these traits, which as noted throughout the text is of paramount importance in defining dispersal kernels. The spatial and temporal scales contemplated by biophysical models, as well as the ways by which particles are tracked (i.e., algorithm used) vary across models and study sites. However, biophysical models excel at providing a mechanistic understanding of the processes underlying larval dispersal (and hence connectivity), and have been used to address a range of important ecological and evolutionary questions (Cowen et al. 2006; North et al. 2008; Kool et al. 2011; Foster et al. 2012; Guizien et al. 2012; Holstein et al. 2014; reviewed in Miller 2007 and Werner et al. 2007).

In recent years, a number of studies have used biophysical models, sometimes combined with complementary approaches, to make inferences about dispersal and connectivity in coral taxa (Baums et al. 2006; Galindo et al. 2006; Andras et al. 2012; Foster et al. 2012; Golbuu et al. 2012; Torda et al. 2013b; Wood et al. 2013; Holstein et al. 2014). For example, Baums et al. (2006) used a Lagrangian stochastic model of larval dispersal to test the occurrence of a hypothetic present day bio-oceanographic filter to gene flow of *A. palmata* across the Mona Passage, as suggested by empirical genetic data (Baums et al. 2005, 2006). The model, which was parameterized with life-history traits of the species (spawning strategy, PLD and pre-competency period), showed that during the reproductive season of *A. palmata* the flow pattern in the Mona Passage, as well as the development of small-scale eddies in the vicinity of Mona Island, acted as a dynamic filter to larval dispersal preventing most exchange between populations located on each side of the Mona Passage.

In a subsequent study focusing on a different species (*O. annularis*), Foster et al. (2012) combined information on larval dispersal derived from a coupled biophysical model with a theoretical matrix-based genetic model to project patterns of gene flow resulting from larval migration over time in the Caribbean, and compared those predictions with empirical genetic data. The authors found that at a large scale (i.e., Caribbean-wide) the predictions of the genetic projection model were largely congruent with the observed patterns of population genetic structure, indicating that at the regional scale larval dispersal could, in general, explain the patterns of evolutionary connectivity in *O. annularis*. Some disparities between the two approaches were however evident, particularly at finer scales, which as noted by the authors suggest other processes may be at play (e.g., local adaptation and small-scale factors affecting larval survivorship). Interestingly, the genetic projection model identified the development of a genetic break between eastern and western populations of *O. annularis* in the Caribbean, as suggested by the empirical genetic data and work on *A. palamata* (Baums et al. 2005, 2006), but the exact location of this break was ambiguous.

In another study in the Caribbean basin, Holstein et al. (2014) used a multi-scale biophysical model to estimate the connectivity network dynamics between Caribbean populations of two coral species (as well as three fish species) with contrasting reproductive modes and larval traits, the broadcast spawner *O. annularis* and the brooder *P. astreoides*. As expected based on life-history traits, the connectivity net-

works (i.e., source-sink dynamics) were largely species-specific, with *P. astreoides* exhibiting the most fragmented network as a result of the shorter time to competency, which enhances self-recruitment and restricts connectivity among distant populations. The network analyses also revealed that the connectivity networks of the two coral species shared highly central regions that act as stepping-stone locations facilitating multi-generational connectivity between regions (e.g., Bahamian and Cuban reefs), which has important implications for management.

While it is unquestionable that biophysical models are powerful tools that have greatly improved our understanding of dispersal in marine systems, as in all modeling their accuracy is circumscribed by both the model and the data used to generate the simulations. The major drawbacks of biophysical models, as well as the advances needed, have been comprehensively discussed in a plethora of recent studies, including several reviews (Gallego et al. 2007; Leis 2007; Pineda et al. 2007; Werner et al. 2007; Metaxas and Saunders 2009; among others). The discussion that follows is a brief account of where we stand.

A well acknowledged limitation in modeling larval dispersal concerns the physical oceanography itself. Many of the processes governing larval dispersal, including both biological and physical processes, operate at spatial and temporal scales that are currently below the resolution of most models. This limits how accurately larval transport (and hence dispersal) can be modeled (Largier 2003; Gawarkiewicz et al. 2007; Werner et al. 2007). For instance, increasing the resolution of the physical forcing (i.e., reducing the grid size of physical models), along with a finer characterization of the bathymetric features, significantly impacts the spatial variation in velocity fields and the proportion of larval retention obtained in model simulations (Guizien et al. 2006).

Increasing mesh resolution is crucial to resolve small-scale processes, but as pointed out by Pineda et al. (2007), it only solves one aspect of the problem as many processes operating at sub-grid levels (e.g., internal and surface waves, turbulence, frontal convergence, etc.) are still poorly understood and rarely incorporated into physical models, particularly those occurring in the shallow nearshore and reef environments where bathymetry fundamentally alters the nature of water circulation (Gallego et al. 2007; Pineda et al. 2007; Werner et al. 2007). This is of particular relevance for those species (e.g., brooding corals) where dispersal is restricted to small spatial and temporal scales. Fine scale variation in hydrodynamics at the substratum affects settlement and recruitment of larvae in other taxa (Reidenbach et al. 2009; Gaylord et al. 2013; Quinn and Ackerman 2013). However, incorporating processes at these scales will require both their inclusion in the modeling, as well as detailed characterizations of physical features such as bottom roughness and behavioral data, all of which are generally not available. These small-scale processes are not independent from those occurring at larger scales, but simultaneously resolving flow fields across all scales of interest remains a major computational challenge (Werner et al. 2007). Nevertheless, considerable progress has been achieved using methods such as multi-scale nesting of grids or unstructured meshes where high resolution is introduced only in areas where flow is particularly complex (e.g., Lambrechts et al. 2008; Andutta et al. 2012; Holstein et al. 2014).

As the oceanographic modeling matures, parameterization of biophysical models with biological information also needs to improve, first in identifying which variables are important and then in obtaining more realistic data. Several modeling studies have shown that traits such as PLD, onset of larval active movement, larval mortality and swimming behavior all significantly affect dispersal and connectivity (Baums et al. 2006; Cowen et al. 2000, 2006; Guizien et al. 2006; North et al. 2008). Yet, we know very little about how those traits vary in the field or how they are regulated over time and space, particularly in coral taxa. For example, larval swimming behavior, as well as sensing and orientation abilities in response to environmental cues, is relatively well studied in taxa such as fishes and lobsters (Kingsford et al. 2002; Leis 2007), and have been successfully incorporated in some biophysical models of larval dispersal (Paris et al. 2007; Kough et al. 2013; Wolanski and Kingsford 2014). Much less is known about those processes in corals, and benthic invertebrates in general (Metaxas and Saunders 2009). This gap in knowledge limits our ability to include such traits in biophysical models. Incorporation of larval mortality is also a crucial step to explicitly determine the spatial extent over which demographic connectivity can take place (Leis 2007). However, quantifying mortality in the field is extremely difficult. Overall, parameterization of biophysical models with the biological traits of interest must account for natural variation both within and among species as most of these traits vary greatly between taxa (Leis 2007; Metaxas and Saunders 2009). The Lagrangian IBM framework of most present-day models allows the inclusion of such detailed parameterizations. However, it is critical that the biological data is as much accurate as possible and preferentially derived from *in situ* estimates.

Finally, proper validation of model outcomes, which will require a collaborative approach between disciplines, should be a standard component of modeling studies and hence an important step toward "best practices" (Hannah 2007; Werner et al. 2007; Metaxas and Saunders 2009). Although critical, this step is notoriously difficult since we lack direct measures of larval dispersal distance for most taxa, and when data are available there is often a mismatch between the scales of the modeling and the empirical estimates of dispersal and connectivity. However, a number of approaches with

varying levels of accuracy have been implemented in several studies (reviewed in Hannah 2007 and Metaxas and Saunders 2009).

19.4 A Heuristic Perspective of Dispersal

The first rule about dispersal is that there are no laws, only guidelines. The morphologic and behavioral ontogeny of planulae larvae creates a template for dispersal that is then modified by the "seascape", creating a diverse array of dispersal patterns. However, the nature of each species' ontogeny constrains the effects of the seascape on dispersal. We expand on this concept and examine how seascape and biologic traits shape dispersal. We start with a distribution of the time to settlement for the propagules (i.e., eggs and larvae) of a species in the idealized state of no predation/mortality and uniformly available substratum for settlement, which we refer to as Ontogenetic Dispersal Curve (ODC) (Fig. 19.2). In general, the ODC is likely to fit a gamma distribution. However, gamma distributions encompass a large range of curves and the designation of a gamma distribution does not by itself identify a single shape. Each curve has definable traits such as mean, mode, median, variance and skewness, but the value of the PLD is not immediately obvious in these distributions. Is it the mean, median, mode or some "maximum" time to development? Is the "maximum" when the cumulative frequency reaches 95 %, 99 % or greater? Since the concept of PLD was created with the purpose of deducing how far larvae might go, we suggest 99.9 % is a useful cutoff, but that designation is arbitrary. Instead of defining a single value as the PLD, we advocate the identification of specific points on the ODC such as PLD_{50} and $PLD_{99.9}$. As in characterizations of physiologic responses this provides an unambiguous meaning, and which "PLD" is used can be varied without confusion.

Figure 19.2 depicts three ODCs. Curve A is for a species without a pre-competency period and in which propagules rapidly settle and metamorphose. Among anthozoans these are species that brood, and release competent, negatively buoyant larvae that quickly settle and metamorphose. The other two hypothetical curves (B and C) are the ODCs of species with a pre-competency period and thus a temporal delay in settlement. Two different patterns of settlement are depicted with one species (C) exhibiting a greater PLD_{50} and substantially greater $PLD_{99.9}$. A broadcast spawning coral whose planulae take several days to develop and subsequently settle over perhaps a week would have an ODC depicted in Curve B, while a species whose planulae were even longer lived would have an ODC similar to Curve C.

The ODC has time as its abscissa. In conditions of a unidirectional, constant current (i.e., flow field) the ODC can be simply translated from a time dependent function to the species' distance dependent dispersal kernel by calculating distance as the product of the time from spawning and the current velocity. However, larvae in the water column are not subject to constant current velocities. Thus, the distance traveled will not be a simple linear function of time. Additionally, flow fields are not unidirectional and the path a larva follows, i.e., the oceanographic distance (larval transport *sensu* Pineda et al. 2007), is inherently different from the Euclidean dispersal distance that would be derived from a map view of where dispersing larvae settle. The numbers of larvae settling at a given distance will also include the effects of mortality and whether or not there is suitable habitat available. Whether a "settling" propagule can delay settlement and metamorphosis will further affect the number settling. In concert with the ODC, current patterns, water column mor-

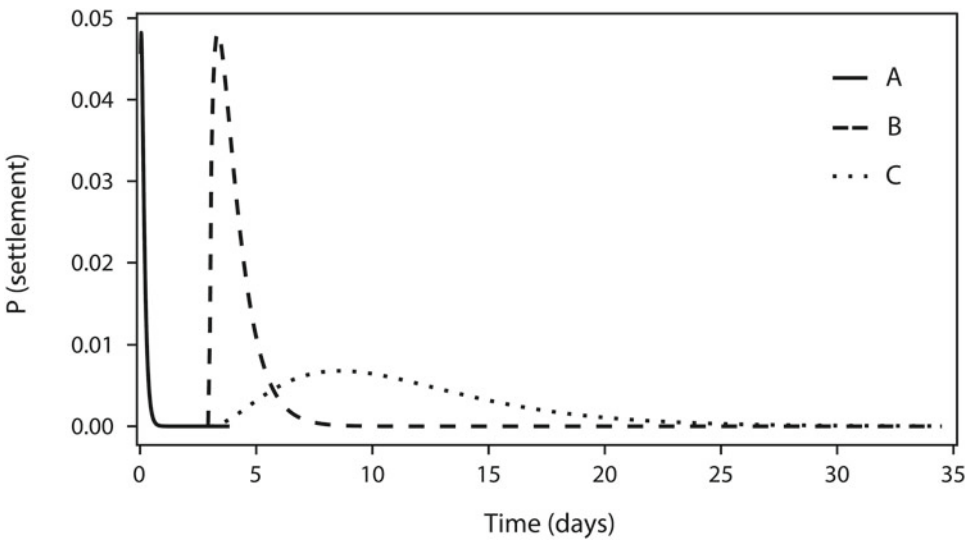

Fig. 19.2 Ontogenic distribution curve, the instantaneous probability of a planula settling as a function of time for three hypothetical species with 0 day (*A*) and 3 day (*B* and *C*) pre-competency periods, and different probability functions. PLD_{50} and $PLD_{99.9}$ for the three curves are: (*A*) 0.1 and 0.7 days; (*B*) 3.8 and 8.7 days; and (*C*) 10.5 and 34.5 days

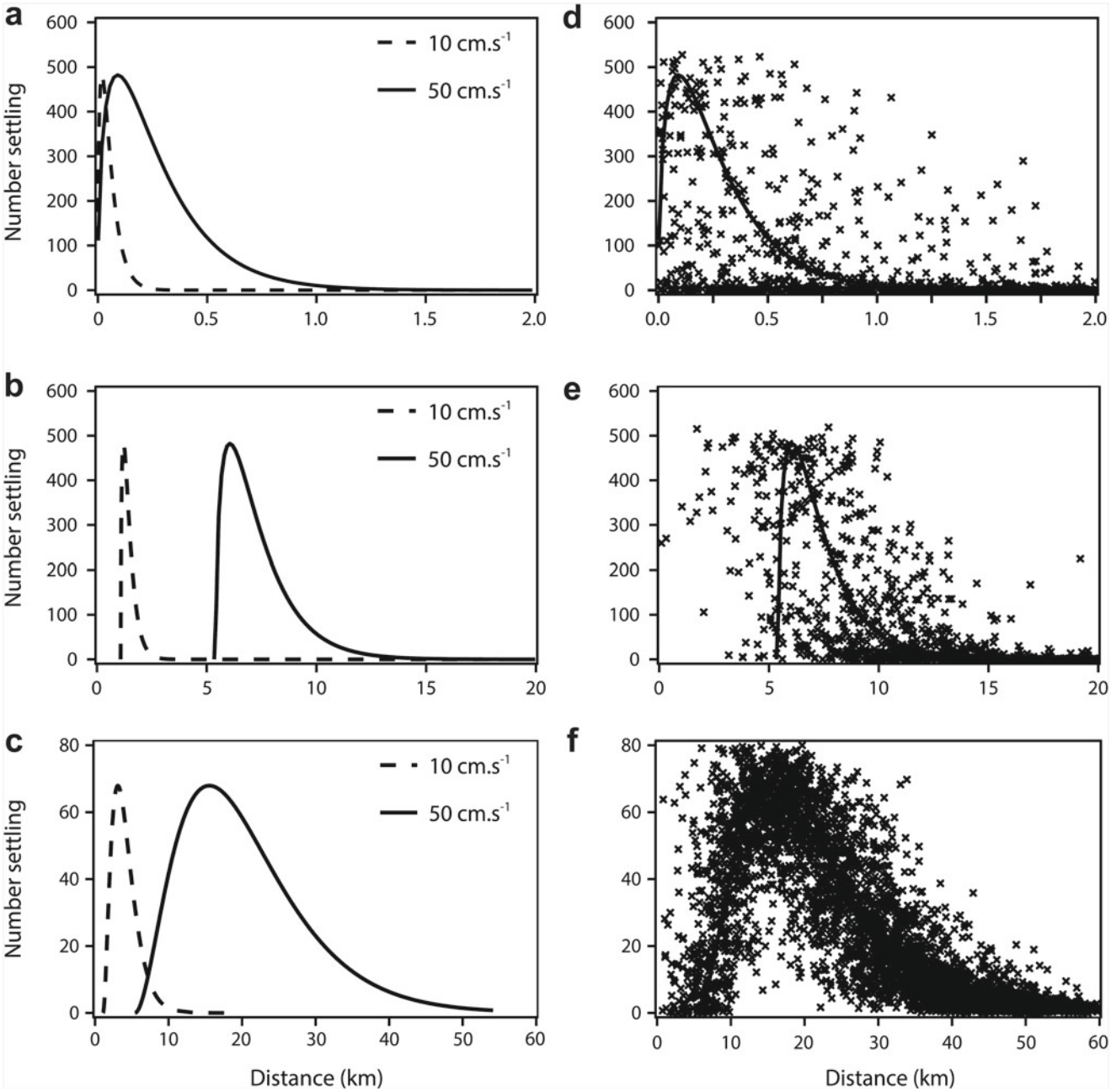

Fig. 19.3 Dispersal of 10,000 larvae based on the gamma distributions from Fig. 19.2. *Left panel* (**a–c**) represent dispersal kernels based on the ODC and assuming constant unidirectional currents of 10 cm•s^{-1} or 50 cm•s^{-1}. *Right panel* (**d–f**) represent the dispersal kernels for 50 cm•s^{-1} flow, with random variation added to both distance traveled and proportion settling. Note the differences in scale

tality, biologic traits, as well as the distribution of habitat and the quality of those habitats create the dispersal kernel. Variation in the environmental factors (i.e., the seascape) means the dispersal kernel for a species can vary dramatically between locations, years and seasons. Although the shape of the dispersal kernel will not necessarily resemble that of the ODC, the ODC constrains the range of modification and creates most of the generalities that are discussed in evaluations of dispersal patterns.

We illustrate the interaction between the seascape and the ODC in Fig. 19.3. Figure 19.3a–c depicts the dispersal kernels of the three hypothetical ODCs from Fig. 19.2 plotted as a function of dispersal distance each under different unidirectional flow velocities (10 and 50 cm•s^{-1}) with all else omitted (i.e., mortality, biological traits, etc.). In Fig. 19.3a, the very short time in the water column translates into short dispersal distances and philopatry, and even at the 2 km scale used it would be difficult to discern the difference in the dis-

persal kernels for 10 and 50 cm•s^{-1} currents. The pattern is further obscured in Fig. 19.3d, in which random variation in distance traveled and in the proportion settling is added to the curves. However, neither different current velocities nor additional random variation obscures the association between rapid development and philopatry.

Among species whose larvae spend more time in the water column (ODC curves B and C; Fig. 19.2), the effects of the seascape are substantially greater. Although the overall shape of the ODC is preserved, larval transport at different current velocities substantially affects the dispersal kernel (Fig. 19.3b, c). The addition of random variation (Fig. 19.3e, f) begins to obscure the curves, even in this overly simplified rendition. Finally, recognizing that subsets of planulae can follow different trajectories with different net flow velocities, is represented in Fig. 19.4, and where a single ODC (Fig. 19.2, Curve B) is used to generate dispersal patterns in which half of the planulae were transported by a net flow velocity of 10 cm·s^{-1} and the other half by a flow of 50 cm·s^{-1}. In nature, the patterns will be even more complex, but the point we wish to illustrate is that complex seascapes can very effectively decouple the dispersal kernel from the pattern suggested by the ODC. Furthermore, reefs are not continuously distributed and gaps in which there can be no settlement will also occur. That will further distort the dispersal kernel. If planulae can delay settlement in the absence of suitable substrate then dispersal will be pushed to greater distances, or alternatively dispersal distances may exhibit a multi-modal pattern that simply corresponds to the distribution of suitable habitats.

In sum, this view of dispersal is consistent with the pattern often observed among brooding species with short-lived larvae that have short dispersal ranges and philopatric distributions. In contrast, for those species with propagules that are not immediately competent to settle, and whose pre-competency periods, as well as the overall shape of the ODC and resultant PLD differ, the range of outcomes will vary dramatically both between species and seascapes. In other words, it is unlikely that we would find a single pattern among all broadcast spawning species, nor a simple relationship between the dispersal kernel and PLD. This leaves open the issue of the extent to which it is variation in the traits of the species or the seascape that accounts for variation among species. Such analyses can only be conducted with careful characterizations of the ODC, development of biophysical models that incorporate the seascape, data-based validation of those models and then finally sensitivity analyses to determine how dispersal is altered by changes in aspects of the ODC or seascape.

19.5 Concluding Remarks

Dispersal in marine systems, how larvae can travel 100s or 1000s of kilometers, and the implications of those events have always been of interest to invertebrate zoologists and biogeographers. It is only in the past 30–40 years that ecologists have considered it as a process that can affect the demographics of populations and metapopulations, and a process that undoubtedly affects their evolution. Further piquing our interest has been the overall concern about the future of coral reef communities and the recognition that dispersal plays a key role in the resilience of populations. Although the volume of work on anthozoans is not as great as that for fishes, there has been a continuously growing body of research assessing connectivity among anthozoan populations based on genetic, and more recently modeling approaches.

The overall status of our understanding of dispersal is that of a glass that is half-empty and half-full. For those inter-

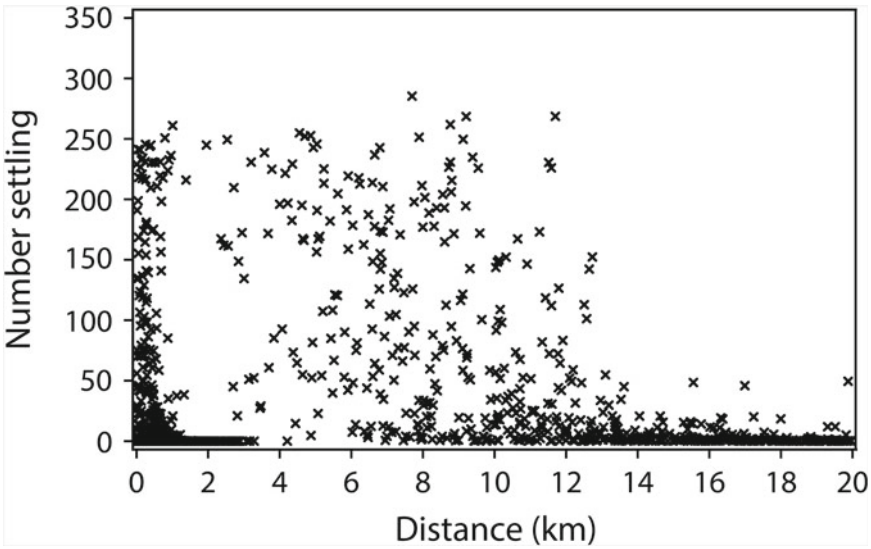

Fig. 19.4 Dispersal of 10,000 larvae of a species with ODC curve B (Fig. 19.2) in which half of the larvae disperse at an average net velocity of 10 cm·s^{-1} and the other half at 50 cm·s^{-1}, with random variation in distance and proportion settled added to each point

ested in the extent to which dispersal affects local population dynamics and population resilience the glass is half empty. The large list of references in this chapter, as well as those in the reviews that have preceded this one, attest to the tremendous amount that we do know. However, it is also clear that conclusions regarding dispersal are often species specific and almost always location specific. Perhaps the greatest illustrations of this are the array of results that have been reported from different locations for the scleractinian *S. hystrix* (e.g., Ayre and Hughes 2000; Maier et al. 2005; Underwood et al. 2007; van Oppen et al. 2008), and the differences in patterns between the sympatric congeners *O. annularis and O. faveolata* (Severance and Karl 2006). We can make broad generalities such as brooders are more likely to be sensitive to population crashes and the loss of stepping stone habitats, but community wide assessments of which species will or will not be affected by such events will require detailed information about the seascape and life-history of individual species.

The glass is half-full for those interested in understanding how life-history traits affect dispersal and how those traits have evolved in different groups. Implicit to the recognition that dispersal and connectivity affect population dynamics is the recognition that it also is a selection pressure. However, connectivity is not itself a trait. It is the product of the developmental, morphologic and behavior traits of individuals, i.e. the life history. Natural selection operates on life-history traits, and the fundamental question is whether there are predictable combinations of traits that promote (or retard) dispersal. The question has long standing in the literature (Thorson 1946, 1950; Vance 1973a, b; Strathmann 1978; Palmer and Strathmann 1981; Strathmann et al. 2002), and there are generalities that start with the expectation that planktotrophic larvae should be able to disperse over greater distances than lecithotrophic larvae. However, that pattern is not universal and as the study of dispersal has grown, the pattern of generalities with many exceptions continues. For instance, the conventional assertion that PLD is a reliable predictor of patterns of dispersal has many exceptions. Among the anthozoans, as briefly discussed here and in the reviews that have preceded us (cf. Jones et al. 2009), there are some common patterns that relate to life histories. Extreme cases of larval philopatry are predominantly more common in brooding species where the very short period of time the larvae spend in the water column (i.e., narrow ODC) constrains the range of distortions the seascape can have on the dispersal kernels. In contrast, moderate to large-scale dispersal is seemingly more common in broadcast spawners in which a pre-competency period and variation in the period over which larvae settle (i.e., $PLD_{99.9}$) provide a template for those processes to act. In between these extremes the divide is less clear (if it exists at all) and complex patterns of connectivity appear widespread among both brooders and broadcast spawners. Data from more species will make the presence or lack of pattern more evident, but in exploring the effects of life history on dispersal, real world data are restricted to the combinations of life-history traits found in nature. Biophysical models have the potential of enabling us to compare *in silico* life histories that do not exist in nature and thus fully explore the adaptive space.

In sum, there still are many gaps in our understanding of dispersal of anthozoan species, both in terms of the processes driving dispersal and in taxonomic coverage. Genetic analyses and biophysical modeling are filling those gaps, but many challenges need to be overcome. With respect to genetic approaches, the outstanding question will be whether the massive amounts of data that will come from next generation sequencing approaches will increase the spatial and temporal resolution of analyses sufficiently to address questions of demographic scale connectivity (the relevant scale for management). Biophysical models have become increasingly sophisticated and powerful, but we often lack the data to fully characterize dispersal in those models, and we often do not know the sensitivity of model outcomes to that lack of information. It will be crucial to match the scales of both approaches, improve the quality of the hydrodynamic and biological data used to parameterize biophysical models, as well as to conduct proper validation of the model outcomes. Parameterization, in particular, will require careful consideration of all the relevant parameters known to influence the outcome of dispersal, including (but not limited to) the onset and duration of competency to settle, timing and frequency of spawning or planulae release, larval mortality, larval swimming and settlement behavior, and distribution of suitable habitat (see Table 19.2). Careful consideration of the seascape will also be critical. While validation of biophysical models is notoriously difficult to accomplish because of the lack of direct evidence of the scale of dispersal in anthozoans (Jones et al. 2009; Metaxas and Saunders 2009), the development and use of fine scale genetic analyses may provide validation of biophysical models at the temporal and spatial scales that we are most interested in.

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Population Dynamics of Temperate Corals in a Changing Climate

20

Erik Caroselli and Stefano Goffredo

Abstract

In contrast with the number of studies on tropical species, analyses of the variation of growth parameters with environmental variables in temperate areas are very scarce. Notwithstanding the importance of obtaining information on coral population dynamics, few studies have quantified demographic parameters of scleractinian corals, partly because of the processes of fragmentation, fusion and partial colony mortality, which cause corals of similar size to be of widely different ages, thus distorting the age-size relationships. Available literature on growth and population dynamics of natural populations of temperate scleractinians is reviewed in the present work. As general trends, it seems that: (1) solitary species have a definite growth pattern, in contrast with colonial species; (2) symbiotic species are more sensitive to increasing temperatures and more vulnerable to global warming; (3) non-symbiotic species are more tolerant to increasing temperature, but may be negatively affected by the indirect effects of increasing solar radiation; and (4) even if the energy resulting from photosynthesis may increase as a consequence of ocean acidification, the growth and abundance of symbiotic corals seem to be negatively affected by acidification and the negative response of non-symbiotic corals is expected to be even stronger.

Keywords

Astrangia • Azooxanthellate • *Balanophyllia* • *Caryophyllia* • *Cladocora* • Definite growth • Demography • Field studies • Global warming • Growth • *Leptopsammia* • Natural populations • Ocean acidification • *Oculina* • pH • Temperate corals • Temperate scleractinians • Temperature • Zooxanthellate

20.1 Introduction

The variation of temperature and solar radiation associated with latitude plays a major role in determining the global distribution of corals and coral reefs (Kleypas et al. 1999). Latitude is the main factor associated to the variation of light and temperature (Kain 1989), which strongly influence the growth, physiology, and demography of coral species (Kleypas et al. 1999; Lough and Barnes 2000). As a general trend, coral growth decreases when moving from the equator to the poles, up to a limit beyond 30°N and 30°S, where erosion exceeds coral growth and reef development no longer occurs (Kinsey and Davies 1979). Coral "growth" is defined as a composite of three related characters: net calcification rate, skeletal bulk density, and linear extension rate (net calcification rate = skeletal bulk density x linear extension rate; Lough and Barnes 2000; Carricart-Ganivet 2004). In order to study the effects of environmental parameters on coral growth, assessing all the three components is necessary, since none of the three is a perfect predictor of the other two (Dodge and Brass 1984). The three variables have been analyzed in the tropical genera *Porites* (Lough and Barnes 2000;

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Cooper et al. 2008; Tanzil et al. 2009, 2013; Elizalde-Rendon et al. 2010; Fabricius et al. 2011; Morgan and Kench 2012; Shi et al. 2012; Crook et al. 2013; Cantin and Lough 2014), *Montastraea* (Carricart-Ganivet 2004; Helmle et al. 2011), *Diploastrea* (Cantin et al. 2010), *Pocillopora*, *Pavona*, *Gardineroseris* (Manzello 2010; Morgan and Kench 2012), *Acropora*, *Lepstastrea*, *Hydnophora*, and *Fungia* (Browne 2012; Morgan and Kench 2012), *Montipora* and *Turbinaria* (Browne 2012), *Siderastrea* (Carricart-Ganivet et al. 2013), and *Orbicella* (Manzello et al. 2015) where their variation has been linked to changes in temperature and solar radiation associated with latitude, or with pH variations at CO_2 vents. While there are many researches about growth parameters in relation to environmental variables in tropical genera, temperate areas are still poorly studied (e.g. Howe and Marshall 2002). The three growth parameters of only four scleractinian species have been studied in the field in temperate regions, all of them along temperature or pH gradients in the Mediterranean Sea (Goffredo et al. 2009; Caroselli et al. 2012a, 2016b; Kružić et al. 2012; Fantazzini et al. 2015).

Since the 1970s, the structure and dynamics of scleractinian populations have been related to environmental parameters and to the symbiosis with zooxanthellae, unicellular algal symbionts of the genus *Symbiodinium* (Grigg 1975; Loya 1976; Buddemeier and Kinzie 1976; Bablet 1985; Hughes and Jackson 1985). In fact, analyzing the demography of coral populations may reveal relationships between the organisms and their environment, and can give information on habitat stability and suitability (Grigg 1975; Bak and Meesters 1998; Meesters et al. 2001; Goffredo et al. 2004, 2008, 2010; Caroselli et al. 2012b). Moreover, actions to restore degraded or damaged coastal areas can benefit of information on population turnover (Connell 1973; Rinkevich 1995; Chadwick-Furman et al. 2000; Goffredo and Chadwick-Furman 2003; Knittweis et al. 2009). Notwithstanding their importance, few studies have quantified population dynamics parameters of scleractinian corals, partly because of the processes of fragmentation, fusion and partial colony mortality, which cause corals of similar size to be of widely different ages, thus distorting the age-size relationships (Hughes and Jackson 1985; Babcock 1991). The scarce studies on population dynamics of scleractinian corals were reviewed in 1973, describing their growth and survivorship (Connell 1973). Since then, demography has been studied for some species in the Red Sea (Loya 1976; Chadwick-Furman et al. 2000; Goffredo and Chadwick-Furman 2003; Glassom and Chadwick 2006; Guzner et al. 2007), Pacific (Fadlallah 1983; Nozawa et al. 2008; Roth et al. 2010; Knittweis et al. 2009; Thresher et al. 2011), Caribbean (Hughes and Jackson 1985; Johnson 1992; Meesters et al. 2001; Vermeij 2006; Goffredo and Lasker 2006, 2008; Green et al. 2008; Vermeij and Sandin 2009; Edmunds 2010, 2015; Vardi et al. 2012), Great Barrier Reef (Babcock 1991; Anderson and Pratchett 2014), Southwestern Atlantic (Lins de Barros and Pires 2006), China and Japan Seas (Zhao et al. 2014; Muko et al. 2014; Bramanti et al. 2015), Persian Gulf (Bauman et al. 2013; Foster and Foster 2013), and the Mediterranean (Goffredo et al. 2004, 2008, 2010; Shenkar et al. 2005; Teixido et al. 2011; Caroselli et al. 2012b, 2016a; Kersting et al. 2014). The growth of modular organisms can be modeled through the replication, growth and death of the modules (Harper 1977), and studies of modular growth have often focused on plasticity of form and the complexity of both the growth and the population dynamics of these organisms (Hughes and Jackson 1985; Babcock 1991; Hughes 1989).

In coral species whose individuals rarely fragment or fuse, and where partial colony mortality can be recognized by growth anomalies, it is possible to reliably determine coral age (Babcock 1991; Chadwick-Furman et al. 2000). The growth and dynamics of modular organisms that match these prerequisites can be analyzed using age-based models applied to colony morphology (Grigg 1975; Hughes 1989; Goffredo and Lasker 2006). Hence, for some species growth and population dynamic models based on age can be applied to describe demographic characteristics (Grigg 1974; Ross 1984; Chadwick-Furman et al. 2000; Goffredo and Chadwick-Furman 2003; Goffredo et al. 2004, 2008, 2010; Caroselli et al. 2012b). In some solitary corals, age estimates can be easily obtained by counting the externally visible growth bands (Chadwick-Furman et al. 2000; Goffredo and Chadwick-Furman 2003). Growth band analysis has been used to determine the age of colonial scleractinian and gorgonian corals (Knuston et al. 1972; Logan and Anderson 1991; Goffredo and Lasker 2006), and in solitary forms (Chadwick-Furman et al. 2000; Goffredo and Chadwick-Furman 2003; Goffredo et al. 2004, 2008, 2010; Caroselli et al. 2012b, 2016a).

20.2 Growth in the Field

The three components of growth have been measured in the field in only four species of temperate scleractinians, all of them from the Mediterranean Sea: *Cladocora caespitosa* (previously included in the Family Faviidae, now changed to Oculinidae; Fukami et al. 2008), *Balanophyllia europaea* and *Leptopsammia pruvoti* (Family: Dendrophylliidae), and *Caryophyllia inornata* (Family: Caryophylliidae). Available literature on field growth of temperate scleractinians is shown in Table 20.1.

Table 20.1 Available literature on the growth of natural populations of temperate scleractinians

Reference	Species	Study site
Fadlallah (1983)	*Balanophyllia elegans*	1
Schiller (1993a)	*Cladocora caespitosa*	2
Miller (1995)	*Oculina arbuscula*	3
Peirano et al. (1999)	*C. caespitosa*	4
Peirano et al. (2001)	*C. caespitosa*	4
Kružić and Požar-Domac (2002)	*C. caespitosa*	5
Goffredo et al. (2004)	*Balanophyllia europaea*	6
Peirano et al. (2004)	*C. caespitosa*	4
Goffredo et al. (2007)	*B. europaea*, *Leptopsammia pruvoti*	6, 7, 8, 9, 10, 11
Dimond and Carrington (2007)	*Astrangia poculata*	12
Goffredo et al. (2008)	*B. europaea*	6, 7, 8, 9, 10, 11
Goffredo et al. (2009)	*B. europaea*	6, 7, 8, 9, 10, 11
Goffredo et al. (2010)	*L. pruvoti*	6
Caroselli et al. (2011)	*B. europaea*, *L. pruvoti*	6, 7, 8, 9, 10, 11
Teixido et al. (2011)	*L. pruvoti*, *Caryophyllia inornata*	13, 14
Kersting and Linares (2012)	*C. caespitosa*	15
Caroselli et al. (2012a)	*L. pruvoti*	6, 7, 8, 9, 10, 11
Caroselli et al. (2012b)	*L. pruvoti*	6, 7, 8, 9, 10, 11
Kružić et al. (2012)	*C. caespitosa*	17
Caroselli et al. (2015a)	*C. inornata*	6, 7, 8, 9, 10, 11
Fantazzini et al. (2015)	*B. europaea*	18
Caroselli et al. (2016a)	*C. inornata*	6, 7, 8, 9, 10, 11
Caroselli et al. (2016b)	*C. inornata*	6, 7, 8, 9, 10, 11

For each reference, the studied species and the codes of the study sites used in Fig. 20.1 are indicated

20.2.1 *Balanophyllia elegans*

The first published record of a temperate scleractinian whose growth has been measured in a natural population is on the species *B. elegans*. The linear extension rate of this solitary, non-zooxanthellate, and dendrophylliid coral was measured in the North Pacific Ocean (Table 20.1; Fig. 20.1), where it is very variable but homogeneous among size classes, with an average value of 0.6 mm year^{-1} (Fadlallah 1983). This contrasts with a previous unpublished investigation of the same species at the same site, where growth rate is reported to decrease with increasing age (Gerrodette 1979). This difference has been interpreted in terms of differential growth between horizontal and vertical growth, and it has been proposed that volume increase, rather than linear extension, is a better estimator to model the growth of this species (Fadlallah 1983). Even if this was true, the measure of volume during field studies may be difficult and more time consuming than the measure of a single linear dimension and was rarely used after this pioneering study.

20.2.2 *Cladocora caespitosa*

The first temperate and colonial scleractinian whose growth has been measured is *C. caespitosa*. The linear extension rate of single polyps of this Mediterranean endemic and zooxanthellate coral that is able to form wide bioherms (Zibrowius 1982; Schuhmacher and Zibrowius 1985; Schiller 1993b; Kružić and Požar-Domac 2003) has been measured in the Adriatic Sea (Table 20.1; Fig. 20.1), where it spans 1.9–6.2 mm year^{-1} (Schiller 1993a; Kružić and Požar-Domac 2002; Kružić et al. 2012). Even if highly variable, similar values (1.3–6.1 mm year^{-1}) have been obtained by analyzing the annual growth bands on X-ray prints of polyps from the Ligurian and Adriatic Seas, and from the Columbretes Islands in Spain (Table 20.1; Fig. 20.1; Peirano et al. 1999; Kružić and Požar-Domac 2002; Kersting and Linares 2012). The species deposits one high density band in winter and one low density band in summer (Peirano et al. 1999), confirming the proposed trend for temperate scleractinians (Highsmith 1979). The summer low density band in *C. caespitosa* is produced when the density of the deposited skeleton, the linear extension rate, and consequently the calcification rate (Lough and Barnes 2000; Carricart-Ganivet 2004) are lower, while the opposite occurs in winter for the high density band (Peirano et al. 1999, 2005). The same pattern is proposed for all temperate scleractinians, but several clues suggest that banding patterns may also be related to factors other than temperature, such as light, the seasonal heterotrophy/autotrophy budget variation, and/or other factors (Miller 1995; Peirano et al. 1999, 2005). The three

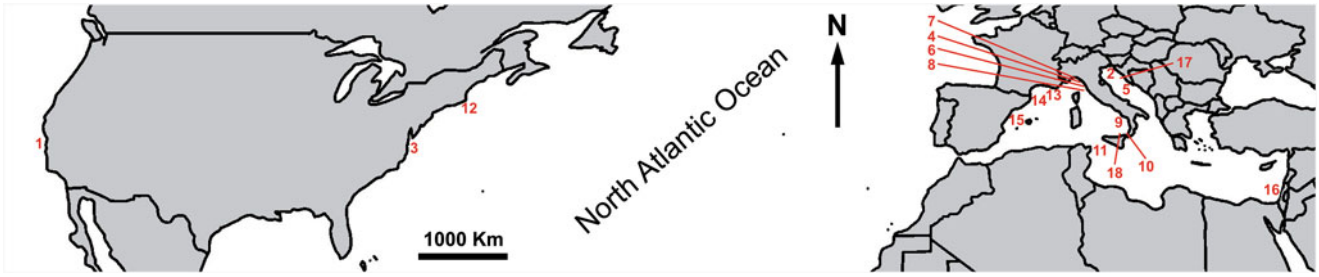

Fig. 20.1 Map of the sites where field studies on growth and population dynamics of temperate scleractinians available in the literature have been performed. ***1*** Hopkins Marine Life Refuge, North Pacific Ocean, California, USA (36° 37′ N, 121° 53′ W); ***2*** Bay of Piran, Northern Adriatic Sea, Slovenia (45° 31′ N, 13° 33′ E); ***3*** Radio Island Jetty, North Atlantic Ocean, North Carolina, USA (34° 42′ N, 76° 41′ W); ***4*** La Spezia region, Ligurian Sea, Italy (44° 05′ N, 9° 45′ E); ***5*** Veliko Jezero, Southern Adriatic Sea, Croatia (42° 46′ N, 17° 21° E); ***6*** Calafuria, Ligurian Sea, Italy (43° 27′ N, 10° 21′ E); **7**: Genova, Ligurian Sea, Italy (44° 20′ N, 9° 8′ E); ***8*** Elba Island, Northern Tyrrhenian Sea, Italy (42° 45′ N, 10° 24′ E); ***9*** Palinuro, Central Tyrrhenian Sea, Italy (40° 2′ N, 15° 16′ E); ***10*** Scilla, Southern Tyrrhenian Sea, Italy (38° 1′ N, 15° 38′ E); ***11*** Pantelleria Island, Strait of Sicily, Italy (36° 45′ N, 11° 57′ E); ***12*** Jamestown and Narragansett, North Atlantic Ocean, Rhode Island, USA (41° 28′ N, 71° 21′ W and 41° 27′ N, 71° 27′ W); ***13*** Riou Archipelago, Balearic Sea, France (43° 10′ N, 5° 23′ E); ***14*** Medes Islands, Balearic Sea, Spain (42° 3′ N, 3° 13′ E); ***15*** Columbretes Islands, Balearic Sea, Spain (39° 53′ N, 0° 41′ E); ***16*** Sdot Yam, Levantine Sea, Israel (39° 29′ N, 34° 53 E); ***17*** 25 locations along the whole Croatian coasts, Adriatic Sea, Croatia (from 45° 6′ N, 13° 36′ E to 42° 45′ N, 17° 25′ E); ***18*** Panarea Island, Southern Tyrrhenian Sea, Italy (38° 38′ N, 15° 6′ E)

components of growth have been recently analyzed in populations of this species spanning three degrees of latitude along the whole Croatian coasts in the Adriatic Sea, characterized by different temperature regimes spanning 4 °C of average annual temperature (Kružić et al. 2012). While skeletal bulk density resulted inversely related to temperature (going from 0.0013 to 0.0012 g mm^{-3}), linear extension (going from 3.0 to 4.0 mm $year^{-1}$) and net calcification rates (going from 0.0034 to 0.0057 g mm^{-2} $year^{-1}$) result positively correlated to temperature (Kružić et al. 2012). *C. caespitosa* seems well adapted to winter conditions, characterized by low solar radiation and high zooplankton availability, while the summer appears as a period of stress and starvation due to the scarce nutrients and lower photosynthetic efficiency of the zooxanthellae, caused by high solar radiation and temperature (Peirano et al. 2005). The net calcification rate of this species (0.004–12.8 Kg $CaCO_3$ m^{-2} $year^{-1}$), even if very variable among sites, depths and colonies, comprises values comparable with the calcification potential of tropical corals, making this species a major carbonate producer among Mediterranean organisms (Peirano et al. 2001; Kružić et al. 2012). Moreover, fossil specimens recorded in their skeleton information on the environmental conditions experienced in the past, making this species a high-resolution proxy for analyzing the changes in Mediterranean climate from short (annual) to long (millennial) timescales (Peirano et al. 2004, 2009; Silenzi et al. 2005). Mass mortality events of this species and other benthic organisms have been observed in the Mediterranean during summer periods characterized by prolonged excessive temperatures (Rodolfo-Metalpa et al. 2000; Cerrano et al. 2000; Garrabou et al. 2001, 2009; Jiménez et al. 2014).

20.2.3 *Oculina arbuscula*

The first study in the Atlantic Ocean (Miller 1995) was performed on the colonial, facultatively zooxanthellate oculinid *Oculina arbuscula*, which is endemic to the North and South Carolina, USA (Ruppert and Fox 1988; Table 20.1; Fig. 20.1). Growth has been measured as wet mass percentage increment, thus resulting in an estimate of the relative net calcification rate (Miller 1995). A higher growth was reported in summer than in winter, and at shallow depths (1 m) relative to deeper sites (6 m), as expected for tropical species (Miller 1995).

20.2.4 *Balanophyllia europaea*

The first study on a Mediterranean solitary scleractinian has been performed on the endemic, zooxanthellate, solitary coral *B. europaea* at Calafuria, on the Italian coasts (Table 20.1; Fig. 20.1; Goffredo et al. 2004). Growth was measured both in the field, by repeatedly measuring linear extension, and by dating corals after counting the annual growth bands on computerized tomography (CT) scans (Kenter 1989; Logan and Anderson 1991; Bosscher 1993; Goffredo et al. 2004). The normal X-ray technique used for tropical massive species (Barnes and Lough 1989) did not allow the visualization of bands in this small, solitary, and common species found in Mediterranean rocky shores, as well as in some tropical corals with complex growth forms (Cantin et al. 2010). For *B. europaea*, growth has been modeled with the von Bertalanffy age-length growth function, which is characterized by a decreasing linear extension rate as coral age increases (von

Bertalanffy 1938; see Pauly 1984 and Sparre et al. 1989 for derivation of the von Bertalanffy curve parameters). In fact, Mediterranean solitary corals have a decreasing linear extension rate as they grow old, as opposed to the common trend of a constant growth rate throughout the entire lifespan for colonial corals (Goffredo et al. 2004, 2008, 2010; Caroselli et al. 2012b, 2016a). While field measurements of linear extension are rather accurate, they consider growth increments on a relatively small timescale (months). Instead, growth band count accuracy is limited by the resolution of the scan, but it records the growth rate during the entire lifespan of the animal. The growth curves resulting from field measurments and from CT scan analysis were very similar, thus indicating that the two growth assessment methods were comparable (Goffredo et al. 2004). This preliminary analysis has shown to be fundamental, since further analyses of growth in different populations (Goffredo et al. 2008) may be performed by using only the CT scan technique, thus saving funds and time that would be required for in situ measurements in distant locations. *B. europaea* was the first temperate scleractinian species whose all growth components (skeletal bulk density, linear extension rate and net calcification rate) have been measured (Goffredo et al. 2009). Along a latitudinal gradient of temperature spanning seven degrees of latitude, populations of this species exibithed a decrease in skeletal bulk density (from 0.0020 to 0.0009 g mm^{-3}; −53.3 %) and net calcification rate (from 0.0029 to 0.0010 g mm^{-2} $year^{-1}$), while the average linear extension rate remained constant (1.16 mm $year^{-1}$), with increasing temperature (Table 20.1; Fig. 20.1; Goffredo et al. 2007, 2008, 2009). A fine-scale analysis of the components of skeletal density (bulk density, micro-density and porosity, sensu Bucher et al. 1998) indicated that the observed decrease of skeletal bulk density with temperature (Goffredo et al. 2007) depends on an increase in skeletal porosity, while the density of calcium carbonate composing it remains constant (Caroselli et al. 2011). Time-domain nuclear magnetic resonance investigations revealed that the increase in porosity is due to the increase of the number and size of larger pores (>10–20 μm; Fantazzini et al. 2013), and nanoindentation techniques measured a consequent decrease in the mechanical resistence (stiffness) of the skeletons to physical stress (Goffredo et al. 2015). Even if this species is zooxanthellate, temperature has always shown a higher effect on the variation of biological parameters than solar radiation did (Goffredo et al. 2007). The main hypothesis to explain these negative effects of temperature on growth of *B. europaea* states that the photosynthesis of the zooxanthellae may be inhibithed by high temperatures (Howe and Marshall 2002; Al-Horani 2005) causing a cascading inhibition effect on the calcification process (Gattuso et al. 1999; Al-Horani et al. 2005), which would result in a lower calcification rate and more porous skeletons (Goffredo et al. 2007, 2008, 2009; Caroselli et al. 2011). This hypothesis has been recently supported by experimental evidence (Caroselli et al. 2015b), but other explanations related to differences in nutrients availability between study sites may contribute to the observed trends (Goffredo et al. 2007, 2008, 2009; Caroselli et al. 2011). Today, there is concern for the future of this endemic species in the light of the expected increase in global seawater temperatures (Goffredo et al. 2008, 2009), and, as well as for *C. caespitosa*, mass mortality events of the species have been observed in the Northwestern Mediterranean during summer periods characterized by prolonged temperatures values above the normal ones (Rodolfo-Metalpa et al. 2000; Cerrano et al. 2000; Kružić and Popijač 2015). Recently, *B. europaea* populations naturally living along an acidification gradient have been analyzed at underwwater CO_2 vents. While linear extension does not vary with acidification, the skeletal bulk density and net calcification rate decrease, as a consequence of the increasing porosity (Fantazzini et al. 2015). The resuting skeletons are less resistant to mechanical stress (Fantazzini et al. 2015), thus partially explaining the decline in population abundance of the species with increasing acidity (Goffredo et al. 2014).

20.2.5 *Leptopsammia pruvoti*

The solitary non-zooxanthellate *L. pruvoti*, closely related to *B. europaea*, was analyzed in the same sites as the latter species. Even if the two species share part of their bathymetric distribution (0–40 m for *B. europaea*, 0–70 m for *L. pruvoti*), *L. pruvoti* lives in the shadows on the vaults of caves and crevices, while *B. europaea* colonizes open and illuminated habitats (Zibrowius 1980). Field and sclerochronological measurements have shown that also *L. pruvoti* has a definite growth pattern with a decreasing growth rate with increasing coral age, suggesting that this may be a general characteristic of solitary corals (Chadwick-Furman et al. 2000; Goffredo and Chadwick-Furman 2003; Goffredo et al. 2004, 2008, 2010; Caroselli et al. 2012b), even if some gorgonian colonies have the same growth pattern as well (Grigg 1974; Goffredo and Lasker 2006). The skeletal bulk density is slightly negatively correlated with temperature in populations arranged along a gradient of environmental conditions spanning seven degrees of latitude, but the difference between the extremes is very low (from 0.0013 to 0.0012 g mm^{-3}; −7.7 %), especially if compared with the sharp decline in *B. europaea* (from 0.0019 to 0.0008 g mm^{-3}; −57.9 % Fig. 20.2; Goffredo et al. 2007). Polyp skeletal growth rate is homogeneous along the latitudinal gradient, averaging about 0.9 mm $year^{-1}$ (derived from Caroselli et al. 2012b) that is very similar to the estimated growth rate after a 5 years monitoring in France and Spain (0.7 mm $year^{-1}$; Teixido et al. 2011). The homogeneous growth rates may indicate a higher tolerance of this species to increasing temperature, with

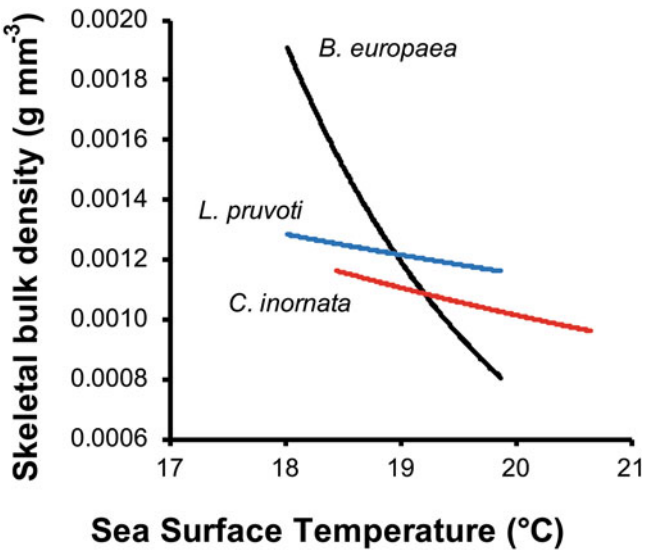

Fig. 20.2 *Balanophyllia europaea* (*black curve*), *Leptopsammia pruvoti* (*blue curve*), and *Caryophyllia inornata* (*red curve*). Variation in skeletal bulk density with sea surface temperature on a latitudinal gradient spanning 7° of latitude along the western Italian coasts. For *B. europaea*: N = 1,257, $y = 2*10^{-8} x^{-8.8319}$, $Rs^2 = 0.514$, Rs = –0.717, $P < 0.001$; for *L. pruvoti*: N = 820, $y = 0.0251\, x^{-1.0277}$, $Rs^2 = 0.007$, Rs = –0.084, $P < 0.050$; for *C. inornata*: N = 568, $y = 0.1588\, x^{-1.6870}$, $Rs^2 = 0.055$, Rs = –0.234, $P < 0.001$. Rs^2 Spearman's determination coefficient; Rs Spearman's correlation coefficient N number of individuals. Derived by Goffredo et al. (2007) and Caroselli et al. (2015a)

respect to the zooxanthellate *B. europaea* (Goffredo et al. 2010; Caroselli et al. 2012b). This is confirmed also by the analyses of skeletal bulk density, micro-density, and porosity (Bucher et al. 1998) that highlighted homogeneous bulk density and porosity, while micro-density even increases with temperature (Caroselli et al. 2011). The net calcification rate is variable along the latitudinal gradient, with an average 0.0010 g mm^{-2} $year^{-1}$, but not correlated with temperature (Caroselli et al. 2012a).

20.2.6 *Astrangia poculata*

This colonial, facultatively zooxanthellate, encrusting rhizangiid coral has been studied on North-western Atlantic coasts, where its growth rate has been measured as increase in polyp number over time (Dimond and Carrington 2007). Zooxanthellate colonies have been found to grow faster than non-zooxanthellate ones, and growth was higher in summer (high temperatures) than in winter, when it stopped for almost 4 months (Dimond and Carrington 2007).

20.2.7 *Caryophyllia inornata*

The growth of this solitary non-zooxanthellate coral was also analyzed in the same sites where *B. europaea* and *L. pruvoti* were studied (Fig. 20.1, Table 20.1). Also this solitary species has a definite growth, which was homogeneous along the whole latitudinal gradient (Caroselli et al. 2016a). Its net calcification rate, skeletal bulk density, and linear extension rate are not related to the thermal and solar radiation regime of the sites (Caroselli et al. 2015a, 2016b). The measured values of linear extension rate (0.63–0.90 mm $year^{-1}$; Caroselli et al. 2016b) are in agreement with the only previous record on this species coming from a 25 years monitoring in two sites in Spanish and French waters (Teixido et al. 2011). Net calcification rate values span 0.63–1.50 mg mm^{-2} $years^{-1}$ (Caroselli et al. 2016b). The recorded values are similar to the ones of the solitary non-zooxanthellate *L. pruvoti*, but lower than the zooxanthellate solitary *B. europaea* and extremely lower than the colonial *C. caespitosa*, confirming the hypothesis that zooxanthellae photosynthesis plays a major role in enhancing the calcification capabilities of their hosts (Gattuso et al. 1999; Al-Horani et al. 2005).

20.3 Population Dynamics

Available literature on population dynamics of temperate scleractinians studied in the field is reported in Table 20.2.

20.3.1 *Balanophyllia elegans*

The first published study on population dynamics of a temperate scleractinian dates back about 35 years, and has been performed on the solitary *B. elegans* in the Pacific Ocean (Fadlallah 1983). According to a population size structure based on polyp volume, a life table has been produced describing parameters such as recruitment, mortality, and lifespan (Fadlallah 1983). Population abundance was around 560 individuals m^{-2} and average lifespan was found to be 6.5–11 year, with high adult mortality (Fadlallah 1983). These results were discordant with the only previous and unpublished study on this species (Gerrodette 1979), possibly because of the different method for class determination (size class vs age class) which strongly influence the shape of the class distribution and all parameters derived from it.

Table 20.2 Available literature on population dynamics of natural populations of temperate scleractinians

Reference	Species	Study site
Fadlallah (1983)	*Balanophyllia elegans*	1
Goffredo et al. (2004)	*Balanophyllia europaea*	6
Shenkar et al. (2005)	*Oculina patagonica*	16
Goffredo et al. (2007)	*B. europaea*, *Leptopsammia pruvoti*	6, 7, 8, 9, 10, 11
Goffredo et al. (2008)	*B. europaea*	6, 7, 8, 9, 10, 11
Goffredo et al. (2010)	*L. pruvoti*	6
Teixido et al. (2011)	*L. pruvoti* and *Caryophyllia inornata*	13, 14
Caroselli et al. (2012b)	*L. pruvoti*	6, 7, 8, 9, 10, 11
Kersting et al. (2014)	*Cladocora caespitosa*	15
Caroselli et al. (2016a)	*Caryophyllia inornata*	6, 7, 8, 9, 10, 11

For each reference, the studied species and the codes of the study sites used in Fig. 20.1 are indicated

20.3.2 *Balanophyllia europaea*

The first population dynamic study on a Mediterranean species was performed on *B. europaea* (Goffredo et al. 2004). The clear dependency between size and age in this species allowed to determine the age distributions in populations along a natural gradient of temperature spanning seven degrees of latitude (Goffredo et al. 2004, 2008). By applying population dynamics models commonly used in coral and fish management (Pauly 1984; Sparre 1989; Chadwick-Furman et al. 2000; Goffredo and Chadwick-Furman 2003; Goffredo and Lasker 2008; Knittweis et al. 2009), it has been possible to assess the effect of temperature and solar radiation on some important demographic parameters such as population abundance and structure stability, instantaneous rate of mortality, average age, percentage of immature individuals, age at maximum biomass, and average age of biomass (Goffredo et al. 2004, 2007, 2008). With increasing seawater temperature, populations of this species are characterized by a decrease of abundance (from 50 to 4 individuals m^{-2}; −91.7 %), structure stability (from 0.935 to 0.423; −54.8 %) and percentage of immature individuals (from 56.9 % to 31.4 %; −44.0 %; Goffredo et al. 2007, 2008). All these parameters relate to the reproductive biology of this species, therefore it has been hypothesised that the same inhibition of zooxanthellae photosynthesis that would affect skeletal growth at higher seawater temperatures may also cause a decrease of energy availability for reproduction and for the zooxanthellate larvae of *B. europaea* (Goffredo and Zaccanti 2004; Goffredo et al. 2008; Caroselli et al. 2015b). In fact, in zooxanthellate corals, most of the energy required for gametogenesis comes from photosynthesis (Rinkevich 1989; Carlon 2002; Baird and Marshall 2002), thus a reduction of photosynthetic efficiency with increasing temperature may explain the observed negative effects on population abundance, stability and presence of young individuals (Goffredo et al. 2008).

20.3.3 *Leptopsammia pruvoti*

To check the above hypothesis, the same analyses have been performed for populations of the non-zooxanthellate *L. pruvoti* in the same sites where *B. europaea* was analyzed (Goffredo et al. 2010; Caroselli et al. 2012b). As expected, this species has an homogeneous abundance across the seven degrees of latitude studied (10,155 individuals per square meter, SD = 5741), while differences in demographic parameters seem related to local factors and are not dependent on seawater temperature (Goffredo et al. 2010; Caroselli et al. 2012b). The fact that the non-zooxanthellate *L. pruvoti* does not present negative effects on growth and demographic characteristics related to high temperatures, while the zooxanthellate *B. europaea* is strongly and negatively affected, suggests that the symbiotic condition may indeed play a major role in determining this different response. In one population of *L. pruvoti* (Calafuria; Table 20.2; Fig. 20.1), by combining the information from growth and population dynamics (Goffredo et al. 2010) with the ones on reproductive biology (Goffredo et al. 2005, 2006), it has been possible to estimate the larval mortality (98 %) of this species at Calafuria (Goffredo et al. 2010). Performing the same analysis on all the populations of *B. europaea* and *L. pruvoti* along the latitudinal gradient will allow to further clarify the reproductive processes most affected by seawater temperature. A long term population dynamics analysis on several benthic Mediterranean species in French and Spanish waters includes *L. pruvoti* (monitored for 5 years; Table 20.2; Fig. 20.1; Teixido et al. 2011). Max lifespan in that population has been estimated as 25–30 years, a higher value than previously reported at Calafuria (13 years; Goffredo et al. 2010) but similar to Palinuro and Pantelleria (26 years) and lower than in Elba, Scilla and Genova (44, 47 and 73 years, respectively; derived by Caroselli et al. 2012b). This indicates that even if the growth of *L. pruvoti* is quite stable in the Mediterranean locations where it has been studied, its population dynamics

traits may be extremely variable, probably due to local enviromental differences (Teixido et al. 2011; Caroselli et al. 2012b).

20.3.4 *Oculina patagonica*

The size frequencies of one Israeli population of the colonial zooxanthellate *Oculina patagonica* (Table 20.2; Fig. 20.1) reveals that its bleaching-related mortality is higher in large colonies than in smaller ones (Shenkar et al. 2005). This result has been considered quite surprising, since mortality is usually inversely related to colony size (Hughes and Jackson 1980; Johnson et al. 1995). It has been hypothesised that large colonies may have a higher probability to be infected by the causative agent of bleaching in this species (the bacterium *Vibrio shiloi*; Kushmaro et al. 1996), confirming the usefulness of population structure analysis to assess the relationships between species and their environment (Bak and Meesters 1998).

20.3.5 *Caryophillia inornata*

The demography of this species has recently been analyzed in the same sites along a latitudinal gradient used also for *B. europaea* and *L. pruvoti* (Goffredo et al. 2007; Caroselli et al. 2016a). Population abundance has resulted unrelated to environmental parameters, with an average value of 833 individuals m^{-2} (Caroselli et al. 2015a). Unexpectedly, while no demographic parameter correlates with temperature, the populations of this species become less stable and present deficits of young individuals with increasing solar radiation (Caroselli et al. 2016a). Since a direct effect of solar radiation is unlikely for this non-zooxanthellate species colonizing shaded overhangs, the reduction of zooplankton availability with increasing solar radiation (D'Ortenzio and Ribera d'Alcalà 2009) seems the most likely cause of the negative effects on population dynamics (Caroselli et al. 2016a). A previous long term population dynamics analysis on several benthic Mediterranean species in French and Spanish waters includes *C. inornata* (monitored for 25 years; Table 20.2; Fig. 20.1; Teixido et al. 2011). Maximum lifespan in that population has been estimated as 25–30 years, a higher value than reported at Genova and Calafuria (10 and 15 years, respectively; Goffredo et al. 2010) but similar to Elba (25 years) and lower than in Palinuro, Scilla, and Pantelleria (34, 52, and 66 years, respectively; derived by Caroselli et al. 2016a). As for *L. pruvoti*, also in this non-zooxanthellate species the growth is quite stable, but its population dynamics traits may be extremely variable (Teixido et al. 2011; Caroselli et al. 2016a).

20.4 Environmental Controls

Climate change is the defining environmental, economic, and social issue of our time, and it is now certain that the rapid increase in CO_2 concentration in the atmosphere since the nineteenth century industrial revolution is significantly changing the physical and chemical environment of the planet (Hoegh-Guldberg 2011). Global climatic changes are accelerating, and the average surface temperature of the Earth is likely to increase by 1–4 °C by the end of this century, with a higher magnitude in temperate areas than in tropical ones (Stocker et al. 2013). Climate change is having higher and faster effects on marine communities than on terrestrial ones (Richardson and Poloczanska 2008). Increasing atmospheric CO_2 not only induces the warming of the planet, but also dissolves in the oceans, lowering seawater pH and aragonite saturation state (a process called ocean acidification; Sabine et al. 2004; Stocker et al. 2013), thus threatening calcifying organisms.

20.4.1 Temperature

Global temperature increase is one of the greatest threats for coral and coral reefs survival (Hughes et al. 2003). However, to date, field analyses of the relationships between temperature and growth and population dynamics of temperate scleractinians are available only for four species from the Mediterranean Sea: the solitary *B. europaea*, *L. pruvoti*, and *C. inornata*, and the colonial *C. caespitosa* (Table 20.3; Fig. 20.1; Goffredo et al. 2007, 2008, 2009; Caroselli et al. 2011, 2012b, 2015a, 2016a, b; Kružić et al. 2012).

The studied zooxanthellate scleractinians, *B. europaea* and *C. caespitosa*, are likely to be negatively affected by seawater warming, since increasing temperature is associated with several negative effects on growth and demographic traits of these species (Rodolfo-Metalpa et al. 2000, 2006; Cerrano et al. 2000; Garrabou et al. 2001; Peirano et al. 2005; Goffredo et al. 2007, 2008, 2009; Caroselli et al. 2011; Kružić et al. 2012). Instead, the studied non-zooxanthellate scleratinians, *L. pruvoti* and *C. inornata*, seem to be quite tolerant to temperature increase, since none of their biological traits studied until now results negatively affected by high temperature (Goffredo et al. 2007; Caroselli et al. 2011, 2012b, 2015a, 2016a, b). The higher tolerance of non-zooxanthellate corals may indeed rely on the absence of symbionts, and thus the lack of an inhibition of host physiological processes by the heat-stressed zooxanthellae. This may be a general difference between symbiotic and asymbiotic corals. However, even if tolerant to high temperatures, the populations of *C. inornata* present deficits of young individuals with increasing solar radiation, likely as a result of

Table 20.3 Available literature on the effects of the environment on growth and/or population dynamics of natural populations of temperate scleractinians

Reference	Species	Study site
Goffredo et al. (2007)	*Balanophyllia europaea*, *Leptopsammia pruvoti*	6, 7, 8, 9, 10, 11
Goffredo et al. (2008)	*B. europaea*	6, 7, 8, 9, 10, 11
Goffredo et al. (2009)	*B. europaea*	6, 7, 8, 9, 10, 11
Caroselli et al. (2011)	*B. europaea* and *L. pruvoti*	6, 7, 8, 9, 10, 11
Caroselli et al. (2012b)	*L. pruvoti*	6, 7, 8, 9, 10, 11
Kružić et al. (2012)	*Cladocora caespitosa*	17
Caroselli et al. (2015a)	*Caryophyllia inornata*	6, 7, 8, 9, 10, 11
Fantazzini et al. (2015)	*B. europaea*	18
Caroselli et al. (2016a)	*C. inornata*	6, 7, 8, 9, 10, 11
Caroselli et al. (2016b)	*C. inornata*	6, 7, 8, 9, 10, 11

For each reference, the studied species and the codes of the study sites used in Fig. 20.1 are indicated

the associated decrease in zooplankton availability (Caroselli et al. 2016a).

20.5 pH

Ocean acidification determines a decrease in the calcification rates of several organisms (Fabry et al. 2008), even if cases of upregulation are observed (Rodolfo-Metalpa et al. 2010). To date, field analyses of the relationships between pH and growth and population dynamics of temperate scleractinians are available only for *B. europaea* (Table 20.3; Fig. 20.1; Goffredo et al. 2014; Fantazzini et al. 2015). The negative response of population abundance and calcification rate of this species with increasing acidification casts concerns for the future of this species in acidified seas (Goffredo et al. 2014; Fantazzini et al. 2015). Considering that this species shows negative effects even if the photosynthesis of its symbionts is likely to be increased by the higher CO_2 partial pressure in acidified waters, the response of non-symbiotic species, which cannot benefit of additional photosynthetic energy, is expected to be even more negative (Fantazzini et al. 2015). However, further studies on temperate non-zooxanthellate species are required to test this hypothesis.

20.6 Conclusion

The present work reviewed the available literature on growth and population dynamics of natural populations of temperate scleractinians, which has considerably increased during the last 20 years. As general trends, it seems that: (1) solitary species have a definite growth pattern, in contrast with colonial species; (2) symbiotic species are more sensitive to increasing temperatures and more vulnerable to global warming; (3) non-symbiotic species are more tolerant to increasing temperature, but may be negatively affected by the indirect effects of increasing solar radiation; and (4) even if the energy resulting from photosynthesis may increase as a consequence of ocean acidification, the growth and abundance of symbiotic corals seem to be negatively affected by acidification and the negative response of non-symbiotic corals is expected to be even stronger.

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Part V

Symbiosis

Microbial Interactions on Coral Surfaces and Within the Coral Holobiont

21

Max Teplitski, Cory J. Krediet, Julie L. Meyer, and Kim B. Ritchie

Abstract

Microbial communities associated with coral surfaces are diverse and complex. They play key roles in nutrient acquisition by coral holobionts and in responses to stressors and diseases. Members of coral-associated microbiota produce antimicrobial compounds, inhibit cell-to-cell signaling, and disrupt virulence in opportunistic pathogens. Characterization of coral-associated microbial communities suggests that metabolic capabilities define the core members of the communities. However, some taxonomic conservation is becoming evident in microbial communities associated with the same coral species and genera in different geographic regions. Even though shifts in the composition of coral microbiota often correlate with the appearance of signs of diseases and/or bleaching, it is not yet clear to what extent these shifts are a cause or a consequence of diseases. This chapter focuses on interactions within coral-associated microbial communities and suggests potentially interesting directions for future research.

Keywords

Coral microbiology • Coral disease • *Halomonas* spp. • Coral mucus • Coral commensal microbiota

21.1 Microbial Partners Within the Coral Holobiont: An Overview

Microorganisms play critical roles in marine, freshwater, and terrestrial ecosystems. Recognition of the unique role of the microbiota in eukaryotic host development and response to stressors has led to the concept of a "holobiont," which is now used to describe a complex co-evolved multi-partner symbiotic entity; a holobiont (and its hologenome) is a unit of evolutionary selection (Rosenberg et al. 2007; Rosenberg and Zilber-Rosenberg 2011; Rohwer et al. 2002). In the case of the coral holobionts, these multi-partite symbiotic organisms are formed by polyp animals, photosynthetic dinoflagellates, and microbial associates of polyps and dinoflagellates (Rohwer et al. 2002). Dinoflagellates from the genus *Symbiodinium* are harbored within membrane-bound vacuoles formed inside gastrodermal cells of the

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polyp. Dinoflagellates play at least two important roles in the holobiont. Over half (and up to 90 %) of photosynthate that they produce is translocated to the coral host, providing much of the host's carbon needs (Tremblay et al. 2012; Muscatine et al. 1981). *Symbiodinium* also produces high levels of dimethylsulfoniopropionate (DMSP), which has multiple functions in the holobiont: DMSP serves as an osmolite and antioxidant for both the alga and the coral (Kirst 1990; Deschaseaux et al. 2014). It is a nutrient source for coral-associated bacteria, although the ability to utilize DMSP is broadly distributed in marine microbes (Cui et al. 2015). DMSP is an important chemical cue for a number of different organisms, from coral pathogens to reef fishes and penguins (Garren et al. 2014; DeBose et al. 2008; Nevitt 2011).

The inter-organismal associations within the holobiont appear to be co-evolved and are quite dynamic. Throughout their lifetime, corals can expel or lose their dinoflagellate symbionts and acquire new strains (or even clades) of *Symbiodinium* (Cunning et al. 2015). This flexibility allows for associations with clades that may be more effective under ever-changing environmental conditions, which may aid in the holobiont's response to environmental stressors.

The evidence presented in this chapter also suggests that coral's associations with bacteria are similarly co-evolved. Studies now show that microbial communities associated with healthy corals are not as diverse as communities associated with diseased corals or seawater. Oceanospirillales have been consistently identified in microbiota from stony corals in the Caribbean, Great Barrier Reef, and the Red Sea, as well as sea fans in the Mediterranean (Bourne et al. 2008; Kvennefors et al. 2012; Littman et al. 2009; Pantos et al. 2015; Raina et al. 2009; Lema et al. 2014; McKew et al. 2012; Meyer et al. 2014; Morrow et al. 2012; Rodriguez-Lanetty et al. 2013; Sharp et al. 2012; Bayer et al. 2013; Vezzulli et al. 2013). This specificity suggests that the coral host selects Oceanospirillales symbionts from a pool of potential colonizers in a more directed fashion than simply setting up a competitive environment with winners that arise stochastically. However, they establish within coral surface microbiome with time, as Oceanospirillales do not dominate microbiomes of coral larvae. Nevertheless, these associations are established at very early stages of larval development. For example, in the brooding coral, *Porites astreoides*, Oceanospirillales were detected just 4 days after larval release (Sharp et al. 2012). In the broadcast spawning coral, *Acropora millepora*, Oceanospirillales were detected in 1-week old juveniles, but not in planulae (Sharp et al. 2010). These studies support earlier findings that bacteria are not associated with eggs released by several different genera and species of broadcast spawners, but are acquired post-settlement (Sharp et al. 2010). Thus, the Oceanospirillales symbionts are not inherited vertically, but rather selected from the environment by the host at very early stages of larval development, regardless of the coral reproductive strategy.

The composition of coral-associated microbial communities appears to be quite conserved. High throughput sequencing revealed that commensal microbiota of *Montastraea*/*Orbicella* corals is dominated by the members of the *Halomonas* spp. (Meyer et al. 2015). Interestingly, even when corals were removed from their natural ecosystem and were maintained in an aquarium for over a year, their microbiota were only modestly changed. As shown in Fig. 21.1, *Halomonas* and *Moritella* spp. were most abundant members of the communities of corals sampled in the wild and then maintained in aquaria, even though an expansion of *Enterobacteriaceae* and other relatively minor members of the microbiome was also observed.

Recently, genomes of representative members of Oceanospirillales isolated from coral surfaces were sequenced. Analysis of the genome of *Halomonas sp*. strain R1t3 recovered from healthy mucus of *A. palmata* revealed that it belongs to the Group 2 of the polyphyletic family Halomonadaceae (Meyer et al. 2015). The small subunit ribosomal RNA gene sequence of *Halomonas* strain R1t3 is nearly indistinguishable from the sequence in type strains of both *H. meridiana* and *H. aquamarina*, while *secA*, *atpA*, and *rpoD* are approximately 99 % identical between the two type strains and strain R1t3. In contrast, gene sequences for the *gyrB* locus are identical in the type strains, but only 87 % similar to the *gyrB* locus in strain R1t3. Furthermore, small subunit ribosomal RNA gene of *Halomonas* sp. R1t3 exhibits high sequence identity with the orthologous genes in the strains RA001 (isolated from *Acropora* coral in India), and in the uncultured *Halomonas* retrieved from *Acropora* corals in Mexico and Indonesia (Meyer et al. 2015).

The genome of the strain R1t3 provides the first glimpse into the functions that are present in this coral commensal. Similarly to other halomonads, strain R1t3 tolerates a wide range of salinities, and this is likely due to the production of osmoprotectants, such as glycine betaine and ectoine. The strain is able to utilize a wide range of carbon sources (Krediet et al. 2009a, b), and this ability is also reflected in its genome, which includes six homologues of various glycoside hydrolases predicted to act on polysaccharides, such as starch, glycogen, and fructan (Meyer et al. 2015).

Interestingly, whole-genome comparisons of *Halomonas* strain R1t3 and *Endozoicomonas montiporae* LMG 24815 (another coral commensal) revealed that the two genomes share only 392 genes (11 % of the *Halomonas* genome) using 60 % sequence identity and 70 % coverage criteria, or 442 genes (12.5 % of the *Halomonas* genome, if 30 % sequence similarity criterion was used). Of the orthologs with at least 30 % sequence identity, four glycoside hydrolases likely involved in the utilization of coral mucus were present in the

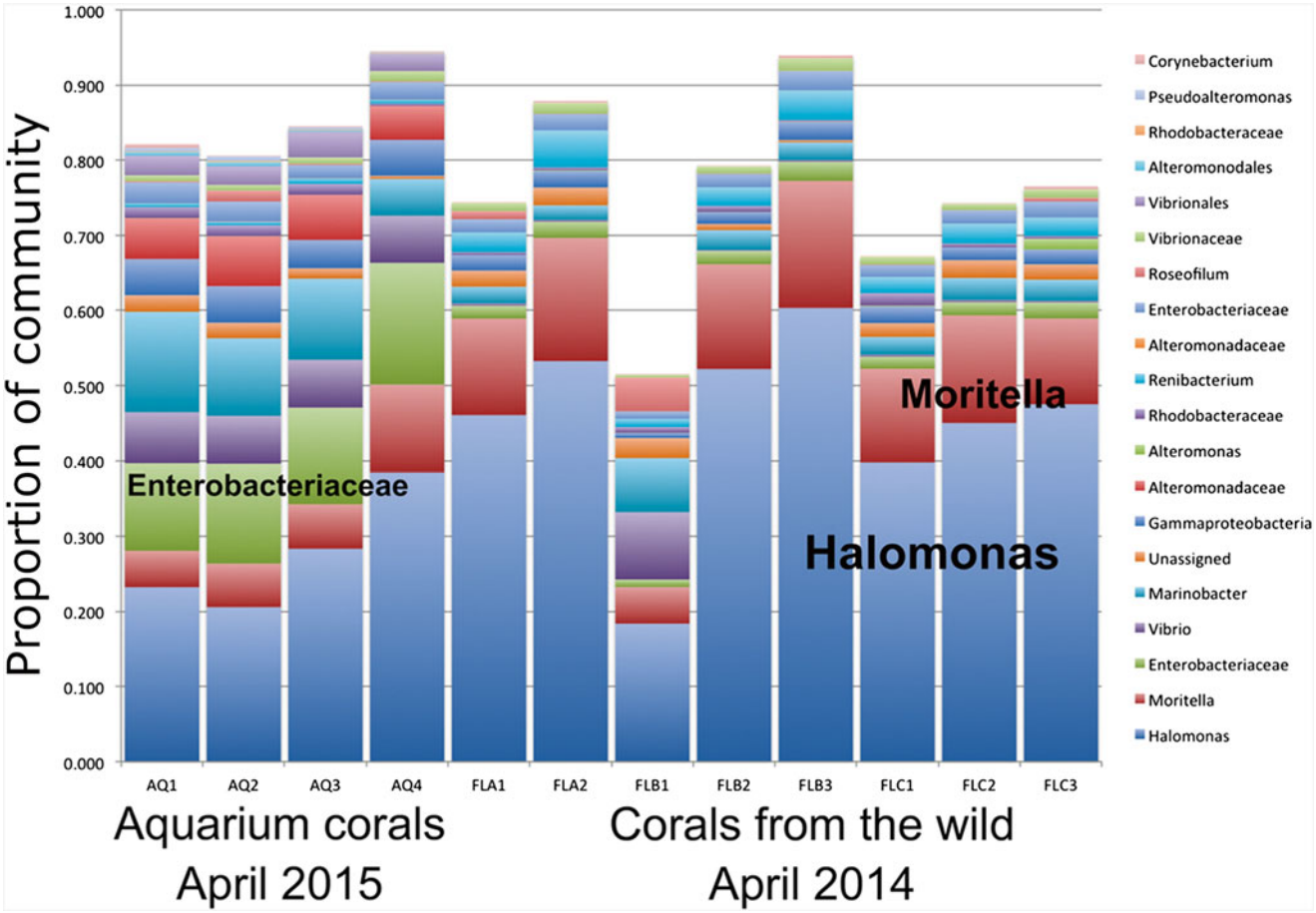

Fig. 21.1 Comparison of dominant bacterial genera in *Montastraea cavernosa* corals sampled in the wild (Florida Keys) and after a year in an aquarium (in Ft. Pierce, FL). The relative abundance of dominant bacterial genera in Illumina MiSeq 16S rRNA gene (V6 region) libraries within coral surface mucus layer (Data were analyzed as in (Meyer et al. 2015))

genomes of *Halomonas* and *Endozoicomonas* (Meyer et al. 2015). Further sequencing of the Oceanospirillales genomes and comparative genomics analyses will likely reveal functions that are common to all members of this group, and those that are more representative of coral isolates.

21.2 Microbiota of the Coral Surface Mucus Layer

21.2.1 Composition of the Coral Mucus

Microbes have been isolated from the endolyth, digestive tracts and endosymbiotic zooxanthellae, however, most commonly studied coral-associated microorganisms have been recovered from the coral surface mucopolysaccharide layer. The surface mucus layer is a selective environment and a setting for both host-microbe and microbe-microbe interactions. It is within this layer the presumed commensal microbiota interact with potential pathogens and environmental organisms. The surface mucus layer of corals is the interface between the coral holobiont and the surrounding environment. As such, it plays an important role in the overall health of the meta-organism. Even though several studies have characterized functions of coral mucus in protection against desiccation and trapping particulates (rev. Brown and Bythell 2005), it is also reasonable to hypothesize – based on the discoveries made in other animal models – that structuring of the associated microbiota is an important function of coral mucus. As discussed below, there are several mechanisms by which mucus is involved in structuring of the associated microbiota. The ability to hydrolyze mucus and utilize its components is one of such mechanisms (Krediet et al. 2013b). In fact, coral commensals and pathogens differ in their ability to hydrolyze mucus and utilize its components (Krediet et al. 2009a, b). The pattern of induction of glycolytic activities also differs between pathogens and commensals (Krediet et al. 2009a, b). Therefore, understanding of the chemical structure of mucus is important to define the spectrum of activities that could be found within coral-associated commensals and pathogens.

The elucidation of the chemical structure of coral mucus is complicated by the fact that it is composed of excretions of

the coral mucocytes and extracellular substances produced by the associated microbiota. It also contains oligomers that result from the degradation of these polymers. Sulfated glycoprotein polymers, made in specialized mucocytes of the polyp, are the main component of coral mucus. It is derived from the photosynthate produced by the endosymbiotic dinoflagellates (Brown and Bythell 2005). The chemical structure of coral mucus components has been reported for *Pseudopterogorgia americana*, *Acropora formosa*, *A. millepora*, *Pachyseris speciosa*, *Fungia fungites*, *Sarcophyton sp*., *Lemnalia sp*., *Cespitularia sp*, *Oculina arbuscula*, *Galaxea fascicularis*, *Pavona cactus*, *Turbinaria reniformis*, *Gonipora djiboutiensis*, and *Montastrea faveolata* (Molchanova et al. 1985; Meikle et al. 1987, 1988; Coddeville et al. 2011; Tremblay et al. 2011; Jatkar et al. 2010). Even though there are differences in the composition of mucus produced by different corals and methods with which mucus was collected and analyzed in these studies, several generalizations could be made based on these reports. The polypeptide backbone of mucus accounts for up 80 % of its mass, with serine, threonine, aspartate, glutamate and glycine being most common amino acids in different coral species (Meikle et al. 1987, 1988; Coddeville et al. 2011). Sulfated oligosaccharide side chains are O-linked to the polypeptide backbone through a mannose residue, which is different from mucins in most other animals (Meikle et al. 1987, 1988; Coddeville et al. 2011). Mannose, N-acetyl-D-glucosamine, galactose, fucose, glucose, and arabinose are most common monomers in mucus, with xylose and N-acetyl-D-galactosamine being minor components of coral mucins (Meikle et al. 1987, 1988; Molchanova et al. 1985; Coddeville et al. 2011; Tremblay et al. 2011). Arabinose and xylose are "plant" monosaccharides, and are not present in mucins from other animals (Meikle et al. 1987, 1988; Molchanova et al. 1985).

Coral mucus is a rich milieu capable of supporting robust microbial populations. Bacterial counts in mucus are an order of magnitude higher than those in the surrounding water (Paul et al. 1986). In the laboratory, coral pathogens and commensals (as well as *E. coli*) can reach 10^6–10^8 cfu ml^{-1} within hours when grown on total coral mucus, its low molecular weight fraction and high molecular weight mucin constituents (Sharon and Rosenberg 2008; Garren and Azam 2010; Krediet et al. 2009a, b; Frydenborg et al. 2013). Interestingly, commensal bacteria reached lower population densities then coral pathogens when grown on mucus *in vitro* (Vine et al. 2004; Krediet et al. 2009a, b). *Vibrio* spp., which are related to coral pathogens, dominated microbial communities formed on mucus of the *Oculina patagonica* coral after an extended incubation in vitro (Sharon and Rosenberg 2008; Rosenberg and Falkovitz 2004), even though vibrios make up less than 5 % of culturable bacteria in the mucus layer of this coral under normal conditions (Koren and Rosenberg 2006). *Vibrio's* ability to hydrolyze mucus is enhanced under elevated temperatures (Frydenborg et al. 2013). Collectively, these observations suggest that in the absence of other factors, opportunistic pathogens can overgrow and dominate coral-associated microbial communities under some conditions. However, in addition to carbon and nitrogen sources, coral mucus also contains potent antimicrobials (Ritchie 2006). Therefore, when crude preparations of fresh mucus are used as growth substrate, declining bacterial viability is sometimes reported (Looney et al. 2010). The presence of antimicrobials, interactions with commensal microbiota, and defense responses of the coral all contribute to the stability of the holobiont and its resistance to invasion by pathogens.

21.2.2 The Role of Commensal Microbiota in Acquisition and Turnover of C, N

The population of heterotrophs in the surface mucus layer of healthy corals contains both high levels of commensal bacteria and low levels of opportunistic pathogens. This community structure, dominated by mutualistic Gammaproteobacteria, Alphaproteobacteria, and Bacteroidetes can be quite stable and is broadly conserved across coral species and large geographic distances. While the dominant taxa vary at the level of genus between different corals, abundant coral commensals are often members of the Oceanospirillales, especially the cultured genera *Endozoicomonas* and *Halomonas*, as well as a clade of uncultured, coral-associated Gammaproteobacteria (Speck and Donachie 2012) that may represent a third genus.

The abundant organic carbon available in the surface mucus layer is in direct contrast to the surrounding oligotrophic tropical seawater and induces stiff competition between heterotrophic bacteria that feed on the mucus. To degrade coral mucus and grow on it, bacteria produce glycosidases, proteases, and esterases (Vacelet and Thomassin 1991; Krediet et al. 2009a). Coral commensals and pathogens appear to possess a similar suite of enzymatic activities, even though their metabolic capabilities differ based on Biolog Ecoplate comparisons (Krediet et al. 2009a; Sharon and Rosenberg 2008). While pathogens and commensals produce essentially the same exoenzymes to degrade coral mucus, they differ in patterns of temporal regulation and levels of activity (Krediet et al. 2009a, b). At least five glycosidases were strongly expressed in the starved cultures of a model coral pathogen *Serratia marcescens*. Upon exposure to coral mucus, mannopyranosidases were strongly induced in *S. marcescens* (Krediet et al. 2009a, b). Mannosidases hydrolyze oligosaccharide side chains from the polypeptide backbone of the mucus glycoprotein. Conversely, mannosidases were not produced by *H. meridiana* cultures, nor were they induced upon growth on coral mucus (Krediet et al.

2009b), suggesting that this commensal is not capable of completely degrading coral mucus. Instead, *H. meridiana* appears to depend on very few glycosidases, mostly α-D-glucopyranosidase, and four additional glycosidases that were induced during growth on the high molecular weight fraction of coral mucus (Krediet et al. 2009b).

In *S. marcescens*, most glycosidases were subject to early catabolite repression, which was relieved upon overnight incubation (Krediet et al. 2009b). This suggests that the ability to efficiently down-regulate enzymatic activities during colonization of coral mucus may help *S. marcescens* establish within the coral mucus layer, and as nutrients become less available, this pathogen becomes more aggressive. This is consistent with the observations in other pathogens that up-regulate their virulence genes when preferred carbon sources become limiting (Gorke and Stulke 2008; Deutscher 2008). Interestingly, catabolite repression effects in commensals were strongest after 18 h of incubation, while in the white pox pathogen, the catabolite repression effects were largely relieved within the same time frame (Krediet et al. 2009b). It may be possible (while not yet experimentally tested) that this catabolite repression by sugars is one of the mechanisms of arrest of overgrowth of commensals that could be detrimental to the coral host (Krediet et al. 2009b).

To outcompete commensals within the coral surface mucus layer, coral pathogens employ strong, constitutively active glycosidases (Krediet et al. 2009a, b). The activities of these glycosidases provide carbon for the bacteria and make the polypeptide backbone of mucins available to proteases. Coral commensals, however, have the ability to inhibit activities of these glycosidases. Approximately 8 % of culturable native coral-associated bacteria (including members of the genera *Exiguobacterium*, *Photobacterium* and *Vibrio*) were capable of inhibiting glycosidases in a coral pathogen (Krediet et al. 2013a). This inhibition resulted in 10- to 100-fold decrease in growth of the coral pathogen *S. marcescens* PDL100 in co-cultures with the inhibitory commensals on coral mucus (compared with the monoculture of *Serratia*) (Krediet et al. 2013a). Even though the study of Krediet et al. (2013a) focused on the inhibition of glycosidases in *S. marcescens*, it is likely that that these inhibitory activities will affect glycosidases in other bacteria as well. The chemical nature of these activities is not yet elucidated, but based on their solvent partitioning behavior, they are likely not imino- or azasugars, known glycosidase inhibitors.

There is also growing interest in understanding whether coral-associated bacteria can carry out nitrogen fixation and then contribute fixed nitrogen to other partners within the holobiont (Fiore et al. 2010). A survey of diazotrophs (defined as those bacteria that encode *nifH* nitrogenase within their genomes) from mucus and tissues of three corals on the Great Barrier Reef revealed that the diversity of the *nifH*+ bacteria in mucus was generally similar to that in the surrounding seawater, however, over 70 % of *nifH* sequences recovered from tissues of corals were most similar to those from rhizobia (Lema et al. 2012). In contrast, *Vibrio harveyi* and *V. alginolyticus* which were shown to be capable of nitrogen fixation in coral mucus, were dominant culturable nitrogen-fixers recovered from the Brazilian coral *Mussismilia hispida* (Chimetto et al. 2008). To determine whether corals can benefit from nitrogen-fixation by the associated microbiota, a recent study (Grover et al. 2014) employed an $^{15}N_2$ enrichment technique (which is preferred to acetylene reduction assays to demonstrate not only N_2-fixation, but also incorporation of the fixed nitrogen into biological molecules). Of the three corals tested, nitrogen fixation rates were higher in microbial communities associated with *Stylophora pistillata* (compared to *Porites sp*. and *Cladopsammia gracilis*), interestingly, samples of *S. pistillata* collected at 40 m had more significant nitrogen fixation rates then the samples of the same coral collected at 5 m (Grover et al. 2014). Rates of N-fixation in these mesocosms were similar to those observed in other oligotrophic environments and ranged from 0.1 to 4 nmol N l^{-1} day^{-1}, demonstrating that nitrogen fixation indeed takes place within the holobiont (Grover et al. 2014). However, all of fixed ^{15}N was found in particles released from corals, and none were detected in tissues of the coral or in zooxanthellae (Grover et al. 2014), indicating that no transfer of fixed nitrogen to other members of the holobiont took place within 24 h of the experiment, and the longer incubation times were not feasible due to the experimental set-up. Earlier experiments which relied on acetylene reduction assays and stable isotope measurements also indicated that nitrogen fixation takes place in coral mesocosms and that the transfer of fixed nitrogen to the coral animal was unlikely (Lesser et al. 2007b). Based on the significant depletion of ^{15}N in the fraction containing *Symbiodinium*, Lesser et al. (2007b) concluded that at least some of the fixed nitrogen is transferred to the zooxanthellae.

21.2.3 Interactions Within Stable and Destabilized Coral Microbial Communities

When the stable relationship between dominant commensal bacteria and the coral host is disrupted, the holobiont is in a state of dysbiosis and susceptible to disease. Indeed, the loss of Oceanospirillales and other abundant commensals has been linked to the appearance of coral disease symptoms (Cardenas et al. 2012; Meyer et al. 2014; Vezzulli et al. 2013). While it is unclear whether the dysbiosis is a cause or consequence of a degraded health state in the host, it may be assumed that the health outcome of the host is directly

influenced by interactions within coral-associated microbiota. These interactions may take the form of competition for resources, antimicrobial activity, or processes that inhibit quorum-sensing pathways. It is reasonable to hypothesize that by providing nutrient-rich mucus, the coral host actively selects commensal bacteria that are both antibiotic-producing and antibiotic-resistant.

Isolates of coral-associated bacteria consistently display antimicrobial activity against potential coral pathogens (Kvennefors et al. 2012; Ritchie 2006; Nissimov et al. 2009; Shnit-Orland and Kushmaro 2009) and against other commensals (Rypien et al. 2010; Kvennefors et al. 2012) in laboratory experiments. The conclusions drawn from these studies are tempered by the fact that many of the isolates used were Vibrionales or Pseudomonadales and not the dominant commensals detected in situ and that some of the test strains utilized would not normally be encountered by coral bacteria. What we can draw from these studies is that many of the microbe-microbe interactions taking place in the dynamic surface mucus layer likely occur between species of Gammaproteobacteria. Consistent with this idea, network analysis of *in situ* bacterial species co-occurrences in *Porites astreoides* has demonstrated negative interactions between dominant strains of *Endozoicomonas* and several different genera of Vibrionaceae (Meyer et al. 2014). Negative correlations between closely related strains of heterotrophic Gammaproteobacteria are not unexpected, as these strains presumably compete for similar resources in the surface mucus layer.

In addition to producing antimicrobial compounds that kill competitors, bacteria in coral mucus are known to both produce and disrupt quorum-sensing (QS) signals. QS is a mechanism of bacterial population density-dependent gene regulation, used by pathogens and commensals to structure interactions within their communities and to interact with the host (Ng and Bassler 2009; Dobretsov et al. 2009). QS was first discovered in bioluminescent bacterial symbionts of squid (Eberhard et al. 1981), and it was recently shown that coral bacteria produce at least two types of QS signals (*N*-acyl homoserine lactone (AHL) signals and the more general autoinducer-2 (AI-2)) (Tait et al. 2010; Alagely et al. 2011; Golberg et al. 2011). Recently, AHL and AI-2 signals were reported in polymicrobial black band disease consortia and Alpha- and Gammaproteobacteria isolated from both healthy coral mucus and disease consortia (Zimmer et al. 2014). Interestingly, the type of AHL produced by coral-associated vibrios changed in response to temperature. Furthermore, one strain of *V. harveyi* disrupted AHL production in other strains when temperatures were elevated (Tait et al. 2010). Genes and functions controlled by QS in coral-associated microbial communities are not yet known, and the consequences of temperature-dependent changes in the profiles of the produced AHLs are also not clear.

There are also plenty of examples of cross-species and cross-kingdom interference with bacterial QS (Teplitski et al. 2011; Dobretsov et al. 2009, 2011). By interfering with QS in other species, bacteria are thought to gain advantage in at least three ways: (1) by taking advantage of the "public goods" (such as exoenzymes and extracellular polymers) produced in a QS dependent manner and (2) by disrupting production of antibiotics and toxins, production of which is regulated by QS and (3) by interfering with QS-dependent attachment, surface spreading and biofilm formation (Dobretsov et al. 2009, 2011). Some of the bacterial strains recovered from coral surfaces were also capable of inhibiting AHL-mediating QS signaling, disrupting biofilm formation and surface spreading (Alagely et al. 2011; Golberg et al. 2013). Inoculation of *Aiptasia pallida* with coral commensals capable of interfering with QS and disrupting QS-regulated behaviors (such as swarming, biofilm formation) abolished the ability of *S. marcescens* to degrade polyps (Alagely et al. 2011). Thus, interference with QS in pathogens was shown to have effects that were beneficial to the entire holobiont.

In addition to the two common QS systems mediated by AHLs and the AI-2 signal, vibrios and closely related genera also utilize the third QS system mediated by the CAI signal (synthesized via CqsA), which is perceived by the CqsS sensor (Henke and Bassler 2004; Ng et al. 2011). Production of the CAI-1 signals was demonstrated in vibrios isolated from healthy corals and from the BBD (Meyer et al. 2015). Cyanobacterial mimic of the CAI-1 QS system (lyngbic acid) was recently isolated from the black band disease consortium and shown to inhibit QS-dependent luminescence in *V. harveyi* reporters as well as in the luminescent coral vibrios (Meyer et al. 2015). How and to what extent this compound affects interactions within microbiota of healthy corals and that of the Black Band Disease is not yet known, however, the production of the CAI signal-mimic may be responsible for the negative interactions observed between cyanobacteria (that produce the CAI signal-mimic) and vibrios (that utilized CAI as one of their QS signaling mechanisms) (Meyer et al. 2015).

21.2.4 Destabilization of the Commensal Microbiota and Coral Diseases

In addition to their functions in promoting coral health and inhibiting coral pathogens, members of the native commensal microbiota appear to be involved in polymicrobial diseases of marine invertebrates and algae (Meyer et al. 2014; Fan et al. 2013; Olson et al. 2014; Apprill et al. 2013; Cardenas et al. 2012). For example, high-density 16S rRNA gene microarray analysis of *Montastraea* (current name: *Orbicella*) colonies infected with white plague, documented shifts in the associated bacterial populations (Sunagawa et al. 2009a). Similar observations were made in *Porites astreoides* with unusual lesions (Meyer et al. 2014). It is possible that under some conditions, members of the coral commensal microbiota escape restrictions imposed on them by the host or other

members of the host microbiota and then multiply to numbers that exceed the carrying capacity and start to degrade host tissues (Lesser et al. 2007a; Krediet et al. 2013b). However, it is not yet clear whether commensals are active contributors in the disease consortium, or whether they take advantage of the host tissue degradation opportunistically.

Environmental factors (temperature, seawater pH, nutrient loading, anthropogenic influences, etc.) have also been implicated in the transitions of coral microbiomes from a stable, "healthy" status to a destabilized disease state (Aronson et al. 2003; Ban et al. 2014; Ben-Haim et al. 2003; Burge et al. 2014; Haapkyla et al. 2011; Harvell et al. 1999; Meron et al. 2011; Voss and Richardson 2006; Kuta and Richardson 2002; Frydenborg et al. 2013), but mechanisms by which these changes occur remain poorly understood. Interactions within the surface microbial community may facilitate resilience to these transitions and the return to pre-disease conditions after an infection (Mumby et al. 2007; Mao-Jones et al. 2010). However, specific physiological and biochemical cues that result in a functional switch of the microbiota from a commensal state to a pathogenic polymicrobial consortium are far from being clear.

21.3 Bacterial Interactions with *Symbiodinium*

The presence of photosynthetic symbiotic dinoflagellates was shown to impact the composition of invertebrate-associated microbiota (Bourne et al. 2013), thus indicating the importance of *Symbiodinium* spp. in the composition and function of the holobiont. Bourne et al. (2013) also hypothesized that DMSP produced by *Symbiodinium* spp. within the holobiont was, at least in part, responsible for the composition of the holobiont's microbiota (Bourne et al. 2013). While most of the studies in coral microbiology focused on the microbiota present within the coral surface mucopolysaccharide layer, several investigators focused on understanding interactions between bacteria and *Symbiodinium* spp.

Bacteria are known to establish mutually-beneficial relationships with algae in general, and members of the genera *Roseobacter* and *Sphingomonas* are most commonly found as commensals of free-living soil and aquatic algae (rev. Teplitski and Rajamani 2011). In association with algae, bacteria were shown to fix atmospheric nitrogen, and produce plant hormones and vitamins that improve growth of their algal partners (rev. Teplitski and Rajamani 2011; Wichard 2015). Studies of the coral holobiont recently led to the appreciation of the roles of microbes that are associated with coral symbiotic *Symbiodinium* and invited interesting hypotheses about the roles of *Symbiodinium*-associated bacteria in nutrient acquisition by the coral host (Radecker et al. 2015).

Bacteria present in cultures of *Symbiodinium* spp. (representing six clades) have been characterized via both culture-based and culture-independent methods (Ritchie 2011). Members of the Roseobacteriales group were recovered from all tested *Symbiodinium* cultures representing seven different clades of this dinoflagellate (Ritchie 2011). It is of note that these *Symbiodinium* cultures were derived from different corals and coral reef invertebrates from different oceans, suggesting that these bacteria may be true mutualists of *Symbiodinium* spp. Furthermore, *Roseobacter* spp. were shown to increase growth rates of *Symbiodinium* (Ritchie 2011). Similarly, growth of another dinoflagellate (*Pfiesteria*) was observed only when it was co-cultured with a strain of α-proteobacteria (Alavi et al. 2001). It is not yet clear what α-proteobacterial nutrients or functions were critical for growth of *Symbiodinium* spp. however, in studies with *Ulva lactuca*, its bacterial partners *Roseobacter sp* and *Cytophaga* sp. were shown to produce substances that had activities similar to the plant hormones auxin and cytokinin, with *Roseobacter* sp. playing an important role of mediating chemical interactions within this tri-partite relationship (Spoerner et al. 2012).

A novel function for a *Symbiodinium*-associated α-proteobacterium as a host of phage-like particles (GTAs, gene transfer agents) was recently demonstrated (McDaniel et al. 2010). GTAs, inducible under some conditions, are able to transfer genes to a range of bacteria in the marine environment (McDaniel et al. 2010, 2012). Furthermore, the gene transfer via GTAs is much higher in the coral reef environment, suggesting an alternate mode of adaptation to environmental changes by propagating potentially beneficial genes within the holobiont (McDaniel et al. 2010).

21.4 Consequences and Mechanisms of the Disruption of the Symbiosis with *Symbiodibium*

A collection of symptoms, broadly known as "coral bleaching", is often interpreted as a sign that corals are distressed. As discussed below, bleaching could be a consequence of bacterial infections, and bleaching itself could cause changes in the coral microbiome. Furthermore, some corals employ "symbiont shuffling" to actively manipulate their associated *Symbiodinium* (Cunning et al. 2015), and the closer examination of the molecular mechanisms involved in this phenomenon may lead to a better understanding of the interactions between corals and their bacterial associates.

Coral bleaching is a visually observable loss of holobiont's green (or brown or red) color, which as described below, could be associated with a variety of environmental factors and could be a consequence of several mechanisms by which zooxanthellae are lost or expelled. Coral bleaching is a consequence of various abiotic and biotic stressors. The role of coral pathogens and their consortia in bleaching is nuanced: some coral pathogens, such as *Vibrio shiloi* have been shown to cause bleaching through inhibition of photosynthesis and

lysis of *Symbiodinium* cells during periods of elevated sea surface temperatures (Kushmaro et al. 2001; Rosenberg and Falkovitz 2004). Similarly, infections of corals with *Vibrio coralliilyticus* lead to bacterially-induced bleaching (Ben-Haim et al. 2003) and white syndrome of Indo-Pacific corals (Sussman et al. 2008). *V. coralliilyticus* produces an impressive suite of proteases (Kimes et al. 2011). The zinc-metalloprotease produced at warm temperatures (>26 °C) is the primary virulence factor leading to the signs of the white syndrome, which include inhibition of the photosystem-II of *Symbiodinium*, paling of coral tissue, and the spread of coral tissue lesions culminating in mortality (Sussman et al. 2009) (Fig. 21.2). While at 24–26 °C proteases of *V. coralliilyticus* appear to target primarily the coral symbiotic dinoflagellates, at 27–29 °C coral tissue is the primary target with tissue necrosis as the only observable disease sign (Ben-Haim et al. 2003). Similar temperature sensitive proteases have been shown to disrupt photosynthesis in other models. Although the exact mechanism by which these proteases inhibit photosystem-II of *Symbiodinium* remains unclear, it resembles the effect of thermolysin of *Bacillus termoproteolyticus* on the outer envelope membrane of chloroplasts (Cline et al. 1984) and a ToxB protein produced by the fungal wheat pathogen, *Pyrenophora tritici-repentis*, which inhibits photosynthesis and results in chlorosis (Kim et al. 2010).

Given the observation that the presence of photosynthetic dinoflagellate symbionts shapes the composition of the holobiont's microbiota (Bourne et al. 2013), it is, perhaps, not surprising that bleaching is also associated with the changes in coral microbiota. During a bleaching event in *A. millepora*, Bourne et al. used DGGE fingerprint analysis to show that the microbial populations shifted drastically and increasing temperatures correlated with the presence of *Vibrio*-associated sequences (Bourne et al. 2008). These shifts were additionally observed on apparently healthy coral tissues during a bleaching event even before coral tissue bleaches (Richier et al. 2006) Therefore, below we briefly review environmental factors that lead to coral bleaching and mechanisms of symbiosis breakdown.

21.4.1 Mechanisms of the Destabilization of the Polyp-*Symbiodinium* Symbiosis

In response to elevating seawater temperatures, the prevalence and intensity of mass coral bleaching events worldwide have increased (Goreau 1964; Jaap 1979; Hoegh-Guldberg et al. 1987, 2007). During periods of stress, the symbiosis between cnidarian hosts and their endosymbiotic dinoflagellate algae breaks down with the loss of the algae from the host

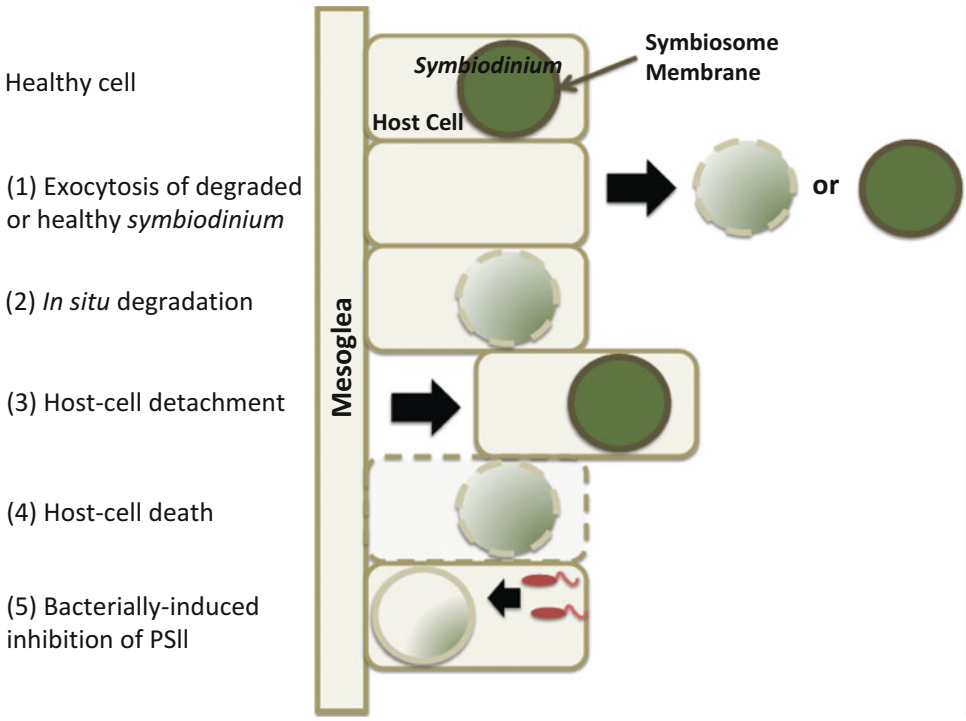

Fig. 21.2 Possible cellular mechanisms of cnidarian bleaching under thermal stress. During stable symbiosis, algae of the genus *Symbiodinium* reside within gastrodermal cells of the cnidarian host and are surrounded by the host-derived symbiosome membrane. Under stress, algae could be lost (*1*) by expulsion of healthy and/or degraded algae, (*2*) by *in situ* degradation (involving fusion of the symbiosome with lysosomes, autophagy, and/or a cell-death reaction of the algae themselves), (*3*) by detachment of algae-containing host cells, (*4*) by death of algae-containing host cells through apoptosis or necrosis, (*5*) by bacterially-produced toxins and/or virulence factors that inhibit photosystem II in *Symbiodinium*, or by some combination of these mechanisms (Figure modified with permission from (Bieri et al. 2016))

(Douglas 2003). Understanding the mechanisms by which this symbiosis is destabilized is important to gain a better understanding of the molecular underpinning of the interactions within the holobiont. If corals have evolved mechanisms to selectively expel or recruit specific members of the genus *Symbiodinium*, is it also reasonable to then hypothesize that similar molecular mechanisms may be at play during structuring of the holobiont-associated microbiota?

The prevailing bleaching paradigm suggests that elevated thermal stress and high irradiances of solar radiation lead to the production of reactive oxygen species (ROS) that cause damage to lipids, proteins, and DNA, as well as act as signal transduction molecules and mediators of cellular damage processes (Richier et al. 2006; Lesser 2007). This oxidative stress is often proximal to (and classically thought to be the driving mechanism of) coral bleaching. Elevated temperatures and light irradiances lead to light energy in excess of what can be processed by photosystem II and leads to the production of ROS and oxidative stress that activates many gene cascades involved in cellular damage, such as apoptosis and autophagy (Dykens et al. 1992; Richier et al. 2006; Lesser 2007).

However, light-induced reactive oxygen species is not the only mechanism of thermal bleaching in Cnidaria. Recent work showed that corals and sea anemones bleach in response to thermal stress alone, independent of photosynthetic activity (Tolleter et al. 2013). Corals and anemones exposed to thermal stress (34 °C) in the light and dark, bleached to the same degree, suggesting that photosynthetically-induced ROS production may not be the only mechanism to induce thermal bleaching.

Besides photosynthetically-induced ROS production and oxidative stress, there are four proposed mechanisms that contribute to cnidarian bleaching. The mechanisms are (1) expulsion of *Symbiodinium* from the host cell, resulting in the release of isolated algae, (2) apoptosis (programmed cell death) and/or autophagy of host cells, both resulting in the release of *Symbiodinium* with remnants of the host cell, (3) *in situ* degradation of *Symbiodinium*, (4) detachment of the host cells that still contain *Symbiodinium* (Gates et al. 1992), and (5) bacterial inhibition of photosystem II (PSII) in the *Symbiodinium* through the production of toxins and other virulence factors (Sussman et al. 2009, see discussion above) (Fig. 21.2). Independent groups using different species of corals and anemones have explored these mechanisms with varied stress conditions, and thus, evidence for all mechanisms, as drivers of bleaching exist. Programmed cell death and autophagy have been extensively studied in both corals (Franklin et al. 2004; Downs et al. 2009; Kvitt et al. 2011; Pernice et al. 2011; Tchernov et al. 2011) and sea anemones (Richier et al. 2006; Dunn et al. 2007; Dunn and Weis 2009; Hanes and Kempf 2013; Paxton et al. 2013) in response to thermal stress. Activation of apoptosis by chemicals such as colchicine leads to host cell death and degradation of algal cells in symbiotic anemones, which were also observed in anemones subjected to thermal stress (Paxton et al. 2013). Activity of caspase-3 protein, which is a downstream protein in the apoptotic pathway, has often been correlated with bleaching during thermal stress (Kvitt et al. 2011; Tchernov et al. 2011; Dunn et al. 2012). Autophagy has also been implicated as a driver of bleaching through ultrastructural examination of *Symbiodinium* cells in sea anemones either exposed to rapamycin (a known inducer of autophagy) or thermal stress (Hanes and Kempf 2013). Both conditions revealed similar ultrastructural characteristics suggestive of host autophagic degradation. However, Dunn et al. (2007) found that bleaching was significantly affected only when apoptosis and autophagy were manipulated in parallel, but not in isolation (Dunn et al. 2007).

Mechanisms of thermal bleaching that are perhaps less studied or potentially less prevalent include *in situ* degradation of *Symbiodinium* and host cell detachment. Degradation of *Symbiodinium* within host cells was observed in *Pocillopora damicornis* in response to thermal stress whereby the symbiosome membrane surrounding the endosymbiotic algae was transformed from a conduit for nutrient exchange to a digestive organelle resulting in the consumption of the *Symbiodinium* (Downs et al. 2009). Rab7 is a known protein marker of autophagy (Chen et al. 2003) and was localized to the membranes surrounding *Symbiodinium* cells in thermally stressed corals (Downs et al. 2009), suggesting that host autophagic mechanisms led to in *situ* degradation of symbiotic algae prior to release from the host cell. *In situ* degradation of *Symbiodinium* prior to release from the host cells was also observed by histology in the intertidal coral *Goniastrea aspera* under thermal stress (Le Tissier and Brown 1996). Gates et al. (1992) concluded that the driving mechanism of thermal-induced bleaching in both corals (*Pocillopora damicornis*) and the sea anemone *Aiptasia pulchella* was detachment of intact host cells containing *Symbiodinium* (either healthy or degrading) by investigation of released material during a cold stress event. Intact gastrodermal cells containing *Symbiodinium* were observed and soon after release, the host cell membrane disintegrated in the environment, leaving isolated algal cells. The authors were unable to conclude that the same mechanism led to bleaching in response to increased thermal stress. This observation led Sawyer and Muscatine (2001) to test the hypothesis that thermal stress led to a membrane thermotropic event leading to an increase in intracellular calcium concentration, resulting in a breakdown of the cytoskeleton and perturbations to cell adhesion. While they did not find support for altered calcium concentrations, thermal stress- and caffeine-treated samples showed different levels of phosphorylated proteins compared to controls, possibly leading to loss of host cells.

The previous discussion focused on the individual proposed mechanisms of thermal bleaching and gave evidence

supporting each mechanism under varied experimental conditions and in different species of corals and anemones. Each of these previous studies tested a specific hypothesis to explain the mechanism driving the loss of *Symbiodinium* from the host, however, to date no single study has set out to test the relative contribution of each proposed mechanism of bleaching in the same experimental system under the same experimental conditions. Using the model system *Aiptasia* sp., Bieri et al. (2016) set out to do just that. Sea anemones were thermally stressed at 34 °C (7 °C above their normal culture conditions, 27 °C with 25 μmol photons m^{-2} s^{-1} on a 12 h: 12 h light : dark cycle) and 150 μmol photons m^{-2} s^{-1} for 7–10 days. The bleaching response of the anemones to thermal stress was measured by determination of the relative number of *Symbiodinium* remaining in the anemone by Guava flow cytometer as previously described (Krediet et al. 2015) using the autoflourescence of the chlorophyll pigments of the algal cells. Experiments performed in parallel under the same conditions demonstrated involvement of all four of the proposed mechanisms introduced above, but concluded that expulsion of algae through exocytosis was the predominate mechanism of bleaching under the conditions tested. A spike in the apoptotic pathway as measured by caspase-3/7 protein activity was observed between 2 and 6 h of thermal stress, but bleaching was not observed until 96 h. Expelled material from bleaching anemones were collected and analyzed by fluorescence microscopy using the autoflourescence of the *Symbiodinium* and Hoescht staining of host and symbiont nuclei. Microscopic analysis indicated that the vast majority of cells in the expelled material were 'naked' algal cells not enclosed by a host membrane, thus ruling out host cell detachment as a driving mechanism of bleaching. Transmission electron microscopy (TEM) of sectioned adult anemones during thermal stress demonstrated that *Symbiodinium* cells were not degraded *in situ* and that within the host cells and in the expelled material, the *Symbiodinium* cells were intact and physiologically comparable to healthy cells under control conditions. In fact, the expelled *Symbiodinium* were photosynthetically active and able to re-infect aposymbiotic anemones (as seen in Bhagooli and Hidaka 2004). While all mechanisms were observed in this study and may play a role in thermally-induced bleaching of *Aiptasia*, under the conditions tested, the driving mechanism of bleaching was expulsion of intact *Symbiodinium* no longer surrounded by a host cell membrane (Bieri et al. 2016).

21.4.2 Bleaching in Response to Other Stressors

Increasing sea surface temperatures (SST) are not the only stressors that threaten coral-dinoflagellate symbiosis. Increased solar radiation is often correlated with elevated seawater temperatures (Lesser 1997; Dunne and Brown 2001; Jones and Hoegh-Guldberg 2001; Smith et al. 2005). The thermal bleaching paradigm (as described above) presumes that with increased light energy, there is photoinhibition of photosynthetic electron transport chain and the consequent photodamage to photosystem II (PSII) as well as the production of damaging reactive oxygen species (ROS) in the symbiotic algae, leading to coral bleaching (Smith et al. 2005). However, it is possible that these events may not be the primary drivers of bleaching but rather only constitute secondary responses to thermal and/or other stressors. When corals and sea anemones were exposed to high light stress (≥500 μmol photons m^2 s^{-1}) in the absence of thermal stress, they bleached relative to control light levels (≤100 μmol photons m^2 s^{-1}), possibly through photoinhibition of algal photosynthesis (Jones and Hoegh-Guldberg 2001; Bieri et al. 2016).

Ocean acidification (OA) is another leading threat to coral reefs worldwide. As pCO_2 in the atmosphere increases due to anthropogenic activities, the oceans absorb more and more CO_2, which ultimately decreases the pH of the ocean (Hoegh-Guldberg et al. 2007; Pandolfi et al. 2011). This acidification of the ocean puts all calcifying organisms in jeopardy as decreased ocean pH favors dissolution of calcium carbonate ions in addition to reducing calcification rates (Andersson and Gledhill 2013). While it is well accepted that ocean acidification poses a significant threat to future of coral reefs, it is less clear what effect it will have on the holobiont itself. Few studies have specifically aimed to directly measure the impact of ocean acidification on bleaching, and the results have often been contradictory (Reynaud et al. 2003; Schneider and Erez 2006; Anthony et al. 2008a; Wall et al. 2014; Bieri and Pringle 2016). Early studies using corals and moderate light levels (approximately 350 μmol photons m^2 s^{-1}) found no effect of pCO_2 (460 μatm vs. 760 μatm) on bleaching after 5 weeks, whereas thermal stress did induce bleaching (Reynaud et al. 2003). Similarly, Schneider and Erez (2006) found that a decrease in pH of 0.2 pH units led to decreased calcification (50% of controls) but did not affect photosynthesis or respiration of the symbiotic algae. Longer (8 weeks) exposure of *Acropora* corals to increased pCO_2 did lead to bleaching (40–50% chlA pigment loss) as compared to control corals (Anthony et al. 2008b). In addition to the longer exposure, corals in this study were exposed to much higher light irradiances (1,000 μmol photons m^2 s^{-1}) than the previous studies, which could explain the exacerbated bleaching response (Anthony et al. 2008b). Of course, in light of increasing SSTs and ocean acidification, it is possible that these mixed stressors may have an additive effect on coral-dinoflagellate symbioses. Indeed, while low pH stress did not affect *Symbiodinium* photophysiology or productivity and did not cause bleaching (Reynaud et al. 2003;

Schneider and Erez 2006; Wall et al. 2014), low pH stress in addition to thermal stress did lead to bleaching in both corals and sea anemones (Wall et al. 2014; Bieri and Pringle 2016).

Eutrophication and runoff from fertilizers and herbicides are additional threats to the health and stability of coral reefs. Chemical inhibitors of photosystem II including the common herbicide diuron (DCMU) and inhibitors found in marine anti-fouling paints (e.g. copper) have also been shown to lead to coral bleaching, independently or synergistically with other stressors (Jones 2004, 2005; Negri et al. 2005, 2011; van Dam et al. 2015). Photoinhibtion and algal bleaching are often measured by F_v/F_m, which is a measure of the overall heath and photosynthetic capacity of photosystem II based on chlorophyll fluorescence (Baker 2008). While these inhibitors are specific to photosystem II and do not appear to be toxic to the coral host at biologically relevant concentrations (Negri et al. 2005), these chemicals can lead to bleaching and decreased photosynthetic potential in the symbiotic algae, even at concentrations as low as 10–30 μg l^{-1} when mixed with high light stress (Jones 2004). Photoinhibtion and subsequent bleaching was increased when corals were incubated with diuron or atrazine at 32 °C compared to control treatments, suggesting that reduced water quality due to runoff of herbicides and fertilizers increases the vulnerability of corals to elevated sea surface temperatures (Wooldridge 2009; Negri et al. 2011).

Of course, corals rarely experience a single stress at a given time and these stressors have synergistic effects as discussed above. Elevated concentrations of a certain nutrient (e.g. nitrogen) on a coral reef will likely lead to a comparative limitation of another nutrient. This potential starvation of one nutrient can destabilize the symbiosis and make corals more susceptible to thermal and other stressors (Wiedenmann et al. 2012). Numerous linkages have been made between multiple stressors and coral bleaching and there is a growing appreciation that studying individual stressors only provides a snap-shot of what is happening on a coral reef and that long-term monitoring and assessment of multiple, synergistic stressors is needed (Hughes and Connell 1997; Takahashi and Murata 2008; Wooldridge 2009; van Dam et al. 2015).

21.5 Current Uncertainties and Potential Directions for Future Research

Growing evidence indicates that key members of coral-associated microbial communities are coral species-specific. How do corals influence the composition of their associated microbiota is not entirely certain. Based on comparisons with other models of host-microbial interactions, hosts have evolved multiple, often overlapping strategies for selecting symbionts, encouraging commensals and fighting off pathogens. The ability to distinguish amongst potential symbionts and other microorganisms based on their surface structures (lipopolysaccharide, peptidoglycan, flagellin, etc.) and/or timing or place of their presentation has been documented during the establishment of *Vibrio fischeri*-bobtail squid and rhizobium-legume (Post et al. 2012; McFall-Ngai et al. 2012). Genomes of Cnidarians (including Hydrozoa and Anthozoa) encode homologues of proteins capable of recognizing microorganisms and their associated molecular patterns (MAMPs): C-type and other lectins, membrane-associated Toll-like receptors and intracellular nucleotide-binding and oligomerization (NOD)-like receptors (Augustin et al. 2010; Shinzato et al. 2011; Reidling et al. 2000; Sunagawa et al. 2009b; Iguchi et al. 2011; Kvennefors et al. 2008; Reitzel et al. 2008; Baumgarten et al. 2015). Two *P. damicornis* lectins (PdC and Concanavalin) were strongly up-regulated following challenge with a virulent strain of *V. coralliilyticus* (Vidal-Dupiol et al. 2011), suggesting that they are involved in the recognition and response to pathogens.

As described above, corals use their immune system to control their symbiotic zooxanthellae. Do corals use similar mechanisms to control their bacterial associates? Kvennefors et al. (2008) reported molecular overlap in the way corals control their algal and bacterial associates. A purified lectin from *A. millepora*, Millectin, bound and coagulated vibrios, Gram-positive bacteria as well as cells of *Symbiodinium* (Kvennefors et al. 2008). However, bacterial and dinoflagellate surface structures recognized by the cnidarian pattern recognition receptors are not yet known.

In addition to the detection of specific MAMPs (microorganism-associated molecular patterns, such as LPS, peptidoglycan, flagellin, etc.), hosts can excrete antimicrobial compounds to select against general environmental organisms and/or release of chemical cues and/or nutrients that attract microorganisms with potentially beneficial functions, among other potential mechanisms (Krediet et al. 2013b). Antimicrobial compounds produced by corals probably function in controlling the associated microbiota. Ritchie (2006) demonstrated strong selective properties of mucus (Ritchie 2006). Antimicrobials from corals appear to have different chemical structures, based on the reported results of bioassay-guided solvent partitioning studies. In *Siderastrea siderea*, substances in organic extracts had selective antimicrobial activity against two of four strains of Gram positive bacteria isolated from coral surfaces (Gochfeld et al. 2006). In Pacific corals *Montipora captitata*, *Porites lobata,* and *Pocillopora meandrina*, antibiotic activities against known coral pathogens and their close relatives were found in the crude aqueous extracts (Gochfeld and Aeby 2008). Extracts of *M. capitata* displayed the most antimicrobial activity, which might be related to the presence of montiporic acids A and B, which are cytotoxic and antimicrobial polyacetylene carboxylic acids found in *Montipora* spp.

(Fusetani et al. 1996). Otherwise, the chemical structures of antimicrobial compounds in corals are not known. Elucidating chemical structures of secondary compounds produced within the holobiont and capable of controlling the associated microbiota should be another research priority.

The role of commensal coral microbiota in coral diseases remains unclear. There are at least half a dozen of coral diseases that are generally recognized (Rosenberg et al. 2007; Bourne et al. 2009), however, signs of pathologies described as coral diseases are quite general. Despite the fact that several coral pathogens have been identified and Koch's postulates for them have been fulfilled (Rosenberg et al. 2007; Bourne et al. 2009), it is possible to hypothesize that at least some coral diseases are a collection of generic symptoms that could be elicited by a number of opportunistic pathogens which attack the host when its defenses are compromised (Lesser et al. 2007a). It is also possible that members of the commensal microbiota escape restrictions imposed on them by the host and then multiply to high numbers and start degrading host tissues under some conditions (Krediet et al. 2013b). Reported shifts in the composition of coral microbiota during disease outbreaks (Bourne et al. 2008; Sunagawa et al. 2009a; Meyer et al. 2014) can be interpreted as evidence to support competing hypotheses of coral diseases. It remains to be established whether these shifts are a cause or a consequence (or both) of the coral diseases.

Commensal microbiota has also been shown to produce activities that inhibit pathogens or disease progression (Ritchie 2006; Alagely et al. 2011; Krediet et al. 2013a). Members of native microbiota produce antimicrobials, inhibitors of QS and enzymatic activities in coral pathogens. Chemical structures responsible for the observed outcomes are not currently known. More broadly, characterization of the functions of native microbiota in coral disease progression will have a major impact on our understanding of interactions on coral surfaces and may very well have biotechnological applications beyond coral reefs.

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Association of Coral-Microbes, and the Ecological Roles of Microbial Symbionts in Corals

22

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Abstract

Corals are sessile marine invertebrates and they form a unique ecosystem, which supports diverse marine organisms. They host a wide variety of microorganisms such as bacteria, archaea and fungi. The interactions between corals and associated microbes are likely to play an important role in coral health. These microbial communities respond and quickly adapt to changes and also have central roles in ecological function. They are also involved in the biogeochemical cycling, particularly nitrogen, sulfur, phosphorus as well as in host defense. A complex web of interactions exists between the coral-associated microbes and each of them is linked with one another. So, characterizing these links is fundamental to understand coral reef resilience and to improve to predict ecological change. The culture-independent methods have recorded high proportions of novel coral-associated microbes and also species-specific microbial communities. Metagenomics-based investigations have also revealed the dynamics in the genomic diversity of the microbes upon environmental changes. Eventhough, their complete roles are not currently accounted and only less culturable microbes have been isolated, especially from the stony corals. So, it is necessary to develop novel techniques to increase the culturables as well as explore the shaping factors of coral-associated microbes and their central functions in corals.

Keywords

Corals • Symbionts • Microorganisms • Nutrient cycling • Host defense • Stress tolerance

22.1 Introduction

Corals (phylum Cnidaria, class Anthozoa) are sessile marine invertebrates and classified into two different types as stony and soft corals. Stony corals are known as reef-building scleractinian corals and the majority of the coral life cycle is spent in the polyp stage, attached to a hard substrate and surrounded by a secreted, calcium carbonate skeleton. The calcium in stony corals is extracted from surrounding seawater for structural organization, protection and growth. Soft corals comprise a variety of species in subclass Alcyonaria or Octocorallia, such as gorgonians and sea pens. Soft corals do not produce a rigid calcium carbonate skeleton and do not form reefs, although they may appear in a reef ecosystem. Soft coral polyps are anatomically similar to their hard coral counterparts, with a few differences. Symbiotic relationship commonly occurs between the coral hosts and their algal and microbial symbionts (Wegley et al. 2007). Corals host diverse array of microorganisms including bacteria, archaea, and fungi and certain microbial processes can influence the physiology of coral hosts as well as coral reef ecosystem, e.g., coral-associated microorganisms play a key role in nutrient cycling, coral health, and coral reef resilience (Fig. 22.1) (Ainsworth et al. 2010; Krediet et al. 2013). A complex web of interactions exists between the microbial holobiont.

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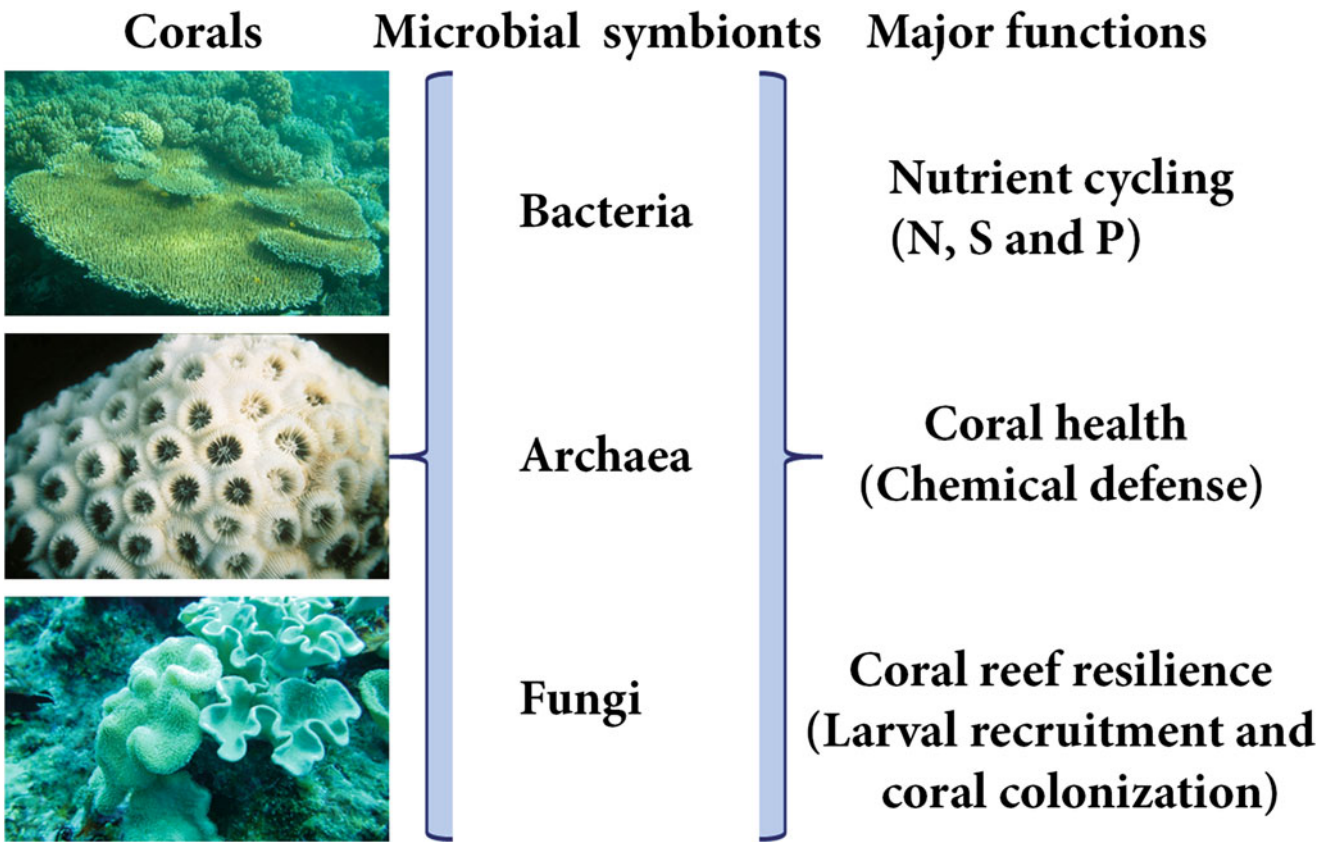

Fig. 22.1 Corals-associated microorganisms and their major ecological roles

Each member is linked with another, so that any change in a member will have impact on the others. A holobiont theory of evolution has been proposed, whereby rapid adaptation of corals to specific pathogens is attributed to change in the composition of the associated microbiota resulting in "evolution of the hologenome" (Rosenberg et al. 2007). As like in the rhizosphere of plants, corals change their surroundings by producing organic molecules to create niches for microbial growth near, on, and within the corals (Brown and Bythell 2005). Coral-associated microbial community shifts have been recorded between healthy and diseased corals in both culture (Ritchie and Smith 1998) and culture-independent based analyses (Cooney et al. 2002; Frias-Lopez et al. 2002; Pantos et al. 2003). A trend of healthy coral having lower diversity than diseased coral have also been reported (Frias-Lopez et al. 2002). The culture-independent methods such as sequencing of 16S rRNA genes have discovered high proportions of novel coral-associated microbial diversity and the dynamics in the genomic diversity of the microbes upon environmental changes has been revealed by the metagenomics (Yokouchi et al. 2006; Dinsdale et al. 2008; Vega Thurber et al. 2009).

Exploring the community structure of coral-associated microbes is critical and significant for understanding their potential ecological roles. Early investigations on coral microbiota based on molecular techniques revealed the presence of diverse and sometimes species-specific microbial communities (Rohwer et al. 2002; Wegley et al. 2004). In recent years, metagenomics-based studies greatly enriched the known diversity of coral microbiota (Tringe et al. 2005). Meanwhile, increasing ecological functions of these microbes were revealed (Krediet et al. 2013). It has been found that involvement of coral-associated microbes in biogeochemical cycling, particularly nitrogen, sulfur and phosphorus cycle, promote coral health and drive the resilience of coral reef ecosystems. This chapter mainly presents the diversity and ecological roles of coral-associated bacteria, archaea and fungi.

22.2 Microbial Symbionts in Corals

22.2.1 Bacteria

It has been documented about 1,000 distinct microbial ribotypes, including the representatives of 16 bacterial divisions from corals (Rohwer and Kelley 2004). The degradation of the mucus constituents in the corals facilitates to the fast growth of microbes. Generally, the microbial community structure is determined by environmental factors as well as

physiological conditions of the coral holobiont (Hong et al. 2009). A molecular-based investigation on the coral *Lophelia pertusa* showed the presence of unique and distinct bacterial community in contrast to the surrounding water (Yakimov et al. 2006; Kellogg et al. 2009; Schottner et al. 2009). Diverse bacterial community was also identified from the coral *Fungia granulosa* by molecular techniques, with most dominant bacterial group belonging to class *Alphaproteobacteria* (Kooperman et al. 2007).

The host species-specific bacteria have been recorded from the mucus of *Mussismilia braziliensis* and *M. hispida* (Reis et al. 2009; de Castro et al. 2010). By using culture-independent methods species-specific bacteria associated with *L. pertusa*, mycoplasma-like bacteria and thiotrophic bacteria were also described (Neulinger et al. 2008; Kellogg et al. 2009). The Caribbean coral *Montastraea annularis* mainly hosted green sulfur bacteria, *Alphaproteobacteria*, *Firmicutes* and *Planctomycetales*, whereas the coral *M. cavernosa*-associated bacterial community was predominated by *Gammaproteobacteria* and *Betaproteobacteria* (Frias-Lopez et al. 2002). Physically adjacent corals also hosted species-specific bacterial community (Rohwer et al. 2002). In the Caribbean coral *Porites astreoides*, the associated bacterial community largely comprised *Gammaproteobacteria* (Rohwer et al. 2002). The soft coral *Alcyonium antarcticum* was collected from the Antarctica and high diversity of coral-associated bacteria was observed, with the detection of *Gamma-*, *Alpha-* and *Beta-Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Actinomycetales*, *Planctomycetes* and *Chlorobi* (Webster and Bourne 2007). The predominance of *Alphaproteobacteria* and *Gammaproteobacteria* has been recorded in different soft as well as stony corals (Bourne and Munn 2005). From the soft coral *Paragorgia arborea*, a *Flavobacterium* species was successfully cultured (Nedashkovskaya et al. 2005) and one *Erythrobacter* species was cultivated from an unidentified soft coral (Ivanova et al. 2005). Another culture-dependent study examined the soft coral *Dendronephthya* sp.-associated bacterial population, in which *Gammaproteobacteria* constituted 55 %, *Alphaproteobacteria* added up to 27 % and *Bacteroidetes* accounted for 18 % (Harder et al. 2003). Based on available culture-dependent and culture-independent surveys (Rohwer et al. 2001, 2002; Bourne and Munn 2005; Lampert et al. 2006; Wegley et al. 2007; Thurber et al. 2009; Chen et al. 2011; Littman et al. 2011; Meron et al. 2011, 2012; Bourne et al. 2013; Yang et al. 2013a, b; Closek et al. 2014; Li et al. 2014a, b), the known diversity of coral-associated bacteria was summarized at the phylum/class level (Table 22.1).

The microbial assemblage in cold-water corals in deep water is totally different from that in the shallow water corals because of different environmental parameters such as darkness, temperature and pressure (Kellogg et al. 2009). Bacteria live in three different sections in one coral including surface mucus layer, coral tissue, and the calcium carbonate skeleton, each of which harbors distinct bacterial community (Bourne and Munn 2005; Nithyanand and Pandian 2009). In the tissue of Great Barrier Reef coral *P. damicornis*, the bacterial community was dominated by *Gammaproteobacteria*, but in the mucus it was dominated by *Alphaproteobacteria* (Bourne and Munn 2005). High bacterial diversity has also been recorded from the black band diseased coral *Siderastrea sidereal* and the bleaching coral *Acropora millepora* (Sekar et al. 2006; Bourne et al. 2008). In contrast to the culture-independent techniques the culture-based methods underestimated the diversity of the coral-associated bacterial communities (Rohwer et al. 2001). Most isolates from the Red Sea solitary coral *F. Scutaria* belonged to *Gammaproteobacteria*, *Alphaproteobacteria* and *Actinobacteria*. Approximately 30 % of the isolates were representatives of putative novel species and one strain was likely to be a representative of a new genus (Lampert et al. 2006).

22.2.2 Archaea

Archaea are widespread in marine environments. In 2004, by using Archaea-specific primers, diverse archaeal 16S ribotypes were detected from the mucus of three reef-building Caribbean corals, which were affiliated with *Crenarchaeota* (Marine Group I) and *Euryarchaeota* (Marine Group II, Marine group III, *Thermoplasma*-like and methanogen) (Kellogg 2004). Meanwhile, by means of fluorescent in situ hybridization, Wegley and coworkers found that Archaea were present at $>10^7$ cells cm^{-2} in *P. astreoides*, accounting for nearly half of the prokaryotic community (Wegley et al. 2004). In the analysis of the *P. astreoides* metagenome (Wegley et al. 2007), the sequences of archaeal origin comprised approximately 1 % of the total sequences and *Crenarchaeotes*-related sequences were detected. Diversity and distribution of Archaea associated with the surface mucus of corals from three genera (*Acanthastrea*, *Favia* and *Fungia*) from the Gulf of Eilat, Israel and from Heron Island, Australia were investigated by Siboni et al. (2008). 16S rRNA gene sequencing showed that *Crenarchaeota* dominated the archaeal community (79 %, on average), most of which were closely related to *Nitrosopumilus maritimus*. Based on currently available studies, *Crenarchaeota* and *Euryarchaeota* represented most common archaeal symbionts associated with corals (Kellogg 2004; Wegley et al. 2004, 2007; Siboni et al. 2008) (Table 22.1). These findings indicated that Archaea are abundant, diverse, and potentially important components of the coral holobionts.

Table 22.1 Known diversity of coral-associated microbes at the phylum/class level

Domain	Phylum/class
Bacteria	*Acidobacteria*
	Actinobacteria
	Alphaproteobacteria
	Aquificae
	Bacteroidetes
	Betaproteobacteria
	Caldithrix_KSB1
	Chlamydiae
	Chlorobi
	Chloroflexi
	Cyanobacteria
	Deferribacteres
	Deinococcus-Thermus
	Deltaproteobacteria
	Epsilonproteobacteria
	Firmicutes
	Fusobacteria
	Gammaproteobacteria
	Gemmatimonadetes
	Lentisphaerae
	Nitrospirae
	OD1
	Planctomycetes
	Spirochaetes
	Tenericutes
	Thermotogae
	TM7
	Verrucomicrobia
	WS3
	Zetapr oteobacteria
Archaea	*Crenarchaeota*
	Euryarchaeota
Fungi	*Ascomycota*
	Basidiomycota
	Chytridiomycota

22.2.3 Fungi

Coral-associated fungi have been described from diverse hosts inhabiting different geographical locations (Kendrick et al. 1982; LeCampion-Alsumard et al. 1995; Freiwald et al. 1997; Bentis et al. 2000; Golubic et al. 2005). For decades the idea that coral-associated fungi are pathogenic to corals or endolithic algae within the coral skeleton has been widely accepted (Priess et al. 2000). However, recent investigation proposed that some fungal groups are potentially beneficial to the corals (Wegley et al. 2007). Consequently, the diversity of the coral-associated fungi attracted increasing research interest. In the analysis of the *P. astreoides* metagenome, fungi were found to be highly prevalent within the coral holobiont (Wegley et al. 2007). Members from three fungal phyla (*Ascomycota*, *Basidiomycota* and *Chytridiomycota*) were identified, with *Ascomycota* as the dominant phylum. Most of the fungi were affiliated with the class *Sordariomycetes*. Others were assigned to three fungal classes including *Eurotiomycetes*, *Saccharomycetes* and *Schizosaccharomycetes* (Wegley et al. 2007). An SSU-based amplicon sequencing/transcriptomic analysis of the fungal community associated with the coral *A. hyacinthus* showed the presence of a diverse, metabolically active, fungal community (Amend et al. 2012). Phylogenetic analysis of the fungal ribosomal DNA amplicons revealed a high diversity of *Basidiomycetes* and *Ascomycetes*, including several clades separated from known fungal taxa by long and well-supported branches. Interestingly, a phylogenetically diverse core assemblage of fungi consistently associated with *A. Hyacinthus* was also found (Amend et al. 2012).

In addition to culture-independent investigations, culture-based studies also significantly enriched known diversity of coral-associated fungi at the species/genus level. The diversity of culturable fungi associated with six species of healthy South China Sea gorgonians were investigated using a culture-dependent method (Zhang et al. 2012), a total of 121 fungal strains were isolated and they were identified to be 41 fungal species from 20 genera. The diversity of fungi associated with *P. pukoensis* was investigated and the results showed the presence of 23 fungal strains belonging to 10 genera and *Aspergillus* was predominant fungal genus (Li et al. 2014a, b). Overall, diverse fungi have been recorded in various corals (Wegley et al. 2007; Amend et al. 2012) (Table 22.1), and the picture of their diversity will be drawn with more and more in-depth investigations.

22.3 Association of Coral-Microbes and Ecological Roles of Microbial Symbionts in Corals

It was reported that corals can adapt to different ecosystems by changing its microbial associates, suggesting potential key roles of coral microbiota (Wegley et al. 2007). In the past decade, our knowledge of ecological significances of coral-associated microbes has been greatly advanced. Within healthy corals, coral microbes are probably involved in diverse processes of recycling inorganic nutrients, based on the analysis of functional genes derived from microbes (Wild et al. 2004; Ritchie 2006). Coral microbial symbionts can protect their coral hosts from pathogens by filling entry niches, or producing bioactive metabolites, and the disruption of this association may lead to coral disease (Mouchka et al. 2010). In addition, microbial processes are also key drivers of many factors that influence the resilience of coral

reef ecosystems, such as larval recruitment and colonization (Ainsworth et al. 2010). Bacteria associated with corals are playing various important roles in both coral hosts and coral reefs ecosystem. The changes in the bacterial community structure may lead to the coral disease and bleaching (Kvennefors et al. 2010). In this part, the ecological roles of coral-associated microbes were described from three major aspects, including biogeochemical cycling (nitrogen, sulfur and phosphorus cycle), coral health (chemical defense) and other functions relatively rarely concerned.

22.3.1 Nitrogen Cycle

Besides carbon, nitrogen is the most important nutrient for life, as it is required for the synthesis of amino acids and proteins. In coral reefs where nitrogen concentration is low, although the coral animals can assimilate organic nitrogen from the water column or their prey, efficiently recycling nutrients are still important and microbial members of the coral holobiont are potentially involved in different process in the nitrogen cycle. The first evidence that microbes contribute to the nitrogen budget of corals via fixation of atmospheric nitrogen was proposed early in 1987. It was estimated that nitrogen-fixing bacteria could supply up to 6 % of the total nitrogen requirements of the coral community, as measured by acetylene reduction (Williams et al. 1987). Shashar et al. (1994) proposed that nitrogen-fixing bacteria in the skeleton of corals benefited from organic carbon excreted by the coral tissue. Their finding suggested the interaction between the nitrogen-fixing microorganisms and the coral may be of importance for the nitrogen budget of the corals. Lesser and coworkers observed high abundance of cyanobacteria cells on the surface of *M. cavernosa* and detected the presence of nitrogenase proteins, which suggested these *Cyanobacteria* were capable of fixing nitrogen (Lesser et al. 2004). In 2007, Beman and coworkers investigated the distribution and diversity of archaeal ammonia monooxygenase genes associated with various coral species, suggesting that *Crenarchaeota* may be involved in ammonia oxidation (Beman et al. 2007). Notably, bacterial ammonia monooxygenase subunit A (*amo*A) gene was semultaneously amplified, however, it could not be obtained from any coral sample. Chimetto et al. (2008) reported that *Vibrio harveyi* and *V. alginolyticus* were capable of nitrogen fixation in coral mucus and dominated in the culturable nitrogen-fixing bacteria of the Brazilian coral *M. hispida*. Kimes et al. (2010) have detected bacterial nitrite reductase (*nir*) and *amo*A genes in the coral *M. faveolata*, suggesting that coral-associated bacteria also potentially participate in the ammonia oxidation outside coral-associated archaea. A comprehensive survey of nitrogen-fixing bacteria (using *nif*H nitrogenase gene as the functional marker) recovered from mucus and tissues of three corals on the Great Barrier Reef revealed that the diversity of the *nif*H in mucus was generally similar to that in the surrounding seawater; however, over 70 % of *nif*H sequences recovered from tissues of corals were most similar to those from rhizobia (Lema et al. 2012). However, the ability of marine rhizobia to fix nitrogen in the association with the coral holobiont has not been confirmed experimentally, related research in future will advance our knowledge of evolution of nitrogen fixation and holobiont ecology. By using *nir*S, *nir*K and *amo*A genes as functional markers, the diversity of bacterial denitrifiers and ammonia oxidizing bacteria (AOB) were revealed in two East China Sea corals *Alcyonium gracillimum* and *Tubastraea coccinea* and it suggested that coral-associated bacteria are potentially involved in the process of denitrification and ammonia oxidation (Yang et al. 2013a, b).

In recent years, metagenomic analysis further supported that several microbial groups within the coral holobiont were potentially involved in nitrogen cycling, including previously reported *Cyanobacteria*, non-photosynthetic *Bacteria* and ammonia-oxidizing *Crenarchaeotes* (Wegley et al. 2007). In the metagenomic library from *P. astreoides*, *Cyanobacteria* added up to 7 % of the bacterial groups and included nitrogen fixers, such as *Chroococcales* and *Nostocales*. Nitrogenase and *nif*-specific regulatory protein were detected in the metagenome as well. In addition, metagenomics-based investigation provided new insights into the potential role of coral-associated fungi. The endolithic fungi potentially participated in two steps of the nitrogen cycle within the coral, including reduction of nitrate/nitrite to ammonia and assimilating ammonia, suggesting that the endolithic fungi play a previously unknown role in the nitrogen cycle within the coral holobiont.

22.3.2 Sulfur and Phosphorus Transformation

In contrast to nitrogen cycle, relatively little is known about sulfur metabolism of coral-associated microorganisms. Sulfur is one of the essential element for the synthesis of proteins. Both inorganic and organic sulfur sources can be assimilated by microbes and used for the biosynthesis of certain amino acids. Glutathione can be used in sulfur metabolism by some bacteria. Wegley et al. (2007) reported the genes responsible for transport and degradation of glutathione in the metagenome library of the coral *P. astreoides*. The abundance of genes related to glutathione utilization was three times higher than that of genes for the assimilation of inorganic sulfur, suggesting that the coral-associated microbes probably use glutathione as a sulfur source, which commonly occurs in eukaryotic cells in quantities.

Marine bacteria are known to be involved in the degradation of dimethylsulfoniopropionate (DMSP) to dimethyl sul-

fide (DMS) and acrylic acid. High concentrations of DMSP and DMS were previously recorded in scleractinian corals by Broadbent and Jones (2004) and Van Alstyne et al. (2006). Since DMSP often occurs as one product of coral symbiotic zooxanthellae (Raina et al. 2010), the question whether coral-associated microbes can degrade DSMP awaits further exploration. To reveal the influence of these organic sulfur compounds on coral-associated bacteria, Raina et al. (2009) investigated the bacterial communities associated with *Montipora aequituberculata* and *A. millepora*, using both culture-dependent and molecular techniques. Strains from four genera (*Roseobacter*, *Spongiobacter*, *Vibrio*, and *Alteromonas*) were isolated on media with either DMSP or DMS as the sole carbon source. Meanwhile, clones representing these genera accounted for the majority of those retrieved from coral mucus and tissue 16S rRNA gene libraries. In addition, genes homologous to dddD and dddL, closely related to DMSP degradation, were characterized from the isolates, confirming that coral-associated bacteria have the potential to metabolize the sulfur compounds present in coral tissues. These findings suggested that DMSP, DMS, and acrylic acid potentially act as a nutrient sources for coral-associated bacteria and that these sulfur compounds were likely to play a key role in the structuring bacterial communities in corals.

Phosphonates are characterized by a stable carbon-phosphorus bond and commonly occur as lipid conjugates in invertebrate cell membranes. Phosphonoacetate hydrolase is encoded by the *phn*A gene, which catalyses the cleavage of phosphonoacetate to acetate and phosphate. Thomas et al. (2010) demonstrated high *phn*A diversity in coral-associated bacteria. A total of 16 bacterial taxa capable of using phosphonoacetate as the sole carbon and phosphorus source were isolated from various tropical and temperate corals, among which 8 had a *phn*A-positive genotype. This study provided enhanced information on the taxonomic and environmental distribution of *phn*A, and highlighted the importance of bacteria in phosphonates transformation in coral ecosystems.

22.3.3 Chemical Defense

Coral-associated microorganisms have the potential to synthesize diverse bioactive metabolites, with significant role in pathogen's catabolic enzymes inhibition and disruption of cell-to-cell communication in pathogens (Ritchie 2006; Shnit-Orland and Kushmaro 2009; Teplitski and Ritchie 2009; Alagely et al. 2011). The compounds isolated from these microbes also exhibited activity against broad spectrum of pathogens, including coral pathogens (Ritchie 2006; Shnit-Orland and Kushmaro 2009). Initially, coral microbiota was supposed to play a protective role for their coral hosts by means of producing antibiotics and competing with pathogens. To confirm this hypothesis, a series of studies were subsequently performed. Shnit-Orland and Kushmaro (2009) proposed that coral mucus-associated bacteria possibly acted as a first line of defense to protect the coral host against pathogens. Their research results showed that between 25 % and 70 % of culturable mucus-associated bacteria from scleractinian corals exhibited antibacterial activity, which were mainly members of *Bacillus* and *Vibrio*. In contrast to those bacteria from branching or soft corals, higher percentages of activity were observed in mucus-associated culturable bacteria from massive and solitary corals (Shnit-Orland and Kushmaro 2009). Nithyanand and Pandian (2009) investigated the antibacterial activity of bacteria associated with *A. digitifera* and they found 37 % of isolates displayed antibacterial activity against different pathogens, including both mucus-associated and tissue-associated isolates. Moreover, they reported for the first time that actinomycetes were present in both the coral mucus and the coral tissue and showed high activity against pathogens. Nithyanand et al. (2011) examined the culturable actinomycetes associated with the mucus of the same coral species. Excitingly, all the actinomycete isolates exhibited antibacterial activity against various pathogens. In recent years, bioactive compounds are increasingly being discovered from marine actinomycetes, however, there are few reports of coral-associated actinomycetes that produced bioactive natural products. All these findings suggested that coral-associated actinomycetes were probably involved in the chemical defense for coral hosts. The commensal bacteria associated with healthy coral showed activity against known coral pathogens. But, the bacteria associated with diseased corals (*V. coralliilyticus* and *Pseudoalteromonas* spp.), exhibited strong activity against native coral bacteria and this also suggested that these bacteria may inhibit the potential probiotic strains under favorable condition (Gantar et al. 2011; Kvennefors 2012). Epibiotic bacteria (*Vibrio* sp., and an unidentified α-Proteobacterium) were isolated from the soft coral *Dendronephthya* sp., exhibited inhibition against the settlement of larvae of tubeworms *Hydroides elegans*. This indicated that, bacteria associated with corals may play a key role in the antifouling mechanisms of the host against bacterial growth and settlement of macrofoulers. Recently, Shnit-Orland et al. (2012) reported that coral mucus-associated *Pseudoalteromonas* may participate in the coral holobiont's defense against Gram-positive coral pathogens. Based on the studies mentioned above, it is proposed that coral mucus layer-derived bacteria probably contribute to the chemical defense of their coral hosts. Even though, several metabolites were isolated from the microbes associated with corals, they were mainly derived from the soft corals (Table 22.2 and Fig. 22.2). It remains unexplored the metabolic potential of reef forming coral-associated microorganisms as well as their roles, and importance in the host health. It is

Table 22.2 Metabolites isolated from coral-associated microorganisms

Host	Microbes	Compounds	Activity	References
Muricella abnormaliz	*Aspergillus* sp.	Penilumamides B–D (**1**–**3**)	–	Chen et al. (2014a)
		22-*O*-(*N*-Me-L-valyl)aflaquinolone B (**4**)	–	
		22-*O*-(*N*Me-L-valyl)-21-epi-aflaquinolone B (**5**)	Anti-respiratory syncytial virus	
		Aflaquinolones A and D (**6**, **7**)	Anti-RSV activity (A)	Chen et al. (2014b)
Sarcophyton sp.	*Eurotium rubrum*	Eurothiocin A and B (**8**, **9**)	α-glucosidase	Liu et al. (2014)
Dichotella gemmacea	*Aspergillus* sp.	Aspergilone A (**10**)	–	Shao et al. (2013)
		Aspergilone B (**11**)	Cytotoxicity and antifouling	
Sarcophyton tortuosum	*Chondrostereum* sp.	Chondrosterins F–H (**12**–**14**)	–	Li et al. (2013)
Dichotella gemmacea	*Aspergillus* sp.	17-epinotoamides Q and M (**15**, **16**)	Antibacterial	
		Cordyols D and E (**17**, **18**)	Antibacterial	Chen et al. (2013)
Sarcophyton sp.	*Pestalotiopsis* sp.	(±)-Pestalachloride D (**19**)	Antibacterial	Wei et al. (2013)
		(±)-pestalachloride C (**20**)	Antibacterial	
Sarcophyton sp.	*A. elegans*	Phenylalanine derivative 4′-OMe-asperphenamate (**21**)	–	
		Aspochalasin A1 (**22**)	–	
		Cytochalasin Z24 (**23**)	–	Zheng et al. (2013)
Sarcophyton sp.	*Alternaria* sp.	Tetrahydroaltersolanols C–F [1–5] (**24**–**27**)	Antiviral (C)	
		Dihydroaltersolanol A [6] (**28**)	–	
		Alterporriols N–R [2–16] (**29**–**33**)	Cytotoxicity (L)	Zheng et al. (2012)
S. tortuosum	*Chondrostereum* sp.	Chondrosterins A–E (**34**-**38**)	Cytotoxicity (1)	Li et al. (2012)
Scleronephthya sp.	*Micromonospora* sp.	Jadomycin B (**39**)	–	Sun et al. (2012)
Cladiella sp.	*A. versicolor*	Cottoquinazoline D (**40**)	Antifungal	Zhuang et al. (2011)
D. gemmacea	*C. lunatus*	Cochliomycins A–C (**41**–**43**)	Antibacterial and antifouling (A)	Shao et al. (2011)
Annella sp.	*A. sydowii*	Aspergillusenes A and B (**44**, **45**)	–	Trisuwan et al. (2011)
		(+)-(7S)-7-O-methylsydonic acid (**46**),	–	
		Aspergillusones A and B (**47**, **48**)	–	
D. gemmacea	*Aspergillus* sp.	(+)-methyl sydowate (**49**)	–	Wei et al. (2010)
		7-deoxy-7,14-didehydrosydonic acid (**50**)	–	
		7-deoxy-7,8-didehydrosydonic acid (**51**)	–	

also not well known that the ability and levels of secondary metabolites produced by these microorganisms and effects on other coral-associated microbial communities.

22.3.4 Other Functions

Besides influencing biogeochemical and ecological processes mentioned above, microbial processes are also able to influence the resilience of coral reef ecosystems, such as larval recruitment and colonization (Dobretsov and Qian 2004). For example, chemical cues from the coral-associated microbial communities can influence the settlement of larvae of many coral species (Webster et al. 2004). In recent years, with the rapid development of metagenomics and its application in the environmental samples including corals, new insights into the functions of coral-associated microbiota were provided. In the metagenomic analysis of the microbial community associated with the coral *P. astreoides*, high abundance of genes involved in DNA repair was observed in the library, suggesting that coral microbes have repair mechanisms to deal with ultraviolet radiation (Wegley et al. 2007). To the better understanding of the molecular mechanisms underlying coral biology, Shinzato et al. (2011) decoded the approximately 420-megabase genome of *Acropora digitifera* using next-generation sequencing technology. They found *Acropora* seemed to lack an enzyme essential for cysteine biosynthesis, implying dependency of this coral on its symbionts for this amino acid. Comparative metagenomics allows the identification of microbial communities that share similar functional and taxonomic composition, and the observation of patterns controlling ecological niche partitioning. By comparing the metagenomes of two coral species with other metagenomes, Carlos et al. (2014) identified some functions that are probably required or advantageous for coral colonization, such as transport proteins, suggesting they may play a role in the establishment and maintenance of bacteria-coral associations. There is no doubt that some pre-

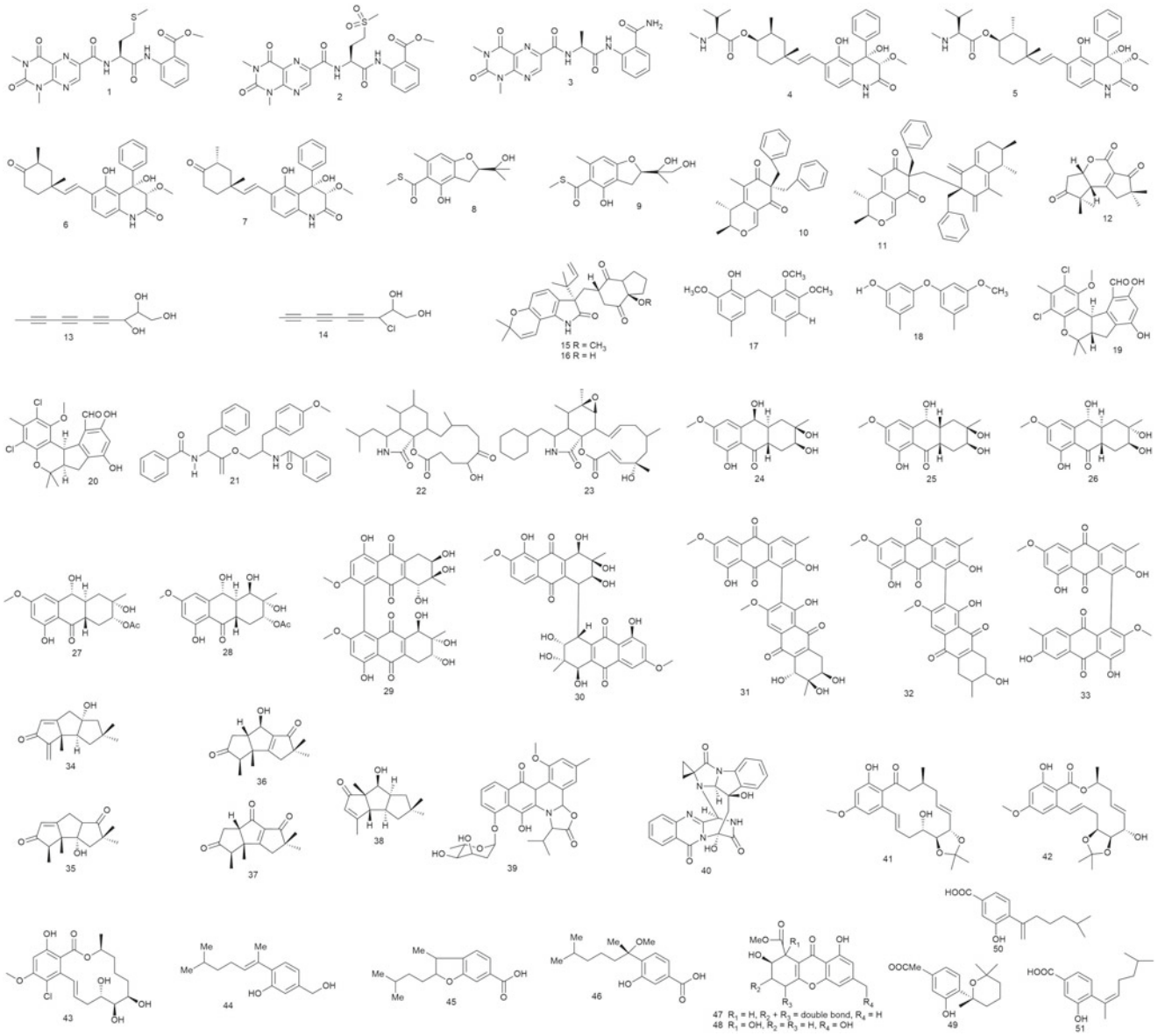

Fig. 22.2 Secondary metabolites isolated from the coral-associated microorganisms

viously unknown roles of coral microbes will be revealed by increasing metagenomic analyses in future researches.

22.4 Summary

Coral reefs, considered as the rainforests of the oceans, are the most diverse and biologically productive of all marine ecosystems. The coral holobiont is a dynamic assemblage of the coral animal, algae (zooxanthellae, and endolithic algae) and microbes (bacteria, archaea, and fungi). The coral-associated microbial community structure and their interactions are important in the health of host. But, the knowledge on the mechanisms and significance of these interactions are poorly explored. Overall, about 30 bacterial phyla (including 4 candidates), 2 archaeal phyla and 3 fungal phyla were detected from corals. The members of the classes *gammaproteobacteria* and *alphaproteobacteria* microbes are most commonly associated with corals. Coral microbiota is suggested to be vital in the biogeochemical cycling (e.g. nitrogen, sulfur and phosphorus cycling) and chemical defense. It is necessary to find out the coral immunity mechanisms and the secondary metabolites of associated microbes to elucidate the mechanisms of host microbial selection. Corals are continuously affected by the increasing environmental changes including climate change, sea level rise and anthropogenic activities. The coral-associated microbes are important in the adaptation of the corals to the changing

environmental conditions. They also involve in the resilience of corals to the stresses like resistance against coral-specific pathogens and other stresses. It is necessary to expand the understanding of coral physiology and interactions of associated microorganisms. Although numerous investigations have been performed on the coral microbial ecology, there is a lack in the whole picture of microbial diversity and their roles. Interactions among host-associated microbial communities are critical for the health of the coral holobiont, but our understanding of the mechanisms and consequences of these interactions is still limited. Metagenomic sequencing projects revealed a great taxonomic diversity of coral-associated microorganisms and in some cases host-specific microbes. The metagenomic analysis also suggested the potential diverse functions of coral-associated microbes, and which should be further confirmed by transcriptomic-based investigations. The continuous investigation is necessary in order to further understand the fundamental mechanisms underlying in the shaping of coral-associated microbial communities and their role in the nutrient cycling, host defense and stress tolerance.

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From One to Many: The Population Genetics of Cnidarian-*Symbiodinium* Symbioses

23

Scott R. Santos

Abstract

The success of the coral reef ecosystem is due in large part to endosymbioses between cnidarians such as scleractinian corals, octocorals and anemones and single-celled dinoflagellates in the genus *Symbiodinium*. While the rise of molecular genetic analyses have offered valuable insight into *Symbiodinium* biodiversity, host and geographic distributions and evolutionary relationships, less well-studied are patterns and processes at the population level. Since populations represent the fundamental unit by which evolution occurs, furthering understanding in this area is paramount towards addressing questions ranging from the basic biology of *Symbiodinium* and their hosts to how anthropogenic-driven global climate change may impact these symbioses in the future. Here, a synopsis of population-level characteristics for *Symbiodinium* and various cnidarian host species are distilled from the current literature. Mutational patterns in the most commonly utilized genetic markers for population-level studies of *Symbiodinium*, nuclear microsatellite loci, are also explored. Substitutions, nucleotide insertions and deletions (indels), alterations to the repeat array structure and non-stepwise changes in repeat number are drivers of both allelic variation and size homoplasy, with the latter of concern due to the potential to bias estimates of genetic structure. Such mutations, however, are also a rich source of information that complement and extend the population-level data inherent to microsatellites and provide additional insight into various facets of these symbioses. Lastly, advents in DNA sequencing technology and genomics are discussed since they offer exciting opportunities to rapidly explore pertinent questions in cnidarian hosts and their *Symbiodinium* populations in a rapidly changing world.

Keywords

Cnidarian • Endosymbiosis • Microsatellite • Population genetics • *Symbiodinium*

23.1 General Overview of Invertebrate-Dinoflagellate Symbioses, with a Focus on *Symbiodinium*

Considered some of the planet's largest structures constructed by living entities, the physical nature of coral reefs is predominately derived from the skeletons of scleractinian corals. Many of these organisms, along with a range of protist and invertebrate species including members of the ciliates, foraminiferans, sponges, sea anemones, octocorals,

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zoanthids, hydrocorals and mollusks, form intimate relationships with dinoflagellate belonging to the genus *Symbiodinium* (Glynn 1996; Lobban et al. 2002). The intra- or intercellular presence of these algal symbionts directly benefits hosts' nutrition and physiology via translocation of photosynthetic products like glycerol and glucose utilized in metabolism, growth, calcification and reproduction (Muscatine et al. 1981, reviewed by Davies 1993, Jones and Berkelmans 2011, Burriesci et al. 2012). Unfortunately, the future viability of coral reefs have been questioned (Bellwood et al. 2004; Parmesan 2006) due to their declining health, integrity and geographic coverage (Bruno and Selig 2007) from anthropogenic activities, disease outbreaks and increased incidences of "bleaching", or the loss of photosynthetic pigments from the algal symbionts or massive reduction of *Symbiodinium* density from tissues, that have been correlated with global climate change and can significantly contribute to host mortality (e.g., Sampayo et al. 2008).

All invertebrate hosts that associate with *Symbiodinium* were once thought to harbor a single species of symbiotic dinoflagellate, *S. microadriaticum* Freudenthal (Taylor 1974). This is most likely due to vegetative (i.e., coccoid) and motile stages of *Symbiodinium* lacking, or having reduced, diagnostic morphological characters that are life stage dependent or impacted by the specifics of cell preparation for analyses (Trench and Blank 1987). However, multiple lines of evidence in the 1980s originating from biochemical (Schoenberg and Trench 1980a), ultrastructural (Schoenberg and Trench 1980b; Trench and Blank 1987), infectivity (Schoenberg and Trench 1980c), motility pattern (Fitt et al. 1981), karyotyping (Blank and Trench 1985), DNA/DNA hybridization (Blank and Huss 1989), and DNA base composition (Blank et al. 1988) studies on *Symbiodinium* cultures began demonstrating heterogeneity within the genus. Subsequently, analyses of various regions (i.e., 18S-, 28S- and/or ITS1 and ITS2) within the ribosomal (rDNA) array have provided a framework to classify diversity within *Symbiodinium*, with isolates from either cultured or field collections typically being placed into one of nine groups, or clades (i.e., *Symbiodinium* clades A, B, C (Rowan and Powers 1991), D (Carlos et al. 1999), E (LaJeunesse and Trench 2000; LaJeunesse 2001), F (LaJeunesse 2001) G, H and I (Pawlowski et al. 2001; Pochon et al. 2001; Pochon and Gates 2010)). Notably, these same clades are recovered from phylogenetic analyses utilizing sequence variation in molecules from the chloroplast and mitochondrial genomes (i.e., Santos et al. 2002; Takabayashi et al. 2004; Pochon et al. 2014), providing independent genetic evidence supporting such divisions within *Symbiodinium*. Given that genetic differentiation between the clades within the genus are comparable to that observed among orders of free-living dinoflagellates (Rowan and Powers 1992), it is not surprising that each *Symbiodinium* clade, in turn, can be comprised of one (i.e., Jeong et al. 2014) to numerous (i.e., LaJeunesse 2005) evolutionary lineages often called "types", with many fulfilling multiple species concepts (Thornhill et al. 2014). In some cases, fine-scale discrimination of unique genetic individuals (i.e., genotypes) within *Symbiodinium* clades and "types" have utilized random amplified polymorphic DNA (RAPDs) (Baillie et al. 2000) or DNA fingerprinting (Goulet and Coffroth 2003). More recently, analyses of microsatellite loci have made a significant impact in understanding the biology of cnidarian-*Symbiodinium* symbioses.

23.2 Microsatellites in *Symbiodinium* and the Importance of Populations

Since the 1990s, there has been an explosion of data regarding the genetic diversity between *Symbiodinium* clades as well as within them, particularly from field isolates. Specifically, the number of nucleotide sequences in the National Center for Biotechnology Information's (NCBI's) GenBank database attributed to *Symbiodinium* has grown to ~500K as of July 2015 (http://www.auburn.edu/~santosr/symblast.htm; Fig. 23.1). This has been largely driven by molecular techniques leveraging the polymerase chain reaction (PCR) to circumvent the need for establishing laboratory cultures of these algal symbionts, which can have low rates of success (Schoenberg and Trench 1980a). One such molecular technique is the analysis of microsatellite loci. Distributed abundantly in the genomes of virtually all organisms (Bennett 2000), microsatellites are segments of DNA in which a specific mono- to hexa-nucleotide motif is tandemly arranged in an array (reviewed in Chambers and MacAvoy 2000). Such loci have been considered highly versatile markers for population genetic studies given their single-locus, multiallelic, and codominant properties. While microsatellite loci have historically been a common tool of population geneticists in other systems for identifying genotypes of individuals, their application to dinoflagellates in general, and cnidarian-*Symbiodinium* symbioses in particular, only began gaining traction in the 2000s. For example, members of *Symbiodinium* in culture were the first among the >2000 extant species within the phylum Dinoflagellata (Gómez 2012) to be analyzed with microsatellites and data regarding the size variability in, or presence/absence of, alleles at multiple loci demonstrated that cultured genotypes maybe representative or non-representative of the populations or host species from which they were isolated (Santos et al. 2001). Along with this, microsatellite analyses confirmed the haploid nature of *Symbiodinium* spp. (and dinoflagellates in general) in the vegetative life stage (Santos and Coffroth 2003), which is essential knowledge for the interpretation of population genetic data. When applied to field populations of *Symbiodinium* and their cnidarian hosts, either individually or comparatively, microsatellites have offered interesting and unexpected insights into these symbioses (discussed in

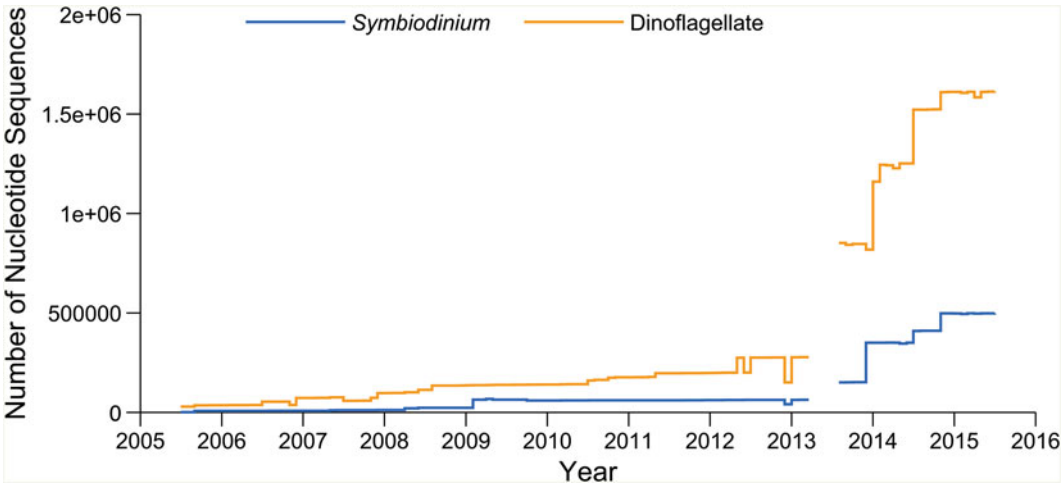

Fig. 23.1 Number of nucleotide sequences attributed to *Symbiodinium* (*blue line*) and dinoflagellates in general (including *Symbiodinium*, *orange line*) archived in the National Center for Biotechnology Information's (NCBI's) GenBank database over the 10-year period spanning July 2005 to July 2015. Values were acquired at the beginning of each month via automated queries to GenBank utilizing the keywords "*Symbiodinium*" and "Dinophyceae". Due to an issue with the computer server running the queries from March 2013 to July 2013, data are missing for that time period (represented by the gap between 2013 and 2014). Sharp increases and decreases in number of nucleotide sequences are typically due to the release of data from genomic or transcriptomic projects or how NCBI indexes those data, respectively

detail below). This is important since the actions of microevolutionary forces such as genetic drift, migration, mutation and natural selection on populations is the manner by which decent with modification (i.e. evolution) occurs. Thus, examination of population-level processes not only offers the capacity to understand current patterns of genetic diversity in cnidarian-*Symbiodinium* symbioses and how they might have come to be but also to potentially anticipate evolutionary trajectories the partners involved in these relationships might take, particularly in the face of continuing environmental perturbations associated with global climate change.

23.3 The Genetics of *Symbiodinium* Populations

As mentioned previously, the base of knowledge on *Symbiodinium* at the level of populations is in its infancy, with only ~40 peer-reviewed and indexed publications on the subject since 2001 (Santos 2014). Given this limited body of work, caution should be applied when attempting to draw overarching conclusions. Thus, population-level characteristics for *Symbiodinium* as presented below are a generalized synopsis from the available literature on the subject.

Symbiodinium populations associated with the octocoral *Antillogorgia* (formerly *Pseudopterogorgia*) *elisabethae* from 12 localities across the Bahamas were the first field isolates to be examined at microsatellite loci (Santos et al. 2003b). Analyses of just two microsatellite loci revealed striking differentiation in these populations of *Symbiodinium* belonging to Clade B (ITS2 "type" B1; nomenclature of LaJeunesse (2001) is utilized for symbiont ITS2 "types" unless otherwise noted), with 62 of 66 (94 %) pairwise comparisons between localities exhibiting significant differences. This high level of genetic differentiation was driven by the fact that a number of the 23 identified *Symbiodinium* genotypes were either unique to a population, or found infrequently in other populations, for 9 of the 12 localities. In cases where no significant differentiation was detected, populations from those localities were spatially close, implying isolation by distance (i.e., IBD), or a positive correlation between genetic and geographic distances (Santos et al. 2003b). Along with this, the vast majority (i.e., 550 of 575; 96 %) of *A. elisabethae* colonies harbored only a single *Symbiodinium* genotype, with that specific genotype generally being locality-specific and of high prevalence (i.e., 66–100 %) among the examined host colonies at a particular locality in 10 of 12 cases (Santos et al. 2003b).

Remarkably similar patterns to those documented for the *Symbiodinium* populations of *A. elisabethae* have been reported from several other Caribbean cnidarian host species. For example, *Symbiodinium* Clade B (ITS2 "type" B1) populations of the octocoral *Eunicea* (formerly *Plexaura*) *flexuosa* were reported to exhibit patterns of IBD between Florida, Panama, Saba and the Dominican Republic (Wirshing et al. 2013, but see Prada et al. 2014). Furthermore, Clade B (ITS2 "type" B1) populations associating with the Caribbean sea fan *Gorgonia ventalina* examined from 16 and 35 localities in the Florida Keys (Kirk et al. 2009) and Caribbean Sea and Western Atlantic (Andras et al. 2011), respectively, exhibited significant differentiation in 54–98 % of pairwise comparisons depending on whether occurrences of host individuals infected simultaneously with two *Symbiodinium* genotypes (~28 % in Kirk et al. (2009) and

~18 % in Andras et al. (2011) of sampled colonies) were included or excluded from analyses. Similar to *A. elisabethae*, localities in close proximity geographically for both *E. flexuosa* and *G. ventalina* tended to account for cases where no significant differentiation was detected between *Symbiodinium* populations (Kirk et al. 2009; Andras et al. 2011; Wirshing et al. 2013). Notably, instances of significant differentiation were also detected between *Symbiodinium* Clade B populations of *G. ventalina* from deep and shallow sites (Kirk et al. 2009) or of different size (i.e., age) classes (Andras et al. 2011) at the same locality, suggesting physical habitat, light regimes and/or temporal variation in the availability of *Symbiodinium* genotypes for larval infection (*G. ventalina*, like *A. elisabethae* and many other hosts, including 75 % of scleractinian corals (Baird et al. 2009), acquire their dinoflagellate symbionts at the larval stage in a horizontal (i.e., environmental) rather than vertical (i.e., maternal) fashion) may influence genetic structuring among *Symbiodinium* populations.

For those examined to date, the population-level patterns in *Symbiodinium* Clade B (ITS2 "types" B1 and B10) from Caribbean scleractinian coral species have also been highly congruent to those of octocorals and sea fans from the same region. Specifically, *Symbiodinium* genotypes were found to be largely reef endemic, with little to no sharing between localities, for the scleractinian corals *Orbicella* (formally *Montastraea*) *annularis* and *O. faveolata* of the Florida Keys and Bahamas (Thornhill et al. 2009). Temporal samplings of these same *O. annularis* and *O. faveolata* colonies identified stability in the structure of their *Symbiodinium* populations over an ~3 year period, including an appreciable bleaching event in 2005 that reduced symbiont densities to levels significantly lower than natural, seasonal fluctuations (Thornhill et al. 2009). The comparison of two closely related and sympatric host species also provided a framework for recognizing strong specificity between *O. annularis* and *O. faveolata* and their *Symbiodinium* Clade B populations in the Florida Keys relative to the Bahamas, with breakdown in the specificity between partners at the latter location likely due to hybridization among the two host species (Thornhill et al. 2010). Similar patterns of strong specificity between partners have been identified for Caribbean octocoral and sea fan species and their *Symbiodinium* belonging to Clade B ITS2 "type" B1 (Santos et al. 2004), which represents the most prevalent lineage of symbiotic dinoflagellates of the Caribbean Sea and Western Atlantic (LaJeunesse 2002).

Along with Caribbean octocorals, sea fans and scleractinian corals, the population genetics of *Symbiodinium* Clade B (ITS2 "type" B1) associating with an anemone species have been investigated. In this case, sampling of the anemone *Exaiptasia* (formerly *Aiptasia*) *pallida* from 16 sites around the world found *Symbiodinium minutum* to have strong specificity with *E. pallida* and populations to typically possess high clonality and low genotypic diversity (Thornhill et al. 2013), analogous to many of the *Symbiodinium* populations of other cnidarian hosts harboring Clade B (see above). In contrast to these previous examples, however, sharing of both alleles and *Symbiodinium* genotypes among localities like Japan, Hawaii and Mexico in the Pacific Ocean, or regions such as the Mediterranean and Red Sea, resulted in minimal levels of differentiation being detected at the global scale (Thornhill et al. 2013). One explanation for this unexpected pattern is the potential anthropogenic vectoring, either intentionally or unintentionally, of the "weedy" *E. pallida* (and its *S. minutum* by association) through routes like fouling communities on ship hulls, ballast waters, aquaculture, the aquarium trade and/or scientific researchers (Thornhill et al. 2013). *Symbiodinium trenchii*, a member of Clade D (formerly ITS2 "type" D1a, or D1-4), is another example where dinoflagellate symbionts have been apparently vectored from one oceanic basin (i.e., the Indo-Pacific Ocean) to another (i.e., the Caribbean), resulting in symbiont populations of low genetic diversity and cases of suboptimal symbiotic relationships as they rapidly spread through resident scleractinian coral communities and displace native *Symbiodinium* from hosts (Pettay et al. 2015).

Although the population genetics from members of *Symbiodinium* Clade B ITS2 "type" B1 have been the most extensively studied to date, comparable work utilizing microsatellite loci have been conducted on lineage "types" within Clades A, C and D as well. For instance, *Symbiodinium* Clade A (ITS2 "type" A3) populations of *Symbiodinium* '*fitti*' from the Caribbean scleractinian coral *Acropora cervicornis* exhibited strong specificity but lacked differentiation at geographic distances <850 km (Pinzón et al. 2011). In contrast, *S.* '*fitti*' populations associating with *A. palmata*, as well as the Clade A (ITS2 "type" A3) symbionts of the Caribbean scleractinian coral *Stephanocoenia intersepta*, possessed significant differentiation at scales of 100s of km (Baums et al. 2014; Pinzón et al. 2011). For members of *Symbiodinium* Clade C, populations of ITS2 "type" C1d were found to have negligible differentiation at short geographic distances (i.e., ~10s of km) that became significant at scales of 100s of km for the scleractinian coral *Pocillopora meandrina* in the South Pacific (Magalon et al. 2006). On the other hand, significant differentiation at 10s of km between populations of *Symbiodinium* ITS2 "types" C1.3a (sensu Goulet et al. 2008) and C3 was reported for the soft coral and scleractinian coral hosts *Sinularia flexibilis* and *Acropora millepora*, respectively, on the Great Barrier Reef (Howells et al. 2009, 2013). In the Caribbean, populations were comprised of unique, locality-endemic genotypes for members of *Symbiodinium* Clade C (ITS "types" C7 and C7a/C12) from *Orbicella* spp. as well as the ITS2 "type" C3 specific to the scleractinian coral *Siderastrea siderea*; while differentiation became significant on the order of 100s of km, symbiont

populations from the same host species had higher genetic similarity over large geographic distances compared to ones from other host species in sympatry, implying each represents a unique species with a distinct gene pool (Thornhill et al. 2014). Lastly, while *Symbiodinium trenchii* of *Symbiodinium* Clade D exhibits little to no differentiation given its likely and recent introduction and subsequent spread throughout the Caribbean, strong differentiation was found between closely situated (i.e., 10s of km) populations of the same symbiont species in the Indian and Pacific Oceans (Pettay et al. 2015). This latter scenario contrasts with the lack of differentiation between populations of *Symbiodinium* ITS2 "type" D1 from the scleractinian coral *Pocillopora* sp. type I of the Eastern Pacific, which vertically transmits symbionts to planulae that have appreciable larval durations and dispersal capabilities (Pettay et al. 2011; Pettay and LaJeunesse 2013).

In summary, molecular analyses of *Symbiodinium* via microsatellite loci have revealed a continuum of genetic structure, ranging from approximately one homogeneous population (e.g., *S. trenchii* in the Caribbean) to many (e.g., the symbionts of *Sinularia flexibilis* and *Acropora millepora* from the Great Barrier Reef) significantly differentiated populations. While exceptions exist, several recurring themes can be gleaned from the population genetic studies of *Symbiodinium* conducted to date that appear independent of the host species under examination. Firstly, >50 % (and >95 % in some cases) of the individuals of a particular host species at a locality possess within their tissues a single *Symbiodinium* genotype. Secondly, *Symbiodinium* genotypes are often endemic to a locality, with the majority of host individuals at a given site harboring that endemic genotype over multiple years and populations thus being generally low in genotypic diversity. Thirdly, the high endemicity displayed by *Symbiodinium* genotypes leads to significant differentiation between populations on the scales of 10s–100s of km and apparent isolation by distance (IBD), implying poor dispersal capabilities, local adaptation via natural selection, or a combination of these (and likely other) factors driving such patterns for these symbionts. Lastly, strong specificity is common between *Symbiodinium* genotypes and their host species, with fidelity occurring across the range of the partners. In instances where the above phenomena are not observed, physical (e.g., oceanographic current, light regimes and depth, etc.) and/or biological (i.e., symbiont transmission mode, reproductive and other life history traits or genetic identity of the host, etc.) characteristics relevant to a specific locality or particular *Symbiodinium*-host species pairing are typically responsible for such deviations. Again, this generalized synopsis of population-level traits for *Symbiodinium* is based on the sparse literature related to the topic and it will be interesting to see how it will be revised and amended as additional data from future studies becomes available.

23.4 The Genetics of Cnidarian Populations

Relative to *Symbiodinium*, the population-level genetics of cnidarians have been more heavily investigated (reviewed by Baums 2008). To illustrate this, a search utilizing the combined keywords "coral" or "scleractinian", along with "population" and "microsatellite", to Thomson Reuters' Web of Science in June 2015 returned 315 and 56 publications, respectively, since 1999. Overall, the primary mechanism of gene flow for sessile marine invertebrates such as scleractinian corals is dispersal of larvae between populations. Larvae can be broadly categorized depending on the reproductive mode of the particular species in question and whether syngamy between egg and sperm occurs internally or externally of the maternal individual. In the case of "brooding" species, planula larvae result from internal fertilization of eggs and undergo significant development within the maternal individual prior to release. In contrast, the planula larvae of "broadcast spawning" species are the product of external fertilization following the release of eggs and sperm into the water column. Of these two reproductive modes, "broadcast spawning" is employed by the majority of reef-building scleractinian species (Baird et al. 2009). Furthermore, pelagic larval duration (PLD) varies between reef-building scleractinian species (Connolly and Baird 2010) and larvae have been shown to delay metamorphosis by modifying their metabolism in the absence of suitable settlement cues (Graham et al. 2008, 2013). Thus, larvae, particularly those produced by "broadcast spawning" species, are theoretically endowed with the opportunity and physiology to disperse widely via ocean currents.

While the larvae of cnidarian hosts such as reef-building scleractinian corals have the potential to maintain connectivity between populations (reviewed in Hellberg 2009) and evidence supporting long-distance dispersal has been reported (e.g., Baums et al. 2005; Severance and Karl 2006; van Oppen et al. 2011b), studies have also simultaneously identified instances of limited gene flow and significantly differentiated populations across 10s to 100s of km (e.g., Ayre and Hughes 2000; Maier et al. 2005; Underwood et al. 2009; Torda et al. 2013). An exemplar highlighting this dichotomy is the scleractinian coral *Seriatopora hystrix* of the Indo-Pacific Ocean and Red Sea. As a "brooding" species, larvae of *S. hystrix* were hypothesized to have low dispersal potential given their large (~1.5 mm) size and typically shorter precompetency period relative to "broadcast spawning" species, leading to self-seeding populations and significant differentiation among reefs (Ayre and Hughes 2000). Consistent with this prediction, nine *S. hystrix* populations examined along the Great Barrier Reef were found to exhibit varying levels of genotypic diversity, low gene flow

and weak connectivity, with significantly differentiated populations on the scale of 10s of km (Ayre and Hughes 2000). An analogous situation was reported for ten *S. hystrix* populations separated by ~0.150–610 km in the Red Sea, where differentiation and patterns of IBD were detected at scales ≤20 km (Maier et al. 2005). In fact, most larvae of *S. hystrix* apparently settle and undergo metamorphosis within 100 m of the maternal colony they are released from (Underwood et al. 2007), which not only influences the genetic structure of populations geographically (i.e., horizontal connectivity) but can lead to significant differentiation amongst populations at different depths (i.e., vertical connectivity) as well (van Oppen et al. 2011a). However, estimates suggest that ~4 % of sexually produced larvae from *S. hystrix* can be exchanged between reefs separated by 10s–100s km over several generations (van Oppen et al. 2008) and larvae of deep water origin have been documented as recruits into shallow water habitats (van Oppen et al. 2011a). Such exchanges, though low and likely sporadic, appear sufficient towards maintaining connectivity for both "brooding" and "broadcast spawning" species to the point that they effectively form more-or-less panmictic populations over wide geographic scales such as the Great Barrier Reef (Ayre and Hughes 2000, 2004) and supports the notion that reproductive mode and larval type are but weak predictors for dispersal potential and population structure in scleractinian corals (Miller and Ayre 2008).

Varying levels of genotypic diversity and population genetic structure have also been reported from various cnidarian species in other parts of the Pacific Ocean as well as the Caribbean Sea, Western Atlantic and elsewhere. For example, scleractinian corals in the genus *Pocillopora* of the Indo-Pacific, Eastern Pacific, Red Sea and Arabian/Persian Gulf can be divided into between five and seven genetically distinct lineages comparable to species because of little to no gene flow between them. Although *Pocillopora* populations within the same lineage, but occurring in different ocean basins, exhibited low levels of differentiation, overall similarities in allelic compositions across populations in general implied gene flow and connectivity among geographically widespread localities (Pinzón et al. 2013). For two populations of one of these lineages, *Pocillopora* sp. type I of the Eastern Pacific, genotypic diversity differed significantly over 10 km due to one population being nearly exclusively comprised of clonal (i.e., possessing the same multilocus genotype) individuals relative to the other (Pinzón et al. 2012). Clonality via vegetative propagation (i.e., fragmentation, fission, etc.) is a common trait among numerous coral reef organisms (reviewed by Lasker and Coffroth 1999) and its prevalence can vary greatly depending on species and locality under examination. At the extremes, populations of the scleractinian coral *Montipora capitata* in the Hawaiian Archipelago and sea fan *G. ventalina* of the Caribbean and Western Atlantic possessed low (<2 %) numbers of, or no, clonal individuals, respectively, implying that the reproductive mode in these species is primarily sexual (Andras et al. 2013; Concepcion et al. 2014). In contrast, a genetic survey of 2352 *Pocillopora damicornis* colonies on a single patch reef in Kaneohe Bay, Hawaii revealed 70 % of the individual colonies belonging to just 7 of the 53–116 clonal lines identified on that particular reef, suggestive of a highly clonal and inbred population (Gorospe and Karl 2013). More typically, levels of clonality differ on a locality-by-locality basis. For instance, the frequency of clonal individuals in *Orbicella annularis* populations at 18 localities around the Caribbean Sea varied and was highest at places with greater incidences of hurricanes (Foster et al. 2013), suggesting fragmentation via storm energy was responsible for the observed differences. Likewise, for 26 localities in the Caribbean where *Acropora palmata* was examined, populations ranged from being composed of primarily clonal to purely sexually produced individuals, with the overall prevalence of clonal structure being statistically different between the genetically isolated western and eastern Caribbean subregions of the species (Baums et al. 2005, 2006). Taken together, the level of clonality exhibited by cnidarian species capable of forming relationships with *Symbiodinium* can vary greatly and is best determined on a case-by-case basis, but should be taken into consideration since it can impact population-level characteristics such as heterozygosity, effective size, and differentiation (Balloux et al. 2003) for both partners.

In the small number of studies where the population genetics of cnidarian hosts and their *Symbiodinium* could be concurrently examined, both coupling and decoupling between the partners have been reported that correlates with symbiont transmission mode. Specifically, vertical symbiont transmission appears to result more often in congruence of host and symbiont population structure while horizontal symbiont acquisition does not. In support of the former, populations of the vertically-inherited *Symbiodinium* Clade D (ITS2 "type" D1) as well as its host *Pocillopora* sp. type I lacked differentiation over thousands of square kilometers in the Eastern Pacific (Pettay and LaJeunesse 2013). Similarly, the presence of identical *Symbiodinium minutum* genotypes in populations of the anemone *E. pallida* from Japan, Hawaii and Mexico has been attributed to anthropogenic vectoring as well as the propensity of this host species to reproduce asexually by pedal laceration, which results in vertical symbiont transmission of the maternal *Symbiodinium* genotype to progeny (Thornhill et al. 2013). On the other hand, comparisons of the population structure from horizontally-acquired *Symbiodinium* Clade C (ITS2 "type" C1:3a) and its soft coral host *S. flexibilis* along the Great Barrier Reef identified no correlation (Bastidas et al. 2001; Howells et al. 2009). Likewise, populations of *Symbiodinium* '*fitti*' (ITS2 "type" A3) and *A. palmata* in the Caribbean exhibit an order of magnitude difference in gene flow and >3X genetic structure in the former compared to the latter (Baums et al. 2014).

While some of the genetic breaks among these horizontally-acquired *Symbiodinium* '*fitti*' populations are due to known barriers and environmental conditions, the biology of the symbiont itself appears to drive much of this differentiation and ultimately leads to seven regionally-distinct populations for *S.* '*fitti*' and just two (i.e., western and eastern Caribbean subregions) for *A. palmata* (Baums et al. 2005, 2014). An analogous situation of differing levels of population structure has been reported between the sea fan *G. ventalina* and its *Symbiodinium* B ITS2 "type" B1 of the Caribbean and Western Atlantic (Andras et al. 2013). Such disparities in population structure between cnidarian hosts and their horizontally-acquired *Symbiodinium* could arise from a number of factors operating singularly or in combination, including intrinsic differences in dispersal abilities and other life history traits, selection for locally- or regionally-adapted host and/or symbiont genotypes (Howell et al. 2012; Pettay and LaJeunesse 2013), or a general inability for migrant symbionts to establish themselves in new populations due to density-dependent barriers (Thornhill et al. 2009). Understanding the underlying mechanisms for these disparate patterns have important implications in anticipating the future of these populations, as well as the coral reef ecosystem, in the face of global climate change.

23.5 Microsatellite Evolution in *Symbiodinium*

Analyses of microsatellite loci have provided a wealth of information on the genetic diversity and structure of cnidarian and *Symbiodinium* populations. However, only a single study to date has explored the mutational behavior of microsatellite loci in cnidarians (i.e., Tang et al. 2010) and no similar work has been done for *Symbiodinium* or any other dinoflagellates, for that matter. Notably, Tang et al. (2010) reported from crossing experiments in the Indo-Pacific scleractinian coral *Acropora muricata* that microsatellite loci with GT-repeats had relatively lower mutation rates then loci with AAT-repeats and cautioned that underestimates in genetic variability could result from the utilization of AAT-repeat loci. Thus, given that the accurate estimation of genetic diversity and population structure using microsatellite data rests in the assumption that alleles identical in state (i.e., size) have experienced a common mutational history (reviewed by Estoup et al. 2002), there is a need to further understand the molecular processes operating on these types of loci in cnidarian and *Symbiodinium* genomes. Here, two microsatellites, CA4.86 and CA6.38, utilized in a number of studies on *Symbiodinium* Clade B ITS2 "type" B1 (e.g., Santos et al. 2003b, 2004; Santos and Coffroth 2003; Kirk et al. 2009; Thornhill et al. 2009, 2013) are examined to illustrate the complex mutational patterns and potential evolutionary processes that can occur at these loci.

The sequence data considered here were originally presented in Santos et al. (2004). Specifically, only the nucleotide substitutions found in the flanking regions around the microsatellite repeat array were included in those analyses, with the other mutations occurring at these loci not discussed in detail. In total, 49 sequences from either cultures ($n=11$) representing a range of hosts and geographical locations or field isolates ($n=15$) from the Caribbean octocorals *Antillogorgia* (formerly *Pseudopterogorgia*) *bipinnata*, *A. elisabethae*, *Briareum asbestinum*, *G. ventalina* and *Plexaura kuna* (Santos et al. 2004) were examined. All symbiont samples belonged to *Symbiodinium* Clade B, and more specifically, to *Symbiodinium* B184, B211 and B223 based on chloroplast large subunit (cp23S)-rDNA length heteroplasmy (nomenclature following Santos et al. 2003a; Coffroth and Santos 2005). Detailed information regarding the samples can be found in Santos et al. (2004) and sequences retrieved from GenBank using accession numbers AF474166-AF474168, AF474170-AF474171, and AY264293-AY264335.

For each microsatellite locus, allele sequences were first aligned by eye and mutations in the flanking regions and repeat arrays of the loci assessed by visual inspection. To describe the mutational patterns of the microsatellite repeat arrays at locus CA4.86 in particular and in the context of its flanking sequence, the number and structure of the nucleotide repeats constituting the array were mapped onto a phylogeny depicting the relationships between alleles. The phylogeny was inferred from the flanking regions of locus CA4.86 following the procedures of Santos et al. (2004). To map the pattern of allele amplifications from loci CA4.86 and CA6.38 onto a phylogeny for *Symbiodinium* clade B, nucleotide data from cp23S-rDNA domain V sequences were acquired from the six lineages of this clade that are currently recognized in phylogenies of *Symbiodinium* based on cp23S-rDNA (Santos et al. 2002). The phylogeny was reconstructed from complete cp23S-rDNA domain V data (including the hypervariable region excluded from Santos et al. 2002) under maximum parsimony (MP) and nodal support based on bootstrap analysis of 1000 replicates. The MP analysis was conducted according to the methods of Santos et al. (2002) and *Symbiodinium* cultures Ua#31 and CCMP 421, which belong to chloroplast lineages C180 and E202, respectively, (Santos et al. 2003a), were utilized as outgroups. To ensure the reliability of the PCR results, allele amplifications (including positive controls) from loci CA4.86 and CA6.38 were repeated at least 3X from independent DNA extractions.

23.6 Complex Mutational Patterns in *Symbiodinium* Microsatellites

The orthologous nature of CA4.86 alleles sampled from *Symbiodinium* B184, B211 and B223 and CA6.38 alleles from *Symbiodinium* B184 and B211 are apparent from their

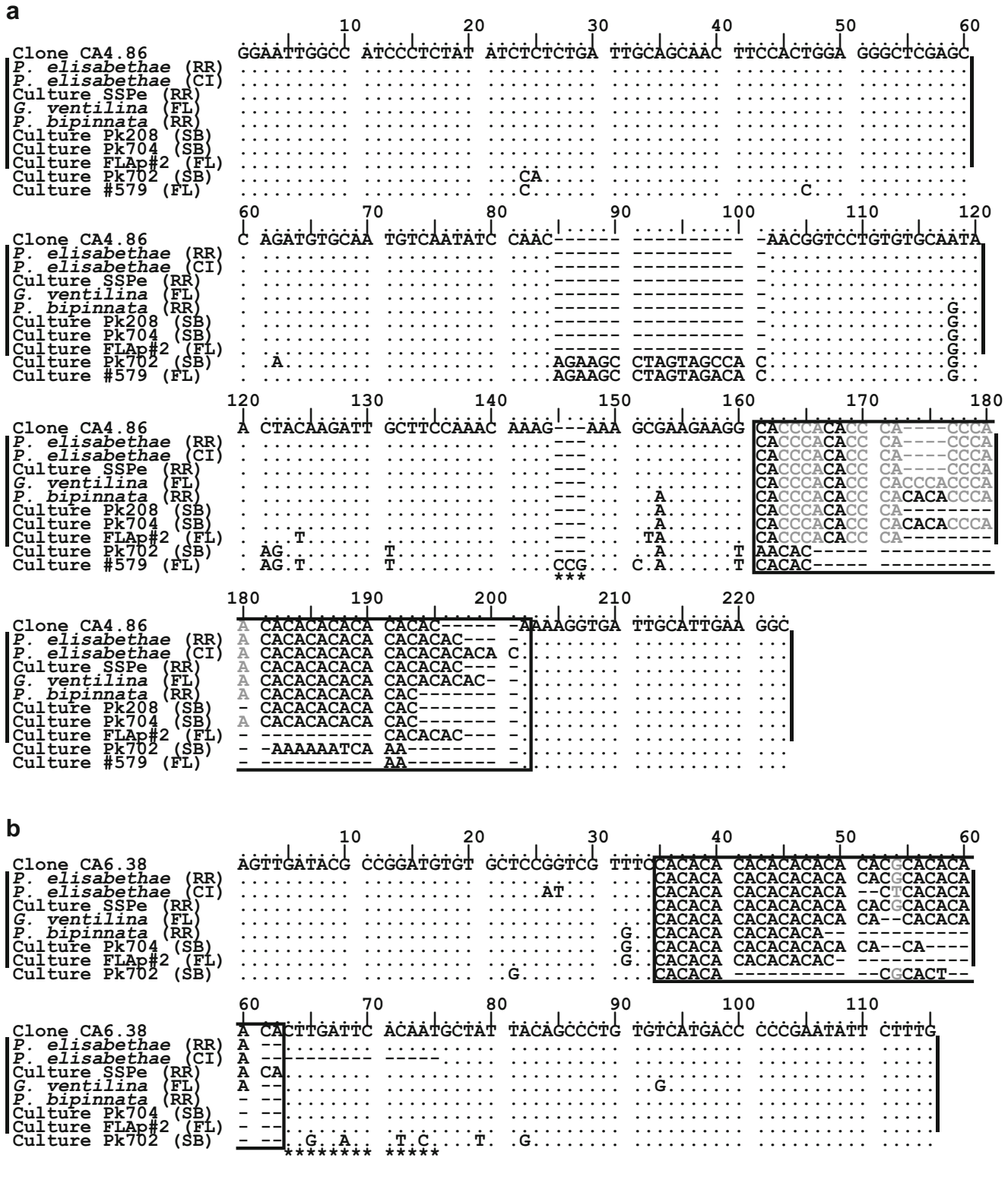

Fig. 23.2 Alignments of representative alleles from *Symbiodinium* microsatellite loci **a** CA4.86 and **b** CA6.38. Samples that are bracketed belong to *Symbiodinium* B184. Cultures Pk702 and #579 represent *Symbiodinium* B211 and B223, respectively. Only those positions differing from the bacterially cloned inserts used in PCR primer construction (first sequence in each alignment) are shown. The scale at the *top* designates site position. A *dot* (.) indicates a base pair identical to that of the bacterial clones while a letter signifies a change in the nucleotide. *Dashes* (-) signify insertion/deletions (indels) and are further differentiated by *asterisks* (*). The boundary of the repeat array for each locus is boxed. Nucleotides within each array represent positions of interest are presented in *grey* (see text for additional details)

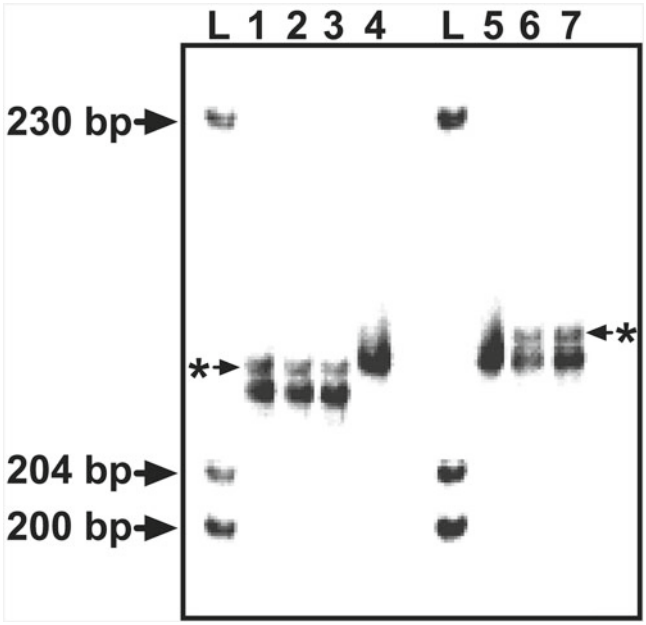

Fig. 23.3 Polyacrylamide gel electrophoresis of alleles from microsatellite locus CA4.86. Lanes #1–3 and #6–7 are from *Symbiodinium* B184 isolates, #4–5 are from *Symbiodinium* B211 isolates and L = DNA ladders. *Asterisks* (*) designate "stutter" artifact that is produced by imperfect replication of the microsatellite allele due to polymerase slippage during PCR

sequence alignments (Fig. 23.2). A notable difference among the alleles was the presence of insertion/deletions (indels) at both loci. For CA4.86, the allele from *Symbiodinium* B211 (culture Pk702) contained a 17 bp indel absent from *Symbiodinium* B184 alleles (Fig. 23.2a). A 17 bp indel was also found in the same position of the *Symbiodinium* B223 allele characterized from culture #579. Although the *Symbiodinium* B211 and B223 alleles differed by a single transversion mutation within the indel (position 98 in Fig. 23.2a), a common trait such as this typically represents a shared history for a group (e.g., Prather and Jansen 1998). This suggests that *Symbiodinium* B211 and B223 are more closely related to each other than either is to *Symbiodinium* B184, a relationship not evident from cp23S-rDNA phylogenies (Santos et al. 2002, 2003a, see below). Along with the single base pair difference in the 17 bp indel, the presence of a second, unique indel of 3 bp further differentiates *Symbiodinium* B223 from *Symbiodinium* B211 (Fig. 23.2a). For CA6.38, alleles of 96 and 98 bp sampled from the symbionts of *A. elisabethae* possessed a 13 bp indel that was absent from all other *Symbiodinium* populations and isolates, including others belonging to B184 (Fig. 23.2b). Since these indels do not share common features, it is difficult to identify the forces responsible for their creation. Along with the indels, *Symbiodinium* B184, B211 and B223 isolates possess point substitutions in the flanking regions of alleles from both loci (Fig. 23.2). These substitutions have been used to identify distinct *Symbiodinium* B184 variants (referred to as phylotypes), reconstructing phylogenies among them and demonstrating fine-scale specificity in octocoral-*Symbiodinium* symbioses (Santos et al. 2004).

Similar to the flanking regions of both loci, complex mutations are also evident within the repeat arrays of CA4.86 and CA6.38. The first indication of disparity between arrays came from polyacrylamide gel electrophoresis of alleles from *Symbiodinium* B211 and B223. In these cases, there was an absence of "stutter" in alleles from *Symbiodinium* B211 and B223 that was present in those from B184 (Fig. 23.3). Since "stutter" is an artifact produced by imperfect replication of the allele due to polymerase slippage across the array during the PCR (Hite et al. 1996; Walsh et al. 1996), such absence suggests a change in the structure of the microsatellite repeat itself. This hypothesis was confirmed upon DNA sequencing: at locus CA4.86, no more than three consecutive dinucleotide (e.g., $[CA]_n$) repeats are present in the microsatellite arrays of the alleles from *Symbiodinium* B211 and B223 (Fig. 23.2a). For *Symbiodinium* B211, besides having only a few repeats, a mononucleotide track was present in the CA4.86 array that was absent from all other alleles (Fig. 23.2a). Likewise, for locus CA6.38, the allele sequenced from *Symbiodinium* B211 had only three consecutive dinucleotide repeats in the array (Fig. 23.2b). Thus, the significant alterations in the microsatellite arrays of CA4.86 and CA6.38 for *Symbiodinium* B211 and B223, namely the reduced number of repeats and the mononucleotide tracts, are responsible for the observed lack of allelic "stuttering". Furthermore, locus CA4.86 in *Symbiodinium* B223 and loci CA4.86 and CA6.38 in *Symbiodinium* B211 should not be considered "microsatellites" since they do not fulfill the standard of a four repeat minimal used to classically define such loci (Chambers and MacAvoy 2000).

The repeat arrays of CA4.86 and CA6.38 alleles from *Symbiodinium* B184 also appear to have experienced complex mutations. At CA4.86, allelic variation resulted from changes in two types of repeats. In addition to a variable number of dinucleotide $[CA]_n$ repeats, the array is also comprised of between one and four tetranucleotide (i.e., $[CCCA]_n$) motifs (grey residues in boxed region of Fig. 23.2a). The evolution of these tetranucleotide motifs in relation to the flanking regions of the same alleles is presented in Fig. 23.4. It is noteworthy that the number of tetranucleotide motifs present in an array does not correlate with the phylogeny based on the flanking regions of the same alleles. As an example, repeats of $[CCCA]_3$ were found in the arrays of (*i*) *Symbiodinium* B184 populations and a culture from *A. elisabethae* as well as (*ii*) populations from *A. bipinnata* and cultures from *B. asbestinum* and *P. kuna* (Fig. 23.4). Since these phylotypes are found on different branches in the phylogenetic tree (Fig. 23.4) and given the propensity of microsatellite repeat arrays to mutate faster then the regions flanking them (Schlotterer 2000), convergent evolution is the most likely explanation for the identical number of tetranucleotide repeats between the

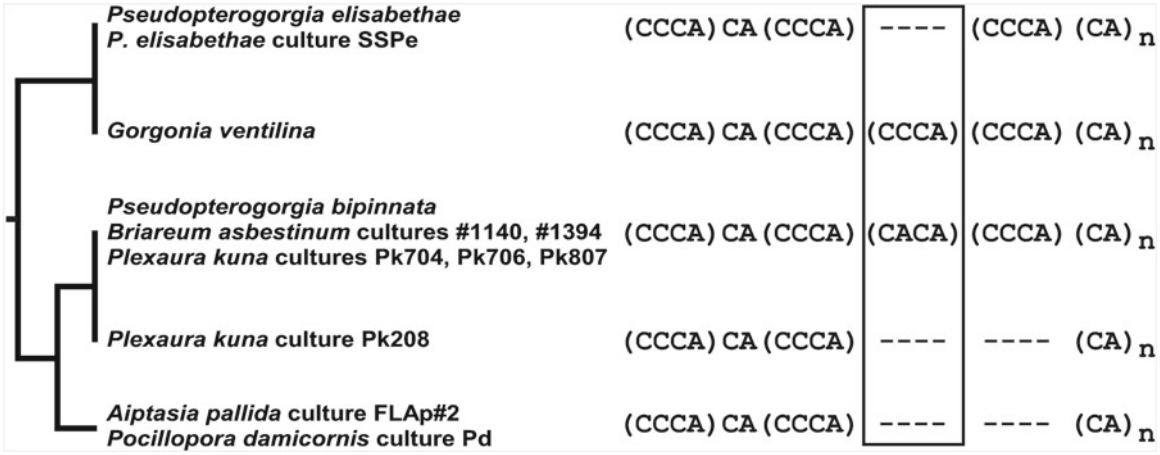

Fig. 23.4 Evolution of *Symbiodinium* microsatellite locus CA4.86 tetranucleotide motifs in relation to the flanking regions of the same alleles. Alleles were either recovered from cultures (indicated in label) or from Caribbean octocoral *Symbiodinium* populations *in hospite* (all others). *Boxed* area designates tetranucleotide repeat of interest (see text for additional details)

groups. In addition, the $[CCCA]_n$ motif is interrupted by a $[CA]_2$ repeat in some *Symbiodinium* B184 phylotypes (boxed in Fig. 23.4). It appears that the intervening $[CA]_2$ repeat has resulted from either a polymerase slippage event leading to the incorporation of a pair of dinucleotide repeats or a substitution of C → A in a [CCCA] motif unit. The idea that the discontinuous nature of this $[CCCA]_n$ motif results from a substitution rather then an incorporation finds support in the fact that a single [CA] repeat has yet to be described from the same region in other *Symbiodinium* cultures or populations *in hospite*. At CA6.38, a change in the otherwise perfect dinucleotide array of some *Symbiodinium* B184 isolates as well as B211 resulted from a point mutation (grey residues in boxed region of Fig. 23.2b).

23.7 Allele Size Homoplasy and Phylogenetic Distribution of Microsatellite Loci in *Symbiodinium* Clade B

Given the substitutions, indels and alterations to the array structure observed at loci CA4.86 and CA6.38, it is not surprising to find cases where alleles identical in size result from independent mutational events. Specifically, from the 49 sequences examined here, four cases of this phenomenon were recognized at CA4.86 and CA6.38, including the alleles from *Symbiodinium* B184 and B211 at CA4.86 depicted in Fig. 23.3. This situation, known as allele size homoplasy, has been previously reported for microsatellites (e.g. Estoup et al. 1995; Angers and Bernatchez 1997; Viard et al. 1998; Culver et al. 2001) and is considered a potentially significant problem when using such markers to estimate genetic diversity and infer population structure (Orti et al. 1997; Colson and Goldstein 1999). Assuming these symbiont isolates and microsatellites do not represent outliners, but instead the mutational potential of such loci across the *Symbiodinium* genome, size homoplasy could be considered a major concern to future studies that utilize these markers. One method to circumnavigate the problem of allele size homoplasy is to use a technique that identifies differences in the primary sequence of the microsatellite alleles themselves. DNA sequencing is the most accurate method of doing this; characterizing alleles in this fashion, however, can become time-consuming and costly. Alternatively, a single stranded conformational polymorphism (SSCP) approach has proven successful in identifying cases of size homoplasy in microsatellite alleles (Angers et al. 2000) since this technique separates DNA fragments predominately by sequence differences rather than be size (reviewed by Sunnucks et al. 2000). Unique alleles identified in this manner can then be sequenced to determine their nucleotide composition. Future investigations are encouraged to utilize DNA sequencing, SSCP or denaturing gradient gel electrophoresis (DGGE) (reviewed by Muyzer 1999) approaches to screen alleles as a way of avoiding the pitfalls of allele size homoplasy in *Symbiodinium* microsatellites. This is particularly relevant in cases were primer pairs designed for the *Symbiodinium* populations of one host are employed on the symbiont populations of another host (see Santos et al. 2004 for an example).

Presented in Fig. 23.5 is the presence and absence screening for microsatellite alleles from loci CA4.86 and CA6.38 relative to a phylogeny of *Symbiodinium* Clade B inferred from cp23S-rDNA domain V sequences. This phylogenetic approach provides further insight into the evolution of these loci, and, by extension, the *Symbiodinium* genome. For example, alleles from CA4.86 were recovered from only three of the six *Symbiodinium* clade B cp23S-rDNA lineages that were screened. Similarly, alleles from CA6.38 were recovered from representatives of only two cp23S-rDNA lineages. In the phylogeny, these *Symbiodinium* B184, B211 and B223 cp23S-rDNA lineages are scattered throughout the tree and do not cluster together (Fig. 23.5). Significantly, the

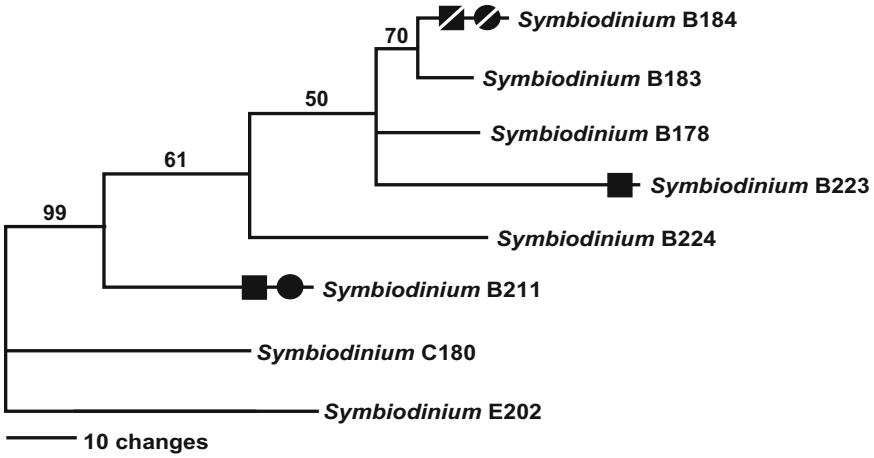

Fig. 23.5 Recovery of alleles from *Symbiodinium* microsatellite loci CA4.86 and CA6.38 relative to the evolutionary history of *Symbiodinium* clade B. The tree was constructed under maximum parsimony (MP) with branch support from 1000 bootstrap replicates (values above nodes). *Solid squares* (■) represent recovery of an allele from CA4.86; *solid circles* (●) represent recovery of an allele from CA6.38; hatched symbols signify cases where alleles were recovered from only a subset of isolates

recovery of alleles from both microsatellite loci for the deepest branching cp23S-rDNA lineage of *Symbiodinium* Clade B in the phylogeny, *Symbiodinium* B211 (Fig. 23.5), suggests loci CA4.86 and CA6.38 should be present in all members of *Symbiodinium* Clade B. Given this, the most parsimonious explanation for the lack of allele recovery (i.e., the presence of null alleles) from some cp23S-rDNA lineages of *Symbiodinium* Clade B are mutations in the flanking regions leading to the loss of one, or both, priming sites and subsequent inhibition of the PCR. This hypothesis of amplification loss due to mutation finds support in the previously described nucleotide substitutions and indels of alleles from microsatellite loci CA4.86 and CA6.38 (see above). In addition, such mutational processes could also lead to the presence of null alleles in a subset of isolates belonging to a particular *Symbiodinium* cp23S-rDNA lineage (e.g., as depicted for B184 in Fig. 23.5). This observation suggests that the phylogenetic identity of an isolate is an inaccurate gauge of microsatellite loci conservation in *Symbiodinium*. The *in hospite* algal populations of the Caribbean gorgonian *P. kuna* exemplify this; although belonging to *Symbiodinium* B184, alleles have yet to be recovered from loci CA4.86 and CA6.38 of these populations (Santos and Coffroth 2003). Thus, the lack of microsatellite loci conservation among symbiont isolates, even those that are closely related, is a testimonial to the mutability of the *Symbiodinium* genome.

23.8 Conclusions and Future Directions

Cnidarian and *Symbiodinium* populations, when examined either individually or in combination, can exhibit genetic diversity and structure spanning a wide spectrum. While differences within and amongst species of the symbiotic partners in organismal properties (i.e., reproductive mode, levels of clonality, dispersal ability, etc.) drive a component of the observed variability, geographic and physical (i.e., spatial scales, depth, currents, etc.) contexts contribute as well. Recognizing the roles and importance of these various factors, as well as how they interplay with one another, is required towards understanding current patterns and anticipating future ones for population-level biodiversity on coral reefs. Analyses of microsatellite loci have been one powerful tool in this endeavor, oftentimes providing unexpected, but exciting, insights into cnidarian-*Symbiodinium* symbioses. Such loci, however, can undergo complex mutational processes, as illustrated by two commonly utilized microsatellites for *Symbiodinium* Clade B ITS2 "type" B1. Notably, cases of alleles homologous in size but resulting from different evolutionary processes (e.g., allele size homoplasy) were identified for these loci. Such situations can inadvertently lead to inaccurate estimates of genetic diversity or inferences regarding population structure and raises the question of whether the complex mutations occurring at microsatellite loci make them inappropriate genetic markers for exploring questions pertaining to these symbioses. Fortunately, the problems associated with allele size homoplasy can be avoided by employing techniques capable of detecting differences in the primary sequence of microsatellite alleles themselves (i.e., SSCP or DGGE in conjunction with DNA sequencing) to ensure that those alleles identical in state are most likely identical by descent. In fact, mutations such as those described previously can act as a source of information that complement and extend the population-level allelic data inherent to microsatellite loci (e.g., Anderson et al. 2000; Santos et al. 2004, see above). Future studies dedicated to assessing evolutionary processes and mutation rates of microsatellite loci from *Symbiodinium* and their cnidarian host will contribute to our understanding of genomic evolution in these important and enigmatic organisms.

In addition to microsatellites, forthcoming efforts towards elucidating the population genetics of cnidarian-*Symbiodinium* symbioses will be able to leverage advancements in high-throughput DNA sequencing and computational pipelines to greatly expand analyses from just a handful to literally millions of loci. For example, techniques like restriction-site-associated DNA sequencing (RAD-Seq) and its derivatives (reviewed by Puritz et al. 2014) offer the opportunity with a modest investment in time and resources to scale population genetic studies to surveying whole genomes from individuals of species with little to no existing genomic data (reviewed by Davey and Blaxter 2010). While such "population genomics" will undoubtedly advance understanding of cnidarian hosts and their *Symbiodinium*, one obstacle they face and need to overcome is the fact that cnidarians like scleractinian corals are complexes of co-occurring organisms, including viral, prokaryotic, protist and fungal species. Commonly referred to as the "holobiont" (e.g., Rohwer et al. 2002; Knowlton and Rohwer 2003), these consortia (as well as the primary partners themselves) have the potential to contribute as sources of variation in genetic assessments of either host individuals or the dinoflagellate symbionts via their own genetic variability, population structure as well as presence or absence, particularly if the given technique is nonspecific in nature or produces data that has low discriminatory power (i.e., short DNA sequences). In the case of RAD-Seq, the simultaneous utilization of taxon-specific genetic markers such as microsatellite loci or the validation of loci to references like annotated genomic or transcriptomic data of the targeted organism (e.g., Combosch and Vollmer 2015) are possible avenues for mitigating this issue. These types of practices should be prudently exercised in all cases as a means of avoiding spurious results and erroneous conclusions in population-level work of cnidarian-*Symbiodinium* symbioses and other systems in general.

Lastly, the ecological and evolutionary importance of populations and the genotypic diversity within them has been longer recognized in terrestrial symbioses (e.g., bacterial symbionts of insects (Russell and Moran 2006); endophytic fungi of plants (Rodriguez et al. 2009)) and is now being appreciated in the coral reef ecosystem, particularly for cnidarian-*Symbiodinium* relationships (reviewed by Parkinson and Baums 2014). Given this, the number of population genetic studies for cnidarians and *Symbiodinium* have been steadily increasing since the early 2000s and should continue to do so as researchers attempt to fill the knowledge gap in this previously neglected area and integrate it into our biological understanding of these systems. Although population-level genetic diversity and processes will play fundamental roles in the response of coral "holobionts" to future environmental perturbations, there have been suggestions from within the scientific community that the rate of continuing global climate change is outpacing the potential for shorter-term ecological acclimatization and longer-term evolutionary adaptation in cnidarian-*Symbiodinium* symbioses and that "(human-)assisted evolution" might be required. In this context, possible avenues put forth include (1) exposing nursery-raised scleractinian corals to sub-lethal environmental stressors in order to promote acclimatization; (2) generating coral genotypes with stress-tolerant phenotypes via selective breeding; (3) modifying the coral "holobiont" by manipulating its community members, and; (4) artificial mutagenesis and selection of *Symbiodinium* towards developing genetically adapted, novel strains (Van Oppen et al. 2014), all of which could one day be utilized in restoring coral reef habitats that have been severally degraded or already lost. These ideas might be considered by some to be novel and forward thinking in regards to conservation and restoration efforts for this ecosystem. However, the human-mediated introductions of individuals into wild plant and animal populations can contribute to adverse loses in genetic diversity and adaptations as well as alterations of population composition and structure (reviewed by Laikre et al. 2010) or the creation of invasive situations (reviewed by Lee 2002). For cnidarians and *Symbiodinium*, the intentional release of new genotypes or strains that have been derived from natural or artificial means into existing populations or non-native habitats could result in a variety of scenarios, ranging from reversing current trends in organismal and habitat loss to no measurable impacts to negative repercussions with long-lasting consequences. If the introduction of *Symbiodinium trenchii* into the Caribbean and resulting spread of maladaptive symbiotic relationships through resident scleractinian coral communities (Pettay et al. 2015) serves as an indicator, "(human-)assisted evolution" of cnidarian-*Symbiodinium* symbioses should be approached with extreme caution since they would likely be irreversible once implemented and could cause more long-term harm then good for coral reef habitats that are already succumbing to other assaults of anthropogenic origin.

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General Ecological Aspects of Anthozoan-*Symbiodinium* Interactions in the Mediterranean Sea

24

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Abstract

The aim of this chapter is to provide a general overview of the main ecological aspects of Anthozoan-*Symbiodinium* mutualisms in the Mediterranean Sea. There are reports of at least twelve species of symbiotic anthozans in the basin. These anthozoans establish symbiotic relations with *Symbiodinium* Temperate A and B2 (*Symbiodinium psygmophilum*), corresponding to the only two species of *Symbiodinium* described in the region. A synthesis of the trophic and biochemical aspects of the interaction between *Symbiodinum* and their cnidarian hosts is given to contribute to the understanding of the mechanisms that maintain this special association. Finally, current knowledge about the ecological importance of this interaction in engineering species is examined. This review is framed to highlight the ecological importance of this symbiotic relationship in ecosystem construction and maintenance on an enclosed, temperate marine basin.

Keywords

Symbiodinium sp. • Anthozoans • Mutualism • Engineer species • Mediterranean Sea

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24.1 Introduction

The Anthozoan-*Symbiodinium* association is of fundamental ecological importance in the marine realm because it allows the holobiont to succeed in nutrient-poor waters, and helps in reef development and the construction of some of the largest bioconstructions on Earth. The symbiotic association between anthozoans and *Symbiodinium* has received extensive attention in tropical seas and is considered an adaptation to life in oligotrophic environments that may have played a crucial role during the development of early (Triassic) coral reefs (Muscatine et al. 2005). Additionally, the disruption of the symbiosis (e.g., bleaching) usually represents a symptom of decline in reef ecosystems (Lough and van Oppen 2009; Baird and Maynard 2008). Anthozoan-*Symbiodinium* associations are also present in temperate environments; however, these temperate partnerships have received less attention. Temperate associations are exposed to fluctuating environmental conditions in comparison with tropical regions; therefore, studying these associations can contribute to our understanding of the physiological limits of the holobiont and each of its components.

The productivity and dynamics of temperate marine environments is characterized by marked seasonal patterns of irradiance, temperature, and storms throughout the year. The Mediterranean Sea is the largest enclosed sea on the planet and a temperate marine ecosystem. The annual mean sea surface temperature is highly seasonal with notorious west-to-east and north-to-south gradients. Seasonal temperature variation creates a period of water column stability, depletion of dissolved nutrients at the surface, and a period of homogenization in which the availability of nutrients in the water column increases productivity. The biological diversity decreases from north to south and from west to east, coinciding with the levels of productivity, and it is inversely related to the increase in temperature and salinity (Turley 1999; Danovaro et al. 1999). In addition to environmental gradients, the Mediterranean diversity and its distribution in the basin have been shaped by the geological events and the narrow connection with the Atlantic Ocean (Boudouresque 2004; Templado 2014). These characteristics have made the Mediterranean Sea a hotspot of biological diversity with a high level of endemism and an assortment of temperate and subtropical elements (Coll et al. 2010). Additionally, the relative small size and fast deep water (15–50 years) renewal of the Mediterranean compare to ocean basins, suggest that this sea may be especially sensitive to the effects of climate change (Calvo et al. 2011).

Even though the Mediterranean basin is important as a biodiversity hotspot, some groups of organisms in the region deserve more attention from the ecological and conservational point of view, including Anthozoans and their companion symbionts. This chapter highlights the current and most relevant information on symbiotic anthozoans in the Mediterranean Sea by presenting a revision of their main ecological characteristics and their symbiotic associations with the dinoflagellate algae of the genus *Symbiodinium*.

24.2 Anthozoan-Dinoflagellate Trophic Relationship: A Costly Mutualism?

The cnidarian body structure is characterized by radial symmetry and displays two epithelial layers (epiderm and gastroderm) separated by an acellular, watery, and collagen-rich layer, the mesoglea. In symbiotic cnidarians, unicellular symbionts (Fig. 24.1) tend to live inside the gastrodermal cells. *Symbiodinium* cells are isolated from the host cytoplasm by a host-derived perisymbiotic membrane, forming the symbiosome. Compared to the free-living *Symbiodinium*, found in sediments or on macroalgae, the *in-hospite* symbiont biomass is high. For example, in Mediterranean sea anemones, one to eight *Symbiodinium* cells per animal cell can be observed and several million of algae cells per mg of animal protein can be measured (Fig. 24.2).

Depending on the species, cnidarians acquire their symbiotic partners through either vertical (parent to embryo) or horizontal transmission (larvae/adults acquire the symbiont cells from the environment). These transmission modes are commonly species-specific, and the involved/responsible cellular mechanisms remain largely unknown. However, several host-recognition receptors (lycan-lectin interactions, the complement C3, the scavenger receptors, or the Nieman Niemann-Pick type C proteins) have been suggested to be implicated during the establishment of the symbiosis (Davy et al. 2012; Dani et al. 2014).

The mutualism offers both the host and the symbiont trophic benefits, improving their survival. The symbiont greatly contributes to increasing the cnidarian energy budget. Algal

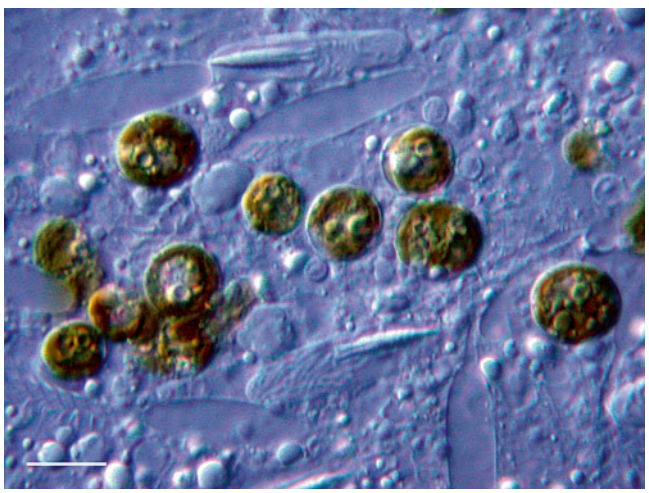

Fig. 24.1 *Symbiodinium* cells within the endoderm of *Balanophyllia europea* (Author: A. Terrón-Sigler) (Scale bar: 10 μm)

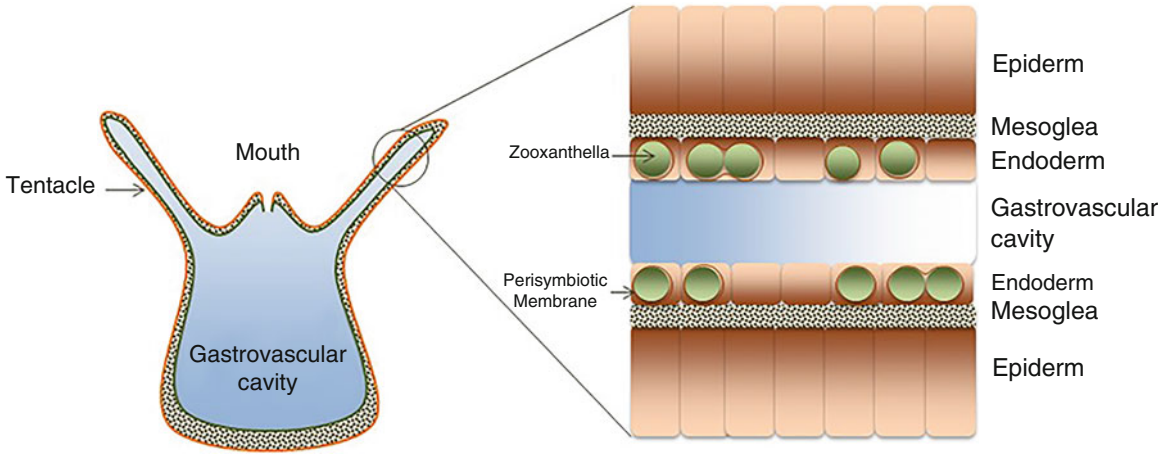

Fig. 24.2 Anatomy of a cnidarian polyp and the symbiotic tissue organization (Author: P. Furla)

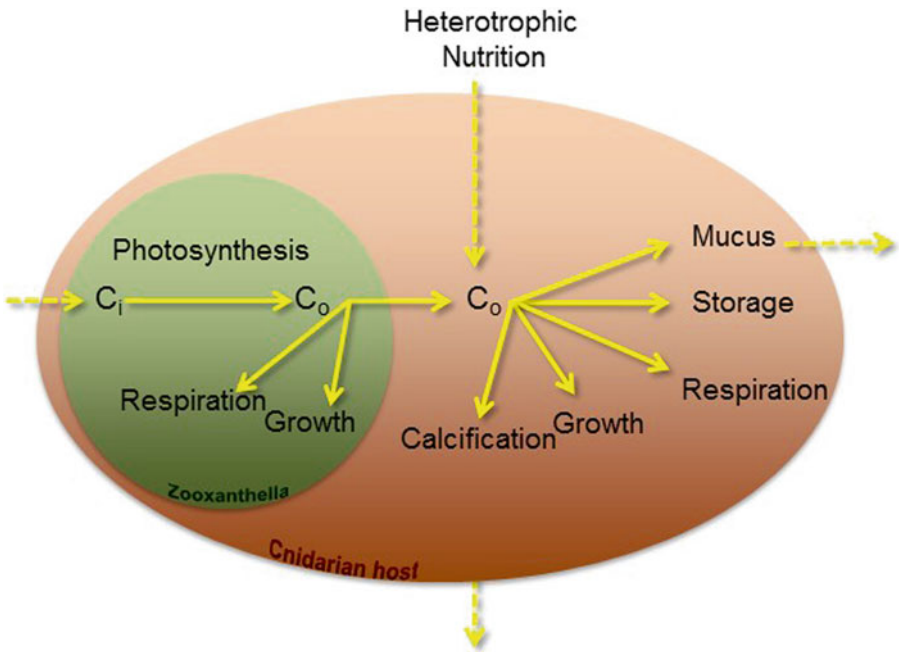

Fig. 24.3 Organic carbon supply and use in a symbiotic cnidarian. *Ci* inorganic carbon (carbon dioxide, bicarbonate), *Co* organic carbon (sugars, lipids, amino acid) (Author: P. Furla)

photosynthesis provides the animal with a new metabolic capacity, making it partially autotrophic. In *Anemonia viridis* up to 97 % of the carbon fixed during photosynthesis is transferred to the host cell (Davy et al. 1996). The transferred organic carbon takes various forms (e.g., glycerol, fatty acids, alanine, glucose, and glutamic acid) and plays several roles (e.g., structural components, metabolisms, reserves, reproduction, or components of the organic matrix) in the coral host. *Symbiodinium* cells can also influence the metabolic respiration of their cnidarian host cells, supplying not only energy but also oxygen (Fig. 24.3).

In oligotrophic waters, algal cells directly benefit from its *in hospite* lifestyle, as the host provides various nutrients that are essential for the symbiont (e.g., nitrogen, phosphorus, and sulfate). This happens through a recycling of the host catabolism products, which are re-metabolized and re-cycled by the algal photosynthesis system and through direct uptake of the nutrients from the seawater by the host (Wang and Douglas 1998). In addition, the intracellular location of algae protects it from predators while providing a stable habitat to grow. The benefits of this symbiosis, however, come with a cost. Both partners face strong constraints that lead to the selection of specific morphological, biochemical, and physiological traits. Optimization of the photosynthesis is a clear example of the restrictions. The dilemma between optimal light exposure and low ultraviolet radiation requires a balance between different species of partners. To favor irradiation, the symbiotic cnidarians exhibit long-term adaptations to shallow waters but morphological plasticity (i.e., flattened coral skeletons or increased symbiotic tissue expansion with

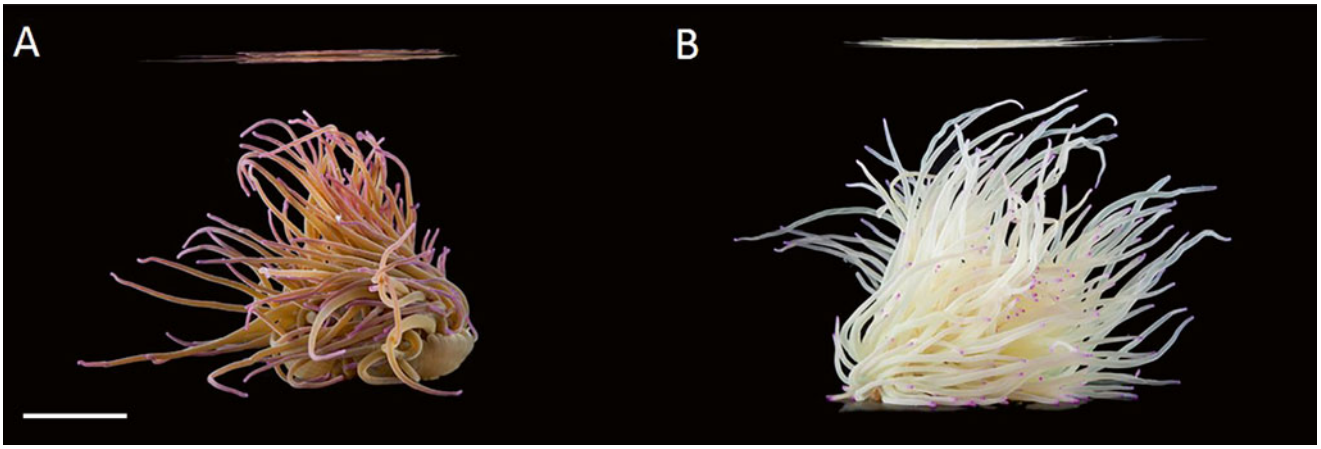

Fig. 24.4 Symbiotic **a** and bleached **b** *Anemonia viridis* morphs (Image copyright: ©A. Amiel/E. Röttinger/Kahi Kai Images) (Scale bar: 5 cm)

depth) to expand the depth range. Behavioral traits could also be involved, as displayed in sea anemones exposed to moderate irradiances, which move or orient their tentacles toward the light source. Protection against damaging radiation in cnidarians includes a battery of photoprotective molecules, such as fluorescent pigments (pocilloporines) and specific amino acids (mycosporine-like amino acids – MAAs). Present in 97 % of the corals of the Great Barrier Reef, pocilloporines absorb excessive or non-photosynthetic radiation (Roth 2014). MAAs are anti-UV molecules present in high concentrations in cnidarian animal symbiotic tissues, providing a wide UV spectrum screen to prevent DNA mutation and cell degradation, among other issues.

In symbiotic cnidarians, the main source of inorganic carbon is bicarbonate, the symbiont has no direct access to the inorganic carbon, and the dinoflagellate inorganic carbon-fixing enzyme, RUBISCO (ribulose-phosphate carboxylase oxygenase) has biochemical characteristics that limit the fixation of CO_2 (the carboxylation reaction). For these reasons, CO_2 fixation in Cnidaria-*Symbiodinium* mutualisms is more constrained than in terrestrial plants. To address these limitations, the symbiotic association shows several adaptive strategies. The host animal has developed mechanisms for bicarbonate absorption, which allow the symbiont to acquire inorganic carbon for photosynthesis (Furla et al. 2005). The inorganic carbon is not only transported but also concentrated within the symbiotic tissues to favor RUBISCO carboxylase activity (Furla et al. 2005). Finally, within the symbiont cells, RUBISCO is isolated from the photosynthetic oxygen production within the pyrenoid, a specialized area deprived of the photosystem.

The photosynthetic activity of the holobiont produces daily oxygen fluctuations. For example, in the sea anemone *A. viridis*, the pO_2 of the gastric cavity increases very rapidly in the light, reaching three times the normoxia pO_2 value (21 %) in only 20 min. In the dark, because of the respiration, pO_2 declines progressively, reaching anoxia in less than 60 min (Richier et al. 2003). In the cnidarian tissues, hyperoxia results in the overproduction of reactive oxygen species, which induce cellular damage. However, such oxidative damage is not observed in any of the partners, suggesting the presence of effective anti-oxidant systems and thus a high level of adaptation of the symbiotic association. Among this oxydative defensive weaponry, enzymes such as superoxide dismutases (SOD), catalases (CAT), and gluthation peroxidases (GPX) play an important role by destroying active oxygen species (Furla et al. 2005) and providing a defense against pathogens (Mydlarz et al. 2006).

Despite the sophisticated adaptations and advantages of the relation, symbiotic cnidarians are some of the most sensitive organisms in the oceans. Indeed, environmental perturbations lead to the disruption of the symbiosis and the expulsion of *Symbiodinium* cells from animal tissue. This phenomenon, known as "bleaching," results in the loss of pigmentation of the affected cnidarians (Fig. 24.4), and while it might be temporal, it can produce mortality and has been linked to other effectors, such as diseases. Climate change is causing an increase in ocean temperatures, which is the main factor in bleaching. Heavy metal pollution, herbicides or pesticides, an increase in visible or UV radiation, changes in salinity, or microbial infection can also synergistically aggravate the symbiosis breakdown. Despite the ecological importance of the phenomenon, the molecular and physiological mechanisms caused by environmental perturbations that lead to bleaching are not yet clearly defined. However, several studies have already identified critical points, such as early photosynthetic machinery dysfunction, excessive accumulation of reactive oxygen species, induction of cell death processes, and reduction of the expression of several putative symbiont recognition molecules in the host, such as lectins (Jones et al. 1998; Lesser 2006; Dunn et al. 2007; Davy et al. 2012; Dani et al. 2014).

In the temperate context of the Mediterranean Sea, the seasonal and diurnal variations of the physicochemical

parameters (e.g., temperature, light, and/or salinity) could influence the cnidarian-*Symbiodinium* association, leading to specific mechanisms of symbiosis adaptations and responses to stress. Moreover, the reduced *Symbiodinium* clade diversity in the Mediterranean Sea (Sect. 24.3.2 and Forcioli et al. 2011) constitutes a unique perspective to understand these important symbiotic relationships.

24.3 Diversity and Ecology of Anthozoan-*Symbiodinium* Mutualisms in the Mediterranean Sea

24.3.1 Diversity

In general temperate anthozoans are characterized by their resilience to variable environmental conditions. Approximately 235 species of anthozoans have been described in temperate regions of the oceans. However, the conditions in these "marginal" regions prevent the formation of reef structures like those seen in tropical seas. In the Mediterranean, there are approximately 150 species of anthozoans (~64 % of described temperate anthozoans), of which 12 species (~6 %) are symbiotic, forming mutualisms with *Symbiodinium* algae. The symbiotic Mediterranean anthozoans are found in the photic zone down to 40 m depth, depending on the location. A majority of these anthozoans are sea anemones (Actiniaria), stony corals (Scleractinia), and a single symbiotic gorgonian species (Alcyonacea) (Fig. 24.5). Anyhow, it has to be denoted that research in this basin accounts for new species (Coll et al. 2010). Symbiotic anthozoans play an important role by means of carbonate production and nutrients fixation, as well as they contribute to the sustainability of other Mediterranean communities and ecosystems.

24.3.2 State of Knowledge Concerning Anthozoa-*Symbiodinium* Interactions in the Mediterranean at the Species Level and Future Challenges

The genus *Symbiodinium* was originally described as a single dinoflagellate species, *Symbiodinium microadriaticum* (Freudenthal 1962). Subsequent studies using molecular markers expanded the diversity of the genus by recognizing numerous evolutionary lineages, phylotypes, or clades (Baker 2003; Coffroth and Santos 2005). The differentiation of these lineages has been supported by several studies using different genetic markers (Sampayo et al. 2009; Thornhill et al. 2013; Pochon et al. 2014). Furthermore, integrative taxonomic approaches have matched molecular genetics through electron microscopy morphological analysis (LaJeunesse et al. 2010). Each *Symbiodinium* clade includes a large diversity of genetic sub-clades (also named sub-phylotypes, or types), each with distinctive biogeographical, ecological, and host-specific patterns (Baker and Rowan

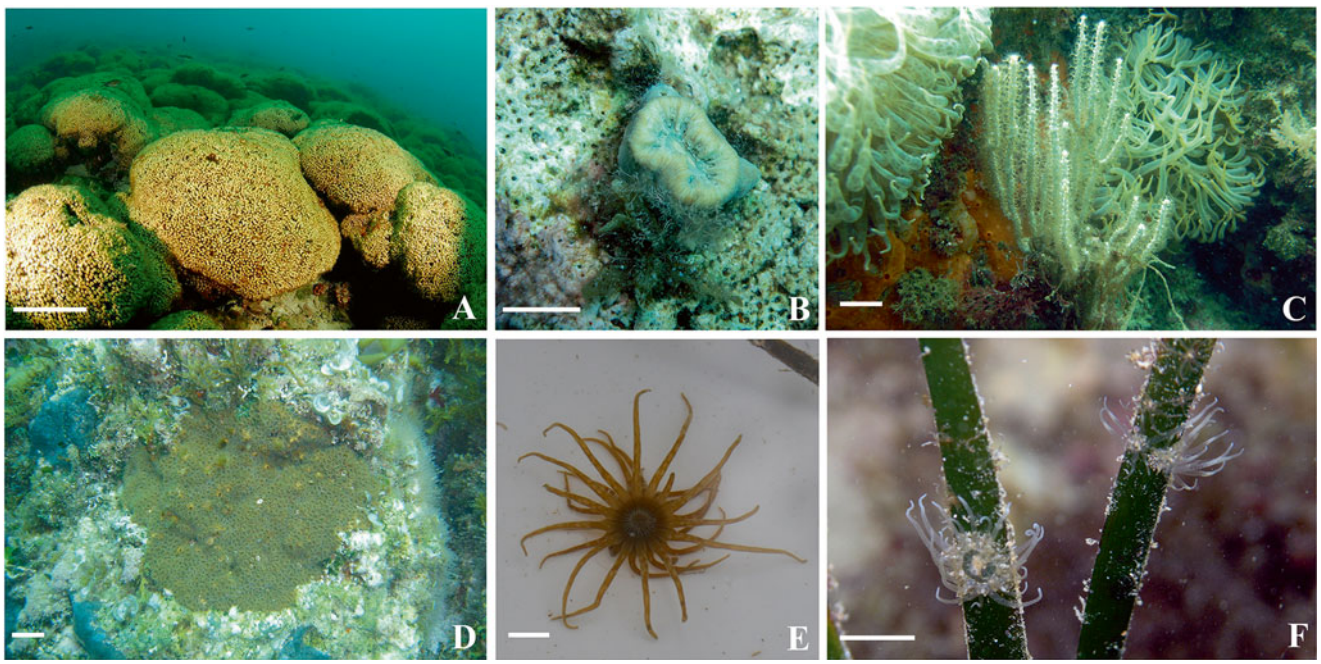

Fig. 24.5 Examples of symbiotic anthozoans from the Mediterranean Sea. **a** One of the largest reef formations of *Cladocora caespitosa*, Mjlet National Park, Adriatic Sea (Author: P. Kružić). **b** *Balanophyllia europea* (Author: A. Terrón-Sigler). **c** *Eunicella singularis* (Author: A. Terrón-Sigler). **d** *Oculina patagonica* (Author: R. Coma). **e** *Paranemonia cinerea*, (Author: P. Casado-Amezúa). **f** *Bunodeopsis strumosa* (Author: A.Terrón-Sigler) (Scale bars- **a** 50 cm; **b**–**f** 1 cm)

1997; LaJeunesse et al. 2003, 2010). Most of the diversity within clades has been assessed using molecular markers such as the internal transcribed spacers (ITS1 and 2) and the large subunit (LSU) and small subunit (SSU) of the ribosomal DNA. Recently, the noncoding region of the psbA minicircle has drawn interest in the study of *Symbiodinium* diversity, ecology, and evolution (LaJeunesse 2001; Rodriguez-Lanetty 2003; Coffroth and Santos 2005; Barbrook et al. 2006). Additionally, the sequencing of microsatellites flanking regions has provided support for the delineation of *Symbiodinium* types (LaJeunesse et al. 2010).

In the Mediterranean, only two groups of *Symbiodinium* have been described up to date: clade "temperate A" and clade B or *Symbiodinium psygmophilum* (Table 24.1). Most studies of Mediterranean anthozoans have found that the prevalent clade in the Western region is *Symbiodinium* "temperate A" (Savage et al. 2002; Barbrook et al. 2006; Visram et al. 2006; Casado-Amezúa et al. 2014). This clade includes a variety of types, currently only described by molecular statistical analysis with the use of ITS markers (Casado-Amezúa et al. 2014). *Symbiodinium* temperate A was first described by Savage et al. (2002) from Mediterranean and Atlanto-Mediterranean sea anemones, and it was defined as a phylotype evolutionary close to clade A elsewhere. Species within clade A are mainly found in tropical and temperate regions of the Western Atlantic (Baker 2003; Pochon et al. 2006). These observations suggest a recent divergence of the clade and a possible adaptation and isolation of the temperate type to the Mediterranean. Physiologically, "temperate A" is regarded as having potential resistance to short-term increases in temperature compared to conspecific types in other basins (Rodolfo-Metalpa et al. 2006). "Temperate A" may have diverged from its sister species after the opening of the Mediterranean to the Atlantic Ocean during the Plio-Quaternary (Blanc 2002), but further studies are necessary to confirm this theory.

Clade B (type B2) is found in the Western and Eastern Mediterranean. LaJeunesse et al. (2012) described the species as *S. psygmophilum*, associated with three species of Scleractinian corals (*Oculina patagonica*, *Cladocora caespitosa*, and *Madracis pharensis*), all from Israel (Eastern Mediterranean Basin). In addition, Meron et al. (2012) found this same type associated with *Balanophyllia europaea* and *C. caespitosa* in the Central Mediterranean (Iscchia Island, Southern Tyrrhenian Sea, Western Mediterranean Basin). *S. psygmophilum* (LaJeunesse et al. 2012) has been previously reported only in northerly coral reef habitats in the Western Atlantic, including the Florida Keys and Bermuda (LaJeunesse 2001; Savage et al. 2002). However, this clade is not restricted to temperate regions and has been found in scleractinian corals and other invertebrates in tropical areas (Loh et al. 1998). This type represents a cold-tolerant lineage able to survive conditions that are inhospitable to most other *Symbiodinium* species (Rodriguez-Lanetty et al. 2011). The presence of both *Symbiodinium* temperate A and *S. psygmophilum* has been described for *Balanophyllia europaea* and *C. caespitosa*. The presence or absence of *Symbiodinium* species in the same host have been related to the environmental conditions of the geographic region where the host lives. These observations suggest that the environment is important in defining the formation of the symbiotic partnerships (Rodriguez-Lanetty et al. 2011; Savage et al. 2002).

It has to be pointed out that earlier studies on the symbiont of *O. patagonica* based on light microscopy, defined the species that the corals host as *S. oculinidae* (Duclaux 1977). Anyhow, given the recent molecular techniques, integrative taxonomic approaches are currently leading to more accurate taxonomical approximations.

The presence of type B2, *S. psygmophilum*, in temperate regions of both the Western Atlantic and Indo-Pacific Oceans may be an indication that this clade is a relic from the ancient Tethys Sea. An additional possible explanation for the presence of type B2 in the Mediterranean basin is the introduction of this species during the Messinian salinity crisis. Proving or disproving these theories requires in-depth studies using novel markers, molecular clocks, wider sampling strategies (including latitudinal and longitudinal gradients), and integrative approaches across the Mediterranean basin and surrounding areas.

24.4 Importance of Anthozoan-*Symbiodinium* Mutualisms for the Health of the Mediterranean Ecosystems and Specific Approaches

Anthozoan-*Symbiodinium* associations are critical to sustain ecosystems dominated by symbiotic species (Yellowlees et al. 2008). In temperate areas, such as the Mediterranean, macroalgae provide most of the three-dimensional structure of shallow subtidal communities (Zabala and Ballesteros 1989). Some anthozoans, however, are capable of overcoming the unfavorable conditions and built biogenic structures. Among the anthozoans in the Mediterranean, the endemic coral, *Cladocora caespitosa*, and the gorgonian, *Eunicella singularis*, are the main contributors to the biogenic structure in the region.

C. caespitosa, with a carbonate production of up to 12.8 kg m^{-2} $year^{-1}$, is the only coral capable of producing a carbonate frame similar to that built by other corals elsewhere (Peirano et al. 2001). It this species is the only shallow-water scleractinian coral that can be considered a reef-building coral in the Mediterranean basin. *C. caespitosa* frameworks are called "banks" of phaceloid colonies and can reach a height of up to about 1 m (Peirano et al. 2001; Morri et al. 2001; Kružić and Benkovic 2008). The fact that the corallites develop vertically with independent walls generates a considerable interstitial space that provides a habitat for a large

Table 24.1 Mediterranean anthozoan species and identified associated *Symbiodinium* type

Order	Species	Location	Basin	*Symbiodinium* type	Molecular marker(s)/ reference(s)
Actiniaria (Sea anemones)	*Aiptasia diaphana*	France. Gulf of Lyon	Western	Temperate A	LSU/Visram et al. (2006)
	Anemonia rustica	France. Gulf of Lyon	Western	Temperate A	SSU, LSU, ITS/Savage et al. (2002)
		Italy. Tyrrhenian Sea	Western	Temperate A	SSU, LSU, ITS/Savage et al. (2002)
					LSU/Visram et al. (2006)
	Anemonia sulcata var. *rufescens*	France. Gulf of Lyon	Western	Temperate A	SSU, LSU, ITS/Savage et al. (2002)
		Italy. Tyrrhenian Sea	Western	Temperate A	SSU, LSU, ITS/Savage et al. (2002)
	Anemonia sulcata var. *smaragdina*	France. Gulf of Lyon	Western	Temperate A	SSU, LSU, ITS/Savage et al. (2002)
					psbA/Barbrook et al. (2006)
		Italy. Tyrrhenian Sea	Western	Temperate A	SSU, LSU, ITS/Savage et al. (2002)
	Anemonia sulcata var. *viridis*	France. Gulf of Lyon	Western	Temperate A	SSU, LSU, ITS/Savage et al. (2002)
					psbA/Barbrook et al. (2006)
	Anemonia sulcata var. *vulgaris*	Italy. Tyrrhenian Sea	Western	Temperate A	SSU, LSU, ITS/Savage et al. (2002)
	Bunodeopsis strumosa	France. Gulf of Lyon	Western	B	LSU/Visram et al. (2006)
					psbA/Barbrook et al. (2006)
		Spain. Algerian Basin	Western	Temperate A	LSU, ITS/Casado-Amezúa et al. (2014)
	Cereus pedunculatus	Spain. Balearic Sea	Western	Temperate A	LSU/Visram et al. (2006)
					psbA/Barbrook et al. (2006)
	Cribinopsis crassa	Italy. Tyrrhenian Sea	Western	Temperate A	LSU/Visram et al. (2006)
	Paranemonia cinerea	Spain. Balearic Sea	Western	Temperate A	LSU, ITS/Casado-Amezúa et al. (2014)
Alcyonacea (Gorgonians)	*Eunicella singularis*	France. Gulf of Lyon	Western	Temperate A	SSU, cp23S, LSU/Forcioli et al. (2011)
		Spain. Baleric Sea	Western	Temperate A	SSU, cp23S, LSU/Forcioli et al. (2011)
		Italy. Tyrrhenian Sea	Western	Temperate A	SSU, cp23S, LSU/Forcioli et al. (2011)
Scleractinia (Stony corals)	*Balanophyllia europaea*	Spain. Balearic Sea	Western	Temperate A	LSU/Visram et al. (2006)
		Italy. Tyrrhenian Sea	Western	Temperate A/	psbA/Barbrook et al. (2006)
				S. psygmophilum	ITS2/Meron et al. (2012)
	Caryophyllia smithii	France. Gulf of Lyon	Western	Temperate A	LSU/Visram et al. (2006)
	Cladocora caespitosa	France. Gulf of Lyon	Western	Temperate A	LSU/Visram et al. (2006)
		Spain. Balearic Sea	Western	Temperate A	LSU, ITS/Casado-Amezúa et al. (2014)
		Italy. Tyrrhenian Sea	Western	*S. psygmophilum*	ITS2/Meron et al. (2012)
		Israel. Levantine Sea	Eastern	*S. psygmophilum*	ITS, mic, cob1, cp23S/ LaJeunesse et al. (2012)
	Madracis pharensis	Israel. Levantine Sea	Eastern	*S. psygmophilum*	ITS, mic, cob1, cp23S/ LaJeunesse et al. (2012)
	Oculina patagonica	Spain. Algerian Basin.	Western	*S. psygmophilum*	LSU, ITS/Casado-Amezúa et al. (2014)
		Spain. Balearic Sea	Western	*S. psygmophilum*	LSU, ITS/ Casado-Amezúa et al. (2014)
		Israel. Levantine Sea	Eastern	*S. psygmophilum*	ITS, mic, cob1, cp23S/ LaJeunesse et al. (2012)

SSU small subunit of the ribosomal DNA, *LSU* large subunit of the ribosomal DNA, *ITS* internal transcribed spacers, *psbA* psbA minicircle, *mic* flanking regions of microsatellites, *cob1* mitochondrial cytochrome b, *cp23S* domain V of chloroplast large subunit ribosomal DNA

variety of associated fauna. It has been estimated that up to 242 macrobenthic species are associated with these coral structures (Koukouras et al. 1998; Antoniadou and Chintiroglou 2010). This associated fauna enhances fragmentation of the coral and has been considered a relevant mechanism contributing to asexual reproduction/dispersion of the coral (Schiller 1993).

The only symbiotic Mediterranean gorgonian, the white gorgonian *Eunicella singularis*, is among the most abundant and widespread species of anthozoan in the Mediterranean. It is an engineer species that, similar to other gorgonians, provides most of the tridimensional structure of the reef community (Ballesteros 2006). It has been shown recently that the canopies formed by this species harbor a large variety of associated epifauna (Carvalho et al. 2014), contributing significantly to the diversity and ecology of the Mediterranean.

The Mediterranean Sea has been highly affected by climate change (Coll et al. 2010; Calvo et al. 2011). In recent decades, *C. caespitosa* and *E. singularis* have been suffering from episodes of mass mortality related to increased seawater temperatures in summer and their effect on the water column (Perez et al. 2000; Rodolfo-Metalpa et al. 2005; Coma et al. 2009; Garrabou et al. 2009; Kružić et al. 2012; Kersting et al. 2013). An increase in the intensity of thermal anomalies diminishes the natural capacity of the species to recover. The effect of these events has been documented to be large, with species losing up to 50 % of their populations (Kersting et al. 2013).

Can the benefits of the symbionts attenuate the effects of thermal anomalies in *C. caespitosa* and *E. singularis*? *C. caespitosa* can feed on a wide variety of food items, from particulate organic matter to zooplankton, representing >50 % of the carbon obtained from photosynthesis (Tremblay et al. 2011). This allows this species to thrive in turbid environments, as it can acquire enough energy from sources other than photosynthesis. With a very high abundance of zooplankton, the species can completely rely on heterotrophy. Additionally, this species can withstand at least 2 months without light and not lose its photosynthetic capacity, making it a very flexible and adaptable coral (Hoogenboom et al. 2010). *C. caespitosa* acquires most of its energy needs from the symbionts during the summer, but during winter, it relies on heterotrophy to supply its energetic necessities (Ferrier-Pagès et al. 2011). It is important to note that the relative contribution of autotrophy during winter increases because of low temperatures, which reduce respiration (Schiller 1993; Ferrier-Pagès et al. 2011). In contrast, the feeding rate does not decrease with high light (i.e., summer conditions) (Hoogenboom et al. 2010). These contrasting but complementary observations indicate that both autotrophy and heterotrophy are crucial to the survival of the species.

Several studies have addressed the nutrition of *E. singularis* and the role of *Symbiodium* in resource acquisition. Studies with stable isotopes have shown an important variability in the relative contribution of heterotrophic and autotrophic nutrition (Gori et al. 2012; Cocito et al. 2013). Despite this variability, experimental and in situ studies have shown that autotrophy can account for the needs of the species in late spring and during the nutritionally poor summer conditions. However, they detected a collapse of the photosynthetic capacity of the symbionts above a warm temperature threshold (Ezzat et al. 2013; Ferrier-Pagès et al. 2015).

Summer conditions in the Mediterranean Sea are characterized by high water column stability and high temperatures, resulting in a strong stratification of the water column. This stratification is responsible for the reduction of dissolved surface nutrients, as well as particles sinking results in the depletion of suspended food resources (Coma and Ribes 2003). Therefore, among the main triggering mechanisms, temperature anomalies are considered the primary underlying cause of changes in energetic shortage and death in *E. singularis* and *C. caespitosa* (Coma et al. 2009; Crisci et al. 2011). In this framework, an assessment of the ingesta of the species and its contrast with respiration suggest that heterotrophic nutrition cannot account for the energy needs of the species in summer and fall (Coma et al. 2015). The limits of heat sensitivity are reached first by the symbiont and then by the host, therefore the low heterotrophic ingesta in summer and fall, together with the collapse of the photosynthetic capacity above a warm temperature threshold, suggests a resource limitation that contributes to the understanding of the mechanisms of tissue mortality (Ezzat et al. 2013; Coma et al. 2015). The negative impact of these mortality episodes is expected to increase because of the increase in the frequency and geographic extent of these climate-induced mortalities (Calvo et al. 2011).

Other two symbiotic anthozoans are of special importance in the Mediterranean, the stony coral *Oculina patagonica* and the snakelock anemones *Anemonia spp*., both the scope of wide number of studies in the last years.

The symbiotic scleractinian coral *Oculina patagonica* has long been described as a non-native species from the Mediterranean Sea (Zibrowius and Ramos 1983). However, a recent genetic study found no evidence supporting the introduction of this species to the Mediterranean, as *O. patagonica* appears to have diverged from *Oculina spp*. from the WN Atlantic ~5 Mya (Leydet and Hellberg 2015). Although *O. patagonica* used to be a scarce species, in recent decades, it has been experiencing range expansion, attributed to the availability of open space (artificial habitats), trophic interactions, and environmental change (Sartoretto et al. 2008; Coma et al. 2011; Salomidi et al. 2013; Serrano et al. 2013; Rubio-Portillo et al. 2014). Although this species is abundant in man-made structures such as harbors, marinas, and dikes, populations are also found in nearby natural environments (Serrano et al. 2013; Rubio-Portillo et al. 2014; Salomidi et al. 2013). *O. patagonica* has the ability to thrive under a wide range of environmental conditions in terms of temperatures, salinity, UV radiation, turbidity, and wave energy (Fine et al. 2001). The species is capable of developing gametogen-

esis even in polluted waters (Armorza-Zvuloni et al. 2011) and can resist repeated bleaching events (Ainsworth et al. 2008). Therefore, it is considered an "opportunistic dominant settler" that can compete with algae and other organisms (Sartoretto et al. 2008). The shift from macroalgal to *O. patagonica* dominance (Serrano and Coma 2012) suggests potential changes at the ecosystem level that might benefit other corals in the future climate. Bleaching events have been recorded in the Israel coasts. These events have been related to a bacterial infection trigger by high water temperature (Ainsworth et al. 2008), nevertheless it has been recorded as a reversible event, and mainly driven by opportunistic bacteria rather than pathogenic ones. The resistance of the species to mortality and bleaching events has been also associated to the symbiont species it hosts, which is *S. psygmophilum* (LaJeunesse et al. 2012; Casado-Amezúa et al. 2014).

Sea anemones in benthic systems promote biodiversity by supporting mutualism and numerous predators, reducing macro- and filamentous-algal recruitment and maintaining primary productivity rates (Fit et al. 1982). Anemones are also important to heavy metal (Cu, Ni, and Zn) uptake from the water column (Mitchelmore et al. 2003). In the Mediterranean Sea, the snakelock anemones, *Anemonia* spp., forms extensive blankets that function as micro-ecosystems, harboring several crustaceans (Fig. 24.6) and some fish (e.g., *Parablennius policornis*, *Gobius xanthocefalus and G. buchichii*) (Fig. 24.6. In some areas, such as in the Andalussia coasts (Northern Alboran Sea), these anemones have economic importance as a fishery resource. The symbiotic algae associated with the anemone are crucial to their performance, as it is the main source of primary productivity and nutrient cycling (Suggett et al. 2012). It has been shown that fields of anemones could be expected to play a major local to regional role in biogeochemical cycles. Anemones can increase CO_2 concentrations, driving the ecosystem equilibrium toward greater net autotrophy and CO_2 sequestration (Suggett et al. 2012). However, their function and relevance remain a focus of future studies (Fig. 24.6).

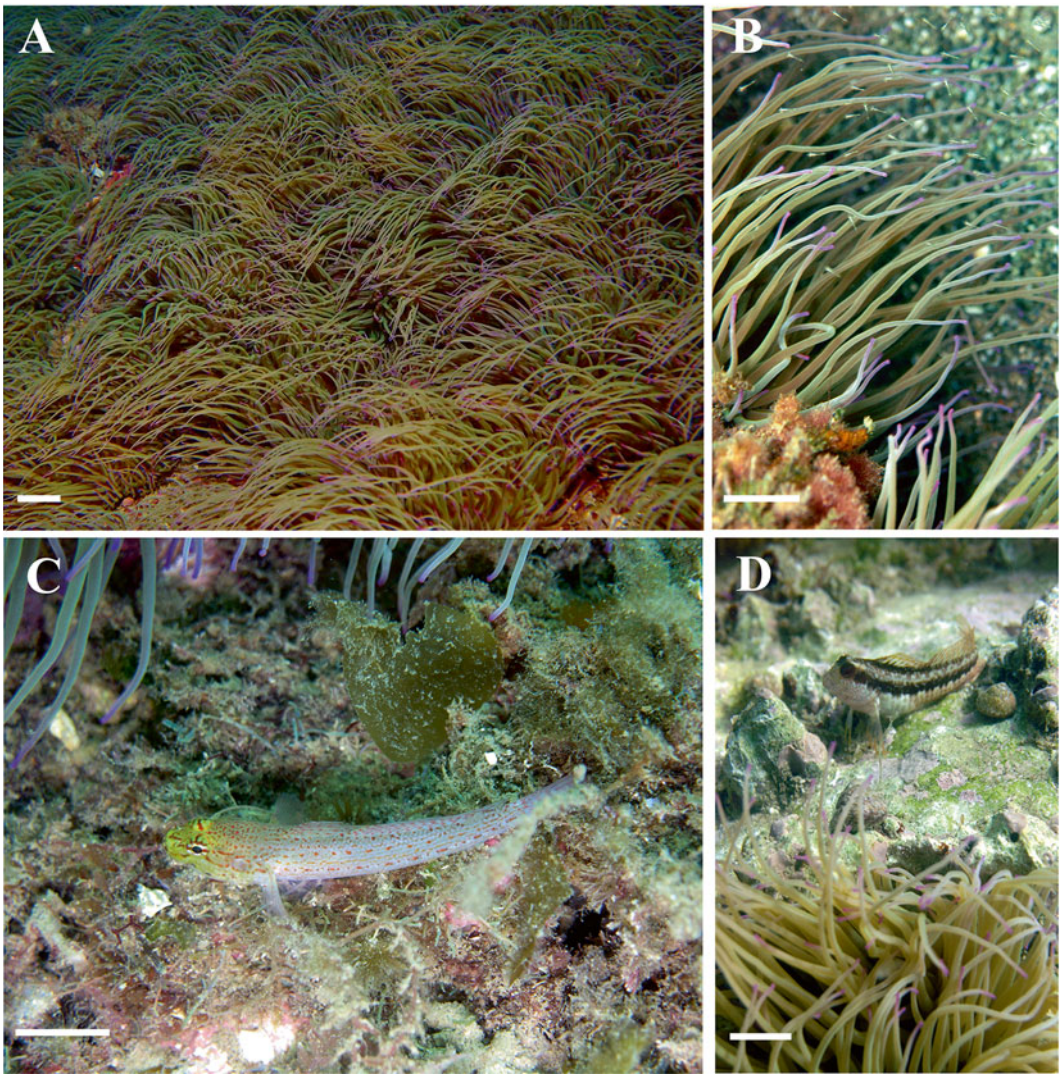

Fig. 24.6 **a** Field of *Anemonia* spp. in the Andalussian coasts, Northern Alboran Sea (Author: C. Aldaya). **b** Amphipods associated to *Anemonia* spp. **c**, **d** *Gobius xanthocephalus* and *Parablennius policornis* inhabiting *Anemonia* spp. field (Author: A. Terrón-Sigler) (Scale bars- all pictures: 5 cm)

24.5 Research Priorities

Anthozoan-*Symbiodinium* mutualisms are in general understudied in the Mediterranean Sea in comparison with tropical regions. Species- and population-specific research needs to be undertaken to better establish the biogeography and ecology of these relationships across the basin.

In general terms, the main factors that deserve further research include the following:

- Taxonomic identification of species clades of symbiotic anthozoans. Genetic identifications need to be expanded and complemented with morphological and ecological approaches to obtain a complete picture of the diversity of *Symbiodinium* species in the Mediterranean.
- Complete assessments along latitudinal and longitudinal distributional gradients of anthozoans and their symbiotic algae, as well as the possible impacts at the local and regional level, are recommended to better understand the biogeographical patterns of *Symbiodinium* species in the Mediterranean basin.
- Further research on *Symbiodinium* acquisition mechanisms, as well as specificity in the association, is necessary to understand the dynamics of both the associations and that of each mutualism component.
- Species-specific studies on thermotolerance and adaptive and acclimatization processes are necessary to understand the impacts of climate change (i.e., sea surface temperature increase) on these symbiotic organisms and the communities that they might support.

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Cnidarians and Their Polychaete Symbionts

25

Tina N. Molodtsova, Temir A. Britayev, and Daniel Martin

Abstract

Cnidarians, especially skeleton-bearing anthozoans and hydrocorals, are known to host abundant and diverse symbiotic fauna encompassing members of the majority of metazoan taxa, ranging from sponges and flat worms to fishes. Members of the class Polychaeta are between the most diverse and perhaps the least studied taxa of coral symbionts. The last revision (Martin and Britayev, Oceanogr Mar Biol 36:217–340, 1998) reckoned about 60 species of symbiotic polychaetes associated with more than 100 species of cnidarian hosts. However, this number is considerably underestimated. Some populations of scleractinians, sea fans and black corals show up to 100 % infestation by symbiotic polychaetes. Close association and inter-relation of highly host-specific symbionts and cnidarian hosts often lead to dramatic changes in the host morphology. At the moment, actual mechanisms of most of mutual relations between host and symbiont in such associations are generally unknown. The objective of the present paper is to summarize data on species composition and ecology of polychaetes associated with cnidarians. In our review, we report 281 species of cnidarian hosts involved in 324 relationships with symbiotic polychaetes. Most polychaete-hosting cnidarians belong to skeleton-bearing taxa, particularly Scleractinia (125 species or 44.48 % of the total cnidarian hosts), Alcyonaria (73 species or 25.97 %) and Hydrozoa (60 species or 21.35 %). About 120 species of symbiotic polychaetes of ten families are reported from cnidarian hosts. Polynoidae include the highest number of cnidarian-associated polychaetes (almost one half of the currently known species), followed by Syllidae and Serpulidae. Host symbiont interrelations, host specificity, location, infestation characteristics and adaptive modifications of symbionts, as well as host reaction on symbionts presence, have been considered. Our review highlights that (1) every group of cnidarians seems to have their own assemblage of symbiotic polychaetes, (2) some deep-sea alcyonaceans and black corals have never been reported without their often undetermined polynoid symbionts so that its presence has been considered as a species-specific, robust taxonomic character, and (3) we certainly expect the polychaete symbionts associated with deep-sea corals to be a hidden hot-spot of diversity, with many species still waiting to be described.

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Cnidaria • Polychaeta • Symbiosis • Host-symbiont interrelations • Adaptations

25.1 Introduction. State of Knowledge

It is now well known and accepted that many marine organisms can harbor rich associated fauna. Cnidarians, and in particular skeleton-bearing corals, are between the most preferred hosts for such associations (see e.g. Buhl-Mortensen and Mortensen 2004; Stella et al. 2011; Watling et al. 2011). The complex three-dimensional network created by the massive skeleton or the characteristic branching pattern of alcyonaceans and black corals may provide food, substrate and shelter for various types of organisms. According to the comprehensive review on symbiotic polychaetes by Martin and Britayev (1998), cnidarians appeared to be the second dominant group harboring symbiotic polychaetes (20 % of the 569 by then known host species) yielding only to Echinodermata (36 % of host species). Buhl-Mortensen and Mortensen (2004) in the review of symbiosis in deep-sea water corals counted 311 and 112 species of obligate symbiotic invertebrates (all taxa) from shallow-water and deep-sea corals, respectively. Seven years later, the number of symbiotic invertebrates recorded in association with scleractinian corals only increased up to 869 species (Stella et al. 2011). Among them, only 29 species of polychaetes were recognized as facultative and obligatory coral symbionts. Little is still known about the biology of deep-sea symbionts, since many species are known only from the type localities, or based on limited number of historical dredge samples when hosts and symbionts were often separated during preliminary on-deck sorting. On the other hand, it is hard to distinguish the organisms inhabiting the coral framework and using it just for shelter from those living in close association with the host coral (i.e. real symbionts).

The purpose of the present paper is to revise the current data on host-symbiont relationships between cnidarians and their associated polychaetes. Also, we try to define main gaps in the present knowledge on the biology of the polychaete-cnidarian symbiosis.

25.2 Cnidarian Hosts Involved in Coral-Polychaete Associations

To date, 281 species of cnidarian hosts involved in 324 relationships with symbiotic polychaetes have been reported (Table 25.1). Most polychaete-hosting cnidarians belong to skeleton-bearing taxa (Fig. 25.1). Among the anthozoans, most belong to the Scleractinia (125 species or 44.48 % of the total cnidarian hosts), Alcyonacea (73 species or 25.97 %), Hydrozoa (60 species or 21.35 %), and Antipatharia (19 species or 6.76 %). The hosts lacking hard skeleton include only two species of Actiniaria: *Bolocera tuediae* associated with *Alentiana aurantiaca* and *Metridium senile* with *Arctonoe vittata*. One non-identified zoanthid hosted *Lumbrineris flabellicola* and several species of not obligate polychaetes are reported from cerianthid tubes. There are no polychaete symbionts reported in association with Corallimorpharia.

25.2.1 Ceriantharia

Species associated with tube anemones (Ceriantharia) generally inhabit the surface or are embedded into their thick felt-like tubes consisting of discharged ptychocyst threads incrusted with particles of mud and sand. About 30 species of polychaetes were reported from tubes of *Pachycerianthus multiplicatus* Carlgren, 1912 (Kilkerrin Bay, Ireland). However, most reported species were in fact associated with polychaetes and sipunculids inhabiting the cerianthid tube or use the cerianthid tube as elevated substrate for settlement. Apparently, *Myxicola infundibulum* is the only polychaete species that is not reported from the surrounding grounds but from cerianthid tubes (O'Connor et al. 1977) and thus this species is considered in the Table 25.1.

25.2.2 Scleractinia

Scleractinians are apparently the most attractive group of marine cnidarians for symbiotic organisms. Dead skeletons of reef-building scleractinians provide a substrate that is actively eroded and occupied by numerous excavating taxa, including many species of polychaetes (Hutchings 2008; Glynn and Enoch 2011). However, only 29 polychaetes (including borers and cryptic species) out of 869 invertebrates have been recently reported as scleractinian symbionts in coral reefs (Stella et al. 2011), while five and nine species have been reported as obligatory coral symbionts from shallow-water and deep-sea habitats, respectively (Buhl-Mortensen and Mortensen 2004).

Despite the relatively low number of symbiotic polychaete species, they are often reported in association with a wide range of scleractinian hosts from several families (Zibrowius et al. 1975; Martin and Britayev 1998). On coral reefs, symbiotic polychaetes more often inhabit massive, slow-growing species of Acroporidae, Poritidae, Faviidae etc. (Rowley 2008). In deep-sea habitats, polychates have been mostly reported during last decades in association with

Table 25.1 List of symbiotic polychaetes reported in associations with Cnidarian hosts

Polychaete	Cnidarian host		Type	Source
	Taxon	Species		
Chaetopteridae				
Spiochaetopterus sp.	SCL	*Montipora* spp.	1	Bergsma (2009)
Eunicidae				
Aciculomarphysa comes Hartmann-Schröder, 1998	ANT	Unid. antipatharian	1	Hartmann-Schröder and Zibrowius (1998), Molodtsova and Budaeva (2007)
Eunice antipathum (Pourtalès, 1867)	ANT	*Distichopathes filix* (Pourtalès, 1867)	1	Hartmann-Schröder and Zibrowius (1998), Molodtsova and Budaeva (2007)
	ANT	*Elatopathes abietina* (Pourtalès, 1874)	1	Hartmann-Schröder and Zibrowius (1998), Molodtsova and Budaeva (2007)
E. cf *dubiata* Fauchald, 1974	SCL	*Madrepora oculata* Linnaeus, 1758	1	Buhl-Mortensen and Mortensen (2004)
E. kristiani Hartmann-Schröder, 1998	ANT	cf. *Antipathes cylindrica* Brook, 1889	1	Hartmann-Schröder and Zibrowius (1998), Molodtsova and Budaeva (2007)
E. marianae Hartmann-Schröder, 1998	ANT	cf. *A cylindrica*	1	Hartmann-Schröder and Zibrowius (1998), Molodtsova and Budaeva (2007)
	ANT	*Cupressopathes* sp.2	1	Molodtsova and Budaeva (2007)
E. norvergica (Linnaeus, 1767)	SCL	*Lophelia pertusa* (Linnaeus, 1758)	1	Zibrowius (1980), Roberts (2005), Mueller et al. (2013)
	SCL	*M. oculata*	1	Zibrowius (1980), Roberts (2005)
	SCL	*Solenosmilia variabilis* Duncan, 1873	1	Zibrowius (1980), Roberts (2005)
	STY	*Errina atlantica* Hickson, 1912	1	Zibrowius and Cairns (1992)
Eunice pennata (OF Muller, 1776)	SCL	Unid. scleractinian	1	Fauvel (1923)
	SCL	*L. pertusa*	1	Fauchald (1992), Jensen and Frederksen (1992)
	SCL	*Primnoa resedaeformis* (Gunnerus, 1763)	1	Buhl-Mortensen and Mortensen (2005)
Eunice sp.	SCL	*M. oculata*	1	Cairns and Zibrowius (1997)
Eunice sp.	SCL	*M. arbuscula* (Moseley, 1881)	1	Cairns and Zibrowius (1997)
Eunice sp.	SCL	*M. minutiseptum* Cairns, Zibrowius, 1997	1	Cairns and Zibrowius (1997)
Eunice sp.	SCL	*M.* cf. *porcellana* (Moseley, 1881)	1	Cairns and Zibrowius (1997)
Eunice sp.	SCL	*Lobophyllia hemprichii* (Ehrenberg, 1834)	1	Chisholm and Kelley (2001)
Leodice antennata ? (Savigny, 1818)	SCL	Unid. coral	1	Gardiner (1976)
Unid. eunicid	STY	*Stylaster cocosensis* Cairns, 1991	1	Cairns (1991)
Unid. eunicid	STY	*S. marenzelleri* Cairns, 1986	1	Cairns (1986), Cairns (1991)
Unid. eunicid	STY	*S. spatula* Cairns, 1986	1	Cairns (1991)
Hesionidae				
Leocrates chinensis Kinberg, 1866		Corals	1	Pettibone (1970)
L. claparedii (Costa in Claparède, 1868)		Corals	1	Pettibone (1970)
Lumbrineridae				
Lumbrineris flabellicola Fage, 1936	SCL	*Balanophyllia cellulosa* Duncan, 1973	1	Zibrowius et al. (1975), Miura and Shirayama (1992)
	SCL	*Balanophyllia* sp.	1	Zibrowius et al. (1975), Cairns and Zibrowius (1997)
	SCL	*Caryophyllia quadrogenaria* Alcock, 1902	1	Miura and Shirayama (1992)
	SCL	*C. grayi* (Milne Edwards and Haime, 1848)	1	Cairns and Zibrowius (1997)
	SCL	*C. sarsiae* Zibrowius, 1974	1	Zibrowius et al. (1975)
	SCL	*C. smithii* Stokes and Broderip, 1828	1	Zibrowius et al. (1975)

(continued)

Table 25.1 (continued)

Polychaete	Cnidarian host		Type	Source
	Taxon	Species		
	SCL	*C. spinigera* (Saville-Kent, 1871)	1	Miura and Shirayama (1992), Cairns and Zibrowius (1997)
	SCL	*C. spinicarens* (Moseley, 1881)	1	Miura and Shirayama (1992), Cairns and Zibrowius (1997)
	SCL	*Caryophyllia* sp.	1	Zibrowius et al. (1975)
	SCL	*C. transversalis* Moseley, 1881	1	Cairns and Zibrowius (1997)
	SCL	*Ceratotrochus duodecimcostatus* (Goldfuss,1826) †	1	Zibrowius et al. (1975)
	SCL	*Conotrochus brunneus* (Moseley, 1881)	1	Cairns and Zibrowius (1997)
	SCL	*Dendrophyllia cornigera* (Lamarck, 1816)	1	Zibrowius et al. (1975)
	SCL	*Eguchipsammia cornucopia* (Pourtalès, 1871)	1	Zibrowius et al. (1975)
	SCL	Dendrophyllid coral	1	Cairns and Zibrowius (1997)
	SCL	*Desmophyllum dianthus* (Esper, 1794)	1	Zibrowius et al. (1975)
	SCL	*Flabellum avicula* Michelotti †	1	Zibrowius et al. (1975)
	SCL	*F. chunii* Marenzeller, 1904	1	Marenzeller (1904), Fage (1936), Bellan (1959), Zibrowius et al. (1975), Miura and Shirayama (1992)
	SCL	*F. fornasinii* Osasco, 1895†	1	Zibrowius et al. (1975)
	SCL	*Flabellum lamellulosum* Alcock, 1902	1	Cairns and Zibrowius (1997)
	SCL	*F. majus* Milne Edwards and Haime†	1	Zibrowius et al. (1975)
	SCL	*F. patens* Mozeley, 1881	1	Cairns and Zibrowius (1997)
	SCL	*F. pavoninum* Lesson, 1831	1	Zibrowius et al. (1975), Miura and Shirayama (1992)
	SCL	*F.* sp.	1	Zibrowius et al. (1975)
	SCL	*Rhizotrochus typus* Milne Edwards and Haime, 1848	1	Cairns and Zibrowius (1997)
	SCL	*Stephanocyathus moseleyanus* (Sclater, 1886)	1	Zibrowius et al. (1975)
	SCL	*Truncatoflabellum candeanum* (Milne Edwards and Haime, 1848)	1	Zibrowius et al. (1975)
	SCL	*T. inconstans* (Marenzeller, 1904)	1	Zibrowius et al. (1975)
	HYD	Unid. hydroids	1	Zibrowius et al. (1975)
	SCL	Unid. scleractinian	1	Miura and Shirayama (1992)
	SCL	*Stephanocyathus moseleyanus* (Sclater, 1886)	1	Zibrowius et al. (1975)
	ZOA	Unid. zoantharian	1	Zibrowius et al. (1975)
L. coccinea (Renier, 1804)	SCL	Unid. coral	1	Gardiner (1976)
Polynoidae				
Acanthicolepis zibrowii Barnich and Fiege, 2010	SCL	*Madrepora oculata*	1	Barnich and Fiege (2010), Núñez et al. (2011)
Alentiana aurantiaca (Verrill, 1885)	ACT	*Bolocera tuediae* (Johnston, 1832)	1	Pettibone (1963)
Antipathipolyeunoa nuttingi Pettibone, 1991	ANT	*Tanacetipathes tanacetum* (Pourtalès, 1880)	1	Pettibone (1991b), Wagner et al. (2012)
Arctonoe vittata (Grube, 1855)	ACT	*Metridium senile* (Linnaeus, 1761)	1	Britayev (1991)
Australaugeneria michelseni Pettibone, 1969	ALC	*Dendronephthya* sp.	1	Britayev and Antokhina (2012)
	ALC	Unid. alcyonacean	1	Pettibone (1969a)
	ALC	Unid. gorgonian	1	Pettibone (1969a)
Australaugeneria pottsi Pettibone, 1969	ALC	Unid. gorgonian	1	Pettibone (1969a, 1969b)

(continued)

Table 25.1 (continued)

Polychaete	Cnidarian host		Type	Source
	Taxon	Species		
A. rutilans (Grube, 1878)	ALC	*Xenia* sp.	1	Okuda (1950), Pettibone (1969a)
	ALC	*Dendronephthya* sp.	1	Britayev and Antokhina (2012)
Bayerpolynoe floridensis Pettibone, 1991	ANT	*Stylopathes litocrada* Opresko, 2006	1	Pettibone (1991b); Wagner et al. (2012), Britayev et al. (2014), present paper
Benhamipolynoe anthipaticola (Benham, 1927)	ANT	*Asteriopathes arachniformis* Opresko, 2004		Molodtsova and Budaeva (2007)
	ANT	*Cupressopathes cf. gracilis* (Thomson and Simpson, 1905)	1	Molodtsova and Budaeva (2007)
	ANT	*Cupressopathes* sp.	1	Molodtsova and Budaeva (2007)
	ANT	*S. tenuispina* (Silberfeld, 1909)	1	Pettibone (1970, 1989)
	ANT	*S. columnaris* (Duchassaing, 1870)	1	Pettibone (1970, 1989); Wagner et al. (2012)
B. cairnsi Pettibone 1989	STY	*Conopora adeta* Cairns, 1987	1	Pettibone (1989), Hanley and Burke (1991b)
Brychionoe karenae Hanley and Burke, 1991	ANT	*Leiopathes secunda* Opresko, 1998	1	Hanley and Burke (1991a), present paper
Eunoe purpurea Treadwell, 1936	ANT	*Bathypathes* cf. *alternata* Brook, 1889	1	Barnich et al. (2013)
	SCL	*Madrepora oculata*	1	Barnich et al. (2013)
E. spinulosa Verrill, 1879	ALC	*Acanella arbuscula* (Johnson, 1862)	1	Buhl-Mortensen and Mortensen (2004)
Gorgoniapolynoe bayeri Pettibone, 1991	ALC	*Narella clavata* (Versluys, 1906)	1	Pettibone (1991a)
G. caeciliae (Fauvel, 1913)	ALC	*Acanthogorgia aspera* Pourtalès, 1867	1	Pettibone (1991a)
	ALC	*A. armata* Verrill, 1878	1	Barnich et al. (2013)
	ALC	*Candidella imbricata* (Johnson, 1862)	1	Pettibone (1991a), Cairns and Bayer (2004), Eckelbarger et al. (2005), Barnich et al. (2013), Britayev et (2014)
	ALC	*Corallium bayeri* Simpson and Watling, 2011	1	Simpson and Watling (2011), Tu et al. (2015)
	ALC	*Corallium johnsoni* Gray, 1860	1	Fauvel (1913), Hartmann-Schröder (1985), Pettibone (1991a), Tu et al. (2015)
	ALC	*C. niobe* Bayer, 1964	1	Hartmann-Schröder (1985), Pettibone (1991a), Simpson and Watling (2011), Tu et al. (2015)
	ALC	*C. secundum* Dana, 1846	1	Stock (1986)
	ALC	*C. tricolor* (Johnson, 1899)	1	Hartmann-Schröder (1985), Tu et al. (2015)
G. cairnsi Pettibone 1991	STY	*Stylaster eguchii* (Boschma, 1966)	1	Pettibone (1991a)
G. corralophyla (Day, 1960)	STY	*S. bithalamus* Broch, 1936	1	Day (1967)
	STY	*Stylaster* sp.	1	Stock (1986)
	STY	*Conopora* sp.	1	Stock (1986)
	STY	*Crypthelia* sp.	1	Stock (1986)
G. galapagensis Pettibone, 1991	ALC	*Narella ambigua* (Studer, 1894)	1	Pettibone (1991a)
G. guadalupensis Pettibone, 1991	ALC	*Corallium imperiale* Bayer, 1955	1	Britayev (1981), Pettibone (1991a)
G. muzikae Pettibone, 1991	ALC	*Acanthogorgia bocki* Aurivillius, 1931	1	Pettibone (1991a)
	ALC	*Candidella helminthophora* (Nutting, 1908)	1	Pettibone (1991a)
	ALC	*Corallium* sp.	1	Pettibone (1991a)
G. uschacovi Britayev, 1981	STY	*Stylaster* sp. 1	1	Britayev (1981)
	ALC	*Callogorgia* sp.	1	Britayev (1981), Pettibone (1991a)
Gorgoniapolynoe sp.	ALC	*Narella vermifera* Cairns and Bayer 2008	1	Cairns and Bayer (2008)
Gorgoniapolynoe sp.	ALC	*N. macrocalyx* Cairns and Bayer 2008	1	Cairns and Bayer (2008)
Gorgoniapolynoe sp.	ALC	*Narella alata* Cairns and Bayer 2008	1	Cairns and Bayer (2008)
Gorgoniapolynoe sp.	ALC	*Candidella helminthophora* (Nutting, 1908)	1	Nutting (1908), Cairns (2009)

(continued)

Table 25.1 (continued)

Polychaete	Cnidarian host		Type	Source
	Taxon	Species		
Harmothoe dannyi Barnich et al. 2013	STY	*Stylaster erubescens* Pourtalès, 1868	1	Barnich et al. (2013)
H. gilchristi Day, 1960	SCL	*L. pertusa*	1	Miranda and Brasil (2014)
	SCL	*S. variabilis*		Miranda and Brasil (2014)
	SCL	*E. rostrata*		Miranda and Brasil (2014)
	SCL	*M. oculata*		Miranda and Brasil (2014)
	STY	*Errina sp*		Miranda and Brasil (2014)
H. oculinarum (Storm, 1879)	SCL	*L. pertusa*	1	Buhl-Mortensen and Mortensen (2004)
H. melanicornis Britayev, 1981	STY	*Stylaster* sp. 2	1	Britayev (1981)
H. ruthae Miranda and Brasil, 2014	SCL	*L. pertusa*	1	Miranda and Brasil (2014)
	SCL	*Solenismilia variabilis*		Miranda and Brasil (2014)
	SCL	*E. rostrata*		Miranda and Brasil (2014)
	SCL	*M. oculata*		Miranda and Brasil (2014)
	STY	*Errina sp*		Miranda and Brasil (2014)
H. vinogradovae Averincev, 1978	STY	Unid. hydrocoral	1	Britayev (1981)
Hemilepedia versluysi Horst, 1915	ALC	*Thouarella hilgendorfi* (Studer, 1879)	1	Horst (1915), Britayev (1981)
Hololepidella nigropunctata (Horst, 1915)	SCL	*Lobactis scutaria* (Lamarck, 1801)	1	Pettibone (1993)
Hololepidella sp.	SCL	*Galaxea astreata* (Lamarck, 1816)	2	Britayev and Antokhina (2012), Britayev et al. (2015)
Lagisca irritans Marenzeller, 1904	STY	*Errina macrogastra* Marenzeller, 1904	1	Marenzeller (1904), Zibrowius (1981), Cairns 1986
	STY	*Stenohelia concinna* Boschma, 1964	1	Marenzeller (1904), Zibrowius (1981), Cairns (1986)
L. zibrowii Hartmann-Schröder, 1992	HYD	*Pseudosolanderia* sp.	1	Hartmann-Schröder (1992)
Malmgreniella dicirra Hartman, 1967	STY	*Stylaster eguchii*	1	Pettibone (1993)
	STY	*S. densicaulis* (Studer, 1878)	1	Pettibone (1993)
	ALC	*Calyptopora reticulata* Boschma, 1968	1	Pettibone (1993)
	STY	*Conopora verrucosa* (Studer, 1878)	1	Pettibone (1993)
	STY	*Lepidopora* sp.	1	Pettibone (1993)
M. pettiti Pettibone, 1993	ALC	Unid. alcyonacean	1	Pettibone (1993)
Medioantenna variopinta Di Camillo, Martin and Britayev, 2011	HYD	*Solanderia secunda* (Inaba, 1892)	1	Nishi and Tachikawa (1999), Di Camillo et al. (2011)
Neohololepidella anthipathicola Hartmann-Schröder, 1998	ANT	*Elatopathes abietina*	1	Hartmann-Schröder and Zibrowius (1998), Molodtsova and Budaeva (2007)
	ANT	*Distichopathes filix*	1	Hartmann-Schröder and Zibrowius (1998), Molodtsova and Budaeva (2007)
Neopolynoe acanellae (Verrill, 1881)	ALC	*Acanella arbuscula*	1	Verrill (1881), Ditlevsen (1917)
	ALC	*Acanthogorgia armata*	1	Verrill (1881)
	ALC	*Pseudoanthomastus agaricus* (Studer, 1890)	1	Ditlevsen (1917), Molodtsova (2013)
	PEN	*Pennatula grandis* Ehrenberg, 1834	1	Pettibone (1963)
N. antarctica (Kinberg, 1858)	HYD	*Thuiaria* sp.	1	Hartmann-Schröder (1989), Barnich et al. (2012b)

(continued)

Table 25.1 (continued)

Polychaete	Cnidarian host		Type	Source
	Taxon	Species		
N. paradoxa (Anon, 1888)	SCL	*Lophelia pertusa*	1	Jensen and Frederiksen (1992)
Paradyte levis (Marenzeller, 1902)	ALC	*Dendronephthya* sp.	1	Okuda (1950), Imajima (1997), Britayev and Antokhina (2012)
P. tentaculata (Horst, 1915)	ALC	*Dendronephthya* sp.	1	Hanley and Morton (1992)
	ALC	*Nephthea* sp.	1	Pettibone (1969a), Hanley and Morton (1992)
Parahololepidella greeffi (Augener, 1918)	ANT	*Tanacetipathes* cf. *spinescens* (Gray, 1857)	1	Britayev et al. (2014)
Parapolyeunoa flynni (Benham, 1921)	STY	*Errina aspera* (Linnaeus, 1767)	1	Barnich et al. (2012b)
	STY	*Inferiolabiata labiata* (Moseley, 1879)	1	Barnich et al. (2012b)
	ALC	*Thouarella* sp.	1	Barnich et al. (2012b)
Polyeunoa laevis McIntosh, 1885	ALC	*Thouarella variabilis* Wright and Studer, 1889	1	Pettibone (1969c)
	ALC	*Thouarella* sp.	1	Hartmann-Schröder (1989), Barnich et al. (2012b)
	ALC	Acanthogorgiidae gen. sp.	1	Hartmann-Schröder (1989), Barnich et al. (2012b)
	ALC	*Primnosis* sp.	1	Stiller (1996), Barnich et al. (2012a, 2012b)
	ALC	*Dasystenella* sp.	1	Stiller (1996), Barnich et al. (2012a, 2012b)
Showascalisetosus shimizui Imajima, 1997	STY	*Stylaster* sp.	1	Imajima (1997)
Subadyte papillifera (Horst, 1915)	SCL	Unid. coral	1	Pettibone (1969a)
Tottonpolynoe symantipatharia Pettibone, 1991	ANT	*Parantipathes* sp.	1	Pettibone (1991b)
	ALC	*Sclerisis macquariana* Bayer and Stefani, 1987	1	Pettibone (1991b)
Uncopolynoe corallicola Hartmann-Schröder, 1960	ALC	Unid. alcyonacean	1	Hartmann-Schröder (1960), Wehe (2006), Britayev and Antokhina (2012)
Unid. polynoid	STY	*Conopora major* Hickson and England, 1905	1	Cairns and Zibrowius (2013)
Unid. polynoid	STY	*C. tenuiramus* Cairns and Zibrowius, 2013	1	Cairns and Zibrowius (2013)
Unid. polynoid	STY	*C. verrucosa*	1	Cairns and Zibrowius (2013)
Unid. polynoid	ALC	*Corallium sulcatum* Kishinouye, 1903		Tu et al. (2012)
Unid. polynoid	ALC	*Minuisis pseudoplanum* Alderslade, 1998	1	Alderslade (1998), Watling et al. (2011)
Unid. polynoid	ALC	*Minuisis granti* Alderslade, 1998	1	Alderslade (1998), Watling et al. (2011)
Unid. polynoid	ALC	*Narella hypsocalyx* Cairns, 2012	1	Cairns (2012)
Unid. polynoid	ALC	*N. vulgaris* Cairns, 2012	1	Cairns (2012)
Unid. polynoid	ALC	*N. mosaica* Cairns, 2012	1	Cairns (2012)
Unid. polynoid	ALC	*N. dampieri* Cairns, 2012	1	Cairns (2012)
Unid. polynoid	ANT	*Hexapathes hivaensis* Molodtsova, 2006	1	Molodtsova (2006)
Unid. polynoid	STY	*Stylaster amphiheloides* Kent, 1871	1	Cairns (1991), Cairns and Zibrowius (2013)
Unid. polynoid	STY	*S. bithalamus*	1	Cairns and Zibrowius (2013)
Unid. polynoid	ALC	*Thouarella affinis* Wright and Studer, 1889	1	Taylor et al. (2013)
Unid. polynoid	ALC	*T. bipinnata* Cairns, 2006	1	Cairns (2006)
Unid. polynoid	ALC	*T. laxa* Versluys 1906	1	Versluys (1906), Watling et al. (2011)
Unid. polynoid	ALC	*T. cristata* Cairns, 2011	1	Cairns (2011)

(continued)

Table 25.1 (continued)

Polychaete	Cnidarian host		Type	Source
	Taxon	Species		
Unid. polynoid	ALC	*Tokoprymno anais* Zapata-Guardiola and López-González, 2010	1	Zapata-Guardiola and López-González (2010)
Sabellidae				
Myxicola infundibulum (Linnaeus, 1767)	CER	*Pachycerianthus multiplicatus* Carlgren, 1912	1	O'Connor et al. (1977)
SERPULIDAE				
Floriprotis sabiuraensis Uchida, 1978	SCL	*Favites abdita* (Ellis and Solander, 1786)	1	Uchida (1978)
	SCL	*Dipsastraea speciosa* (Dana, 1846)	1	Bailey-Brock (1985)
	SCL	*Goniastrea pectinata* (Ehrenberg, 1834)	1	Bailey-Brock (1985)
	SCL	*Hydnophora* sp.	1	ten Hove (1989)
	SCL	*Platygyra* sp.	1	ten Hove (1989)
Propomatoceros sulcicarinata Ware, 1974†	SCL	*Glomerula lombricus* (Defrance, 1827)†	1	Garberoglio and Lazo (2011)
	SCL	*Mucroserpula mucroserpula* Regenhardt, 1961†	1	Garberoglio and Lazo (2011)
Pseudovermilia madracicola ten Hove, 1989	SCL	*Madracis pharensis* (Heller, 1868)	1	ten Hove (1989)
	SCL	*M. decactis* (Lyman, 1859)	1	ten Hove (1989)
Serpula sp.	SCL	*Echinopora gemmacea* (Lamarck, 1816)	1	ten Hove and Land (1994)
Spirobranchus corniculatus (Grube,1862)	SCL	*Acropora* sp.	1	ten Hove and Land (1994)
	SCL	*Acropora digitifera* (Dana, 1846)	1	Rowley (2008)
	SCL	*A. humilis* (Dana, 1846)	1	Rowley (2008)
	SCL	*A. hyacinthus* (Dana, 1846)	1	Rowley (2008)
	SCL	*A. loripes* Brook, 1892	1	Rowley (2008)
	SCL	*Astreopora cucullata* Lamberts, 1980	1	Rowley (2008)
	SCL	*Dipsastraea amicorum* (Milne Edwards and Haime, 1849)	1	Rowley (2008)
	SCL	*Coeloseris mayeri* Vaughan, 1918	1	Rowley (2008)
	SCL	*Cyphastrea chalcidicum* (Forskål, 1775)	1	Rowley (2008)
	SCL	*Cyphastrea microphthalma* (Lamarck, 1816)	1	Rowley (2008)
	SCL	*C. serailia* (Forskål, 1775)	1	Rowley (2008)
	SCL	*Dipsastraea favus* (Forskål, 1775)	3	Ben-Tzvi et al. (2006)
	SCL	*D. lax* (Klunzinger, 1879)	3	Ben-Tzvi et al. (2006)
	SCL	*D. pallida* (Dana, 1846)	1	Rowley (2008)
	SCL	*D. speciosa*	1	Rowley (2008)
	SCL	*Favites abdita*	1	Rowley (2008)
	SCL	*F. pentagona* (Esper, 1795)	1	Rowley (2008)
	SCL	*Galaxea astreata*	1	Rowley (2008)
	SCL	*Gardineroseris planulata*	1	ten Hove and Land (1994)
	SCL	*Hydnophora microconos* (Lamarck, 1816)	1	ten Hove and Land (1994)
	SCL	*Merulina ampliata* (Ellis and Solander, 1786)	1	Rowley (2008)
	MIL	*Millepora platyphylla* Hemprich and Ehrenberg, 1834	1	Rowley (2008)
	MIL	*M. tenera* Boschma, 1949	1	Rowley (2008)
	MIL	*Millepora* sp.	1	ten Hove and Land (1994)
	SCL	*Montipora aequituberculata* Bernard, 1897	1	Rowley (2008)
	SCL	*M. foliosa* (Pallas, 1766)	1	Rowley (2008)
	SCL	*M. foveolata* (Dana, 1846)	1	Rowley (2008)
	SCL	*M. grisea* Bernard, 1897	1	Rowley (2008)

(continued)

Table 25.1 (continued)

Polychaete	Cnidarian host		Type	Source
	Taxon	Species		
	SCL	*M. informis* Bernard, 1897	1	Rowley (2008)
	SCL	*M. spongodes* Bernard, 1897	1	Rowley (2008)
	SCL	*M. spumosa* (Lamarck, 1816)	1	Rowley (2008)
	SCL	*M. tuberculosa* (Lamarck, 1816)	1	Rowley (2008)
	SCL	*M. undata* Bernard, 1897	1	Rowley (2008)
	SCL	*M. venosa* (Ehrenberg, 1834)	1	Rowley (2008)
	SCL	*M. verrucosa* (Lamarck, 1816)	1	Rowley (2008)
	SCL	*Montipora* sp.	1	ten Hove and Land (1994)
	SCL	*Mycedium elephantotus* (Pallas, 1766)	1	Rowley (2008)
	SCL	*Pavona maldivensis* (Gardiner, 1905)	1	ten Hove and Land (1994)
	SCL	*Porites annae* Crossland, 1952	1	Rowley (2008)
	SCL	*P. lichen* Dana, 1846	1	Rowley (2008)
	SCL	*P. lobata* Dana, 1846	1	Rowley (2008)
	SCL	*P. lutea*	1	ten Hove and Land (1994), Rowley (2008)
	SCL	*P. nigrescens* Dana, 1848	1	Rowley (2008)
	SCL	*P. porites* (Pallas, 1766)	1	DeVantier et al. (1986)
	SCL	*P. rus* (Forskål, 1775)	1	Rowley (2008)
	SCL	*Porites* sp.	3	DeVantier et al. (1986)
	SCL	*Porites* sp.	1	Bailey-Brock (1985), Hunte et al. (1990)
	SCL	*Siderastrea siderea* (Ellis & Solander, 1768)	1	Hunte et al. (1990)
	SCL	*Seriatopora hystrix* Dana, 1946	1	Rowley (2008)
	SCL	*Stylocoeniella armata* (Ehrenberg, 1834)	1	Rowley (2008)
	SCL	*Stylophora pistillata* Esper, 1797	1	Rowley (2008)
	SCL	*Stylophora* sp.	1	ten Hove and Land (1994)
S. gardineri Pixell, 1913	SCL	*Gardineroseris planulata* (Dana, 1846)	1	ten Hove and Land (1994)
	SCL	*Porites lutea* Quoy and Gaimard, 1833	1	ten Hove and Land (1994)
	SCL	*Pavona varians* Verrill, 1864	1	ten Hove and Land (1994)
S. giganteus (Pallas, 1766)	SCL	*Agaricia* spp.	1	Hunte et al. (1990)
	SCL	*Diploria labyrinthiformes* (Linnaeus, 1758)	1	Hunte et al. (1990)
	SCL	*Pseudodiploria strigosa* (Dana, 1846)	1	Hunte et al. (1990)
	SCL	*Madracis* spp.	1	Hunte et al. (1990)
	MIL	*Millepora alcicornis* Linnaeus, 1758	1	Pallas (1766) in ten Hove (1989)
	MIL	*M. complanata* Lamarck, 1816	1	Hunte et al. (1990), Marsden (1992)
	SCL	*Orbicella annularis* (Ellis and Solander, 1786)	1	Hunte et al. (1990)
	SCL	*Montastraea cavernosa* (Linnaeus, 1767)	1	Hunte et al. (1990)
	SCL	*Porites astreoides* Lamarck, 1816	1	Hunte et al. (1990)
S. cf. nigranucha (Fischli, 1903)	SCL	*Porites* sp.	1	ten Hove (1989)
S. polycerus (Schmarda, 1861)	MIL	*Millepora complanata*	1	Marsden (1992)
	SCL	*Porites asteroides*	1	Marsden (1992)
S. richardsmithi Pillai, 1990	SCL	*Podabacia motuporensis* Veron, 1990	1	Hoeksema and ten Hove (2014)
S. tetraceros (Schmarda, 1861)	MIL	*Millepora exaesa* Forskål, 1775	1	ten Hove (1970), ten Hove and Land (1994)
	SCL	*Pavona maldivensis*	1	ten Hove (1970), ten Hove and Land (1994)
Sphaerodoridae				
Sphaerodoridium guilbaulti Rullier, 1974	ALC	*Paragorgia arborea* (Linnaeus, 1758)	1	Rullier (1974)
Spintheridae				
Spinther arcticus (M. Sars, 1851)	HYD	Unid. hydroids	1	George and Hartmann-Schröeder (1985)

(continued)

Table 25.1 (continued)

Polychaete	Cnidarian host		Type	Source
	Taxon	Species		
Spionidae				
Dipolydora armata (Langerhans, 1880)	SCL	*Leptastrea purpurea* (Dana, 1846)	3	Okuda (1937)
	MIL	*Millepora complanata*	2	Lewis (1998)
Polydora alloporis Light, 1970	STY	*Stylaster californicus* (Verrill, 1866)	2	Light (1970a), Lindner et al. (2004)
	STY	*Stylantheca papillosa* (Dall, 1884)	2	Cairns and Lindner (2011)
P. cf. alloporis Light, 1970	STY	*Distichopora robusta* Lindner, Cairns and Guzman, 2004	2	Lindner et al. (2004)
P. villosa Radashevsky and Hsieh, 2000	SCL	*Montipora angulata* (Lamarck, 1816)	2	Liu and Hsieh (2000)
	SCL	*Montipora hispida* Dana, 1846	2	Liu and Hsieh (2000)
	SCL	*M. informis* Bernard, 1897	2	Liu and Hsieh (2000)
	SCL	*Porites lichen*	2	Liu and Hsieh (2000)
	SCL	*P. lobata*	2	Liu and Hsieh (2000)
	SCL	*P. lutea*	2	Liu and Hsieh (2000)
	SCL	*Hydnophora exesa* (Pallas, 1766)	2	Radashevsky and Hsieh (2000)
	SCL	*Cyphastrea chalcidicum*	2	Radashevsky and Hsieh (2000)
P. wobberi Light, 1970	ALC	*Lophogorgia* sp.	3	Light (1970b)
Polydora sp.	STY	*Stylaster brochi* (Fisher, 1938)	2	Cairns and Lindner (2011)
Unid. spionid	STY	*S. parageus parageus* (Fisher, 1938)	2	Cairns and Lindner (2011)
Unid. spionid	STY	*S. repandus* Cairns and Lindner 2011	2	Cairns and Lindner (2011)
Unid. spionid	STY	*S. stejnegeri* (Fisher, 1938)	2	Cairns and Lindner (2011)
Unid. spionid	STY	*S. trachystomus* (Fisher, 1938)	2	Cairns and Lindner (2011)
Unid. spionid	STY	*S. venustus* (Verrill, 1870)	2	Cairns and Lindner (2011)
Unid. spionid	STY	*S. verrillii* (Dall, 1884)	2	Cairns and Lindner (2011)
Syllidae				
Alcyonosyllis aidae Álvarez-Campos, San Martín and Aguado, 2013	ALC	*Dendronephtya* sp.	1	Álvarez-Campos et al. (2013)
A. bisetosa (Hartmann-Schröder, 1960)	ALC	Unid. alcyonacean	1	Hartmann-Schröder (1960), Glasby and Aguado (2009)
A. exiliformis (Imajima, 2003)	ALC	*Verrucella* sp.	1	Imajima (2003)
A. glasbyi San Martín and Nishi. 2003	ALC	*Melithaea flabellifera* (Kükenthal, 1908)	1	San Martín and Nishi (2003), Kumagai and Aoki (2003)
A. gorgoniacolo (Sun and Yang, 2004)	ALC	Orange-red gorgonia	1	Sun and Yang (2004), Lattig and Martin (2009), Glasby and Aguado (2009)
A. hinterkircheri Glasby and Aguado, 2009	SCL	*Goniopora* cf. *stokesi* Milne Edwards and Haime, 1851	1	Glasby and Aguado (2009)
A. phili Glasby and Watson, 2001	ALC	*Dendronephthya* sp.	1	Glasby and Watson (2001)
	ALC	*Melithaea* sp.1	1	Glasby and Watson (2001)
	ALC	*Melithaea* sp.2	1	Glasby and Watson (2001)
	ALC	Nephthyidae gen. sp.	1	Britayev and Antokhina (2012)
	ALC	*Carijoa* sp.	1	Britayev and Antokhina (2012)
A. xeniacola (Hartmann-Schröder, 1993)	ALC	*Xenia viridis* Schenk, 1896	1	Hartmann-Schröder (1993), Glasby and Aguado (2009)
Bollandiella antipathicola (Glasby, 1994)	ANT	*Antipathes* sp.	1	Glasby (1994), Molodtsova and Budaeva (2007), Glasby and Krell (2009)

(continued)

Table 25.1 (continued)

Polychaete	Cnidarian host		Type	Source
	Taxon	Species		
Brania pusilla (Dujardin, 1851)	SCL	Unid. coral	1	Gardiner (1976)
Epigamia alexandri (Malmgren, 1867)	HYD	*Abietinaria turgida* (Clark, 1877)	2	Britayev and San Martín (2001)
	HYD	*Orthopyxis integra* (MacGillivray, 1842)	2	TA Britayev personal observations
Haplosyllides sp.	SCL	Unid. coral	1	Hartman (1954), Rullier and Amoureux (1979)
Haplosyllis anthogorgicola Utinomi, 1956	ALC	*Anthogorgia bocki* Aurivillius, 1931	1	Utinomi (1956), Imajima and Hartman (1964), Martin et al. (2002), Lattig and Martin (2009)
H. chamaeleon Laubier, 1960	ALC	*Paramuricea clavata* (Risso, 1826)	1	Laubier (1960), López et al. (1996), Martin et al. (2002)
	ALC	*P. grayi* (Johnson, 1861)	1	Lattig and Martin (2009)
H. villogorgicola Martin, Núñez, Riera and Gil, 2002	ALC	*Villogorgia bebrycoides* (Koch, 1887)	1	Martin et al. (2002)
Imajimea draculai (San Martín and López, 2002)	PEN	*Funiculina quadrangularis* (Pallas, 1766)	2	Nygren and Pleijel (2010)
Pachyprocerastea hydrozoicola (Hartmann-Schröder, 1992)	HYD	*Pseudosolanderia* sp.	2	Hartmann-Schröder (1992)
Proceraea cornuta Agassiz, 1862	HYD	Unid. hydroid	1	Pettibone (1963)
	SCL	Unid. coral	1	Gardiner (1976)
P. fasciata (Bosc, 1802)	HYD	*Eudendrium carneum* Clarke, 1882	2	Cruz-Rivera personal communication
Procerastea halleziana Malaquin, 1893	HYD	*Syncoryne eximia* (Allman, 1859)	2	Allen (1915,1921), Spooner et al. (1957), Alós (1989)
	HYD	*Tubularia indivisa* Linnaeus, 1758	2	Caullery (1925), Spooner et al. (1957)
	HYD	*Ectopleura crocea* (Agassiz, 1862)	2	Genzano and San Martín (2002)
P. simpliseta Hartmann-Schröder, 1990	HYD	*Pseudosolanderia* sp.	2	Hartmann-Schröder (1992)
Syllis gracilis Grube, 1840	ALC	*Elisella paraplexauroides* Stiasny, 1936	1	López et al. (1996)
	HYD	Unid. hydroid	1	López et al. (1996)
	ALC	*Paramuricea clavata*	1	Alós (1988), López et al. (1996)
Syllis onkylochaeta Hartmann-Schröder 1991	ALC	*Xenia* sp.	1	Hartmann-Schröder (1991)
Typosyllis sp.	SCL	*Cyphastrea microphthalma* (Lamarck, 1816)	1	Randall and Eldredge (1976)
	SCL	*Dipsastraea favus*	1	Randall and Eldredge (1976)
	SCL	*Dipsastraea speciosa* (Dana, 1846)	1	Randall and Eldredge (1976)
	SCL	*Favia* sp.	1	Randall and Eldredge (1976)
	SCL	*Favites pentagona*	1	Randall and Eldredge (1976)
	SCL	*Favites valenciennesi* (Milne Edwards and Haime, 1849)	1	Randall and Eldredge (1976)
	SCL	*Favites* sp.	1	Randall and Eldredge (1976)
	SCL	*Symphyllia recta* (Dana, 1846)	1	Randall and Eldredge (1976)
Unid. polychaete	ALC	*Sphaerasclera flammicerebra* (Williams, 2003)	1	Williams (2003)
Unid. polychaete	STY	*Inferiolabiata africana* Cairns and Zibrowius, 2013	1	Cairns and Zibrowius (2013)
Unid. polychaete	STY	*Errinopsis fenestrata* Cairns, 1983	1	Cairns and Zibrowius 2013

† – fossil record

In table: *ACT* Actiniaria, *ALC* Alcyonacea, *ANT* Antipatharia, *CER* Ceriantharia, *HYD* Hydrozoa without calcified skeleton, *MIL* Milleporidae, *PEN* Pennatulacea, *SCL* Scleractinia, *STY* Stylasteridae, *ZOA* Zoanthacea, association, 1 -commensalism, 2 – parasitism, 3 – mutualism

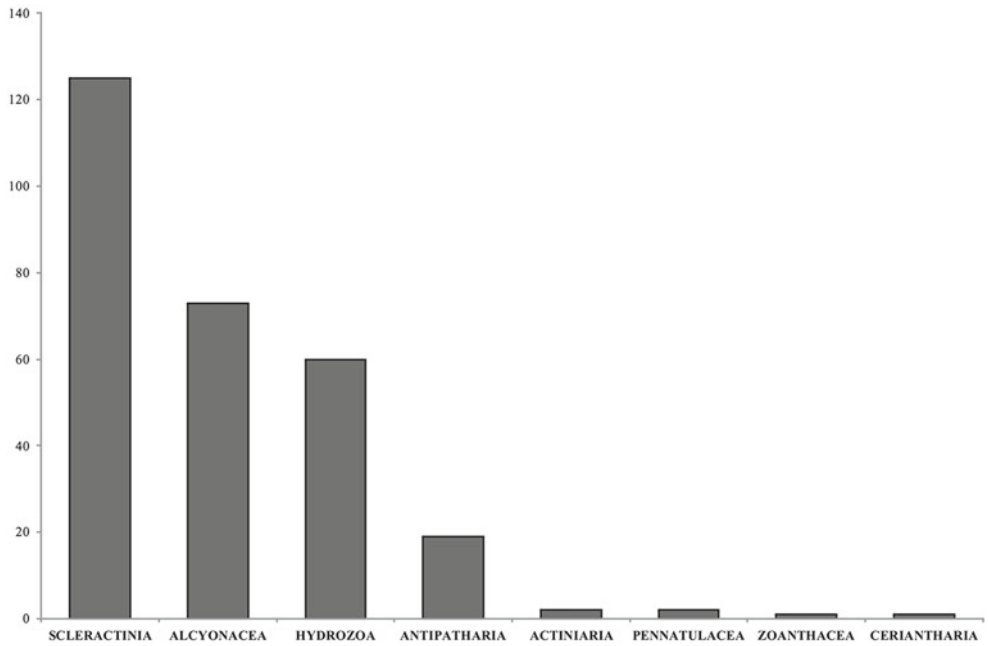

Fig. 25.1 Number of species in each cnidarian taxa hosting symbiotic polychaetes

intensively studied frame-building corals such as *Lophelia pertusa* (Fig. 25.2b), *Solenosmilia variabilis* and *Madrepora oculata* (Buhl-Mortensen and Mortensen 2004). However, solitary cup-corals (Fig. 25.2a) may be also involved in symbiotic associations (Zibrowius et al. 1975).

25.2.3 Antipatharia

Nineteen species of black corals hosting 14 species of symbiotic polychaetes are known to date (Martin and Britayev 1998; Opresko 2006; Molodtsova and Budaeva 2007; Wagner et al. 2012; Britayev et al. 2014), but the number of hosts is clearly underestimated. The genera of black corals infested by polychaetes (i.e., *Cupressopathes* Opresko, 2001, *Tanacetipathes* Opresko, 2001 (Fig. 25.3d), *Stylopathes* Opresko, 2006 (Fig. 25.3c), *Asteriopathes* Opresko, 2004, *Antipathella* Brook, 1889) often have bottle-brush colonies that seem to be more favorable for polychaete symbionts. However, the coral growth form may also be somehow influenced by the symbiont presence (Molodtsova and Budaeva 2007). Some species of black corals, like *Tanacetipathes spinescens* (Britayev et al. 2014), often host polychaete symbionts, while in all species of *Stylopathes* Opresko, 2006 (Stylopathidae) a symbiotic polynoid was always present living on the main stem of the monopodial colony.

25.2.4 Octocorallia

Among the 73 octocorals harboring symbiotic polychaetes, only two species of Pennatulacea (Pettibone 1963; Nygren and Pleijel 2010) and one Helioporacea (Martin et al. 2009) were reported. The remaining 70 hosts belong to the Alcyonacea. The shallow water octocorals hosting polychaetes (mainly Nephtheidae, Xeniidae and Melithaeidae) are relatively scarce and, when reported, they are rarely determined to species level. In turn, the alcyonaceans are the most important deep-sea cnidarians harboring polychaetes (Buhl-Mortensen and Mortensen 2004; Watling et al. 2011), particularly Primnoidae (24 species, mostly belonging to *Narella* Gray, 1870, *Candidella* Bayer, 1954 (Fig. 25.3a) and *Thouarella,* Gray 1870), Coralliidae (8 species), Nephthyidae (8 species), Isidiidae (6 species), Acanthogorgiidae (4 species) and Plexauriidae (3 species). Among the species of *Narella*, for instance, the presence of a symbiotic polychaete is considered as specific at the species level (Cairns 2012).

25.2.5 Hydrozoa

More than two thirds of the hydroids reported in association with polychaetes possess massive calcified skeleton and belong to the families Stylasteridae and Milleporidae.

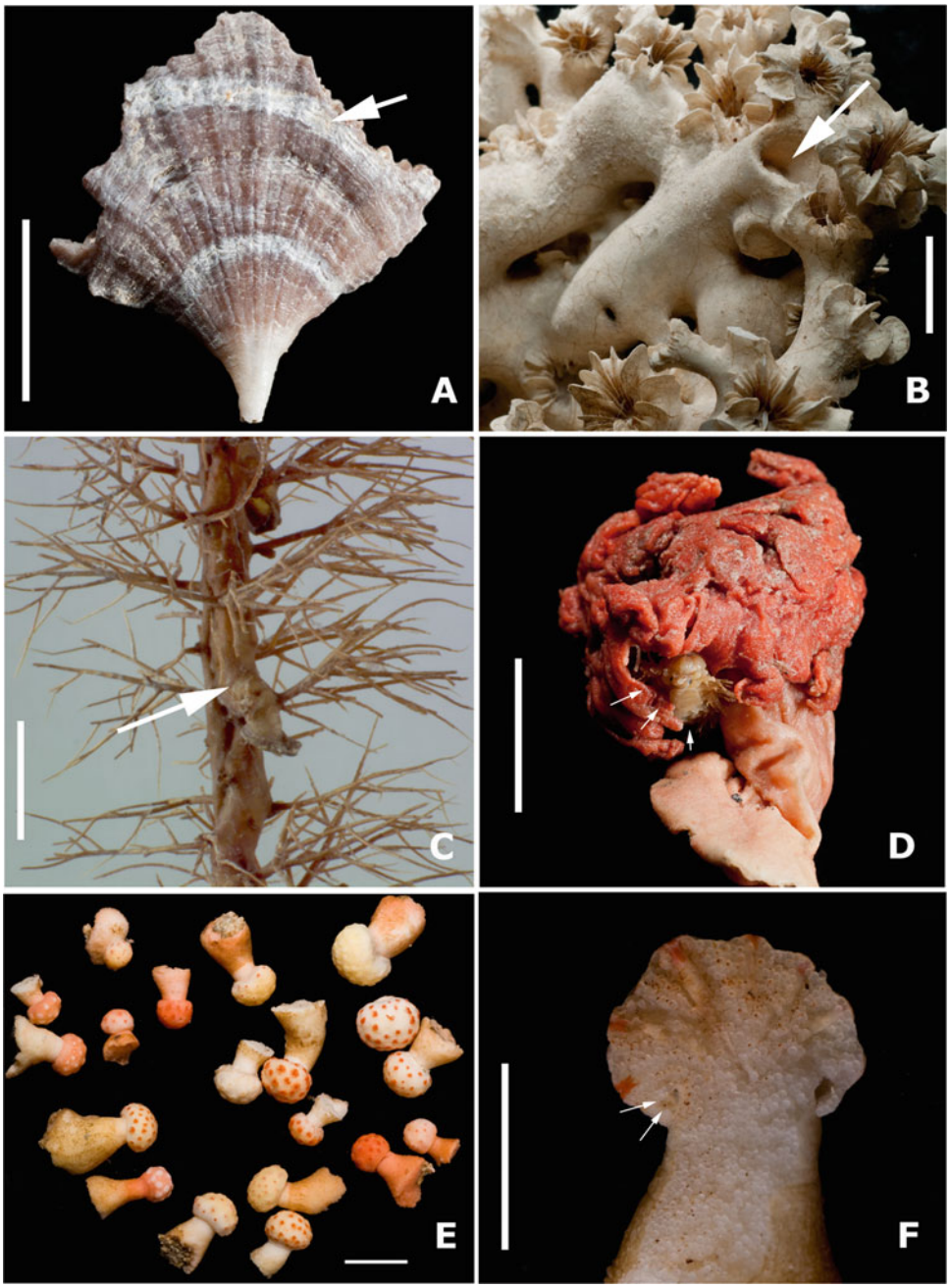

Fig. 25.2 Morphological modifications of cnidarian hosts. (**a**) *Flabellum chunii* with scars (*arrowhead*) from *Lumbriconeris flabellicola*; (**b**) tube of *Eunice* sp. (*arrowhead*) overgrown by *Lophelia pertusa*; (**c**) syntype of *Antipathes cylindrica* with eunicid tube (*arrowhead*) overgrown by coral tissue (Photo courtesy P. Lozouet); (d) *Pseudoanthomastus agaricus* hosting *Neopolynoe acanellae* (*small arrowheads*); (**e**) mushroom-like colonies of *Sphaerasclera flammicerebra*, (**d**) cross section of one specimen of *S. flammicerebra* showing a groove with symbiotic polychaete (*small arrowheads*). Scale: (**a**–**c**, **e**) 10 mm, (**d**, **f**) – 5 mm

Stylasterids or 'lace corals' (Anthoathecata: Filifera), are known from all oceans (i.e., from the Arctic circle to the Antarctica and from 0 to 2,789 m depth) but are more common from 200 to 400 m depth (Cairns 2011). Nearly 40 species of Stylasteridae have been reported to harbor polychaete symbionts (mainly the genera *Conopora* Moseley, 1879, *Errina* Gray, 1835 and *Stylaster* Gray, 1831). Particularly, *Conopora adeta* is known to live exclusively in association with the symbiotic polynoid *Benhamipolynoe cairnsi* (Cairns 1987).

The species of Milleporidae (Anthoathecata: Capitata) inhabit exclusively tropical shallow waters, being among the most conspicuous skeleton-forming coral reef organisms (Lewis 2006). The Milleporidae comprises only one genus, *Millepora* Linnaeus, 1758, with 15 valid species, 6 of them being reported as hosts of symbiotic polychaetes (Serpulidae and Spionidae).

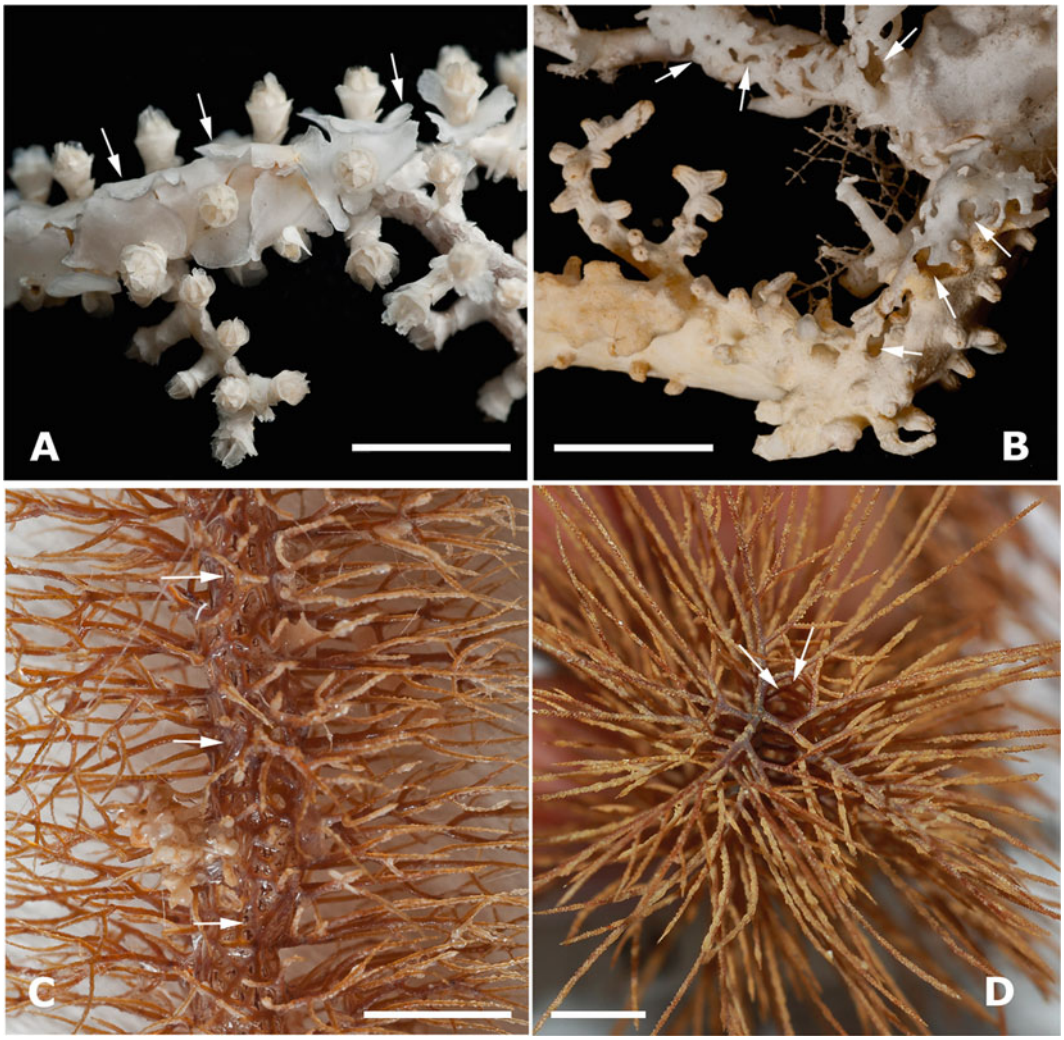

Fig. 25.3 Morphological modifications of cnidarian hosts. (**a**) *Candidella imbricata* with enlarged basal scales forming a tunnel (*arrowheads*) harboring *Gorgoniapolynoe caeciliae*; (**b**) tunnels (*arrowheads*) in branches of *Corallium* cf. *niobe* induced by *G. caeciliae*; (**c**) worm-run (*arrowheads*) in colony of *Stylopathes* sp. formed by densely anastomosed pinnules; (**d**) worm-run (*arrowheads*) along the stem of *Tanacetipathes* cf. *spinescens*. Scale: (**a**, **c**–**d**) – 5 mm, (**b**) – 10 mm

25.3 Polychaetes Involved in Associations with Cnidarians

About 120 species of symbiotic polychaetes of 10 families are reported from cnidarian hosts (Table 25.1, Fig. 25.4). It is hard to approximate the exact number of species as many host descriptions (e.g. Williams 2003; Cairns and Bayer 2004; Opresko 2006; Cairns 2012) report on symbionts that are not determined, even to the genus level. Apparently, the Polynoidae include the highest number of cnidarian-associated polychaetes: almost one half of the currently known species. They are followed by Syllidae, Serpulidae, Eunicidae and Spionidae. Spionids are generally considered as parasitic, however there are some indications that at least some cnidarian hosts can benefit from presence of these particular symbionts (see the next section). The families Hesionidae, Lumbrineridae, Spintheridae, Spherodoridae and Chaetopteridae comprise each only one or two species reported as symbiotic with cnidarians. The species of Sabellidae are known exclusively from cerianthid tubes (Table 25.1) and it is not clear if they can be considered as symbiotic.

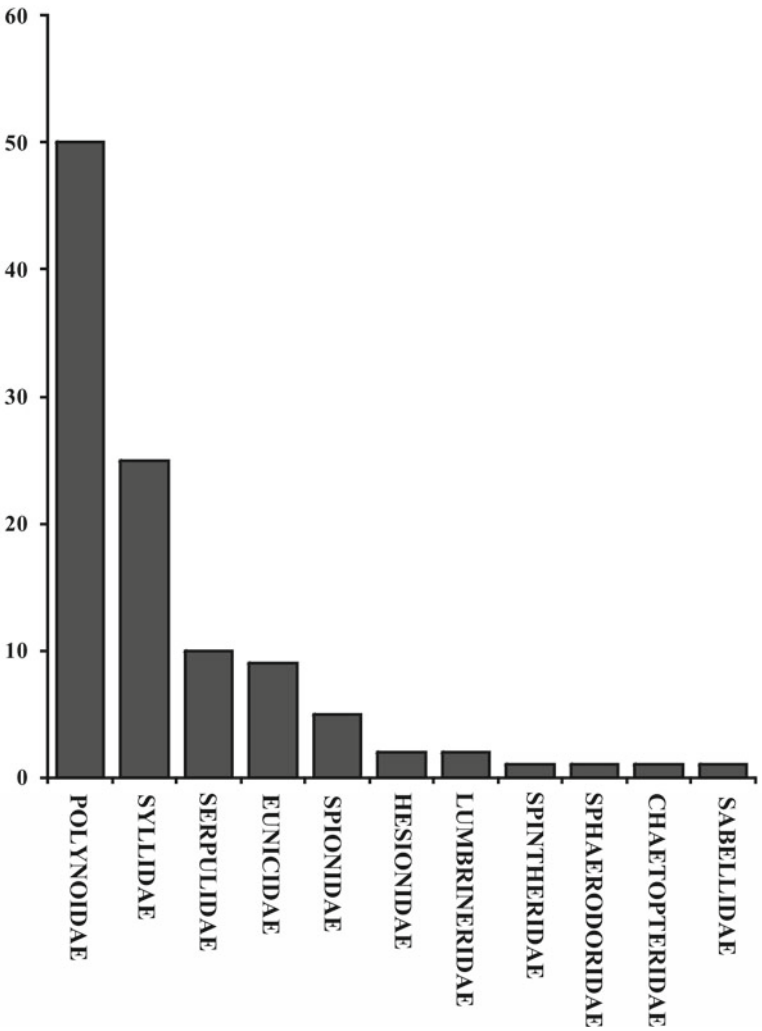

Fig. 25.4 Number of species in each polychaete family associated with cnidarian hosts

25.4 Host-Symbiont Interrelations

Symbiosis sensu De Bary (1879) is currently considered as a general term including close long-term associations between organisms of different species (Margolis et al. 1982), which are subsequently characterised according to the cost/benefit for partners (host:symbiont): commensalism (+:0), parasitism (+:−), and mutualism (+:+). More than 90 % of the relationships between coral hosts and symbiotic polychaetes are commensalisms (Table 25.1). However, the low level of knowledge and scattered available information on the biology of symbionts may artificially exaggerate their relevance (Martin and Britayev 1998). It is evident that coral colonies provide safety shelter for symbiotic polychaetes hidden among their three-dimensional network of branches, inside the skeleton, on the surface of coral branches beneath sclerites, or in tubular galls induced by the polychaetes themselves. So, if there are no clear evidences on negative (parasitism) or positive (mutualism) feedbacks for the host, the association is considered as commensalism.

The number of parasitic associates is significantly lower, while mutualists are virtually negligible (7.3 % and 1.8 % respectively). Among symbionts, some species from different families (i.e., spionids, syllids, polynoids) affect host growth (see section below). For instance, readdressing their energy resources to repair the damages induced by polychaetes instead to somatic growth and reproduction indicates a clear negative effect of the symbionts on their hosts. Moreover, there are indications on polychaetes feeding by stealing host food (Buhl-Mortensen 2001; Mueller et al. 2013) or by consuming coral mucus and tissues (Britayev and San Martín 2001; Britayev et al. 2014). At the same time symbionts may clean coral hosts from detritus, bacteria, fungi and algae, thus increasing their competitiveness, as

well as protect them from predators attacks (e.g. Stewart et al. 2006; Bergsma 2009). However, the net outcome of these different processes on the metabolism and survivorship of corals is unknown. Accordingly, the status of some of these associations may need further re-evaluation depending on the appearing of new information on host symbionts relationships.

Nevertheless, there are some well studied associations between polychaetes and coral hosts, which are briefly summarized in the next sections.

25.4.1 Polynoidae

Polynoids are the most diverse group of polychaetes associated with gorgonian and antipatharian corals, but only one species has been well documented (Table 25.1). The unique species whose relationships with a host coral was studied is the scleractinian associate *Hololepidella* sp. (Britayev et al. 2015). Most studied specimens of *Hololepidella* sp. had up to one third of the gut length filled with mucus containing unicellular algae and cnidocysts, and a few of them also had copepod fragments. The algae were very similar in size, shape and color to the zooxantella living in host tissues, indicating that *Hololepidella* sp. was trophically related to the host coral and, thus, the species can be considered as parasite. In turn, the copepods closely resembled those obligatory associated with the scleractinian corals of the genus *Galaxea* Oken, 1815. The feeding of *Hololepidella* sp. on the parasitic siphonostomatoid copepod evidence that one symbiont may control the density of another one living in the same host. The high prevalence, specific location on the host, and feeding strategy clearly suggest that *Hololepidella* sp. is a specialized scleractinian symbiont, closer to a parasite. In turn, other scleractinians associates (i.e. crabs and shrimps) are known as mutualistic due to their cleaning or guarding activity (Stewart et al. 2006). However, like *Hololepidella* sp., they also feed on coral mucus and tissues. Therefore, further clarification of the ecological role (parasitism *vs.* mutualism) of *Hololepidella* sp. will require detailed studies including more field observation and experimental approaches.

25.4.2 Serpulidae

The single well-documented example of coral–polychaete relationship among serpulids is that of the filter-feeding species of *Spirobranchus*. In this case, the association is considered as mutualism, since the current created by the branchial crown of *Spirobranchus* spp. draws water up from the coral surface (Strathmann et al. 1984), enhancing the arrival rate of food particles to the coral polyps, improving the water circulation close to coral surface and, consequently, decreasing the susceptibility of the host corals to bleaching (Hunte et al. 1990; Nakamura et al. 2003). An additional advantage for the coral is that the worms may defense the host from the attaks of the carnivorous starfish *Acanthaster planci*. When contacted by the starfish, the worms hosted by *Porites* spp. immediately retract and reappear, pushing against the tube feet and arms of the starfish with the ornamented operculum and the branchial crown, forcing the predator to move away (DeVantier et al. 1986).

25.4.3 Spionidae

Data on the impact of spionids on their respective coral hosts are rare and controversial. In fact, polydorid worms may affect their hosts by weakening their branches and drawing energy to repair the skeletal tissue damaged by polychaete boring activity. For example, the burrow openings of *Dipolydora armata* on the surface of *Millepora complanata* develop distinctive, erect spines caused by the combined growth of worm tubes and host tissue. The zooids of *Millepora* were absent in the vicinity of tube openings and on spines and, thus, the potential feeding surface of the coral is reduced in heavily colonized branches. Burrows and openings were densest at the bases of the branches of *Millepora* where the skeleton weakening may easily occur (Lewis 1998).

In contrast, indirect evidences prove that the presence of spionids may enhance tissue growth/calcification rate in *Astreopora myriophthalma* (Wielgus and Levi 2006). In fact, the capture of particulate organic matter from the water column and the adjacent substrate and the production of nitrogen enriched metabolic waste products may affect primary production in coral reefs by influencing the physiology of the coral/zooxanthella association.

Another spionid, *Polydora villosa*, often inhabits the branched morph of *Montipora* spp., while is rare in encrusting or columnar morphs. Accordingly, this has been considered as an indirect evidence on the symbiont-mediated modification of *Montipora* spp. from an encrusting or columnar morph to a branched one (Liu and Hsieh 2000). Interestingly, similar morphological changes induced by a symbiotic amphipod apparently enhanced the resistence of *Montipora* spp. to predation by pincushion (*Culcita novaeguineae*) and crown-of-thorns (*Acanthaster planci*) sea stars. The fingers of the branched colonies of *Montipora* spp. were both less susceptible to be attacked and more likely able to survive to an attack than the colonies without fingers. Furthermore, the presence of fingers altered the preferences of *A. planci* prey, as the sea star preferred *Montipora* spp. without fingers over other common corals, but preferred these other corals when the specimens of *Montipora* spp. had fingers (Bergsma 2012).

25.4.4 Chaetopteridae

Finger-like skeletal modifications in the scleractinian coral *Montipora* spp. induced by the chaetopterid *Spiochaetopterus* sp. have been recently described by Bergsma (2009). Fingers inhabited by worms were similar in size and shape to those inhabited by amphipods in the same host, while their frequency (29.2 % of colonies and 9.3 % of fingers) and length (up to 122 mm) was lower. The fingers induced by chaetopterids are considerably longer than the 50 mm reported for the otherwise identical structures induced by spionids on *Montipora* spp. in Taiwan (Liu and Hsieh 2000). These fingers were frequently found detached from their parent colony, which evidences that symbionts may reduce the ability of *Montipora* spp. to withstand physical disturbances. However, detached coral fingers are able to survive and reattach to form new colonies, which can be helpful for coral dispersal. Morphological changes may also affect corals' ability to utilize resources and to compete for space. These observations indicate that symbiont-induced growth forms may enhance the reproductive potential and competitive ability of *Montipora* spp. in Moorea (French Polynesia).

25.4.5 Host Specificity

The host specificity of symbiotic polychaetes associated with corals is relatively high (Fig. 25.5): 69 out of 107 species determined to a species level (64.48 %) are known from a single cnidarian host, 17 from 2, 8 from 3, and 2 from 4. However, among the "monoxenous" species occurring in only one cnidarian host, some occur also in association with other non-cnidarian taxa, such as echinoderms or mollusks. For instance, *Arctonoe vittata*, a symbiont of *Metridium senile*, is also known from at least 30 more hosts including echinoids, asteroids, polychaetes and mollusks. *Hololepidella nigropunctata*, a symbiont of *Lobactis scutaria* in the Red sea, commonly occurs in association with at least 20 species, mostly asteroids and brittle-stars. Nevertheless, polyxenous cnidarian-symbiotic polychaetes are relatively scarce: Only 11 species are reported from more than 5 hosts, which usually belong to closely related taxa or inhabit the same ecological niche. For instance, *Spirobranchus corniculatus* (Serpulidae) occurs in association with 52 cnidarian hosts, all them belonging to the Scleractinia, except for three Milleporidae. *Lumbrineris flabellicola* (Lumbrineridae), reported in 31 associations, lives mostly with ahermatypic scleractinians (29 species), but also with one hydroid and one zoanthid (Zibrowius et al. 1975; Martin and Britayev 1998). Symbiotic polychaetes inhabiting black corals seem to be strict associates of antipatharians (Wagner et al. 2012), except *Tottonpolynoe symantipatharia*, which was also reported from *Sclerisis macquariana* (Alcyonacea: Isidiidae). Finally, some cnidarian taxa have no specific symbionts. For instance, all polychaetes reported from *Millepora* are also known from scleractinian hosts (Lewis 2006).

25.4.6 Location on the Host

Polychaetes are generally found on the surface of their cnidarian hosts (either on colonies or on individual polyps). Alternatively, tubes of symbiotic polychaetes can be embedded in the hard skeleton, with only part of the animal appearing at the surface, such as the precious Christmas tree worm *Spirobranchus* spp. (Serpulidae), whose calcareous tubes are deeply embedded inside the host (Scleractinia or Milleporidae) skeleton as they are overgrown by the coral skeleton while being formed.

Some polychaete symbionts live inside tunnels or galleries (formed by modifications of the coenenchyme or the sclerites) on branches of the host colony as, for example, *Gorgoniapolynoe* spp. on colonies of Primnoidae (Fig. 25.3a) and Corallidae (Fig. 25.3b) (Eckelbarger et al. 2005; Simpson and Watling 2011; Britayev et al. 2014). Alternatively, galleries may be formed (apparently excavated by the symbionts) inside the host coenenchyme, as in the case of *Haplosyllis anthogorgicola* (Syllidae) on *Anthogorgia bocki* (Martin et al. 2002).

Only few reports of life cycle stages of polychaete symbionts living inside cnidarian hosts are known to date. Among them, the larvae of the syllid *Epigamia alexandri* (reported as *Proceraea rzavskyi*) develop inside hydrothecae of *Abietinaria turgida*. At an early juvenile stage, they apparently begin to feed on the tissues of the hydranth, to the extent that they finally occupied the whole space inside of the zoothecae. When the juveniles reach about 1 mm, they leave the zootheca and start building a mucus tube attached to the main stem of the hydroid colony (Britayev and San Martín 2001). Early larval and juvenile stages of at least some symbiotic polychaetes are planktonic free-living (Eckelbarger et al. 2005; Rowley 2008), whereas in other species eggs are hatched inside the host (Britayev and San Martín 2001).

25.4.7 Prevalence of Infestations

The relationship between number of infested and total number of hosts, also known as "prevalence of infestation" (Martin and Britayev 1998), is not often discussed in literature. Such information for cnidarian hosts is even more rare and mostly available for easy to spot symbionts. As discussed by Martin and Britayev (1998), this value cannot be considered as characteristic at the species level. Each population of a given commensal species is usually characterized by a dif-

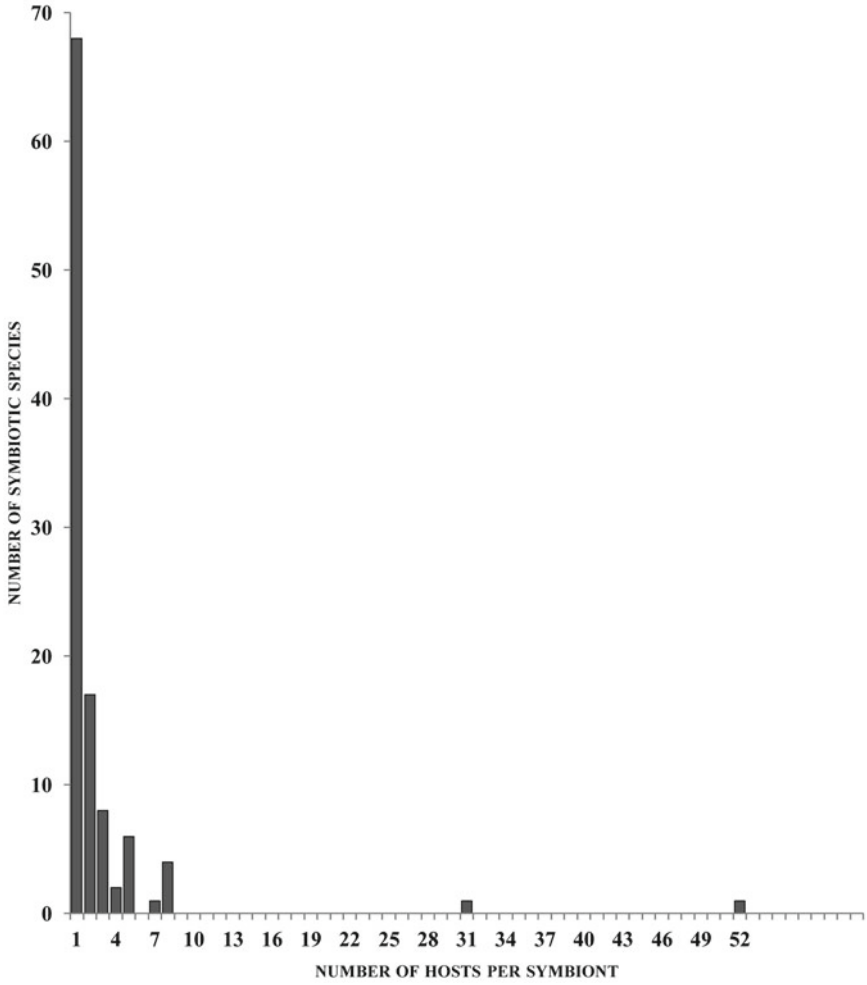

Fig. 25.5 Number of cnidarian hosts reported for symbiotic polychaetes

ferent prevalence. Prevalences may range from extremely low to considerable high percentages. For instance, the infestation by *Imajimaea draculai* reaches 10 % in the population of *Funiculina quadrangularis* (Pennatulacea) from the Bratten area (Skagerrak) (Nygren and Pleijel 2010). About 50 % of nephtheids from the Darwin region (Northern Australia) harbor *Alcyonosyllis phili*, while a 100 % of the colonies of some species of *Narella* (Primnoidae) are infested, to the extent that the presence of the symbiotic polynoid is considered a species level indicator (Cairns 2012). Also a 100 % of *Conopora adeta* (Stylasteridae) were reported to host the polynoid *Benhamipolynoe cairnsi* (Cairns 1987), and all hitherto known species of *Stylopathes* (Antipatharia) host polynoid polychaetes (Opresko 2006; Molodtsova and Budaeva 2007).

Factors that can be crucial for prevalence of infestation include bathymetry, spatial variability and hydrology (Martin and Britayev 1998). Another factor that can be important is the antropogenic disturbance. For instance, Wielgus et al. (2006) showed that the infestation of reef-building stony corals by spionids was significantly correlated with the total oxydized nytrogen in the water column in the vicinity of organic waste discharges.

If a symbiont occurs on different hosts, the prevalence may vary even within the same locality (Martin and Britayev 1998) depending on how suitable are the different hosts. For instance, *Spirobranchus polycerus* in Barbados occurs on several species of scleractinians and milleporids, but is most common on *Millepora complanata* and only occasionally occurs on scleractinian corals (Lewis 2006).

25.4.8 Intensity of Infestation

The number of symbionts per coral host is also highly variable. Martin and Britayev (1998) reported at least seven species of symbiotic polychaetes associated with cnidarian hosts with known intensities of one symbiont per host.

Apparently, the intensity is closely related to a territorial behavior, but no direct evidences have been reported for cnidarian symbionts. There are no references to isolated heterosexual pairs inhabiting the same coral host individual. On the other hand, some coral symbionts show very high intensities. For instance, 18 specimens of *Brychinoe karenae* were collected from a single relatively small colony of *Leiopathes secunda* (Hanley and Burke 1991a). About 120 specimens of *Gorgoniapolynoe caeciliae* were recovered from one-fourth of a single colony of *Candidella imbricata* (Eckelbarger et al. 2005), while intensities of about 0.2–0.4 of symbionts per 1 cm of the host were reported for the co-generic *G. uschacovi* (Britayev 1981) and *G. guadaloupensis* (Pettibone 1991). The intensity of the serpulid *Spirobranchus giganteus* range from 0.2–12 symbionts per 1 cm^2 of living coral surface (Martin and Britayev 1998).

Even closely related species can differ in their intensity. For instance, the maximum intensity reported for *H. chamaeleon* and *H. villogorgicola* are about ten symbionts per host colony, whereas *H. anthogorgicola* can reach up to 15 symbionts per 1 cm of colony (Martin et al. 2002).

25.5 Host Reactions to Symbiont Presence

The number of studies reporting changes in host morphology caused by the presence of symbiotic polychaetes is very limited. Quite often, these modifications are not attributed to the symbiont presence because the two partners are studied separately. In few cases, cnidarian host did not exhibit any morphological reactions to the presence of symbiotic polychaetes. *Haplosyllis chamaeleon* inhabiting the surface of the branchlets of *Paramuricea clavata* did not induce significant changes to the coral host morphology. However the symbiont usually occurred on parts of the colony with high number of living polyps (Martin et al. 2002). No changes in the host morphology were recorded for the *Alcyonosyllus phili* (Fig. 25.6b, d) (Glasby and Watson 2001; Britayev and Antokhina 2012) or in *Funiculina quadrangularis* hosting *Imajimaea draculai* (Nygren and Pleijel 2010).

Scars or grooves induced by *Lumbrineris flabellicola* were reported on the outer surface of the skeleton in several azooxanthelate scleractinians (Fig. 25.2a) (Zibrowius et al. 1975; Miura and Shirayama 1992; Cairns and Zibrowius 1997). This lumbrinerid polychaete inhabits soft membranous tubes attached to the external surface of the coral skeleton. However, the calcareous skeleton became partly dissolved beneath the tube, giving rise to a grove causing the tube to become partly embedded into the coral skeleton. In turn, the depth of the groove is highly dependent of the host species, being more pronounced in *Caryophyllia* spp. and *Flabellum chunii* (Zibrowius et al. 1975).

Frame-building scleractinian corals, such as *Lophelia pertusa*, *Madrepora oculata* or *Solenosmilia variabilis*, often overgrow the tubes of the symbiotic polychaete *Eunice* spp. (Fig. 25.3b) (Zibrowius 1980; Cairns and Zibrowius 1997). A similar effect was reported for *E. norvegica* on the stylasterid *Errina atlantica* (Zibrowius and Cairns 1992). Aquarium experiments with *E. norvegica* (Buhl-Mortensen 2001; Roberts 2005) showed that their tube-building stimulates the production of coral skeleton. The parchment-like tubes of *Eunice* spp. are used as cores for calcification and may serve as the main stem for the skeleton, supporting longer branchlets >25 cm long (Buhl-Mortensen 2001). In the presence of eunicid symbionts, calcification rates in *Lophelia pertusa* increase up to four times (Mueller et al. 2013). Molodtsova and Budaeva (2007) reported overgrowth of eunicid tubes by the chitinous skeleton of the black coral *Antipathes* cf. *cylindrica* Brook 1889 (Fig. 25.3c).

Overgrowth of symbiont tubes by host tissues and skeleton leading to changes in the cnidarian host morphology have also been reported from shallow-water reefs. Accordingly, the chaetopterid polychaetes *Spiochaetopterus* sp. inhabiting colonies of *Montipora* spp. induce the formation of finger-like branchlets in the host (Bergsma 2009). The spionid *Dipolydora* sp. from the Gulf of Eilat (Red Sea) was reported (Wielgus et al. 2002, 2006) to induce skeletal aberrations in 10 % of its host scleractinians, resulting in the formation of 5–25 mm high cones. *Dipolydora armata* can stimulate formation of distinctive, erect spines at the bases of branches in *Millepora complanata* on Barbados coral reefs (Lewis 1998).

Surprisingly, symbiotic polychaetes rarely induce gall formation. Apparently, the single case was reported for *Proceraea penetrans*, which builds calcified blister-like galls on the host *Stylaster californicus* (Wright and Woodwick 1977, Zibrowius 1981). On the other hand, the formation of tunnels and galleries is characteristic for practically all known taxa of cnidarian hosts harboring symbiotic polychaetes which, to some extent, may be considered as a particular case of gall formation. Symbiotic syllids and polynoids generally produce tunnels or galleries. In soft corals, such galleries can be distinguished by a grove at the edge of capitulum, as that in *Pseudoanthomastus* spp. induced by *Neopolynoe acanellae* (Molodtsova 2013), or by a rolled margin, as that in *Sphaerasclera flammicerebra* induced by an unidentified polychaete (Williams 2003) and giving a distinctive mushroom-shape look to the colony (Fig. 25.2d–f). In both cases, the groove is formed from overgrowths and processes of the soft tissue, and there are no indications in the literature on skeletal elements' affectation.

Galleries in primnoids are generally attributed to the presence of symbiotic polynoids of the genus *Gorgoniapolynoe* (Cairns and Bayer 2004; Eckelbarger et al. 2005; Cairns 2012; Britayev et al. 2014). In primnoids, the gallery is

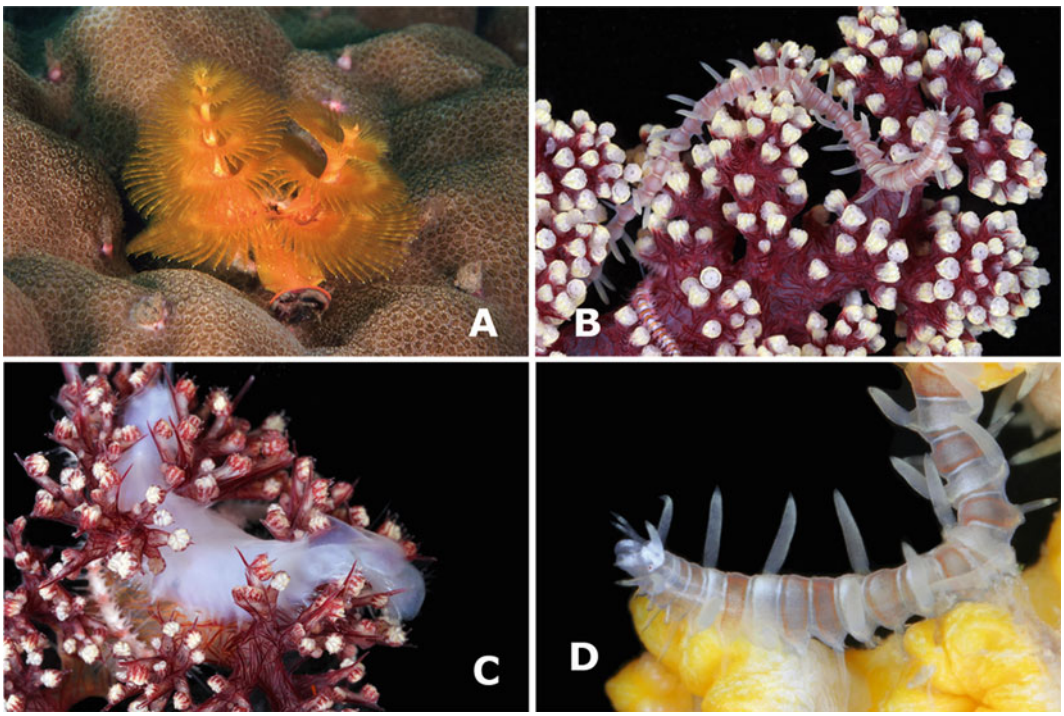

Fig. 25.6 Symbiotic polychaetes with their hosts (photos courtesy O. Savinkin). (**a**) *Spirobranchus corniculatus* on colony of *Porites* sp.; (**b**) *Alcyonosyllis phili* on undetermined nephtheid; (**c**) – *Paradyte levis* on *Dendronephthya* sp. (**d**) *A. phili* on *Carijoa* sp. (**a**) – in situ, (**b**–**d**) lab photos

formed by highly modified polyp scales: the basal ones of successive adjacent polyps became enormously enlarged and curved to meet together forming a tube that can attain up to 3 mm in diameter (Cairns and Bayer 2004, 2008; Cairns 2012) (Fig. 25.3a).

Galleries induced by symbiotic polynoids were reported on branches of several species of *Corallium* Cuvier, 1798 (Fig. 25.3b) and *Paracorallium* Bayer and Cairns, 2003 (Bayer 1964; Britayev 1981; Pettibone 1991a; Simpson and Watling 2011; Britayev et al. 2014). The gallery formation involves not only soft tissues but also the underlying calcareous axis. The mechanism of gallery formation in Corallidae is not really known. Obviously it does not result from any boring activity of the symbiont, but from a gradual formation as a response to its presence. Taking into account that the axial epithelium and free scleroblasts in *Corallium* have the same cellular origin (Grillo et al. 1993), we can speculate that the free scleroblasts of coenenchymal lobes involved in the gallery formation are induced somehow to form an additional layer of axial epithelium that begins to produce solid axis instead of loose sclerites.

Some symbiotic syllids also produce a kind of galleries in the coenenchyme of cnidarian hosts. For instance, *Alcyonosyllis glasbyi* is reported to form tubular nests or shelter-like structure on the surface of the host *Melithaea flabellifera* (San Martín and Nishi 2003). *Haplosyllis anthogorgicolla* forms galleries inside the coenchym of *Anthogorgia bocki*, opening near base of polyps as minute tube-like projections. The galleries are located between the surface of the host covered by spicules and inner axis, and appear as well-structured tubes, with tissue-built walls that can be easily distinguished from the remaining unaltered tissue (Martin et al. 2002). The species *H. villogorgicola* is assumed to induce fusion of two adjacent branchlets in the host *Villogorgia bebricoides* which forms a cavity inhabited by the symbiotic worm (Martin et al. 2002). The only case of gallery formation in scleractinians was reported for *Typosyllis* sp. forming so-called groove-and-tube structures in several species of reef-building scleractinian corals in Taiwan (Randall and Eldredge 1976).

Several species of symbiotic polynoids induce malformations in black corals, mainly of the genus *Stylopathes* (e.g. *Bayerpolynoe floridensis* inhabiting *S. litocrada*). These are the so-called "worm runs" (Totton 1923; Opresko 2006; Molodtsova and Budaeva 2007): tubular reticulated structures formed near the base of primary pinnules of numerous short highly anastomosing and fusing secondary and tertiary branchlets and pseudo-lateral pinnules that connect the stem with the worm run, but rarely extend beyond its surface (Fig.

25.3c). Feebly developed worm-runs with few or no anastomoses can be also found in species of the genus *Tanacetipathes* Opresko, 2001 (e.g. *Paraholotepidiella greeffi* inhabiting *T. spinescens*) (Molodtsova and Budaeva 2007; Britayev et al. 2014) (Fig. 25.3d) and *Asteriopathes* Opresko, 2005 (Molodtsova and Budaeva 2007; Molodtsova unpublished data).

There are some other modifications that can affect the length of individual branchlets or the skeletal structures of individual zooids. For instance, the hydrothecae modification by the symbiotic syllid *Epigamia alexandri,* which caused the formation of an elongated tubular distal part in hydrothecae of *Abietinaria turgida* (Britayev and San Martín 2001). The spionid *Polydora wobberi* associated with *Lophogorgia* sp. apparently affect the length of branches. Worms inhabit narrow U-shaped burrows that open to the exterior at the tips of short stubby 20–30 cm long branches of the gorgonian, whose uninfested branches may reach up to 70 cm long. Molodtsova and Budaeva (2007) reported that presence of symbiotic polychaetes can alternate size and morphology of skeletal spines in black corals.

Changes of the host branching pattern induced by symbiotic polychaetes have also been reported. The presence of the spionid *Polydora villosa* may result in modifications of the growth form in hosts *Montipora* spp. from the encrusting or columnar morph to the branched one, but does not affect *Porites* spp. hosts (Liu and Hsieh 2000). Cairns (2011) described development of the third row of pinnules in a typically uniplanar colony of *Thouarella cristata* in the presence of undetermined commensal polynoid.

25.6 Adaptive Modification of Symbiotic Polychaetes

Symbiotic polychaetes have more or less defined morphological features allowing distinguishing them from their free-living relatives (Martin and Britayev 1998), which can be grouped in different categories: coloring, morphology, life cycle and behavioral. Also, these modifications can be adaptive or non-obviously adaptive. 'Non-adaptive modifications', either morphological or not, allow to differentiate the symbionts from their free-living relatives, but lack obvious adaptive significance. Cnidarian polychaete symbionts are not an exception. However, only two out of the nine families including cnidarian associates, Syllidae and Polynoidae, are sufficiently represented to evaluate trends in adaptive modifications. Moreover, as very little is currently known on life cycle and behavior adaptations, we have here considered only those affecting morphology and coloration.

25.6.1 Syllidae

Color adaptive modifications in Syllidae generally imply a correspondence with the host color. Worms inhabiting the surface of corals often have cryptic coloration, mimicking that of the host. Thus, the pale-yellowish *Haplosyllis villogorgicola* and orange *H. anthogorgicola* match exactly the color of their hosts; *H. chamaeleon* can demonstrate a range of colorations from yellow to dark red or violet, with dark violet dorsal marks, but generally matches exactly the color of their hosts *Paramuricea clavata* and *P. grayi*, which may exhibit all this color range in the same or in different colonies, respectively (Martin et al. 2002; Lattig and Martin 2009). An alternative color adaptation providing protection when living on the host surface is the disruptive coloration of *Alcyonosyllis phili* (Fig. 25.6b, d). This cream colored syllid with transverse brown bands can be hardly visible on brightly colored nephtheid or xeniid hosts (Glasby and Watson 2001; Britayev and Antokhina 2012).

The main morphological modifications reported in cnidarian associated Syllidae affect chaetal arrangement and shape (Fig. 25.7). For instance, the number of chaetae per

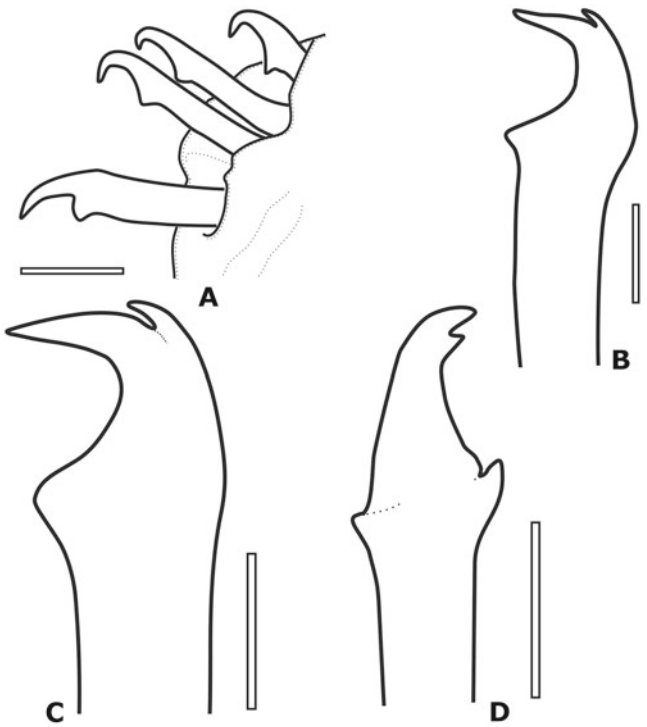

Fig. 25.7 Hooked chaeta in *Haplosyllis* spp (Syllidae) (After Martin et al. 2002). (**a**, **b**). *H. chamaeleon*: (**a**) Chaetae from the first chaetiger; (**b**) Tip of a chaeta from posterior-most chaetigers; (**c**) *H. villogorgicola*: Tip of ventral mid-body chaetae. (**d**) *H. anthogorgicola*: tip of pseudocompound chaeta of the first chaetiger. Scale: (**a**) – 15 mkm, (**b**) – 6 mkm, (**c**) – 4.3, (**d**) – 5 mkm

bundle tended to be reduced and their morphology tended to be simplified from the typically articulated type of most free-living forms to either pseudocompound chaeta (*H. anthogorgicola*), simplified, or hooked forms (*H. chamaeleon*, *Alcyonosyllis* spp., symbiotic Autolytinae) (Martin and Britayev 1998; Martin et al. 2002), which probably serve better to allow the worms to remain attached to the host surface.

25.6.2 Polynoidae

The adaptive modification in coloration of the Polynoidae strongly depends on the position of the symbiotic polychaete on the host. Polynoids inhabiting the host surface usually have cryptic colorations. For instance, *Australaugenira rutilans*, was described from *Xenia* sp. (Octocorallia Xeniidae) and reported as to be of 'exactly the same red color as host' (Wehe 2006) and mimic also the color of another host, *Dendronephthya* sp. (Britayev and Antokhina 2012). The special case of the almost transparent *Uncopolynoe corallicola* and *Paradyte laevis* (Fig. 28.8c) which are hardly visible on the bright surface of their nephtheid hosts *Dendronephthya* spp. (Britayev and Antokhina 2012), can also be considered as an example of cryptic coloration. Polynoids inhabiting galleries and tunnels formed by skeletal elements or tissues of cnidarian hosts are mostly whitish or fleshy in color as, for instance, the species of *Gorgoniapolynoe* inhabiting different species of Corallidae and Primnoidae (Eckelbarger et al. 2005; Simpson and Watling 2011; Britayev et al. 2014).

A true case of mimicry was described for *Medioantenna variopinta* associated with *Solanderia secunda* (Hydrozoa, Capitata). The orange body of this symbiotic worm mimics the large orange eumedusoids of the host colony, while the white pigmentation on the cephalic appendages and on dorsal cirri and the finger-like macropapillae of the elytra mimic the coloring of the host polyps (Di Camillo et al. 2011).

Developing hooked chaetae on anterior segments seems also to be a clear adaptive trend in symbiotic polynoids. *Uncopolynoe corallicola* has stout, strongly bent hooks on segments 2–4, while *Australaugeneria rutilans* has strongly hooked neurochaetae on the anteriormost segment (Wehe 2006).

While chaetal modifications seems to be an obvious adaption to the symbiotic mode of life, most of morphological modifications of elytra and parapodia in symbiotic polynoids cannot be easily considered as adaptive. The elytra of many commensal scale-worms are small, often leaving much of the dorsal surface uncovered, thin and smooth, and usually lack ornamentations (i.e. papillae and tubercules). *Paraholepidella greeffi* inhabiting colonies of *Tanacetipathes* cf. *spinescens* has soft transparent elytra lacking tubercules and papillae, which are large, covering mid-dorsum on the first 11–12 segments, and then become very small, leaving dorsum and parapodia uncovered (Fig. 25.8a–c) (Britayev et al. 2014). *Gorgoniapolynoe* spp. inhabiting mostly octocorals of the families Primnoidae, Corallidae and Acanthogorgidae also have relatively small transparent elytra without ornamentation, except on the first pair that is larger and completely cover the prostomium (Pettibone 1991; Britayev et al. 2014). However, these elytra shows an additional adaptive modification: just above the eyes, each elytron of the first pair has a crescent shaped, transparent, chitinized area with scattered microtubercules (Fig. 25.8d) that is apparently connected to the ability of the worm to distinguish between light and dark even from when lied inside the coral gallery. Taking into account the depth range of *Gorgoniapolynoe* spp. (300–2,000 m), it is hard to expect enough light at these depths. However, there is a number of reports about biolumenescence of corals and polynoids (see e.g. Nicol 1953; Herring 1991; Plyuscheva and Martin 2009; Johnsen et al. 2012) and apparently such adaptation may be connected with bioluminescence ability of host or symbiont. Parapodial modifications are most often reductions in size. Thus *P. greeffi* has small digitiform notopodia (Britayev et al. 2014) and *U. corallicola*, associated with undetermined alcyonaceans, completely lacks notopodia (Wehe 2006).

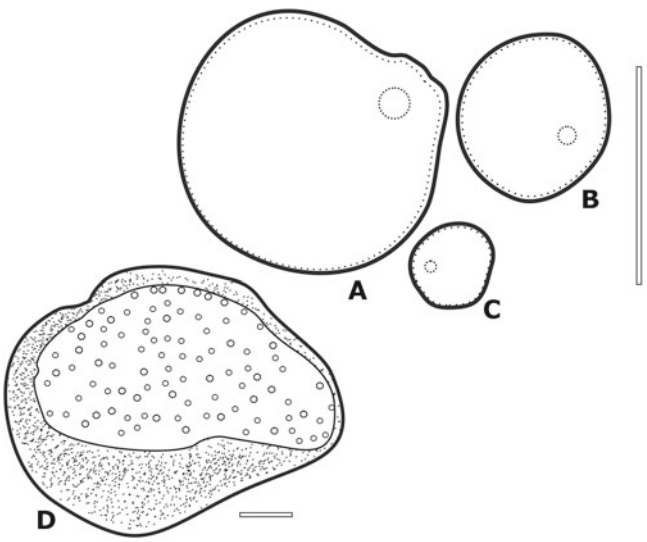

Fig. 25.8 Elytra modifications in Polynoidae (After Britayev et al. 2014). (**a**–**c**) *Paraholepidella greeffi*: elitra of 9th (**a**), 32nd (**b**) and 87th (**c**) segments; (**d**) *Gorgoniapolynoe caeciliae*, elytron of the first pair. Scale (**a**–**c**) – 2 mm; (**d**) – 0.1 mm

25.7 Conclusions. Main Gaps in Our Present Knowledge in Biology of Polychaete-Coral Symbiosis

One of the most interesting results revealed by our review is that every group of cnidarians seems to have their own assemblage of symbiotic polychaetes. Accordingly, scleractinian corals more often harbor lumbrinerids, serpulids and spionids, which include a few symbiotic species inhabiting a wide range of hosts. On the other hand, the alcyonaceans and antipatharians are more often associated with polynoids and syllids, which include numerous symbiotic species. It is interesting to notice that the members of these families are among the symbionts inducing the most dramatic changes in host morphology. Also, the species of host genera such as *Narella* (Primnoidea) or *Corallium* (Corallidae), or even whole genera of deep-sea alcyonaceans or black corals such as *Stylopathes* (Antipatharia, Stylopathidae) have never been reported without their polynoid symbionts. Despite these hosts are so deeply involved in symbiotic associations that the presence of symbionts has been considered as a species-specific, robust taxonomic character, quite often little is known about their symbiotic partners, which usually remain undetermined even at the genus level. Taking this into account, as well as the high diversity in the host morphology, we certainly expect the fauna associated with deep-sea corals to be a hidden hot-spot of diversity, and many species of polychaete symbionts are waiting to be described in deep sea environments.

Symbiotic associations, particularly parasitic and mutualistic ones, are clearly bidirectional and, thus, a well-known source of co-evolution. This means that not only the symbionts may acquire modifications due to their mode of life, but also the hosts may tend to develop specific adaptations. However, very few studies have been addressed to understand the mechanisms leading the symbionts to influence on the host morphology, which quite often involve particular elements of the host skeleton (e.g., the extra-large sclerites of primnoids or the slow-growing processes of the central axis of corallids, the changes in spine morphology of antipatharians). Also, no studies have been addressed to assess how long it takes to produce the worm tubes or worm runs, neither on the mechanisms and chemical cues that influence skeletogenesis in cnidarian hosts. Implicitly, there are no studies on the extend of the associations or, in other words, whether the symbiont grows in parallel with the host or, if not, when the colonization occurs (i.e., larval settlement, juvenile or adult migration) and on the mechanisms leading the symbionts to recognize the presence of their hosts, and this is particularly true in deep-sea environments. Last, but not least, there are no studies focusing on how symbiotic worms affect the host fitness, as well as their metabolism, growth rate or reproductive potential.

Despite more than 15 years passed since Martin and Britayev (1998) published their review on the whole symbiotic polychaetes, one of their main conclusive statements still holds for the particular subset of coral associates: more than a closing analyses on a well-developed matter, our review still reveals many gaps in the study of polychaete-coral relationships, meaning that we highly encourage further work to be done on these highly interesting symbiotic associations.

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Cassiopea and Its Zooxanthellae

26

Kathrin P. Lampert

Abstract

As many cnidarians, the upside-down jellyfish *Cassiopea* spec. lives in an obligate symbiosis with its zooxanthellae: Dinoflagellates of the genus *Symbiodinium*. The symbiosis seems mutual and both partners have adapted to suit the partner's needs. Despite the very close co-operation the zooxanthellae are not transmitted vertically but are taken up from the water column during the polyp stage. Polyps seem flexible which clades to internalize and usually take up all clades available but medusae seem much more restrictive and as far as we know only co-operate with a single *Symbiodinium* clade (the clade however can vary between individuals). The *Cassiopea*-symbiont interaction is especially interesting for researchers as *Cassiopea* occurs in shallow lagoon waters meaning a quite stressful environment with high temperatures and high levels of irradiation as well as potentially drastic changes in salinity and sedimentation rates. In other cnidarian-zooxanthellae partnerships these conditions would lead to a severe disturbance of the symbiosis often ultimately leading to the death of the holobiont (bleaching). The *Cassiopea*/*Symbiodinium* interaction, however, is an example of a successful co-operation under stressful environmental conditions and therefore interesting also in the context of climate change. This chapter summarizes our knowledge of the symbiosis of *Cassiopea* and its zooxanthellae focusing on uptake and choice of symbiosis partners by *Cassiopea* as well as the adaptations making the co-operation possible.

Keywords

Cnidarian/algal symbiosis • Stressful environment • Model system • Morphological adaptations • *Symbiodinium* clade uptake • Upside-down jellyfish

The mutualistic relationship between scleractinian corals and their symbiotic algae (mostly dinoflagellates of the genus *Symbiodinium*) is the basis for a globally important and at the same time severely threatened ecosystem: Coral reefs (Done et al. 1996; Kennedy et al. 2013; Knowlton et al. 2010; de Groot et al. 2012). The biological as well as economic value of this symbiosis has led to a great scientific interest and triggered a large number of studies investigating many different aspects of the relationship (e.g. Baums et al. 2014; Frade et al. 2008a; Hoegh-Guldberg et al. 2007a; Hofmann et al. 1996; Roth 2014; Pernice and Levy 2014).

Currently, nine different clades (A to I) of *Symbiodium* are recognized that can be subdivided even further depending on the molecular marker used for genotype determination (Coffroth and Santos 2005; LaJeunesse 2001; Pochon and Gates 2010; Pochon et al. 2012; Rowan and Powers 1991; Baums et al. 2014; Howells et al. 2013). Clades A to D are most commonly found in the cnidaria (Baker 2003; LaJeunesse 2001). These symbionts are especially adapted to the co-existence with their hosts and can reach very high densities within the host tissue (Berkelmans and Van Oppen

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2006). Some of the clades/subtypes are generalists (infecting a wide range of hosts) while others are specialized and only infect certain host organisms (LaJeunesse 2004; LaJeunesse et al. 2003, 2009). *Symbiodinium* clades also differ in their ecological requirements e.g. irradiation tolerance and temperature optimum (Abrego et al. 2008; Baker 2003). As algae, *Symbiodinium* are photosynthetically active. They provide nutrients (sugar, fatty acids) for their host and facilitate the building of the calcareous skeleton. In return they are provided with inorganic waste from the host's metabolism and a sheltered environment (Hoegh-Guldberg et al. 2007a; Mortillaro et al. 2009; Muscatine 1967; Stat et al. 2006; Stat and Gates 2008; Falkowski et al. 1984; Muscatine et al. 1984; Muscatine and Porter 1977; Pearse and Muscatine 1971). At least for the corals the symbiosis is obligate and at least in the long term they are unable to survive without endosymbionts even though some species might be able to switch their symbiosis partner (Hoegh-Guldberg and Bruno 2010; Hoegh-Guldberg et al. 2007b; Jokiel and Coles 1977; Woolridge 2013).

Reef building corals, however, are not the only cnidarians that live in close co-operation with endosymbiotic algae. A similarly close relationship exists between jellyfish of the genus *Cassiopea* and zooxanthellae.

26.1 *Cassiopea*

Cassiopea, the up side down jellyfish, is a worldwide distributed genus of the Scyphozoa in the family of Rhizostomidae (Bigelow 1900; Gohar and Eisawy 1960). It was first described in the Red Sea by Forskal from Thor in 1775. Eschscholtz then placed it in the genus *Cassiopea* in 1829 (Gohar and Eisawy 1960; Hofmann et al. 1996). The analysis of the systematic status of *Cassiopea* based on morphology is very complex and the number of different species present in the genus was rather controversially discussed (Dawson 2003; Hofmann and Hadfield 2002; Hummelinck 1968). Recently, a study based on molecular data (COI) found six clearly differentiated and well supported haplotype groups that closely correspond to the geographic region the animals were found (Holland et al. 2004). Three of these haplotype clusters confirm the described species *C. andromeda*, *C. ornata* and *C. xamachana*. In addition three new species (*C.* sp. 1–3) were proposed that still need to be described (Holland et al. 2004) (Table 26.1).

As many cnidarians, *Cassiopea* lives in a close symbiosis with zooxanthellae: Dinoflagellates of the genus *Symbiodinium* (Freudenthal 1962; Trench 1993; Trench and Blank 1987). The *Cassiopea*/*Symbiodinium* symbiosis is very close and *Cassiopea* shows many adaptations to suit its symbiont partner. The most obvious adaptations are in its morphology, habitat and life history.

26.1.1 Morphology

In some aspects *Cassiopea* are "typical" Medusae with a smooth and soft round umbrella and tentacles. *Cassiopea* medusae can become quite large with diameters up to 24 cm (Bigelow 1900; Lampert et al. 2012). *Cassiopea* can vary in the number of rhophalia, sensory channels and tentacles/arms. In our laboratory population medusae had 4 and 8 arms, between 6 and 18 rhophalia and 13–20 sensory channels (unpublished data). All *Cassiopea* have identical cnidomes but the toxicity of their sting varies between species (Jensch and Hofmann 1997; Radwan et al. 2001).

Endosymbionts of the genus *Symbiodinium* can be found intracellularly in all jellyfish tissues (Colley and Trench 1983; Hofmann et al. 1978) and *Cassiopea* morphology is closely adapted to harbouring those symbionts. Instead of long straight tentacles for capturing prey, *Cassiopea* have several short feeding arms (oral tentacles) that have a highly enlarged surface area (Bigelow 1900). These arms are used to capture small prey e.g. crustaceans and mollusc larvae, but are also colonized by zooxanthellae (see below) (Fitt and Trench 1983) (Fig. 26.1). The large surface area provides room for high numbers of zooxanthellae.

To enhance the symbionts access to light for photosynthesis *Cassiopea* have adopted a (for a jellyfish) rather unusual

Table 26.1 Geographic distribution of *Cassiopea* species. For each of the six currently recognized *Cassiopea* species the regions where they can be found are marked with X (Holland et al. 2004)

Location\species	*Cassiopea frondosa*	*C. andromeda*	*C. ornata*	*C.* sp. 1	*C.* sp. 2	*C.* sp. 3
Western Atlantic	X	X				
Hawaiian islands		X				X
Red Sea		X				
Indonesia			X			
Palau			X			
Fidji			X			
Eastern Australia				X		
Papua New Guinea					X	X

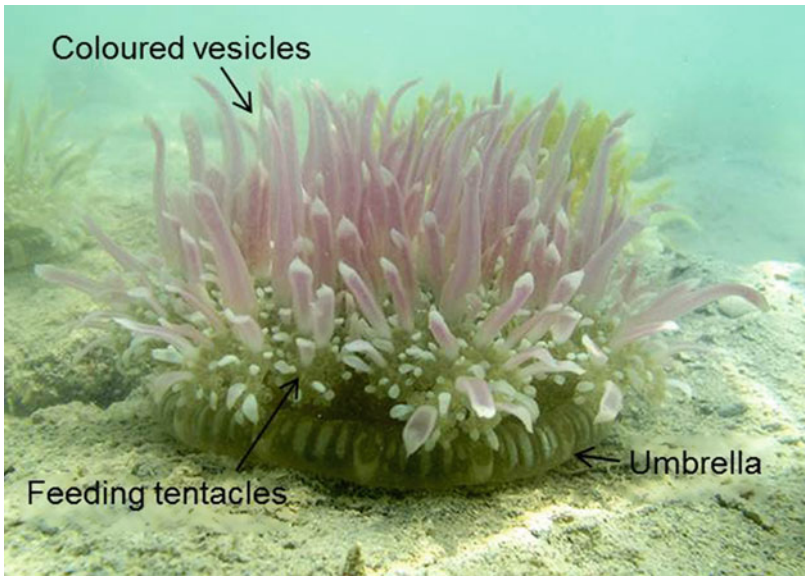

Fig. 26.1 *Cassiopea* morphology. The animal lies on its umbrella with the tentacles pointing up towards the light. The *brownish* colour of the feeding tentacles and the umbrella is due to high densities of zooxanthellae

life style: Instead of swimming with the umbrella up, they lie with the umbrella on the ground and rise their tentacles towards the light (upside-down) (Fig. 26.1).

One of the most striking features of *Cassiopea* is a second type of tentacles: the coloured vesicles (Figs. 26.1 and 26.2). They occur in a large range of shapes (from slender and pointed to very broad and round) and even more noticeable differ in colour. While in most areas the colours brown and green are predominant, specimen in the Red Sea (Nabq) can show many different colours e.g. white, yellow, brown, green, blue, red, purple and pink are found (Fig. 26.2).

The biological significance of this polymorphism is still unknown. Colour is not associated with sex and as far as we know it is not an indicator of environmental condition. In our laboratory experiments adult vesicle colour could not be changed by varying temperature, light intensity or salinity (unpublished data). In addition, in the field differently coloured adults occur in close proximity thereby sharing the same environmental conditions (Fig. 26.2i) (Lampert et al. 2012). Juveniles on the other hand do not always immediately present in their final colour. Initially blue individuals remain blue as adults however, primarily colourless, green or brown phenotypes seem to be invertible to some degree (unpublished data).

Only the blue colour was so far identified and named Cassioblue (Blanquet and Phelan 1987; Phelan et al. 2006). The chemical identity of the other colours and their physiological production are still unknown.

Pigments, however, have been shown to influence the radiation environment of their producers and are often discussed in the context of wave length filtering (UV protection) (Bandaranayake 2006). Different colours could therefore create different light environments. Light, however, is the main source of energy for the zooxanthellae (photosynthesis) (Roth 2014), and in corals differences in light regime have been shown to influence *Symbiodinium* performance (Dove et al. 2008). Host pigments can be protective and shade the zooxanthellae from potentially damaging radiation forms/levels or they can enhance light availability of certain wavelengths and thereby benefit photosynthesis (Salih et al. 2000; Schlichter et al. 1986). *Symbiodinium* react to changes in their light environment and for corals a correlation of water depth (light availability), host colour and *Symbiodinium* clade has been found (Frade et al. 2008b; Iglesias-Prieto et al. 2004). We therefore hypothesized that the vesicle colour of *Cassiopea* could impact the light conditions in the host thereby influencing or being influenced by the *Symbiodinium* clade present in the host. To test this hypothesis we determined the *Symbiodinium* clade present in different colour morphs of *Cassiopea* but found that all *Cassiopea* from the Red Sea only comprise *Symbiodinium* clade A1 irrespective of vesicle colour (Lampert et al. 2012).

26.1.2 Life Cycle and Development

In the Red Sea *Cassiopea* can reach high densities (Fig. 26.2i) (Lampert et al. 2012). Population size, however, is very variable and fluctuates largely between years (personal observation). The factors that influence population growth are still unknown. Environmental effects are certain to play a role and the specific habitat requirements of *Cassiopea* need to be met. *Cassiopea* preferably lives in shallow lagoons. Adults prefer a sandy ground in water depths between 1 and 5 m. Polyps on the other hand prefer smooth, more stable surfaces to grow and strobilate. In the field mangrove leaves are thought to be their preferred habitat type (Hofmann et al. 1996; Fleck and Fitt 1999).

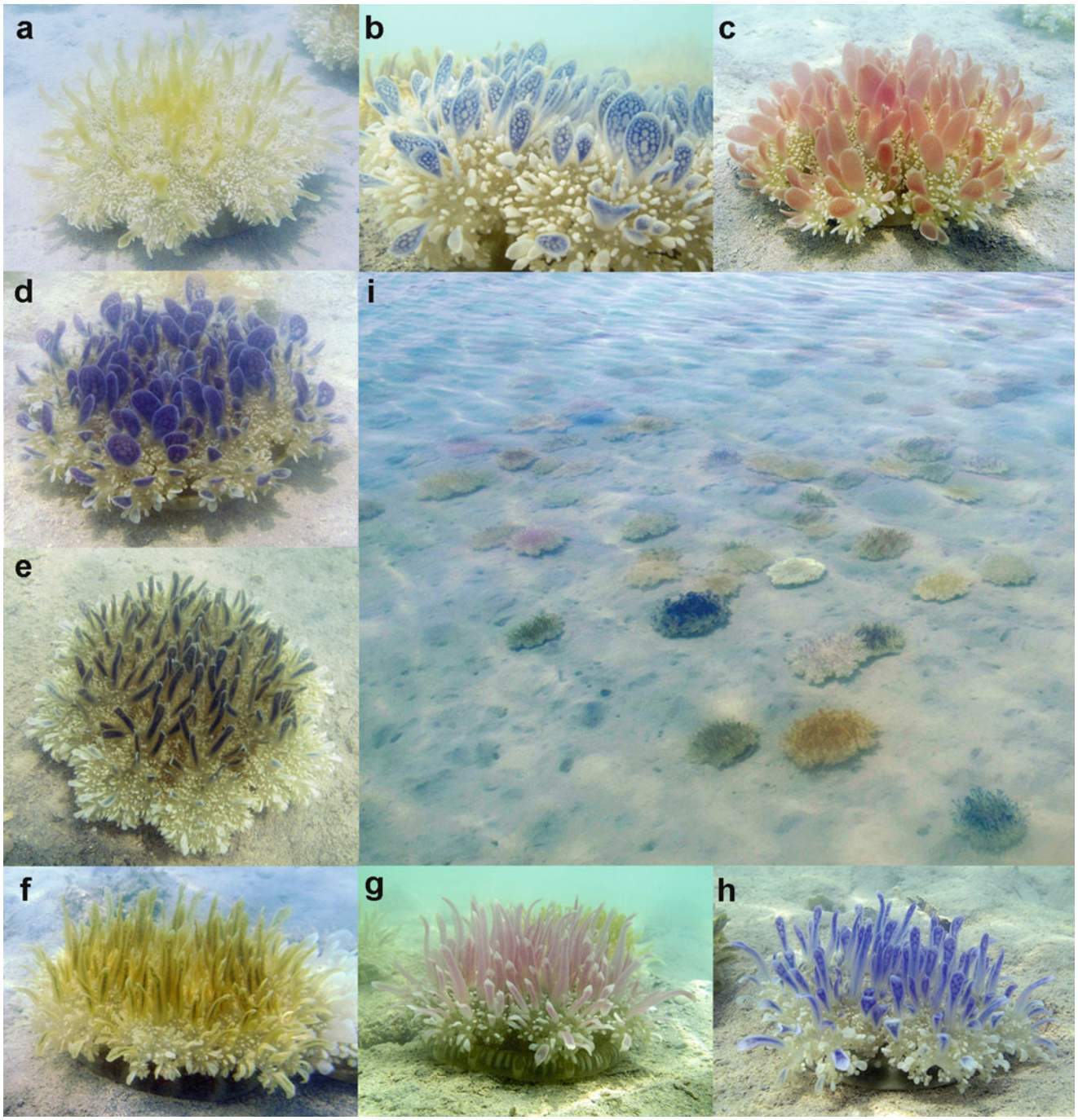

Fig. 26.2 Examples for *Cassiopea* colour morphs and tentacle shapes. All individuals were found in the Nabq lagoon (Red Sea, Egypt) (**a**) *white* morph with slender and pointed tentacles, (**b**) *blue* and *white* morph with round tentacles, (**c**) *red* morph with round tentacles, (**d**) *purple* morph with round tentacles, (**e**) *dark blue* morph and flattened tentacles, (**f**) *green* and *yellow* tentacles, slender and pointed shape, (**g**) *pink* morph with slender and pointed tentacles, (**h**) *blue* morph with flattened tentacles, (**i**) field example for co-occurrence of different colour morphs (and tentacle shapes) in close proximity

Cassiopea have a typical cnidarian life cycle. The medusae have separate sexes (gonochoristic). Sperm is released into the water and fertilizes the eggs inside the female medusa. Fertilized eggs are kept at the basis of tentacles close to the oral disk and only the ciliated planula larvae leave the medusa (Hofmann et al. 1996). Larvae exclusively settle on specific substrates. Their settlement seems to be dependent on proteins produced by microbial growth (Hofmann et al. 1978, 1996; Fleck and Fitt 1999). If the larvae can settle successfully it metamorphoses into a polyp. Polyps develop feeding tenta-

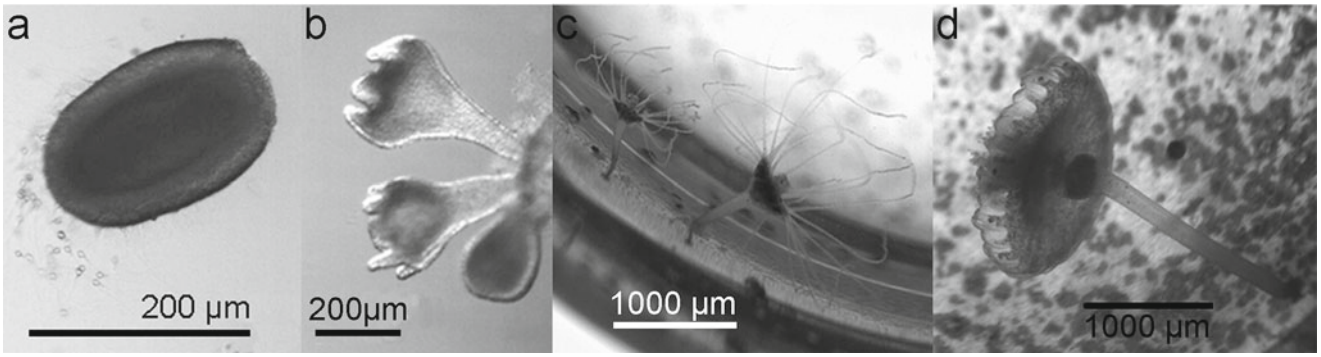

Fig. 26.3 Developmental stages of *Cassiopea andromeda* (**a**) Planula larvae still followed by sperm (*lower left side*) (**b**) two stages of very young polyps: Scyphula stage without tentacles below, the other two are young polyps with eight tentacles (**c**) mature polyps with well-developed tentacles. The dark coloration of the polyp heads indicates the presence of *Symbiodinium* (**d**) strobilation. The new medusa is almost ready to leave the polyp (All pictures taken by Joana Stiehl)

cles, grow for some time and can split via budding, producing genetically identical polyps. At a specific age/size polyps strobilate and release ephyra (juvenile medusae) into the water. These medusa grow and reproduce sexually by producing eggs or sperm when they reach maturity (Bigelow 1900; Gohar and Eisawy 1960; Trench and Colley 1983) (Fig. 26.3). Interestingly, the polyp strobilation depends on the presence of zooxanthellae (see below), while budding does not (Hofmann et al. 1996; Hofmann and Kremer 1981).

26.2 Symbiont Uptake

Even though *Cassiopea* still feeds on small prey it depends on the nutrients provided by the endosymbiotic algae to thrive. Adult medusae lose size and weight when they are bleached or deprived of light (inhibiting photosynthesis) even when prey is available (McGill and Pomory 2008; Verde and McCloskey 1998). In addition, strobilation does only occur when the polyp is capable of symbiont uptake (Hofmann et al. 1996; Hofmann and Kremer 1981).

As *Cassiopea* is so clearly dependent on their symbiont it is surprising that the transfer of *Symbiodinium* is not vertically ensured, but happens horizontally making it seemingly insecure and random (Coffroth and Santos 2005). In *Cassiopea* eggs and planula larvae are free of endosymbionts and only the polyps aquire *Symbiodinium* during growth.

In addition, polyps seem to be very flexible in their symbiont uptake. We found that polyps readily took up what was available to them (Fig. 26.4). If more than one clade was present in the water all available clades could be found in the polyps. A polyp even had better chances of survival if more clades were present than if only a single clade was present in the water (even though overall symbiont cell density was equal) (Fig. 26.4a). All four clades presented were taken in, however, if only one single clade of algae was present, polyps survived best with clades A or B and died rather quickly if only clade D was available for symbiosis (Fig. 26.4b).

In contrast to the flexibility of the polyps, adults are more consistent and usually harbour only a single clade of *Symbiodinium*. We found that animals from the field (Red Sea) all harboured only clade A1. In laboratory animals from the zoo of Cologne, however, we detected exclusively *Symbionium* D1 (Lampert et al. 2012). A possible explanation might be that clade D is often recognized as the most resistant against environmental stress as high temperatures and changing salinity (Berkelmans and Van Oppen 2006) which might be a problem in artificial environments. However, *Cassiopea* individuals from a different zoo (Aquazoo Düsseldorf) contained exclusively *Symbiodinium* clade A1. Interestingly, even when kept in the same aquarium the difference in *Symbiodinium* clade between the adults was stable and no switches were recorded even though the "other" clade should have been available to them as *Cassiopea* as all symbiotic cnidarians constantly releases zooxanthellae into the surrounding water (Hoegh-Guldberg et al. 1987; Stimson and Kinzie 1991).

Our finding of flexibility during juvenile stages and selective stability in adults confirm other studies. This patterns has been observed in cnidarians in general (Abrego et al. 2009; Cumbo et al. 2013) and for *Cassiopea* in particular (Mellas et al. 2014; Thornhill et al. 2006). Mellas et al. (2014) concluded also that even though *Cassiopea* polyps were flexible in their symbiont uptake a certain symbiont type might be advantageous for development. Our data support this finding as polyps infected with clade D did less well than polyps infected with clade A. Interestingly clade D is the *Symbiodinium* clade with the highest tolerance against high temperature but also the lowest nutrient production that might benefit the host (Jones and Berkelmans 2010). The high mortality rate in our experiments might therefore be due to the lower nutrient availability, On the other hand adult medusae harbouring only clade D1 exist and do well (at least in an artificial surrounding). Obviously clade D1 therefore can provide a valuable symbiosis partner for *Cassiopea*. In addition, the positive effect of high *Symbiodinium* clade

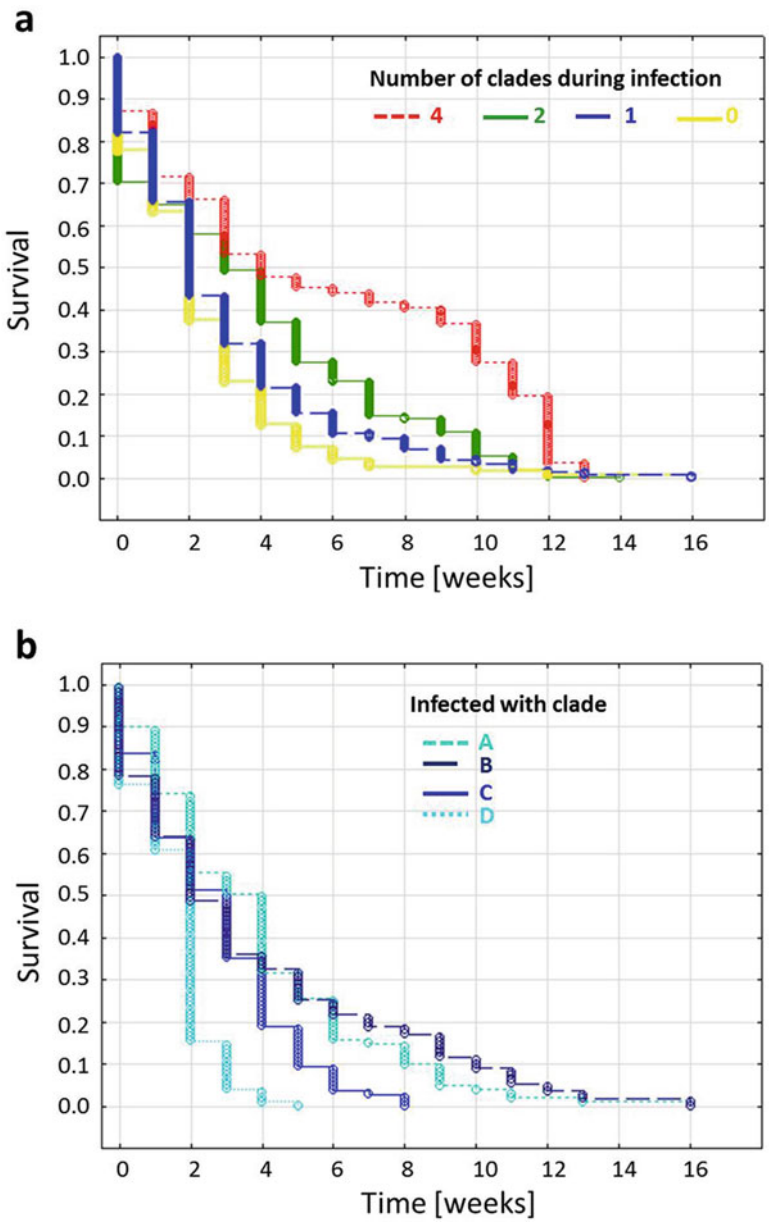

Fig. 26.4 Survival of *Cassiopea* polyps under different *Symbiodinium* availabilities. (**a**) Different numbers of clades were present in the water while the *Symbiodinium* density was kept constant. *Red* – four clades present, *green* – two clades present, *blue* – a single clade present, *yellow* – no *Symbiodinium* present in the water. Polyp survival was highest when all four clades were available. (**b**) Infection experiments with only a single clade present in the water. Different clades are shown in different shades of *blue*. Highest polyp survival was observed in treatments containing clades A or B while clade D led to high polyp mortality

variability during early host developmental stages could not be explained.

Obviously we are only beginning to understand symbiont uptake and maintenance in *Cassiopea* (and other cnidarians). It seems clear though that the combination of early flexibility with later stability is a general pattern and might have an adaptive value, for example in unpredictable environmental conditions.

26.3 A Model Symbiosis?

While *Cassiopea* themselves are fascinating and extraordinarily beautiful research objects they are also often investigated as models for cnidarian/algal symbiosis in general.

When doing so, one has to admit that due to the lack of calcification in jellyfish, *Cassiopea* might not be a perfect model for all cnidarian/zooxanthellae symbioses and all potential effects climate change might have on coral reefs. However, in many aspects the *Cassiopea*/*Symbiodinium* symbiosis is very typical for the mutualistic relationships between cnidarians and algae including the reef building corals.

As corals *Cassiopea* is strongly adapted to harboring zooxanthellae which is reflected in its morphology, habitat and life cycle. For *Cassiopea* the symbiosis seems obligate as nutrition and reproduction depend on the presence of the symbiont and several studies have shown that *Cassiopea* suffers when the dinoflagellates are incapacitated or expelled (Hofmann et al. 1996; McGill and Pomory 2008).

The symbiosis seems to be mutual as *Symbiodinium* receive metabolic waste necessary for photosynthesis and *Symbiodinium* densities within hosts are much higher than outside in the water column. Similar relationships can be found in other cnidarian/zooxanthellae symbioses and *Cassiopea* are therefore very good models to investigate host and symbiont adaptations.

In addition, the *Cassiopea* method of symbiont uptake (horizontally rather than vertically) is realized in the majority of cnidarian/zooxanthellae relationships. Eighty-five percent include an asymbiotic phase during their development and have to acquire their symbiont partners from the surrounding water some time during development (Richmond 1997). Even the combination of juvenile flexibility with adult selective stability can be found in many different symbiosis systems. *Cassiopea* are therefore optimal models to investigate symbiont uptake and maintenance.

At the same time, *Cassiopea* inhabits a rather harsh environment. Shallow water means high levels of (potentially damaging) irradiation and in lagoons water temperature and salinity can become quite high and/or vary strongly depending on water currents. This makes *Cassiopea* a good model to investigate possible reactions and solutions to environmental stress (climate change) (Mellas et al. 2014).

They are also much easier to keep in the laboratory than most coral colonies and therefore make experimental approaches e.g. on nutrient exchange possible.

Many questions still remain. Future studies should e.g. focus on the genetic diversity of hosts as well as symbionts (Baums et al. 2014; Howells et al. 2013; Santos 2014). Understanding the genetic basis for the symbiosis and the fitness consequences of particular associations might enable us to understand the resistance, resilience and survival potential of cnidarian/algal associations.

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Sea Anemones and Anemonefish: A Match Made in Heaven

27

Karen Burke da Silva and Anita Nedosyko

Abstract

Sea anemones are amongst the most venomous organisms on earth and yet there are species of fish and crustacea that are known to tolerate anemone venoms and live in association with them in a mutually beneficial relationship. One of natures most compelling displays of symbiotic behavior is found in the relationship between anemonefish and their sea anemone host. This relationship was first described more than a century ago and despite it being widely studied since, our understanding of the evolution of the relationship and the mechanisms and behaviors involved remains shrouded in mystery. Anemonefish (Family: Pomacentridae) comprise of a distinct group of 28 species that are able to live within sea anemones. Despite the large diversity of anemones in the tropics, only ten species are suitable as hosts for anemonefish. Within these species, only certain pairs of anemone and anemonefish are compatible and found in the wild together. This relationship is obligatory for the fish and in some cases for the anemone, meaning that the symbionts are entirely or heavily dependent on each other for survival. Symbioses between the two groups provide the following benefits: mutual protection from predators, an exchange of nutrients, improved reproductive and lifetime fitness. While past studies have explored the different patterns of host species that fish use and multiple authors have examined the mechanisms involved in protecting fish from anemone venom, how fish acquire immunity from the anemone's stinging tentacles and why only certain anemone species are found associated with some anemonefish more often than others still remains uncertain.

Keywords

Anemonefish • Clownfish • Anemone • Symbiosis • Mutualism

27.1 Patterns of Association Between Anemones and Anemonefish

The association between sea anemones and anemonefish is a classic textbook example of a mutualistic interaction where both symbionts appear to benefit from living with each other, but the relationship is complex. Since its discovery by Collingwood in 1868, biologists and aquarists alike continue to observe these endearing little fish that form close bonds with anemones on coral reefs in order to understand how they live unharmed in the toxic environment of their host. For apart from only a few other fish and crustacean species, the venom contained in the nematocysts of anemones is toxic enough to kill other fish that make contact with their tentacles. What has been discovered is that out of the approximate 1,200 species of sea anemones (Actinaria) (Dalay et al. 2008) only ten form symbiotic relationships with anemonefish in the wild (Fautin and Allen 1997; Hobbs et al. 2013). These ten 'host' anemone species are phylogenetically diverse arising from three different families (Actiniidae,

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Stichodactylidae and Thalassianthidae) and five genera (*Entacmaea*, *Macrodactyla*, *Stichodactyla*, *Heteractis* and *Cryptodendrum*) (Astakhov 2002). Some of these species are more closely related than others, with three species in the *Stichodactyla* genus and four species within the *Heteractis* genus.

In comparison to anemones, their symbiotic anemonefish are phylogenetically similar. There are currently 28 defined species of anemonefish that have historically been divided into two sister genera, *Amphiprion* and *Premnas*, belonging to the subfamily Amphiprioninae (Perciformes: Pomacentridae) (Allen 1991; Jang-Liaw et al. 2002; Hobbs et al. 2013). Recent studies of nuclear and mitochondrial DNA analysis show a monophyletic origin of anemonefish, indicating the two-genus partition is incorrect (Santini and Polacco 2006) and all anemonefish fall within the *Amphiprion* genus. Although *Premnas biaculeatus* is morphologically distinct with cheek spines and large body size, we agree that it warrants re-classification as an *Amphiprion* species (Elliott et al. 1999). In addition, two known hybrids exist (*Amphiprion leucokranos* and *Amphiprion thielli*) that are no longer recognized as distinct species but do hybridize naturally in the wild (Ollerton et al. 2007) as well as two recently identified anemonefish species (A*mphiprion barberi* and *Amphiprion pacificus*) (Allen et al. 2008, 2010).

Host anemones and anemonefish can be found throughout the Indian Ocean and across the western Pacific Ocean (Fig. 27.1). In these locations, anemones and anemonefishes usually occur in relatively shallow waters (<40 m) on or near coral reefs and associated sandy bottoms (Mariscal 1970; Dunn 1981; Fautin 1991; Fautin and Allen 1997) but some host species such as *Entacmaea quadricolor* and *Heteractis crispa* have also recently been discovered at depths of 50–65 m (Bridge et al. 2012). Throughout their distribution, only certain symbiotic pairings are observed. Some host anemones have a large number of anemonefish species that they form symbiotic relationships with, whereas other host anemone species have only a single anemonefish symbiont. For example, *E. quadricolor* has the highest number of fish associates (16 species), whereas *Heteractis malu* and *Cryptodendrum adhaesivum* both have only a single known anemonefish symbiont, *Amphiprion clarkii* (Fautin and Allen 1997; Ollerton et al. 2007). Host anemones may also vary in the number of individual fish symbionts that they have at any given time, with some having a single resident breeding pair and others having a breeding pair plus multiple non-reproductive individuals. Fautin and Allen (1997) and Ollerton et al. (2007) suggest that the variable numbers of fish that form associations with anemones are due to differences in host quality because geographical distribution alone does not sufficiently explain these patterns. These different patterns of association vary so understanding how each symbiont supports the other in the relationship is worth investigation.

27.1.1 Benefits of This Symbiotic Relationship

Whilst the exact benefits of the symbiotic partnership for both anemonefish and anemones continues to be explored, it is generally agreed that the relationship for the fish is obligate, as they are never found in the wild without a sea anemone host. Suggested benefits for the fish include protection from predators (Fautin 1991; Wilkerson 1998; Buston 2003), removal of external parasites (Allen 1972), nourishment from the anemone tentacles (Allen 1972) and reproductive benefits gained through the protection of eggs (Saenz-Agudelo et al. 2011).

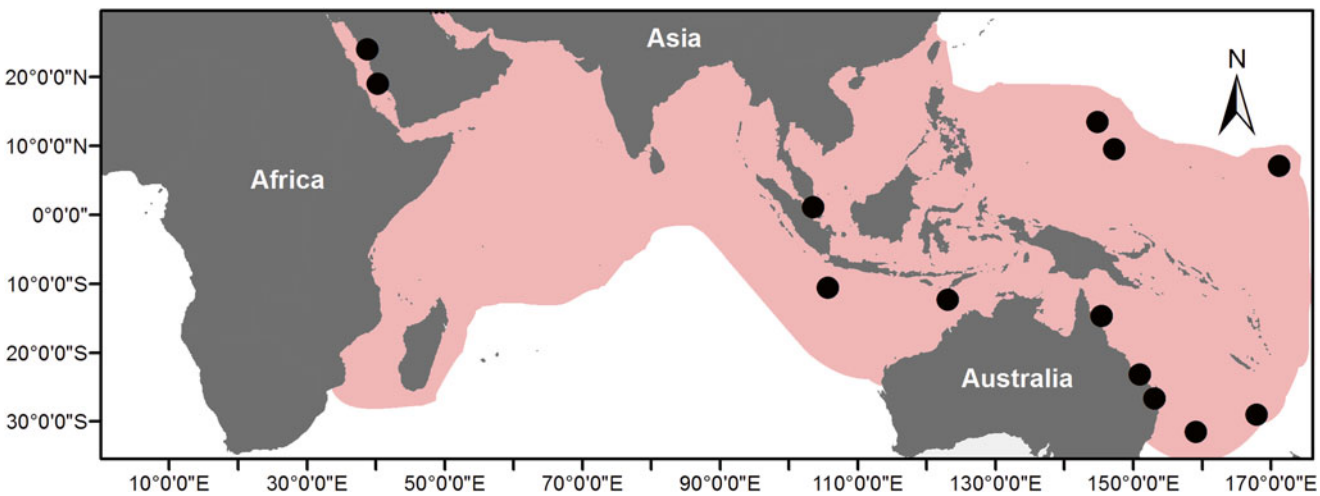

Fig. 27.1 Geographical distribution of anemone and anemonefishes and locations where host anemone bleaching has been documented (Fautin and Allen 1997; Hobbs et al. 2013; K. Burke da Silva unpublished data)

Host anemones benefit by being fiercely defended by their anemonefish associates, particularly against predators such as butterflyfish (Family: Chaetodontidae) (Godwin and Fautin 1992). It has also been shown that having one or two anemonefish in attendance can enhance survivorship, foster faster growth and increase asexual reproduction of host anemones (Holbrook and Schmitt 2005). Holbrook and Schmitt (2005) also found that host anemones without anemonefish suffered greater mortality than those with fish in attendance. Another advantage to an anemone living with an anemonefish is that fish oxygenate their host at night (Szczebak et al. 2013) and metabolize much more rapidly than anemones resulting in large quantities of metabolic waste, in the form of dissolved ammonia readily available and assimilated by their host (Roopin et al. 2008). Thus anemonefish are a major contributor to the fitness of their cnidarian host (which also harbour photosynthetic zooxanthellae that use the fish's ammonia to produce food that is then taken up by the anemone) through the use of their metabolic wastes (Roopin et al. 2008).

The specific fitness advantages of living symbiotically with another organism (e.g. increased longevity and fecundity) are difficult to measure and would require long term studies which currently have yet to be undertaken. Scientists have been unable to accurately age sea anemones with current technology, although there are records of very long-lived individuals living within aquaria. While Holbrook and Schmitt (2005) found a link to increased asexual reproduction in anemones that host anemonefish, lifetime reproductive success has not been ascertained. Anemonefish also live surprisingly long lives with predicted lifespans of more than 30 years, which is twice as long as any other damselfish and up to six times longer than marine fish of comparable size that live only between 5 and 10 years (Buston and Garcia 2007). Clearly the protection provided to anemonefish through their association with host anemones and the decreased predation risk that follows represents a life history strategy that is highly advantageous. How the association evolved is a question that needs to be asked next.

27.1.2 Evolution of This Unique Association

It is estimated that this partnership has likely been in existence for at least 10 million years, with the first anemonefish diversifying in the Central Indo-Pacific area (Litsios et al. 2014). How the relationship was established is based mostly on speculation. The closest relative of anemonefish are the damselfish (*Dascyllus spp*) that also associates with the same species of host anemones. However, they tend to be outcompeted by anemonefish (Holbrook and Schmitt 2004) and only form mutualistic partnerships with anemones as juveniles. Randall and Fautin (2002) describe a number of reef fish that form loose associations with anemones whereby they spend considerable amounts of time in close proximity of the anemone but they avoid making contact with the tentacles. A move from close association followed by full contact and immersion into the tentacles may have been the evolutionary pathway that anemonefish undertook which eventually gave them protection against the stinging nematocysts of host anemones.

Anemonefish have diversified through an adaptive radiation process that has been driven by the symbiotic association with anemones (Timm et al. 2008; Litsios et al. 2013) and the availability of different ecological niches may have contributed to speciation and geographic spread. As anemonefish diversified and spread geographically, it is likely that more potential host anemone species would have been encountered and new combinations of anemone-anemonefish relationships would have evolved. Over time, a larger number of different associations formed and these varying combinations may have shaped the evolution of anemonefish (Timm et al. 2008; Litsios et al. 2014) through adaptation that enabled them to use different species of anemones living in different ecological niches.

The degree of specificity in a symbiotic relationship depends on the number of host species that an organism will live and interact with in nature (Miyagawa 1989). It was originally proposed that ancestral anemonefish were host generalists, living in multiple species of host anemones (Allen 1972; Futuyma and Moreno 1998). However, an analysis using molecular phylogenetics by Elliott et al. (1999) supports the hypothesis that ancestral anemonefish probably lived with a very limited number of anemones and were likely host specialists. As they spread further geographically increasing the numbers of associations with anemones, this would have given rise to generalists and other specialist fish species with the associated morphological characteristics and behaviours that enabled successful speciation.

An optimal host anemone toxicity range may be biologically significant for the establishment of anemonefish and anemone associations as the potency of anemone venom differs (Nedosyko et al. 2014). This may be the limiting factor in anemonefish niche expansion but as shown in at least one anemonefish species, *A. clarkii*, utilization of an anemone species with moderately higher toxicity, as found in *C. adhaesivum*, may be an adaptation that will allow this unique symbiotic relationship to continue expanding to use more anemone species in the wild. Hence, the mechanism by which anemonefish use that enables them to live within the toxic environment of host anemones may be the limiting factor in the number of different associations found. An understanding of this mechanism may help understand the evolution and establishment of the association.

27.1.3 Mechanisms for Living Within a Toxic Environment

Probably the most intriguing aspect of this symbiosis is that anemonefish are unharmed by the otherwise lethal sting of anemone nematocysts. Although sea anemones harbor powerful venom that is composed of haemolytic activity (Lanioa et al. 2001; Uechi et al. 2005; Nedosyko et al. 2014), immunomodulating activities (Tytgat and Bosmans 2007; Pento et al. 2011), neurotoxic properties (Gondran et al. 2002; Nedosyko et al. 2014), and cardiotoxic properties (Bruhn et al. 2011) that are capable of killing prey and keeping predatory fish at bay, anemonefish manage to settle and live within anemones without negative effect. Numerous studies have sought to understand the mechanisms underlying the protective elements that allows anemonefish to remain unharmed from the stinging nematocysts of the host anemones (e.g. Caspers 1939; Davenport and Norris 1958; Mariscal 1969; Mariscal 1970a, b, 1971; Schlichter 1975, 1976; Lubbock 1980, 1981; Miyagawa 1989; Mebs 1994; Elliott et al. 1994; Elliott and Mariscal 1996). Most research has come to a general conclusion indicating that a protective mucus coat acting as camouflage or as a molecular mimic prevents anemones from recognizing anemonefish as being different from self and may be responsible for not eliciting nematocyst discharge when in contact. Interestingly, immunity from anemone toxicity is not necessarily innate for the fish. A specific acclimation procedure is performed by anemonefish when making contact with a host anemone for the first time (Mariscal 1970b). In addition, when anemonefish are removed from their host and reintroduced after a given period of time they lose their resistance and will be stung (Mariscal 1970b, *pers. obs*). They must slowly re-acclimate to the toxic environment before reestablishing the partnership.

Not all anemonefish species seem to require time to re-acclimate as Lubbock (1980, 1981) found with *A. clarkii* that were able to settle into *Stichodactyla haddoni* immediately. He assumed this was due to the fish producing it's own mucus that differs significantly from other non-symbiotic fishes which elicit nematocyst discharge without *A. clarkii*'s "special" mucus. Follow up studies by Miyagawa and Hidaka (1980) and Miyagawa (1989) also support this finding and suggest that *A. clarkii* has an innate protection produced in their mucus coat. However, we have observed that after a period of 2 months without a sea anemone host *A. clarkii* can be stung by a host anemone species as indicated by white sting marks on their black skin. Elliott et al. (1994) found that within the mucus coat, *A. clarkii* held anemone antigens that were not found in individuals that were not living in association with a host anemone, moving the argument more toward an acquired resistance than innate. Mebs (1994) also found that anemonefish can acquire resistance to a host anemone when exposed to low concentrations of mucus from that particular anemone species in the water. It is still unresolved how different species of anemonefish manage to live within the toxic environment of their host anemone but it is clear that compounds within the mucus of the fish are inhibiting nematocyst discharge and thus preventing the fish from being harmed. The ability of an anemonefish to locate and recognize a host anemone is hard to imagine and will be discussed next.

27.1.4 Recognizing and Locating a Host

Upon hatching from the egg, anemonefish larvae disperse and have a brief pelagic stage before juveniles return back to the benthic habitat in search of a host anemone. Juvenile anemonefish have an incredible ability to recognize and locate the same host anemone species that they were born near. The ability to recognize a natal host provides an evolutionary advantage for vulnerable juveniles by enabling them to locate a safe refuge quickly and efficiently.

Past research has focused on the question of how newly settling juveniles detect their host anemone (Fricke 1974; Miyagawa and Hidaka 1980; Murata et al. 1986; Miyagawa 1989; Konno et al. 1990; Elliott et al. 1995; Arvedlund and Nielsen 1996; Arvedlund et al. 1999; Miyagawa-Kohshima et al. 2014). Their findings indicate that smell rather than sight enables host anemone recognition during their first encounter however, visual cues may be important to find anemones later in life (Fricke 1974). Anemones release chemical cues secreted in the mucus on the tentacles and the oral disc (Murata et al. 1986; Konno et al. 1990) which anemonefish are strongly attracted to (Miyagawa 1989; Miyagawa and Hidaka 1980; Elliott et al. 1994). The attraction intensifies in the later stages of larval development, which reflects the period when larvae settle into the benthic habitat (Dixson et al. 2011).

Host anemone choice experiments manipulating the breeding environments of anemonefish larvae in association with or without an anemone indicate that innate preference (genetic) plays a role in identifying their symbiotic host anemone, a preference that is enhanced by imprinting (learned) (Arvedlund and Nielsen 1996; Arvedlund et al. 1999, 2000; Dixson et al. 2011; Miyagawa-Kohshima et al. 2014). This influences where females choose to lay their eggs, preferring sites that encourage the greatest amount of chemical release from the anemones to carry over and imprint upon the developing embryos (Miyagawa 1989; Mitchell 2003; Arvedlund et al. 2000). When given a choice, larvae show a clear innate preference for the smell of their natal anemone species and recognize the chemical quicker if hatched normally next to their host anemone (Miyagawa-Kohshima et al. 2014). Some anemonefish such as *A. ocellaris* can be imprinted to live within anemones that they do not

commonly associate with in the wild through early exposure to these anemone species in a hatching aquarium. It is not possible however to imprint *Amphiprion melanopus* larvae to their non-host anemone species *H. malu* (Arvedlund et al. 2000) which suggests that anemonefish imprinting on host anemones may be rather restricted.

The ecological relevance of anemone host recognition in the laboratory however, differs when compared to anemone host choice by anemonefish larvae in their natural environment. Elliott et al. (1995) found that in field experiments where juveniles were released near other potential host anemone species, the following anemonefish species, *A. polymnus*, *A. perideraion*, *A. sandarcinos* and *A. crysopterus*, were all attracted to 'unnatural' host anemone species. Similarly, genetic parentage analysis relating larval recruits to parents of a population of *A. percula* at Kimbe Bay, Papua New Guinea, indicate that recruits do not preferentially return to their natal host anemone species (Dixson et al. 2011). An explanation for this could be that in areas where anemone choice is limited, larvae may settle in less preferred host anemones when encountered or when required. However, the distinct specialization behavior that certain anemonefish species such as *P. biaculeatus* display suggests that anemone host choice is more complex than just random selection. The authors of these conflicting data suggest that the mechanisms for host recognition may be species specific and larvae may be responding to a hierarchy of innate and imprinted cues that relate to different components of the environment that might influence settlement habitat selection in the wild. Choice and acquisition of high quality host anemones could have significant effects on anemonefish behavior, reproductive success and lifetime survival. Understanding what makes a host anemone more preferred by anemonefish is clearly important.

27.1.5 Host Choice and Acquisition of High Quality Anemones

The number of anemonefish species that are found inhabiting particular species of anemones can be found in Table 27.1. Observed patterns of anemone usage by anemonefish in the wild indicate that some anemone species are used more often as hosts than others. More popular host anemones have been found to host up to 16 species of anemonefish compared to less popular host anemones that may only have a single anemonefish species symbiont. Fautin (1986) inferred from this pattern that host anemones with the highest number of fish symbionts such as *E. quadricolor* must be more desirable and preferred over others.

Hypotheses explaining the different patterns of relationships between anemonefish and host anemone species have been proposed by Fautin (1985, 1986) and Murata et al. (1986) and include olfaction and innate preference (by fish), competitive exclusion (between fish), and environmental requirements of the symbionts (both fish and anemone). Authors have argued that anemone use by different anemonefish species can be largely explained by innate preference and environmental requirements (Elliott et al. 1995; Fautin and Allen 1997; Ollerton et al. 2007) however there must be other contributing factors involved as some anemonefish are known to move from one anemone species as juveniles to a different anemone species as adults (Moyer and Bell 1976; Dunn 1981; Chadwick and Arvedlund 2005; Huebner et al. 2012). The fact that some anemone species are used as nurseries (as they only have immature anemonefish using them in some locations) (Moyer 1976; Dunn 1981; Chadwick and Arvedlund 2005; Huebner et al. 2012) from which an anemonefish must move if it is to reproduce, provides evidence that choice of anemone can influence fish fitness. What qualities anemonefish deem as desirable however remains somewhat unclear.

Considering that anemonefish benefit primarily from the protection that a host anemone provides, Huebner et al. (2012) suggests that anemone morphology may play a role. Specifically, the shelter that the tentacles provide could be an indicator of host quality. Anemone morphology does vary amongst the ten host anemone species, primarily in body size and in tentacle length (Table 27.2). Some species have long tentacles whereas others have very short tentacles such as in the carpet anemones (Fig. 27.2). The most preferred anemone, *E. quadricolor* has long tentacles that form bubbles on the tips and anemonefish can immerse their whole bodies within the tentacles when hiding or sleeping thus avoiding predators. The carpet anemone *Stichodactyla gigantea* also has a large number of fish symbionts and whilst anemonefish are not able to immerse themselves completely within their short tentacles, they can be found concealed within the sizeable folds of the protective anemone blanket. This indicates that tentacle length alone is not the only indicative measure of host anemone quality. Huebner et al. (2012) suggests that because adult *A. bicinctus* are competitively excluding conspecific juveniles from living within the highly desired host anemone *E. quadricolor*, this species of anemone must be preferred over *H. crispa*, which is used only by juveniles at their study area in the Gulf of Aqaba, northern Red Sea.

An anemone characteristic that has not had much consideration, but would clearly influence anemone quality is toxicity. A large variance in toxicity exists amongst host anemone species and anemones with high haemolytic characteristics typically have high neurotoxic characteristics as well (Nedosyko et al. 2014). The study by Nedosyko et al. (2014) proposes that anemonefish may be forming associations with host anemones based on the potency of toxins, and that this could be a critical factor in determining the

Table 27.1 Combinations of host anemone and anemonefish associations (Updated from Fautin and Allen 1997)

	Anemone species										
Anemonefish species	*Entacmaea quadricolor*	*Heteractis crispa*	*Stichodactyla mertensii*	*Heteractis magnifica*	*Heteractis aurora*	*Stichodactyla haddoni*	*Stichodactyla gigantea*	*Macrodactyla doreensis*	*Heteractis malu*	*Cryptodendrum adhaesivum*	Total anemone associates
Premnas biaculeatus	X										1
Amphiprion akallopisos			X	X							2
Amphiprion akindynos	X	X	X	X	X	X	X				7
Amphiprion allardi	X		X		X						3
Amphiprion bicinctus	X	X	X	X	X		X				6
Amphiprion barberi	X[a]	X[a]									2
Amphiprion chagosensis	X										1
Amphiprion chrysogaster			X	X	X	X		X			5
Amphiprion chrysopterus	X	X	X	X	X	X					6
Amphiprion clarkii	X	X	X	X	X	X	X	X	X	X	10
Amphiprion ephippium	X	X									2
Amphiprion frenatus	X										1
Amphiprion fuscocaudatus			X								1
Amphiprion latezonatus	X[b]	X									2
Amphiprion latifasciatus			X								1
Amphiprion mccullochi	X										1
Amphiprion melanopus	X	X		X							3
Amphiprion nigripes				X							1
Amphiprion ocellaris			X	X			X				3
Amphiprion omanensis	X	X				X					2
Amphiprion pacificus											?
Amphiprion percula		X		X			X				3
Amphiprion perideraion		X		X			X	X			4
Amphiprion polymnus		X				X		X			3
Amphiprion rubrocinctus	X						X				2
Amphiprion sandaracinos		X	X								2
Amphiprion sebae						X					1
Amphiprion tricinctus	X	X	X		X	X[c]					5
Total fish associates	16	14	12	11	7	8	7	4	1	1	

[a]Described in Allen et al. (2008)
[b]Described in Rushworth et al. (2011)
[c]Described in Hobbs et al. (2014)

Table 27.2 Morphological characteristics of host anemone species (Fautin and Allen 1997)

Anemone species	Tentacle length (mm)	Oral disc diameter (mm)
Entacmaea quadricolor	100	50
Heteractis aurora	50	250
H. magnifica	75	1,000
H. crispa	100	500
H. malu	40	200
Macrodactyla doreensis	175	500
Stichodactyla gigantea	10	500
S. haddoni	10	800
S. mertensii	20	1,000
Cryptodendrum adhaesivum	5	300

quality of host anemones for anemonefish survival. This study demonstrated that the most preferred host anemones, in terms of the number of anemonefish symbionts, have a venom toxicity value that falls within the mid range of potency, suggesting that moderate toxicity may be optimal for anemonefish survival and reproduction. Considering that most anemonefish are not innately protected from anemone venom but have to acquire protection through a process of acclimation (Mariscal 1970b, *pers. ob.*), it is possible that an upper toxicity threshold exists for anemonefish species to establish tolerance to venom without being harmed. Low anemone toxicity levels are also unlikely to be optimal for anemonefish survival as anemonefish will not be able to obtain the protective benefits from the association. Little research has been done to ascertain whether higher quality anemones afford their symbionts greater fitness benefits. This may be the true indicator of anemone quality and would require an extensive field-based project to determine the outcome.

Among the preferred hosts such as *E. quadricolor*, competition amongst fish dictates who lives where, as demonstrated in two localities by Fautin (1986, 1992) but who gets the best anemone is the question to be asked next.

27.1.6 Competitive Exclusion: Who Gets the Best Anemone?

Anemonefish species may look alike but when examined closely not only do they vary in color and shape but a big difference exists in size as well. The largest anemonefish species *P. biaculeatus* is 160 mm in length compared to the smallest species, *A. percula*, at only half the size, 80 mm in length (Fautin and Allen 1997). With most competitive species, size plays a major role in determining dominance and resource holding potential. Studies on anemonefish competition have found that a hierarchy exists amongst anemonefish species (Fautin 1986; Hirose 1995) with body size being a major contributor to competitively dominant species (Srinivasan et al. 1999; Hattori 2002). But what are anemonefish competing for? Clearly, the most important element for their survival and reproduction is their host anemone and as indicated above host anemones vary in their morphology and likely quality. Therefore competition should exist amongst anemonefish for the highest quality anemones; indeed this is the case as reported by Fautin (1986), Huebner et al. (2012), and Srinivasan et al. (1999). Some anemonefish species are known to be competitively superior to others and therefore would be predicted to access and hold the most valuable host anemone resource. Fautin (1986) demonstrated in aquaria that interspecific competition between *P. biaculeatus* and *A. akindynos*, would require the latter species to be 175 % larger in body length to outcompete *P. biaculeatus* for an anemone host. Hirose (1995) also observed larger *A. frenatus* displacing smaller individuals after a typhoon event. Body size in these cases was the predictor for resource holding success of these species.

Anemonefish are classified as either generalist or specialist based on the frequency of interactions fish species have with host anemone species (Miyagawa 1989). In the case of the extreme anemone specialists such as *P. biaculeatus* that only uses *E. quadricolor*, there is probably strong selection for individuals to out-compete those of other anemonefish species for the single host anemone species with which it lives. Indeed *P. biaculeatus* in particular has also evolved enhanced morphological characteristics such as large cheek spines that we propose increase its competitive advantage for occupying the most preferred host anemone. Large specialist anemonefish such as *P. biaculeatus* can always monopolise their host anemonefish species to such an extent that they are almost always found as a single breeding pair occupying a large solitary *E. quadricolor* while smaller species are not only pushed into the less desirable anemones but also often forced to form size hierarchies within their host anemones where individuals form queues of up to nine individuals for a reproductive position within their anemone (Fricke 1979). Aggressive behavior in the form of chasing, biting and continual harassment can limit anemonefish size and prevent smaller individuals from challenging the position of individuals above them in the queue (Buston 2003). However,

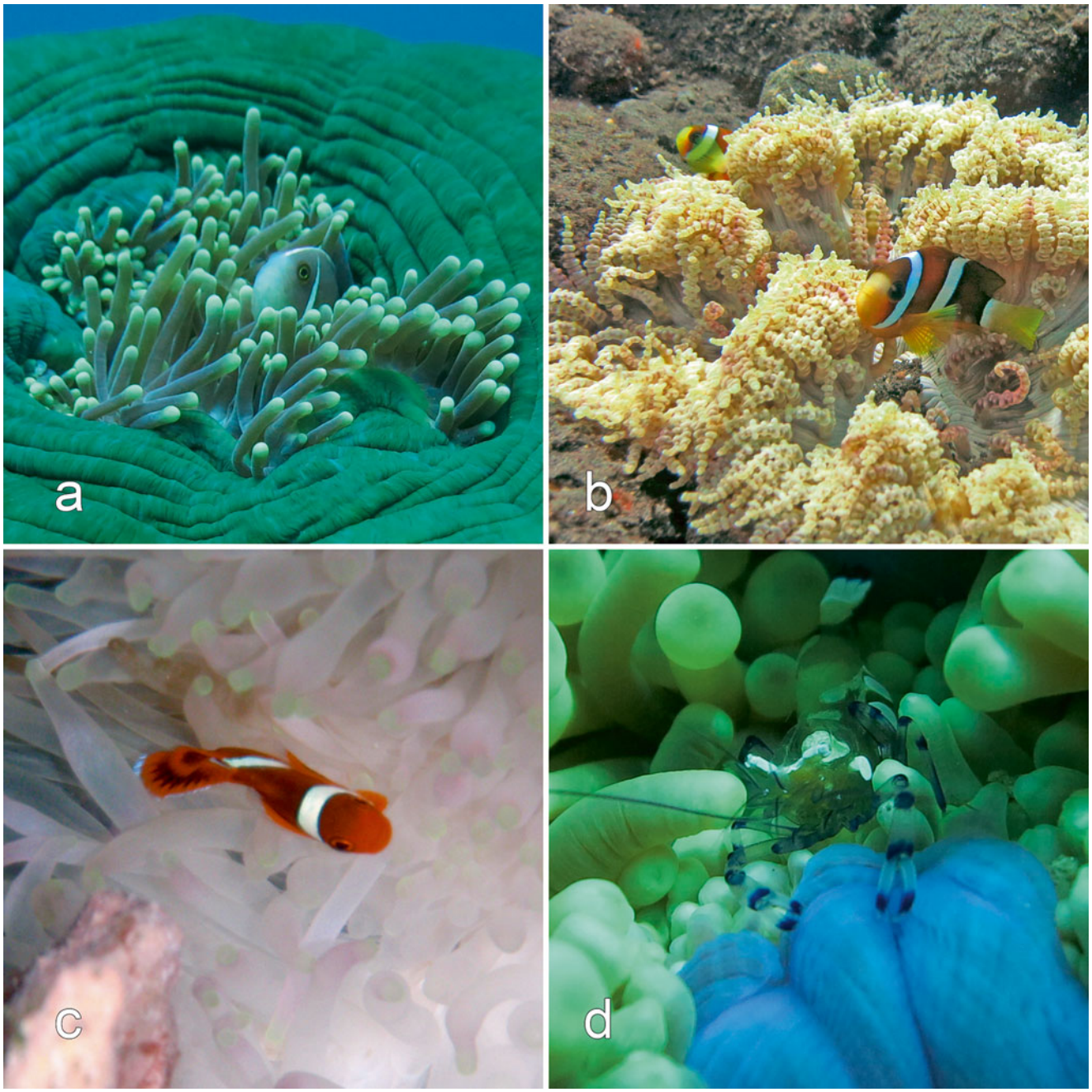

Fig. 27.2 Host anemones with (**a**) long tentacles *Heteractis magnifica* (**b**) short tentacles *Heteractis Aurora* (**c**), a bleached *Entacmaea quadricolor* (Lizards Island, Australia) (**d**) and *E. quadricolor* with a shrimp (*Periclimenes brevicarpalis*) (Photograph credits: A. Rocconi (**a**, **b**, **d**), C. Burke da Silva (**c**))

perhaps increasing group size might be an evolutionary strategy to maintain acquisition of host anemone resource against larger congeners.

Limitation or reduction in the availability of host anemones can increase competition and affect community structure. Competition is expected to increase as coral reef habitats come under threat from anthropogenic disturbances. In areas where multiple anemonefish species coexist, species that have greater resource-holding potential, such as those gained from large body size, will be favored, thus driving natural selection of competitively dominant morphology and behavior. Monitoring changes in anemonefish population structure in threatened areas will be important to elucidate which species are most vulnerable to extinction.

27.2 Conservation Issues: Threats to the Anemone Symbiosis and Hope for the Future

Organisms that are dependent on anemones are diminishing in numbers on reefs impacted by disturbances largely due to anemone bleaching, but also from coastal process such as run-off from flooding. There is now evidence that ocean acidification, as a result of increased atmospheric carbon dioxide, could also threaten the survival of some anemonefish species by affecting their ability to locate a host anemone during a critical life history stage. As popular target species for commercial aquarium collectors, anemonefish are also at risk due to the compounding effects of unsustainable harvesting for trade. Anemonefish and their host anemones have several life-history characteristics that make them vulnerable to localized population decline (1) anemones are long-lived, slow growing and have relatively low reproductive rates (i.e. they spawn infrequently, have low spawning success, and have short larval lifespan) (2) anemonefishes have limited dispersal capabilities, are habitat specialists and have long life spans (e.g. 30 years, Buston 2007) and (3) both groups of organisms are mutually dependent on each other for survival (Fautin and Allen 1997; Wilkerson 1998; Jones et al. 2008; Shuman et al. 2005; Almany et al. 2007). Here we explain these threats further and discuss why there is hope for the conservation of anemones and their symbiotic partners with the establishment of marine protected areas, aquaculture initiatives and reintroduction programs.

27.2.1 Anemone Bleaching

Similar to corals, anemones are susceptible to bleaching (Fig. 27.2), due to the sensitivity of their symbiotic zooxanthellae to environmental stressors such as increased temperature of the ocean and high irradiance. These stressors cause anemones to bleach by expelling their algal symbionts resulting initially in shrinking and often followed by death due to lack of nutrition (Jones et al. 2008; Saenz-Agudelo et al. 2011). The occurrence of host anemone bleaching has now been documented at twelve different reef locations (Fig. 27.1) and is expected to increase in frequency and extent. Examining the thermal tolerance of a common host anemone species, *E. quadricolor*, at Solitary Island, Hill and Scott (2012) found that at temperatures even 1 °C above the summer average of 27 °C will cause these anemones to expel their symbiotic algae. The same study found that at temperatures 3 °C above the summer average, anemones experience severe bleaching that can lead to mortality. The effect of anemone bleaching on host anemones and their associated anemonefish has only recently been discussed in the literature and very little is known about the impacts to other symbiotic species that associate with anemones. We know that host anemone bleaching can severely degrade habitat quality for anemonefish and can also affect recruitment of juveniles and decrease reproductive fitness (Saenz-Agudelo et al. 2011) and survival (Lönnstedt and Frisch 2014). As a result of decreased anemone abundance and reduced size of bleached anemones, anemonefishes are decreasing in numbers due to their dependence on host anemones for habitat and protection (Hattori 2005; Jones et al. 2008; Frisch and Hobbs 2009; Saenz-Agudelo et al. 2011; Hill and Scott 2012; Hobbs et al. 2013).

Anemonefish have been observed abandoning a bleached host anemone in search of another suitable anemone (Hattori 2005) but other studies have shown that fish will stay associated with a bleached host anemone and that anemones can regain full color within a year after a bleaching event (Saenz-Agudelo et al. 2011; Hobbs et al. 2013). Abandoning a host anemone is risky for an anemonefish due to exposure to predators during relocation however we now know that staying associated with a bleached anemone has fitness costs. Saenz-Agudelo et al. (2011) demonstrated that egg production of female *A. polymnus* living in a bleached anemone was reduced by 38 % during a bleaching event. A behavioural study by Lönnstedt and Frisch (2014) demonstrated that *A. akindynos* living in bleached anemones also display altered avoidance behaviors (e.g. they uncharacteristically continue feeding and don't seek refuge within the tentacles of their anemone) in the presence of a common predator and suggest being eaten as a possible explanation for the observed reduction in anemonefish numbers on bleached anemones compared to those living in healthy unbleached ones in the southern Great Barrier Reef, Australia. We found that when anemonefish are given a choice in a captive situation of a bleached and unbleached anemone, they always chose to associate with the unbleached anemone (K. Burke da Silva, *unpublished data*). The choices fish make in response to this habitat disturbance may be dependent on the severity of the bleaching event, mobility of the fish species and availability of vacant non-bleached host anemones in the area. Considering further temperature increases of 2.8–3.6 °C predicted in waters around coral reefs this century (Brainard et al. 2011) the frequency of anemone bleaching is expected to increase, which will have adverse impacts on both anemones and their resident anemonefish.

27.2.2 Ocean Acidification

Ocean acidification is the process of the ocean's pH decreasing as carbon dioxide is absorbed from the atmosphere into the water (Munday et al. 2009). One of the major impacts of acidification is the inability for marine organisms like corals to calcify their skeletons, shells and coccoliths because

minerals, calcite and aragonite become less available (Fabry et al. 2008). This threatens the coral reef habitat of anemones and their symbionts by diminishing coral abundance and reef-building capabilities (Hoegh-Guldberg et al. 2007) and making them more vulnerable to degradation by erosion, storms, predation and other disturbances. In addition to causing habitat loss, the adverse effect of acidification directly threatens the survival of anemonefish as a result of disturbances to behavior and other biological functions. Recent laboratory studies have found that ocean acidification at levels predicted mid to later this century (~pH 7.8–7.6) disrupts the sensory capacity and behavior of anemonefish during a critical life history stage (Munday et al. 2009, 2010; Dixson et al. 2010; Simpson et al. 2011; Nilsson et al. 2012; Nowicki et al. 2012). These studies indicate that extended exposure to lower pH levels of seawater affects the olfactory and auditory system of larval anemonefish, which is likely to have negative effects on their ability to locate settlement sites and avoid predators. Munday et al. (2009) also found that when *A. percula* larvae are reared in seawater at pH levels expected to occur at the end of this century, they completely lose their ability to discriminate the smell of their host anemone. While it appears that some sea anemone species are predicted to grow larger and much more abundant in a higher CO_2 environment due to an increase in the energy output of their symbiotic algae (Suggett et al. 2012), without anemonefish to protect them, it is unlikely that obligate anemone hosts will survive. Disruption to these processes as a result of acidification will have significant consequences to larval settlement, population replenishment and likely population-level and species decline of both host anemones and anemonefishes as early as mid-century.

27.2.3 Collection for the Aquarium Trade

Anemones and anemonefish are particularly vulnerable to collection for the marine aquarium trade compared to other targeted species because they are constantly in demand and dealers can virtually guarantee a sale (Edwards and Shepherd 1992; Wood 2001; Wabnitz et al. 2003). In general, the family Pomacentridae, consistently dominate the global marine aquarium market and account for 43 % of all marine fish traded (Zajicek et al. 2009). With the release of the Disney film 'Finding Nemo' in 2003, the global demand and trade of anemonefish increased dramatically, particularly for the orange clownfish *A. percula* and sister species *A. ocellaris* that resemble the star character (Prosek 2010). Commercial fishers also harvest anemones but the extent of collection is not well documented. Several international studies indicate that anemones and anemonefish are threatened due to overexploitation from wild harvesting. In the Philippines, which is the largest supplier of marine ornamental species and accounts for ~55 % of global exports (Bruckner 2005; Rhyne et al. 2012), anemonefish and anemones in this region have been diminishing in numbers due to aquarium fishing activities (Shuman et al. 2005). On the Great Barrier Reef, Australia where habitat loss has been compounded by thermal stress and low salinity from coastal run-off, there is evidence that anemone and anemonefish populations in areas subject to harvesting have also declined in numbers and failed to recover (Jones et al. 2008; Frisch and Hobbs 2009). Fisher's logbooks can be a useful tool to determine catch records, however, Jones et al. (2008) reported that the global marine aquarium industry is almost entirely self-regulating and collectors are reticent to reveal collection numbers and location data due to competition. This is combated by weak governance capacity in major source countries such as the Philippines and Indonesia and high international demand that offers few incentives to strengthen trade policies or management practices. Even in some of the best-managed reef areas of the world, such as the Great Barrier Reef, where quota restrictions, voluntary stewardship agreements and no-take zones are implemented, populations of both anemone and anemonefishes are still declining in some areas (Jones et al. 2008; Frisch and Hobbs 2009; Scott and Baird 2014).

It is common for collectors to remove a breeding pair of adults or sub-adults, leaving at least one anemonefish behind (Jones et al. 2008). Contrary to sustainable principles, collectors are removing breeding adults on the assumption that this will foster faster growth of sub-adults. Hattori (1991) however, found that it could take 1.5 years for the next fish in line to mature to a breeding position. Even though a study has indicated that post-larval anemonefish may rapidly settle onto an unoccupied anemone (~30 days) after the removal of adult fish residents (Fautin 1992), Sale et al. (1986) found that removal of fish can cause a total cessation of recruitment in some areas. It is apparent that long-term monitoring needs to occur in areas where anemonefish are being collected to inform a sustainable management approach.

27.2.4 Hope for the Future

We are faced with a real challenge to fundamentally change the way we are living to reduce the synergistic impacts of both ocean acidification and global warming to protect reef habitats. Growing evidence suggests that if we continue to emit CO^2 into the atmosphere at the same rate as we are now, reefs are predicted to experience rapid and terminal declines worldwide before mid-century (Hoegh-Guldberg et al. 2007; Wilkinson 2008). However, with technological progress into the use of renewable energy resources, investment in aquaculture and better conservation management approaches, including more targeted restrictions and in some cases complete cessation of the harvest of anemones and

anemonefishes, there is hope to safeguard species in threatened coral reef habitats (Jones et al. 2008; Frisch and Hobbs 2009; Scott et al. 2011). Recent studies reveal the positive impacts of implementing protective measures. For example, no-take zones around north solitary island, far north Queensland and Keppel Islands off the coast of Australia (Jones et al. 2008; Scott et al. 2011) and also in the Maldives where a cap on exports for all allowable coral reef species has been implemented (Edwards and Shepherd 1992), which has increased the abundance of different species of both anemones and anemonefishes in these areas. Larger body size of host anemones is another observed benefit in areas closed to fishing compared to open areas (Frisch and Hobbs 2009). With considered planning, protected areas may also be vital for sustained larval recruitment of anemonefish (Jones et al. 2009). In consideration of future ecological scenarios, we agree with the recommendation of a suspension of commercial harvest in some areas to relieve the additional pressure that over-collecting has placed on these species (Jones et al. 2008).

Captive breeding is the most viable option to meet the increasing demand for aquarium species particularly for anemonefish but also of anemones. Currently, there are records of successful asexual propagation of host anemones in captivity such as *E. quadricolor* by cutting healthy specimens longitudinally in half and quarters and attaching them to a suitable substrate (Scott et al. 2014). Recent studies have also advanced our understanding of the sexual reproductive cycle of two highly collected anemones for marine aquariums (*E. quadricolor* and *H. cripa*) (Scott and Harrison 2007a, b, 2008, 2009). This information may one day enable the supply of captive-bred anemones for the aquarium trade. Captive breeding programs of several anemonefish species are already well established in different parts of the world (Le et al. 2011; Dhaneesh et al. 2012a, b; Ghosh et al. 2012; Kumar et al. 2012). The 'Saving Nemo' project based at Flinders University in Adelaide, South Australia, aims to reduce the harvest of anemonefish from the Great Barrier Reef through a captive breeding and education outreach program (The Saving Nemo Organisation 2015). Captive breeding is most likely the only sustainable means to replenish the already depleted natural source (Dhaneesh et al. 2012a). In some areas where overexploitation has occurred, captive breeding can serve as a mechanism for the reintroduction of captive bred species back into the wild thus supporting coral reef biodiversity conservation. In 2002, a program guided by Dr. Thon Thamrong-nawasawat was established to begin a reintroduction of captive bred false anemonefish *A. ocellaris* into its natural environment at Mu Koh Ha Yai in Krabi Province, Thailand. The first few years of the project had low success rates with the majority of introduced anemonefish eaten by predators. However, fish survival rates increased during subsequent years after host anemones were protected with mesh wire cages. This enabled released anemonefish to swim freely in and out of the anemone but inhibited larger predatory fish. This is the first program of its kind to investigate and establish suitable methods to increase survival rate of reintroduced anemonefish.

27.3 Shrimps, Crabs and Other Fish Symbionts

While no other fish lives as closely with an anemone as an anemonefish, other fish form facultative associations with sea anemones such as damselfishes (Pomacentridae), cardinalfishes (Apogonidae), wrasses (Labridae), hawkfishes (Cirrhitidae), butterflyfishes (Chaetodontidae), blennies (Clinidae and Blenniidae), parrotfishes (Scaridae), gobies (Gobiidae) and greenlings (Hexagrammidae) amongst others (Fautin and Allen 1997; Randall and Fautin 2002; Arvedlund et al. 2006). Arvedlund et al. (2006) provides a review of 51 species of fishes that are assumed to be facultative symbionts of sea anemones, mainly in tropical waters. These fish often dwell within the area of the tentacles of sea anemones, mostly as juveniles and appear to avoid the tentacles with only few making contact. The damselfish *Dascyllus spp* and the painted greenling *Oxylebius pictus* have been observed living within the anemones tentacles as juveniles for protection (Elliott 1992; Fautin and Allen 1997). For example, juvenile painted greenlings sleep amongst the tentacles of the same individual anemone *Urticina lofotensis* each night but leave during the daytime until they are mature enough to live independently (Elliott 1992). These fish often associate with an anemone on their own but damselfish will sometimes share a host anemone with an anemonefish (Fautin and Allen 1997). The relationship of these fish to anemones is considered facultative because while the benefits to the fish are obvious the complete behavioral and physiological facets of these relationships remain to be studied.

Several shrimp and crab species such as *Periclimenes* (Fig. 27.2), *Stenorhynchus*, *Mithraculus*, *Neopetrolisthes*, *Allopetrolisthes* spp., are also associated with different sea anemone species. For symbiotic crustaceans, sea anemone hosts act as their reproductive habitat (Baeza et al. 2001), food source (Viviani 1969) and a refuge against predators such as fishes and birds (Va'squez 1993). Laboratory experiments suggest that the mechanisms that enable crustaceans to live unharmed amongst the sea anemone are similar to those of the anemonefish–anemone mutualism (Giese et al. 1996). For example the crustacean species, *Mithraculus sculptus*, requires acclimation to a new host to acquire a protective mucus coating from the anemone otherwise it will be immediately stung and ingested (Mebs 2009). Similar to anemonefishes, crustaceans are also found with only some of the anemone species within their distribution (Nizinski 1989;

Gwaltney and Brooks 1994). It is understood that at least for some species, crustaceans are able to distinguish and locate potential hosts primarily by olfactory cues but it is likely that more than one sensory modality is involved (Guo et al. 1996).

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Part VI

Immune System

Cnidarian Immunity: From Genomes to Phenomes

28

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Abstract

Cnidarians rely on the innate immune defenses based on self/non-self recognition, signaling and effector responses to kill pathogens and heal wounds. Like other invertebrates, the immune system of cnidarians can be classified into several functional components and many of these elements have now been described in various cnidarian model systems. These include recognition receptors, toll-like receptors and peptidoglycan binding proteins, pro-phenoloxidase and melanin synthesis for an impermeable melanin barrier, anti-microbial proteins and molecules, reactive oxygen-producing and scavenging systems and wound repair and cellular systems. This chapter will summarize the current state of knowledge of cnidarian immune pathways and mechanisms. We will summarize the available data, guiding the reader through the steps of initiating and executing an immune response: recognition, signaling, effector and repair mechanisms. We will also explore the current research of constitutive immunity observed in healthy organisms and elicited immune responses seen in naturally infected organisms or organisms exposed to live pathogens or pathogen associated molecular patterns. We will connect these known immune pathways with how they are expressed and regulated and how this may influence the wide variation in disease resistance observed in the field, both within and between species. The overarching goal of this chapter is to take the reader from a genomic to phenotypic perspective, while keeping pathways and mechanisms in a whole organism and ecological context, whenever possible.

Keywords

Innate immunity • Pathogen associated molecular pattern • Antimicrobial peptide • Coral disease • Pathogen recognition receptor

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Abbreviations

AMP	Antimicrobial peptide
C3	Complement protein
LPS	Lipopolysaccharide
LRR	Leucine rich repeat
MACPF	Membrane-attack complex–perforin protein
MAPK/ERK	Mitogen activation protein kinases/extracellular signal-regulated kinases
MASP	Mannose-binding lectin associated serine protease
Myd88	Myeloid differentiation primary response protein 88

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NF-κB	Nuclear factor kappa light chain of activated B cells
PAMP	Pathogen/microbe-associated molecular pattern
PO/PPO	Phenoloxidase/prophenoloxidase
PRR	Pathogen recognition receptor
ROS	Reactive oxygen species
SML	Surface mucus layer
SOD	Superoxide dismutase
TGF-β	Transforming growth factor beta
TIR	Toll/interleukin-1 receptor
TLR	Toll-like receptor

28.1 Introduction

Coral diseases are important processes that affect coral reef structure and function (Ruiz-Moreno et al. 2012). Not only can coral mortality be directly due to disease, but the effects on coral physiology, such as reproduction, could have long term effects on coral populations. Therefore understanding how corals fight disease is more critical than ever. It is likely that immunity, and consequentially disease resistance, play large roles in the varying temporal, spatial and species-specific patterns of disease distribution (Pinzon et al. 2014a).

The study of cnidarian immunity has benefited from advances in cell biology and genomics, enabling researchers to discover pathways and processes and compare them among the Cnidaria and other phyla (Miller et al. 2007, 2011). Full genome and transcriptome sequencing and analyses, along with complementary DNA (cDNA) microarrays and quantitative real-time polymerase chain reaction (qPCR), have opened the doors for discovery of immune-related genes, especially in non-model organisms. At this time, there are certainly gaps in our knowledge regarding how and when these pathways are controlled and the downstream and phenotypic effects of many of these pathways. However, the aforementioned resources have primed the field of cnidarian immunology to better piece together immune pathways and begin placing them into the context of coral disease ecology.

As invertebrates, cnidarians have to rely on the innate arm of immunity (Mydlarz et al. 2006; Palmer and Traylor-Knowles 2012) and do not appear to have the ability to develop resistance to a particular pathogen. In essence, they must use the same arsenal to repeatedly fight pathogens and prevent infections. However, currently the classic dichotomy between innate/no memory and adaptive immunity is being challenged as evidence of immune memory is found in cnidarians (Netea et al. 2011). To date, two lines of evidence support the existence of memory in the cnidarian immune system. For one, tissue transplantation of conspecifics and rejection among heterospecifics has long been attributed to immunological memory (Rinkevich 2004). A caveat here is that the mechanisms behind allorecognition may differ from those of 'training' an immune system. The second line of evidence is the discovery of many classes of receptors that recognize molecular patterns of different pathogens (Netea et al. 2011). Recognizing the difference between pathogen types and responding with the appropriate binding receptor seems like the first step in acquiring memory to that pathogen. In fact, heightened immune responses to a second exposure of the same or different pathogen is seen in other invertebrate models (Netea et al. 2011).

Cnidarian immunity is comprised of four categories of processes that enable the organism to recognize that it is under attack, kill the foreign perpetrator, and repair the wound. There are four major functions of the cnidarian immune system: (1) immune recognition, (2) intracellular signaling, (3) effector response and (4) tissue repair. The first step, recognition of a pathogen, leads to the activation of the immune system. This is accomplished by membrane bound receptors that bind directly to molecular patterns in pathogens (Rast and Messier-Solek 2008). For example, pattern recognition receptors, or PRRs, recognize pathogen/microbe-associated molecular patterns (PAMPs), such as lipopolysaccharides (LPS) from bacteria or β-glucans from fungi, and transmit the signal internally. The signal transduction mediators, signaling molecules and transcription factors that communicate the signal within the cell, comprise the intracellular signaling component of immunity (Fig. 28.1). The endpoints of the signaling cascades are the proteins and molecules that kill the pathogen. These can comprise antibacterial proteins and small molecules, reactive oxygen and nitrogen molecules, and cellular activity and barriers. These effectors can be variable and novel among taxa (Rast and Messier-Solek 2008). Finally, the organism must seal off the infection and detoxify the tissue in order to heal the wound.

There are several unique aspects of cnidarian and anthozoan physiology that influence immunity and health of the organism. Many cnidarians live with intracellular dinoflagellate symbionts from the genus *Symbiodinium* and extracellular microbial communities that reside in a host-derived mucus layer. This entire group of organisms (cnidarian host, algal symbionts and microbial associates) is typically referred to as a holobiont (Rosenberg et al. 2007). This term has been used primarily in anthozoans, especially for reef-building scleractinian corals, but can be applied to many species of cnidarians and other taxa. Although the host is thought to be the primary contributor to cnidarian immune responses, there is mounting evidence for the important roles that the symbiont algal and microbial communities play. For example, the mechanisms used by the cnidarian host to establish and maintain these symbioses include many immune processes used to protect the host from pathogens. Thus, these components are of great interest when considering the immune system of cnidarians.

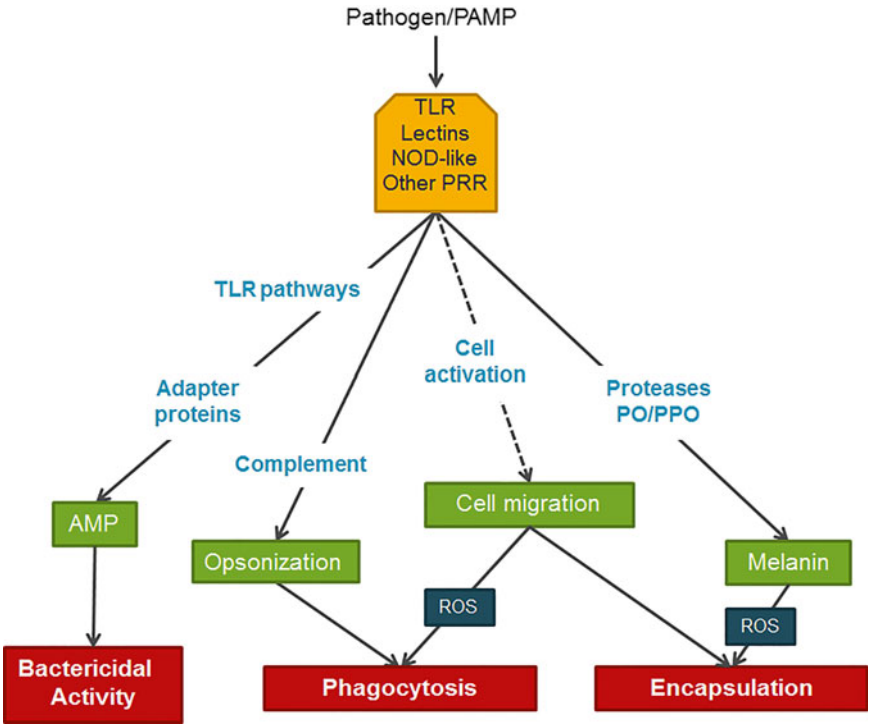

Fig. 28.1 General scheme of signaling pathways (*blue font*) and related effector responses (*green* and *red boxes*) in cnidarians. *Dashed line* at Cell Activation indicates that not much is known about signaling controlling cell migration and phagocytosis in this group

The health of the cnidarian holobiont relies on balance within these delicate symbioses and disruption of the symbiosis can lead to coral death. Breakdown of the symbiosis between the dinoflagellate symbiont and the host is referred to as bleaching. Bleaching can occur when the holobiont is stressed due to elevated temperature, excess ultraviolet radiation or pathogens (Baird et al. 2009). Additionally, shifts in the microbial communities of corals as a result of abiotic stressors (temperature, dissolved organic carbon, pH and nutrients) can lead to an increase in disease causing pathogens. Many of the cnidarian host responses to bleaching and shifting microbial communities can elicit immune responses, and we acknowledge the overlapping mechanisms throughout this chapter.

This chapter summarizes the current state of knowledge of cnidarian immune pathways and mechanisms by guiding the reader through the steps of initiating and executing an immune response as explained above. We draw examples from the presence of constitutive immunity observed in healthy organisms and elicited immune responses seen in natural infections or experimental exposures to live pathogens or PAMPs. We explore the functionality and expression/regulation of known immune pathways and their relation to wide variation, within and between species, in disease resistance observed in nature. The overarching goal of this chapter is to take the reader from a genomic to phenotypic perspective, while discussing pathways and mechanisms within a whole organism and ecological context, whenever possible.

28.2 Immune Recognition in Cnidarians

The activation of innate immunity in both vertebrates and invertebrates depends on pathogen recognition by various families of proteins known collectively as pattern recognition receptors, or PRRs. The term PRR is used to describe a diverse family of proteins that function in the recognition of potential pathogens by binding extracellular domains to proteins or other compounds characteristic of bacterial, fungal, protist, or parasitic pathogens (Akira et al. 2006). The cnidarian immune system is believed to include three main families of PRRs: Toll-like receptors (TLRs), lectins, and nucleotide oligomerization domain (NOD)-like receptors (NLRs) (Miller et al. 2007; Schwarz et al. 2007, 2008b; Shinzato et al. 2011). Here we will discuss what is known of the presence and function of these three PRR groups across the Cnidaria as well as their expression patterns and importance to cnidarian immunity.

28.2.1 Toll-Like Receptors (TLRs)

Toll-like receptors (TLRs) are known to play an important role in the activation of innate immunity in many species (Aderem and Ulevitch 2000; Akira and Takeda 2004). These receptors are glycoproteins embedded within the host membrane which possess domains specific for binding to unique molecular patterns of microbes/pathogens, commonly

referred to as pathogen-associated molecular patterns, or PAMPs, as the ligand.

There is an abundance of genomic data suggesting the presence of a wide repertoire of TLR type receptors in the Cnidaria. Binding of TLRs to PAMPs induces activation of both inflammatory and antimicrobial immune responses (Medzhitov 2001). Many TLR type proteins have been predicted from *Nematostella vectensis* genomic data (Miller et al. 2007). These include NvTLR-1, which is closely related to known Toll/TLR proteins, and three Toll/interleukin-1 (TIR) receptor proteins with immunoglobulin (Ig) domains, NvIL-1R1, NvIL-1R2, and NvIL-1R3. Finally, *Nematostella vectensis* also contains a MyD88 protein homolog, which functions in downstream transmission of TLR signaling (Miller et al. 2007).

In the gorgonian soft coral, *Gorgonia ventalina*, transcriptomic analysis contains annotations for three TLRs (TLR-2, -4, and -6; Burge et al. 2014). In scleractinian corals, several TLR type proteins were initially described from the genus *Acropora*. These acroporid TLRs are similar in sequence to NvIL-1R1, but they currently lack domain description (Miller et al. 2007). Subsequent genomic studies of *Acropora digitifera* have resulted in the discovery of many more putative TLR proteins (Shinzato et al. 2011) and the most recent studies have determined that up to 27 TIR proteins may be present in certain lineages of scleractinian corals (Poole and Weis 2014). Despite this knowledge, the exact functions of TLR proteins in corals and anemones are still not well understood. It can be assumed, based on sequence similarities, that cnidarian TLRs participate in recognition and activation of the immune system.

While components of TLRs have been detected in cnidarians, experimental evidence documenting their role in cnidarian immunity is still emerging. Two studies have documented conflicting patterns of TLR expression in cnidarians exposed to immune challenge (Burge et al. 2013; Libro et al. 2013). While the transcriptome of the sea fan *Gorgonia ventalina* includes annotations for three Toll-like receptors (TLR-2, -4, and -6), none of these were differentially expressed in response to immune challenge with the pathogen *Aplanochytrium* (Burge et al. 2013). In contrast, *Acropora palmata* displaying symptoms of White Band Disease show higher expression levels of two TLR-2 transcripts than do corals with signs of disease (Libro et al. 2013).

In contrast to *Nematostella*, *Gorgonia ventalina*, and acroporids, relatively few TLR proteins have been found in hydractinians. The *Hydra magnipapillata* genome includes two TIR domain-containing proteins, Toll-receptor-related (HyTRR)-1 and HyTRR-2 (Miller et al. 2007; Bosch et al. 2009) Both proteins contain an extracellular domain lacking characteristic leucine-rich repeats (LRRs). However, additional transcripts containing TLR-associated LRRs, HyLRR-1 and HyLRR- 2, have been discovered in the *Hydra magnipapillata* genome. HyLRR-2 is reactive to flagellin in the presence of human TIR domains, and is capable of modifying expression of HyTRR-1. Therefore it has been concluded that HyLRR-2 likely acts in tandem with HyTRR-1 to bind bacterial flagellin and induce innate immunity in *Hydra magnipapillata* (Bosch et al. 2009). TLRs in *Hydra*, particularly the combination of HyTRR-1 and HyLRR-2, are likely important in recognition of bacterial pathogens and activation of immunity.

Additional TIRs have been identified in a variety of cnidarians (Poole and Weis 2014). TIR domains have been identified in three anemone species, *Anthopleura elegantissima*, *Aiptasia pallida* (Lehnert et al. 2012), and *Nematostella vectensis* (Putnam et al. 2007), and six coral species, *Acropora digitifera* (Shinzato et al. 2011), *Acropora millepora* (Moya et al. 2012), *Fungia scutaria* (Poole and Weis 2014), *Montastraea cavernosa* (Poole and Weis 2014), *Pocillopora damicornis* (Traylor-Knowles et al. 2011), and *Seriatopora hystrix* (Poole and Weis 2014).

Even though TLR pathways are evolutionarily basal and present in most cnidarians, some lineages (*Hydra magnipapillata*) have since lost many major components of the pathways (Miller et al. 2007; Bosch et al. 2009). Other genomic studies have found the number of TLR and TIR proteins present in cnidarians to be lineage specific (Poole and Weis 2014); however, it is difficult to determine whether observed patterns are the result of gene expansion or gene loss (Miller et al. 2007; Bosch et al. 2009). Regardless of the mechanism of origin, it is believed that the wide variety of TLR proteins present in many coral genomes is important for the maintenance of the diverse microbial communities associated with corals (Poole and Weis 2014). Further experimental data will likely clarify the mechanisms driving TLR diversification between cnidarian lineages as well as the exact roles of Toll/TLR recognition in cnidarian immunity.

28.2.2 Lectins

Lectins are carbohydrate-binding proteins that act as important pattern recognition receptors (PRRs). Different classes of lectins have been described in cnidarians (Schwarz et al. 2007; Wood-Charlson and Weis 2009; Hayes et al. 2010; Kvennefors et al. 2010). Binding of lectins to potential pathogens is believed to trigger activation of the complement cascade and opsonization (Dunn 2009). Here we will focus on four main types of cnidarian lectins (tachylectin-2, millectin, rhamnospondin, and C-type lectins) and their roles in the activation of immune responses.

Tachylectin-2 was originally isolated from the Japanese horseshoe crab, *Tachypleus tridentatus*, and is capable of binding to N-acetylglucosamine and N-acetylgalactosamine

(Beisel et al. 1999) and agglutinating bacteria during infections (Kawabata and Iwanaga 1999). Tachylectin-2 homologues have been well documented as PRRs in multiple lineages of octocorals, scleractinians, and hydractinians (DeSalvo et al. 2008; Hayes et al. 2010; Burge et al. 2013). Tachylectin-2 expression increased in *Gorgonia ventalina* following inoculation with the parasite *Aplanochytrium* (Burge et al. 2013). Furthermore, tachylectin-2 homologues have been identified in the scleractinian coral *Orbicella* (formerly *Montastraea*) *faveolata* (DeSalvo et al. 2008) and from the genus *Oculina* (Hayes et al. 2010). Interestingly, the *Oculina* homologue shows high amino acid diversity and a strong positive selection signal, indicating that it is not only part of the immune system, but is also undergoing rapid diversifying selection. This rapid selection indicates adaptive evolution, potentially as a result of rapidly changing microbial challenges (Hayes et al. 2010). Finally, *Hydractinia echinata* also contains a tachylectin-2 homologue gene named CTRN. However, no change in expression of CTRN was detected following exposure to LPS, suggesting that, although similar in sequence to immune functioning tachylectins, CTRN may not play a role in pathogen recognition (Mali et al. 2006).

Millectin, a mannose-binding lectin, has been well described in *Acropora millepora* as an important PRR and elicitor of immune response. Millectin is capable of binding and agglutinating a wide variety of Gram-positive and Gram-negative bacteria (Kvennefors et al. 2008). Millectin also shares a high degree of sequence similarly with other animal C-type lectins known to have immune functions. Due to these attributes, it is believed that millectin both recognizes and initiates opsonization and phagocytosis of potential pathogens (Kvennefors et al. 2010). Interestingly, the sensitivity of millectin depends upon the type of PAMP encountered. Millectin can exist in many different isoforms, enabling it to bind to a wide range of pathogens, thus increasing immune system efficiency (Kvennefors et al. 2008). Millectin is expressed in both nematocysts and the gastroderm, therefore coral pathogens are likely exposed to this PRR at some point (Kvennefors et al. 2010). Its diverse sensitivity, localization and expression, makes millectin a primary PRR and elicitor of immune response in *Acropora millepora*.

Several cnidarians express at least one rhamnose-binding lectin (RBL) gene, rhamnospondin, which contains multiple PRR-type domains (Lopez et al. 2011). The RBL domain of the rhamnose-binding lectin recognizes rhamnose, a component of the cell walls of some Gram-negative bacteria, and LPS in some Gram-positive bacteria. Expressed mainly in the mouth of *Hydractinia symbiolongicarpus*, rhamnospondin could prevent potential pathogenic bacteria from entering the organism through opsonization or agglutination, although there is no experimental evidence to support this (Schwarz et al. 2007). Since rhamnospondin and millectin are both highly expressed in areas that come into contact with the environment, they may serve homologous functions as the primary PRRs preventing infection in *Hydractinia symbiolongicarpus* and *Acropora millepora*, respectively.

In addition to the three main lectins described above, experimental and genomic studies have resulted in the discovery of a number of other potential C-type lectins (CTLs) in cnidarians. The *Nematostella vectensis* genome encodes a diversity of CTLs, many of which have extracellular domains suggesting their role in recognition and immune function (Reitzel et al. 2008; Wood-Charlson and Weis 2009). Furthermore, in *Acropora palmata* colonies infected with White Band Disease, three CTLs (C- type mannose receptor 2, macrophage lectin 2, and collectin-12) exhibited greater expression than in coral colonies with no signs of disease. These three CTLs can bind to a variety of bacterial, viral, and fungal compounds and likely trigger activation of innate immunity (Libro et al. 2013). While not well understood, it is likely that cnidarian CTLs function in recognition and activation of immune responses in a manner similar to the other lectins, tachylectin-2, millectin, and rhamnospondin.

Cnidarians possess a diverse and lineage-specific repertoire of lectins capable of recognizing pathogens and activating innate immunity. Localization of expression of some of these genes further suggests that lectins may serve as the first line of defense against pathogens in many cnidarians (Schwarz et al. 2007; Kvennefors et al. 2010). While lectins are essential to innate immunity in cnidarians, further research is necessary to understand the mechanistic basis of the involvement of these genes in activation of immunity.

28.2.3 NOD-Like Receptors

Nucleotide oligomerization domain (NOD)-like receptor (NLR) proteins are a type of PRR in vertebrates characterized by the presence of three domains: a NAIP, CIIA, HET-E and TP1 (NACHT) domain, one of three types of N-terminal effector domains, and a C-terminal leucine rich repeat (LRR) domain. While the genomes of several cnidarians have been found to contain NLR-like genes, the diversity of these genes is highly variable across the different lineages (Lange et al. 2011; Hamada et al. 2013). For example, very few identifiable NLRs were found in the *Nematostella vectensis* genome (Hamada et al. 2013). In contrast, the *Acropora digitifera* genome includes nearly 500 putative NLR loci that include NODs, one of the highest numbers among sequenced animal genomes. Furthermore, these putative NLRs have a wider diversity of protein domains compared to those seen in vertebrates (Hamada et al. 2013). In *Acropora digitifera*, the NACHT domain contains proteins with novel domain structures not seen in other organisms (Shinzato et al. 2011). The *Acropora millepora* genome also contains an unexpected

number of NOD proteins, many of which have unique domain combinations (Lange et al. 2011).

Similar to *Nematostella*, *Hydra* appears to lack a wide diversity of NLR genes. Those NLRs that have been identified lack functional LRRs. Instead, NLRs in *Hydra magnipapillata* may function as a combination of two distinct proteins, the second of which contains a necessary LRR. A *Hydra* NLR protein has been shown to be capable of interacting with DD-Caspase, suggesting that it may be involved in the induction of apoptosis or downstream immune pathways (Lange et al. 2011). The lack of NLR diversity in *Nematostella* and *Hydra*, particularly in comparison to scleractinian corals, is still poorly understood. One hypothesis suggests that the diversity of NLRs in corals may serve as an adaptation to account for a lack of adaptive immunity; however, that does not explain observed differences in diversity among cnidarian lineages, all of which lack adaptive immunity (Hamada et al. 2013). The need to maintain a diverse microbial community may have driven the expansion and subsequent diversity of NLRs (Hamada et al. 2013). Further genomic and experimental studies are necessary to understand the distribution of NLR diversity in cnidarians and to elucidate their function in immune activation.

28.2.4 Other PRR's

A variety of immunoregulatory receptors that do not fall into one of the three major categories previously discussed have also been documented in cnidarians through a variety of molecular methods. These include LRR candidate PRRs (Schwarz et al. 2008b) and a transforming growth factor β (TGF-β) receptor (Detournay et al. 2012). Four LRR proteins, which may be important PRRs, were identified in expressed sequence tags (ESTs) from *Orbicella faveolata* and *Acropora palmata*. These may serve roles in recognition of and response to microbial infection (Schwarz et al. 2008b). Additionally, the presence of these receptors suggests that cnidarians possess a variety of other miscellaneous receptors, which contribute to recognition as well as immune activation and regulation.

28.3 Immune Signaling in the Cnidarians

Critical to the receptor binding detailed above is transmission of the signal and activation of the responses that will result in a successful immune response. These pathways are associated with the receptors and include many of the intracellular domains that propagate the signal though the cell. Some pathways are matched with specific receptors, whereas some are activated downstream from many potential sources. The TIRs introduced above, the mitogen-activated protein kinases (MAPK), the prophenoloxidase cascade and complement will be discussed in this section.

28.3.1 TLR Pathways and TIR Domains

Intracellular toll/interleukin-1 receptor (TIR) domains are found in several proteins associated with innate immunity signaling pathways. TIR domains are part of three classes of Toll receptor families: IL-1Rs, TLRs Myd88-dependent, and TLRs Myd88-independent (Poole and Weis 2014). These signaling pathways are most commonly initiated by cytokines or PAMPs. Upon activation, TIR proteins will employ downstream signaling and activate transcription factors. Transcription factors can then induce a variety of other pathways, including the melanin synthesis pathway and transforming growth factor beta (TGF-β) pathway (Medzhitov 2001).

As described above, several components of the Toll pathway have been identified in cnidarians. Four TIR domain-containing proteins were identified by Poole and Weis (2014), including TLR-like, IL-1R-like, Myd88 and TIR-only proteins. IL-1R-like and Myd88 proteins were identified in three anemone species, and in both Indo-Pacific and Caribbean scleractinian corals. However, no TIR domain-containing proteins have yet to be identified in gorgonians, although they are likely to exist (Miller et al. 2007). Myd88 proteins have also been identified in the freshwater hydrozoan, *Hydra magnipapillata*, in which they have been shown to be highly involved in bacterial recognition (Franzenburg et al. 2012).

One of the most commonly described transcription factors associated with Toll pathways in cnidarians has been the nuclear factor kappa light chain (NF-κB) transcription factor signaling pathway. NF-κB activation alters expression of target genes, including innate immune factors such as cytokines or antimicrobials (Hayden and Ghosh 2004). NF-κB gene homologs and proteins have been characterized in the anemone *Nematostella vectensis* (Wolenski et al. 2011) and identified in the scleractinian *Acropora millepora* and the hydrozoan *Hydra magnipapillata* (Miller et al. 2007). The role of NF-κB as an important immunity transcription factor has been demonstrated in *Hydra*, in which exposure to flagellin caused up-regulation of NF-κB (Bosch et al. 2009). However, functionality of the NF-κB transcription factor in relation to immunity has yet to be demonstrated in other cnidarian systems.

28.3.2 MAPK/ERK Pathway and GTPase Immune-Related Proteins

The multi-tiered kinase pathway consisting of mitogen-activated protein kinases (MAPK) and extracellular signal-regulated kinases (ERK) have also been identified in

cnidarians as a component of TLR pathways (Miller et al. 2007). MAPK cascades direct many cellular responses and are likely involved in many of the immune pathways in cnidarians. To date, identified involvement and function of MAPK signaling have been limited to wound healing. In *Nematostella vectensis*, MAPK signaling-related genes were shown to be critical components of wound healing (Dubuc et al. 2014).

GTPases make up a large family of hydrolase enzymes that regulate a variety of cellular functions, including signaling and gene expression. GIMAPS, also known as immune-associated nucleotide-binding proteins, are a smaller family of GTPases. GIMAPs were once thought to only exist in vertebrates and play a role in T cell maturation (Filen and Lahesmaa 2010). Recently, however, several gene families of GIMAPs have been annotated in the transcriptome of the scleractinian coral *Acropora millepora*, where they are up-regulated when challenged with bacterial and viral PAMPs (Weiss et al. 2013). Unlike many of the other immune signaling pathways examined here, no GIMAP genes have been detected in either *Nematostella vectensis* or *Hydra magnipapillata*.

28.3.3 Prophenoloxidase Signaling Pathways

Melanin synthesis is a critical component of invertebrate immunity and is largely involved with pathogen encapsulation and wound healing. This cascade is initiated by a variety of PRRs, including TLRs and lectins, which subsequently trigger several proteolytic reactions that result in the synthesis of melanin. Upon activation, pro-phenoloxidase (PPO) enzymes are cleaved into phenoloxidases (PO) by trypsin-like serine proteases. POs then oxidize phenols in two steps: monophenols ➔ *o*-phenols ➔ *o*-quinones. Quinones are then polymerized to synthesize the final melanin product (Nappi and Ottaviani 2000).

Unlike many of the other signaling pathways, several components of the melanin synthesis cascade have been detected in cnidarians through both molecular and biochemical methods. Tyrosinase genes have been identified in the genomes of *Nematostella vectensis* and *Hydra magnipapillata* (Esposito et al. 2012) and a laccase-3 like gene was found in *Pocillopora damicornis* (Vidal-Dupiol et al. 2014). In addition, expression of a trypsin-like serine protease was highly up-regulated in *Acropora millepora* when challenged with bacterial and viral PAMPs (Weiss et al. 2013) and a gene homologous to a shrimp prophenoloxidase activating enzyme was also up-regulated in *Pocillopora damicornis* exposed to bacteria (Vidal-Dupiol et al. 2014). PPO/PO activity and deposits of melanin have been detected biochemically and/or histologically in many cnidarians, including sea anemones, gorgonian, alcyonacean and scleractinian corals, and hydrozoans (Palmer et al. 2008; Mydlarz et al. 2008; Mydlarz and Palmer 2011; Gimenez et al. 2014). Furthermore, Mydlarz and Palmer (2011) demonstrated that several classes of PO enzymes exist in scleractinian corals, including laccase, cresolase and catecholase, indicating the potential presence of different signaling cascades. In both gorgonian and scleractinian corals, higher PPO activity is found in infected versus healthy colonies (Mydlarz et al. 2008).

28.3.4 Complement Pathway

The complement pathway is a highly conserved component of innate immunity throughout metazoans. Activation of the pathway can occur through three different proteolytic pathways: classical, alternative, and lectin pathway. All pathways ultimately activate the complement protein (C3) complex to increase inflammatory responses such as phagocytosis, cell lysis, and coagulation. The lectin pathway has been most widely detected among cnidarians (Hemmrich et al. 2007). In this pathway, a lectin binds to a sugar on the pathogen, activating mannose-binding lectin associated serine proteases (MASPs), which then activate C2 and C4-like proteins to begin formation of the C3 complex (Endo et al. 2006). After the complex is formed, membrane-attack complex–perforin (MACPF) effector proteins are secreted to create a hole in the microbial membrane resulting in lysis of the pathogen.

In cnidarians, several components of the lectin complement pathway have been identified, including MASP, C3, and MACPF proteins. Mannose binding proteins have been found in the transcriptomes of *Nematostella vectensis* and two scleractinian corals, *Acropora millepora* and *Orbicella faveolata* (Brown et al. 2013; Pinzon et al. 2014a). Homologous genes for the C3 complex are found in the transcriptomes of numerous cnidarians, including the anemones *Nematostella vectensis* (Kimura et al. 2009) and *Aptasia pallida* (Sunagawa et al. 2009b), the octocorals *Swiftia exserta* and *Gorgonia ventalina* (Dishaw et al. 2005; Burge et al. 2013), and the scleractinian corals *Acropora millepora* (Miller et al. 2007) and *Orbicella faveolata* (Jorge et al. 2015).

Several additional complement effectors have been proposed and described among cnidarians. A gene matching human and abalone macrophage-expressed gene protein (MPEG-1) was identified in the *Nematostella* genome, but not in the *Hydra* genome (Miller et al. 2007). Although the function of MPEG in cnidarians has not been described, it is believed to represent an MACPF effector related to the complement system (Hemmrich et al. 2007). Toxic nematocyst venoms (TX-60A) from the anemones *Actineria villosa* and *Phyllodiscus semoni* belong to the MACPF protein family, form pores in target cell membranes and play roles in feeding

and antipredator defense (Nagai et al. 2002; Oshiro et al. 2004), but could also kill potential pathogens. Two *Nematostella vectensis* MACPF proteins, one *Acropora millepora* transcript related to this protein type, and Hy-Mac from *Hydra magnipapillata* have been identified (Hemmrich et al. 2007; Miller et al. 2007). In *Acropora millepora*, expression of two complement pathway components, C3 and mannose-binding C-type lectin, were up-regulated when colonies were challenged with bacteria (Brown et al. 2013). Additional pore-forming toxins have been isolated from hydrozoans, scyphozoans and other anemones (Narat et al. 1994; Otero-Gonzalez et al. 2010), although antimicrobial functions have not been described. One other *Hydra magnipapillata* MACPF-domain protein, Hy-Apextrin, has an ortholog in *Acropora millepora* but not in *Nematostella* and is related to protein families in echinoderms and protozoans, although its function in *Hydra* remains unknown (Hemmrich et al. 2007; Miller et al. 2011).

28.4 Effector Responses in Cnidarians

A diversity of effector responses has been identified in cnidarians. These include a surface mucus layer that inhibits access of pathogens to cnidarian hosts, and antimicrobial activity due to antimicrobial peptides and secondary metabolites, reactive oxygen species (ROS) released as byproducts of various metabolic processes in both the host and symbiont, antioxidants that protect the host from those processes, and compounds produced as intermediates in various signaling pathways (e.g., during melanization activation). Melanin, the final product of this activation pathway, has antimicrobial activity, and provides a mechanism to encapsulate foreign cells and enhance structural support for impaired tissue. Foreign cells are also encapsulated, phagocytosed and expelled by cells that migrate to areas of tissue damage or infection.

28.4.1 Surface Mucus Layer

The first line of defense against invading pathogens in sedentary cnidarians, including anthozoans and hydrozoans, is the surface mucus layer (SML), a dynamic polysaccharide protein lipid complex covering the animal's exterior (reviewed by Mullen et al. 2004; Brown and Bythell 2005). Mucous secretory cells are abundant in the epidermis and release mucus, which varies in composition among species. The mucus acts as a physical barrier, and the presence of cilia on the ectodermal cells aids in mucuciliary transport of particles, including foreign cells, towards the polyp mouth where they can be digested, or removed by sloughing of the mucus layer. In general, gorgonians have fewer mucous secretory cells than scleractinian corals, however, cilia on epidermal cells, mesenterial filaments, and the pharynx may also produce currents to remove wastes and potential foreign cells. Mucus production can vary in response to various stressors, including disease. Reneger et al. (2008) observed an increase in the size of mucocytes in *Siderastrea siderea* tissue affected by Dark Spot Syndrome.

The SML also serves as a nutrient source for diverse microorganisms, which can reach densities orders of magnitude higher in the mucus than in the surrounding seawater (Ritchie and Smith 1995; Krediet et al. 2009, 2013). The SML represents the natural coral surface microbiota, but may also include pathogenic species. Putative pathogens can take advantage of coral mucus as a nutritional resource and outcompete other commensals (Krediet et al. 2009). The surface microbial communities of healthy and diseased conspecifics vary considerably, representing not only changes in abundance of pathogens, but also in potential competitors and opportunistic commensals (Santavy 1995; Pantos et al. 2003; Gil-Agudelo et al. 2006; Bourne et al. 2008; Sunagawa et al. 2009a; Garcia et al. 2013; Meyer et al. 2014).

The ability of the cnidarian to control production and composition of the SML and its associated bacteria may serve as an important part of immunity. Antibacterial compounds produced by the cnidarian host, as well as its associated microbial consortium, are released into the mucus and play important roles in controlling the mucus-associated microbial community (Brown and Bythell 2005; Reshef et al. 2006; Ritchie 2006; Rosenberg et al. 2007; Shnit-Orland and Kushmaro 2009; Rypien et al. 2010; Krediet et al. 2013). In addition, the presence of quorum sensing molecules within the mucus also point to mechanisms by which the bacteria themselves can control the microbial community structure (Tait et al. 2010; Golberg et al. 2011). Quorum sensing molecules and activities have been detected in the polymicrobial consortium associated with Black Band Disease (Zimmer et al. 2014) and in gorgonian corals (Hunt et al. 2012).

28.4.2 Antimicrobial Activity

28.4.2.1 Antimicrobial Peptides

Antimicrobial peptides (AMPs) are a vital component of the innate immune system in both vertebrates and invertebrates, and several representatives have been identified in cnidarians (Zasloff 2002). AMPs are typically cationic membrane-bound peptides and have bactericidal activity against a diversity of microbes, largely due to their ability to disrupt microbial cell membranes. AMPs are often inducible and can be translocated where needed (Vidal-Dupiol et al. 2011).

The first AMP reported from a scleractinian coral is damicornin (Vidal-Dupiol et al. 2011), a compound of similar

structure to the jellyfish AMP aurelin that exhibits antibacterial activity against Gram-positive and Gram-negative bacteria (Ovchinnikova et al. 2006).

Damicornin is constitutively transcribed in ectodermal granular cells in *Pocillopora damicornis*, where it is stored, probably as a precursor molecule subject to post-translational processing following immune challenge. In response to non-pathogenic *Vibrio coralliilyticus* (which is non-virulent at 25 °C), damicornin is released into the coral's mucus. When exposed to virulent *Vibrio coralliilyticus* (at 28–32 °C), damicornin gene expression increased transiently at day 6 and was later repressed. Damicornin exhibited antimicrobial activity against Gram-positive bacteria and a fungus, although not against Gram-negative bacteria, including the coral pathogens *Vibrio shiloi and Vibrio coralliilyticus* (Vidal-Dupiol et al. 2011). More recently, mytimacin-like and LBP-BPI (lipopolysaccharide-binding protein and bactericidal/permeability-increasing protein) genes, which code for AMPs in other invertebrates (Krasity et al. 2011; Gerdol et al. 2012) were reported in *Pocillopora damicornis* (Vidal-Dupiol et al. 2014), and these were down-regulated in response to thermal stress and exposure to pathogens.

Three families of AMPs have been identified in *Hydra* by a combination of biochemical methods and by mining existing genomes (reviewed in Augustin et al. 2010; Bosch 2012, 2014). In *Hydra* spp., AMPs are sequestered into the epithelium and pathogen invasion causes increased expression of genes encoding for AMPs, which induces their production (Bosch et al. 2009; Augustin et al. 2010). Exposure to a culture filtrate of *Pseudomonas aeruginosa*, a potent immune inducer containing LPS and flagellin, induced expression of Hydramacin-1 (Bosch et al. 2009), which exhibits strong antibacterial activity against a diversity of human pathogens (Bosch et al. 2009; Jung et al. 2009). Hydramacin-1 promotes bacterial aggregation at low concentrations, representing the initial step of its bactericidal mechanism, which includes permeabilizing membranes of viable bacteria, although the bacteria die with intact cell membranes, suggesting that programmed cell death may be induced (Jung et al. 2009). However, Hydramacin-1 did not inhibit *Pseudomonas aeruginosa*, even though *Hydra* spp. tissue extract is active against this bacterium, suggesting that additional components of the *Hydra* tissue extract also possess antibacterial activity (Bosch et al. 2009).

The genomes of *Hydra* spp. contain genes for numerous arminins (Franzenburg et al. 2013). The first of these described was arminin 1a, an AMP with no sequence homology in other taxa (Augustin et al. 2009). Arminin 1a has strong broad-spectrum activity against human drug resistant pathogens, and kills bacteria by disrupting the bacterial cell wall without harming eukaryotic cells (Augustin et al. 2009). The expression of other arminims is species-specific (Franzenburg et al. 2013). Using arminin knockouts, Franzenburg et al. (2013) demonstrated that arminins function as species-specific AMPs, enabling *Hydra* spp. to control their associated bacterial communities.

The periculins represent a third group of AMPs in *Hydra* spp. with no known orthologs to other AMPs. Periculin-1 responds rapidly to bacterial flagellin and LPS, both of which cause a dose-dependent increase (Bosch et al. 2009). Periculin-1 may also respond to viral inducers (Bosch et al. 2009). Its strong bactericidal activity suggests that periculin is involved in *Hydra* spp. host defense (Bosch et al. 2009). Like the arminins, Perculin-1a also reduces overall bacterial load in embryonic *Hydra vulgaris*, and its strong selectivity against certain bacterial species highlights its importance in controlling bacterial community structure (Fraune et al. 2010). At least five members of the periculin family have been identified in *Hydra* spp. (Fraune et al. 2010).

28.4.2.2 Antimicrobial Secondary Metabolites

Antimicrobial secondary metabolites, also called "small molecules" or "natural products" are vital to the survival of sessile marine invertebrates. The need to protect surfaces from colonization by fouling organisms and pathogens is a critical role of these compounds (reviewed in Slattery and Gochfeld 2012). Although largely studied from a drug discovery perspective, recent studies have taken a more ecological approach to evaluating the roles of chemical extracts and, in some cases, purified compounds, within their host organisms or against ecologically relevant microbes. Unfortunately, little is known about the receptor mediated activation and signaling pathways associated with the biosynthesis of these compounds in cnidarians.

Whereas anemones produce highly toxic venoms that are released by their nematocysts and are important in feeding and providing protection from predators and competitors, these compounds probably play minimal roles in defense from microbial pathogens (Bosch 2008). However, toxic nematocyst venoms from the anemones *Actineria villosa*, *Phyllodiscus semoni*, and *Stichodactyla helianthus* include a class of compounds called the actinoporins, which form pores in target cell membranes and are suspected to be responsible for killing prey and deterring predators (Narat et al. 1994; Nagai et al. 2002; Oshiro et al. 2004; Otero-Gonzalez et al. 2010), but could also kill potential pathogens. Mucus and tissue extracts from anemones have shown only limited antimicrobial activity against microbes (Williams et al. 2007; Gunasundari et al. 2013), whereas mucus from the surface of *Metridium senile* stimulated the growth of 11 marine bacterial strains, suggesting that antibacterial compounds are not an effective defense against pathogenesis in this species (Astley and Ratcliffe 1989).

In addition to broad surveys of antimicrobial activity in gorgonians (reviewed in Slattery and Gochfeld 2012), some studies have targeted particular gorgonian pathogens.

Nonpolar extracts of the sea fans *Gorgonia ventalina*, *Gorgonia flabellum* and 13 other Caribbean gorgonians inhibited spore germination of *Aspergillus sydowii*, the fungal pathogen of sea fans, at natural concentrations (Kim et al. 2000a, b; Dube et al. 2002). However, since *Gorgonia* spp. are the known hosts of *Aspergillus sydowii*, this suggests that other sources of immune function are necessary to inhibit pathogenesis. Extracts from *Gorgonia marinae*, which is less susceptible to aspergillosis than either of its conspecifics, had the strongest antifungal activity against *Aspergillus sydowii* (Mullen et al. 2006).

Because of their greater interest in the pharmaceutical arena, more effort has been directed towards identifying specific compounds associated with antimicrobial activity in the soft corals than in other cnidarians. *Sinularia flexibilis* produces the antibacterial diterpenes sinulariolide and flexibilide, which inhibit the growth of Gram-positive bacteria associated with human health (Aceret et al. 1998). The Antarctic soft coral *Gersemia antarctica* releases potent antimicrobial compounds into the surrounding seawater (Slattery et al. 1997). The most active of these compounds was identified as homarine, which is widespread in marine invertebrates, including cnidarians (e.g., gorgonians *Leptogorgia virgulata*, *Leptogorgia setacea* and *Eunicella sinuglaris*, hydrozoan *Tubularia crocea*, and scleractinian corals *Montipora capitata* and *Porites* spp.; Affeld et al. 2006; Targett et al. 1983; Maldonado et al. in review). In addition to *Gersemia antarctica*, homarine also exhibits antibacterial activity in *Leptogorgia virgulata*, *Montipora capitata* and *Porites* spp. (Shapo et al. 2007, Gochfeld unpublished data).

There is evidence that some of these antimicrobial metabolites are inducible during infections. Kim et al. (2000b) found antifungal activity to be higher in tissues closest to aspergillosis lesions and in diseased vs. healthy *Gorgonia ventalina* colonies. Antifungal activity also increased in *Gorgonia ventalina* following experimental inoculation with the fungal pathogen *Aspergillus sydowii*, providing strong evidence for an inducible defense (Dube et al. 2002). However, in broader geographic surveys, antifungal activity did not correlate with sea fan disease status or history, suggesting selection for resistance over time (Dube et al. 2002; Kim and Harvell 2004; Couch et al. 2008).

In addition, stimulation of immune effectors by disease can affect the production of other types of chemical defenses. For example, in the sea fan *Gorgonia ventalina*, the feeding deterrent compound julieannafuran decreased following fungal infection, resulting in increased levels of predation on affected fans (Slattery 1999). Likewise, colonies of *Sinularia* spp. affected by *Sinularia* Tissue Loss Disease were more palatable to butterflyfishes than healthy conspecifics (Slattery et al. 2013), suggesting a reduction in defenses in these corals.

Antimicrobial activity in scleractinians has received far less attention than in soft-bodied sessile taxa, largely due to the supposition that their hard calcareous skeletons precluded the need to synthesize chemical defenses. Clearly, that supposition undervalues the importance of immunity in scleractinian corals, and studies have demonstrated antimicrobial activity in scleractinian coral tissues, their mucus and in compounds released into the surrounding water. As in octocorals, broad surveys of extracts from scleractinian corals documented widespread but selective antibacterial activity (reviewed in Slattery and Gochfeld 2012). For example, aqueous extracts from 18 species of Caribbean corals (Pappas 2010; Gochfeld unpublished data) and 3 species of Hawaiian corals (Gochfeld and Aeby 2008) showed highly selective antibacterial activity against a panel of known coral pathogens, potential marine pathogens from human waste, and coral-associated bacteria. In contrast, Kelman (2009) found minimal antibacterial activity from nonpolar extracts of Red Sea corals, compared with widespread activity among the soft coral species tested. Some of these differences may reflect the overall greater antibacterial activity of aqueous extracts in the scleractinian corals, as compared to the more active nonpolar fractions in soft corals (Gochfeld unpublished data). Likewise, due to differences in extraction methods, antibacterial activity in protein extracts of corals may include activity due to AMPs (such as damicornin mentioned above), which would not necessarily be present in aqueous extracts. Protein extracts from tissues of the Caribbean corals *Orbicella faveolata*, *Stephanocoenia intersepta* and *Porites astreoides* also exhibit antibacterial activity (Mydlarz et al. 2009; Palmer et al. 2011b).

The isolation techniques and manipulation of the corals may have underestimated the antimicrobial compounds in scleractinian corals. For example, Geffen and Rosenberg (2004) described the rapid release of coral antibacterial compounds into the surrounding water following mechanical disturbance of several species of Red Sea corals. The release was fast and potent, killing Gram-negative and Gram-positive bacteria, including coral pathogens, within 1 min of release. In a follow-up study, antibacterial activity was released upon repeated disturbance, suggesting that it is constitutively present rather than inducible upon infection or damage (Geffen et al. 2009).

While antimicrobial activity now appears to be widespread in corals, there is significant intraspecific variability in activity due to a diversity of stressors, including disease, suggesting either that healthy corals remain healthy due to different constitutive levels of chemical defenses or that pathogenesis induces up- or down-regulation in the production of these defenses. Differences in antibacterial activity in extracts from colonies of *Siderastrea siderea* affected by Dark Spot Syndrome compared to healthy colonies may be related to differences in the concentrations of two uncharacterized compounds, suggesting the potential for induced antimicrobial defenses (Gochfeld et al. 2006; Pappas 2010).

Extracts from healthy colonies of *Montipora capitata* exhibited greater antimicrobial activity against certain bacteria than did healthy tissues on colonies affected by *Montipora* White Syndrome, suggesting that greater constitutive levels of antibacterial defense in healthy corals may protect them from pathogens (Gochfeld and Aeby 2008). In addition, extracts from diseased tissues on *Montipora* White Syndrome-affected colonies exhibited higher levels of antibacterial activity against the coral pathogen *Aurantimonas coralicida* than did healthy tissues on those diseased colonies, indicative of a locally induced defense (Gochfeld and Aeby 2008). Protein extracts from *Orbicella faveolata* colonies affected by Yellow Band Disease had higher levels of antibacterial activity in both healthy and infected tissues of diseased colonies than in healthy colonies, suggesting a systemic response to infection (Mydlarz et al. 2009).

Surprisingly, in spite of their toxicity towards humans, little is known about antimicrobial activity in hydrozoans beyond their reported AMPs. Two species of fire coral, *Millepora* spp., from the Red Sea exhibited minor antibacterial and antifungal activity against human, animal and plant pathogens (Al-Lihaibi 2005).

28.4.2.3 Other Antimicrobial Mechanisms

Protease inhibitors counteract the impacts of proteases produced by many pathogenic microbes, which aid in colonization of host tissue and evasion of host immune defenses. By inhibiting these processes, serine protease inhibitors reduce pathogen growth or kill them outright (Augustin et al. 2010). *Hydra magnipapillata* produces a kazal-type serine protease inhibitor, Kazal-2, which has potent anti-staphyloccocal activity (Augustin et al. 2009). *Gorgonia ventalina* produces protease inhibitors that are active against proteases secreted by the fungal pathogen *Aspergillus sydowii* (Mann et al. 2014).

Lysozyme-like activity in sea fans is facilitated by a small lysozyme-like protein commonly grouped with AMPs due to its small size and ability to hydrolyze the peptidoglycans in bacterial cell walls (Couch et al. 2008). Lysozyme-like activity was greatest in colonies of *Orbicella faveolata* affected by Yellow Band Disease (Mydlarz et al. 2009). Mucus from the anemone *Anthopleura elegantissima* also contains a lysozyme-like enzyme that lyses bacteria (Phillips 1963, cited in Mullen et al. 2004).

Chitinases are enzymes that hydrolyze chitin, a major component of fungal cell walls. Exochitinase is found in healthy *Gorgonia ventalina*, but only a transient increase in exochitinase activity was observed in response to fungal infection, suggesting that it serves primarily as a constitutive defense (Douglas et al. 2006). Exochitinase was released into the water surrounding the sea fan and did exhibit limited antifungal activity against *Aspergillus sydowii* (Douglas et al. 2006). Chitinases have also been reported from three species of hydrozoans, *Hydra attenuata*, *Hydra circumcincta* and *Podocoryne carnea* (Klug et al. 1984). The first chitinase cDNA from a cnidarian was cloned from *Hydractinia echinata* by Mali et al. (2004), and in-situ hybridization indicated localized expression of this gene in the ectoderm and hyperplastic stolons, which are induced during allorecognition of histoincompatible colonies, suggesting a potential function in immunity.

28.4.3 Cellular Responses

Maintenance of an intact epithelium layer is essential to protection from pathogens and other stressors. In contrast to the hydrozoans, anthozoans have circulating populations of amoebocytes that can migrate to sites of injury and phagocytose foreign cells resulting in exposure to proteolytic enzymes and free radicals (Mullen et al. 2004). Many cnidarians can mount an inflammation response, which consists of an attempt to destroy or isolate the source of damage and any affected host cells. This typically involves rapid infiltration of injured tissue by immune cells and initiation of phagocytosis. Phagocytic cells can engulf and destroy pathogens or other foreign cells, often with the aid of ROS or antimicrobial compounds produced by the phagocytes, or encapsulate foreign cells and thereby provide a barrier between affected and healthy tissue (Palmer et al. 2008). In cnidarians, phagocytosis is also a primary means of acquiring nutrition and of maintaining host-symbiont relationships (Mullen et al. 2004).

28.4.3.1 Mobile Cytotoxic Cells and Reactive Oxygen and Nitrogen

In the anemone *Actinia equina*, amoebocytes are stored in the mesenterial filaments. Whereas only hyaline amoebocytes were observed to phagocytose and kill Gram-negative bacteria, mixed cultures of hyaline and granular amoebocytes also produced antibacterial activity upon stimulation by LPS, likely via the production of superoxide anions and other ROS through an oxidative burst (Hutton and Smith 1996).

In gorgonians, immediately following injury, a significant increase in amoebocytes was observed in the immediate vicinity (<1 mm) of the damaged tissue, with a decline in amoebocytes in adjacent tissues, suggesting that amoebocytes migrate from adjacent tissues to the site of repair (Meszaros and Bigger 1999). Following tissue damage in *Swiftia exserta*, granular cells staining positive for peroxidase were associated with phagocytosis of foreign particles, but the population of phagocytic cells was not uniform, indicating that multiple cell types may be involved in this process (Olano and Bigger 2000).

Amoebocytes also play critical roles in the cellular response to disease in gorgonians. In the sea fan *Gorgonia ventalina*, an inflammatory response is characterized by massive increases in granular acidophilic amoebocytes.

Amoebocyte aggregation was associated with both naturally occurring infections and in the immediate area of experimental inoculations with the fungal pathogen *Aspergillus sydowii* (Mydlarz et al. 2008; Couch et al. 2013). However, there was no evidence that amoebocyte migration from immediately adjacent tissue was responsible for this increase (Mydlarz et al. 2008). Fungal hyphae infiltrate the gorgonin axial skeleton, where host-produced melanin is deposited, and melanosomes were detected in amoebocytes adjacent to these melanin bands in infected sea fans, pointing to granular amoebocytes as the sites of melanin synthesis. Since amoebocytes are responsible for phagocytosis and melanin production, they likely play a role in the antifungal activity observed in or adjacent to fungal lesions (Mydlarz et al. 2008).

In colonies of the hybrid soft coral *Sinularia maxima* x *polydactyla* affected by *Sinularia* Tissue Loss Disease, diseased tissue was also characterized by a proliferation of amoebocytes, which were frequently observed to envelop unidentified foreign eukaryotic cells (Slattery et al. 2013). Although not confirmed, these foreign cells may represent the disease agent, as all of these cells were engulfed by amoebocytes. Mesogleal amoebocyte area was also significantly increased in diseased tissue, although this appeared to be a localized response, as healthy tissue on diseased colonies did not differ in amoebocyte area from healthy colonies (Slattery et al. 2013).

In scleractinian corals, amoebocytes containing acidophilic granules have been observed in *Montastraea cavernosa* (Vargas-Angel et al. 2007), in which a rapid migration of granular amoebocytes was observed during tissue repair (Renegar et al. 2008). Chromophore cells infiltrating the calicoblastic epithelium in skeletal tissue anomalies of *Porites compressa* (Domart-Coulon et al. 2006) and melanin-containing granular cells in the epidermis or gastrodermis of several species of Indo-Pacific corals (Palmer et al. 2008, 2010) likely also represent amoebocytes.

An important role of mobile amoebocyte or phagocytic cells is the release of reactive oxygen and nitrogen species to aid in pathogen killing. Hydrogen peroxide as part of an oxidative burst has been identified in several gorgonian corals (Mydlarz et al. 2006), or as a constitutive release into the surrounding water (Shaked and Armoza-Zvuloni 2013; Armoza-Zvuloni and Shaked 2014). In a process called ETosis, chromatin is expelled from cell nuclei in response to an oxidative burst, LPS, or bacteria (Robb et al. 2014). To date, this has been studied in only a few invertebrates, but phagocytes in the mesoglea of the anemone *Actinia equina* produce ROS via a respiratory burst, which releases chromatin that may combine with the mucus to trap microbes, where they may come into contact with antimicrobial compounds or ROS that destroy them (Robb et al. 2014).

Nitric oxide synthase (NOS) has been measured in the sea anemone *Aiptasia pallida*, the scleractinian coral *Acropora millepora* and the soft coral *Lobophytum pauciflorum*, indicating that it is widespread among cnidarians (Safavi-Hemami et al. 2010). Nitric oxide (NO) was induced in response to LPS in *Aiptasia pallida* (Perez and Weis 2006), suggesting a potential role in response to pathogens.

Other cellular responses to infection have been observed in scleractinian corals. For example, zymogen granules were concentrated in the calicodermis in response to endolithic fungi associated with Dark Spot Syndrome in *Siderastrea siderea*, and mucocyte size increased in Dark Spot Syndrome-affected tissues (Renegar et al. 2008), suggesting the need for greater mucus production, a common sign of stress in corals. *Hydra* spp. do not have mobile phagocytes, and thus the epithelium serves as the sole center of the innate immune system, employing complex signaling pathways and defensive molecules, including AMPs (Bosch et al. 2009). However, upon exposure to culture filtrates of *Pseudomonas aeruginosa*, ectodermal epithelial cells of *Hydra magnipapillata* changed morphology and acquired a large number of granules, and endodermal epithelial cells were observed to engulf bacteria (Bosch et al. 2009).

28.4.3.2 Encapsulation

Scleractinians and gorgonians can also encapsulate invading microbes by increasing deposition of skeleton and proteinaceous organic material, such as collagen or gorgonin, to form a nodule surrounding the foreign organism (Mullen et al. 2004). Gorgonians may separate the host from the parasite by formation of a proteinaceous capsule (Goldberg et al. 1984). *Gorgonia ventalina* secretes gorgonin to surround invading algal filaments (Morse et al. 1977) and an unidentified parasite (Mullen et al. 2004). Scleractinians may exhibit accelerated growth in response to the presence of foreign organisms, such as epibionts or parasites (Aeby 2003; D'Angelo et al. 2012), which may encapsulate and attempt to minimize their damage to host tissues.

An increase in purple-pigmented calcium carbonate sclerites in gorgonians occurs in response to biotic stressors, including fungal infection (Alker et al. 2004; Mydlarz and Harvell 2007), and in response to predation (Puglisi et al. 2002). The purple pigment appears to be due to a carotenoid, some of which have antioxidant or antifungal activity (Leverette et al. 2008), but otherwise the function is unknown.

Although less studied than in corals, the melanin-synthesizing pathway has been found to occur in several species of anemones. The Argentinian anemones *Aulactinia marplatensis* and *Bunodosoma zamponii* exhibited PO activity in all tissues, and the high constitutive levels in *B. zamponii* were considered likely to provide resistance against disease (Gimenez et al. 2014). Upon exposure to the polymicrobial consortium associated with Caribbean Yellow Band Disease in corals, PPO activity in *Aiptasia pallida* polyps was up-regulated 8.5-fold over healthy polyps (Zaragoza

et al. 2014). To corroborate the end product of this pathway in anemones, melanin granules were observed in *Aiptasia pallida* following infection with *Serratia marcescens* (Zaragoza et al. 2014).

In *Gorgonia ventalina*, melanin is deposited as a localized band along the axial skeleton in areas adjacent to fungal hyphae, and acts both to strengthen the skeleton and to encapsulate the fungal hyphae so they are unable to penetrate surrounding tissue (Petes et al. 2003; Mullen et al. 2004; Mydlarz et al. 2008). An increase in PPO activity was observed in conjunction with this increase in melanin deposition and granular amoebocytes in fungal-infected tissues (Mydlarz et al. 2008; Couch et al. 2013). Melanin deposition in *Gorgonia ventalina* was also observed in response to an unidentified parasite (Mullen et al. 2004) and to aggregations of Labyrinthulomycetes (Burge et al. 2012). PO, PPO and melanin were found constitutively in colonies of the soft corals *Sarcophyton* sp. and *Lobophyton* sp., with *Sinularia* sp. having the highest level of enzyme activity (Palmer et al. 2010, 2012).

The melanin synthesis pathway is also active in scleractinian corals. Interspecific constitutive differences in immune responses may play a role in relative susceptibility to disease. For example, *Acropora millepora* had constitutive levels of PO that were half those of *Porites* sp., with no melanin deposition, whereas *Porites* sp. had higher constitutive levels of PO and an inducible melanin response. These factors may contribute to the greater susceptibility of *Acropora* spp. than *Porites* spp. to diseases (Palmer et al. 2008). A more extensive survey of scleractinian coral species from the Great Barrier Reef found that all contained PO, PPO and melanin-containing granular cells, and that these constitutive indicators of immunity, along with fluorescent protein concentration, were good predictors of relative susceptibility to bleaching and disease (Palmer et al. 2010, 2012). Likewise, *Porites astreoides* had significantly higher PPO activity and melanin concentration than *Orbicella faveolata* (Palmer et al. 2011b). Melanin concentration was significantly correlated with phylogeny at the species level in Caribbean corals and was higher in corals that experienced less disease pressure (Pinzon et al. 2014a).

Palmer et al. (2008) found up-regulation of PO activity in abnormally pigmented tissues of *Acropora millepora*, although no melanin deposition was observed, confirming the inducibility of PO activity in corals, but also indicating that PO activity does not necessarily lead to melanin deposition. In contrast, a fourfold increase in the density of granular cells containing melanin was observed in pigmented regions of *Porites* sp. colonies and assumed to be involved in encapsulation (Palmer et al. 2008). Melanin-containing granular cells are also present in the epidermis of pigmented tissues in *Porites* trematodiasis (Palmer et al. 2009b). These studies confirmed that melanin represents the biochemical content of the granular cells observed in anthozoan cellular responses (i.e., amoebocytes) to invasion. Since abnormally pigmented tissues are commonly observed in corals and are due to a diversity of causes, it appears that melanization is a generalized defensive response to localized stress.

Exposure to commercial LPS, in combination with elevated seawater temperature, induced a significant increase in PPO activity, but not melanin concentration, in *Stephanoecoenia intersepta* and *Porites astreoides*, although not in *Orbicella faveolata* (Palmer et al. 2011b). PO activity was significantly reduced in colonies of *Acropora millepora* affected by White Syndrome relative to healthy colonies, although PO activity in diseased colonies was induced at the border of the lesion (Palmer et al. 2011a). *Porites compressa* affected by *Porites* Bleaching and Tissue Loss Disease also showed a decline in melanin containing granular cell densities in affected tissues (Sudek et al. 2012).

28.5 Repair Mechanisms in Cnidarians

Pathogenic infections and physical injuries trigger cellular and tissue repair mechanisms in all organisms. These responses are critical for individual survival and species fitness (Reitzel et al. 2008). In cnidarians, responses to stressors and repair mechanisms are complex and dynamic (Olano and Bigger 2000), integrating several cellular components and events (Reitzel et al. 2008). Among the best characterized repair mechanisms are antioxidants, apoptosis, and wound healing (Fig. 28.2). These mechanisms are an essential part of an efficient immune response in Cnidaria (Wenger et al. 2014).

28.5.1 Antioxidants

Continuous production of exposure to oxygen radicals can result in oxidative stress (Tarrant et al. 2014), an imbalance between ROS production and antioxidant defenses (Sies 2000; Betteridge 2000; Lesser 2006). Common sources of ROS in the immune response include respiratory burst and other disruptions of cellular and physiological processes (Betteridge 2000; Landis and Tower 2005; Tarrant et al. 2014). High levels of ROS affect cellular integrity, lead to tissue damage, diminish whole organism defensive responses and/or cause death (Betteridge 2000; Palmer et al. 2009a; Tarrant et al. 2014). To limit ROS levels and prevent oxidative stress, the cell uses various enzymatic and non-enzymatic antioxidants (Halliwell 1994; Hawkridge et al. 2000; Kim et al. 2011; Tarrant et al. 2014). The enzymes superoxide dismutase (SOD), catalase and peroxidase play important roles in controlling free radical levels and preventing oxidative stress. SOD catalyzes superoxide oxidation to molecular

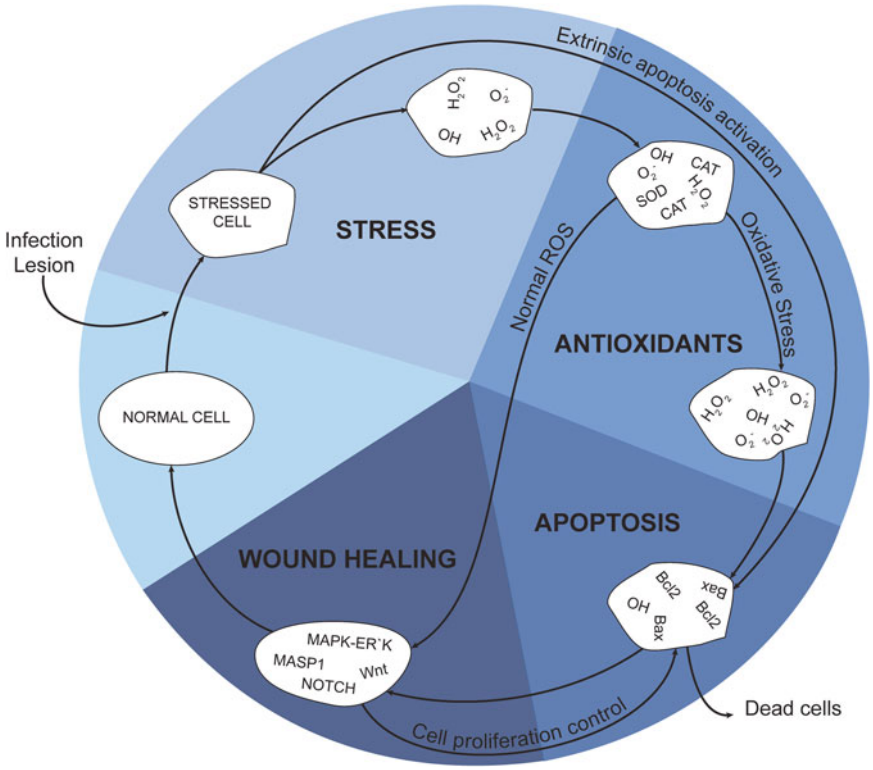

Fig. 28.2 Schematic representation of the steps involved in tissue repair and wound healing after a pathogen attack and lesion

oxygen and reduction to hydrogen peroxide, while catalases and peroxidases convert hydrogen peroxide to oxygen and water (Halliwell 1994; Betteridge 2000). Together, these three enzymes control ROS levels; however, during stress, up-regulation of heat shock proteins also contributes to regulating ROS and repairing cells (Feder and Hofmann 1999; Tarrant et al. 2014).

Through genome and transcriptome sequencing, many antioxidant genes have been identified in the Cnidaria. Antioxidant genes have been found in the sea anemones, *Nematostella vectensis* (Goldstone 2008; Reitzel et al. 2008) and *Aiptasia pallida* (Sunagawa et al. 2009b). An analysis of the *Nematostella vectensis* genome described the "defensome" (the battery of genes in charge of immune production), in which antioxidants play a key role in immune responses (Goldstone 2008). Two kinds of SOD (CuZnSOD and MnSOD) and several catalase genes were identified in the genomes and transcriptomes of the corals *Acropora digitifera* (Shinzato et al. 2011), *Acropora palmata* (DeSalvo et al. 2010) and *Orbicella faveolata* (DeSalvo et al. 2008). Antioxidant genes were also part of the immune responses of the sea fan *Gorgonia ventalina* to the unicellular parasite *Aplanochytrium* (Burge et al. 2013). Genes for catalase have been identified in *Hydra vulgaris* (Wenger et al. 2014). These sequences have helped characterize the protein structure of cnidarian SOD and catalases (Dash et al. 2007; Dash and Phillips 2012).

Protein activity levels of antioxidants, such as SOD, catalase and peroxidase, have been measured in many different species. *Anemonia viridis* has two kinds of SOD (CuZnSOD and MnSOD) enzymes, both of which are found in the ectoderm and the endoderm of this anemone. In the ectoderm, SOD activity is low and catalase activity is high, in an apparent discordance between the functions and relation between these enzymes (Richier et al. 2003). The reason could be that catalase acts against external stressors in addition to scavenging hydrogen peroxide produced by SOD (Merle et al. 2007). SOD and catalase activity were observed in 24 cnidarians from the Great Barrier Reef (Shick and Dykens 1985), and demonstrated a positive correlation across most of the collected species, implying that catalase activity is related to the production of hydrogen peroxide by SOD.

Hawkridge et al. (2000) used immunocytochemical techniques to establish the presence, location and distribution of SOD, catalase and glutathione peroxidase in the sea anemone *Anemonia viridis* and in the coral *Goniopora stokesi*. Antioxidants in these two cnidarians are associated with granular vesicles and accumulation bodies, indicating the conformation of peroxisomes (Hawkridge et al. 2000). Five peroxidase isoforms occur in the sea fan *Gorgonia ventalina* and were induced following experimental inoculation with the fungal pathogen *Aspergillus sydowii* (Mydlarz and Harvell 2007). Furthermore, peroxidase was localized in granular amoebocytes in the process of phagocytosis in the gorgonian *Swiftia exserta* (Olano and Bigger 2000). Peroxidase, along with other antioxidant enzymes, occurs constitutively in seven species of healthy Caribbean sclerac-

tinian corals (Mydlarz and Palmer 2011). Peroxidase activity varied at the genus and species level in Caribbean corals and was correlated with disease prevalence (Pinzon et al. 2014a).

There are several other antioxidant systems that may serve as supplemental systems when enzymatic antioxidant systems are overwhelmed. Fluorescent proteins (FPs) have multiple functions in anthozoan symbioses, primarily in photoprotection (Salih et al. 2000). With respect to immunity, their most important role is to scavenge hydrogen peroxide, and thereby contribute to the host's antioxidant defense (Palmer et al. 2009a). Coral tissue often develops abnormal pigmentation in response to injury or infection, and red FPs, which exhibit significantly greater total fluorescence and hydrogen peroxide scavenging activity, are often associated with this pigmentation response (Palmer et al. 2009a, b). FPs are locally induced in *Porites compressa* tissue harboring parasitic trematodes (Palmer et al. 2008), with the resulting pink tissue attracting coral-feeding butterflyfishes that consume the parasite and enable the coral polyps to recover (Aeby 1992).

Mycosporine-like amino acids and dimethylsulfoniopropionate represent other antioxidant molecules and mechanisms identified in cnidarians (Lesser 2006; Van Alstyne et al. 2006). Their roles have mainly been characterized in response to environmental stress, but like FPs, they may be important in immunity as supplemental antioxidant systems.

28.5.2 Apoptosis

Apoptosis is a key biological process important in many aspects of the development and functionality of all organisms, including defense against diseases (Zmasek et al. 2007; Di Pietro et al. 2009). In fact, as a testament to the critical role of apoptosis in immunity, some pathogens develop regulating factors to control apoptosis as a way to increase virulence (Hedrick et al. 2010). During apoptosis, old, damaged and/or infected cells are destroyed. Cell death starts with signaling recognition and activation of the initiation caspases, and is followed by the activation of executing caspases and cell deletion (Dunn et al. 2006). This process is highly conserved in nature and is regulated by pro- (e.g., Bax) and anti- (e.g., Bcl2) apoptosis proteins. Apoptosis-related proteins include NB-ARC, caspases, caspase recruitment domain (CARD), death domain (DD) and death domain effectors (DDE) (Zmasek et al. 2007). These proteins form the same complexes, with similar domain organization (i.e. CARD-NB-ARC-repeats) among cnidarians and across vertebrates. Genomic data from *Nematostella vectensis* and *Hydra magnipapillata* indicate that cnidarians possess some of the most variable apoptosis-protein cores among animals, with 4–11 proteins (Zmasek et al. 2007).

Several genes associated with apoptosis, including both pro- and anti-apoptotic regulators were differentially affected in White Band Disease infected *Acropora cervicornis*. Tumor necrosis factor receptor superfamily member 1A (TNFRSF1A) and caspase 3 (CASP-3) genes were up-regulated, while caspase 8 (CASP-8) was down-regulated in White Band Disease infected corals (Libro et al. 2013). Similarly, in Indo-Pacific acroporid corals, individuals infected with White Band Disease had cellular markers indicative of apoptosis. The proportion of apoptotic cells was dramatically increased in diseased corals; that, combined with the absence of a clear pathogen, suggested that apoptosis was part of the disease etiology (Ainsworth et al. 2007). Characteristic blebbing of apoptotic cells was also detected in the wound-healing clot of *Porites cylindrica* (Palmer et al. 2011c).

28.5.3 Wound Healing

Wound healing is important as it re-establishes the function(s) of injured areas and prevents infections (Poss 2010; Galliot 2012). In sessile organisms, including most cnidarians, which are continuously exposed to physical damage by both abiotic and biotic stressors, including disease, wound healing is extremely important (Reitzel et al. 2008). Immediately after injury, the host response is activated to prevent infection and start the removal of cellular debris around the wound area (Peiris et al. 2014). Wound healing involves several phases: plug formation, inflammation, tissue proliferation and maturation (DesVoigne and Sparks 1968; Fontaine and Lightner 1973; Sherratt and Murray 1990; Theopold et al. 2004; Palmer et al. 2011c). The coordination and synchronization of the different components of this process is complex and involves several pathways (Godwin and Brockes 2006; Gurtner et al. 2008; Godwin and Rosenthal 2014). Differences in the healing and regeneration capabilities across taxa have been linked to the complexity of the immune system (Godwin and Brockes 2006; Godwin and Rosenthal 2014; Peiris et al. 2014). In general, organisms with "simple" immune systems (e.g., cnidarians) have a higher regeneration capacity, while higher animals with "complex" immune systems (e.g., mammals) usually show low regeneration capabilities (Peiris et al. 2014).

Genomic analyses are now revealing details of the wound healing process in cnidarians (Pang et al. 2004; Reitzel et al. 2008). In *Nematostella vectensis*, the cells around an injury tend to cluster together, triggering the expression of genes related to wound healing and regeneration. The MAPK/ERK and Wnt pathways are actively involved in this process (Pang et al. 2004). MAPK/ERK regulates cell movement and recognition while Wnt regulates axis formation (Pang et al. 2004). In *Hydra magnipapillata*, Wnt3 signaling appears to be the primary tissue regeneration mechanism (Lengfeld et al. 2009; Galliot and Chera 2010). In these two cnidarians,

regeneration and wound healing act in conjunction with apoptosis to control cell proliferation (Pang et al. 2004; Galliot and Chera 2010).

Several cell types with wound healing potential are known in the anemone *Actinia equina* (Hutton and Smith 1996) and the gorgonians *Swiftia exserta* and *Plexaurella fusifera* (Meszaros and Bigger 1999; Olano and Bigger 2000). Amoebocytes play a key role by aggregating near wound sites and initiating the production of connective mesogleal fibers to seal the wound (Meszaros and Bigger 1999; Mydlarz et al. 2008). This process was detailed in higher resolution studies of wound healing in *Porites cylindrica* and *Acropora millepora*. A sequence of four main events was characterized (Palmer et al. 2008, 2011c). First, plug formation via degranulation of melanin-containing granular cells occurs, indicated by a lack of melanin-containing granular cells and an increase in extracelluar melanin. This degranulation likely releases antimicrobial and cytotoxic material, including melanin, which can kill foreign organisms, and may also explain an immediate decline in zooxanthellae in the wound vicinity. In stage 2, immune cells, including eosinophilic (acidophilic) granular amoebocytes infiltrate the area as part of an inflammatory response, and spread out along the lesion edge. Agranular hyaline cells and fibroblasts with extensive pseudopodia help to connect the tissue components, and are assumed to secrete collagen. Stage 3 is characterized by granular tissue formation (proliferation) with an increase in melanin, possibly to provide structural support. Stage 4, maturation, is characterized by tissue differentiation, re-melanization of eosinophilic granular amoebocytes and apoptosis or programmed cell death of some immune cells.

Finally, another wound-healing related pathway in cnidarians is the Notch signaling pathway. This pathway regulates cell fate and differentiation, controls proliferation, and has been associated with wound healing (Chigurupati et al. 2007; Marlow et al. 2012). In *Nematostella*, Notch is fundamental for cellular differentiation during development and for cnidocyte cell fate (Marlow et al. 2012). In *Hydra vulgaris*, Notch signaling is essential to control the differentiation of interstitial cells (Kasbauer et al. 2007). All of these functions play significant roles during the final stages of wound healing.

28.6 Overlap Between Immunity and Symbiosis

Many Cnidarians, predominantly anthozoans (such as anemones and corals), form mutualistic relationships with dinoflagellate symbionts that provide much of the nutritional resource for the host in exchange for protection (Baird et al. 2009). Since the symbiont is intracellular, both the establishment and breakdown of the symbiosis involves host immunity (Vidal-Dupiol et al. 2009). Even though this review has focused on the immune responses of cnidarians to pathogens, we will briefly highlight the overlap and integration of immune functions in both facilitating the formation of the symbiosis and protecting the host from pathogens. A primary function of innate immunity is discrimination of self vs. non-self. The cnidarian immune system must thus be able to differentiate between non-self cells that can be tolerated, including the symbionts, and pathogens that need to be eliminated.

Cellular and physiological processes need to be modified to facilitate the formation of the symbiosis. A number of changes in gene expression are observed during the establishment of symbiosis in corals. These changes involve genes regulating cellular processes, such as those involved in cell cycle progression and apoptosis, and genes coding for lectin and other PRR domains (Schwarz et al. 2008a). Many of these patterns of gene expression are similar to those that occur during host-pathogen interactions (Rodriguez-Lanetty et al. 2006). Therefore, there is strong evidence for the involvement of multiple recognition genes that have shared function in both host defense and symbiont recognition (Rodriguez-Lanetty et al. 2006; Schwarz et al. 2008a).

28.6.1 Establishment of Symbiosis

The recognition of symbionts by cnidarian hosts is believed to be largely dependent on lectin/glycan interactions. In order for symbionts to be recognized by the host, they must possess an intact cell surface. Furthermore, α-mannose/α--glucose and α-galactose residues have been identified as potential *Symbiodinium* cell-surface ligands essential in recognition (Wood-Charlson et al. 2006). One of the first lectins described as having a potential role in the recognition of symbionts was SLL-2, a D-galactose binding lectin isolated from the soft coral *Sinularia lochmodes*. SLL-2 is highly expressed near the surface of symbionts, suggesting its role in the maintenance of symbiosis (Jimbo et al. 2000). A second lectin important in symbiosis is CecL from the scleractinian coral *Fungia echinata*, which binds to melibiose, lactose, and D-galactose. Exposure of symbionts to CecL triggers phenotypic changes, such as reduced motility and growth, suggesting that CecL, may play an important role in regulating symbiotic relationships in cnidarians (Jimbo et al. 2010). Finally, *Pocillopora damicornis* C type lectin (PdC-Lectin), which is specific for mannose, is believed to mediate molecular interactions between the host and symbiont. PdC-Lectin is highly expressed in endodermal tissue that is in contact with transient free zooxanthellae. Furthermore, expression of this protein has been shown to decrease just prior to bleaching, suggesting a role in the maintenance of symbiosis (Vidal-Dupiol et al. 2009).

Other lectins that have been identified as crucial to symbiotic processes in cnidarians also have immune functions. The

best documented of these molecules is millectin (Kvennefors et al. 2008, 2010), which binds both bacterial pathogens and certain members of the genus *Symbiodinium*. Millectin binds *Symbiodinium* clades C1, C2, and A1, leading to agglutination and opsonisation of the potential symbionts (Kvennefors et al. 2008). Furthermore, millectin has been observed in coral epithelial cells and surrounding *Symbiodinium* (Kvennefors et al. 2010). Based on these findings, it is hypothesized that millectin plays a role in triggering the phagocytosis of *Symbiodinium*, while other proteins determine whether the phagocytosed cells will become resident in host cells (Kvennefors et al. 2008). In addition to millectin, the complement protein C3 is also believed to play a role in symbiosis. C3 is expressed in areas of the gastroderm in close association with symbionts. C3 may serve a similar role to millectin, mediating phagocytosis of *Symbiodinium* to initiate symbiosis; however, further evidence is needed to fully understand its role in mediating this symbiosis (Kvennefors et al. 2010).

In addition to the dual use of cnidarian receptors for immune function and establishment of symbiosis, a number of effector responses may also affect both immunity and symbiosis. The sphingosine rheostat, a homeostatic cell regulatory pathway consisting of the sphingolipids sphingosine (Sph) and sphingosine 1-phosphate (S1P), impacts a number of cellular functions, including immunity, cell fate, and cell differentiation (Spiegel and Milstien 2000). Transcriptomic analysis has identified the sphigosine rheostat as a potential pathway involved in the regulation of symbiosis in cnidarians (Rodriguez-Lanetty et al. 2006). Furthermore, excess S1P may reduce the prevalence of heat-induced bleaching in cnidarians, while excess Sph may increase bleaching frequency in response to heat stress (Detournay and Weis 2011). Whereas evidence indicates that the sphigosine rheostat plays a role in regulating symbiosis, the potential impacts of this system on immunity are unknown.

In contrast, recent studies have suggested that TGF-β may have effects on both establishment of symbiosis and immunity in cnidarians (Detournay et al. 2012). Binding of TGF-β to its cellular receptor results in the production of SMAD intermediate compounds which can regulate expression of immune genes, resulting in immunosuppression (Massague 1998). Genes encoding for both TGF-β and its receptor have been found in *Aiptasia pallida*, in which *Symbiodinium* may up-regulate host expression of TGF-β, thereby resulting in immunosuppression and tolerance of symbiosis (Detournay et al. 2012). While most known cnidarian immune receptors are designed to elicit an up-regulation of immune response, immunosuppression by a TGF-β receptor suggests that other mechanisms may exist in cnidarians in which immunity may be suppressed via receptor action.

In conclusion, the significant systemic overlap in processes involved in both the establishment of symbiosis and the immune system suggests that many of the genes and molecules essential to the establishment and maintenance of symbioses have been evolutionarily co-opted from existing immune pathways. Therefore, when considering questions of cnidarian immunity, it is essential to also consider the potential effects of symbiotic processes on immune functions of the host organism.

28.6.2 Breakdown of Symbiosis

The breakdown of symbiosis (known as bleaching) also involves many overlapping processes with immunity. ROS are produced as part of metabolism in photosynthetic symbionts (Lesser 2006, 2007; McGinty et al. 2012). During stress, the levels of ROS can exceed constitutive antioxidant systems and become harmful to the host coral. This is believed to be one of the drivers of coral bleaching (Lesser 2006, 2007). Since ROS is also a powerful signaling molecule and part of the oxidative burst in phagocytes, cross-talk between algal ROS and host immune pathways may occur (Mydlarz et al. 2009).

Antioxidants, such as SOD, are expressed by both the cnidarian host and the algal symbiont, and can aid in regulating oxidative stress during pathogen-induced host responses (Mydlarz et al. 2006) and during thermal and UV stress in corals (Lesser 1997; Lesser and Farrell 2004). In the anemone *Anemonia viridis* and the coral *Goniopora stokesi*, SOD and catalase were found throughout the tissue in both species, but were localized in the cnidae, intracellular granules throughout the host, and the symbiont itself (Hawkridge et al. 2000). Downs et al. (2002) and McGinty et al. (2012) also showed that various *Symbiodinium* clades produce antioxidants and can increase production at elevated temperatures.

Apoptosis is a key player during the breakdown of symbiosis in cnidarians. The activation of apoptotic pathways (along with autophagy) during bleaching may remove symbiont cells identified as dysfunctional (Yu et al. 2006). This is essentially an activation of immune pathways to reject and remove foreign invaders ("symbiosis gone wrong"). During thermal stress, the corals *Acropora aspera* and *Stylophora pistillata* regulate pro- (Bax-like) proteins and anti- (Bcl2-like) apoptosis genes by differentially expressing them in different areas of the polyps. Apoptosis is initially active in the gastrodermis. Later, as stress continues, and *Symbiodinium* cell density is reduced, the activity is centered in the epithelium (Ainsworth et al. 2008; Kvitt et al. 2011). In *Acropora millepora*, up-regulation of Bcl2-like genes may be a protective response in the unaffected or surviving cells during high-temperature stress (Pernice et al. 2011). Bcl2 up-regulation might also help surviving corals to recover faster from thermal related bleaching events (Kvitt et al. 2011).

In addition to the intrinsic Bax-Bcl2 controlled activation, apoptosis can be activated by extrinsic mechanisms such as the tumor necrosis factor (TNF) pathway (Hedrick et al. 2010). Several TNF receptors are critical for the onset of apoptosis in corals (Quistad et al. 2014). Activation of the TNF pathway is important against biological stressors (Ainsworth et al. 2007; Libro et al. 2013) and may also play a significant role in the survival and adaptation of corals to climate change (Barshis et al. 2013; Palumbi et al. 2014). Additionally, MAPK signaling is likely involved in exocytosis of *Symbiodinium* in two scleractinians, *Acropora palmata* and *Orbicella faveolata*, and in *Nematostella vectensis* (Granados-Cifuentes et al. 2013).

Immune responses in some coral diseases seem to originate from *Symbiodinium*. Caribbean Yellow Band Disease appears to be caused by *Vibrio* spp. that infect the symbionts and cause chlorosis (Mydlarz et al. 2009). The coral *Orbicella faveolata* up-regulates immune effector responses when infected with Caribbean Yellow Band Disease, illustrating the crossover of immune and symbiosis signaling. In several other disease systems, such as *Oculina* bacterial bleaching (Rosenberg et al. 2009) and *Acropora* White Syndrome (Sussman et al. 2008), bacterial virulence factors affect the *Symbiodinium* directly, eliciting disease etiology in the coral. Since the health of the symbiont is inextricably linked to health of the coral host, adopting a 'your fight is my fight' approach against symbiont diseases would be advantageous to both members of the symbiosis.

28.7 General Conclusions and Discussion

Despite their basal phylogenetic placement, cnidarians possess a highly complex innate immune system. To recognize invaders, three main groups of receptors (TLRs, lectins, and NLRs) likely initiate downstream immune responses. The diversification and variation in the PRRs across different cnidarian lineages may be attributed to each lineage's unique ecological needs, primarily the demand for maintenance of diverse holobionts (Miller et al. 2007; Schwarz et al. 2007, 2008b; Shinzato et al. 2011). The need to recognize and distinguish between commensal and pathogenic microorganisms, including algal symbionts, may be a selective force driving this diversity of PRRs in cnidarians (Hayes et al. 2010; Hamada et al. 2013; Poole and Weis 2014). Signaling cascades in the Cnidaria are among the most complex components of the immune response. Many of the cascades are highly conserved within invertebrate innate immunity (i.e., PPO and TLR), while others are homologous with mammalian cascades (e.g., GIMAP GTPases). Since many of these cascades work behind the scenes, identifying effector responses that have clear phenotypes has been more fruitful. Antimicrobial activity (e.g., AMPs and secondary metabolites) and encapsulation (cellular and skeletal) have been identified in cnidarians, and many of these cellular mechanisms play roles in would healing and tissue repair.

One of the goals of this chapter was to connect what is known about the presence of immune genes to phenotypes of either immunity itself or disease resistance. It was clear after compiling the data that for many of the known immune pathways, information providing this direct connection either exists in fragmented form or does not exist at all. There is also a disparity in the type of information available on each of the four stages of immunity we described. For example, receptor identity and signaling cascades are heavily reflected in genomic data, with the presence of gene homologs in genomes and transcriptomes, while information on effector responses and, to some extent wound repair, rely predominantly on observations of cellular, biochemical and antimicrobial activity.

In addition to understanding the genome to phenotype links within one arm of immunity, we still need more information on the identities of and mechanisms by which PRRs lead to specific effector responses. For example, in other invertebrate systems, activation of TLRs and subsequent signaling pathways lead to up-regulation of AMPs (Aderem and Ulevitch 2000; Akira et al. 2006). While these elements have been identified in the Cnidaria, especially within the Anthozoa, the connections between the entire cascade are not fully understood (Fig. 28.3). The discovery of the AMP damicornin in the coral *Pocillopora damicornis*, and identification of its genetic sequence, cellular localization, antibacterial activity and response to pathogen challenge (Vidal-Dupiol et al. 2011) bring us a few steps further to this goal. Likewise, while it is clear that amoebocytes and other mobile cytotoxic cells respond to infections and aggregate near lesions or wounds (Mydlarz et al. 2008; Palmer et al. 2008, 2011c), it is less clear what genes or upstream processes control differentiation and movement of these cells. If this process is analogous to other invertebrates, it is likely the integrin or lectin/complement pathway are involved, but again, this is still unknown. In many cases, as the tools and experimental techniques advance, so will our understanding of the connection between genotypes and phenotypes.

Another main goal of understanding cnidarian immunity is elucidating which elements lead to organismal disease resistance. Diseases affecting scleractinian corals, one of the cnidarian groups hardest hit by disease, are caused by a diversity of etiological agents (e.g., bacteria, fungi, viruses, etc.), increasing the challenge of this endeavor. It is unlikely that one cascade or PRR is responsible for resistance against all types of pathogens, but identifying the genetic basis of variation in susceptibility to a particular disease will help aid in predicting population-wide consequences of a disease outbreak. To date, several phenotypes have been linked to species-specific disease resistance, including PO/PPO

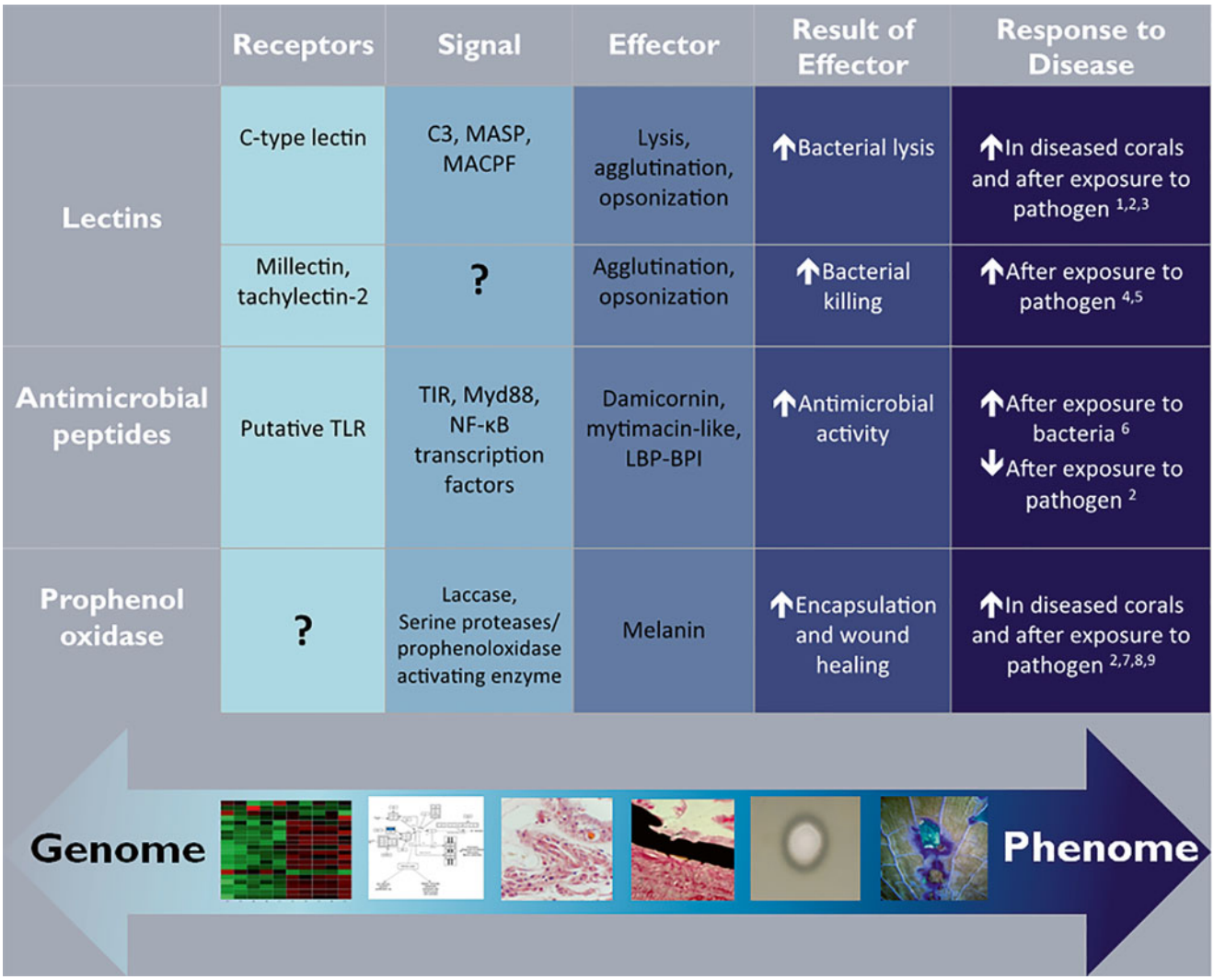

Fig. 28.3 Details of three coral immune pathways (lectins, antimicrobial peptides and prophenoloxidase) and the known and (yet) unknown linkages between the genome (i.e. gene identity) and phenotype (e.g., effectors and role in disease or pathogen exposure). Select examples and references for Response to Disease are as follows: (1) *Acropora cervicornis* with White Band Disease (Libro et al. 2013), (2) *Pocillopora damicornis* exposed to bacteria (Vidal-Dupiol et al. 2014), (3) *Acropora millepora* exposed to bacteria (Brown et al. 2013), (4) *Gorgonia ventalina* following inoculation with the parasite *Aplanochytrium* (Burge et al. 2013), (5) *Acropora millepora* following exposure to many pathogens (Kvennefors et al. 2008), (6) *Pocillopora damicornis* exposed to bacteria (Vidal-Dupiol et al. 2011), (7) *Gorgonia ventalina* infected with *Aspergillus sydowii* (Mydlarz et al. 2008), (8) *Acropora millepora* exposed to PAMPs (Weiss et al. 2013), (9) Wound healing in *Pocillopora damicornis* (Palmer et al. 2012). *Two-headed arrow* signifies the interaction between genome and phenome that can go in either direction, images are generic representations of genome to phenome in coral immunity (*left* to *right*): generic heat map of gene expression, generic cell signaling pathway, ameobocytes aggregating in *Gorgonia ventalina*, melanin deposition in *Gorgonia ventalina*, clear zone of inhibition showing antibacterial activity, *Gorgonia ventalina* showing whole organismal immune response to *Aspergillus sydowii*

(Palmer et al. 2010; Pinzon et al. 2014a) and antimicrobial activity (Pinzon et al. 2014a), but the basis for this variation is not well understood (Pinzon et al. 2014b).

Despite significant progress in the last 10–15 years, much is still unknown about cnidarian immunity, mainly with respect to how it is coordinated and executed, and how key processes are translated from receptor to effector. Confirming these specific functional linkages will require empirical controlled experiments in which cnidarian host responses (both genotypic and phenotypic) to specific stressors, such as pathogen challenge, are evaluated. Advances in the field of cnidarian immunity are expected to increase exponentially in the coming years as more genomic and molecular resources become available. With tools in place, discerning and linking gene networks to their function in fighting pathogens and their roles in disease resistance will become possible.

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Part VII

Photobiology and Circadian Clock

Corals and Light: From Energy Source to Deadly Threat

29

Zvy Dubinsky and David Iluz

Abstract

The bathymetric distribution of reef-building, zooxanthellate corals is constrained by the attenuation of underwater light. The products of algal symbiont photosynthesis provide a major share of the energy, supporting the metabolic needs of the animal host. The two orders of magnitude in the underwater light field spanned by corals are overcome by photoacclimative responses of both the algae and the animal host. The corals decrease the photosynthetic fraction of their energy budget as light dims with depth, increasing their heterotrophic dependence on predation. In shallow water, corals are exposed to photodynamic dangers to which both host and symbiont are susceptible. These effects are mitigated by an array of antioxidative mechanisms that wax and wane in a diel periodicity to meet the concomitant fluctuation in oxygen evolution. The nocturnal tentacular extension seen in many corals is terminated at dawn by light, probably mediated by the photosynthesis of the zooxanthellae.

The Goreau paradigm of "light-enhanced calcification" is summarized while recently documented exceptions are discussed. The role of light as an information source, besides its acknowledged role as an energy source, is evident in the poorly understood role of lunar periodicity in triggering the spectacular mass-spawning episodes of pacific corals.

The characteristics of the underwater light field, its intensity and directionality, control the architecture of zooxanthellate coral colonies, favoring the optimization of the light exposure of the zooxanthellae.

This rejview summarizes the main gaps in our understanding of the interaction of corals and light, thereby suggesting promising future research directions.

Keywords

Photoacclimation • UV • Light-enhanced calcification • Photodynamic damage • Moonlight • Tentacle extension • Mass spawning • Zooxanthellae

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29.1 The Bathymetric Distribution of Coral Reefs and Underwater Light

The relationship of coral reefs to depth and light has been suggested and documented as early as 1825 by Quoy and Gaimard (1825), and there is a figure showing the parallel course of light and reef development in a paper by Wells (1957) (Fig. 29.1). In general, in most locations, reefs decline at depths close to the euphotic depth (z_{eu}), where the light intensity is down to 1 % of its subsurface values. However,

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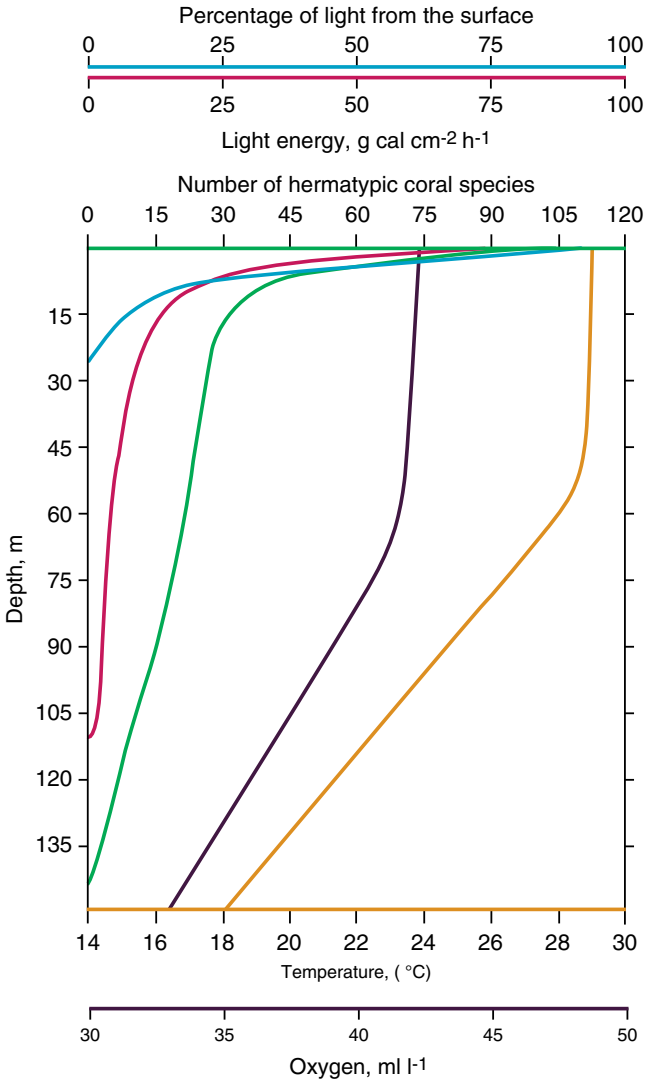

Fig. 29.1 The generalized bathymetric distribution of coral species and of the environmental parameters temperature, oxygen, and irradiance. The numbers of coral species decrease concomitantly with light energy (After Wells (1957))

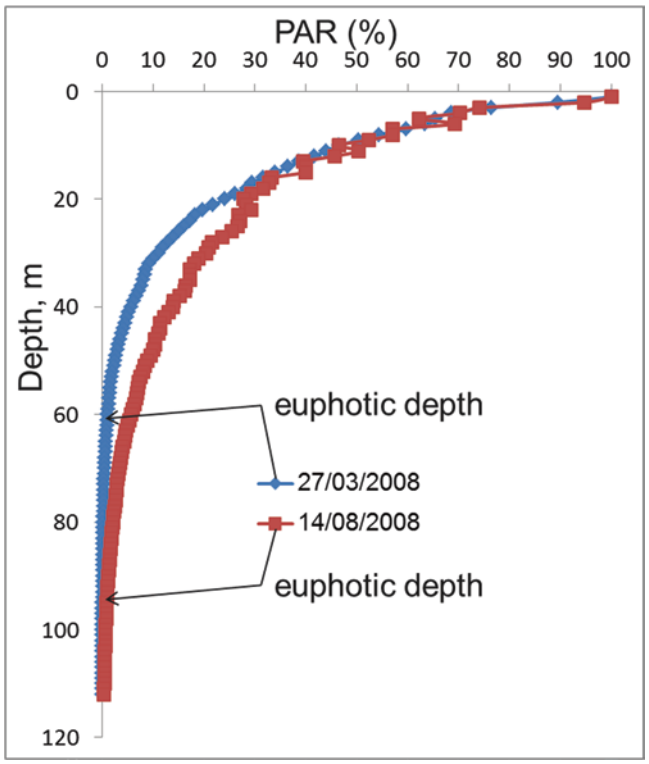

Fig. 29.2 The attenuation of light in the waters of the Gulf of Aqaba (Eilat), Northern Red Sea. The euphotic depth (1 % of subsurface irradiance) ranges from a winter minimum of ~61–120 m in summer

that cannot be translated into depth, since, depending on water transparency, it may be anywhere from a few meters to well over 100 m. For instance, in the well-developed reefs of the Red Sea, the euphotic depth ranges from a winter minimum of ~80–120 m in summer (Fig. 29.2). This fluctuation is caused by the combination of reduced solar irradiance in winter and a seasonal increase in cloud cover, with nutrient-driven proliferation of light-absorbing phytoplankton assemblages (Iluz et al. 2008). In the paper by Loya and Slobodkin (1971) describing the reefs of the Gulf of Aqaba (Eilat), Northern Red Sea, reef morphology and distribution change with depth between its peak distribution at 1–20 m, petering out towards 70 m (Loya and Slobodkin 1971). Nevertheless, isolated colonies and stands of *Leptoseris hawaiiensis* (Fricke et al. 1987) abound as deep as 120 m (Fig. 29.3). Most of that light-limited distribution is attributable to the dependence of the coral animal host on the photosynthate produced by the algal symbionts that decreases with depth, as is discussed in the following sections. This notion is in agreement with Morse et al. (1988) and Babcock and Mundy (1996), who provided evidence of the ability of at least some coral planulae to detect light directionality and intensity and settle accordingly, optimizing the underwater distribution of the light field for symbiont photosynthesis.

In a global survey of reefs, Bigot et al. (2000) summarized as follows: "The number of coral species, their production, and growth rates decrease with depth, due to the decreasing light levels. Therefore, coral species continue to thrive up to irradiance levels of 30–40 %, reaching a maximum just below the surface of the water. At lower irradiance levels, some species such as with only 1 % of the surface light; but in this case, only small colonies develop and coral reefs are never formed." In general, an array of photoacclimative mechanisms, discussed below, enables zooxanthellate corals to exceed their respiration needs for substrate by products of photosynthesis at irradiances even below the euphotic depth (Dubinsky et al. 1984; Stambler and Dubinsky 2007).

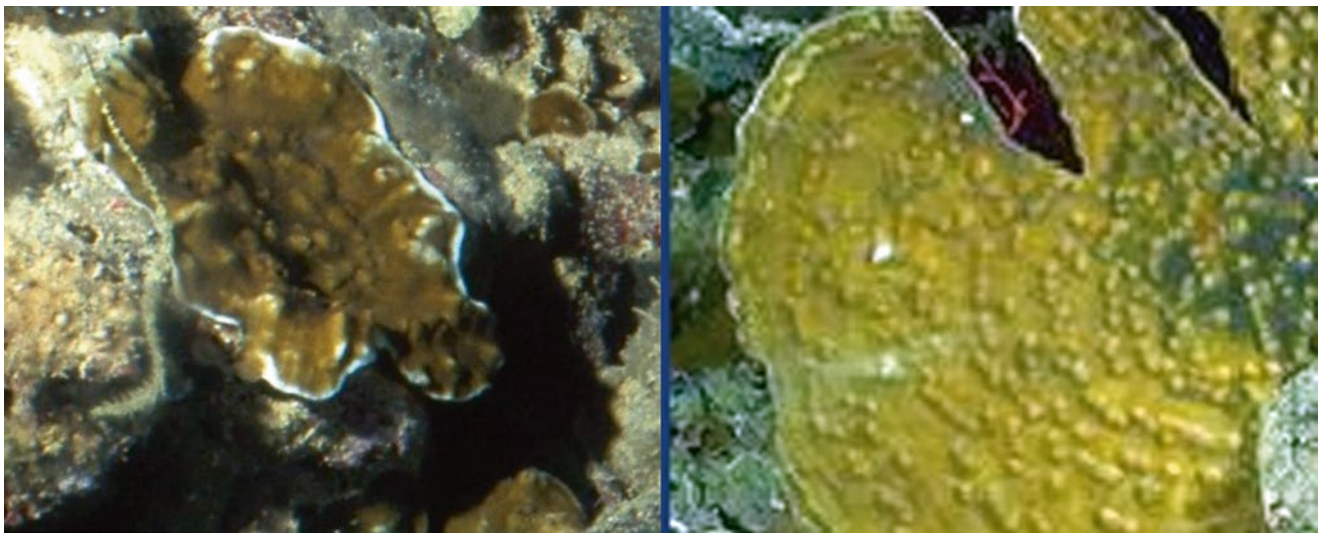

Fig. 29.3 *Leptoseris hawaiiensis* in Eilat, Red Sea, abounds as deep as 120 m. The planar, horizontal morphology is characteristic of deep-water low-light corals

29.2 Zooxanthellae: Providers of Energy

The term zooxanthellae for algal symbionts living within aquatic invertebrates was coined by Brandt (1881), who documented their presence in several aquatic invertebrate taxa (Fig. 29.4). In subsequent experiments with starved anemones, Brandt reached the conclusion that the animals obtained nutriment from the zooxanthellae when deprived of animal food (Brandt 1881). The widespread distribution and role of the zooxanthellae in corals were discussed in reviews by Yonge (1931), Droop (1963), and, more recently, by Stambler (2011a). These symbionts were isolated and cultured [e.g., for methods, see McLaughlin and Zahl (1959), and their taxonomy was defined (e.g., Trench 1987). Subsequently, it was discovered that zooxanthellae were an array of eight clades (A-H) differing in their affinity to host species and their geographic and bathymetric distribution (for a detailed discussion, see, e.g., Baker (2003), Chen et al. (2005), Berkelmans and van Oppen (2006), Lampert-Karako et al. (2008), and Stambler (2011b)).

Brandt's suggestions regarding the role of zooxanthellae as a source of food for their hosts were subsequently confirmed in several studies [to mention just a few: Yonge (1931), Odum and Odum (1955), and Muscatine et al. (1983, 1984)]. In these studies, it was shown that high-energy products of the photosynthesis of the zooxanthellae, including sugars, lipids, and amino acids, were "translocated" to their animal host (Pearse 1971; Eden et al. 1996; Titlyanov et al. 2000). Depending on coral species and, as discussed below, also ambient light, the zooxanthellae may provide as much as 96 % of the respiratory requirements of the coral host (Falkowski et al. 1984).

29.3 Photoacclimation of the Zooxanthellae

The capability of zooxanthellate corals to thrive over a bathymetric range of 0–120 m requires them to be able to cope with an irradiance range from the intense sunshine at the reef table down to ~0.5 % of that, or for example, from 2000 to 10 μmole quanta $m^{-2}.s^{-1}$. This is accomplished by a series of phenotypic, photoacclimative adjustments of the zooxanthellae, including optical, photobiological, ultrastructural, biochemical, and physiological changes, similar to those reported for free-living phytoplankton (Dubinsky et al. 1986). These strategies and mechanisms mitigate the 200-fold reduction in available solar energy, while preventing photodynamic damage to zooxanthellae exposed to the full, near-surface intense insolation. The first in the sequence of photoacclimative responses is the change in cellular pigment content, which ranged from 2 pg chlorophyll in shallow-water colonies of the common Red Sea coral *Stylophora pistillata* to 8 pg chlorophyll in dim-light colonies Table 29.1 (Falkowski and Dubinsky 1981). This fourfold increase in pigmentation is accompanied by a concomitant expansion of the thylakoid surface area required for accommodating the light-harvesting antennae and associated structures (Dubinsky et al. 1983) (Fig. 29.5). This phenotypic acclimation took place within 1 week, when high-light–acclimated colonies were transplanted to low light. Interestingly, the converse transplantation – low to high light – failed and resulted in colony mortality, until Cohen et al. (2013) succeeded in such transplants, allowing a few weeks of acclimation at intermediate depths and irradiance levels. These studies underscore the dangers facing corals that are acclimated to a

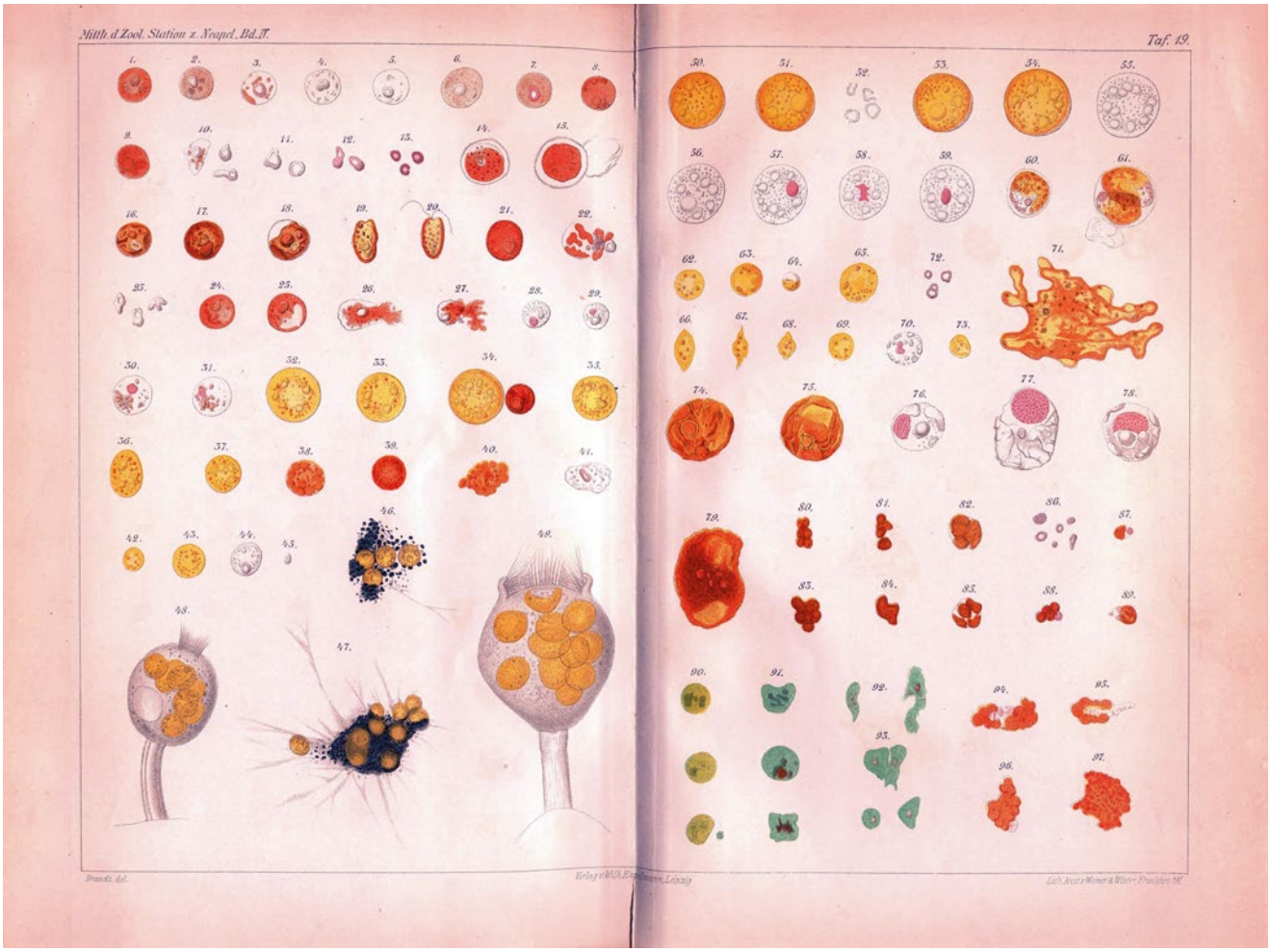

Fig. 29.4 Brandt's original plate showing algae symbiotic with diverse aquatic invertebrates. The term zooxanthellae appeared in that paper (From Brandt 1881)

Table 29.1 High- and low-light acclimation in *Stylophora pistillata* zooxanthellae (After Falkowski and Dubinsky (1981)). Shallow water, high-light-growing colonies (HL) of *S. pistillata* compared to colonies from deeply shaded crevices (LL), where irradiance amounted to ~1 % of surface intensity. Zooxanthellae harvested from measured coral surface were counted microscopically, to determine areal cell densities. From extracted chlorophyll and cell counts from the same colony surfaces, the cellular chlorophyll concentration was calculated

Treatment	μg chl a cm^{-2}	10^6 cells cm^{-2}	pg chl a $cell^{-1}$
HL	3.6 ± 1.1	1.7 ± 0.3	2.2 ± 0.3
LL	14.2 ± 4	1.6 ± 0.1	8.3 ± 0.5
LL/HL	3.9	1.1	3.7

low-light environment, poised to harvest as much light as possible, when experimentally transplanted to high light. When that happens, rates of photon harvesting vastly exceed those of their utilization, which is impeded by the relatively slow throughput rates via the quinone pool. Under such a combination, free radicals are generated with deadly consequences. To counter the dangers imposed by high-light–triggered free radicals, both the zooxanthellae and the host corals developed an array of defenses, of which several have been described. These include the antioxidant enzymes catalase and superoxide dismutase (Levy et al. 2006). In these studies of the corals *Favia favus*, *Plerogyra sinuosa*, and *Goniopora lobata*, it was evident that the levels of both enzymes closely followed the diel light pattern in response to that of the photosynthetic activity of the zooxanthellae (Fig. 29.6). The location of the zooxanthellae inside the symbiosome organelle in the host tissue necessitated, in addition to the response of the algae, a host mechanism in order to avoid potential photodynamic damage. The results of photoacclimation include major differences in ϕ, the quantum yield of photosynthesis, ranging from the highest possible one mole of oxygen evolved (or CO_2 assimilated) for 8 mol of photons absorbed (ϕ = 1/8) in corals grow-

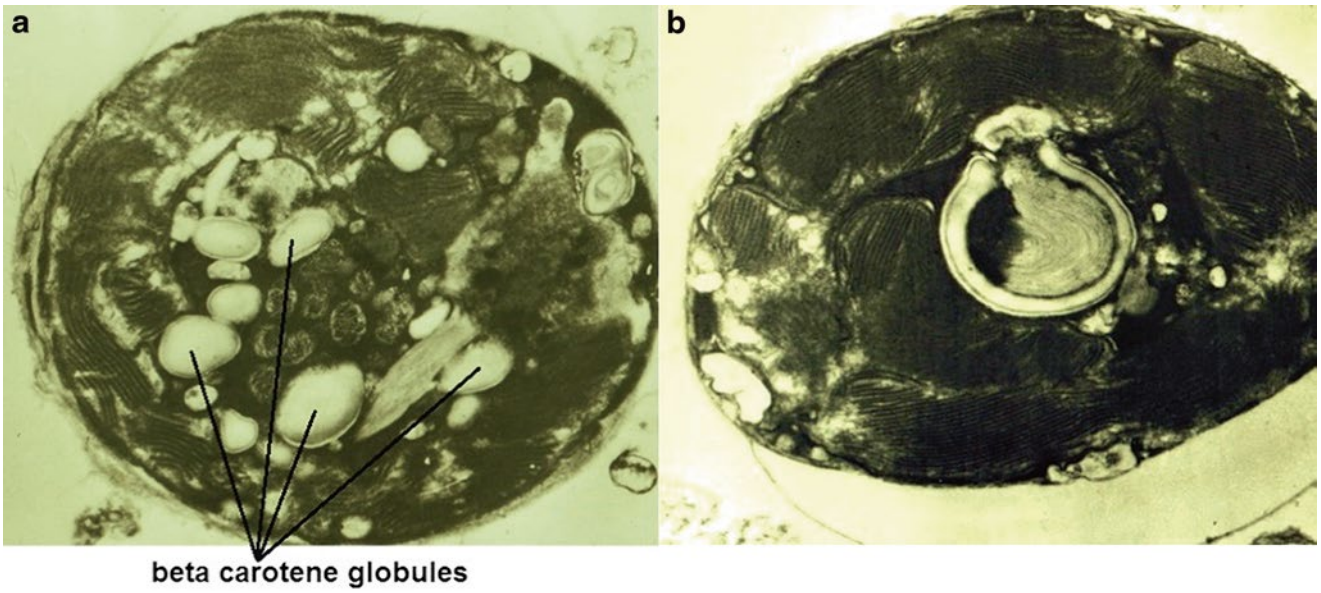

Fig. 29.5 (**a**) Transmission electron micrograph of zooxanthellae from high-light *Stylophora pistillata*. Note the photoprotective carotenoid globules and the paucity of thylakoids. (**b**) Electron micrograph of zooxanthellae from low-light *S. pistillata*. The entire cross-section of the cell is occupied by thylakoid membranes, intercepting virtually every photon (Dubinsky et al. 1983). Corals as in Table 29.1. The diameter of the zooxanthellae is ~10 μm

ing at dim 10 μmoles quanta $m^{-2}s^{-1}$ to ϕ ~1/50 in shallow-water, high-light colonies (Dubinsky et al. 1984). The change in pigmentation resulted in the corresponding four to fivefold differences in areal chlorophyll concentrations (Table 29.1) and in colony absorptivity ranging from 5 to 10 % in high-light, ivory-colored colonies to ~95 % in nearly black-shaded ones (Fig. 29.7). It is noteworthy that Red Sea zooxanthellate corals display a strong hysteresis pattern (Fig. 29.8), whereby afternoon values were significantly higher than morning ones for the same irradiance levels (Levy et al. 2004). This phenomenon, even though ubiquitous, does not yet have a satisfactory explanation.

While the multiple effects of UV radiation are outside the scope of the present review, Cohen et al. (2013) found that UV accelerates photoacclimation processes in corals in ways similar to those of the effects of light.

29.4 Light and Trophic Status of Corals

The abundant light available to the high-light colonies of *S. pistillata* and the high concentrations of RuBisCo (Fisher et al. 1989) more than compensate for their low quantum yield and for using light "wastefully". Hence, the high-light–acclimated colonies of that coral provide translocated photosynthates amounting to ~95 % of the metabolic requirements of the coral holobiont (host and symbionts) against only some 30 % in the mesophotic colonies (Falkowski et al. 1984). This results in a significant shift from near-energy autotrophy in the high-light colonies to predominant dependence on heterotrophic zooplankton predation for most of their energy budget (Fig. 29.9). However, surprisingly, the high-light colonies of virtually all zooxanthellate corals invest structures and energy, a costly effort to capture zooplankton, as can be seen in their abundant tentacles armed with stinging cells, extended at sunset for the ensuing nighttime emergence of the vertically migrating zooplankton (*Lobophyllia*, *Favia*). Thus, under low light, corals depend on capturing zooplankton as a source of energy to supplement the meager trickle provided by the light-limited zooxanthellae. Under high light, the situation is reversed; there, corals rely on zooplankton as a source of nutrients, such as nitrogen, to match the abundant flux of otherwise "empty" carbon, of "junk food" provided by the photosynthetic activity of the algal symbionts, and to satisfy the universal Redfield ratios allowing growth (Falkowski et al. 1984, 1993). Indeed, the light-dependent difference in trophic status between corals living under high light, that are predominantly autotrophic, and low light colonies, which depend on, predation on zooplankton, was demonstrated by Muscatine and Kaplan (1994). These authors demonstrated that difference between dependence on photosynthesis and on predation, using ratios of stable isotopes of nitrogen. That difference between predominantly autotrophic shallow-water corals and deep-water ones feeding on zooplankton, was confirmed (based on ^{14}C chromatography) by Titlyanov

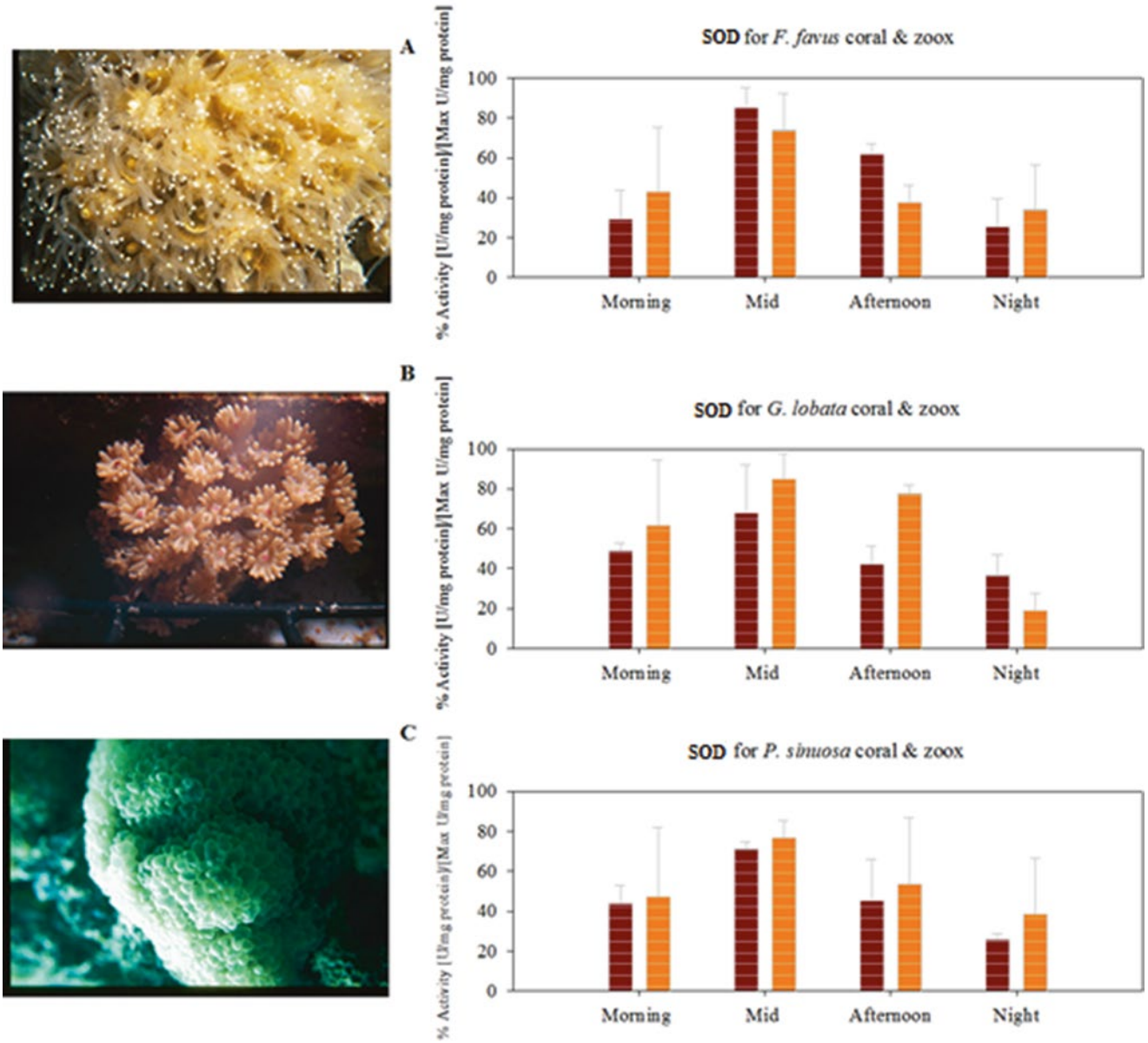

Fig. 29.6 The diel levels of the antioxidant enzyme SOD in the corals *Favia favus*, *Plerogyra sinuosa*, and *Goniopora lobata*, in both host and zooxanthellae. It is evident that the levels of the enzyme closely followed the diel light pattern, in response to that of the photosynthetic activity of the zooxanthellae (After Levy et al. 2006)

et al. (2000). This divergence was also evident in the high C:N ratios of high-light corals compared to lower ones in deep-water colonies (Rahav et al. 1989). It is noteworthy that the residence time of carbon acquired by the zooxanthellae and translocated to animal hosts is significantly shorter than that stemming from predation on zooplankton. The photosynthetically derived carbon is readily and primarily used by the animal host as a substrate for respiration, while that obtained from prey digestion is incorporated in cellular structures (Bachar et al. 2007).

29.5 Colony Morphology

Coral colonies are known for their plasticity, which posed difficulties in their taxonomy, as was already pointed out by Bernard (1902) and discussed in detail by Veron (1995). This phenotypic variability is controlled by the following environmental forces: light, current (Chindapol et al. 2013), and gravity (Meroz et al. 2002), with light playing the main role (Graus and MacIntyre 1976). The effect of light is a dramatic flattening of subspherical colonies (Fig. 29.10) and a horizontal fan-

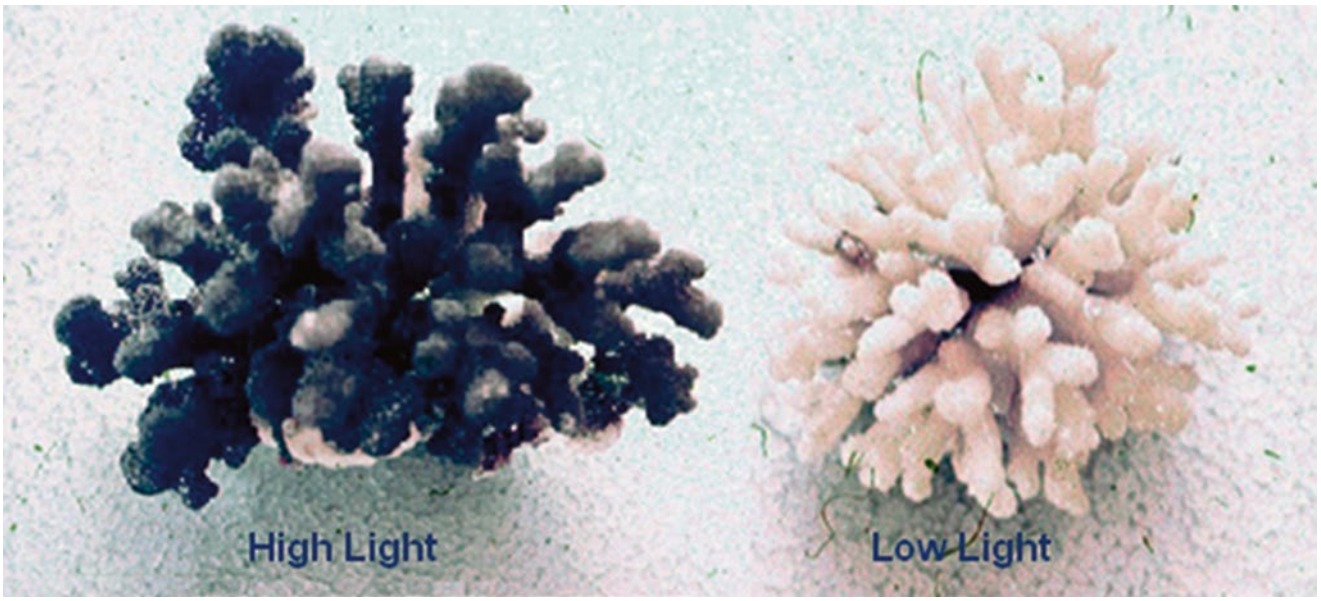

Fig. 29.7 High- and low-light colonies of *Stylophora pistillata* with absorptivity ranging from 5 to 10 % in the high-light ivory-colored colonies to ~95 % in the nearly black-shaded ones

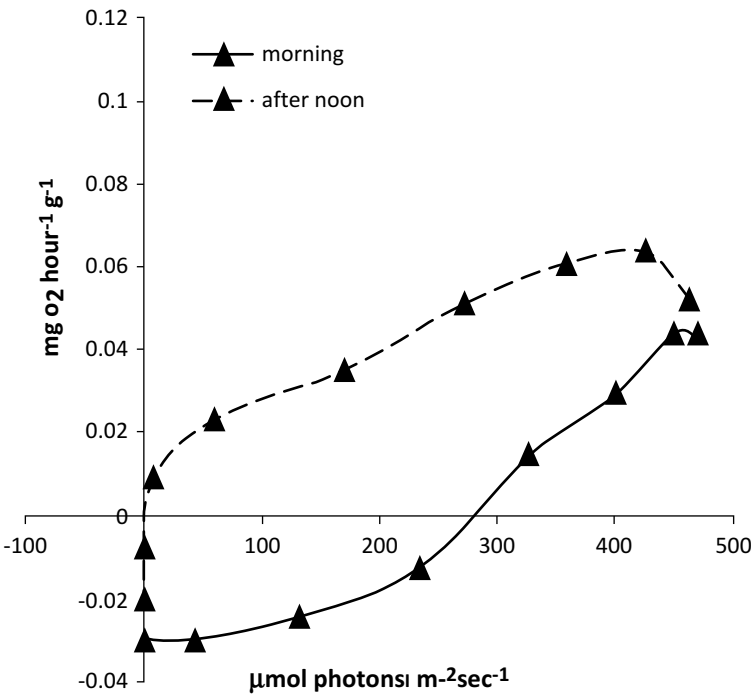

Fig. 29.8 Red Sea zooxanthellate corals display a strong hysteresis pattern, whereby for the same irradiance levels, afternoon values were significantly higher than the morning ones (After Levy et al. 2004)

ning of rounded, branching species (Fig. 29.11). This change in colony architecture results in a dramatic reduction in the ratio between live tissue and harvested light, expressed as reduction in the height-to-diameter ratio of *S. pistillata* colonies {Mass et al. 2007; Einbinder et al. 2009). The amount of light available for the photosynthetic activity of a zooxanthellate, shallow water colony is 'diluted' over a large branched or spherical tissue area, exposing the zooxanthellae to a far lower average light intensity than that impinging on a flat area, thereby avoiding potential high-light damage. The horizontally flattened architecture characteristic to low-light colonies ascertain the opposite, i.e., the live-tissue area is similar to the optical cross section of the colony, namely, the shadow cast by it, maximizing access of the algal symbiont to much of the dim light reaching the colonies. The response of corals to transplantation between light extremes results in dramatic reorgani-

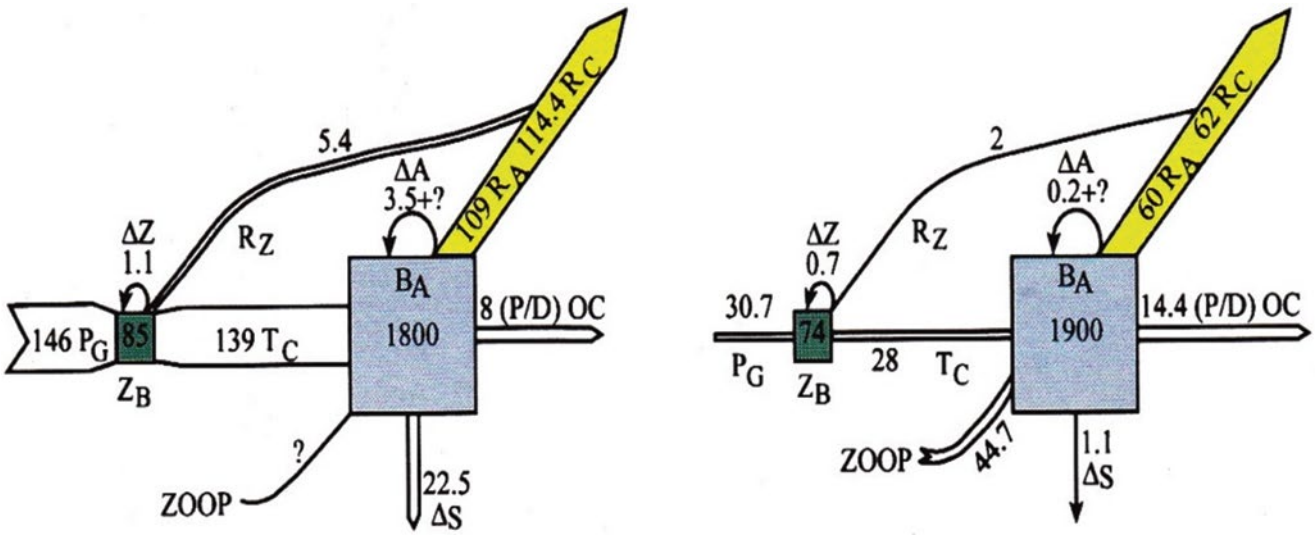

Fig. 29.9 Model of carbon (-energy) flow and budget in corals expressed in mg carbon per cm^{-2} per day. HL corals should have no need for predation (*left*), in contrast to LL corals (*right*), which have to capture zooplankton (ZOOP) as a source of energy (Z_B = biomass of zooxanthellae (zoox), B_A = biomass of animal host, P_G = gross photosynthesis, Tc = carbon translocated from zooxanthellae to animal, Δz = growth of zoox. biomass, Rz = respiration of zoox., Δs = skeletal growth, Δ_A = growth of animal, R_A = respiration of animal, (P/D) DC = particulate and dissolved organic carbon, Rc = respiration of coral holobiont) (After Falkowski et al. (1984))

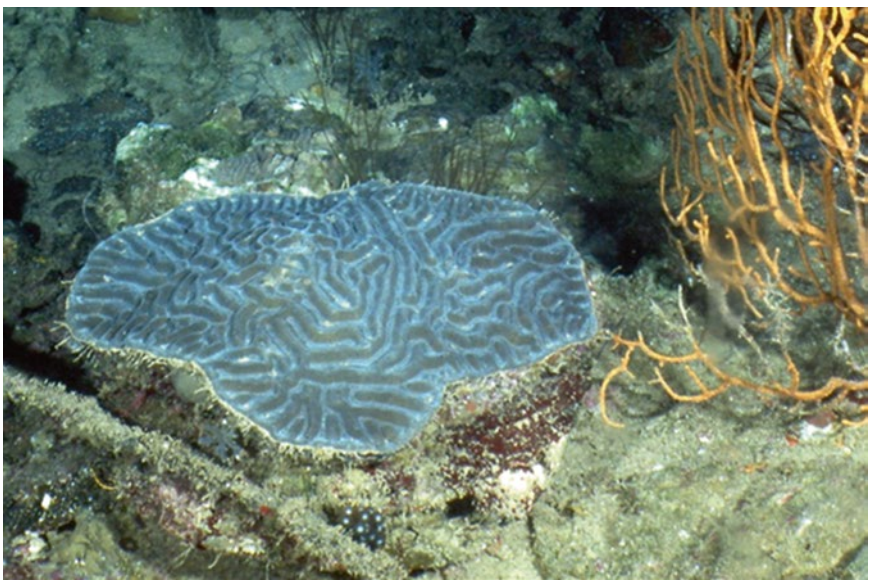

Fig. 29.10 The effect of low light is a dramatic flattening of subspherical colonies. Deep *Platygyra* colony from the submarine Yago, at ~100 m depth

zation of the growth patterns, as shown in the reciprocal transplantation experiments on *Montipora verrucosa* in Hawaii (Fig. 29.12), where, within 3 months, the low-light, horizontal, flat colony transferred to high light 'sprouted' an upright branching growth. An opposite response was seen in the high-light colony that developed a horizontal, flat 'shelf' following its transplantation to low light. Since both colonies were originally taken from a massive colony spread out vertically from a high-light upper part to a dim-light region at its bottom, they were a clone, with the same genetic constitution showing the large range of phenotypic plasticity.

A parallel flattening response is evident in Red Sea *S. pistillata* colonies along a depth gradient (Mass et al. 2007) as they flatten in response to the decrease in irradiance (Fig. colonies (29.13). In experiments where both water flow and irradiance were manipulated experimentally, the dominance of light was evident in controlling colony modifications (Shwartsberg et al. 2012).

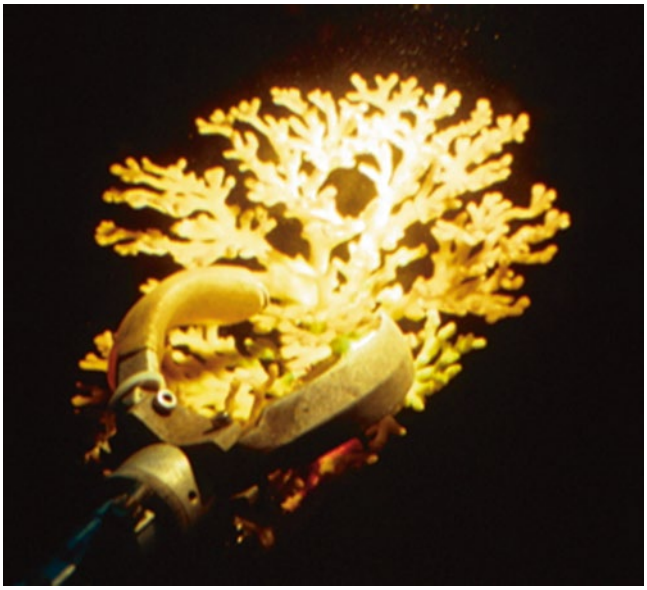

Fig. 29.11 *Stylophora pistillata* at 70 m, collected by the submarine Yago. The fan-shaped, horizontally flattened colony architecture is typical of low-light environments

29.6 Host Factor and Light

There is ample proof that in the cells of the coral animal, there are compounds that accelerate, facilitate, or stimulate the secretion, excretion, or leakage of photosynthate from the zooxanthellae to the host. These were termed collectively "host factor" (HF) or "host release factor" (HRF) (Malkin et al. 1996; Muscatine 1967; Grant et al. 2006; Biel et al. 2007). They differ in composition, show species-specificity, and vary in the translocated compounds. However, it turns out that the light history of the zooxanthellae determines the sensitivity or responsiveness of zooxanthellae to HF presence. In *S. pistillata*, nutrient-limited, high-light zooxanthellae readily responded to the host homogenate by releasing ~2.5 times more ^{14}C-labeled photosynthate than the controls kept in seawater (Fig. 29.14). However, zooxanthellae freshly isolated from 30-m–deep, shade-acclimated colonies, which were relatively nutrient-replete due to the limited light-driven carbon influx, showed no response to the host homogenate (Fig. 29.15).

29.7 Tentacle Expansion

Many corals expand their tentacles at dusk and these remain extended until sunrise (Fig. 29.16 of *Plerogyra sinuosa*, *Favia favus*, and *Lobophyllia corymbosa*). However that behavior weakens under dim light, and deep-water corals may remain with partially extended tentacles at all times. It seems that the daytime extension serves a few purposes: it aims to capture demersal zooplankton during their nocturnal raise, and to avoid diurnal fishes that prey on coral tissue and tentacles. Besides these goals, the extension of tentacles vastly increases the exposed surface of the corals and allows tissue oxygenation at night, while controlling algal exposure to light and, by that, regulating oxygen evolution rates. Any illumination of a coral at night, while its tentacles are extended, causes a tentacle contraction response. If a single polyp is illuminated, it contracts, and the contraction spreads within minutes throughout the colony. Using monochromatic light, Levy et al. (2003) illuminated single polyps of *Favia favus* at night, when the tentacles of the entire colony were extended, and documented the spectrum of the tentacles' withdrawal (Fig. 29.17). It turned out that the response was similar to the spectral response of photosynthesis, leading the authors to suggest that the response of the host was mediated by the photosynthesis of the zooxanthellae that oxygenated the animal tissues or changed their pH. This view is supported by the lack of response to illumination of the azooxanthellate coral *Cladopsammia* sp. (Fig. 29.18), whose tentacles remain constantly extended and it does not respond to light induced tentacle closure.

29.8 Light Enhanced Calcification (LEC)

Based on the convincing evidence of stimulation of calcification by light, Yonge (1931) and Kawaguti and Sakumotu (1948) already suggested a causative relationship between the photosynthetic activity of the zooxanthellae and coral growth. This relationship is clearly evident whenever the diel course of both processes is examined (Barnes and Chalker 1990; Mass et al. 2007), with a diurnal peak of calcification around noon and its cessation at night, occasionally even accompanied by some skeletal dissolution tracking nighttime respiration (Fig. 29.19). When Goreau (1959) and Goreau and Goreau (1959), using ^{45}Ca and ^{14}C radioisotopes, showed the correlation between the diel patterns of the photosynthetic activity of the zooxanthellae and that of the light-enhanced calcification of the coral, it became a nearly universally accepted paradigm. Explanations of that phenomenon were summarized by Pearse and Muscatine (1971). Using ^{14}C-labeling, these authors demonstrated the contribution of photosynthates such as glycerol, lipids, and various amino acids 'translocated' to the host, to the calcification process. Additional support for the importance of products of algal photosynthesis was offered by Crossland and Barnes (1974), who claimed that algal photosynthesis provides energy that, is ATP-generated, according to a scheme they suggested, through the citrulline-ornithine pathway. Pearse and Muscatine (1971) also acknowledged the effect of symbiont photosynthesis, namely, that the removal of CO_2 raises the pH, thereby facilitating calcification according to the equations proposed by Goreau (1959) and reevaluated by Gattuso et al. (1999) and Allemand et al. (2011). This aspect

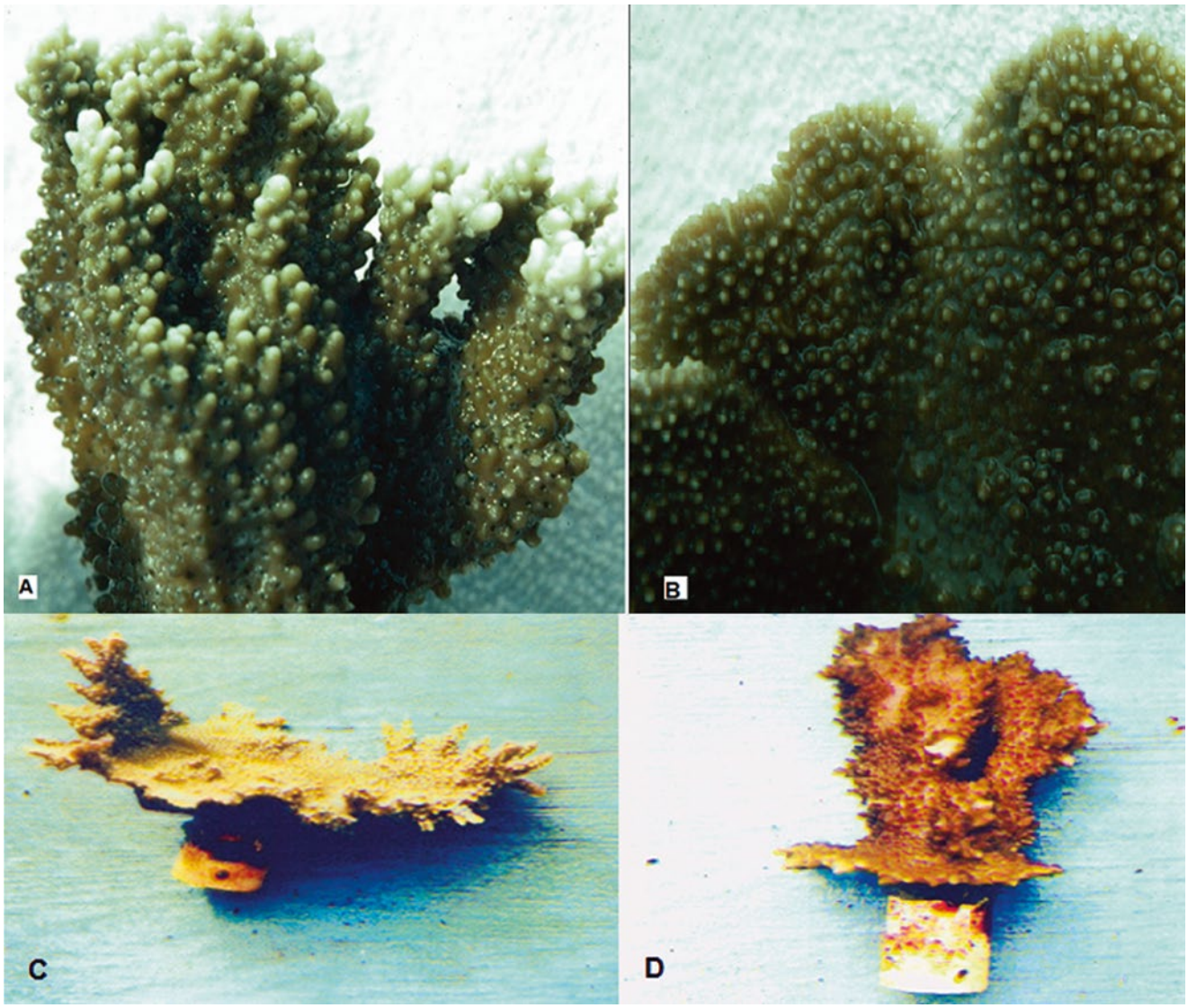

Fig. 29.12 High-light (**a**) profusely branched, upper part of a *Montipora verrucosa* colony in Hawaii; (**b**) lower part, flat, horizontal, shaded part of the same colony; (**c**) Deep water *M. verrucosa* colony following 3 months after transplantation to high light. Note the vertical branch; (**d**) High-light colony of the same species after 3 months at low light, showing the development of a flat, horizontal growth

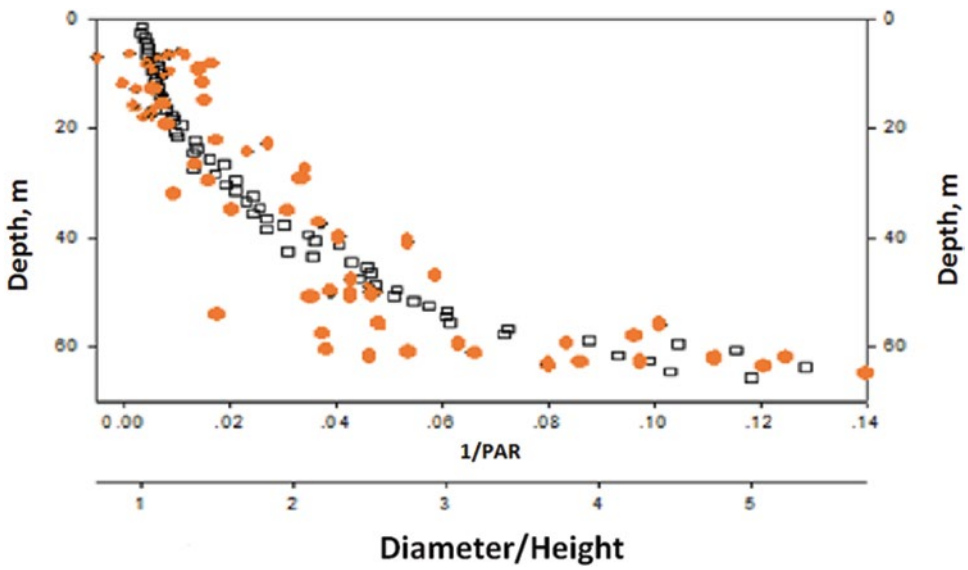

Fig. 29.13 As irradiance decreases (open squares – 1/PAR), the colonies of the coral *S. pistillata* became progressively flattened with the diameter/height ratio (*filled circles*) changes from ~1 in the high light subspherical colonies to ~5 in flatttened, fan-shaped low-light, deep-water colonies (After Mass et al. 2007)

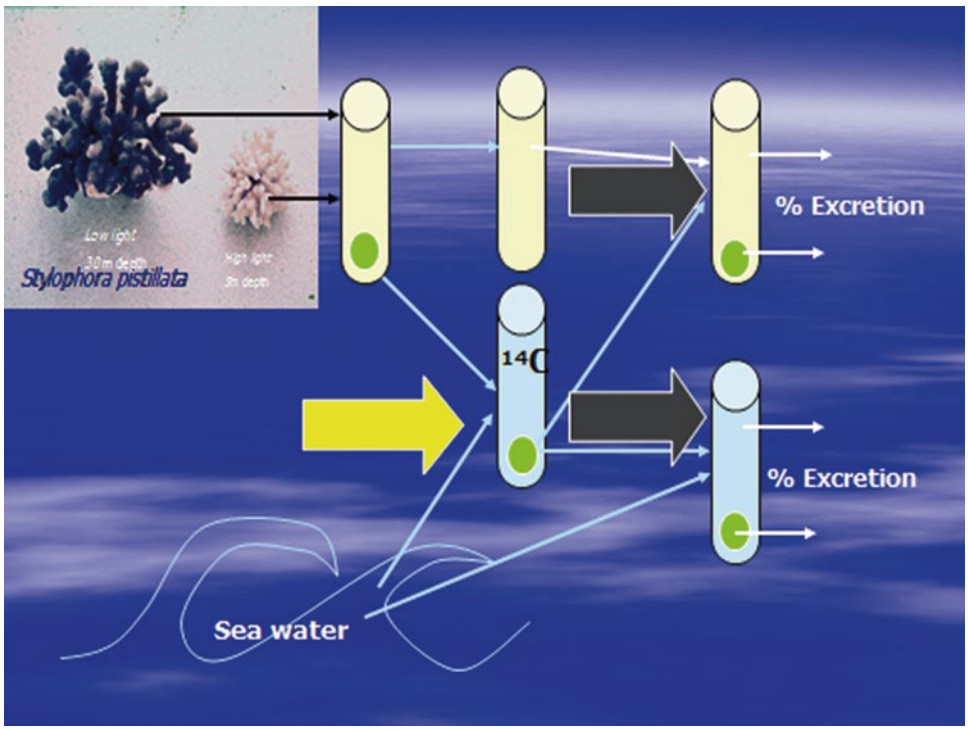

Fig. 29.14 The test for the "host factor". The coral tissue is removed from the skeleton, the homogenate is centrifuged, and the algae are separated from the host homogenate. The zooxanthellae are incubated in the light with ^{14}C, and subsequently incubated in the dark with the animal homogenate, and with seawater as a control. The percentage of ^{14}C excreted in the dark is determined

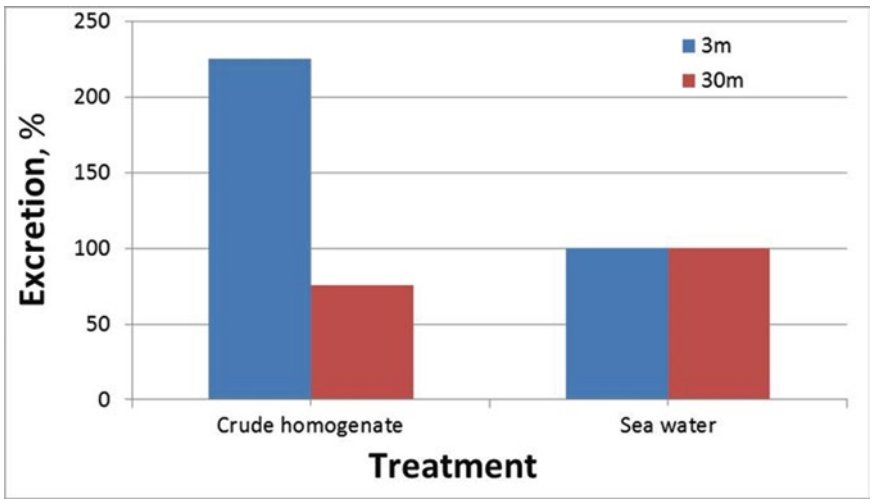

Fig. 29.15 The host-factor effect on excretion of ^{14}C-labeled photosynthate. *Blue* HL zooxanthellae from 3 m, 2000 µmole q m^{-2} s^{-1}. *Red* LL zooxanthellae from 30 m, 500 µmole q m^{-2} s^{-1}. Excretion in seawater is taken as 100 % (After Dubinsky et al. 1986). Only the zooxanthellae from the high-light coral responded to the host homogenate by increased excretion

has been studied in great detail by several researchers and reviewed by Barnes and Chalker (1990) and Erez et al. (2011) in relation to seawater chemistry, and it is continuously being reexamined and refined (Jokiel 2011). However, the equations of calcification and photosynthesis can be interpreted as indication that the two processes compete for CO_2:

Photosynthesis $CO_2 + H_2O -> CH_2O + O_2$

Calcification $Ca^{2+} + CO_2 -> CaCO_3 + 2H^+$

Or, conversely, that calcification actually lends support to photosynthesis by releasing CO_2 by a process called "transcalcification":

$$Ca^{2+} + 2HCO_3 -> CaCO_3 + CO_2 + H_2O,$$

proposed by McConnaughey and Whelan (1997). This hypothesis has since been rejected by most workers (Allemand et al.

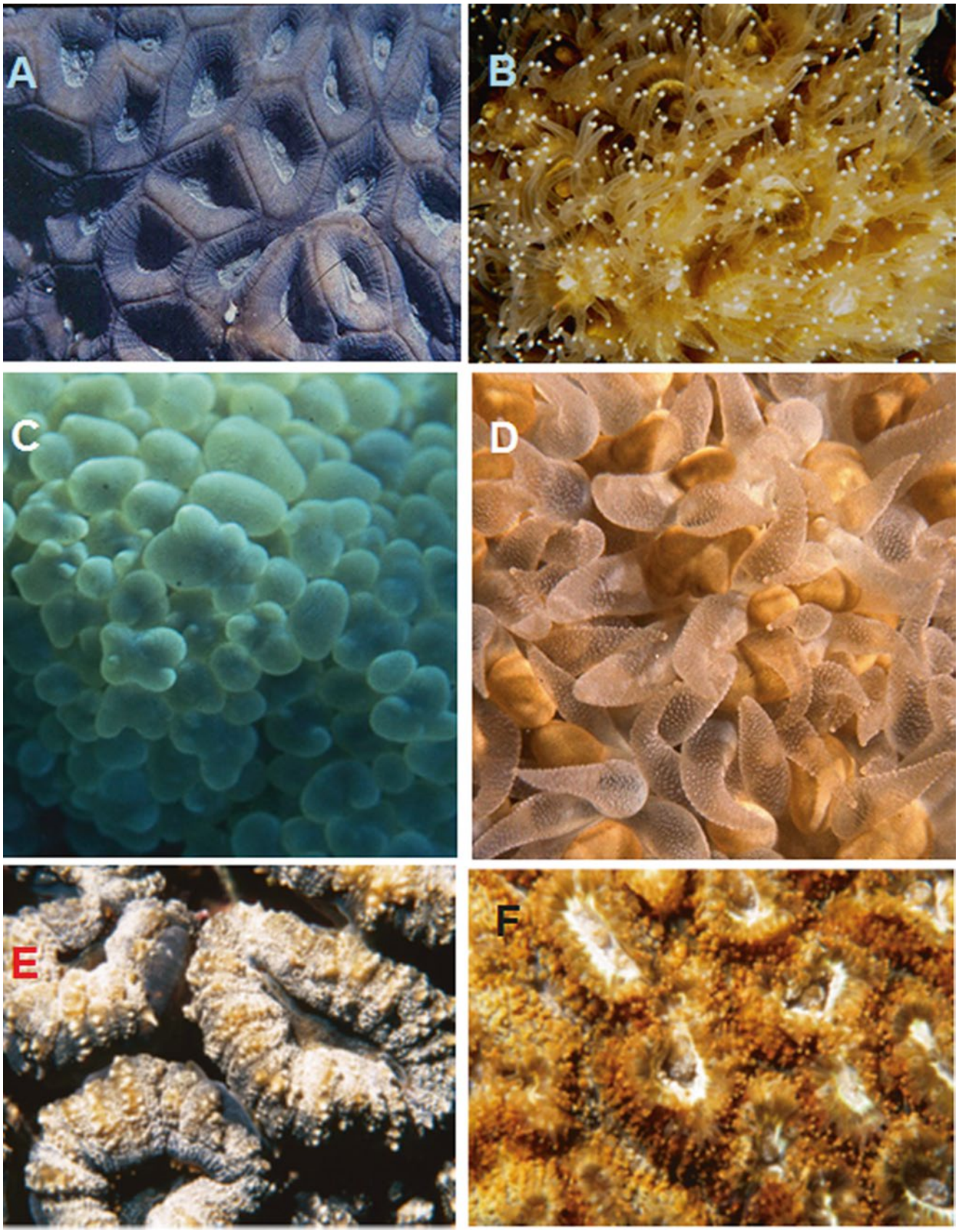

Fig. 29.16 Light control of tentacle expansion in the Red Sea corals *Favia favus* (**a**, **b**), *Pleurogyra sinuosa* (**c**, **d**), and *Lobophylia corymbosa* (**e**, **f**) in the Red Sea. *Left*, daytime, tentacles retracted; *Right*, at night, tentacles expanded

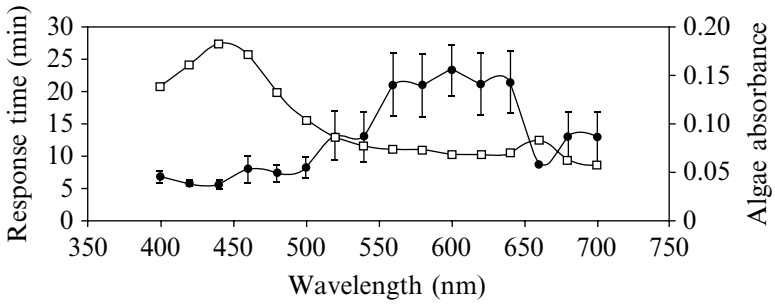

Fig. 29.17 The time required for tentacle withdrawal in *Favia favus* (*filled circles*) following illumination of a spot, as a function of the light wavelength. That action spectrum closely resembles the absorptance of the zooxanthellae (*open squares*). Values are means ± S.D., N=5. Comparison of the action spectrum of *F. favus* tentacle contraction to the light spectrum absorbed by the zooxanthellae at an irradiance level of 30 µmol quanta m-2 s-1 (Levy et al. 2006)

Fig. 29.18 The azooxanthellate Red Sea coral *Cladopsammia* sp. does not respond to light stimulation

2011). Schneider and Erez (2006) report yet another difficulty in the LEC paradigm: they were able to increase coral calcification rates by manipulating water chemistry but failed to see any concomitant increase in zooxanthellate photosynthesis. An interesting aspect of the link between photosynthesis and calcification got support from the results of Goffredo et al. (2012), working on the Panarea CO_2 vent. They showed that the zooxanthellate coral *Balanophyllia pruvoti* is more tolerant of ocean acidification than the azooxanthellate species *Astroides calycularis* and *Leptopsammia pruvoti*. species *Astroides calycularis* and *Leptopsammia pruvoti*. An additional proposed contribution of algal metabolism to calcification is the removal of phosphate that interferes with the formation of the skeletal aragonite crystals (Simkiss 1964). Several studies have been examining various facets of the relationship between symbiont photosynthesis and coral calcification, progressively adding to the cellular (Schneider and Erez 2006; Holcomb et al. 2014); and chemical (Jokiel 2011) aspects of the relationship between the two concomitant processes. Nevertheless, the theory is not without theoretical difficulties. Pearse and Muscatine (1971) state: "Light enhancement of calcification rates is, paradoxically, greatest in the algae-poor tips of branches." This dilemma is evident in most fast-growing, branched corals as well as in the zooxanthellate hydrocoral *Millepora dichotoma* (Fig. 29.20). Based on their double-label experiments with Calcium-45 and Carbon-14, they suggested – as a solution to that dilemma – that light enhancement of calcification in the algae-poor tip results from photosynthesis by zooxanthellae further down the branch. Fang et al. (1989) also raise the same issue, suggesting that the support of calcification by symbiont photosynthesis is based on spatial separation between the sites where photosynthesis and calcification take place and the products of photosynthesis reach the calcification sites. Further difficulties emerged upon examination of cases where no clear support of calcification by photosynthesis could be detected, for instance, under dim light, as light does stimulate calcification but with no quantitative correlation (Mass et al. 2007, Fig. 29.21).

The current situation regarding LEC is succinctly summarized by Allemand et al. (2011): "…at the macroscale level, it is most probable that zooxanthellae enhance calcification under light conditions, at the microscale level many studies have to be performed in order to understand how zooxanthellae play a role in LEC…". They continue to justify their conclusion: "calcification itself is still a black box" and "we have to admit that the major steps of coral calcification remain to be discovered". Obviously the relationship of light and coral calcification still remains a main challenge for future research.

29.9 Internal Light Gradients Within Coral Colonies

Already Shibata and Haxo (1969) examined the millimeter scale of light penetration into the massive coral *Favia*, and described the resulting layering of the algal symbionts (Fig. 29.22), as well as the underlying stratum of the boring Chlorophyte *Astreobium*. With the advent of spectral microsensors such analyses became far more rigorous and detailed

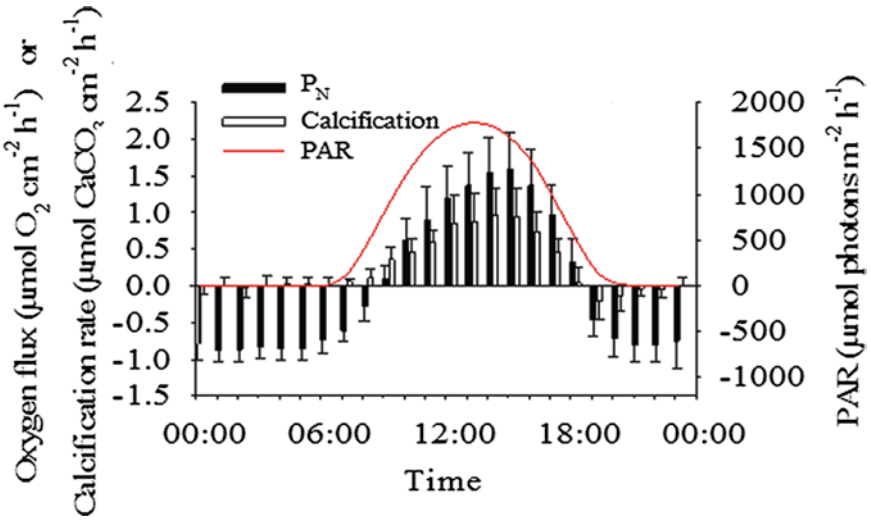

Fig. 29.19 Diel course of calcification vs. light and photosynthesis (P_N- net photosynthesis, PAR = photosynthetic available radiation) (After Mass et al. 2007). Both photosynthesis and calcification follow the diel pattern of irradiance intensity, clearly supporting the light enhanced calcification paradigm. Both calcification and photosynthesis follow the pattern of light- but with a delay. During the night, both respiration and some skeletal dissolution are evident

(Kuhl et al. 1995). Furthermore, these tools allowed relating the depth profiles of light intensity and its spectral distribution to the concomitant photosynthesis profile (Fig. 29.23). That study, and subsequent works (Wangpraseurt et al. 2012; Roth 2014; Fig. 29.24). Marcelino et al. (2013) underscore the limitations and over simplification inherent in the standard practice of studying coral photobiology nearly exclusively in relation to the light impinging on the coral surface (Iluz and Dubinsky 2015). A further attempt towards a microscale charting of the internal light fields within coral skeletons was accomplished by applying fractals to the task (Marcelino et al. 2013). However, the interesting realization that skeletal elements function as light guides has yet to be linked to the concomitant, detailed spatial resolution of the photosynthesis of zooxanthellae.

Fig. 29.20 The hydrocoral *Millepora dichotoma* shows the pattern typical to corals, where the fastest growing tips lack zooxanthellae

29.10 Light and Reproduction

The reproductive activities of several corals, including both broadcasting and brooding species, are synchronized with lunar phases (Fig. 29.25a). Atoda (1947) timed the spawning of *Pocillopora damicornis* to nights 15–24 of the lunar month. This author listed additional species that correspond to that pattern, which also coincides with the spectacular mass spawning of most coral species on the Australian Great Barrier Reef (GBR). However, some GBR species, as well as Red Sea corals, spawn at new moon (Zakai et al. 2006; Fig. 29.25b). Harrison and Wallace 1990) review the above patterns, as well as such differing from the seemingly predominant one.

Vize et al. (2008) report that "In the Gulf of Mexico corals undergo mass spawning on just one or two nights per year, peaking on the eighth evening after the August full moon. This event occurs with extraordinary consistency and is predictable to within a few minutes from year to year". Based on currently available information, we may summarize that the timing of coral spawning during the year, in terms of months, is determined by seasonal patterns such as temperature and food availability, on which gonad maturation depends. However the remarkably precise timing within the month, even though variable among species and localities, is

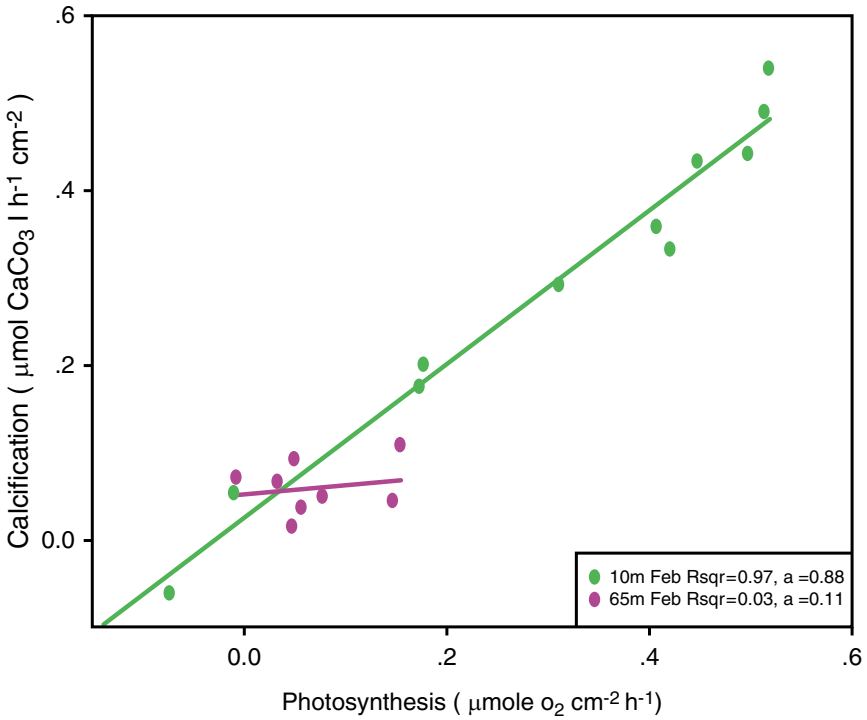

Fig. 29.21 The relation between calcification and photosynthesis at 10 m (*green*) and at 65 m (*pink*) in the Red Sea coral *S. pistillata*. While at 10 m there is a clear stoichiometric relationship between the two processes, no such correlation is evident under dim light (After Mass et al. 2007)

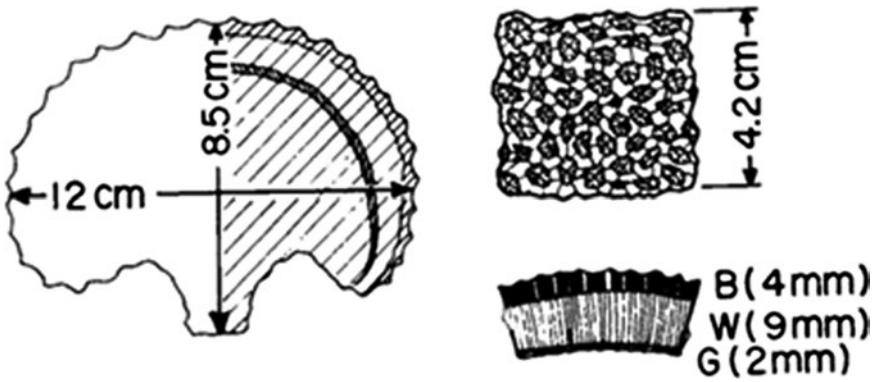

Fig. 29.22 A section through the coral *Favia pallida* (From Shibata and Haxo 1969). (*B* The tissue layer containing the zooxanthellae; *W* white skeletal zone; *G* green layer of the boring filamentous alga *Ostreobium reineckei* layer)

correlated to the lunar phases, probably mediated by cryptochromes (Levy et al. 2007), in ways yet to be elucidated. While the date of spawning is set by the lunar cycle, the hour/min of spawning is set by the spectral quality of light around sunset. Boch et al. (2011), and Sweeney et al. (2011) show that shifts in twilight color and intensity on nights both within and between evenings, immediately before and after the full moon, are correlated with the observed times of synchronized mass spawning, and that these optical phenomena are a biologically plausible cue for the synchronization of these mass spawning events. That view is supported by Boch et al. (2011) who conclude that "spawning synchrony on a particular lunar night and specific time of night is a threshold response to differential periods of darkness after twilight that is primarily influenced by lunar photoperiod and secondarily by discrete optical components of early nocturnal illumination". Nevertheless, the widely accepted lunar control dogma is far from universally accepted. Brady et al. (2009), working in the laboratory with *Montastrea franksi* indicate that spawn timing is not controlled by a circadian rhythm and that it is directly controlled by local solar light cycle. Alternative explanations, based on regional differences in wind patterns were also suggested (van Woesik 2010).

The notion that the lunar control of coral reproduction is somehow synchronized by light is supported by the documented interference of coastal "light pollution" with the patterns of coral reproduction (Jokiel et al. 1985).

In summary, the cellular mechanisms responsible for the lunar light triggering and timing of coral reproduction still await further research and elucidation.

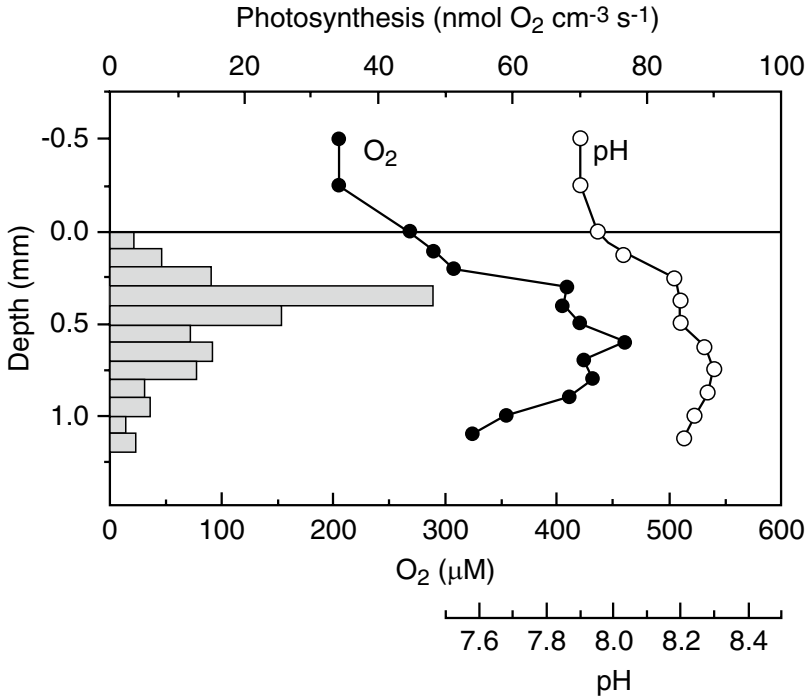

Fig. 29.23 Photosynthesis (*bars*), O_2 and pH in the tissue of Acropora sp. from 5 to 6 m water depth. Incident photon irradiance (400–700 nm) was 535 µmole quanta m-2 s-1 (From Kuhl et al. 1995)

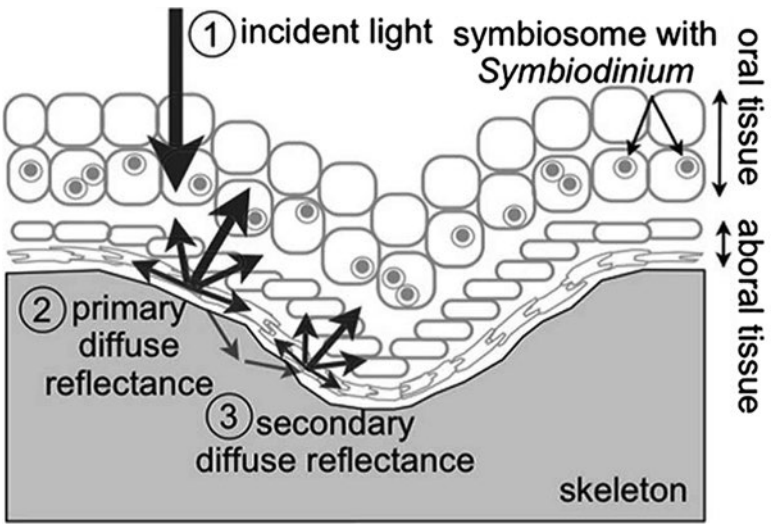

Fig. 29.24 Light dynamics in the coral–algal symbiosis. (*1*) Downwelling sunlight. Light not absorbed by the coral or *Symbiodinium* is (*2*) reflected by the coral skeleton, and (*3*) enters the porous skeleton, re-emerging as diffuse reflectance. The multiple scattering by the skeleton amplifies the light *Symbiodinium* is exposed to (From Roth 2014)

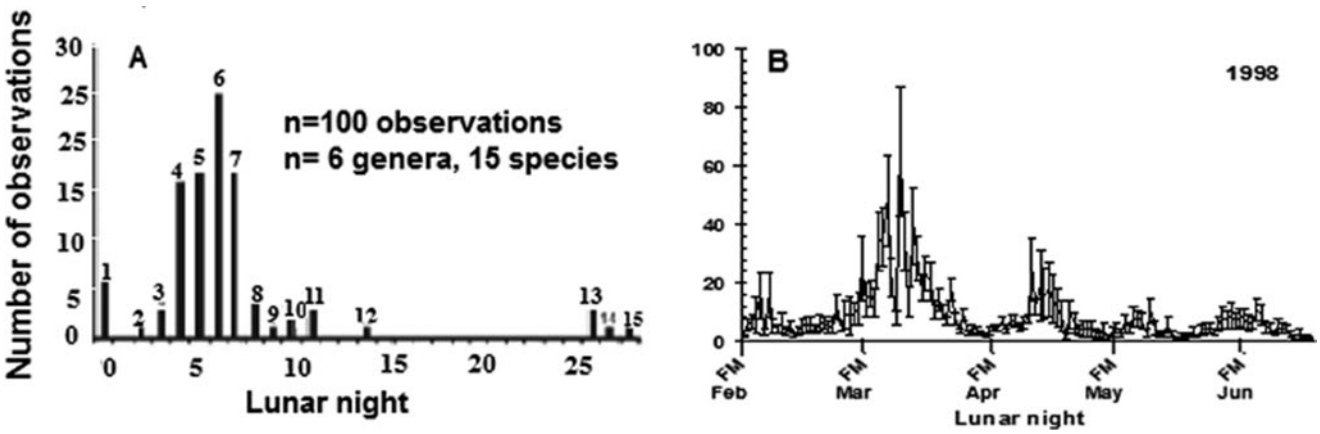

Fig. 29.25 **a** From Zakai Lunar periodicity of planulae release by 20 *Stylophora pistillata* colonies maintained in the laboratory in Eilat, Northern Red Sea. peaking on night 6 after full moon. Numbers on the y axis representing the total number of planulae released each lunar night. Data were pooled from three lunar cycles during 1998 (After Zakai et al. 2006). (**a**) Boch et al. (2011) show corals spawning, based on data pooled from several coral species, peaking on nights 4–7 after the full moon. That pattern is similar to the planulae released by *S. pistillata* in relation to the lunar cycle (**b**)

29.11 Conclusions

The main foci for future research in the vast field of coral photobiology are likely to be the following:

1. Furthering the molecular, chemical and cellular mechanisms underlying LEC.
2. Elucidating the pathway linking the light regime to coral colony architecture.
3. Understanding the mode of action of lunar light on coral reproductive temporal patterns.
4. Mapping the internal light fields within coral skeletons and their physiological effects

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The Photobiology of *Symbiodinium* spp.: Linking Physiological Diversity to the Implications of Stress and Resilience

30

Mark E. Warner and David J. Suggett

Abstract

Over the past two decades, our knowledge of *Symbiodinium* genetic diversity and ecological distribution has grown at an incredible pace, while the physiological diversity and how it may be compared to the phylogenetic and evolutionary constraints of these dinoflagellates is still catching up. Our knowledge of the photobiology of *Symbiodinium* has been driven largely by the desire to explain cellular mechanisms of stress and reaction to global events driven by climate change. We tend to focus more on the former in this chapter since this is where the majority of work has been performed to date. However, it is imperative that we return to the first principles of phytoplankton physiological ecology to form a more complete understanding of the photobiology of these algae and the physiological constraints expected in an algal cell capable of living benthic, pelagic and symbiotic life styles. In order to understand not only the pathways of cellular dysfunction, but also resilience, we address specific patterns of light harvesting balance, reaction centre turnover, inorganic carbon acquisition and utilisation, and alternative electron transport across this genus. *Symbiodinium*-focused research, and physiology in particular, poses several challenges that demand a broad perspective gleaned from working with these algae in culture as well as *in hospite* in controlled 'model' and natural cnidarian hosts across many different environments. We outline several key processes related to photosynthesis as well as new directions integrating many of these processes that are required to more fully understand how environmental change shapes the role of photobiology in governing the ecological success of these important algae.

Keywords

Symbiodinium • Photosynthesis • Reaction centre • Light harvesting • Photoacclimation • Photoinactivation • Alternative electron flow

30.1 Introduction

The endosymbiotic dinoflagellates in the genus *Symbiodinium* are a cornerstone of the success of reef building corals and numerous other invertebrate taxa in the tropical marine realm. In particular, these symbioses have played a significant role in supporting the productivity, growth, reproduction and hence evolution of the scleractinian corals over millions of years (Stanley 2003). Over the past 25 years, numerous efforts describing the taxonomy of this genus, largely grounded in molecular phylogenetics, have culminated in a refined description of a highly divergent

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group of microalgae. Phylogenies based on nuclear, chloroplast, and mitochondrial DNA sequences divide the genus into nine primary lineages or "clades," presently designated "A" through "I" and, depending on the resolution of a particular molecular marker used, each of these clades are broken down further into a number of sub-clades or other categories (Coffroth and Santos 2005).

As noted by LaJeunesse (2005) and Thornhill et al. (2014) given the adaptive radiation that has occurred within each clade, it is often difficult if not impossible to map important questions concerning the ecology and physiology of these algae to the "clade-level" taxonomy. For example, while patterns of thermal tolerance are apparent for the many algal samples studied to date in at least one major lineage, clade D, this appears to be the exception rather than the rule (see also Abrego et al. (2008) and LaJeunesse et al. (2014) for even exceptions for some D-*Symbiodinium*), and there is a high range of physiological plasticity to thermal perturbation among several of the algae within other clades (Robison and Warner 2006; Sampayo et al. 2008; Tchernov et al. 2004). Hence, further taxonomic distinctions are necessary before trends concerning the physiological ecology across these taxa may be fully realized. Indeed development of several molecular markers with faster rates of evolution (as compared to nuclear level markers such as the nr18s) provide greater intra-cladal resolution and better detail concerning the ecological distribution of *Symbiodinium*, their particular host associations across spatial and temporal scales, as well as physiological differences that can be related to their photobiology. Such distinctions have further ramifications for how *Symbiodinium* diversity is quantified, as grouping clusters of sequences into operational taxonomic units (OTUs) based on sequence similarity alone when the members of such groups may represent a mixture of intragenomic variants, true species or species clusters (Stat et al. 2013) may not result in accurate categorization of functional diversity (Baird et al. 2009). Further refinement of molecular markers, e.g. detecting allelic variation at microsatellite loci, or the incorporation of several independent markers with different levels of resolution, are now paving the way toward differentiating populations of closely related *Symbiodinium* species as well as clonal variation within a species (LaJeunesse et al. 2014; Pettay and LaJeunesse 2009; Baums et al. 2014; Finney et al. 2010). Hence, we are getting closer to identifying many different species of *Symbiodinium* and relevant strains and are thus closer than ever to being able to map physiological diversity to species diversity and population distributions. Such progress should open a broader understanding of realized niche space for particular symbionts across various host taxa and geographic ranges (LaJeunesse et al. 2010; Finney et al. 2010).

We are now on the cusp of discovery for determining much more detail in the taxonomy and specificity of these symbioses and how possible physiological functional diversity relates to such patterns. However, important questions to consider are what are the physiological constraints facing different *Symbiodinium*, and with regard to the physiology of these algae, is there a balance or trade-off between functional diversity and functional redundancy? Likewise, one must consider the context of the specificity of a particular symbiosis, as some host coral species are capable of harboring several different species of *Symbiodinium* at once, while other hosts appear to be relatively fixed with one particular dominant symbiont. Hence, there may be a range of variability in a particular functional trait for these algae, but the specificity of the symbiosis itself can impart a significant constraint such that a particular coral/symbiont combination may be 'locked' into a specific functional redundancy. For a photochemical example, several shallow water symbionts may be able to maximise light energy capture and light energy dissipation but some may have a minimal ability to adjust photosystem II reaction centre turnover (discussed in detail below), which could in turn play a significant role during periods of high water temperature stress.

Trait-based approaches, similar to those investigated in the community ecology of other phytoplankton (Litchman and Klausmeier 2008), are quite germane and applicable to broadening our understanding of *Symbiodinium* photobiology and physiology. The allure of determining functional traits is to form a better understanding of how they may contribute to algal (and symbiotic host) fitness across biological and environmental scales. Importantly, some traits may be driven by genes that have evolved independently across multiple groups, thereby allowing a further investigation of such traits alone with less focus on phylogeny (Barton et al. 2013). However, a key challenge with such an approach is teasing apart which traits define a particular symbiont niche and the associated hurdles with how to measure a particular trait. This last point is especially relevant to this system, as the majority of naturally occurring *Symbiodinium* are not easily cultured, nor should we expect all aspects of cellular physiology to be the same when comparing a symbiont *in* and *ex hospite* (Stochaj and Grossman 1997).

This chapter attempts to summarize some key aspects of the photobiology of the different algae within *Symbiodinium* in the context of specific cellular traits used in the balance of light harvesting and light utilization. Next, we focus on several elements of *Symbiodinium* photosynthesis that have served as primary focal points in examining the response to environmental perturbation, such as elevated water temperature related to coral bleaching, and carbon supply related to ocean acidification. Several functional traits are common across a number of classes of phytoplankton yet also display notable differences that can play a significant role in determining the ecological success and niche space for a particular alga. Similarly, symbiosis research is reliant on a

range of subjects from uni-algal cultures, to 'model' symbiotic cnidarians that are amenable to laboratory manipulations, to reef corals that harbor a dizzying array of morphological and additional layers of complexity. A primary point of our synthesis here is that using photobiological traits to begin to define functional diversity will enable movement away from the reliance of using *Symbiodinium* phylotype as a primary determinant of the response capability and/or pre-determinacy of ecological success.

30.2 Balancing Light Harvesting and Light Utilisation

Microalgal cells within both benthic and pelagic environments are continually subjected to highly variable light fields. Light availability is largely stochastic and thus cells have evolved a variety of mechanisms, which operate over a continuum of time scales from microseconds to hours (Falkowski and LaRoche 1991; MacIntyre et al. 2002) that ensure physiological and biochemical plasticity to continually optimise photosynthetic rates, i.e. photoacclimation. Upper and lower thresholds of photosynthetic optimization to changes in light availability, as either light intensity or spectral quality (Falkowski and LaRoche 1991; Hickman et al. 2009; Moore et al. 2006), are set according to genotypic constraints in cellular functioning, i.e. photoadapation; as such, photoacclimation potential can be highly variable between microalgal species (Kulk et al. 2011; Lavaud and Lepetit 2013; Six et al. 2008) and even between different genetic variants of the same species (Hennige et al. 2009; Suggett et al. 2007). Photoadaptation thus ensures dynamic changes to microalgal community and population structure over space and time according to the light niche to which species are best optimized. *Symbiodinium* as a genus indeed appears highly plastic in both photoacclimation and photoadaption potential (Frade et al. 2008; Hennige et al. 2009; Iglesias-Prieto and Trench 1994), which no doubt is a major contributor towards its broad ecological success of inhabiting a dynamic range of spatial and even temporal light environments, e.g. shallow reef flats to mesophotic reefs, but also of life history, i.e. free living benthic and pelagic as well as *in hospite* within invertebrate hosts.

Light-niche optimization imposed through photoacclimation is almost universally characterized through use of the so-called photosynthesis-light response curve (PE curve). The shape and magnitude of the PE-curve reflect the underlying cellular processes that interact to regulate photosynthesis as a function of light availability (MacIntyre et al. 2002); as such, both shape and magnitude are inherently dependent upon availability of environmental factors that regulate microalgal growth, in particular temperature and inorganic nutrient availability (including CO_2), as well as the "currency" used to describe photosynthesis (sensu Suggett et al. 2009), i.e. O_2 evolution, CO_2 uptake, or linear electron transport. Applying models describing the light-dependency of photosynthesis (Sakshaug et al. 1997; Silsbe and Kromkamp 2012) to PE curves thus provide an objective means to quantify how environmental history influences: (i) extent of light absorption under "sub-saturating" light intensities, and so the light-dependent photosynthesis rate (termed α); (ii) the rate of maximum photosynthetic turnover under "saturating" light intensities, and so the light-independent maximum photosynthesis rate (termed P^{max}); and (iii) extent of photoinhibition under "supersaturating" light intensities. These various parameters are not static but continually undergo "fine tuning", or dynamic balancing (Geider et al. 1998), based on the scales of operation of cellular processes relative to those of environmental variability. The saturation light intensity of photosynthesis (termed E_K) is particularly useful in understanding the mechanism behind dynamic balancing. In a classic experiment by Escoubas et al. (1995), inhibitors were used to mimic the process of changes in light availability as either $E > E_K$ (high light) or $E < E_K$ (low light) by either reducing or oxidising the plastoquinone (PQ) pool. As such, cells underwent photoacclimation via "signaling" according to the redox state of the PQ pool where $E \neq E_K$ (i.e. E/E_K was greater or less than a value of 1). Analysing PE curves where E is normalised to the corresponding value of E_K thus provides a powerful means to differentiate patterns of photoacclimation from adaptation (MacIntyre et al. 2002; Hennige et al. 2008, 2011; Suggett et al. 2012b), in particular where inter-comparing data from across different light fields using easily collected active-fluorescence based light curves (Hennige et al. 2008). In this context of the PE curve, the extent and timescale that E_K is effectively exceeded will ultimately govern the nature by which photoacclimation potential can maintain cellular fitness according to (i) relatively long term changes in light availability (e.g. hours to seasons) that predominantly influence the rate of macromolecular synthesis, notably of key proteins associated with light harvesting and CO_2 uptake; and (ii) relatively rapid light variability (e.g. seconds to hours) that predominantly influences physiological fine tuning to light utilization pathways and minimisation of photodamage.

Photosystem II (PSII) and photosystem I (PSI) harvest light in concert to provide the electrochemical energy required for linear electron transport, and so evolve O_2 and generate the energy (ATP) and reductant (NADPH) required for cellular growth and maintenance (Fig. 30.1). As such, the product of absorption by PSII and PSI determine the total light harvested for photosynthesis according to: (i) the extent of photon absorption by the type and arrangement of chromophoric pigment-protein complexes, (ii) the efficiency with which energy is funneled to the photosynthetic reaction centres, and the number of reaction centres present to then

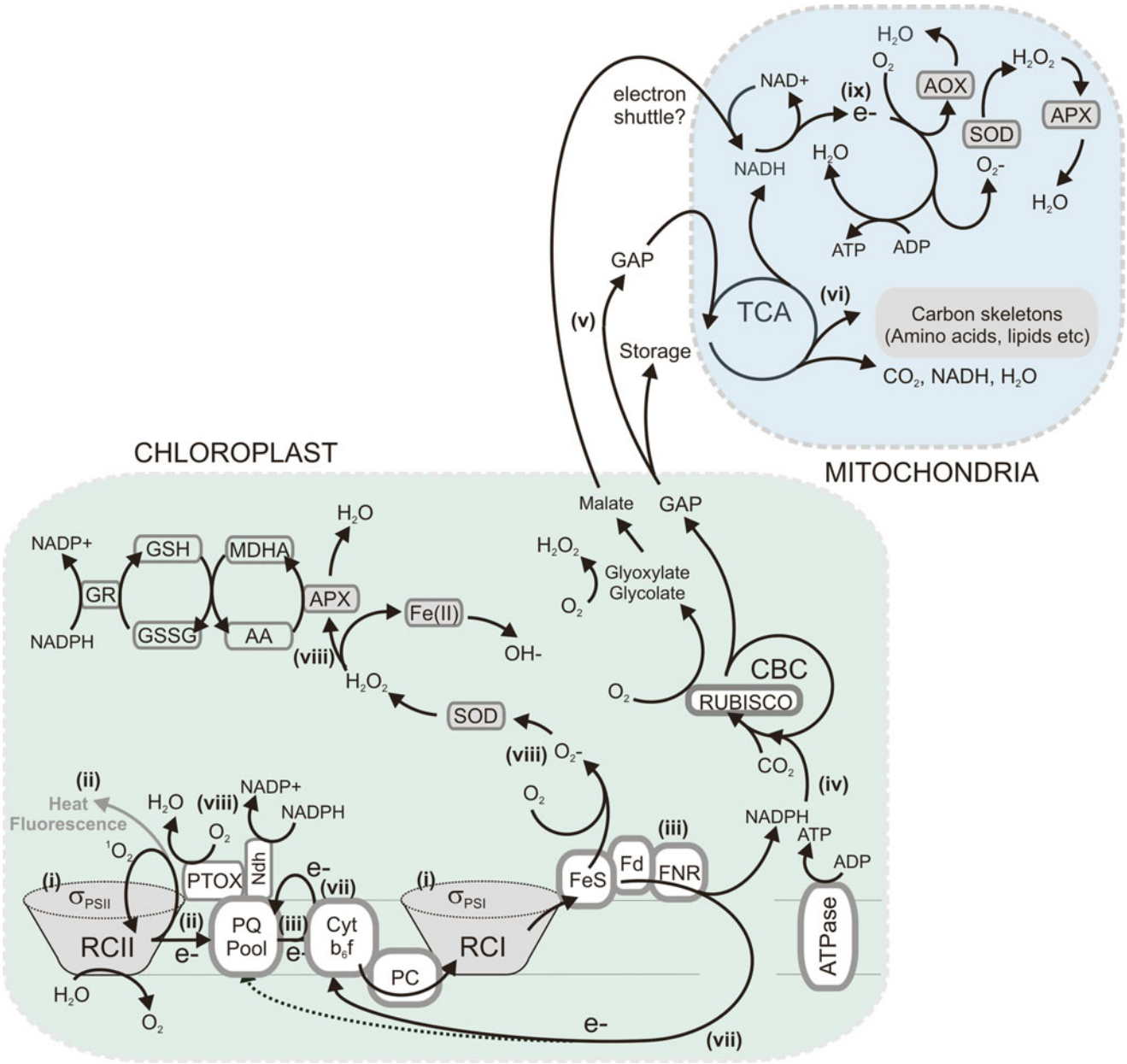

Fig. 30.1 Photochemistry and associated pathways within the chloroplast and mitochondria acting as intracellular electron sinks and/or sources of Reactive Oxygen Species (ROS) (Modified from Behrenfeld et al. (2008) and Suggett et al. (2011); see also main text): (i) light harvesting by the light harvesting complexes (the coupled antennae, σ_{PSII} and σ_{PSI}, and reaction centres, RCII and RCI); (ii) competing pathways for absorbed photons, photochemistry (splitting water to generate an electron) within, and fluorescence and heat emission from, the PSII complex; (iii) electron flow via the linear electron transport chain from RCII via the plastoquinone pool (PQ pool), Cytochrome b_6f (Cyt b_6f) and plastocyanin (PC), to generate the transthylakoid proton motive force required for ATP formation via ATPase. Also, linear electron flow from PSI from RCI via iron-sulphur complex (FeS), Ferrodoxin (Fd) and Ferrodoxin-NADP+ reductase (FNR) to generate reductant (NADPH); (iv) consumption of ATP and NADPH from competing pathways of RuBisCO within the Calvin-Benson-Cycle (CBC) of carboxylation (CO_2) through to glyceraldehyde 3-phosphate (GAP) – CO_2 assimilation – versus oxygenation through to malate – photorespiration; (v) substrate connectivity of the chloroplast to the mitochondria – GAP to fuel the tricarboxylic acid cycle (TCA) and export/storage to the cytosol, and/or possible energetic shuttling via malate- oxaloacetate inter-conversion; (vi) fates for an 'incomplete' TCA cycle: drawing off reductant (NADH) prior to mitochondrial electron transport or drawing off carbon skeletons (where the cycle is 'complete' ATP is generated by mitochondrial electron transport); (vii) cyclic electron flow around PSII (via Cyt b_6f to the PQ pool) and/or around PSI (via the PSI electron carriers back to either Cyt b_6f or the PQ pool); (viii) non-linear (alternative) electron flow to convert O_2 back to H_2O: plastoquinone terminal oxidase activity (PTOX) (as well as associated "chlororespiration" from a plastid NAD(P)H dehydrogenase (Ndh) complex via the PQ pool). and Mehler-Ascorbate-Peroxidase (MAP) activity. MAP involves a cascade of electron flow via superoxide dismutase (SOD) and ascorbate peroxidase (APX) that result in intermediate ROS superoxide (O_2^-) and hydrogen peroxide (H_2O_2), with possible catalysis of H_2O_2 to hydroxyl radicals (OH^-) by iron (Fenton's Reaction). Activity of APX is inherently coupled to glutathione cycling (Foyer-Halliwell-Asada cycle) via glutathione reductase (GR) and inter-conversion of glutathione disulphide (GSSG), reduced glutathione (GSH), monodehyroascorbate (MDHA) and ascorbate (AA). Other potential sources of ROS within the chloroplast are from inter-conversion of glycolate to glyoxylate (H_2O_2) via photorespiration and generation of singlet oxygen ($^1O_2$) from energy transfer within the antennae and reaction centers; and (ix) mitochondrial respiration via cytochrome-c or alternative oxidase (AOX) leading to non-linear electron flow to convert O_2 back to H_2O via SOD and APX. To date, how these various pathways and processes interact as a connected network remains unexplored for *Symbiodinium*

actively "trap" energy for photochemistry. A particularly useful parameter in describing this process is the "effective absorption cross section" (termed σ, see Mauzerall 1972), which parameterizes the effective antennae size (i.e. the target area) of the light harvested for photosynthesis, and in the case of PSII is easily measured using active chlorophyll *a* fluorometry (σ_{PSII}, Kolber et al. 1998; Gorbunov et al. 2001; Szabó et al. 2014). Absorption-specific to PSII (a_{PSII}) can then be calculated from the product of σ_{PSII} and the number of PSII reaction centers, [RCII] (Suggett et al. 2007, 2011). Whilst the same principle applies to PSI, i.e. $a_{PSI} = \sigma_{PSI} \cdot [RCI]$, measuring σ_{PSI} is extremely challenging (e.g. Ryan-Keogh et al. 2012). Even so, this biophysical-based consideration of light harvesting capacity has provided a primary framework for improving our understanding as to how microalgal cells (Strzepek and Harrison 2004; Six et al. 2008; Suggett et al. 2007), including some *Symbiodinium* genotypes (Suggett et al. 2015; Hennige et al. 2009; Iglesias-Prieto and Trench 1997; Falkowski and Dubinsky 1981), have adapted different strategies for allocating resources and light niche optimization.

Photoacclimation reflects a trade-off between modifying the pigment bed (σ), "photosynthetic unit size", versus reaction centre content (n), "photosynthetic unit number" (Falkowski and Dubinsky 1981; Falkowski and Owens 1980). Cellular resources required for production of reaction centers far outweigh those for pigment-protein antenna complexes; consequently, σ_{PSII} often appears to be larger, and preferentially modified over [RCII], in resource limited environments (Moore et al. 2006; Strzepek and Harrison 2004; Suggett et al. 2009). In contrast, dynamic environments where light is highly variable but nutrients in greater supply, appears to favour photoacclimation through modification of [RCII] over σ_{PSII} (Six et al. 2008). Interestingly, *Symbiodinium* predominantly appears to photoacclimate according to this "n-type" strategy more associated with dynamic environments (Hennige et al. 2009; Iglesias-Prieto and Trench 1994), including the highly variable light fields of many reefs (Roth 2014). Whilst such a strategy is potentially at odds with the very static environmental conditions with which *Symbiodinium* cultures have been used to derive these photoacclimation properties, this response is in fact not universal with some genotypes preferentially modifying σ_{PSII} (Falkowski and Dubinsky 1981; Hennige et al. 2009). As such, persistent life in laboratory cultures does appear to conserve adaptive diversity in photoacclimation strategy amongst isolates, although whether inherent strategy after years in culture actually reflects the strategy at the time originally isolated is untested. However, we now know that this variation in strategy amongst *Symbiodinium* genotypes is not consistent with phylogenetic variation but best explained by differences in cell size (Suggett et al. 2015). These authors demonstrated that changes of a_{PSII} amongst 18 genotypes (covering five clades) of *Symbiodinium* could be closely related to changes in cell size; this finding would appear highly consistent with the notion that *Symbiodinium* chloroplasts appear to be arranged as reticulated plastids encircling the cell, and thus light harvesting will be under selective pressure form cell size/volume (LaJeunesse et al. 2010). Interestingly their study also confirmed that co-variability of a_{PSII} with cell size appears largely (but not exclusively) under control from [RCII] (n-type) rather than from σ_{PSII} (σ-type) patterns. Such biophysical explorations of light harvesting have thus begun to establish a broad operational basis to explain adaptive differences in light niche partitioning ("ecotypes") observed for this alga (Falkowski and Dubinsky 1981; Frade et al. 2008; Iglesias-Prieto et al. 2004).

Understanding how absorbed light is subsequently utilised (dissipated) is especially important when considering photobiological responses to short term environmental variability. Cells must minimise transient excessive exposure to conditions that induce photoinhibition via "photoprotective pathways", and thus represent an important component of overall photoacclimation strategy and associated resource investment. The overall photosynthetic yield that is generated from the total quanta absorbed is referred to as the quantum yield (identified by the symbol ϕ); again, as with the PE curve, a quantum yield can therefore be generated for different photosynthetic currencies (O_2 evolved, CO_2 fixed or extent of photochemistry). In the case of PSII photochemistry, the quantum yield (termed ϕ_{PSII}) is easily measured using active chlorophyll *a* fluorometry and predominantly describes the balance of absorbed light that is dissipated as photochemistry versus heat, relative to the quantum yield of fluorescence itself. The balance of photochemical versus heat dissipation is therefore regulated by (i) longer-term acclimation processes that determine "σ-type" versus "n-type" strategies, and so govern the maximum achievable PSII quantum yield, and (ii) transient short term photoprotective process that occur under light exposure to increase heat dissipation (decrease photochemical dissipation), to decrease the maximum PSII quantum yield in the light. As such, the extent with which changes in the PSII quantum yield under transient light exposure reflect alterations to heat versus photochemical dissipation provides a secondary framework to evaluate photoacclimation strategies (Lavaud et al. 2007; Suggett et al. 2007). For example, diatom species evolved to more stable oceanic environments appear to have invested in cellular machinery that drives a lower heat-to-photochemistry ratio than for diatom species evolved to more variable coastal waters (Lavaud et al. 2007; Lavaud and Lepetit 2013). *Symbiodinium* genotypes appear to similarly express differences in their inherent heat-to-photochemistry ratio (Suggett et al. 2015). Whilst these differences appear to show some relationship with phylogenetic designation (Suggett et al. 2015), they also appear to relate to niche

separation in terms of shallow versus deeper corals (Iglesias-Prieto et al. 2004) as well as tolerance to transient heat stress (Hennige et al. 2011; Suggett et al. 2012b); however, such relationships are still largely unexplored. Similarly, how these differences in light utilization strategy may ultimately reflect a trade off against corresponding light absorption strategy is still unresolved.

Whilst the balance of heat versus photochemical dissipation of absorbed excitation energy provides a useful means to identify patterns of photoacclimation strategy, it is in reality difficult to interpret since cells can have a number of "safety valves" to maintain photochemical electron turnover that may or may not feedback to whether/how energy is transiently dissipated as heat, i.e. "non-photochemical quenching". Linear electron transport through the photosynthetic apparatus is required to generate a proton gradient across the thylakoid membrane, which is used to drive energy (ATP) formation but also acts as the trigger for heat dissipation. However, up-regulation of alternative (non-linear) electron flow (AEF) pathways can sustain linear electron flow and in turn maintain the accumulation of the proton gradient across the thylakoid membrane/extent of heat dissipation (Fig. 30.1). An important basis for these pathways is not only to photoprotect the electron transport chain against excessive excitation but also balance ATP and reductant (NADPH) supply needed for optimum cellular functioning (e.g. Cardol et al. 2011); we return to the importance of AEF later in this chapter. An additional shortcoming to RUBISCO is especially important in the context of AEF and photoprotection (see Sect. 30.3.4).

In spite of the numerous pathways that exist to "photoprotect", it is inevitable that many microalgae are still periodically subjected to light stress-induced photoinhibition given the dynamic nature of light availability. Energy transfer from the triplet state of excited chlorophyll molecules within both the antennae and reaction centers (Telfer 2014) results in formation of the Reactive Oxygen Species (ROS) singlet oxygen ($^1O_2$). Ordinarily, carotenoid molecules quench $^1O_2$; however, when this quenching capacity is exceeded the excess $^1O_2$ causes "acceptor side" inhibition by directly damaging the reaction centre complex integrity (Yadav and Pospíšil 2012), although evidence suggests that in some cases $^1O_2$ may actually be beneficial by modifying the redox state of the PQ-pool to signal down-regulation (Kruk and Szymańska 2012; Triantaphylidès and Havaux 2009). Such loss of reaction center integrity (notably the PSII reaction centre) has been frequently observed for *Symbiodinium* under stress (Warner et al. 1999, see Sect. 30.3.2) and appears linked with $^1O_2$ targeting of the light harvesting complexes (Takahashi et al. 2008). However, the very nature of photoprotection via AEF makes cells additionally vulnerable to exposure by other ROS. Both the chloroplast and mitochondria (Lesser 2006; Rhoads et al. 2006), employ AEF pathways that result in transient formation of superoxide (O_2^-) and/or hydrogen peroxide (H_2O_2) (see Fig. 30.1). Whilst the very nature of reduced quinone molecules within these organelles can act as antioxidants (Lutz et al. 2014), excessive ROS production will occur where the key quenching molecules such as superoxide dismutases and peroxidases cannot match O_2^- and H_2O_2 production rates (Das et al. 2015; Lesser 2006). At face value therefore, use of AEF pathways would seem a risky strategy to minimize photodamage since highly reactive O_2^- and/or H_2O_2 can attack numerous cellular components, including proteins, nucleic acids and lipid membranes (see Lesser 2006); for example the thylakoid membranes of *Symbiodinium* can become particularly destabilized during ROS-induced stress (Tchernov et al. 2004). However, these ROS are also increasingly recognized as important signaling molecules that not only play a role in maintaining optimum photosynthetic operation via redox homeostasis in other photosynthetic organisms (Das et al. 2015; Rhoads et al. 2006; Triantaphylidès and Havaux 2009), and thus are effectively part of the photoacclimation process, but also the wider cellular network for population control (autophagy) (Pérez-Pérez et al. 2012). Not only is this ROS component of photoacclimation and photoprotection still largely unexplored for most microalgae, but how it links to cellular processes via metabolites and signaling, i.e. the ROS production "network" that is well established in terrestrial plants, unknown. This latter point is particularly important given the increasingly recognized role of volatile biogenic gases, which are produced in abundance by many microalgae, but especially *Symbiodinium* (Exton et al. 2015), in the antioxidant network.

Given the dynamic and complex network of processes inherent to photoacclimation and adaptation, we still know surprisingly little as to how these processes operate and interact to determine the net measureable outcomes, e.g. photosynthesis- and quantum yield-light response curves. Instead most detailed studies to date have focused on specific pathways and processes that appear to govern photosynthetic optimisation and thus how they act as 'pinch points" under stressors and/or low resource availably. In our next section we consider these main areas of focus for *Symbiodinium* to date.

30.3 Specific Cases

30.3.1 The Role of the Light-Harvesting Complex in Photosynthetic Downregulation

As with other dinoflagellates, the light-harvesting complex (LHC) that is coupled to the photosynthetic reaction centres of *Symbiodinium* binds Chlorophyll*a* and Chlorophyll*c2*,

with peridinin as the primary "accessory" carotenoid (Venn et al. 2006; Boldt et al. 2012). Peridinin is associated with both water-soluble peridinin-Chl*a* protein complex (PCP) located on the lumen side of the thylakoid membranes as well as the integral Chl*a*-Chl*c2*-peridinin protein complex (acpCP) (Boldt et al. 2012; Kanazawa et al. 2014, and references therein). Together these harvest light across a relatively broad spectrum but with primary absorption across the blue green region of the visible spectrum (ca. 420–520 nm, Venn et al. 2006). An array of secondary carotenoids, e.g. dinoxanthin, diatoxanthin, diadinoxanthin, β-carotene and phaoephytin*a*, are also present to enhance absorption across the blue-green absorption band (see Venn et al. 2006). Importantly, these various pigments govern the transfer efficiency with which absorbed light is passed to photochemistry or re-emitted as heat; for example, the transfer efficiency for chlorophylls, peridinin and diadinoxanthin is higher than that for diatoxanthin and β-carotene, so these two pigment groups are often referred to as "photosynthetically active" and "photoprotective" respectively. However conformational changes in binding of the "photosynthetically active" pigments and protein complexes with one another can further influence their overall transfer efficiency (Kanazawa et al. 2014; Suggett et al. 2004).

The type of pigments present and how they are arranged within the thylakoids (packaged, binding state), together determine the effective antennae size of light harvesting for photochemistry (σ_{PSII}, Sect. 30.2). Growth under higher light intensities typically increases the ratio of photoprotective-to-photosynthetically active pigments (as well as reduced packaging) and thus reduced σ_{PSII} (Hennige et al. 2009; Suggett et al. 2004, 2007, 2009); however two key properties of *Symbiodinium* play fundamental roles in further modifying σ_{PSII} within the LHC:

Firstly, transient exposure to high light induces "xanthophyll cycling" (Brown et al. 1999; Ragni et al. 2010; Warner and Berry-Lowe 2006). This process up-regulates the proportion of absorbed light re-emitted as heat (non-photochemical quenching or NPQ) and is signaled by increases in transthylakoid pH (ΔpH). An enzymatic process removes epoxy groups from diadinoxanthin (DD) to produce diatoxanthin (DT) (Goss and Jakob 2010) and hence the extent of xanthophyll cycling is often referred to as the "de-epoxidation state" (DPS = DT/[DD+DT]); as such, the transfer efficiency of the LHC (and therefore σ_{PSII}) is lowered. Many studies have now established that xanthophyll cycling is an important means of photoprotection for *Symbiodinium*, whereby xanthophyll activity tracks natural light-dark cycles for cells both in culture (Sorek et al. 2013) and *in hospite* (Brown et al. 1999; Ferrier-Pagès et al. 2013; Ulstrup et al. 2008; Warner and Berry-Lowe 2006) and remains high where cells are exposed to continuous light (Sorek et al. 2013). The extent with which this activity operates in response to transient light availability is not the same for all *Symbiodinium* sp. *in hospite* (Warner and Berry-Lowe 2006; Ulstrup et al. 2008; Venn et al. 2008). Xanthophyll cycling capacity does appear to differ between *Symbiodinium* genotypes (Krämer et al. 2013), which may in part reflect differences in pigment pool sizes pre-transient exposure (e.g. Hennige et al. 2009; Ragni et al. 2010) and thus cell size (Suggett et al. 2009, 2015); however, the modification of the light field by host properties incident to *Symbiodinium* when *in hospite* clearly plays a major role as to why extent of xanthophyll activity cannot be easily reconciled with *Symbiodinium* genotype present (Brown et al. 1999; Dove et al. 2006; Venn et al. 2006; Warner et al. 2006; Ulstrup et al. 2008; Krämer et al. 2012) . Enhanced xanthophyll cycling is also observed when cells experience greater excitation pressure associated with transient heat stress (Dove et al. 2006; Venn et al. 2006; Roth et al. 2012; Krämer et al. 2013) as well as cold stress (Roth et al. 2012). Importantly, such enhancement is not observed where thermal stressors are applied relatively slowly/gradually (e.g. Brown et al. 2002; Middlebrook et al. 2010), presumably since cells invest in other downregulation pathways that operate across longer time scales.

Secondly, peridinin itself has been proposed as a "photoprotective" pigment under some circumstances. Not only can peridinin instantaneously quench Chl*a* triplet excitation, which otherwise forms $^1O_2$ (Niedzwiedzki et al. 2013), but also acts to dramatically lower the transfer efficiency during stress. Early evidence for the latter (McCabe Reynolds et al. 2008) resulted in a "detachment model" whereby the PCP complex was thought to dissociate or move between different membrane components. Such dissociation, however, is not analogous to the subsequent binding and redistribution of light toward the PSI reaction centre or state transition noted in terrestrial plants and until recently, chlorophytes (Ünlü et al. 2014; Lemeille and Rochaix 2010). This model was developed from observations of increased 77 K fluorescence emission attributable to PCP (672–675 nm) from both free living and *in hospite Symbiodinium* genotypes undergoing light (McCabe Reynolds et al. 2008) and heat (Hill et al. 2012) stress. However, the "detachment model" has recently been rejected in favour of a "quenching model" (Kanazawa et al. 2014), whereby the enhanced emission appears to in fact originate from strong non-photochemical quenching (NPQ) of longer wavelength band(s) (686 nm) by the membrane antenna complexes associated with acpPC and reaction centers. Just how acpCP operates in *Symbiodinium* as a strong quencher is still unknown but may relate to conformational changes associated with the inherent pigment protein complexes (e.g. Ünlü et al. 2014); indeed, acPCP sequences appear highly variable both within and between algal genotype (Boldt et al. 2012; Jiang et al. 2014). De novo synthesis of acpCP is impaired by heat stress in some *Symbiodinium*,

further highlighting the key role this complex plays in photoprotection (Takahashi et al. 2008).

30.3.2 The Role of Reaction Centre Stability and Turnover

The PSII reaction centre has been a primary focal point in previous and current investigations of environmental stress in reef corals and in the study of coral bleaching in particular. Bleaching, a common phenomena whereby corals lose a significant number of symbionts (and less frequently a loss in chlorophyll algal cell^{-1}) following periods of exposure to high water temperature, has long been linked to a loss in photosynthetic activity as measured by traditional oxygen exchange and PE curve analyses (Coles and Jokiel 1977) (described above). Subsequent work utilizing active chlorophyll *a* fluorescence noted a substantial loss in the maximal PSII fluorescence quantum yield (Φ_{PSII}^{max} or F_v/F_m) and established a link between the loss of symbionts and the decline in PSII photochemistry (Hill et al. 2004; Iglesias-Prieto et al. 1992; Jones et al. 2000; Warner et al. 1996). In many cases, PSII function declines prior to the significant loss of algal cells (Warner et al. 2002), however, such evidence is reliant on high temporal sampling, which is not always logistically possible. A common key feature of natural mass coral bleaching is that seawater heating is accompanied by extended periods of calm weather and hence greater light dose (Mumby et al. 2001). This factor leads to the notion that patterns of photodamage are similar or analogous to pathways of photoinhibition in terrestrial plants and other phytoplankton (Lesser and Farrell 2004). In particular, this process occurs when the rate of light absorption and photochemical reactions in the PSII reaction centre exceed the rate of energetic utilization for carbon fixation and/or rates of other sources of non-assimilatory electron flow; importantly, here the strict definition of photoinhibition is used to refer to reactions driven by high light alone and does not require damage to other components of photosynthesis (e.g. the Calvin Benson Cycle) or any manipulation of temperature per se (Adir et al. 2003). PSII damage and repair, whether related to high light alone or in combination with several stressors, involves a suite of possible triggers such as different reactive oxygen species as well as several modes of reaction centre degradation and assembly. For a more exhaustive review on the many facets of photoinhibition and the current debated topics therein, we direct the reader to other reviews (Roach and Krieger-Liszkay 2014; Nishiyama and Murata 2014). However, given the evidence for multiple stress pathways, and the dependence of heat and light in the context of coral bleaching, we use the broader term 'photoinactivation' to describe the general disruption to photosynthesis in *Symbiodinium*, while still acknowledging some clear analogs to the loss in PSII function by high light alone.

Earlier investigations with *Symbiodinium* noted a significant loss in a primary structural PSII reaction centre protein, known as D1 (also denoted as PsbA), during natural and experimental bleaching in thermally sensitive algae *in hospite* as well as in culture (Warner et al. 1999). By following the rate of photoinactivation at ambient and elevated temperature by chlorophyll *a* fluorescence and in the presence and absence of the chloroplast protein synthesis inhibitor lincomycin, Warner et al. (1999) noted a much higher rate of PSII turnover in a *Symbiodinium* isolate possessing a higher thermal tolerance, thus providing evidence that the reaction centre repair cycle was a key determinant for heat tolerance as well as a possible weak point for heat-sensitive algae. This hypothesis was further corroborated by studies with corals (Takahashi et al. 2004). For some *Symbiodinium*, there is an interesting link between their ability to withstand acute shifts to high light and their thermal tolerance. Comparing the rate of loss and recovery in PSII photochemistry following high light exposure in the absence and presence of lincomycin permits calculation of an index of net vs. gross photoinhibition (Ragni et al. 2010). Such comparisons have indeed shown how one cultured alga (*Symbiodinium microadriaticum*, ITS2-type A1) sustains a higher rate of PSII repair as compared to a closely related yet thermally sensitive alga *Symbiodinium necroappentens* (formerly ITS2-type A13; LaJeunesse et al. 2015; Ragni et al. 2010), and these differences match the previously determined respective thermal tolerances of these two isolates (Robison and Warner 2006). Subsequent studies using similar protocols with various corals also provide a close link between more rapid PSII repair rates and historical resistance to thermal bleaching (Hennige et al. 2011). In contrast to one study, where PSII repair declined in a cultured isolate held above 31 °C (Takahashi et al. 2009), other studies to date with *in hospite Symbiodinium* across a range of coral species have demonstrated substantial elevated PSII repair under elevated light and/or temperature (Hennige et al. 2011; Hill et al. 2011; Krämer et al. 2013). Thus, in several thermally sensitive *Symbiodinium* spp., rather than chloroplast protein repair pathways being directly damaged, the rate of PSII repair cannot keep pace with the simultaneous rate of photodamage during thermal stress. However, it should be stressed that it is not easy to compare results across many of the aforementioned studies, given the use of cultures vs. intact corals and different species of corals, as well as different heating and light treatments. Thus, there is a strong probability that some thermally sensitive algal species may suffer from direct damage to protein repair pathways, while other algal species are essentially losing a race to maintain adequate repair.

Further examination of the photochemical response by active chlorophyll *a* fluorometry and tracking core PSII reaction centre proteins has led to a suite of reactions across different corals and *Symbiodinium*, most likely reflecting the fact that a range of damage and repair processes are occurring and there is not a "one-size-fits-all" model of photodamage across this genus. For example, Jeans et al. (2014) noted a significant drop in PsbA and PsbD cell^{-1} (the D1 and D2 proteins of the PSII reaction centre respectively) in *Symbiodinium* remaining within a coral subjected to short-term heating, yet F_v/F_m remained unchanged. However, expelled algae over this time period displayed the opposite trend. By 14 h of heating, net F_v/F_m declined while PSII reaction centre protein content of the remaining *in hospite* symbionts increased, possibly representing a portion of inactive reaction centres. Some work has shown a close correlation between the rate of loss in photochemistry and D1 protein abundance in heated corals and algal cultures (Hill et al. 2011; Robison and Warner 2006). However, continued efforts to measure recovery in the presence/absence of PSII reaction centre repair following thermal and light stress have shown that well over half of the photochemical recovery was independent of de novo chloroplast protein repair in one temperature sensitive coral (*Pocillopora damicornis*), while protein repair was responsible for over 90% of PSII photochemical recovery in symbionts within another bleaching-sensitive species (*Acropora millepora*). Further, there was minimal increase in D1 protein content in either of these corals in the absence of protein synthesis inhibitors.

Interestingly, while the PSII core and its de novo assembly are incredibly conserved from cyanobacteria to terrestrial plants, there are marked differences in comparison to PSII repair pathways (Nickelsen and Rengstl 2013). For example, in plants several light harvesting and core PSII proteins, including D1 and D2, have a redox regulated phosphorylation cycle that is critical for proper migration of damaged PSII complexes from grana to stromal lamellae (Tikkanen et al. 2008), while such biochemical pathways and segregation of chloroplast thylakoids are not present in dinoflagellates. Further, in the chlorophyte, *Chlamydomonas reinhardtii*, repair-related synthesis and de novo assembly synthesis of D1 are two very different pathways that are spatially segregated, and de novo assembly is controlled at the level of translation initiation while synthesis for repair seems to be regulated at the peptide elongation step (Nickelsen and Rengstl 2013). Similarly, recent studies with cyanobacteria and plants have noted a myriad of specific proteases, including ATP-dependent FtsH metalloproteases and ATP-independent Deg endoproteases required for the proper degradation and clearance of D1 (Kato et al. 2012; Nixon et al. 2010). Recent efforts have noted an excellent correlation between the rate constant for removal of D1 and the content of an $FtsH_6$ protease in the diatom *Thalassiosira pseudonana* (Campbell et al. 2013). Furthermore, an offshore strain of *T. pseudonana*, which had evolved from a more stable light environment, displayed a lower FtsH content and slower rate constants for removal of D1 as compared to a coastal strain which had evolved in a much more variable light environment (Campbell et al. 2013). Given some similarity in thylakoid topology between diatoms and dinoflagellates, these results point to the possibility that perhaps similar protease systems could function in an analogous fashion in the PSII repair cycle in *Symbiodinium* spp.

Substantial gaps are still evident for our knowledge of how dinoflagellates maintain chloroplast protein assembly and repair and these are grounded in the current mystery surrounding the cellular and molecular level of their control. Like all peridinin-based dinoflagellates examined to date, *Symbiodinium* possess a chloroplast genome that is encoded on several small minicircles approximately 2–3 kbp in size, with typically one gene per minicircle (Mungpakdee et al. 2014; Howe et al. 2008). While the majority of the original chloroplast encoded genes have been transferred to the nucleus, a set of 14 genes remain in these minicircles within the *Symbiodinium minutum* chloroplast that encode core functional proteins involved in the photosynthetic electron transport (PET) chain (Mungpakdee et al. 2014). An enticing hypothesis for why these particular genes have been retained in the chloroplast while so many more reside in the nucleus is that they are needed for rapid regulation in response to environmental change that also affects the redox state of the PET chain (Allen 1995). This Co-location for Redox Regulation (CoRR) hypothesis would seem germane, especially in light of the rapid loss in electron transport and plastoquinone reduction noted in *Symbiodinium* undergoing thermal and light stress. However, attempts to test co-regulation of specific PET components by manipulating the redox poise of the plastoquinone pool in cultured *Symbiodinium*, either by light or with specific chemicals (sensu Pfannschmidt et al. 2009), has provided mixed results at best (McGinley et al. 2013; McGinley 2012). Despite evidence for thermal loss in *Symbiodinium* chloroplast encoded genes (e.g. *psbA* and *psaA*) (McGinley et al. 2012), and while posttranscriptional control of such genes likely plays a larger role, substantial differences in gene expression were recently noted across two different types of *Symbiodinium* (Barshis et al. 2014).

Single turnover chlorophyll *a* fluorescence techniques such as Fluorescence-Induction and Relaxation (FIRe) and Fast Repetition Rate fluorometry (FRRf) have provided yet more detail regarding the possible change in energy balance between light harvesting and photochemistry during thermal stress. In some cases the functional absorption cross section of PSII may not change while F_v/F_m is stable or declines under thermal treatment (Jeans et al. 2014; Lesser and Farrell 2004), thus light harvesting may remain balanced with PSII

photochemistry or a substantial rise in nonphotochemical quenching (NPQ) of chlorophyll *a* fluorescence may be the result of damaged or down-regulated PSII reaction centers acting as quenchers (Matsubara and Chow 2004). Likewise, there is evidence for substantial xanthophyll de-epoxidation even after prolonged dark acclimation and hence this may also contribute to long-lived nonphotochemical quenching and confound the interpretation of a decline in PSII photochemistry (Middlebrook et al. 2010). While no significant change in σ_{PSII} suggests minimal contribution from antennae-based down regulation (e.g. xanthophyll cycling; see Sect. 30.3.1) other studies have noted a significant rise in σ_{PSII} and a decline in photochemistry (F_v/F_m) within cultured and *in hospite Symbiodinium* spp. (Ragni et al. 2010; McGinley et al. 2012). This particular pattern of damage likely reflects a greater loss of core PSII reaction centre proteins while the antennae bed remains more intact, thereby enhancing the interactive effects of combined thermal and light stress, as light harvesting complexes continue to move excitation energy toward damaged reaction centers. Importantly, such differences in photochemical metrics may also be driven by the constraints inherent in a particular experimental design, such as the range and rate of heating, as well as the different response. How such trade-offs between LHC and reaction centre maintenance during thermal stress relate to the fundamental constraints of "σ-type" versus "n-type" strategies for photoacclimation (see Sect. 30.3.1) remain an open area ripe for new discoveries that could provide more detail in forecasting the resilience of a particular *Symbiodinium*/host combination.

30.3.3 Interactions with Nutrient Limitation

An emerging theme amongst studies examining *Symbiodinium* photochemistry in the context of a changing environment is possible interacting roles from different sources of limiting compounds, including inorganic carbon as well as organic and/or inorganic nitrogen and phosphorous. Despite earlier assertions (Burris et al. 1983), there is now considerable evidence for some form of carbon limitation in many organisms harboring *Symbiodinium*. For example, Goiran et al. (1996) noted that the half saturation constant (K_m) for HCO_3^- was considerably lower in *Symbiodinium* freshly isolated from a coral as compared to the alga following isolation and culture (71 vs 178 μM, respectively); however, it bears noting that it was not confirmed that the alga in culture was indeed representative of the dominant alga *in hospite*. Other work has identified a significant increase in gross photosynthesis (normalized to chlorophyll or algal cell number) when comparing *in hospite* vs. freshly isolated algae from a high light acclimated hydroid (Fitt and Cook 2001), and elevated photosynthesis in several coral species when HCO_3^- is supplied above typical levels of ambient seawater DIC (~2 mM) (Herfort et al. 2008). Whilst photosynthesis may be saturated above ambient DIC, greater sensitivity of *in hospite Symbiodinium* as compared to cultured algae is evident when DIC is experimentally limited, leading to a reduction in PSII electron flow and net oxygen production (Buxton et al. 2009).

Further evidence for the high carbon demands of these symbioses stems from the fact that the dissolved CO_2 necessary for carbon fixation in the Calvin-Benson cycle (CBC) is a small constituent in seawater and cannot meet the demands of photosynthesis by passive diffusion alone. This problem is solved, in part, from the enhanced transcriptional up-regulation, elevated activity and cellular localization of the host-derived carbon concentrating enzyme carbonic anhydrase (CA), an enzyme that catalyses the interconversion of HCO_3^- and CO_2 (Bertucci et al. 2013, 2011; Weis and Reynolds 1999; Weis 1993). Like many algae, *Symbiodinium* also minimises CO_2 limitation by investment in several possible carbon concentrating mechanisms (CCMs), including internal and external CAs, an H^+-ATPase pump, and Na^+/HCO_3^- transporters (Al-Moghrabi et al. 1996; Leggat et al. 1999; Yellowlees et al. 1993; Bertucci et al. 2010). Attempting to understand the possible role(s) of C-limitation is further confounded by variability or alternative preference in DIC utilised within the intact symbiosis as well as for algae in vitro and how such preferences affect particular CCMs. Strong preference for HCO_3^- as well as CO_2 has been exemplified in some *Symbiodinium* in corals (Goiran et al. 1996), while algae within the giant clam, *Tridacna gigas*, utilise CO_2, but then rapidly covert to a greater dependence for HCO_3^- shortly after going into culture (Leggat et al. 1999). Brading et al. (2013) further recently documented different preferences of carbon type amongst two *Symbiodinium* genotypes in culture.

The importance of CO_2 limitation as well as DIC utilization in *Symbiodinium* symbioses has been highlighted in two areas related to global climate change: thermal anomalies that result in coral bleaching and increased human production and dissolution of CO_2 resulting in ocean acidification. For coral bleaching, Jones and colleagues (1998) proposed that some unknown aspect of the CBC, possibly at the point of carboxylation, was the initial site of thermal perturbation in the algal chloroplast, which then leads to over-reduction of the PET chain and eventual damage to the PSII reaction centre as detailed above. Subsequent investigations have attempted to further validate this hypothesis by other measures such as by tracking Rubisco content and the photochemical response following chemical inhibition of the CBC as well as electron transport inhibitors. No studies to date have documented a significant loss in total RbcL content or Rubisco enzyme activity in *Symbiodinium* that corresponds to ecologically relevant temperatures for many reef locations

(inhibition of activity is typically >34 °C) (Hill et al. 2011; Leggat et al. 2004; Lilley et al. 2010). However, studies inhibiting carbon fixation by low levels of inhibitors (e.g. glycolaldehyde or potassium cyanide) or exogenous competitive electron acceptors (e.g. methyl viologen) have corroborated the original hypothesis of some limitation to the dark reactions leading to a loss of photochemistry and a bleaching response for the same coral species (*Stylophora pistillata*) and collection location investigated by Jones et al. (1998) (Bhagooli 2013); however, such a response has not been replicated for other coral-symbiont associations (Hill et al. 2014). As such, the role of the CBC in regulating photobiological stressors during heat stress is still largely unresolved, but may be important for a small number of host-*Symbiodinium* combinations. Additional hypotheses concerning C-limitation and bleaching are that either the symbiont and/or host CCM pathways are damaged by excess temperature or the loss of photosynthate itself would limit the host energetic demands to maintain high CCM activity (Wooldridge 2009). All of these scenarios could lead to a similar sink limitation resulting in eventual PSII photoinactivation as previously outlined in Jones et al. (1998), nevertheless, direct evidence for thermal inhibition of host CCM mechanisms is currently lacking, and Oakley et al. (2014) recently demonstrated a decline in the half-saturation constant of photosynthesis vs. DIC concentration (a good predictor of CCM function) with elevated temperature among several cultured *Symbiodinium*; thus algal CCM components do not appear to be likely candidates for the point of initial thermal liability. While determining the ultimate vs. proximal cues of photostress in the context of bleaching remains a formidable challenge, the exacerbation of temperature with high light and common links to ROS formation (see Sect. 30.3.4 below) suggests several shared physical and chemical pathways across many different symbioses.

Ocean acidification has driven renewed recent interest in carbon assimilation processes in *Symbiodinium* and how they are potentially connected to photochemical performance. Laboratory culture studies have demonstrated that genotypes within clade A exhibit improved photosynthesis or growth rates when grown under "future" CO_2 scenarios (Brading et al. 2011), which appears to reflect differences in inorganic carbon acquisition and assimilation pathways (Brading et al. 2013, above); specifically under elevated pCO_2, some isolates within clade A undergo enhanced growth, interpreted as a relief of the energetic costs imposed through use of a CCM, whilst others undergo enhanced rates of productivity, which is interpreted as relief of carbon limitation within the CBC (see Brading et al. 2011). Similarly, ITS2-type A19 shows enhanced electron transfer and CO_2-uptake rates when *in hospite* in the anemone *Anemonia viridis* under naturally elevated CO_2 conditions, leading to enhanced ecological success of the host anemone (Suggett et al. 2012a). Not surprisingly, such a response is not solely restricted to algae within a particular clade, with enhanced symbiont productivity and host growth recently observed for an ITS2-type B1 (most likely *Symbiodinium minutum*) within the sea anemone *Aiptasia* sp. (Gibbin and Davy 2014).

An important element of these OA-induced responses appears to be the interactive role of light availability (Suggett et al. 2013). Whilst several recent studies from free-living phytoplankton indicate an inhibitory role of OA on photosynthesis (Gao et al. 2012) or, in some cases, enhanced photosynthesis (Trimborn et al. 2014; Hutchins et al. 2007; Fu et al. 2007), Suggett et al. (2013) demonstrated that higher light availability increases the symbionts' demand for CO_2 when *in hospite* in common Indo-Pacific coral species, which is met through the supply of elevated CO_2. In turn, higher light promotes host calcification and dampens the effect of OA on coral growth. O_2 competes with CO_2 at the active site of RUBISCO such that cells undergo "photorespiration" where O_2 availability is high (CO_2 low). Not only does this process lower the quantum yield of carbon fixation but also is energetically expensive (five ATP and three NADPH per oxygenation event) (Badger et al. 2000), exceeding carboxylation events by two ATP and one NADPH. Thus, photorespiration also provides a means to consume excessive energy produced under high light (Silva et al. 2015) and hence can act as another source of alternative electron flow (detailed further in the next section below). Although *Symbiodinium* minimize CO_2 limitation at the site of RUBISCO through the use of CCM's as discussed above, several studies have documented biochemical and molecular evidence for a photorespiratory pathway (e.g. demonstration of phosphoglycolate phosphatase, as well as glycolate production and release) (Crawley et al. 2010; Trench 1993). In the context of OA, Anthony et al. (2008) and Crawley et al. (2010) have hypothesized declined photorespiration whilst under high light and excess CO_2 leads to a loss in the pathway's AEF-associated photoprotective role. Together these various lines of evidence to date would suggest that OA potentially relieves carbon limitation of steady state growth but at a cost how well *Symbiodinium* can respond to transient light stress; however, considerably more evidence is needed in order to confirm the general importance of photorespiration in *Symbiodinium*.

Overall, there appears to be a range in the photosynthetic response of *Symbiodinium* to OA, but in several cases, there is minimal change to PSII photochemistry (Wall et al. 2014), while elevated photosynthesis leads to enhanced carbon translocation to the host (Tremblay et al. 2013). However, in the few studies that have tested the combined affects of elevated temperature and OA with regard to photochemical response, excess CO_2 rather than alleviating or enhancing the negative affect of high temperature to *Symbiodinium* photosynthesis, largely has a minimal affect (Wall et al. 2014;

Sinutok et al. 2014; Hoadley et al. submitted, but see also Anthony et al. 2008).

Nutrient limitation, often in the context of nitrogen, has been inferred in many studies with animals hosting *Symbiodinium* (Dubinsky and Jokiel 1994; Muller Parker and D'Elia 1997). Historically, considerable focus has revolved around the physiological response of reef corals to large changes in inorganic nitrogen and phosphorus availability, and questions of nutrient demand between the host and the alga in the context of algal population control. In many cases, when a coral is exposed to excess nitrogen, chlorophyll *a* cell^{-1} as well as algal cell number increase significantly, providing clear evidence for nutrient limitation. For a typical healthy coral, the high density of *Symbiodinium*, low specific algal growth rate (doubling times ~60–100 days), yet sustained productivity means that algal cell growth is uncoupled from photosynthesis and *Symbiodinium* are essentially living in a state of extreme unbalanced growth (Dubinsky and Berman-Frank 2001). However, it is important to recognize two important points when considering nutrients such as nitrogen or phosphorus in the context of these symbioses: (i) nutrient limitation is not equivalent to nutrient starvation (see MacIntyre and Cullen (2005) and Moore et al. (2013) and references therein for a more thorough summary on this point) and (ii) *Symbiodinium* appear to be fully acclimatised to living in a unique state of unbalanced growth. While *Symbiodinium* release a significant portion of their photosynthetically fixed carbon to the host, they are still able to utilise the organic nutrients released by the host as well as some photosynthetically fixed carbon in order to carry out energetically expensive pathways of photoacclimation, protein repair and typical daily homeostatic functions. Photochemistry often declines in phytoplankton maintained in batch growth, as cells approach stationary phase and transition from N-limited to an N-starved state (Parkhill et al. 2001; Suggett et al. 2009). In contrast to previous assertions that N-limitation may decrease F_v/F_m in *Symbiodinium* (Falkowski et al. 1993), other work has shown that F_v/F_m is independent of external nitrogen concentration and influenced to a larger extent by light acclimation (Rodriguez-Roman and Iglesias-Prieto 2005). Thus, from a photobiological perspective, *Symbiodinium in hospite* appear more like a nutrient-limited alga in steady-state growth (Parkhill et al. 2001) despite the unbalanced growth, which they are clearly under while within the host. However, surprisingly few studies have yet examined *Symbiodinium* physiological adjustments and how they potentially feedback to photochemical operation, when under nutrient limitation in *ex-hospite* culture experiments.

While *Symbiodinium* appear to be well-acclimatised to living under nutrient limitation, recent evidence has shown that elevated inorganic nitrogen but low phosphate, i.e. imbalances in the N:P (see Moore et al. 2009) increase the susceptibility to light and thermal stress, and in turn coral bleaching (Wiedenmann et al. 2012). Specifically, as the N:P ratio falls out of balance under excess N supply, *Symbiodinium* numbers increase but the algae transition to a P-starved state as evidenced by elevated levels of alkaline phosphatase as well as the lipid sulphoquinovosyldiacylglycerol (SQDG) (Wiedenmann et al. 2012). Wiedenmann et al. (2012) hypothesised that this shift in structural lipids may create an ion imbalance in the thylakoid membrane thereby leading to a loss in PSII activity under high light or temperature. Intriguingly, trace metal limitation, such as by iron, also appears to increase photoinactivation in *Symbiodinium* in heated corals, possibly by destabilising pathways of ROS detoxification (Shick et al. 2011). Conversely, there is also an important link between nutrient availability and photoinactivation and coral bleaching, as many studies have documented higher photoprotection in fed vs. starved corals (Borell and Bischof 2008; Borell et al. 2008; Ferrier-Pagès et al. 2010; Tolosa et al. 2011). However, as Fabricius et al. (2013) note, the alleviation of thermal stress by nutrients is determined in part by the quality of the nutrient source, whereby high-quality food may provide an excellent source, while organically enriched water may also include a number of possible confounding components (e.g. sediments, detritus, microbial flocs or excess DOC) that are detrimental to the host animal. Clearly, the interplay of C, N, and P quotas and how they affect the photobiology of different *Symbiodinium* is yet another important avenue requiring more detailed research.

30.3.4 The Growing Importance of Alternative Electron Flow

The capacity to process absorbed excitation energy through the photosynthetic electron transport chain is a major determinant of how well microalgae can tolerate rapid shifts in light availability. In the marine environment, this paradigm appears to be particularly the case where nutrient limitation places high excitation pressure in PSII and thus large selective pressure for alternative electron flow (AEF) pathways in microalgae (Kana 1993; Behrenfeld et al. 2008; Mackey et al. 2008; Grossman et al. 2010). AEF decouples light harvesting from carbon fixation and so comes with a cost to the growth efficiency of cells (Wagner et al. 2006; Suggett et al. 2009; Brading et al. 2013) but provides the benefit of enhanced buffering against "light stress". AEF can be defined according to a number of central processes (Cardol et al. 2011; Fig. 30.1): (i) Regulation of electron flow at the PSI acceptor side by Mehler Ascorbate Peroxdase (MAP) activity, photorespiration (oxygenase activity of RUBISCO) and cyclic electron transport from PSI back to PSI donor side molecules (CET-PSI); (ii) Alternative electron flow processes around the PSII donor side by plastoquinone terminal

oxidase and cyclic flow amongst the PSII electron carriers (CET-PSII); and (iii) metabolic interactions between organelles, notably the chloroplast and mitochondria whereby photosynthetically generated reducing equivalents are consumed by the mitochondria (mediated by "shuttles") and can induce the operation of mitochondrial alternative oxidase (AOX). Whilst these various processes act to balance the availability of energy and reductant available for nutrient assimilation and reduction they can act as a direct sink for electrons. For MAP, PTOX and AOX the electrons convert photosynthetically generated O_2 back to water (and for photorespiration the O_2 is cycled back to CO_2 and NH_3). In contrast, CET-PSII reduces PSII charge capacity without loss of O_2 evolution capacity whereas CET-PSI maintains both O_2 evolution and PSII charge separation capacity.

Symbiodinium typically thrives in relatively nutrient limited environments and so it is perhaps unsurprising that considerable AEF is often observed for *Symbiodinium*; up to 50 % of gross O_2 produced by PSII is consumed (respired) in the light by *Symbiodinium* (Leggat et al. 1999; Badger et al. 2000), although this rate can be highly variable between types (Suggett et al. 2008; Brading et al. 2011; Roberty et al. 2014). However, perhaps most importantly, light-dependent O_2 consumption via AEF activity appears further up-regulated under high light conditions (Brading et al. 2013; Roberty et al. 2014) and under heat stress (Suggett et al. 2008; Oakley et al. 2014), strongly indicating an important role for AEF in regulating how *Symbiodinium* responds to stressors that are known to induce oxidative cascades and autophagy (Lesser and Farrell 2004, Sect. 30.3.2).

Recent studies have attempted to dissect the specific nature of the substantial light-dependent O_2 consumption inherent to *Symbiodinium* via pathway-specific inhibitors. Roberty et al. (2014) provided the first compelling evidence from several cultured *Symbiodinium* genotypes that maintenance of high PSII flow under high light conditions was sustained by high PSI activity and hence that the light-dependent O_2 consumption was largely from Mehler Ascorbate Peroxidase (MAP) activity. Such an outcome seems entirely logical since *Symbiodinium* is a high emitter of H_2O_2 in the light, which would be consistent with operation of MAP (e.g. Tchernov et al. 2004; Suggett et al. 2008) and how it interacts with the peroxidase and glutathione system activity via the Foyer-Halliwell-Asada cycle (see Krueger et al. 2014). However, it is somewhat contrary to additional evidence for significant operation of mitochondrial alternative oxidase (AOX) activity (Oakley et al. 2014) and chlororespiration in *Symbiodinium* (Badger et al. 2000; Hill and Ralph 2008; McCabe-Reynolds et al. 2008). In the latter case, chlororespiration acts to oxidise NADPH and catalyse the reduction of O_2 to water via reduction of the PQ pool and subsequent use of a terminal oxidase, and thus is analogous to plastoquinone terminal oxidase that operates in the light for some microalgae when PSII becomes over-reduced (PTOX, see Mackey et al. 2008; Cardol et al. 2011). Activity of AOX and PTOX would also be consistent with identification of domains for both pathways in ESTs of *Symbiodinium* (Oakley et al. 2014; Roberty et al. 2014). Similarly, PSI-CET has been observed for *Symbiodinium* via post-illumination PQ pool reduction (McCabe Reynolds et al. 2008) thereby suggesting not all PSI non-linear flow is directed to MAP.

Activity of AOX, CET and chlororespiration (PTOX) have in fact only been evidenced to date in the dark for *Symbiodinium* (Hill and Ralph 2008; McCabe-Reynolds et al. 2008; Oakley et al. 2014) and thus raises the question of why these pathways do not appear to significantly occur in the light. The answer may simply reflect AEF to MAP far outweighs that to other electron and/or O_2 consuming pathways (Roberty et al. 2014). That said, the few studies to date that have examined specific AEF pathways have largely utilized very different symbiont genotypes and growth conditions (including media of different nutritional status) making reconciliation virtually impossible. Potential differences in the nutrient status of cells across studies as a source of variability in AEF observed is particularly intriguing: Lewitus and Kana (1995) demonstrated using mixotrophic microalgae that the extent of reliance on chloroplast versus mitochondrial activity to meet cellular energy demands governed the light-dependent O_2 consuming pathway at play. Recent evidence suggests that some *Symbiodinium* can feed heterotrophically on bacteria (Jeong et al. 2012), which in turn would influence the metabolic balance of the cells. Similarly, nitrogen and sulphur assimilation pathways clearly represent important energy/reductant sinks that can require up regulation of light dependent O_2 consumption (e.g. Weger and Turpin 1989) and in turn potentially influence production of additional ROS such as nitric oxide (Bouchard and Yamasaki 2008); therefore, the nutrient status ("quality") of the culture media used could further influence the nature and extent of AEF pathway expression. Such points certainly resonate when considering an additional role of photorespiration in AEF and light-dependent O_2 consumption. In culture, *Symbiodinium* does not appear to show any evidence of reduced light-dependent O_2 consumption in response to elevated CO_2 suggesting photorespiration is not significant (Leggat et al. 1999; Brading et al. 2011), an outcome that is perhaps not surprising given that *Symbiodinium* employs carbon concentrating mechanisms (CCMs) (Leggat et al. 1999; Brading et al. 2013). However, regulation of photorespiration is considered important in the *Symbiodinium*-cnidarian symbiosis (Yellowlees et al. 2008; see also Crawley et al. 2010; Mayfield et al. 2012), and appears to account for substantial light-dependent O_2 consumption when measured on intact corals (Schrameyer et al. 2014). As such, the predominant role of MAP observed from cultures may not accurately reflect how AEF ultimately

operates *in hospite*, i.e. where light and nutrient availability is very different compared to free living cultures. Both photorespiration (Fahnenstich et al. 2008) and AOX (Rhoads et al. 2006) can be significant producers of ROS, such as H_2O_2, suggesting that AEFs other than MAP could still potentially contribute to the central role of ROS production in governing the stress susceptibility of *Symbiodinium*.

30.4 Conclusions

Technical and conceptual advances that have predominantly stemmed from microalgal and terrestrial plant research fields have undoubtedly advanced our understanding of photobiological diversity and how this confers niche specialization in *Symbiodinium*. Even so, major uncertainty remains as to how adaptive variability in many photobiological traits as well as phenotypic plasticity can be reconciled (and how this variance potentially confer resilience to environment stressors), which in part is likely a consequence of the predominantly reductionist nature of past studies. Whilst experiments focusing on specific cellular components or pathways have been, and continue to be, at the forefront of advancing our state of knowledge, they inevitably can only capture the net outcome of the wider "operational network" at play (Fig. 30.2). Importantly, an array of signals, interactions and feedbacks govern the measurable outcome of any one component. At the cellular scale this operational network represents a con-

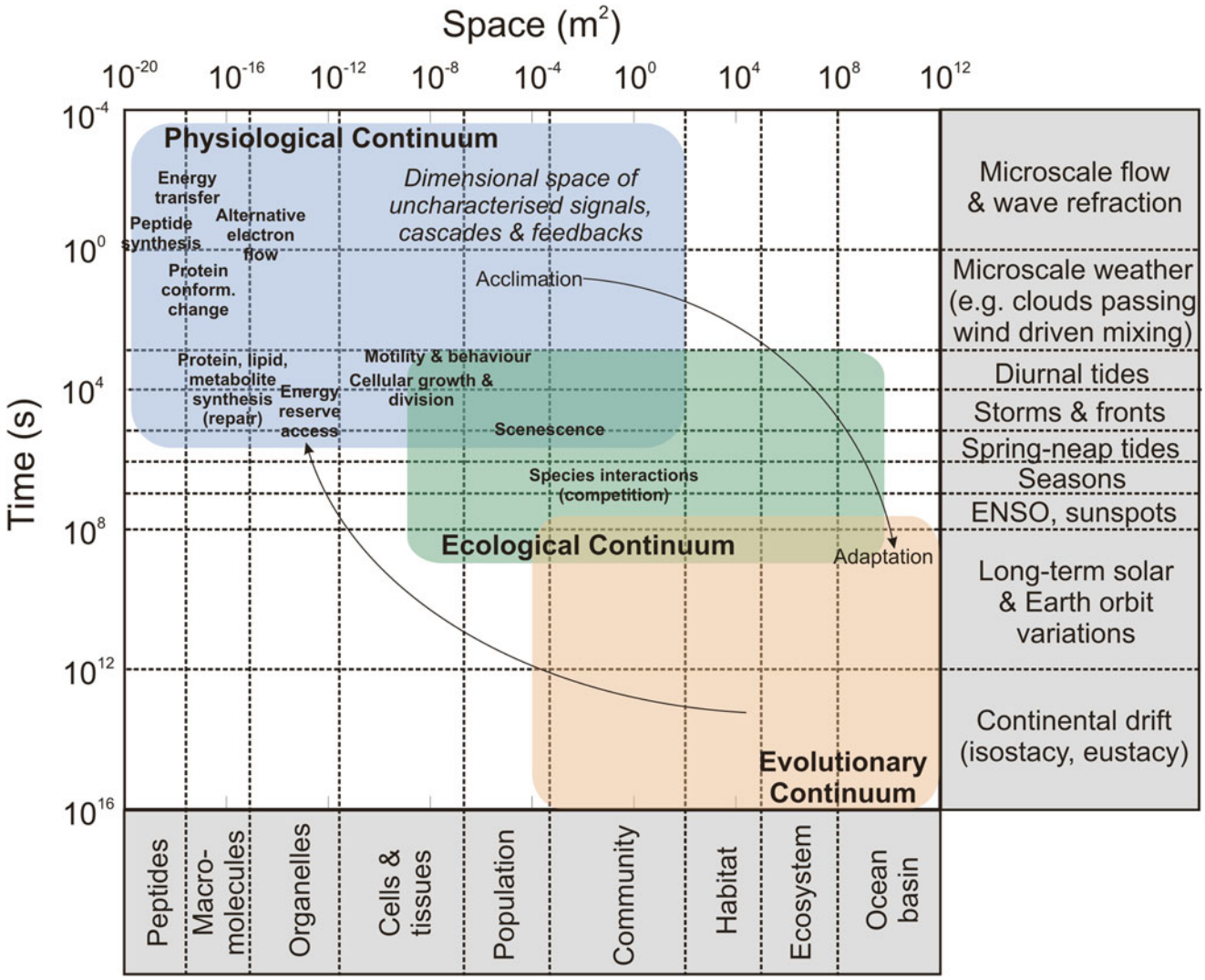

Fig. 30.2 Representation of the time- and space-scales that govern processes driving *Symbiodinium* physiological acclimation and genetic adaptation: Acclimation and adaptation processes occur across physiological, ecological and evolutionary continua within the space-time domain. Whilst scales of operation are shown for the predominant physiological and ecological components, each continuum box portrays the inherent network of (as yet largely uncharacterized signals, cascades and feedback) processes within and between each component. The time-space scale by which these various components are examined will therefore inevitably determine the net response via the extent to which other positive (synergy, cumulative) and/or negative (antagonist) interactions operate

tinuum of physiological processes (the "physiological continuum"), sensu Yellowlees and Warner (2003) that operate across various time-space scales, but ultimately couple across broader scales by ecological and evolutionary processes (Fig. 30.2); mutation rates serve as the feedback loop from the evolutionary continuum to the physiological continuum to alter resilience, and inherently reflects changes to the "topology" of the operational network itself (Chae et al. 2012; Fig. 30.2). Consequently, many fields have turned to 'systems' based approaches to overcome such fundamental limitations. Systems biology provides the computational means to model complex operational networks within a biological system, reducing the dimensionality of the complex layers of physiological and molecular information (Weston et al. 2012b). Central to this approach are network (re)construction tools, which have already transformed understanding of how metabolic pathways respond to environmental change in other microalgae (e.g. Chang et al. (2011) for *Chlamydomonas reinhardtii*; and Ashworth et al. (2013) and Park et al. (2014) for *Thalassiosira pseudonana*) but requires the means to annotate the complex "omics" data required. Transcriptomics (Baumgarten et al. 2013; Barshis et al. 2014; Palumbi et al. 2014), proteomics (Weston et al. 2015, 2012a) and metabolomics (Gordon et al. 2013) are all gathering pace, and it is becoming increasingly clear that these tools are needed in tandem, both with one another but also alongside key physiological "response metrics", to more fully resolve acclimation and acclimatisation processes (and their adaptive variability) within *Symbiodinium* and their hosts. Taking a systems approach for *Symbiodinium* in the near future will not be trivial, and certainly not possible to capture the vast phylogenetic diversity that exists; consequently, distilling *Symbiodinium*'s ever growing complex phylogenetic diversity into more meaningful functional groups may be a critical, if not an essential first step.

In parallel with systems biology, phenomics has emerged as a means to effectively capture the complex systems network at play through the net expression of traits that result from genome-environment interactions (e.g. Furbank 2009; Houle et al. 2010). In principal, phenomics operates as a platform to identify variability of both intrinsic (e.g. mechanistic properties) and/or extrinsic (emergent properties) expression of traits (Houle et al. 2010), and has become a key in industrial applications screening genotypes amongst a species to identify properties of commercial and/or societal interest (Furbank and Tester 2011). To date, this is perhaps best exemplified for algae from the wealth of studies now searching for high lipid yield mutants for biofuel applications. Importantly phenomics represents the practical step to screen for properties that define not only commercial value, but also ecological success where traits inherently impact or reflect ecosystem service provision (Matsubara et al. 2012), including key functional traits. On that basis and in the context of this chapter, *Symbiodinium* phenomics has in essence been growing in practice for decades, notably through screening genotypes for adaptive differences in photobiological traits (Hennige et al. 2009; Iglesias-Prieto and Trench 1994; Robison and Warner 2006; Suggett et al. 2015). However to effectively facilitate the use of phenomics as a platform in *Symbiodinium* research will first require that physiological-based studies begin to (i) inherently screen a much greater representation of the pool of possible *Symbiodinium* genetic diversity and (ii) identify properties that can not only be easily screened for (high throughput) but can accurately define ecological success. Such data will ultimately be pivotal for much-needed model development and parameterization into the future. For example, recent work by van Woesik et al. (2010) provides an exciting example where symbiont population fluctuations were predicted by a discrete time optimal-resource model that generated data similar to empirical results for some Caribbean coral species capable of hosting multiple types of *Symbiodinium*. Similarly, utilizing data for phytoplankton temperature optima for growth in eco-evolutionary as well as mechanistic species distribution models (i.e. models that use specific physiological traits to predict species abundance across environmental gradients) allowed Thomas et al. (2012) to show how algal distributions are driven by adaptive evolution to global temperature as well as the possibility for substantial range size reduction in tropical phytoplankton.

Appreciably, these challenges we have briefly summarized are not trivial to overcome and will only be solved through further technical innovation; for example, traits that define ecological success under steady state conditions may not be the same as those needed to define success under specific stressors. However, relatively easily measured photobiological traits have certainly proved central in explaining how *Symbiodinium* cell lines persist across ambient and stressed environments and thus seem a good candidate to provide a platform in some form for *Symbiodinium* phenomics and establish a basis for functional diversity. Integrating these layers of information into the development of better predictors of the success of particular *Symbiodinium* symbioses will be one of the grand challenges in coming years.

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Current Understanding of the Circadian Clock Within Cnidaria

31

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Abstract

Molecularly-based timing systems drive many periodic biological processes in both animals and plants. In cnidarians these periodic processes include daily cycles in metabolism, growth, and tentacle and body wall movements and monthly or yearly reproductive activity. In this chapter we review the current understanding of biological clocks in the cnidaria, with an emphasis on the molecular underpinnings of these processes. The genes that form this molecular clock and drive biological rhythms in well-characterized genetic systems such as *Drosophila* and mouse are highly conserved in cnidarians and, like these model systems, display diel cycles in transcription levels. In addition to describing the clock genes, we also review potential entraining systems and discuss the broader implications of biological clocks in cnidarian biology.

Keywords

Circadian rhythms • Biological clocks • Reproductive timing • Non-visual photodetection • Light perception

31.1 Overview

Entrainment of physiological rhythms to environmental cues is ubiquitous among living organisms and allows coordination of biology and behavior with daily environmental changes. This coordination improves survival and reproductive fitness, and, thus, it is not surprising that an endogenous "clock" has evolved to maintain rhythmicity over a circadian (24 h) period. From the identification of the first circadian gene (Konopka and Benzer 1971), the past 40 years have produced thousands of studies focusing on the molecular basis of the circadian clock. Across species, from bacteria, to fungi, to plants and animals, this molecular circadian clock involves transcription and translation feedback loops with a self-sustained period of about 24 h (reviewed in Dunlap 1999). Investigation in the model genetic species, mouse and fly, has identified a core set of genes that form the central oscillator in animals (reviewed in Panda et al. 2002). Input pathways to this central oscillator reset the circadian clock to environmental cues, such as sunlight, and output pathways direct changes in biology and behavior, such as sleep-wake cycles. Coordination of behaviors, like reproduction and migration, to seasonal environmental changes, such as photoperiod (day length) also utilizes components of the circadian clock (reviewed in Hut and Beersma 2011, Ikegami and Yoshimura 2012, and Coomans et al. 2014). This core set of genes appears conserved among animals including, as revealed by very recent work (reviewed here), cnidarians. These findings are evidence of the very early evolutionary origin of the molecular circadian clock within the animal lineage (Young and Kay 2001).

The existence of a molecular circadian clock within cnidarians is not unexpected. Many cnidarian species display

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behavioral and reproductive changes that are synchronized to diel solar or lunar light cycles. Tentacle retraction during the day to avoid predation and expansion at night to feed is a well-known behavior observed within many shallow water scleractinian coral species (Yonge 1940; Sweeney 1976; Sebens and DeRiemer 1977) and rhythmic body wall contractions in response to light have been described in Hydra (Passano and McCullough 1962). In addition, rates of calcification, photosynthesis, and respiration also display diel rhythmicity and are thought to utilize the daily light cycle as a principle entrainment cue (Lasker 1979; Moya et al. 2006; Sorek and Levy 2012). Importantly, these behaviors maintain their rhythmicity even under conditions of constant dark, an important feature suggesting regulation by an intrinsically generated molecular circadian clock. Behavioral patterns over longer time periods, such as gamete release during annual mass spawning events or monthly coordinated larval release in brooding corals species, may also be entrained responses, utilizing environmental cues to synchronize reproductive efforts (Harrison et al. 1984; Szmant-Froelich et al. 1985; Baird et al. 2009). Although synchronous coral spawning is well-characterized, little is known about the molecular signaling pathways responsible for such precise timing and entrainment of reproductive effort has not been demonstrated. As with tentacle retraction, an important environmental entrainment cue is thought to be light, both solar and lunar. Even small changes in low intensity light can induce behavioral responses, such as tentacle retraction or larval release in scleractinian corals, indicating sensitivity to small changes in light such as those experienced throughout the lunar cycle (Jokiel et al. 1985; Gorbunov and Falkowski 2002).

This chapter discusses what is currently known about the molecular mechanisms governing environmentally regulated behavior in cnidarians. The chapter begins with a brief description of the molecular mechanisms elucidated from model mammalian and insect species to build a framework for understanding the cnidarian molecular circadian clock. In describing the cnidarian circadian clock, emphasis is placed on outlining the molecules that form the circadian clock, describing what is known about the input pathways that set the clock, and then discussing possible output pathways that direct entrained behaviors. The chapter concludes with a discussion of the work that still needs to be done to understand more fully the molecular basis of the circadian clock in cnidarians and how it regulates behavior.

31.2 The Molecular Circadian Clock as Revealed from Model Metazoan Species

The first investigations of molecular circadian clocks in cnidarians were naturally guided by knowledge gained from well-studied model systems, and, therefore, it is useful to review briefly the molecular basis of circadian clocks as elucidated in the genetically tractable organisms mouse (*Mus musculus*) and fly (*Drosophila melanogaster*). A number of excellent reviews much more extensively outline the current molecular understanding of clocks in these species (Ko and Takahashi 2006; Hardin 2011; Ozkaya and Rosato 2012) and are summarized below. Importantly, early work unraveled the molecular mechanisms within the central oscillators, which are contained within a specific set of neurons known as the superchiasmatic nucleus (SCN) in mammals (reviewed in Hastings et al. 2014) or the lateral and the dorsal neurons within the fly brain (reviewed in Nitabach and Taghert 2008). Circadian clocks are now known to be composed of a hierarchy of oscillators that function not just centrally but also peripherally at the cellular, tissue, and systems level (reviewed in Mohawk et al. 2012). The molecular mechanisms regulating the synchrony and coherence of these various oscillators as part of a larger circadian "system" is the focus of intense investigation and may prove informative to understanding how circadian clocks operate in cnidarians (as discussed later in this chapter). Finally, the molecular understanding of circadian clocks has expanded to a variety of other organisms, including a diversity of animals, plants, and even unicellular prokaryotes and eukaryotes (reviewed in Crane and Young 2014). Whereas a similar molecular mechanism appears conserved among animals, now reinforced by our knowledge of these mechanisms in cnidarians, circadian clocks in plants (*Arabidopsis thaliana*, see Nakamichi 2011), fungi (*Neurospora crassa*, see Baker et al. 2012), and bacteria (*Synechococcus elongates*, see Mackey et al. 2011) utilize different cores sets of molecules, suggesting the independent evolution of circadian clocks among these different lineages (reviewed in Young and Kay 2001 and Bell-Pedersen et al. 2005).

31.2.1 The Molecular Circadian Clock: The Central Players

The period of oscillation of the molecular clock (approximately 24 h) is set by two interacting transcription/translation feedback loops. This periodicity is set autonomously and maintained even in the absence of external cues. At their simplest examination, these feedback loops involve two activators, CLOCK and BMAL1 in mouse and CLOCK and CYCLE in fly, and two repressors, PER and CRY in mouse and PER and TIM in fly. Once transcribed, CLOCK and BMAL1/CYCLE form a heterodimer that translocates to the nucleus and acts as a transcriptional activator by binding to specific DNA elements, especially E-box regulatory elements within the promoter region of additional clock genes or genes under clock-regulated control (Hardin 2004). As expected for transcriptional activators, binding increases

transcription rates. In particular, CLOCK/BMAL1 (mouse) or CLK/CYC (fly) initiate the transcription of the *Per* and *Cry* genes (mouse) or *per* and *tim* genes (fly). Following transcription and heterodimerazation, PER/CRY or PER/TIM translocate to the nucleus and suppress CLOCK/BMAL1 or CLK/CYC activity, resulting in reduced transcription of *Per* and *Cry* or *per* and *tim* as well as other clock-controlled genes. The degradation of PER and CRY or PER and TIM are necessary to terminate suppression. Thus, the stability and rates of degradation of these repressors determine the period of the central oscillator. CLOCK/BMAL1 and CLK/CYC also initiate transcription of a second transcription/translation feedback loop (reviewed in Hardin and Panda 2013). In brief, in flies, this feedback loop is controlled by the transcription factor VRILLE, which represses *Clk* activation. In mammals, this feedback loop is controlled by the proteins ROR (α, β, and γ) and REV-ERB (α and β), which bind to regulatory elements and serve to regulate *Bmal1* transcription (Yang 2010). This second transcription/translation feedback loop serves to improve the robustness and fidelity of the circadian clock.

These feedback loops form an endogenous rhythm that enables genes to be turned on and off at a regular cycle, thereby regulating behavioral and/or physiological outputs regardless of the presence or absence of environmental signals. Both the positive and negative feedback loops undergo significant post-translational modification (Gallego and Virshup 2007), including ubiquitination and phosphorylation which help set the approximate 24 h cycle of the molecular circadian clock. In addition to these core genes, mutants have identified various accessory genes that serve to enhance the robustness of periodicity.

31.2.2 Input Pathways: Setting the Clock

Because the period of the molecular clock is only approximately 24 h and because shifts in environmental cues can occur (such as changing day length in different seasons), mechanisms must exist to synchronize circadian rhythms with important environmental cues. (The best example of a mismatch in biological and environmental time occurs during jet lag.) These cues are called "zeitgebers" or time givers and can include light, temperature, food availability, day length, as well as social parameters. The strongest entrainment cue appears to be light, and especially the daily light cycle, and, because it is also easy to manipulate experimentally, it is the most well-studied entrainment cue. It has long been appreciated that light pulses reset circadian activity (Pittendrigh and Minis 1964): light pulses in the early night (or dusk) delay circadian activity whereas light pulses in the late night (or dawn) advance circadian activity. Light induces these shifts in circadian activity by molecularly resetting the oscillation of the transcription/translation feedback loops.

In mammals the role of opsin, melanopsin, and cryptochrome based systems in the entrainment of biological clocks and light responses have been extensively studied. Mice lacking either opsin-based light detection (via either retinal degeneration that causes loss of the opsin-containing rod and cone photoreceptors or by targeted mutagenesis of the transducin and the cyclic GMP-gated channel activated by transducing) or melanopsin still show pupillary light responses and can still be entrained to light (reviewed in Lucas et al. 2012). However, in the absence of both opsin- and melanopsin-based photoreception pupillary light responses and photoentrainment is eliminated (Hattar et al. 2003). These findings suggest that cryptochromes do not have a light-detecting role in the retina as had been suggested previously in mice lacking *Cry1* and *Cry 2* (Vitaterna et al. 1999; Van Gelder et al. 2003). Thus, at least in mice, the central pacemaker clock is synchronized by opsin- and melanopsin-based photoreception, and cryptochromes function as transcription factors in the clocks they entrain. This central clock, in turn, regulates peripheral clocks whose synchronization is driven by the SCN and not light itself.

In the fly, environmental light syncs the molecular clock in part via CRY (reviewed in Ashmore and Sehgal 2003). CRY within the nucleus competes with PER to bind with TIM, forming a heterodimer. Formation of the CRY/TIM heterodimer leads to proteosomal degradation of the TIM protein, and CRY itself is degraded in a light-dependent manner. In addition, the disassociation of the PER/TIM heterodimer exposes PER to phosphorylation and subsequent degradation. The reduction of the PER/TIM heterodimer releases the CLOCK/CYCLE heterodimer from inhibition, which, in turn, increases the transcription of gene products with E-box motifs within their promoter regions. In this way, CRY resets the molecular clock. Importantly, the original *cryb* mutant flies do retain some light-driven entrainment (Stanewsky et al. 1998), indicating the existence of redundant circadian photoreceptors. Furthermore, CRY activity is also dependent on the redox state of the cell (Lin et al. 2001). Thus, in fly, CRY acts as both a sensor of multiple cellular parameters and also a transcription factor within the core circadian clock.

31.2.3 Output Pathways: Timing Biological Processes

The molecular mechanisms linking the circadian clock to circadian rhythmicity are very poorly understood in any species. The most readily observed circadian outputs include, in fly, eclosion (the emergence of the adult fly from the pupa case) and locomotor activity, and, in mammals, sleep-wake

and fasting-feeding cycles. However, as is being increasingly appreciated, the circadian clock regulates a range of metabolic processes, including but not limited to glucose and lipid metabolism, body temperature, and endocrine hormone secretion. Complicating research is the fact that metabolic processes both receive input and also return input to central as well as peripheral clocks (Rutter et al. 2002; Bass and Takahashi 2010). For example, feeding behavior is regulated by the circadian clock and yet food can also act as a zeitgeber (reviewed in Challet 2013). Not surprisingly then, many mutants of core components of the circadian clock also exhibit metabolic disorders (reviewed in Marcheva et al. 2013 and Richards and Gumz 2013). In general, output pathways require neuroendocrine circuits to link central clocks with central and also peripheral targets that direct activity. For example, in the mouse, the SCN targets (among other structures) the hypothalamus (reviewed in Li et al. 2012). Via the pituitary gland, the hypothalamus serves as an important link between the nervous system and the endocrine system and regulates a number of circadian behaviors. Also via the hypothalamus (and other intermediate structures), the SCN triggers the increased release of the signaling molecule cAMP from cells of the pineal gland during the dark. Increased cAMP increases the nighttime production of the hormone melatonin (reviewed in Pevet and Challet 2011). Melatonin receptors are expressed throughout the body. Temporal patterns of melatonin levels encode not only day-night but also photoperiod; thus, melatonin functions in both the regulation of circadian and also seasonal behaviors (see Coomans et al. 2014). Output pathways from the central clocks have been reviewed in animals in greater detail elsewhere (Hardin 2000; Bass and Takahashi 2010).

31.3 Investigating the Cnidarian Molecular Circadian Clock

When applying principles derived from work on mammalian and insect systems to the cnidarian molecular clock, major physiological differences between these taxa become immediately apparent. For mammals and insects alike, the central oscillator is located within specialized cells of the central nervous system and provides master control of peripheral oscillators. In contrast, most cnidarian nervous systems revolve around a nerve-net-like system with many small ganglia but no clear centralization (Marlow et al. 2009). Thus, the location of oscillators and the presence of central or peripheral oscillators in cnidarians is unclear. Furthermore, in mammals and insects, complex light entrainment pathways may be necessary since the core molecular clock exists within the central nervous system and is unlikely to be directly exposed to environmental light. Cnidarians however are largely translucent organisms and for those species living within the photic zone, solar irradiance likely penetrates through most of their living tissues. This is especially true of scleractinian coral species where highly reflective calcium carbonate skeletons greatly increase the light field for symbiotic algal cells living within the host (Enriquez et al. 2005).

31.3.1 Molecular Basis of the Cnidarian Circadian Clock: The Players So Far

The molecular clock machinery in cnidarians has been elucidated from genomic (Shoguchi et al. 2013) and transcriptomic (Vize 2009; Brady et al. 2011) analyses aimed to identify clock genes and also investigation of diel (and lunar) differences in expression of smaller sets of presumed clock genes (Levy et al. 2007; Reitzel et al. 2010; Hoadley et al. 2011). Genetic information is presently limited to anthozoan coral species.

Utilizing the framework established in fly and mouse, *Clock* and *Cycle* (sometimes referred to as *Bmal/Cycle* in the literature), the positive elements of the molecular clock feedback loop, have been identified in the genomes of *Acropora digitifera* (Shoguchi et al. 2013) and *Nematostella vectensis* (Reitzel et al. 2010) and transcript expression has been verified in *Acropora millepora* (Levy et al. 2007, 2011; Vize 2009; Brady et al. 2011), *Favia fragum* (Hoadley et al. 2011), and *Nematostella vectensis* (Reitzel et al. 2010, 2013; Peres et al. 2014). In genomic analyses, *A. digitifera* expresses three clock and one clock-like gene compared to one clock and one clock-like gene identified in *N. vectensis* (Shoguchi et al. 2013). In both species, one *Bmal/Cycle* gene has been identified. Comparison of expression profiles of these genes among studies is complicated by experimental differences in sampling frequency, light regimes, and life stages of the species utilized. Nonetheless, molecular clock expression profiles for *A. millepora* (Levy et al. 2007, 2011; Brady et al. 2011), *F. fragum* (Hoadley et al. 2011) and *N. vectensis* (Reitzel et al. 2010, 2013; Peres et al. 2014) appear to share many of the same features both among cnidarians and in comparison to model (fly and mouse) systems. Under a 12:12 h light:dark (LD) cycle, expression profiles for *Clock* and *Cycle* are similar for *N. vectensis* and *F. fragum.* Moreover, only *Clock* shows cyclical expression, with peak gene expression occurring near the LD transition (Reitzel et al. 2010; Hoadley et al. 2011). For *A. millepora*, both *Clock* and *Cycle* appear to peak in expression a few hours after the LD transition, with the lowest expression values occurring during the subjective day (Brady et al. 2011). However, because the *A. millepora Clock* and *Cycle* genes were only profiled within a 22-h window within this study, a cyclic diel pattern of expression is difficult to identify.

The negative elements of the molecular clock feedback loop have also been examined. The *Cry* genes have been

most extensively examined. Three *Cry* gene variants have been identified within the cnidarian genomes: *Cry1*, *Cry2*, and *Cry-DASH*. In addition, *N. vectensis* contained more than twice as many *Cry* variants (seven) as compared to the three identified in *A. digitifera* and *A. millepora* (Shoguchi et al. 2013). It is unclear if this suggests a gene loss or gene duplication between the scleractinian and actinarian taxa and/or if all seven of the *N. vectensis Cry* genes are important to the molecular clock mechanism. Peak *Cry* expression within the two scleractinian coral species, *A. millepora* and *F. fragum*, occur during the daytime, with *Cry1* expression peaking earlier in the day than *Cry2* (Levy et al. 2007; Hoadley et al. 2011). The *Cry2* ortholog in *N. vectensis, Cry1b*, also peaks during mid to late daytime hours. However, in *N. vectensis*, peak expression of *Cry2* (which is orthologous to *Cry1* in *F. fragum* and *A. millepora*) occurs just a few hours prior to the dark:light transition (Reitzel et al. 2010). The offset in peak expression for this *Cry* variant can potentially be explained by differences in the life history and physiology of the three species. Whereas *F. fragum* and *A. millepora* are calcifying scleractinian corals harboring symbiotic dinoflagellate species, the anemone *N. vectensis* is aposymbiotic and does not secrete a calcium carbonate skeleton. The presence of photosynthetic alga, which presumably express their own light-entrained molecular clock, within the cells of *F. fragum* and *A millepora*, may influence expression patterns within the host (Sorek et al. 2013). Regulation of cnidarian circadian clocks by symbiotic algae will be discussed more later on.

An additional negative feedback gene in model organism clocks is the transcription factor VRILLE (Cyran et al. 2003). In other model systems VRILLE binds to the promoter of the CLOCK gene and inhibits its expression (Hardin 2005). The *A. millepora Vrille* gene displays a very strong diel cycle of transcription and shows 200-fold higher levels of expression at night versus day (Brady et al. 2011) and is an excellent candidate as a repressor of the *Clock* mediated positive elements of the coral network.

Considering these negative regulatory elements, a very surprising and yet consistent finding has been the absence of the *Per* genes within the cnidarian genomes (Hoadley et al. 2011; Shoguchi et al. 2013; Reitzel et al. 2013). Cnidarian genomes contain PAS family members but the overall level of conservation is modest and none of the three family members analyzed in *A. millepora* larvae showed any differential expression over diel cycles (Brady and Vize, unpublished findings). Another feature is that a single *Timeless* gene is present within all three cnidarian species, and may be orthologous to *Timeless2/Timeout*, although the N-terminal Timeless domain alone clusters with *Timeless* and not *Timeless2* (Oldach and Vize, unpublished findings). The *Drosophila timeless2/timeout* gene plays two roles, one in chromosome stability and one in circadian timing (Benna et al. 2010) while *timeless* is a dedicated clock gene (Hunter-Ensor et al. 1996). Although it is unclear whether TIM is involved in the negative feedback loop of the cnidarian molecular clock, its transcription does follow a 24 h cycle suggesting that it may be under clock regulation (Reitzel et al. 2010; Brady et al. 2011). It is possible that *Timeless2/Timeout* also acts in the negative feedback loop within the cnidarian molecular clock or, as in fly, binds with CRY in a light sensitive fashion to serve as a repressor of transcription. However, physical protein interactions would need to be studied in order to confirm this interaction.

Another striking contrast between adult cnidarians and fly and mammal clock gene expression patterns is the loss of circadian rhythmicity in conditions of constant dark in some studies, suggesting that there may be no (or at least reduced) intrinsic maintenance of rhythmicity. Intrinsic rhythmicity in gene expression may be masked by the sampling techniques utilized when sampling from adult colonies: most expression studies have ground up relatively large portions of coral tissue. Thus, the resulting mRNA samples represent the combined expression profiles for multiple tissue layers. Because larvae are small and relatively undifferentiated in terms of tissue types, an intrinsic rhythm may be easier to observe in larval samples. In support of this hypothesis, differences in expression profiles are observed between larval and adult coral colonies (Brady et al. 2011; Peres et al. 2014) and rhythmic expression under constant dark conditions has been documented over 24 h for *A. millepora* planulae (Brady et al. 2011). *N. vectensis* larvae also displayed dark:dark rhythmic expression of the molecular clock genes *Clock*, *Timeout*, *Cry1a*, *Cry1b* and *Cry2* but lost this effect after 96 h of constant darkness (Peres et al. 2014). Interestingly, mouse and fly *Per* mutants display disrupted free running rhythms (Hardin et al. 1992; Zheng et al. 2001). Perhaps the absence of *Per* in the cnidarian genome contributes to the degraded free running rhythms observed in adult cnidarians, suggesting that function of the core clock mechanism to maintain intrinsic transcriptional rhythmicity may differ between cnidarians and other animal (mammalian and insect) groups.

In addition to the core elements of the circadian molecular clock, a number of other clock genes have been identified in cnidarians. Based on transcriptomic analysis in *A. millepora*, matches with strong confidence levels were found to 22 key insect and mammalian circadian clock genes have been identified (Vize 2009). Both positive and negative elements of the feedback system were identified, along with key phosphatases and ubiquitinases important to posttranslational modifications of the core clock proteins. As discussed above, in fly and mouse these mechanisms are important regulators of the approximate 24 h periodicity of the clock. A comparison of clock genes across the three available cnidarian genomes, *A. millepora, N. vectensis*, and *A. digitifera* revealed differences in gene copy numbers and duplications

for several of the core molecular clock components (Shoguchi et al. 2013). This research proposes various hypotheses on the evolutionary origin of clocks within these groups.

31.3.2 Input Pathways: Light as an Entrainment Cue in Cnidarians

Cnidarians respond to a wide range of different light wavelengths and intensities (Levy et al. 2003; Mason and Cohen 2012). A variety of potentially light-sensitive molecules have been identified in cnidarians and presumably the co-expression of multiple photosensitive molecules enables light detection over a broad range of wavelengths. The likely candidates currently include cryptochromes, which are discussed in more detail below. In addition, genome-wide searches have identified opsins in *N. vectensis* (Suga et al. 2008) and *A. digitifera* (Shoguchi et al. 2013), melanopsin in *A. millepora* (Vize 2009), as well as rhodopsin-like G protein-coupled receptors in *A. millepora* (Anctil et al. 2007; Mason et al. 2012).

Cryptochromes were introduced earlier as part of the core circadian clock, and, based on their known role as blue light flavoprotein photoreceptors (photosensors) in plants (Chaves et al. 2011), have been the most investigated potential molecular photosensors in cnidarians. Unlike the vitamin-A derived chromophore used by opsins and melanopsins, cryptochromes use FAD as their chromophore. When light strikes the FAD and reduces it to FADH, a conformational shift in the cryptochrome occurs in plants and changes its transcriptional activity (reviewed in Christie et al. 2015). A potential role for cryptochromes as cnidarian photosensors has been proposed (e.g. Levy et al. 2007), but, importantly, no data demonstrating intrinsic photosensitivity of cnidarian cryptochromes has yet been published. Although the transcription of *Cry1* and *Cry2* appears to depend on light in *A. millepora* and *F. fragum* (Levy et al. 2007; Brady et al. 2011; Hoadley et al. 2011), this observation indicates only that these genes are a part of the light-mediated response and not necessarily the photosensor per se.

In cnidarians cryptochromes display diel cycles in transcription (Levy et al. 2007; Reitzel et al. 2010; Brady et al. 2011; Hoadley et al. 2011), with high levels of expression in the daytime and low levels of expression at night. In *A. millepora* planulae, the cycle of transcription for *Cry2* can continue in complete darkness, indicating that expression of this gene is, in fact, driven by the circadian clock and not directly by light (Reitzel et al. 2010; Brady et al. 2011). Thus, cryptochromes in *A. millepora* (analogous to those of mammals) may serve as components of the core circadian clock rather than as photosensors. Similar results have been obtained in the anemone, *N. vectensis* (Reitzel et al. 2010), and possibly also in the coral *F. fragum* (Hoadley et al. 2011). Results vary somewhat between studies, and these differences may arise from differences in tissue- or cell-specific expression and/or as the result of expression profiles that vary between central versus peripheral clocks. In other systems, cryptochromes are targeted for proteosomal degradation after shifting to their light-activated configuration (Ashmore and Sehgal 2003). Thus, if cnidarian cryptochromes are intrinsically photosensitive, light-triggered degradation may be essential to their diel changes in expression. On the other hand, or additionally, redox state can regulate the transcriptional activity of the CLOCK and NPAS proteins (Rutter et al. 2002) and presumably a similar form of regulation may operate for cryptochromes independently of any intrinsic light sensitivity (see Lin et al. 2001).

Much less is known about the physical structures that house the photosensors important for the entrainment of cnidarian circadian clocks. In other animals, both visual and non-visual light detection systems convey information about environmental light to the central circadian clock. Visual systems include specialized light sensing organs known collectively as eyes, though variations in structure and function can vary widely. Eyes with a single lens are known as camera eyes, single simple eyes with no lens are known as ocelli, and eyes with multiple lenses are known as compound eyes. Within cnidarians the most highly developed eyes are found in cubozoan medusae, which have both camera eyes capable of detecting specific features for hunting and navigation (Kozmik et al. 2008). Cubozoan planulae also have pigment spot ocelli. These are individual specialized cells that contain a pigmented pit that allows for directional light detection. These cells also contain a cilium with a standard 9+2 axoneme, the activity of which is thought to be directly controlled by light striking the photoreceptor containing villi within the pigment pit (Nordstrom et al. 2003). This arrangement allows for directional motility in response to light without any relay via a nervous system.

Detailed studies on cnidarian light-induced signal transduction have only been performed in detail in cubozoans. In the camera type eyes of these animals ciliary-type opsins and a cyclic nucleotide pathway is utilized, followed by a transient receptor potential channel triggered response that initiates an action potential in camera eyes (Koyanagi et al. 2008). Cubomedusae also contain less well-developed ocelli. The opsins and transduction pathway in the ocelli have been shown to lack ciliary opsins (Ekstrom et al. 2008), and they likely use rhabdomeric-type opsins and calcium-mediated transduction pathways. In coral planulae treatment with compounds that alter cytoplasmic calcium levels induce changes matching those triggered by light, providing at least indirect evidence that light triggers a calcium-mediated response (Hilton et al. 2012). A separate series of experiments identified opsins in *A. palmata planulae* that localized to the aboral end of the larvae and were capable of in vitro

GDP-GTP exchange in response to light (Mason et al. 2012). These results are all consistent with the pigment pit ocelli of cubozoans and both adult and larval scleractinians using a classical rhabdomeric opsin and G protein- and calcium-mediated transduction cascade. If and how these visual systems convey information to the cnidarian molecular clock has yet to be elucidated. Moreover, the contribution of non-visual systems, using cryptochromes and/or melanospin, to the entrainment of the cnidarian molecular clock has yet to be functionally investigated.

31.3.3 Output Pathways: Molecular Mechanisms That Regulate Circadian and Seasonal Physiology in Cnidarians

As introduced earlier, very little is known about the molecular mechanisms that link the circadian clock to circadian rhythmicity, even in model genetic systems. Nonetheless, recent research has begun to investigate output pathways of circadian clocks in cnidarians using a variety of strategies. At the genetic level, E-box motifs, specific DNA elements found within the promoter region of many clock-regulated genes, have been identified within the promoter region of several cnidarian genes, including those known to be core clock components (Reitzel et al. 2013). At the transcriptional level, previous research (discussed above) has uncovered additional genes with cyclical transcriptional profiles. These types of investigations should, without a priori assumptions, identify genes that are under clock control and, therefore, may comprise the output pathways that regulate circadian behaviors.

More targeted approaches to examine output pathways in cnidarians have also been utilized. Specifically, melatonin, the circadian and output signaling molecule important in vertebrates (see discussion above), has been examined using immunoreactivity, histology, and HPLC (Mechawar and Anctil 1997; Roopin and Levy 2012a, b; Peres et al. 2014). Although there are conflicting findings on the diel expression patterns of melatonin, there is agreement on the likely involvement of melatonin in reproductive maturation. In the most recent of these investigations, Peres and others found *e*xpression that peaked at the end of light period in *N.* vectensis adults maintained under diel conditions (2014), expression patterns that would be expected from vertebrate systems. This study also provided data indicating that exogenously applied melatonin may restore circadian oscillation of clock transcripts that have lost transcriptional rhythmicity after being transferred to conditions of constant darkness. This research suggests that melatonin may serve as an intermediate connecting input and output pathways. Such dual roles for clock signaling molecules have been observed in fly and mouse as well (as discussed above) and may be an inherent feature of circadian clock regulation. Much work remains to be done to determine the mechanism by which melatonin functions to regulate circadian and seasonal behaviors in cnidarians. Importantly, previous work in *N. vectensis* (Roopin and Levy 2012b) used in-situ hybridization to localize transcripts of two representative melatonin receptor orthologs (identified previously by Anctil 2009) and found abundant expression of these putative melatonin receptors in both reproductive tissues and also highly neuralized areas. These findings begin to uncover the circuitry of the melatonin signaling pathway in (at least select) cnidarians and suggest interesting hypotheses on the role of melatonin signaling in anthozoans.

Transcript differences in clock genes have also been investigated during development as well as during reproductive cycles, offering insight into the genetic mechanisms regulating these behaviors. For *N. vectensis*, diel periodicity in molecular clock expression profiles is absent in larvae at 48 h, with rhythmic expression only emerging later (Peres et al. 2014). When sampled at 1 week of age, *A. millepora* larvae displayed similar diel periodicity (Brady et al. 2011) as do 1–2 week old *N. vectensis* larvae (Peres et al. 2014). Importantly, the spatial expression of *Cry1a*, *Cry1b*, *Clock* and *Cycle* changes considerably within the initial 168 h of embryonic development, with diffuse expression first observed within the blastula stage, localizing mostly within the endoderm by the planulae stage (Peres et al. 2014). As pointed out by the authors of that study, expression of clock genes during the early stages of development has been well-documented within fly and mouse (Curran et al. 2008; Dekens and Whitmore 2008), where there expression may be important to the proper timing and formation of organs and tissues. In cnidarians, developmental and spatial differences in localization suggest additional roles for these genes directing the initial stages of development.

The earliest investigations of clock genes in cnidarians actually investigated the role of these genes in regulating seasonal reproductive events (Levy et al. 2007). Increases in *A. millepora Cry 2* were observed during a full moon (Levy et al. 2007) and these authors have suggested, therefore, that cryptochromes may play a role in reproductive synchronization. However, peaks in cryptochrome expression for the brooding coral *F. fragum* did not correlate with either larval or gamete release (Hoadley et al. 2011), both of which are known to follow the lunar light cycle (Szmant-Froelich et al. 1985) and very different nightly profiles of these genes have been described by others (Brady et al. 2011). Thus, the role of clock genes and cryptochromes in coordinating reproductive cycles is unclear. However, all work on cnidarian molecular clock rhythms thus far has been mostly focused on expression levels of core clock genes. Although informative, this assay requires peaks in expression to be synchronized with reproductive outputs in order to conclude a positive correlation.

Capturing such a correlation requires precise timing between sampling regimes and the biological/behavioral output of interest and interactions at the protein and post-translational rather than transcriptional levels may be informative. Moreover, events downstream of the core clock genes may be involved in the timing of reproductive cycles and, therefore, not captured by assays that examine expression patterns of the core clock genes over reproductive cycles.

Finally, recognizing that metabolic processes are not only a product of circadian clocks but also provide important entrainment cues in other animals (Rutter et al. 2002; Bass and Takahashi 2010), the importance of translocation of metabolites and signaling molecules between the host coral and its endosymbiont (Whitehead and Douglas 2003; Yellowlees et al. 2008) need to be considered when discussing output and input pathways in cnidarains. Adult scleractinian coral colonies may contain up to four million *Symbiodinium* cells per cm^2 and are, therefore, under significant influence from their symbiotic algae via photosynthesis and translocation of glucose rich photosynthates (Fitt et al. 2000). Additionally, in contrast to aposymbiotic corals such as *N. vectensis*, major changes in both oxygen saturation and carbonate ion concentrations occur within the host as a direct result of daily photosynthesis by their symbionts (Sorek and Levy 2012; Sorek et al. 2014). Several behavioral and physiological outputs of the host/symbiont (holobiont), including respiration, photosynthesis, calcification, tentacle retraction and feeding are known to exhibit 24 h peridocitiy (Moya et al. 2006; Sorek and Levy 2012). Additionally, endosymbiotic algae, such as *Symbiodinium*, have molecular clock mechanisms of their own (Levy et al. 2003; Sorek and Levy 2012; Sorek et al. 2013). The degree to which there is cross talk between host and symbiont clock mechanisms is also largely unknown and certainly worthy of further investigation.

31.4 The Road Ahead

The last few years have seen a transformative increase in our understanding of the molecular basis of circadian clocks in cnidarians, largely through the use of genomic and transcriptomic approaches. As is typical in science, this increased understanding presents many new questions. Some of the most exciting questions get at fundamental differences in the circadian clock between cnidarians and other animals. For example, in which tissues are clock genes located and expressed and to what extent are their central versus peripheral clocks in cnidarian species? Is the periodicity of this clock intrinsic or does it require entrainment cues? What, then, are the most salient entrainment cues (zeitgebers) in cnidarians and what are the receptive molecules detecting these cues? What are the anatomical circuits that direct input to and output from the cnidarian circadian clock and how do these pathways converge? Finally, what role do symbionts play in entraining the clock?

Answering these questions will require the development and application of a variety of new tools. Firstly, the utility of genomic approaches will be greatly improved as the number and accessibility of full cnidarian genomes increases. Currently only four cnidarian genomes are available, and three of these four are sessile cnidarians within the Anthozoan class. A jellyfish genome would be particularly useful and would allow investigation of the mechanisms regulating other circadian behaviors, like diel vertical migrations. Secondly, moving the clock mechanism from gene to transcript to protein will require a better understanding of the direct physical interactions among the identified cnidarian molecular clock proteins. Presently, only the core molecular clock proteins CLOCK and CYCLE have been examined and confirmed by coimmunoprecipitation to form a heterodimer in *N. vectensis* (Reitzel et al. 2010). More systemic application of biochemical techniques will be required to validate the protein interactions predicted in cnidarians based on knowledge obtained from model systems. These techniques also promise to identify the physical location of the cnidarian circadian clock. Interestingly, recent investigation of developing *N. vectensis* embryos and planulae suggest that the major site of expression of clock genes is the endoderm, rather than the nervous system or specialized sensory cells (Peres et al. 2014). Finally, the development of techniques to eliminate gene expression via genetic modification or protein interference will allow testing of the necessity and sufficiency of predicted clock proteins to the circadian molecular clock in cnidarians.

Decoding the cnidarian circadian clock is an important piece of understanding the evolutionary origin of fundamental mechanisms that coordinate physiological rhythms to environmental cues and improve survival and reproductive fitness. In turn, the cnidarian circadian clock is essential to understanding the basic metabolic processes among of these animals and may prove useful to their future conservation.

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Part VIII

Global Climate Change

Survey of Cnidarian Gene Expression Profiles in Response to Environmental Stressors: Summarizing 20 Years of Research, What Are We Heading for?

32

Keren Maor-Landaw and Oren Levy

Abstract

Coral research has come a long way since the pioneering coral biology studies of thermal tolerance dating back to the turn of the previous century. In great contrast, at the present time, the currently available in silico technologies enable the entire transcriptome to be surveyed in a high-throughput manner following an array of stress manipulations. Deep-sequencing is expected to revolutionize the way we study gene expression and holds the potential to answer prominent questions regarding cnidarian cellular pathways following global change scenarios. In this review we focus on cnidarian responses to environmental stressors in general and to global climate change in particular, focusing on the gene expression levels. A wide characterization of studies conducted in cnidarians following environmental stress revealed that most of the studies investigated a single stress factor and mostly thermal stress, were short-term and focused on branching corals. Subsequently, there is a lack of gene expression knowledge concerning massive corals that are known to be less susceptible to bleaching comparing to branching corals. In this review, we present a detailed list of differentially expressed genes in branching/massive corals under eight types of environmental stress. A conceptual model was constructed of the main processes occurring within the coral host cell under heat, ocean acidification and UV stress. The tables and the pathways of this review emphasize gaps in knowledge and can assist in guiding future research as they suggests which genes/processes one should look at in order to achieve a greater understanding of the cnidarians molecular processes affected by global anthropogenic stress.

Keywords

Cnidaria • Coral • Coral bleaching • Environmental stress • Gene expression • In silico • Molecular pathways

32.1 Introduction: The "Chronology" of the Methods Toolbox

Coral research has come a long way since the pioneering coral biology studies of thermal tolerance dating back to the turn of the previous century (Mayer 1917, 1918; Edmondson 1928). It appears that the very first suggestion that the coral bleaching phenomena is correlated with high temperatures was made by Yonge in 1931 (Yonge 1931). In great contrast, at the present time, the currently available in silico technologies enable the entire transcriptome to be surveyed in a high-throughput manner following an array of stress manipulations.

Until 1974 thermal studies among corals were restricted to laboratory experiments of minutes to hours of incubations under lethal temperature conditions (such as studies on corals calcification under elevated temperatures: (Yamazato

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1970; Clausen 1971; Clausen and Roth 1975)) (Jokiel and Coles 1974) and focused on a single factor effect (Coles and Jokiel 1978). The first seasonal coral bleaching was reported by Jokiel and Coles (1974), they witnessed zooxanthellar pigment loss in summer and recovery in winter. The novelty was the quantifying of chlorophyll concentrations and plant carotenoids in naturally bleached versus normal colonies. Following that study, the same group (Coles and Jokiel 1978) studied the synergetic effect of interactions between salinity and light parameters along with high temperatures on the coral *Montipora verrucosa*.

However, it was not until the mass bleaching events which accompanied the 1982–1983 and 1987–1988 El Niño events that brought about a heightened awareness into studying how environmental parameters and primarily temperature, effect coral reefs in general and corals' well-being in particular. Bleaching events reported prior to the 1980s were restricted to small areas with low bathymetric depth range, were less frequent and less severe. In 1983 Glynn described an extensive coral bleaching event on the Pacific coast of Panama with an outcome of 70 % coral mortality. Consequently Glynn (1983) was the first to draw attention to the mass coral bleaching events of 1982–1983. Subsequently, widespread bleaching and coral deaths were reported in the Java Sea (Indonesia) (Brown 1987; Brown and Suharsono 1990), French Polynesia, Southern Japan, Tokelau Islands [see review by Brown (1987)], the Great Barrier Reef (GBR) (Harriott 1985), Puerto Rico (Goenaga et al. 1989), Bermuda (Cook et al. 1990) and Jamaica (Gates 1990; Goreau and Macfarlane 1990).

These aforementioned studies" tool box" was primarily comprised of descriptive ecology approaches. The extent of the mass coral bleaching events was described via coral community surveys of bleached and dead colonies, coral cover, species diversity (Brown and Suharsono 1990), corals growth rates (Goreau and Macfarlane 1990), mortality rates (Harriott 1985) and Echinoid bioerosion (Glynn 1988), during several months or years [1981–1988 (Brown and Suharsono 1990)], and along a depth transect up to 18 m (Gates 1990). An approach of histological photomicrographs showed that bleaching was accompanied by a drastic decrease in zooxanthellae densities in the gastrodermis coral tissue (Lasker et al. 1984).

Estimations assed that the 1982–1983 El Niño events caused coral mortality of 50–99 % over the eastern Pacific in a bathymetric range of all hermatypic corals (Glynn 1993). The widespread mass bleaching events and their severity contributed greatly to the motivation to search for causative factors (Harriott 1985). Mass bleaching was attributed to radiation since it was restricted to colonies in shallow waters or to colonies on upper un-shaded surfaces in Puerto Rico and the Great Barrier Reef (example: Goenaga et al. 1989; Harriott 1985). In contrast, others have proclaimed that even though coral bleaching can be induced by a number of environmental factors mass bleaching is mostly related to rising seawater temperatures (Brown and Suharsono 1990; Cook et al. 1990; Goreau 1990). Nonetheless, during the early 1990s some argued that efforts to explain large scale bleaching events in terms of global change were not convincing due to a lack of standardized methods evaluating coral bleaching (Glynn 1993). The next phase in the chronology of the methods "toolbox" progress met this need by introducing quantitative physiology tactics (Fig. 32.1).

Physiological characterization of coral-host relations during bleaching is essentially based on quantifying parameters such as: zooxanthellae cell count, zooxanthellae mitotic index and expulsion rate, protein and lipids contents, algae photosynthesis and chlorophyll quantities (e.g. Glynn and D'Croz 1990; Hoegh-Guldberg et al. 1987; Hoegh-Guldberg and Smith 1989; Porter et al. 1989; Szmant and Gassman 1990; Fitt and Warner 1995). Some of these studies illustrated that the paling of coral is through either loss of zoo-

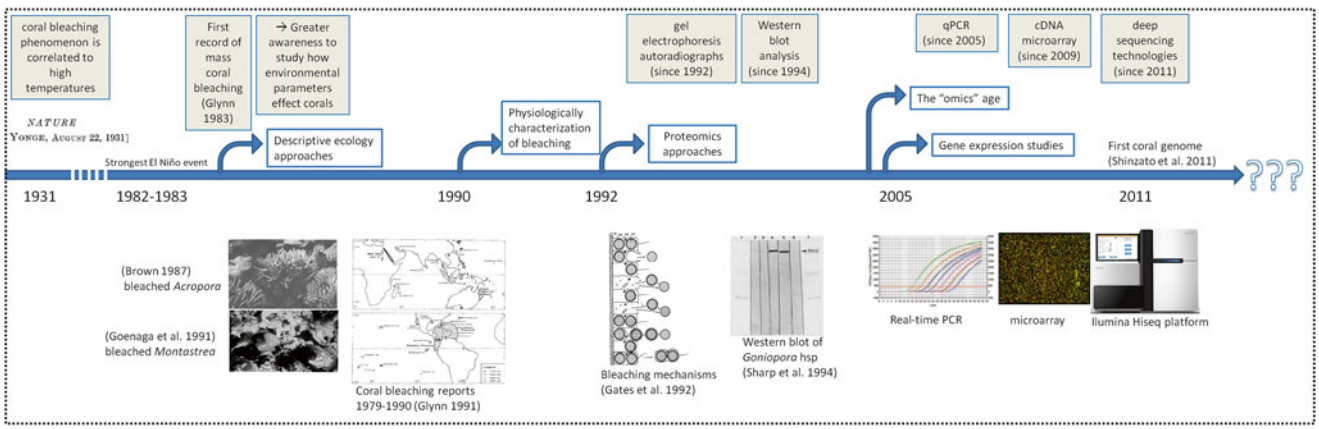


Fig. 32.1 A time line illustrating the chronology of methods in corals responses to environmental stress studies

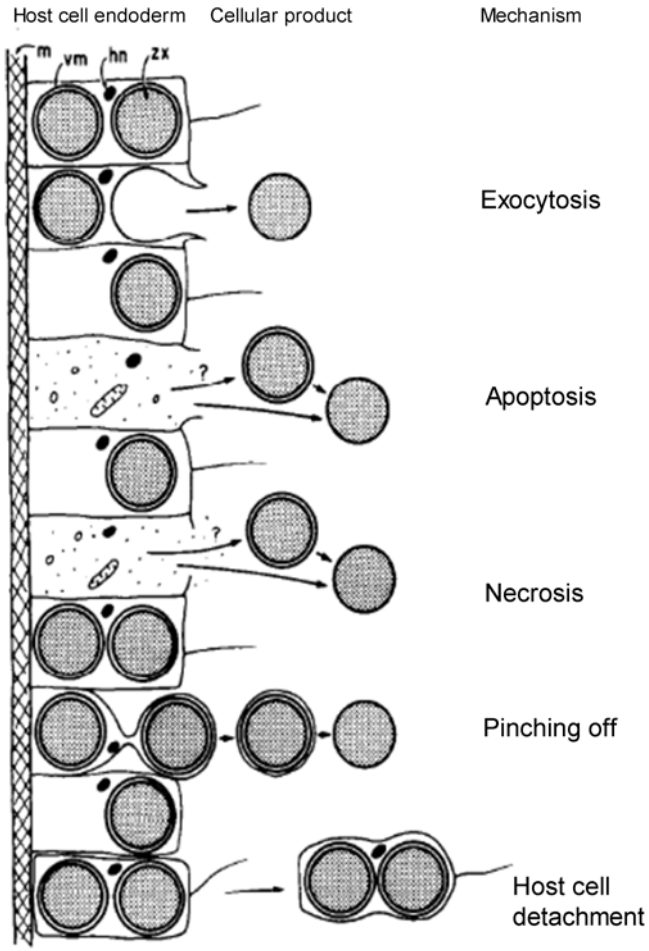


Fig. 32.2 A schematic representation of potential mechanisms by which zooxanthellae could be released from the endoderm of cnidarians, and the cellular entities associated with each mechanism. *m* mesoglea, *vm* host vacuolar membrane, *hn* host cell nucleus, *zx* zooxanthella (Taken from Gates et al. 1992)

xanthellae and/or the loss of photosynthesis pigment per zooxanthellae cell. Moreover, a decrease in photosynthesis and photosynthesis efficiency were measured following increased temperatures (Fitt and Warner 1995; Warner et al. 1996). Over time, several potential mechanisms of bleaching were proposed: (a) exocytosis of isolated zooxanthellae from the host cell (Steen and Muscatine 1987); (b) apoptosis (programmed cell death) and (c) necrosis, in both cases zooxanthellae are released with the dead cell; (d) release of zooxanthellae surrounded by the vacuolar and pinched off plasma membrane; and (e) detachment of endoderm cells (including zooxanthellae inside) from the host (Gates et al. 1992) (see Fig. 32.2).

Proteomics arose during the beginning of the 1990s due to the emerging need for new protein analysis tools. The proteome is essentially the catalogue of all proteins translated from a genome, at a certain time point or under particular environmental conditions. As opposed to a transcriptome, the proteome includes the dynamic changes within the proteome that can occur in response to different stimuli, such as post-translational modifications (Lopez 2007). Since the early 1990s, the use of proteomic tools in Cnidaria began to be more prevalent in studies focusing on heat shock proteins (Hsps) induction as response to temperature increase. Hsps have a crucial role as molecular chaperones, in protein folding, assembly, localization, secretion, regulation and degradation of proteins (Gething 1997). They were studied most commonly upon gel electrophoresis autoradiographs in cnidarians (Miller et al. 1992; Black et al. 1995). Alternatively, Hsps were studied using Western blot analysis that essentially enabled the detection of specific proteins with the aid of primary antibodies (Sharp et al. 1994; Hayes and King 1995; Fang et al. 1997; Gates and Edmunds 1999; Downs et al. 2000; Brown et al. 2002).

Proteomics studies can also utilize enzymes activity assays in order to deduce on the cellular condition. Activities of antioxidants enzymes, such as: superoxide dismutase, catalase and ascorbate peroxidase, which are responsible for detoxifying reactive oxygen species (ROS), can indirectly indicate on ROS elevated concentrations and oxidative stress within the cell [(Lesser et al. 1990) and reviewed by: Lesser (2006, 2011)]. The oxidative stress theory proposes a mechanism elucidating coral bleaching; when elevated seawater temperatures increase ROS production in zooxanthellae chloroplasts and mitochondria leading to cellular damage, bring about the expulsion of symbionts (Downs et al. 2002; Weis 2008) and trigger programmed cell death in their hosts cells [such as: (Dunn et al. 2004; Richier et al. 2006; Kvitt et al. 2011)]. Similarly, coral's programmed cell death can be studied via Caspase 3 enzyme activity as an executioner Caspase that cleave a variety of cellular substrates leading to apoptosis programmed cell death (Dunn et al. 2004; Richier et al. 2006; Kvitt et al. 2011). Here, in this review we would like to focus on the different responses to environmental stressors in general and to global climate change in particular, focusing on the gene expression levels.

32.2 Gene-Expression Profile Studies in Cnidarians Using High-Throughput Tools

Why study gene expression? Transcriptomic analysis provides a reliable snapshot of cellular activity and it is suitable as a proxy to detect cellular events. A transcriptome is essentially defined as the complete set of transcripts (messenger RNA-mRNA) generated by a cell or a population of cells, and their quantity, for a specific developmental stage or a physiological condition (Wang et al. 2010). However, it is important to emphasize that the correlation between mRNA quantities and their respective proteins in the cells was found to be poor, since gene expression does not take into account

regulatory steps that follow mRNA synthesis (Sealfon and Chu 2011; Bramanti et al. 2013).

Quantitative real-time polymerase chain reaction (qPCR) has been and still is a useful tool for quantifying specific transcripts at a particular time or environmental condition in a reproducible and accurate manner. qPCR is fundamentally a modification of PCR, but based on quantifying cDNA molecules after every cycle of amplification using fluorescent dyes (Hofmann and Place 2007; Bustin et al. 2009). Utilizing this technique requires knowledge on the genes of interest in order to design specific primers for a successful amplification. Several studies have applied qPCR for following heat stress experiments in examining apoptotic transcripts (Ainsworth et al. 2011; Kvitt et al. 2011; Pernice et al. 2011) or stress-related transcripts in corals (Császár et al. 2009; Seneca et al. 2010; Kenkel et al. 2011; Leggat et al. 2011; Souter et al. 2011; Yuyama et al. 2012), in sea anemone (Sunagawa et al. 2008) and *Scyphozoa* (Schroth et al. 2005).

The development of complementary DNA (cDNA) microarrays of different cnidarians has enabled major advances in coral biology research, enabling large-scale studies of expression patterns for thousands of genes simultaneously. So far, transcriptomic databases have been developed for the Caribbean corals *Montastraea faveolata* and *Acropora palmata* (Schwarz et al. 2008), *Stylophora pistillata* (Karako-Lampert et al. 2014) and based on next generation technologies for *Acropora millepora* (Meyer et al. 2009a), *Pocillopora damicornis* (Traylor-Knowles et al. 2011) and *Acropora palmata* (Polato et al. 2011). Unpublished transcriptome assemblies for *Acropora hyacinthus*, *Acropora tenuis* and *Porites astreoides* are available online (Matz Lab 2012).

The rapidly developing "omics" (genomics, proteomic, transctiptomic) techniques have brought on a transition from studies of single specific genes (like Western blot and qPCR) towards researching simultaneously many components resulting with large data sets (Johnson and Browman 2007). RNA microarray is based on probes of fragmented cDNA that are immobilized on a glass slide. Each cDNA fragment represents a gene of interest, so the array can be printed with a focused selection of genes or alternatively with a whole genome, often containing tens to hundreds of thousands of genes on a single chip. Experimental RNA samples are labeled with fluorescent markers. Typically each treatment/control samples are tagged with two different colors. After mixing all the labeled samples, the RNA is hybridized to the microarray. The intensity of the emitted fluorescence from a target spot on the array represents an estimation for the relative amount of each transcript in the different samples (Hofmann and Place 2007; Sealfon and Chu 2011).

RNA microarrays have been used to study the transcriptomic response to heat stress in the corals *Montastraea faveolata* (DeSalvo et al. 2008, 2010), *Acropora palmata* (DeSalvo et al. 2010b), the larvae of *Montastraea faveolata* (Polato et al. 2010), *Acropora millepora* (Rodriguez-Lanetty et al. 2009) and *Acropora palmata* (Portune et al. 2010). Other coral studies have utilized this technique to detect responses to darkness (Desalvo et al. 2012), ultraviolet radiation (Aranda et al. 2011), and to study coral-algal symbiosis (Voolstra et al. 2009) and phenotypic plasticity (Bay et al. 2009).

Today, in silico technology moved us into the 'omics' age and since 2008 (McGettigan 2013) researchers are increasingly applying a high throughput sequencing, termed RNA-Seq, which has considerable advantages over microarrays. However, thus far, only a few studies have employed this emerging tool for coral transcriptome studies following climate change scenarios; elevated temperatures (Meyer et al. 2011; Barshis et al. 2013) and ocean acidification (Moya et al. 2012b; Vidal-Dupiol et al. 2013). Other RNA seq projects have utilized this technology to study corals immune response (Burge et al. 2013; Libro et al. 2013).

As opposed to Sanger sequencing which is considered as first generation, the fast developing Next generation/deep sequencing technologies are based on repeated sequencing of a DNA fragment in a very short time, by means of high sensitivity and accuracy (Malone and Oliver 2011). RNA sequencing, termed RNA-seq, is a recently developed approach to transcriptome profiling that utilizes deep-sequencing technologies. Firstly, extracted RNA is converted to a library of cDNA fragments and sequencing adaptors are added to each cDNA fragment. Then each molecule is sequenced in a high-throughput manner to acquire short sequences from one end (single-end sequencing) or both ends (pair ends sequencing). The resulting output is typically 30–400 bp reads which are subsequently aligned with a reference genome, transcriptome or alternatively assembled *de novo* generating a transcription map. Finally, the expression level of a certain gene is assessed by the number of mapped reads to the reference/de novo transcriptome (Wang et al. 2010; Malone and Oliver 2011). Currently, the Illumina HiSeq platform is the most commonly used next generation technology of RNA-Seq, yielding up to three billion reads per sequencing run (Metzker 2010; McGettigan 2013; Van Verk et al. 2013).

RNA-seq is expected to revolutionize the way we study transcriptomes and is highly suitable for profiling non-model organisms with no available genome (Johansen et al. 2010; Malone and Oliver 2011). The major advantage of RNA-seq over microarrays is that there no need for prior genetic knowledge (probes etc.). This makes RNA-seq ideal for researching different cnidarian species, where there is no single model organism that is being studied and only a few genome sequencing projects *Nematostella vectensis* (Putnam et al. 2007), *Hydra magnipapillata* (Chapman et al. 2010) and *Acropora digitifera* (Shinzato et al. 2011) are available.

Other prominent advantages of RNA-seq over first generation approaches and microarrays are: (1) very low, if any,

background signal. (2) A large range of transcript expression levels that can be detected, while microarray lacks sensitivity to distinguish low or high levels of expressed genes. (3) Requires less sample RNA (4) Highly accurate, has high levels of reproducibility for technical and biological replicates (Wang et al. 2010). (5) Less expensive comparing to Sanger-based sequencing technologies and costs will probably decrease with increasing sequencing output (Malone and Oliver 2011). (6) The single base resolution enables RNA-seq to reveal precisely the location of transcription boundaries (Wang et al. 2010). (7) Better at quantifying transcript isoforms generated by alternative splicing (Malone and Oliver 2011).

RNA-seq still confronting a few challenges, some are novel analytical problems concerning how to store, manage, retrieve and process large amounts of data and some are bioinformatic, such as: misalignment of reads to closely related genes and challenges in library construction (Wang et al. 2010). Another important issue is the applied depth of sequencing that is required to effectively sample the transcriptome, or the sequence coverage of the library (Wang et al. 2010; Malone and Oliver 2011).

32.3 Categorizing Gene Expression Studies in Cnidarians in Response to Environmental Stressors

With the purpose of evaluating current knowledge and reviewing recent publications on molecular level responses to environmental stressors in cnidarians, we have generated a designated catalog. The Catalog that is presented in Table 32.1 lists relevant studies and the following details about each paper: type of stress (heat/pH/UV), duration of experiment, the studied species, if a scleractinian coral; branching/massive and robust/complex, geographical location, zooxanthellae clade and method of study. Seventy one papers describing proteomic or transcriptomic studies of cnidarian responses to environmental stressors are recorded in this catalog, while 48 of them are transcriptome studies.

Heat has certainly been the most studied environmental stressor as 75 % of the papers investigated responses to elevated temperatures. Gene expression following UV or ocean acidification scenario produced each almost 10 % of the listed publications (the rest are macroalgae, darkness, salinity and hypo-salinity). Only very few gene expression studies were aimed at investigating the combined effect of pH and temperatures (Ogawa et al. 2013; Putnam et al. 2013), which can lead to a synergetic effect and is more realistic under the predicted upcoming global change.

Most cnidarians gene expression studies relating to global climate change focused on branching corals, as described in the pie chart in Fig. 32.3. The majority of coral studies have been conducted on *Acropora* sp. (for example: Rodriguez-Lanetty et al. 2009; Seneca et al. 2010; Pernice et al. 2011), while only about a quarter focused on massive corals; *Porites* and *Montastrea*. Subsequently, there is a lack of knowledge concerning massive coral gene expression under stress. The same trend can be witnessed in Fig. 32.4 as the number of proteomic studies in massive corals is similar to that of branching ones but publications utilizing gene expression techniques in massive corals are significantly smaller comparing to branching ones.

It appears that *Acropora* is the most studied coral worldwide, with variations regarding the species; *A. millepora* is the organism of choice in Australia and the Pacific, while in the Caribbean *A. palmata* is the most commonly studied species (see Table 32.2) (Weis et al. 2008). Conversely, gene expression studies on massive corals of the genus *Montastraea* and *Porites* are exclusively conducted in Caribbean coral reefs. This matter makes it problematic and complicated to evaluate and compare results from different publications in order to accomplish a more comprehensive perspective of the molecular processes. Reasons for this issue are intriguing due to the fact that massive corals are distributed in all coral reefs around the world (Veron 2000). One of the major considerations in choosing an organism to study is its abundance in the reef, so that it will be a suitable representative of the local corals and the required permits will be feasible. Another important factor in choosing an appropriate model coral is how easily it can be cultivated in aquaria (fragmentation etc.). A branched colony, such as *Acropora*, is relatively easy to fragment and to establish duplicate "micro-colonies" from. On the other hand, massive coral colonies often require a core-forming drill against the massive skeleton and are in general far more difficult to fragment successfully. Moreover, adjusting fruitful molecular tools and experimental methodologies, such as species-distinctive RNA extraction protocols or records of genes/primers, results in greater advantages of the chosen species and makes them essentially even more appealing to new studies (Weis et al. 2008). However, a greater emphasis should be placed on investigating the gene expression responses of numerous diverse coral species in a particular geographical location. This is all the more important given what we know about the vast interspecies variation of sensitivities to environmental stressors among coral species (examples: Cook et al. 1990; Goenaga et al. 1989; Wooldridge 2014). Furthermore, the responses of a few model species do not necessarily reflect on most reef corals. The new in silico technologies can assist in doing so, as previous knowledge in the form of primer sequences/designated microarray, is no longer needed. Hence, theoretically gene expression responses of every single coral species in the reef can be studied from scratch via RNA-seq methodologies.

Table 32.1b Catalog of publications on molecular level responses to environmental stressors in cnidarians

Authors	Year	Stress	Duration of experiment	ΔT	Species	b/m	r/c	Geographical location	Zoox clade	Method
Bosch and Praetzel	1991	Heat	2 h	15	*H. vulgaris*					Western blot
Miller et al.	1992	Heat and heavy metal ions	2 h	8, 12, 13						Proteomics
Sharp et al.	1994	Heat	8 w	11	*G. djiboutiensis*, *A. Viridis*	m	c	Indian Ocean, Thailand and UK		Western blot
Black et al.	1995	Heat	2 h	13	*M. Faveolata*, *A. pallida*	m	r	Caribbean, Bahamas		Stable isotopes and gel electrophoresis for proteins
Choresh et al.	2001	Tidal pools	7 d	8	*A. viridis*			Mediterranean, Israel		Western blot
Sawyer and Muscatine	2001	Heat, cold			*A. pulchella*, *P. damicornis*			Hawaii		Proteomics
Brown et al.	2002	Heat	4 d	4.2	*G. aspera*	m	r	Indian Ocean, Thailand		ELISA/western blotting
Kingsley et al.	2003	heat, cold	1, 2 h	16	*L. virgulata* (Octocorallia)			North Carolina		Western blot
Choresh et al.	2004				*Hydrozoa*, *Scyphozoa*, *A. viridis* and *Scleractinian*					Western blot
Snyder and Rossi	2004	Tidal zones			*A. elegantissima*					Western blot
Edge et al.	2005	Heat	4 h	6	*M. faveolata*	m	r	Caribbean, Florida		Microarray
Edge et al.	2005	salinity	4 h		*M. faveolata*	m	r	Caribbean, Florida		microarray
Edge et al.	2005	Uv	4 h		*M. faveolata*	m	r	Caribbean, Florida		Microarray
Schroth et al.	2005	Heat			*Aurelia*			Mediterranean, Adriatic Sea		qPCR
Smith-Keune and Dove	2008	Heat	7 d	5	*A. millepora*	b	c	GBR		qPCR
Choresh et al.	2007	Heat	6 d	9	*A. viridis*					Immunoblotting and sequencing
Merle et al.	2007	Heat			*A. viridis*			Mediterranean, France		Catalase activity, western blot
Leutenegger et al.	2007	Natural bleaching			*A. sulcata var. smaragdina*, *A. rustica*			Mediterranean, France		Real time PCR and antioxidants activity
DeSalvo et al.	2008	Heat	17 h, 10 d	3	*M. faveolata*	m	r	Caribbean	A	Microarray
Richier et al.	2008	Heat	24 h	8	*A. elegantissima*			central Oregon coast		Microarray
Richier et al.	2008	Uv	24 h	8	*A. elegantissima*			Central Oregon coast		Microarray
Sunagawa et al.	2008	Heat	8 d	5	*A. pallida*					qPCR
Rodriguez-Lanetty et al.	2009	Heat	0, 3, 10 h	4, 7	*A. millepora* larvae	b	c	GBR		Microarray
Meyer et al.	2009	Heat	5 d	4	*A. millepora* larvae	b	c	GBR		qPCR

Császár et al.	2009	Heat	6 d	9	*A. millepora*	b	c	GBR	D	qPCR
Vidal-Dupiol et al.	2009	Heat	15 d	4	*P. damicornis*	b	r	Indonesia	A/B	SSH approack led to qPCR
Voolstra et al.	2009	Heat	12, 48 h	4	*M. faveolata* embryos	m	r	Caribbean, Mexico		Microarray
Downs et al.	2009	Hypo-salinity	24 h		*S. pistillata*	b	r	Red Sea		ELISA
Starcevic et al.	2010	Uv	36 h		*A. microphthalma*	b	c	GBR	D1	cDNA library based on PCR-SSH
Seneca et al.	2010	Natural bleaching	2 natural summers		*A. millepora*	b	c	GBR		qPCR
DeSalvo et al.	2010	Heat	58 d	3	*A. palmata*	b	c	Caribbean, Panama	A	Microarray
Carpenter et al.	2010	Heat, flow	1, 9 d	1.5–2	*M. annularis*	m	r	Caribbean, Florida		Western blot
Woo et al.	2010	Heat	24 h	4	*S. gracillimum* (soft coral)			Korea		DD-PCR
Liñán-Cabello et al.	2010	Uv	32 h		*P. capitata*	b	r	Caribbean, Mexico		Antioxidants activity
Polato et al.	2010	Heat	24, 48 h	4.5	*M. faveolata* larvae	m	r	Caribbean, Mexico and Florida		Microarray
Portune et al.	2010	Heat	6 d	3.5	*A. palmata* embryos and larvae	b	c	Caribbean, Porto Rico		Microarray
Leggat et al.	2011	Heat	8 d	6	*A. aspera*	b	c	GBR	C1/C3	qPCR
Ainsworth et al.	2011	Heat	6 d	8	*A. aspera*	b	c	GBR		qPCR
Pernice et al.	2011	Hear	5 d	10	*A. millepora*	b	c	GBR		qPCR, Tunel, caspase 3 activity
Souter et al.	2011	Heat	9 d		*A. millepora*	b	c	GBR		qPCR
Meyer et al.	2011	Heat	4 h, 5 d	3.7	*A. millepora* larvae	b	c	GBR		RNA seq
Tchernov et al.	2011	Heat	2 m	6	*S. pistillata and S. hystrix*	b	r	Red Sea		Microscopy, caspase activity, western blot
Detournay and Weis	2011	Heat	24 h	8	*A. pallida*					Caspase activity, nitric oxide production
Pey et al.	2011	Heat	52 d	8	*E. singularis* (Octocorallia)			Mediterranean, Marseille		Dot blot, western blot, total oxyradical capacity
Aranda et al.	2011	Uv	6 h		*M. faveolata* larvae	m	r	Caribbean, Mexico		Microarray
Kvitt et al.	2011	Heat	24 h	10	*S. pistillata*	b	r	Red Sea		qPCR, TUNEL, capsase activity
Kenkel et al.	2011	Heat-exposure	2 d	8.2	*P. astreoides*	m	c	Caribbean, Florida		qPCR
Dunn et al.	2012	Heat	24 h	7–8	*A. pulchella*					qPCR, microscopy
Kaniewska et al.	2012	pH	28 d		*A. millepora*	b	c	GBR		Microarray
Bellantuono et al.	2012	Heat	29 d	10	*A. millepora*	b	c	GBR		Microarray
Downs et al.	2012	Diverse natural stressors			*P. damicornis*	b	r	Pacific Ocean, Guam		ELISA
Moya et al.	2012	Heat, uv, heat + uv	21 d	10	*A. viridis*			Mediterranean, France	A	Microarray
Yuyama et al.	2012	Heat, chemical	27 h	8	*A. tenuis* juvenile	b	c	Pacific Ocean, Okinawa		qPCR

(continued)

Table 32.1b (continued)

Authors	Year	Stress	Duration of experiment	ΔT	Species	b/m	r/c	Geographical location	Zoox clade	Method
DeSalvo et al.	2012	Dark	9, 10 d		*M. faveolata* and *A. palmata*	m, b	r, c	Caribbiean, Panama	A, C	Microarray
Shearer et al.	2012	Macroalgae	20 d		*A. millepora*	b	c	Pacific Ocean, Fiji		microarray
Moya et al.	2012	ph	3 d		*A. millepora* larvae	b	c	GBR		RNA seq
Nakamura et al.	2012	pH	7 d		*A. digitifera* larvae	b	c	Pacific Ocean, Okinawa		qPCR
Ogawa et al.	2013	Heat, pH	14 d	9	*A. aspera*	b	c	GBR		qPCR
Edge et al.	2013	Natural bleaching	16 m	8	*M. cavernosa*	m	r	Caribbean, Florida		Microarray
Kenkel et al.	2013	Heat	6 w	3.7	*P. astroides*	m	c	Caribbean, Florida	A	RNAseq and qPCR
Vidal-Dupiol et al.	2013	pH	3 w		*P. damicornis*	b	r	Indonesia		RNA seq
Barshis et al.	2013	Heat	53 d	3	*A. hyacinthus*	b	c	Pacific Ocean, American Samoa	D/C	RNA-seq
Putnam et al.	2013	Heat, pH	9 d	4	*P. damicornis* larvae	b	r	Pacific Ocean, Taiwan		qPCR, western blot
Ross et al.	2012	Heat, oxidative stress	24 h	3	*P. astreoides* larvae	m	c	Caribbean, Florida		Antioxidants activity
Olsen et al.	2013	Heat	4, 24, 48 h	3.7	*P. astreoides* larvae	m	c	Caribbean, Florida		Antioxidants activity, qPCR
Hawkins et al.	2014	Heat	9 d	4.5	*A. millepora*	b	c	GBR	C3	Total oxidised NO assay, caspase activity
Hawkins et al.	2014	Heat	9 d	4.5	*M. digitata*	b	c	GBR	C15	Total oxidised NO assay, caspase activity
Hawkins et al.	2014	Heat	9 d	4.5	*P. damicornis*	b	r	GBR	C42-2	Total oxidised NO assay, caspase activity
Carreiro-Silva et al.	2014	pH	8 m		*D. dianthus*		c	Atlantic ocean, Azores		qPCR
Maor-Landaw et al.	2014	Heat	13 d	10	*S. pistillata*	b	r	Red Sea		Microarray
Seveso et al.	2014	Heat	1.5 d	7	*S. hystrix*, *M. monasteriata*, *A. echinata*	b	c			Western blot

The following details are indicated for each relevant paper: type of stress, duration of the stress (*h* hour, *d* day, *w* week, *m* month), if a heat stress experiment; the elevation in temperatures (ΔT), species, if a Scleractinian coral; branching (b)/massive (m) and robust (r)/complex (c), geographical location, zooxanthellae clade and method of study. For further details see relevant paper

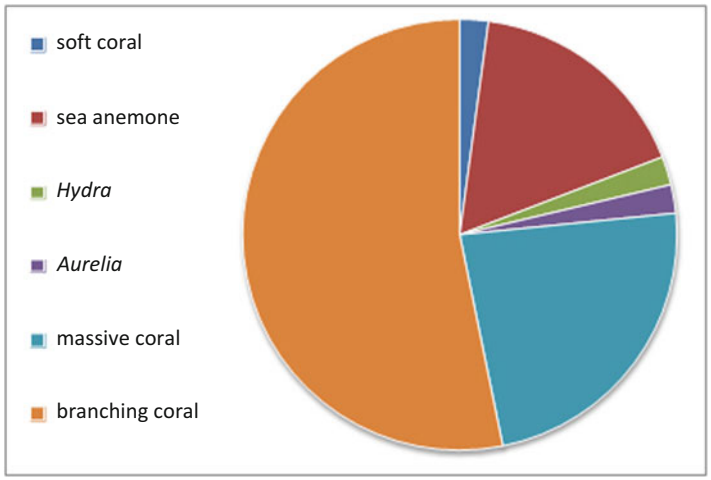


Fig. 32.3 A pie chart of the studied species of gene expression studies (real-time PCR, microarray and RNA-seq)

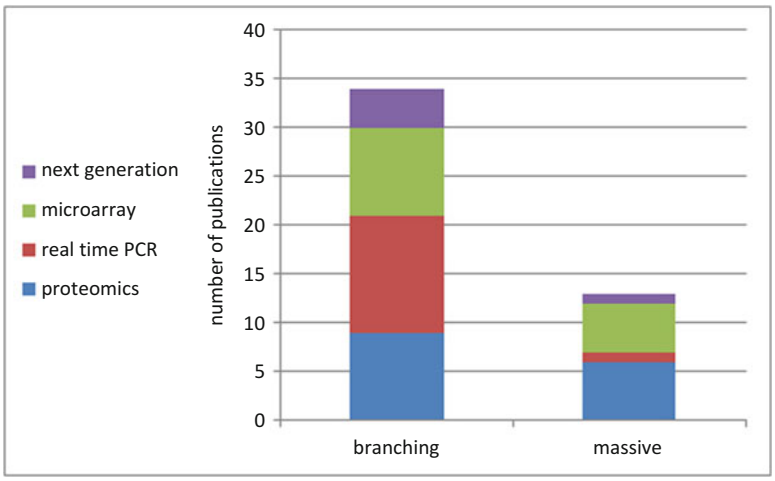


Fig. 32.4 Number of publications in branching and massive corals related to global climate change with proteomic techniques, real time PCR, microarray and next generation sequencing

Table 32.2 Geographical location of gene expression studies and the studied species

Geographical location	Number of gene expression publications	The studied species
Great Barrier Reef	15	*Acropora millepora*, *Acropora aspera*
Caribbean	13	*Montastraea faveolata*, *Montastraea cavernosa*, *Porites astroides*, *Acropora palmata*
Pacific	7	*Pocillopara damicornis*, *Acropora tenuis*, *Acropora hyacinthus*,
		Scleronephthya gracillimum (soft coral), *Acropora millepora*
Red sea	2	*Stylophora pistillata*

There is little agreement with regard to experimental procedures for corals stress experiments. This is manifested by different experiment durations and the temperature profile of the particular experiment. Most of the experiments are short-term, as in almost 75 % of them the organisms were exposed to the stressor for no longer than 10 days. There is a lacking in long-term experiments of several weeks to months (see Fig. 32.5) which are not less important to investigate, considering that in nature stress can last for several months during the summer time.

32.4 Profiling Differentially Expressed Genes in Response to Environmental Stressors: Branching Versus Massive Corals

It was well established that different coral species have different susceptibilities during heat stress (example:(Harriott 1985; Glynn 1988; Cook et al. 1990)), as branching, especially acroporids, are known to be more susceptible to bleaching comparing to massive corals (examples: (Jokiel

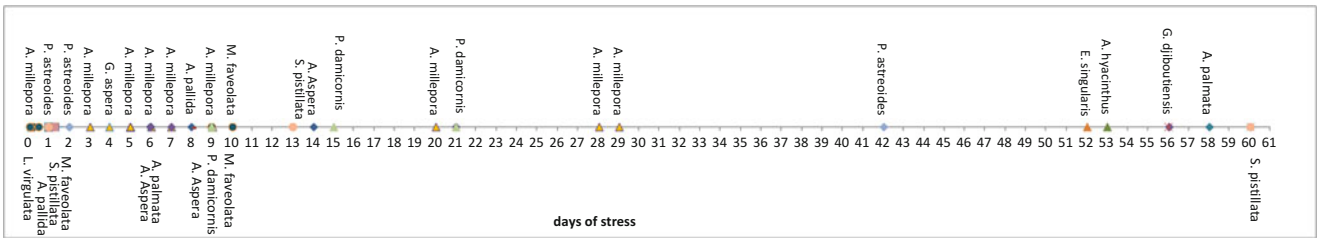


Fig. 32.5 Duration of environmental stress in cnidarians transcriptome and proteome studies. Each *dot* represents a publication and the studied species is indicated

and Coles 1974; Brown and Suharsono 1990; Loya et al. 2001). There have been an attempt to explain this phenomenon physiologically: It seems that massive corals, on average, have a thicker tissue (Loya et al. 2001) which posses more photoprotective abilities by self-shading especially when polyps are retracted (Brown 1997; Hoegh-Guldberg 1999). Another hypothesis is based on colony morphology; flat and massive corals experience higher flow regimes and smaller boundary layer. Under these conditions transfer of mass, such metabolites and ROS, is more efficient (Loya et al. 2001; DeSalvo et al. 2010b). Recently, Wooldridge (2014) reviews and argues that the differences in susceptibilities are due to different strategies of ensuring a continuity of CO_2 for photosynthesis. However, still we cannot explain these changes in bleaching susceptibilities in a gene expression manner and cellular pathways. Only one attempt was made by DeSalvo et al. (2010b) to compare microarray results of massive and branching corals after similar thermal stress experiments; the massive *Montastrea faveolata* (DeSalvo et al. 2008) and the branching *Acropora palmata*. Despite that the gene overlap between the two microarrays was very small: about 10 %, and the percentage of annotated differentially expressed genes was different, they reported on a core response for the two species. In addition, they identified few elements in *A. palmata* that didn't appear in *M. faveolata*: osmotic stress, p53 and NF-κB signaling, sensory perception, the glyoxylate cycle and nitric oxide signaling.

In Table 32.3 we summarized differentially expressed genes in cnidarians following different environmental stressors based on the publications described in Table 32.1. We categorized the genes into those that are differentially expressed both in branching and massive corals and in addition genes which are unique and were up- or down-regulated only in one group of the two. We have divided the genes by their functional role in the cell: oxidative stress and heat shock, protein biosynthesis, apoptosis, cell cycle, cytoskeleton and extracellular skeleton, DNA damage, calcium homeostasis, carbon metabolism, lipid metabolism, cellular transport, RNA processing and cellular energy, as these are the main processes occurring within the cell during environmental stress (Hahn et al. 2004) (e.g. from a whole yeast genome study). Different environmental stressors are indicated in the table (see figure legend): heat, UV, pH, macroalgae, hypo-salinity, salinity and heat combined with pH.

As presented in Table 32.3, there appear to be some points of concurrence with branching and massive corals following heat stress; heat shock proteins, genes related to oxidative stress, such: thioredoxin, catalase, glutathione transferase, protein degradation with Ubiquitin and Ubiquitin ligase, cell adhesion and collagen, DNA damage, metabolism, histone and GFP, were found to be commonly up-regulated genes/processes. Several genes such as: ribosomal proteins, apoptosis regulation, elements of the cytoskeleton and extracellular matrix: actin, carbonic anhydrase, calcium homeostasis proteins and oxidative phosphorylation were commonly down-regulated. Many others were differentially expressed only in branching species and were not indicative in massive corals, for example: proteins related to ER unfolded protein response (UPR) that were up-regulated in branching corals following heat stress and dark conditions and TCA, glycolysis and Krebb cycles that are not indicative at all in massive corals. On the other hand, genes such as: recombination repair protein 1 (DNA damage), malate synthase and indication of transposon activity are examples of changes unique to massive corals.

32.5 A Model for Cellular Pathways Following Environmental Stress in Corals

The compiled data was employed to generate a summarizing pathway illustrating known cnidarians molecular environmental responses to global climate change (Fig. 32.6). The pathway describes processes within corals cells following three major environmental stressors: thermal stress, ocean acidification stress and UV stress. It was constructed based on DeSalvo et al. (2008), Aranda et al. (2011), Kaniewska et al. (2012), and Maor-Landaw et al. (2014) and data from Table 32.3. The pathway emphasizes gaps in knowledge that are still missing and what is yet to be investigated in order to achieve a greater understanding of the molecular processes effected by global anthropogenic stress.

Table 32.3 Differentially expressed genes following environmental stressors in branching and massive corals

	common branching-massive		unique branching		unique massive	
	↑	↓	↑	↓	↑	↓
oxidative stress and heat shock	heat shock proteins		heat shock proteins	heat shock proteins		
			heat shock proteins			
			small heat shock proteins (shsps)			
			small heat shock proteins (shsps)			
				heat shock transcription factor 1	heat shock transcription factor 1	
			Mn superoxide dismutase			
	thioredoxin		Superoxide dismutase (copper/zinc)			
			peroxiredoxin			
	catalase		catalase			
			catalase			
	glutathione-s-transferase		glutathione-s- transferase			glutathione -s-transferase
			protein carbonyl			
			glutathione transferase			
			glutaredoxin			
			peroxidase			
			hypoxia-inducible factor-1a			
			Metallothionein			
			Metallothionein			
			ferritin			ferritin
nitric oxide				nitric-oxide synthesis		
protein	protein degradation		protein degradation			protein degradation
					protein biosynthesis	
			permease - protein transport	protein transport		protein transport
			ER unfolded protein response (UPR)			
			ER unfolded protein response (UPR)			
			protein processing in the ER			
			ER-associated degradation (ERAD)			
		ribosomal proteins	Ribosome-binding protein 1			
	ubiquitin		ubiquitin			
	ubiquitin ligase		ubiquitin			
			PERK (UPR)			
			calreticulin	calreticulin		calreticulin
			Protein disulfide-isomerase A3 (pdia3)			Protein disulfide-isomerase A3 (pdia3)
				Translocon-associated protein subunit delta (protein secretion)		
				cyclophilin-type (facilitate protein folding)		
				Methionine aminopeptidase 2 (tissue repair and protein degradation)		
				calumenin		calumenin
			Hypoxia up-regulated protein 1			Hypoxia up-regulated protein 1
			calnexin			
				prefoldin 2		endoplasmin
apoptosis		apoptosis regulation	cell death and regulation			
			caspase			
			caspase			

(continued)

Table 32.3 (continued)

			caspase			
			proapoptotic Bcl-2 family: Bak, Bax.		proapoptotic caspase adapter protein	
			TNF receptor			TNF receptor
			TRAF			
			TRAF			
			Antiapoptotic: BIR			
			Antiapoptotic: Bcl-2			
						voltage-dependent anion-selective channel 2 (VDAC)
				API-5 (apoptosis inhibitor)		
			p53 inducible protein 11			autophagy
cell cycle				cell cycle regulation		
			BTG (growth arrest)			
			BTG (growth arrest)			
cytoskeleton and extracellular skeleton		actin		actin		
				actin		
			tubulin			
			tubulin			
		extra-cellular matrix	extra-cellular matrix			
			cysteine-rich peptide activity			cysteine-rich peptide activity
			lectin			cysteine-rich peptide activity
	collagen		lectin			
			collagen	collagen		
				collagen	cytoskeleton related gene dynein light chain roadblock - type 2	
				peroxidasin	peroxidasin	
				Vitellogenin		
				Cadherin	matrix metalloprotein	matrix metalloprotein
		carbonic anhydrase	carbonic anhydrase		carbonic anhydrase	
			carbonic anhydrase	carbonic anhydrase		
				galaxin		galaxin
			galaxin			
			HCO3- transporter			
				radixin		
			Thymosin			
				integrin		
			Profilin			
					myosin 7,9	tropomyosin
						lethat giant larvae 2
	cell adhesion					neurofascin homolog
DNA damage	DNA damage					
					recombination repair protein 1	
			rad51			rad51
			DNA topoisomerase I			
			MutS protein			
						double strand break repair
				DNA-3-methyladenine glycosylase		DNA mismatch repair
			MutY			
calcium		Ca homeostasis proteins				

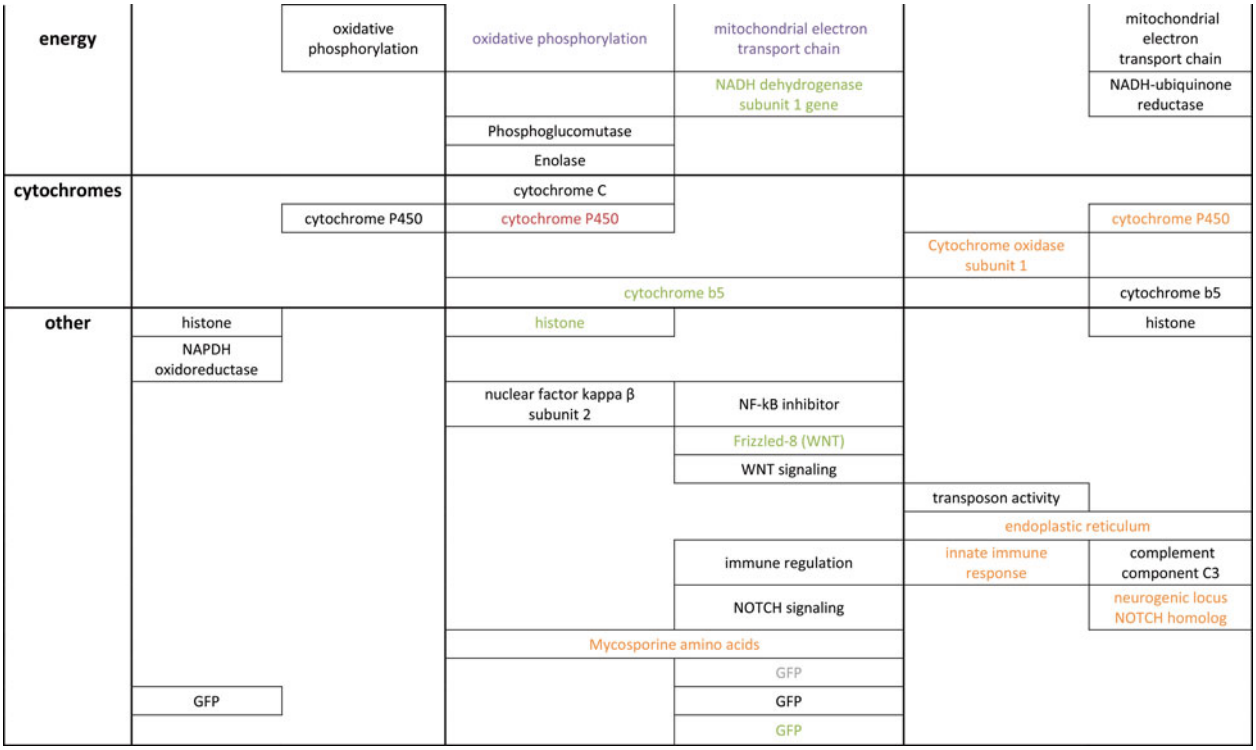
(continued)

Table 32.3 (continued)

homeostasis		Ca bionding synapotagmin like protein effector of SNARE assembly exocytosis			
			calmodulin	calmodulin	
			calmodulin		calmodulin
		calpain			
			calcium binding receptors	calcium ion binding	
		calcium channel			calcium channel
		calcium channel			
		Ca2+ plasma membrane ATPase			
carbon matabolism	matabolism				
		glycolysis			
		Krebb cycle			
		GAPDH			
		glycogen synthase and glycogen phosphorylase			
		glycogen synthase and glycogen phosphorylase			
		a-ketoglutarate dehydrogenase			
		glyceraldehyde-3-phosphate dehydrogenase			
				phosphoenolpyruvate carboxykinase	
				pyruvate carboxylase	
				malate synthase	
		amino acid metabolism			amino acid metabolism
		protein phosphatase 1 (PP1) - glycogen metabolic process			
		Type I inositol-3,4-bisphosphate 4-phosphatase			
		Glutamate carboxypeptidase			
			F16P protein		
lipid metabolism		llipid catabolic process	lipid biosynthesis process		
		lipid peroxidation		lipid peroxidation	
				perixisome organization	
				phospholipase A2	
		lipolysis			
		lipid transporters			
		triglyceride lipase			
		Acyl-CoA dehydrogenase		Acyl-CoA dehydrogenase	
cellular transport		Rab/Ras GTPases families		Ran	
		Rab/Ras GTPases families			
		proton channels			
		transport ions			
			phosphate transport		
		sodium and potassium transporter			
		ABC transporter			
		Charged multivesicular body protein 2a (endosomal transport, establishment of protein localization)			endosome to lysosome transport
			Importin-5 (transfet to nucleus)		
		vesicle/ion/macromolecule transport			
RNA		RNA binding protein			
			splicing factor		
			nuclear ribonucleoprotein A3		
cellular		Mitochondrial ATP synthase	Mitochondrial ATP synthase		Mitochondrial ATP synthase

(continued)

Table 32.3 (continued)



The genes are divided into those that are common to both branching and massive corals and in to genes which are unique and were up- or down-regulated only in one group of the two. The genes are furthermore divided by their functional role in the cell, into: oxidative stress and heat shock, protein biosynthesis, apoptosis, cell cycle, cytoskeleton and extracellular skeleton, DNA damage, calcium homeostasis, carbon metabolism, lipid metabolism, cellular transport, RNA processing and cellular energy. The environmental stressors are indicated in black – heat, orange – UV, purple – pH, green – macroalgae, red – hypo-salinity, blue – salinity, gray – darkness and black and purple – heat and pH

All stressors appear to have a common oxidative stress phase which leads to increasing calcium concentrations and essentially disrupt calcium homeostasis in the cell. The latter is an intersection that serves as a cell messenger for several processes in the cell, some are accountable for one or two types of stress but one is shared by all three; cell death. Having said that, although many of the processes are common to the environmental stressors they are not necessarily governed by the same expressed genes. For example after UV stress, double strand break repair and DNA mismatch repair genes were differentially expressed, but following heat stress DNA topoisomerase I and Muts protein were up-regulated, these are also correlated with DNA damage. This could be a result of sampling or reporting effort or alternatively and more complicatedly different mechanisms within the same similar overall process.

Ocean acidification stress challenges cellular pH homeostasis, which is crucial for a range of functions in the cell. Membrane transporters have a critical role in maintaining pH homeostasis and pH stress resulted in their differential expression. Down-regulation of proton channels, phosphate transport and protein transport along with up-regulation of sodium-potassium transporter, cell membrane receptors and ABC transporters, were reported in an ocean acidification scenario causing pH imbalance in the cell (Kaniewska et al. 2012).

Following both pH and thermal stress, the chloroplast and mitochondria of the host and zooxanthellae, are prime sources for reactive oxygen species (ROS) (Lesser 2006). Accumulation of ROS is known to damage lipids and membranes, proteins and DNA in the cell and is correlated with up-regulation of antioxidant defense agents (reviewed by (Lesser 2011)), such as: Mn superoxide dismutase, peroxiredoxin, thioredoxin and catalase. Interestingly, stress and particularly thermal, is known to trigger up-regulation of heat shock proteins, but after manipulations in pH they were not differentially expressed in coral larvae (Nakamura et al. 2011) or down regulated (Kaniewska et al. 2012). This phenomena either suggests that coral larvae can buffer the external pH changes (Nakamura et al. 2011) or alternatively is a sign that the coral tissue no longer has the capacity to maintain protein folding services (Kaniewska et al. 2012).

Nitric oxide (NO) production in the cell after heat stress has shown to be associated with the breakdown of symbiosis (Trapido-Rosenthal et al. 2005; Perez and Weis 2006) and combined with ROS, it can be altered to reactive nitrogen species (RNS) which are highly reactive as ROS. ROS, NO and RNS can lead to changes in metabolism in the cell, as

many genes related to carbon metabolism are differentially expressed. Metabolic suppression is a common outcome of environmental stress, as well as pH stress (Kaniewska et al. 2012). Moreover, NO inhibits NADH-ubiquinone reductase (DeSalvo et al. 2008) which was found to be down-regulated following heat stress, and by doing so it disrupts oxidative phosphorylation and ATP production in the mitochondria. In order to satisfy the energetic demands within the cell, for processes such as: protein degradation, protein refolding (chaperoning) and DNA repair, genes related to energy metabolism are up-regulated (Maor-Landaw et al. 2014). Furthermore, metabolic enzymes generate reductive elements, such as NADPH and NADH, in order to cope with the oxidative damage (Kültz 2005) in the cell.

Oxidative stress triggers release of Ca^{2+} ions from intracellular storage, such as the Endoplasmatic Reticulum (ER), and essentially to a breakdown of Ca^{2+} homeostasis. This may be indicated from calcium-related differentially expressed genes in corals environmental stressors studies, such as calmodulin which plays a role in calcium signaling and is crucial for various cellular processes (DeSalvo et al. 2008). Ca^{2+} homeostasis disruption is a mediator for cell death, extracellular matrix changes, cytoskeleton rearrangements and cell adhesion disruptions. In pH stress Ca^{2+} homeostasis disruption is thought to trigger changes in cell reception and signaling due to a down-regulation of calpain and other calcium binding proteins (Kaniewska et al. 2012). In the UV stress cascade, changes in the ER as well as direct UV interfere with NOTCH and WNT signaling pathways which are essential for developmental processes in larvae cells (Aranda et al. 2011).

In addition to discharging Ca^{2+}, the ER has an important function in the assembly, folding and post posttranslational modifications of newly synthesized peptides. Correctly folded proteins will be shuttled via transport vesicles to the Golgi body for final processing and assembly (Barlowe 1998; Springer et al. 1999), however misfolded proteins tend to accumulate in the ER lumen and may trigger the unfolded protein response (UPR). The UPR signal is transduced to the nucleus resulting in cell cycle arrest (Brewer and Diehl 2000) and programmed cell death (Hetz 2012), as reported in coral thermal stress experiments (Maor-Landaw et al. 2014). Terminally misfolded proteins undergo ER-associated degradation (ERAD) and with the assistance of cytosolic heat shock proteins (HSPs), target proteins for degradation via the attachment of ubiquitin molecules (Smith et al. 2011; Kriegenburg et al. 2012). Finally, ERAD substrates are degraded into small peptides by the multi-catalytic 26S proteasome.

Cytoskeleton elements, such as: actin, tubulin, tropomyosin, myosin, thymosin and profiling, were found to be differentially expressed following environmental stressor experiments in cnidarians (examples: (Richier et al. 2008; DeSalvo et al. 2010b; Barshis et al. 2013)). Suggesting that the cytoskeleton undergoes rearrangement when under stress. Some of these elements are regulated by levels of intracellular Ca^{2+} that as stated, are out of balance during stress. Heat stress impairs the ability to repair the cytoskeleton, but also it has been discussed that it can also directly damage the cytoskeleton integrity (DeSalvo et al. 2008), or lead to osmotic stress and result in changes in cell volume and fracturing of cytoskeleton elements (Mayfield and Gates 2007). Integrin (Shearer et al. 2012), Lethal giant larvae 2 and neurofascin homologue (DeSalvo et al. 2008) are cell adhesion molecules whose expression was down-regulated during stress. Cell adhesion interruptions could facilitate detachment of endoderm cells with zooxanthellae, which is one of the proposed mechanisms of coral bleaching (Gates et al. 1992).

Drake et al. (2013) identified 36 coral skeletal organic matrix predicted proteins, using proteomic analysis of liquid chromatography-tandem mass spectrometry from a protein extraction of *S. pistillata* skeleton. More than a half of these proteins, which were defined as a constitutive part of the biomineralization toolkit of Scleractinia, were identified as differentially expressed following environmental stress. These proteins and other extracellular matrix related ones, such as: galaxin and peroxidasin are crucial for calcification as they are involved in the synthesis of the organic matrix. Carbonic anhydrase (CA) catalyzes the hydration of CO_2 to HCO_3^- and is thought to play a significant role in the calcium carbonate assimilation of Scleractinian corals (Tambutté et al. 2006; Bertucci et al. 2011). CA was suggested to promote coral resilience against heat stress (Barshis et al. 2013) and there are indications of changed expression following heat stress (example: (Edge et al. 2005), pH stress (Vidal-Dupiol et al. 2013), darkness (Desalvo et al. 2012) and salinity (Edge et al. 2005).

Eventually all stressors lead to cell death, triggered by changes in metabolism or via the uncontrolled release of Ca^{2+}. Prolonged ER stress or mobilization of intracellular Ca^{2+} stimulates pro-caspase 12 activation, which is localized to the ER membrane (Orrenius et al. 2003). Activated caspase 12 cleaves pro-caspase 9 which in turn activates the executioners caspase 3, 6 and 7 (Chowdhury et al. 2008). The latter cleave a variety of cytoplasmic proteins, cytoskeletal elements and cell adhesion molecules throughout the cell and within the nucleus, ultimately resulting in apoptosis (Hengartner 2000). Alternatively, during heat stress Ca^{2+} channels upon the mitochondria membrane are altered causing Ca^{2+} increase and mitochondrial rapture (DeSalvo et al. 2008). This permeabilization results in the release of pro-apoptotic agents, such as: cytochrome C, which is of the essence in the apoptosis intrinsic pathway (Boatright and Salvesen 2003), and reported to be up-regulated following corals heat stress. Among apoptosis-related genes indicated

as differentially expressed following corals stress, are: caspases that are the core effectors of apoptosis, pro-apoptotic regulators as: Bak and Bax, anti-apoptotic agents as: Bcl2, BIR and API-5, TNF receptor, TRAF and voltage dependent anion selective channel 2 (VDAC). Some researches focused on understanding the apoptotic network of cnidarians and its crucial regulation mechanisms (Ainsworth et al. 2011; Kvitt et al. 2011; Pernice et al. 2011) as they appear to be comparable in theirs complexity to that of vertebrates (Zmasek et al. 2007). Oxidative stress and Ca^{2+} homeostasis disruption can alternatively lead to necrotic cell death when mitochondria is severely degraded and cellular energy levels are low (Nicotera et al. 1998). Necrosis cell death was observed in stressed cnidarians (for example: (Lasker et al. 1984; Brown et al. 1995; Dunn et al. 2004)), but as opposed to apoptotic programmed cell death there is no indication of differentially expressed genes and the identification is largely based on cell morphology.

The box on the bottom right hand corner of Fig. 32.6 represents processes or genes that are not incorporated in the pathway and at this stage constitute question marks. It is not clear how these are integrated in the cellular cascade following stress and their significance is not well defined. One of these is autophagy which is a conserved process of degradation of proteins, organelles, cytoplasmic contents and pathogens. Autophagy was suggested to play a role in coral bleaching in a model where it acts together with apoptosis in a see-saw mechanism and also in the regulation of symbiont population (Dunn et al. 2007; Downs et al. 2009b). However, with regard to coral gene expression studies following thermal stress, only one publication reported on changes in autophagy levels and unexpectedly, found a decrease (DeSalvo et al. 2010b). There was a suggestion that autophagy plays a role in chronic stress while apoptosis and necrosis in acute stress, but also this theory doesn't settle with the absence of autophagy gene expression data.

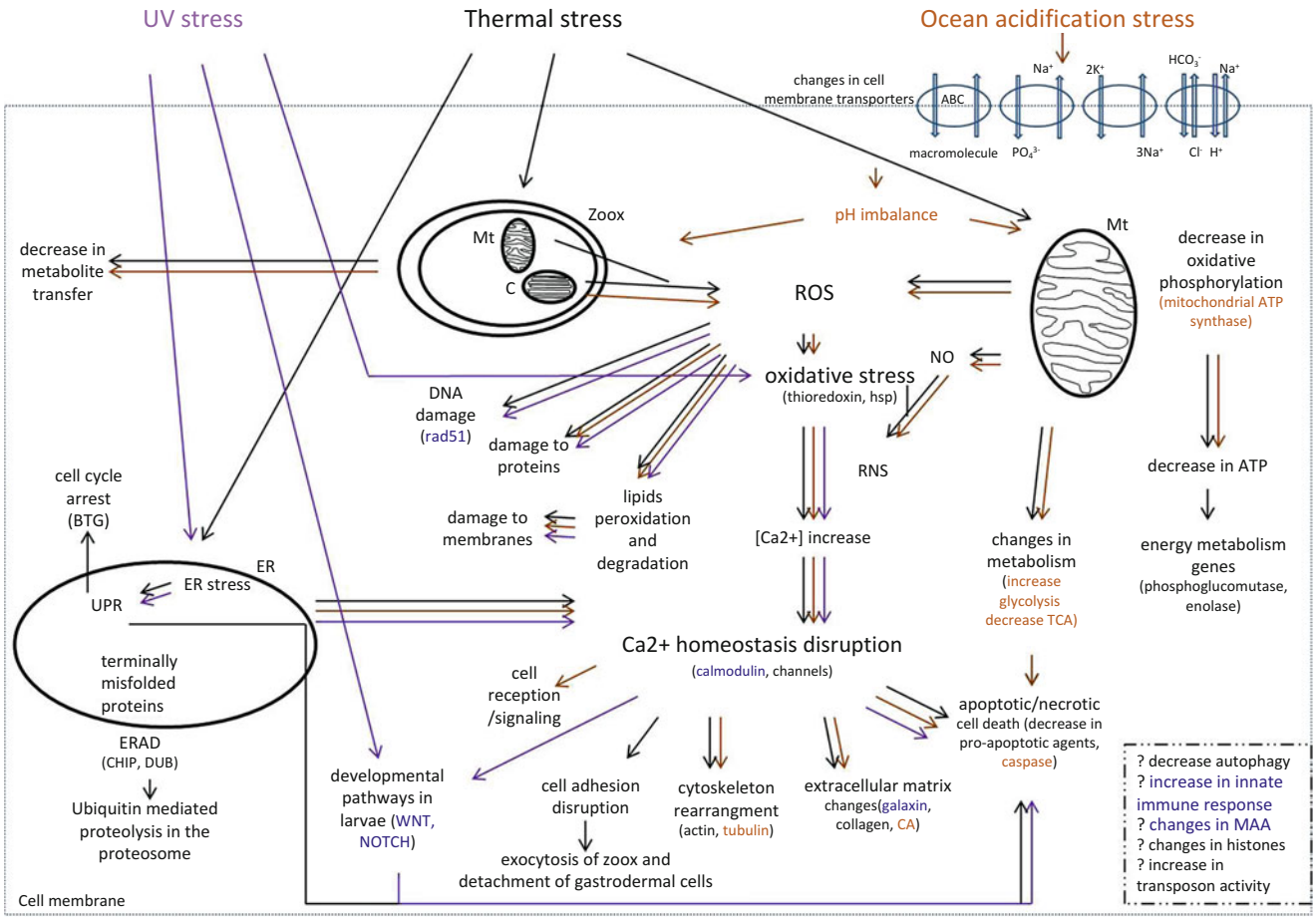


Fig. 32.6 A model of cellular processes following environmental stress in corals. *Brown arrows* and genes titles – ocean acidification stress, *black* – thermal stress and purple – UV stress. In *brackets* are examples of differentially expressed genes from Table 32.3. The *box* in the bottom right hand corner represents processes or genes that are not incorporated in the pathway and at this stage constitute unanswered question marks (The pathway is based on DeSalvo et al. (2008), Aranda et al. (2011), Kaniewska et al. (2012), Maor-Landaw et al. (2014) and data from Table 32.3)

Bleaching was shown to have similarities with an innate immune response to pathogens (Dunn et al. 2007; Weis et al. 2008). One of these is NO which increases in the cell following stress and was previously identified as a signaling molecule during host-microbe interactions. Transcription factor NF- κB was described as the innate immune gatekeeper which is induced to trigger innate immune response via high ROS levels (Weis et al. 2008). This could be corresponding to changes in expression in innate immune response and complement component C3 in a few gene expression studies and the documented changes in nuclear factor kappa β. Another example is the importance of lectin as part of the recognition mechanisms at the onset of algae-coral symbiosis (Wood-Charlson et al. 2006) which resemble a common detection mechanism of microbes (Dunn et al. 2007). As presented in Table 32.3 changes in lectin were documented following heat and UV stress.

Another set of question marks can be presented in the form of absence of arrows with regard to certain environmental stressors. For example: (1) DNA damage was recognized following heat and UV stress, while ocean acidification stress cascade did not result in damage to the DNA or alternatively it has never been looked at. (2) ER stress and UPR processes have been identified in a UV and heat stress experiments, but ERAD process only as regard to heat stress. (3) Changes in metabolisms were never documented after ocean acidification stress. These issues represent examples for gaps in knowledge and what is yet to be investigated in the field of gene expression following environmental stress in corals.

32.6 Conclusions

In this chapter we reviewed differentially expressed genes following environmental stress in cnidarians, and constructed a conceptual model of the main processes occurring within the coral host cell under stress. Table 32.3 presents a detailed list of differentially expressed genes in branching/massive corals under eight types of environmental stress. This table can assist in guiding future research as it suggests which genes/processes one should look at in environmental stress studies, what is already known and what data is still missing. The cellular pathways summarized in Fig. 32.6 emphasize what we know thus far and what is still lacking in the cnidarians stress related literature. Although it seems that we know the guidelines of what is occurring in the cell during stress, the picture is still general and not detailed with high resolution. Most processes such as: DNA damage, cell reception, changes in metabolism and more, are thought to occur because a representative key gene or genes from this category were differentially expressed, without a full understanding of the details in the process. Wide scale research is required in order to fully understand the details of each process, such as: what are the main controlling agents, activators and regulators that drive it.

Here we provided a wide characterization of studies conducted in cnidarians showing responses to environmental stress. Our concerns are related to the fact that most of the studies investigated a single stress factor and mostly thermal stress, were short-term, i.e. less than 10 days and focused on branching corals. This highlights the lacking in long-term experiments studying chronic effects and in studies that investigate the combined effect of pH and temperatures which can lead to a synergistic effect and present more realistic scenarios. These scenarios are more realistic under the predicted long-term and chronic upcoming global change. Considering that mass coral bleaching events are expected to increase in frequency, it is all the more relevant study coral recovery in a gene expression manner. To date, there is a great lacking in coral gene expression profiles covering a recovery period, after stress is implemented.

Furthermore, there is a lacking of studies displaying a complete proteomic response to environmental stress in corals. The majority of proteomic studies focus on several proteins, mostly on heat shock proteins, antioxidant agents and apoptotic enzymes, while neglecting the broad view perspective of pathways occurring within the cell, as we presented in Fig. 32.6. Proteomic high-throughput approaches are not less notable, bearing in mind the imperfect correlation between mRNA quantities and their respective proteins in the cells. The relatively new tool of in silico technologies enables us to study the entire transcriptome in a high-throughput manner and hold the potential to answer these kinds of prominent questions regarding cnidarian cellular pathways following global change scenarios. A deeper understanding of the cellular mechanism of stress in corals will help to develop high-resolution biomarkers, which may help policy makers and scientists to anticipate the future in one of nature's largest ecosystems on earth.

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Thermal-Stress Response of Coral Communities to Climate Change

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Abstract

With the current rapid rate of climate change, coral communities are being repeatedly subjected to anomalously high thermal-stress events. Most global models predict that within the next 100 years, few reef corals will survive in tropical oceans. Yet thermal stresses have long been spatially and temporally variable across the oceans, and coral communities in different geographic regions are likely to be inherently different in their capacity to tolerate thermal stress. Using a spatially explicit Bayesian approach, we examined the response of coral communities to the hazards of climate-change associated thermal stress. We used the rates of change in sea-surface temperatures and the maximum sea-surface temperatures from 1980 to 2012 as predictive covariates of global records of coral bleaching over the same time period. There were negative relationships between the rates of change in sea-surface temperatures and coral bleaching, although the results were misleading because the highest rates of change in sea-surface temperatures were recorded at high latitudes, where average sea-surface temperatures are characteristically cooler than at low latitudes. Also, the results suggested that the most hazardous localities for corals, which experienced the highest maximum sea-surface temperatures, were in the northern hemisphere, particularly in the northern and western Indian Ocean, and along the rim of the eastern and western Pacific Ocean. Coral populations in these localities have suffered the greatest mortality. When a capacity to adapt to thermal stress was considered in the model, several localities responded positively, particularly corals in the Hawaiian Islands, the northern Marshall Islands, Micronesia, the Line Islands, the Cook Islands, the southern Great Barrier Reef, and along the coast of Brazil.

Keywords

Corals • Temperature • Stress • Climate • Communities • Populations • Adaptation

33.1 Introduction

33.1.1 A New Era: Coral Bleaching

Modern scleractinian corals have been building coral reefs since the Triassic, for some 250 million years (Veron 2000; Stanley 2003). Both their longevity and their capacity to build coral reefs through the ages are closely linked with their capacity to house symbiotic dinoflagellates (Pearse and Muscatine 1971). The symbiotic dinoflagellates, within the genus *Symbiodinium*, photosynthesize and transfer photosynthates to the host corals; in turn, the corals produce organic wastes that are utilized by the symbionts (Muscatine and Cernichiari 1969). Recently, however, the symbiotic relationship between corals and their symbionts has become intermittently dysfunctional. The disruption of this symbiosis is referred to as coral bleaching, which is the loss of pigmentation and symbiotic algae from the coral hosts.

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Coral bleaching was first described at least a half-century ago as a response to osmotic shock during heavy rainfall (Goreau 1964), and during cold-water events. Most recently however, coral bleaching has become associated with anomalously high seawater temperatures that result from climate-change-driven ocean warming (Glynn 1993; Brown 1997; Hoegh-Guldberg 1999; Hoegh-Guldberg et al. 2007; IPCC 2013). Although coral bleaching is a sign of symbiotic dysfunctionality, subtle paling also occurs seasonally (Fitt et al. 2000). Seasonal paling of corals is a consequence of high seasonal irradiance that leads to the over reduction of the light-reaction centers in the corals' symbionts (Warner et al. 1999). Such conditions can lead to dynamic photoinhibition, which is reversible; however, when water temperatures are anomalously high, and persist for an extended duration, dynamic photoinhibition can lead to chronic photoinhibition. Recovery from chronic photoinhibition is problematic (Iglesias-Prieto 1997), especially when the seasonal rates of change in temperature are rapid (Chollett et al. 2014). Under combined temperature and irradiance stress, coral symbionts attempt to down-regulate the reaction centers by: (i) reducing pigments, including peridinin, xanthophylls, chlorophyll c_2, and chlorophyll *a* (Brown 1997); (ii) reducing the density of the thylakoids within the chloroplasts (Brown 1997); and (iii) reducing the density of the symbionts (Titlyanov et al. 1996). These reductions decrease the area of the photosystem antennae, allowing the symbionts to avoid chronic photoinhibition, and avoid the resulting buildup of damaging oxygen radicals when the photosystem gets overloaded (Lesser 1997).

33.1.2 Different Tolerances to Temperature Stress

Coral species differ considerably in their tolerance to chronic photoinhibition, which results from the combined stress of high irradiance and high temperature (Warner et al. 1999; Takahashi et al. 2004). These differences in tolerance depend upon the symbiont types (LaJeunesse et al. 2010), and on the physiological and morphological differences of the host corals (Loya et al. 2001; Baird et al. 2009). Encrusting and massive morphologies are among the most thermally tolerant coral morphologies, whereas branching morphologies generally are the most thermally susceptible (Marshall and Baird 2000). But there are exceptions to these patterns, which can change through selective pressure over time (Thompson and van Woesik 2009; Guest et al. 2012). For example, Guest et al. (2012) reported a switch in thermal susceptibility of various coral morphologies on the reefs of Singapore. In 1998, the most susceptible corals were branching *Acropora* and *Pocillopora* colonies, whereas 12 years later, during a thermal-stress event in 2010, the most susceptible corals were massive *Porites*. Guest et al. (2012) suggested that the contrasting responses were a result of the 1998 thermal-stress event removing the susceptible *Acropora* and *Pocillopora* genotypes from the populations. By contrast, large, massive *Porites* colonies tend to bleach but survive thermal-stress events (Loya et al. 2001). Therefore the reefs of Singapore most likely retained the same *Porites* genotypes, over the 12-year time period, which bleached in 1998 and in 2010, whereas the reefs lost the most thermally susceptible *Acropora* and *Pocillopora* genotypes.

Susceptibility to thermal stress also varies in accordance with colony size (Loya et al. 2001), the habitat type in which the coral colonies are located (van Woesik et al. 2012a), water depth (Wagner et al. 2010), and water-flow rates (Nakamura and van Woesik 2001). In addition, thermal susceptibility is a consequence of daily and seasonal exposure to temperature conditions (McClanahan and Maina 2003). Certainly, the history of exposure to high irradiance and elevated temperature plays a central role in the response of corals to regional thermal-stress events (McClanahan and Maina 2003; Thompson and van Woesik 2009; Ateweberhan and McClanahan 2010; McClanahan et al. 2007; Brown et al. 2014). Coral colonies in localities that are subjected to regular thermal stress do better under regional temperature-stress events than corals in localities that have not been exposed to these high background temperatures (McClanahan and Maina 2003; Barshis et al. 2013). For instance, Barshis et al. (2013) showed that some corals in a lagoon in Samoa, which were constantly exposed to high water temperature, had naturally elevated expressions of genes that were an important component of the thermal-stress response, priming the corals' protein pool to handle frequent thermal stress.

Over the last three decades, most coral populations have suffered some bleaching, and many populations have suffered subsequent mortality (Baker et al. 2008). The El Niño-Southern Oscillations in 1982–1983, in 1997–1998, and in 2015–2016, produced particularly memorable thermal-stress events. From 1980 to 1998, thermal-stress anomalies were primarily evident during El Niño-Southern Oscillations (Glynn 1993). Yet more recently the increase in global ocean temperatures associated with climate change seem to have reached a threshold, and recent coral-bleaching events have become independent of El Niño-Southern Oscillations (Baker et al. 2008). An increase in the frequency and intensity of coral-bleaching and mortality events are changing the composition of many reef assemblages (Loya et al. 2001; Hughes et al. 2003; Hoegh-Guldberg et al. 2007; Pandolfi et al. 2011).

33.1.3 Objectives

Although projecting coral–population trajectories under homogenously warming oceans is laudable (Donner et al. 2005; Frieler et al. 2012), the oceans are not homogenous

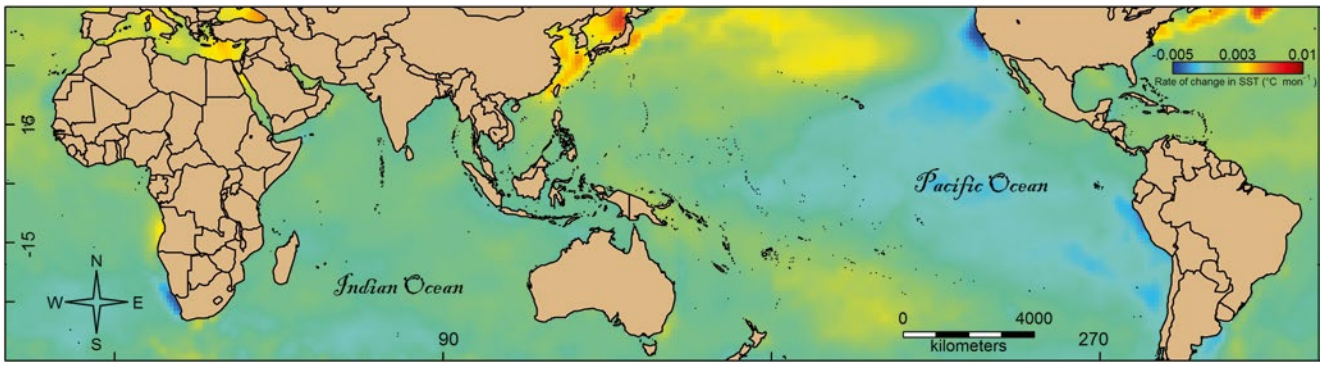

Fig. 33.1 Rate of change in sea surface temperature (°C mon^{-1}) from 1980 to 2012 using 1° by 1° spatial resolution HadISST data

(Thompson and van Woesik 2009), nor are they warming at the same rates (Fig. 33.1). Therefore, projecting the spatial response of coral populations to heterogeneous ocean warming is necessary, albeit challenging (van Hooidonk et al. 2013; Bivand et al. 2013). As a first approximation toward such a spatially-explicit framework, this study examined the spatial extent of thermal hazards across the globe using: (i) the rates of change in sea-surface temperatures (SST) from 1980 to 2012, and (ii) the maximum SST from 1980 to 2012, as predictors of coral bleaching from 1980 to 2012. Our objectives were to: (1) construct a global framework to quantify the response of coral communities to environmental stress, and (2) hindcast the global hazards of thermal stresses on reef-coral communities over the last three decades.

33.2 Methods

33.2.1 A Spatially Explicit Format

We were interested in: (1) globally representing the distribution of temperature hazards for corals, and (2) synthesizing the spatial relationship between coral bleaching and sea-surface temperature data. The coral communities were defined in 141 non-overlapping ecoregions that were identified by Veron et al. (2009). An additional ecoregion was added in the Atlantic Ocean to include Ascension Island. An ecoregion is a realistic geographic marine unit that is large enough to encompass oceanographic 'climates', but small enough to agree with genetic evidence of local adaptation and connectivity (Veron et al. 2009). With comprehensive data, the ecoregions can be divided into strata to include data from different depths, or from different habitats. For convenience we did not stratify the ecoregions in the present study. These ecoregions were prepared as digital shapefiles (and we thank J.E.N. Veron for making the shapefiles available to us), which are polygons outlining the ecoregion boundaries, and stored in vector format.

33.2.2 Variables

We used coral bleaching as a response variable to temperature. Categorical estimates of coral bleaching were compiled from the global ReefBase data using 6191 bleaching records (www.reefbase.org) from 1980 to 2012. There were four bleaching categories, defined as: (i) no bleaching, (ii) low-severity bleaching, (iii) moderate-severity bleaching, and (iv) high-severity bleaching. Temperature hazards were defined as: (i) the rates of change in monthly sea-surface temperatures (SST) over the last 33 years (1980–2012) in each ecoregion *i* using monthly HadISST data at a spatial resolution of 1° by 1° (Rayner et al. 2003) (http://www.metoffice.gov.uk/hadobs/hadisst/) (Fig. 33.1), and (ii) the maximum monthly sea-surface temperatures from 1980 to 2012 in each ecoregion (Fig. 33.2), also using HadISST. With more comprehensive data, the response of each coral species can be examined, however, for the present study, as a proof of concept, we examined the bleaching response of the coral communities to thermal stress.

33.2.3 The Bayesian Model

We employed a spatially explicit Bayesian model to examine, firstly, coral bleaching as a binomial process:

$$b_i \sim \text{binomial}(p_i, n_i) \tag{33.1}$$

where b_i is the extent of coral bleaching at ecoregion *i*, n_i is the maximum extent of bleaching at ecoregion *i*, and p_i is the coral (species, or higher taxa) response at ecoregion *i*. The coral response was initially assessed with a uniform prior distribution (0, 1). The response variable was then hierarchically linked to a hazard function, set over a predetermined time interval, h_i:

$$p_i = 1 - \exp^{(-h_i \cdot h_i)} \tag{33.2}$$

where *hi* is the hazard at site *i*. Three time intervals were examined for coral response to thermal hazards: (i) from

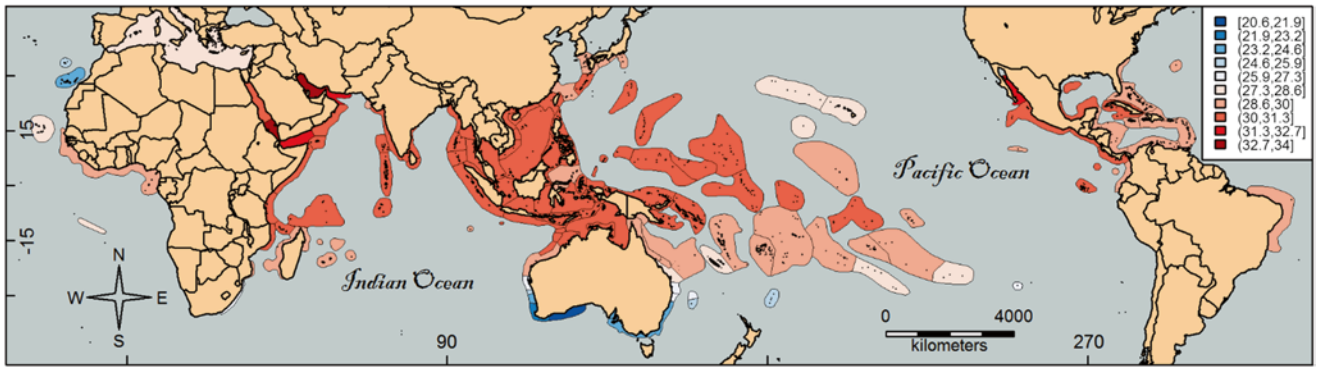

Fig. 33.2 Maximum sea surface temperatures (°C) per ecoregion from 1980 to 2012 using 1° by 1° spatial resolution HadISST data

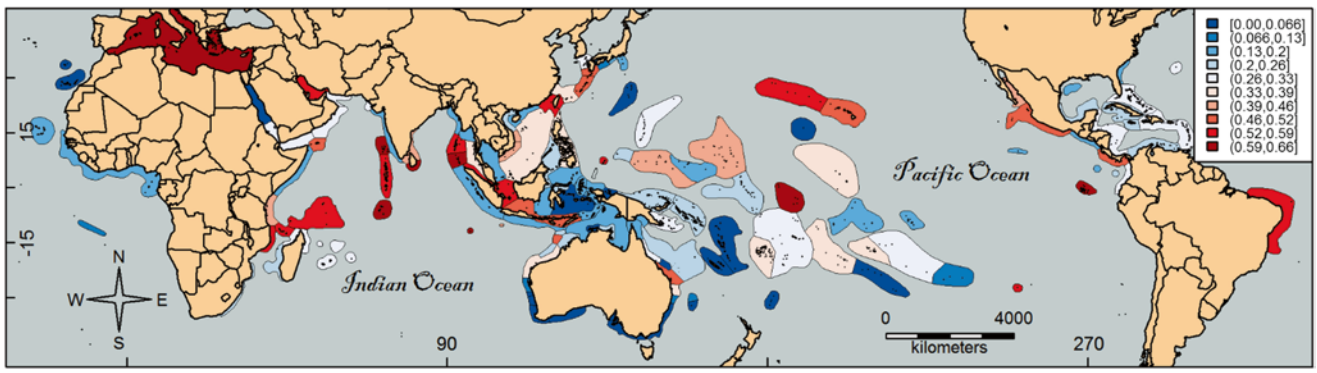

Fig. 33.3 Relative likelihood of coral stress (p_i in Eqs. 33.1 and 33.2) in response to thermal hazards (in this example maximum sea surface temperature) from 1980 to 2012, using Eqs. 33.1, 33.2, and 33.3

1980 to 1998, to include the global thermal-stress event associated with the El Niño-Southern Oscillations in 1998; (ii) from 1999 to 2012 to determine whether the bleaching responses were different after 1998, and (iii) from 1980 to 2012. A link function was implemented to connect the hazard function with a generalized linear model that included the standardized temperature covariate as:

$$\log(\mathrm{h}_i) = \beta_1 + \beta_2.(\mathrm{temp}_i - \mu\_\mathrm{temp}) / \sigma\_\mathrm{temp} + \mathrm{error}_i \quad (33.3)$$

where β_1 is the intercept of the linear response, β_2 is the slope of the relationship with the main covariate of interest, temp_i is the temperature at ecoregion i, μ_temp is the mean temperature across all ecoregions, σ_temp is the standard deviation of temperature across all ecoregions, and error_i is a stochastic environmental error term for each ecoregion i. The β_2 term was treated as a normal distribution with non-informative priors. Equation 33.3 can be expanded to include other covariates, such as turbidity and land-use change. We also considered adaptation as a reduction of p_i for thermal hazards, expressed as:

$$p_i = 1 - \exp^{(-hi.ht.adapti)} \quad (33.4)$$

where adaptation was treated as a fractional shift in the log-normal distribution of phenotypic traits (μ, σ) (Gingerich 2009). Research effort is necessary to determine the potential adaptive response of coral populations in each ecoregion, i, which will depend on different thermal histories, on local adaptations, and on life-history traits. The adaptive response also can be adjusted to dynamically and incrementally shift the expected population response, per successive generation, as a proportion of the standard deviation of a given phenotype of interest. We approximated the posterior probabilities of p and $\beta 2$ (in Eqs. 33.1 and 33.3), at each ecoregion i, using Markov Chain Monte Carlo simulations and Gibbs sampling in OpenBugs (Thomas et al. 2006; Lunn et al. 2012). All Bayesian models were implemented in OpenBugs through R

Table 33.1 Results of the Bayesian analysis, solving Eq. 33.3, where DIC is the Deviance Information Criterion, which is essentially a goodness of fit criterion that includes model complexity (where low numbers show a better model fit); CI are the 95 % credible intervals; mean $\beta 2$ is defined in Eq. 33.3 as the slope of the relationship with the main covariate of interest; and SST is the sea-surface temperature

	DIC	mean $\beta 2$	CI (2.5 %)	CI (97.5 %)
33-year SST slope	2623	–0.711	–1.030	–0.468
Phase 1 (1980–1998)	2168	–0.258	–0.410	–0.105
Phase 2 (1999–2012)	2012	–0.130	–0.342	0.067
33-year maximum SST	2205	0.787	0.014	0.926
Phase 1 (1980–1998)	2191	0.529	0.422	0.638
Phase 2 (1999–2012)	2077	0.654	0.496	0.815

(R Core team 2014) using the R package *R2OpenBugs* (Sturtz et al. 2005).

33.3 Results and Discussion

33.3.1 Regional Differences

The most thermally hazardous localities for reef corals were in the northern hemisphere, particularly in the northern and western Indian Ocean, along the rim of the eastern and western Pacific Ocean, and in the center of the Pacific Ocean at low latitudes (Fig. 33.3). These localities experienced the most extensive coral bleaching between 1980 and 2012. Some localities have been prone to repeated and intensive thermal stress, such as Kenya and the Maldives in the Indian Ocean, the Phoenix Islands in the central Pacific Ocean, the north-western Philippines, the Ryukyu Islands, Costa Rica, and Panama in the eastern Pacific Ocean, and Brazil in the Atlantic Ocean. Other localities have rarely experienced bleaching, such as Cenderawasih Bay and the Moluccas Islands in Indonesia at low latitudes, and the Austral Islands in the Pacific Ocean at high latitude (Fig. 33.3).

In the present study, bleaching data were somewhat biased toward well studied ecoregions including the Great Barrier Reef (n=1149), Belize (933), the Gulf of Thailand (n=98), Fiji (n=85), and East Africa (n=54). By contrast, there were some remote ecoregions that have been poorly studied, such as the Myanmar coast in the Indian Ocean, the Gulf of Tomini, Halmahera, and the Lesser Sunda Islands in Indonesia, and the Marquesas, the northern Cook Islands and the Pitcairn Islands in the central Pacific Ocean. The spatial models are fairly robust at coping with missing data, because the spatial models borrow strength from the likelihood contributions for all of the ecoregions (Best et al. 1996); however, to improve the predictions it would be useful to acquire more information on the response of corals to thermal-stress events from these remote regions.

33.3.2 Thermal Stress

There was an unexpected negative relationship between the rate of increase in SST and coral bleaching over the last 33 years (Table 33.1). These negative relationships are somewhat misleading, however, because the localities with the highest rates of change in SST were at high latitudes, where temperatures were generally cooler and therefore less likely to cause thermal stress (Fig. 33.1). There was a clear and expected strong, positive relationship between coral bleaching and the maximum SSTs within each ecoregion over the 33-year time period in which coral bleaching was recorded (Table 33.1).

In the present study thermal stress was considered to be the primary stress to corals. Yet there are other environmental conditions and interacting variables that also need to be included in future models. For instance, the present models do not consider differences in irradiance that are caused by cloud cover and high turbidity (Mumby et al. 2001; Wagner et al. 2010). Low irradiance during times of temperature stress reduces photoinhibition, and therefore reduces the potential for coral bleaching and subsequent coral mortality. For example, high-cloud cover in the Society Islands in 1998 reduced coral bleaching when the regional temperatures were anomalously high (Mumby et al. 2001). Notably, high volcanic islands are more likely to support consistent cloud cover through orogenic effects, which may potentially protect corals on the prevailing windward sides of the islands. It would be tempting to hypothesize that high islands will be more likely to support climate-change refugia through local, orographic cloud-formation effects, than low islands, but more research is needed in this area. Similarly, high turbidity reduces irradiance and therefore reduces the extent of coral bleaching (Wagner et al. 2010; van Woesik et al. 2012a). These local and regional conditions need to be incorporated into spatial models in the near future.

33.3.3 Adaptive Capacity

When a capacity to adapt to thermal stress was considered in the model, several localities responded positively, particularly the Hawaiian Islands, the northern Marshall Islands, Micronesia, the Line Islands, the Cook Islands, the southern Great Barrier Reef in the Pacific Ocean, and reefs along the coast of Brazil (Fig. 33.4). However, the reefs that suffered thermal stress and bleaching in the Indian Ocean (Fig. 33.3)

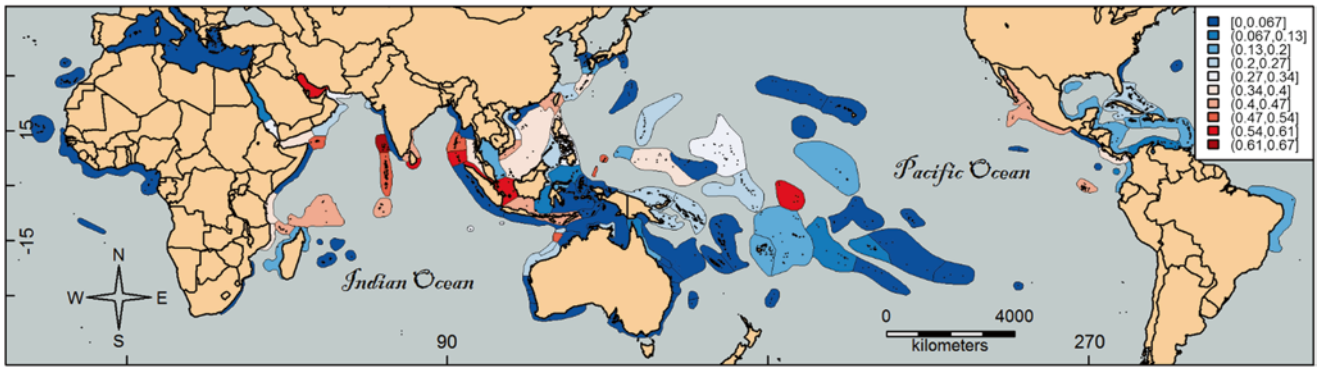

Fig. 33.4 Relative likelihood of coral stress in response to thermal hazards from 1980 to 2012, using Eqs. 33.1, 33.2, 33.3, and 33.4, which included a capacity to adapt to thermal stress; the *red* localities are highlighting regions where the thermal hazards most likely exceeded the corals' capacity to adapt to the thermal-stress events

still responded strongly to thermal stress even when an adaptive capacity was included in the model. The longitudinal differences between the potential adaptive capacities may be a consequence of the more extreme thermal stress and subsequent coral bleaching experienced in the Indian Ocean from 1980 to 2012, where six of the ten most extensively bleached ecoregions were located. Although coral populations in these localities have suffered the greatest mortality, the consistent selective pressure of thermal stress, through differential mortality of thermally-susceptible genotypes, suggests that coral populations in these localities might be also undergoing rapid evolutionary change.

Although more extensive research is necessary in this area, persistent thermal stress has the capacity to 'prime' the extant coral colonies to cope with temperature stress (Barshis et al. 2013). By contrast, extremely harsh thermal-stress events act as genetic filters (Thompson and van Woesik 2009; Guest et al. 2012), removing the susceptible genotypes from coral populations. Still, without persistent selective pressure, in the form of frequent thermal-stress events, the coral-allele frequencies will revert back to Hardy-Weinberg equilibrium, and any given thermal event will have no long-lasting effect on the thermal tolerance of the populations. The conundrum of adaptation to climate change is that repeated thermal-stress events are necessary to fix thermally resistant alleles in the coral populations, yet repeated thermal stresses may have other hidden consequences on the remaining coral populations. For example, repeated thermal-stress events may lead to shifts toward small coral colonies because: (i) large colonies tend to experience disproportionately high mortality compared with small colonies (Loya et al. 2001), and (ii) bleaching can lead to partial mortality, reducing colony size (Roth et al. 2010). If thermal-stress events result in repeated setbacks toward small, supposedly sexually-immature colonies, then adaptation is unlikely because these small colonies will not produce offspring. Furthermore, offspring that are produced during periods of elevated temperature often experience high rates of mortality (Bassim and Sammarco 2003; Randall and Szmant 2009a, b). It is not unreasonable, however, to suspect that the age or size of coral maturation may shift toward younger or smaller corals, under frequent climate-change associated stresses, as has been documented for coral-reef fishes under fishing pressure (Olsen et al. 2004). It is also possible that the timing of mass-spawning of coral gametes will shift, as the climate changes (van Woesik 2010; Nozawa 2012). Still, we know little about how maturation, gametogenesis, spawning, and other life-history processes will play out in a rapidly changing climate (van Woesik et al. 2012b). It is also unknown how those processes may influence the capacity of reef-coral populations to recover from thermal-stress events and persist.

33.4 Conclusions

This study was a first attempt at a spatially explicit synopsis of thermal stresses and bleaching globally, using 6191 data records collected since 1980. Yet, the approach we took examined the overall coral-community response to thermal stresses. We did not consider the subtleties and differential responses of the more than 800 coral species to thermal stress (Carpenter et al. 2008). We know that coral species differ in their responses to thermal stress (Loya et al. 2001; van Woesik et al. 2011), and the present spatially-explicit approach can be adopted for individual species. Not only do coral species differ in their thermal tolerances, but populations also differ locally in the extent of their genotypic and phenotypic variance (Darwin 1859; Gingerich 1983), and in the extent of local adaptation (Baums et al. 2006; van Oppen et al. 2011). Therefore genetic heritability, upon which selection acts, varies considerably across geographic regions. Weedy, fast-growing, thermally-sensitive species may do

best in frequently stressed environments, because they reach maturation quickly and their high turnover may facilitate adaptation. Yet, weedy species suffer from high genetic drift which prevents adaptive traits from infiltrating population-gene pools (Mosblech et al. 2011). By contrast, species that live in marginal environments, and are adapted to thermal stress, may have the advantage in a frequently-stressed future. For example, research on the response of plants to climate-change induced stress suggests that plants with conservative life-history traits will outperform plants that rapidly acquire nutrients (Ikeda et al. 2014). Therefore, two key questions remain: (i) which biological traits will have an adaptive advantage for corals in a warming ocean? and (ii) where will those traits be most advantageous to reef corals, considering the oceans are warming heterogeneously? It is critical that we continue to examine the detailed effects of a heterogeneously warming ocean on coral populations in a spatially explicit framework so that we can identify coral-reef refugia (Cacciapaglia and van Woesik 2015), and then focus our conservation efforts on those localities.

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Ecological and Evolutionary Considerations Regarding Corals in a Rapidly Changing Environment

34

Paul W. Sammarco and Kevin B. Strychar

Abstract

Coral reefs have been threatened for ≥35 years, primarily by global warming, disease, and unwanted inter-oceanic species introductions. Here we discuss differences between the evolution of corals and other organisms in the Atlantic vs. the Pacific Oceans through natural selection caused by oceanic cooling in the Atlantic and the resultant differential extinctions. We will also consider the implications of differential Pacific vs. Atlantic adaptations for invasive species and how it makes the former formidable predators and competitors. The effects of climate change and global warming on corals will also be considered, including the poleward movement of our current climatic zones at the expense of the polar and subpolar zones. We also predict the creation of a new "Hyper-Tropical Zone" in the center of the Equatorial Zone, characterized by mass mortalities of zooxanthellate organisms, causing both local endemic and global pandemic extinctions. The effects of global warming on the coral-zooxanthellar symbiotic relationship examines how zooxanthellae may be the "weak" link in the system, explaining why they are having difficulty keeping up with the pace of global warming. We also consider the possibility of a replacement symbiont arising. In this context, we examine the evolution of coral immunity as it affects the host, symbiont replacement, and disease. Such studies will help us to better understand the evolution of innate and adaptive immune systems, and ultimately better understand vertebrate model systems for human health studies.

Keywords

"Hyper-Tropical Zone" • Adaptations • Adaptive immune system • Atlantic Ocean • Climate change • Climatic zones • Competitors • Coral immunity • Coral reefs • Coral-zooxanthellar symbiotic relationship • Disease • Equatorial zone • Evolution • Extinctions • Global warming • Innate immune system • Invasive species • Mass mortalities • Natural selection • Oceanic cooling • Pacific Ocean • Polar and sub-polar zones • Predators • Species introductions • Symbiont replacement • Zooxanthellae • Zooxanthellate organisms

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Abbreviations

CLRs	C-type lectin receptors
DAMPs	Danger/damage-associated molecular patterns
DNA	Deoxyribonucleic acid
DSMs	DNA sensing molecules
HMGB1	High-mobility group box 1 proteins
IG	Immunoglobulin

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LRR	Leucine rich repeat
MBL	Mannose binding lectins
Mhc	Major histocompability complex
NLRs	NOD-like receptors
PAMPs	Pathogen associated molecular patterns
PRR	Pattern-recognition receptors
RLRs	RIG-I-like receptors
TCR	T-cell receptors
TIRs	Toll/IL-1 receptors
TLR	Toll-like receptors

34.1 Introduction

Coral reefs occur throughout the world's tropical and sub-tropical oceans. At least partially because of their natural beauty, let alone their social and economic importance for the people associated with them, they have captured the imagination of both scientists and non-scientists alike for centuries. Their beauty is unsurpassed in the marine environment. In terms of scientific research, they continue to draw the attention of numerous marine scientists, perhaps now more than ever. This is because of rapid changes in the Earth's environment which are currently occurring and which threaten to drive this group of organisms, and those parts of the ecosystem which are dependent upon them, extinct – either locally or globally. These recent threats have been happening, in the context of geological time, in the blink of an eye – over the past 35–150 years.

All organisms living on Earth today represent a snapshot of life-forms which have survived a very long walk along an evolutionary pathway. This pathway is the result of a long history of adaptive change – hundreds of millions if not billions of years. Along that path, there have been many experimental failures which could not keep up with environmental changes occurring on the earth (Jackson 2010). Indeed, most species which once existed on earth are now extinct, and what we are left with is a few extraordinary successes, of which we humans are a part. Scleractinian corals are one of these groups.

Scleractinian corals, octocorals, and their close taxonomic relatives, are now surviving their current environments due to adaptation and evolution, with the weaker or less adapted individuals or colonies of a population within a species being weeded out through natural selection. This occurs as conditions change to move outside their range of tolerance – be they physical, chemical, or biological boundaries (Futuyma 1998). Some species became isolated in time and/or space, exposed to different environmental conditions – some of which became selective factors. And some populations over time became adapted to their new environment through natural selection as that environment slowly changed through time, eventually exhibiting new characters – either physiological, morphological, or behavioral – to deal with that new environment.

In this paper, we shall review and discuss several different aspects of the ecology and evolution of corals. We shall cover the following areas:

- Evolution of corals in the Atlantic vs. the Pacific Oceans, including vicariance, oceanic cooling, and differential extinctions.
- Implications of these different adaptations for invasive species
- Climate change and global warming on the Earth, effects on corals, and impacts on global oceanic zones
- Effects of global warming on the coral-zooxanthellar symbiotic relationship, the weak link in the system, why zooxanthellae are having difficulty keeping up with the pace of global warming, and the probability of a replacement symbiont arising for corals.
- The evolution of immunity in corals and how their immune systems may actually relate to our own and those of other higher organisms.

34.2 Comments on the Evolution of Corals in the Atlantic Versus the Pacific Oceans

During the early development of the Earth, its oceans were continuous and pan-equatorial. We have called this ocean the Tethys Sea (Veron 1995; Thurman 1978). It has been determined through fossil evidence that scleractinian corals were distributed in a pan-Tethyan manner prior to the end of the Oligocene/beginning of the Miocene (Fig. 34.1); i.e., the fossil remains of the same species of coral have been found in terrestrial finds in the tropics and sub-tropics around the world (Stanley 1979; Veron 1986, 1995; Rosen 1988). During this period, however, sea level fell by ~50–75 m (Kominz 2001). Through this negative change in sea level, the Isthmus of Panama emerged and severed the Tethys Sea into two separate oceans – the Atlantic and the Pacific (Stanley 1979, 1984; Veron 1986; Rosen 1988). These two new oceans became isolated from each other at all but the highest latitudes – the Southern Ocean and the Arctic Ocean, both of which were too cold to permit the successful exchange of tropical and sub-tropical marine fauna and flora – adults or larvae. This sea level drop was accompanied by oceanic cooling in the Atlantic, particularly in the Caribbean, but the cooling was less severe in the Pacific. The cooling in the Atlantic caused mass extinctions of marine fauna in this part of the newly formed Atlantic, particularly in bivalves and corals (Fig. 34.2; Wells 1956). Some of the coral community in South America survived, and this region

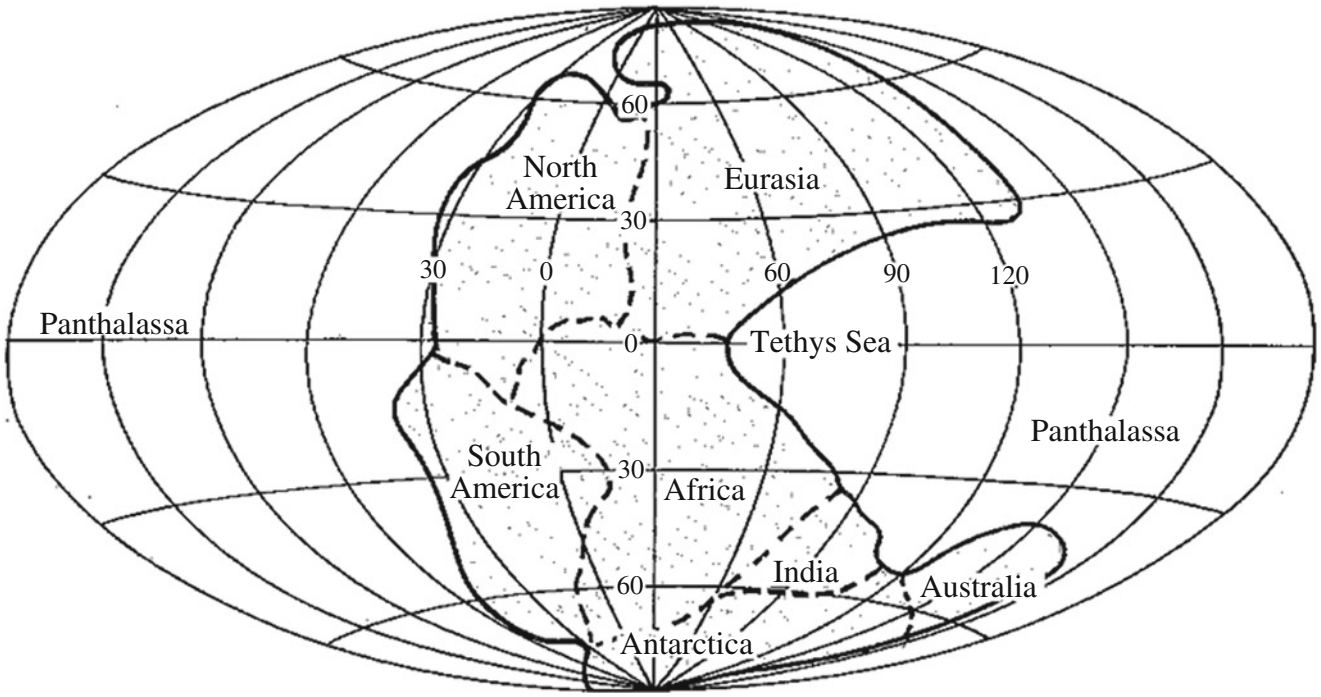

Fig. 34.1 A diagram of the earth and the predecessors of its continents – joined as Pangaea – during the pre-Mesozoic, 200 M years BP. The larger ocean we refer to as Panthalassa. The Tethys Sea contained the ancestors of our present day corals (Thurman 1978, Fig. 5.5, C.E. Merrill Publ., Bell and Howell Co., Pearson Higher Education, used with permission)

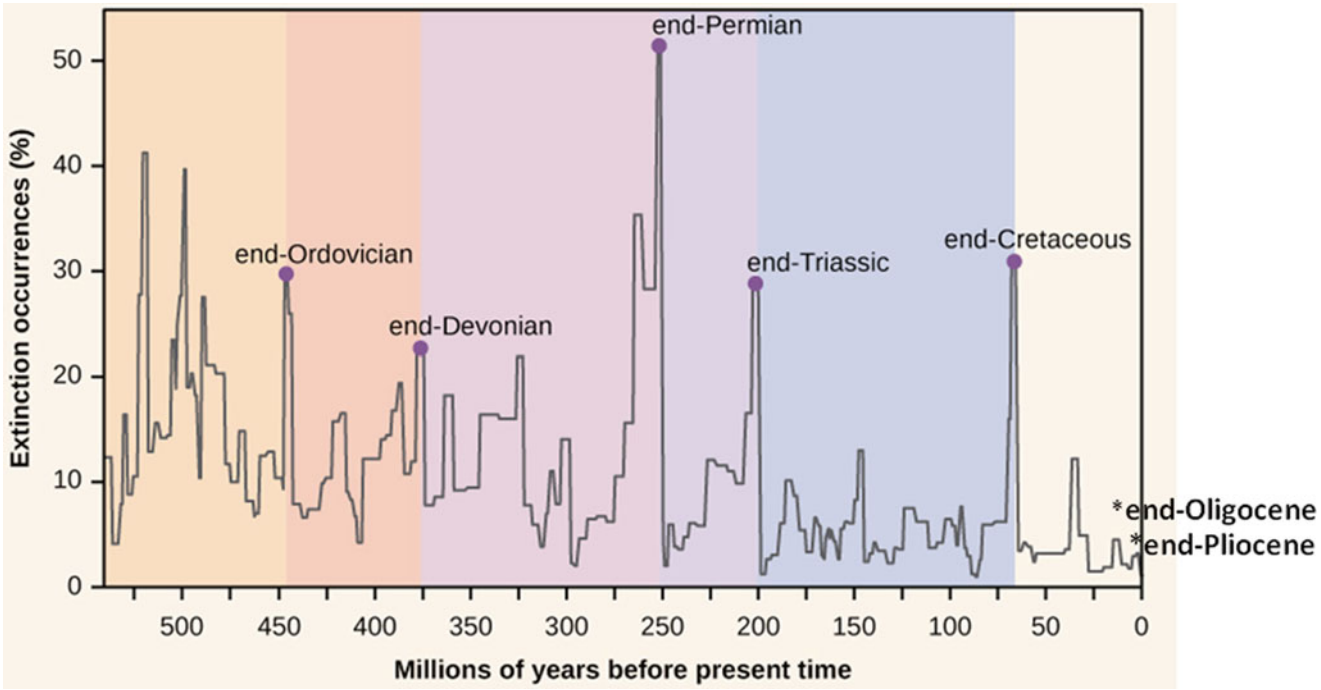

Fig. 34.2 A graph of the rate of extinction occurrences which have occurred through geological time from ~550 M YBP to the present. Note the smaller extinction events at the end of the Oligocene and Pliocene within the past 20 M years, which caused major extinctions in the Caribbean and tropical western Atlantic. Derived from https://www.boundless.com/biology/textbooks/boundless-biology-textbook/introduction-to-animal-diversity-27/the-evolutionary-history-of-the--animal-kingdom-165/post-cambrian-evolution-and-mass--extinctions-640-11862/ Post-Cambrian Evolution and Mass Extinctions. Boundless. "Post-Cambrian Evolution and Mass Extinctions." *Boundless Biology*. Boundless, 03 Jul. 2014. Retrieved 09 Feb. 2015 from https://www.boundless.com/biology/textbooks/boundless-biology-textbook/introduction-to-animal-diversity-27/the-evolutionary-history-of-the-animal-kingdom-165/post-cambrian-evolution-and-mass-extinctions-640-11862/

appears to have served as a refuge for coral species during this period. Later, when the Earth entered a warming phase again, the remaining coral species, depauperate in species richness, were able to recolonize the Caribbean from there (J.E.N Veron, pers. commun.). The region then remained relatively stable for a while, as the Earth continued to warm.

Later, the Earth went through another major cooling period with the onset of a major glaciation at the end of the Pliocene/beginning of the Pleistocene (Stanley 1979, 1981, 1984, 1985, 1986). At this time, there were mass extinctions of bivalves and corals in the Atlantic again (Fig. 34.2; Dana 1975; Frost 1977), but, once again, parallel extinctions did not occur to this extent in the Pacific. During this period of climatic cooling, glaciers in the northern hemisphere in the Atlantic longitudes moved southward from the North Pole towards the equator. They did not, however, proceed as far south in the Pacific as in the Atlantic (Fig. 34.3). Kuhlmann (1985) believes that the geomorphology of their respective mountain ranges were different. In the Pacific, the Himalayas and their associated mountain chains are generally oriented east-west. This served as an obstacle to the southerly movement of the glaciers over Asia. On the other hand, the Appalachian mountain range is generally oriented north-south, allowing the glaciers to move more easily southward towards the Caribbean and the Gulf of Mexico, stopping at latitudes of ~37°N in North America.

These two extinction events in the Atlantic decimated the coral populations twice over a period of ~23 million years. This caused two sequential phylogenetic bottlenecks to occur in this region which the Pacific did not experience. During this period when the phylogenetic radiation of corals was arrested and set-back repeatedly, leaving a species-depauperate ecosystem (Fig. 34.4a), the Pacific fauna was permitted to continue its phylogenetic radiation without these same genetic bottlenecks or restrictions (Fig. 34.4b). Thus, the species diversity of the Pacific corals increased, genera and species diverged morphologically and with respect to their adaptations to their environment, niches grew smaller due to species packing, competitor interactions became more intense as resources became more limited, predator-prey interactions became more intense and more finely co-evolved, as did anti-competitor and anti-predator adaptations.

The coral populations in these two regions have been isolated for ~23MY and this was primarily a vicariance or isolation effect (Veron 1995). This combined with the mass extinctions most likely contributed to the divergence of their species and differences in their respective species richness. The added perturbation of extinctions in the Atlantic exacerbated this situation, amplifying the isolation effect. Figure 34.4b illustrates how the phylogenetic tree was allowed to "grow" and expand in the Pacific, where it was severely "trimmed" twice in the Atlantic, with a concomitant marked loss of species during each cooling event (Fig. 34.4a). This left the Atlantic not only with an impoverished coral species diversity, but with, on the average, a comparatively "old" fauna in comparison to the more mature, recently evolved fauna of the Pacific. A good example of this is the *Acropora.*

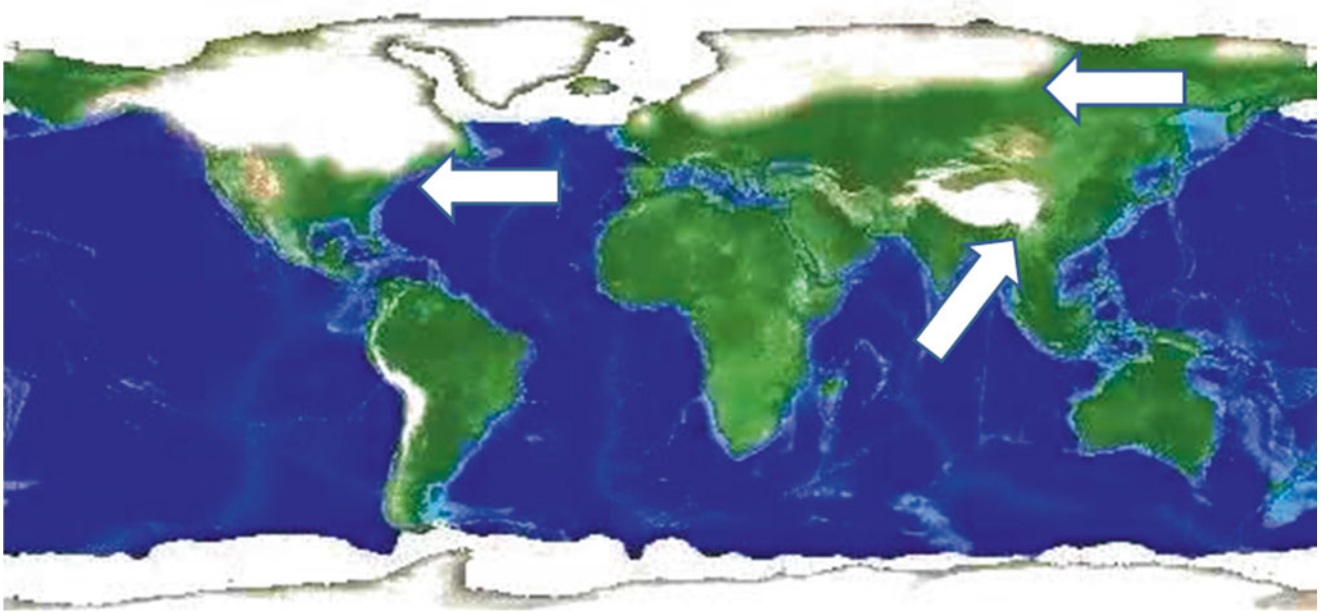

Fig. 34.3 A diagram depicting extension of the glaciers from the north pole to the south, and the south pole to the north. *Arrows* indicate the lower latitudes to which the northern glaciers extended towards the equator in North America vs. in Eurasia (Adapted from https://water.usgs.gov/edu/earthglacier.html Original diagram from the US Geological Survey, Washington, DC)

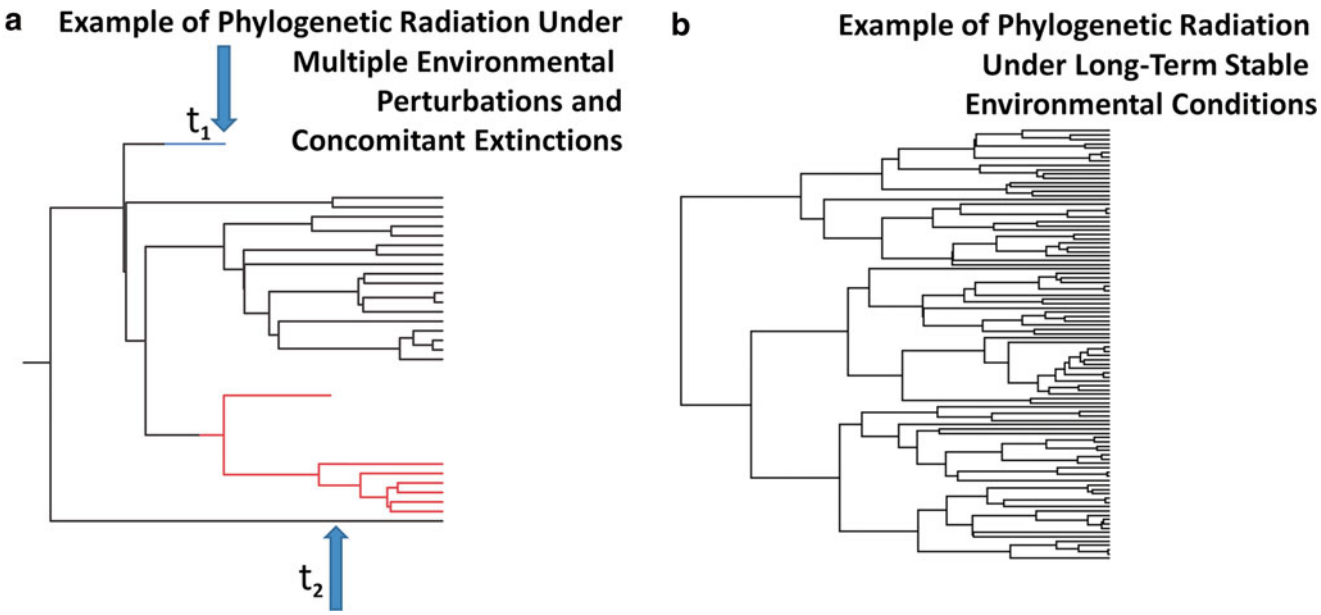

Fig. 34.4 (**a**) A dendrogram demonstrating the consequences to phylogenetic radiation and resultant species diversity in a region where several successive extinction events have occurred through time. Note the resultant low species diversity. (**b**) A dendrogram demonstrating the consequences when phylogenetic radiation has been permitted to expand, left undisturbed by extinction events over a similar period of time

There are ~70 species of *Acropora* in the Indo-Pacific; there are only three in the Caribbean.

With a high species diversity comes narrower and more specialized niches. In the Indo-Pacific, with an estimated total of 80 genera and 700 species of corals, it may be assumed that there has been considerable niche specialization, probably depleting most open niches which had existed. By comparison, the Caribbean is known to have ≥60 spp (Wells 1957, 1973; Goreau and Wells 1967). Assuming that this is the case, it would also be logical to presume that the niches for coral species in the western Atlantic/Caribbean are broader than those in the western Indo-Pacific and that some niches may still be open.

34.2.1 Inter-Oceanic Invasions of Coral Species and Comparative Adaptations

One way to assess this is to examine the behavior of a species when it is introduced from one of these environments to the other. A good example of this is the case of *Fungia scutaria* which was brought from somewhere in the western Pacific to the Discovery Bay Marine Laboratory in Jamaica, W.I. (Fig. 34.5). The coral was introduced into the seawater tables of the laboratory and kept there for several years. It was believed that they were isolated there. In reality, the tables are flow-through seawater tables, with seawater being pumped into them and inadvertently allowed to be discharged directly into the lagoon of the bay. Although the adult corals were isolated, this species is a brooder and releases planulae monthly for several months per year. The larvae were most likely flushed into the lagoon and successfully colonized it.

Fungia scutaria is a member of the Fungiidae. There are no naturally occurring corals from this family in the Caribbean. Fungiids have an unusual life-history and also utilize an unusual habitat. They are not sessile for their entire lifespans; they are only attached to the bottom for a short time after they settle as planulae. After secreting a small skeleton, they dissociate from the hard bottom and become vagile, living predominantly on soft sand-bottom but also on hard-bottom. They move across the bottom freely on soft or hard bottom and can right themselves if turned over. There is no equivalent coral in the Caribbean which lives on soft bottom and is vagile. This was most likely an open niche, which *F. scutaria* is now filling in this region.

Along with high species diversity in the western Pacific come more strongly adapted and narrowly focused characters, specialized for survival in individuals living in this long-term stable environment. This includes adaptations for predation and anti-predator defenses. For example, in the Indo-Pacific, corals feed on zooplankton, phytoplankton, detritus through mucus production, bacteria, mesoplankton, and sometimes larger prey such as the scyphozoan *Aurelia aurita* (Alamaru et al. 2009). In the Caribbean, corals primarily feed on zooplankton unless strongly autotrophic. Perhaps the best documented example regarding the adaptive escalation of predator-prey interactions comes from the work of Vermeij (1976, 1977, 1978). He examined comparative shell thicknesses of gastropods from numerous sites throughout the Indo-Pacific and the Caribbean. By quantifying the

Fig. 34.5 *Fungia scutaria*. An Indo-Pacific scleractinian coral, member of the Fungiidae. A solitary, vagile coral which is a brooder with respective to reproduction. This species was accidentally introduced into Jamaica via its larval production in the late 1960s/early 1970s, and has become established there (Photograph courtesy of Dieter Kloessing; used with permission; http://www.dieter-kloessing.de/Malediven-Reisen/112-Pilzkorallen.html)

Fig. 34.6 Gastropod shells colonized by hermit crabs. Note the shell on the right (see *arrow*) which exhibits evidence of having been attacked by a crab which attempted to chip away at the aperture of the shell in order to access its gastropod prey. The frequency and severity of these types of attacks vary significantly between the Indo-Pacific and the Atlantic, indicating higher predation pressure in the Indo-Pacific (see text) (Photo by Anne Fawcett, Small Animal Talk, Australia; used with permission; http://www.smallanimaltalk.com/2013/12/three-things-i-learned-about-hermit.html)

frequency of evidence of attack on the shells and the degree of damage done to them by the predators (scrapes on shells and shell breakage; Fig. 34.6), he demonstrated that intensity of predation as measured by these parameters was significantly higher in his Indo-Pacific sites than in the Caribbean ones. He also compared the thickness of the Indo-Pacific shells with those of the Caribbean and found that the Indo-Pacific ones were thicker and stronger. This indicated that the defenses of the gastropods were stronger there, most likely in response to heavier predation pressure than in the Caribbean. This was consistent with the findings of Palmer (1979).

Another example of a well-adapted predator which has been recently introduced from the Indo-Pacific from the Atlantic is the lionfish *Pterois volitans* (Whitfield et al. 2002; Hamner et al. 2007). A number of specimens were introduced apparently purposely into the waters of southeast Florida by an aquarist. It is a demersal fish and has since promulgated along the coasts of the western Atlantic Ocean. It possesses long, sharp, toxin-filled spines which serve as an excellent predator defense (Fig. 34.7). There are very few predators which have been able to successfully prey upon these fish in the Caribbean. Thus far, it appears that only the grouper (Maljkovic et al. 2008) can, and these predators were most likely already adapted or exapted to deal with these new species as a prey item. In addition, *P. volitans* uses a predatory technique similar to that of the Indo-Pacific gracile lizard-fish *Saurida gracilis*. Both species are sit-and-wait predators. In the case of the lionfish, it swims or drifts slowly in a non-threatening manner, until ready to attack. In the case of the lizard-fish, it sits on the bottom quietly without movement, camouflaged by the coloration of its body, awaiting its prey. Both species have large mouths capable of consuming large prey in a single movement, and both species attack their prey by darting rapidly with no warning, sucking their prey into their mouths, and consuming their prey whole. The introduction of *Pterois volitans* was followed by its range expansion from Florida north to Massachussetts, south to Venezuela, and west to Central America. In addition, it has expanded its vertical range to ≥150 m depth and has had negative impacts on the demersal, territorial pomacentrid fish populations of these regions. Small demersal fish like the Gulf of Mexico and Caribbean pomacentrids have probably never encountered such predators before and have readily succumbed to *Pterois* as a group.

34.2.2 Comparative Abilities in Competition for Space

The same principle of evolution which applies to predation also applies to competition. In the case of corals, one critical component for survival is success in competition for space, because these are sessile epibenthic organisms and, except in the case of the fungiid scleractinian corals (Fig. 34.5; Chadwick 1988; Elahi 2008) and some alcyonacean octocorals (LaBarre and Coll 1982), cannot move to secure additional space. It has been known for quite some time that corals can protect themselves against aggressive competitors plus expand their space against their sessile epibenthic neighbors by utilizing extracoelenteric digestion (Lang and Chornesky 1990). That is, upon encountering another sessile organism which it immunologically does not recognize as "self" (Bigger and Hildemann 1982), the coral extrudes its mesenterial filaments from its mouth and extends them

Fig. 34.7 *Pterois volitans*, the lionfish (North Solitary Island, New South Wales, Australia). This Indo-Pacific predatory fish was introduced into the western Atlantic Ocean supposedly purposely during the early 2000s and may now be found in abundance from Rhode Island, USA to Venezuela, South America. It is well-defended against most predators and voraciously and effectively feeds on other small fish in its new environment, that are not adapted to avoid its predation (Photo by Ian V. Shaw; provided by ReefLifeSurvey of Australia, CC by Attribution; http://www.fishesofaustralia.net.au/Home/species/2113)

towards the competitor and digests its neighbor. The neighbor may do the same. A "win" in this contact situation may go either way.

One way to determine the efficacy of competition in Indo-Pacific vs. Caribbean corals is to examine interactions between species from one region in contact with the other, either through laboratory experimentation or by making observations in the field. Invasive species provide an excellent opportunity to do so. Data exist on competition for space on two Indo-Pacific coral species which have successfully invaded the tropical/sub-tropical western Atlantic Ocean. These are two congeneric, azooxanthellate, ahermatypic, scleractinian corals – *Tubastraea coccinea* and *T. micranthus* (Hennessey and Sammarco 2014). The first was introduced from the Indo-Pacific to the Caribbean in the 1940s, most likely being carried on the hull of a ship which traversed the Panama Canal (Cairns 2000). This species has been quite successful in enhancing its distribution, having spread from Puerto Rico (Humann and deLoach 1996) to as far south as Brazil (de Paula and Creed 2004), as far west as Panama and other Central American countries, throughout the Greater and Lesser Antilles, and north to the Gulf of Mexico and Florida Keys (Fenner and Banks 2004; Shearer 2008). It has been very successful in increasing both its abundance as well. Its populations on a single oil production platform in the Gulf of Mexico can reach into the hundreds of thousands, and there are thousands of such platforms in that region (Sammarco et al. 2004, 2012). It has the capability to not just dominate the substrate but to monopolize it. *Tubastraea micranthus*, on the other hand, was only discovered around 2007 (Sammarco et al. 2010). It has now been documented to spread to 9 out of 14 platforms surveyed (Sammarco et al. 2014), and its populations are spreading, both horizontally and with respect to depth (Sammarco et al. 2013). Competition experiments have been run on both of these species against several Caribbean species – *Ricordia florida* (Cnidaria, Corallimorpharia), *Epicystis crucifer* (Cnidaria, Actinaria) and *Condylactis gigantea* (Cnidaria, Actinaria) (Hennessey and Sammarco 2014). These experiments demonstrated that both of these Indo-Pacific coral species were aggressive and formidable competitors for space. In most cases, the *Tubastraea coccinea* and *T. micranthus* exhibited extrusion of mesenterial filaments and killed or caused extensive tissue necrosis in their competitors via extracoelenteric digestion within several days (Fig. 34.8).

If *Tubastraea coccinea* is so successful at competition for space against its Caribbean neighbors and colonizing the hard substratum of the coasts of numerous countries in the western Atlantic, why has it not dominated the natural coral reefs there? *T. coccinea* is extraordinarily successful at colonizing and dominating the jacket pilings of offshore oil production platforms in the Gulf of Mexico, and has distributed itself over thousands of kms there. It is likely that the reason for this is that this species reverts to its native habit when in a natural setting; i.e., it becomes cryptic, occurring only under ledges and in caves (PWS pers. obs.; E. Hickerson pers. comm.). This species is adapted best to colonize and thrive on artificial substrata. *T. micranthus*, although it also grows well on artificial substrata and on the upper surfaces of coral reefs – is exposed. It is not yet known how well it will thrive on natural reefs once it colonizes them. This could be a major concern regarding its introduction.

Codium fragile (Suringar; Hariot 1889) is another example of an Indo-Pacific species which was successfully introduced into the Atlantic Ocean, most likely using the vector of the hull of a ship. Originally from Japan, it was introduced into to the western Atlantic and spread rapidly there (Chapman 1999). Other introductions from the Indo-Pacific to the Atlantic include the scyphozoan *Phyllorhiza punctata* which has had negative effects on the ichthyoplankton and invertebrate larval densities in the Gulf of Mexico (Graham et al. 2003; Graham and Bayha 2008) and the didemnid ascidian *Didemnum perlucidum* which has proven itself to be a formidable competitor for space on aritificial hard-bottom in the Gulf of Mexico (Culbertson and Harper 2002; Lambert 2002; Sammarco 2007).

34.2.3 Control and Eradication of Inter-Oceanic Invasive Species

The problem here is that species introduced to the Atlantic from the Pacific may well have themselves few to no local predators that can keep them under control or eliminate

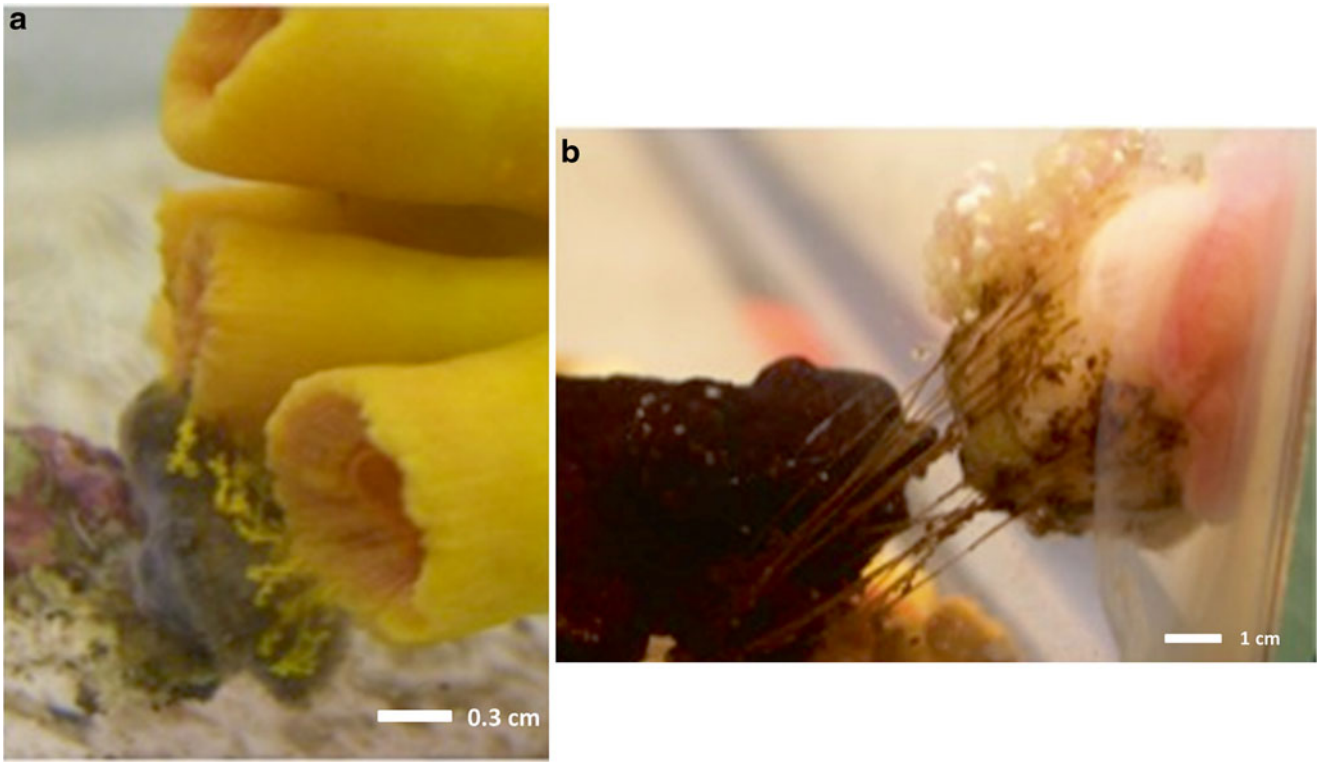

Fig. 34.8 (**a**) Competitive interaction between the Indo-Pacific invasive scleractinian coral – *Tubastraea coccinea* – and the Caribbean corallimorpharian *Ricordea florida*. Note the extrusion of mesenterial filaments and concomitant extracoelenteric digestion by the former species. (**b**) Similar competitive interaction between the Indo-Pacific invasive scleractinian coral – *T. micranthus* – and the Caribbean *Epicystis crucifer*, exhibiting similar responses (Reprinted from Hennessey and Sammarco 2014; reproduced with permission from the Journal of Experimental Marine Biology and Ecology, Elsevier Publishers, The Netherlands)

them. Some species may be adapted or exapted to prey on the newly introduced species, but the local predator populations may not be large enough to keep the new species under control. This is the case with the groupers which have been preying upon the lionfish (Maljkovic et al. 2008). This concept probably applies to new sessile epibenthic competitors for space as well; the Atlantic species generally do not possess the adaptations to specifically deal with the newly introduced Indo-Pacific competitors.

By comparison, one must ask, how many successful introductions have there been of Atlantic species into the western Indo-Pacific? Marine game fish have a history of being purposely introduced from one region to another. Bass have been moved around within the waters within the US (e.g., Philipp and Whitt 1991). With respect to the eastern Pacific, however, one successful introduction has been the striped bass (*Morone saxatilis*), introduced from its original western Atlantic habitat (Fig. 34.9a) to the San Francisco Bay, California region (and many other regions), where the introduction took hold (Fig. 34.9b; Nichols et al. 1986; Feyrer et al. 2007). This success of this introduction is not, however surprising; this introduction was made into the eastern Pacific, which has a much lower species diversity than the western Pacific. Regarding introductions from the Atlantic to the western Indo-Pacific, it would appear that there are few successful ones (Coles et al. 1999). One must presume, as can be surmised from the lack of studies published on such, that these introductions – whether accidental or purposeful – simply did not take. Some western Pacific fish species have been introduced into Oahu, Hawaii (Mooney and Drake 1986; Englund et al. 2000). The scientific literature is filled with reports of successful introductions but not failed ones.

With the knowledge of the difference between the respective evolutionary histories of marine species in the western Indo-Pacific Ocean vs. the Atlantic, what are the implications for the future? International maritime commerce has expanded greatly over the past century (Fig. 34.10; Hummels 2007). Despite the fact that we are now building and utilizing ships larger than have been used in the past and which have a much greater capacity for carrying cargo (Fig. 34.11), the number of these vessels making the trans-oceanic western Pacific to all points in the western Atlantic and elsewhere is still increasing (Fig. 34.12; Wonham and Carlton 2005). If an invasive species is introduced multiple times, control or eradication, even if successful initially, will be nearly impossible (Sammarco et al. 2013). In that case, there will always be a new source of larvae to colonize the target region. The only effective means by which to control or eradicate the

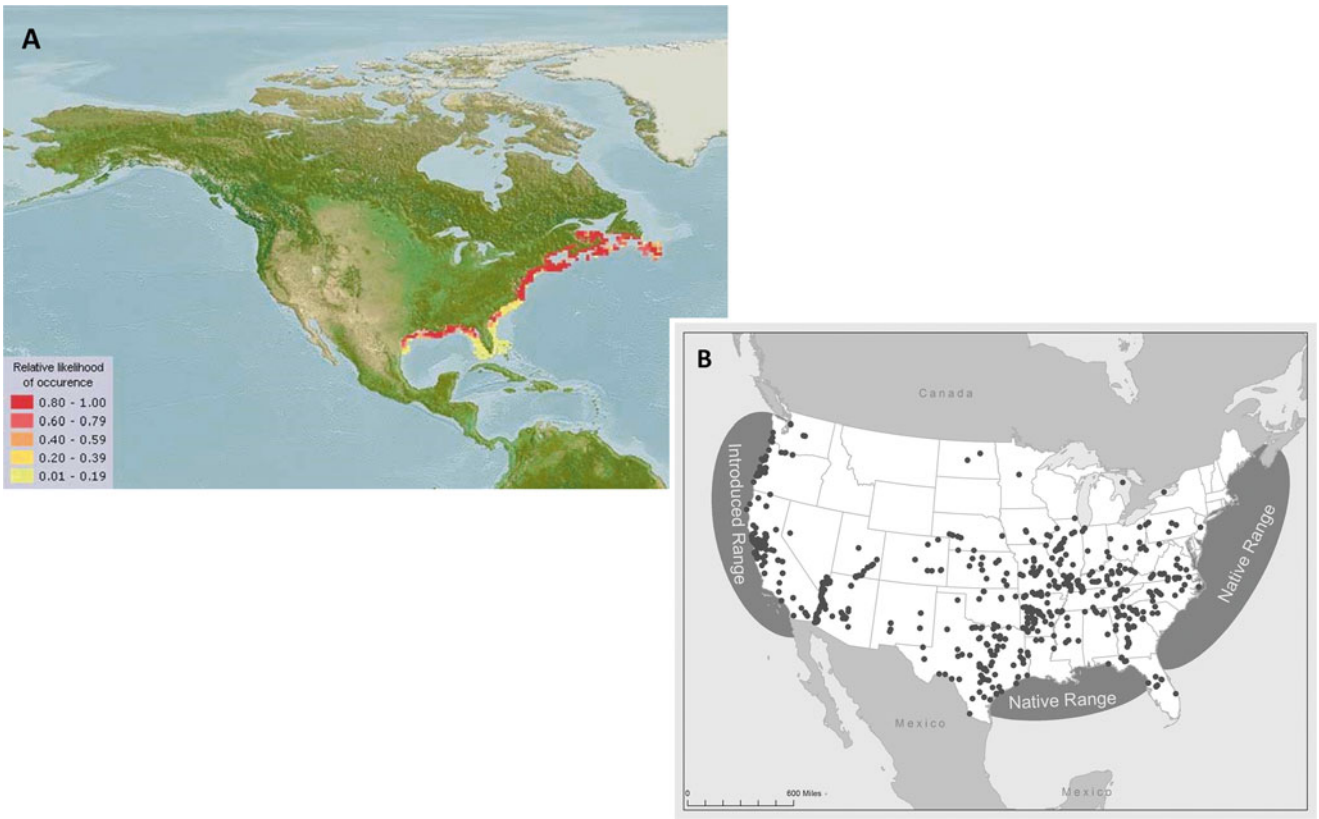

Fig. 34.9 (**a**) Early natural distribution of *Morone saxatilis* (striped bass) in the US. This species is native to Atlantic and Gulf of Mexico coastal areas and major coastal rivers that flow to those areas (http://ww2.odu.edu/sci/cqfe/striped_bass.html; reproduced with permission from the Center for Quantitative Fisheries Ecology, Old Dominion University, Norfolk, Virginia, USA; also see http://www.aquamaps.org/main/home.php). (**b**) Map showing the current distribution of native and introduced *Morone saxatilis*. It has been introduced into many inland waters and is also found on the Pacific coast and is now distributed in inland waters, as well as on the Atlantic and Gulf coasts, and on the Pacific coast (Map prepared by Matt Cannister and Pam Fuller (USGS, Gainesville, FL) and provided courtesy of the Nonindigenous Aquatic Species Program, US Geological Survey, Gainesville, FL)

potential invasive is to do so by controlling the invasive at the vector level – the vessel level – not the colonization level. That is, the hulls and ballast water of incoming ships must be monitored and treated for potential invasives, not the environment which they are invading (Carlton 1996; Miller 2007). In addition, it would appear that greater vigilance and stronger action is required on commercial vessels traveling from the western Pacific to the Atlantic than vice versa, as the probability of a western Indo-Pacific species successfully colonizing the eastern Pacific would be higher (Osman and Shirley 2007).

34.3 Climate Change, Changes in the Oceanic Climatic Zones, and Their Effects

That climate change is occurring has become an inescapable fact. Associated with that is the phenomenon of progressive global warming. NOAA data clearly demonstrate that both our atmosphere and our oceans have been warming at an increasing rate for the past 150 years. It has also been determined that under these conditions of an increasingly warming atmosphere, our oceans have absorbed an estimated extra 25×10^{22} J of heat since 1957 (Church et al. 2011, pers. comm.). Even if we ceased introducing greenhouse gases into the atmosphere today, the oceans would not begin to cool until at least 2075. Here, we will discuss the possibility of projected changes in the global oceanic climatic zones. Then we will discuss some ways in which corals may be expected to respond to these changes, including an expansion in latitudinal range and extinction in certain regions.

The oceanic climatic zones do not correspond precisely to those over land. The two overlap, but do not exhibit the same patterns over the Earth. The oceanic climatic zones are, progressing with respect to latitude from the equator towards the poles, the Equatorial, Tropical, Sub-Tropical, Temperate, Sub-Polar, and the Polar Zones (Fig. 34.13). With global warming progressing at the rate it is, it is likely that the borders of these oceanic climatic zones will change. For exam-

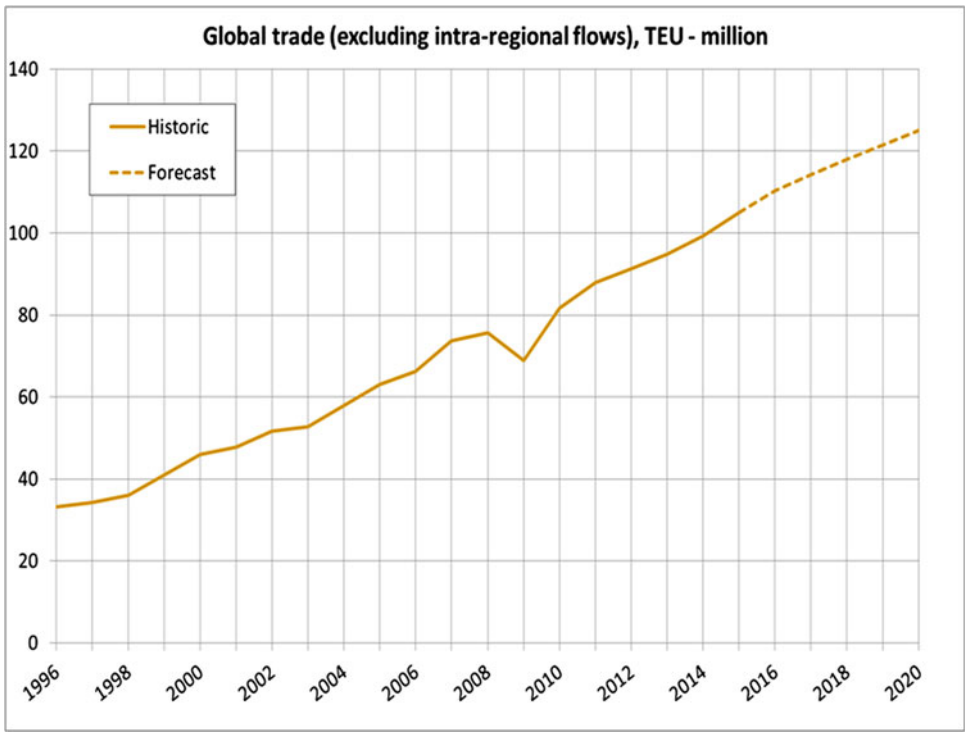

Fig. 34.10 A graph depicting the steady increase in global trade via oceanic shipping since 1996, with projections through 2020. Data presented in millions of TEUs (20-ft Equivalent Units, intermodal shipping containers). Based on the MDS Transmodal world cargo database, Feb. 25, 2015 (Graph provided courtesy of MDS Transmodal, UK)

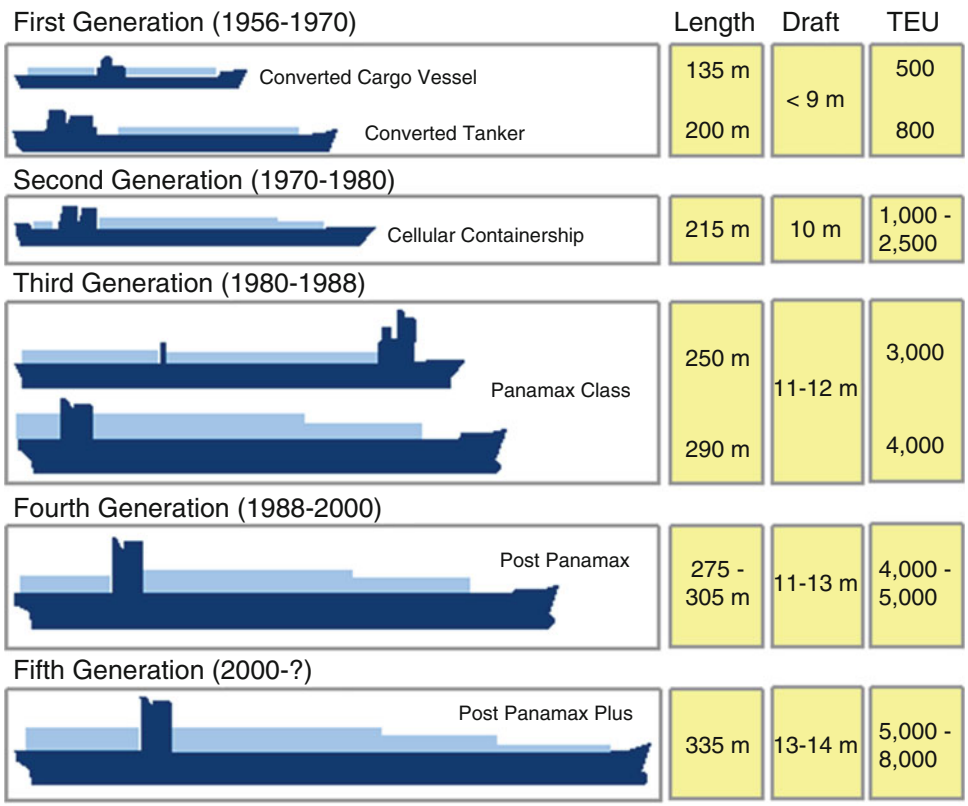

Fig. 34.11 A diagram indicating the steadily increasing size of oceanic cargo vessels from 1956 to 2000. With increasing vessel size comes increasing surface area of the hull and increased capability of the vessel carrying potential invasive species into a new environment. This combined with increased vessel numbers (see Fig. 34.15), multiplies the probability of successful species invasions (Diagram provided courtesy of Logistics Group Eurans, Ukraine. http://www.eurans.com.ua/eng/faq/containerships/)

ple, it is likely that the Equatorial Zone in the northern hemisphere will shift north, and its counterpart in the southern hemisphere, south. The next zone, the Tropical Zone, may also be expected to shift north, with a symmetrical mirror-image shift in the southern hemisphere. There will be a cascading effect, with most of these zones. These expansions, however, will be at the expense of the size of the subpolar and polar zones, which will most likely shrink.

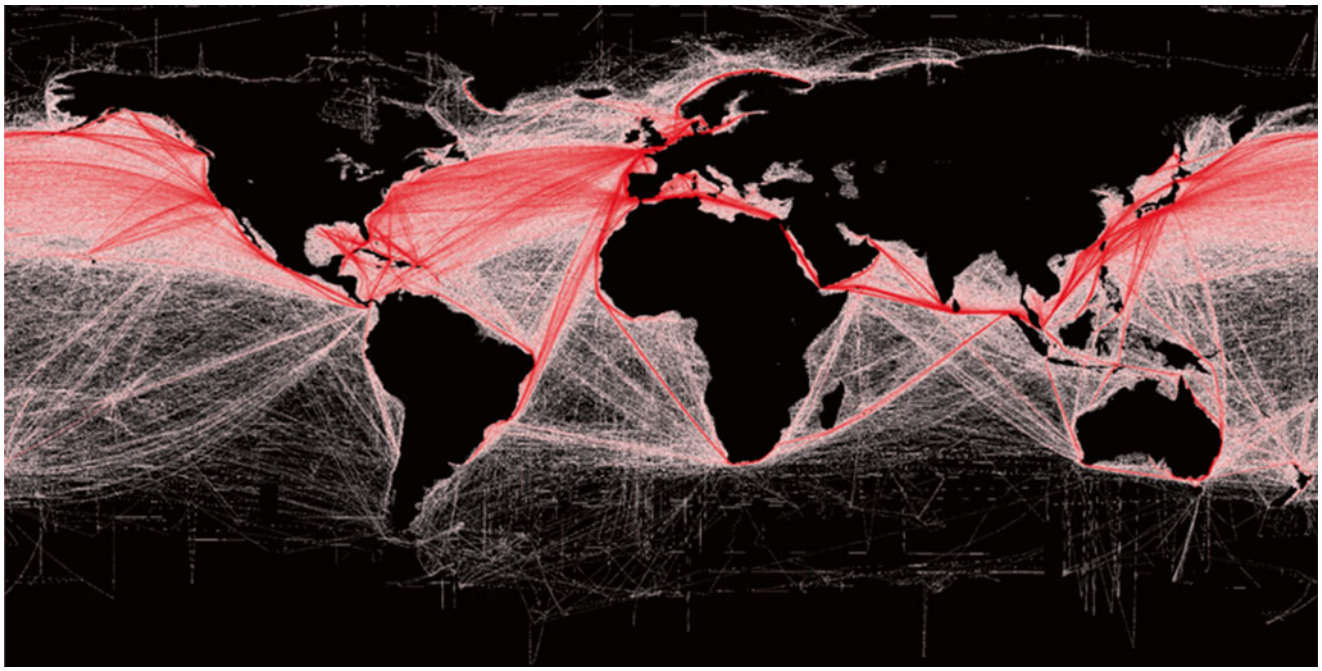

Fig. 34.12 Major oceanic shipping routes of the world. Figure by Grolltech (Wikipedia), derived from the work of T. Hengle, http://www.nceas.ucsb.edu/globalmarine/impactssrc, as well as Halpern et al. (2008). doi:10.1126/science.1149345src. Date: 2008src. Licensed under CC by-SA 3.0 via Wikimedia Commons – http://commons.wikimedia.org/wiki/File:Shipping_routes_red_black.png#mediaviewer/File:Shipping_routes_red_black.png (Figure provided courtesy of B.S. Halpern and colleagues, National Center for Ecological Analysis & Synthesis, University California at Santa Barbara, Santa Barbara, CA, USA. http://en.wikipedia.org/wiki/Sea#mediaviewer/File:Shipping_routes_red_black.png)

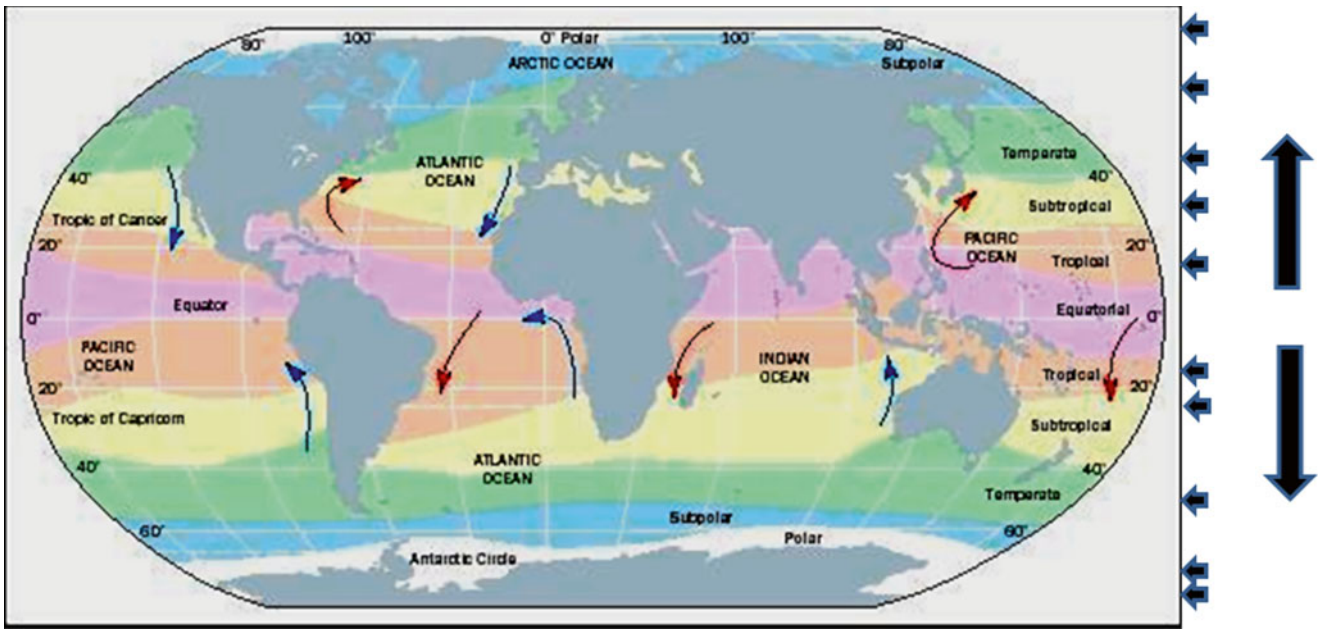

Fig. 34.13 A diagram of oceanic climatic zones as defined in 2015. Oceanic climates as they are predicted to shift with climate change/global warming in coming decades. The Equatorial Zone may be expected to expand northward and southward. The Tropical, Sub-Tropical, and Temperate Zones may be expected to most likely maintain their size, but move northward and southward, respectively, at the expense of the Sub-Polar and Polar Zones, which may be expected to move northward and shrink in size. Adapted from the Water Encyclopedia (see original image at http://www.waterencyclopedia.com/images/wsci_01_img0100.jpg)

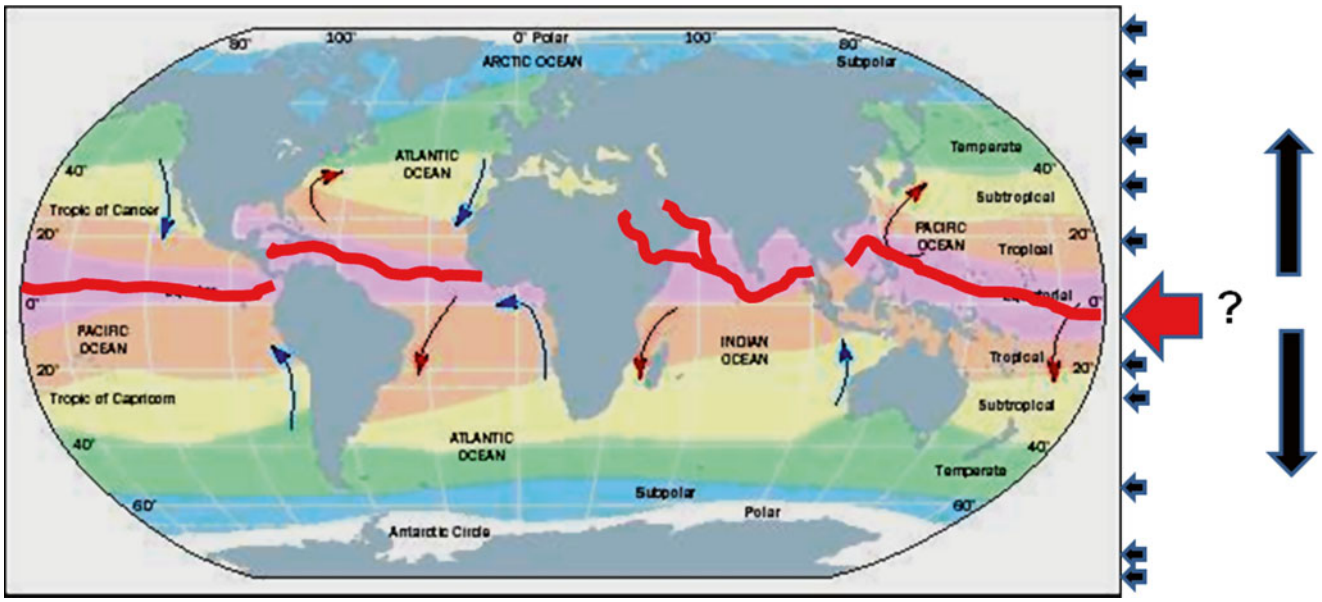

Fig. 34.14 It is also predicted that a new zone may appear at the center of the Equatorial Zone, here named the Hyper-Tropical Zone. Seawater temperatures are expected to increase at such a rapid rate and reach such high levels that mass mortalities may be expected to occur in zooxanthellate organisms, causing local if not global extinctions of some species and few adapted species remaining (Adapted from the Water Encyclopedia (see http://www.waterencyclopedia.com/images/wsci_01_img0100.jpg))

What do we have to look forward to? It is likely that the temperatures will become so warm in the center of the Equatorial Zone that they will become lethal to a variety of zooxanthellate organisms, including corals (Fig. 34.14). We predict that there may well be a new zone which is created at the center of the Equatorial zone. This zone will be one where the temperatures are extraordinarily high, compared to those of today's tropical zone – so high that they will cause the mortality and possible extinction of at least some zooxanthellate organisms. This would include scleractinian and octocorallian corals – particularly those whose zooxanthellae are very sensitive to high temperatures. This will have a particularly strong impact on zooxanthellate organisms that are rare, endemic or have limited local distributions. This will be an area of local and possibly global extinction for certain species. We have termed this "the hyper-tropical zone".

With this warming and the change in latitudinal borders of the oceanic climatic zones, it is probable that regions like the Gulf of Mexico will progress from being sub-tropical to tropical. In that case, coral reefs can be expected to expand and additional coral reefs to develop there with time. At present, the northern portion of the Gulf of Mexico possesses banks, such as Stetson Bank, which are shallow enough to support reefs, but the winter conditions occasionally drive seawater temperatures down to ≤16 °C (Pulley 1963; but see Debose et al. 2013), which is not conducive to facilitating substantial reef development. Such conditions are not conducive to substantial deposition of $CaCO_3$ by corals. This may be expected to change, however, as temperatures rise with global warming. There is already evidence of latitudinal expansion of coral reefs in the Ryuku Islands, Japan and in the sub-tropical region of southeastern Florida, USA. In the former case, Yamano et al. (2011) estimate that coral reefs in this region have been expanding northward at a maximum rate of 14 km/year. In the latter case, coral reef communities have developed offshore from Fort Lauderdale, Florida – 75 km north of Key Biscayne (Vargas-Angel et al. 2003; Precht and Aronson 2004). The Florida reefs are dominated by the threatened and endangered coral species *Acropora cervicornis* which has experienced mass mortality in the western tropical and sub-tropical Atlantic due to bleaching (Eakin et al. 2010) and disease (Williams and Miller 2005) over the past 35 years.

34.3.1 Failure of the Coral Symbiotic System Under Thermal Stress

Symbiotic zooxanthellate corals are apparently not keeping up with the pace of global warming, at least at this point in time. A critical question has arisen repeatedly over the decades regarding which component of the symbiotic partnership is most sensitive to these thermal stresses – the host animal tissue or the zooxanthellae (NOAA 2011). Recent experimental evidence has shown that the zooxanthellae are more sensitive to increased seawater temperatures than the coral tissue (Strychar et al. 2004a, b, 2005; Strychar and Sammarco 2009). This has now been demonstrated to be the case in three western Indo-Pacific scleractinian coral species

from the Great Barrier Reef – *Acropora hyacinthus, Favites complanata*, and *Porites solida* – representing three scleractinian families – the Acroporidae, Faviidae, and the Poritidae. It has also been shown in three species of western Indo-Pacific octocorals from the Great Barrier Reef – *Sarcophyton ehrenbergi, Sinularia lochmodes*, and *Xenia elongata*, representing two families – the Alcyoniidae and the Xeniidae. We believe this is a general phenomenon associated with the symbiotic zooxanthellate scleractinians and octocorals. In fact, we believe that this relational principle may be applied to all zooxanthellate cnidarians, including organisms such as *Cassiopaea xamachana* (Scyphozoa; Verde and McCloskey 1998) and *Aiptasia patella* (Anthozoa, Actinaria; Taylor 1973; Lindquist and Hay 1995). In addition, this zooxanthellate symbiotic relationship is not unique to the Cnidaria. It may be extended to zooxanthellate organisms from other phyla as well (Rowan 1998), such as the Platyhelminthes (flatworms; e.g., *Amphiscolops langerhansi;* Schoenberg and Trench 1980a, b, c; Fig. 34.15) and the Mollusca (e.g., *Tridacna gigas, T. maxima, T. crocea*, and other *Tridacna* spp; Fig. 34.16; Muscatine and Greene 1973; Fitt and Trench 1981). Most of these species – within and outside of the Cnidaria – are known to bleach under conditions of increased seawater temperatures (Perez et al. 2001; Dunn et al. 2002; Norton et al. 1995; Addessi 2001; see above figures).

Another question arises. Can natural selection serve to allow the zooxanthellae to adapt to global warming? Can these organisms keep up with the rate of change in seawater temperatures and the frequency, intensity, and duration of positive temperature "spikes" which have recently been occurring? The answer lies at least partially in the mutation rates of the zooxanthellae (Sammarco and Strychar 2009; Sachs et al. 2011). Are they sufficient? Or are there any strains of zooxanthellae which are already adapted, or exapted, to these new high-temperature conditions (Buddemeier et al. 1997; Baker 2001; Huertas et al. 2011)? We have already demonstrated that a number of the coral hosts are adapted, or exapted, to these temperature increases (Strychar and Sammarco 2009; Sammarco and Strychar 2013). In addition, the host corals can function well for a certain period of time without their zooxanthellae (Szmant and Gassman 1990). The problem is that, on average, the zooxanthellae have lower temperature tolerances than their host corals and readily succumb to this perturbation. This is indicated by the zooxanthellae being mostly dead or dying (necrotic or apoptotic) when they are expelled from the host under increased seawater temperature and bleaching conditions (Strychar et al. 2004a, b; Sammarco and Strychar 2009).

Fig. 34.15 *Amphiscolops langerhansi* (Platyhelmenthes). A flatworm, also possessing zooxanthellae and another example of an organism which may be susceptible to increasing seawater temperatures under global warming conditions (Photo by Richard Parisot, courtesy of Nanozine Blogspot; Nanozine.blogspot.com/2007_05_01_archive.html)

Fig. 34.16 *Tridacna gigas* (Mollusca, Bivalvia). Much of the color in the mantle of this giant clam was derived from zooxanthellae in the organism's tissues. This bivalve is shown here bleached due to increased seawater temperatures (Photograph by James W. Fatheree. Provided courtesy of AdvancedAquarist.com. www.advancedaquarist.com/2010/8/inverts)

34.3.2 Is Adaptation by Zooxanthellae the Answer?

Are multiple clades part of the answer? We now know that certain clades of *Symbiodinium* have different temperature tolerance ranges. This is an important point and suggests that one or more of these clades will predominate under conditions of increasing seawater temperatures through time, as the others whose temperature tolerances are lower or more restricted, will eventually become extinct – locally or globally. The problem, however, is that specific clades of *Symbiodinium* are known to be associated most commonly with one or several species of scleractinian host coral species (Fabricius et al. 2004). Even if they do infect a less preferred coral host, they do not operate at their optimal level, nor do their populations reach maximum levels (see Abrego et al. 2008). Thus, if one strain or clade of *Symbiodinium* were to become adapted to higher seawater temperatures, it would probably benefit only a suite of coral species. There would have to be a second set of mutations regarding the immune-recognition systems, possibly in the host coral and the symbiont, to allow this new clade to penetrate and infect the new host coral species. This would have a much higher probability of success, particularly in the short or medium term, than substitution of another symbiont (e.g., *Prochloron;* Munchhoff et al. 2007; see below), because the immune systems are already at least partially adapted for the host and symbiont to accept each other. It would still require a series of mutations and adaptations, however, before it would be beneficial to zooxanthellate scleractinian corals as a whole. The combination of low mutation rates, multiple parallel requirements for mutations and adaptations, and the rate at which our environment is changing make the probability of survival of various species of temperature-sensitive zooxanthellate scleractinian corals low (Sammarco and Strychar 2009).

34.3.3 Comparative Population and Genetic Characteristics of the Host and Its Symbionts

In order to gain a better understanding of whether the zooxanthellae may be able to adapt to these increasing seawater temperatures, one has to consider comparative generation times, age of first reproduction, life-expectancy, and comparative mutation rates in both the host and symbiont (Hughes 1984; Hughes and Jackson 1985; Caley et al. 1996; van Oppen et al. 1999; Hellberg 2006; Sammarco and Strychar 2009). Determining generation times can be estimated by comparing body lengths and comparing them with those of a number of other species McNaughton and Wolf 1979). For this comparison, we have sometimes used data pertaining to dinoflagellates, which are closely related to zooxanthellae, (since data were not always available for *Symbiodinium*). Figure 34.17 indicates that the host corals and their symbiotic *Symbiodinium* have widely disparate generation times. With respect to the coral host, in terms of lifespan, some coral hosts can live for tens, hundreds or even thousands of years (Boto and Isdale 1985). Because corals

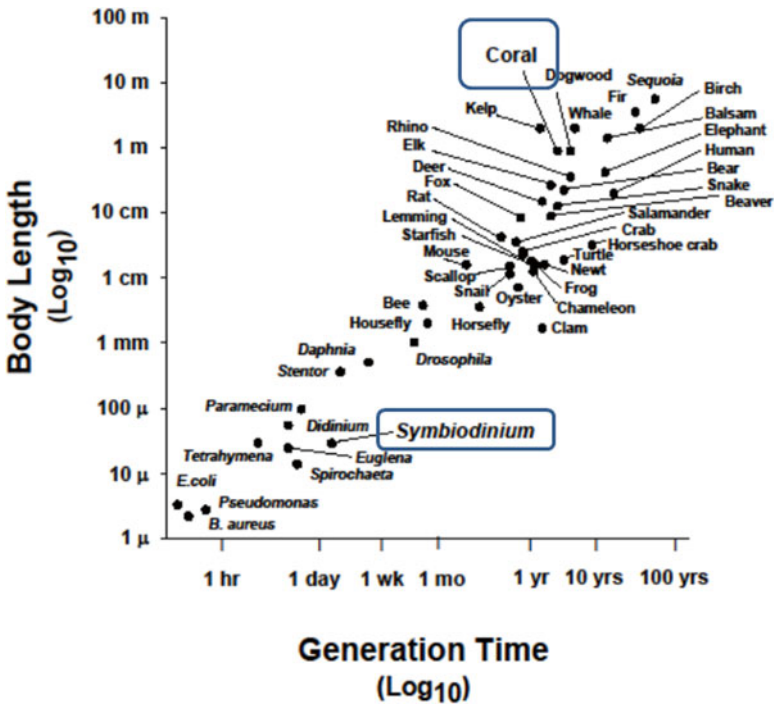

Fig. 34.17 The relationship between body length and generation time in numerous terrestrial, marine, and freshwater organisms. Both X and Y axes are presented with a log transformation. Note the large differences not only in size but in generation times between host corals and their symbiotic zooxanthellae (*Symbiodinium*) (From Sammarco and Strychar 2009, provided courtesy of Taylor and Francis, Publ., Philadelphia, PA, USA; Adapted from Bonner, 1964; McNaughton and Wolf 1979)

are colonies and utilize asexual reproduction/budding abundantly, the generation time or time to first sexual reproduction life expectancy can be 2–3 years (Harrison and Wallace 1990). *Symbiodinium* are known to reproduce asexually frequently (Correa and Baker 2009), (which raises the question of somatic mutations). There is evidence that *Symbiodinium* also reproduces sexually, permitting recombination – via observations of linkage disequilibria and high allelic diversities (LaJeunesse 2005; Stat et al. 2006; Porto et al. 2008). *Symbiodinium*'s order, the Suessiales, is also known to exhibit nuclear cyclosis and meiosis, evident through both nuclear and mitochondrial DNA (Parrow and Murkholder 2004; LaJeunesse et al. 2005; Zhang et al. 2005).

In the host corals, the frequency of sexual reproduction is annually in broadcast species (Harrison and Wallace 1990; Richmond and Hunter 1990). It is monthly through more than half of the year for brooders (*ibid*; also see McGuire 1998). Figure 34.18 shows the relationship between generation time and mutation rates in a number of organisms. This information can then be translated into a comparison between the coral host and its symbiont, based upon both theoretical and empirical data. The empirical data indicate that *Symbiodinium* may be projected to have the same mutation rates as would be projected from theoretical information (Fig. 34.19). The host corals, however, fall well below the mutation rates predicted for their size. It then becomes evident which member of this symbiotic partnership has the highest probability of adaptive change: The zooxanthellae. This group has higher rates of reproduction and more opportunities for mutation during meiosis and recombination than do the host corals.

On the other hand, comparative mutation rates between the two partners are also important. If one has a high reproductive rate but a low mutation rate on a per generation basis, one may counteract the other in terms of producing an adaptation such as higher temperature tolerance. Here we find that mutation rates are comparatively low in the host corals on a per generation basis and in absolute terms, and comparatively high in the zooxanthellae. One possible reason they may be low in the coral is that it is suspected that they have a DNA repair mechanism (Snell et al. 1998; Baruch et al. 2005; Hellberg 2006). This makes the host coral an even more conservative member of the symbiotic pair. The zooxanthellae, of course, have a higher mutation rate on a per generation basis. This, combined with the low generation time, low first age of reproduction, and high reproductive rate suggests that the zooxanthellae have better potential to adapt to increasing temperatures. Nonetheless, observations thus far regarding survivorship of corals and their zooxanthellae exposed to increased seawater temperatures raise the question as what adaptation may or may not be occurring at a fast enough rate (Hoegh-Guldberg 1999; Hughes et al. 2003; Baker et al. 2004, 2008). On the average, the zooxanthellae simply cannot deal with current increases in the intensity, frequency, and duration of thermal stresses/perturbations to which they are being subjected. Some strains of zooxanthellae clearly survive; others do not.

34.3.4 What About Other Types of Photosynthetic Symbionts?

Is it possible that another surrogate or substitute symbiont could replace *Symbiodinium* in this partnership. *Symbiodinium* is not unique in terms of symbiotic partnerships between marine invertebrates and plants. Examples

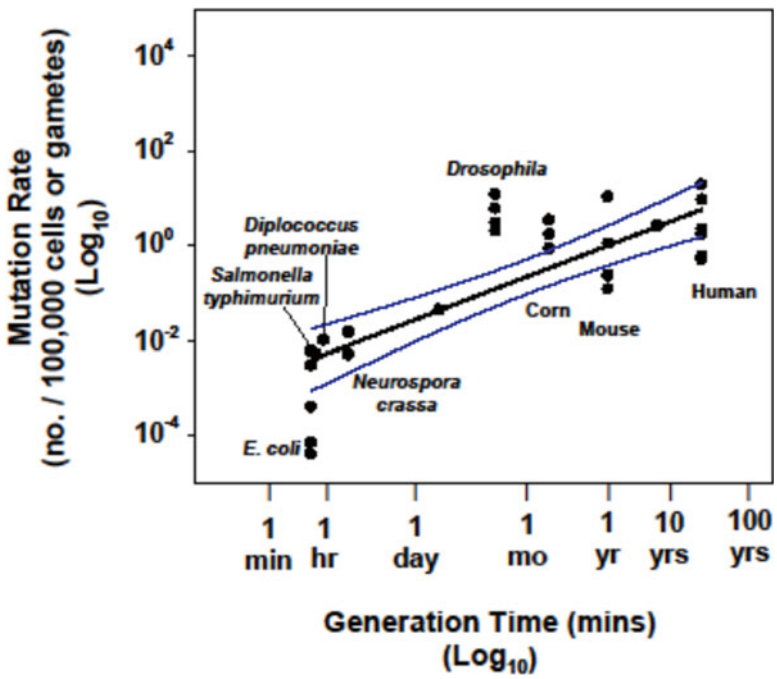

Fig. 34.18 The relationship between generation time (in min) and mutation rate (in no./100,000 cells or gametes), derived empirically, in a number of different organisms, ranging from bacteria to humans. Both X and Y axes are log-transformed. Model II regression line shown along with 95 % confidence limits (From Sammarco and Strychar 2009, courtesy of Taylor and Francis, Publ., Philadelphia, PA; data derived from Strickberger 1968; Dobzhansky 1970; Drake 1974; Futuyma 1998)

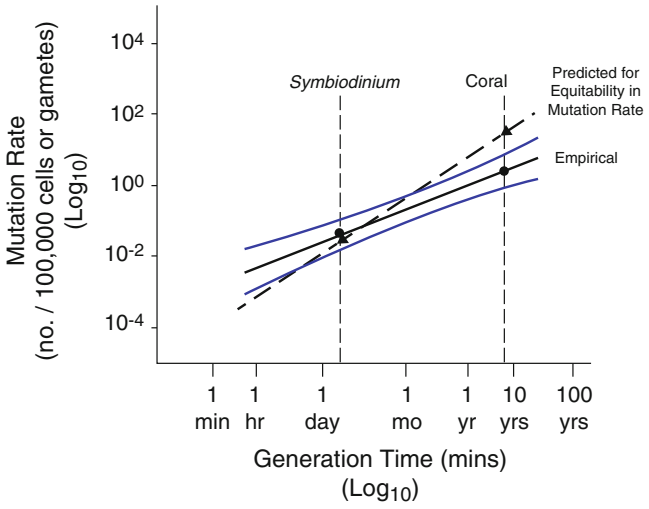

Fig. 34.19 The relationship between generation time (in min) and mutation rate (in no. per 100,000 cells or gametes). Comparison between host corals and their symbiotic *Symbiodinium* (zooxanthellae). Note that *Symbiodinium* falls directly on the regression line, but the empirical mutation rate for corals, although falling directly on the empirical line, falls well below (and significantly below) the mutation rate predicted if there were equitability in mutation rates between the two, once standardized for generation time (From Sammarco and Strychar 2009, courtesy of Taylor and Francis, Publ., Philadelphia, PA)

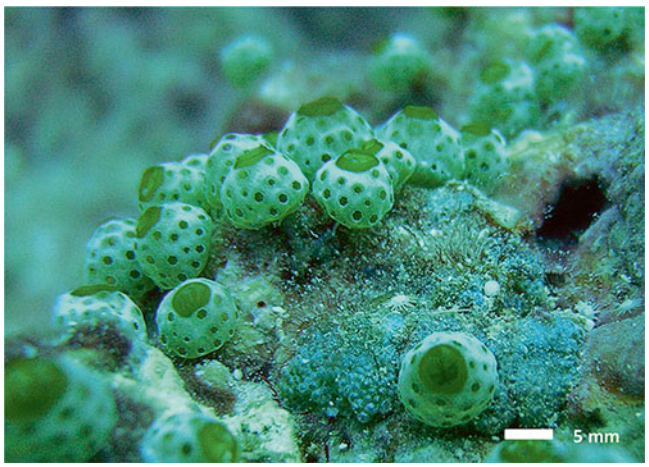

Fig. 34.20 *Didemnum molle* (Ascidea, Didemnidae). This ascidian possesses a symbiotic cyanobacterium – *Prochloron didemni*, which gives it its green color (Photo courtesy of Dr. Chris Lobban, Department of Botany, University of Guam; http://university.uog.edu/botany/474/cyano/prochloron.html)

which come to mind include *Prochloron didemni* (Munchhoff et al. 2007) and *Chlorella* (Dolan 1992; Johnson 2011). *Prochloron didemni* is a cyanobacterium which is symbiotic with the ascidians *Didemnum molle* (Olson and Porter 1985; Cox 1986; Fig. 34.20) and *Lissoclinum patella* (Houssen and Jaspars 2010). *Chlorella* sp. is a green algae which is symbiotic within the sponge *Spongilla lacustris* (Sand-Jensen and Pedersen 2003; Fig. 34.21). Would such a development be possible? Yes. Would such a development be probable? No. Here is the reason why. One of the reasons that corals and zooxanthellae can function well as a symbiotic unit is because they possess a number of co-adaptations. These include, firstly, the ability of the coral's immune system to recognize *Symbiodinium* as "self", and not attempt to attack the cells attempting to enter their tissues as foreign cells (e.g. pathogens) or "non-self". Secondly, the alga must also be able to recognize the coral as "self" to allow itself to penetrate the host coral's outer layer of cells. Thirdly, the *Symbiodinium* cells must be adapted to survive in a living, gel-like medium. Fourthly, the *Symbiodinium* must be adapted to find a mate if it uses sexual reproduction while in vivo. In a new relationship with *Prochloron*, similar adaptations would have to arise in both partners. The probability of any one of these arising is small but calculable. The probability of all of these mutations/adaptations occurring within a short period of time in both the host coral and the symbiont is the product of each of these single adaptations arising individually – multiplied together. This produces an extraordinarily small probability.

So, could it happen? Yes – but with throwing this many dice at the same time, the time required to successfully come up with this combination of adaptations across two phyla would be enormous, since the probability of all adaptations would be miniscule. The same mathematical principles would apply to other potential substitute symbionts.

34.4 Comments on Evolution of the Immune System in Corals

34.4.1 Background

As climate change continues to warm atmospheric and aquatic environments around the globe, some pathogens are rapidly expanding into areas they never existed in the past, compromising many organisms. In some places, e.g. the Caribbean, coral reefs have experienced major losses due to habitat degradation, over-fishing, and pollution confounded by increased sea surface temperatures (Hoegh-Guldberg et al. 2007); but disease has resulted in the highest loss of coral cover (Weil 2002). Today we consider the Caribbean as a disease "hot spot" due to the fast emergence and virulence of new/novel diseases and syndromes, their wide distribution and ability to affect/infect different host organisms, and the high frequency of epizootic events (Weil 2004). Despite such problems, some corals appear to be adapting by modifying their cell death mechanisms and immune responses. In so doing, we are at a pivotal point in history where our understanding of how immunity evolved and what exists in-

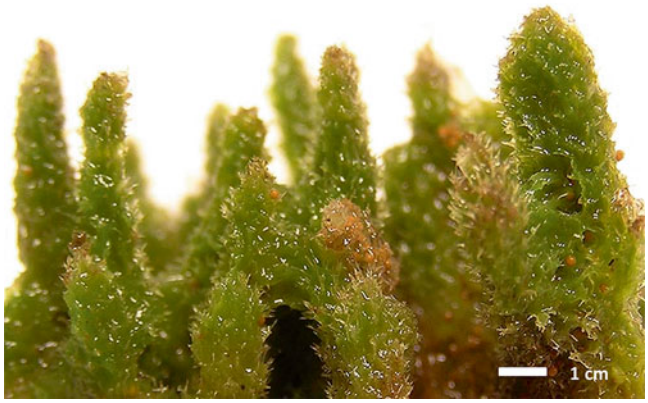

Fig. 34.21 *Spongilla lacustris* (Porifera). This freshwater sponge possesses the chlorophyte *Chlorella* as a symbiont (Photo courtesy of Jirka Novak and biolib.cz; www.biolib.cz/en/image/id18059/)

between the two major players (innate versus adaptive immunity) may be revealed.

34.4.2 Innate Versus Adaptive Immunity

All vertebrates possess adaptive immunity – a system that allows new responses to pathogens on the basis of past interactions (McFall-Ngai 2007). Adaptive immune responses are considered superior to other immune responses because of their system's ability to "learn" from past experiences. Immunological memory within invertebrates, however, is considered to be absent because they lack T-cell receptors (TCR), immunoglobulin (Ig), and major histocompatibility complex (Mhc) molecules (Arala-Chaves and Sequeira 2000). Considered simpler in design, invertebrates rely on a less advanced system called innate immunity. This type of system is considered non-adaptive, induced, and specific, and is based upon immediate responses to, for instance, a stressor or pathogen. Herein, however, lies an interesting conundrum. How can simple invertebrates, comprising >96 % of all animal species on earth, be similarly challenged by the microbial world, and yet remain healthy? If their immune systems can't "remember" past experiences (Paredes Juárez et al. 2014), why are they still so prevalent? Compared to vertebrates whose immune responses are slow (days to weeks) and dependent upon prior exposure (Kumar et al. 2013), recent evidence suggests that we may have overlooked possible connections between the two phyolgenies – and that technologies which did not exist a few decades ago are now opening up Pandora's Box.

The big question, then, is – what is the connection between vertebrates and invertebrates? The answer is that the innate immune system preceded adaptive immunity in the evolution of immuno-recognition, and it provides the common thread that ties together both lineages (Iwasaki and Medzhitov 2004; Litman and Cooper 2007). In vertebrates, the innate system is "first on the scene" responding through pattern recognition and initiating an inflammatory response, including cytokine production and activation of cells to kill pathogens thereby alerting the adaptive immune system (Fig. 34.22) (Janeway et al. 2001; Valiante et al. 2003; Staros 2005).

Over millions of years, innate immune cells (e.g. dendritic cells, macrophages) evolved receptors, termed pattern recognition receptors (PRR). PRRs recognize PAMPs (pathogen-associated molecular patterns), which are structurally and chemically diverse (e.g. lipopolysaccharide, flagellin, mannan, peptidoglycan) but are conserved in pathogens (Kumar et al. 2013; Paredes Juárez et al. 2014; Toledo-Hernández and Ruiz-Diaz 2014). When PAMPs are detected, PRR's stimulate host responses to cause cellular lysis, phagocytosis, and opsonization (Toledo-Hernández and Ruiz-Diaz 2014). Host receptors via leucine-rich repeat (LRR) motifs, Toll/IL-1 receptor (TIRs) domains (Bosch 2008), and soluble proteins bind to specific lectins (e.g. Mannose Binding Lectins (MBL); Kvennefors et al. 2010) as a process of recognition. PRRs not only recognize proteins that exist between cells (intercellular) but also recognize intra-cellular components associated with dying cells called DAMPs (danger/damage-associated molecular patterns; Bianchi 2007; Kono and Rock 2008). In healthy systems, DAMPs help alert other cells of impending dying or damaged cells and stimulate an inflammation cascade (i.e. healing). This involves complement factor C3 (Sahu and Lambris 2001), TLRs (Paredes Juárez et al. 2014), heat shock protein 70 (HSP70; Land 2011), and high-mobility group box 1 proteins (HMGB1; Castiglioni et al. 2011; Daly et al. 2012).

So why is all of this important? It is important because even though scientists in the past had not identified adaptive immunity in invertebrates and suggested that these pathways did not exist, today's technology is beginning to show the opposite. For example, in vertebrates PRRs are formed by various receptor-type families, such as C-type lectin receptors (CLRs), NOD-like receptors (NLRs), DNA sensing molecules (DSMs), RIG-I-like receptors (RLRs), leucine-rich repeat domains (LRRs), and Toll-like receptors (TLRs; Kawai and Akira 2010, 2011; Kumar et al. 2013; Paredes Juárez et al. 2014). In fact, 10 TLR families have been identified in humans (Kumar et al. 2013), each with specific functions. In comparison to coral (e.g. *Acropora digitifera*, Miller et al. 2007; *A. palmata*, *A. millepora*, and *Montastraea faveolata*, Miller et al. 2007; Schwarz et al. 2008), these animals also show different types and concentrations of TLRs when stressed (e.g. climate change, infection). Similarly, complement factor C3 homologues have been identified (e.g. *Swiftia exserta*; see Dishaw et al. 2005) consisting of C2, C3, C4, and C5 (Dishaw et al. 2005; Hemmrich et al. 2007).

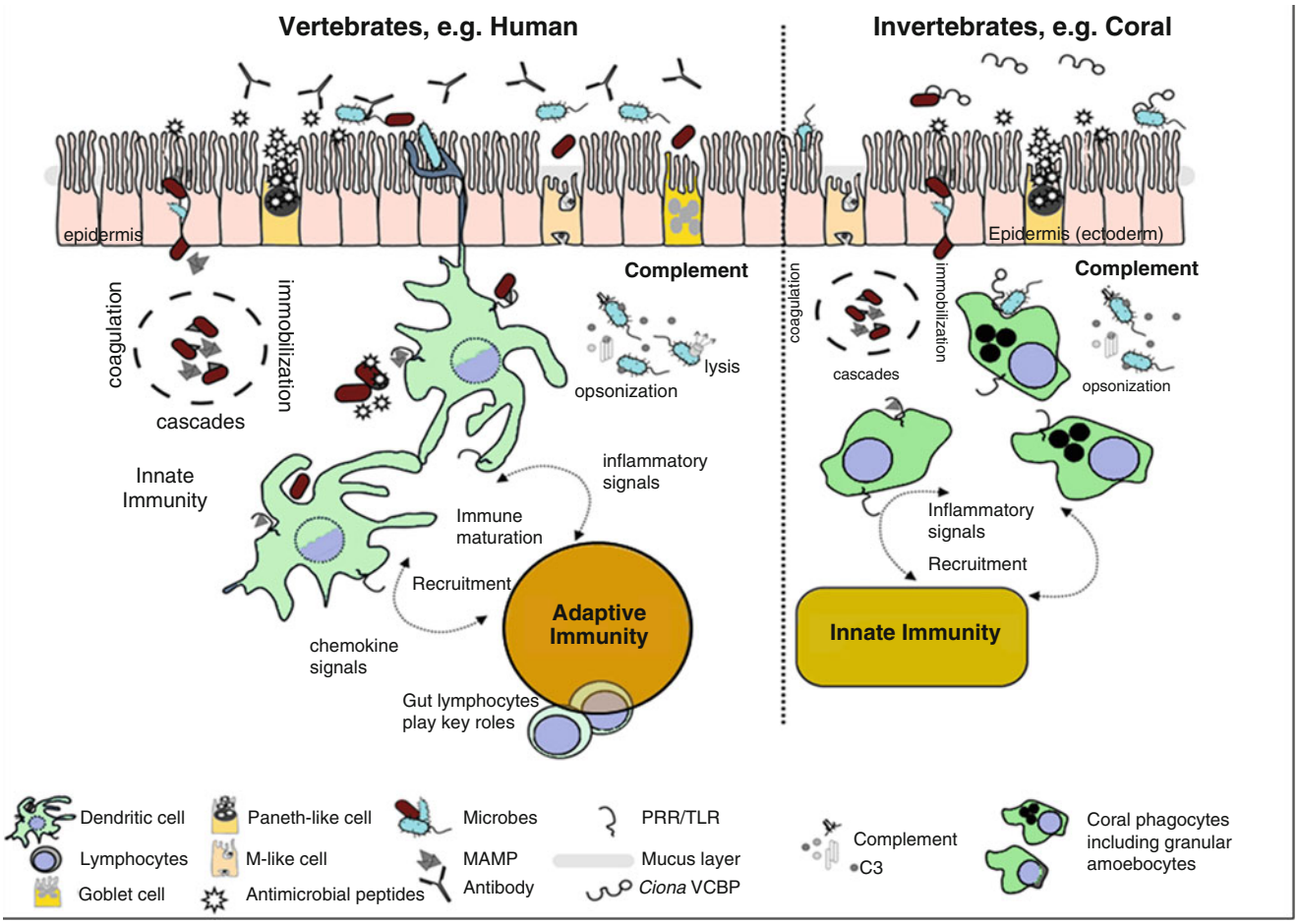

Fig. 34.22 An overview comparing vertebrate (e.g. human) vs. invertebrate (e.g. coral) immunity. Defense systems of both lineages begin with an epidermal-type barrier governed by mucus, immune molecules, and antimicrobials. Despite different cascades involved with each type of immunity, both are 'linked' via the use of complement defense pathways. Some obvious distinctions include dendritic cells in vertebrates vs. phagocytes in invertebrates. Antigens, lymphocytes, and T and B cell lineages further differentiate the adaptive and innate lineages. However, immunological competence in invertebrates is believed to include pro-inflammatory signals, PRRs and PAMPs, and the recruitment of granular ameobocytes – many of which may involve memory and/or a less-specialized type of adaptive immunity (Modified from Dishaw et al. 2012; http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3342567/)

The immune system 'complement' in vertebrates was assumed to be confined to deuterostome lineage, i.e. chordates and echinoderms such as sea urchins and starfish. This indicates that the complement cascade pathway known only from higher animals, predates divergence of the simplest of true animals (Hemmrich et al. 2007). Because complement also occurs in lower invertebrates like coral, such animals may recognize "self" vs. "not-self" and perhaps, also have some type of adaptive memory albeit primitive in design. For instance, although cnidarians are believed to lack specialized immune cells, they do display allorecognition (or xenorecognition; Miller et al. 2007). This occurs when the animal's tentacles protect colonies from fusion with genetically different individuals, preventing germ-line parasitism (Miller et al. 2007), as has been observed in *Stylophora pistillata* and *Montipora verrucosa* (Hildemann et al. 1980; Müller et al. 1984; Chadwick-Furman and Rinkevich 1994).

34.4.3 The Influence of Zooxanthellae on the Coral's Immune System

It is well known that the host coral and symbiont *Symbiodinium* Freudenthal (zooxanthellae) exchange nutrients, however, it is not known how the host accepts and permits an "invader" to infect and take-up residency within its endodermal tissues. Kvennefors et al. (2008) speculated that this may occur via a coral lectin called "Millectin". Millectin has been observed in both bacteria and *Symbiodinium* in vitro, indicating its potential role in both immunity and possibly symbiont recognition. If Millectin is present in both bacteria and zooxanthellae, this poses a problem for coral with multiple clades, bacterial infection, and loss of homeostasis. Another possibility, however, is that *Symbiodinium* are recognized during coral gastrulation using integrins, which are surface receptors

highly conserved throughout the animal kingdom. Integrins function across many physiological processes, including growth and development, and wound repair and immunity (Toledo-Hernández and Ruiz-Diaz 2014). Integrins have been found to occur in *Acropora millepora* (Brower et al. 1997; Knack et al. 2008). Non-symbiotic corals, on the other hand, possess no symbiotic zooxanthellae, do not require zooxanthellae for survival, and thus have no need to accept them into their tissues. Thus, *Symbiodinium* must be recognized as "non-self", foreign, or "not acceptable". Comparatively speaking, their recognition system may be more robust with fewer allowances for "infections". Considered simple in structure and yet "old" in terms of evolutionary age, the host coral must possess a functional blocking mechanism for dinoflagellates and pathogens – and the differences between these two sets of taxa (symbiotic vs. non-symbiotic) may hold the key to better understanding the foundation of immune systems in coral and other invertebrates.

34.4.4 The Influence of Multiple Clades on the Immune System: Ability to Accept Multiple Clades

Symbiodinium sp. occurs in the form of many clades, varying from each other in their DNA sequences. These clades thus far number 12–14 (Sammarco and Strychar 2009), with numerous sub-clades/types (~1,700) being described using various methods (see review by Riddle 2007). The immune system of the individual clade must produce one or more proteins for recognition by the host coral in order for successful colonization of the host to occur. Likewise, the coral must have the ability to recognize more than one clade's proteins at the same time, and this capability is probably genetically based. We speculate that "self" vs. "non-self" recognition in symbiotic corals is based upon an immune-neuroendocrine systems optimized for gross but efficient host-symbiont recognition (Arala-chaves and Sequeira 2000) which is similar to, but less complex than, that in vertebrate immune systems (Ottaviani et al. 1998). Janeway (1989) speculated that invertebrates may have low concentrations of immuno-stimulant receptors and elicit intense responses to foreign contaminants. In coral, it is highly probable that such immuno-stimulants exist in the mesoglea. This is analogous to hemolymph in some invertebrates where investigators such as Arala-Chaves and Sequeira (2000) observed significant increased hemocyte proliferation rates (HPRs) when exposed to foreign molecules.

If we assume that the immune system can be over-taxed by too many propagules (as vertebrate immune systems can be), then the question arises regarding how many clades is actually optimal for survival (via nutrient exchange), but not overwhelming (i.e. taxing the immune system). We postulate that having more than one clade confers better nutritional fitness but poorer immunity. This is because more clades require more immuno-recognition proteins to address the presence of benign symbionts. For example, *Acropora hyacinthus* (Great Barrier Reef) can possess up to three clades simultaneously in a colony and yet, this species is highly susceptible to bleaching – and today is considered an endangered species. On the other hand, *Favites complanata* possesses two clades of zooxanthellae and is less susceptible to bleaching than *A. hyacinthus*. Further, *Porites solida* has only one clade of zooxanthellae and is the most resistant to bleaching. To take this one step further, many non-symbiotic corals show no signs of disease. In this case, the immune system need only recognize "self" cells, as there is no need to allow other symbiotic cell types to enter the organism. This would imply that necessity for a relatively sophisticated immune system is diminished in a symbiotic coral, whereas in an azooxanthellate coral, immunity is focused on self-defense.

34.5 Closing Comments

Coral reefs are very sensitive, integrated, fragile, and complex systems. They operate like a Nineteenth Century Swiss watch – where some of the cogs are small and some are large, but they are all inter-connected. Turning one affects all of the others. Turning a large cog turns all of the others rapidly; turning a small one turns them slowly – but all move. All are affected.

Our coral reef ecosystems are not interchangeable. Those from different parts of the world have species with adaptations to deal with their specific environments the members of their specific communities. The geological age of these isolated systems has affected the types and intensities of interactions they have with other species. One size does not fit all. And one species will not fit easily into the niche of another from another ocean; something has to give. Organisms from older, more stable, diverse systems, having more aggressive behaviors and better predatory or anti-competitor adaptations, may be apt to drive one or more species out in a younger, less stable system. Reverse introductions are probably less successful.

Will coral reefs be salvageable over the next few decades in the face of climate change and global warming, given their present set of tools for adaptation? Yes, it is possible, but the probabilities of successful change in the face of the rapidly intensifying thermal stress points to the extinction of those coral species and zooxanthellar clades that simply cannot keep up with the pace. Predicted latitudinal shifts in the distribution of coral reefs globally imply that these reefs will survive. Endemic species, however, and particularly those

with restricted distribution to the center of the tropics and limited dispersal capabilities may be lost if a hyper-tropical zone is generated through the ever-increasing seawater temperatures near the equator. Basically, some species (probably extant) will survive, while others won't.

One last point. The reason we are even discussing these problems is due to atmospheric carbon that we have been introducing into the atmosphere for approximately 160 years. The real answer to all of these problems is to greatly reduce our use of carbon as an energy source and move to renewable, sustainable energy sources (wind, hydro, geothermal, wave, ocean currents, ocean thermal, etc.). There is no question that this needs to be done. On the other hand, our economic, political, and social systems are so dependent on carbon as an energy source that the probability of any rapid change occurring in our usage is near zero. A shift will happen – but it will require a long period of time. In addition, even if we stopped introducing greenhouse gases into the atmosphere today, it would take another ~75 years for the heat which we have added to our oceans and is now stored there, to begin reversing itself. This should not, however, discourage us from starting the reversal of carbon-usage as an energy source. After all, if you never begin a journey, the only thing that is absolutely certain is that you will never reach your destination.

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The Impact of Climate Change and the Environment on Coral Growth

35

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Abstract

Knowledge of factors that are important in reef growth and resilience helps us understand how reefs react following major environmental disturbances including overfishing, destructive fishing practices, coral bleaching, ocean acidification, sea-level rise, algal blooms, agricultural run-off, coastal and resort development, marine pollution, increasing coral diseases, invasive species, hurricane/cyclone damage and bleaching. Research in both the Indo-Pacific and in the Caribbean show how temperature and environmental extremes have influenced coral growth, recruitment and mortality. Three dimensional topography and complexity is important for reef vitality and viability in the face of environmental stressors. Within the narrow temperature range for coral growth, corals can respond to rate of temperature change as well as to temperature *per se*. A rational polynomial function model for coral colony growth appears as the best-fitting model for coral growth, closely followed by exponential logistic, Gompertz, and von Bertalanffy models. Models have also been developed for many varieties of coral morphologies, as well as for polyp spacing in those morphologies. The chapter concludes with the suggestion that developing large Marine Protected Areas (MPAs) as part of an overall climate change policy for a country may be the best way of integrating climate change into MPA planning, management and evaluation.

Keywords

Modelling • Resilience • Hurricanes • Bleaching • SST • Sedimentation

35.1 Introduction

Coral reefs throughout the world are under severe challenges from a variety of anthropogenic and environmental factors including overfishing, destructive fishing practices, coral bleaching, ocean acidification, sea-level rise, algal blooms, agricultural run-off, coastal and resort development, marine pollution, increasing coral diseases, invasive species, and hurricane/cyclone damage (Gardner et al. 2003; Bellwood et al. 2004; Crabbe et al. 2008). Maintaining coral reef populations in the face of large scale degradation and phase-shifts on reefs depends critically on recruitment and coral growth (Hughes and Tanner 2000; Coles and Brown 2007), maintenance of grazing fish and urchin populations (Mumby et al. 2007), clade of symbiotic zooxanthellae (Stat et al. 2008) and management of human activities related to agricultural land use and coastal development (Mora 2008; Curnick et al. 2015). Small changes in environmental parameters (e.g. a 2° C change in temperature) can cause significant (up to 50 %) changes in growth rates (Meesters et al. 1998; Kaandorp 1999; Crabbe and Smith 2002; Macdonald and Perry 2003; Jimenez and Cortes 2003; Lirman et al. 2003). Often, environmental parameters influencing growth can be multifactorial, and result in complex cellular changes (Luo et al. 2009) so that

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high energy and high sedimentation together can reduce growth (Cruz-Pinon et al. 2003), while changes in temperature, salinity, and sedimentation can influence not only growth but also diversity and abundance of corals (Lirman et al. 2003).

Sea surface temperature (SST) across much of the tropics has increased by 0.4–1 °C since the mid-1970s. A parallel increase in the frequency and extent of coral bleaching and mortality has fuelled concern that climate change poses a major threat to the survival of coral reef ecosystems worldwide. Steadily rising SSTs, not ocean acidification, are already driving dramatic changes in the growth of an important reef-building coral in the central Red Sea (Cantin et al. 2010). Three-dimensional computed tomography analyses of the massive coral *Diploastrea heliopora* revealed that skeletal growth of apparently healthy colonies declined by 30 % since 1998. The same corals responded to a short-lived warm event in 1941/1942, but recovered within 3 years as the ocean cooled. The authors predicted that should the current warming trend continue, this coral could cease growing altogether by 2070.

On the Great Barrier Reef an extensive time series on reef condition (2258 surveys of 214 reefs over 1985–2012), showed a major decline in coral cover from 28.0 to 13.8 % (0.53 % y^{-1}), a loss of 50.7 % of initial coral cover. Tropical cyclones, coral predation by crown-of-thorns starfish (COTS), and coral bleaching accounted for 48, 42, and 10 % of the respective estimated losses, amounting to 3.38 % y^{-1} mortality rate. Importantly, the relatively pristine northern region showed no overall decline (De'ath et al. 2012). This has now changed with the long El-nino occurring from 2015–2016. Aerial survey in 2016 found that middle stretches of the 2300-kilometer-long GBR system were, on the whole, moderately bleached, while eighty percent of the northern reefs were severely bleached; in-water surveys confirmed 50 % mortality in some reefs, a percentage that could eventually exceed 90 % (Hughes, 2016).

Atmospheric carbon dioxide concentration is expected to exceed 500 ppm and global temperatures to rise by at least 2 °C by 2050–2100, values that significantly exceed those of at least the past 420,000 years during which most extant marine organisms evolved. Under conditions expected in the twenty-first century, global warming and ocean acidification will compromise carbonate accretion, with corals becoming increasingly rare on reef systems. The result will be less diverse reef communities and carbonate reef structures that fail to be maintained. Climate change also exacerbates local stresses from declining water quality and overexploitation of key species, driving reefs increasingly toward the tipping point for functional collapse (Hoegh-Guldberg et al. 2007). Scaled-up management intervention and decisive action on global emissions are required if the loss of coral-dominated ecosystems is to be avoided.

Documenting variability and detecting change in global and regional climate relies upon high-quality observational records of climate variables supplemented, prior to the mid-nineteenth century, with reconstructions from various sources of proxy climate information. Annual density banding patterns that are recorded in the skeletons of massive reef-building corals have been used to document environmental change and impacts within coral reefs (see e.g. Lough and Cantin 2014). Massive corals provide a historical perspective of continuous calcification processes that pre-date most ecological observations of coral reefs. High-density stress bands, abrupt declines in annual linear extension, and evidence of partial mortality within the skeletal growth record reveal signatures of catastrophic stress events that have recently been attributed to mass bleaching events caused by unprecedented thermal stress. Comparison of recent trends in annual calcification with century-scale baseline calcification rates reveals that the frequency of growth anomalies has increased since the late 1990s throughout most of the world's coral reef ecosystems. Table 35.1 shows measurements of some representative coral growth rates by a number of researchers in the late twentieth century.

Continuous coral growth histories provide valuable retrospective information on the coral response to environmental change and the consequences of anthropogenic climate change. Co-ordinated efforts to synthesize and combine global calcification histories will greatly enhance our understanding of current calcification responses to a changing ocean. Chapters in the 'Physiology and Biomineralization' section of this book cover calcification and growth through time. This chapter presents studies on modelling coral growth and the influence of environmental conditions on current growth patterns of a number of species.

35.2 Measurements in the Indo-Pacific and the Caribbean

35.2.1 Indo-Pacific

The Wakatobi Marine National Park is situated in the Tukang Besi archipelago, a remote island group of about 200,000 ha off SE Sulawesi in Indonesia (Fig. 35.1), in the Coral Triangle, a global centre of marine biodiversity. Research has concentrated on three different reef sites in the Park, each separated by about 1.5 km. The first site, Sampela, has high turbidity and experiences high human activity, the second site, Kaledupa, has the lowest turbidity and least human activity in the park, and the third site, Hoga, represents intermediate water clarity and human use conditions. The Sampela site is next to a human population (c. 1300 Bajau people) living in huts built on fill of mined corals, while the other sites have less than 100 people living immediately near them.

Using digital videophotography and computer image analysis, as well as physical measurements, surveys have been conducted on reefs near the island of Hoga, where a Marine Research Station run by Operation Wallacea is situated. There

Table 35.1 Coral growth rates. Note that *Montastrea* sp. is synonymous with *Orbicella* sp

Coral	mm/year	References
Montastrea. annularis	6.3–11.2	Hudson (1981) (1); Huston (1985)
	6.6–8.9	Gladfelter et al. (1978) (2)
	8	Hubbard and Scaturo (1985) (3)
	6.8–7.3	Bak (1976) (4)
	(24)	see 4
	4.5–12.8	see 4
	5–11.3	Hudson et al. (1994)
	5.6–7.0	Miller and Cruise (1995)
Montastrea cavernosa	4.5	3
Porites astreoides	3–3.5	2
	3	3
Porites lutea	7–15	Nie et al. (1997) (6)
	15–25	Scoffin et al. (1992)
Agaricia agaricites	1.5	3
	17.2–24.9 diam	see 4
Sidastrea siderea	3	3
Diploria clivosa	3.5	3
Acropora cervicornis	71	2
A. prolifera	59–82	2
A. palmata	47–99	2
	60–100	4
A. elseyi	127	Atkinson et al. (1995)
A. pulchra	(206)	Atkinson et al. (1995)

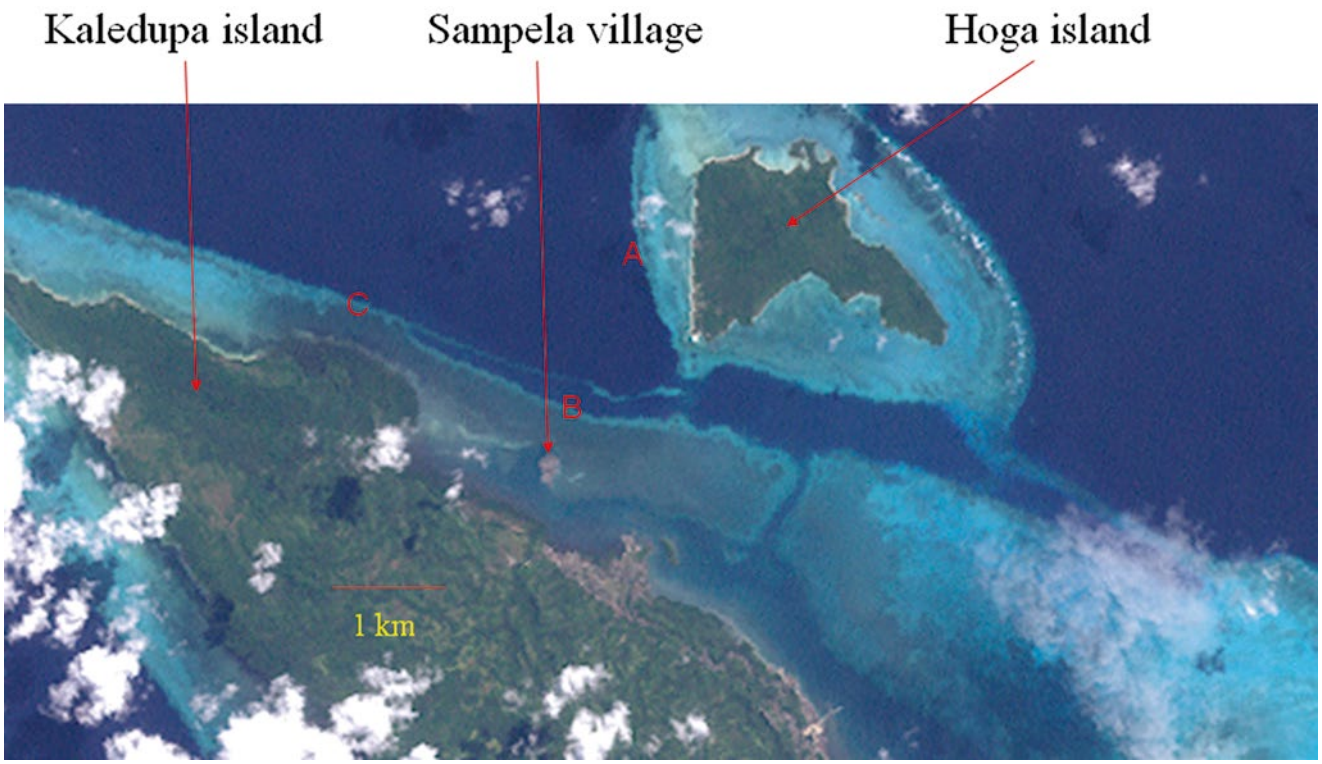

Fig. 35.1 Diagram showing the sites studied in the Wakatobi Marine National Park. (**a**) Hoga reef study site (low sedimentation rate; 7.54 ± 0.76 g dry weight m_2 d_1, n = 22); (**b**) Sampela reef study site (high sedimentation rate; (mean = 20.16 ± 1.71 g dry weight m_2 d_1, n = 26)); (**c**) Kaledupa reef study site (low sedimentation rate; mean = 5.35 ± 0.68 g dry weight m_2 d_1, n = 22) (Modified from Crabbe and Smith 2005)

Table 35.2 Regional climatic characteristics from remote sensing and meteorological sources. Modified from Crabbe and Smith 2005

	Wet season	Dry season
Cloud cover[a]	25 ± 4.1	8 ± 3.2
Rainfall[b]	3300 ± 400	850 ± 90
SSTs[c]	28.9 ± 0.3	27.2 ± 1.1

The slightly cooler SST in the dry season is probably due to a period of upwelling in the Banda Sea
[a]% age monthly cloud cover (±standard deviation) averaged from satellite data (Wet October 2001–March 2002; Dry April 2002–September 2002), over the whole of the Wakatobi Marine Park
[b]Rainfall (mm ± standard deviation) (Wet October 2001–March 2002; Dry April 2002–September 2002), from Government of Indonesia data
[c]Sea surface temperatures (monthly means ± standard deviation) in the Banda Sea (Wet October 2001–March 2002; Dry April 2002–September 2002)

Table 35.3 *Acropora valenciennesi* linear extension rates. Growth rates are given in mm/year, estimated from growth in wet and dry seasons. Modified from Crabbe and Smith 2005

	Wet season	Dry season	p value
Sampela	71 ± 5	113 ± 22	0.016
Hoga	135 ± 5	282 ± 19	0.000003
Kaledupa	142 ± 9	333 ± 42	0.0008

p values were calculated using nested ANOVA for variations between site and season. n = 18 for each site ±S.D

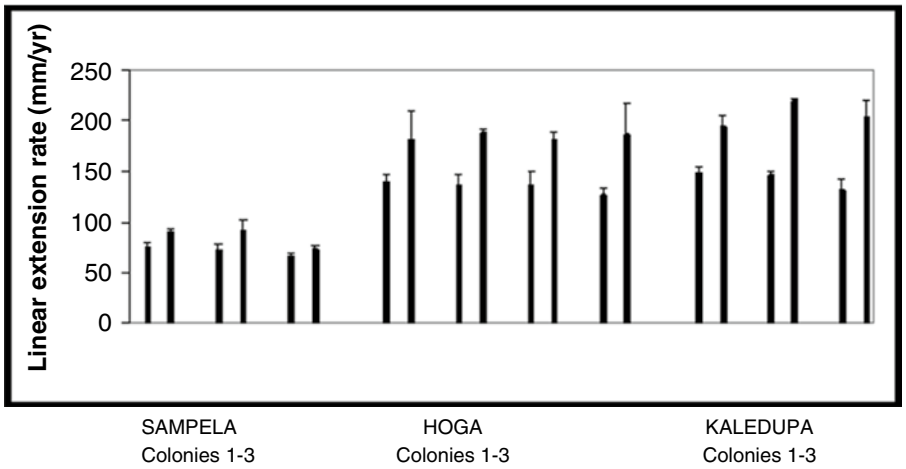

Fig. 35.2 *Acropora valenciennesi* linear extension rates, each colony measured from July 2001 to March 2002, and from July 2001 to July 2002. n = 6 for each colony. Three colonies were measured at Sampela (high sedimentation rate; mean = 20.16 ± 1.71 g dry weight m_2 d_1), and Kaledupa (low sedimentation rate; mean = 5.35 ± 0.68 g dry weight m_2 d_1), and four at Hoga (low sedimentation rate; 7.54 ± 0.76 g dry weight m_2 d_1). Error bars represent standard deviations (Modified from Crabbe and Smith 2005)

is a demarcation in cloud cover and rainfall in the area from October to March (the wet season), and from April to September (the dry season). Table 35.2 presents derived climatic characteristics. The slightly cooler SST in the dry season is probably due to a period of upwelling in the Banda Sea.

Table 35.3 and Fig. 35.2 show that *Acropora valenciennesi* linear extension rates (given as mm/year) were significantly higher in the dry season than in the wet season for all three sites. The relative increase in linear extension rates was significantly less at Sampela ($p < 0.004$) than at Kaledupa or Hoga, even though the sites were only about 1.5 km apart. These results are consistent with the hypothesis that the high sedimentation levels at Sampela would limit light availability. The radial growth rates for three genera of nonbranching corals (n = 3 for each genera [*Porites lutea*, *Montipora* sp. and *Favia* sp.] at each site) at the three sites at 10 m depths are shown in Table 35.4. Radial growth rates were also lower at Sampela than at the other sites for *Porites lutea* ($p = 0.004$), *Montipora* sp. ($p < 0.01$), and *Favia* sp. ($p < 0.01$).

Coastal development at Sampela impacted on this natural variability by decreasing the coral growth rates. At this site, and under light-limiting conditions, the coral *Galaxea fascicularis* has developed strategies to optimise energy utilization from heterotrophic feeding and photosynthesis (Crabbe and Smith 2006).

35.2.2 Caribbean

The fringing reefs around Discovery Bay in Jamaica constitute one of the best documented areas of reef decline in the Caribbean, where loss of corals and macroalgal domination has been due to hurricanes (Woodley et al. 1981; Crabbe et al. 2002), overfishing (Jackson 1997; Hawkins and Roberts

Table 35.4 Radial growth rates of non-branching corals. Corals (*Porites lutea*, *Montipora* sp. and *Favia* sp.) are at 10 m depths at Sampela, Hoga and Kaledupa. From Crabbe and Smith 2005

	Porites lutea	*Montipora* sp.	*Favia* sp.
Sampela	3.98 ± 1.32	1.75 ± 0.7	2.86 ± 2.5
Hoga	10.04 ± 3.34	9.23 ± 1.1	9.23 ± 1.3
Kaledupa	15.26 ± 4.83	9.74 ± 1.2	12.73 ± 4.1

n = 3 at each site. Growth rates are given as mm/year ± S.D

2004), die-off of the long-spined sea urchin *Diadema antillarum* in 1983–1984 (Hughes 1994), and coral disease (Aronson and Precht 2001). Nutrient enrichment does not appear to have been a causal factor in the development of the reef macroalgal communities (Greenaway and Gordon-Smith 2006).

These fringing reefs have seen a number of climate-related challenges in recent years, notably several hurricanes as well as a mass bleaching event in the Caribbean in 2005 (Jones et al. 2004, 2008). Despite all these negative factors, there is evidence that prior to 2005 some Discovery Bay reefs were recovering (Idjadi et al. 2006), although a study subsequent to the 2005 bleaching event is not so positive (Quinn and Kojis 2008).

The North Jamaican fringing reefs, unlike elsewhere in Caribbean, for example the reefs of Tobago (Mallela and Crabbe 2009), have shown some apparent resilience to acute disturbances from hurricanes and bleaching, in addition to the recurring chronic stressors of overfishing and land development (Idjadi et al. 2006; Crabbe 2009). Factors that can improve coral reef resilience include species and functional diversity, connectivity to larval sources, appropriate substrates for larval settlement, and protection from other anthropogenic effects. Rugosity (reef three dimensional complexity) (Dunnand and Halpin 2009) has been linked to reef resilience in the South Central Pacific (Adjeroud et al. 2009) and decline in reef complexity in the Caribbean (Alvarez-Filip et al. 2009). Viability of small coral colonies over time can used as a measure of reef resilience (Loya 1976; Connell 1978).

Figure 35.3 shows that there are significant relationships between mean rugosity values at fringing reef sites near Discovery Bay on the North coast of Jamaica and changes in both small (<250 mm^2) and medium-large sized (>250 mm^2) *Colpophyllia natans* colony numbers from 2006 to 2009. R^2 values were 0.77 for colonies <250 mm^2 and 0.91 for colonies >250 mm^2. From 2006 to 2009, after the mass bleaching event of 2005, there was an increase in small colony numbers, with a greater increase at sites with high rugosity (Crabbe 2010). For medium-large sized colonies, there was a decrease in colony numbers from 2006 to 2009 at sites where the rugosity was <1.8. Only at Dairy Bull, where the rugosity was highest at 2.3, did medium-large sized *C. natans* numbers increase between 2006 and 2009.

The continued presence of small coral colonies over time can indicate reef resilience, and increases in colony numbers of both the smallest size class (<250 mm^2) and the medium-large size class (>250 mm^2) for *C. natans* were significantly correlated with reef rugosity at all the sites studied around Discovery Bay on the North coast of Jamaica since the mass bleaching event of 2005. The major difference between the size classes is that where the rugosity is <1.8, i.e. for most of the sites studied, there is a decrease in colony numbers of the medium-large sized *C. natans*. This suggests that while recruitment and initial growth since 2005 has been successful for this species at the sites studied in North Jamaica, medium and large sized colonies of this species have decreased in numbers since the bleaching event at most sites, except where the rugosity is highest, at Dairy Bull reef. At Dairy Bull, the bleaching event in 2005 led to significant loss of coral cover, which allowed space for the development of new colonies. These findings suggest that the three dimensional topography and complexity is important for reef resilience and viability in the face of extreme environmental stressors. Interestingly, rugosity also correlates well with fish abundance on other reefs, for example with parrotfish (Scarid) abundance on reefs of Oahu, Hawaii, and rugosity has been used in regional modelling of coral habitats for marine conservation (Howard et al. 2009).

Increase in *A. cervicornis* cover was one of the main reasons for suggesting a phase-shift reversal at Dairy Bull reef (Idjadi et al. 2006). There was a significant linear relationship between percentage coral cover of *A. cervicornis* colonies with rugosity values for Dairy Bull reef, M1, New reef, Dancing Lady, Rio Bueno and Pear Tree Bottom (Fig. 35.4). The line is a best-fit linear regression, with $R^2 = 0.94$. The two reefs with the highest rugosity values, Dairy Bull reef and New reef, exhibited the highest cover of the urchin *D. antillarum* (measured at 0.4 ± 0.1 Diadema m^{-2} for both reefs), and no macroalgal cover in the vicinity of *A. cervicornis* colonies.

In contrast, the other reefs with lower rugosities exhibited extensive macroalgal cover and no *D. Antillarum* (Crabbe 2010).

Figure 35.5 shows mean percentage cover +1 S.E. of total live coral, and dominant species *Acropora cervicornis*, *Porites astreoides*, and *Acropora palmata* at Dairy Bull reef from 2002 to 2012. Live coral cover increased from 13 ± 5 % in 2006 to 31 ± 7 % in 2008, while live *Acropora cervicornis* increased from 2 ± 2 % in 2006 to 22 ± 7 % in 2008. Coral cover levels were maintained until 2012. Statistical analysis of the recovery showed that only *Sidastrea siderea* and

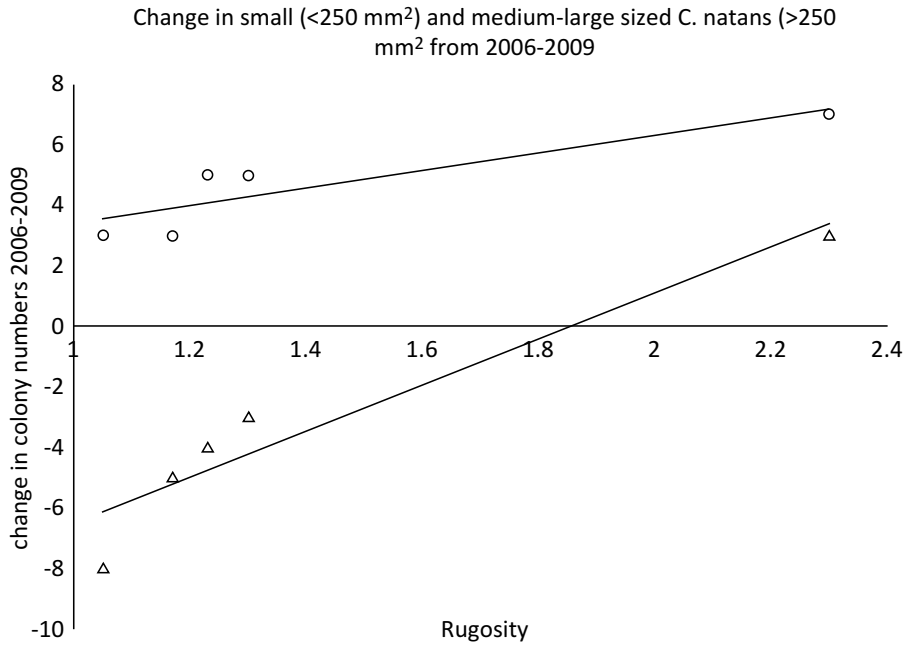

Fig. 35.3 Relationships between mean rugosity values on the North coast of Jamaica near Discovery Bay and changes in both small (0) (<250 mm^2) and medium-large sized (Δ) (>250^2) *Colpophyllia natans* colony numbers from 2006 to 2009. R^2 values were 0.77 for colonies <250 mm^2 and 0.91 for colonies >250 mm^2

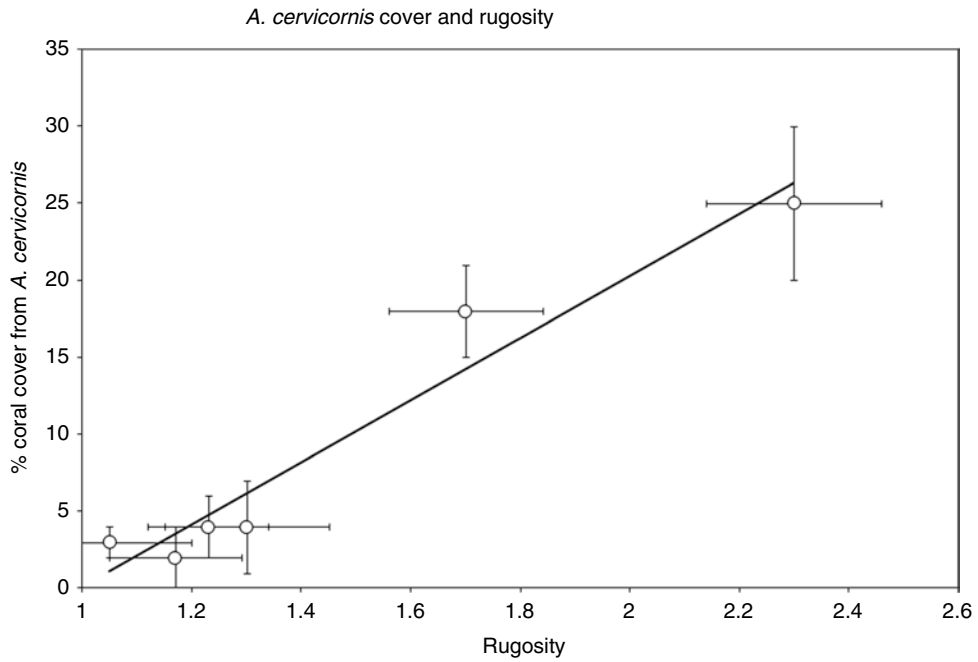

Fig. 35.4 Percentage coral cover ±1 standard error of *A. cervicornis* colonies against rugosity values ±1 standard error, for Dairy Bull reef (2.3), M1 (1.17), New reef (1.7), Dancing Lady (1.3), Rio Bueno (1.05) and Pear Tree Bottom (1.23). Mean rugosity values are in brackets. The line is a best-fit linear regression, with $R^2 = 0.94$. The six sites were: Rio Bueno (18° 28.805′ N; 77° 27.625′ W), CREWS (Coral Reef Early Warning Station) (18° 28.375′ N; 77° 24.921′ W), Dancing Lady (18° 28.369′ N; 77° 24.802′ W), M1 (18° 28.337′ N; 77° 24.525′ W), Dairy Bull (18° 28.083′ N; 77° 23.302′ W), and Pear Tree Bottom (18° 27.829′ N; 77° 21.403′ W). New reef is between Dancing Lady and M1, where *A. Cervicornis* cover was comparable to that at Dairy Bull reef (Modified from Crabbe 2010)

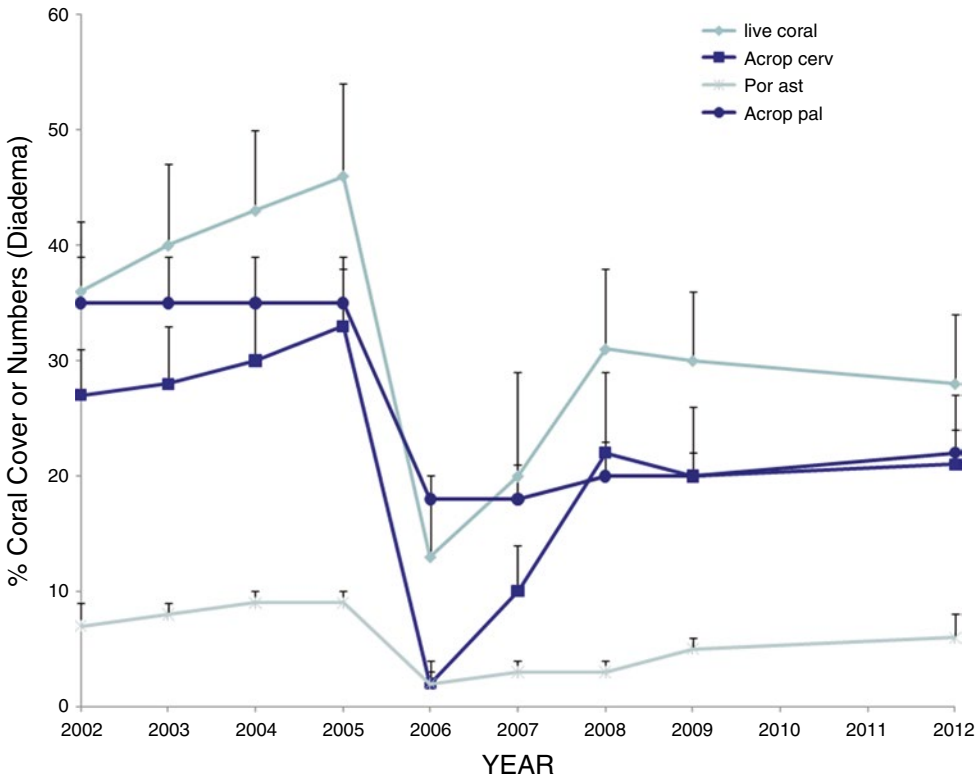

Fig. 35.5 Coral cover at the Dairy Bull reef site from 2002 to 2012, for: live coral (♦); *Acropora cervicornis* (■, Acrop cerv); *Porites astreoides* (▲, Por ast); *Acropora palmata* (●, Acrop pal). Values are shown + 1 S.E

Diploria strigosa had coral cover in 2012 that was statistically significantly similar to coral cover in 2002 and 2005 before bleaching. Interestingly, growth rates of both branching and non-branching corals showed similar values throughout the study period, with trends, not significant, for slightly higher values at Dairy Bull reef. It is encouraging that coral cover and the rapidly growing *A. cervicornis* colonies have returned to the reef at levels approaching pre-bleaching values, even over a fiftenn-year period (Crabbe 2016). This site shows relatively high rugosity (Fig. 35.4) and the influence of *M. annularis (Orbicella)* colonies on the reef, acting as structural refugia (Idjadi et al. 2006) and maintenance of biological legacies and reasonably fast growth (Díaz-Pulido et al. 2009) may have facilitated this recovery. Coral resilience has also been noted on the reefs of Bonaire (Bruckner 2012). Interestingly, there are a number of different clades of zooxanthellae, including clade C, in corals at Dairy Bull reef (Crabbe and Carlin 2007), and that may also be a factor in their recovery (Stat et al. 2008). Macroalgal density in the surrounding habitat has a strong influence on species driving the process of macroalgal removal on reefs post-disturbance (Chong-Seng et al. 2014).

A number of hurricanes that had the potential to impact the reef sites during the study period shown in Fig. 35.5. Only one of these storms caused any significant damage on the reefs, Ivan in 2004, a category 4 hurricane as it passed south of the island. Visually, the damage was minimal as far as reef destruction was concerned, with some *Acropora palmata* colonies being fragmented and overturned, notably at Pear Tree Bottom (personal observation). Although hurricane Emily in 2005 was also a category 4 hurricane, the eye passed sufficiently south of the island so that the impact involved sediment transfer owing to the high winds and rain (Crabbe and Carlin 2007). Tropical storms Iris (category 1 hurricane, 2001), Lili (tropical storm, 2002), Bonnie (tropical wave, 2004), Charley (category 1 hurricane, 2004), Dennis (category 3 hurricane, 2005), Olga (tropical storm, 2007) and Dean (hurricane category 4, 2007) did not cause significant damage to the reef sites.

The only bleaching event that significantly impacted the reef sites during the study period was the mass Caribbean bleaching event of 2005 (Eakin et al. 2010). Analysis of satellite data showed that there were 6° heating weeks (dhw) for sea surface temperatures in September and October 2005 near Discovery Bay, data which was mirrored by data loggers on the reefs (Quinn and Kojis 2008).

What is apparent is that despite the chronic and acute disturbances between 2002 and 2008, demographic studies suggest coral resilience on some fringing reefs around Discovery Bay in Jamaica. The bleaching event of 2005 resulted in mass bleaching but relatively low levels of mortality (Quinn and Kojis 2008), unlike corals in the US Virgin islands where there was extensive mortality (Miller et al. 2006; Whelan et al. 2007), possibly because of their greater degree heating week values. Marine park managers may need to assist coral

recruitment and settlement in years where there are severe acute disturbances, including hurricanes and bleaching events, by setting up coral nurseries (Forsman et al. 2006) and/or natural or artificial high rugosity substrate on the reef. In addition, where the insults to coral reef are of a long term human-induced chronic nature, such as overfishing and land development (Adger et al. 2005; Mora 2008; Mumby and Hastings 2008), engagement with fisherfolk and land developers is vital to minimise the human-induced threats which are so damaging to coral reefs. Unfortunately, previously successful efforts to engage the local fisherman in controlling catches around Discovery Bay (Sary et al. 1997) have not been maintained, and it may that development of a legally enforced and extensive Discovery Bay Marine Park is the only solution.

35.3 Modelling of Coral Growth and Morphology

35.3.1 Modelling Coral Growth

Classically, exponential, logistic, Gompertz or von Bertalanffy equations, or modifications of them, have been used in growth studies of many species (Ricklefs 1967, 1968; Reiss 1989). Rational polynomial functions have been used to model many biological processes, including the catalysis of enzyme reactions, membrane transport, and other molecular processes (Crabbe 1984, 1988). The general rational polynomial function as a model for coral growth may be described as:

$$dW/dt = \frac{a_1W + a_2W^2 + a_3W^3 + Ka_nW^n}{1 + b_1W + b_2W^2 + b_3W^3 + K\,b_mW^m} \qquad (35.1)$$

where dW/dt is the increase of coral weight with time; W is the variable (coral weight), up to the power of n in the numerator and m in the denominator; $a1 \ldots an$ and $b1 \ldots bm$ are parameters that relate to the 'rate constants' for growth. The values for n and m represent the degree of the polynomial. Usually in this model n will equal m, as there will be little decline in growth that cannot be explained by an equal number of terms in the numerator and denominator. Although in the equation above coral weight is used as an indicator of growth, other parameters, length, or surface area, may be equally applicable.

In an extensive study, Bak (1976) investigated the growth of a number of coral colonies in reefs on the south-west coast of Curaçao. Those data have been fit to a number of growth models (Crabbe et al. 2002). Curvefitting was performed using the SIMFIT package (Bardsley 1993; Bardsley et al. 1996), a suite of 40 programs dedicated to statistical analyses of datafitting. Bak (1976) fitted the data he obtained from weighing corals underwater to exponential function(s), the simplest way to fit the results of his growth experiments.

Table 35.5 shows the best fit parameters (sum of squares and F statistic) for the five species (six data sets) studied, with weight ranges of 0–1000 g (*Acropora palmata*), 0–250 g (*Agaricia agaricites*), 0–170 g (*Montastraea annularis*), 0–350 g (*Meandrina meandrites*) and 0–50 g (*Madracis mirabilis*), and the best fit functions at the 5 % level. Table 35.6 shows a comparison of best fits to data for polynomial, logistic, Gompertz and von Bertalanffy models for the data sets. The equations used were as follows:

Polynomial: Eq. (35.1), where n = m = 1, 2 or 3

Logistic: $dW/dt = \left(A / \left[1 + B\exp\{-CW\}\right]\right) + D$

Gompertz: $dW/dt = A\exp\left(-B\exp\left[-CW\right]\right) + D$

Von Bertalanffy: $dW/dt = A\left(1 - B\exp\left[-CW\right]\right)^D$

where W = weight, exp = exponential, and A, B, C and D are constants (not equivalent for all models).

Fitness of the model was judged by a combination of low values of sum-of-squares, and % coefficient of variance. For all the models tested, the rational polynomial model was the most consistent best fit throughout, when both the sum of the

Table 35.5 Fits of growth data from individual coral species to rational polynomial functions judged by sums of squares of residuals (SS), and the F test statistic (FS) for improvement of it of the more complex model over the previous model

	1:1	2:2		3:3		Best fit	
Species	SS	SS	FS	SS	FS	(5 %)	Morph.
Ac. Palm.	1.95E5	3.21E3	4.77E2	1.58E3	7.25	3:3	B
Mad. Mir.	1.64E4	1.45E3	1.03E2	6.59E2	1.08E1	3:3	B
Ag. agar.(dp)	5.85E2	9.64E1	4.31E1	7.03E1	2.79	2:2	P
Ag. agar.(sh)	1.83E3	1.10E2	1.17E2	9.45E1	1.09	2:2	P
Mean. Mean.	1.44E2	5.62E2	3.89		–	1:1	M
Mon. ann.	2.60E2	2.54E2	1.85E1		–	1:1	M

The table also gives the best fit function at the 5 % level. Growth data from Bak 1976

For species abbreviations *Ac. palm* Acropora palmata, *Ag. agar* Agaricia agaricites, (*dp*) deep, 24 m, (*sh*) shallow, 13 m, *Mon. ann* Montastrea annularis, *Mean. mean* Meandrina meandrites, *Mad. mir Madracis mirabilis. Morph* coral morphology, *B* branching coral, *P* plate coral, *M* massive coral

Table 35.6 Comparison of best fits to data for rational polynomial, logistic, Gompertz and von Bertalanffy models for data sets

	Rational polynomial		Logistic		Gompertz		von Bertalanffy	
Species	SSQ	co.var.%	SSQ	co.var%	SSQ	co.var.%	SSQ	co.var%
Ac. Palm.	1.58E3	3.02	3.08E3	3.83	1.81E3	2.94	2.2E5	32.33
Mad. Mir.	6.58E2	4.51	1.79E3	6.99	1.26E3	5.77	3.58E4	30.77
Ag. agar. (dp)	7.03E1	1.88	7.58E2	5.64	3.59E2	3.88	6.49E3	16.49
Ag. agar. (sh)	9.45E1	2.63	2.81E2	4.09	1.10E2	2.56	7.27E3	20.81
Mean. Mean.	1.44E2	2.89	3.42E2	6.51	1.69E2	4.58	1.68E3	14.46
Mon. ann.	2.60E2	2.63	1.07E2	5.50	5.93E2	4.09	4.23	10.93

For species abbreviations *Ac. palm* Acropora palmata, *Ag. agar* Agaricia agaricites, *Mon. ann* Montastrea annularis, *Mean. mean* Meandrina meandrites, *Mad. mir* Meandrina meandrites

Table 35.7 Comparison of best fits to data for rational polynomial, exponential, logistic, and von Bertalanffy models for *Orbicella* (*Montastrea*) *annularis*. (3 data sets)

	Rational polynomial		Logistic		Gompertz		von Bertalanffy	
Species	SSQ	co.var.%	SSQ	co.var%	SSQ	co.var.%	SSQ	co.var%
Mon.ann. 1	2.76E0	1.56	2.62E2	14.84	3.81E1	2.94	2.06E2	13.34
Mon.ann. 2	1.19E0	1.16	1.92E2	14.10	1.67E1	4.24	9.33E1	10.02
Mon.ann.3	3.42E-1	0.81	8.96E1	13.11	1.31E1	5.09	1.08E2	14.62

squares and the coefficient of variation were taken into account. An exponential fit produced similar fits to the logistic fit, and the logistic relationship when surface area and volume were taken into consideration, but there was no significant improvement in the fit.

It is interesting that for *A. agaricites*, the maximum projected size attainable from Eq. (35.1) was slightly greater in deep water than in shallow water, although the time taken to achieve it is longer, as has been found experimentally for *Diploria* sp. in reefs in Bermuda (Logan et al. 1994). The parameters themselves influence the curve shapes as well as the rates of growth and the maximum size achievable. For the six sets of data studied here, four of them show evidence of sigmoidicity. While none shows evidence of passing through a maximum, that may reflect on the time scale used to determine growth, and on the environment of the study. Bak (1976) reported that some individual growth curves of *A. palmata* did show damage during the observation period, resulting in a sigmoid curve passing through a maximum; this too can be modelled using Eq. (35.1) where $m > n$.

In another data set, three *M. annularis (Orbicella)* colonies, ca 40 years old, were collected from a reef off Barbados, from ca 12.5 m depth, sectioned, X-rayed to reveal the skeletal density bands, and X-ray photographs taken as described (Dodge and Vaisnys 1975; Carricart-Ganivet et al. 2000). Table 35.6 is a comparison of best fits to three data sets for rational polynomial, exponential, logistic, and von Bertalanffy models for 40 years of growth (see Crabbe et al. 2002). The Gompertz fit produced similar results to the von Bertalanffy fit. Curvefitting using SIMFIT showed that in each data set Eq. (35.1) gave significantly better fits to the growth band data than the other models (Table 35.7).

The rational polynomial model produced significantly better datafitting to reef coral growth, whether by weight or by growth bands, than classical models. The model is for all corals, not just the massive or the branching species. Previous models, whether exponential, logistic, von Bertalanffy etc. have also been used to model coral growth independent of species. Interestingly, the model produced parameters that related to coral morphology, where branching corals have more complex 3:3 functions than non-branching corals, at least on the small numbers of data sets used. The plate coral *A. agaricites* gave a best fit 2:2 function, while the massive corals gave best fit 1:1 functions. For these five species of coral, there was good correlation ($r = 0.92$) between rates of growth (judged by the time taken to achieve near maximum growth) and the degree ($n = m$) of the rational polynomial function. Both *M. annularis (Orbicella)* and *M. meandrites*, which showed 1:1 function growth, are non-branching, and have a lower surface area than the other four types of coral, which are plate or branching corals. The plate coral gave a best fit 2:2 function, while the highest surface area branching corals gave best fit 3:3 function growth curves.

Growth of undamaged *A. palmata* colonies over a 400 days period showed best fit to a 3:3 function, as illustrated in Fig. 35.6 where the 3:3 fit was significantly ($p < 0.002$) better than a 2:2 fit. A 4:4 function gave no significant improvement over the 3:3 function. This was also the case for other *Acropora* species, including *Acropora valenciennesi* colonies in Indo-Pacific fringing reefs of Sulawesi, Indonesia.

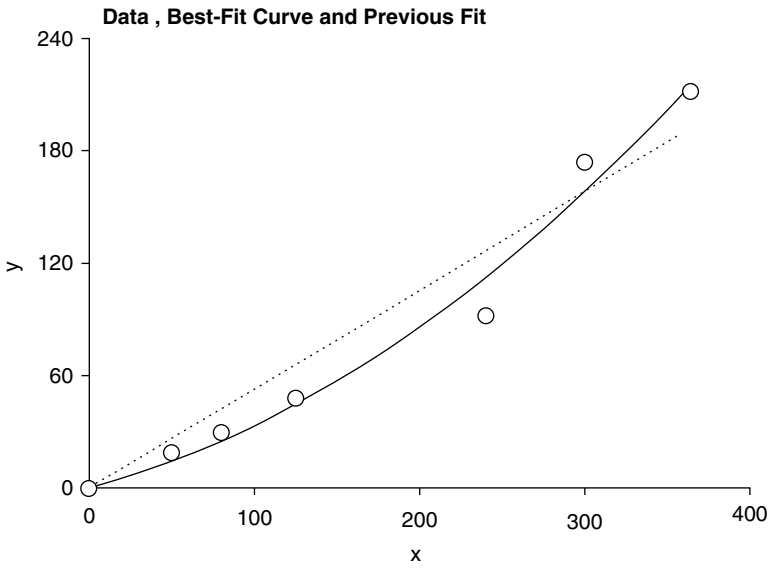

Fig. 35.6 Growth of an undamaged *Acropora palmata* colony, with best fit 2:2 (*dotted line*) and 3:3 (*solid line*) rational polynomial function. The x axis shows the time in days; the y axis the growth in cm (Modified from Crabbe 2007)

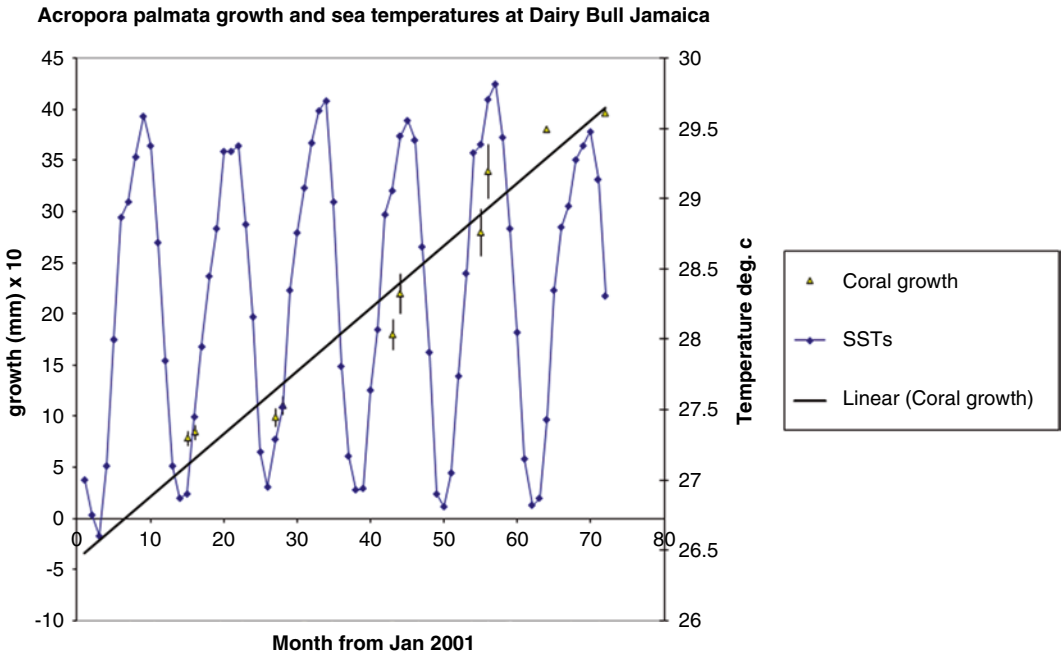

Fig. 35.7 Mean growth, on a logarithmic scale, of two *A. palmata* colonies from the Dairy Bull fringing reef of Discovery Bay, Jamaica, and the SSTs for the area, from 2001 to 2007. •, Sea surface temperatures (SSTs) °C; ▲, mean growth on log scale of two *A. palmata* colonies ($n=5$ for each colony) (Modified from Crabbe 2007)

Figure 35.7 shows the mean growth, on a logarithmic scale, of two *A. palmata* colonies ($n=5$ for each colony) from the Dairy Bull fringing reef of Discovery Bay, Jamaica, and the SSTs for the area, from 2001 to 2007. Over this period, growth could be modelled on a logarithmic scale. The high SSTs around September 2005, which occurred throughout the Caribbean, caused bleaching and mortality of some *Acropora* colonies (both *palmata* and *cervicornis*) at Dairy Bull, but less mortality at Rio Bueno and Pear Tree Bottom.

Figure 35.8 shows a plot of logarithmic rate of growth of *A. Palmata* against rate of change of SSTs, over the period 2002–2007. The relationship is predominantly linear, with $R^2=0.935$. This shows that within the temperature limits for coral growth, that rate of colony growth is proportional to rate of temperature change.

Coral growth rates can depend on both maximum and minimum seasonal temperatures (Slowey and Crowley 1995). What is apparent, at least for *Acropora palmata* branching corals, while growth can be approximated to a

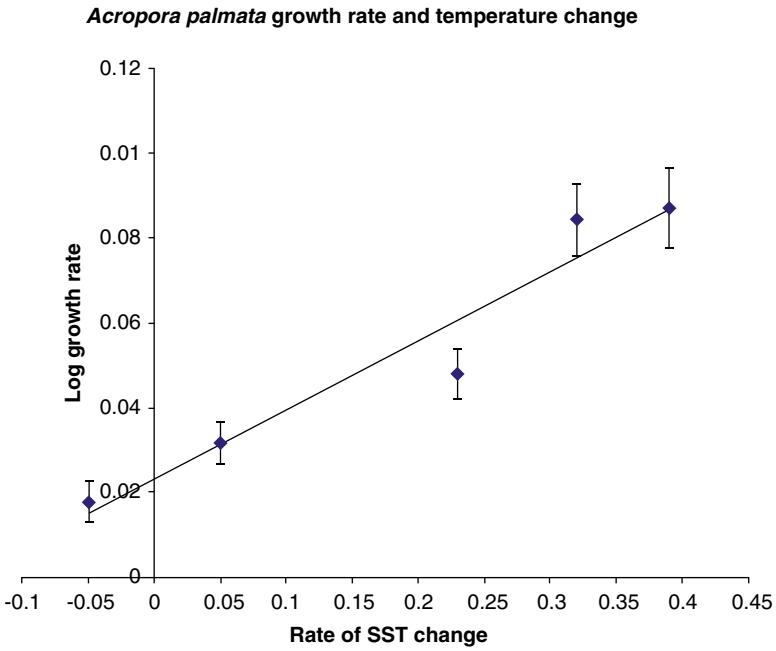

Fig. 35.8 Logarithmic rate of growth (mm) of *A. palmata* ± S.E. ($n = 10$) per 30 days against rate of change of SSTs per 30 days, over the period 2002–2007 (Modified from Crabbe 2007)

logarithmic function, particularly over several years growth, a rational polynomial function provides a more accurate model for growth. Accurate growth modelling is important as it allows more accurate and timely investigation of the role of anthropogenic and climate effects on corals, which in turn enables more accurate prediction and management of coral reef ecosystems and the fisheries derived from them. Over the period 2002–2007, involving several cycles of SST change, rate of growth of *A. palmata* is largely proportional to rate of change of SST. However, as temperatures approach those where bleaching can occur, i.e. the maximum sustainable temperatures for coral growth, rates of growth can fall. This was shown in a study of *A. Palmata* colonies on a fringing reef of Curacao (Bak 1976) modelled using a smoothing spline to produce a nonparametric fit to the data (Crabbe et al. 2008). Taken together, these results show how sensitive *A. palmata* colonies are to fluctuations in temperature. Coral reefs are disturbance-adapted ecosystems (Connell 1997) with a 'shifting steady-state mosaic' of large scale reef structure (Done 1999). We need to continue to develop models of how non-steady-state processes such as global warming and climate change will effect coral reefs.

35.3.2 Modelling Coral Morphology

The morphogenesis of colonial stony corals is the result of the collective behaviour of many coral polyps depositing coral skeleton on top of the old skeleton on which they live. Yet, models of coral growth often consider the polyps as a single continuous surface. Each polyp takes up resources, deposits skeleton, buds off new polyps and dies. In a polyp-oriented model, spontaneous branching occurs. One model argues that branching is caused by a "polyp fanning effect", by which polyps on a convex surface have a competitive advantage relative to polyps on a flat or concave surface. The fanning effect generates a more potent branching mechanism than the Laplacian growth mechanism that studied previously (Merks et al. 2003). In this model, the spacing of polyps influences the thickness of coral branches and the overall compactness of the colony. Density variations in the coral skeleton may also be important for the whole colony morphology (Merks et al. 2004).

Methods from fractal geometry are applied to model growth forms, taking as a case study a type of growth process which can be found among various taxonomic classes such as sponges and corals. These models can be used, for example, to understand the amazing variety of forms to be found in a coral reef and to simulate their growth with 2D and 3D geometrical objects. Models which mimic the growth of forms and the environmental influence on the growth process are also useful for ecologists, as a combination of simulation models together with the actual growth forms can be used to detect the effects of slow changes in the environment (Kaandorp 2012).

This model, called PORAG (Polyp Oriented Radiate Accretive Growth) considers each polyp as an individual, fending for itself. This is not entirely true, as it is known that many coral species have polyps which are connected by a gastrovascular cavity (which runs through the coenosarc), allowing them to transfer nutrients between each other. The point is that direct food capture may still be beneficial, and therefore there still is some competition going on. The model

considers all polyps as individuals, which take up nutrients, deposit skeleton, are budding off new polyps and die occasionally. In this polyp-oriented model, spontaneous branching of colonies occurs without pre-programming.

One can also model the effect of polyp spacing – the amount of space which exists between individual polyps. In the PORAG model, the size of inter-polyp space drastically altered a coral colony's shape. The larger the inter-polyp distance, the thicker and more compact the colony's branches become. Merks et al. (2004) also experimented with diffusion of nutrients between polyps, which is known to occur in many species such as *Stylophora pistillata*. The higher the diffusion in the model, the more branching occurred.

While genes programme how different coral species grow (branching, plate-like, massive, encrusting), the environment can fine-tunes this process. For example, abiotic factors such as water flow and light availability will alter a coral's shape. More flow seems to stimulate thicker branches, as seen with *Stylophora pistillata*, and more light seems to stimulate vertical instead of horizontal growth (low light may cause plate corals such as *Montipora* spp. to create a larger surface area by growing horizontally). The PORAG model has helped scientists understand why factors such as polyp competition may have caused many species to be programmed to grow into branching colonies. This strategy may enable the individual polyps to take up more nutrients. Further details and modeling of calcification can be found in this volume in the chapter on *Modelling and analysis of growth and form of the scleractininan corals using a multi-scale approach*.

35.4 Protecting Coral Growth: Integrated Coastal Zone Management (ICZM) and Marine Protected Areas (MPAs)

Against a backdrop of natural and anthropogenic insults, an important question is: how can management practices maintain sustainable coral reef ecosystems? Integrated Coastal Zone Management (ICZM) is a complex worldwide governance issue requiring an integrated and coordinated approach. It involves many relevant stakeholders and policy initiatives need to be developed over long time scales once conservation priorities are established (Curnick et al. 2015). Ideally, marine ecosystems (i.e., corals, seagrass beds) should be closely linked to terrestrial ecosystems such as mangroves and coastal forests. In developing management policies, education and training to enhance human skills and institutional capacity in resource management is critical (Wescott 2002; Cho 2005). Both developed and developing countries have used capacity-building programs (Kaplan et al. 2006). While many, if not all, of these programs involve building competencies and empowerment in local communities, few of them involve policy makers or government officials (Mequanent and Taylor 2007). Partnerships can be vital for ICZM, particularly where government policies link to local stakeholders (e.g., beach clean-up groups and marine wildlife associations) to produce collaborations that can involve people with vested interests in the coastal ecosystem (e.g., fishers, tour operators) and in ongoing management frameworks (Gray and Hatchard 2008).

The effective application of ICZM to coral-reef ecosystems should address a number of themes including: (1). Use of ecosystem and economic parameters to quantify the needs of marine reserves. (2). Development of tactics for leading, educating, and supporting issues regarding sustainable development of coral reef ecosystems. (3). Incorporation of all relevant stakeholders into the formulation of policy issues pertaining to marine resource management zoning plans. Table 35.8 identifies a set of 12 management operational needs identified as part of a capacity-building exercise in Belize (see Crabbe et al. 2010) as a united action plan that involved partnerships among government, non-governmental organizations (NGOs), and communities to improve ICZM.

The 2012 IUCN report on Caribbean coral reefs points to the decline of Caribbean reefs from c. 50 % cover in the 1970 to just 8 % today. They call for strictly enforced local action to improve the health of corals, including limiting fishing through catch quotas, an ex- tension of MPAs, a halt to nutrient run-off from the land, and a reduction on the global resilience on fossil fuels. Protection using *fully protected* MPAs is one of the few management tools that governments and local com- munities can use to combat large scale environmental impacts. In an environment where carbon dioxide concentrations predicted to occur at the end of this century would significantly reduce coral settlement and crustose coralline algae cover, and would so reduce successful coral recruitment and larval settlement, establishing and maintaining fully-protected marine parks in Jamaica and elsewhere in the Caribbean is one tool to help the future of the fishing industry in developing countries.

Where protection in MPAs was not found to reduce the effect of warm temperature anomalies on coral cover declines, then shortcomings in MPA design, including size and placement, may have contributed to the lack of an MPA effect. It may be that the benefits from single MPAs may not be great enough to offset the magnitude of losses from acute thermal stress events. Although MPAs are important conservation tools, their limitations in mitigating coral loss from acute thermal stress events suggest that they need to be complemented with policies aimed at reducing the activities responsible for climate change. One way forward is to have networks of MPAs and they could be more effective in con-

Table 35.8 A set of 12 management operational needs identified as part of a capacity-building exercise in Belize (see Crabbe et al. 2010) as a united action plan that involved partnerships among government, nongovernmental organizations (NGOs), and communities to improve ICZM

1. Ecosystem zonation redesignated to balance stakeholders' wishes and evidence-based fisheries catches
2. A community-based research program developed via participants. This involved local fishers, with qualitative and/or quantitative research methods
3. Data of high accuracy recorded. Quantitative ecosystem data needs to be verified statistically
4. Co-management plans between NGOs, communities and fisheries departments to address problems of illegal fishermen from states or countries outside the governance of the MPAs. This is a significant problem in reef areas close to Belize
5. Regular public meetings of stakeholders fostered, as well as regular education events. Action plans were developed and monitored by staff and stakeholders alike
6. Effectiveness of zoning monitored and quantified. This relates to fishing practices as well as ecosystem health
7. Alternative livelihoods for fishers (e.g., in the tourist industry) fostered and maintained. Government agencies were involved in linking tourism and economic development
8. Tourists monitored and encouraged sustainably. All stakeholders were involved, with penalties for unsustainable practices
9. Effective management linked to the country's economy. This is helped in Belize as fishing, and tourism are both important parts of the country's gross domestic product (GDP)
10. NGOs and MPAs link together. In areas where different NGOs are responsible for MPA management, as in the MesoAmerican Barrier Reef, and where MPAs are distant from one another, it was helpful to link both NGOs and MPAs so that a greater area of reef could be managed
11. Regular information to all stakeholders, from the politicians to the local communities, maintained. Communication linked to the communities served (e.g. some oral, some printed, some via internet)
12. Management plans passed into law. The involvement of government officers –e.g., fisheries officers– as partners is key to this important outcome, to ensure appropriate policing if resources are made available

junction with other management strategies, such as fisheries regulations and reductions of nutrients and other forms of land-based pollution. Developing large and connected MPAs (see Crabbe 2015) as part of an overall climate change policy for a country may be the best way of integrating climate change into MPA planning, management, and evaluation.

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Cold-Water Corals in an Era of Rapid Global Change: Are These the Deep Ocean's Most Vulnerable Ecosystems?

36

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Abstract

Cold-water corals create highly complex biogenic habitats that promote and sustain high biological diversity in the deep sea and play critical roles in deep-water ecosystem functioning across the globe. However, these often out of sight and out of mind ecosystems are increasingly under pressure both from human activities in the deep sea such as fishing and mineral extraction, and from a rapidly changing climate. This chapter gives an overview of the importance of cold-water coral habitats, the threats they face and how recent advances in understanding of both past and present cold-water coral ecosystems helps us to understand how well they may be able to adapt to current and future climate change. We address key knowledge gaps and the ongoing efforts at national and international scales to promote and protect these important yet vulnerable ecosystems.

Keywords

Cold-water coral • Ocean acidification • Aragonite saturation horizon • *Lophelia pertusa* • Conservation

36.1 Introduction

Corals are a highly diverse and evolutionarily adaptable group, with more than 5000 species described to date. Of these around 65 % are found in waters greater than 50 m deep (Cairns 2007; Roberts et al. 2009) and among this hidden coral diversity are the habitat forming cold-water corals. Given suitable substrate and food supply conditions cold-water corals can develop elaborate framework reefs (primarily corals from the order Scleractinia) or extensive coral gardens (primarily corals from the orders Octocorallia and Stylasteridae) which provide highly complex biogenic habitats for other species and thus play critical roles in deep-water ecosystem functioning across the globe.

Targeted research on cold-water corals began to grow exponentially in the late 1990s, as technological developments brought their deep-sea habitats within the range of underwater remotely operated vehicles (ROVs). This revealed physically complex cold-water coral ecosystems that promote and sustain a biological richness in the deep sea unrivalled by many other ecosystems, and produced a step change in scientific understanding and public awareness of these remote areas (Fig. 36.1). Cold-water corals support a rich variety of lifeforms. At a microbial level bacterial and fungal biofilm communities encrust coral surfaces. Grazers, deposit feeders and small benthic predators forage among the polyps and branches of corals. Larger coral surfaces are colonized by sessile active and passive suspension feeding

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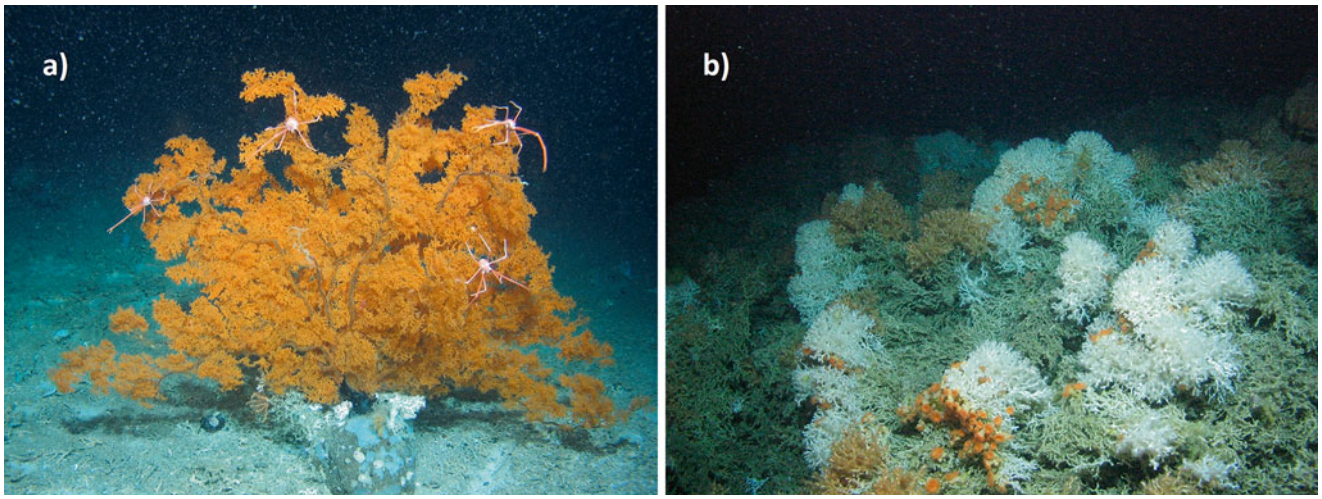

Fig. 36.1 Examples of (**a**) anomuran crustaceans on a *Leiopathes* sp. (Order Antipatharia) in a coral garden and (**b**) a *Lophelia pertusa* reef both at the Logachev mounds West of the UK and Ireland (Photographs taken during the 2012 Changing Oceans Expedition, RRS *James Cook* cruise 073 (Roberts and shipboard party 2013))

epifauna and a variety of teleost and elasmobranch fishes use cold-water coral habitats to forage, to seek refuge and to breed. Many species have mutualistic or commensal symbiotic relationships with the corals or other associated fauna, such as the well-known mutualistic relationship between the scleractinian cold-water coral *Lophelia pertusa*, the dominant framework building coral in the North Atlantic and the eunicid polychaete *Eunice norvegica* (Roberts 2005). Actinarians live on the glass sponge *Aphrocallistes* sp. in a commensal relationship in cold-water coral reef habitats constructed by *Lophelia pertusa* and *Enallopsammia profunda* in the Campeche Bank area in the southern Gulf of Mexico (Hebbeln et al. 2014), and further research may identify a mutual benefit to the hard corals themselves such as protection of coral surfaces from grazers. Just over half of symbioses with cold-water corals tend to be obligate parasites, a relationship most strongly pronounced with gorgonian hosts (Buhl-Mortensen and Mortensen 2004).

The biodiversity hotspots provided by cold-water corals are in part explained by the close interactions between seafloor topography and impinging water currents that drive enhanced food flux (Davies et al. 2009). This biophysical coupling supplies high quality food to deep-sea organisms that is otherwise scarce or erratic in supply. Critically, the combined persistence of this physical complexity, stability, and the flow of carbon and nutrients through these habitats is vital to maintaining such high levels of biodiversity. The significantly higher levels of biogeochemical cycling and respiration taking place in these habitats (Cathalot et al. 2015; Rovelli et al. 2015) in comparison to the surrounding seafloor contributes to the wider ecosystem functioning of the deep-sea biome and global carbon budgets, highlighting the importance of these largely unseen ecosystems. However, whilst studies have provided glimpses into this incredible world, they have also revealed the extensive damage they have suffered from deep-sea bottom trawling and the vulnerability of cold-water corals and the biodiversity they support to climate change, in turn prompting international efforts towards their management and conservation (Roberts et al. 2009).

This chapter will give a brief overview of our understanding of cold-water coral biology and ecology in a changing environment (with a primary focus on the Scleractinia), and how the longevity of the corals and the structures they produce opens windows into the environments they experienced in the past. With research 20 years ago largely focused upon geological mapping and habitat characterization we are now entering an era when sound biological and ecological understanding of these habitats must underpin management across local, regional and global scales. With an ever expanding human population, the pressures on global resources are leading to increased fishing, mining and hydrocarbon extraction in the deep sea. Alongside the unprecedented threat of climate change, cold-water corals will be exposed to multiple stresses from anthropogenic activities and may be amongst the most vulnerable of marine ecosystems.

36.2 Cold-Water Corals in an Era of Rapid Global Change: Present-Day

It is now well established that anthropogenic carbon emissions are driving an unprecedented rise in global temperatures (IPCC 2013). For marine life this is coupled with ocean acidification, raising concerns for the future of calcifying species, such as corals. Much of the research to date has

focused on tropical corals and implies that rising seawater temperatures are the more immediate threat to shallow reefs, leading to wide scale bleaching events (Frieler et al. 2013). For deeper dwelling cold-water corals however, the effects of elevated CO_2 on seawater carbonate chemistry are likely to have more impacts in the immediate future than for their shallow water counterparts (Hennige et al. 2014a). This is because the distribution of the coral reefs of the deep sea is highly dependent, at the global scale, on seawater carbonate chemistry (Guinotte et al. 2006), as corals require aragonite and calcite, polymorphs of calcium carbonate, to build their skeletons. Both these compounds are supersaturated in surface waters but become less saturated with depth largely due to the effect of pressure on the $CaCO_3$ solubility constant. Variations in in situ CO_3^{2-} concentration result in the saturation horizon for $CaCO_3$ being found at different depths in different ocean basins, and due to the different solubility of $CaCO_3$ polymorphs, seawater becomes undersaturated firstly for aragonite (termed the aragonite saturation horizon, ASH) and then for calcite (the calcite saturation horizon) as depth increases (Cairns 2011). In ocean basins with higher calcium carbonate saturation states the depths to which aragonitic corals can persist are far greater, helping in part to explain the proliferation of scleractinian reef framework forming corals in the Atlantic (Guinotte et al. 2006; Roberts et al. 2006). Areas of the global ocean with naturally lower CO_3^{2-} and hence calcium carbonate saturation states, such as the north Pacific, support far fewer aragonitic corals, but corals which build their skeletons from calcite can persist and also provide important structural habitats (Stone 2014). As atmospheric CO_2 levels rise, the oceans absorb more CO_2 and become more acidic (Zeebe and Wolf-Gladrow 2005). As a result, ocean pH has already fallen by 0.1 pH units and will likely fall another ~0.3 units by the end of the century (Caldeira and Wickett 2003). Such a pH decline shifts the distribution of dissolved carbon species away from CO_3^{2-} and hence directly impacts $CaCO_3$ saturation states (Feely et al. 2004; Orr et al. 2005). By 2100 the ASH will become sufficiently shallow to expose approximately 70 % of known aragonitic cold-water corals to corrosive waters undersaturated with respect to aragonite (Guinotte et al. 2006). Eventually the calcite saturation horizon will also shallow, exposing calcitic corals to corrosive conditions, but aragonitic corals face the more immediate threat. Given the importance of seawater carbonate chemistry for cold-water coral reef frameworks, and the direct relationship between aragonite saturation and atmospheric CO_2, they appear to be one of the most vulnerable marine ecosystems to present-day anthropogenic climate change.

Concerns about global CO_2 emissions were first raised in the 1950s and the Intergovernmental Panel on Climate Change (IPCC) was established in 1988. Whilst it was first identified in the late 1950s that changes in atmospheric CO_2 alters seawater carbonate chemistry (Revelle and Suess 1957), ocean acidification research only gathered momentum from 2000 onwards. There is now a large body of research examining how ocean acidification will impact tropical corals but there has been far less effort focused on understanding its effects on cold-water corals (Wicks and Roberts 2012), even though they are arguably more at risk than tropical corals, due to their depth range and thus proximity to corrosive waters. It remains unclear how coral calcification rates and other metabolic processes (e.g. feeding, respiration, reproduction) may be affected by ocean acidification, but several cold-water coral species are already periodically exposed to waters undersaturated with respect to aragonite (Langdon et al. 2000; Guinotte et al. 2006; Thresher et al. 2011; Henry et al. 2014a) and these populations are characterized by slow growth rates and therefore may have a limited ability to withstand the harsher environmental conditions that will become prevalent in a warmer and acidified future ocean.

Furthermore, whilst scleractinian corals can up-regulate their internal pH at the sites of calcification through energy intensive processes (Venn et al. 2009; Anagnostou et al. 2012; McCulloch et al. 2012a, b), the regulation only applies for coral skeleton that is covered by living coral tissue. Cold-water coral framework reefs are typically composed of a significant amount of bare, dead skeleton beneath the living material (Fig. 36.2) and this exposed dead skeleton will start to dissolve in undersaturated conditions, a process hastened by the increased efficiency of bio-eroding sponges in acidified seawater (Wisshak et al. 2012). Net reef accretion in aragonite-undersaturated conditions will thus only occur if coral calcification exceeds dissolution and bioerosion of exposed dead skeleton. This is critical to understand since the coral's skeletal framework provides important ecosystem functions and can persist for millennia after the coral has

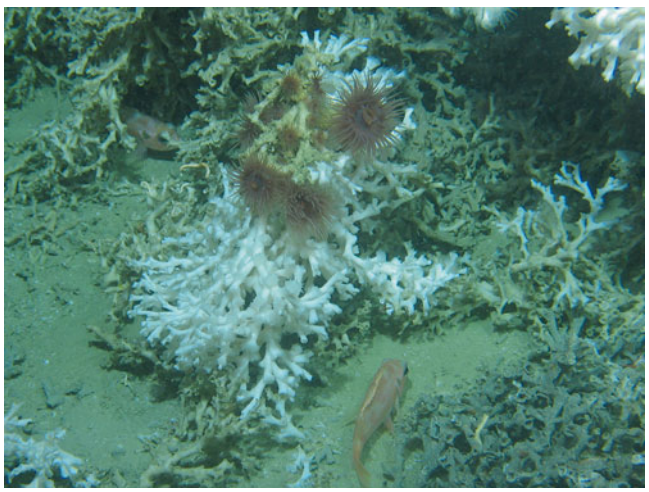

Fig. 36.2 Live *Lophelia pertusa* (*white*) growing on dead skeletons (*grey*) (Photograph taken during the 2012 Changing Oceans Expedition, RRS *James Cook* cruise 073 (Roberts and shipboard party 2013))

died. The ability and long-term sustainability of cold-water corals to survive and thrive below the ASH is currently a topic of active research.

36.2.1 Impacts of Climate Change on *Lophelia pertusa*

Lophelia pertusa is the most widespread and dominant reef framework building coral of the North Atlantic, and as such has been the focus of many of the cold-water coral studies to date. It is arguably the best understood cold-water coral species, but there are still significant gaps in the literature regarding the physiology and biology of this species. The general consensus from studies examining the response of *L. pertusa* to single stressors of ocean acidification or warming, and to a combined increased temperature and CO_2 treatment, is that considerable variability exists between individuals, but *L. pertusa* has the ability to acclimatize (Dodds et al. 2007; Form and Riebesell 2012; Hennige et al. 2014a, 2015; Lunden et al. 2014; Maier et al. 2009, 2013a, b; Movilla et al. 2014).

However, time scales are important when considering whether corals can acclimatize or not, as short-term experiments may produce results (including a detrimental impact of ocean acidification upon key processes) which may not appear in long-term studies, as organisms have undergone alterations in key regulatory processes to acclimatize (Widdicombe and Spicer 2008). This makes it very useful to compare both short and long term research and, with regard to *L. pertusa*, Fig. 36.3 highlights that the most significant changes in respiration and calcification only occur in the short term, from 24-h experiments to 4 weeks. Beyond 4 weeks, decreases in calcification and respiration (with regard to ocean acidification) are not observed (Form and Riebesell 2012; Maier et al. 2013a, b; Movilla et al. 2014). Likewise, for temperature, *L. pertusa* respiration increases following a 24-h exposure (Dodds et al. 2007) but is either unaffected or even decreases in 3–12 month exposures (Hennige et al. 2014a). Given that *L. pertusa* has only been demonstrated to be significantly impacted by ocean acidification and temperature in short term exposures (Form and Riebesell 2012; Hennige et al. 2014a; Lunden et al. 2014; Maier et al. 2009), we can infer from non-significant differences in longer term studies that corals readily acclimatize to their new undersaturated conditions. It is likely that in the days to months following a perturbation, energetic pathways in this species may prioritize protective or acclimation pathways as recorded in tropical coral species (Gates and Edmunds 1999).

Even when acclimatization has been demonstrated, it may come at a cost to other processes and may therefore not be sustainable in the long-term, in particular with regards to maintaining growth under ocean acidification. With this in mind, recent research has demonstrated that although growth rates continue as normal under low pH conditions, skeletal biomineralization, molecular-scale bonding, and skeletal structure all change (Hennige et al. 2015). The breakdown in the recognized positive linear correlation between respiration and calcification (Maier et al. 2013a) at the end of long term experiments (Hennige et al. 2015) may also indicate that 'normal' energetic strategies no longer apply in the long-term, possibly due to other processes using energetic reserves, and highlights that many processes may be occurring of which we have poor understanding, and/or cannot easily measure.

In addition to potential energetic implications for the live coral, the dead, exposed skeletal framework which supports the reef itself and provides habitat for a range of associated species (Roberts et al. 2009) may be at risk from ocean acidification. Exposed skeleton cannot acclimatize or adapt to future conditions, and its dissolution is a purely biogeochemical process (Eyre et al. 2014; Fig. 36.4). The dissolution and weakening of the exposed skeleton observed after long term ocean acidification exposure (Hennige et al. 2015) when combined with bio-erosion (Silbiger and Donahue 2015; Wisshak et al. 2012), may mean that reefs of the future may be smaller than they are currently, and consequently unable to support the rich biodiversity present today. While the ecologically significant ability of adult *L. pertusa* to skeletally fuse (Hennige et al. 2014b) helps strengthen the framework as a whole, the fact that over 95 % of cold-water coral reefs are found above the ASH depth (Guinotte et al. 2006) infers that, in the long-term, net reef growth cannot normally be maintained in undersaturated water.

36.2.2 Impacts of Climate Change on Other Cold-Water Coral Species

In studies to date the cosmopolitan solitary cold-water coral *Desmophyllum dianthus* has generally shown the ability to acclimate to ocean acidification (McCulloch et al. 2012a; Rodolfo-Metalpa et al. 2015; Naumann et al. 2013). Unlike *L. pertusa*, the geographic and bathymetric distribution of *D. dianthus* includes corrosive waters, for example in upwelling regions where the pH can be as low as 7.4 (Thresher et al. 2011; Anagnostou et al. 2012; McCulloch et al. 2012a; Jantzen et al. 2013; Fillinger and Richter 2013). This suggests that *D. dianthus* may be better adapted to deal with ocean acidification than *L. pertusa* and for this species ocean warming may be the greater threat from rising atmospheric CO_2. Indeed, bulk growth and calcification did not significantly differ in corals incubated for 2–11 months in acidified seawater (Movilla et al. 2014; Rodolfo-Metalpa et al. 2015). However,

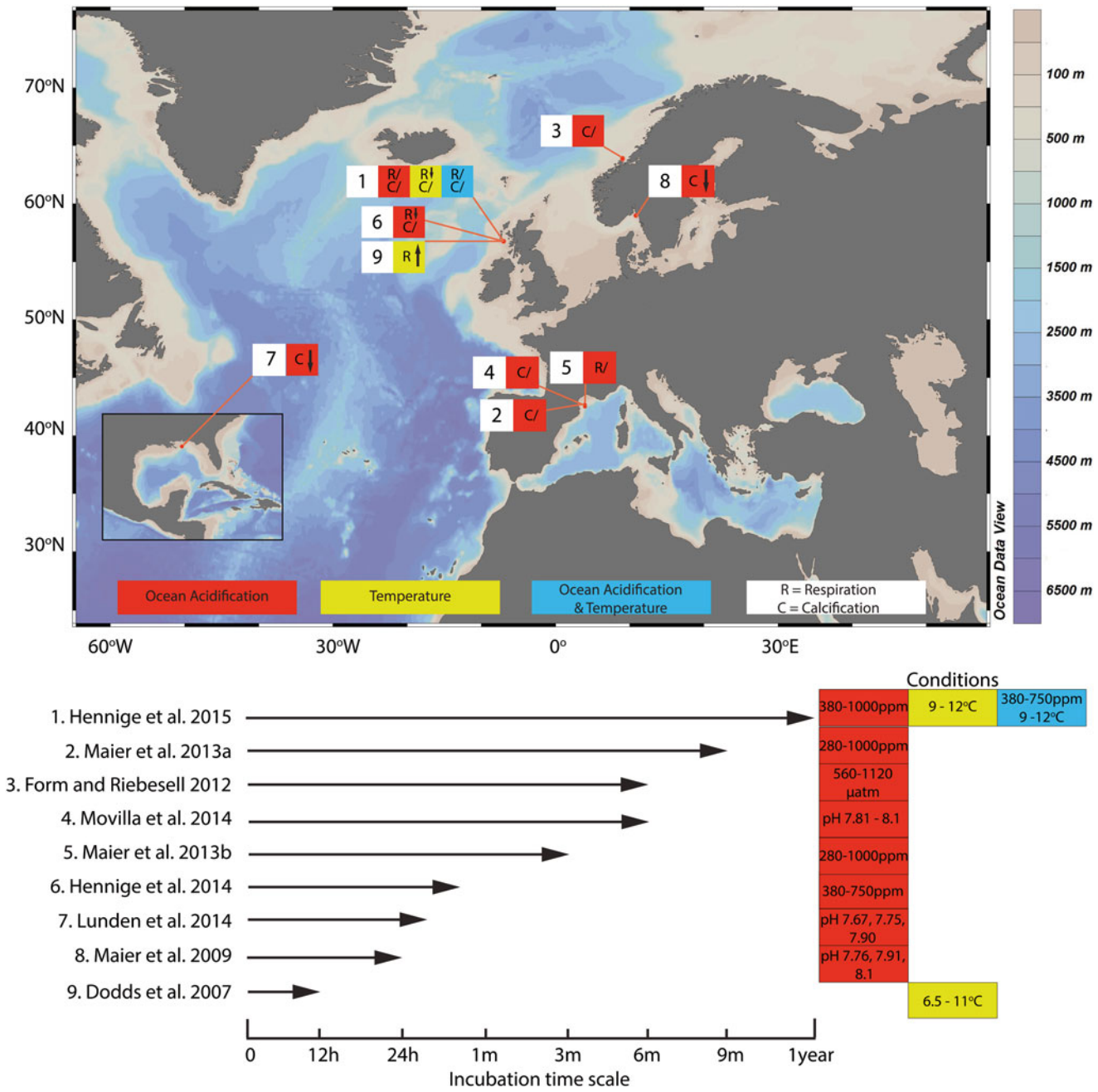

Fig. 36.3 Chart showing locations and summarizing physiological results from research on projected future impacts of temperature and ocean acidification on *Lophelia pertusa*. 'R', Respiration; 'C', calcification; '↑', an increase; '↓', a decrease; '/', no statistically significant change. *Symbols* represent experiment endpoint results, pH is recorded in the total scale (Adapted from Hennige et al. (2015))

these studies used mature polyps and younger, fast-growing polyps have been observed to reduce their growth rate after long incubations in acidified seawater (Movilla et al. 2014), possibly due to their energetic requirements for growth. In mature polyps, neither respiration nor ammonium excretion rates have been found to respond to seawater acidification (Carreiro-Silva et al. 2014), suggesting coral metabolism is unaffected. However the upregulation of genes involved in cellular calcification and energy metabolism in acidified seawater (Carreiro-Silva et al. 2014) imply that *D. dianthus* may maintain growth and metabolism by switching from a mixed use of protein and carbon-based substrates, to a carbohydrate or lipid-dominated metabolism. This highlights the need for more fully integrated assessments that consider the full implications of ocean warming and acidification on coral metabolism, energy budget and resource allocation.

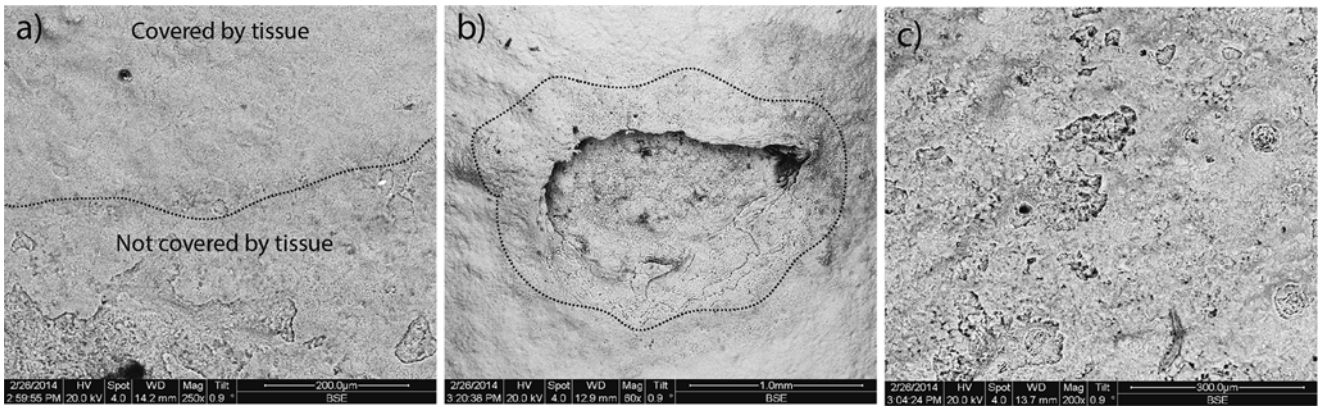

Fig. 36.4 Back Scattered Electron emission (BSE) of *Lophelia pertusa* skeleton fragments maintained in $\Omega_{Aragonite}$ <1. (**a**) The interface between tissue-protected skeleton (*top*) and exposed skeleton (*bottom*). The interface is highlighted with the *dashed line*. (**b**) A site of tissue damage on *L. pertusa*, and subsequent dissolution of skeleton in an otherwise protected area. *Dashed line* indicates the interface between tissue-protected and exposed skeleton. (**c**) Exposed section of skeleton (Adapted from Hennige et al. (2015))

36.3 Cold-Water Corals in an Era of Rapid Global Change: Looking to the Past to Predict the Future

Scleractinian corals arose in the Triassic 237 million years ago (Stanley 2003) with examples of today's characteristic cold-water coral fauna emerging from the Cretaceous onwards (Fig. 36.5). However, the coral fossil record is punctuated by several mass extinction events related to major perturbations in the global carbon cycle (Knoll et al. 1996; Veron 2008). Recent work is attempting to understand coral resilience to past and present climate change using an array of geochemical tools and proxies of seawater acidification, temperature and nutrient regimes as the geochemistry of the skeletons of cold-water corals may provide unique insights into the effect of ocean acidification and other stressors on coral survival. Scleractinian corals can be used as archives of seawater chemistry, as they deposit carbonate skeletons from a semi-isolated pool created and controlled by the coral, yet influenced by the external seawater environment (Tambutté et al. 2011; Comeau et al. 2013; Gagnon 2013). The isolation of the coral's calcifying space is made possible by the presence of cellular membranes, modifying seawater chemistry at the site of calcification (McConnaughey 1989; Adkins et al. 2003; Cohen and McConnaughey 2003) and also presenting a physical barrier to less saturated ambient conditions (Cohen et al. 2009). By looking to the past it may be possible to predict how cold-water corals will respond to the rapidly changing climate of today.

Boron isotopes ($\delta^{11}B$) are one such geochemical tool which have recently been used to study cold-water coral resilience to ocean acidification. As a tracer, boron is directly linked to seawater pH because its speciation (borate ion and boric acid) and the isotopic composition of the aqueous species are pH dependent (Zeebe and Wolf-Gladrow 2005; Klochko et al. 2006; Nir et al. 2015). There are a number of studies in cold-water corals that have used this proxy to study past ocean acidification events, coral calcification mechanisms and resilience (Blamart et al. 2007; Rollion-Bard and Erez 2010; Anagnostou et al. 2012; McCulloch et al. 2012b). Coral $\delta^{11}B$ composition is a recorder of calcifying fluid pH, as verified by microelectrode measurements (Al-Horani et al. 2003) and the use of a pH sensitive fluorescent indicator (Venn et al. 2011), and it has been suggested that cold-water corals record elevated pH to that of ambient seawater (Anagnostou et al. 2012; McCulloch et al. 2012b). This upregulation is not perfect because corals are unable to completely control their internal pH (if they were their $\delta^{11}B$ composition would have to be constant and independent of ambient seawater pH), however corals have been shown to elevate their calcifying fluid pH more when external pH is lower (Fig. 36.6). This ability is vital in allowing calcification to continue in conditions where carbonate ion concentrations are extremely low (aragonite saturation <1).

Although disentangling the mechanism of coral biomineralization is currently an active research area, using the available models (McConnaughey 1989; Zoccola et al. 2004) first approximations can be made to identify the energetic thresholds for maintaining the required calcifying fluid pH such that calcification can take place (Cohen et al. 2009; McCulloch et al. 2012a; Venn et al. 2013). Further development and expanding application of biological and geochemical tools to probe the pH of calcification may be key to understanding the chemical response of coral resilience to ocean acidification (Gagnon 2013). Further, the energetically demanding process of calcification could be assisted by elevated food availability counteracting the cost of ocean

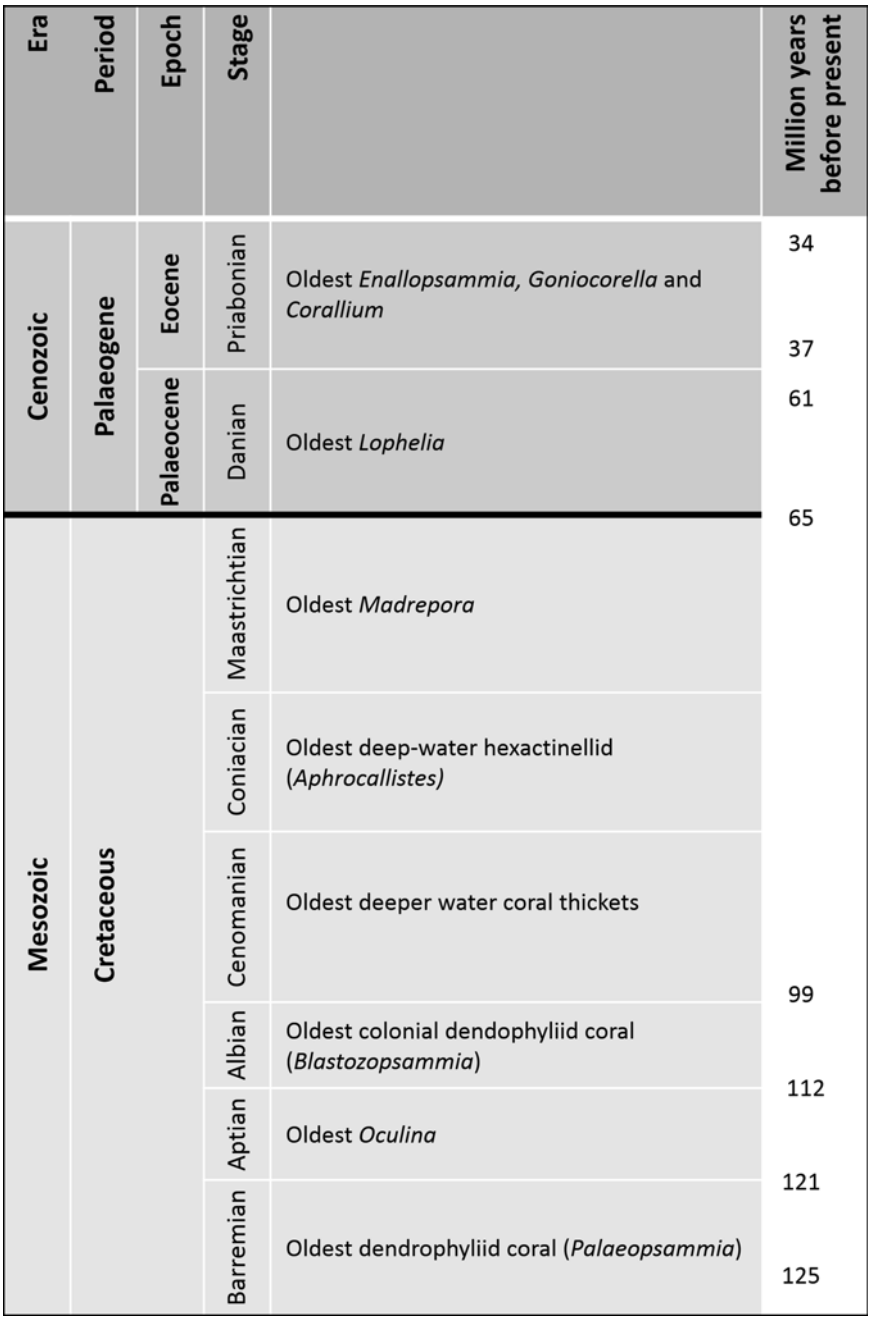

Fig. 36.5 Temporal evolution of cold-water corals. Age indications derived from Gradstein et al. (2004). The bold line at 65 Ma marks the position of the end-Cretaceous mass extinction event (Adapted from Roberts et al. (2009))

acidification. Coral survival likely depends on a complex array of factors including the interaction of deep-water currents with surface primary productivity and seafloor topography to supply organic rich particles (Duineveld et al. 2004, 2007; Kiriakoulakis et al. 2004; Davies et al. 2009; Dodds et al. 2009), seawater oxygen concentrations (Thiagarajan et al. 2013), and temperature (e.g. McCulloch et al. 2012b). Therefore, to produce robust conclusions on the sensitivity of cold-water corals to ocean acidification, the roles of not only pH, but also nutrients, temperature, and oxygen all need to be considered in combination.

There is a long history of developing temperature proxies from scleractinian cold-water corals. Two emerging proxies with great promise are the Li/Mg ratio and clumped oxygen isotopic composition of coral skeletons. The Li/Mg proxy (Case et al. 2010; Montagna et al. 2014) is based on systematic patterns observed between Mg/Ca and Li/Ca in the various microstructural components of scleractinian cold-water corals. The combination of the two ratios, in the form of the Li/Mg ratio, seems to produce a relatively pure environmental signal, which in this case is principally temperature. Nevertheless, paleo-temperature reconstructions carry large uncertainties on

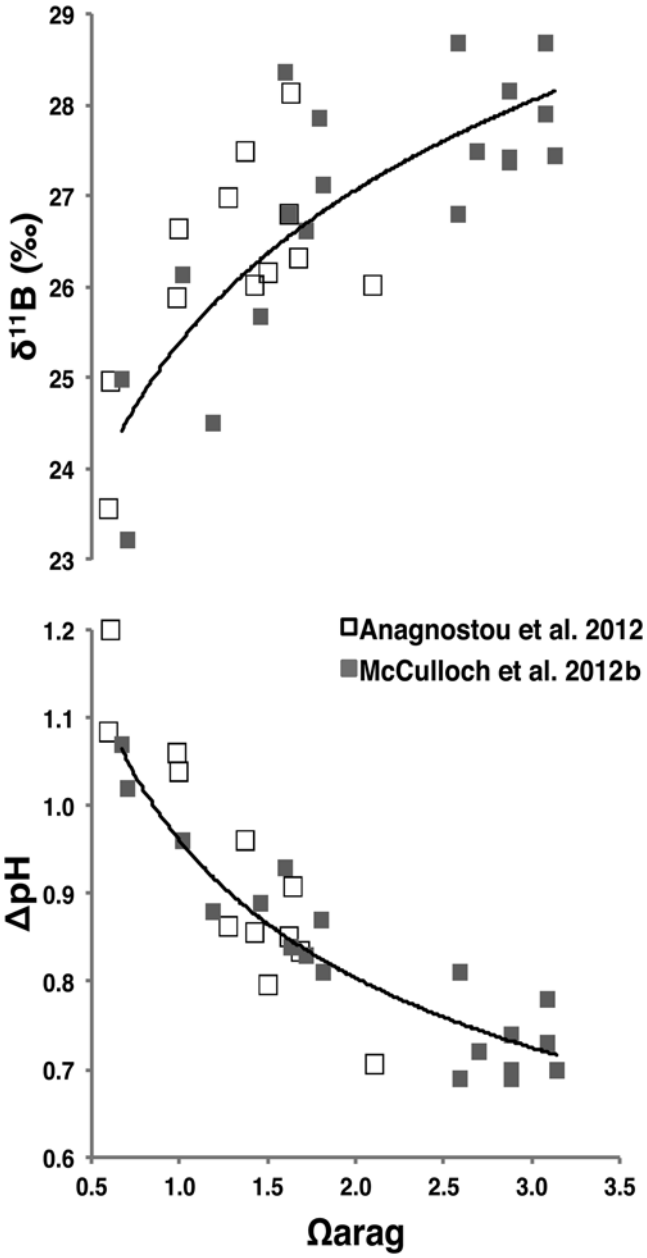

Fig. 36.6 pH upregulation as a function of aragonite saturation in scleractinian cold-water corals. *Top* panel shows the $\delta^{11}B$ measurements and *bottom* panel the pH elevation (=pH recorded – pH seawater)

the scale of ±1.6–1.8 °C and the current uncertainty regarding the exact nature of the relationship between Li/Mg and temperature (i.e. either linear (Case et al. 2010) or exponential (Montagna et al. 2014)) could lead to even larger errors. An alternative temperature proxy is the Δ_{47} (clumped isotopes of C and O in the carbonate skeleton (Thiagarajan et al. 2011)). This approach exploits the degree of disorder in the isotopologues of the C-O bonds of carbonates, and has been calibrated over a wide range of temperatures, although it is currently characterized by poor precision (up to ±2.8 °C equivalent).

In addition to these temperature proxies, the skeletons of *D. dianthus* have been proposed as a reliable recorder of seawater phosphate, a key nutrient. Coral carbonate P/Ca ratio is the only direct nutrient proxy described to date, and as such it holds great promise. An earlier study on this proxy (Montagna et al. 2006) was revised to obtain reliable deep-water nutrient compositions with an uncertainty of ±0.4 μmol kg^{-1} for the majority of intermediate oceanic waters (Anagnostou et al. 2011). Despite good field calibrations, the mechanism of phosphorus incorporation is still debated with NMR studies suggesting that the majority of phosphorus in corals is in the form of the inorganic phosphate in aragonite crystal defects (Mason et al. 2011). Application of the proxy requires fine scale sampling and thorough data filtering to account for the presence of different structural features and phosphate contaminants (Anagnostou et al. 2011; Mason et al. 2011), but novel sampling strategies offer high precision. Nonetheless, further work is needed to study the validity of the proxy in waters undersaturated for aragonite and in controlled incubation experiments.

Past environments hold a plethora of information on the effect of climate change on coral proliferation and demise. The skeletons of fossil scleractinian corals can be accurately dated using radiometric techniques (Lomitschka and Mangini 1999; Cheng et al. 2000; Douville et al. 2010; McIntyre et al. 2011; Longworth et al. 2013), and a number of proxies have been developed to reconstruct the corals' paleo-environment. When geochemical reconstructions are combined with records of coral density and distribution, insights into the synergistic effects among ocean acidification, nutrients and temperature on coral survival and growth can be revealed (Thiagarajan et al. 2013), assisted by genetic connectivity data providing further insights into cold-water coral dispersal and extinction at ocean basin scales (Henry et al. 2014b).

36.4 Knowledge Gaps

Despite the recent research advances, there are still major knowledge gaps to be explored before any certain inferences can be made as to the long-term survival and ecological role of cold-water coral reefs (Rodolfo-Metalpa et al. 2015).

36.4.1 Acclimatization Versus Adaptation

Whilst the ability of *L. pertusa* to acclimatize to ocean acidification conditions in laboratory mesocosms, albeit with subsequent impacts on its physiology, has been established; its ability to evolve and adapt to future conditions has not been addressed. *Lophelia pertusa*, like many long lived species has high levels of phenotypic plasticity which allow it to

thrive over a wide geographic distribution (Roberts et al. 2009) and research to date has focused on this species' ability to cope with ocean acidification within this existing plasticity. This acclimatization is important because although adaptation to changing conditions can occur over subsequent generations, the slow growth of cold-water corals coupled with the projected rapid change in ocean acidification and warming (CBD 2014a), means that reef survival will depend heavily on the acclimatization capacity of currently living cold-water corals. There is, however, a disconnect between laboratory observations where *L. pertusa* can survive and grow in acidified seawater and field observations as *L. pertusa* reefs have not been observed below the ASH (Guinotte et al. 2006), raising further questions over the ability of *L. pertusa* to acclimate or adapt to ocean acidification. Furthermore there is some debate as to whether the plasticity of long lived species like *L. pertusa* aids adaptation by extending the persistence of populations long enough to allow time for local adaptation (Jump and Penuelas 2005) and possibly by supplying an initial step in the evolutionary process (Pfennig et al. 2010; Reed et al. 2011) or if it impedes long-term adaptation to environmental change by delaying the onset of evolutionary responses (Ghalambor et al. 2007). Since the most likely future climate scenario involves changes in both temperature and CO_2 (IPCC 2013), it is vital to understand whether cold-water corals can acclimatize to multiple stressors simultaneously, what the cost is to other processes (Gates and Edmunds 1999), and whether they have the potential to adapt in the longer term. To assess the acclimatization and adaptation abilities of organisms, it is vital to conduct long-term experiments.

36.4.2 Reproduction

No experiments to date on cold-water corals have considered the impacts of climate change on reproductive fitness or connectivity. Early life stages are particularly vulnerable in a number of species (CBD 2014a), and this needs to be investigated in cold-water corals as a matter of urgency. However, there are serious logistical constraints with regard to this, as it is not feasible to collect fresh reproductive material at the time of spawning due to the often-unsuitable weather, and reliably harvesting gametes from aquaria-kept cold-water coral specimens has only recently been achieved (Larsson et al. 2014, Sect. 36.4.3). In the tropical coral *Acropora tenuis*, it has been demonstrated that fertilization success was negatively impacted by increased CO_2 (Albright and Mason 2013). It is therefore feasible to assume that similar effects may occur in cold-water corals, and acclimatization may not ensure the long-term survival of corals if reproductive fitness decreases.

36.4.3 Connectivity Between Populations

The only mechanism available for gene flow between geographically separate cold-water coral populations and for provision of new recruits to colonize new sites or recolonize damaged sites is the production of pelagic larvae. This connectivity, an important part of coral ecosystem function and resilience, is poorly understood, but the use of emerging genetic techniques integrated with geochemistry has provided some information on current and historical connectivity (Henry et al. 2014b). Gene flow between large ocean regions, Gulf of Mexico, coastal southern US, New England Seamounts and NE Atlantic, is restricted in North Atlantic *L. pertusa* populations (Morrison et al. 2011). Within regions however, connectivity has been shown across large distances, suggesting some larvae are widely dispersed. While there is evidence of long-range gene flow between some *L. pertusa* populations (Flot et al. 2013), there can be poor connectivity between reefs that are relatively close together, for example within the Norwegian fjords and the Skagerrak (Dahl 2013). To understand the fine detail of the connectivity suggested by genetic data, and predict how this connectivity might change under climate change we need to understand both the behavior of the larvae and the local hydrography. To date larval behavior remains the largest knowledge gap in attempts to understand cold-water coral population connectivity.

Lophelia pertusa larvae have not been observed in ocean plankton samples, possibly due to their small size and fragile nature, so knowledge of the larval behavior is restricted to laboratory studies. Successful fertilization of *L. pertusa* gametes and subsequent description of embryogenesis and larval development has recently been achieved in the laboratory (Larsson et al. 2014). The larvae survived for several weeks and showed a negative geotactic behavior, accumulating exclusively in the upper half or one third of experimental aquaria, even when kept in the dark. Measurements of swimming speed suggest the ability to move vertically in the water column sufficiently fast to reach the surface waters, even from deep cold-water coral reefs. If larvae can reach the surface they have the potential to travel hundreds of km before settling and models of larval dispersal in other species (e.g. Corell et al. 2012; Coscia et al. 2012) show dispersal, and hence connectivity, to be crucially dependent on larval depth distribution and vertical movement. Genetic connectivity studies of *D. dianthus* populations revealed that the genetic differentiation with depth is consistent with the stratification of water masses, suggesting that, for some coral species, larvae will be retained within, and rarely migrate between, different water masses (Miller et al. 2011). This implies that as the aragonite saturation horizon shallows, deep populations may be unable to colonize shallow water. Likewise, deep populations are unlikely to be able to act as refuges for

shallower populations impacted by fishing or mining activities. Furthermore, in addition to temperature rises and ocean acidification, changes in ocean and atmospheric circulation patterns are expected. Numerical model projections suggest that the Atlantic Meridional Overturning Circulation (AMOC) is likely to weaken over the twenty-first century (e.g. Cheng et al. 2013) while, in the northern hemisphere extratropics, models indicate changes to atmospheric circulation including a poleward shift and strengthening of the Atlantic storm tracks (Pinto et al. 2007; Gillett et al. 2013; IPCC 2013), although there is a great deal of uncertainty in these predictions (IPCC 2013). Any changes in ocean circulation are likely to impact on cold-water coral larval dispersal, and thus connectivity, and it remains unclear what the implications of this may be for the resilience of these species in a changing ocean.

36.5 Conservation and Policy

Conserving cold-water coral habitats is challenging, in part because of the nature of the threats they face, from deep sea trawling to climate change, and in part because many of the known reefs and coral gardens occur beyond the limits of national jurisdictions and thus require international legal frameworks and efforts to manage and protect these ecosystems. Furthermore, deep-sea habitats are often out of sight and out of mind and therefore "invisible" to the public and policy makers alike who are not readily aware of the goods and services they supply.

Cold-water coral reef frameworks can attract and concentrate commercially valuable fish species but are highly susceptible to damage from heavy fishing gear and very slow to recover (Ramirez-Llodra et al. 2011). The extent of the damage caused by fishing activities has not been widely quantified, however trawling on seamounts off Tasmania (Australia) has resulted in a reduction in cold-water coral reef cover (*Solenosmilia variabilis*) by two orders of magnitude resulting in threefold declines in the richness, diversity and density of other benthic fauna (Althaus et al. 2009), with no sign of recovery 5 years after the area was closed to trawling. Even where heavy fishing gear has not directly come into contact with corals, fishing activity can cause high mortalities. In the northwestern Mediterranean, intensive trawling between 400 and 700 m has caused substantial sediment erosion leading to sediment gravity flows down to 1200 m which can suffocate cold-water corals in submarine canyons far from the immediate reach of the trawling fleet (Palanques et al. 2006). Consequently, closing cold-water coral habitats to fishing may not be enough to protect them from the impacts of trawlers.

Less well understood are the potential impacts of subsea cabling, mineral and fossil fuel exploration and extraction, bioprospecting and indeed scientific sampling and survey activities for cold-water coral ecosystems. However, the depletion of terrestrial resources combined with technological advances making the deep sea more accessible means that anthropogenic pressures on cold-water coral habitats are likely to increase. These activities can be highly profitable and difficult to regulate in international waters. Combined with unfavorable conditions brought by the rising ASH (Sect. 36.2), which is predicted to shallow to depths above which 70 % of present day aragonitic cold-water coral reefs live by 2100 (Guinotte et al. 2006), cold-water corals face an uncertain future.

Nevertheless, progress has been made towards safeguarding these vulnerable ecosystems, most notably in the north Atlantic. The value of cold-water coral habitats, in particular reefs and gardens, is well known and has been recognized in conservation legislation since the 1990s. The Earth Summit held by the UN in Rio de Janeiro in 1992 was the first conference of its kind and the legally binding Convention on Biological Diversity (CBD) with 168 signatories was one of its major outputs (CBD 2015). The CBD explicitly categorizes cold-water coral reef and coral garden habitats as ecologically and biologically significant areas (EBSA). Countries which have ratified the CBD are obligated to take measures to protect cold-water coral habitats. The EU made steps towards fulfilling its obligations with the Habitats Directive (also from 1992) that lists cold-water corals as priority habitats (EC 1992).

The most recent international efforts by the CBD to protect cold-water corals fall under the Nagoya Protocol (2010) where cold-water corals fall under three Aichi targets: Target 5 "By 2020, the rate of loss of all natural habitats, including forests, is at least halved and where feasible brought close to zero, and degradation and fragmentation is significantly reduced"; Target 6: "By 2020 all fish and invertebrate stocks and aquatic plants are managed and harvested sustainably, legally and applying ecosystem based approaches, so that overfishing is avoided, recovery plans and measures are in place for all depleted species, fisheries have no significant adverse impacts on threatened species and vulnerable ecosystems and the impacts of fisheries on stocks, species and ecosystems are within safe ecological limits"; and Target 10: "By 2015, the multiple anthropogenic pressures on coral reefs, and other vulnerable ecosystems impacted by climate change or ocean acidification are minimized, so as to maintain their integrity and functioning". However at the halfway point none of these Aichi targets are on track to be achieved by 2020. Some progress has been made towards Targets 5 and 6, but anthropogenic pressures on coral reefs have continued to increase (CBD 2014b).

Nevertheless, at national levels protection for cold-water coral ecosystems is coming into effect. Norway was the first country in Europe to take measures to protect their cold-

water coral reefs when they closed an area of about 978 km^2 in 1999 to protect *L. pertusa* reefs (CBD 2008). This banned bottom trawls but did not affect fishers using static gear (Armstrong and van den Hove 2008). Subsequently the USA, Canada and New Zealand have closed areas to bottom trawling to protect cold-water coral habitats on seamounts (CBD 2008). The EU closed the Darwin Mounds (Northwest of Scotland) to bottom fishing in 2003 in the first example of a conservation area for cold-water corals in an area fished by several nations, namely the UK, France and Spain (Roberts et al. 2009).

Outside of national jurisdictions, protection measures are more difficult to instigate and enforce. Globally a number of structures and mechanisms have been developed at international levels to support the implementation of global conservation policies including United Nations Convention on the Law of the Sea (UNCLOS), and efforts from the Food and Agriculture Organization of the United Nations (FAO) and the CBD at national and international levels. Of particular importance for protecting cold-water corals is their designation as Vulnerable Marine Ecosystems (VMEs) with respect to fishing by the FAO (FAO 2009), which gained momentum after UN Resolution 61/105 (Sustainable Fisheries) was passed in 2006. In 2009 the FAO published guidelines on the management of deep-sea fisheries aimed at ensuring sustainable fisheries and long term conservation of deep-sea habitats which includes measures to "prevent significant adverse impacts on VMEs". These guidelines apply both to individual states and to regional fisheries management organizations and arrangements (RFMO/As) and require contributions to compiling the FAO global database on VMEs through the identification of known and likely locations of VMEs. They recommend that such areas should be closed to fishing activity until suitable management and conservation structures can be established which could include closures or the introduction of gear specifications, catch limits and measures to limit both bycatch and ghost fishing through lost equipment (FAO 2009). They also require states to establish policies and legal frameworks which would enable them to control the fishing efforts of any vessel flying their flag in the high seas and stop their vessels fishing in VMEs.

Whilst these guidelines are voluntary, there have been international efforts made to protect VMEs outside of national jurisdictions, such as the establishment of six MPAs, a network covering 286,200 km^2, in the international waters of the North Atlantic under the OSPAR convention (O'Leary et al. 2012). The countries that surround the North Atlantic are all developed nations and it may be more difficult to establish such networks in other parts of the world, nevertheless it provides a precedent that could be implemented elsewhere.

36.6 Conclusions

Cold-water corals remain relatively poorly understood ecosystems and key questions about their biology and ecology are yet to be answered. It is clear however, that they are increasingly at risk from growing anthropogenic pressures and their slow growth and stable environments may render them exceptionally vulnerable. Headway has been made and is continuing towards protecting cold-water coral habitats although the global distribution of efforts is highly skewed and much more is required to safeguard these ecosystems. The CBD has had some success both in the number of countries which have signed and ratified the convention, and in progress made towards the Aichi targets (if not as substantial as hoped for). The FAO too has had successes in embedding VMEs into management regimes for deep-sea fisheries in areas beyond national jurisdiction. However, the UN Framework Convention on Climate Change (UNFCCC), which also emerged from the 1992 Earth Summit has been less successful. The Kyoto Protocol (1997) largely failed as major polluters refused to sign up. The subsequent Copenhagen Agreement (2009) did not seek to be legally binding in the same way and was not ambitious enough even in its aims to limit global warming to 2 °C above pre industrial levels (Stern and Taylor 2010). At the time of writing, the outcomes of the Paris 2015 climate talks may prove critical in determining future carbon emissions. Whilst protecting cold-water corals from trawling and other physical impacts is important, without similar advances in curbing carbon emissions, the future of cold-water coral habitats remains uncertain.

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Part IX

Medical Aspects and Applications

Unusual Cnidarian Envenomations

37

Özgür Deniz Tezcan

Abstract

This chapter is a collection of unusual cnidarian envenomations. Cnidarian envenomations are common all around the world. According to the World Register of Marine Species database, there are 10,762 accepted cnidarian species and about 100 of these are considered harmful to humans. There is a great diversity of cnidarian envenomations. At one end of the spectum, there are serious envenomations, such as that of *Chironex fleckeri*, which can kill a man in minutes; while at the other end, there are minor conditions, such as an annoying itch. While the more serious or frequent envenomations are well-studied, others are rarely reported and poorly studied. Only a fraction of cnidarian toxins have been identified and throughly investigated. Rare cnidarian envenomations, such as fulminant hepatitis, autonomous nervous system dysfunction, or stroke are not only interesting, but they also provide a wider perspective to cnidarian toxicology. Here, there is a total of 65 cases. The cases are grouped according to the clinical outcomes and underlying physiopathology. To facilitate our understanding of these unusual and sometimes bizarre envenomations, some physiopathological data is also included with each group.

Keywords

Unusual cnidaria envenomation • Seastroke • Guillan-Barre • Neuropathy • Mononeuritis multiplex • Autonomic neuropathy • Priapizm • Dysphonia • Amnesia • Myocardial infarction • Tako-tsubo • Renal failure • Fulminant hepatitis • Mondor's disease • Palytoxin • Anaphylaxis • Dermographism

37.1 Introduction

Cnidaria is one of the oldest animal phylums. They have existed since the Cambrian period (541 million years ago). The *sine qua non* of cnidaria is the toxic organelle, nematocyst. Nematocyst is one of the most complex intracellular secretion products. The diversity of cnidarian toxins is immense. The venom encapsulated in the nematocyst is a complex mixture of polypeptides, proteins, purines, quaternary ammonium compounds, biogenic amines, and betaines. These toxins may induce necrotic, hemolytic, neurotoxic, cardiotoxic, nephrotoxic, hepatotoxic reactions, or hypersensitivity reactions (type I or type IV). On the cellular level, cnidarian toxins act through three basic mechanisms: pore formation, sodium channels, and potassium channels. Their action on calcium channels is controversial.

Observations suggest that overfishing, eutrophication, climate change, translocations, and habitat modifications will cause more frequent jellyfish blooms. Cnidarian toxins are considered to be a rich source for the development of new drugs or biomedical materials. The risk of more severe jellyfish blooms and hopes for new drug discoveries are the main motives underlying the growth of interest in cnidarian toxins.

Even today, the area of cnidarian toxicology may still be defined as *terra incognita*. In the conclusion of her detailed

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review, Frazao et al. (2012) states, "*the venom from each species of cnidarians is supposed to contain around 100 compounds, but not more than 1 % is currently known even in the better studied species*". There are many reasons for this.

Cnidarian toxicology is not an easy area to study. For the clinician, it is hard to identify an envenomation which has happened underwater. Most of the victims don't even see the responsible organism. Only a fraction of the victims seek medical advice, and even the most interesting cases are rarely reported. The work of the toxicologist is even harder, and the difficulties begin with specimen collection. Laborious venom extraction, instability and inconsistency of the toxins, variation of the toxin composition of an organism's venom in different stages of its life cycle, and the difference between the toxin composition of the same species in different geographic localisations adds to this difficulty.

37.2 Neurotoxicity

The rapid paralysis of prey is vital for fragile, slow moving cnidaria. This makes neurotoxins the most important component of the venom. Neurotoxins act on voltage-gated ion channels, which are critical to normal neuromuscular transmission. Disruption of the voltage-gated ion channels can lead to rapid paralysis, which is why they are excellent targets for venomous organisms. In humans, cnidarian neurotoxins may cause direct toxicity (local or systemic), autonomic nervous system dysfunctions, or immune-mediated neuropathies.

37.2.1 Seastroke

Meyer (1993) reported three previously healthy young male patients who suffered brain stem infarction while swimming off the southeastern North Carolina coastline between the years 1990 and 1992. He called the condition "seastroke". These cases are unique, as none had a predisposing factor for a stroke, and the only probable offender was an unidentified neurotoxin.

The first case was a 15-year-old boy. After swimming in the ocean for 30–45 min, he experienced severe dizziness. He complained of a sting, feeling strange, and a mild headache. He began to laugh, cry and moan, and started sweating profusely. He experienced seizure-type activity, and lapsed in and out of consciousness. Initial cranial computerized tomography (CT) and cerebrospinal fluid examination, cervical spine films, electroencephalography and bloodwork were all normal. The drug screen was negative. Brain magnetic resonance imaging (MRI) showed a hyper-intensive spot within the central pontine region. The cerebral arteries which supply the area were all open. The patient remained intellectually unimpaired. In retrospect, the patient definitely remembered stepping on something in the sea 30–45 min before the onset of the symptoms. With his own words, it was "sort of rough and prickly; I wouldn't have remembered it, but it surprised me and caused an eerie feeling."

The second case was a 13-year-old boy who was playing in the shallow surf off Topsail Beach. He told his brother that something had stung him on the arm. He left the water and, approximately 30 min later, he was found by his father: alert but unable to move, paralyzed from the neck down. CT, lumbar puncture, cerebral arteriogram, roentgenograms of chest and cervical spine, and echocardiogram were normal. Three days later, he was transferred to a university center. Brain MRI showed "evidence of edema and abnormal enhancement within the cervical cord and lower medulla, consistent with myelitis. Infraction could not be excluded." After 9 weeks hospitalization, the patient was still ventilator dependent. He was transferred to a spinal rehabilitation center.

The third case was a 39-year-old man. While swimming off Wrightsville Beach, he experienced a sudden onset of severe dizziness and imbalance. His wife reported that he staggered out of the water, vomited, nearly passed out, and complained of severe dizziness. The patient was observed in the emergency department for 5 h. CT, lumbar puncture, and bloodwork were normal. As soon as he was well enough to walk on his own, he was discharged. Two days later, he experienced swallowing difficulty. A barium swallow study showed an inappropriate swallowing mechanism and poor motor function. MRI showed an abnormal signal in the inferior and posterior aspect of each cerebellar hemisphere extending on the left, involving the inferior lateral aspect of the medulla, suggesting ischemic infarcts. In retrospect, the patient remembered stepping on an object in the ocean that he describes "like seaweed; it was very quick, like when you get bit by a crab, but it didn't hurt, it didn't faze me." His symptoms came on within minutes of stepping on the object.

There is no information on whether any investigation was conducted to identify the causative organism in the area.

37.2.2 Peripheric Neuropathies

Cnidaria may induce peripheric neuropathies in three ways: autoimmune mechanism, direct toxicity, and indirect injury. Cnidarian toxins may trigger a cascade of immune reactions, which may end up as an autoimmune disorder, such as Guillain-Barre syndrome or mononeuritis multiplex. Direct toxicity is caused by the damage of a cnidarian toxin on a nerve underlying a sting site. Indirect injury is caused by mechanical factors, such as edema or inflammation, in the adjacent tissues caused by the sting.

37.2.2.1 Guillain-Barre

Guillain-Barre syndrome is an autoimmune disease which causes ascending polyneuropathy. The main symptom is muscle weakness, starting in the feet and hands, and progressing towards the trunk. Some subtypes can also affect sensation or pain. Guillain-Barre syndrome is usually triggered by an infection. The autoimmune process damages the myelin sheets of peripheral nerves leading to the blockage of signal transmission.

Pang and Schwartz (1993) reported a 39-year-old man who had multiple jellyfish stings over both legs on the north coast of Majorca. The victim described the jellyfish as red, palm-sized, and translucent.

One week later, a tingling sensation started in both heels. The following week, the tingling spread to his hands and he developed mild proximal muscle weakness in all limbs. He was unable to work as a van driver. Over the next 2 months, there was a general improvement in his symptoms. He had minimal proximal muscle weakness in the legs and mildly diminished sensation to light touch in a glove-and-stocking distribution. He also had an unsteady gait. All tendon reflexes were absent, except for a barely elicitable left knee jerk. Position and vibration sense were intact. Nerve conduction studies showed a prominent demyelinating neuropathy with conduction block.

The second case was reported by Devere (2011). The victim was a 66 year old woman who was stung by a jellyfish while swimming in the Atlantic Ocean off Charleston, South Carolina. She felt a stinging sensation on her right thigh and she saw a jellyfish in the water. A red rash arose in the same area. The sting and rash improved in 4 days.

Ten days after the sting, she developed severe low back pain radiating to the right thigh. In 24 h, progressive numbness, muscle weakness, burning and tingling pain began in all extremities. Her neuromuscular symptoms peaked 1 month after the onset. In her neurological examination: muscle strength in biceps, triceps, wrist extensors, finger flexors, quadriceps, and distal leg muscles were reduced to 3/5 (Medical Research Council scale); reflexes were absent; and she had hyperesthesia and decreased vibration/position senses in a glove-and-stocking distribution. Hip and shoulder muscles were normal. The nerve conduction studies 4 months after onset revealed sensory motor polyneuropathy compatible with demyelination.

Ten months after onset, the muscle strength, position and vibration senses were normal, and nerve conduction studies proved that there was continued improvement. After 4 years, her neurologist contacted her and heard that she was fine, and the only thing left was minimal numbness in the first two fingers of both hands and middle toes of both feet.

37.2.2.2 Mononeuritis Multiplex

Mononeuritis multiplex is a painful asymmetric peripheral neuropathy in at least two separate nerve areas. One of the mononeuritis multiplex cases was reported by Filling-Katz (1984). The patient was a 25-year-old man who was stung by a jellyfish across the right forearm off the coast of Norfolk, Virginia. At the time of injury, Physalia and Cyanea species were present in the region. Erythematous wheals developed immediately, and "purple blotches" developed progressively at the site of contact. Next morning, he woke up with weakness and numbness in the right hand and wrist, nausea, vomiting, generalized myalgias, sore throat, and low grade fever. While the weakness in his right hand started improving after a week, he noticed acute diffuse weakness, this time in his left hand and arm and a feeling of numbness extending to his elbow. His neurologic examination of the right and left upper extremity showed sensory and motor dysfunctions. The electromyography showed denervation at the right radial and left radial, ulnar, axillary nerves. The patient improved slowly and the electromyography made 2.5 months later showed re-innervation of the muscles.

There are other cases reported by Burnett et al. (1994). The first case was an experienced underwater photographer who lay on a corallimorpharia while photographing reef fish. Although he was wearing a thick lycra "skin suit", he realised a non-painful irritation on his left arm and knee. Shortly after his second dive, he observed itchy papillo-erythematous lesions over his left knee, elbow, and shoulder. That evening, muscle weakness developed in his left arm. Two days later, he woke up from his night sleep with back pain which worsened with movement. His left arm was noticeably weak. The paresthesia on his arm developed during sleep, but dispersed on walking and moving the arm. Six weeks later, he returned to normal vigorous diving activity, slept normally, and had full arm movement. Twelve weeks later, he recovered completely.

The second case was a healthy 20-year-old woman who was stung by an undefined jellyfish on her right arm and hand. She felt instant pain and came to the shore to receive first aid. Her arm swelled up immediately, and she had extreme pain at the sting site. She felt shortness of breath. Over the next few hours, she noticed numbness, paresthesia, and cooling of the distal right arm together with nausea and faintness. She developed pain on her trunk and became agitated. Pain, paresthesia, coolness, numbness and muscular weakness progressed. The neurological examination after 5 weeks showed considerable weakness in the dorsiflexors of the right hand and arm, profound weakness of the opponens pollicis muscle, and mild weakness in the interossei muscles and decreased sensation in the right median and radial nerve distribution areas. Over the next 4 months, the patient's condition improved spontaneously without residual damage.

Another case was reported from southern Florida, by Moats (1992). A 52-year-old woman touched a fire coral (*Millepora sp*.) with her right wrist. She experienced a local burning sensation for 2–3 days followed by general weakness and high fever (39 °C). In 6 weeks, she developed weakness and difficulty moving her right shoulder and arm. Winging of the right scapula was detected, and electromyography showed delayed latency of the right long thoracic nerve. After 8 months, the symptoms disappeared with a physical therapy programe.

37.2.2.3 Acute Peripheric Neuropathy

Peel and Kandler (1990) reported an 18-year-old female who was stung on her right wrist off the coast of Penang. An urticarial rash developed immediately and it was followed by painful swelling of the forearm. She noticed progressive numbness and difficulty in moving her fingers. By the third day, there was little active movement of the fingers, accompanied by complete anesthesia distal to the wrist. Nerve conduction studies carried out in Singapore showed that sensory action potentials could not be recorded from the ulnar, median or radial nerves. When the patient returned to England 2 weeks later, there was no clinical improvement. The findings implied a combination of degeneration and conduction block. Ten months after the sting, she had made a complete clinical recovery, apart from minimal sensory loss.

The second case was reported by Laing and Harrison (1991). A 21-year-old British student, who was on a hockey tour in Thailand, was stung by *C. fleckeri* on his back and right arm. On return to the shore, he developed wheezing and generalized edema. He was treated in the local hospital for anaphylaxis. After some days, his systemic illness improved, but he was left with right ulnar nerve palsy. His examination showed incomplete autonomous, sensory and motor weakness in the ulnar nerve distribution. Surgical exploration of the elbow was carried out to rule out a possible compression. The surgeons observed that the ulnar nerve was free in the ulnar tunnel, but the nerve was edematous and the adjacent muscles were ischemic.

There are two possible explanations for this case. Although the first explanation for this case is direct damage of *C. Fleckeri* toxins on the ulnar nerve segment, a second explanation could be derived from the surgeon's observation of "adjacent muscle ischaemia". It is known that cnidarian toxins may induce local vascular spasms. A local ischemic event may well damage the nerves crossing through the affected area.

37.2.3 Autonomic Nervous System Toxicity

The autonomic nervous system controls the visceral functions (digestion, perspiration, respiratory rate, heart rate, blood pressure, urination, etc.), unconsciously and involuntarily. It is diffused throughout the body with many interconnected centers and ganglions. The system keeps its balance with the help of two counteracting mechanisms: the sympathetic nervous system and the parasympathetic nervous system. While the sympathetic nervous system is responsible for the "*fight or flight*" response, the parasympathetic nervous system is responsible for the "*feed and breed*" and "*rest and digest*" responses.

37.2.3.1 Parasympathetic Dysautonomia

A reversible dysautonomia case after a *Chironex fleckeri* sting was reported by Chand and Selliah (1984). The victim was a 52-year-old fisherman who presented with acute abdominal distension and urinary retention. He was stung by a large (0.3 m wide, 1 m long), white-colored jellyfish while wading in the sea. He felt a sudden pain in both of his thighs. In a few minutes, he developed dyspnea, cough, wheezing, palpitations, and profuse lacrimation that lasted 30 min. Two hours later, his abdomen started bloating and he could not pass urine. Physical examination of the patient revealed linear purple vesicular sting marks on both thighs, abdominal distension, and urinary bladder distension 4 cm above the pubic bone. Direct x-ray of the abdomen showed gas distension in the large intestine. Bladder function returned to normal after 2 days and he improved enough to take oral food after 4 days. He lost nocturnal erection for a week after the sting. He recovered totally in the space of a week.

37.2.3.2 Urinary Incontinence and Biliary Dyskinesia

Burnett (2006) reported a 16-year-old girl who was stung on her abdomen by Chrysaora fuscescens while surfing. She developed biliary dyskinesia and urinary incontinence. The morning after the jellyfish contact, she started to complain of a burning sensation and cramps in the right upper quadrant. Eating exacerbated the symptoms. Tests revealed a healthy liver and a non-functioning gallbladder. The second main complaint was urinary incontinence. Urological examination showed inhibited detrusor contractions with urge incontinence.

The abdominal pains persisted. Ten weeks later, the gallbladder had to be removed surgically. The intraoperative cholangiogram documented rapid free flow of bile into the duodenum. The pathological examination of the gallbladder was compatible with biliary dyskinesia. After the operation, the abdominal pain resolved completely. The urinary incontinence resolved with the help of remedial bladder exercises.

Although there are some case reports concerning autonomic dysfunctions after cnidarian envenomations, this case is especially interesting, because two completely different organs, conducted by different parts of the autonomic nervous system, were affected.

37.2.3.3 Ileus

Digestion, (from regulation of the peristaltic movements, to the secretion of the digestive enzymes) is controlled by the enteric nervous system. Although the enteric nervous system can operate independently, it receives innervation from the parasympathetic and sympathetic nervous systems. Ileus is a condition when the gastrointestinal passage stops, either because of mechanical (stricture or occlusion) or neurological (paralytic) causes. Paralytic ileus is the loss of normal propulsive ability of the gastrointestinal tract (peristalsis) which causes bloating, abdominal pain, and vomiting.

A 31-year-old man from Medan, Sumatra, with paralytic ileus was reported by Ponampalam (2002). The patient picked up and handled a large jelly like mass while he was on a beach. While handling the mass, he felt a sharp pain in his left forearm. He noticed a linear urticarial lesion in that area. Within half an hour, he started to feel general weakness, joint pains, and lethargy. Abdominal distension followed shortly after. When he was admitted into Singapore General Hospital, 24 h after the incident, he had a distended abdomen, vomiting, and no bowel sounds. "Paralytic ileus", was diagnosed. Blood chemistry and abdominal radiography confirmed the diagnosis. He received conservative, supportive treatment and the intestines gained normal function after 4 days without any complication. Another case from India was reported by Das et al. (2002).

Some of the cnidarian toxins which affect intestinal motility are caissarone, reported by Cooper et al. (1995), pCrTX from the jellyfish *Carybdea rastonii*, reported by Nagase et al. (1987), and toxin II from *Anemonia sulcata* reported by Tazieff-Dapierre et al. (1977).

37.2.3.4 Priapism

Priapism is a medical condition characterized by erection of the penis and not returning to the flaccid state for more than 4 h, despite the absence of physical or psychological stimulation. Vascular, endocrine, and neurological factors may mediate priapism. Penile tumescence is determined by the balance between mediators of contraction (by norepinephrine) and relaxation (by nitric oxide, vasoactive intestinal peptide, and prostanoids) of the corpus cavernosum's vascular smooth muscles. Fenner and Carney (1999) and Nickson et al. (2009) reported cases of priapism caused by Irukandji syndrome.

Nickson et al. (2010) suggests that priapism caused by C. barnesi envenomation could be mediated by a neural sodium channel activator that releases nitric oxide.

37.2.3.5 Blurred Vision

Burnett and Burnett (1990) reported a 17-year-old patient who was stung on the knee by *Linuche unguiculata* in Nassau, The Bahamas. Three hours after the sting she developed nausea, dizziness, sweating, faintness, and feeling very hot in a cool room. At the same time, she realized that she could not focus on distant objects. She said, "Everything looked grey and blurred". The blurred vision continued for 8 days.

Accomodation reflex which maintains focusing on near or distant objects is controlled by the parasympathetic nervous system. Although most of the patient's symptoms can be attributable to systemic autonomic nervous system toxicity, the long-lasting and prominent symptom was confined to the eye, which was far away from the sting site.

37.2.4 Other Neurological Disorders

37.2.4.1 Dysphonia

Burnett (2005) reported a dysphonia case from Ft Lauderdale, Florida. The patient, who was a fit 20-year-old man, felt a sudden stinging irritation in his mouth after swimming through a swarm of small Physalia physalis medusae. When he returned to the companion boat, he realized he could not talk properly. He could make sounds and mumble, but no words. This lasted 30–45 min. While standing on the boat, he noticed a stinging, linear, papular eruption on his left pectoral and anterior deltoid areas. The condition was interpreted as toxin-induced paralysis of tongue muscles and lingual nerves.

Burnett et al. (1996) mention three other patients who had temporary loss of voice in their renowned book, *Venomous and Poisonous Marine Animals*. The patients were stung by unidentified jellyfish in the waters off Hua Hin, near Bangkok. Two weeks after being stung, two Thai and one American scuba diver developed aphonia for 3–4 days.

An explanation for these cases could be a super selective neurotoxin affecting a specific ion channel which is only found at the neuromuscular junction of the laryngeal muscles. A similar mechanism could also explain the biliary dyskinesia with urinary incontinence case and the blurred vision case. Until such toxins and ion channels are demonstrated, these cases will retain their mystery.

37.2.4.2 Coma and Death

Rhodactis howesii, a sea anemone-like corallimorph, is known to be cooked and eaten by the Samoans without any health hazard. But when eaten raw, it is known to cause fatal envenomations. Martin (1960) described this unusual envenomation from Pago Pago, capital of American Samoa. The envenomations started shortly after ingestion, with stupor that lasted 8–36 h. During this period, knee jerk and pupillary light reflexes were absent, but blood pressure and pulse rate stayed normal. After this period, patients went into a prolonged shock and eventually died of pulmonary edema.

37.2.4.3 Memory Impairment

Williamson et al. (1996) described a 49-year-old healthy man who had temporary memory impairment after contact with anemones. While he was collecting mussels from Hokimai Bay, Kawau Island, New Zealand, his chest came into contact with numerous small anemones. Within minutes, he developed a painful rash, swelling, and burning on his chest. He felt dizzy, weak, and hot. In the next 24 h, hemorrhagic vesicles erupted on his chest, and over the following days, he could not sleep or concentrate, and he felt dizzy and nauseated. For 4 weeks, he had memory impairment and muscle incoordination that restrained him from his professional work.

37.3 Cardiovascular Disorders

37.3.1 Cardiac Complications

37.3.1.1 Myocardial Infarction

Salam et al. (2003) reported a 45-year-old professional diver who developed myocardial infarction shortly after a jellyfish sting. The patient was stung by an unidentified jellyfish in the Gulf Sea, Qatar. Four hours after the contact, he started to feel chest pain. Twenty-two hours after contact, the patient presented to the hospital, distressed, diaphoretic and in pain. He was mildly hyperthermic (37 °C), tachypneic (20/min), and he had an erythematous area (10×5 cm) on his left forearm (site of contact). The electrocardiogram (ECG) and blood chemistry results suggested an acute myocardial infarction. The patient received routine thrombolytic and supportive treatment in the coronary care unit. The coronary angiography, which was made on the fourth day of his treatment, showed normal coronary arteries and hypokinetic right ventricle. The conclusion of the authors was a toxin-induced coronary vasoconstriction.

Konya and Elliott (1996) showed that the toxin from anemone *Tealia felina* produced marked reduction in the coronary flow (82 %) in the rat heart. Their findings showed that the toxin had a marked and irreversible constrictor action on coronary circulation. Elliott and Konya (1984) had previously shown that the venom extract was a potent vasoconstrictor of the isolated mesenteric artery. Lee et al. (1985) showed that equinatoxin, from *Actinia equina*, also produced coronary vasoconstriction.

37.3.1.2 Tako-Tsubo Cardiomyopathy

Tako-tsubo cardiomyopathy takes its name from a Japanese octopus fishing-pot, "takotsubo". It is characterized by acute chest pain, electrocardiographic changes which mimic an acute myocardial infarction, ballooning of the apical left ventricle, and absence of significant stenosis on the coronary arteries. It is known to be triggered by severe psychological stress and catecholamine discharge (Akashi et al. 2008).

Bianchi et al. (2011) reported the case of a patient who developed Takotsubo cardiomyopathy after a *Pelagia noctiluca sting*. The patient was a 53-year-old woman, swimming off the Calabrian coast of Italy, who was stung on her right forearm. She rushed to the shore and lost consciousness due to pulseless electrical activity. The lifeguard resuscitated her successfully. When the patient arrived at the emergency room, she had chest pain and the ECG showed signs of acute myocardial infarction. Echocardiogram showed apical akinesia with severe left ventricular dysfunction. She received thrombolytic treatment.

After successful thrombolysis, the chest pain and the ECG signs should improve. In this woman's case, none of these happened, as a result, an urgent coronary angiography was performed. The coronary angiography showed that there was no stenosis in the coronary arteries, but ejection fraction was low, and there was an aneurism in the left ventricle. She received supportive treatment. After 7 days, she was discharged from the hospital with improved EF and completely asymptomatic.

A similar case was reported from the far north of Queensland, Australia, by Tiong (2009). The 26-year-old diver was admitted to a local hospital following a jellyfish sting on his body. The description of the jellyfish was similar to that of Carukia barnesi. On admission to hospital, he developed symptoms consistent with Irukandji syndrome (pain, restlessness, agitation, palpitation). His serial ECGs revealed persistent generalized hyper-acute T waves. His Troponin I peaked at 5.5 μg 14 h after the sting. The serial echocardiograms showed mid-ballooning stress cardiomyopathy with poor mid-regional wall motions. The apex was spared. No invasive cardiac investigation was performed. This was the first documented case of mid-ventricular stress cardiomyopathy induced by jellyfish sting.

37.3.1.3 Heart Muscle Injury

Troponin I is used as a highly specific diagnostic marker for myocardial infarction and heart muscle death. Raised levels indicate cardiac muscle damage. In the medical literature, there are few reports of elevated troponin levels after jellyfish stings.

McD Taylor et al. (2002) reported a case with persistent cardiovascular symptoms and raised troponin levels after an unknown jellyfish sting. The 34-year-old female tourist was stung while snorkeling in 12 m of water on the outer portion of the Great Barrier Reef in North Queensland in April 2000. Shortly after seeing a jellyfish, she experienced intense burning pain in the right arm, leg, and flank, followed by dysphoria, anxiety, stomach cramps, vomiting, chest pain, dyspnea, and hyperventilation. When the patient arrived at

the hospital 3.5 h after the sting, her ECG showed non-specific T wave flattening and her cardiac troponin I level was high (12.4 μg/L). The echocardiogram was normal. Three days after the sting, the patient was transferred to a hospital in Melbourne with continuing symptoms. Troponin I elevation persisted for 2 weeks. The patient was discharged from the hospital still symptomatic with breathlessness, chest tightness, weakness, and fatigue, but over 5 months there was gradual improvement of symptoms. The most persistent symptoms were occasional chest pains and anxiety which remained for 17 months post-sting.

Little et al. (2001) reported another case in which the patient came into contact with an unidentified jellyfish off the Great Barrier Reef. The patient had initially normal troponin I levels (3.5 h post-sting) which peaked to 72 mg/L within the next 10 days. The patient developed left ventricular dysfunction and reduced ejection fraction 6 h after the sting.

37.3.2 Vascular Complications

Cnidarian envenomations may cause vascular complications. El Khatib and Al Basti (2000) reported a case who had ischaemia in her hand after a jellyfish sting in the Gulf Sea. The 15-year-old girl got stung by an unidentified jellyfish. She presented a few hours after the incident with skin eruption and erythema on the dorsum of her left hand. The index and middle fingers were swollen, cold, and cyanosed. The patient complained of numbness, pain, and itching in the area. Eighteen hours later, despite the anticoagulant and steroid treatments, the symptoms got worse. Surgical treatment was planned and fasciotomies were performed on the mid-ulnar and mid-radial aspects of the index and middle fingers. An immediate relief of ischemia was observed during the surgery.

Wiliamson et al. (1988) reported three jellyfish envenomation cases which caused impaired circulation of the stung limb from the Indian Ocean and the Andaman Sea. Distal arterial pulses were absent and there was regional cyanosis, suggesting the threat of distal gangrene. None of the patients had a previous history of vascular disease. Two patients underwent surgical fasciotomy, and surgical exploration was performed in one patient. The affected arterial segment appeared to be under the actual sting site. One of the patients was seriously and permanently handicapped with bilateral upper limb numbness and paresis, one had permanent sensory loss, while the third made an uneventful recovery.

37.3.2.1 Irukandji-Like Syndrome

Irukandji syndrome is characterized by abdominal pain, backache, vomiting, neuralgia, delayed systemic effects, and, cardiotoxicity. It is caused by *Carukia barnesi*. The envenomation sets of cathecolamine-like effects, ECG changes, troponin I elevation, hypertensive crisis, systolic dysfunction, or segmental hypokinesis of myocardium, and pulmonary edema. In Huynh et al.'s (2003) series, 22 % of the patients had evidence of myocardial injury.

The classical Irukandji syndrome is caused by Carukia barnesi; however, some cases with a similar clinical profile were reported from different locations. This entity is named as "Irukandji-like syndrome". Little et al. (2006) reported *Alatina nr mordens*, *Malo maxina*, *Carybdea alata* and *Carybdea xaymacana* as other cubozoan jellyfish which can cause this complex syndrome.

Grady and Burnett (2003) reported three US military combat divers who suffered from Irukandji-like syndrome during their diving exercises in Fleming Bay, off Key West, Florida. All cadets were in excellent physical condition before their dives. Envenomations happened during night dives in shallow water (10 ft). The patients experienced a stinging sensation, which was followed by extreme pain and spasm in the lower back. About 20 min later, cough and dyspnea started. They received basic first aid with 5 % acetic acid, according to the first aid instructions of the navy. They were found to be mildly hypertensive and tachycardic. Two of the patients experienced headache, cough, salivation, lacrimation, abdominal cramping, nausea, vomiting, anxiety, restlessness and severe muscle pain, and cramps in major muscle groups. One of the patient's blood chemistry revealed mild myoglobinemia and raised cardiac enzymes. The third patient's symptoms were less severe. Most symptoms resolved after 4 h with supportive, symptomatic treatment. This was the first report of Irukandji-like syndrome from US coastal waters.

37.3.2.2 Fatal Irukandji Syndrome

The first death from Irukandji syndrome was reported by Fenner and Hadok (2002). The victim was a 58-year-old tourist from the UK who was stung on the face and chest soon after he entered shallow water at a beach on Hamilton Island, Queensland, Australia. He did not see the creature, but he said, "something has got me," to his wife. Over 20 min, he developed muscle pains, cramps, sweating, anxiety, and nausea. He presented to the resort doctor. He was hypertensive (260/160) and tachycardic (142/min). Forty-five minutes after the sting, his condition suddenly deteriorated and he became unresponsive. A provisional diagnosis of cerebrovascular accident was made and an urgent transfer to a mainland hospital was requested. When the medical flight team arrived, the patient was unconscious with fixed dilated pupils. When he arrived at Mackay Base Hospital, brain CT showed an 8×5×7 cm hemorrhage centered on the basal ganglia, causing 1 cm midline shift. The hemorrhage was not considered salvageable by the neurosurgeon. The next day, the patient's pupils remained fixed and dilated, and brain death was confirmed.

37.4 Other Unusual Envenomations

37.4.1 Renal Complications

37.4.1.1 Acute Renal Failure

Guess et al. (1982) reported a Portuguese mano'war sting which caused acute renal failure in a 4-year-old girl. The envenomation caused a hemolytic reaction which led to the renal injury. In the same year, a similar case was reported by Spielman et al. (1982). Deekajorndech et al. (2004) reported a 7-year-old boy who developed acute renal failure following a jellyfish sting. The patient came into contact with the unidentified jellyfish at the Pattaya Beach, Thailand, 3 days before hospitalization. He developed hemoglobinuria and the renal biopsy revealed acute tubular necrosis.

Mizuno et al. (2000) reported an acute renal failure case after contact with the anemone, *Phyllodiscus semoni*. The 27-year-old man accidentally touched the anemone with his right arm while snorkeling in the Sulu Sea around Cebu Island. When the patient was admitted to the hospital, he had severe dermatitis on his right arm and signs of acute renal failure. The renal failure improved with supportive treatment. The renal biopsy revealed acute tubular necrosis.

P. semoni is found in the Western Pacific ocean. It is known as the "sea wasp anemone" in Japan. It is known to induce severe dermatitis. Nagai et al. (2002) identified three different toxins (PsTX-20A, PsTX-60A and PsTX60B) in the venom of *P. semoni*. Mizuno and colleagues identified another toxin (PsTx115), and carried on searching for the toxin which induced renal failure. They showed that the toxin bound directly to the glomeruli and induced endothelial injury in the glomeruli extending into the glomerular epithelial cells. The reaction was an acute thrombotic microangiopathy with complement activation and decreased membrane complement regulatory protein expression in rat glomeruli (Mizuno et al. 2007, 2012). Mizuno and colleagues' studies started with an unusual envenomation case and extended to the identification of the causative toxin and the nephrotoxicity mechanism. Their work deserves admiration.

37.4.1.2 Nephrotic Syndrome

Prasad et al. (2006) reported a 45-year-old woman who developed nephrotic syndrome after contact with *Milepora sp*. The renal biopsy showed minimal change disease.

37.4.2 Hepatic Complications

A fatal hepatotoxicity after contact with an anemone was reported by Garcia et al. (1994). The 28-year-old diver touched an anemone while free-diving in 6–10 m deep waters of St. Thomas, Virgin Islands. Ten to fifteen minutes after the contact, vesicular eruption and severe pain developed on his back and arms. He was admitted to a local hospital and followed for 24 h. After discharge he became progressively weak and lethargic. He was admitted to the hospital again, this time with jaundice and elevated liver enzymes. His condition deteriorated and after 5 days, he was intubated and transferred to Jackson Memorial Hospital in Miami, Florida. Four days after the transfer, he received liver and kidney transplants. His liver biopsy showed massive necrosis with peripheral lobular regeneration. The kidneys showed mild tubular necrosis. The patient's dive partner identified an anemone from *Condylactis sp*. The patient's serum was positive in 1:450 dilution against *Condylactis sp*.

Burnett (1992) reported another case from Florida who had elevated liver enzymes for 16 days after a presumed cnidarian sting. The serum of this patient was tested for *Condylactis sp*. antigens and found them to be similar to the other patient.

There are a number of studies which demonstrated the hepatotoxic effects of cnidarian toxins. Muhvick et al. (1991) encountered hepatic injury in rats injected by *Chrysaora quinquecirrha* venom while studying the effects of two antidotes (hyperbaric oxygen and verapamil). Wang et al. (2013) injected *Cyanea capillata* tentacle extracts into the rats and demonstrated destruction in the lobular structure, severe coagulation necrosis, extensive hemorrhage in the liver, as well as a dose-dependent increase of the liver enzymes. Ramkumar et al. (2012) observed occlusion of central veins with hemolyzed blood and vacuolation with pleomorphic nuclear material in the liver of mice which were injected with *Anthopleura asiatica* venom intraperitoneally. Houck et al. (1996) investigated the toxicity of *Chrysaora quinquecirrha* venom on cultured liver cells of rats and found that the hepatotoxin acted through Ca channels and that there was a large inter-animal variability. They also found that the hepatocytes cultured from old rats were more vulnerable to toxicity.

Some of the cnidarian hepatotoxicities could be related to complement activation. Ishikawa et al. (2004), who worked with *Chrysaora quinquecirrha* venom, found that the venom could induce complement activation, suggesting a role of complement activation in the tissue damage secondary to jellyfish stings.

37.4.3 Others

37.4.3.1 Gastrointestinal Bleeding

Fenner et al. (2010) reported a 40-year-old British diver who felt a sharp pain on the back of his head while ascending from a recreational dive near Pattaya in 2008. The burning

pain was very severe. On the boat, he developed nausea and started vomiting. He had abdominal cramps, shivering, headache, dizziness, chest tightness, and dyspnea. For a short while he fell unconscious. Three hours after the sting, the patient arrived at the hospital. He was hypertensive and still had abdominal cramps. After 18 h, he was discharged from the hospital with partial resolution of symptoms. Four hours after discharge, his abdominal cramps relapsed and he started vomiting blood. He returned to the hospital and his complaints settled with spasmolytic, anti-emetic, narcotic analgesic, and proton pump inhibitor treatments.

37.4.3.2 Pancreatitis and Pneumonia

Nickson et al. (2009) mention a 10-year-old girl who experienced pancreatitis, pneumonia, and ileus after jellyfish contact. There is not much detail except she was hospitalized for 11 days.

37.4.3.3 Mondor's Disease

Mondor's disease is a rare condition which causes superficial venous thrombophlebitis of the breast and anterior chest wall. Although it is usually benign and self-limiting, it is rarely associated with malignancy.

Ingram et al. (1992) reported two cases who developed Mondor's disease after a jellyfish sting. The first case, who was a 30-year-old woman, had a jellyfish sting while swimming off Rottnest Island, Western Australia. One week after the sting, a palpable 5–8 cm linear indentation developed in the breast. The second case who was a 50-year-old woman presented with a palpable cord extending from the nipple to the axilla. There were some visible marks from a jellyfish sting in the area. She reported as being stung by a jellyfish a month before.

37.4.3.4 Palytoxin Related Envenomations

Not all cnidarian envenomations take place in the sea. The ocean aquariums have brought the risk of cnidarian envenomation into homes.

Palytoxin is a powerful toxin found in soft corals or dynoflagellates. According to Moore and Scheuer (1971) and Khoo (2002), it is 60 times more potent than tetrodotoxin and 113 times more potent than stonustoxin of stonefish, with an intravenous LD50 (mice) of 0.15 mcg/kg. Intoxication with palytoxin can induce myalgias, weakness, neuromuscular dysfunction, wheezing, respiratory distress, hemolysis, and cardiac conduction abnormalities. Palytoxin may also cause Haff disease, which is characterized by massive rhabdomyolysis.

The first palytoxin intoxication from an aquarium tank case was reported from Europe by Hoffman et al. (2008). The patient was a 32-year-old man who was admitted to the emergency department of the University Hospital of Heidelberg, Germany. He had cut three fingers of his right hand while cleaning his sea aquarium which inhabited some zoanthid colonies. Two hours after the cut, shivering, myalgia, and general weakness appeared in all extremities. About 16 h later, he had collapsed in his workplace, exhibiting dizziness, speech disturbance, and glassy eyes. The palytoxin assay in the zoanthid colonies in the aquarium (*Parazoanthus sp*. and *Palythoa sp*.) was positive for *Parazoanthus sp*.

Another case was reported by Snoeks and Veenstra (2012) from the Netherlands. A family got intoxicated in their house by the soft coral in their ocean aquarium. The aquarium was getting invaded by the soft corals And to get rid of the invading coral, the father poured boiling water in the tank. This produced an offensive smelling fume. The 36-year-old man, his wife, and their 10 year old twins found themselves in an emergency department with nausea, headache, shivering, a metallic taste in their mouth, severe muscle cramps, hypotension, and high fever. The symptoms started right after they inhaled the offensive smelling steam coming out of the aquarium tank.

A third paper was published in 2013, this time from New York. Sud et al. (2013) reported six cases who were intoxicated by palytoxin vapor. The first case was a 32-year-old man who was trying to clean the mucous secretions of the *Palythoa* coral by boiling water. When he arrived at the emergency department he was tachycardic (120/min), tachypneic (24/min), and he was wheezing at chest oscultation.

The other cases mentioned in the paper were from another incident. A professional fish tank cleaner, the owner of the tank, the owner's wife and two children were intoxicated by palytoxin vapour. The tank cleaner poured boiling water over *Palythoa* coral. The man who inhaled the vapor developed shortness of breath almost instantly. The tank owner who was near the tank during the cleaning procedure developed a dry cough shortly after exposure. He presented to the emergency department 8 h later, when his cough got worse and he developed chills, myalgia, and fatigue. The wife of the patient also had similar symptoms, and paresthesia in both upper extremities. Their 3-year-old boy developed a dry cough, vomiting, and extreme fatigue. Their 2-month-old baby, who had been further away from the tank, was asymptomatic and had normal findings in the emergency department.

37.5 Hypersensitivity

The physiopathological mechanisms of hypersensitivity reactions and envenomations are completely different. In the case of hypersensitivities, the complex mixture of nematocyst constituents that enter the human skin sets off a complicated system of cytokine interactions. The symptoms are not direct actions of toxins.

The key cellular mediators of hypersensitivity are keratinocytes, mast cells, tissue macrophages, and dendritic cells.

Mast cells are the most potent drivers of inflammation. According to Tibals et al. (2011), cnidarian stings may activate mast cells in three ways. Primary envenomation may activate mast cells directly by porins or secretagogues (bioactive lipids, amines) in the venom; components of the nematocyst tubule or venom may activate innate or pattern recognition receptors on mast cells; physical changes (hypoosmolarity, acidification, reactive oxygen species) at the sting site may activate mast cells.

Cnidarian stings may induce type I or type IV hypersensitivity reactions. Type I hypersensitivity reactions can range from simple urticaria to lethal anaphylaxis. In this kind of hypersensitivity, an antigen stimulate the production of specific IgE antibodies and these bind to mast cells and basophils, producing a sensitized state against the antigen. Re-exposure to the same antigen results in degranulation of active mediators like histamine, prostaglandin, and leukotriene. These mediators produce vasodilatation, smooth muscle spasm, and leucocyte extravasation; causing an acute, sometimes life threatening reaction.

Type IV (delayed) hypersensitivity reactions develop days or weeks after the sting. This is a cell mediated response. Activated CD8+ T cells destroy target cells on contact and activated macrophages produce hydrolytic enzymes.

37.5.1 Anaphylaxis

Anaphylaxis is a severe, type I hypersensitivity reaction. It is characterized by fatal edema, low blood pressure, and rash. Anaphylaxis was discovered during the studies on anemone toxins. The French biologists, Portier and Ricket (1902), were investigating the toxicity of jellyfish in the early 1900s. After demonstrating the dose-related toxicity of *Physalia physalis*, they sought to demonstrate the same phenomenon with anemones. The toxin from the anemone proved to be less potent. But when they used the same animals again, they observed that a much smaller second dose was enough to kill them. They named this phenomenon anaphylaxis; in contrast to phylaxis (protection). This accurate observation provided the first insight into type I allergic reactions and brought them a Nobel Prize in 1913.

There are few reports of anaphylaxis caused by cnidaria. One of these was reported by Garcia Bara et al. (2006). The 34-year-old underwater swimmer with an allergic medical history (mite allergy with rhinitis, and asthma) came into contact with anemone *Actinia equina*. In a few minutes he developed local edema and papules at the affected skin area, followed by generalized flushing, dyspnea, hypotension, and he finally lost consciousness. When he arrived in the emergency room, he was under cardiopulmonary arrest. Fortunately, the resuscitative efforts were successful, and after only 24 h, he was discharged from the hospital with complete resolution of symptoms.

In this patient, a detailed allergy work-up was performed to find the triggering antigen. It was known that the patient had contact with anemones with minor symptoms before. The skin prick test was positive for mites and strongly positive for *Ac. equine* anemone. Gel electrophoresis for *Ac. equina* and *A. viridis*, which are prevalent anemones in the region, revealed a common 14.4 kDa band and the IgE immunoblotting with the patient's serum revealed an IgE binding band for this common 14.4 kDa protein. The conclusion was an IgE-mediated sensitization to *Ac. equina* and in vitro cross reactivity for another anemone (*A. viridis*).

Another case who had an anaphylactic reaction after contact with anemone was reported by Nagata et al. (2006). The 24-year-old man, working in an aquarium shop, presented to an emergency department with dyspnea and systemic urticaria. Before the incident, (at about 10 o'clock) he had started cleaning an aquarium tank in which there were many dead and decomposed Haddon's carpet anemones (*Stichodactyla haddoni*). At about 12 o'clock, he developed systemic eruptions and shortness of breath. When the patient presented to the hospital at 12:30, he was in visible distress and he had a cold sweat. There was marked urticaria on his face, trunk, and extremities, and he was drowsy. His physical examination revealed tachycardia (pulse: 116/min), hypotension (blood pressure 60 mmHg systolic) and wheezing. The patient received subcutaneous epinephrine, intravenous methyl prednisolone, dopamine infusion, and oxygen inhalation which improved his condition. The patient stated that several months before, he had been exposed to anemones and noticed local urticaria. The detailed allergy work-up of the patient and Western blotting with the homogenate of the sea anemones revealed specific IgE-mediated reaction against an 86 kDa anemone protein.

There are two cases of anaphylaxis caused by ingestion of jellyfish. The first of these was reported by Imamura et al. (2013). A 32-year-old female developed wheals, oral stinging sensation, dyspnea, hypotension, nausea, vomiting, and abdominal pain after eating salt-preserved jellyfish. As the patient was a surfer and frequently stung by jellyfish, the authors speculated that she could have been sensitized through the previous skin contacts.

The other case was reported by Inomata and colleagues (2014). The 45-year-old man presented with two episodes of anaphylactic reaction after eating jellyfish salad. He had a medical history of asthma and food allergies to natto (soybeans fermented by *Bacillus subtilis*), crab, and shrimp. In both instances, 2 h after the ingestion of jellyfish, he developed dyspnea, chest tightness, abdominal cramps, palpita-

tions, vomiting, dizziness, headache, and loss of consciousness. Interestingly, poly-gamma-glutamic acid, which is the major allergen of natto, is also found in cnidarian nematocyst capsules. The authors mention that 10 out of 12 natto allergy cases in their hospital were surfers who were frequently stung by jellyfish and speculate that their sensitization against natto could have been mediated by cnidarian stings.

37.5.2 Delayed Hypersensitivity Reactions

Nobody would expect a rash 1 week after a jellyfish contact. That was exactly what happened to a 57-year-old woman who returned from a trip to the Red Sea. Veraldi and Carrera (2000) reported a case who developed widespread papulonodular eruption, itching, and burning pain 1 week after contact with a shoal of unidentified jellyfish. At the time of contact, she did not have any symptoms. She claimed she had previous contacts with jellyfish during other trips to exotic seaside resorts. A histopathological examination revealed some necrotic keratinocytes in the upper and middermis, edema, and predominately perivascular and periadnexal lymphohistiocytic infiltrate with numerous neutrophils and eosinophils. The patient was treated with antihistamine and steroid, which resulted in the rapid disappearance of pruritus and burning symptoms, but the cutaneous lesions persisted for 3 weeks.

Another delayed hypersensitivity reaction was reported by Miracco et al. (2001). The 56-year-old woman was injured by coral while snorkeling off the coast of Saudi Arabia. She had an acute urticarial reaction which disappeared in 4 days with topical steroid treatment. But after 1 month, streaks of red papules appeared in the same area. The biopsy taken from the area revealed features similar to those of pityriasis lichenoides and persistent insect bite reaction. Daily topical corticosteroid application gave some symptomatic relief but it took 4 months for the skin lesions to clear completely. Some residual hyper-pigmentation remained.

An even more surprising case was reported by Rallis and Limas (2007) from Greece. A 4-year-old girl was referred to a dermatology clinic in Athens with recurrent dermatitis after a jellyfish sting. She had no previous allergies or other disease. The patient came into contact with a jellyfish on her left shoulder and cheek. She was initially treated with topical steroids and oral amoxicillin. However, the lesions reappeared after 15 days, and a second relapse followed within 1 month. Within 5 months, the child had nine relapses at gradually increasing intervals. The relapses always involved the sites of the primary lesions and consisted of erythematous, pseudo-vesicular, urticarial papules with mild edema. The biopsy taken after the second relapse revealed spongiotic vesiculation, dermal edema, and perivascular lymphohistiocytic infiltration. A search for nematocyst tubules was negative. 0.1 % tacrolimus ointment was used to control the relapses with partial success.

37.5.3 Dermographism

Dermographism is a skin disorder in which the skin gets raised and inflamed, like an urticarial rash, when scratched, rubbed, or stroked. Dermographism has simple or symptomatic forms. While simple dermographism has no apparent cause, symptomatic dermographism is linked to a cause, such as infection or medication. Wu et al. (2006) reported a coral induced symptomatic dermographism case. The 23-year-old perfectly healthy woman who had no personal or familial allergic condition presented to a dermatologist with pruritic hives appearing 5 min after she scratched her skin. The only unusual thing she could remember was a coral injury which happened 2 weeks ago. She cut her left foot on a coral, while scuba diving in the Florida Keys, which resulted in edema in the area. Her dermatologist prescribed a medium strength topical steroid and the wound was healed with scarring after 7 days without any complication.

37.6 Discussion

This chapter is a collection of unusual cnidarian envenomations in the medical literature. The diversity of these cases should remind us of the complexity of cnidarian envenomations and the variety of cnidarian toxins. We should be alarmed, as the oceans are transforming to be better habitats for most cnidaria, and activities, such as shipping and ballast water transfer, have facilitated the inoculation of alien species to distant seas more than ever (Richardson et al. 2009). Cnidaria can threaten human activities in the sea with their wide range of staggering envenomation syndromes, making it a potential public health care problem. The medical community's awareness of the unpredictable nature of cnidarian envenomations is an important issue. Cases in this chapter are plotted on a world map in Fig. 37.1. Distribution of the cases suggest a zone with better awareness against cnidarian envenomations. It could be hypothesized that there should be similar cases round the globe in tropical, subtropical and temperate zones which have not been diagnosed or reported.

With the current sum of knowledge, only some of these rare conditions can be explained. For others, such as the sea-

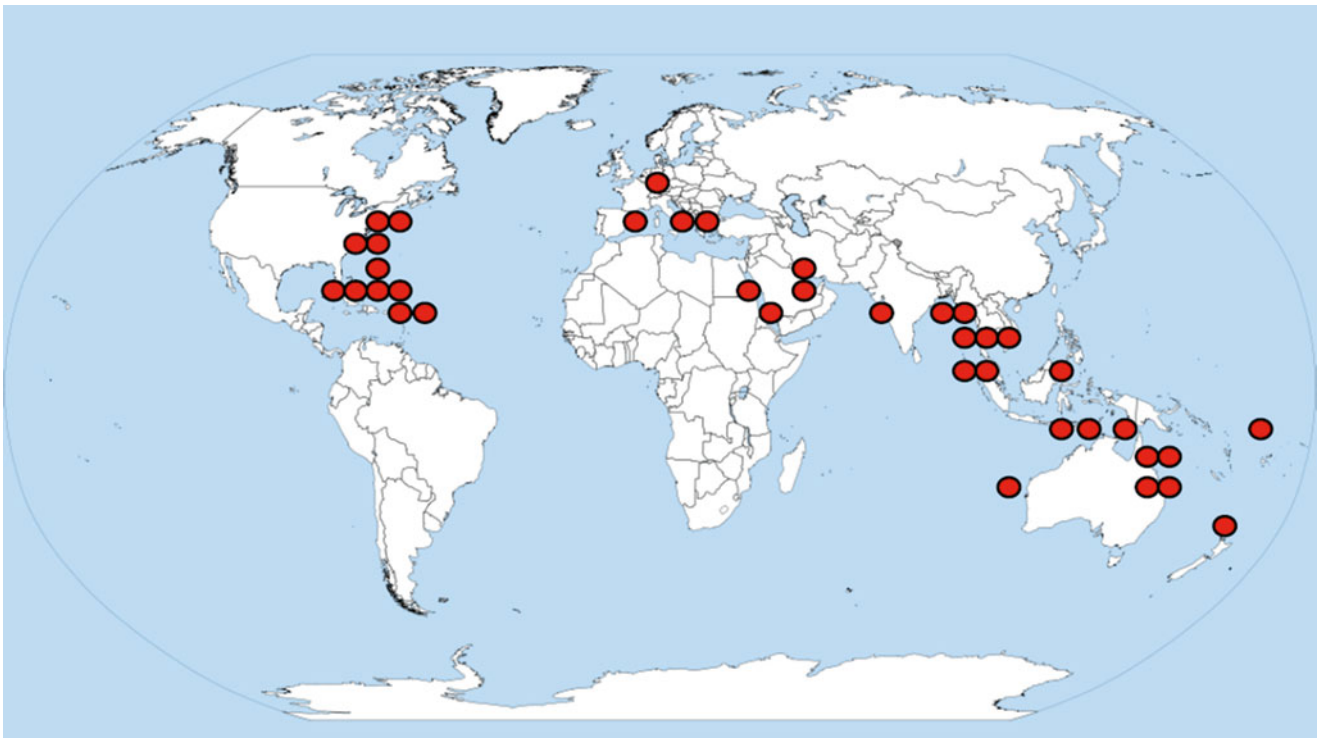

Fig. 37.1 Distribution of unusual cnidarian envenomations around the globe

stroke cases, there is still a dearth of sufficient data. Cnidaria are a rich source of bioactive compounds, making it an interesting and potentially beneficial area. As a result, both the number of scientists dealing with cnidarian toxins, and the annual number of studies being published is increasing, which should one day be able to provide data to enlighten these enigmatic cases.

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Envenomation by Cnidarians and Renal Injuries

38

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Abstract

Natural toxins are among the most important causes of acute kidney injury in humans. Several marine animals produce toxins that are nephrotoxic for humans. Cnidarians produce toxins in nematocysts that offer protection from potential enemies and are used in feeding. Envenomation by these toxins through stings can be toxic for humans, with injuries typically comprising skin eruptions and ulcers. However, the stings of several species induce more severe symptoms such as shock syndromes and organ failure, particularly renal failure. Here I summarize the current state of knowledge concerning renal injuries resulting from envenomation by coelenterates and describe the expected mechanisms, possibilities for therapeutic approaches, and strategies for preventing human diseases in future.

Keywords

Acute kidney injuries • Kidney disease • Nephrotoxin

38.1 Introduction

Many natural toxins induce tissue damage in the human kidney. Some toxin-related injuries cause acute kidney injury (AKI; acute renal failure) (Bacchetta et al. 2009), while others cause chronic kidney injury (Martinez et al. 2002; Liu et al. 2003). Envenomation by natural toxins occurs by one of three routes, i.e., bites, stings, and food poisoning, and the pathologies associated with envenomation include dermatitis, anaphylaxis, diarrhea, shock, and organ failure, including AKI (Mizuno et al. 2012). Among terrestrial organisms, clinical cases of AKI have been reported due to snake bites, bee stings, scorpion stings, spider bites and food poisoning, with symptoms typically manifesting as severe skin lesions and failure of other organs (Sitprija and Sitprija 2012; Mizuno et al. 2012). In Africa, Asia and Latin America, the risk of being bitten by a snake is a serious medical and social problem; for example, more than 120,000 deaths are attributed to snake bites in Asia and Africa every year (Sitprija 2006). Envenomation by vipers in India and around would has been reported to induce AKI in up to 30 % of affected patients (Chugh 1989). Other studies have reported AKI occurs in 5–80 % of snake-bite cases, with most of the damage attributed to the massive release of inflammatory mediators, hemolysis and intravascular coagulation (Warrell 1993; Barsoum 2004). The situation is complicated by the fact that envenomation can induce different types of AKI, including acute tubular necrosis, glomerular nephritis, interstitial nephritis and papillary necrosis (Chugh 1989). Natural toxin induced AKI sometimes requires renal replacement therapy (Cheng and Currie 2004; Cruz et al. 2009; Jha and Parameswaran 2013; Albuquerque et al. 2013), and it is one of the major causes of death in the developing countries (Cruz et al. 2009).

In aquatic environments, a wide variety of organisms have been reported to cause renal injury in humans following envenomation (Mizuno et al. 2012). For example, envenomation caused by poisonous aquatic animals can be occurred after bites of sea snakes and blue-ringed octopuses and stings of lion fishes, stingrays, cone-shells and sea urchins as poisonous aquatic animals in human (Karalliedde 1995; Brush

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2006; Mizuno et al. 2012). It is known that parts of cnidarians are also harmful in human. The phylum Cnidaria consists of the following five classes: Staurozoa (stalked jellyfish); Scyphozoa (true jellyfish); Hydrozoa (Portuguese Man O' War, fire corals and hydroids); Cubozoa (box jellyfish); and Anthozoa (sea anemones and true corals) (Cegolon et al. 2013). Although most cnidarians are harmless to humans, a few are extremely dangerous and are capable of causing dermatitis, systemic shock, respiratory failure, hepatic failure and renal failure (Mizuno et al. 2012; Cegolon et al. 2013). Some patients that have been stung by Cnidarians have either primarily or secondarily developed kidney injuries. However, compared to terrestrial animals, AKI following envenomation by aquatic organisms is rare. The main reason for this decreased incidence is likely to be that encounters with potentially dangerous aquatic organisms are less common than encounters with terrestrial animals. In addition, deaths resulting from envenomation at sea are likely to be underestimated as victims often drown due to cardiovascular shock and respiratory failure. If these cases could be identified, then true incidence of AKI attributable to cnidarians would likely be greater than the number of cases that have been reported. In Australia, more than more than 60 lethal outcomes reported in children (Fenner and Williamson 1996). This high incidence is partly because venomous species, such as the box jellyfishes *Chironex fleckeri* is well represented in Australian. In Japan, *Chiropsalmus quadrigatus* (Habu-kurage in Japanese) is similarly another deadly specie called as a sea wasp (Nagai et al. 2002b). Indeed, given the threat posed by these box jellyfishes, anti-venom has been developed to neutralize envenomation by *Chironex fleckeri* in Australia (Andreosso et al. 2014).

No general and effective treatment has yet been developed for treating the stings of other cnidarians (Junghanss and Bodio 2006). However, supportive therapies, such as the administration of corticosteroids or antihistamines for severe dermatitis, intravenous drip infusions of liquid substances, and/or dialysis therapies for AKI, are usually sufficient for local/systemic reactions. To date, the only anti-venom that has been developed to treat envenomation by a marine organism in Australia is the anti-venom for the deadly *Chironex fleckeri* (Andreosso et al. 2014). On the other hand, several anti-venoms have been developed to treat the bites from poisonous terrestrial species. For example, anti-venoms have been developed to treat bites from the Habu snake, *Trimeresurus flavoviridis* and the Mamushi snake, *Gloydius blomhoffii* (Kondo and Murata 1972; Fukuda et al. 2006). However, the toxins from some cnidarians as well as products derived from the toxins have been found to possess bioactivities that could potentially be useful in the fields of biochemistry and medicine.

In this chapter, I focus on cnidarian envenomation and various aspects of renal injuries that have been described in clinical case reports and basic research, including reports published by our group.

38.2 Natural Toxins and Suspected Mechanisms of Kidney Injuries in Kidney

Acute kidney injury (AKI) is an important example of organ failure caused by natural envenomation (Table 38.1; modified from (Mizuno et al. 2012)). Well-known examples of food-related poisons that result in AKI include ingestion of the alkaloids in magic mushrooms (e.g., *Amanita sp.*) (Courtin et al. 2009; Iwafuchi et al. 2003; Kirchmair et al. 2012) ; toxins in pufferfish meat ("fugu" in Japanese) (Nakashima et al. 2007), and dinoflagellate toxins in ciguatera poisoning (Caillaud et al. 2010). Biting injuries that cause AKI include envenomation by poisonous snakes such as vipers and cobras (Sitprija and Boonpucknavig 1977). Similarly, toxins from terrestrial invertebrates, such as hornets (*Vespa crabro*), honey bees (*Apis mellifera*), wasps (*Vespa magnifica*), Iranian scorpions (*Hemiscorpius lepturus*) and Lonomia caterpillars (*Lonomia obliqua*) have also been reported to cause AKI in humans (Xuan et al. 2010; Vetter et al. 1999; Vikrant et al. 2005; Vachvanichsanong and Dissaneewate 2009; Pipelzadeh et al. 2007; Valavi and Ansari 2008; Burdmann et al. 1996; Gamborgi et al. 2006).

AKI induced by these natural toxins exhibits a wide variety of pathologies, including acute tubular necrosis (ATN), renal tubular occlusions, renal ischemia and thrombotic microangiopathies (TMA) such as hemolytic uremic syndrome (HUS) and disseminated intravascular coagulation (Sitprija 2008; Bacchetta et al. 2009; Mizuno et al. 2012). The mechanism of development of ATN, which is a common cause of AKI, likely involves changes in the systemic hemodynamics induced by systemic shock or changes in the local hemodynamics within the kidney; rhabdomyolysis and hemolysis are also implicated (Sitprija 2008; Bacchetta et al. 2009; Mizuno et al. 2012). Direct nephrotoxicity by venom can also cause tubulointerstitial and glomerular injuries in the kidney and TMA and renal injuries resulting from immunological mechanisms have also been reported (Sitprija 2008; Bacchetta et al. 2009; Mizuno et al. 2012) (Fig. 38.1).

Cnidarian envenomation is typically caused the stings of nematocysts, but parts of toxins are derived from ectodermal gland cells (Frazão et al. 2012). The severity, type, and amount of toxin, as well as the area of envenomation are important factors in deciding whether severe injuries follow envenomation by cnidarians. Cnidarian stings have the potential to induce kidney injuries due to systemic shock, and hemodynamic alterations can lead to renal ischemia due to vasodilation, inflammation and direct nephrotoxicity.

Table 38.1 Natural toxins capable of inducing acute kidney injury in humans and potential animal models

Organisms	Type of renal injuries/pathology	References
1. Marine envenomation		
(1) Biting		
Sea snakes (*Hydrophis cyanocinctus*, *Laticauda semifasciata*)	ATN[a], renal ischemia	Sitprija and Sribhibhadh (1971), Schmidt et al. (1976), Tu (1987), and Ali et al. (2000)
(2) Sting		
Lionfish (genus *Pterois*)	ATN	Balasubashini et al. (2006)
Cnidaria		
Jelly fishes *Aurelia aurita*	ATN	Wiersbitzky et al. (1973)
Portuguese man-of-war (*Physalia physalis*)	ATN with hemolysis, shock kidney	Guess et al. (1982), Spielman et al. (1982), and Stein et al. (1989)
Lion's mane jellyfish (*Cyanea capillata*)	ATN with hemolysis	Guess et al. (1982) and Wang et al. (2013a), (2014)
Box-jellyfish (*Chirodropids* sp.)	ATN	Fenner et al. (2010)
Sea nettle (*Chrysaora quinquecirrha*)	ATN, change of renal hemodynamics	Muhvich et al. (1991)
Non-specified jellyfish	ATN	Deekajorndech et al. (2004)
Fire coral (*Millepora* spp.)	Minimal change nephrotic syndrome	Ramesh Prasad et al. (2006)
Sea anemone *Phyllodiscus semoni*	ATN, TMA[b], renal ischemia	Mizuno et al. (2000, 2007)
Condylactis sp.	ATN	Garcia et al. (1994)
Heteractis magnifica	ATN with hemolysis	Ravindran et al. (2010)
Stichodactyla haddoni	Renal ischemia	Ravindran et al. (2010)
Stichodactyla gigantea	Hemorrhage and hyaline casts in renal insterstitium	Hu et al. (2011)
Paracodylactis sinensis	Hemorrhage and hyaline casts in renal insterstitium	Hu et al. (2011)
Anthopleura asiatica, Anthopleura midori, *Bunodosoma caissarum*	ATN with hemolysis, abnormality of renal perfusion injuries	Ramkumar et al. (2012)
(3) Food poisons		
Puffer (Globe) fish (*Lagocephalus*, *Lactoria*)	ATN with rhabdomyolysis, renal ischemia	Nakashima et al. (2007), Shinzato et al. (2008), and Saoudi et al. (2010)
2. Land envenomation		
(1) Biting		
Snakes: viper (Viperidea) and cobra (Elapidae)		
Habu snakes (*Trimeresurus*)	Mesangial proliferative glomerulonephritis, mesangial injuries	Kubo et al. (2002), Matsumoto et al. (2002), Masuda et al. (2001), and Yasunaga et al. (2011)
Mamushi snake (*Gloydius blomhoffii*)	ATN with hemolysis	Yasunaga et al. (2011)
Tiger snake (*Notechis scutatus*)	TMA, ATN with rhabdomyolysis	Casamento and Isbister (2011) and Hood and Johnson (1975)
"Fer-de-Lance" pit viper (*Bothrops* (*B.*) *lanceolatus*)	TMA	Malbranque et al. (2008)
(*B. jararaca*, *B. jarararacussu*, *B. moojeni*)	Renal cortical necrosis, rhabdomyolysis	Amaral et al. (1985), Burdmann et al. (1993), and Barbosa et al. (2002)
Brazilian rattlesnake (*Crotalus durissus*)	Rhabdomyolysis and hemolysis related renal injuries	Azevedo-Marques et al. (1985), Martins et al. (2002), and Pinho et al. (2005)
Russell's viper (*Daboia* (*Vipera*) *russellii*)	Cortical necrosis, ATN with rhabdomyolysis, mesangiolysis	Ratcliffe et al. (1989) and Willinger et al. (1995)
Lansberg's pit viper (*Porthidium lansbergii*)	ATN, glomerular and tubular injuries	Otero et al. (2002) and Vargas et al. (2011)
Taipan (*Oxyuranus scutellatus*)	HUS[c]	Cobcroft et al. (1997)
Spider		
Brown recluse spider (*Loxosceles intermedia*)	Hemolysis and rhabdomyolysis related renal injuries, Lung and glomerulonephritis	Luciano et al. (2004) and Lung and Mallory (2000)
(2) Sting		
Honey bee (*Apis mellifera*)	ATN with hemolysis and rhabdomyolysis, renal ischemia	Vetter et al. (1999)

(continued)

Table 38.1 (continued)

Organisms	Type of renal injuries/pathology	References
Hornet (*Vespa crabro*)	ATN with hemolysis and rhabdomyolysis	Xuan et al. (2010)
Wasp (*Vespa magnifica*)	ATN with hemolysis and rhabdomyolysis, or by direct toxic effects	Vikrant et al. (2005) and Vachvanichsanong and Dissaneewate (2009)
Iranian scorpion (*Hemiscorpius lepturus*)	HUS[c]	Pipelzadeh et al. (2007) and Valavi and Ansari (2008)
Lonomia caterpillars (*Lonomia obliqua*)	Hemodynamic changes and disseminated intravascular coagulation related renal injuries	Burdmann et al. (1996) and Gamborgi et al. (2006)
(3) Food poison		
Mushroom		
Cortinarius sp.	Chronic interstitial nephritis	Frank et al. (2009) and Calviño et al. (1998)
Amanita (*A.*) *phylloides*, *A. proxima*, *A. smithiana*, *A. pseudoporphyria*, *A. gracilior*, *A. boudierim*, *A. Echinocephala, etc.*	ATN, interstitial nephritis	Garrouste et al. (2009), Courtin et al. (2009), West et al. (2009), Iwafuchi et al. (2003), and Kirchmair et al. (2012)
Lepiota sp. (no detail pathology)	Acute renal failure	Paydas et al. (1990)
Squirting cucumber (*Ecbalium Elaterium*)	Renal failure (no detail pathology)	Vlachos et al. (1994)
Herb		
Chinese herb (*Aristolochia* sp.)	Chinese harb nephropathy, ATN, interstitial nephritis	Martinez et al. (2002) and Liu et al. (2003)

Table modified from Table 3 in Mizuno et al. (2012)
[a]Acute tubular necrosis
[b]Thrombotic microangiopathy
[c]Hemolytic uremic syndrome

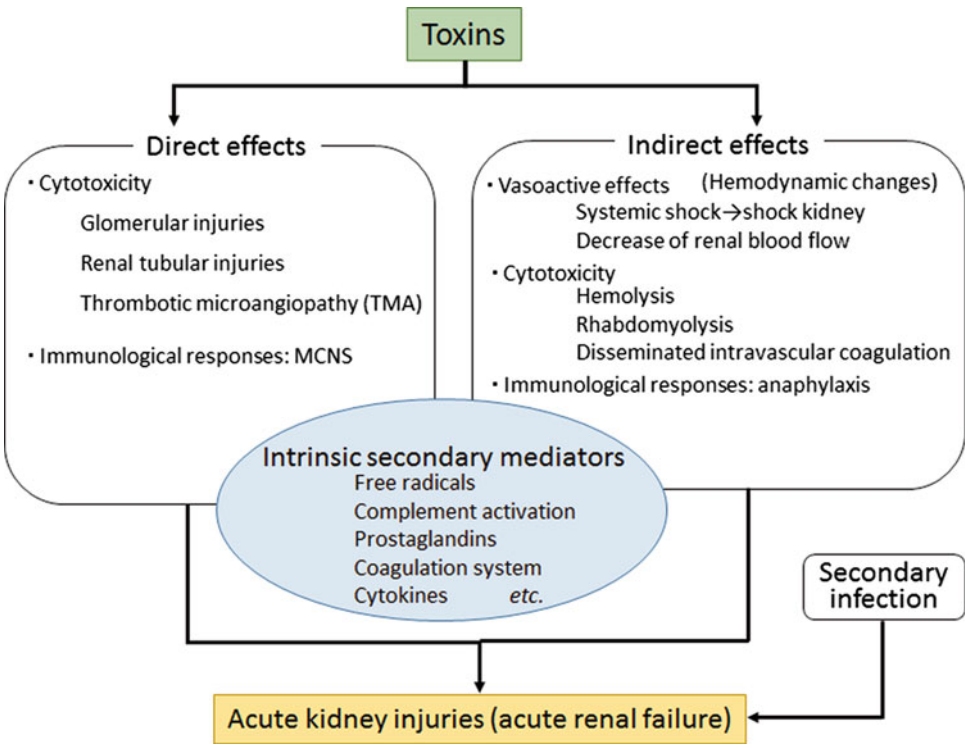

Fig. 38.1 Mechanisms for inducing acute kidney injury using a natural toxin. Mechanisms for inducing AKI are divided into direct and indirect toxin effects. Intrinsic factors associated with natural toxins exacerbate kidney injuries locally or systemically, resulting in AKI

38.3 Acute Kidney Disease and Stings of Cnidarians in Human

38.3.1 Jellyfishes

The poisons of several species of jellyfishes are toxic to humans. Most envenomation events cause local reactions that are typically associated with general symptoms such as pain, edema and skin eruptions, but skin necrosis has been observed in severe cases (Giordano et al. 2005). In addition, some events are accompanied by severe systemic reactions and organ failure such as coronary vasospasm, cardiac arrhythmia, pulmonary edema and AKI (Burnett and Calton 1987a; Burnett et al. 1987). In extremely severe cases, envenomation by jellyfishes can be fatal due to respiratory and/or cardiac arrest (Burnett et al. 1987).

Although jellyfishes in the phylum Cnidaria are categorized into three classes, Cubozoa, Scyphozoa and Hydrozoa, the class Cubozoa, which contains the box jellyfishes *Chironex fleckeri* and *Chiropsalmus quadrigatus*, have received considerable attention because they are extremely poisonous to humans. Stings from either of these species can be fatal within minutes, with death resulting from rapid cardiovascular collapse (Tibballs et al. 2011). In addition to *Chironex fleckeri*, human fatalities have also been attributed to *Chiropsalmus quadrigatus* (sea wasp, or habu-kurage in Japanese), *Carybdea arborifera* and *Alatina moseri* (Nagai et al. 2002b; Tibballs et al. 2011). Other highly venomous cnidarians include *Physalia physalis* (Portuguese man-o'-war), *Physalia utriculus* (blue bottle), *Chrysaora quinquecirrha* (sea nettle), *Stomolophus meleagris* (cabbage head jellyfish), *Cyanea capillata* (lion's mane jellyfish), *Carukia barnesi* (Irukandji jellyfish), *Morbakka* (Moreton Bay Carybdeid medusa) and *Pelagia noctiluca* (mauve blubber) (Burnett and Calton 1987b).

Death following envenomation typically occurs due to cardiac arrest and respiratory failure (Burnett and Calton 1987a; Burnett et al. 1987), which in vivo experiments have suggested is due to cardiotoxins that induce coronary vasospasm (Ramasamy et al. 2005) and/or arrhythmias which can cause cardiogenic shock (Kim et al. 2006). These in vivo experiments might support the presence of cardiotoxins that induce coronary vasospasm (Ramasamy et al. 2005) and/or arrhythmias, developing cardiogenic shock (Kim et al. 2006). Most severe jellyfish injuries occur in young children, because of their relatively lower body mass compared to adults because they lack body hair which can offer some protection by limiting direct contact of the jellyfish tentacles with exposed skin (Currie 2003; Currie and Jacups 2005).

Very few studies have been conducted describing jellyfish envenomation accompanied by AKI. The first report of a fatality was due to lethal shock and AKI resulting from envenomation by *Physalia physalis* envenomation (Stein et al. 1989). Another report of an individual stung by *Physalia physalis* described the induction of AKI (Spielman et al. 1982). Lethal reactions associated with AKI have also been described in patients that have been stung by chirodropid and Irukandji jellyfishes (Fenner and Lippmann 2009). Under conditions of circulatory shock, AKI may develop secondarily and is referred to as "shock kidney".

Few primary AKI cases have been reported in the literature; *Cyanea capillata* envenomation caused AKI associated with intravascular hemolysis and hemoglobinuria in a 4-year-old girl stung on three extremities (Guess et al. 1982). In another case of AKI following envenomation by a non-specified jellyfish, development of ATN was reported in a young child (Deekajorndech et al. 2004).

Envenomation by jellyfish can also induce immunological reactions, which can manifest as anaphylactic shock (Togias et al. 1985). Another immunological reaction to jellyfish envenomation is delayed hypersensitivity, which occurs in approximately 50 % of cases that have been stung by jellyfishes (Nimorakiotakis and Winkel 2003). Delayed injuries following jellyfish stings can include severe organ injury or systemic reactions, termed the delayed jellyfish envenomation syndrome (DJES) (Wang et al. 2013a), which can develop into organ failure, including AKI. In an animal model of DJES, crude venom extracts from *Cyanea capillata* caused vasoconstriction, suggesting a cause for development of renal ischemia (Wang et al. 2013a).

38.3.2 Sea Anemones

Sea anemones, which belong to the class Anthozoa, are armed with venom-secreting nematocysts that are used to capture prey and offer protection from predators. While most sea anemone injuries are limited to skin rash and edema, some species are extremely poisonous to humans; for example, *Actinodendron plumosum* (hell's fire sea anemone (Fig. 38.2a)), *Stichodactyla haddoni*, *Anemonia sulcata*, *Condylactis sp.* and *Phyllodiscus semoni* (Fig. 38.2b) (Ulrich et al. 2008; Haddad et al. 2009; Mizuno et al. 2000; Brush 2006; Nakamoto and Uezato 1998; Nagata et al. 2006; Maretic and Russell 1983; Garcia et al. 1994). Stings from these species may induce skin necrosis at the wound site and require prolonged treatment periods in hospital (Ulrich et al. 2008; Maretic and Russell 1983; Mizuno et al. 2000). Fortunately, only a few case reports describing AKI in patients stung by sea anemones have been reported to date. In one case, a patient developed AKI with severe acute tubular necrosis after contact with *P. semoni* (Mizuno et al. 2000) which can be difficult to identify because it can be resemble other organisms under some circumstances (Hoeksema and Crowther 2011). In this case, direct nephrotoxicity of the venom was considered to be responsible as there was no evidence of organ failure or a state of shock. In another case of envenomation by the sea anemone, *Condylactis sp.*, the

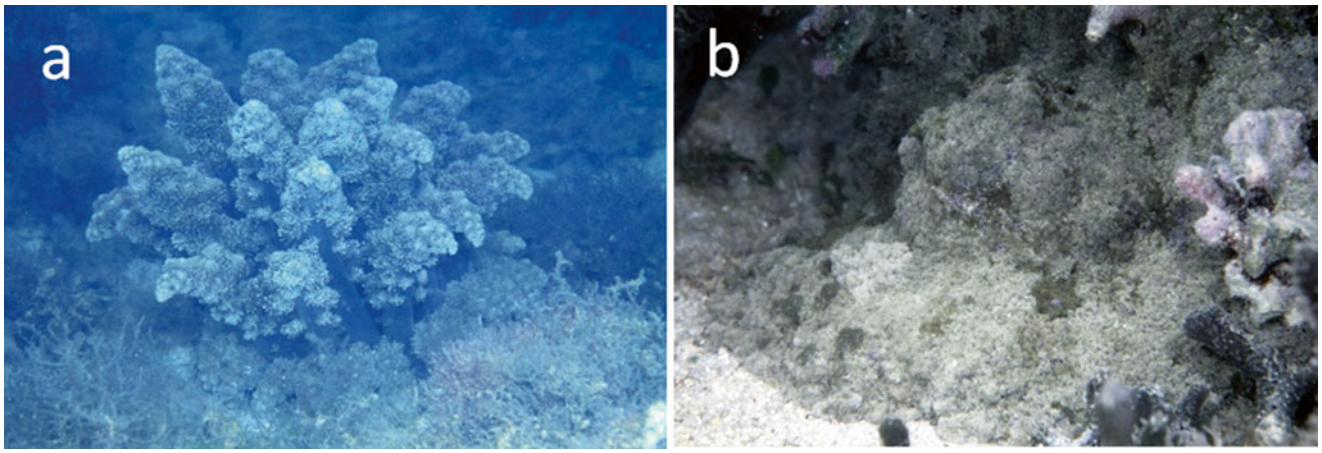

Fig. 38.2 Two of potent harmful sea anemones, *Actinodendron plumosum* and *Phyllodiscus semoni* to be able to see in Okinawa. **a** *Actinodendron plumosum*, or hell's fire anemone ("Hanabusa-isogintyaku" in Japanese); **b** *Phyllodiscus semoni*, or sea wasp ("Unbachi-isogintyaku" in Japanese) (Both pictures were taken by the author around Okinawa Island, Japan)

patient developed AKI with acute tubular necrosis accompanied with hepatic failure (Garcia et al. 1994).

As with envenomation by another poisonous creature, a report has described the onset of systemic shock in patients following envenomation by sea anemones that the toxin of *Stichodactyla haddoni* induced anaphylactic shock in one (Nagata et al. 2006). Although the report did not clarify whether the patient developed AKI, a patient in shock state may experience shock kidney due to disruption of the hemodynamics in the kidney.

38.3.3 Other Causes of Kidney Injuries Related to Envenomation by Cnidarians

38.3.3.1 Soft Coral

In a rare case of kidney injury following exposure to fire corals (*Millepora* spp.), minimal change nephrotic syndrome (MCNS) and AKI with pulmonary edema were reported (Ramesh Prasad et al. 2006). MCNS is usually diagnosed by the presence of a nephrotic state accompanied by minor glomerular abnormalities, as a typical pathological finding under light microscopy and no deposits in glomeruli under electron microscopy. In this case, corticosteroid therapy improved symptomatic proteinuria. Physicians should therefore consider MCNS when injured patients exposed to cnidarians present with proteinuria. A prompt diagnosis by renal biopsy may be helpful for selecting an appropriate therapeutic approach and potentially decrease the possibility of developing complications.

38.3.3.2 Secondary Infections in Wounds Caused by Cnidarians

Injuries sustained after being stung by a cnidarian may lead to the development of secondary bacterial infections, which can cause or exacerbate AKI. Causative microorganisms include *Vibrio (V.)* spp., *Aeromonas hydrophila*, *Mycobacterium marinum* and *Streptococcus iniae* (Haenen et al. 2013). Pathogenic vibrio species can cause sepsis and lethal shock (Ruppert et al. 2004), occasionally accompanied by severe organ failure and kidney injury. Managing wounds to prevent secondary bacterial infection can be achieved by administration of prophylactic antibiotics (Kuo et al. 2007). Cases of wound infection following consumption or handling of fresh raw seafood have been reported, with severe septicemia observed in patients infected with *V. cholera*, *V. parahaemolyticus* and *V. vulnificus* (Johnston et al. 1985; Ruppert et al. 2004); in these cases, AKI was reported as a complication (Eko et al. 1994; Wang et al. 2000: Mascarello et al. 2013). The risk of secondary infection following cnidarian envenomation, combined with the potential for the development of AKI (Maretic and Russell 1983; Oscherwitz 1994), means that wound management involving the treatment of local wounds, pain control, tetanus prophylaxis and supportive therapy is important for preventing systemic reactions.

38.4 Basic Research on Acute Kidney Injury Caused by Toxin of Cnidarians

To perform detailed in vivo and in vitro analyses of the mechanisms of human diseases caused by cnidarian envenomation, extracts of nematocysts and purified toxins are required.

38.4.1 Toxins from Jellyfishes

In 1968, a report described renal toxicity associated with envenomation by the jellyfish, *Chrysaora quinquecirrha* (Burnett et al. 1968), and in 1973, it was reported that extracts

from *Aurelia aurita* induced ATN in rabbits (Wiersbitzky et al. 1973). Previously, pathogenesis of AKI following envenomation by jellyfishes was considered to be secondary to changes in systemic hemodynamics (shock kidney), or to changes in the local hemodynamics of the kidney, such as vascular occlusion caused by hemolysis and rhabdomyolysis, which can induce ATN. Direct effects of the toxin could also damage the glomeruli, tubuli and/or small vessels. In addition, other possibilities such as immunogenic reactions against the toxin should also be considered. Cases of renal injury are partly summarized in Table 38.1.

Considering the detailed mechanisms by which toxins cause AKI, the following effects of the toxin need to be considered: vasoactivity of the toxin and its affect on hemodynamics, enzyme dynamics and inflammatory mediators involved in hemolysis and cytotoxicity, and neurotoxicity and arrhythmia and how these affect neurotransmitters and ion transport (Sitprija and Suteparak 2008). Hemodynamic changes due to the endogenous vasoactivities of toxins, or the release of vasoactive mediators from the host, can induce local ischemia and/or systemic shock, leading to AKI. The effect of toxins on enzyme functioning and inflammatory responses can cause hemolysis and cytotoxicity in kidneys. For example, some cnidarian toxins can induce pore formation in the cell membrane by effecting enzymes such as phospholipase A2 (PLA2), and induce cytotoxicity. PLA2 has been shown to cause hemodynamic changes with decreased renal blood flow (de Sousa Alves et al. 2005). In an in vitro study, tentacle extract from the jellyfish *Cyanea capillata* directly triggered cytotoxicity in rat renal tubular epithelial cells by inducing mitochondrial dysfunction (Wang et al. 2013b). In an animal model, tentacle extract from *Cyanea capillata* caused kidney injuries with a hemorrhagic appearance (Wang et al. 2014). Experimental administration of venom from the sea nettle (*Chrysaora quinquecirrha*) induced necrosis of renal proximal tubular epithelium in renal cortical structures, possibly due to cytotoxic effects in the cell membrane and by change in the hemodynamics of the kidney (Muhvich et al. 1991).

Another possible mechanism for inducing AKI, allergic reactions, can cause shock and circulatory failure; high-titer cross-reactive antibodies were detected in sera from some patients injured by *Chrysaora quinquecirrha* or *Physalia physalis* (Russo et al. 1983).

These findings showed that toxins, directly or indirectly, cause intravascular hemolysis, hemorrhage, disseminated intravascular coagulation, myonecrosis, complement activation, and the generation of free radicals which then contribute to hemodynamic changes.

38.4.2 Toxins from Sea Anemones

Although numerous extracts of nematocysts and purified toxins have been analyzed to date, few venoms and purified toxins have shown direct nephrotoxicity. The sting of *P. semoni* caused severe necrotic dermatitis and AKI in affected patients (Mizuno et al. 2000; Araki et al. 1994). Crude venom (PsTX-T) extracted from the nematocysts of *P. semoni* severely injured the renal cortex, outer medulla and inner medulla in rats (Fig. 38.3) (Mizuno et al. 2007, 2012). A 115 kDa extract, PsTX-115, purified from PsTX-T showed specific nephrotoxic effects without injuring other organs in the rat (Mizuno et al. 2007) ; however, other purified toxins from *P. semoni*, PsTX-20A, PsTX-60A and PsTX-60B, all showed hemolytic activities (Nagai et al. 2002a). In fact, PsTX-60B acted as a kind of perforin, causing pore formation in cell membranes (Satoh et al. 2007), which could in turn exacerbate AKI induced by PsTX-115. The venom directly attacked the kidney and the nephrotoxicity was manifested as thrombotic microangiopathy, which has a similar pathology to hemolytic uremic syndrome and acute tubular necrosis (Mizuno et al. 2007, 2012).

As with venom from jellyfishes and snakes (da Cruz Höfling et al. 2001), nematocyst-derived extracts from some sea anemones also contain phospholipases (PLA). For example, PLA2 proteins derived from *Bunodosoma caissarum* (a sea anemone from Brazil) increased perfusion pressure, renal vascular resistance, glomerular filtration and excretion of electrolytes such as sodium and potassium in an isolated kidney (Martins et al. 2009). Hypotension associated with envenomation can markedly decrease local circulation in the kidney, inducing AKI and renal ischemia (Chen et al. 1984). Envenomation by *Cyanea capillata* induced shock kidney and decreased blood flow in the kidney (Xiao et al. 2010).

In addition to neurotoxic, cardiotoxic and hepatotoxic effects, venom from other sea anemone species, such as *Heteractis magnifica*, *Stichodactyla haddoni*, and *Paracodylactis sinensis*, all showed nephrotoxic effects in animal models; the observed renal pathologies were mainly tubular epithelial degeneration, vascular congestion in the cortical region, glomerular tuft shrinkage, suspected occlusions of hemoglobin in renal tubuli, and/or renal ischemia (Ravindran et al. 2010). Venom of *Anthopleura asiatica* also induced AKI in a mouse model through vaso-occlusion in conjunction with other organ injuries (Ramkumar et al. 2012). Other hemolytic toxins, cytolysin (Ucl and Upl), sticholysin II (St II) and equinatoxin II (EqT-II), have been isolated from *Urticina crassicornis* and *Urticina piscivora* (Razpotnik et al. 2009), *Stichodactyla helianthus* (Celedon et al. 2005) and *Actina equine* (Macek et al. 1994). Although these hemolytic toxins were not observed to cause AKI, they could potentially induce AKI from the predictive block

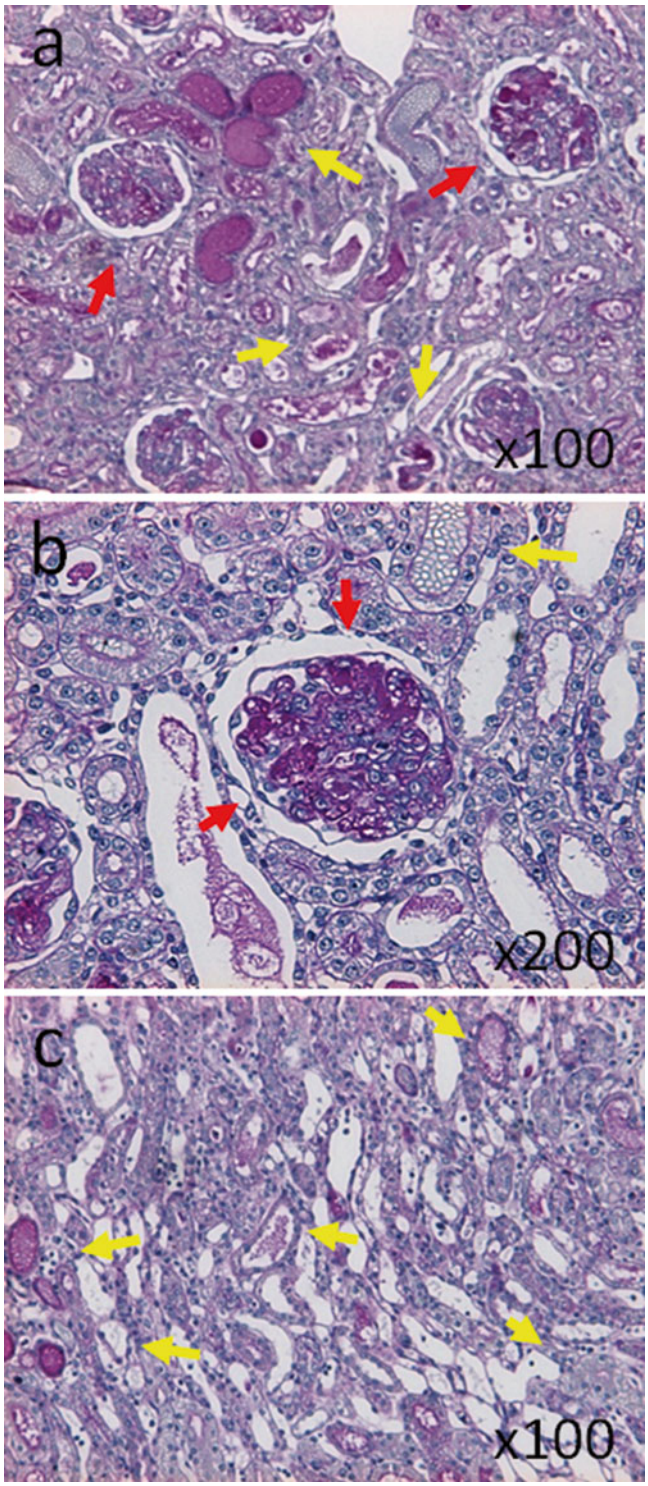

Fig. 38.3 Histological changes after intravenous injection of crude venom from *Phyllodiscus semoni*. Typical pathological changes 3 days after injection with crude venom extracted from *Phyllodiscus semoni* in a rat. Frames **a**–**c** show the cortex, glomerulus and medulla of the rat kidney. *Red arrows* show mesangiolysis, endothelial injuries with bleeding in glomeruli. *Yellow arrows* indicate interstitial damage, such as tubular epithelial damage with casts and tubular occlusion by red blood cells. Original magnification is shown at *bottom right*

mechanism such as occlusions with hemolysis and bleeding in renal tubuli.

Intravenous injection of gigantoxin-4, an actinoporin toxin extracted from *Stichodactyla gigantea*, induced pore formation in the cellular membrane and directly caused AKI due to sporadic hemorrhage and the formation of hyaline casts in mice kidneys (Hu et al. 2011).

38.4.3 Others

Few studies related to renal injuries induced by venoms derived from cnidarians other than jellyfish and sea anemones have been published. Toxins from soft corals have been shown to induce renal disease; for example, palytoxin from the soft coral *Palythoa vestitus* induced shock or uremia with renal bleeding and renal injuries (Wiles et al. 1974).

38.5 Therapeutic Approach for AKI Caused by Cnidarian Envenomation

Vaccinations for protection from deadly jellyfishes such as *Chironex fleckeri* are occasionally used in Australia (Andreosso et al. 2014; Currie and Jacups 2005). In most cases of cnidarian envenomation, only symptomatic treatment can be performed after first-aid (Fenner et al. 1993; Burnett et al. 1983; Fenner 1998; Little 2008; Australian Resuscitation Council 2010; Cegolon et al. 2013). Common symptoms, such as skin eruptions, are often observed, and treatment with antihistamine tablets and creams combined with local and/or systemic steroid therapy is administered (Burnett et al. 1987; Burke 2002). For severe skin reactions, oral steroids and/or pulse systemic therapy can be administered (Araki et al. 1994; Mizuno et al. 2000; Binnetoglu et al. 2013). For highly toxic cnidarians, surgical approaches such as relaxing incisions or fasciotomy may be required to prevent the risk of compartment syndrome in the injured extremities (Bucaretchi et al. 2014).

As described above, although rare, kidney injury has been reported in several cnidarian envenomation cases. In such cases, therapeutic approaches to treating AKI include correcting hypovolemia and renal ischemia, as well as vascular ischemia with adequate infusion therapy. Other supportive therapies, such as adequate usage of vasoregulators such as Carperitide (hANP) and limited usage of renal toxic agents should be considered (see the clinical practice guidelines for AKI, 2012 Kidney Disease: Improving Global Outcomes (Palevsky et al. 2013)). In severe cases of AKI, induction of renal replacement therapy should be considered until recovery from uremic complications such as azotemia, hyperkalemia and oliguria (urinary output, <400–500 mL/day)/anuria (urinary output <50–100 mL/day) (Palevsky et al. 2013).

As a possible new strategy, inhibition of PLA2 may be a target for therapeutic approaches involving PLA2-dependent renal injuries. Antibodies against PLA2 have been shown to prevent renal injury due to ischemia and reperfusion in rats (Takasaki et al. 1998). For kidney injuries by *P. semoni*, evidence from our published studies suggests that anti-complement therapies could also be considered (Mizuno et al. 2007, 2012). It is therefore expected that agents with specific and direct blocking effects may be developed for cnidarian toxins in the future.

38.6 Potential Applications of Cnidarian Toxins and Their Derivatives in the Medical Field

In the future, new cnidarian toxins could potentially be applied to the development of therapeutic agents and experimental materials. The venom and purified toxins from cnidarians are not only useful for analyzing the detailed mechanism of their pathogenesis in AKI, but they may also be useful in in vivo experiments involving disease models and toxin pathologies. Since the extracted toxins could potentially be used as therapeutic tools, further detailed analysis of their mechanisms of action are warranted.

Venoms and/or purified toxins from cnidarians may also be useful for developing therapeutic agents and for analyzing the pathogenic mechanisms of different diseases; indeed, numerous therapeutic agents have been developed from natural products and their derivatives (Mizuno et al. 2012; Frazão et al. 2012). To date, basic research on purified agents derived from cnidarian venoms, has yielded interesting and clinically relevant data. For example, the toxin (CqTX) of *Chiropsalmus quadrigatus* induced apoptosis in glioma cell lines (Sun et al. 2002). Similarly, the toxin RTX-A from *Heteractis crispa* (*Radianthus macrodactylus*), which is an actinoporin, also induced apoptosis in human tumor cell lines and may have potential as an anticancer agent (Fedorov et al. 2010). The pore-forming proteins Bc2 and equinatoxin (EqTx-II) that were originally extracted from the sea anemones, *Bunodosoma caissarum* and *Actinia equina* respectively, are also cytotoxic for glioblastoma cell lines (Soletti et al. 2008). Another lethal pore-forming toxin, membrane-attack complex/perforin (MACPF)-domain toxin, which was extracted from the nematocyst venom of the Okinawan sea anemone *Actineria villosa* (Oshiro et al. 2004), may also be developed as a cytotoxic agent for targeting some malignant tumors. It was recently reported that extracts of marine soft corals, such as *Sarcophyton sp.*, *Lobophytum sarcophytoides* and *Asterospicularia laurae*, inhibited HIF-2 gene expression in renal carcinoma cells (Grkovic et al. 2011). Several other toxin-derived agents including *Cassiopea xamachana* have also been shown to exhibit antitumor activities and have been proposed for use as therapeutic agents (Orduña-Novoa et al. 2003; Tejuca et al. 2004; Fedorov et al. 2010).

As examples of toxins affecting other targets, the toxin APETx2 from the sea anemone *Anthopleura elegantissima* has been used to inhibit $Na_v1.8$, a voltage-gate sodium channel, in rat dorsal root ganglion neurons (Blanchard et al. 2012) to prevent and treat inflammatory and postoperative pain (Deval et al. 2008, 2011; Karczewski et al. 2010). Products of the sponge, *Plexaura homomalla*, include some bioactive prostaglandin substances (PGA2) with anti-inflammatory effects (Reina et al. 2013).

ATX II, a toxin component from the sea anemone, *Anemonia viridis*, has been used in the long QT syndrome model (Shimizu and Antzelevitch 1999; Frazão et al. 2012) and has been shown to possess an anti-arrhythmic action (Platou et al. 1986). The ShK toxin from the sea anemone *Stichodactyla helianthus* is a potent Kv1.3 potassium channel blocker that has been shown to inhibit T lymphocyte proliferation (Beeton et al. 2008). It has been proposed for use as a therapeutic agent for treating autoimmune diseases such as multiple sclerosis (Chi et al. 2011).

It is also possible that animal AKI models, in which AKI has been induced by venom/toxins from cnidarians, could be used as experimental models for in vivo analysis of the pathogenesis and development of therapeutic agents. For example, in our recent studies on the toxic sea anemone, *P. semoni*, severe TMA was induced in the glomeruli of rats when crude venom or the purified PsTX-115 was administered (Mizuno et al. 2007). The resulting impairment of membrane complement regulators resembled a pathology characteristic of the human disease, atypical HUS. The complement system is important for maintaining immunological homeostasis in hosts, but dysregulated complement activation can cause a variety of pathologies (Mizuno and Morgan 2004, 2011; Caprioli et al. 2006; Mizuno et al. 2007). An animal model produced by treatment with *P. semoni* venom may therefore be useful for analyzing the mechanism of human renal diseases such as aHUS and other TMA, as well as for developing new therapeutic agents.

38.7 Conclusion

Few reports describing AKI resulting from cnidarian envenomation have been published to date. However, based on the large number of case studies and in vivo and in vitro experiments involving toxins or toxin components, it is considered that numerous possibilities exist for kidney injuries arising from cnidarian envenomation. In some patients, immediate catastrophic reactions to the toxins resulted in poor prognosis and severe pain, and the risk of drowning due to systemic reactions is considered to be high among divers and swimmers. In cases of drowning, the development of AKI among

those affected may not be clear. It is also important to recognize that, for many cnidarian species, cases of envenomation have a reasonable potential for developing kidney injuries, and also that effective supportive therapy may be required in order to prevent renal failure. Very few direct therapeutic approaches are available for patients injured by cnidarians, although tetracycline administration has recently been reported to prevent jellyfish venom-related skin necrosis in a rabbit model (Kang et al. 2013). These potent toxins not only induce serious reactions in humans, but they also have potential for developing useful therapeutic agents and methods of analysis. Indeed, it is expected that specific therapeutic agents will be developed to treat severe injuries resulting from cnidarian envenomation. In addition, studies on cnidarian toxins and their derivatives might also provide new therapeutic approaches for treating malignant tumors, autoimmune diseases, and other pathologies.

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Cubozoan Envenomations: Clinical Features, Pathophysiology and Management

39

Angel Anne Yanagihara, Christie Wilcox, Jason Smith, and Gerald Wayne Surrett

Abstract

Cubozoa-class cnidarian (box jellyfish) envenomations constitute a complex medical challenge and public health threat. There are currently no validated standards of care for life-threatening stings. Increasing numbers and reported geographic ranges of serious cubozoan envenomations underscore the urgent need for mechanism-based emergency therapeutic options for lifeguards, first responders and clinicians. For the most part, treatment and management approaches have been symptom-driven, utilizing therapeutics familiar to the practitioner and often based on extrapolation from other envenomation sequelae, rather than cubozoan-specific standardized protocols. Newly elucidated mechanisms of pathogenesis provide a context for directed clinical research to test novel therapeutic approaches that could greatly diminish human suffering and improve outcomes. The current state of our understanding about the pathophysiology of cnidarian envenomation and emerging therapeutic options for the medically relevant cubozoans will be presented.

Keywords

Cubozoa • Box jellyfish • Irukandji syndrome • Cnidarian envenomations • Porins

39.1 Introduction

Accurate assessments of the presence of hazardous cubozoans, avoidance and effective mitigation of sting events are critical to reducing morbidity and mortality of cubozoan envenomations. Cubozoan stings may cause a range of outcomes from mild site pain to life-threatening sequelae. Potentially fatal responses include acute cardiopulmonary collapse, as well as Irukandji syndrome, which is characterized by a delayed cytokine storm and endogenous catecholamine surge triggered onset of transient hypertension accompanied by episodic restlessness and agitation, cramping muscle pains, nausea, and ultimately may result in cardiac failure, intracranial bleeding and, occasionally, death.

This chapter will review the clinical features of cubozoan envenomation and the recent advances in pathophysiological concepts, as well as therapeutic options, including recent mechanistic based approaches. Importantly, we have demonstrated that rapid acting pore-forming proteins, or porins, may account for the clinical spectrum and outcomes of

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cubozoan envenomation. Moreover, inhibitors of these porins appear to be effective in ameliorating sting-site pain and reducing mortality from severe envenomation in experimental animal models.

39.2 Clinical Features of Cubozoan Envenomation

Immediate symptoms of cubozoan envenomation include mild to severe sting-site pain or numbness, which can intensify over the first few minutes and may last for hours or even days (Burnett and Calton 1987; Burnett 2001; Tibballs 2006). However, in many cases, there is no sting-site pain and the first symptom is pain in the torso such as bilateral back pain radiating to the groin with increasing nausea and abdominal cramping. Systemic reactions to box jellyfish stings can be acute or delayed. Acute systemic reactions include cardiopulmonary collapse evidenced by pulseless electrical activity within minutes (Burnett and Calton 1987; Burnett 2001; Tibballs 2006). Loss of life may also result from delayed systemic reactions (even without initial sting-site pain) from Irukandji syndrome, a well-described but poorly understood clinical complex characterized by catecholamine surge, histamine overload, cytokine storm and cardiopulmonary instability (Fenner and Hadok 2002; Huynh et al. 2003).

39.2.1 Local Dermal Reactions

Stings by Chirodropid family cubozoans, like the Australian box jellyfish (*Chironex fleckeri*), may produce large, swollen blisters that become necrotic, leaving permanent scars. Lesions may also appear in areas distal to the site of envenomation (Burnett and Calton 1987). Other cubozoan stings are marked by pain and inflammation, erythematous vesicular eruptions and persistent cutaneous hyperesthesia. There are two stages of dermal pathology: an initial reaction triggered by venom compounds characterized by acute inflammation and tissue damage; and a delayed dermatitis resulting from immunological responses to embedded tubules (Fig. 39.1), which have been known to persist for months, often

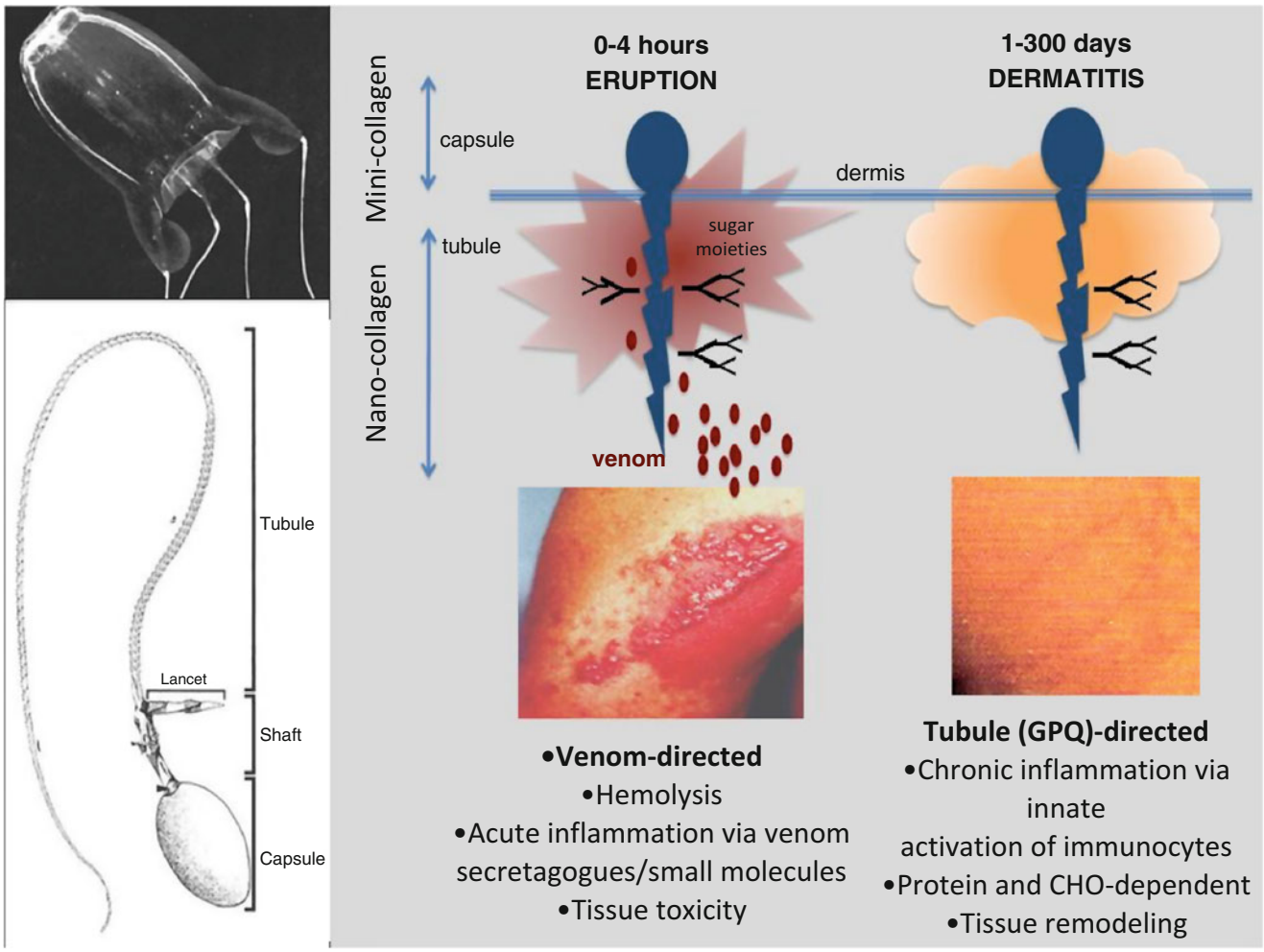

Fig. 39.1 Cubozoan tentacle and dermal pathology. *Upper left*: box jellyfish (*Alatina alata*). *Lower left*: illustration of cnidocyte and tubule. *Right*: schematic of tubule impaling skin and hypothetical pathophysiological scenarios (short- and long-term)

accompanied by swelling and itching (Burnett and Calton 1987). While many Carybdeid family cubozoans such as *Tamoya* species may cause intense immediate pain, others also known to cause life-threatening Irukandji syndrome, may present with little to no dermal pathology, and thus may initially be mistakenly interpreted to be mild stings. For this reason, all cubozoan envenomations should be carefully monitored for at least 1 h.

39.2.2 Acute Cardiopulmonary Collapse

Chirodropid envenomations occasionally result in death attributed to acute cardiopulmonary collapse (Tibballs 2006), often within minutes. Due to the speed of lethality, detailed clinical data on individuals fatally stung by *Chironex fleckeri* are sparse, but available information suggests that death is a result of primary cardiac arrest (Tibballs 2006; Cegolon et al. 2013). The number of deaths from Chirodropid stings exceeds that of deaths from shark attacks in the world (National Science Foundation 2008; Florida Museum of Natural History 2015).

39.2.3 Irukandji Syndrome

Anywhere from 10 to 50 % of certain species of cubozoan stings can result in Irukandji syndrome (Southcott 1967; Fenner and Hadok 2002; Huynh et al. 2003; Tibballs et al. 2011; Gershwin et al. 2013), a constellation of systemic symptoms including protracted headache, sweating, anxiety, backache, myalgia, muscle cramping, chest and abdominal pain, nausea and vomiting. Irukandji syndrome is marked by catecholamine excess and cytokine storm, with death in severe cases due to intracerebral hemorrhage (Southcott 1967; Fenner and Hadok 2002; Huynh et al. 2003; Tibballs et al. 2011; Gershwin et al. 2013). Clinical sequelae commonly include hypertension and tachycardia, occasionally followed by hypotension, bradycardia, cardiac failure, pulmonary edema, cerebral edema and, in two cases, intracerebral hemorrhage. Major sequelae may be delayed by up to 24 h and in the setting of supportive treatment deaths have been delayed by several days (Williamson et al. 1996).

The deaths in 2002 of two tourists in Far North Queensland, Australia, highlighted the potential risk and severity of this syndrome (Fenner and Hadok 2002; Huynh et al. 2003). A particularly worrisome complaint of patients suffering from Irukandji syndrome is the catecholamine surge-associated certainty of "impending doom". Although symptoms generally abate within 24 h or after pharmacological management of pain, muscle spasms, nausea, anxiety and cough (unpublished clinical observation, J. Smith and G. Surrett), some patients experience persistent symptoms for weeks over even months after the sting. These longer term sequelae are inadequately documented but have been anecdotally observed, and are in need of further characterization.

39.3 Pathophysiology of Cubozoan Envenomation

The cnidome of cubozoa is rich in penetrant cnidae, called nematocysts. When a nematocyst fires, its tubule is explosively propelled into the skin, and can penetrate the epidermis and dermis, as well as nerves and capillaries. Venom can be injected intraepidermally, intradermally and intravascularly simultaneously (Fig. 39.2). The pathology of the sting depends on the number and kind of nematocysts that fire, the location of envenomation, skin thickness at the site, the health of the patient, and the condition of the envenomated tissue(s).

Since the mid twentieth century, investigators have sought to elucidate the pathophysiology underlying the complex range of box jellyfish envenomation sequelae (Gershwin et al. 2013). Currently known toxins include a porin, lipases, proteases, small molecular weight compounds and biologically active lipids (Fig. 39.3).

Of these, the fastest-acting agent in the venom is a pore-forming protein, or porin (Chung et al. 2001; Yanagihara and Shohet 2012). Negative-staining electron microscopy demonstrates that these porins self assemble to form rivet-like transmembrane pores that perforate red blood cells, white cells and platelets, resulting in a dose-dependent release of the respective intracellular contents (Fig. 39.4; Yanagihara and Shohet 2012). Full amino acid sequence analysis of the porin and molecular modeling demonstrates structural motif homology to self-assembling bacterial porins, namely anthrolysin O and streptolysin O (Bernheimer et al. 1979). Since the report of the first cubozoan hemolytic porin from *Alatina sp*. (previously reported as *Carybdea alata*; Chung et al. 2001), all cubozoan venoms investigated to date have been found to contain close homologs of this porin; two isoforms are present in *Chironex fleckeri* venom (MW 43 and 45 kDa; Nagai et al. 2000a, b; Yanagihara et al. 2002; Brinkman and Burnell 2009). The conserved nature of the porins in all Cnidarian species further supports the hypothesis that these proteins are essential venom constituents and play a key role in the clinical presentation of cubozoan stings.

Prior to erythrocyte swelling sufficient to allow release of the large tetrameric hemoglobin molecule, porin-based perforation of erythrocytes results in very rapid, and potentially lethal, loss of potassium from red cells into the plasma. Similarly, ex vivo whole blood studies demonstrate that within seconds, porin exposure can lead to rupture of platelets, and within minutes, foam cell formation and other

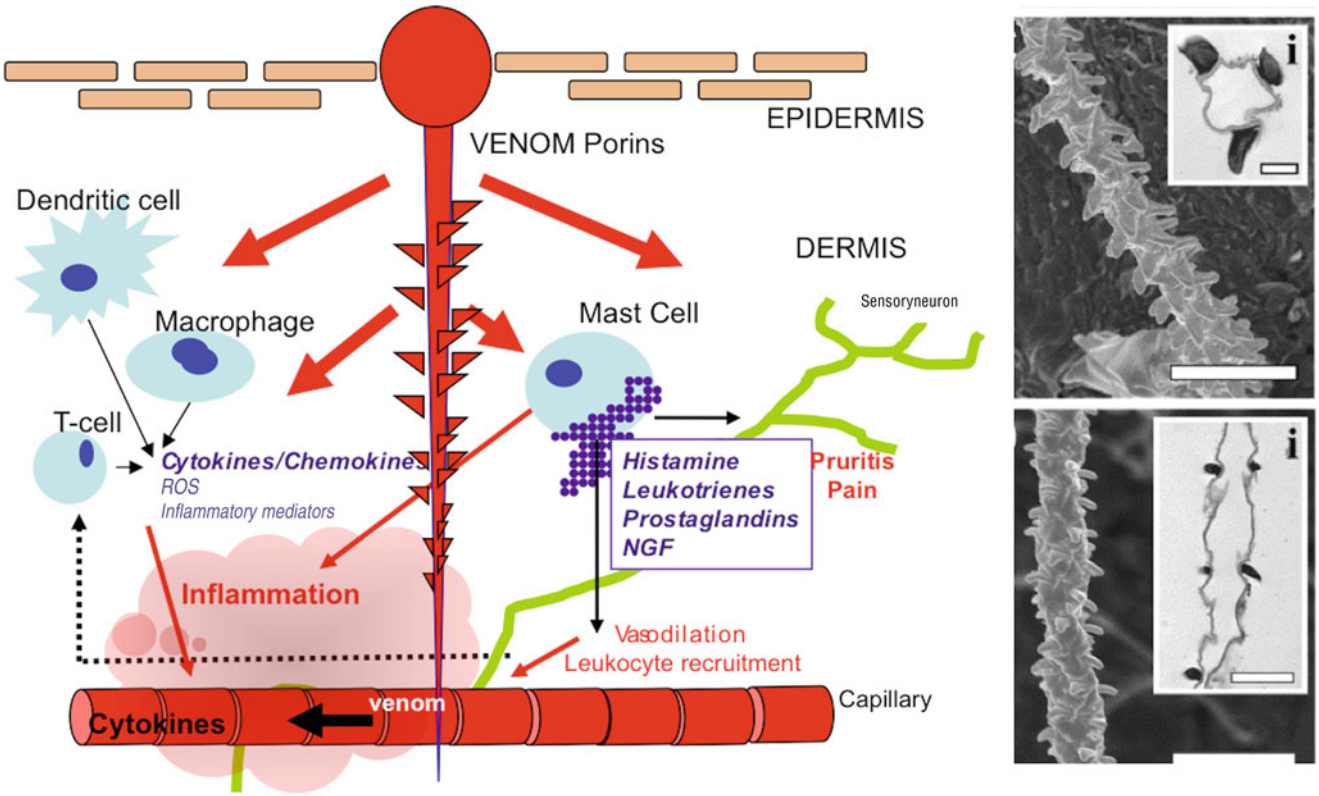

Fig. 39.2 Illustration (*left*) and scanning electron microscopy (SEM, *right*) of *Alatina alata* discharged eurytele nematocyst structures. *Top*, *right*: SEM of proximal tubule showing spines along the tubule and transverse section (*inset*). *Bottom*, *right*: SEM of mid-tubule and TEM longitudinal section (*inset*). Bars = 6 μm

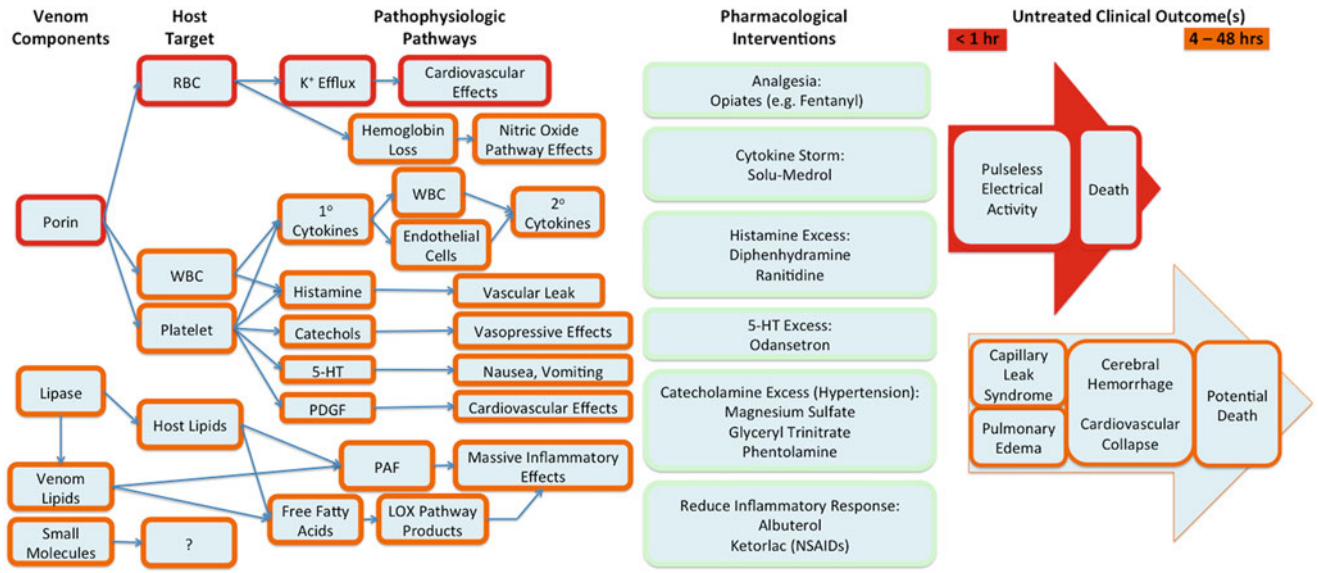

Fig. 39.3 A united pathophysiological model which explains the pathway by which high doses of venom cause acute cardiopulmonary collapse (ACC, in *red*) while lower doses lead to Irukandji syndrome (in *orange*)

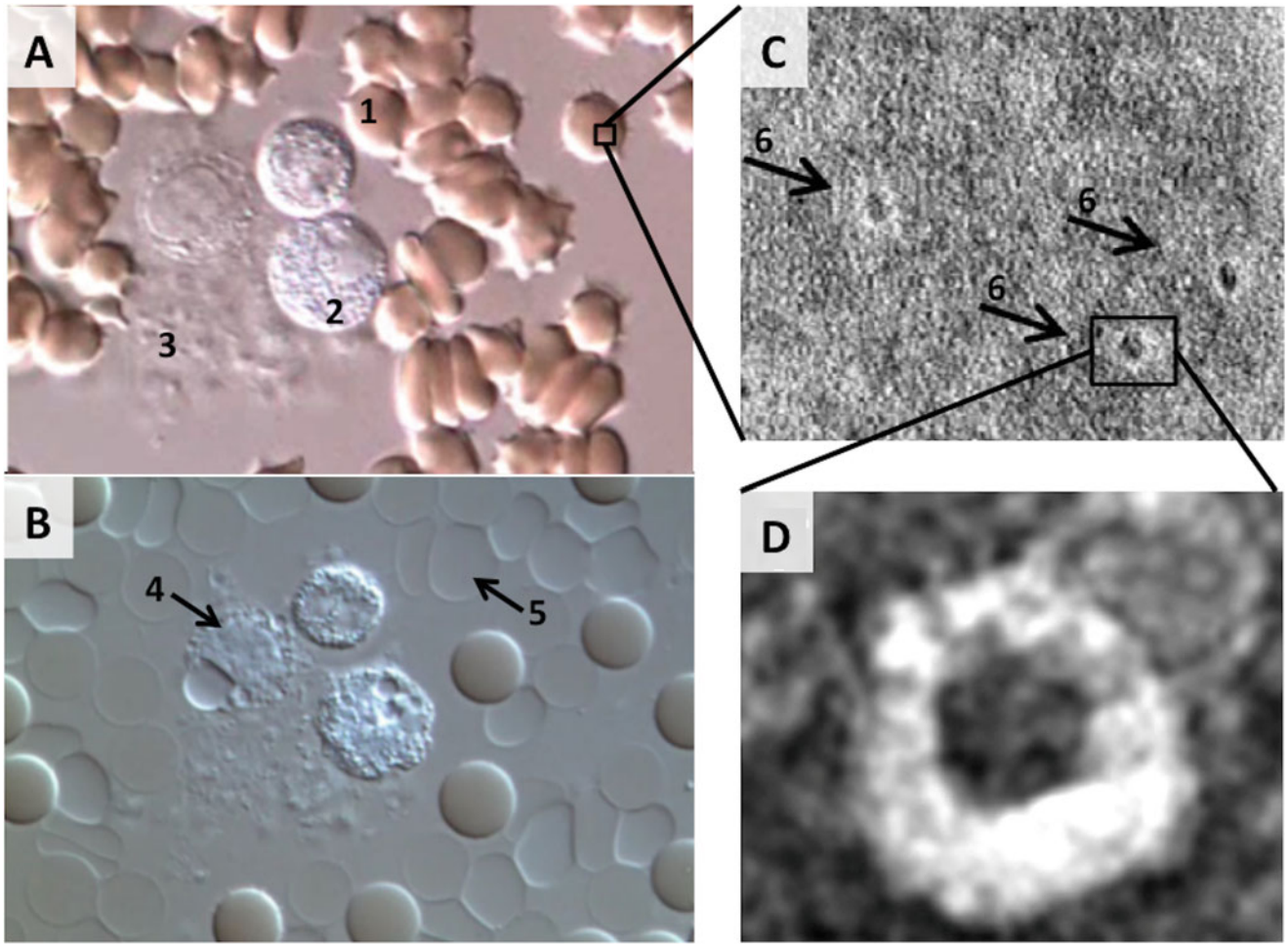

Fig. 39.4 Human whole blood at (**a**) 10 s and (**b**) 5 min after exposure to box jelly venom. Immediately, red cells *1* are already crenulated. White cells *2* and platelets *3* are also affected; foam cell pathologies can be detected within 5 min *4*, and hemoglobin loss leaving red cell ghosts *5*. (**c**) Transmission electron micrographs of 2 % ammonium molybdate negative-stained purified venom porin pretreated red cells showed the presence of self-assembled porin ring structures *6*. (**d**) Highest magnification of a single polymeric porin structure

monocyte cytopathology, with concomitant explosive rise in histamine, inflammatory cytokines and chemokines to life-threatening levels. For these reasons, we suspect that white cells and platelets likely represent the source of the catecholamines, biogenic amines and cytokine surge. Platelets and their granules in particular store a diversity of more than 300 distinct molecules that are uncontrollably released upon porin-induced lysis (Whiteheart 2011). These compounds include small biogenic amines such as histamine, serotonin and catecholamines (dopamine, epinephrine and norepinephrine), as well as cytokines, including platelet-derived growth factor (PDGF), tumor necrosis factor-alpha (TNF-α), interleukin-7 (IL-7), and endothelial growth factor (EGF). In addition, white cells contain high levels of inflammatory cytokines and various biogenic amines that can be liberated through porin-induced lysis.

Recent work has shown that the cubozoan porin can invoke rapid, acute cardiopulmonary collapse and pulse-less electrical activity in both mouse and piglet animal models (Yanagihara and Shohet 2012), and at lower doses induce Irukandji syndrome-like pathologies. In addition, elevation of a suite of inflammatory cytokines and chemokines together with catecholamines occur in plasma when peripheral blood mononuclear cells and platelets are exposed to purified porin; This is noteworthy since Irukandji syndrome has been described clinically as an outcome of “catecholamine excess”, “histamine overload” and “cytokine storm” of unknown etiology.

Together, these data suggest a parsimonious hypothesis that the essential if not sole driving force behind the diversity of pathophysiological responses to cubozoan venoms are the cubozoan porins (Fig. 39.5). This hypothesis is based on

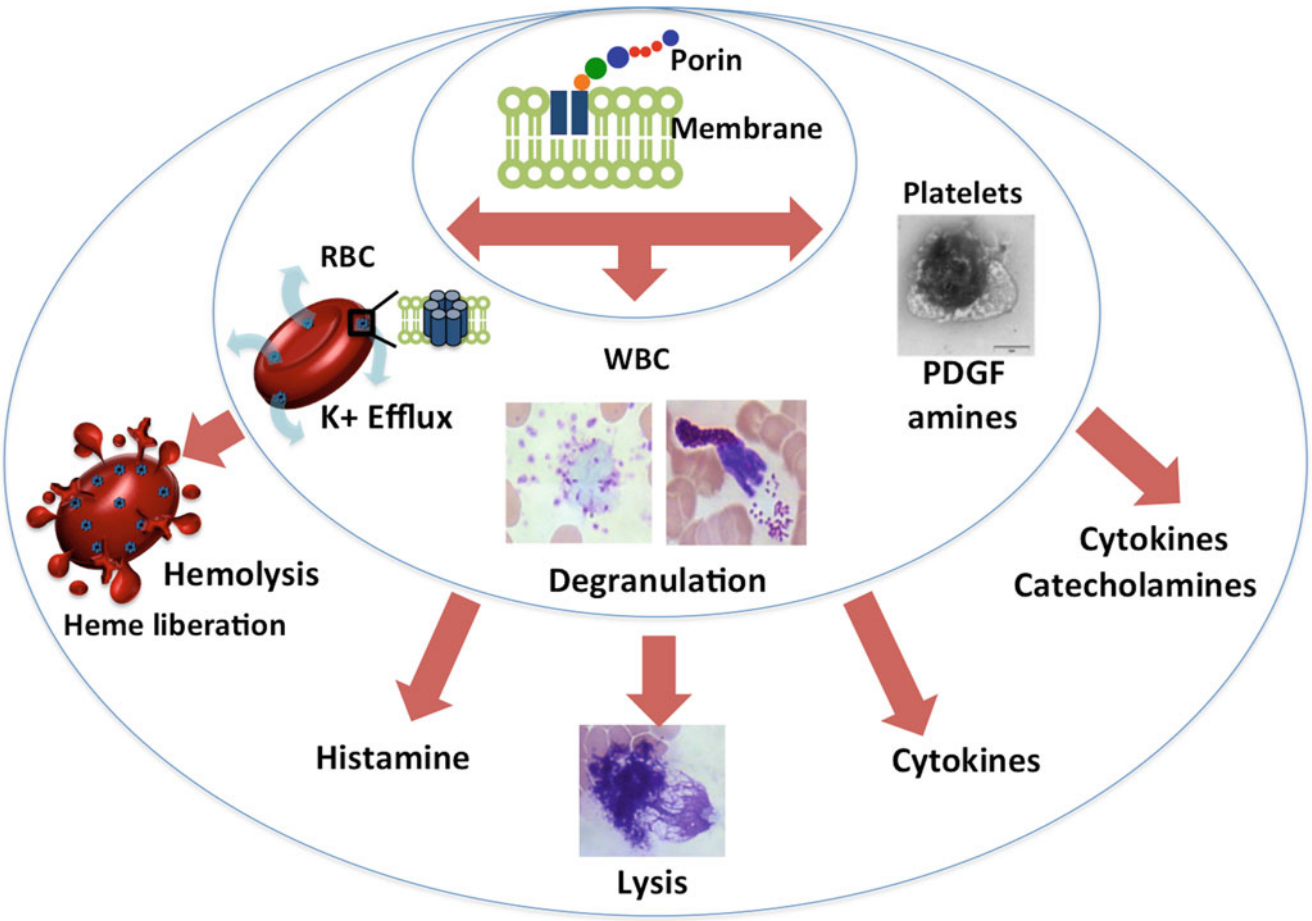

Fig. 39.5 Schematic of the hypothesis of venom-porin initiated mechanism of potassium, catecholamine and cytokine release. Cubozoan venom porin injected into the blood stream inserts and self assembles to form pores on target cell plasma membranes causing target cell effects, such as platelets: a release of catecholamine-rich storage granules; peripheral blood monocytes: cytokine release; and red cells: K+ then hemoglobin release. Geimsa Wright stain blood smear images of white cells and transmission electron microscopy of platelet are shown as *insets*

systematic studies aimed at attempting to recapitulate complete disease states with single venom fractions. We considered that acute cardiopulmonary collapse and Irukandji syndrome could be the result of distinct or synergistic effects of multiple venom component(s) given the complexity of cubozoan venom. However, evaluation of venom fractions suggests that the porin was both the fastest-acting fraction and sufficiently lethal. Both in vitro and in vivo animal modeling work demonstrated that at high doses of porin, perforation of red cells led to systemic hyperkalemia with pulseless electrical activity and rapid death. In contrast, exposure of whole blood or piglets to intermediate doses of porin below that causing hyperkalemia-driven acute cardiopulmonary collapse demonstrated a massive increase in plasma levels of platelet- and white blood cell-derived catecholamines and cytokines, the hallmarks of Irukandji syndrome. Taken together these data suggest that the porin is the most important venom component when it comes to clinical symptoms, and thus it is essential that porin blockade is a treatment priority.

39.3.1 Local Dermal Reactions

Ultimately, the pathophysiology of envenomation results from a combination of factors. Envenomation comprises both physical trauma, with perforation of the epidermis and dermis by thousands of venom-delivering hollow tubules that explosively discharge from stinging cells upon tentacle contact, as well as physiological response to the complex constituents of the highly lipidic venom (Tibballs et al. 2011). Hundreds to thousands of mineralized spine-covered, hollow, nematocyst tubules (Brinkman and Burnell 2009) explosively perforate the skin of sting victims per linear centimeter of contacting tentacle, releasing venom. If the organism is exposed to a dose of venom below the threshold required to lead to lethal hyperkalemia from red cell perforation, there is still the profound physiological effects due to porin-based damage to white cells and platelets, which lead to uncontrolled release of catecholamines and cytokines (McIntire et al. 2009). Venom proteins, proteases, lipases, peptides and small molecules also activate or damage dermal mast cells

and nerve pain receptors in skin. Porin-perforated and venom-activated mast cells degranulate to release histamine and factors which elicit white cell diapedesis and rapid immune cell recruitment (extravasation) with resultant swelling, pain and redness.

Lastly, in cases with or even without severe systemic reactions, dermal pathologies can persist for weeks to months after the sting. Jellyfish sting patients with chronic dermal symptoms have presented with localized fat atrophy, hyperhidrosis, vasospasm, hyperpigmentation, keloids, lymphadenopathy and scarring (Gunn 1949; Mansson et al. 1985; Burnett et al. 1987; Fisher 1987; Mandojana and Sims 1987; Pierard et al. 1990; Tamanaha and Izumi 1996; Veraldi and Carrera 2000; Loredana Asztalos et al. 2014; Putra et al. 2014). Persistent local reactions have included cutaneous hypersensitivity, recurrent cutaneous eruptions, granulomatous nodules, and lesions which sometimes appear days after a primary exposure (Gunn 1949; Mansson et al. 1985; Burnett et al. 1987; Fisher 1987; Mandojana and Sims 1987; Pierard et al. 1990; Tamanaha and Izumi 1996; Veraldi and Carrera 2000; Loredana Asztalos et al. 2014; Putra et al. 2014). Unlike the initial dermatological symptoms, prolonged dermal pathologies are believed to have an immunological basis, likely from intracutaneous tubule components which remain long after the venom is injected (Fisher 1987; Burnett 2001). Histological studies have shown significant perivascular inflammatory infiltrates of lymphocytes and eosinophils (Pierard et al. 1990; Loredana Asztalos et al. 2014; Putra et al. 2014). Finally, experimental data have shown that jellyfish collagens trigger enhanced immunoglobulin production by lymphocytes in vitro and in vivo as well as promote cytokine production and phagocytotic activity of macrophages (Putra et al. 2014). Cytokine production stimulated by jellyfish collagen was reduced by inhibitors of a Toll-like receptor 4 and a c-Jun N-terminal kinase (JNK). Thus, it is likely that both acquired and innate immune responses (though especially innate ones) to nematocyst-deposited structural components, such as collagens, play important roles in recurrent dermal pathologies.

39.3.2 Acute Cardiopulmonary Collapse

In vitro, the toxic components of cnidarian venoms show direct effects on muscle and nerve tissue (Ramasamy et al. 2003; Cuypers et al. 2006). The venom has also been recognized to create pores in myocytic membranes (Bailey et al. 2005). Yet, the identity of the causative toxin and the mechanism of these acute deaths remained elusive until the discovery of cubozoan porins. Recent work demonstrated that biochemically purified porin alone could cause acute cardiopulmonary collapse in vivo and that this was associated with significant hyperkalemia (Yanagihara and Shohet 2012). Echocardiographic (ECHO) and electrocardiographic (EKG) measurements of venom-injected mice sought to elucidate the mechanism by which *Chironex fleckeri* venom induced both immediate and delayed systemic reactions and mortality. Rapid death within 5–20 min was observed at doses over 10 U/mL/% (HU_{50} units/blood volume/percent red blood cells) or roughly over 1000 Units/adult mouse with the chirodropid venom porin or at matched dosage levels using carybdeid venom porin (Fig. 39.6; Yanagihara and Shohet 2012). Lesser cubozoan porin doses resulted in death at 6–24 h. Further testing of this dose range in freshly drawn human whole blood revealed that the platelets and white cells exhibited profound morphological changes as assessed by time course live-cell light microscopy and end-point Wright-Geimsa stain analysis.

Hyperkalemia is a well known cause of pulseless electrical activity. It is our hypothesis that extremely acute cardiac arrest is caused by elevated potassium levels due to porin disruption of red blood cell membrane integrity. In such cases, death can occur in minutes because porin-induced potassium efflux leads to severe hyperkalemia, which directly leads to cardiac myocyte instability and consequent inability to properly contract, electromechanical disassociation, which results in the pulseless electrical activity detected by an EKG. However, even if this further degenerates into ventricular fibrillation or asystole, patients have survived after appropriate CPR, suggesting that the envenomation effect is transient and does not involve direct cardiac damage. This observation of transient and reversible cardiac insufficiency does not support an alternative possibility that the porins directly damage the cardiac myocytes or endothelial cells to cause the collapse rather than a potassium-mediated collapse. Still it is noteworthy that troponin elevation has been clinically observed so it is clear that some cardiomyocyte damage may occur. While the hypothesis of profound red blood cell derived hyperkalemia-driven PEA is supported by high dose envenomation animal study (5–20 min survival), our hypothetical model also includes porin effects upon platelets and white blood cells. It has been shown that mediators released from these cellular populations can also contribute to acute phase effects in animal models at doses where survival was at least 20 min. More work is needed to elucidate these, but it is critical to note that some patients presenting with cardiovascular collapse (surviving at least to hospital transport and care) do not present with clinically significant hyperkalemia. In these cases, it is of interest to further elucidate the role of certain white blood cell and platelet mediators such as platelet derived growth factor which can be cardiotoxic.

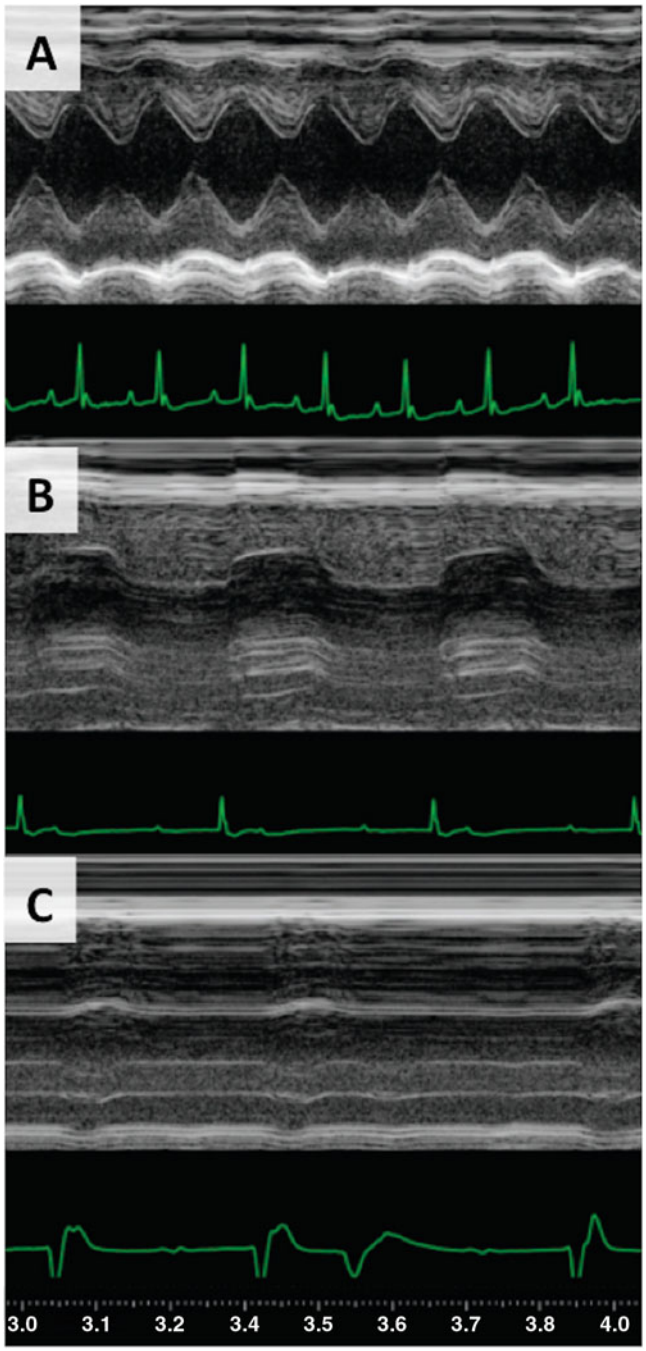

Fig. 39.6 Representative ECG and ECHO recording of *Chironex fleckeri* venom-injected mouse. (**a**) Preinjection recording is shown together with (**b**) 525 s, and (**c**) 840 s recording after injection with 25 U/mL/% *Chironex fleckeri* venom. Left ventricular function was markedly impaired, progressing to electromechanical dissociation. Contractility briefly recovered slightly, but not sufficiently to maintain perfusion. ECG showed second-degree block with progression to nodal- and ventricular-escape arrhythmia before death

39.3.3 Irukandji Syndrome

The pathophysiological mechanism underlying Irukandji syndrome remains to be determined. Theories include ischemia due to vasoconstriction of arterioles as a result of excess catecholamines, or sodium channel-dependent activation of afferent pain pathways (Tibballs et al. 2012). Indeed, although Irukandji syndrome-associated cubozoan stings may cause local pain, the characteristic severe muscular pain of delayed onset cannot be explained by the involvement of local nociceptive effects alone. Electrolyte imbalance due to purported interference with sodium channels would have profound effects on nerve signaling and muscle function (both skeletal and cardiac muscle). Thus, the characterization of electrolyte profile changes after toxin exposure is needed for a better understanding of the mechanisms behind pain and peripheral muscle spasm and cardiac muscle dysfunction.

Over the past decade, the role of cytokine interaction (Szelenyl 2001) with the central nervous system (CNS) is being increasingly appreciated, such that cytokines have been shown to lead to increased plasma catecholamines based on adrenal responses. This would be in addition to direct porin-induced release of sequestered catecholamines. Animal experiments and human clinical studies of African and American scorpion envenomation have implicated both cytokines, including interleukin-1alpha (IL-1α), interleukin-6 (IL-6), interleukin-8 (IL-8), interferon-gamma (IFN-γ), granulocyte macrophage colony-stimulating factor (GM-CSF) and TNF-α, as well as nitric oxide release as contributors to the underlying pathophysiology (Fenner et al. 1988; Magalhães et al. 1999; Abdoon and Fatani 2009). Indeed there are many common clinical features when Irukandji syndrome and severe scorpion envenomations are compared including hypertension, cardiac failure and pulmonary edema (Abdoon and Fatani 2009). Such stimulation of cytokine release could also affect the hypothalamic-pituitary-adrenal axis regulation of CNS interaction with the immune system, and thus stimulation of adrenocorticotropic hormone (ACTH) and resultant effect on adrenal gland production of cortisol or catecholamines is possible. In addition, hypothalamic vasopressin also acts as a co-stimulator of ACTH release, and it is likely that the blood pressure changes seen with toxin exposure also stimulate vasopressin release. Therefore, exploration of the possible role of evoked cytokine and vasoactive hormone responses may provide new therapeutic leads for the treatment of this cubozoan envenomation.

Transiently elevated serum adrenaline and noradrenaline levels have been found in experimentally envenomated animals and cardiac sympathetic nerve activation demonstrated in human cardiac tissue (Winkel et al. 2005). Further, venom exposure has been linked to increased histamine, serotonin and cytokine levels. Observations in case series of progressive cardiac failure, with pulmonary edema, ischemia and hypotension, are consistent with prolonged exposure to elevated circulating biogenic amines and cytokines.

Cytokine panels of human blood treated at doses titrated to match survival time of Irukandji syndrome in venom-injected mice show an extraordinarily pronounced release of certain highly bioactive cell signaling molecules (Yanagihara, unpublished data). Specifically, PDGF levels were elevated by two orders of magnitude from ~100 to over 10,000 pg/mL, indicating a "cytokine storm". This specific cytokine released by perforated platelets can result in life-threatening vascular pathologies at that level (Raines 2004; Barst 2005; Villagra et al. 2007).

Because the symptoms of envenomated patients may seem similar to classical IgE mediated anaphylaxis (histamine-mediated respiratory insufficiency), it is worth noting that anaphylactoid reactions caused by cubozoan venom porins occur in IgE naïve individuals. Classical hymenoptera type anaphylaxis is mediated by the adaptive immune response, specifically the production of immunoglobulin E (IgE) to a previously encountered antigen. IgE induces mast cells to release histamine, leading to a swift and significant rise in histamine levels, which are the cause of life-threatening symptoms. True anaphylaxis is accompanied by severe hypotension in which epinephrine injection is a well-established and life-saving intervention.

Alternatively, while cubozoan envenomated victims may present with perceived respiratory insufficiency, the toxin is novel and IgE is not responsible for histamine release. Most importantly, because the clinical presentation includes a catecholamine surge, the typical acute presentation includes a severe hypertensive phase. Porin-induced lysis of cells leads to substantial increases in circulating levels of endogenous norepinephrine and epinephrine as well as histamine. Indeed, additional epinephrine on top of the release of endogenous norepinephrine and epinephrine liberated by the porin has the potential to lead to life threatening hypertension. Extremely elevated catecholamine levels can lead to end organ damage including hemorrhagic stroke, cardiac arrest, or acute kidney injury, and death. Thus, in the context of a cubozoan venom-induced catecholamine surge, if a sting victim is presenting with shortness of breath, it is critical to ascertain whether the victim is hypertensive, and in the case where hypertension exists, the therapeutic decision should include potential exacerbation of the hypertensive state to the end point of organ damage. It is interesting to note clinical similarities to Takotsubo cardiomyopathy, which has a demonstrated association with elevated catecholamines.

39.4 Clinical Management of Cubozoan Envenomation

The primary and most important first-aid for cubozoan stings is to remove the victim from the water, administer basic or advanced life support as warranted by the sting victim's condition, and remove tentacles from the skin. Victims may experience intense pain and acute effects of histamine overload with respiratory insufficiency. Once the victim is out of the water, further evaluation should be performed to determine the most appropriate emergency treatment steps.

While conventional neutralizing antibodies or "antivenoms" work well in snake envenomations, the clinical efficacy of the Commonwealth Serum Laboratory (now bioCSL Limited) box jellyfish antivenom remains controversial (Tibballs et al. 1998; Yanagihara and Shohet 2012; Ramasamy et al. 2003, 2004; Winkel et al. 2003; Hughes et al. 2012). Previous in vivo studies using isolated toxins from snake venoms showed that once platelets and WBC had been lysed by venom components, treatment with antivenom was unable to halt cardiopulmonary disturbances, suggesting that endogenous stored cellular compounds released by the toxins, and not the toxins themselves, were the proximal cause of these clinical envenomation symptoms (Prado-Franceschi et al. 1981). Similarly, the failure of jellyfish antivenom to reduce morbidity and mortality in animal models may be due to its ineffectiveness to mitigate the effects caused by the rapid-acting porin lysis of blood cells. Given the discovery of the extremely rapid pathophysiological effects of cubozoan porin exposure, previous methods of bacterial porin inhibition were examined as potential treatment options, including the use of certain metal salts before the advent of antibiotics (Avigad and Bernheimer 1976). Accordingly, a novel therapeutic has been developed that markedly inhibits cubozoan venom-induced pathologies.

Systematic experiments, conducted to identify rapidly acting porin blockers that interrupt porin-induced pathologies in human whole blood and marked enhancement in survival time, from 5 to 20 min to more than 12 h (Fig. 39.7), in mice injected intravenously with lethal doses of *C. fleckeri*

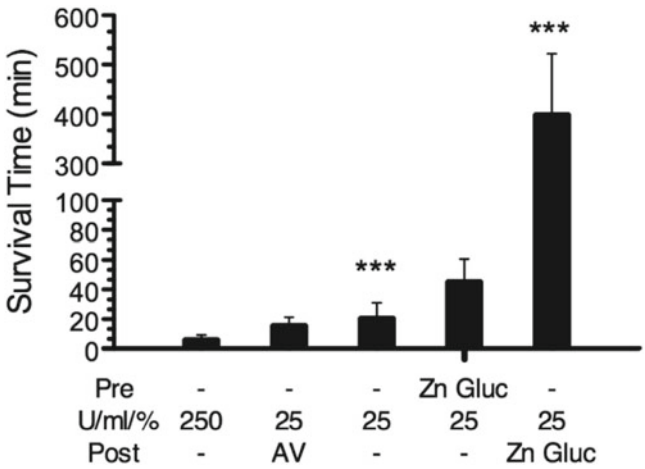

Fig. 39.7 Mean survival times of mice lethally injected with cubozoan porin alone or with pre or post treatment of CSL antivenom, Zn Gluconate, or nitrites

venom, have demonstrated that copper gluconate was about 50 times more potent than zinc gluconate (Yanagihara, USPTO PCT/US 62010330, PCT/US10/50061).

39.4.1 Local Dermal Reactions

Primary care for mild stings consists of (1) removing and inactivating tentacles and (2) treating for pain and inflammation. Vinegar is the ideal solution for rinsing off remaining tentacles and unfired nematocysts as laboratory studies have shown that it permanently prevents discharge (Hartwick et al. 1980; Yanagihara et al. 2016). Magnesium salts have been shown to cause tentacle relaxation (Williamson et al. 1996). Tentacles should be rinsed off if possible, avoiding direct pressure. If rinsing solutions are unavailable, responding personnel should take care to protect themselves from secondary stings. If vinegar is unavailable, nematocysts can be rinsed with seawater. Fresh water, ethanol or other solutions lead to immediate firing of nematocysts, and thus can increase injury and venom load (Yanagihara et al. 2016).

After saltwater or vinegar wash and tentacle removal, the victim should be monitored for systemic effects with later onset and can be treated for pain and dermal pathologies. Topical or oral pain medications may be considered for pain management. There is some debate whether cold or heat therapy is more effective at pain relief. Ice packs can help temporarily relieve pain and inflammation of other injuries. However, multiple studies including three randomized, controlled trials show that cold therapy is not effective while hot water emersion is highly effective at relieving pain from cnidarian envenomations (Thomas et al. 2001; Nomura et al. 2002; Bowra et al. 2002; Loten et al. 2006; Wilcox and Yanagihara 2016). In vitro assays have demonstrated that the venom porins are irreversibly inactivated by heat (Yoshimoto and Yanagihara 2002; Atkinson et al. 2006) and magnesium salts. Others note that heat may also directly modulate pain receptors, thus relieving the symptom (Cegolon et al. 2013). Topical relief may include steroids such as triamcinolone or soon-to-be marketed commercial products that contain copper gluconate.

The U.S. Army Special Forces Underwater Operations (SFUWO) protocol ensures a suitable rinse and hot immersion solution are available on every dive in a simple, cost-effective manner (empirical observation, J. Smith): the unit fills black hard-plastic jugs with a 4:1:1 saltwater, freshwater, vinegar saturated with Epsom salts. The jugs are then left in the sun, and are thus heated because of their dark coloration. Such innovative thinking could be easily adopted by beach first-responders in areas known to have frequent cubozoan envenomations.

39.4.2 Acute Cardiopulmonary Collapse

Currently available treatment options for cubozoan stings are suboptimal and limited to antivenom combined with supporting hemodynamics and treating symptoms (Table 39.1). Due to the rapid action of venom toxins, the therapeutic efficacy of *Chironex* antivenom (bioCSL Limited, Parkville, Australia), which is not a specific pharmacological antagonist, remains contentious (Tibballs 2006; Yanagihara and Shohet 2012).

Further, as calcium entry into cardiac myocytes has been observed after experimental application of *Chironex fleckeri* venom, calcium-channel blockade has been proposed as treatment for such stings. However, pharmacological calcium-channel blockers do not prevent calcium influx (Mustafa et al. 1995) and do not prevent acute cardiopulmonary collapse (Tibballs et al. 1998; Ramasamy et al. 2004). Moreover, calcium-channel blockers can exacerbate hypotension and could be counterproductive in resuscitating sting victims.

Given the mechanistic model presented in this chapter, emergency personnel should consider treatment even if there is no pulse. If plasma potassium is at lethal limits, and it is feasible, therapeutic methods to rapidly lower potassium such as glucose/insulin and bicarbonate may be attempted. If attempted, cardiopulmonary resuscitation (CPR) and fluid support should be maintained until plasma potassium reduces to normal levels. In cases of pulseless electrical activity when box jelly envenomation is suspected, then concurrent treatment for hyperkalemia should be attempted alongside the standard advanced cardiac life support protocols. A "C BIG K DROP" protocol may be attempted alongside epinephrine administration in "non-shockable" PEA arrest: the administration of calcium gluconate ("C"), sodium bicarbonate ("B") and/or beta-agonists, insulin ("I") glucose ("G"), and kayexylate ("K"). Finally diuretics ("D") and dialysis ("ROP", i.e. "Renal unit for dialysis Of Patient") may be considered to further accelerate the decrease of high potassium levels. Once the patient is stabilized, treatments for Irukandji syndrome or general dermal symptoms may be necessary.

39.4.3 Irukandji Syndrome

The most effective current therapy for Irukandji syndrome seems to be an intravenous infusion of magnesium sulfate (Corkeron 2003; Corkeron et al. 2004). There are no Irukandji syndrome species-specific (e.g., *Carukia barnesi*, *Alatina sp.* and various carybdeids) antivenoms available. *Chironex* antivenom does not appear to be effective in the treatment of this syndrome (Winkel et al. 2003). The rationale for the use of magnesium therapy is based on the in vivo evidence of

Table 39.1 Chirodropid acute cardiopulmonary collapse

1. Extreme pain with "catecholamine surge" features: anxiety, nausea, sweating
2. Whip-like violaceous lesions
3. Dyspnea
4. Death within minutes
5. Postmortem findings
a. Multisystem cardiopulmonary event
b. Lungs distended with frothy serous material
c. Blood in heart dark and fluid
d. Viscera congested

Range of Current Clinical Management Methods: Maintain airway, breathing and circulation support (ABCs), fluid support, antivenom treatments are contentious. Magnesium Sulfate bolus and IV (Queensland Government Protocol), zinc gluconate or copper gluconate, diuretics, extracorporeal membrane oxygenation (ECMO) until serum potassium levels are reduced

catecholamine excess and sympathetic and parasympathetic involvement (Tibballs et al. 2001). Certainly, to date, anecdotal clinical evidence suggests that magnesium is successful in reducing both hypertension and pain associated with Irukandji syndrome (Corkeron et al. 2004).

The most important clinical assessment when a patient presents with potential Irukandji syndrome is blood pressure. Hypertension has been reported as a result of greatly elevated plasma catecholamines. Deaths from Irukandji syndrome occur after a hypotensive state that had been preceded by extreme hypertension and involve cerebral hemorrhage, thus the control of hypertension may be of life-saving importance. Opiates alongside glyceryl trinitrate and magnesium sulfate is currently the recommendation of the Queensland Government Irukandji Taskforce (Queensland Government 2015). Nitrates are suggested for the management of hypertension because in both animal models and clinical settings, they have shown promise for Irukandji treatment (Fenner and Lewin 2003). Glyceryl trinitrate (GTN) may be administered sublingually initially, followed by GTN infusion using standard cardiac dilutions and doses.

Alternatively, the U.S. Army SFUWO protocol is to administer morphine and valium together with antihistamines (both H1 and H2 antagonists) and an anti-inflammatory glucocorticoid (Solu Medrol). Controlling the primary symptoms of pain, muscle spasms, nausea, and anxiety may be enough to normalize blood pressure, respiratory rate, and pulse (personal clinical observation, J. Smith). In addition, phentolamine, a nonselective alpha-adrenergic antagonist, may also be useful to combat hypertension caused by excess catecholamine release during porin-induced platelet lysis (Fenner et al. 1986), and is suggested as a possible treatment option in the Townsville Hospital protocols for Irukandji treatment (Queensland Government 2015).

Low blood pressure may occur because of vascular leak caused by a cytokine storm and histamine overload. Oxygenation is key (Corkeron 2003; Corkeron et al. 2004; Queensland Government 2015). Pulmonary edema has been managed by furosemide (loop diuretic, Lasix), morphine, nitroglycerin and oxygen support as well as positive-pressure ventilation while magnesium sulfate and zinc or copper gluconate can be used to combat porin activities.

Histamine overload may be a problem in many sting cases, and thus antihistamine use should be considered generally good practice (J. Smith, personal communication). A combination of H1-receptor antagonists (e.g., diphenhydramine) and H2-receptor antagonists (e.g., ranitidine or famotidine) may be used to combat histamine-overload sequelae related to Irukandji syndrome according to the U.S. Army Special Forces protocols.

Pain management is also an important concern. Historically, analgesia for both Irukandji syndrome and *Chironex* box jellyfish stings has required large doses of intravenous opiates (Lippman et al. 2011). Aggressive management of pain and muscle spasms through opiates and benzodiazepines should be considered, though caregivers should be cognizant of the risks of opiate use, including respiratory depression. The Queensland protocol (Queensland Government 2015) includes fentanyl and morphine, while the U.S. Army SFUWO protocol includes administration of morphine and valium. Anecdotally peripheral nerve blocks and neuraxial blockade have been successfully used for local pain following Chironex envenomation, though Irukandji syndrome requires the use of systemic analgesia as described above.

Other pharmaceuticals for symptom management may include anti-emetics (e.g., Zofran, a 5HT3 antagonist), bronchodilators (e.g., albuterol), anti-inflammatories (e.g., ketorolac or other a nonsteroidal anti-inflammatory drugs) or cough suppressants (e.g., benzontate) (Table 39.2).

39.5 Future Research

While the porin-based integrated pathophysiological model of cubozoan envenomation is sufficient to induce the most dire envenomation pathologies, there are other venom com-

Table 39.2 Irukandji syndrome

Characteristics: extreme pain with "catecholamine" surge features: anxiety, sweating, nausea (serotonin), respiratory affects (histamine)
Clinical case definition:
1. Swimming in salt water environment
2. May present with minimal skin findings
3. Delayed symptom onset
4. One or more of the following symptoms:
a. Muscular pain, backache, cramps, tremors
b. Nausea, vomiting
c. Cardiac dysrhythmias
d. Anxiety, agitation
Clinical observations:
Hypertensive then hypotensive phase
Signs and symptoms of intracerebral hemorrhage (uncommon), pulmonary edema, death (rarely)
Elevated troponin, creatine kinase, creatine phosphokinase, WBC
Electrocardiogram (EKG)
a. Ventricular ectopy
b. AV conduction defects
c. ST segment depression
d. T-wave inversion (troponin leak)

Range of Current Clinical Management Methods: IV or IM opiates for pain (morphine, fentanyl), benzodiazepines (valium), intravenous infusion of magnesium sulfate, crystalloid oxygen therapy, diuretics, albuterol, phentolamine to control blood pressure, glyceryl trinitrate, inotropic support, mechanical ventilation or application of continuous positive airway pressure (CPAP)

ponents, which may work additively or synergistically with venom porins, particularly at lower-dose exposures where hyperkalemia is not swiftly fatal. Investigations into whether venom lipases and bioactive lipids contribute to pathophysiological states that result in cerebral hemorrhage are sorely needed. The presence of increasing levels of pro-inflammatory bioactive lipids generated by venom lipase together with cytokines is consistent with the occurrence of profound capillary leak syndrome. Ultimately, cerebral hemorrhage as the most frequent cause of death in Irukandji syndrome is consistent with a cumulative level of both venom- and host-derived bioactive agents that promote increased vascular permeability and capillary leak.

39.5.1 Venom Lipases

Lipases are common venom components in a diversity of species. For example, in reptiles, where such enzymes have been studied in depth, they exhibit antiplatelet, myotoxic and neurotoxic activities (Fry et al. 2009). Studies suggest that lipases, specifically phospholipase A_2 (PLA_2) enzymes, which catalyze the hydrolysis of the ester bond at the *sn*-2 position of 1,2-diacyl-*sn*-phosphoglycerides, have been convergently recruited into cephalopods, insects, arachnids and reptiles. Thus, their presence in cnidarians would not be surprising (Fry et al. 2009). Several enzymes with PLA_2 activity have been isolated, cloned and sequenced from anemone species. While they share structural features with other PLA_2, they are phylogenetically distinct from all other venom phospholipases (Nevalainen 2008). In addition to anemones, high PLA_2 activity has been reported in 22 different species representing the classes Anthozoa, Hydrozoa, Scyphozoa and Cubozoa (Nevalainen et al. 2004). In particular, PLA_2 activity has been reported in the tentacles of *Chironex fleckeri* (184 U/g) and *Carybdea rastonii* (91 U/g), as well as whole extracts from the Irukandji syndrome-inducing *Carukia barnesi* (130 U/g). However, the underlying lipases were not isolated or sequenced.

Previous studies investigating lipase activities have relied on kits that use marker lipids to specifically test for PLA_2-bond cleavages. While such methods allow for the rapid detection of this activity, they are unable to distinguish specific PLA_2 enzymes from multi-action lipases like those found in bacteria and other lower invertebrates. Thus, with such kits, it is possible to mistake non-specific lipases or pluripotent lipases for specific single-cleavage type PLA_2, or alternatively to miss other lipases in the venom. Recent investigations have shown that crude venom and venom fractions from *Chironex fleckeri* exhibit PLA_2 and phospholipase C (PLC) activities, and digestion of fatty acids. Further studies are planned to isolate and characterize this enzyme(s).

The contribution of lipases associated with the generation of potent pro-inflammatory lipids in the pathophysiology of cubozoan venoms is not well understood. While PLA_2 activity has been reported in cubozoan and other venoms, the extent to which these lipases contribute to pathologies is not well resolved. It is unlikely that the lipase activity

is a major factor in acute cardiopulmonary collapse because the reaction kinetics was determined to be too slow. However, PLA_2, PLC, lipid peroxidases or potential pluripotent lipases likely amplify inflammatory mediator-related Irukandji syndrome symptoms by increasing plasma levels of bioactive lipids. Further, liberated free fatty acids, such as arachadonic acid, can be rapidly metabolized in plasma by lipoxygenases to become pro-inflammatory lipid mediators (Nigam and Schewe 2000). Thus, further studies are warranted to understand the contribution of venom lipases to the pathophysiology of cubozoan envenomation.

39.5.2 Bioactive Lipids

Lipids are a significant chemical component in the body of cnidarians, from corals to jellyfish. Several bioactive lipids have been isolated from cnidarians which exhibit a wide variety of activities, including cytoxic, hypotensive, inflammatory, antibacterial and antiviral actions (Rocha et al. 2011). However, such research has focused on the class Anthozoa, with surprisingly little attention paid to the other classes. Studies have also rarely looked specifically at cnidae venom lipids. What is known is that the total lipid content of the lyophilized saline-soluble venom extract of Portuguese man-of-war (*Physalia physalis*) was reported at 3.44 % with about a third of those lipids comprised of free fatty acids (Stillway 1974). Phosphonolipids may be responsible for the toxicity of the mauve stinger (*Pelagia noctiluca*; Nakhel et al. 1988), a jellyfish known to cause serious human injury.

Preliminary investigations into cubozoan venoms have revealed that they are extremely lipid-rich. Published reports, as well as discussions with investigators in the field of venom toxinology, indicated a general consensus that the lipids act as an incipient to facilitate delivery or bioavailability of amphipathic or hydrophobic toxins. However, fractions from organic extracts of total *Alatina alata* cnidae content were found to retain potent inflammatory properties, suggesting the presence of yet-undiscovered bioactive lipids.

Incubation of total crude venom demonstrated autolysis of *Alatina* venom lipids by venom lipases. Time-dependent PLA_2-like generation of lyso-PAF was documented at levels that would constitute a massive pro-inflammatory effect. Lyso-PAF though was further cleaved in whole venom demonstrating that the lipolytic activity of crude *Alatina* venom is not limited to specific PLA_2 single site cleavage of phospholipid. The venom lipids were also characterized using mass spectrometry and found to comprise long chain fatty acids from C20 to C36. The pharmacological properties of long chain lyso-PAF have yet to be elucidated fully but preliminary work suggests that these PAF species have longer half-lives and greater potency. Further research may elucidate not only the composition of bioactive venom lipids, but may also shed light on how these lipids contribute to clinical sequelae.

39.5.3 Experimental Priorities for Animal Modeling

Currently, there is no standardized protocol for the treatment of cubozoan envenomations. Thus, practitioners have tried a diverse set of treatments to varying degrees of efficacy. Many of these potential therapeutic options should be studied in further depth with the aid of an appropriate animal model to determine whether they have the potential to be valuable therapeutics.

39.5.3.1 Porin Blockers

As the cubozoan porins have emerged as the primary venom component responsible for the diverse pathologies associated with envenomation, potential therapeutics which effectively reduce or halt porin activities are a primary research concern. Although inactivation of venom components is the goal of antivenoms, the evidence for the effectiveness of the CSL *Chironex fleckeri* antivenom as a polyspecific porin blocker remains mixed. While some studies have suggested it can relieve pain and provide systemic benefits for *Chironex fleckeri* stings (Baxter and Marr 1974; Winkel et al. 2003), others have demonstrated no reduction in morbidity and mortality and even less utility against other cubozoan species (Tibballs et al. 1998; Winkel et al. 2003; Ramasamy et al. 2004; Cegolon et al. 2013).

However, an antibody-based approach to cubozoan envenomation may still be useful, particularly now that porins can be purified. Future box jellyfish antivenom development should be taken as seriously and carefully as snake antivenoms (Nakhel et al. 1988). Proteomics-based immunochemical analyses (antivenomics) can help determine whether crude venoms, venom fractions or purified porins produce the best antibodies that bind and inactivate the most medically relevant venom components (Gutiérrez et al. 2009). Similarly, these methods can also evaluate the cross-reactivity between the antivenom and toxins from different cubozoan species. Detailed efforts to optimize immunogenic mixtures and adjuvants have the potential to lead to the holy grail of venom treatment: a polyspecific or 'universal' box jelly antivenom.

However, in the absence of an effective antivenom, porin blockade may still be achieved. As the cubozoan porins demonstrate structural motif homology to bacterial porins (Bernheimer et al. 1979), metal salts, which were used to treat infections before the advent of antibiotics (Avigad and Bernheimer 1976), are promising (and inexpensive) candidates for porin-blockers. Preliminary studies suggests that zinc and copper gluconate are highly effective in animal models, however, further research is needed to confirm their

clinical relevance. Further studies need to investigate doses, timing and outcomes in animal models that better mimic envenomations in humans, including whether these gluconate salts act synergistically or antagonistically with other currently used sting treatments, like magnesium sulfate.

39.5.3.2 Topical Agents

Research into topical agents has largely focused on the most effective means removing tentacles and of preventing unfired nematocyst discharge to reduce overall venom load. Relatively little research has investigated topical agents that may ameliorate pathologies by deactivating venom components. There is strong evidence that hot water immersion is effective at reducing pain from cnidarian envenomations (Thomas et al. 2001; Bowra et al. 2002; Nomura et al. 2002; Loten et al. 2006; Wilcox and Yanagihara 2016), likely through its ability to inactivate porins (Yoshimoto and Yanagihara 2002; Atkinson et al. 2006). However, comparison of heated solutions is warranted to determine the most effective relief. Future research could investigate whether Epsom salts, magnesium sulfate, vinegar or urea increase or decrease the efficacy of immersion. In addition, further research into whether immersion reduces overall morbidity would be helpful.

Topical creams may help ameliorate dermal pathologies either through direct inhibition of venom components in the skin or by specific targeting of adverse immunological pathways. Topical application of the gluconate salts suggested as porin blockers should be a top priority, as well as evaluation of topical steroids (e.g., triamcinolone, hydrocortisone, prednisone) to reduce sting-site inflammation.

39.5.3.3 Catecholamine Surge Treatment

As Irukandji syndrome is marked by a surge in catecholamines, further research should investigate therapeutics that either slow their release or prevent their downstream physiological effects. Magnesium sulfate is the current recommended option, as magnesium decreases both catecholamine release as well as receptor sensitivity (Corkeron 2003). However, its efficacy in all cases of Irukandji syndrome has been debated (McCullagh et al. 2012; Rathbone et al. 2013). Further research in animal models could also evaluate whether magnesium sulfate exhibits synergistic or antagonistic effects with other commonly administered treatments, such as morphine. Another therapeutic that may help reduce the effects of catecholamine excess is phentolamine, a non-selective alpha-adrenergic antagonist (Fenner et al. 1986). Finally, randomized, placebo-controlled clinical trials would help settle the matter more definitively as well as determine most effective dose(s).

In addition, further research is needed into the effects of epinephrine administration after cubozoan envenomation. As porin-induced lysis of platelets leads to high circulating levels of norepinephrine and epinephrine as evidenced by a severely hypertensive state, further administration of epinephrine, as is standard treatment for airway constriction, may exacerbate an already life-threatening condition. Further research is necessary to evaluate the risk of epinephrine in such cases, and to identify alternative or concurrent therapeutic options, such as GTN administration.

39.5.4 Cytokine Storm Treatment

There has been relatively little research evaluating the most effective methods to counteract the 'cytokine storm' that is characteristic of Irukandji syndrome. Cytokines are profoundly pro-inflammatory, thus anti-inflammatory agents may help alleviate Irukandji syndrome symptoms. Steroids have yet to be evaluated in any depth for the treatment of cubozoan stings, however, intravenous corticosteroids have been suggested as a potential avenue for treatment (Holmes 1996).

Cytokines are not only liberated upon porin lysis of platelets and white cells, they are also released through histamine-mediated mechanisms, as blood cell lysis releases massive stores of histamine. Antihistamines may prove helpful in counteracting histamine-induced cytokine release and general histamine-mediated inflammation. Currently, the U.S. Army's SFUWO uses a cocktail of antihistamines, which includes an H2-receptor antagonist (generally ranitidine) as well as an H1-receptor antagonist (diphenhydramine). However, the clinical efficacy of this mixture has not been tested. Thus, animal modeling of antihistamine use to treat cubozoan envenomations is another potential avenue for future research.

39.5.4.1 Other Important Research Questions

Other important avenues of research include the review of current treatments and novel, previously untested therapies. Studies of scorpion envenomation, which shares similarities to Irukandji syndrome, have found inotropic agents, such as calcium gluconate, increase survival times (Ismail et al. 1992), and thus the use of these agents to treat Irukandji syndrome may be worth testing. The usefulness of diuretics like furosemide or the osmotic diuretic mannitol may also be worth investigating. Given the lipid solubility of a number of venom components the use of lipid emulsions, as used in a variety of toxidromes, is a potential line of research in Irukandji syndrome.

There are several current medications that could use further analysis. For example, the analgesic effectiveness of morphine versus fentanyl has yet to be tested. Furthermore, given the potential for morphine to increase hypotension in patients that may already be hypovolemic from capillary leak, animal models should be used to evaluate whether morphine or other analgesics improve or worsen morbidity and

mortality. The use of magnesium sulfate awaits further definition in either well conducted randomized trials or database analysis and is an area of active clinical interest. Similarly, while GTN is suggested by several emergency protocols, it has not been tested fully in in vitro or in vivo models. But most importantly, the combined effects of concurrent treatments have not been well modeled. As vital as it is to ensure the safety and effectiveness of different treatments individually, it is of the highest priority to determine whether the use of multiple treatments simultaneously is equally safe and effective. The use of multiple medications may have unknown synergistic effects, or independently useful therapeutics may prove antagonistic in vivo.

Increases in cubozoan envenomations represent a critical clinical concern in tropical medicine (Grady and Burnett 2003). Rapid advances in biochemical characterization of cubozoan venoms together with cell and animal model based mechanistic studies have begun to provide important insights into core mechanisms which in turn suggest therapeutic approaches. Continuation of this iterative approach will undoubtedly answer many of the remaining questions about venom components, their physiological effects, and the best way to reduce morbidity and mortality from sting events.

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The Role of Cnidaria in Drug Discovery

40

Gian Luigi Mariottini

Abstract

A lot of organisms, including microbes, algae, plants and animals, are known to produce substances of pharmacological concern provided with different biological activity. At present, several bioactive natural substances have application as drugs, pigments, biocides or have been utilized to synthesize biologically active molecules. Oceans are inhabited by an enormous amount of species. For this reason the marine environment is thought to be the richest and the most complex ecosystem. Nevertheless, despite the 50-year-long research activity and interest in this subject, natural compounds from marine organisms have been considered slightly in pharmacology and have had limited therapeutical application. Cnidarians are gelatinous invertebrates, in large part inhabiting seawaters, which play an important role among venom-producing organisms and are viewed with particular concern owing to the implications of their stings on human health and on economy. Cnidarian tentacles and/or oral arms, and at times the whole body, are armed with nematocysts, specialized venom-containing structures provided with a tightly wrapped and spiralized thread used to inject the venom. Taking into account that during the last decades a lot of interesting bioactive substances have been isolated from Cnidaria and demonstrated cytotoxic, hemolytic, anti-inflammatory, antitumoral, anti-infective, anti-parasite, as well as other interesting properties, this chapter aims to review the recent data about cnidarian extracts that could be of concern for drug discovery as potential sources for pharmaceuticals.

Keywords

Cnidaria • Jellyfish • Sea anemones • Corals • Drug discovery

40.1 Introduction

Living organisms are viewed as a significant source of substances useful to fight human diseases being a lot of utilized drugs derived directly from natural sources or based on their structures. During the last century many natural products provided with pharmacological properties were discovered, mainly in terrestrial plants and in microorganisms (Newman et al. 2000). Several of these compounds are currently used for therapeutic purposes, mainly in oncology and in infectivology (Newman et al. 2003), others have potential application in cosmetic industry, as molecular tools, fine chemicals, nutraceuticals and agrochemicals (Kijjoa and Sawangwong 2004; Fusetani 2010). The oceans, covering most of the Earth's surface, are an enormous resource for the discovery of bioactive molecules and the development of potential chemotherapeutic agents. As a matter of fact, waters are inhabited by species belonging to almost all animal phyla; 32 of them are represented in aquatic environments and 15 are exclusively marine (Cragg and Newman 2013). Despite their abundance and variety, marine organisms have been scarcely considered in studies aimed to develop substances of pharmacological concern (Proksch

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et al. 2003). The difficulties to develop new drugs from marine natural products are mostly due to the difficulty to collect specimens, to the scarce amount of extracts which can be obtained by marine organisms, as well as to the structural diversity of marine compounds (Leone et al. 2013). In spite of this, several active compounds against cardiovascular, nervous, endocrine, immune, infective, inflammatory disorders or having antitumoral properties have been reported (Mayer and Lehmann 2001; Mayer and Gustafson 2004, 2006, 2008, Mayer et al. 2009). Nevertheless, several compounds produced by marine organisms are known to be venomous. They play a role in ecosystem functioning as well as in the competition among species but are important also in human pathology, having a serious impact on public health, as well as in economy being able to affect some human activities (Nastav et al. 2013; Mariottini 2014).

The production of natural venoms is an interesting aspect characterizing the physiology and the ecology of Cnidaria (jellyfish, corals and anemones) which use them for defence/offence and for trophic purposes to get the food. Cnidaria store venoms into specialized structures, the nematocysts, secretory products of the Golgi apparatus, provided with a double-walled proteinaceous capsule containing a tightly spiralized filament (Mariscal 1974) which after stimulation injects the venom into the prey or the attacker acting as a syringe needle. Cnidarian venoms are thought to be synthesized into tissues (Allavena et al. 1998). Cnidarian envenomations largely occur during jellyfish outbreaks which are recurrent phenomena in coastal marine environments at a global scale and at present are viewed as an emergent problem (Duarte et al. 2013; De Donno et al. 2014). The venomous properties of cnidarians have been studied starting from early twentieth century obtaining results useful in both biological and medical fields, such as the formulation of anaphylaxis which was defined studying the damage induced on dogs by sea-anemone toxins (Portier and Richet 1902). In most cases cnidarian stinging induces only nuisance and local symptoms but some species can pose serious threats to humans causing dermonecrotic, cardiotoxic, neurotoxic and hemolytic effects, with possible serious atopic or anaphylactic phenomena. Some highly venomous jellyfish are able to induce lethal envenomations. Cnidarian venoms are complex proteinaceous mixtures (Lassen et al. 2011) which induce pore formation or oxidative stress in cells (Morabito et al. 2012), affect cell membrane permeability and ion exchange, or have phospolipase activity. Nevertheless, despite their toxicity, Cnidaria are thought to be a promising source of compounds useful to develop biomedical materials or new drugs (Rocha et al. 2011). In this connection, this paper aims to review, in a pharmacological perspective, the published data about the compounds derived from Cnidaria dividing them on the basis of the main biological activity.

40.2 Does Cnidaria Play a Role in Drug Discovery?

Cnidaria were indicated as early as the 1970s to be a source of bioactive compounds useful to develop new drugs or materials of biomedical concern (Mariscal 1974). During the subsequent decades a number of different compounds and metabolites, several of them having features useful to develop new drugs were discovered and isolated. Notably, during the first decade of the twenty-first century over 2000 molecules were described (Rocha et al. 2011) making cnidarians a research subject of pre-eminent interest. As a matter of fact, cnidarians, and particularly corals, are known to play an important role in the traditional medicine of Asian countries of ancient civilization, such as in India (De Zoysa 2012), to treat several respiratory (coughs, pulmonary tuberculosis, asthma, chronic bronchitis) and genito-urinary (spermatorrhea, gonorrhea) diseases, as well as headache, vertigo and anemia (Gopal et al. 2008). Jellyfish have been used traditionally in China to treat hypertension (Hsieh and Rudloe 1994) and several presumed therapeutic or therapeutic-like jellyfish properties are known to be described in Chinese non-scientific publications (Hsieh et al. 2001). Dried powder of jellyfish was used by Australian Aborigine shamans as a remedy for burns and in South Korea jellyfish are used to improve the beauty of skin and to loss weight. Notably, collagen was hypothesized to have beneficial effects in the treatment of bronchitis and arthritis, as well as for dermatological purposes (Hsieh and Rudloe 1994). The employment against ulcers, asthenia, swelling, to improve digestion and to soften skin as well as to stimulate blood flow during the menstrual cycle was also reported (Hsieh et al. 2001). Notwithstanding these efforts and the numerous non-scientific indications, the efficacy of the bulk of cnidarian extracts has remained an hypothesis and only the diterpene glycosides pseudopterosin derived from the sea whip *Pseudopterogorgia elisabethae* (Kohl et al. 2003), eleutherobin derived from the soft coral *Eleutherobia* sp. and subsequently isolated from *Erythropodium caribaeorum*, and the diterpenes sarcodictyns extracted from corals have been exhaustively studied for their therapeutic properties in preclinical studies (Mariottini and Pane 2013).

40.2.1 Anti-inflammatory Activity

The inflammation is a process involved in the response to infections, injuries and to variously originating damages. It is known to be part of the pathogenesis of several important affections, such as arthritis, cancer, stroke, neurodegenerative and cardiovascular diseases (Ricciotti and FitzGerald 2011). Even though in therapeutic practices inflammation is

often fought, it is an advantageous process aimed at the eradication, by natural way, of the pathogenetic causes of disease and consequently to the restoration of the anatomical and physiological features of tissues. The mitigation of the inflammation by pharmacological tools has a rationale in the intent to reduce the unpleasant effects of the process.

Notwithstanding cnidarian stinging causes, among other consequences, also inflammation, several extracts have demonstrated anti-inflammatory properties. During the 1980s the pseudopterosins, diterpene-pentoseglycosides found in the Caribbean sea whip *Pseudopterogorgia elisabethae* (Gorgoniidae), were shown to have anti-inflammatory properties exceeding that of indomethacin. Pseudopterosins were stated to modify the arachidonic acid cascade and, in particular pseudopterosin A, to block phorbol myristate acetate-induced topical inflammation in mice skin (Look et al. 1986); 10–15 mg/kg pseudopterosins inhibited 50 % (ED_{50}) of writhing after intraperitoneal injection (Fenical 1987). Another interesting compound, the diterpenoid pseudopterolide, isolated from *Pseudopterogorgia acerosa*, was recognized to potently inhibit the topical inflammation. In the same paper the isolation of two potent anti-inflammatory cembrenes from *Pseudopterogorgia bipinnata* was described and the activity of pseudopterosin A in reducing (75–95 %) the ear edema in laboratory animals (ED_{50} = 8.3 μg/ear) was emphasized (Fenical 1987). Subsequently, pseudopterosin E (PSE) and pseudopterosin A (PSA) were found to reduce ear edema by blocking neutrophil infiltration and degranulation during the early stages of the inflammatory response. The effect was observed after both topical (ED_{50} = PSE 38 μg/ear, PSA 8 μg/ear) or systemic (ED_{50} = PSE 14 μg/ear, PSA 32 mg/kg, i.p.) administration. In the same research pseudopterosins have been also shown to be inactive in vitro as inhibitors of phospholipase A, cyclooxygenase and cytokine release, as well as regulators of adhesion molecule expression (Mayer et al. 1998). The biosynthesis of pseudopterosins, involving the cyclization of geranylgeranyl diphosphate to elisabethatriene and then the dehydrogenation and aromatization to erogorgiaene, was explained by Kohl and Kerr (2003). Other compounds, amphilectosins A and B, were subsequently found to have a role in the biosynthesis of pseudopterosin Y and F, respectively (Ferns and Kerr 2005). Further attempts to synthesize pseudopterosins lead to the purification of the enzyme responsible for the biosynthesis which was identified as the elisabethatriene synthase, and subsequently purified (Kohl and Kerr 2004; Brueck and Kerr 2006). Seco-pseudopterosins from *Pseudopterogorgia elisabethae* and the biosynthetic intermediate elisabethadione were indicated to have similar anti-inflammatory activity (Kohl et al. 2003). To date, the University of Prince Edwards Island (2014) lists 26 different identified derivatives of pseudopterosin (PsA-PsZ) and indicates that a derivative of pseudopterosin A, methopterosin, has completed a Phase II clinical trial as a topical anti-inflammatory agent.

Several studies on cnidarians concerned the evaluation of the activity of their extracts on two pro-inflammatory proteins, the inducible NO-synthase (iNOS), which is known to be regulated by mediators of inflammation, such as cytokines and LPS, and to be implicated in the inflammatory response (Putra et al 2012a), and cyclooxygenase (COX-2) which is known to be a key-enzyme in the conversion of arachidonic acid to prostaglandins. Octocorals *Sinularia flexibilis* were reported to contain cembranoid diterpenes. One of them (sinularin) is able to inhibit the up-regulation of iNOS and COX-2 and to upregulate the production of TGF-β in lipopolysaccharide (LPS)-stimulated RAW264.7 murine macrophages. Notably, 80 mg/kg sinularin injected s.c. in rats inhibited carrageenan-induced tissue inflammatory responses, redness, edema, and leukocyte infiltration (Huang et al. 2012b). Chen et al. (2012b) from cultured *Sinularia flexibilis* obtained 11-dehydrosinulariolide, another compound able to induce the upregulation of iNOS and COX-2 as observed after exposition of LPS-stimulated RAW264.7 murine macrophages to 10–50 and 25–50 μM, respectively, and the suppression of iNOS expression.

Other studies on LPS-stimulated RAW264.7 macrophages demonstrated that the diterpenoids flexibilisolide C and D inhibit the accumulation of iNOS, while the accumulation of COX-2 was reduced by flexibilisolide C (Shih et al. 2012). Sinulasulfoxide and a furanosesquiterpenoid extracted from an unidentified species of *Sinularia* were evaluated for the inhibition of iNOS considering the production of a stable NO metabolite (NO_2^-) as a parameter of macrophage activation and iNOS induction. After 24 h LPS (1 μg/mL)-stimulated J774 mouse macrophages gave dose-dependent release of NO_2^- (15.6 nmol/mL) (Putra et al. 2012a). The sesquiterpenes Lochmolin A, B, C and D from *Sinularia lochmodes* were found to reduce the levels of LPS-induced COX-2 in RAW264.7 macrophages to 36.6 % with 1 μM of Lochmolin A, to 8.7 %, 61.0 %, and 83.4 % with 10 μM of Lochmolin A, C and D, respectively and to 1.7 %, 17.6 %, 32.8 %, and 71.3 % with 100 μM of Lochmolin A, B, C and D, respectively. The authors concluded that Lochmolin A could be a promising COX-2 inhibiting agent (Tseng et al. 2012). *Sinularia crassa* yielded a sterol, crassarosterol A, and four steroidal glycosides, crassarosterosides A–D, which showed diversified activities against iNOS and COX-2 expression in stimulated macrophages; 10 μM crassarosterosides A and C inhibited iNOS expression, while the same concentration of crassarosterol A and crassarosteroside B stimulated the expression of COX-2 (Chao et al. 2012). Two secosterols from *Sinularia granosa* (compounds 1 and 2), at 10 μM induced significant reduction of iNOS levels (66.1 % and 19.4 %, respectively) in LPS-stimulated RAW264.7 macrophages. Compound 1 was

also able to reduce COX-2 expression to 42.7 %. Thanks to these results and to the absence of cytotoxic effects, the Authors stated that these compounds, and particularly Compound 1, might be promising antiinflammatory agents (Huang et al. 2012a). Two compounds (gyrosanols A and B) from *Sinularia gyrosa* exhibited ambiguous anti-inflammatory activity at 10 μM, evidenced by the suppression to 19.6 % and 29.1 %, respectively of the expression of COX-2 in RAW264.7 macrophages and a stimulating activity on iNOS expression (118.5 % and 171.7 %, respectively) (Cheng et al. 2010). The same amount of scabralin A from *Sinularia scabra* caused inhibition (to 39.1 %) of iNOS accumulation in LPS-stimulated RAW264.7 macrophages but had no activity on COX-2 (Su et al. 2012b). The methanol extract of *Sinularia maxima* yielded 12 diterpenoids which were studied for their inhibition activity on the production of pro-inflammatory cytokines in LPS-stimulated bone marrow-derived dendritic cells. Notably, three compounds (sinumaximol B, sinumaximol C, and isomandapamate) resulted active. Sinumaximol C had a strong inhibitory effect on interleukin(IL)-12 and IL-6 production (IC_{50}s = 4.35 and 17.72 μM, respectively), sinumaximol B and isomandapamate had effect mainly on IL-12 (IC_{50}s = 15.20 and 18.04 μM, respectively), but also on IL-6 with respective IC_{50} values of 59.77 and 54.32 μM. TNF-α production was moderately inhibited only by sinumaximol C (IC_{50} = 62.33 μM) (Thao et al. 2012).

The steroids 24-methylene-3β,5α,6β,19-tetrahydroxy-5α-(cholestane) and 24-methylene-5α-cholesten-3β,7β,19-triol provided with anti-inflammatory activity were isolated from *Nephthea brassica* (Amir et al 2012). Four ergostanoids extracted from the affine species *Nephthea erecta* were shown to have in vitro anti-inflammatory activity at 10 μm, significantly reducing the levels of iNOS to values ranging from 15.6 % to 62.8 % in *Escherichia coli* LPS-stimulated RAW264.7 cells. Two of these compounds were also able to inhibit COX-2 expression to 68.1 % and 10.3 % (Cheng et al. 2009a). The same research group reported the sesquiterpene erectathiol isolated from *Nephthea erecta* and chabrosterol isolated from *Nephthea chabroli* exhibited anti-inflammatory activity in vitro at 10 μM reducing the levels of iNOS to 58.0 % and 12.4 %, respectively in RAW264.7 macrophages. Otherwise, the levels of COX-2 were significantly reduced by chabrosterol (45.2 %) but not by erectathiol which was stimulant (108.7 %) (Cheng et al. 2009b). Eight methylated steroids, nebrosteroids A–H, from *Nephthea chabroli* were tested for the anti-inflammatory activity. Results showed that 10 μM of nebrosteroid D, E and G reduced the levels of iNOS to 0 %, 43.1 %, and 76.9 %, respectively and of COX-2 to 63.9 %, 57.4 %, and 72.0 %, respectively in LPS-stimulated RAW264.7 cells. iNOS protein expression was reduced also by nebrosteroids A, B, C, and H (10.6 %, 9.6 %, 0 %, 32.8 %, respectively). Of particular concern was the activity of nebrosteroids C and D which completely inhibited iNOS production (Huang et al. 2008).

Studies on other Alcyonacea showed that 10 μg/mL of lobocrassin F extracted from *Lobophytum crassum* significantly inhibited (58.29 %) the release of elastase by human neutrophils with IC_{50} of 6.27 μg/mL. At the same concentration the sesquiterpenoid menelloide E from *Menella* sp. caused lower inhibition (26.99 %) of elastase release (Lee et al. 2012). Pregnane-type metabolites (sclerosteroids A, B, E) from *Scleronephthya gracillimum* were indicated to be effective anti-inflammatory agents significantly inhibiting the accumulation of iNOS and COX-2 in RAW264.7 macrophages stimulated with LPS, reducing their expression to below 30 % and 20 %, respectively (Fang et al. 2012). Chang et al. (2012) isolated from *Cespitularia taeniata* three compounds (cespitulin E, F, and G) which were tested for inhibitory effects of superoxide anion generation and elastase release by human neutrophils in response to N-formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B. Only Cespitulin G induced a significant inhibition of superoxide production (IC_{50} = 6.2 μg/ml) and elastase release (IC_{50} = 2.7 μg/ml). A concentration of 10 μM of the cembranoids sarcocrassocolides M-O isolated from *Sarcophyton crassocaule* reduced iNOS levels to 4.2 %, 52.9 %, and 22.7 %, respectively in LPS-stimulated RAW264.7 macrophages but only sarcocrassocolide M reduced COX-2 expression (to 62.8 %) (Lin et al. 2012).

From *Eunicea fusca* Marchbank et al. (2012) extracted and identified three diterpenes (eunicidiol, fuscol and eunicol) which showed significant anti-inflammatory activity when applicated topically at 100 μg/ear in mouse, reducing phorbol myristate acetate-induced edema. The reduction of edema ranged from 44 % using eunicidiol, to 54 % using eunicol. The reduction using fuscol showed an intermediate activity (46 %). The Authors stated that the activity of these diterpenes surpassed that of indomethacin. The methanolic extract of *Briareum asbestinum* yielded three diterpenes (seco-briarellinone, briarellin S, and seco-asbestinin) two of which were found to have anti-inflammatory properties inhibiting nitric oxide production in LPS-stimulated macrophages with IC_{50}s of 4.7 μM and 20.3 μM, respectively (Gómez-Reyes et al. 2012). The diterpenoid echinohalimane A and the steroid derivative 6-*epi*-yonarasterol B isolated from *Echinomuricea* sp. were found to inhibit the release of elastase by human neutrophils at 10 μg/mL with IC_{50}s of 0.38 and 1.13 μg/mL, respectively. The steroid induced 95.54 % inhibition and showed also significant effects on the generation of superoxide anions by human neutrophils with IC_{50} = 2.98 μg/mL and inhibition 89.76 % (Chung et al. 2012a, b). Other data about the antiinflammatory activity of compounds isolated from Taiwanese soft corals can be found in a recent review (Wei et al. 2013).

The antiinflammatory activity of the crude hydroalcoholic extract of the hexacoral *Palythoa caribaeorum* was assessed in carrageenan-induced edema of rat hind paw but scarce antiinflammatory activity was observed at 200 and 400 mg/kg of extract (Soares et al. 2006).

Jellyfish collagen has been tested in rats to study its beneficial effects against arthritis. Rats with experimentally induced arthritis fed daily with 10 μg of jellyfish collagen evidenced delayed (5–10 days) onset of arthritis in comparison to positive controls joined with a significant ($p < 0.05$) lower incidence of redness, swelling and deformity (Hsieh et al. 2001).

40.2.2 Antimicrobial Properties

The development of new antimicrobial compounds is a challenge for the modern pharmacology due to the recurrent antibiotic-resistance observed in the treatment of several infectious diseases. Rresistant microorganisms include bacteria, fungi, viruses, and parasites which are able to withstand to antimicrobial drugs (antibiotics, antifungals, antivirals, etc.) having developed resistance as a result of natural causes such as erroneous replication, exchange of resistant traits, etc.

Among hydrozoans, *Hydra* uses an epithelial cells-mediated 'innate immune response' to respond to external antigens, releasing antimicrobials or antiproteinases as a result of increased expression of encoding genes (Bosch 2008). Arminins, gene family extensions for antimicrobials, were detected in four species of *Hydra* and have specific profiles for the production of species-specific antimicrobial peptides (Franzenburg et al. 2013). Furthermore, a protein discovered in *Hydra*, hydramacin-1, the amino acid sequence differing from other antimicrobial proteins (Jung et al. 2009), demonstrated strong antimicrobial properties against a broad spectrum of Gram-positive and Gram-negative bacteria, including multiresistant strains, but evidenced less activity against fungi. The best results were obtained against Gram-negatives *Citrobacter freundii*, *Enterobacter cloacae*, *E. coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Salmonella typhimurium* and *Yersinia enterocolitica* with Minimal Bactericidal Concentration (MBC) of 0.9 μM killing ≥99.9 % of bacteria. LD_{90} values for hydramacin-1 were interesting for multiresistant Gram-negatives, such as *E. coli* (MBC = 0.4–0.9 μM, LD_{90} = 0.2–0.4 μM), *K. oxytoca* (MBC = 0.9 μM for all strains, LD_{90} = 0.2–0.4 μM), and *K. pneumoniae* (MBC = 0.9–3.6 μM, LD_{90} = 0.4–0.9 μM) (Bosch et al. 2009). A subsequent study aimed at elucidate the occurrence of an 'immune system' in *Hydra* demonstrated that gland cells produce a serine protease inhibitor, kazal2, having strong activity against *Staphylococcus aureus* ATCC12600 with Minimal Inhibitory Concentration (MIC) of 0.7–0.8 μM (Augustin et al. 2009). In addition, also recombinant proteins (domains 1–3) resulted active against *S. aureus*. Kazal2 did not affect the growth of *E. coli*. The Authors stated that kazal2 inhibits a protease essential for *S. aureus* but not for *E. coli* with consequent possible utilization as anti-staphylococcal agent (Augustin et al. 2009). The observation of a strong activity against *Bacillus subtilis* and *E. coli* of extracts from nerve-free tissues of *Hydra magnipapillata* sf-1 mutants in comparison to that from normal tissues, suggested the antibacterial properties could depend on the amount of neurons into tissues (Kasahara and Bosch 2003). Furthermore, a receptor-mediated defense system able to recognize pathogen-associated molecules (flagellin, LPS) through TLR and to produce antimicrobial peptides was described in *Hydra* (Bosch et al. 2009).

Considering the available literature, soft corals (Octocorallia) are undoubtedly the main cnidarian source of antimicrobials. In late 1990s, during a research aimed to screen the anti-tuberculosis activity of marine natural products, two diterpenoid alkaloids (pseudopteroxazole and *seco*-pseudopteroxazole) isolated from *Pseudopterogorgia elisabethae* were found to be active against *Mycobacterium tuberculosis* $H_{37}Rv$. Notably, 12.5 μg/mL pseudopteroxazole induced 97 % inhibition, whereas *seco*-pseudopteroxazole had a lower but noticeable (66 %) inhibitory activity on mycobacterial growth (Rodríguez et al. 1999). Subsequently a minor constituent, the alkaloid homopseudopteroxazole, was isolated from the hexane extract of *Pseudopterogorgia elisabethae* which induced 80 % growth inhibition on *M. tuberculosis* $H_{37}Rv$ (MIC = 12.5 μg/mL) (Rodríguez and Rodríguez 2003). Further studies about synthesized pseudopteroxazoles, pseudopteroquinoxalines and pseudopterosin congeners showed that some of them exhibited antimicrobial activity against mycobacteria (*M. tuberculosis* $H_{37}Rv$, *M. smegmatis* and *M. diernhoferi*) and Gram-positive bacteria such as methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococcus faecium*. Notably, the semi-synthetic compound 21-((1H-imidazol-5-yl)methyl)-pseudopteroxazole showed remarkable activity against replicating and non-replicating persistent forms of *M. tuberculosis* and pseudopteroxazole exhibited activity against antibiotic-resistant strains (McCulloch et al. 2012). Another study tested several diterpenes (pseudopterosins and seco-pseudopterosins) from *Pseudopterogorgia elisabethae* against *S. aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Candida albicans* showing a selective activity against Gram-positive bacteria. Satisfactory results were obtained against *S. aureus* with PsU, PsQ, PsS, seco-PsK and PsG (IC_{50}s ranging from 2.9 to 4.5 μM), while the best values against *E. faecalis* ranged between 3.1 and 3.8 μM by using PsG, PsU and seco-PsK (Correa et al. 2011).

Alcyonacean soft corals were indicated to exert strong antimicrobial activity against bacteria suspended in seawater,

which was exhibited by 83 % of examined cases and mainly by *Xenia macrospiculata*. An antibiotic compound, desoxyhavannahine, having MIC of 48 μg/mL was isolated (Kelman et al. 2006). The alkaloids sinularioside, sinulasulfoxide and sinulasulfone extracted from *Sinularia* sp. were found to moderately inhibit LPS-induced Nitric Oxide (NO) release (Putra et al. 2012a, b), a molecule implicated in the response to infections (Wang et al. 1996). From the affine species *Sinularia gyrosa*, the diterpenoids gyrosanol A, B, and C were isolated. Gyrosanol A and B showed antiviral properties affecting human cytomegalovirus (HCMV) at IC_{50}s of 2.6 and 3.7 μM, respectively but were unactive at 100 μg/disk against bacteria *Enterobacter aerogenes*, *Salmonella enteritidis*, *Serratia marcescens*, *Shigella sonnei*, and *Y. enterocolitica* (Cheng et al. 2010). Sinularosides A and B, two 9,11-secosteroidal glycosides isolated from *Sinularia humilis*, exhibited remarkable activity comparable to that of ketoconazole against fungi *Microbotryum violaceum* and *Septoria tritici*, as well as antibacterial activity, comparable to that of streptomycin, against *Bacillus megaterium* (Gram-positive) but had no effect on *E. coli* (Sun et al. 2012).

The sesquiterpene erectathiol from *Nephthea erecta* exhibited moderate antimicrobial activity against *E. aerogenes*, *S. marcescens*, *Y. enterocolitica*, and *S. sonnei* and satisfactory results, surpassing those of ampicillin, against *S. enteritidis* (Cheng et al. 2009b). Parathyrsoidins A–D, sesquiterpenoids isolated from *Paralemnalia thyrsoides* did not induce antiviral activity in human embryonic lung (HEL) cells infected with HCMV (Tseng et al. 2013).

Litosterol, nephalsterol B and nephalsterol C from *Nephthea* sp. were studied for their antitubercular activity observing that litosterol and nephalsterol C strongly inhibit *M. tuberculosis* growth (90 % and 96 %, respectively) with MICs of 3.13 and 12.5 μg/mL, respectively. Nephalsterol B induced 69 % inhibition (El Sayed et al. 2000). Michaolide O, a non-cytotoxic compound from *Lobophytum michaelae* was found to be inactive against HCMV (IC_{50} >200 μM/mL) (Wang and Duh 2012).

A lactone cembrane diterpene (sarcophytolide) was isolated from alcohol extract of the Red Sea soft coral *Sarcophyton glaucum*. It showed antimicrobial activity towards Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacteria, and yeasts (*Saccharomyces cerevisiae*) but was inactive towards *E. coli* (Badria et al. 1998). Three cembranoids, (+)-12-ethoxycarbonyl-11Z-sarcophine, ehrenbergol A and B isolated from acetone extracts of *Sarcophyton ehrenbergi* displayed activity against HCMV resulting in IC_{50} values of 60, 46, and 5.0 μg/mL, respectively (Wang et al. 2012a). Other diterpenoids obtained from acetone extracts of *Sarcophyton ehrenbergi* (ehrenbergol C and acetyl ehrenberoxide B) showed activity towards HCMV (EC_{50}s = 20 and 8.0 μg/mL, respectively) (Wang et al. 2013). *Sarcophyton trocheliophorum* yielded seven cembranolides (sartrolides A–G), bissartrolide, and three analogues one of which, named cembranolide 11, resulted active in paper disk diffusion test (24 h) against *S. aureus* (MIC = 125 μg/mL) (Liang et al. 2013).

Antimicrobial activities have been reported for *Alcyonum digitatum*, whose extract was tested on two Gram-negative bacteria, *E. coli* and the fish pathogen *Listonella anguillarum* serotype O2, and two Gram-positive bacteria, *S. aureus* and *Corynebacterium glutamicum*, as well as on *C. albicans* and *S. cerevisiae*. The main activity was observed against *S. aureus* and *C. glutamicum* (MICs ranging from 0.08 to 0.63 mg/mL for both bacteria), while *L. anguillarum* (MIC = 0.31–1.25 mg/mL) and *E. coli* (MIC = 2.5 mg/mL) exhibited resistance. The antifungal activity was remarkable particularly against *S. cerevisiae* (MIC = 0.16–1.25 mg/mL) and less pronounced against *C. albicans* (MIC = 0.63–1.25 mg/mL) (Tadesse et al. 2008).

Chen et al. (2012a) isolated 15 guaiazulene-based terpenoids (anthogorgienes A–O), as well as eight analogues from the lipophilic extract of the gorgonian *Anthogorgia* sp. After experimental tests using bacteria (*S. aureus*, *Streptococcus pneumoniae*) and fungi (*Aspergillus fumigatus*, *Aspergillus flavus*, *Fusarium oxysporum*), three compounds (1,7-guaiazulenequinone, an aldehydic analogue, and anthogorgiene G) induced moderate growth inhibition. Notably, 1,7-guaiazulenequinone resulted active against *A. fumigatus* (IC_{50} = 13.34 μg/mL), *A. flavus* (IC_{50} = 18.68 μg/mL), *F. oxysporum* (IC_{50} = 15.04 μg/mL), *S. aureus* (IC_{50} = 12.30 μg/mL), and *S. pneumoniae* (IC_{50} = 15.66 μg/mL), the aldehydic analogue against *A. fumigatus* (IC_{50} = 19.50 μg/mL), *A. flavus* (IC_{50} = 37.90 μg/mL), and *F. oxysporum* (IC_{50} = 35.50 μg/mL), and anthogorgiene G selectively inhibited *S. aureus* (IC_{50} = 18.03 μg/mL), and *S. pneumoniae* (IC_{50} = 12.67 μg/mL).

New briarane-type diterpenoids (briacavatolides A–F) and two known briaranes (briaexcavatolide U and briaexcavatin L) have been recently isolated from the acetone extract of *Briareum excavatum* and described by Yeh et al. (2012) and Wang et al. (2012b). Only briacavatolides C and F demonstrated moderate anti-HCMV activity with IC_{50} = 18 μM and ED_{50} = 22 μM, respectively.

Briarane diterpenoids gemmacolides T–Y and the analogs juncenolide J and praelolide isolated from the sea-whip *Dichotella gemmacea* showed weak antimicrobial activity against *E. coli* (inhibition zone: 11.0–34.0 mm diameter) and the fungi *M. violaceum* (inhibition zone: 9.5–15.0 mm diameter) and *S. tritici* (inhibition zone: 9.5–17.0 mm diameter), but were inactive against *B. megaterium* (Li et al. 2012a).

Four steroids (echrebsteroids A–D) extracted from the gorgonian *Echinogorgia rebekka* did not show antibacterial activity, but epimers 2 and 3 showed strong activity against respiratory syncytial virus (RSV) (IC_{50} = 0.19 μM) and epimer 1 furnished IC_{50} value of 0.78 μM (Cao et al. 2014).

Shapo et al. (2007) published the first report of the antimicrobial activity of crude aqueous methanol extract of *Leptogorgia virgulata*. The growth of *Vibrio harveyii* and *Micrococcus luteus* was inhibited by extracts of >0.5 g of tissue, while 2.0 g of tissue were required to inhibit the growth of *E. coli*.

Cultures of marine and non-marine bacteria (*V. harveyi*, *P. aeruginosa*, *S. marcescens*, *S. aureus*, *B. megaterium* and *E. coli*) were used to test the activity of extracts from some Plexauridae (*Plexaura homomalla*, *Pseudoplexaura flagellosa*, *Eunicea clavigera*, *Eunicea tourneforti*, *Plexaurella fusifera*, *Eunicea laciniata* and *Eunicea calyculata*) evaluating the antimicrobial activity measuring the zones of inhibition around disks embedded with extracts. The main antimicrobial activity was exhibited by *P. homomalla* and *P. flagellosa* (Kim 1994). Calyculones A, B, C and H were isolated by Wei et al. (2012) from *Eunicea* sp. and tested for antitubercular activity aganst *M. tuberculosis* which was inhibited (23–43 %) by 6.25 μg/mL of calyculones.

As concerns the hexacorals, Koh (1997) studied the defense mechanisms against marine microbes in 100 scleractinian corals whose extracts were tested against bacteria *Alteromonas rubra*, *Photobacterium damsela*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *V. harveyi*, and *S. aureus* observing a moderate activity against *S. aureus* (1.8 mm of inhibition zone) exerted by 27 corals. A research by Marquis et al. (2005) studied the inhibiting properties of bacterial attachment and growth exerted by eggs from *Acropora elseyi*, *A. tenuis*, *A. hyacinthus*, *A. valida*, *A. cerealis*, *A. millepora*, *Echinopora lamellina*, *Goniastrea favulus*, *Favia pallida*, *Lobophyllia pachysepta* and *Montipora digitata*. Only the eggs of *Montipora digitata* were able to inhibit the growth of *V. harveyii* and *B. subtilis*. On the whole, recent data indicate that stony corals of the Red Sea have scarce antimicrobial activity (Kelman et al. 2006).

Methanol, dichloromethane, ethanol, acetone and butanol extracts from the sea anemones *Stichodactyla mertensii* and *Stichodactyla gigantea* were tested for antimicrobial activity against a battery of bacteria (*S. aureus*, *Salmonella typhi*, *Salmonella paratyphi*, *P. aeruginosa*, *K. oxytoca*, *K. pneumoniae*, *Vibrio cholerae*, *E. coli*, *Proteus mirabilis*) and fungi (*Aspergillus niger*, *Aspergillus fumigatus*, *Botrytis cinerea*, *Cladosporium cucumerinum*, *Penicillium expansum*, *Rhizopus oryzae*, *Trichoderma harzianum*, *Trichoderma koningii*, *Pneumocystis jirovecii*, *Stachybotrys chartarum*). Nine pathogens, mainly *E. coli* and *P. mirabilis* (8.3 mm inhibition), were moderately inhibited by *Stichodactyla mertensii* butanol and acetone extracts, while *P. aeruginosa* (12 mm inhibition) was inhibited by butane extract of *Stichodactyla gigantea*. As concerns fungi, methanol extracts from *S. mertensii* and *S. gigantea* induced 10.3 mm inhibition in *A. niger* and 7.3 mm inhibition in *B. cinerea*, respectively (Thangaraj et al. 2011).

The antimicrobial properties of the anthomedusan *Porpita porpita* were assessed on 20 pathogens (10 bacteria and 10 fungi). The extract was active against *K. pneumoniae* and *A. niger* with 16 mm and 13 mm inhibition zone, respectively (Fredrick and Ravichandran 2010).

Morales-Landa et al. (2007) studied the antimicrobial (against *Streptococcus faecalis*, *B. subtilis*, *M. luteus*, *E. coli*, *K. pneumoniae*, *Serratia marcescens*, and *S. typhi*), and antifungal (against *C. albicans*) activities of crude extracts from jellyfish *Cassiopea xamachana*, *Carybdea marsupialis*, and *Linuche unguiculata*, and sea anemones *Bartholomea annulata*, *Lebrunia danae*, and *Stichodactyla helianthus*; 0.01 mg/mL of extracts produced no damage to bacteria. Otherwise, the extract from *Linuche unguiculata* affected the growth of *C. albicans* (24 mm inhibition zone). Furthermore, in the framework of a research aimed to study the innate response in invertebrates, two fractions (<500 Da and >500 Da) of the extract of the scyphozoan *Aurelia aurita* were studied. The <500 Da fraction was active after 10 h treatment on Gram-positive and Gram-negative bacteria, mainly on *P. aeruginosa* (34.7 % bacterial mortality), *Enterobactor aerogenes* (33.6 %), *S. marcescens* (31.6 %) and *S. aureus* (28.3 %). The fraction >500 Da induced less damage with maximum against *M. luteus* (13.4 %) (Grant et al. 2010). From the mesoglea of *Aurelia aurita* Ovchinnikova et al. (2006) isolated and purified aurelin, an antimicrobial peptide active against both Gram-positive (*Listeria monocytogenes*) and Gram-negative (*E. coli* strain ML-35p) bacteria with MIC values of 22.64 μg/ml and 7.66 μg/ml, respectively. The complete amino acid sequence of aurelin was also determined observing no structural homology with any previously identified antimicrobial peptide. The fractionated n-butanol extracts of the venom of another jellyfish, *Chrysaora quinquecirrha*, showed moderate antimicrobial activity against *S. paratyphi* (8.0 mm) and *A. niger* (9.0 mm) (Suganthi and Bragadeeswaran 2013).

In conclusion, on the basis of what reported above and considering the antibiotic-resistance of bacterial strains, the increased occurrence of nosocomial infections and the consequent increasing request of new antimicrobials for the treatment of infectious diseases, the research about antiviral and antibacterial peptides originating from cnidarians may be in the coming future a valuable support in the antimicrobial therapy or may open new perspectives for the utilization of new and still unexploited sources of drugs.

40.2.3 Anti-parasitic Effects

Sea anemone venoms were seen to be toxic for human intestinal parasites (*Giardia duodenalis*), as verified using the cytolysins sticholysin I (St I) and II (St II) from *Stichodactyla helianthus* and equinatoxin II from *Actinia equina*. All tested

cytolysins showed acute toxicity and IC_{50} values of approximately 10 ng/ml (St I) and 40 ng/ml (St II). A small amount (2 %) of *Giardia duodenalis* were observed to be resistant to the toxins (Tejuca et al. 1999). These results could have in perspective an importance in the therapy of intestinal parasitoses. The antiprotozoan activity of the the extract from the jellyfish *Linuche unguiculata* against *G. lamblia* (IC_{50} = 63.2 μg/mL) was demonstrated by Morales-Landa et al. (2007).

Cnidarian extracts can play a role also as antileishmanial drug candidates: methanolic crude extracts from the octocorals *Carijoa riisei*, *Heterogorgia uatumani* and *Leptogorgia punicea*, the hydroid leptomedusan *Macrorhynchia philippina*, the zoanthid *Palythoa caribaeorum*, the siphonophore *Physalia physalis* and the hexacorals *Aiptasia pallida* and *Zoanthus sociatus* and a modified steroid (18-acetoxipregna-1,4,20-trien-3-one) isolated from *Carijoa riisei* were studied for their activity against microorganisms responsible for leishmaniasis (*Leishmania chagasi*) and Chagas disease (*Trypanosoma cruzi*). *Carijoa riisei* and *Heterogorgia uatumani* extracts furnished the most effective IC_{50} values (2.84 and 4.40 μg/mL, respectively) able to kill 50 % of *L. chagasi* promastigotes. The crude extracts were less effective against *T. cruzi*; the best result was shown by *Macrorhynchia philippina* extract with IC_{50} value of 40.96 μg/mL. Crude extracts from *A. pallida*, *P. caribaeorum*, *P. physalis*, and *Z. sociatus* didn't show antiparasitic activity. A very interesting antileishmania activity was shown by the steroid from *Carijoa riisei* against promastigotes (IC_{50} = 5.5 μg/mL) and intracellular amastigotes (16.88 μg/mL), while the antitrypanosomal activity was scarce (IC_{50} = 50.5 μg/mL) (Reimão et al. 2008).

The diterpenoid cristaxenicin A was isolated by Ishigami et al. (2012) from the deep water gorgonian *Acanthoprimnoa cristata*. This compound showed antiparasitic activity at IC_{50}s of 0.088 and 0.25 μM against *Leishmania amazonensis* and *Trypanosoma congolense*, respectively but had only moderate antimalarial activity against *Plasmodium falciparum* (IC_{50} = 11 μM). Calyculones A, B, C and H from *Eunicea* sp. were tested for antimalarial activity resulting in activity on *P. falciparum* with IC_{50}s values ranging from 5 to 11 μg/mL (Wei et al. 2012).

40.2.4 Antitumor Activity

Starting from the 1990s, several cnidarian-derived antitumor compounds, mainly from corals, have been discovered and described (Mayer and Gustafson 2004, 2006, 2008). Subsequenly, the evaluations of these properties on cell cultures grew up, making possible the publication of recent extensive reviews (Mariottini and Pane 2013, 2014).

During the 1980s, pseudopterolide was isolated from the sea plume *Pseudopterogorgia acerosa*. It was seen to inhibit cell division of fertilized sea urchin eggs at 8 μg/mL (ED_{50} value), but was inactive on nuclear division (Fenical 1987). Subsequently, pseudopterosins (G, P, Q, S, T, U) and seco-pseudopterosins (J, K) from *Pseudopterogorgia elisabethae* were tested for cytotoxicity against four human tumor cell lines, HeLa (cervical adenocarcinoma), PC-3 (prostate adenocarcinoma), HCT 116 (colorectal carcinoma), and MCF7 (breast adenocarcinoma) as well as against normal fibroblasts (BJ). Pseudopterosin Q showed the best activity (GI_{50} ranging from 4.47 to 8.44 μM against BJ and MCF7, respectively) (Correa et al. 2011). Several semi-synthetic derivatived of compounds from *Pseudopterogorgia elisabethae* induced moderate to scarce growth inhibition in Vero cells (IC_{50} values ranging from 12 to 102 μg/mL) (McCulloch et al. 2012).

The methanol extract of Australian collections of *Sinularia* sp. yielded a diterpene and some lobanes and cembranes. Seven of them were tested for cytotoxicity against SF-268 (glioblastoma), MCF7, and H460 (Lung-large cell carcinoma) tumor cells. Excepting the diterpene, all compounds showed moderate cytotoxicity with GI_{50} values ranging from 6.8 to 18.5 μM (Wright et al. 2012). The norcembranoid diterpenes 5-episinuleptolide acetate and scabrolide D from *Sinularia* sp. were assessed for cytotoxicity against human cancer cells K562 (myelogenous leukemia), MOLT-4 (acute lymphoblastic leukemia), DLD-1 (colorectal adenocarcinoma), T-47D (breast ductal carcinoma), and MDA-MB-231 (breast adenocarcinoma). 5-episinuleptolide acetate resulted cytotoxic with IC_{50} values of 0.67, 0.59, 4.09, 0.92, 3.09, and 2.95 μg/mL, respectively, while scabrolide D was not effective when tested at 20 μg/mL for 72 h (Yen et al. 2012). Six steroids extracted from *Sinularia crassa* were tested on five cancer cell lines (HepG2, HepG3, MCF7, MDA-MB-231, and lung carcinoma A-549). Crassarosterol A and crassarosteroside C resulted cytotoxic to hepatocellular carcinoma HepG2 (IC_{50} = 14.9 μM), and to HepG2 and HepG3 (IC_{50}s = 17.6 and 18.9 μM, respectively) after 72 h of treatment (Chao et al. 2012). Another compound (crassalone A) from *Sinularia crassa* was tested on human HL60 (promyelocytic leukemia), MDA-MB-231, HCT-116 and DLD-1 cancer cells but 20 μg/mL did not affect significantly cell viability (IC_{50}s >20 μg/mL) after 72 h exposition (Cheng et al. 2012). The dose-dependent activity on cell growth and migration of the diterpenoid sinulariolide from *Sinularia flexibilis* was demonstrated on TSGH bladder carcinoma cells. Mitochondrial-mediated apoptosis via caspase-dependent pathways was induced (Neoh et al. 2012). Chen et al. (2012b) reported the reduction of the 6-hydroxydopamine-induced cytotoxicity and apoptosis on human neuroblastoma cells (SH-SY5Y) by 11-dehydrosinulariolide from *Sinularia flexibilis* and a neuroprotective effect was supposed (Chen et al. 2012b). The steroids 8αH-3β,11-dihydroxy-5α,6α-expoxy-24-methylene-9,11-secocholestan-9-one, and 3β,11-dihydroxy-

5β,6β-expoxy-24-methylene-9,11-secocholestan-9-one extracted from *Sinularia granosa* induced cytotoxicity to MCF7 (ED_{50} = 4.99 and 9.98 μg/mL, respectively), and cerebellar medulloblastoma Daoy (ED_{50} = 5.53 and 7.07 μg/mL, respectively) cells. The first steroid was cytotoxic also to HeLa (ED_{50} = 8.21 μg/mL) and Hep (ED_{50} = 6.21 μg/mL) cells (Huang et al. 2012a). Three diterpenoids (gyrosanol A, B, and C) extracted by Cheng et al. (2010) from *Sinularia gyrosa* were studied for cytotoxicity in a preliminary screening; lack of cytotoxicity against HT-29 (human colon adenocarcinoma), P-388 (mouse lymphocytic leukemia), and A-549 cancer cells was emphasized. The glycosides sinularosides A and B isolated from *Sinularia humilis* and already reported above, did not exhibit cytotoxicity against Caco-2 (human epithelial colorectal adenocarcinoma) and HepG2 cancer cells (Sun et al. 2012). The dose-dependent decrease of proliferation and migration induced by 11-dehydrosinulariolide isolated from *Sinularia leptoclados* was observed in A2058 melanoma cells at 2–8 μg/mL. This compound induced also mitochondrial- and endoplasmic reticulum-mediated apoptosis through caspase-dependent and partially caspase-independent pathways (Su et al. 2012a). Pavidolides A–E were extracted from *Sinularia pavida*. Two of these compounds (pavidolides B and C) inhibited the growth of human leukemic cells HL-60 with IC_{50}s of 2.7 and 5.3 μg/mL, respectively (Shen et al. 2012). Two terpenoids isolated from *Sinularia polydactyla* were found to be cytotoxic to tumor cells. Notably, durumolide C was active against HepG2 cells (IC_{50} = 1.0 μg/mL), and 24-methylcholestane-3β,5α,6β,25-tetrol 25-monoacetate against Hep2 and HCT cells (IC_{50}s = 6.1 and 8.2 μg/mL, respectively) (Aboutabl et al. 2013). The sesquiterpene scabralin A isolated from *Sinularia scabra* was studied for cytotoxicity against WiDr (colon carcinoma), HEp 2 (epithelial carcinoma), MCF7, and Daoy cells and was found to be moderately/weakly cytotoxic (ED_{50}s = 10.7, 13.8, 9.6, and 7.6 μg/mL, respectively) (Su et al. 2012b).

The cembranoids (+)-12-ethoxycarbonyl-11Z-sarcophine, ehrenbergol A and B from *Sarcophyton ehrenbergi*, already mentioned above, showed in vitro cytotoxicity towards P-388 cells with ED_{50} values of 5.8, 7.4, and 4.7 μg/mL, respectively, while less activity had towards A549 cells, and practically no influence had on HT-29 cells, as well as on human embryonic lung (HEL) cells (Wang et al. 2012a). Sarcophytolide from *Sarcophyton glaucum* exhibited cytostatic activity. The ED_{50} determined on L5178y mouse lymphoma cells was 3.4 μg/mL (Badria et al. 1998). The semi-synthetic derivative sarcodiol from *Sarcophyton glaucum* was observed to affect cell viability and growth of B16F10 mouse melanoma cells, inhibiting DNA synthesis, inducing DNA fragmentation, and enhancing p53 and caspase levels in treated cells. Cell division blockade in G0 quiescent phase and apoptosis were also observed (Szymanski et al. 2012). The cembranoids sarcocrassocolides M-O isolated from *Sarcophyton crassocaule* induced moderate cytotoxicity against Daoy (ED_{50} = 5.0–6.6 μM), HEp-2 (ED_{50} = 10.4–12.4 μM), MCF7 (ED_{50} = 6.4–10.6 μM) cells, and scarce effects on WiDr (ED_{50} = 30.1–>40 μM) cells (Lin et al. 2012).

The CH_2Cl_2 extract of *Lobophytum michaelae* yielded seven cembranolides (michaolides L-Q and lobomichaolide) which, excepting Michaolide O, resulted all strongly cytotoxic to A549, HT-29, P-388 tumor cell lines and to HEL cells with ED_{50} values ranging from 0.3 to 4.9 μM/mL (Wang and Duh 2012). A cembrenolide diterpene (LS-1) from *Lobophytum* sp. inhibited the growth of HT-29 cells and induced apoptosis through generation of ROS and damage of mitochondria. The damage was seen to be blocked by the ROS inhibitor N-acetylcysteine (Hong et al. 2012a). Four diterpenes, cyclolobatriene, lobatriene, eunicol, and fuscol from *Lobophytum pauciflorum* induced cytotoxicity to A431 (human epidermoid carcinoma) cells with IC_{50} values ranging from 0.35 to 0.64 μM (Govindam et al. 2012).

Five eunicellin diterpenes, pachycladins A–E, and other known compounds were isolated from *Cladiella pachyclados* by Hassan et al. (2010). Two pachycladins (A and D) showed strong antimigratory activity against PC-3 cells.

Microtubules, polymers playing a fundamental role in several cell functions and in mytosis, can be damaged by specific agents which result in alteration of the mytotic process with cell cycle arrest and consequent apoptosis (Mollinedo and Gajate 2003). Sarcodictyins A–D, diterpenoids extracted from *Sarcodictyon roseum*, seem to have such properties and could be candidates to act as antiproliferative agents in cancer therapy (Haefner 2003). The same activity was reported for eleutherobin from *Eleutherobia* sp. (Nobili et al. 2009).

During extensive research on some species of *Nephthea* Cheng et al. (2009a) studied five compounds among which an oxygenated ergostanoid having molecular formula $C_{28}H_{46}O_3$ resulted cytotoxic to P-388 cells with ED_{50} of 3.7 μg/mL, while HT-29 cells did not suffer cytotoxicity. A research on other seven compounds (elongatols A–G) from *Nephthea elongata* showed that Elongatol A and Elongatol E affected survival and growth of P-388 cells (ED_{50}s = 3.8 and 3.6 μg/ml, respectively) (Wang and Duh 2007). Other diterpenoids (pacificins K–Q) from *Nephthea elongata* were tested for cytotoxicity; two of them (pacificins K and L) resulted cytotoxic to P-388 cells (ED_{50}s = 3.2 and 2.6 μg/ml, respectively) (El-Gamal et al. 2007). Wang et al. (2012c) studied the acetone extract of *Nephthea chabrolii* and demonstrated that three isolated compounds (nebrosteroids N–P) are cytotoxic to P-388 cells resulting in ED_{50} values of 0.9, 1.2, and 1.7 μg/mL, respectively, while less effect were observed on A549 (ED_{50} = 5.9–7.2 μg/mL), HT-29 (ED_{50} = 5.9–9.5 μg/mL), and HEL (ED_{50} = 15.4–23.5 μg/mL) cells.

The evaluation of the cytotoxicity of four sesquiterpenoids (parathyrsoidins A–D) isolated from *Paralemnalia thyrsoides* to cancer cells showed that P-388 leukemia suffered moderate cytotoxicity (ED_{50}s = 2.32–13.2 μM), while HT-29 and A-549 cells did not suffer damage (Tseng et al. 2013). From another Nephtheidae, *Litophyton viridis*, Iguchi et al. (1989) isolated 5,6-epoxylitosterol, a 19-oxygenated sterol which showed antileukemic activity in vitro against P-388 cells with IC_{50} of 0.5 μg/mL.

A briareolate ester diterpenoid isolated from the methanolic extract of *Briareum asbestinum* was able to inhibit the growth of human embryonic stem cells (BG02) at EC_{50} = 40 μM (Meginley et al 2012). Briacavatolides A–F, briaexcavatolide U, and briaexcavatin L from *Briareum excavatum* were not cytotoxic to P-388, HT-29, A-549, and HEL cells (Yeh et al. 2012; Wang et al. 2012b).

Ethanol extracts of *Subergorgia suberosa* yielded six sterols whose cytotoxicity was assessed against Bel7402 (hepatocellular carcinoma), MCF7, Hep G2, and HeLa cells. Three steroids resulted moderately active mainly against MCF7 and HeLa, with IC_{50} values of 3.57–13.75 μg/mL. Bel7402 resulted scarcely sensitive to all compounds (Liao et al. 2012).

Roy et al. (2012) isolated six diterpenoids from *Cespitularia* sp.; one of them, alcyonolide, was cytotoxic to HCT 116 cancer cells with IC_{50} of 5.85 μM. Other compounds resulted in IC_{50}s ranging from 28.18 to 91.35 μM.

Echrebsteroid A and D from sea whip *Echinogorgia rebekka* induced cytotoxicity to K562 cells resulting in IC_{50}s of 0.85 and 4.19 μM, respectively. Echrebsteroid B resulted cytotoxic to HL-60 and K562 cells (IC_{50}s of 8.99 and 6.36 μM, respectively) (Cao et al. 2014).

Some briarane diterpenoids and analogs (gemmacolides T–Y, juncenolide J, praelolide, and junceellolide C) were isolated by Li et al. (2012a) from *Dichotella gemmacea*. Excluding junceellolide C all compounds induced cytotoxicity to MG63 (human osteosarcoma) and A549 cells. Notably, gemmacolides V and Y strongly inhibited the growth of A549 cells (IC_{50}s <1.5 and <0.3 μM, respectively) and gemmacolide Y was active in inhibiting the growth of MG63 (IC_{50} <0.3 μM) with stronger activity than adriamycin.

The diterpenoid calyculone A isolated from the sea-whip *Eunicea* sp. was screened for cytotoxicity to 60 human tumor cell lines. Three of them, SR (leukemia), UACC-62 (melanoma), and RXF 393 (renal cancer) resulted sensitive with GI_{50} values of 1.94, 1.88, and 2.94 μM, respectively (Wei et al. 2012).

Echinohalimane A from *Echinomuricea* sp. exhibited cytotoxicity particularly toward LoVo (human colorectal adenocarcinoma), MOLT-4, HL-60, and DLD-1 cells with IC_{50} values of 0.6, 2.1, 2.1, and 1.0 μg/mL, respectively. Less effect had against the leukemia cell line K562 (IC_{50} = 6.3 μg/mL), and was not cytotoxic to DU-145 (human prostate carcinoma) cells at 20 μg/mL (Chung et al. 2012b).

Rumphellaone A, extracted from the gorgonian *Rumphella antipathies* showed moderate cytotoxicity, with IC_{50} value of 12.6 μg/mL, against human T-cell acute lymphoblastic leukemia cells (CCRF-CEM) (Chung et al. 2010).

The tetraprenylated alkaloids nuttingins A–F and malonganenones A–H were isolated from the sea-whip *Euplexaura nuttingi* and tested for cytotoxicity against human leukemic cell lines K562 and UT7. Nuttingins A–E and malonganenones D–G were tested on cultured cells for 24 and 48 h. K562 cells were exposed to concentrations ranging from 0.04 to 1 μg/mL, while the more sensitive UT7 cell line was exposed to concentrations ranging from 0.0004 to 0.4 μg/mL. All tested compounds induced dose- and time-dependent decrease of cell growth. In particular, UT7 and K562 cells exposed for 48 h to 0.4 μg/mL of nuttingins C, D and E showed approximately 50 % and 30 % inhibition. Furthermore, 1.25 μg/mL of nuttingins A–E and of malonganenones D–H induced apoptosis (Sorek et al. 2007).

Palyosulfonoceramides A and B extracted from the Brazilian zoanthids *Palythoa caribaeorum* and *Protopalythoa variabilis* were studied for cytotoxicity against HCT-116 cells and were observed to be inactive at maximum concentration of 50 μg/mL (Almeida et al. 2012).

As concerns stony corals, three diacetylene compounds isolated from the methanolic extract of *Montipora* sp. were tested on A549 (lung), SK-OV-3 (ovarian), SK-MEL-2 (skin), XF498 (CNS), and HCT15 (colon) human cancer cells. One of these compounds, having β-hydroxy ketone functionality, showed activity resulting in ED_{50} values ranging from 3.85 μg/mL on SK-MEL-2 to 7.24 μg/mL on XF498 (Alam et al. 2002).

Some extracts from jellyfish have been supposed to play a role, in perspective, against cancer. The experimental induction of CNS tumors in rats by administration of ethylnitrosourea was seen to decrease (27 %) when animals were treated with subtoxic doses of venom from the jellyfish *Cassiopea xamachana*. The antitumor effects were supposed to be due to the cytolytic and cytostatic properties of nematocyst venom (Orduña-Novoa et al. 2003). Concentrations of 2, 4 and 8 μg/mL of a 9 kD MW peptide isolated from *Chrysaora quinquecirrha* were evaluated at 12, 24 and 36 h for apoptosis-inducing in HeLa and HEp2 tumor cells. Dose- and time-dependent cytotoxicity, apoptosis, caspase activation, down regulation of Bcl-2 antibody and DNA fragmentation were recorded. 4 μg/mL of venom peptide induced strong growth inhibition of both cell lines due to apoptosis induction (Balamurugan et al. 2009). In vivo experiments showed that injection of venom peptide improved the survival of mice bearing Ehrlich ascite carcinoma as well as the hematological and antioxidant parameters (Balamurugan et al. 2010). Recently the immunomodulatory role of extracts of *Chrysaora quinquecirrha* was emphasized after the observation of the

immunostimulating effect (25–40 %) at concentration up to 1000 μg and immunosuppressive effects at 20 μg (Suganthi and Bragadeeswaran 2013). The crude venom from the mauve-stinger *Pelagia noctiluca* was assessed for cytotoxicity against human SH-SY5Y neuroblastoma cells at doses 0.05–0.5 μg/mL, resulting in 29.4 % dose- and time-dependent cell viability decrease, ROS production, and mitochondrial damage (Morabito et al. 2012).

It's well known that cancer has close relationship with tissue inflammation. In this connection, considering that the deregulation of Nuclear Factor κB (NF-κB) activation is known to be associated to cancer and inflammation, and NF-κB activation is known to be a factor increasing the proliferation of cancer cells (De Simone et al. 2014), the inhibitory effect of NF-κB activation by extracts from the soft corals *Sarcophyton* sp. and *Sinularia* sp., and from the gorgonian *Subergorgia* sp. was tested on K562 human leukaemic cells. The extracts were demonstrated to inhibit NF-κB (Folmer et al. 2009a). Therefore, compounds able to inhibit NF-κB activation could have employment, in perspective, in cancer therapy (Blunt et al. 2014).

Furthermore, increased levels of COX-2 are commonly found in a variety of malignant tumors, such as colorectal cancers, gastric cancers, hepatocellular carcinomas, and non-small-cell lung cancers (Dannenberg et al. 2001). On the whole, COX-2 is overexpressed in several tumors (Greenhough et al. 2009; Maeng et al. 2014). On this basis, the role in cancer therapy of the above reported compounds acting on COX-2 can be supposed.

In addition, considering that the production of abnormal gap junction intercellular communications (GJIC) or their disfunctions seems to be a pre-eminent aspect in carcinogenesis, causing disregulation of growth control and apoptotic processes (Leone et al. 2013) and that chemopreventive compounds, such as natural compounds and anticancer drugs, seem to be able to promote GJIC up-regulation (see Leone et al. 2013 and references herein), the development of compounds having up-regulating properties based on cnidarian venoms could be a way to find new approaches in cancer therapy. Furthermore, the role of ROS-inducing compounds and the consequent induction of apoptosis by some compounds, such as LS-1 from *Lobophytum* sp. (Hong et al. 2012a) can be supposed a practicable way for the treatment of sensitive tumor cells.

40.2.5 Anticoagulant Effects

The research on anticoagulant compounds is very important in the pharmacological research and in drug discovery, due to the high incidence of thrombotic phenomena in the modern societies, leading to high mortality events. Natural compounds having anticoagulant properties and platelet aggregation inhibitors have been recognized in snake venoms or produced and designed on the basis of such compounds (O'Shea and Tcheng 2002) but, unfortunately, anticoagulants from marine sources have been rarely isolated.

It's well known that fibrinogen-related proteins play an essential role in blood coagulation of vertebrates after conversion of fibrinogen into fibrin. Otherwise, invertebrate fibrinogen-related proteins have been supposed to have different functions. Recent results show that invertebrate genomes have high numbers of fibrinogen-like loci and several molecules containing fibrinogen seem to be linked to the defense response against pathogens (Hanington and Zhang 2011).

The first demonstration of anticoagulant activity in cnidarian extracts was reported after the recognition of a non-dialyzable anticoagulant factor in the sea anemone *Rhodactis howesii* (Martin 1966). The anticoagulant factor was observed to be resistant to extreme pH values (2.0 and 10.5) as well as to heating and to treatment with acetone and chloroform and demonstrated more efficiency than heparin in prolonging the coagulation time.

A recent paper reported the anticoagulant activity of extracts from moon jellyfish (*Aurelia aurita*) tentacles. Tentacle extracts incubated with bovine fibrinogen were able to digest the Aα and Bβ chains of the fibrinogen molecule within 3 h, as well as to completely liquefy fibrin clots after 24 h (Rastogi et al. 2012). The fibrinogenolytic activity of *Aurelia aurita* venom was found to be stronger than that of some anticoagulants of snake venoms. High molecular weight fractions have been indicated to be the main responsible of fibrinogenolysis. On the basis of the observed rapid auto-degradation of proteins and considering the different activity of: (I) a metalloprotease inhibitor (EDTA) which delayed the digestion of Bβ but not Aα chain; (II) a serine protease inhibitor (PMSF) which significantly inhibited the digestion of both chains and (III) heating which totally abolished the digestion, the Authors suggested that small peptides resulting from protein degradation retained the fibrinogenolytic activity and that different fibrinogenolytic toxins, such as serine proteases or metalloproteases could occur in the nematocyst extract. Dose-dependent but unlinear inhibition of ADP-dependent platelet aggregation was also observed after treatment with *Aurelia aurita* extract (Rastogi et al. 2012).

Another study detected the fibrinolytic activity of *Aurelia aurita* venom as well as that of other jellyfish (*Nemopilema nomurai*, *Rhopilema esculenta*, *Cyanea nozakii*) nematocyst extracts. The highest activity against fibrin was showed by *C. nozakii* venom (Lee et al. 2011).

40.2.6 Other Activities

Other possible employment of cnidarian extracts have been reported in the literature. Some compounds were demonstrated to be antioxidant (H_2O_2 scavenging) with possible

implications in the immune response (Palmer et al. 2009). The cembranoid yalongene A isolated from *Sarcophyton trocheliophorum* showed cytoprotective effects at 1 μM in H_2O_2-injured SH-SY5Y (human neuroblastoma) cells (Yao et al. 2012). Briarenolide E, a 2-ketobriarane diterpenoid extracted from *Briareum* sp., was found to induce weak inhibition of superoxide anion generation (23.7 %) and elastase release (28.3 %) after treatment of human neutrophils with 10 μg/mL (Hong et al. 2012b). Other studies on fractionated organic extract from *Briareum* sp. showed that 10 μg/mL produced 36.8 % inhibition of superoxide anion generation and 90.3 % inhibition of elastase release. Briarenolide F (diterpenoid) was inhibitory at 10 μg/mL (IC_{50} = 3.82 μg/mL; 76.6 % inhibition) on superoxide anion generation (Hong et al. 2012c). Furthermore, 6-epi-Yonarasterol B from *Echinomuricea* sp. inhibited the generation of superoxide anions (IC_{50} = 2.98 μg/mL; 89.8 % inhibition) and elastase release (IC_{50} = 1.13 μg/mL; 95.5 % inhibition) (Chung et al. 2012a). The protein SmP90 provided with strong superoxide anion radical-scavenging activity (EC_{50} expressed as half-scavenging concentration ≈ 16 μg/mL) was recently isolated from nematocyst venom of *Stomolophus meleagris* and suggested for the production of antioxidants (Li et al. 2012).

Cnidarian products have been shown to have analgesic activity. The analgesic activity in vivo of pseudopterosin E and pseudopterosin A from *Pseudopterogorgia elisabethae* in mouse models (ED_{50}s of 14 mg/kg i.p. and 32 mg/kg s.c., respectively) was demonstrated (Mayer et al. 1998). The crude hydroalcoholic extract of *Palythoa caribaeorum* exhibited analgesic activity at 200 mg/kg in writhing test in mice (Soares et al. 2006). Methanol and aqueous crude extracts from the sea anemones *Stichodactyla mertensii* and *Stichodactyla gigantea* showed moderate analgesic effects on mice (Thangaraj and Bragadeeswaran 2012).

Sarcophytolide, a diterpene isolated from the alcohol extract of *Sarcophyton glaucum*, was found to be neuroprotective at concentrations 1 μg/mL (44 % viability) and 10 μg/mL (92 % viability) in rat embryo primary cortical cells exposed to glutamate, and to suppress the increase of intracellular Ca^{++} levels after 30-min preincubation of neuronal cells. The neuroprotection provided by sarcophytolide was suggested to be partially the result of bcl-2 (proto-oncogene) overexpression and the utilization of this compound in the therapy of neurodegenerative diseases was hypothesized (Badria et al. 1998). The above reported anti-inflammatory properties of 11-dehydrosinulariolide from *Sinularia flexibilis* which upregulates iNOS and COX-2, have been suggested to have a possible utilization in the therapy of neurodegenerative diseases such as Parkinson's disease. The neuroprotection was stated to be mediated by phosphatidylinositol 3-kinase signaling (Chen et al. 2012b).

Pseudopterosin has been indicated to be active as cicatrizant (Gerwick and Moore 2012) and to be useful in the treatment of contact dermatitis being commercially used in the formulation of skin creams (Coleman and Kerr 2000). Pseudopterosin A was also found to enhance (25 %) the proliferation of endothelial cells ($EC_{50} = 1.34 \times 10^{-8}$ M) useful in conditions of required angiogenesis, such as in ischemia and in wound healing (Day et al. 2013).

At last, the nutritional properties of some cnidarians should be considered (De Zoysa 2012; Armani et al. 2013), taking into account the high concentration of essential elements (Hsieh et al. 1996) and the low content of protein and cholesterol (Hsieh et al. 2001; Hsieh and Rudloe 1994).

40.3 Conclusions

In conclusion, considering that a lot of the best selling drugs derive from natural compounds or were developed starting from natural sources, to view the sea as a still largely unexploited source of useful compounds is a challenge for the modern pharmacology. As a matter of fact, the extracts from marine organisms can be supposed to play a fundamental role in the future of drug discovery taking into account the present difficulties to develop new tools to fight the pressing problems consequent to the antibiotic resistance, the production of new chemotherapeutics, and the increased incidence of diseases linked to life conditions, environmental contamination, increase of life expectancy, such as cancer, inflammation and degenerative diseases. Hence, the marine environment can be considered as an exceptional reservoir of bioactive products having unique characteristics, often different from that found in the terrestrial environment, which wait for being discovered and utilized.

At present, after more than 40 years of research, the literature concerning the cytotoxic properties of cnidarian extracts is enormous and several mechanisms of cytotoxicity, such as the activity on cell growth, the interference with cell metabolism, the inhibition of DNA synthesis, the blockade of cell cycle, the induction of apoptosis have been emphasized in normal and transformed cells and could be useful tools against malignancies. Even though the cytotoxic activity of cnidarian extracts was shown to be effective mainly against some defined tumor cells, e.g. leukemic, colorectal, breast adenocarcinomas, further research are needed to improve the knowledge about their effectiveness also on other transformed cells.

From the anti-infective point of view, several cnidarian extracts have demonstrated their effectiveness against viruses, bacteria and parasites; the improvement of the chemotherapeutic activity of existing drugs already reported in other reviews could be an important tool for the reduction of unpleasant effects of some therapies. The management of the inflammation which occurs in several infectious, neoplastic, neurologic, and in other diseases could be also a therapeutic

opportunity for some cnidarian extracts which exhibited their usefulness as anti-inflammatory agents. At last the nutritional properties of cnidarians, already experimented as food in Asian countries and also in other regions are to be considered carefully in order to use them as low-caloric, low-fat and cholesterol-free food able to prevent and to treat food-related diseases such as cardiovascular, diabetes, hypercholesterolaemia, etc.

The main perspective of this research is supposed to be the increase of studies about coastal benthic cnidarians (coral reefs, sea-anemones), which are logically more available owing to their life habits that facilitate a relatively unexpensive and easy collection, but equally very important will be the development of research about extracts from pelagic cnidarians which to date are still an almost unknown and unexploited source of bioactive compounds within that natural pharmacy which the oceans can be considered.

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How Venom from the Magnificent Sea Anemone, *Heteractis magnifica*, Kills Breast and Lung Cancer Cells

41

Barbara J.S. Sanderson, Karen Burke Da Silva, and Mahnaz Ramezanpour

Abstract

The sea anemone *Heteractis magnifica*, also known as the "magnificent one", is a friendly host to anemonefish. However, it has venom which acts as a chemical defence against predators that helps it to acquire prey in the marine environment. *Heteractis magnifica* produces venom with multiple biological activities. We have shown killing activity against human lung and breast cancer cells that was concentration-dependent (5–40 μg/ml). The mechanism of cancer cell killing by the venom was also uncovered. Apoptosis (programmed cell death) resistance is a hallmark of cancer. *Heteractis magnifica* venom induces apoptosis in human lung and breast cancer cell lines. Apoptosis occurs via two pathways and the investigation of these pathways has been discovered. Another hallmark of cancer is cell cycle deregulation, and *H. magnifica* venom was shown to modulate cell cycle progression in the cancer cell lines mentioned above. Thus the types and levels of breast and lung cancer cell killing by venom from the "Magnificent" sea anemone is explored as well as the mechanistic pathways underlying those effects.

Keywords

Heteractis magnifica • Anti-cancer activity • Venom extract • Breast cancer cells • Lung cancer cells • Apoptosis induction • Cell cycle deregulation

41.1 Introduction

Scientists, clinicians and other health care professionals have waged war on cancer for over 40 years since President Nixon and the US congress coined the phrase and war was declared in 1971. This has resulted in the development of surgery, radiation therapy and single and multiple chemotherapeutic agents for use as treatment regimens (Hanahan 2014). The chemotherapeutic agents developed during this time often miss the mark however, in that they are not always efficacious enough. At the doses at which they kill the cancer cells, they bring with them many side effects. This chapter will explore the potential of a marine source of anticancer agents, specifically venom from the sea anemone, *Heteractis magnifica*. Venom from this anemone has been shown to be a novel source of bioactive agents with activity that significantly impacts against breast cancer and lung cancer cells grown in an in vitro model in the laboratory.

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41.2 Cancer

41.2.1 Origin of Cancer

Cancer is an acquired disease, which may or may not have inherited risk factors. It develops from a damaged progenitor (starting) cell as part of carcinogenesis. Carcinogenesis is a multistep process of normal cells evolving gradually to malignant cells with the accumulation of multiple genetic alterations. These alterations frequently include mutations that alter specific regions of our genetic code (Beckmann

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et al. 1997; Vogelstein and Kinzler 1993; Sugimura 1992). For example turning on of Proto-oncogenes promotes tumor growth. In contrast, activation of tumor-suppressor genes results in stopping tumor growth (Barrett and Wiseman 1987) and if sea anemone venom can be found to interact with DNA we are likely to be able to arrest tumour growth. However, with the process of carcinogenesis, it is predicted that three to six genetic alterations would be required as part of that process (Vogelstein and Kinzler 1993). The process can be nominally be separated into three stages, which are termed initiation, promotion, and progression (Barrett and Wiseman 1987). Vogelstein and Kinzler (1993) demonstrated that genetic alterations are involved in each of the three stages of carcinogenesis (Vogelstein and Kinzler 1993). The first stage, initiation, is generally rapid and involves the development of altered cells, which is usually caused by an irreversible mutation (DNA damage). The second stage, promotion, may involve a latency period of years and involves the expansion of altered cells into a tumour, which can be benign (pre-cancerous) or cancerous. Genetic alterations that promote the proliferation of abnormal cells, via clonal expansion, can lead to a pre-cancerous lesion. The third stage is the progression of these cells to malignant cancer cells, by undergoing a range of different heritable changes. The stage of progression includes angiogenesis and the development of tumor heterogeneity (Barrett and Wiseman 1987). Therefore, development of therapeutic strategies that can target genetic alterations and the tumor cell characteristics acquired during carcinogenesis, including the hallmarks of cancer are highly sought after.

41.2.2 Hallmarks of Cancer

Cancer cells can acquire up to ten characteristics that enable carcinogenesis to progress or persist (shown in Fig. 41.1).

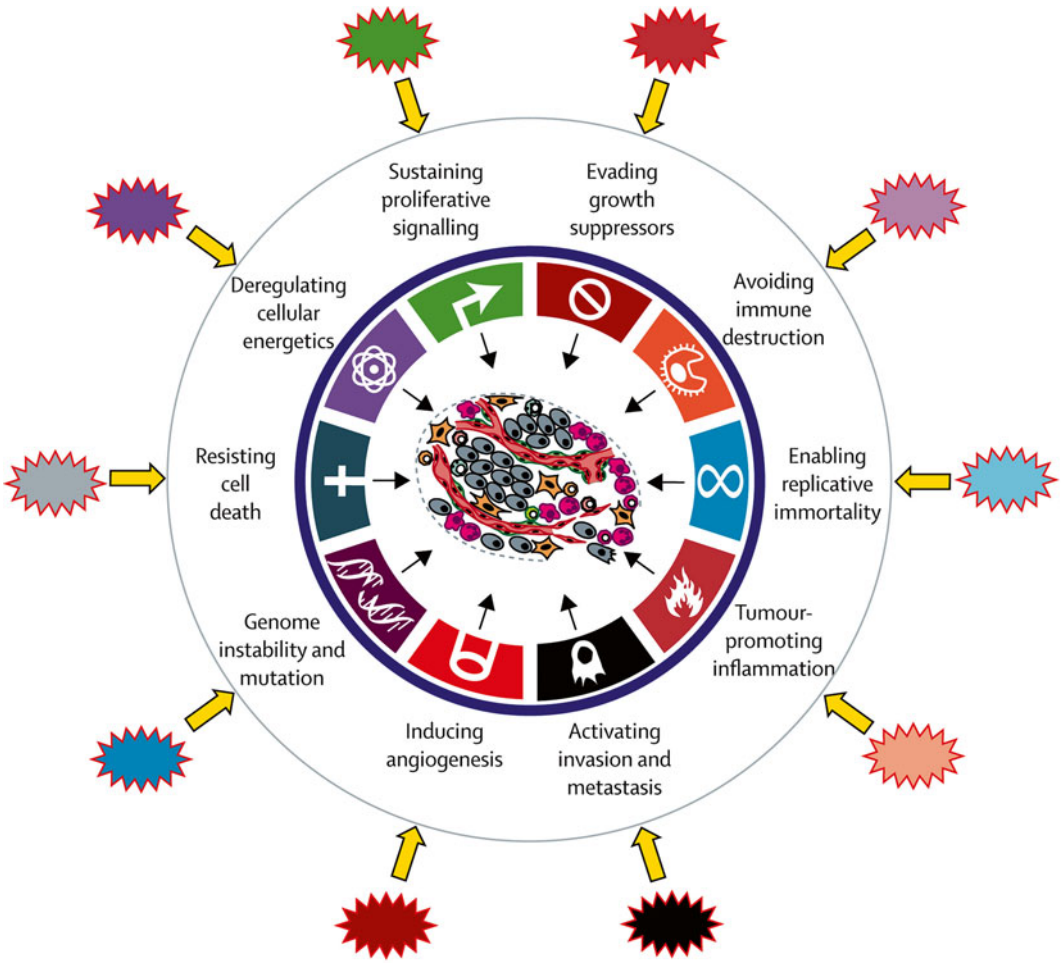

Fig. 41.1 Cancers acquire ten damaging functional capabilities and facilitators (shown in *white ring* and indicated by *symbols* in *coloured ring*). These collectively manifest their effects via successful attacks on the affected individual. Each capability can be counteracted by various mechanism-targeted treatments (shown figuratively as explosion shapes). These generally do not act as curative magic bullets because cells develop advanced strategies of resistance (Hanahan 2014)

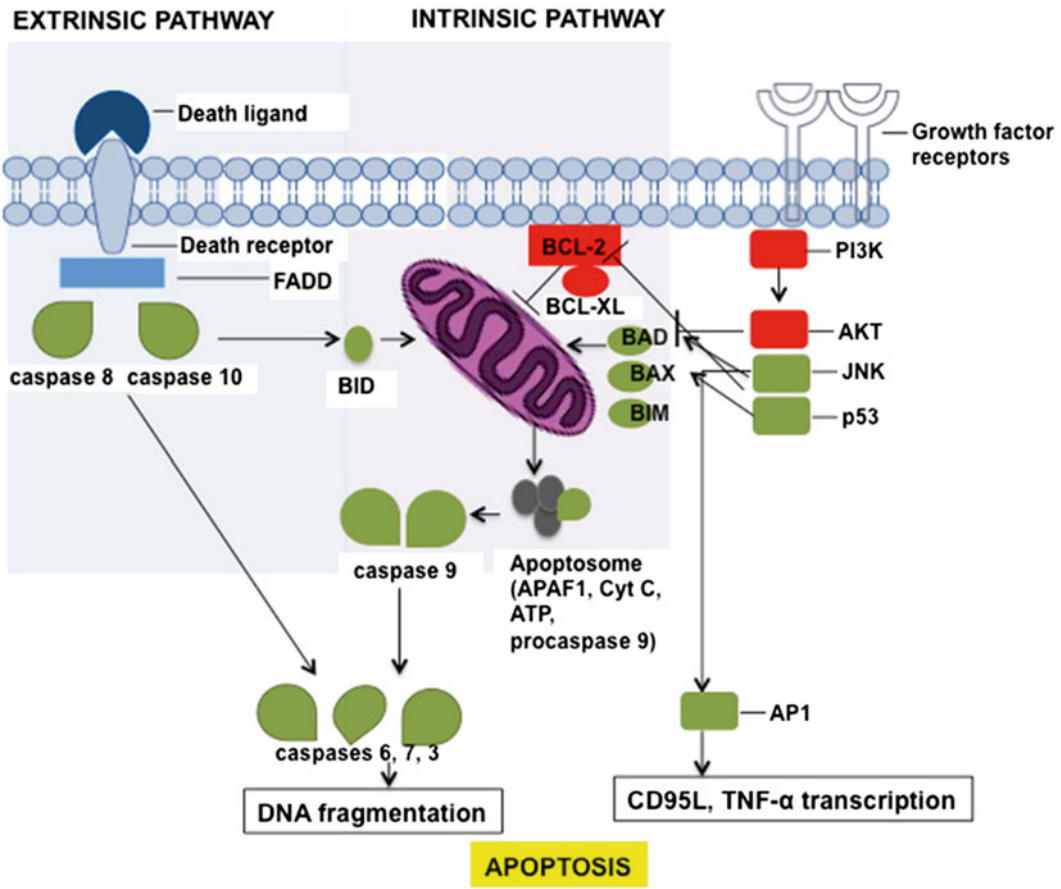

Fig. 41.2 A schematic representation of the main molecular pathways leading to apoptosis and their main regulators. Pro-apoptotic regulators are in *green* and anti-apoptotic in *red*. The intrinsic pathway and extrinsic pathway for apoptosis are shown (Giussani et al. 2014). The *purple* boat shaped organelle represents mitochondria which are influenced by intracellular stress and the status of which is monitored as an endpoint to indicate apoptosis induction. The named Caspases (enzymes), which change levels when apoptosis is induced, are also monitored as indicators of apoptosis induction

These are acquired step by step during the process of carcinogenesis (as described above) such that the symptoms are observed progressively. An example of this would be where a change results in the development of a primary tumour site through to invasion of blood vessels followed by additional symptoms and the development of secondary tumours. Alternatively the development of the cancer cells may be in a timeframe which is so rapid that they collectively manifest their symptoms together as an aggressive cancer via successful attacks of multiple capabilities on the affected individual. Each functional capability that has been recognised is important not only because it helps us understand the hallmarks of cancer and the capabilities and characteristics of cancer cells, but because it provides a target for therapeutic attack. Each uncovered capability can be counteracted by various treatments targeted at the mechanism of action (shown in Fig. 41.1 as the star shaped explosion shapes). As observed in Fig. 41.1, there are multiple hallmarks of cancer, therefore one treatment is generally not going to act as a single curative magic bullet because of the other capabilities that may override the effect of the treatment including the potential development of resistance, particularly drug resistance to current related therapies (Hanahan 2014). That is why we must seek novel sources for therapeutics and sea anemone venom that has both cytolytic and neurotoxic elements is of high interest. However, it is important to keep in mind that one of the most important cancer hallmarks is the capability to evade apoptosis (programmed cell death) (Johnstone et al. 2002). Alterations of apoptosis can promote cancer development (Carson and Ribeiro 1993). Gerl and Vaux (2005) confirm that induction (switching on) of the apoptosis program (see Fig. 41.2) has been observed in some forms of cancer treatment (Gerl and Vaux 2005) including tumours treated with cytotoxic drugs and radiation. It could be implied that inducing apoptosis is a useful target for cancer treatment (Pore et al. 2013) so it was important to determine whether sea anemone venom was not just toxic but actually killed cells through this process.

41.2.3 Background to Cancers That Are the Focus of This Chapter

41.2.3.1 Breast Cancer

Breast cancer is the most commonly diagnosed cancer in women, apart from skin cancer, among American women. In 2014, in Australia and the USA, approximately 12 % (1 in 8) of women developed invasive breast cancer in their lifetime. Over 40, 000 women are expected to die from breast cancer in the US in 2015. The death rate however, has been decreasing in some countries, which may be due to treatment advances and/or improved screening and increased awareness leading to earlier detection. Breast cancer remains one of the highest cancer death rates for American women second only to lung cancer (Breast Cancer Statistics 2015).

Breast cancer can be categorized in a number of ways, including using clinical features; the expression of tumour markers; and the histological type. Based on histological type, the two most common types of breast cancer are ductal and lobular carcinomas. Most breast cancers are ductal carcinoma, occurring in the ducts of the breast. A second, but less common form of breast cancer, is lobular carcinoma (Li et al. 2005).

Breast cancers can be broadly categorized as occurring with hormonal and non-hormonal risk factors. Estrogen exposure is directly associated with an hormonal risk factor for developing breast cancer (Weinberg 2013). Although the exact mechanisms remain to be fully elucidated, the alkylation of cellular molecules and the generation of active radicals that can damage DNA, together with the potential genotoxicity of estrogen and some of its metabolites (the catechol estrogens) have been implicated in causing breast cancer. Reducing exposure to estrogen is thought to be protective against breast cancer, whereas a prolonged exposure to estrogen is associated with an increased risk for developing breast cancer (Martin and Weber 2000). In addition, a family history has been estimated to explain approximately 20–25 % of observed familial breast cancer risk. In addition to *BRCA1* and *BRCA2*, genes, *PALB2*, *CHEK2* and *ATM* are also considered breast cancer susceptibility genes (Antoniou and Easton 2006; Nickels et al. 2013).

41.2.3.2 Lung Cancer

Lung cancer is the second most common cancer among men and women in the world. It accounts for 13 % of all new cancer cases in the USA, with estimates of new cases for men of over 115,000 and women of over 105,000 (American Cancer Society 2015). Lung cancer has the highest death rate for American women of any cancer in current years. The projected death rates for lung cancer for 2015 are 27 % of all cancer deaths in the USA and 19 % in Australia (Lortet-Tieulent et al. 2014) studied populations in 22 countries and found that the trends in lung cancer reflect the trends in smoking. This reflects both the amount of tobacco smoked and the composition of the cigarettes smoked. There was an increased incidence in lung cancer among women in many countries, and in Spain there were increasing rates for both men and women (Lortet-Tieulent et al. 2014). It is clear that targeting prevention is important for lung cancer, however understanding the disease for targeting treatments is still a real scientific and clinical area to be addressed.

Structurally, the lungs are composed of lobes that are reasonably small units. The right lung is divided into three lobes (upper, middle and lower), and the left lung has two lobes (no middle lobe) (Kobashi et al. 2011). The left lung is slightly smaller than the right lung because more space is required for the accommodation of the heart (Albert and Hubmayr 2000). Compared with left lung, the right lung is more frequently diagnosed with lung cancer; however the survival rates are similar regardless of which side of the lung has cancer (Lag et al. 2007).

The two main types of lung cancer are small cell lung carcinomas (SCLC) and non-small cell lung carcinomas (NSCLC). SCLC accounts for 15 % of all bronchogenic malignancies and follows a highly aggressive clinical course (Toyooka et al. 2001). Less than 5 % of SCLC patients currently survive 5 years after the initial diagnosis. In contrast, the 5-year survival rate for patients diagnosed with NSCLC is much higher at 85 % (Cancer Council 2004). SCLC is distinguished from NSCLC by clinical presentation, response to chemotherapy and radiation therapy. Non-small-cell lung Cancers (NSCLC) is the major histological type of lung cancer and includes adenocarcinoma (and the bronchoalveolar carcinoma [BAC] subset), squamous cell carcinoma and large cell carcinoma. In the USA, the most frequent lung cancer subset is adenocarcinoma (ADC) (Mitsudomi et al. 2005; Ray et al. 2010).

41.2.4 Current Breast and Lung Cancer Treatments

41.2.4.1 Current Breast Cancer Treatments

Surgery (a mastectomy) can be performed as one the therapies for breast cancer and can reduce the risk of breast cancer in women with strong genetic links to breast cancer (Domchek et al. 2010). There are a number of variants of surgery, but all forms of surgery are almost always used in conjunction with other types of treatments. Radiotherapy uses high-energy x-rays to destroy breast cancer cells. However, radiotherapy may result in fatigue, dry or itchy skin, swelling, loss of appetite, nausea, digestive problems and dry or sore throat (Cancer Council 2010). Chemotherapy is another important approach to treat breast cancer and an area where venom extracts from sea anemone can make a contribution. Current treatments for breast cancer and their side-effects are shown in Table 41.1.

Table 41.1 Summary of common treatments for breast cancer

Current therapy	Class/agent	Side effect(s)
Surgery		Negative change in body image
		Loss of normal breast functions
Radiation therapy		Fatigue, dry or itchy skin, swelling, loss of appetite, nausea
Chemotherapy	Alkylating agents (Cyclophosphamide)	Bladder irritation, fatigue, hair loss, lowered blood counts, nausea/vomiting
	Anthracyclines (Doxorubicin, Epirubicin)	Cardiac toxicity, fatigue, lowered blood counts, mouth ulcers, nausea/vomiting
	Antimetabolites (Capecitabine, Gemcitabine, 5-fluorouracil, methotrexate)	Diarrhoea, fatigue, hand-foot syndrome, nausea, stomatitis
	Taxanes (Docetaxel, Paclitaxel Nab-paclitaxel)	Allergic reactions (paclitaxel), fatigue hair loss, lowered blood counts muscle aches, neurological damage
	Vinorelbine	Fatigue, injection site pain, hair loss (moderate), lowered blood counts, neuropathy
Targeted hormone therapies	Tamoxifen	Causing endometrial cancer and thromboembolism
	Fulvestrant	Headaches, hot flushes, nausea, disturbance of menses and mood swings
Targeted therapies	Bevacizumab	Cerebrovascular ischemia, fatigue, headache, hypertension, infection, sensory neuropathy, proteinuria
	Lapatinib	Diarrhoea, dyspepsia, fatigue, hand-foot syndrome, nausea/vomiting, rash
	Trastuzumab	Anaemia, cardiac dysfunction, infection, leukopenia, neutropenia

Darby et al. (2011), Fisher et al. (1994), Young et al. (2008), Cancer Council (2014), Pritchard (2003), Gunduz and Gunduz (2011), National Breast and Ovarian Cancer Centre (2010), Geyer et al. (2006), Bonadonna et al. (1995), Meijers-Heijboer et al. (2001), and Domchek et al. (2010)

41.2.4.2 Current Lung Cancer Treatments

Current clinical based therapies for lung cancer vary according to the histologic type, histologic grade, and anatomic extent, or stage, of the lung tumor (Carmichael et al. 1998). There are three commonly used treatments, that of surgery, radiotherapy and chemotherapy. A summary of the main current lung cancer treatments is presented in Table 41.2. It has been estimated that 76 % of all new cases of lung cancer can utilise radiotherapy as an optimal initial therapy. Retreatment rates for radiotherapy of up to 27 % have been observed for some groups (Estall et al. 2007). There are, however, side-effects from radiotherapy, as shown in Table 41.2. Chemotherapy is another treatment option for lung cancer. Research efforts in the area of chemotherapy for lung cancer are focused on developing novel agents that target apoptosis, angiogenesis and tumour growth pathways in particular. We tested the apoptosis induction potential of sea anemone venom extract on lung cancer cells.

41.2.4.3 Summary of Current Treatments

Although surgery and radiotherapy have their place in treating both breast cancer and lung cancer, they need to be supplemented by chemotherapy for adequate efficacy and sufficient flexibility to cover the heterogeneity that make up the two cancer groups. As can be seen from Tables 41.1 and 41.2, there is a need for new anticancer drugs that target breast and lung cancer cells without the unwanted side effects that the current chemotherapy treatments give rise to. Thus there is clearly a role for drug discovery not only to fill in gaps in current treatment applications, but to offer novel agents with mechanisms of action that are well characterised and understood. This chapter will include the description and characterisation of the mechanism of action of such novel anticancer bioactives from sea anemone venom extracts.

41.3 Drug Discovery from the Marine Environment

Natural products are an exceptional source of bioactive compounds with cytotoxicity activity which can be utilised for the development of therapeutics (Carvalho and Ferreira 2001). More than 50 % of drugs in clinical trial have been related to natural products (Majumdar and Siahaan 2012). Although biological diversity in the terrestrial environment is tremendous, by far the highest diversity of life is in the ocean which is estimated to contain approximately one to two million species (Simmons et al. 2005). Moreover, the ocean constitutes approximately 70 % of the earth's surface and thus possesses a potential for the discovery of anticancer

Table 41.2 Summary of common treatments for lung cancer

Current treatment	Target	Most common drugs used	Limitation	Side effect
Surgery			Only for early stage	Pain
				Tiredness fatigue
Radiotherapy			Lack of local control	Tiredness
				Nausea
				Sore skin
				Hair loss
Chemotherapy		Cisplatin	Drug resistance	Cause severe toxicities
		Carboplatin		
		Paclitaxel		
		Albumin-bound-paclitaxel		
		Docetaxel		
		Gemcitabine		
		Vinorelbine		
		Irinotecan		
		Etoposide Vinblastine Pemetrexed		
Targeted therapies (The target is indicated in column 2)	Epidermal growth factor receptor (EGFR)	Erlotinib	Resistance to receptor inhibition	Toxic effect
	Anaplastic lymphoma kinase (ALK)	Gefitinib	Resistance to receptor inhibition	Toxic effect
		Crizotinib		

Bergot et al. (2013), Ray et al. (2010), Wykosky et al. (2011), Shepherd et al. (2000), Shaw et al. (2013), and Kominsky et al. (2002)

Table 41.3 Toxicity effects of selected marine bioactive extracts or compounds on lung cancer cells/models

Extract/compound	Marine source	Mechanism of action
Discodermolide	Discodermia dissolute sp.	Microtubule stabilization and induction of cell cycle arrest in SCLC cell lines A549
Dolastatin 15	Cyanobacterium Symploca sp.	Inhibition of microtubule function and induction of intrinsic apoptosis against four SCLC cell lines (NCI-H69, NCI-H82,NCI-H345, NCI-H446)
Dolastatin 10	Cyanobacterium Symploca sp.	In vitro: inhibition of microtubule assembly and induction of apoptosis by inactivating bcl-2 in SCLC cell lines (NCI-H69, -H82, -H446, -H510)
		In vivo: inhibition of tumor formation in metastatic SCLC xenograft mice model
Dictyostatin-1	Spongia sp.	Disturbance of microtubules spindle formation and induction of cell cycle arrest at G2/M phase in SCLC cell lines A549
NVP-LAQ824	Psammaplysilla sp.	Induction of apoptosis in Lung cancer cells (A549) and inhibition of tumour growth metastasis in a SCLC xenograft mice model
Psammapalin derivative		

Simmons et al. (2005)

agents (Monks et al. 2002; Cragg et al. 1997). However, getting sufficient amounts of finite sources of required compounds for ingredient identification and isolation appears to be an obstacle for the development of marine derived anticancer drugs (Rocha et al. 2001). Furthermore, synthesizing structurally complicated compounds of interest is also a problem (Monks et al. 2002). Recently, with tremendous improvements in deep-sea collection techniques, aquaculture technology and synthesis techniques, many of these problems have been resolved. Table 41.3 summarizes examples marine source derived bioactives (the compounds shown are secondary metabolites of the marine species indicated) using lung cancer as an example. These have been tested on human in vitro cell models or animal models.

41.4 Types of Sea Anemone Discussed in this Chapter

The venom of sea anemones has attracted considerable interest since the 1970s as a potential source of bioactive therapeutic drug compounds (Frazão et al. 2012). Various studies have demonstrated that at least 32 species of sea anemones are able to produce lethal cytolytic peptides and proteins (Anderluh and Macˇek 2002). This is accomplished through a variety of active mechanisms as indicated in Tables 41.1, 41.2, and 41.3, which includes haemolytic (Lanioa et al. 2001; Uechi et al. 2005), immunomodulation (Pento et al. 2011; Tytgat and Bosmans 2007), neurotoxic (Gondran et al. 2002), and cardiotoxic properties (Bruhn et al. 2011). Sea anemones venom also contains complex biologically active proteic substances and neurotransmitters that they use to protect themselves from predators and for acquiring food and are also used in competitive interactions amongst conspecifics (Andreev et al. 2008).

To date a variety of sea anemone venoms have been examined and classified according to their primary structure and functional properties. They can be divided into four different classes: (a) 5–8 kDa peptides as found in two diverse species, *Tealia felina* and *Radianthus macrodactylus* with antihistamine activity, (b) ~20 kDa pore-forming proteins, are most common and have been found to be inhibited by sphingomyelin. These have been found in a variety of anemone species including, *Actinia equina*, *Stichodactyla helianthus*, and *Heteractis magnifica*, (c) ~30–45 kDa cytolysins with or without phospholipase A_2-activity as found in *Aiptasia pallida*, and finally (d) 80 kDa proteins represented by *Metridium senile* cytolysin (Anderluh and Macˇek 2002).

There are only ten species of sea anemones out of approximately 1200 species known to exist world-wide that host anemonefish in a symbiotic relationship (Fautin and Allen 1997). These species are phylogenetically diverse and are found in three different families (Actiniidae, Stichodactylidae and Thalassianthidae) and five different genera (*Entacmaea*, *Macrodactyla*, *Stichodactyla*, *Heteractis* and *Cryptodendrum*) (Astakhov et al. 2008). What makes these ten species particularly unique to host anemonefish has not be ascertained but work by (Nedosyko et al. 2014) found that they range in toxicity and that this may be a key feature in the evolution of their symbiotic relationship with anemonefish. In our laboratory we have previously studied venom extracts from five of the ten host sea anemones, which were *H. magnifica*, *H. crispa*, *H. malu*, *C. adhaesivum* and *E.quadricolor*; and examined their effects on lung, breast and skin cancer cell lines. The anemones are listed as they range from the least toxic to the most toxic of the group of symbiotic species as well as the anemone species that is most preferred by anemonefish (see symbiosis chapter). Each of the venom extracts had varying effects on each of the cell lines, with skin cancer being the least sensitive (Ramezanpour et al. 2012). Another point of note from the earlier work was that *H. magnifica* was the most preferred species because it showed a highly cytotoxic effect on the breast and lung cancer cell lines studied, and because it is a large anemone that is fairly easy to obtain and to keep alive in captivity. Large amounts of venom can be acquired repeatedly from *H. magnifica* through milking, which allows us to use methodology that does not harm the anemones. Thus the work presented here, focussed on understanding the effects of *H. magnifica* venom extract on human breast and lung cancer cell lines.

41.5 Cancer Cell Killing by Sea Anemone Venom

The model to screen for killing of human breast and lung cancer cell lines involved obtaining crude venom samples, which were concentrated, then used to treat the human cell lines, which were subsequently screened for viability using two colourimetric bioassays. Briefly, venom samples from *H. magnifica* that were obtained by milking, were frozen immediately, freeze-dried using a bench-top lyophilizer and ground into a fine powder. Samples were re-solvated with distilled water and the concentration of total protein in the crude extracts that were adjusted to 400 µg/ml. The bioassays used were the Crystal Violet assay and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The crystal violet assay is based on the principle that it stains and fixes the cells which retain the ability to adhere to a surface. Cells were seeded in 96-well flat-bottom plates and incubated for 24 h, during which time the cells attach to the bottom of the wells. Cells are then treated with different concentrations of venom and any dead cells that detach are washed away. The remaining live cells in the wells are then stained with crystal violet and the resulting optical density reading is directly proportional to the number of remaining viable adherent cells (Kueng et al. 1989). The MTT assay set-up is similar to that of the Crystal Violet assay in that cells are added to plates; allowed to adhere; treated and washed. Then the yellow MTT substrate (a tetrazolium salt) is added, which live metabolically active cells convert to a purple product that can be detected on an ELISA plate reader (Mosmann 1983; Young et al. 2005).

41.5.1 Breast Cancer Cell Killing

As discussed above, the past decade has seen a dramatic increase in the number of preclinical anti-cancer lead compounds from diverse marine life entering human clinical tri-

als (Simmons et al. 2005). Hence, the current study of the potential cytotoxicity of sea anemone venom is an important contribution to this area. To screen for breast cancer cell killing, the venom was tested on human adherent breast cancer cells T47D (ductal carcinoma, endogenously expressing mutant p53) and MCF7 (adenocarcinoma, a p53 wild type cell line). A control non-cancer derived cell line, 184B5 cells (human non-cancer breast cell line), was also used. When the treated populations of breast cells were screened for relative survival following 24 h treatment by the venom using the MTT assay, the three cell lines all showed sensitivity to the killing effect of the venom (data not shown). When screened with the Crystal Violet assay (Fig. 41.3), dose-dependent killing was also observed for all breast derived cell lines, but all doses of 10 µg/ml or greater significantly killed T47D and MCF7 cancer cells compared to those in the 0 µg/ml control ($p < 0.05$). This was not the case for the non-cancer breast cell line that was not as sensitive to the venom as either of the cancer cell lines (Fig. 41.3). This implies that the breast cancer cells are more sensitive than the non-cancer cells and that the sea anemone venom has the possibility of development as a selective agent with less effect on normal cells and thus with fewer side-effects. However, this is a long way down the development pathway.

41.5.2 Lung Cancer Cell Killing

Cytotoxicity, as indicated by the number of viable adherent cell, was measured by the crystal violet assay following 24 h treatment of human lung cell lines. The treatment significantly decreased the number of cells retaining the adherent phenotype for a lung cancer (A549) and a lung non-cancer (MRC5) derived cell line when screened with this assay (data not shown). The response of the two lines were at similar levels however it was not very sensitive at detecting a high level of killing and showed cell numbers that decreased to plateau at approximately 50 %. However, when screened for relative viability using the MTT assay for metabolic activity as an indicator of cell killing a greater level of sensitivity was observed. With this assay, *H magnifica* venom decreased the viability of A549 cells to a greater extent than the non-cancer derived MRC5 cell line for the seven concentrations of 10 µg/ml and higher (Fig. 41.4). As found for the breast derived cell lines (Fig. 41.3), these findings, show the lung cancer cell line is more sensitive than its non-cancer partner cell line to cell killing by the venom. This observation suggests that you could develop bioactives from *H magnifica* venom as anticancer agents that may have less harmful effects on normal cells and therefore fewer side-effects. The potential continuation of further development of the venom will depend on whether it displays useful mechanisms of action in the way it kills the cells.

41.6 Mechanism of Action of Sea Anemone Venom on Cancer Cells

Apoptosis is a major target of anti-cancer therapeutics; therefore, it is important to discover novel therapeutics with this mechanism of action (Reed 2003). Apoptosis is triggered through intrinsic and extrinsic signalling pathways as shown above (Elmore 2007). Stimuli of the intrinsic pathway induce changes in the inner mitochondrial membrane that result in the loss of transmembrane potential, causing the release of pro-apoptotic proteins into the cytosol (Adams and Cory 2001). Pro-apoptotic proteins activate caspases that mediate the destruction of the cell through multiple pathways. Caspases have been broadly classified by their known roles in apoptosis and in inflammation (McIlwain et al. 2013). Mitochondrial dysfunction is another key event of apoptosis. Mitochondria play the pivotal roles in integrating and directing the death signal towards the caspase cascade (Liu et al. 2004). The permeabilization of the mitochondrial outer

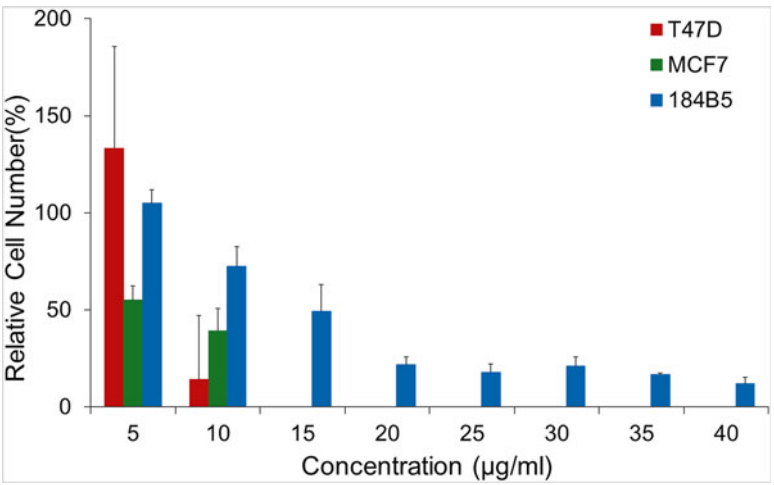

Fig. 41.3 Relative cell number (%) of T47D, MCF7 and 184B5 cells estimated by the crystal violet assay following 24 h exposure to *Heteractis magnifica* venom (µg/ml). Data, indicated as surviving cell numbers compared to the untreated control, are shown as the mean ± SEM, n = 3

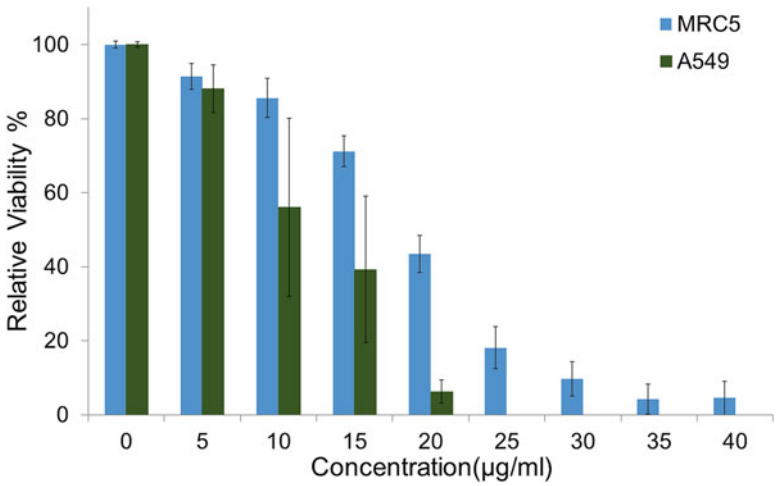

Fig. 41.4 Relative survival (%) of A459 and MRC5 cells estimated by the MTT assay following 24 h exposure to *Heteractis magnifica* venom (µg/ml). Data are shown as survival compared to the untreated control and are shown as the mean ± SEM, n = 3

membrane and the release of intermembrane space proteins, such as cytochrome *c*, Smac/DIABLO and the apoptosis-inducing factor (AIF) are central events in apoptosis (Gogvadzea et al. 2006).

41.6.1 Mechanism of Breast Cancer Cell Killing

Apoptosis was assayed using a flow cytometer and screening of 20,000 cells for each treatment condition. *Heteractis magnifica* venom induced increases in early and late apoptosis in T47D and MCF7 in a dose-dependent manner. The most significant increases were observed at 40 µg/ml, which included approximately 19 % early apoptosis compared with 1.2 % in untreated MCF7 cells. In T47D cells, the most significant increase was 16 % early apoptosis compared with 5.1 % in untreated cells. Thus *H. magnifica* clearly induces apoptosis in both human breast cancer cell lines tested.

Cell cycle progression and mitochondria membrane potential were also studied via flow cytometry (using different staining systems). Related to this, treatment with *H. magnifica* venom enhanced the levels of cleaved caspases-8,-9,-7, and -3 (Fig. 41.5). There were additional apoptosis-related changes, including loss of mitochondria membrane potential. The cell cycle progression was found to be altered by venom treatment. The cell cycle consists of a number of phases through which the cells progress at a regular steady rate to grow and proliferate. However, the treated human breast cell lines T47D and MCF7 had proliferation inhibition from the *H. magnifica* venom and it involved cell cycle arrest. The treatment induced the accumulation of a sub-G1 cell population with a concomitant decrease in the number of cells in the G1 phase. In T47D and MCF7 cells, venom treatment led to the accumulation of 11 % and 8 % in the sub-G1 phase, respectively, with a corresponding decrease in the percentage of cells in the G1 phase fraction as compared with the control untreated cells. This change observed where the cells in the population are in the different phases of the cell cycle indicates induction of apoptosis.

This range of findings demonstrate that *H. magnifica* venom mechanism of action definitely involves the induction of apoptosis in T47D and MCF7 human breast cancer cells, and that this effect is mediated via the activation of caspases and the mitochondria-mediated apoptotic pathway (increasing permeability of the outer mitochondrial membranes). Also, clearly, the mechanism of action involves inhibition of proliferation of breast cancer cells by the venom. This means that if breast cancer cells in the laboratory are treated with the venom, they stop proliferating (growing) at the rate they were before treatment. This is a good thing, because one marked characteristic of cancer cells is their rapid proliferation rate and it is this rapid proliferation rate that can lead to uncontrolled tumor growth. Hence there are at least two mechanisms of action that are potential benefits for the treatment of breast cancer cells with the *H. magnifica* venom: it can arrest cell growth and it can induce apoptosis (programmed cell death) in cells in the treated population. These represent two of the hallmarks of cancer shown in Fig. 41.1 ("evading growth suppressors" and "resisting cell death").

41.6.2 Mechanism of Lung Cancer Cell Killing

Lung cancer is particularly resistant to the induction of apoptosis. Apoptosis was assayed as described above in the section on mechanisms of breast cancer cell killing. The *H. magnifica* venom at 40 µg/ml induced 32 % early apoptosis compared to 2 % in untreated A549 lung cancer cells. In contrast, for MRC5 non-cancer origin lung cells, following venom treatment, a decrease was found in the proportion of early apoptotic cells relative to the untreated control (Fig. 41.6). Apoptotic cell death in the human lung cancer cell line is mediated by the activation of caspases. Caspase-3 assay and JC-1 staining were conducted to detect increases in the

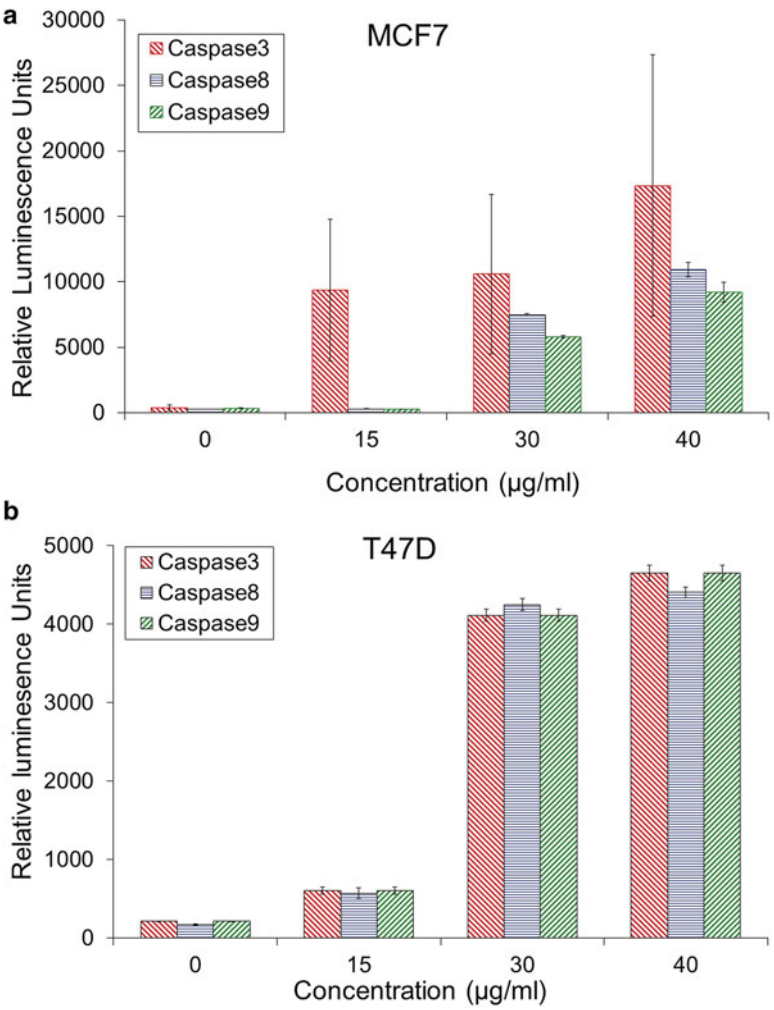

Fig. 41.5 Caspase activities. Determined using a luminescent method on MCF7 and T47D cells (as indicated above each graph) after 24 h treatment with venom (µg/ml) from *Heteractis magnifica*. Data are presented as relative luminescence units (RLUs). Data are shown as means ± SEM; n = 3

levels of apoptosis-regulating proteins and mitochondrial membrane permeability, respectively. It was found that caspase increased in a dose-dependent manner. Early apoptosis increased significantly in A549 cancer cells but not in MRC5 non-cancer derived lung cells, compared to late apoptosis/necrosis which increased in both cell lines (Fig. 41.6). This implies that *H. magnifica* venom induces apoptosis in the lung cancer cells A549, but may act principally through necrosis in the non-cancer cell line MRC5.

Mitochondrial dysfunction is another key event of apoptosis. The loss of mitochondrial membrane potential following treatment of A549 cells was significant at all doses of venom tested. Thus the mechanism by which the venom extract from *H. magnifica* induces apoptosis in A549 human lung cancer cell line is mediated via both the death receptor-mediated and the mitochondria-mediated apoptotic pathways.

Addition of *H. magnifica* venom to A549 cancer cells delayed progression through the cell cycle with a marked reduction in the number of cells in the S phase and an associated accumulation of cells in the G0/G1 phases. Control of the progression of the cell cycle of cancer cells is an effective strategy for cancer therapy because deregulated cell cycle control is a fundamental aspect of cancer for many common malignancies (Senderowicz 2003), including lung cancer. The study of the mechanism of sea anemone venom on lung cancer cells are consistent with the findings with the breast cancer cells. These are that there are at least two mechanism of action that work on hallmarks of cancer and are potential benefits for the treatment of cancer cells with the *H. magnifica* venom.

41.7 Summary of the Application of Sea Anemone Venom in the Anti-cancer Field

The sea anemone *H. magnifica* has been shown to be a candidate of high potential for development as an anticancer therapeutic drug. We have also tested several other sea anemones that are hosts to anemonefish and each has shown cytotoxic activity. Developing anticancer drugs from this group

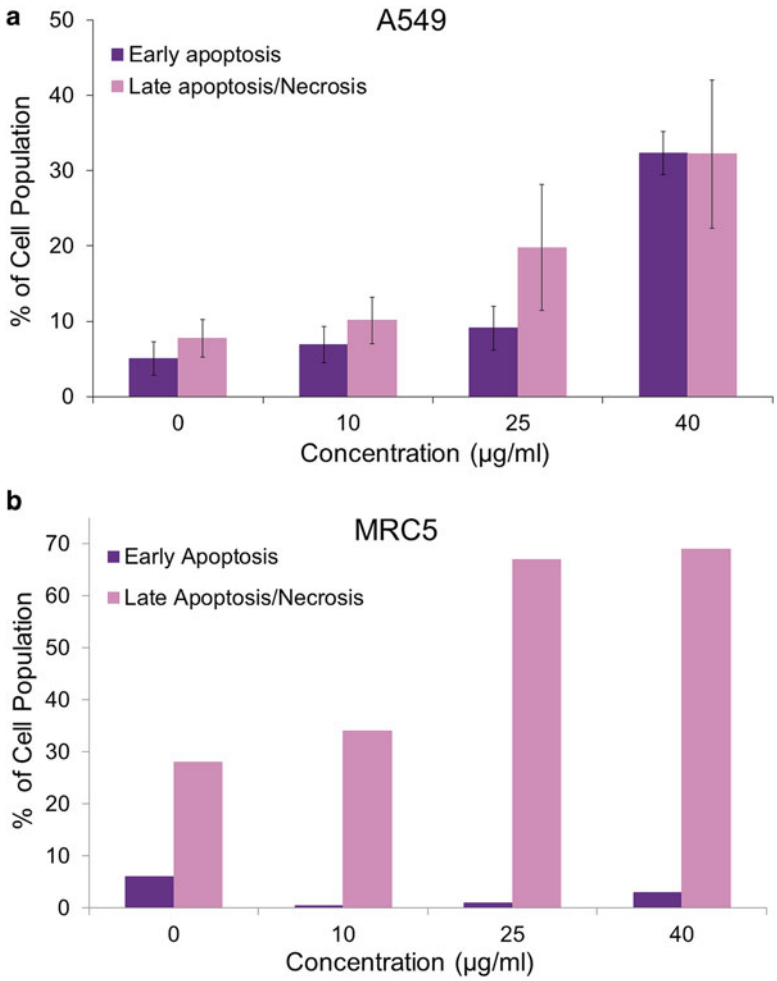

Fig. 41.6 Effect of *Heteractis magnifica* venom on apoptosis in A549 and MRC5 cell lines. Data determined by annexin V-conjugated PI staining followed by flow cytometry. Data were obtained from 10,000 events (screened cells) for each treatment of the cell population. For A549, n = 3; for MRC5, n = 1

of organisms has high potential as there is something about the potency of the venom that is both strong enough to provide food and protection for the anemone but not so toxic that it kills the symbiotic fish living within its tentacles. Consequently, our belief that led us to test sea anemone venom on a variety of human cancer cell types has produced significant anti-cancer results and yet at the anti-cancer doses the venom does not harm human non cancer cells at the same level. This is a highly advantageous finding worth further consideration in the cancer drug development field.

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Leveraging Nematocysts Toward Human Care

42

Tamar Lotan

Abstract

The stinging cells of cnidarians contain nematocysts, which are a sophisticated ultra-fast injection system that can penetrate the skin immediately. This chapter will review two nematocyst-based biotechnology applications. The first hinders nematocyst discharge and thereby prevents jellyfish envenomation. The development of the inhibitory formulation and the demonstration of its effectiveness by clinical trials will be described. The second application utilizes isolated nematocysts as micro-devices for active drug delivery. The advantages of this novel approach will be demonstrated by the results of in vitro and in vivo pharmacokinetics studies.

Keywords

Nematocyst discharge • Jellyfish envenomation • Sting inhibition • Active transdermal drug delivery • Micro drug device

42.1 Introduction

The Cnidaria phylum is characterized by its distinctive stinging cells, known as cnidocytes or nematocytes, which comprise a sophisticated venom delivery system designed for both prey capture and defense against predators (Tardent 1995; Beckmann and Özbeck 2012). Inside the stinging cell, a rigid capsule termed cnidocyst or nematocyst functions as the venom injection apparatus (Lotan et al. 1995). Although there are more than 10,000 species in the Cnidaria phylum, only about 100 of them are known to be toxic to humans (Cegolon et al. 2013). The most toxic cnidarians, such as the *Chironex fleckeri* (box jellyfish) and the *Carukia barnesi* (Irukandji), belong to the Cubozoa class and may cause mortality (Fernandez et al. 2011). However, even when less severe, cnidarian envenomation occurs all over the world and is considered a public health hazard (Purcell 2012). It has been estimated that 150 million people worldwide are exposed to jellyfish annually (Boulware 2006). An extended study conducted in the central Mediterranean found that jellyfish stings are the main cause of human pathologies resulting from contact with marine organisms (De Donno et al. 2014). In the United States, 500,000 jellyfish stings are estimated to occur in the Chesapeake Bay and up to 200,000 stings in Florida waters annually (Burnett 1992). Envenomation may result in local erythema, swelling, itching and pain, and in extreme cases may cause systemic effects such as allergic manifestations, cardiac failure, pulmonary edema and mortality (Cegolon et al. 2013).

The ability of the cnidarian stinging mechanism to penetrate the skin and to deliver toxins into the body poses a great challenge to the design of protection means at sea; on the other hand, it may open new possibilities for developing novel syringe-like injectors. These opposite challenges have been addressed by two biotech companies, namely Nidaria Technology and StarletDerma, in whose establishment I was fortunate to take part. In this chapter, the innovations resulting from our efforts will be reviewed. First, I will show how the stinging mechanism can be inhibited in the marine environment, providing protection to bathers, swimmers, divers and fishermen. I will then discuss how the stinging

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mechanism can be activated, in a controlled pharmaceutical setting, for the purpose of transdermal drug delivery.

42.2 The Stinging Mechanism Principle of Operation

The stinging mechanism of cnidarians has been a target of research since it was first identified by Antonie van Leeuwenhoek at the beginning of the eighteenth century. Here, I will describe in short the basic principle of operation of the nematocyst's explosive delivery machinery.

The basic structure of the nematocyst consists of a capsule, a long tubule and a matrix. The assembly of the capsule is a three-step process, which is derived from the post-Golgi secretory vesicle (Engel et al. 2001). First, the capsule is formed, then a tubule starts to grow and elongate outside the capsule within the cell cytoplasm (Adamczyk et al. 2010). Once the capsule reaches its final size, the tubule invaginates into the capsule to form a highly packed structure. At the last stage, the capsule is filled with high concentrations of poly-γ-glutamate, which is associated with a high concentration of cations, generating an extreme osmotic pressure within the capsule (Weber 1989, 1990; Szczepanek et al. 2002). Finally, the capsule is sealed (Reft and Daly 2011).

This type of condensed structure requires the capsule wall to have a high elasticity and tensile strength (Holstein et al. 1994; Ozbek et al. 2002). The walls of the capsule, as well as the tubule, are made of fibrils of short collagens known as minicollagens (Ozbek et al. 2002; Adamczyk et al. 2008; David et al. 2008). The distribution of these fibrils along the capsule provides the tensile strength necessary to withstand the high osmotic pressure in the capsule. Nevertheless, owing to its fibrillary structure, the capsule wall is permeable to solutions and essentially performs like a porous net. This permits free movement of small molecular weight solutions (Lubbock and Amos 1981), whereas the large matrix of poly-γ-glutamate is trapped within the capsule.

In nature, the nematocyst is embedded in the cell and is triggered through a cell-sensor mechanism (Thurm et al. 2004; Hwang et al. 2008; Anderson and Bouchard 2009; Tang and Watson 2014). Upon activation, the discharge is controlled by osmotic balance (Fig. 42.1). Water flows into the capsule through the porous wall, causing the poly-γ-glutamate/cation matrix to dissociate (Tardent 1995). The resulting osmotic pressure increases to 150 bars, causing the discharge of a long, folded thin tubule at an ultrafast acceleration of 5×10^6 g (Holstein and Tardent 1984; Nüchter et al. 2006; Ozbek et al. 2009). The pressure also causes the continuous injection of the capsule contents and venom, until all the poly-γ-glutamate is swept out of the capsule (Tardent 1995; Lotan et al. 1996). This discharge process, which is one of the fastest events in cell biology, is typically completed within 3 ms (Holstein and Tardent 1984).

Among the different cnidarian species, there are about 30 types of capsules. While they differ in size, shape and length of the tubule, all capsules types share the same mechanism of action (Mariscal 1974; Fautin 2009).

42.3 Inhibition of the Stinging Mechanism

42.3.1 The Designing of an Inhibitory Formulation

Protection against the stinging mechanism can be achieved by mechanical barriers such as personal wet suits or stinger suits. However, besides the fact that the face is often unprotected, these suits are in use mostly in waters known to be inhabited by lethal jellyfish and are not commonly used by bathers in areas of less toxic jellyfish. To provide more practical and convenient protection, a protective formulation has

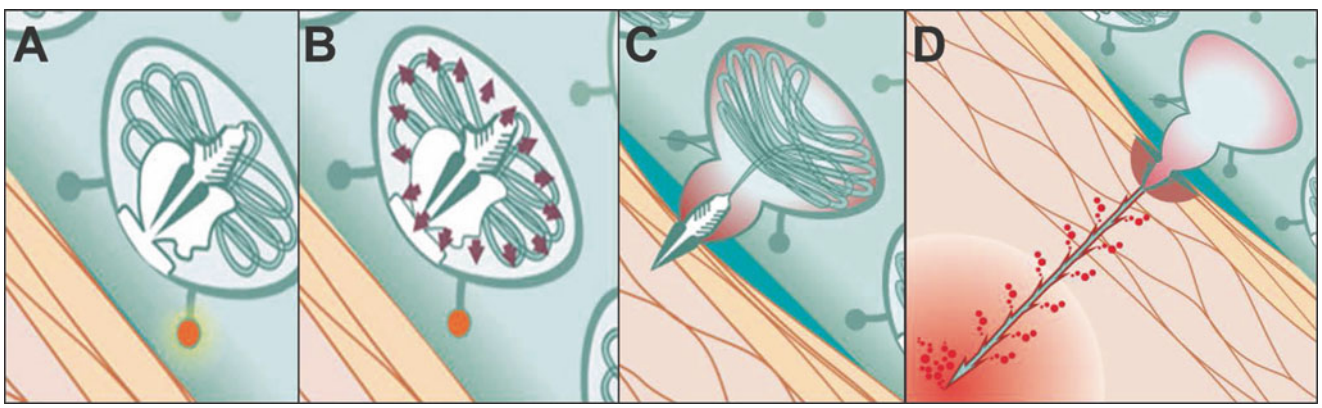

Fig. 42.1 Schematic model of the cnidarian stinging mechanism. (**a**) Stimulation from the skin initiates the stinging cell discharge process. (**b**) High internal pressure of 150 atm develops within the stinging capsule. (**c**) The resulting high acceleration drives the tubule shaft to drill a hole in the skin. (**d**) The tubule follows the shaft and injects poison into the body

been developed. For the ease of use, the protective lotion was combined with sunscreen filters, avoiding the need to apply two different lotions. To prevent envenomation, we aimed to hinder the triggering and the activation cascade of the stinging cell and not to treat the wide toxic arsenal of the nematocysts. Additionally, as the protective lotion had to be waterproof, a basic water-in-oil formulation was chosen. The protective lotion is designed to impede the activation of the stinging mechanism in several ways:

- The water-in-oil formulation, together with the use of different silicon compounds, creates a highly hydrophobic lotion, which prevents tentacle adhesion to the skin and reduces physical contact with the stinging cells.
- Competitive antagonists to nonselective receptors that bind to amino acids and sugar secretions of the prey block the sensor of the stinging mechanism and thus prevent activation (Thorington and Hessinger 1988).
- Positive ions, such as calcium and magnesium, reduce the development of the osmotic force within the nematocyst, which is necessary to create the firing action (Weber 1989).

42.3.2 Clinical Trials

To evaluate the protective effects of the sting inhibitor formulation, ethically approved, double-blind clinical trials were conducted. These trials involved application of either the inhibitor lotion (SafeSea, Nidaria Technology Ltd, Jordan Valley, Israel), or conventional, widely used waterproof sunscreens (such as Coppertone or Nivea SPF 15) as placebo (Kimball et al. 2004); in one trial, a third control group received no prophylaxis (Tønseth et al. 2012). All lotions were applied to the forearms according to a randomization protocol. Tentacles were removed from live jellyfish in storage tanks and were placed on the skin for different time intervals. Subjects were medically examined and queried about pain during the tentacle application and up to 2 h afterwards. Pain was scored and inflammation was evaluated by a dermatologist. The clinical trials were conducted by several teams to investigate the effect against the sting of different jellyfish species (Cegolon et al. 2013):

- The effect against *Chrysaora fuscescens* (sea nettle) sting was clinically tested at Stanford University School of Medicine, CA (Kimball et al. 2004). *C. fuscescens* is well known for stinging swimmers along the west coast of the United States.
- *Chiropsalmus quadrumanus* (box jellyfish or sea wasp) is found in the west Atlantic Ocean, the Gulf of Mexico and the Pacific Ocean and is considered to be specially dangerous to humans and even life-threatening to small children (Bengston et al. 1991; Haddad et al. 2002; Cegolon et al. 2013). The protection against this species was tested at Bert Fish Medical Center, New Smyrna Beach, FL (Kimball et al. 2004).
- The effect against *Cyanea capillata* (lion's mane jellyfish) was tested at the Marine Biological Station in Drøbak, University of Oslo, Norway (Tønseth et al. 2012). *C. capillata* is confined mostly to cold waters of the Arctic, northern Atlantic and Pacific Oceans and is most common along the North Sea and the Norwegian coast. It causes minor to severe skin pain/discomfort (Cegolon et al. 2013).
- *Rhopilema nomadica* was tested in Rambam Health Care Campus, Haifa, Israel (Ramon et al. in preparation). *R. nomadica* is an invasive jellyfish that within 30 years has spread all over the East Mediterranean to form large swarms during summer . It has become a major threat to bathers and fisherman causing painful stings, which in certain cases required hospitalization (Lotan et al. 1996; Uri et al. 2005; Deidun et al. 2011; Yahia et al. 2013).

The above-mentioned clinical trials showed that the application of the inhibitor lotion significantly reduced the frequency and severity of stings, in comparison to regular sunscreen. Additionally, application of regular sunscreen had no clinical advantages over bare skin exposure (Kimball et al. 2004; Tønseth et al. 2012).

A clinical field trial was carried out in the Gulf of Mexico and in the Caribbean, where *C. quinquecirrha*, *C. quadrumanus* and *Lunuche ungulate* (thimble) predominate (Boulware 2006). During the double-blind, randomized, placebo-controlled trial, participants applied either the inhibitor or placebo lotions to opposite sides of their body and went swimming for 45 min (Fig. 42.2). Upon exiting the water, participants were examined for stings. The results from over 80 water exposures showed that the application of inhibitor lotion (SafeSea) reduced jellyfish stings by over 80 % (Boulware 2006).

Through 2013 and 2014, the inhibitory lotion helped breaking several world sea records including the crossing of Monterey Bay, the Night Train from Santa Barbara to San Diego (221 miles) and the Cyprus-Israel Swim (123 h), and is used regularly by marathon swimmers and bathers all over the world.

42.4 Harnessing Nematocysts for Drug Delivery

42.4.1 Transdermal Drug Delivery

Nematocysts are capable of puncturing arthropod coticule, fish scales, but also human keratinous tissue such as skin,

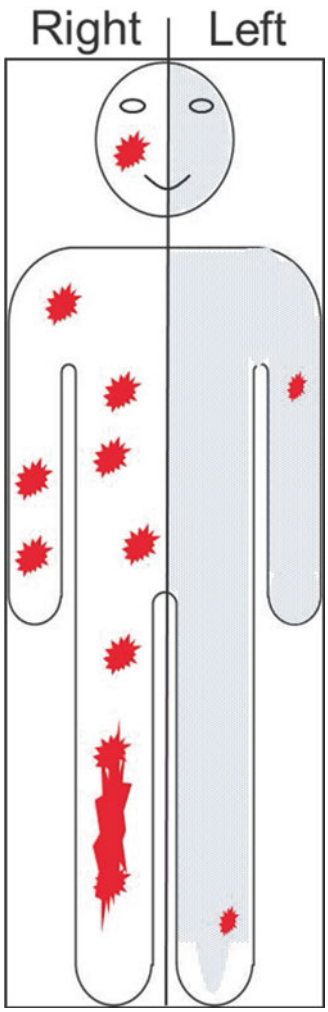

Fig. 42.2 Schematic illustration of the results of the clinical field trial. After 45 min of swimming, the number of stings and inflammation sites were compared between the right half of the body, where a common sunscreen (Coppertone SPF 15) had been applied, and the left side of the body that had been treated with the inhibitor lotion (SafeSea SPF 15)

hair and nail plate (Lotan 2008). The human skin is the most accessible site for drug delivery, providing attractive solutions for local and systemic administration. Transdermal delivery has the advantages of avoidance of first-pass metabolism, prevention of adverse side effects, and a general increase in patient compliance. Nevertheless, after three decades of extensive research, only a relatively small number of drugs, mostly small lipophilic substances, can be introduced transdermally, whereas larger or hydrophilic compounds cannot (Barry 2004; Prausnitz and Langer 2008; Cevc and Vierl 2010; Subedi et al. 2010). This is mainly due to the intrinsic structure and lipophilicity of the most outer layer of the skin, the stratum corneum, which acts as an almost impenetrable barrier for the transdermal route. Nematocysts can overcome this barrier because they are not dependent on passive diffusion, but rather on active penetration via the hollow tubule directly into the viable epidermis. The thin, submicron penetration point makes the nematocyst system almost noninvasive in comparison to other devices. For example, sono/electroporation creates entry points of more than 200 μm in diameter, whereas minimally invasive microneedles have varied diameter of 25–200 μm and are relatively long, able to reach the dermis (Cevc and Vierl 2010; Donnelly et al. 2010; van der Maaden et al. 2012).

In order to harness the potential of nematocysts as an injection system, several preconditions need to me bet. They need to be non-toxic to humans, stable in their intact state, ready to discharge upon controlled activation, and to be able to deliver external molecules as drugs. These requirements and how they have been met will be discussed in the following.

42.4.2 Nematocyst Formulation for Topical Application

Nematocysts were isolated from sea anemones (*Aiptasia diaphana*, *Nematostella vectensis*), exploiting their high density and high stability in salts (Ayalon et al. 2011; Tal et al. 2014). During the purification process the cationic content was changed to Na+ ions, as it was previously shown that the internal osmotic pressure is higher in the presence of monovalent than of divalent cations (Weber 1989; Szczepanek et al. 2002), thus facilitating fast activation during topical application.

Following purification nematocysts were desiccated, all under sterile conditions, yielding a powder of intact micro-devices whose potential activation is unimpaired (Ayalon et al. 2011). The purified nematocysts were then formulated in an anhydrous gel, which kept them in an intact state, but allowed their spreading over the skin. Activation was done by hydration, through the addition of a hydrophilic formulation containing the drug (Fig. 42.3). Because of the nematocyst's intrinsic attributes, the water containing the soluble drug is pumped into the capsule and through the tubule. This process continues until the poly-γ-glutamate, the driving force of the system, is washed out through the tubule. Thus, administration of the drug comprises two steps: first, spreading of the anhydrous gel formulation containing the intact micro-device system over the skin, and second, application of the hydrophilic drug formulation, which immediately triggers capsule activation and drug delivery (see movie in (Ayalon et al. 2011)).

The nematocyst injection system was tested for safety in clinical trials on more than 100 human volunteers, demonstrating no irritation or allergenic reaction. Moreover, studies in pigs showed that the penetrating tubules can be wiped off the skin after application (Lotan 2005, 2008).

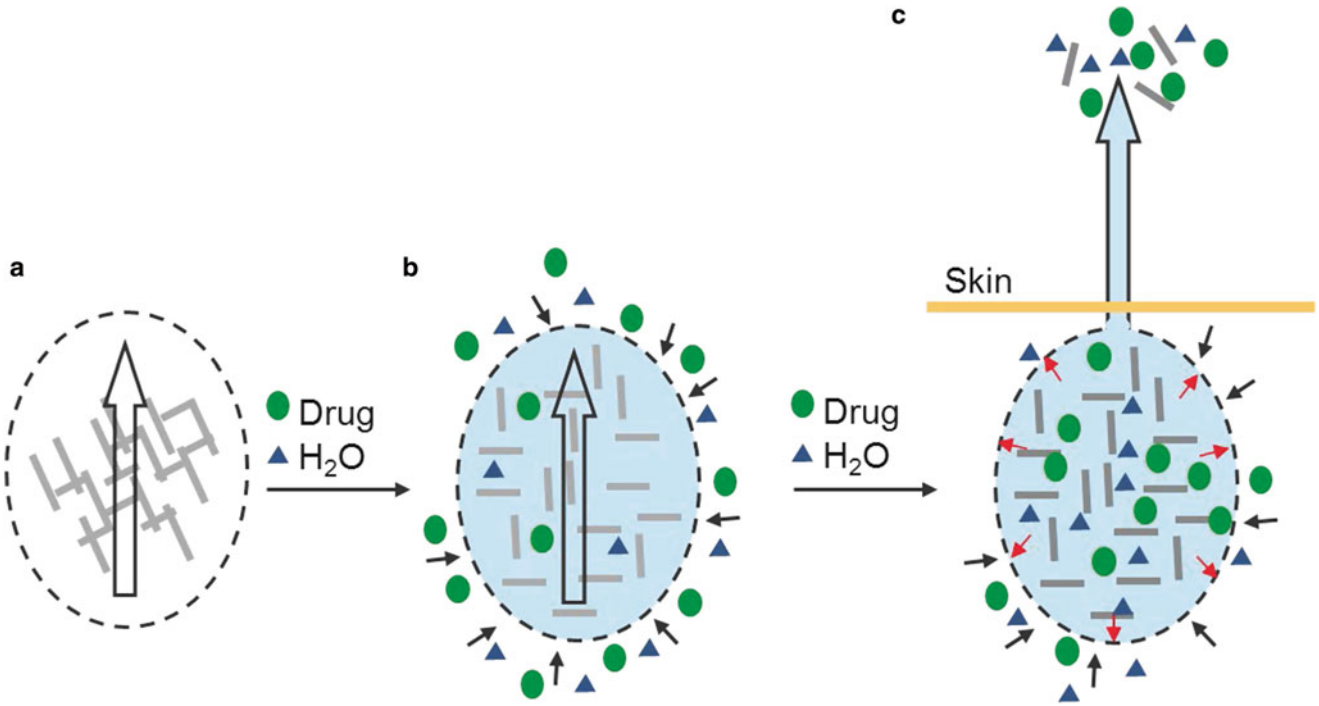

Fig. 42.3 Mode of action of the nematocyst injection system. (**a**) The intact, desiccated capsules are formulated in anhydrous gel. (**b**) Activation is achieved by a hydrophilic drug formulation. Water molecules (*triangles*) and the soluble drug (*circles*) penetrate the porous wall of the capsule, causing the dissociation of the poly-γ-glutamic matrix. (**c**) The resulting high osmotic pressure causes the ejection of the tubule, which delivers the capsular contents into the skin

42.4.3 Drug Delivery Results

Owing to the characteristics of the skin, hydrophilic compounds are the most difficult molecules to deliver by passive topical application. The nematocyst system is specifically designed to address this obstacle and hence to facilitate the delivery mainly of hydrophilic compounds. Using a Franz diffusion cell system, which enables in vitro measurements of compound permeability and kinetics under full thickness skin, different organic compounds and peptides were demonstrated to be delivered locally into the skin (Lotan 2008; Ayalon et al. 2011; Tal et al. 2014). The quantity of drug delivered by the nematocyst injection system can be controlled by regulating four parameters: the size of the application area, the number of capsules, drug concentration and exposure time. The size of the applied area can vary according to the need. When the number of capsules increases, more drug can be delivered as long as the capsules are well spread to make contact with the skin (Ayalon et al. 2011). This was also demonstrated in a study where the delivery of diphenhydramine, an antihistamine drug, was tested with a control formulation devoid of capsules, and two capsule-containing formulations, one of which with a double concentration of capsules than the other. A comparison between the formulations showed not only that the delivered amounts of diphenhydramine were higher with capsules, but also that drug accumulation was proportional to capsule concentration (4–8 times higher than in the control) (Fig. 42.4).

In most drug delivery systems, increased drug concentration raises the amount of drug passing through the skin. However, this is not the case with peptides and proteins whose delivery through the stratum corneum is limited. Although numerous advanced formulations and physical techniques are being developed for this purpose, only a few are currently available (Kalluri and Banga 2011). We tested the delivery of desmopressin acetate, a 9-amino-acid synthetic replacement for the hormone vasopressin. This peptide is relatively stable and can be traced under the skin however it does not passively diffuse through the skin (Cormier et al. 2004). Our results showed that the delivery of this peptide is enhanced using the nematocyst injection system. Furthermore, there was a correlation between drug concentration and peptide concentration under the skin (Fig. 42.5). In these experiments, exposure time was limited to 5 min, in order to demonstrate immediate topical drug delivery. However, longer exposure times also resulted in higher drug concentration under the skin (Tal et al. 2014), indicating that the nematocyst system can be used as submicron open channels for continuous drug release (Fig. 42.6).

The delivery with the nematocyst system can be either local or systemic, depending on the drug chemical properties. To demonstrate systemic delivery, we used the potent

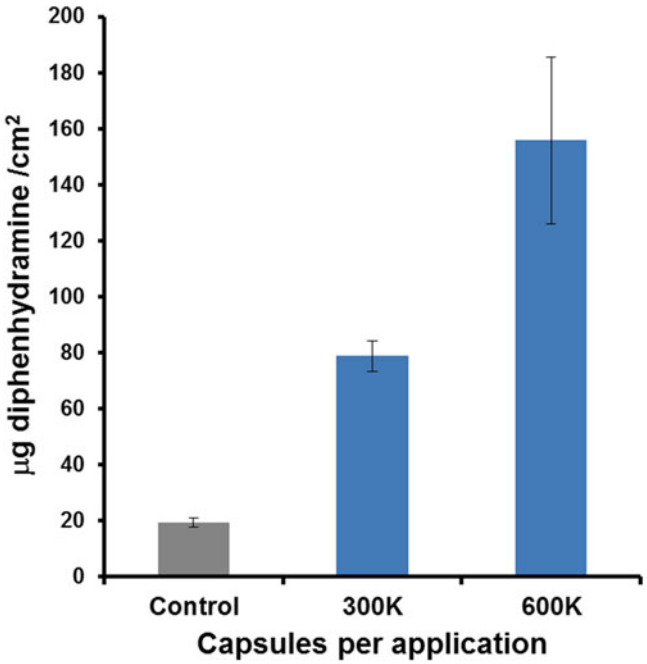

Fig. 42.4 Accumulation of delivered diphenhydramine as a function of capsule number. Diphenhydramine HCl (5 % solution) was added to gel formulations containing 300×10^6 or 600×10^6 capsules per application, or, as a control, to the same gel formulation without any capsules. After exposure to diphenhydramine HCl for 5 min, the drug was removed and the skin samples were left in the diffusion cell for 5 h to allow subcutaneous diffusion of the delivered diphenhydramine. Error bars represent the mean ± SE. Capsules were isolated from *A. diaphana*

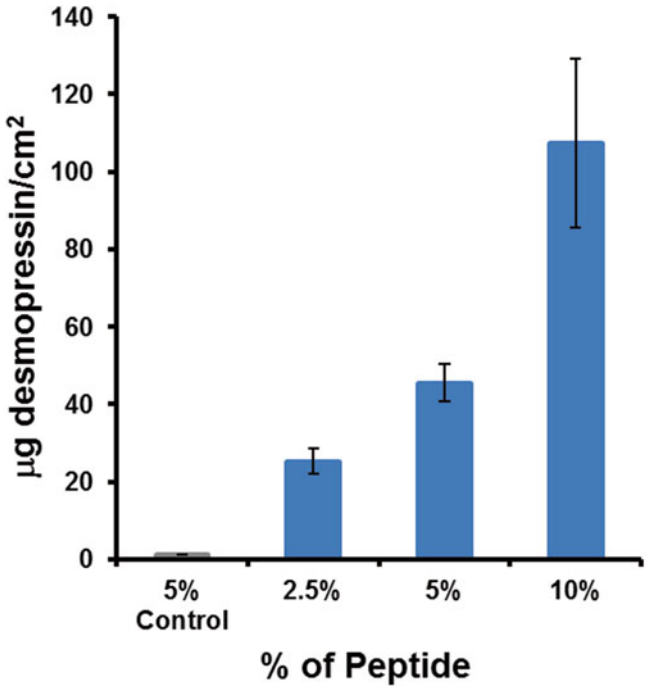

Fig. 42.5 Cumulative permeation of desmopressin acetate delivered by the nematocyst system or by control gel without capsules, as a function of drug concentration. Desmopressin acetate solutions (2.5 %, 5 % or 10 %) were applied over the tested and control (5 %) formulations ($n \geq 8$). After exposure to desmopressin acetate for 5 min, the drug was removed and the skin samples were left in the diffusion cell for 5 h to allow subcutaneous diffusion of the delivered desmopressin acetate. Error bars represent means ± SE. Capsules were isolated from *A. diaphana*

muscarinic receptor antagonist scopolamine, which is one of the most effective single agents used to prevent motion sickness (Spinks and Wasiak 2007). It was also among the first drugs to be incorporated into a transdermal patch. Because the activity of the nematocyst system is immediate, we expected fast drug delivery resulting in rapidly detectable plasma drug levels. Therefore, we tested the pharmacokinetics in a swine model upon 5 min of skin exposure, after which the sites of application were thoroughly washed and blood samples were collected (Shaoul et al. 2012). Our finding of a short T_{max} (time after administration of maximum plasma concentration) of 30 min (Fig. 42.7) indicates that despite the topical mode of administration, the scopolamine pharmacokinetic characteristics are comparable to those reported for subcutaneous injections (Renner et al. 2005). Furthermore, reaction time is much shorter as compared to patch application, where the drug does not exert its effect until 6–8 h after application because of its characteristically slow and incremental transdermal diffusion (Nachum et al. 2006; Apfel et al. 2010).

The use of the nematocyst biological system as a drug delivery platform allows rapid delivery that is self-activated via internal osmotic pressure, without the need to apply external energy. The compatibility of the system with hydrophilic and peptide compounds is attractive, as there is an unmet need for formulations that can deliver these molecules across the skin. The findings thus far suggest that this 700 million-year-old natural system may offer a novel alternative for therapeutic applications.

42.5 Discussion

The nematocyst is the most evolutionarily ancient injection system. Its unique physical characteristics make its discharge one of the fastest biomechanical cellular processes in nature. The nematocyst has been studied for over 200 years, and its regulation, synthesis and many of its proteins building blocks have been characterized. Being an autonomous system, which acts as an independent micro-injection device, has inspired us to explore its implementation as a drug delivery system. However, the tight folding of the long tubule, its invagination at high acceleration, and the fluid dynamics in sub-micron dimensions are still open questions that may stimulate interdisciplinary research. Future exploration of this unique biological apparatus may yield important contributions to and innovations in biotechnology, material sciences, and fluid dynamics, providing another example of how the wisdom of Nature can be transformed into technological advances.

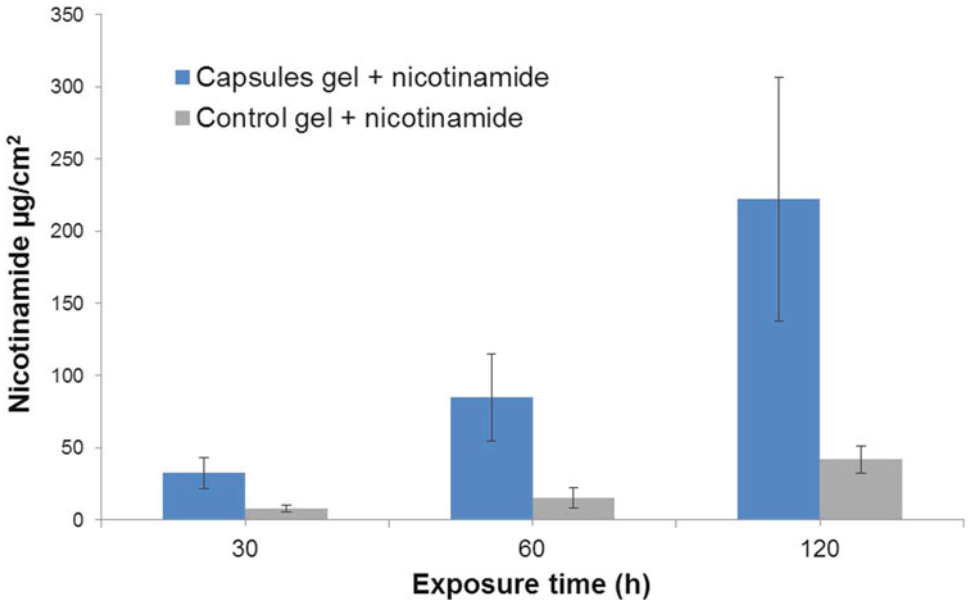

Fig. 42.6 Cumulative permeation of nicotinamide as a function of exposure time. Nicotinamide (4 % in activator solution) was added to capsule-containing gel or to control gel without capsules for 30, 60, or 90 min ($n \geq 6$). The drug was then removed and the skin samples were left in the diffusion cell for up to 5 h. Error bars represent means ± SE. The amount of delivered nicotinamide was significantly greater in the skin treated with capsules gel than in the control ($p < 0.001$). Capsules were isolated from *N. vectensis* (Modified from Tal et al. (2014))

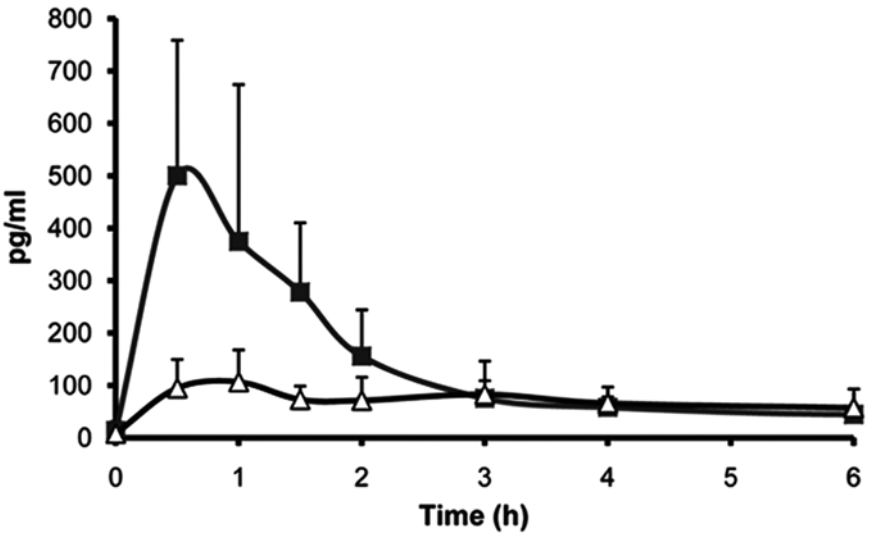

Fig. 42.7 Plasma levels of scopolamine in pigs after short topical exposure. Test and control groups were exposed for 5 min to the same solution of 5 % scopolamine HBr. Averaged values (means ± SD) of the test group (■) treated with gel formulation containing isolated nematocysts and of the control group (Δ) treated with gel formulation only. Capsules were isolated from *A. diaphana* (Modified from Shaoul et al. (2012))

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Coral Scaffolds in Bone Tissue Engineering and Bone Regeneration

43

Mathieu Manassero, Adeline Decambron, Nane Guillemin, Hervé Petite, Rena Bizios, and Véronique Viateau

Abstract

Coral exoskeleton, which consists of $CaCO_3$ and has an interconnected-pore structure that resembles that of natural human bone, has been used as scaffold material to fill bone defects in both animal models and humans since the early 1970s. This natural material is biocompatible, osteoconductive, and biodegradable. Most importantly, the possibility of seeding coral scaffolds with either stem cells or loading them with growth factors has provided novel alternatives for bone tissue engineering. *In vitro* studies have demonstrated that (1) seeded cells adhered and proliferated in a time-dependent manner, and (2) loaded growth factors were absorbed on coral scaffold and subsequently released. Moreover, when coral scaffolds containing either cells and/or growth factors were implanted *in vivo*, a significantly increased amount of newly-formed bone was observed. Most importantly, timely resorption of the scaffold material *in vivo* was associated with full bone regeneration in a clinically-relevant sheep model of bone defect. Although sometimes inconsistent, such outcomes provided evidence that bone regeneration, which matches, and even supersedes, the efficacy of autologous bone graft, is achievable with coral scaffolds.

Use of coral scaffolds for bone tissue regeneration purposes is thus an appealing strategy for the following reasons: (1) these materials are biocompatible and bioresorbable; (2) have three-dimensional structure and porosity; (3) have material surface chemistry which promotes stem cell differentiation and function of differentiated cells which are pertinent to new tissue formation; and (4) they can be used as carriers for growth factors.

Keywords

Coral exoskeleton • Bone regeneration • Bone resorption • Animal models • Tissue engineering • Bone cells • Stem cells • Growth factors

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43.1 Introduction

Bone healing in mammals is generally considered to be a biologically optimal process since most fractures and small defects in this tissue heal spontaneously with minimal treatment. In contrast, clinical management of massive, segmental, bone defects resulting from high-energy trauma, tumor resection, and debridement of infected bone non-union and osteomyelitis represent a challenge to reconstructive surgery because endogenous regenerative mechanisms are often insufficient to achieve complete bone tissue healing. Among the six million fractures occurring every year in the United States, 5–10 % require further treatment resulting from compromised healing outcomes (Preaemer et al. 1992). Currently, bone is the second most transplanted tissue second only to blood transfusions (Giannoudis et al. 2005).

In clinical practice, autologous cancellous bone grafts, harvested from the iliac crest of patients, are the most effective materials for promoting healing and are widely considered as the "gold standard" in compromised bone healing situations (De Long et al. 2007; Gardiner and Weitzel 2007; Garrido et al. 2011; Perry 1999; Sen and Miclau 2007). Autografts possess all the characteristics necessary for the growth of new bone because they are osteoconductive, osteoinductive, osteogenic and compatible; in addition, implanted autografts do not cause immune activation and disease transmission. Osteoconductivity refers to the situation in which the graft material supports attachment of bone-forming cells (such as osteoblasts and osteoprogenitor cells), and acts as a scaffold on which bone cells can subsequently migrate, proliferate, and deposit extracellular matrix; these cell functions result in new tissue formation. Osteoinductivity is the process that supports proliferation and differentiation of undifferentiated cells into osteoblasts. Osteogenicity refers to the presence of skeletal stem cells and progenitor cells whose functions result in new bone formation.

In some patients, however, autologous bone graft collection can be either associated with morbidity (such as blood loss, post-operative pain, risk of infection and fracture), or can be limited by the availability of adequate graft material specifically in children and in other patients when repeated grafting surgical procedures are required (Sen and Miclau 2007). Moreover, autologous bone grafts increase operative time, hospital stay and associated costs, and often do not promote bone union in segmental bone defects longer than 60 mm in length (Ward et al. 1990).

These aforementioned disadvantages have prompted extensive research focused on the development of alternative methods for promoting new bone formation in non-union defects. For many years, surgeons used banked bone and either natural or synthetic bone substitutes to augment or replace autologous bone grafts. To date, calcium phosphate ceramics specifically hydroxyapatite (HA), tricalcium phosphate (TCP), biphasic calcium phosphate (BCP) and coral exoskeleton are widely used as scaffolds for tissue engineering and regenerative medicine applications (De Long et al. 2007; Gardiner and Weitzel 2007; Garrido et al. 2011; Sen and Miclau 2007). Among these bone substitutes, coral exoskeleton is an interesting scaffold substrate because it is a natural material with remarkable similarities to bone. Researchers first started evaluating coral exoskeleton as a potential bone graft substitute in the early 1970s in both animal models and in human patients (Demers et al. 2002a).

43.2 Brief Review of Bone Composition and Structure

Bone contains several types of special cells including osteocytes (specialized cells), osteoblasts (bone forming cells), mesenchymal stem cells (MSCs) (osteoprogenitors), and osteoclasts (bone resorbing cells). Each one of these cells types has specific functions and participates in either the bone forming, bone resorption or bone remodeling processes.

In terms of its extracellular matrix, bone is a composite material consisting of an inorganic (mineral) phase, and an organic phase (Polo-Corrales et al. 2014; Rey et al. 2009). The mineral, or inorganic, phase of bone mainly contains calcium and phosphorus, and is an analog of HA ($Ca_{10}(PO_4)_6(OH)_2$). Ions such as sodium, magnesium, calcium carbonate, citrate, and fluoride are also associated with the mineral phase of bone.

The organic phase of bone consists mostly of type I collagen (90 % of dry matter), non-collagenous proteins (8 % of dry matter), water, and lipid. Non-collagenous proteins include proteoglycans, fibronectin and several proteins (specifically osteonectin, osteocalcin and bone sialoprotein) which account for a small proportion of the bone extracellular matrix, but serve important functions in cell signaling and metabolism as well as in extracellular matrix organization and mineralization. In addition, several growth factors (including the Bone Morphogenetic Proteins (BMPs) which play a role in inducing bone formation), are also present in the extracellular matrix of bone.

From a structural point of view, bone is a porous material with interconnecting porosity. This characteristic structure is used to classify the type of bone tissue in two categories: cortical or compact bone and cancellous or trabecular bone. Cortical bone is the hard outer layer of bone while cancellous bone is found at the core of vertebral bones in the spine and inside the ends of the long bones. The major differences between cortical and cancellous bones are the pore sizes (which range from 1 to 10 and 200 to 400 μm, respectively) and the extent of porosity (which is 5–30 % and 50–90 %,

respectively). The size and the interconnection of pores in bone are critical for the diffusion of oxygen, nutrients, cells attachment, migration and differentiation and thus for cell functions pertinent to new tissue formation. From a mechanical point of view, the bone porosity is also responsible for the strength of the tissue, which is in the ranges of 1–12 MPa and 150–200 MPa for cancellous and cortical bone, respectively (Demers et al. 2002a).

In summary, the unique physicochemical properties of bone include (i) interconnecting porosity, (ii) constant remodeling by synchronized bone resorption and new bone formation processes throughout human life after birth, (iii) osteoconductivity, and (iv) osteoinductivity (promoted by the bioactive growth factors present in the extracellular matrix of bone). These properties contribute to the most striking characteristics of bone: its ability to heal and/or to regenerate in several cases of bone injury. In some situations, such as a massive segmental long-bone defect (defined experimentally as a defect length greater than 1.6 times the diameter of bone (Toombs et al. 1985), however, the natural bone-healing capability is insufficient to achieve healing and a bone substitute is needed to promote the desired clinical outcomes.

43.3 Properties of an "Ideal" Bone Substitute

An ideal bone substitute should mimic the natural structure and physicochemical properties of bone. Moreover, bone substitutes, like any other surgical implant, should be biocompatible and sterilizable before implantation by a process that does not alter their structure and their physic-chemical properties.

43.3.1 Biocompatibility

An ideal bone substitute should be biocompatible: it should promote cell functions pertinent to new tissue formation and tissue ingrowth and, it should not induce adverse responses (either by direct contact or by release of degradation products) locally and systemically.

43.3.2 Pore Geometry, Porosity and Permeability

Pore size, total porous volume, and interconnecting porosity are important and necessary characteristics of the "ideal" bone graft substitutes. Microporosity (pore diameter size of 1–10 μm) and interconnecting porosity are needed for ingrowth of blood vessels. Macroporosity (pore diameter size > 100 μm) and total porous volume are needed to allow cell migration (throughout the scaffold structure) as well as circulation of biological fluids which distribute life-sustaining oxygen and nutrients necessary for cell functions pertinent to new tissue formation (Amini et al. 2012; Polo-Corrales et al. 2014; Blokhuis et al. 2000). In fact, osteoid formation requires a minimum pore diameter size of 100 μm; moreover, pore diameter sizes of 300–500 μm were reported to be ideal for osseous ingrowth (Kuhne et al. 1994).

"Permeability" is another important aspect for bone grafts because it critically affects biological fluid circulation and new tissue ingrowth. Permeability is the result of the combination of (i) porosity, (ii) pore diameter size and pore distribution, (iii) pore interconnectivity, and (iv) orientation of pores with respect to the flow direction of biological fluid flow (Wu et al. 2009). Although a material scaffold with high porosity is usually highly permeable, scaffolds with similar porosity may have vastly different permeabilities (Li et al. 2003; Wu et al. 2009).

43.3.3 Osteoconductivity

Osteoconductivity, a very important property of a bone substitute material, promotes and supports migration, attachment, proliferation, and also phenotypic expression of bone-forming cells from the surrounding host tissue. Osteoconductivity is directly dependent on the porosity and biocompatibility of the material used as bone substitute.

43.3.4 Osteoinductivity

Osteoinductivity is a growth-factor-mediated process which supports the proliferation and differentiation of undifferentiated cells into osteoblasts, the bone-forming cells. Moreover, osteoinductivity of a bone material substitute is also defined as the ability of a material to induce *de novo* bone formation when implanted in non-bone forming sites (i.e. subcutaneously, intramuscularly and intraperitoneally). The osteoconductivity of a bone substitute may be promoted by one of the following approaches: (i) by incorporating osteoinductive factors (e.g., demineralized bone matrix, platelet-rich plasma, purified growth factors such as BMPs) into the bone substitute, and (ii) by using select materials and specific geometry (shape and size) for the bone substitute that enable entrapment of endogenous circulating growth factors, when implanted *in vivo*. Despite interesting literature reports regarding bone formation in calcium phosphate ceramics implanted in ectopic locations in animals (such as rats and sheep), the definitive answer to the question of material type and geometry that confer intrinsic osteoinductivity to the implanted graft is still, at best, partially understood and, therefore, under current research investigation (Kuboki et al. 1998; LeGeros 2008; Le Nihouannen et al. 2005; Yuan et al. 2010).

43.3.5 Resorption

Material scaffold resorption synchronous to new tissue formation has been identified as a critical aspect for achieving successful outcomes pertinent to bone regeneration (Hing et al. 2007). *In vivo*, scaffold resorption is mediated by physical, chemical and cell-related processes.

In addition to its ability to be resorbed, a scaffold that enables complete bone regeneration must also exhibit a rate of material resorption that is finely-tuned to new bone formation throughout the material graft. In fact, too fast a resorption rate can lead to (i) destabilization of early bone formation and distribution because of scaffold disintegration, and/or (ii) stimulation of an inflammatory response by *in situ* accumulation of increased levels of material degradation products. Alternatively, non-resorbed scaffold material remnants may prevent further new bone deposition and may impair either long-term biocompatibility or the ultimate mechanical competence of the regenerated bone tissue (Schliephake and Kage 2001).

43.3.6 Bioactivity

Bone bioactivity is defined as the capability of a bone-substitute material to promote, and achieve, direct adhesion and strong bonding with the surrounding host bon surface tissue (LeGeros et al. 2008; Blokhuis et al. 2000). In this respect, formation of a carbonate apatite layer on the implant material surface *in vitro*, is one of the most important criteria for determining the bioactivity of material substitutes for bone (LeGeros 2008; LeGeros et al. 2008).

43.3.7 Mechanical Properties

An ideal bone substitute implanted in a long-bone defect should fulfill the daily functional loading requirement of the specific anatomical sites. For this reason, the mechanical properties of such material should be close to those of native bone. The mechanical properties of any material under consideration as a bone substitute depend on the chemical composition, porosity, structure and architecture of the graft. After implantation in bone-defect sites, any subsequent material resorption that may take place *in vivo* decreases the mechanical properties of these implants, jeopardizing the mechanical stability of the tissue regeneration process; subsequent surgical intervention and osteosynthesis may be required in such cases.

43.4 Intrinsic Properties of Coral

Natural coral bone graft substitutes (Fig. 43.1) are derived from the exoskeleton of marine madreporic corals. The coral genera most studied and used both *in vitro* and *in vivo* for medical applications are *Porites* and *Acropora* and, to a lesser extent, *Lobophyllia*, *Goniopora*, *Polyphyllia*, *Favites* and *Pocillopora*. These corals belong to the phylum Cnidaria, the subclass Hexacorallia, to the class Anthozoa, and the order Scleractinia (also called "stony coral").

Geography sites for harvesting coral scaffolds are the Caribbean Sea, New Caledonia, the Red Sea, the East Coast of Africa, the Thailand Sea, the coast of Hainan Island and the coastline of Australia.

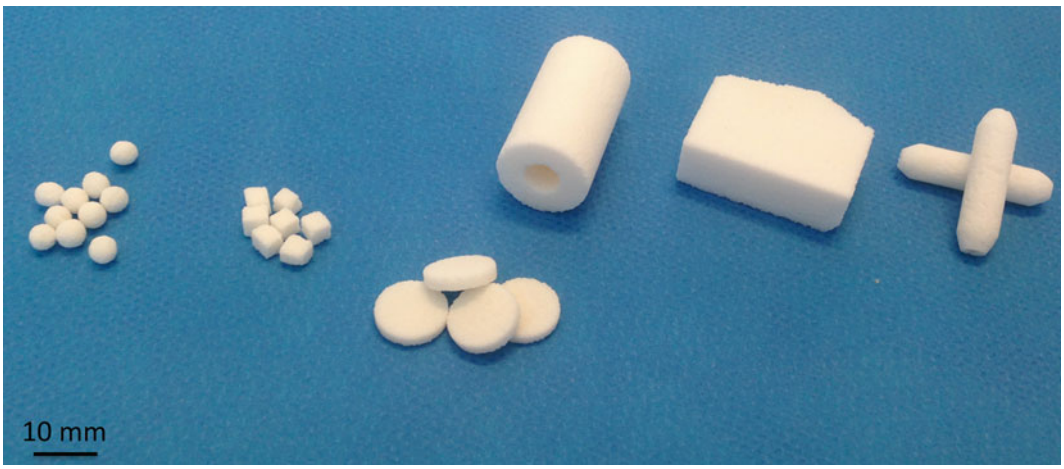

Fig. 43.1 Examples of various coral implants used for bone regeneration purposes

43.4.1 Composition

The coral exoskeleton consists of 97–99 % calcium carbonate ($CaCO_3$) in the form of aragonite (a naturally occurring crystalline form) with 1–3 % organic material and trace elements (specifically, magnesium, sodium, amino-acid, potassium, strontium, fluorine, and phosphorus) (Guillemin et al. 1989; Mainard et al. 2011; Ramos-Silva et al. 2014) (Table 43.1). The trace elements found in coral are essential in stabilizing its aragonite structure and in aiding its natural calcification process (Braye et al. 1996; Irigaray et al. 1993a, c). Moreover, these elements play a role in new bone formation when corals are implanted *in vivo*: specifically fluorine promotes osteoblast proliferation and strontium contributes to the mineralization process (Demers et al. 2002a).

43.4.2 Structure

In coral, the aragonite crystal is about 100 μm long and is prismatic in shape (Guillemin et al. 1989). Coral exoskeletons are porous structures with open, longitudinally- and transversally-interconnected communicating pores and a rough surface (Puvaneswary et al. 2013). The size of the macropores varies from 150 to 1000 μm depending on the coral type; the porosity varies from 20 % to 60 % in these genera of corals (Table 43.2). The porous structure varies among corals (Fig. 43.2). *Porites*, *Acropora* and *Goniopora* have intercommunicating pores ("open" porosity) throughout the exoskeleton. The extent of porosity for *Porites* and *Goniopora* is similar to that observed in trabecular bone (50–80 %) (Wu et al. 2009). It should be noted that about 30 % of the pores in *Porites* are below 100 μm in diameter (Wu et al. 2009).

The structure of *Acropora* is similar to that found in either *Porites* or *Goniopora*. Compared to *Goniopora* and *Porites*, however, *Acropora* has many irregularly shaped, but similarly oriented pores (Wu et al. 2009). In addition, *Acropora* has the largest pore diameter size, and the highest permeability; the latter aspect allows fluid transport in vitro and, thus contributes to successful outcomes of these constructs when used for tissue engineering applications.

Favites, *Pocillopora*, *Lobophyllia* and *Polyphyllia* have a "closed" porosity because their pores are not completely interconnected. The structure of *Favites*, *Pocillopora* and *Lobophyllia* is similar to that of cortical bone because a dense and compact outer wall (theca) surrounds thin inner septa. The structure of *Polyphyllia* is similar to that of trabecular bone.

From the scaffold point of view, the size and interconnectivity of pores contribute for cell invasion, space for new blood vessel formation and for coral resorption; all these aspects contribute to new bone formation. From the material mechanics perspective, however, large pores and extended porosity may weaken the scaffold performance under mechanical loading for long-term applications.

Coral exoskeleton is available for medical applications as granules (particles, spheres, cubes, disks), and massive, three-dimensional (blocks and shaped implants) forms of different sizes (Fig. 43.1).

Coral granules are used in pre-clinical and clinical studies, in which the size and shape of the patients' bone defects are highly variable, because granules can be used for filling purposes and, thus, accommodate defects of any size and shape.

Table 43.1 Composition of the natural coral exoskeleton (Demers et al. 2002a; Guillemin et al. 1989)

Calcium carbonate	97–99 %
Magnesium	0.05–0.2 %
Sodium	0.4–0.5 %
Amino-acids	0.07 %
	Aspartic acid: 155 ppm
	Serine: 55 ppm
	Proline: 125 ppm
	Alanine: 95 ppm
	Methionine: < 0.5 ppm
	Leucine: 70 ppm
	Phenylalanine: 30 ppm
	Theonine: 25 ppm
	Glutamic acid: 110 ppm
	Glycine: 125 ppm
	Valine: 35 ppm
	Isoleucine: 40 ppm
	Tyrosine: 1.5 ppm
	Arginine: 40 ppm
Potassium	0.02–0.03 %
Strontium	Trace
Fluorine	Trace
Phosphorus (in phosphate form)	Trace

43.4.3 Mechanical Properties

The constraint of rupture of massive coralline implants used as bone substitutes is between 20 and 400 MPa (Table 43.2) indicating that coral has initial mechanical properties either similar or superior to those of cancellous bone in compression (Mainard et al. 2011). In order to fill a bone defect, however, coral implants in the form of granules, instead of in a massive form, are used; this aspect affects the mechanical properties of the implant. Moreover, since natural coral is resorbable *in vivo*, its mechanical properties change over time because of material degradation.

Table 43.2 Physical and mechanical properties of several coral species[a]

	Porites	*Acropora*	*Lobophyllia*		*Goniopora*	Trabecular bone	Cortical bone
			Interior	*Exterior*			
Macroporosity (µm)	154 ± 53	412 ± 212	500–1000		314 ± 116	NA	NA
	[89–435][b]	[124–941]			[145–651]		
Microporosity (µm)	5–15	5–20	NA		NA	NA	NA
Global Porosity (% volume)	47–64	12–60	45–60		23–80	50–80	5–30
Permeability (m^2)	0.12×10^{-9}	4.46×10^{-9}	NA		0.35×10^{-9}	$0.4–11 \times 10^{-9}$	
Resistance to compressive strength (MPa)	12.1	7.1	NA		2.6	1–12	131–283
Constraint to rupture (MPa)	20–31	78–142	6–10	315–365	NA	1–12	150–200
Deformation at rupture (%)	0.22–0.30	0.41–0.65	0.22–0.28	0.45–0.51	NA	NA (*because bone is viscoelastic*)	
Young's modulus (MPa)	7620–8360	21,300–27,900	NA		NA	50–400	17,000

Notes
It should be noted that structural characteristics and morphological variations of the same genus of coral could result from the different habitat types of its provenance
NA Not Assessed
[a]From (Demers et al. 2002a; Guillemin et al. 1987, 1989; Mainard et al. 2011; Vuola et al. 1998, 2000)
[b]About 30 % of the *Porites* pores have diameters smaller than 100 µm

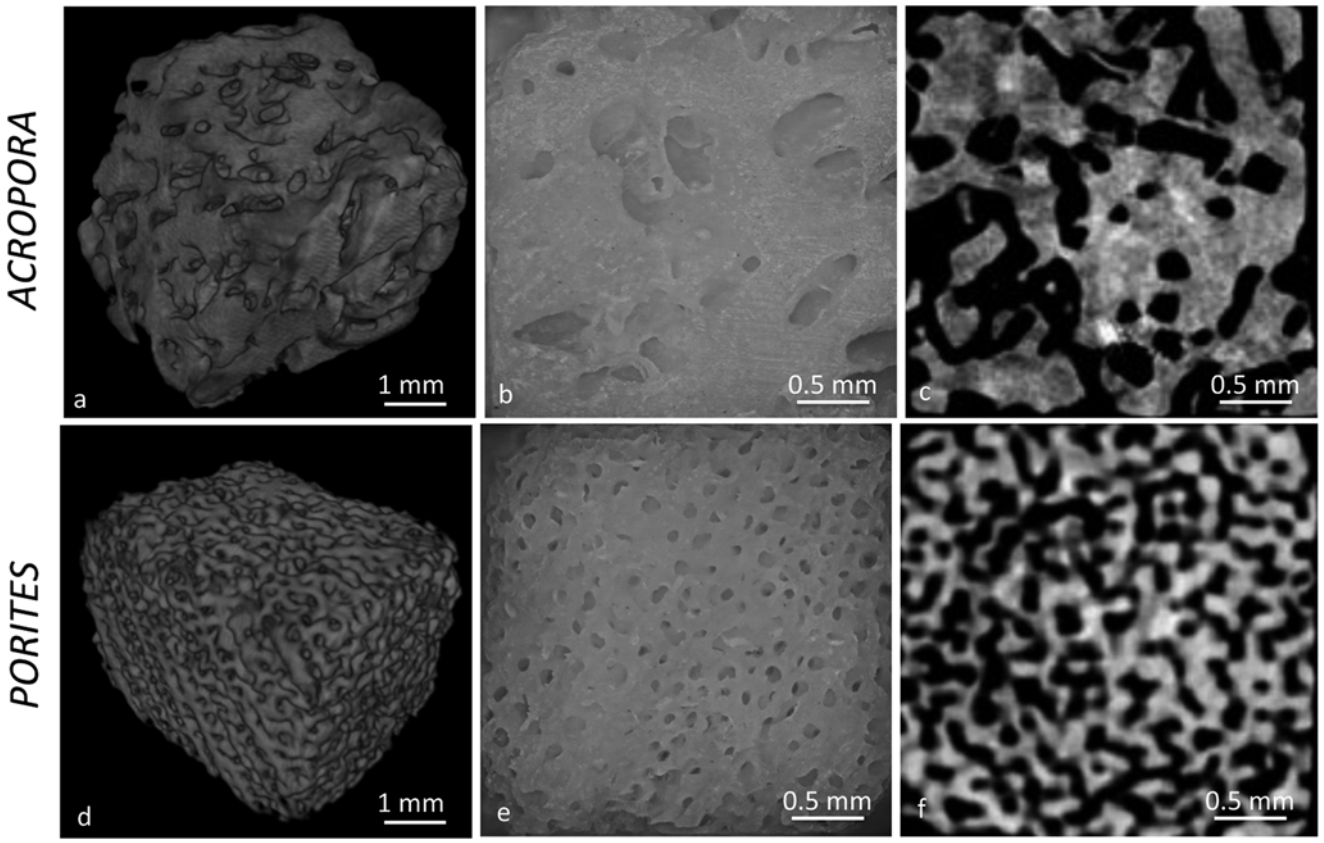

Fig. 43.2 Structure and aspects of *Acropora* and *Porites* scaffolds Micrographs illustrating the microstructure of *Acropora* (**a**, **b**, **c**) and *Porites* (**d**, **e**, **f**) scaffolds. Micro-CT reconstruction of *Acropora* and *Porites* scaffold (**a**, **d**), light microscopy (**b**, **e**) and micro-CT cross-sectional (**c**, **f**) images showing total porosity and the interconnected porosity of these two types of corals scaffolds used for bone regeneration

From a mechanical point of view, implantation of natural coral in a load-bearing bone defect requires (i) use of osteosynthesis, for stress shielding purposes, and (ii) knowledge of the scaffold material resorption behavior. For these reasons, the mechanical properties of coral exoskeleton is a limitation of this type of material scaffolds.

43.4.4 Manufacturing, Quality Control, and Sterilization Processes

After harvesting, the corals are cleaned to remove macroscopic impurities and organic contents by using ultrasound treatment and immersion in a 5 % sodium hypochlorite solution for 30 min before drying at 90 °C (Papacharalambous and Anastasoff 1993; Fricain et al. 1996). The composition of the resulting coral exoskeleton is then determined by various established methods (including titrimetry, atomic absorption, chromatography, etc.). These approaches ensure consistency of material quality and the absence of foreign, and potentially adverse, entities (Clarke et al. 2011; Guillemin et al. 1989).

A requirement before use of all medical material devices, including coral bone substitutes, is sterilization in a way that does not alter the coral structure and chemical composition.

Sterilization of the coral exoskeleton can be achieved by gamma radiation (a 2.5 Mrad, 25 KGy dose) (Damien et al. 1994; Roux et al. 1988a; Mainard et al. 2011), ethylene oxide (a 4-h process followed by an 8-h period to assure desorption of adsorbed ethylene oxide on coral) (Jammet et al. 1994; Gao et al. 1997) as well as by steam sterilization (using autoclaving conditions).

When the effects of temperature were studied by X-ray diffraction, the analysis revealed that high temperature affects coral structure and composition; specifically, mineral transformation of aragonite to calcite was observed at temperatures above 270 °C while complete conversion occurred during exposure to 400 °C for 30 min (Irigaray et al. 1993b; Wu et al. 2009). For these reasons, coral scaffolds are thus mainly sterilized by autoclaving (for 20 min at 121 °C) (Manassero et al. 2013; Viateau et al. 2007, 2016; Petite et al. 2000; Bensaid et al. 2005; Guillemin et al. 1989; Wu et al. 2009), because under these conditions, the coral composition and structure are not affected (Irigaray et al. 1993b).

For clinical applications, and prior to implantation, soaking coral in an antibiotic solution was proposed to prevent subsequent bacterial colonization (Patat et al. 1992; Patel et al. 1980).

43.5 Properties of Coral Post Implantation, *In Vivo*

43.5.1 Resorption

In vivo, natural coral scaffolds are extensively resorbed by a combination of physicochemical dissolution processes and cell-mediated degradation (Demers et al. 2002a, b; Guillemin et al. 1987, 1989, 1995) (Fig. 43.3).

When implanted *in vivo*, in the presence biological fluids, a carbonate apatite layer is formed on the coral surface (Blokhuis et al. 2000). The presence of such a layer makes the coral a bioactive biomaterial because it attracts macro-

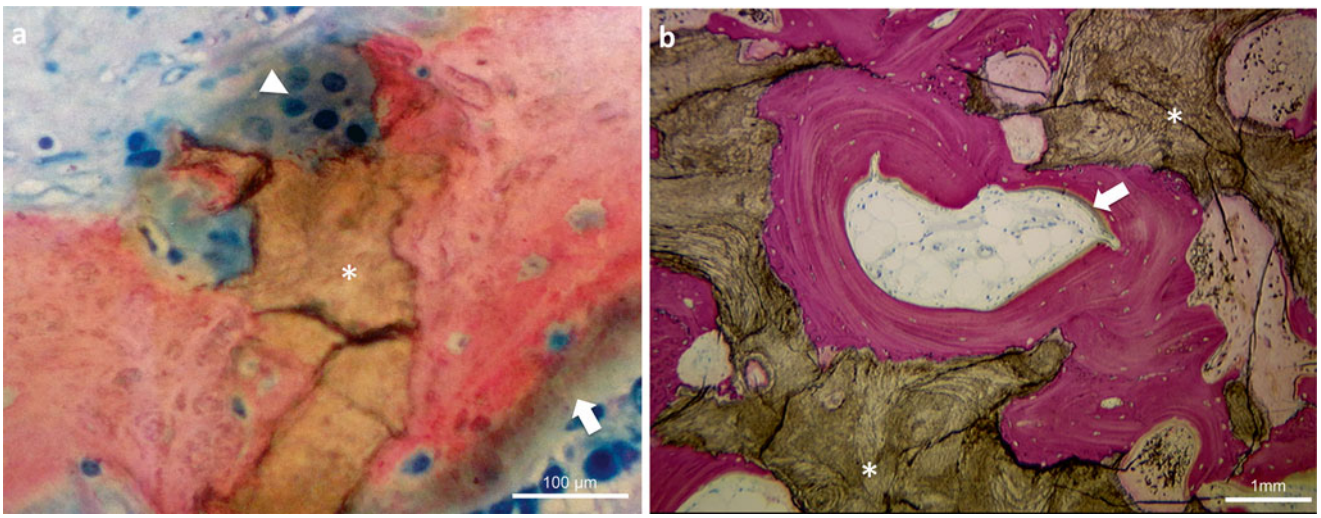

Fig. 43.3 Cell-mediated coral resorption and osteoconductive bone formation on coral scaffolds implanted in sheep metatarsal bone. Frame (**a**): cell-mediated coral resorption. The *white arrowhead* points to a multinucleated giant cell which is degrading the coral scaffold (*white star*) while newly-formed bone is deposited (*white arrow*). Frame (**b**): illustration of the osteoconductive property of coral scaffolds. Abundant, newly-formed bone was present on direct apposition to the coral scaffold (*white star*), which is progressively resorbed. The *white arrow* points to osteoid deposition and, thus, to areas of bone apposition. Bone, cells, and coral were stained red, blue, and brown, respectively. Stains: Stevenel Blue and von Gieson picrofuschin

phages, multinucleated giant cells, fibroblasts and osteoclasts which interact on the modified coral surface (Gao et al. 1997; Fricain et al. 1998); these interactions initiate the cell-induced degradation of coral. For this reason, coral resorption *in vivo* is most active in the parts of the coral implant which are in contact with native bone and then proceeds towards the periphery (Braye et al. 1996; Demers et al. 2002a).

The exact mechanisms of coral resorption and the respective role of the surrounding biological fluids and osteoclast-like cells remain incompletely understood and are still subject of scientific debate and research. Interesting pertinent aspects include that carbonic anhydrase (an enzyme which converts CO_2 and H_2O into HCO_3^- and H^+) plays a key role in the coral resorption process (Guillemin et al. 1987). In fact, production of H^+ lowers the pH either at the host-implant interface (by dissolving the coral exoskeleton) or intracellularly (by degrading the coral inside cells) (Fricain et al. 1998; Guillemin et al. 1987, 1989, 1995; Gay and Mueller 1974; Demers et al. 2002a). Although the exact mechanism of coral resorption is not fully understood, it is established that only about 5 % of the released calcium remains locally at the degradation site and 95 % ends up in the circulation (Irigaray et al. 1993a). Interestingly, the released calcium at the degradation site favors local osteoblast proliferation, contributes to extracellular matrix mineralization, and promotes increased expression of growth factors that enhance new bone formation (Hoppe et al. 2011; Maeno et al. 2005; Marie 2010; Valerio et al. 2009; Yuan et al. 2010; Zaidi et al. 2004).

The rate of coral resorption depends on many parameters such as coral chemical composition, structure (porosity and crystallinity), geometry (size and shape of the coral graft) as well as on host characteristics (such as animal species and anatomical site of implantation). In fact, compared massive scaffolds, resorption is faster for coral with high porosity and for granular scaffolds; for example *Acropora* coral scaffolds degrade more slowly than *Porites* coral scaffolds when implanted into either diaphyseal or metaphyseal bone defects in sheep and pigs. A very intriguing observation is that the coral resorption rates differ among species, making comparison between different animal models and clinical studies challenging if not impossible (Table 43.3).

Several studies using various animal models provided evidence that coral resorption can be pharmacologically modulated (Guillemin et al. 1987; Arnaud et al. 1999; Gao et al. 1997; Cunin et al. 2000). Although some studies reported decreased coral resorption in either direct binding or association with BMP-2 (Arnaud et al. 1999; Sciadini et al. 1997b), most studies agree that coral resorption is increased in the presence of BMP-2 (a potent chemical osteoinductor which plays a role in osteoclast formation, activation and bone resorption activity) (Gao et al. 1997; Boden et al. 1995a, b, 1997, 1999, Demers et al. 2002a). Such enhanced coral resorption could be the consequence of BMP-2-mediated increase of either the number or the bioactivity of osteoclasts as well as of an exaggerated inflammatory environment at the implantation site (Huang et al. 2014; Kim et al. 2014; Zara et al. 2011). Enhanced coral resorption was also reported with fibrin glue in a sheep model of vertebroplasty 2 months post implantation (Cunin et al. 2000).

In contrast, coral resorption can be attenuated by either adhering and functioning stem cells (Manassero et al. 2013), or type-I collagen (Ma et al. 2000a, b) or by the administration of diuretic acetazolamide (a known inhibitor of carbonic anhydrase) (Guillemin et al. 1987, 1989, 1995). Interestingly, in some of the animals models tested, the bone healing process was altered in the acetazolamide-treated cases; corroborating the explanation that coral resorption is a prerequisite for successful bone healing.

For the aforementioned reasons, several research groups have suggested that resorption of coral scaffolds could be one of the key advantages of their use as a bone substitute (Louisia et al. 1999; Manassero et al. 2013; Petite et al. 2000). In contrast, other synthetic bone substitutes widely used such as calcium sulfate, β-TCP, and HA, are resorbed either too quickly (to support bone cell attachment and function pertinent to new bone growth) or too slowly, remaining unresorbed at the implantation site for several years post-implantation (von Doernberg et al. 2006; Cook and Cook 2009; Kon et al. 2000; Marcacci et al. 1999).

To date and despite the advantages of material biodegradation and resorption *in vivo*, the optimal conditions that assure consistent bone regeneration when coral scaffolds are used remain unknown.

Alternatives to using natural coral, as tissue engineering scaffolds, are either total or partial conversion of coral into HA either by replication, microwave treatment, or, most frequently, hydrothermal processes (Roy and Linnehan 1974; Murugan and Ramakrishna 2004; Sivakumar et al. 1996; Vecchio et al. 2007; White and Shors 1986; White et al. 1972; Clarke et al. 2011; Nandi et al. 2015). By using these processes, either a partial or total conversion of coralline calcium carbonate to HA is achieved while preserving the interconnecting pores originally present in the coral materials. The resulting material, known as "coralline HA" or "coralline hydroxyapatite/calcium carbonate composite" (CHACC), has similar porosity as the natural coral but exhibits limited resorption (due to the outer layer of HA, which slows down the material resorption process). Compared to synthetic HA, CHACC has small size crystals and contains small amounts of CO_3^{2-} and of Mg-substituted β- TCP (LeGeros et al. 1991, 2008). One serious concern regarding CHACC is that, when the HA layer is either compromised or lost, fast resorption of the core of the coral results (Clarke et al. 2011). *In vivo*, however, CHACC scaf-

Table 43.3 Resorption rates of various natural coral scaffolds post-implantation in various species and implantation sites

Coral genera	Species	Implantation site	Coral implant form	Coral resorption rate post-implantation
Porites	Mice	Subcutaneous	Particles (600–1000 μm in diameter) within fibrin gel	Almost complete by the end of 1 month (personal data)
	Rabbits	Femoral condyle	Plugs (either 4×4 mm or 20×10 mm×10 mm)	Complete within 4–6 months (Shahgaldi 1998; Roudier et al. 1995)
		Vertebrae	Particles (500 μm in diameter)	Complete by 12 weeks (Tho and Krishnamoorthy 1996)
	Dogs	Ulnar bone (diaphysis)	Cylinders (5 mm diameter; 8 mm height)	Complete by 8 weeks (Guillemin et al. 1987)
	Pigs	Femoral and tibial bone (metaphysis and diaphysis)	Cylinders (8 mm diameter; 10 mm height)	Almost complete by the end of 1 month (93 %) and complete by the end of 2 months (Guillemin et al. 1989)
	Sheep	Vertebrae	Granules (1.7 mm diameter)	Almost complete by 4 months (Cunin et al. 2000)
		Femoral and tibial bone (metaphysis and diaphysis)	Cylinders (8 mm diameter; 10 mm height)	64 % by 1 month and 90 % by 2 months (Guillemin et al. 1989)
		Metatarsal bone (diaphysis)	Granules (3×3×3 mm) in 25×15 mm bone defects	Almost complete by 2 months (Viateau et al. 2007)
			CHACC Granules (3×3×3 mm) in 25×15 mm bone defects	Almost complete by 4 months (Bensaid et al. 2005)
			Cylinders (25×15 mm)	Almost complete by 2–4 months (Petite et al. 2000)
		Subcutaneous	Granules (3×3×3 mm) in a 25×15 mm pouch	80 % by 2 months (Viateau et al. 2016)
	Humans	Cranium	Plug (10 mm in diameter)	40 % within 8 months; almost complete after 1 year (Roux et al. 1988a, b, 1995)
		Iliac bone	Wedge-shaped blocks (30×30×4–12 mm)	Partial (approximately 50 %) after 1.6–2.3 years (Vuola et al. 2000)
		Tibial bone	Wedge-shaped blocks (20×10×10 mm or 15×5×5 mm)	Mostly peripheral resorption after 28 months (de la Caffiniere et al. 1998; de Peretti et al. 1996)
		Calcaneus bone	Wedge-shaped blocks (20×10×10 mm or 15×5×5 mm)	Mostly peripheral resorption after 12–28 months (de Peretti et al. 1996)
Acropora	Mice	Subcutaneous	Particles (600–1000 μm in diameter) within fibrin gel	Almost complete by 1 month (personal data)
		Subcutaneous	Cylinders (3.5×2 mm)	12–16 % by 10 weeks (personal data; (Manassero et al. 2012))
		Femoral bone (diaphysis)	Cylinders (3.5×2 mm)	20–28 % by 10 weeks (personal data; (Manassero et al. 2012))
	Dogs	Vertebrae	Granules (6×6×6 mm)	Almost complete by 8 weeks (Fuller et al. 1996)
	Pigs	Femoral and tibial bone (metaphysis and diaphysis)	Cylinders (8×10 mm)	42 % by 1 month and 64 % by 2 months (Guillemin et al. 1989)
	Sheep	Femoral and tibial bone (metaphysis and diaphysis)	Cylinders (8×10 mm)	33 % by 1 month and 56 % by 2 months (Guillemin et al. 1989)
		Subcutaneous	Granules (3×3×3 mm) in a 25×15 mm pouch	60 % by 2 months (Viateau et al. 2016)
		Metatarsal bone (diaphysis)	Granules (3×3×3 mm) in a 25×15 mm bone defect	Almost complete by 4–6 months (Manassero et al. 2013)
	Humans	Face	Granules of various sizes	5/54 implants fully resorbed after 1–3 years; very little resorption of the other implants (Marchac and Sandor 1994)
Favites	Dogs	Mandible and femoral bone	Granules of various sizes	Complete by 6–8 months (Zeng et al. 1997)
		Femoral bone	Cylinders (10×28 mm)	Almost complete by 12–18 months (Guillemin et al. 1987)

(continued)

Table 43.3 (continued)

Coral genera	Species	Implantation site	Coral implant form	Coral resorption rate post-implantation
Lobophyllia	Humans	Vertebrae	Granules of various sizes	Almost complete by 1 year in children; little resorption by 2 years in adults (Patat et al. 1992; Pouliquen et al. 1989)
	Dogs	Femoral bone	Cylinders (10×28 mm)	Almost complete by 12–18 months (Guillemin et al. 1987)
Polyphyllia	Sheep	Nose and mandible	Plugs (20×10×5 mm)	None by 12 months (Jammet et al. 1994)
Montastrea	Rabbits	Femoral condyle	Plugs (20×10×10 mm)	Partial (<50 %) by 20 weeks (Roudier et al. 1995)
Dichocoenia	Rabbits	Femoral condyle	Plugs (20×10×10 mm)	None by 20 weeks (Roudier et al. 1995)

folds exhibit very little resorption; moreover, formation of new bone is not superior to that obtained with natural corals (Bensaid et al. 2005; Coughlin et al. 2006; Shors 1999).

43.5.2 Osteoconduction

The composition, three-dimensional (3D) structure and porosity (microporosity, macroporosity as well as open and interconnected pores) of coral scaffolds are critical and necessary aspects to allow blood vessel formation and distribution as well as cell infiltration throughout the volume of material scaffolds. The aforementioned aspects make coral exoskeleton an excellent osteoconductive material for clinical applications.

In fact, when either *Goniopora*, *Favites*, *Lobophyllia*, *Porites* or *Acropora* coral implants were used in cases of either cortical or cancellous defects in sheep or dogs (Guillemin et al. 1987, 1989), the newly-formed bone apposed directly on the carbonated coral exoskeleton, as well as on the native bony edges. At the same time, newly-formed bone progressively filled the empty regions resulting from coral resorption. These findings provide evidence regarding both the resorption and osteoconductivity of coral scaffolds and justify their use as filling material in the surgical treatment of bone defects (Fig. 43.3). Interestingly, despite extensive research regarding coral osteoconductivity, it is still debated among researchers in this field whether coral resorption occurs before new bone deposition or whether new bone deposition occurs on the coral which is subsequently resorbed (Richard et al. 1998; Shors 1999; Guillemin et al. 1989; Manassero et al. 2013).

A very interesting pre-clinical model for studying the efficiency of bone substitutes in massive segmental bone defects is the sheep model of metatarsal bone defect (Viateau et al. 2004, 2006, 2007, 2010). In this model, a 25-mm long mid-diaphyseal metatarsal segmental bone defect is created and, subsequently, stabilized using plate osteosynthesis. This model creates bone defects of clinically-relevant volumes under load-bearing conditions which mimic those occurring in humans. Moreover, this model uses straight bone, an aspect that results in reproducible surgical creation of cylindrical defects of clinically-relevant volume and dependable plate fixation. When these defects remained unfilled, consistent atrophic bone non-union was observed 6 months post-operatively (Viateau et al. 2006, 2007). In contrast, when these defects were filled with morselized autologous cortico-cancellous grafts (the "gold standard" for bone defect in humans), bone healing was consistently observed 6 months post-operatively (Viateau et al. 2004, 2006). This ovine metatarsal defect model provided evidence that a critical-size defect healed when grafted with the "gold standard" material, and has proven most suitable in studying bone replacement strategies.

When either *Porites* or *Acropora* coral implants, in either massive or granular form (Bensaid et al. 2005; Manassero et al. 2013; Petite et al. 2000; Viateau et al. 2007), were used to fill the aforementioned critical-size metatarsal bone defects in sheep, radiographic, micro-tomodensitometric and histological analyses revealed that the newly-formed bone was present only in the vicinity of the bone resection edges. Once the coral was resorbed (within 2 and 4 months post-operatively) in this sheep model, fibrous tissue (similar to that observed when metatarsal critical-size defects were left empty in the control sheep) was observed; moreover, bone non-union was observed in all animals tested (Bensaid et al. 2005; Manassero et al. 2013; Petite et al. 2000; Viateau et al. 2007). These findings indicate that, despite their demonstrated osteoconductive properties, coral scaffolds alone fail to achieve bone union in critical-size bone defects; this evidence emphasizes the requirement for either osteoprogenitor cells, osteoinductive growth factors, or both to obtain bone union in such cases.

43.6 The Coral Scaffold as Cell– and Drug-Delivery Systems for Bone Regeneration

43.6.1 The Coral Scaffold as a Cell Carrier

Tissue-engineered bone constructs containing osteoprogenitor cells, such as bone marrow MSCs, with osteoconductive scaffolds have been explored as potential alternatives to

autologous bone grafts that promote bone healing in massive bone defects (Petite et al. 2000; Viateau et al. 2007). In fact, the 3D architecture of scaffolds in which the seeded cells are distributed and interact with other cells and with the extracellular matrix provides a supportive milieu that enhances cellular functions pertinent to new tissue formation (Cukierman et al. 2001; Puvaneswary et al. 2013). Several studies performed using clinically-relevant animal models (such as dogs, pigs and sheep) provided the "proof of concept" for a strategy which uses MSCs loaded within several types of osteoconductive scaffolds (Bensaid et al. 2005; Bruder et al. 1998; Petite et al. 2000; Viateau et al. 2007). Although these studies demonstrated that MSCs significantly enhanced new bone formation, the results were inconsistent and did not match the osteogenic capability, and clinical success, of autologous bone grafts; the latter still remain the "gold standard" for bone repair clinically.

A critical parameter identified as a cause of such failure is insufficient tissue regeneration around the biomaterial soon after implantation. Since adhesion of cells on biomaterials and subsequent function pertinent to new tissue formation is crucial, a lot of research endeavors focused on designing and formulating biomaterial structures that facilitate the aforementioned cell-material interactions and promote new tissue regeneration (Chatzinikolaidou et al. 2015). Moreover, the scaffolds for tissue engineering applications must be three-dimensional (to simulate the structure of natural tissue), as well as highly macroporous and interconnected (to support cell attachment, migration/distribution, proliferation, etc.) and to assure delivery of the nutrient and oxygen supplies needed for cell survival, viability, and function (Chatzinikolaidou et al. 2015; Karageorgiou and Kaplan 2005; Shor et al. 2007).

An ideal scaffold as delivery system for cells should thus meet the following requirements: it must (i) be easy to formulate and prepare; (ii) be non-cytotoxic and biocompatible; (iii) allow cell adhesion, migration, proliferation and other functions pertinent to new tissue formation; and (iv) promote new bone formation synchronous to the scaffold material resorption *in vivo*. Coral scaffolds meet these criteria and, for this reason, are promising cell-carrier biomaterials. In this respect, the ability of coral scaffolds, combined with osteoprogenitor cells, to enhance bone healing and new bone formation has been the subject of several studies and literature reports (Bensaid et al. 2005; Manassero et al. 2013; Petite et al. 2000; Viateau et al. 2007).

43.6.1.1 Cytocompatibility of Coral Scaffolds

Several studies reported that cells of different sources (such as MSCs, dental pulp stem cells, adipose-derived stem cells, and human osteoblasts) adhere, proliferate and function in a time-dependent manner *in vitro* when loaded on either natural coral scaffolds (Bensaid et al. 2005; Manassero et al. 2012, 2013; Petite et al. 2000; Viateau et al. 2007, 2016; David et al. 2011; Becquart et al. 2012; Wu et al. 2009; Puvaneswary et al. 2013; Al-Salihi 2009; Zhu et al. 2010a; Tran et al. 2011; Damien et al. 1993) or CHACC scaffolds (Mygind et al. 2007; Mangano et al. 2011). In fact, a two to five fold increase in MSCs proliferation was observed after 6–8 days under either static (Manassero et al. 2013) or dynamic cell culture conditions (in either perfusion bioreactors (David et al. 2011) or spinner flasks (Mygind et al. 2007; Viateau et al. 2007)). These studies confirmed that coral is both non-cytotoxic and biocompatible.

Furthermore, microscopic and histological analyses revealed that MSCs reached confluence and covered only the outer surfaces of the coral scaffold, but had not infiltrated the volume of the scaffold and had not reached its center, 1 week after cell seeding (Fig. 43.4) (Manassero et al. 2013; Viateau et al. 2007). Another study bypassed this limitation by using a fibrin matrix and achieved uniform distribution of cells within the coral scaffold (Zhu et al. 2010a).

Furthermore, other studies provided evidence that MSCs seeded on coral scaffolds, and cultured *in vitro* in either standard or osteogenic differentiation medium (containing ascorbic acid, β-glycerophosphate and vitamin D2), expressed osteospecific markers (such as alkaline phosphatase, osteocalcin and osteopontin) and also exhibited calcium-containing nodules in their extracellular matrices (Al-Salihi 2009; Al-Salihi and Samsudin 2004; Mygind et al. 2007; Puvaneswary et al. 2013; Mangano et al. 2011; Tran et al. 2011). A noteworthy observation in these studies is that stem cell differentiation occurred without exogenous addition of any differentiation factor.

The pore size of the scaffolds affects both the proliferation and differentiation of the seeded cells. In this respect, a study highlighted that, despite a slower proliferation rate, human MSCs exhibited a faster rate of osteogenic differentiation when seeded on CHACC scaffolds with an average 200-μm pore diameter compared to results obtained when these cells were seeded on scaffolds with an average 500-μm pore diameter (Mygind et al. 2007). This outcome may be explained by the fact that the cells on the 200-μm scaffolds reached confluence earlier, a condition which promoted earlier cell differentiation (Mygind et al. 2007). Whether the results obtained using CHACC scaffolds can be extrapolated to what is to be expected when using natural coral scaffolds, remains unknown. A very promising, and exciting, result was provided by an *in vitro* study, which showed that the osteogenic differentiation of MSCs on *Porites* coral scaffolds is better than that obtained using dried corticospongious bone graft (Puvaneswary et al. 2013).

In summary, the results reported in the literature validate the biocompatibility of coral and, most importantly, provide evidence that this material is non-cytotoxic and supports functions (such as adhesion, migration, proliferation, etc.) of

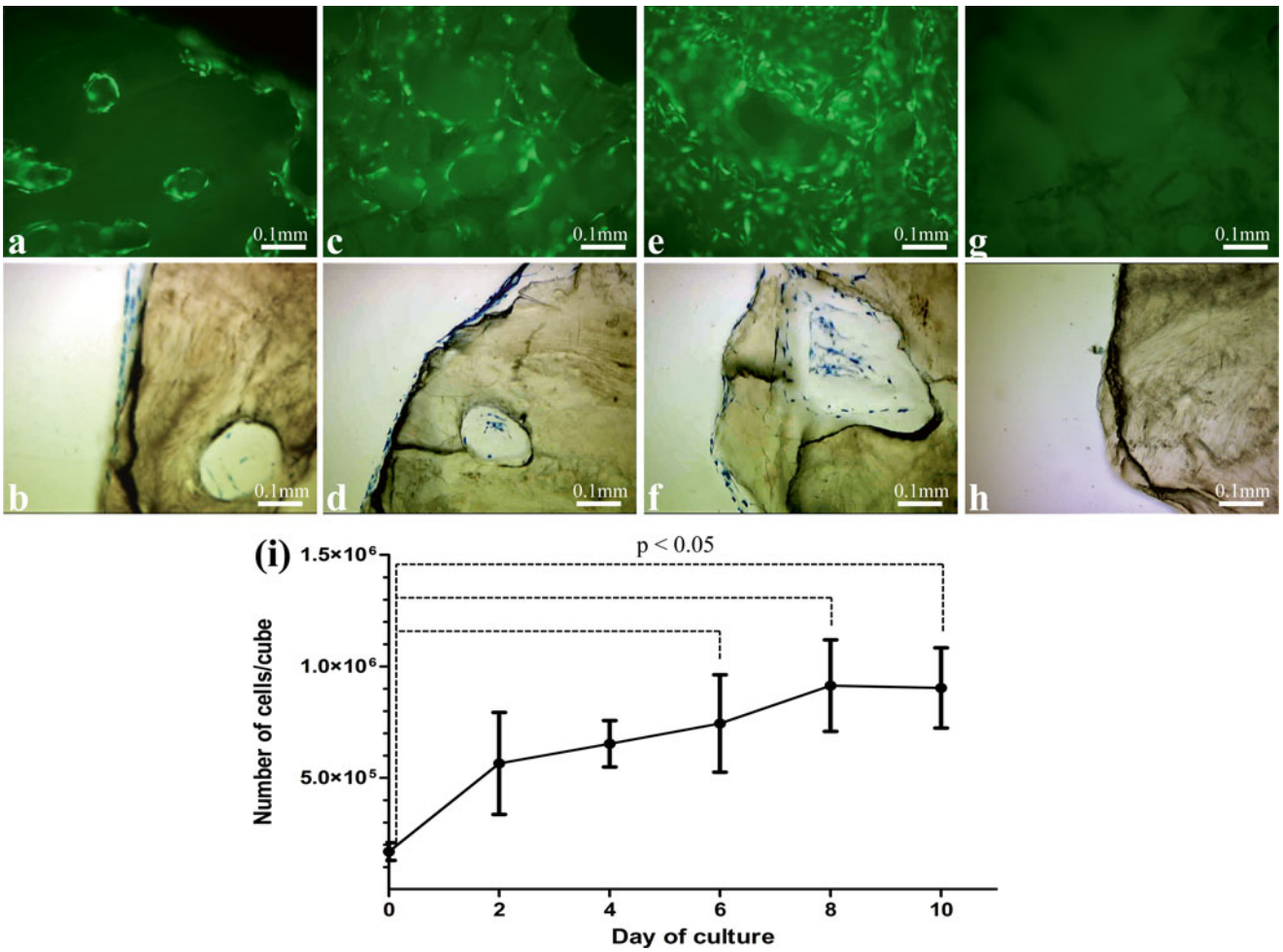

Fig. 43.4 Representative fluorescent and histological micrographs of sheep MSCs seeded on the outer surface of *Acropora* coral scaffolds. Representative micrographs of a coral scaffold seeded with CFDA – MSCs after two (frames **a**, **b**), five (frames **c**, **d**), and eight (frames **e**, **f**) days of culture, compared to results obtained using a coral scaffold without MSCs (frames **g**, **h**). Stains: CFDA labeling green (**a**, **c**, **e**, **g**); and Stevenel Blue staining blue (frames **b**, **d**, **f**, **h**). Scale bar = 0.1 mm. The time course of MSCs proliferation in the coral scaffold is shown in frame (**i**). The seeded cells adhered on the scaffold (frames **a**, **b**) and proliferated (frames **c**, **d**, **e**, **f**, **i**) in a time-dependent manner. Compared to the number of cells seeded at the beginning of the experiment (day 0), cell proliferation was statistically significant on day 6, and remained unchanged till the end of the experiment (10 days). After 8 days, the cells reached confluence (five fold increase compared to the seeding density) and covered the outer surface of the scaffold. Histological analysis revealed the presence of MSCs within a depth of only 1-mm from the outer surface of the *Acropora* scaffolds (frames **e**, **f**) (Reprinted with permission from Tissue Eng Part A, 2013, 19(13–14), 1554–1563)

differentiated cells as well as differentiation of stem cells *in vitro*. Taken together, these data establish the potential of this biomaterial, in combination with appropriate cells, for bone tissue-engineering and for tissue regeneration applications.

43.6.1.2 Osteogenicity of Constructs Containing Coral Scaffolds and Cells

A study assessed and compared the osteogenicity of clinically-available, calcium-based, scaffolds (specifically *Porites*, *Acropora*; β-TCP and banked bone) containing autologous MSCs in an ectopic (i.e., subcutaneous implantation site) sheep model (Viateau et al. 2016). The most important result obtained from this study was that the observed new bone formation was higher in the *Acropora* and *Porites* corals than in either the β-TCP or banked bone constructs; this outcome corroborated the results of a pertinent *in vitro* study (Puvaneswary et al. 2013) and established that coral scaffolds are a promising material for tissue engineered and bone regeneration applications.

When tissue-engineered constructs, combining either *Porites* or *Acropora* (in either massive or granular form) and MSCs, were used to fill segmental, sheep, metatarsal-bone defects, full bone regeneration was observed in some of the tested animals (Bensaid et al. 2005; Petite et al. 2000; Viateau et al. 2007; Manassero et al. 2013). In these cases, and, in contrast to results obtained with other material scaffolds, the

newly formed bone exhibited recorticalization and contained mature bone without remnant coral scaffold material 6 months post implantation. This significant result provided evidence that bone regeneration, which matches and even supersedes, the efficacy of autologous bone graft is achievable using MSCs and coral scaffolds (Fig. 43.5). These results, however, were not consistent most probably because the premature resorption of the coral scaffold material interfered detrimentally with the bone regeneration process.

43.6.1.3 Concluding Remarks

The data published established that coral scaffolds, specifically *Acropora* and *Porites* corals, are fully resorbable *in vivo* and provide appropriate templates for MSCs differentiation and cell functions pertinent to new tissue formation while maintaining the physical and mechanical properties of the scaffold material to provide the necessary milieu during new bone formation and regeneration. The material resorption rates, however, were still too fast and fail to finely synchronize the processes of scaffold resorption and new bone formation. An alternatives approach to achieve consistent bone regeneration using coral scaffolds in order to enhance the rate of osteogenesis in these cell-containing constructs is to incorporate osteoinductive growth factors (Zhu et al. 2010b).

43.6.2 Coral Scaffolds as Drug-Delivery Systems

Growth factors (proteins secreted by cells that act on appropriate target cells to induce specific functions) are part of the chemical processes that stimulate tissue formation and/or repair via well-established physiologic mechanisms, including angiogenesis, cell functions (such as proliferation), stem cell differentiation, and extracellular matrix synthesis (Aryal et al. 2014). Because several growth factors are expressed during different phases of the fracture-healing process, they have been considered, and used, as potential therapeutic agents to enhance bone repair (Aryal et al. 2014; Lieberman et al. 2002).

Since bolus administration of growth factors is not always an effective method for their delivery (because of rapid diffusion and quick deactivation), several approaches in regenera-

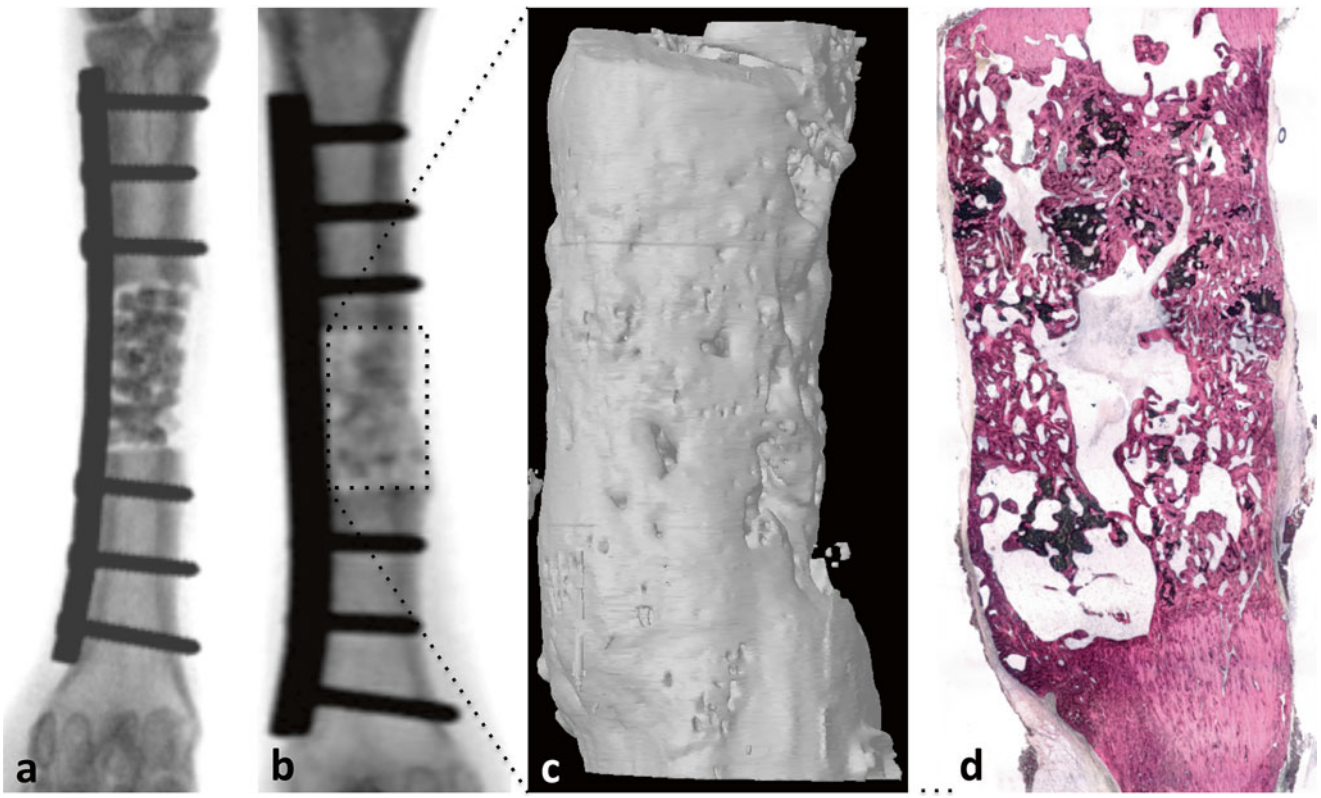

Fig. 43.5 Repair of critical-size bone defects in sheep using constructs containing coral scaffolds and autologous MSCs. Representative radiographs (frames **a**, **b**), 3D tomodensitometric reconstruction (frame **c**), and histology (frame **d**) of a 25-mm-long, critical-size, metatarsal bone defect in sheep implanted with a construct containing *Acropora* coral scaffolds and autologous MSCs, on the day of surgery (frame **a**), and 6 months post implantation (frames **b**, **c**, **d**). Bone regeneration with recorticalisation and mature bone filled the original defect size when the coral scaffold containing MSCs was used. At that time, resorption of the coral scaffold material was almost complete (6 months post implantation). Bone, cells, and coral were stained red, blue, and brown, respectively. Stains: Stevenel Blue and von Gieson picrofuschin

tive medicine have focused on biomaterial-mediated delivery of growth factors. The materials chosen for these purposes are critical aspects for the success of such applications because materials may control the drug deliver mode including reaching the target site within the requisite time, controlling the time course of protein release, and preventing unnecessary dispersion of the growth factor(s) from the targeted delivery site (Boerckel et al. 2011). In fact, the appropriate material scaffold for such applications should provide protection to growth factors against degradation in the *in vivo* milieu and to deliver desired doses effectively and dependably over the time period required for tissue regeneration and repair.

Coral is an attractive biomaterial for bone-tissue engineering applications because of its mechanical properties, biocompatibility, osteoconductivity and resorbability, but it can also be an efficacious delivery system for growth factors which may be needed to overcome osteoinductivity limitations of this material. Among growth factors pertinent to bone, BMPs (specifically recombinant human BMPs, for example, rhBMP-2 and rhBMP-7) are currently used in orthopedic applications such as spinal fusions, non-union defects and oral surgery (Hou et al. 2007; Kujala et al. 2004; Ma et al. 2000a).

The ideal material delivery system for growth factors should meet the following specifications: (i) be easy to formulate and prepare; (ii) have great affinity (i.e., adsorption) for the deliverable protein; (iii) allow release of growth factors; (iv) protect proteins from degradation; (v) function as a substrate for target-cell functions pertinent to new tissue formation (e.g., adhesion and migration); and (vi) be resorbable. Coral scaffold meets these criteria. Despite the potential of coral as delivery system for growth factors for clinical applications, only three studies investigated and reported the advantages of coral as an interactive matrix for the adsorption, and release, of growth factors *in vitro* (Demers et al. 2002a, b; Volpi 1999, 2002); there are no reports of *in vivo* studies.

43.6.2.1 Incorporation of Growth Factors onto Coral Scaffolds

Many processes for coating biomaterials with bioactive molecules have been developed and usually involve surface treatment of the scaffold material. In order to "coat" coral with osteoinductive proteins, the process used in the past mixed proteins in either collagen or fibrin gels and then applied these formulations onto coral scaffold particles (Demers et al. 2002a; Kujala et al. 2004; Ma et al. 2000a, b; Sciadini et al. 1997a, b). Such treatments, however, slowed the kinetics of coral resorption because the gel modified the structure of the material scaffold (specifically, blocked interconnected pores and/or reduced the size of pores); such conditions affected angiogenesis and cell migration into the scaffold (Hou et al. 2007). Because of such limitations, this material-coating technique is not used anymore (Demers et al. 2002a). An alternative coating-technique used freeze-drying and a vacuum freeze-dryer, but formulations thus prepared have not been characterized yet (Hou et al. 2007).

An effective strategy for loading growth factors on materials scaffolds is based on the fact that proteins bind on many materials (including coral) which are used in bone tissue engineering (Yu et al. 2014). For this purpose, coral scaffolds are immersed in a solution of the chosen growth factor in a controlled temperature and pH environment overnight. Growth factors are proteins which require specific conditions (such as temperature and pH) in order not to denature and to remain bioactive. In addition, since electrostatic interactions (involving calcium ions), play a major role in the processes of protein adsorption onto coral, the capacity of protein adsorption on coral depends on the charge type and charge density of the protein and the pH of the environment (Demers et al. 2002a, b; Volpi 1999, 2002). In the appropriate milieu, growth factors exhibit affinity for coral: for example, at 4 °C and pH = 3, 90 % of transforming growth factor β1 (TGF-β1) adsorbed at onto *Porites* particles (Demers et al. 2002b) while at 4 °C and pH 4, 70 % of rhBMP-2 adsorbed onto either *Porites* particles (with sizes in the range of 200–300 μm) or *Acropora* granules (3 × 3 × 3 mm cubes) and 65 % rhBMP-2 adsorbed onto coral (3 × 3 × 3 mm cube) granules (unpublished data). Since neither the size of the coral particles nor the genera of coral (e.g., *Acropora* versus *Porites*) affected growth factor adsorption, this process depends only on the chemical composition (unpublished data). The initial concentration of the growth factor solution did not affect the protein adsorption rate on the coral; moreover, the amount of the adsorbed protein on the coral material correlated linearly with the growth factor concentration (Fig. 43.6). In contrast, adsorption of glycosaminoglycans onto coral exhibits an S-shaped adsorption profile and reaches a saturation level; such adsorption patterns allow more precise evaluation of the affinity of glycosaminoglycans to coral than either TGF-β1 or rhBMP-2 (Volpi 1999, 2002). Most importantly, adsorption of growth factors onto coral does not affect their bioactivity (Logeart-Avramoglou et al. 2006) (unpublished data).

To date, no study comparing the efficacy of various calcium-containing biomaterials as growth-factor delivery systems has been published; preliminary unpublished data, however, showed that rhBMP-2 adsorption was better on coral than on HA/TCP scaffolds.

43.6.2.2 Release of Growth Factors from Coral Scaffolds

Assessment of the capability of coral to provide time-dependent release of growth factors (such as TGF-β1 and rhBMP-2) and identification of the parameters that play a role in modulating growth factor release are the subject of current research investigations.

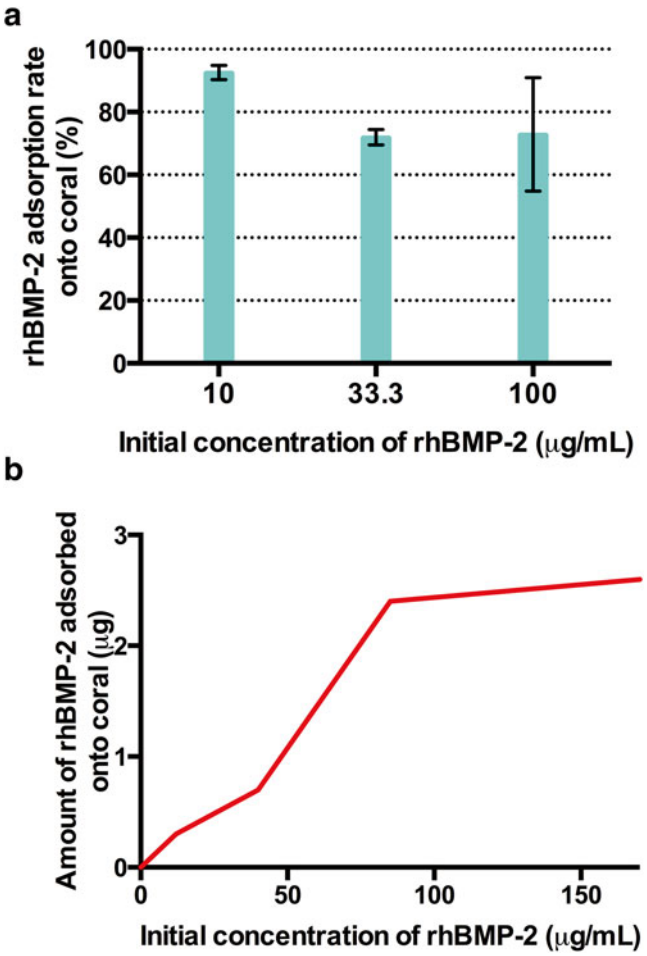

Fig. 43.6 Adsorption of various concentrations of rhBMP-2 onto 50 mg of *Porites* particles (with sizes in the range of 200–300 μm), in hydrochloric acid (pH = 4) at 4 °C overnight. The rhBMP-2 adsorption rate was determined indirectly by either protein dosage in the supernatant (frame **a**) or from the adsorption profile, that is, quantity adsorbed versus the respective initial concentration curves (frame **b**). These results provided evidence for the great affinity of coral for rhBMP-2

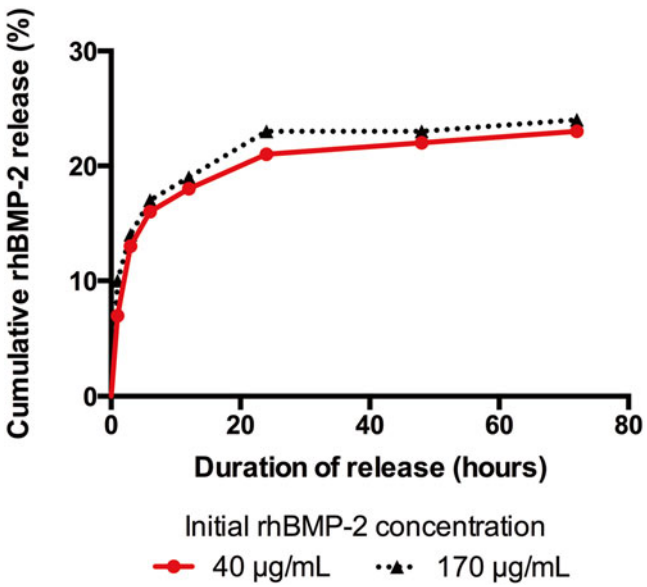

Fig. 43.7 Effect of rhBMP-2 concentration on its time course release from coral particles. These results show the amount of growth factor released as the percentage of the initial amount of rhBMP-2 in the solution at 37 °C. The data was obtained using an ELISA method

Predicting post-implantation outcomes based on *in vitro* data is not a reliable approach; moreover, *in vivo* follow-up techniques (using, for example, fluorescence and radioactivity labelling) are hardly used and reported in the literature (Boerckel et al. 2011; Zhang et al. 2013). Unquestionably, "environmental" aspects (such as pH, temperature, charge density, etc.) impact both protein affinity for, and their subsequent release potential from, the substrate materials (Demers et al. 2002a, b; Yu et al. 2014). In the case of biodegradable materials, release of the contained growth factors depends on the resorption rate of the substrate scaffold, a process which depends *inter alia*, on the coral structure, on the anatomical site of implantation, and a difficult to predict material dissolution process (Yu et al. 2014). For all the aforementioned reasons, few data are available in the literature regarding the release kinetics of growth factor from coral scaffolds. Studies of TGF-β1 and rhBMP-2 reported similar release kinetics profiles, which exhibited an initial (during the first hour) high release, followed by a decrease in the release rate, and reached a plateau (approximately 20 % of the initial protein amount) after 24 h (Fig. 43.7) (unpublished data).

The results of growth factor release rates from coral are similar to those obtained *in vitro* with other mineral scaffolds (Yu et al. 2014; Zhang et al. 2013). It should be noted that the release kinetic of growth factors is dependent on the rate of resorption of the carrier material (Boerckel et al. 2011). Currently, the most widely used carrier for rhBMP-2 is collagen which, in the physiological milieu, resorbs very quickly compared to coral (Boerckel et al. 2011). In addition, sustained delivery of rhBMP-2 increases bone formation (Boerckel et al. 2011; Arnaud et al. 1999). These findings suggest that use of coral as a carrier for rhBMP-2 could result in greater new bone formation than other carriers with less affinity for this protein and faster resorption rate. At present, however, further studies are needed to elucidate pertinent aspects of growth factor adsorption and release from coral, including the role of various coral material properties (such as size of particles, chemistry, pore size, porosity, etc.).

43.6.2.3 *In Vivo* Implantation of Bone-Tissue Engineering Constructs Composed of Coral Scaffolds Containing Growth Factors

Several studies used growth factors incorporated in material scaffolds to promote cell functions pertinent to bone regeneration. Some of the growth factors chosen and used in bone

tissue engineering applications are chemo-attractants for, and induce differentiation of, progenitor cells into osteoblasts (the bone forming cells). Coral has been used as an *in vivo* delivery system of various growth factors, including an extracted BMPs mixture, TGF-β1, and rhBMP-2 (Tables 43.4 and 43.5). Some angiogenic factors (specifically VEGF) have also been used in association with osteoinductive factors to induce formation of vascularized tissues (Geiger et al. 2007).

To date, rhBMP-2 is the most effective osteoinductive growth factor; constructs containing rhBMP-2 on coral scaffolds promoted healing of bone defects in several animal models (such as rats, rabbits and dogs) (Sciadini and Johnson 2000; Zhang et al. 1998; Arnaud et al. 1999) and in clinical studies (Kujala et al. 2002, 2004; Arnaud et al. 1998).

43.6.2.4 Specific Case: Platelet-Rich Plasma Added onto Coral Scaffolds

Platelet-rich plasma (PRP) is an autologous source of various growth factors released from activated platelets in freshly drawn venous blood.

Because activated platelets deliver growth factors (including PDGF, TGF, VEGF, FGF all of which promote bone healing) they have been used as a source of osteoinductive growth factors. The results of such practice, however, remain controversial. In one study, natural coral was described as a good carrier for PRP, and promoted bone regeneration in critical-size defects in a rabbit model (Parizi et al. 2012). In contrast, in another study, PRP-containing coral constructs resulted in less bone formation than PRP alone; these results raised concerns regarding the suitability of coral as PRP-

Table 43.4 Overview of experimental *in vivo* studies evaluating the effectiveness of bone-tissue engineering constructs containing growth factors on coral scaffolds

Growth factors	Coral scaffold details; coating method	Experimental model	Results	Reference
rhBMP-2	15 × 1 mm disks[a]; Freeze-drying	Rabbit critical-size cranial defect	Healing but did not match the results obtained with autograft	Hou et al. (2007)
Bovine BMP-2	Cylinder (9 × 20 mm[a]; Mixed in type I collagen	Canine ulnar defect	Healing but did not match the results obtained with autograft	Tuominen et al. (2000)
Bovine BMP-2	*Porites* particles (size 630–1000 μm); direct binding	Rat critical-size cranial defect	More bone formation than that obtained with coral containing bone marrow cells	Arnaud et al. (1999)
Bovine BMP-2	*Porites* particles (size 630–1000 μm); Mixed in type I collagen	Canine segmental critical-size radial defect	Bone union and improvement of the mechanical properties of the newly-formed bone	Sciadini et al. (1997a)
Moose BMP-2	Cylinder (15 × 16 mm[a]; mixed in type IV collagen	Sheep segmental tibial defect	Healing with large external callus (abnormal bone tissue). No improvement compared to coral alone 6 weeks post implantation	Gao et al. (1997)
Extracted mixture of BMP and bFGF	*Porites* particles (size 630–710 μm); Mixed in type I collagen	Rat subcutaneous model	Endochondral bone formation and mineralization 4 weeks post-implantation	Damien et al. (1993)
TGF-β1	*Porites* Particles (size 500 μm); mixed in Fibrin glue	Rabbit critical-size cranial defect	Increased bone formation compared to results obtained with coral alone	Arnaud et al. (1994)

[a]Coral genera not mentioned

Table 43.5 Overview of clinical studies evaluating the effectiveness of bone-tissue engineering constructs containing growth factors and coral scaffolds

Growth factors	Coral scaffold details; coating method	Clinical defects; number of patients	Results	Reference
Bovine BMP	Cylinders (15 × 1–2 mm[a]; mixed in type I collagen	Ulnar non-unions; five patients	Solid union in all cases	Kujala et al. (2004)
Bovine BMP	Coral granules[a]; mixed in type I collagen	Scaphoid non-unions; ten patients	Two (out of ten) complete unions	Kujala et al. (2002)
TGF-β1	Particles (size 500 μm)[a]; mixed in fibrin glue	Craniofacial and maxillofacial defects; six patients	Healing in 75 % of the patients tested	Arnaud et al. (1998)

[a]Coral genera not mentioned

delivery vehicle (Shafiei-Sarvestani et al. 2012). Moreover, other studies showed that PRP-containing coral constructs were not effective alone, but needed the mediation of MSCs to achieve effective bone regeneration (Zhang et al. 2011). Other literature reports described increased coral resorption and impaired new bone formation, 6 weeks after implantation, when PRP-containing coral constructs were used to fill critical-size femoral defect in rabbits (Soffer et al. 2006).

43.6.2.5 Concluding Remarks

Coral scaffolds are suitable delivery systems for growth factors, especially rhBMP-2, a potent osteoinductive factor. The results of *in vivo* use of such a construct showed increased new bone formation, but are inconsistent and disappointingly ineffective in large defects, especially ones exhibiting limited vascularization; moreover, in some cases, such constructs have been associated with side effects such as abnormal bone formation secondary to use of high growth factor dosages (Gao et al. 1997; Sciadini and Johnson 2000). For these reasons, the clinical applications of high dosage rhBMP-2 are a topic of scientific debate. Other growth factors (such as, TGF-β, VEGF, and PDGF) combined with coral scaffolds had fewer side or negative effects; however, the effectiveness of these growth factors is limited.

Interestingly, it has been shown recently that natural coral physiologically produces proteins that are similar to human and mammalian growth factors (specifically BMP-2 and TGF-β) (Green et al. 2013). For this reason, advances in marine invertebrate cell culture have the potential to produce these sustainable growth factors, supply them dependably and even produce them in large quantities (Green et al. 2013). If this process succeeds, coral could become a widely used osteoinductive scaffold for tissue engineering and other pertinent applications.

43.7 Clinical Applications of Coral Bone Substitutes

The first implantations of natural coral scaffolds as bone graft substitutes were performed in experimental trials in animals (such as dogs, pigs and sheep) in the early 1970s (Demers et al. 2002a; Guillemin et al. 1987). Subsequent use of coral in clinical situations began in 1979 (Demers et al. 2002a). Since then, several clinical trials using either natural coral or CHACC were conducted in fields of surgery where bone grafting is commonly used. Most of these clinical trials used either coralline or CHACC scaffolds in the form of granules, blocks, and, occasionally, pastes. Moreover, these coral materials have been used either alone or in combination with autografts (thus acting as a bone graft extender), PRP or demineralized bone matrix (Thalgott et al. 2001) in orthopaedic surgery, craniofacial, maxillo-facial, spine surgery and neurosurgery.

43.7.1 Orthopaedic Surgery

CHACC has been used for filling bone voids resulting from articular surface depression as observed in tibial plateau fractures. The results of such practices have been similar to those obtained with autologous bone grafts (Bucholz et al. 1989). CHACC has also been used for bone augmentation to fill bone defects resulting from tumor resections (Demers et al. 2002a). CHACC is an excellent bone graft material which induced visible callus formation 1 month post implantation and clinical bone healing after 4 months in all humans patients tested (Fu et al. 2013). Most of the implanted CHACC was degraded within 18–24 months post implantation (Fu et al. 2013).

CHACC as bone graft substitution in hind foot arthrodesis had satisfactory clinical results (Coughlin et al. 2006). In these cases, the graft material was difficult to contain within the surgical sites; consequently, extrusion occurred in all patients. Moreover, and although in the long-term the material *in vivo* did not show any adverse effects at the 6-year follow-up, slow resorption of the scaffold material (associated with the hydroxyapatite component) raised concerns regarding intra-articular migration (Coughlin et al. 2006).

43.7.2 Spine Surgery

Spine arthrodesis is a major indication of bone grafting in spine surgery. Coral and CHACC either alone or combined with PRP or bone autografts have been used for both anterior and postero-lateral intervertebral arthrodesis (fusion) with variable outcomes in several clinical trials (Lowery et al. 1999; Ramzi et al. 2008; Thalgott et al. 2001). Among these studies, a prospective longitudinal study was performed to determine the long-term efficacy of coral grafts in anterior cervical discectomy and fusion (Ramzi et al. 2008). In that study, cervical discectomy, subsequent arthrodesis with coral, and stabilization with anterior cervical locking plates lead to satisfactory clinical results. While 95.5 % of patients expressed overall satisfaction with the surgery outcome, only 70.5 % stated that they had returned to their previous level of physical activity. Moreover, the coral yielded low (45 %) fusion rates. For the aforementioned reasons, the use of coral grafts when fusion is one of the post-operative endpoints is not recommended. In contrast, when CHACC (with or without demineralized bone matrix), was used as a bone graft extender in instrumented postero-lateral fusion in difficult-to-fuse patients in another study, the clinical outcomes were good and an overall fusion rate of 92.5 % was observed (Thalgott et al. 2001). Because of the small number of reported such clinical studies (and because of the small numbers of patients involved), the differences between biomaterials, anatomical implantation sites, surgical techniques used

for fusion intervention and variable length of follow-up time, definitive conclusions regarding the benefit of either natural coral alone or coral-derived composites as bone substitutes in spine surgery cannot be drawn at this time.

43.7.3 Craniofacial Surgery

Porites coral scaffolds were successfully used in several clinical trials of craniofacial surgery to obliterate burr holes, to repair skull defects, and to reconstruct the floor of the anterior cranial fossa (Loty et al. 1990; Roux et al. 1988a; Soost et al. 1998). One of the largest studies in the field of craniofacial surgery used coral grafts as bone substitute in 183 patients; 80 of these cases involved cranial base bone defect repair (Roux et al. 1995). In the latter cases, partial resorption (approximately 40 %) of the initial graft volume occurred in almost all cases within 8–10 months, with complete coral graft resorption after about 1 year post implantation. Interestingly, the recorded 4 % local infection rate was lower than the 20 % infection rate documented with autologous bone autografts (Roux et al. 1995). For all the aforementioned reason, coral is considered a satisfactory bone substitute in cranial surgery.

43.7.4 Maxillofacial Surgery

Adequate bone volume is imperative for the osseointegration of endosseous implants. Bone resorption and bone alveolar remodeling post-extraction may challenge implant placement. Coral grafts have been successfully used as bone augmentation materials to enhance primary implant stability during osseointegration in both maxillofacial surgery and dentistry.

A surgical procedure, preceding graft implantation, is elevation of the base of the maxillary sinus (sinus lift). Coral, combined with PRP, has been successfully used for sinus lift surgery (Papa et al. 2009). Clinical experience on 47 sinus lifts in 34 patients using a mixture of coral and autologous PRP demonstrated that the newly formed bone exhibited morphologic and structural characteristics similar to those of autologous bone. Although all grafting materials used yielded good results of maturation of the newly formed bone, the best results were achieved with autologous bone. Another 5-year period study, which compared the frequency of failure (specifically, either graft material resorption or implant loss) after sinus lifts filled with various graft materials (including a gel-containing coral) showed that the incidence of implant loss after the augmentation procedures were similar. The gel-coral, however, exhibited the highest (40 %) resorption rate among all biomaterials tested (Velich et al. 2004).

In another study, coral was used to fill extraction sites and to enhance bone healing in eight patients. The bone sites in which the coral had been implanted, exhibited good primary stability at implant insertion, implant rigidity, and no adverse effects post implantation (Molly et al. 2008).

43.7.5 Concluding Remarks

During the past 40 years, the number of published clinical trials using either coral or CHACC as graft material was small and involved a small number of patients. The results of these studies clinical confirmed those obtained in experimental studies using laboratory animals: coral and CHACC are well tolerated *in vivo* at all bone sites (i.e., long bone, spine, skull, mandible and maxilla) where these scaffolds were implanted.

New bone is formed as progressive resorption of the coral grafts takes place. Although the safety of coral and CHACC is now well-established, their superiority, regarding new bone formation, compared to other widely used bone substitutes (such as calcium phosphate-derived materials), remains to be established. Most importantly, use of coral and CHACC grafts is limited to the filling of small bone defects but is not recommended for use in either massive, segmental bone defects, or vertebral arthrodesis because in those cases, addition of growth-factors and/or cell-based therapies are needed to provide the additional osteogenic and osteoinductive stimulus required for successful outcomes in clinical applications.

43.8 Conclusions and Future Directions

Coral exoskeleton is a natural three-dimensional material that resembles the architecture and porosity of mammalian bone. For these reasons, coral has attracted the attention of the biomaterials community, and proven to possess characteristics that are important advantages for bone graft substitutes; moreover, coral has been successfully used in clinical practice and shown effective in promoting bone union in small defects.

Use of coral scaffolds for regeneration of massive bone defects is an appealing strategy because they: (1) are biocompatible, osteoconductive and bioresorbable; (2) have three-dimensional architecture and porosity that allow adequate diffusion/delivery of oxygen and nutrients and removal of cell metabolic waste as well as promote cell infiltration and functions pertinent to new tissue formation; (3) have material surface chemistry that supports differentiated cell attachment, proliferation, etc., as well as stem cell differentiation; and (4) can be used as carriers for growth factors

whose bioactivity can compensate for any limitations of the intrinsic osteoinductive properties of the material substrate.

Several animal studies investigated and reported on the efficiency of constructs composed of coral and containing either stem cells or growth factors; despite encouraging results, the outcome of such approaches have not been as effective as those obtained using bone autografts. For these reasons, current research focuses on evaluation of the efficiency of coral constructs containing both stem cells and growth factors. The initial results were promising because the combination of MSCs, rhBMP-2, and coral scaffolds produced new bone formation similar to that obtained with autologous bone grafts (Arnaud et al. 1999; Burastero et al. 2010); such outcomes established that the combination of rhBMP-2, MSCs, and coral scaffold is an attractive option for bone regeneration at large segmental bone defects (Fig. 43.8).

Other research investigations focused on the delivery of polynucleotides that encode growth factors (e.g., RNA, DNA) as an alternative to growth-factor-based therapies (Geiger et al. 2007; Yu et al. 2014; Zhang et al. 2007). This approach provides a novel method to address the need for continuous delivery of growth factors, because the polynucleotides reach target cells, induce local production of the desired growth factors, do not require supra-physiological doses, have no secondary side effects, and are not costly (Geiger et al. 2007; Zhang et al. 2007).

An important issue to be considered when using coral, and also when comparing the results of published studies, is the natural origin of this material, which makes them widely variable (Table 43.2), specifically compared to manufactured-synthetic scaffolds. Explanations for the structural characteristics and morphological variations of the same genus of coral include the habitat types of its provenance, the degree of petrification as well as variations due to environmentally-induced phenotype and/or genetic polymorphism (Wu et al. 2009; Hemond et al. 2014).

Focus on the medical use of corals, should not divert attention from their role in the global ecological system: corals are the main constituent of reefs that provide habitat for many marine organisms. For this reason, coral use (even for clinical applications) should be justified and rationalized especially in view of current global environmental concerns: for example, Australian reefs have already declined by 14.2 % since 1990 and, if current destructive trends continue unchanged, more than half of the coral reefs in the world may be destroyed by the year 2030 (Wu et al. 2009). Innovations in long-term marine invertebrate cell culture, as well as scientific advances that provide pertinent alternatives, may prevent such ecological catastrophe and also guarantee that the coral reef habitats are not damaged during collection of specimens needed for medical applications (Green et al. 2013).

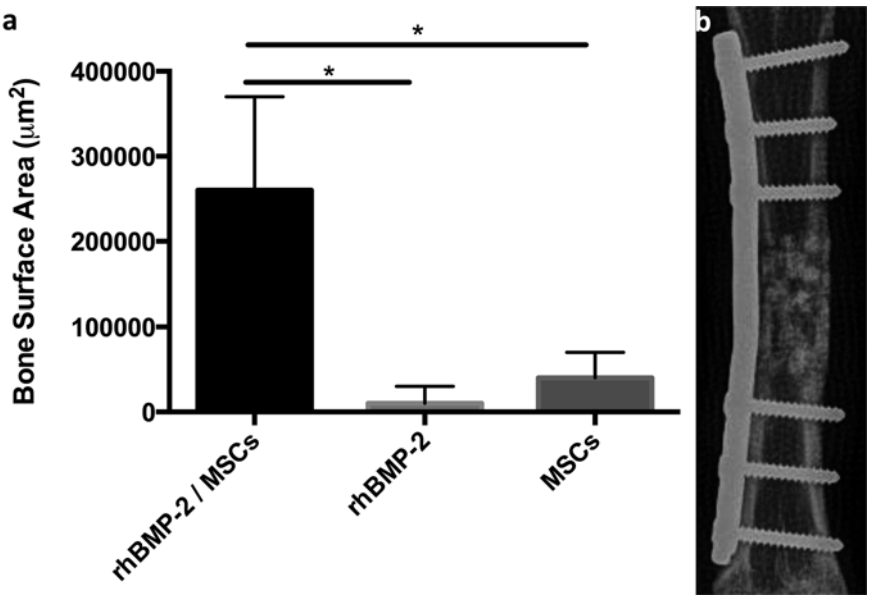

Fig. 43.8 *In vivo* osteogenic capability of (**a**) coral constructs containing either rhBMP-2, or MSCs or both following ectopic implantation in mice, or (**b**) a coral construct containing rhBMP-2 and MSCs implanted in a critical-size bone defect in sheep. Histomorphometric quantification of newly-formed bone associated with *Porites* coral particles containing either MSCs, rhBMP-2, or both, and implanted ectopically in mice for 6 weeks, showed that the highest new bone formation was observed in the constructs containing both MSCs and rhBMP-2 (frame **a**). A representative radiograph of a sheep metatarsal, critical-size defect 4 months post-implantation of *Acropora* granules containing rhBMP-2 and autologous MSCs (frame **b**). The resultant full bone regeneration with recorticalization was similar to that obtained using autograft bone

Boxes

- ***Commercially - available coral bone substitutes:***
  - *Natural coral* ($CaCO_3$): Biocoral®; Inoteb.
  - *Coralline HA* (*complete conversion of $CaCO_3$ to HA*): Interpore200®; ProOsteon200®; ProOsteon500®; Interpore International.
  - *Coralline HA*/*$CaCO_3$* (*partial conversion of $CaCO_3$ to HA*): ProOsteon200R®; ProOsteon 500R®; Interpore International.
- The physical properties of coral are similar to those of mammal bone.
- Natural coral is biocompatible, osteoconductive, and resorbable.
- Coral scaffolds provide an appropriate template for cell functions (such as, adhesion, migration, proliferation, formation of extracellular matrix, etc.) pertinent to new tissue formation as well as for stem cell differentiation.
- Coral scaffolds are appropriate substrates for growth-factor adsorption and subsequent release.
- Coral scaffolds are substrate materials of great promise for bone tissue formation because they have the potential to induce complete bone regeneration which matches, and even supersedes, the efficacy of the autologous bone grafts currently used in clinical practice. The main advantages of coral scaffolds are:
  - Support of MSCs adhesion, proliferation and differentiation;
  - Support of growth factor adsorption and release; and
  - They are natural and resorbable materials.

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Part X

Management and Conservation

44 Population Genetic Structure of *Corallium rubrum* in the Mediterranean Sea: Diversity, Phylogeography, and Bathymetric Patterns

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Abstract

Among Mediterranean cnidarians, the octocoral *Corallium rubrum* is the most harvested species, mostly owing to the exploitation of its red skeleton for jewellery purposes. Red coral colonies can be found on vertical cliffs, in caves and in crevices from 20 to 100 m depths. Red coral also occurs in deeper water where it dwells on scattered boulders, rocky outcrops and seamounts down to about 1,000 m depth. Most red coral banks above 80 m depth have been overharvested all over the Mediterranean Sea. Nowadays commercial harvesting occurs mainly between 80 and 130 m, where older, large-sized and highly valued colonies still occur. Habitat features affect larval dispersal and genetic structuring of populations across spatial scales and depth gradients. In this chapter, knowledge on population genetic structure and diversity in *C. rubrum* is summarized. In Octocorallia, mitochondrial markers have extremely low polymorphism levels and do not reveal genetic patterns in shallow water populations. Conversely, they reveal weak phylogeographic patterns of structure in deep water populations. Microsatellite loci showed strong genetic structure in shallow water populations from Mediterranean basin scale down to populations separated by 10 m. In the Western Mediterranean the pattern of genetic structure reflects a combination of regional clustering and isolation by distance. Marked chaotic structuring was detected when downscaling the studies to very small spatial distances (1 m). Between colonies within a 0.5 m^2 square, significant spatial genetic structure was found. Based on this result, the estimated effective larval dispersal ranges between 20 and 30 cm. This also suggests that breeding units are restricted in space and are composed of related individuals. A similar small-scale heterogeneity pattern of genetic structure was also found among populations inside submarine caves. Depth appeared to be one of the factors affecting genetic diversity, with lower diversity observed in Tyrrhenian and Ligurian deep populations. A threshold in genetic diversity occurs across 40–50 m depth.

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Moreover, patterns of structure differ between shallow and deep-water populations. Genetic data clearly show that connectivity in *C. rubrum* is limited, and suggest that deep red coral banks cannot act as refugia for shallow water populations as hypothesized for some tropical species. Studies on the response to thermal stress suggest that genetic structure could be related to differences in adaptive abilities, with major implications concerning the ability of the species to respond to future climate change. Conservation of red coral in the Mediterranean Sea must take into account that the species consists of an array of metapopulations, structured both geographically and by depth, which have to be considered as discrete management units.

Keywords

Red coral • Temperate biogenic reefs • Genetic diversity • Depth gradient • Phylogeography • Adaptation • Management unit • Conservation

44.1 Introduction

The red coral (*Corallium rubrum*) is the major harvested gorgonian species in the Mediterranean Sea, mostly owing to the exploitation of its red skeleton for jewelry purposes (Tescione 1973; Santangelo and Abbiati 2001; Tsounis et al. 2010; Tsounis et al. 2007; Bruckner 2014) (Fig. 44.1).

Historically, red coral fisheries followed 'boom and bust' cycles, whereby newly discovered precious coral beds were overexploited to depletion. In the 1950s harvesting by SCUBA diving was introduced and nowadays is the only legal harvesting method in the Mediterranean Sea (GFCM 2010). SCUBA diving harvesting is a selective but very effective method and lead initially to the exhaustion of the shallow banks (30–50 m deep), and later, with the widespread use of mixed-gas SCUBA diving, of the deeper banks (to about 130 m depth). Despite the expansion of the harvesting depth range, landings declined over the last decades and rose again in 2006 after the widening of the harvesting geographic area (Fig. 44.2).

Major concern has been expressed about the possible extinction or exhaustion of this fragile resource (Bruckner 2009, 2014, Santangelo and Abbiati 2001; Tsounis et al. 2013). A debate on conservation strategies, contrasting harvest management versus trade control (Bussoletti et al. 2010; Chang et al. 2013), was raised by GFCM and CITES, and involved stakeholders including fishermen, traders, industry, scientists and conservationists.

Other than harvesting, mass mortality events impacting shallow water populations and linked to thermal anomalies have been reported (Cerrano et al. 2000; Garrabou et al. 2001, 2009), raising the question of the impact of environmental fluctuations and climate change on red coral populations.

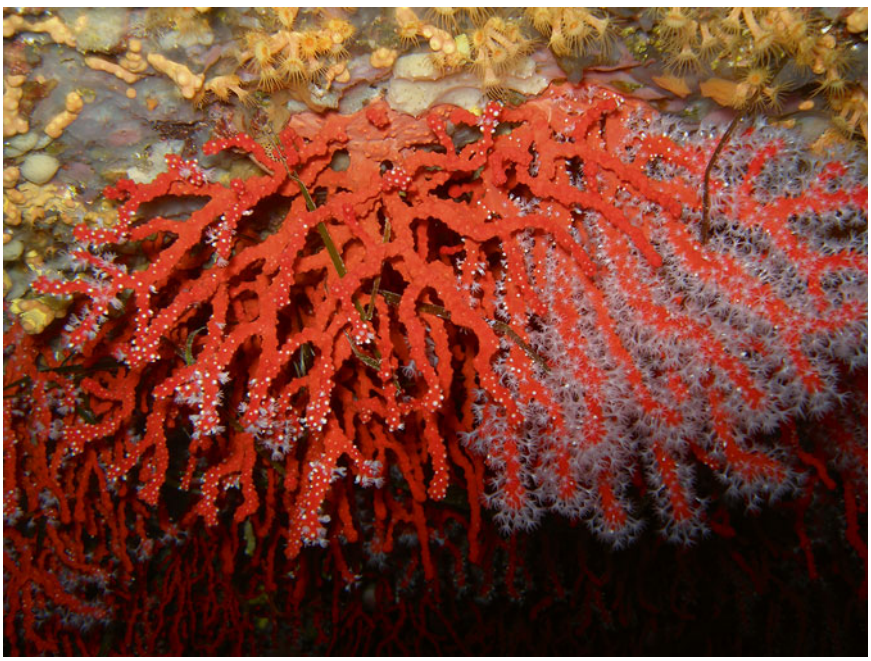

Fig. 44.1 *Corallium rubrum* (red coral)

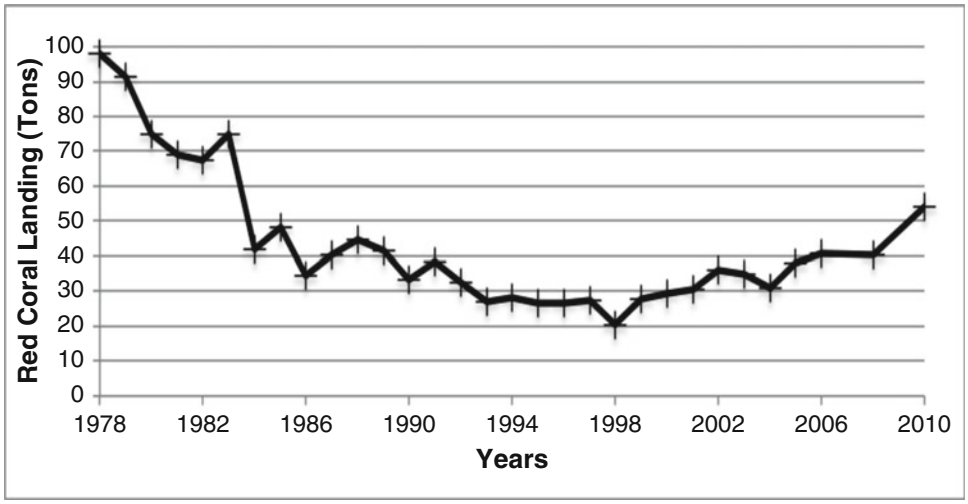

Fig. 44.2 Mediterranean red coral landing data (Source GFCM- FAO)

Knowledge on biology, ecology, and connectivity among populations is essential to define appropriate and effective strategies for the conservation of this species. In recent years a growing number of scientific publications on reproductive biology, feeding, distribution, and population dynamics as well as manipulative experiments on processes driving the ecology of this species in shallow water have been published (RAC/SPA 2014). A multitude of studies also addressed the population genetic structure of the species in different habitats, at a variety of geographical scales, and at different depth ranges.

44.2 Genetic Diversity and Connectivity

Population genetic studies are an effective complement to traditional field studies, providing estimates of genetic differentiation among populations over multiple spatial scales, which can be used to estimate ranges of species dispersal, to locate population boundaries, and to assess levels of gene flow between them (Thorpe et al. 2000). Population genetic data are not limited to two-dimensional spatial information; they can provide information regarding temporal changes in community structure, for example before and after a major disturbance, while studies along depth gradients provide important data concerning the role of deep-water populations in the recovery of threatened, shallow populations.

Genetic connectivity represents the degree to which gene flow affects evolutionary processes among populations, and genetic structure estimates provide a proxy of larval exchange between populations (Lowe and Allendorf 2010). The understanding of current levels of connectivity between populations is relevant to management and conservation, since connectivity is a key element governing population resilience following disturbance (Palumbi 2003; Bellwood et al. 2004). Other than connectivity, genetic diversity is an important parameter to take into account for the management of populations. Genetic diversity within a population can affect productivity, growth and stability, response to disturbance, as well as inter-specific interaction within communities, and ecosystem-level processes (Hughes et al. 2008).

The bulk of available genetic knowledge on *Corallium rubrum* refers to the more accessible shallow water populations (15–60 m depth) (Abbiati et al. 1993, 1997; Costantini et al. 2003, 2007a, b, 2011; del Gaudio et al. 2004; Calderòn et al. 2006; Casu et al. 2008; Ledoux et al. 2010a, b, 2013; Aurelle et al. 2011; Aurelle and Ledoux 2013; Haguenauer et al. 2013; Cannas et al. 2014). The first population genetic research on red coral was conducted using allozymes (Abbiati et al. 1993, 1997) and AFLPs (del Gaudio et al. 2004). They demonstrated significant genetic divergence among samples at a distance of tens of kilometres (Abbiati et al. 1993; del Gaudio et al. 2004). At shorter distances (about 200 m), no significant genetic structuring was observed using allozymes (Abbiati et al. 1997). However, allozyme markers, due to their low mutation rate and low polymorphism, have limited power to resolve genetic structuring at small spatial scales.

Mitochondrial DNA in Octocorallia is highly conserved compared to other taxa (Costantini et al. 2003; Calderòn et al. 2006; Costantini et al. unpublished data; Ledoux et al. this book). Low evolutionary rates in Octocorallia were observed by Shearer et al. (2002) and Hellberg (2006) and might be related to the presence of an apparent homolog of the bacterial mismatch repair gene, the *mtMutS* gene (mitochondrial MutS homolog, Pont-Kingdon et al. 1995; McFadden et al. 2006; see Bilewitch and Degnan 2011 for a discussion on the origin and functionality of *mtMutS*).

The recent sequencing of the whole mitochondrial genomes of two precious coral species (*Paracorallium japonicum* and *C. konojoi*; Uda et al. 2011) allowed the design of primers flanking one of the major noncoding and

variable regions of the mitochondrial DNA, the Control Region (CR). Costantini et al. (2013) amplified the CR for the first time in *C. rubrum* and revealed its higher variability (see below), as observed in tropical and deep-sea corals (e.g. Bongaerts et al. 2010; Miller et al. 2010; Palumbi et al. 2012).

The development of species-specific microsatellite loci (Costantini and Abbiati 2006; Ledoux et al. 2010b) allowed important advances in the study of the genetic structure of red coral populations. These markers revealed significant deviations from Hardy-Weinberg equilibrium in all red coral populations due to elevated heterozygote deficiencies consistent with the occurrence of null alleles, Wahlund effect and inbreeding (mating between relatives) (Costantini et al. 2007b; Ledoux et al. 2010a). These results confirm a predominance of local recruitment (Abbiati et al. 1993, 1997; Ledoux et al. 2010a) in accordance with the reproductive biology and larval ecology of red coral (Santangelo et al. 2003). In red coral embryo development takes place within the female polyps and lasts for about 30 days, and leads to the release of planula larvae. Planulae have a reduced swimming ability and geonegative behaviour suggesting that, once released, larvae settle in close vicinity to parental colonies (Vighi 1972). Therefore, reproduction events in red coral are more likely to involve related individuals compared to broadcasting species (Costantini et al. 2007b; Ledoux et al. 2010a).

44.3 Phylogeography

The first studies on the genetic structure of *Corallium rubrum* assessed phylogeographic patterns in populations between 10 and 50 m depth in the north-western Mediterranean using microsatellite markers and the ribosomal internal transcribed spacer 1 (ITS-1) region (Costantini et al. 2007a). A high degree of genetic differentiation between samples was observed throughout the north-western Mediterranean Sea, with no correlation between genetic differentiation and geographical distance among the analysed samples (Fig. 44.3a) possibly related to the sampling scheme. A partitioning of the total genetic variability into four groups was observed: one main group including samples belonging to the western Mediterranean/Ligurian Sea (Medes, Plane, San Fruttuoso, Calafuria), a second main group of samples from the Tyrrhenian/Adriatic Seas (Tuscany archipelago, Palinuro and Korcula samples), and two other groups representing island samples from Menorca and Sardinia. The Balearic Islands seem to represent an important "hotspot" of genetic diversity and divergence for *C. rubrum* populations, showing higher genetic variability compared to the other Mediterranean populations (Costantini et al. 2007a; Ledoux et al. 2010b; Cannas et al. 2014). The Balearic region is defined as a transition region between the Liguro-Provencal and the Algerian basins, characterized by two contrasting hydrodynamic regimes that induce highly variable circulation patterns (Garcìa et al. 1994). These conditions could have led to frequent mixing of different gene pools from the south and the north of the islands, contributing to the high level of observed genetic diversity as found in other species in the area (Rozenfeld et al. 2008; Ledoux et al. 2010b).

Ledoux et al. (2010b) investigated patterns of spatial genetic structure in the north-western Mediterranean in a larger set of samples from four different regions. Additionally, Aurelle et al. (2011) included populations from the North African Mediterranean coast. Both studies showed regional clustering and isolation by distance in the Western Mediterranean Basin (Fig. 44.3b). Moreover, an additional nuclear sequence marker, an intron from the Elongation Factor 1 (EF1) gene, confirmed the genetic differentiation between populations detected by microsatellites. Surprisingly, the sharing of EF1 haplotypes between distant locations and the low level of differences between most haplotypes suggest no evidence of long-term isolation between regions and no apparent break across the Almeria–Oran front.

In summary, spatial patterns of population structure in red coral are complex and driven by the limited dispersal ability of the larvae. Weak genetic breaks between regions and isolation by distance also contribute to the observed phylogeographic patterns (Aurelle and Ledoux 2013), as could relatively recent diversification or the occurrence of sporadic long distance dispersal events.

44.4 Small-Scale Population Genetic Structure

All studies on genetic structuring in red coral also revealed an extremely high level of divergence at small spatial scales (tens of meters), raising the question of the effective larval dispersal ability of this species and the genetic patch size. Costantini et al. (2007b) used a replicated sampling design to test local scale differentiation and found that strong genetic structuring occurred at the smallest scale investigated: 10 m (Fig. 44.4a). After downscaling the studies, by analysing genetic structure in populations at 1-m distances along a 10-m transect, strong genetic structuring between samples was found. Spatial autocorrelation (Moran coefficient) showed that neighbouring colonies are the most genetically similar, and larvae tend to settle within a patch of about 1 m (Fig. 44.4). Such localised genetic heterogeneity could result from spatiotemporal heterogeneity in the genetic composition of recruits, larval ecological processes (e.g. pre/post-settlement or recruitment mortality), or stochasticity of reproductive success. Similar significant genetic differentiation between populations separated by 10 m was found in populations in the Gulf of Lion (Ledoux et al. 2010b).

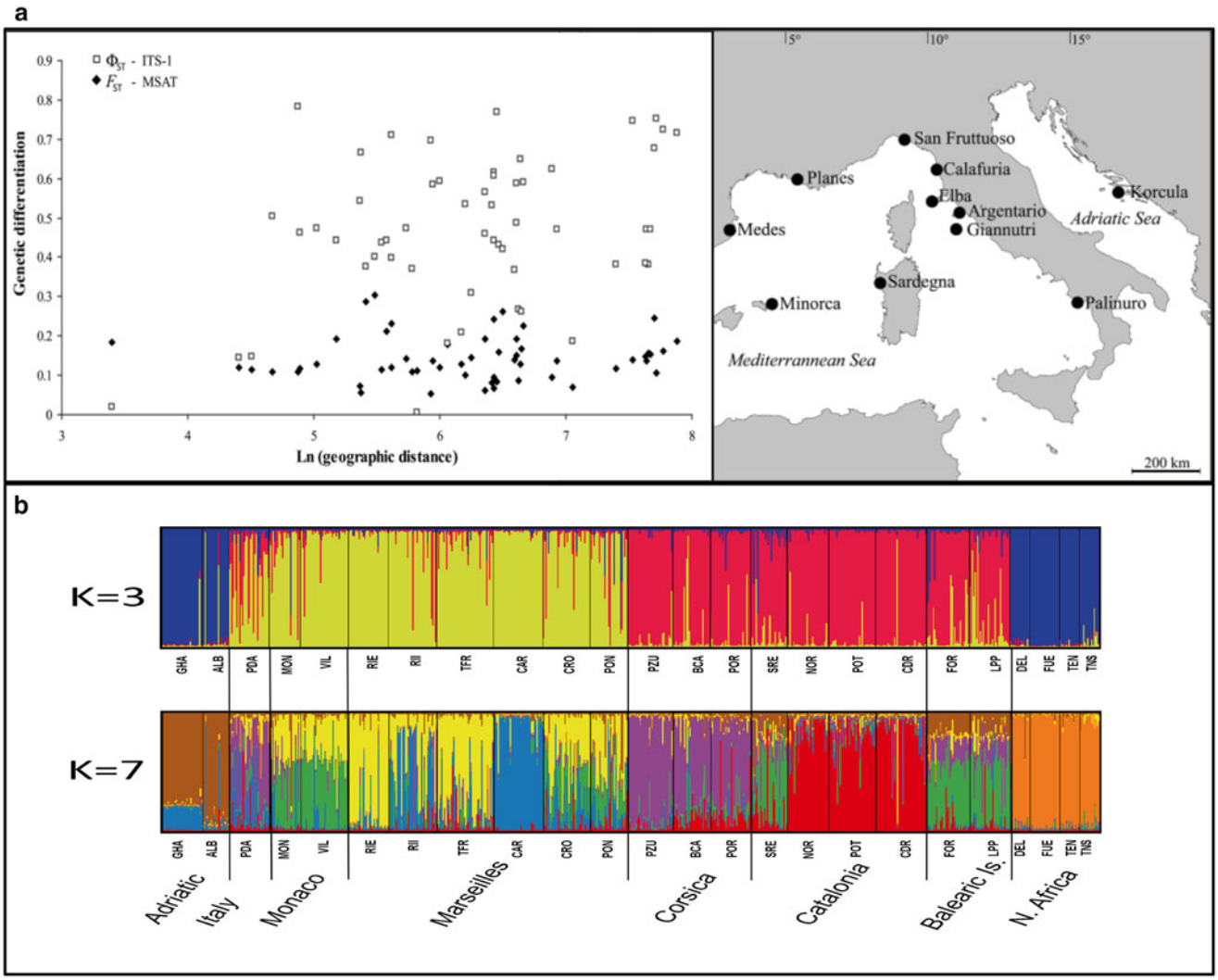

Fig. 44.3 (**a**) Results of the spatial genetic structuring observed in *Corallium rubrum* populations (Sources (Costantini et al. 2007a)). The first graph represents the Relationship between genetic differentiation estimates and the logarithm of geographical distance among *Corallium rubrum* samples for both microsatellite and ITS-1 markers. The map shows the location of the 11 *Corallium rubrum* samples (represented by *black dots*) as in Costantini et al. (2007). (**b**) Results of a Bayesian clustering analysis with microsatellite loci in *Corallium rubrum* covering the Western Mediterranean Basin. Each *bar* corresponds to an individual and the *colours* correspond to the genetic groups to which they were assigned (three and six groups (K) respectively). The proportion of each colour reflects the probability of membership to each group (Aurelle et al. 2011)

Using a geo-referenced sampling design of red coral colonies within half a square meter, Ledoux et al. (2010a) investigated demographic processes acting within red coral populations, showing the occurrence of correlation between geographic and genetic distances between individuals. Based on this result, the mean dispersal within a population was estimated to range between 20 and 30 cm (Fig. 44.5). A kinship analysis on these samples showed the occurrence of a half-sib family structure within the studied quadrat, along with various parent-offspring relationships. These results suggested that breeding units in the red coral are highly restricted in space. In addition to restricted dispersal, a high level of self-recruitment also characterized the functioning of these populations.

44.5 Bathymetric Patterns of Genetic Structuring

Empirical evidence demonstrating small genetic patches along horizontal gradients then raised the question about the possible effect of a depth gradient on patterns of genetic structure in red coral. The possible effect of depth on patterns of genetic structure in marine sessile invertebrates has never been formally addressed using multifactorial sampling designs. In several studies, samples collected at different depths have been analysed, leading to confounding effects in the results and to speculations on the possible role of depth as evolutionary factor. However, to asses if depth affects population genetic structure, replicated comparisons between

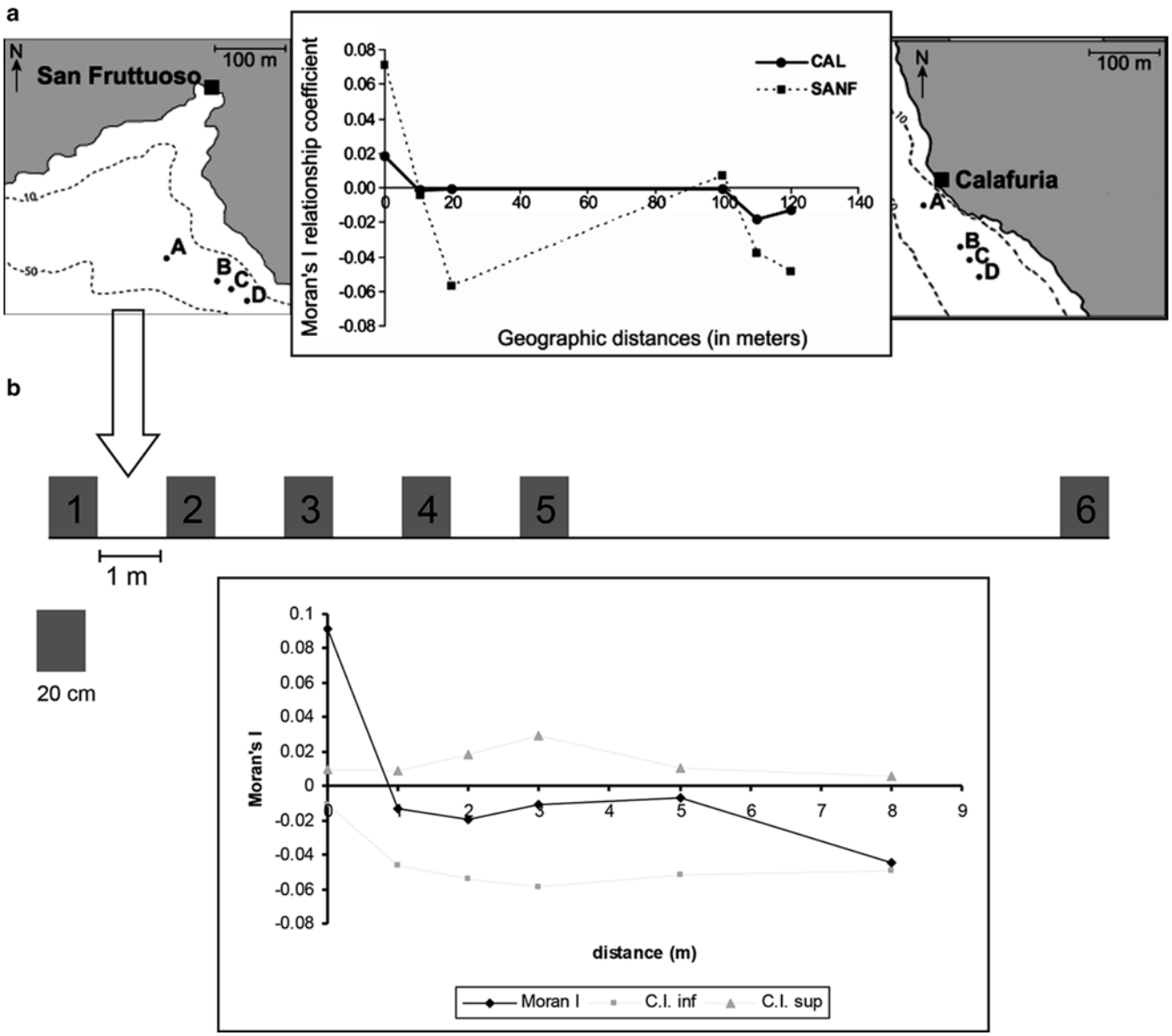

Fig. 44.4 Summary of the major findings on small scale spatial genetic structuring in *Corallium rubrum*. (**a**) Spatial autocorrelograms based on Moran's I coefficients estimated over all microsatellite loci (Costantini et al. 2007b), (**b**) analysis of genetic structure in San Fruttuoso (Italy) populations sampled at 1 m distances along a 10 m transect. Significant Moran's I coefficients were only observed in the 0 m class ($p < 0.01$)

samples collected at different depths are required. The hypotheses to test include: whether red coral shows genetic structuring along a depth gradient, and what the patch size would be along depth gradients. Costantini et al. (2011) addressed these questions by investigating the genetic structure among samples collected between 20 and 70 m depth in two locations in the Western Mediterranean. In both locations, strong patterns of genetic structuring among samples were found. The lack of connectivity observed between shallow- and deep-water populations suggests that deep-water populations are unlikely to act as refugia and/or a larval source (sensu Bongaerts et al. 2010) for shallow water populations, nor vice versa. Cannas et al. (2014) analysed shallow and deep populations on the Sardinian coasts and confirmed these results. They analyzed deep-water colonies from Western Sardinia (between 84 and 121 m depth) using five microsatellite loci, and found high genetic structure between populations. Moreover, Sardinian samples clustered in several distinct groups, according to their geographical origin (northern, central western and south-western Sardinian coasts) with a pattern of isolation by distance.

A consistent reduction in genetic variability from shallow to deep samples was also observed, with a threshold across 40–50 m depth, suggesting isolation between shallow- and deep-water red coral populations. Several explanations could be provided for the reduced genetic variation of deeper popu-

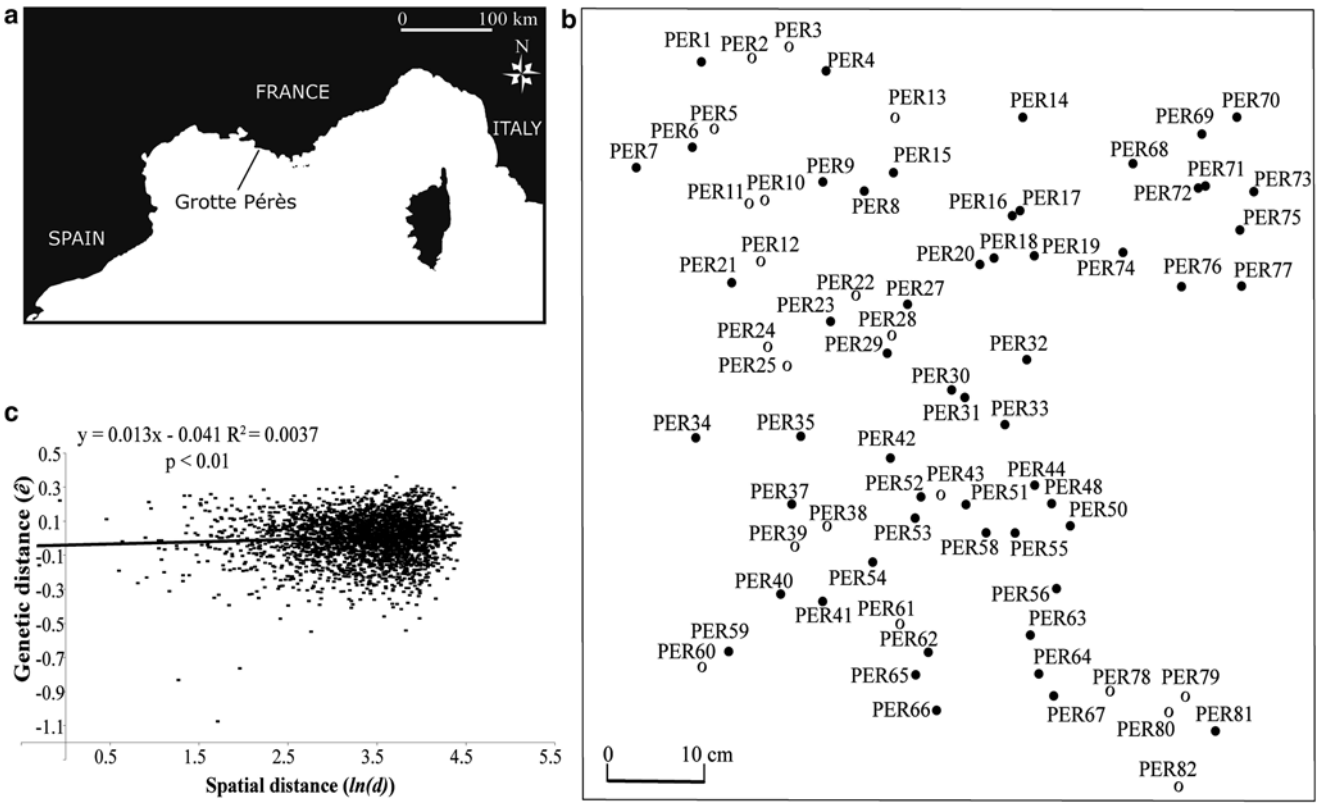

Fig. 44.5 Study of the population genetic structure between individuals within half a square meter. (**a**) This study was conducted in the Grotte Peres in France. (**b**) Geo-referenced sampling of 82 different colonies. (**c**) Significant correlation between geographic and genetic distances between individuals was reported (Ledoux et al. 2010a)

lations, such as physical forcing, environmental factors and/or biological processes (e.g. reproductive biology, recruitment, and selective mortality). However, in Sardinian deep coral populations high genetic diversity was found (Cannas et al. 2014) in contrast with the low diversity typically expected in harvested populations (Allendorf et al. 2008). This suggested that Sardinian red coral populations, despite being severely commercially harvested, are probably large, stable and not yet overexploited (Follesa et al. 2013).

The analysis of the red coral colonies collected below 600 m depths in the Strait of Sicily is also in contrast with the deep refugia hypothesis. In fact, ITS-1 and *mtMutS* sequences of deep-water samples differed from those of shallow water colonies, supporting the occurrence of genetic isolation between shallow- and deep-water populations (Costantini et al. 2010). Costantini et al (2013) also investigated genetic spatial structure of *Corallium rubrum* populations between 58 and 118 m depth in the Tyrrhenian Sea. Significant spatial genetic structure was observed, suggesting that populations are likely to be isolated. Moreover, mitochondrial markers revealed significant genetic structure at spatial scales greater than 100 km, suggesting the occurrence of a geographic pattern in deep-water populations that was not found in shallow water populations (Fig. 44.6).

44.6 Adaptation to Heterogeneous Environments: When Genetics Meets Ecology

The distribution range of the red coral encompasses highly contrasted ecological conditions, especially so when considering thermal regimes. These differences can correspond to different depths (from 5 to more than 800 m; Costantini et al. 2010), to different oceanographic basins (e.g. Northern to Southern Mediterranean, Atlantic) or to differences within basins (e.g. Bensoussan et al. 2010). Several environmental parameters, such as food resources or microbial communities are likely to change in correlation with temperature and depth. However, seawater temperature has been recognized as a source of environmental stress for the red coral (Torrents et al. 2008), and in the framework of climate change studies it has been investigated in different sites and at various depths (Bensoussan et al. 2010). Mortality events in red coral and in other octocoral populations observed in the northwestern Mediterranean (Cerrano et al. 2000; Garrabou et al. 2001, 2009) have been attributed to positive thermal anomalies. Differences in thermal regimes (including the frequency of stressful events) may drive local selection. Whereas the deepest red coral populations experience stable tempera-

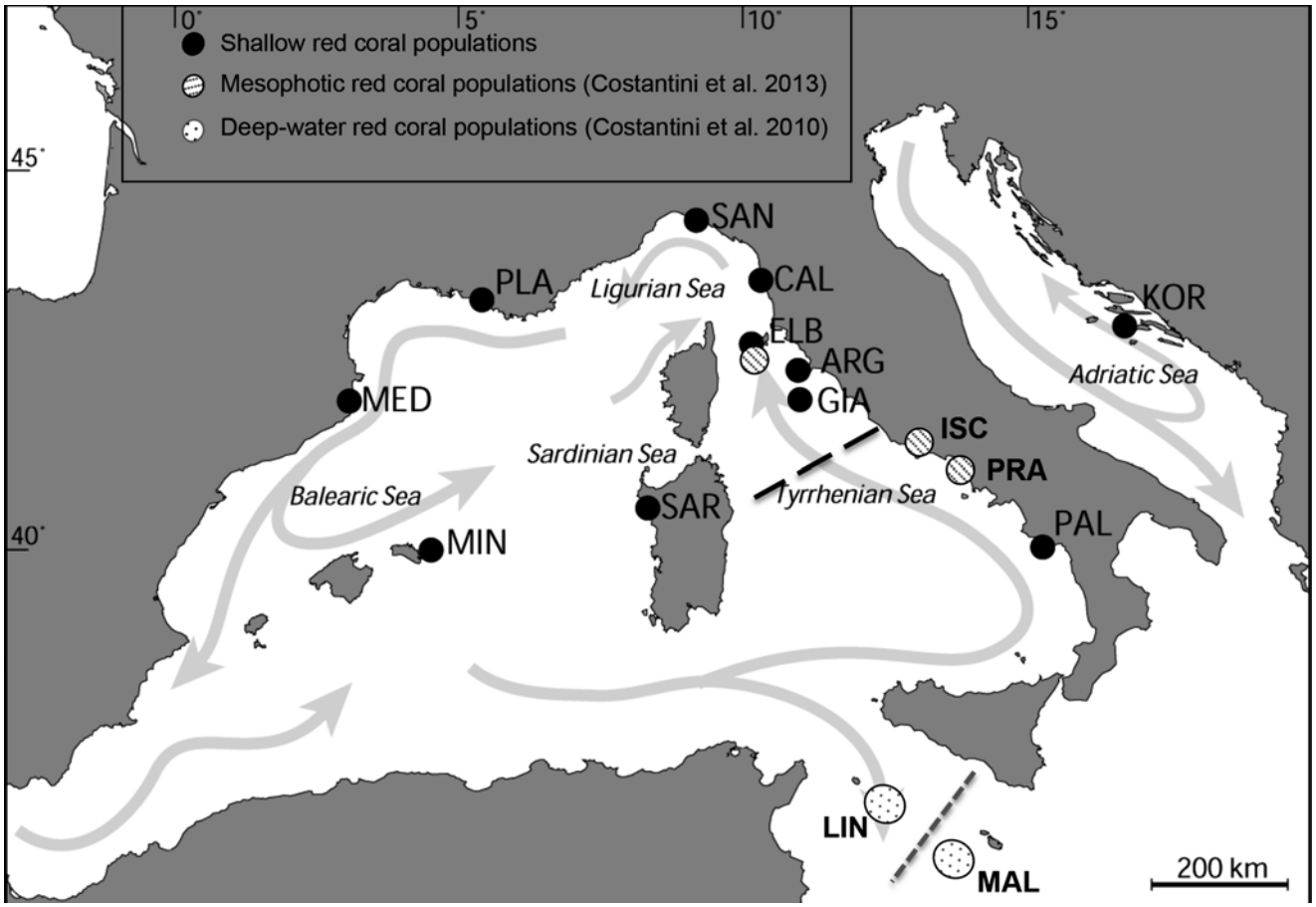

Fig. 44.6 Patterns of spatial genetic structure in *Corallium rubrum* populations at different depths. The *dashed lines* indicate the main genetic boundaries (Source Costantini et al. 2010, 2013)

tures, the shallowest populations face higher and more variable temperatures. For instance, in 2010, a red coral shallow population (5 m depth near Marseille) was exposed to temperatures reaching 25 °C and to fluctuation rates of about 10 °C in a 24 h period (Haguenauer et al. 2013).

The persistence and evolution of red coral populations in such heterogeneous environments raise important questions related to its adaptive abilities. The high genetic structuring observed in red coral populations suggests that different genetic pools are present in different environments. Genetic isolation is favorable to the evolution of local adaptation (i.e. the adaptation to local environmental conditions; Blanquart et al. 2013). Adaptive phenotypic plasticity could also explain the persistence of red coral in different environments (e.g. Palumbi et al. 2014). Experimental studies have shown the occurrence of phenotypic differentiation at various levels in response to thermal conditions in shallow populations (above 50 m). Experiments in aquaria (Torrents et al. 2008) have shown higher thermal tolerance in red coral colonies from 11 m compared to colonies from 40 m depth, with higher polyp activity, calcification rate, and delayed necrosis. Ledoux et al. (2015) combined reciprocal transplant and common garden experiments with population genetics analyses to disentangle the interactions between red coral populations and their local environment conditions. Reciprocal transplant experiments were conducted in two different locations involving populations dwelling at two depths (20 and 40 m) and thus exposed to different environments in situ. Using growth rate as a measure of fitness, this study revealed contrasted populations by environment at a distance of approximately 100 m. This suggested that red coral populations might be locally adapted to their habitat even at a small geographic scale. An in situ common garden experiment was conducted to further investigate how adaptive divergence could impact population viability in the context of an increase in water temperature. Individuals from two populations dwelling at 20 and 40 m were transplanted to 5 m depth to simulate thermal stress. After four summer months, differential responses between colonies were observed on the basis of the level of tissue necrosis and survival. The percentage of survival was significantly higher for the colonies coming from the shallow habitat than from colonies coming from the deep habitat. This experiment demonstrated differential phenotypic buffering capacities against thermal stress between

the colonies dwelling at different depths. Complementary population genetic analyses suggested that these differences might be due to divergent selection. Nevertheless, these results should be confirmed by increasing the number of populations tested and by considering the environmental differences linked to depth. Transcriptomic approaches should help understand the mechanisms explaining this adaptive diversity. Functional genomic approaches have already shown differential expression of heat shock protein (HSP 70) candidate genes in colonies exposed to thermal stress (Haguenauer et al. 2013).

Several experimental approaches should be used to further explore the response of red coral to thermal stress and climate change. New insights into the link between genetic diversity and environmental variables might come from next generation sequencing (e.g. Lundgren et al. 2013). The role of the microbiome should also be taken into account in a holobiont perspective (Parkinson and Baums 2014). *Vibrio* pathogens have been demonstrated as pathogens for another Mediterranean octocoral exposed to thermal stress (Bally and Garrabou 2007; Vezzulli et al. 2010) and *Vibrio* spp. have been isolated from colonies of red coral (Pasquale et al. 2011). An integrated study of the correlation between the genetic diversity of *C. rubrum* and its associated bacteria would prove highly beneficial.

44.7 New Perspectives by Next Generation Tools

To date, the red coral genome and its complex variability remains mostly a mystery. So far, the complete mitochondrial genome of two *Corallium* species has been sequenced (Uda et al. 2011). This facilitated discovery of more variable regions useful for population genetic studies (e.g. Costantini et al. 2013), but also clarified phylogenetic relationships within the genus, such as placing red coral in an evolutionary context, may aid its conservation and management (see below).

The recent technological advances in the "-omics" fields and their extension to non-model species (i.e. those species for which no genomic background information is available) give unprecedented opportunities to improve scientific knowledge at the multiple levels of biodiversity. Massive sequencing technologies (Next Generation Sequencing; NGS) allow to simultaneously characterize large fractions of a genome or transcriptome in multiple individuals or samples. Several research methods can be developed to take advantage of the NGS potential, from the development of new molecular markers for a high-resolution genome scan, to the comparative transcriptome analysis of individuals living in different habitats (in terms of biotic and abiotic parameters). The most straightforward contribution of genomics to conservation will be to greatly increase the precision and accuracy of estimation of parameters that require neutral loci (e.g. effective population size, Ne; migration rate, m) by genotyping hundreds to thousands of neutral loci in numerous individuals. Moreover, they allow investigation of specific genomic regions responding to selection, such as adaptation to local conditions and/or to particular environmental stress, or genes that are turned on/off during various stages of development. For instance, a new useful genomic approach to characterize the ecology and evolution of traditionally non-model species (Rowe et al. 2011) is the restriction-site-associated DNA sequencing (RAD-seq), which combines enzymatic fragmentation of the genome with high-throughput sequencing for the generation of large numbers of SNP markers (Baird et al. 2008).

44.8 Management and Conservation Issues

Genetic data on Mediterranean *Corallium rubrum* populations suggest that populations are 'closed' (supporting the assumptions made in population dynamic studies), and that the effective larval dispersal in this species is limited to very short distances. This information sheds a new light on management needs, and on the limited resilience of overexploited and exhausted red coral banks. Several workshops have been held on the management of red coral aiming to define sustainable exploitation thresholds of the resource. GFCM (2014) successfully promoted the definition of a Regional Management Plan for the Mediterranean Sea. However, experimental data show that *Corallium rubrum* is a slow growing species, with a patchy distribution, low recruitment rates and limited larval dispersal (Santangelo et al. 2012). These biological and ecological features suggest that red coral cannot be managed sustainably. Red coral is not a species at risk of biological extinction thanks to the abundance of small sized, crowded shallow water populations (Santangelo and Abbiati 2001). However, the history of the Mediterranean red coral fishery shows that economic extinction is likely since over-harvesting has boomed at the discovery of new banks and collapsed as soon as the bank was exhausted. There is evidence that banks that have been exhausted in the 1950s by the first SCUBA harvesters have still not recovered. Harvesting can also lead to reduced genetic diversity even with high initial population size, which could have important consequences on the long-term evolution of the red coral populations (Pinsky and Palumbi 2014). Recently, red coral harvesting has been mainly supported by the discovery of new banks (nowadays mainly along the Mediterranean coast of North Africa), and by broadening the harvesting depth to the physical constraints of human physiology (about 130 m) using mixed gas SCUBA diving. Currently a major pressure

is made by the harvesters and traders to adopt the use of Remotely Operated Vehicles (ROV) to access pristine banks below 130 m depth. Genetic data already provided a strong scientific basis for the recommendation made by GFCM (2011) to ban *Corallium rubrum* harvesting from 0 to 50 m depth. Recent data show that genetic diversity of red coral over its actual depth range distribution is shaped by complex interactions between geological, historical, biological and ecological processes, and private haplotypes of deep populations are an important part of the genetic diversity of the species. Moreover, climate change scenarios forecaste an increase of the seawater temperature, which is a new threat to shallow water populations. These findings provide a scientific background for the management of the species. Preservation of the pristine deep red coral banks, and rejection of the use of ROVs for harvesting are current conservation priorities. ROVs, however, do indeed represent a very effective conservation tool for a non-destructive approach for assessing the status of deep red coral banks (Bavestrello et al. 2014).

Moreover, there is an urgent need to improve coral fishery management with an effective enforcement of strict roles on fishing efforts. Finally, to preserve the diversity of the species, establishment of networks of Marine Protected Areas (MPAs) in the deep sea and on off-shore reefs is needed. Creation of MPAs in deep red coral banks is recommended as a precautionary conservation tool. The use of red coral as a "flagship species" could be also beneficial to promote the conservation of coralligenous reefs: a reservoir of Mediterranean marine biodiversity.

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Molecular Forensics into the Sea: How Molecular Markers Can Help to Struggle Against Poaching and Illegal Trade in Precious Corals?

45

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Abstract

Precious corals encompass various species belonging to three different orders (Alcyonacea, Zoanthidea and Antipatharia) of the Anthozoan class. These sessile cnidarians are one of the most valuable marine resources due to the use of their skeleton for jewelry and handcrafted artifacts. The exploitation of precious corals beds generally follows a boom and bust cycle resulting in a worldwide overexploitation of this natural resource. The sustainability of coral fisheries is therefore unambiguously questioned. Discussions regarding international regulations on harvesting or trade have regularly risen in the last decades. As an example, the genus *Corallium*, which includes some of the most harvested and valuables precious coral species, was unsuccessfully proposed for listing in Appendix II of the Convention on the International Trade in Endangered Species (CITES) in 2007 and 2009. To date, there is no international consensus on the management of coral beds (but see 2011 FAO-GFCM recommendations for the Mediterranean Sea). Each country manages independently its stocks of precious corals inducing contrasted conservation policies. Considering the benefit in trading precious corals and the low enforcement of existing regulations, poaching is very attractive and globally expanding, principally within marine protected areas where populations are healthier and colonies are generally bigger. In this context, innovative management tools and strategies should be developed to ensure the protection of these species. Recent advances in wildlife forensics

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sciences and more particularly in molecular forensics methods and associated statistical analyses open new avenues to struggle against poaching and illegal trade in precious corals.

We will introduce this chapter with general considerations regarding the exploitation of biological resources and accordingly the aims of molecular forensics applied in conservation biology. Then, we will present the methods used for species identification with particular emphasize on DNA barcoding methods. We will discuss the application of these methods for precious coral identification. Considering the genetic data available in precious corals, we will illustrate our discussion with an example of DNA barcoding in *Corallium/Paracorallium* species. We will present the atypical evolutionary features of the mitochondrial genome of these species and the implications for species identification. Once species have been identified, the identification of the geographical origin of individuals or populations may be of interest in order to distinguish between legal and illegal (e.g. within marine protected area) harvesting. In a third part, we will therefore expose different molecular and statistical methods that can be used to assign individuals to their populations of origin. As a case study, we will present the data resulting from the genotyping of nuclear microsatellite loci in the red coral, *Corallium rubrum*, a precious coral inhabiting the Mediterranean Sea and part of the Eastern Atlantic. The genetic structure and the repartition of the genetic diversity of *C. rubrum* are thoroughly characterized over the Western Mediterranean Basin. We will discuss the potential of this database of genotypes to characterize the origin of red coral colonies using different assignment methods. An empirical evaluation will be performed to test the statistical power of this dataset. The last part of the chapter will be focused on the perspectives offered by the development of next generation sequencing methods for the molecular forensics in precious corals.

Keywords

Molecular forensics • Precious corals • *Corallium* • Species identification • DNA barcode • Mitochondrial DNA • Assignment tests • Microsatellites • Next generation sequencing

45.1 The Uses of Biological Resources: From a General Perspective to the Specific Case of Precious Corals

45.1.1 General Perspective on the Uses of Biological Resources

Worldwide impacts of human activities resulted in an unprecedented loss of biodiversity, considered by many as the sixth mass extinction (Leakey and Lewin 1995). Uses of biological resources for human consumption are emphasized as a major factor driving the biodiversity crisis (Gavin et al. 2010). Direct exploitation of species for hunting, trade and collection has been identified by the International Union for Conservation of Nature (IUCN) as the second greatest threat for endangered species behind habitat destruction (IUCN 2004). The overharvesting and poaching of wildlife thus leads to a global biodiversity decline and to local extinctions of species. Poaching is defined as any act intentionally contravening the laws and regulations established to protect wild renewable resources (Muth and Bowe 1998). Unsustainable uses and trades of biological resources thus threaten the functioning and persistence and the whole eco-evolutionary dynamics of the ecosystems. They have been identified as one of the main challenges for wildlife conservation (Gavin et al. 2010).

45.1.2 Illegal Uses of Biological Resources to Wildlife Trade: The Rise of Molecular Forensic Analyses

Profit is the only motivation for wildlife traders. The main driver behind overharvesting and poaching of biological resources is the increasing global demand for wildlife products, which is estimated to billion of dollars each year (Wyler and Sheikh 2008; Barber-Meyer 2010). Both, the demand for wildlife and the resulting trade are intrinsically linked to the increase of human population, the economic growth of emerging countries (e.g. in Asia, South America) and the globalization (Nijman 2009; Baker et al. 2013). The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the most important treaty on wildlife trade, was adopted in 1975 to ensure that "international trade of specimens of wild animals and plants does not threaten their survival" (from http://www.cites.org/eng/disc/what.php). Thirty thousand species are listed in three appendices according to their levels of protection: from prohibited (except in

some circumstances; Appendix I) to different levels of controlled (Appendix II and III) trade. Based on CITES records, Roe et al. (2002) estimated that the global legal trade in wildlife (including fisheries and timber) was about US$ 160 billions and is rapidly expanding. Nevertheless, the whole scientific and manager community agrees that these estimations based on CITES-listed species reflect only a small portion of the global wildlife trade (Phelps et al. 2010). Indeed the vast majority of wildlife trade is illegal escaping from regulations and controls (Baker et al. 2013). Due to its cryptic nature, illegal wildlife trade is difficult to estimate and characterize (Liberg et al. 2012). This traffic, just after drugs and arms, is increasing worldwide and was estimated over $20 billions a year in 2008 (Baker 2008; Wasser et al. 2008). Illegal trade impacts a wide variety of species (e.g. large cat, bears, whales, corals) across all ecosystems (see Frankham 2010). One of the main factors explaining the current magnitude of illegal wildlife trade is the low level of associated risk in comparison with the economical benefits. The risk-to-reward ratio for illegal traders is low due to the minimal penalties and goal sentences (Leader-Williams and Milner-Gulland 1993; Johnson 2010; Baker et al. 2013). In this context, recent advances in wildlife forensic sciences and specifically in molecular forensics and associated statistical analyses enhanced by basic research in evolution and ecology (Tomberlin et al. 2011), were emphasized as crucial improvements to struggle against poaching and associated illegal trade (Manel et al. 2002; DeSalle and Amato 2004; Baker 2008; Alacs et al. 2010; Johnson 2010). These DNA based technologies can be applied to any kind of organic samples (from cook or dried meet to egg shell or animal hairs, bones or ivory) as far as DNA can be efficiently extracted (see Box 45.1). They are mainly used for two purposes: identification of species and, once species has been identified, identification of the geographical origin of individuals/populations (Alacs et al. 2010).

45.1.3 The Case of Precious Corals: An Overview of the Ecology of These Species

Precious corals are one of the most valuable marine resources. Their skeleton is handcrafted into various artifacts ranging from amulet to jewelry that are sold in ethnic and luxury fashion markets (Torntore 2002; Tsounis et al. 2010; see Fig. 45.1). Precious corals encompass various Anthozoan species belonging to three different orders (Alcyonacea, Zoanthidea and Antipatharia) such as gold corals (e.g. *Primnoa resedaeformis*, *P. willeyi*), bamboo corals (*Acanella* spp., *Keratoisis* spp.), black corals (genus *Antipathes*) or red and pink corals (*Corallium rubrum*, *Corallium secundum*). Zoanthidea and Antipatharia belong to the Hexacorallia whereas the Alcyonacea is included in the Octocorallia. Hexacorallia and

Box 45.1 DNA Extraction

The rise of molecular forensics to identify species or to determine the geographic origin of a sample is intimately linked to our ability to extract a sufficient amount of good quality DNA for subsequent analyses (PCR, sequencing, genotyping). This may be particularly challenging in forensic sciences because the DNA should usually be extracted from highly processes or degraded samples commonly encountered in wildlife trade markets (Baker 2008; Alacs et al. 2010). In this context, various methods of DNA extractions have been developed and DNA can now be successfully extracted from various products ranging from cooked and dried meats (Baker et al. 2000) to bones (Prado et al. 2002) and including ivory (Mailand and Wasser 2007) or dried shark fins (Chapman et al. 2003) (see Alacs et al. 2010). Regarding precious corals (e.g. *Corallium rubrum*), there is only one study that aimed to test the feasibility of DNA extraction using dried colonies (Ledoux et al. 2013). The main objective of this study on red coral was to test the feasibility of DNA extraction using dried tissues such as those that can be obtained from traded or seized colonies. This study demonstrated the feasibility of DNA extractions and the reliability of subsequent microsatellite genotyping using colonies dried for more than 2 years. In combination with available datasets (Costantini et al. 2007; Ledoux et al. 2010), this study opens new perspectives for the conservation of the red coral as discussed below. Complementary studies focused on the improvement of DNA extractions protocols are needed in order to fulfill the requirements of molecular forensic analyses in precious corals. More particularly, the reliability of the study conducted by Ledoux et al. (2013) remains to be tested for the amplification of mitochondrial or nuclear loci (i.e. barcode) usually used for species identification. Then, extractions of DNA directly from the skeleton of the colonies should also be implemented to allow for molecular forensic analyses of processed samples (e.g. amulet, pieces of jewelry, handcraft). Various methods may be tested such as those applied for extraction of ancient DNA from bones (Rohland and Hofreiter 2007) or methods already used for extracting DNA from ivory (Mailand and Wasser 2007). Finally, these methods should be expanded to all precious coral species. The validations of the methods of DNA extractions using processed or degraded samples are a prerequisite for an efficient development of molecular forensics in these species.

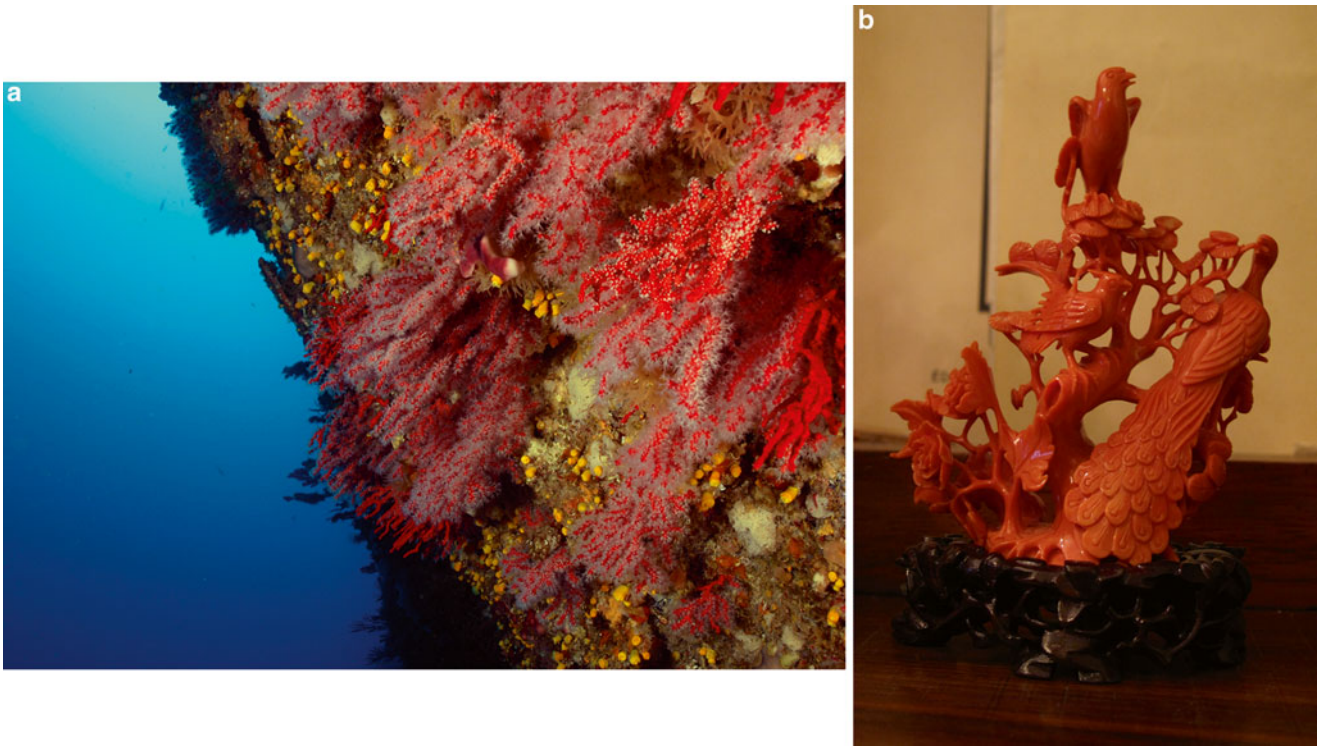

Fig. 45.1 Living (**a**) and handcrafted (**b**) colonies of the precious coral *Corallium rubrum*

Octocorallia represent the two subclasses of Anthozoan. The red and pink corals (including *Corallium* species), which phylogenetic relationships remain under discussion (Ardila et al. 2012; Figueroa and Baco 2014), are the most valuable species. Excepting the red coral (*C. rubrum*) and the Hawaiian black corals (*Antipathes grandis* and *A. griggi*), precious coral are usually found in deep marine environment (below 200 m).

The biology and ecology of precious coral species remain relatively poorly know except for the Hawaiian black corals (Grigg 1965, 1976; Wagner et al. 2012) and the Mediterranean red coral (Garrabou and Harmelin 2002; Torrents et al. 2005; Santangelo et al. 2012). They are considered habitat-forming species because of their arborescent form that increases the structural complexity of the environment. Precious coral are suspension feeder and do not host *Symbiodinium* photosynthetic symbionts. They are gonochoric species that brood their larvae. Asexual reproduction is rare in these species. Precious corals are long-lived species (tens of years) with relatively low population dynamics due to low growth and recruitment rates and late sexual maturity. Accordingly, they show the slowest growth rates of any know past or present fisheries (Grigg 1994; Roark et al. 2006); and their conservation is a central concern for scientists and managers.

45.1.4 Harvesting and Trade in Precious Corals

First records of the use of precious corals date 25,000 years ago, with fragments of the Mediterranean red coral (*Corallium rubrum*) found in Paleolithic human remains (Tescione 1965). For the majority of other precious corals, the fisheries began during the nineteenth century with the development of powered fishing vessels making accessible the deep banks (usually below 100 m) (Tsounis et al. 2010). From the use of iron hook 5000 years ago to the use of Remotely Operated Vehicle (ROV) or SCUBA diving and including a dredging device known as Saint Andrews Cross, the exploitation of precious coral bed always followed a boom and bust cycle (Bruckner 2009, 2014). The discovery of a new coral bed is immediately followed by a sharp increase in the yield and few years later by a strong decline once the bed is depleted (Bruckner 2009). According to the FAO, the harvesting of precious corals reached a peak of 450 t in 1984 and dramatically decreased to tens of tones in 2011 (Tsounis et al. 2010; Bruckner 2014). The decrease of 75 % of the annual yield in the red coral since the 1980s illustrated this trend. Despite this decrease, demand and trade in precious corals are increasing (Grigg 2004; CITES 2007). The combination between this high demand and the depleted stocks induces that the prices are currently rising. For instance, 1 kg of red coral, *C. rubrum* that was traded between US$ 100–900 can now reach US$ 2900 (FAO 1983; Tsounis et al. 2010). The strong demand promotes trades of juvenile colonies and small-sized branches, that were not sold few years ago, and are now traded for up to US$ 300 per kilogram, therefore enforcing the harvesting pressure on the species. Around US$ 230 millions per year are generated by the coral jewelry manu-

factures situated in Italy (Torre del Greco) and more than 1,800,000 of precious corals products were imported into the U.S. in 2006 (Tsounis et al. 2010). There is no international regulation for harvesting or trade of precious corals, and species management is mainly done at the national level (Tsounis et al. 2010). Each country is thus in charge of its stock of precious coral. For instance, the harvest of the Hawaiian black coral (*A. griggi*) is controlled by the Division of Aquatic Resources from the Department of Land and Natural Resources or by the National Marine Fisheries Service of the National Oceanic and Atmospheric Administration (NOAA). In this case, beds are classified in different categories corresponding to the expected yield and the harvesting methods that can be used (Griggs 1994). Moreover in this particular case, the management of the fishery is adaptive, based on the ecological characteristics of the species (harvest minimum size), and the number of licensed fishermen is low. The fisheries of the Hawaiian black coral may thus be considered the only sustainable precious coral fishery (Tsounis et al. 2010). On the contrary, the sustainability of the Mediterranean red coral fisheries is unambiguously questioned (Bruckner 2009, 2014). Red coral has been included in various international conventions (Annex V of the European Union Habitats Directive (92/43/CEE), Annex III of the Bern Convention and Annex III of the Protocol concerning Special Protected Areas and Biological Diversity in the Mediterranean (under the Barcelona Convention). A minimum size of 7 mm in diameter for harvest, the banishment of the dredges and the definition of quota were the first measures to manage the harvesting of the red coral (FAO 1983, 1988). The General Fisheries Commission for the Mediterranean (Recommendation GFCM/35/2011/2) recently recommends the full protection from fishing of population above 50 m depth. Besides these measures, the recommendations linked to red coral fishing differ between countries. The red coral is thus fully protected in Gibraltar and Malta. In Sardinia (Italia), 30 yearly licenses are distributed to professional fishermen. The situation is more alarming for populations of precious coral that dwell in international waters in which harvesting may be conducted without license. Considering that local management represents to date the main way to protect precious coral populations, Marine Protected Areas (MPAs) where harvesting is totally forbidden were emphasized as a central tool for the protection of these species. Focusing on the Mediterranean red coral, the efficiency of MPAs was demonstrated based on different demographic parameters such as the basal diameter and the height of the colony as well as the size structure of the population. Red coral populations within the MPAs showed higher values than populations outside the MPAs (Linares et al. 2010, 2012).

As for the harvesting, the trade of precious coral is only slightly controlled. Various proposals to include precious corals in Appendix II of CITES have been attempted. This results in the addition of black corals. Nevertheless, *Corallium* species which are amongst the most overharvested precious coral species, are still out of the CITES lists despite two attempts promoted in 2007 and 2009 by the US and EU.

In this context, two main points should be emphasized. First, there is a strong mismatch between the harvesting pressure and the population dynamics of precious coral species (growth rates, recruitment rates) with important consequences such as dramatic shifts of the population structure and local extinction. Then, the lack of international consensus regarding the management of these species combined with the low enforcement of existing regulations cannot counteract the increasing demand for precious coral products. Consequently, harvesting of small sized or dead colony is a common practice and poaching and illegal trades are increasing. Black market may represent up to 50 % of the trade (Tsounis et al. 2010). Regarding the red coral, poaching was reported in various parts of the Mediterranean and particularly within MPAs where colonies are generally characterized by larger size (Tsounis et al. 2007; Linares et al. 2012). Poaching has also been reported in other *Corallium* species with recurrent intrusions of Japanese and Taiwainese coral vessels in the US Economic Exclusive Zone (Tsounis et al. 2010). Accordingly, there is a need to develop new tools to enhance the conservation and management of these species. Molecular forensics analyses represent an interesting alternative for trade regulation and law enforcement. These analyses allow for the identification of species even on processed samples. This may help to monitor and to discriminate legal and illegal wildlife trade. Then, molecular forensics may be used to assign the geographic origin of trade products, which can be used to distinguish between legal harvesting and poaching. In this chapter, our aim is therefore to present the interest of these analyses in precious corals focusing more particularly on species identification and identification of the origin of individuals.

45.2 Species Identification Using DNA-Based Approaches

45.2.1 Interest of the DNA Barcoding

The identification of species using morphological characters is not always straightforward. For precious coral species, morphological characters include colony branching pattern, polyp structure, skeletal spine morphology or form and distribution of sclerites. Nevertheless, their diagnostic potential may be limited or even inexistent in certain comparison involving congeneric species (e.g. see Uda et al. 2011, 2013

for limitations regarding the morphological characters in the Coralliidae family). Indeed, morphological characters may show strong phenotypic plasticity and vary within colonies or among habitats (Ardila et al. 2012). The limitations linked to the use of morphological characters are even higher when working with degraded or processed fragments of coral colonies such a commonly encountered in forensics studies. In this context, the identification of species based on biochemical or molecular analyses is an appealing alternative that is increasingly receiving attention from the wildlife forensics community. Regarding biochemical analyses, the pattern and characterization of organic matrix has been proposed as a tool for the identification of *Corallium* species (Debreuil et al. 2011). Nevertheless, this approach may currently be limited by the number of species considered. Regarding molecular approaches, the first attempt to identify species date from the early 1980s (Kangethe et al. 1982). Nevertheless, this is after the works of Hebert et al. (2003a, b) that the molecular identification of species, so-called DNA barcoding, became widely used. DNA barcoding corresponds to the rapid and accurate identification of species based on the use of a standard DNA region (Valentini et al. 2009). The two declared aims of this technique were to assign an individual to a known species and to facilitate the discovery of new, potentially cryptic, species (Hebert et al. 2003a). Because mitochondrial DNA was widely recognized as a powerful tool for species identification (Avise et al. 1987; Moritz et al. 1987; Moritz 1994), a fragment of 685 bp from the 5′ end of the mitochondrial cytochrome oxydase 1 (COI) was proposed as standard barcode for animal species identification. The accuracy of this gene in barcoding relies on the occurrence of a barcoding gap (see Fig. 45.2a), which corresponds to the difference between the intraspecific variation and the interspecies divergence (Meyer and Paulay 2005). The occurrence of this gap allows the definition of a threshold value to distinguish conspecifics from other taxa. The corresponding analysis was named distance-threshold analyses. As a general recommendation, Hebert et al. (2004) proposed a 10 times threshold of the mean intraspecific variation to differentiate species. In complement, a character-based analysis was also developed for DNA barcoding. This method transfers the information of the sequence into diagnostic character (Bergmann et al. 2013). Whatever the method used, barcoding technique supposes that the evolutionary relationships of taxa are known as well as the availability of a reference database of authenticated sequences that enables the unknown samples to be matched on known DNA sequences. The matching can be done through DNA sequence similarity search (e.g. Altschul et al. 1997) or using phylogenetic reconstruction (e.g. Baker and Palumbi 1994). DNA barcoding based on distance-threshold analyses has been successfully applied in various taxonomic groups such as birds (Hebert et al. 2004) or butterflies (Hajibabaei et al. 2006), whereas character-based analyses were used on odonates (Damm et al. 2010) or turtles (Reid et al. 2011). Its range of applications increases rapidly and DNA barcoding is now implemented for the description of cryptic (Smith et al. 2006) and invasive (Siddall and Budinoff 2005) species or for animal diet analysis (Valentini et al. 2009) (see Frézal and Leblois 2008; Bucklin et al. 2011). From a forensics perspective, DNA barcoding has been applied in many different cases such as illegal hunting (Yan et al. 2005), illegal trade (DeSalle and Birstein 1998; Hsieh et al. 2003; Abercrombie et al. 2005), bushmeat trade (Gaubert et al. 2015) or food mislabeling (Marko et al. 2004; Ogden 2008; Rasmussen and Morrissey 2008; Barbuto et al. 2010; Huxley-Jones et al. 2012).

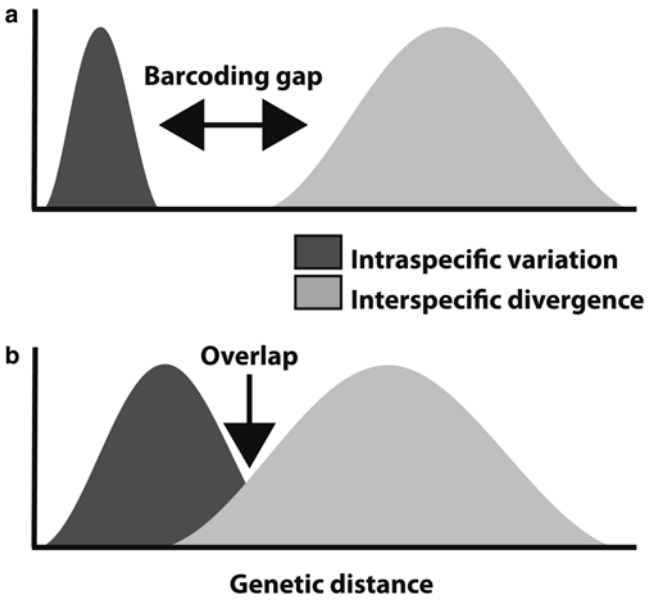

Fig. 45.2 The use of DNA barcode relies on the occurrence of a barcoding gap, which corresponds to the difference between the levels of intraspecific variation and interspecific divergence (**a**). Nevertheless, the barcoding gap may be missing due to an overlap between the two levels of genetic differentiation questioning the use of barcode approach (**b**) (Adapted from Meyer and Paulay 2005)

The diversification of DNA barcoding applications came along with a diversification of the molecular barcodes mainly driven by a lack of barcoding gap (Fig. 45.2b) or diagnostic character reported in some species when using the standard COI (see below). In this context, Valentini et al. (2009) propose to distinguish between the barcoding *sensu stricto* (the identification of species using a single standardized DNA fragment) and the barcoding *sensu lato* (the identification of any taxa using any DNA fragment).

45.2.2 DNA Barcode and Precious Corals

To date, there is no specific study that aimed to validate an approach based on a barcoding *sensu stricto* and using the standard COI barcode in precious corals. This is likely due to the fact that precious corals cover different orders within the two subclasses of Anthozoan. Moreover, Anthozoan phylogeny is still a matter of debate (McFadden et al. 2006; Daly et al. 2007; Kayal et al. 2013; Keshavmurthy et al. 2013; Schmidt-Roach et al. 2014). Nevertheless, various works were conducted to evaluate the interest of the standard COI barcode in octocorals (France and Hoover 2001, 2002; Mcfadden et al. 2011) or in different genera (*Antipathes* Wagner et al. 2010) or orders (Scleractinian Shearer and Coffroth 2008; Zoanthidea Sinniger et al. 2008) of hexacorals. Focusing on Scleractinians, Shearer and Coffroth (2008) demonstrated a null to low level of variations for the COI barcode both at the intraspecific and interspecific levels. This induces an overlap between the two levels of differentiation (Fig. 45.2b) that limits the ability to distinguish different species. In their survey, which involved more than 150 COI sequences from 90 species covering 44 genera and 14 families from the Atlantic, the Caribbean and the Indo-Pacific regions, the range of confamilial divergence overlapped intraspecific variations. Moreover, 21 COI haplotypes were shared between species (ten by species belonging to different genera and three by species belonging to different families) restraining the implementation of a character-based analysis. Thus, the distinction between most of the scleractinian species may not be successfully achieved with this barcode (Shearer and Coffroth 2008). For the Zoanthidea, more promising results were reported. Despite some overlap between intraspecific divergence and interspecific variation, the COI may be useful in some cases (Sinniger et al. 2008, 2010). Focusing more particularly on black corals (genera *Antipathes*), Wagner et al. (2010) revealed similarity between sequences including the COI barcode ranging from 95 to 100 % for congeneric species. In octocorals, 106 species from 46 genera belonging to 16 families were used to test the standard COI barcodes (McFadden et al. 2011). The obtained results were in line with previous studies on Anthozoan species with an absence of a well-defined barcoding gap and various species sharing the same haplotype (see also France and Hoover 2002; Calderón et al. 2006). Character-based species assignment was informative only in some cases (McFadden et al. 2011). Focusing on the genus *Corallium* and *Paracorallium*, we used the COI sequences published by Ardila et al. (2012) to evaluate the levels of intra and interspecies divergences in these precious species. As for other Octocorals, the lack of barcoding gap limits the use of COI as a barcode in these species (see Box 45.2).

Different hypotheses have been proposed to explain the lack of barcoding gap in the standard barcode and more

Box 45.2 Case Study Standard and Extending Barcode in *Corallium* and *Paracorallium* Genus

In a recent study focused on the phylogeny of precious Octocoral species, Ardila et al. (2012) analyzed 127 sequences corresponding to 519 bp of the mitochondrial COI: 112 and 15 from *Corallium* and *Paracorallium* genus respectively (see Table b1-1). Based on this dataset, we tested the occurrence of a barcoding gap in theses species. To achieve this aim, we estimated the levels of intraspecific variation and interspecific divergence by computing the pairwise distance between sequences. We used the *p-distance*, which is the proportion of nucleotide sites at which the two sequences compared are different (Nei and Kumar 2000). We computed the within and between group distances considering the different species from the two distinct genus.

Overall, the mean distance (standard deviation) was 0.023 (0.004). When considering the genus *Corallium*, the mean distance within species varied from 0 for *C. ducale*, *C. eliatus*, *C. kishinouyei*, *C. konojoi*, *C. secundum* to 0.006 for *C. laauense* (mean ± SD = 0.002 ± 0.002). The pairwise distance between species ranged from 0 for *C. abyssale* vs. *C. ducale*, *C. sulcatum* vs. *C. abyssale*, *C. sulcatum* vs.

Table bII-1 Samples used to test for the universal barcode (COI) in *Corallium* and *Paracorallium* genus (Data from Ardila et al. 2012)

Genus	Species	Number of sequences
Corallium	*Corallium abyssale*	1
	Corallium ducale	4
	Corallium elatius	6
	Corallium imperiale	45
	Corallium kishinouyei	4
	Corallium konojoi	4
	Corallium laauense	24
	Corallium medea	1
	Corallium niobe	3
	Corallium niveum	10
	Corallium secundum	5
	Corallium sulcatum	1
	Corallium sp.	4
Paracorallium	*Paracorallium japonicum*	1
	Paracorallium sp.	5
	Paracorallium tortuosum	5
	Paracorallium thrinax	2
	Paracorallium nix	1

(continued)

Box 45.2 (continued)

C. abyssale to 0.051 for *C. eliatus* vs. *C. imperiale*, *C. laauense* vs. *C. eliatus* and the mean distance was 0.028 (0.016). For *Paracorallium*, the within group distance varied from 0.004 for *P. thrinax* to 0.023 for *P. sp.* (mean ± SD = 0.012 ± 0.010). The pairwise distance between species ranged from 0.003 for *P. japonicum* vs. *P. tortuosum* to 0.044 for *P. thrinax* vs. *P. tortuosum* (mean ± SD = 0.027 ± 0.015).

The pairwise distance between species belonging to the two different genus ranged between 0.004 for *P. thrinax* vs. *C. konojoi* and 0.048 for *P. thrinax* vs. *C. laauense*, *P. thrinax* vs. *C. imperiale* and *P. nix* vs. *C. medea* (mean ± SD = 0.029 ± 0.013). The results are shown on Figure bII-1. This analysis demonstrates the overlap between the levels of intra and interspecies variations mainly due to a lack of sequence variation between species (e.g. *C. abyssale* vs. *C. ducale*). The barcoding gap at the COI locus is not present in the precious corals belonging to *Corallium* and *Paracorallium* genus. These results emphasize the need to develop an extended barcode that may be used for forensics analyses in these species.

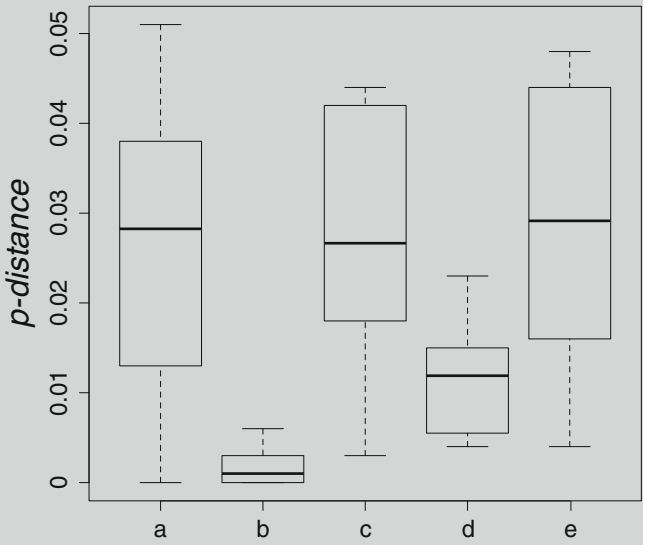

Figure bII-1 Boxplots of the *p-distance* computed at different levels: (a) between *Corallium* species; (b) within *Corallium* species; (c) between *Paracorallium* species; (d) within *Paracorallium* species; (e) between *Corallium* and *Paracorallium* species. The dark lines correspond to the means, the boxes represented the first and third quartiles and the whiskers represented the minimal and maximal values. There is a clear overlap between all the different boxplots

generally, the lacks of polymorphism in the Anthozoans mitochondrial DNA (Shearer et al. 2002; Hellberg 2006; Bilewitch and Degnan 2011; see Box 45.3). These studies demonstrated that COI is usually not informative for species identification in Anthozoans and accordingly for forensic analyses in precious corals. Nevertheless, considering the contrasted results obtained within Anthozoans, the evaluation of the standard COI barcode should continue on a case-by-case basis (McFadden et al. 2011). Indeed, in many cases the COI allowed for the assignment of samples to higher taxa (genus or family), which is already a significant output considering the relatively poor state of Anthozoan taxonomy. Moreover, to circumvent the limitations linked to the COI barcode for species identification, alternate molecular barcodes have been tested alone or in combination. These alternate barcodes include different mitochondrial loci such as the *16S* (Sinniger et al. 2008), the *mtMutS* gene (McFadden et al. 2011), intergenic regions (IGR) (e.g. *trnW-IGR-nad2* and *cox3-IGR-cox1* Wagner et al. 2010; *cytb-IGR4-nad6* van der Ham et al. 2009) as well as different nuclear genes such as the 28S nuclear ribosomal gene (28S rDNA; McFadden and van Ofwegen 2013; McFadden et al. 2014a, b), Internal Transcribed Spacers (*ITS1* or *ITS2*; Wagner et al. 2010) or intronic sequences (*SRP54*; Concepcion et al. 2008). The combination between *COI*, *IGR1* (between *COI* and *COII*) and *mtMutS* including more than 1800 bp has been recommend as extended barcode for Octocorals (McFadden et al. 2011). Combined to the nuclear *28S rDNA* (around 3000 bp), this extended barcode identified more than 90 % of the Indonesian xeniid species sampled in McFadden et al. (2014b). Nevertheless, it remains to be tested in precious octocorals and to be validated for forensics purpose (Dawnay et al. 2007). Moreover, considering that this barcode relies on the sequencing of *mtMutS*, a gene exclusively found in Octocorals, the quest for a unique barcode in Anthozoan allowing the discrimination among all precious coral species remains open. We posit that the input of Next Generation Sequencing (NGS) analyses should open new perspectives on this topic (see below) by refining the phylogeny of Anthozoans and by allowing for the identification of relevant barcode(s).

Meanwhile, we suggest the evaluation of a two steps barcoding method for the forensic identification of the precious corals. The first step would assign the unknown sample to one of the three different orders of precious coral using COI (i.e. Alcyonacea, Zoanthidea and Antipatharia). Once the order assignment has been achieved, identification of the

Box 45.3 The Mitochondrial Genome of Anthozoans

In most of the animal species, mitochondrial genomes are small (<20 kb) compared to the nuclear genome. It is strongly conserved and corresponds to a single double-helical circular molecule (haploid) that contains typically 37 genes including 13 protein-coding and two ribosomal protein genes, 22 t-RNAs and a large non-coding control region. This genome is characterized by a lack of intron and the presence of very short intergenic regions (few nucleotides). Mitochondrial DNA is usually characterized by a high mutation rate that takes place at the third codon. Rates of substitution are several times higher than those for nuclear DNA and induce a high polymorphism in natural populations. Because of its clonal (maternal) inheritance, the mitochondrial genome is considered as non-recombining. Nevertheless, this relative homogeneity is questioned when accounting for non-bilaterian species (Osigus et al. 2013), which are characterized by an important sequence and genome organization variability (Lavrov 2011). Focusing on Anthozoans, around 70 mitochondrial genomes have been sequenced to date, including various precious corals such as *Corallium rubrum* and *C. eliatus* (Uda et al. 2013) or *C. konojoi* and *Paracorallium japonicum* (Uda et al. 2011). These mitochondrial genomes present other characteristics such as additional protein coding genes (e.g. mtMutS in octocorals (Pont-Kingdon et al. 1995)), a reduced number of tRNA, the occurrence of intronic sequences and a high level of gene reorganization between species. Hexacorals display a mitochondrial genome between 14.8 and 21.3 kb with intergenic regions, 13 protein coding genes with *COI* and *ND5* genes containing an intronic sequence and usually two *tRNAs*. The mitochondrial genome in octocorals is around 19 kb and is usually composed by 14 protein-coding genes and one *tRNA*. Variations in genes organization were reported in various octocoral species (e.g. between *C. konojoi* and *P. japonicum* (Uda et al. 2011)). In addition, the mitochondrial genome of octocorals is the only metazoan genome to show the occurrence of a gene putatively involved in DNA mismatch repair function, the *mtMutS* gene (Pont-Kingdom et al. 1995). This mitochondrial gene would result from a horizontal gene transfer from a non eukariotic origin (giant virus or epsilonproteobacteria) (Bilewitch and Degnan 2011). Another striking characteristic of Anthozoan mitochondrial DNA is the slow rate of gene evolution compared to their nuclear genome (five times slower) and to the mitochondrial genome of other animal species (10–100 times slower) (Hellberg 2006; Chen et al. 2009). This has important consequences for the use of mitochondrial DNA as a molecular marker in DNA barcoding or phylogeographic studies (see main text). Various hypotheses have been proposed to explain this result such as constraints on the mitochondrial genome to accumulate mutations due to unusual mutation rate or DNA mismatch repair (particularly in Octocorallia), molecular convergence due to selective pressure or hybridization with introgression (Shearer et al. 2002). Complementary works are needed to formally test these hypotheses.

(continued)

species should be attempted using one of the three following extended barcodes: (1) *COI*, *IGR1* and *mtMutS* for Alcyonacea (McFadden et al. 2011), (2) *trnW-IGR-nad2, cox3-IGR-cox1*, *ITS1* and *ITS2* for Antipatharia (Wagner et al. 2010) and (3) *16S-COI* for Zoanthidea (Sinninger et al. 2008).

45.3 Population of Origin of Individuals: Microsatellites and Assignment Tests

Seizures of illegal wildlife products are usually conducted far from the locations where plants or animals were poached. Moreover, hunting, fishing or harvesting are in numerous cases submitted to local legislation. Accordingly these activities can be allowed in some areas and prohibited in others as a function of the stock abundance or of the occurrence of protected areas or natural parks (Baker 2008). The identification of the geographical origin of individuals or populations is therefore a central topic in forensics science (Baker 2008; Alacs et al. 2010). Inferring the origins of wildlife products should allow distinguishing between legal or illegal use of biological resources and in turn, to target poaching hot spots and to adjust enforcement efforts (Ishida et al. 2013). In this context, DNA based analyses of the origin of individuals received particular attention from wildlife forensics community (Linacre et al. 2011; Johnson et al. 2014). These analyses rely on the refinement of molecular identification of individuals due to the combination between highly variable markers such as microsatellites (Box 45.4) and assignment tests (Paetkau et al. 1995; Cornuet et al. 1999; Manel et al. 2002).

Based on the high level of information contained in individual's multilocus genotypes, assignment tests estimate population membership of individuals or group of individuals (Manel et al. 2005). Briefly, expected genotypic probabilities are computed for each potential source population

Box 45.4 Microsatellites

The nuclear genomes of most taxa are characterized by the occurrence of repeated sequences. Within these repeated sequences, microsatellites (Short Tandem Repeats (STR), Simple Sequence Repeats (SSR), Variable number tandem Repeats (VNTR)) are short repeated sequences of one to six nucleotides (Selkoe and Toonen 2006). The number of tandem repeats usually varies between 5 and 40. In molecular ecology, di-, tri- or tetranucleotides are the most commonly used. Nevertheless, Linacre et al. (2011) recommended the use of tetranucleotide repeat for forensic purposes to avoid various ambiguities arising from genotyping mono-, di- or trinucleotide (e.g. scoring errors due to stuttering). Despite a significant increase of the number of studies using these markers, our knowledge regarding their evolution and their function in the genome is still poor. Their occurrence can vary between related species. Microsatellites are found both in the coding and non-coding regions. They are characterized by a high mutation rate (10^{-3}–10^{-7} per locus per generation) inducing variations in the number of tandem repeats. Because of this mutation rate, microsatellites show a high polymorphism (allelic diversity) allowing studies at the individual level with a high statistical power. Microsatellites are supposed to be neutral. They are usually species specific, which means that they should be develop on a case-by-case basis. Nevertheless, cross species amplifications are possible in some cases (e.g. one microsatellite from *C. laauense* was successfully genotyped in *C. rubrum*; see Costantini et al. 2013).

based on the corresponding allelic frequencies. The individuals of unknown origin are then assigned to the population from which their genotypes are most likely to occur (Manel et al. 2005). The two main applications of assignment tests are classification problems and assignments of individuals to their population of origin. Several assignment procedures based on different statistical methods (frequentist or Bayesian) and methodological improvements (including exclusion methods) are now available (Paetkau et al. 1995, 2004; Rannala and Mountain 1997; Cornuet et al. 1999; Pritchard et al. 2000; Wasser et al. 2004; Kalinowski et al. 2007; see Manel et al. 2005 for details). Overall, the performances of these methods are positively linked to the number of loci, their level of polymorphism as well as the number of sampled individuals (Cornuet et al. 1999; Manel et al. 2002). Their efficiency also relies on the level of differentiation between the different putative populations of origin to be tested: the distinction between the candidate origins is made easier with higher genetic differentiation. The underlying genetic structure should thus first be investigated through F_{ST} estimates, differentiation tests and clustering analyses. Assignment tests were successfully applied in wildlife forensics since the end of the 1990s (e.g. Primmer et al. 2000; Spencer and Hampton 2005; Frantz et al. 2006). Regarding more particularly the struggle against poaching, the number of studies using assignment tests is constantly increasing (Manel et al. 2002; Millions and Swanson 2006; Wasser et al. 2007; Lorenzini et al. 2011; Nielsen et al. 2012; Glover et al. 2012). The study conducted by Wasser et al. (2007) is one of the most significant examples of the strength of assignment tests based on microsatellite genotyping. This method allowed Wasser et al. (2007) to accurately identify the geographic origin of a large shipment of contraband elephant ivory seize in Singapore. This study was followed by important political consequences such as the replacement of the Zambian director of wildlife and significant law enforcement in this country (Wasser et al. 2007).

45.3.1 Assignment Tests and Precious Corals

Regarding precious corals, approaches to define the geographic origin of traded colonies should be urgently implemented considering the harvesting and poaching pressures acting on these organisms. As previously mentioned the management of precious corals is mainly local (Griggs 1994; Bruckner et al. 2010) leading to the definition of no-take zone within Marine Protected Areas (MPAs). These protected coral beds or populations are particularly attractive for poachers. For instance, recent studies in *C. rubrum* demonstrated an increase of poaching within MPAs (Hereu et al. 2002; Linares et al. 2003, 2012; Tsounis et al. 2007) mainly due to the better conservation status leading to bigger colonies, higher densities and thus higher economic value of the protected populations compared to harvested ones (Linares et al. 2010). Assignment tests may allow convicting poachers by demonstrating that seized colonies came from a population submitted to harvested restriction. Various recommendations exist for the use of microsatellites in a forensic context (Alacs et al. 2010; Linacre et al. 2011; Johnson et al. 2014). Focusing on precious coral, the first step would be the development of microsatellites or others highly polymorphic markers (e.g. Single Nucleotide Polymorphism, SNP; see below) for each species. Indeed, to our knowledge, microsatellite markers remain scarcely available in precious corals. A survey of GENBANK (December 2014) revealed that microsatellites are only developed in three species: *C. laauense* (6 microsatellites; Baco et al. 2006), *C. rubrum* (15 microsatellites; Costantini and Abbiati 2006; Ledoux et al. 2010), *Primnoa pacifica* (12 microsatellites; Morrison & Springmann, unpublished). Once genetic markers are avail-

able, their use for forensics purpose should be validated (e.g. Andreassen et al. 2012). Guidelines for markers validation have been proposed by different forensic groups such as the SWGDAM (Scientific Working Group on DNA Analysis Methods) or the ISFG (International Society for Forensic Genetics). Briefly, the validation of molecular markers for forensic purpose means that markers specificity, sensibility, conditions and qualities of amplifications should be characterized (Dawnay et al. 2008; Ogden 2008). When using microsatellites, genotyping procedure (particularly allele scoring) should be standardized between laboratories in order to ensure compatibility between datasets (see Moran et al. 2006; Ellis et al. 2011 for details). Then the identification of the origin of individual samples required a reference database of genotypes to conduct assignment tests. Ideally, this database should include a geographically extensive survey of the genetic diversity of the studied species (i.e. allele frequencies of the populations over the whole distribution area). Such databases are available for some domesticated mammal species such as dogs, cats or cows but remain rare for wild animals (but see Caniglia et al. (2010) for wolf or Andreassen et al. (2012) for brown bears). Regarding the three precious coral species for which microsatellites have been developed, databases are missing. Nevertheless, the studies focused on the characterization of the pattern of spatial genetic structure in *C. laauense* (Baco and Shank 2005) and *C. rubrum* (Costantini et al. 2007, 2011, 2013; Ledoux et al. 2010; Aurelle et al. 2011; Cannas et al. 2015) are promising. For instance, the data from Ledoux et al. (2010) encompassed more than 1200 individuals genotyped at 10 microsatellites and coming from 40 populations from the North-Western Mediterranean Basin. When accounting for the data produced in Aurelle et al. (2011) the databank grouped more than 1300 individual genotyped at 7 microsatellites (3 of the previous ones did not amplified in distant populations; see Aurelle et al. 2011 for details) and coming from 47 different populations distributed all around the Western Mediterranean Basin. The preliminary evaluation of the efficiency of these microsatellites for forensic purpose is promising (see Box 45.5) as 92 % of the colonies where assigned to their region of origin with a mean probability of 0.91. The strong genetic differentiation observed in *C. rubrum* combined with the large geographic range covered by genetic analyses (Box 45.5; Costantini et al. this book) should improve the efficiency of these approaches for the identification of the origin of red coral samples.

A standardization of the available datasets among the different teams working on the red coral should be the next step for this species. This would increase the geographic area under survey by adding Sardinian (Cannas et al. 2014) and deep (Costantini et al. 2013) red coral populations. Regarding *C. laauense*, the amount of available data is smaller as 134 individuals collected from 8 sites in the Hawaiian Islands

Box 45.5 Preliminary Assessment of Assignment Tests in the Red Coral, *Corallium rubrum*

Ledoux et al. (2010) described the spatial genetic structure at large geographic scale of the red coral, *Corallium rubrum*. Their hierarchical sampling scheme covered four different regions: the Provençe-Liguria, the North-Western Corsica, the Catalonia and the Balearic Islands. Within these four regions, around 30 individuals from 40 different populations dwelling between 14 and 60 m depth were sampled. In total, 1222 different colonies were genotyped at 10 different microsatellites. The global *FST* value was 0.097 and significant pairwise genetic differentiations were reported in most of the comparisons even for populations separated by 10 m. Overall, the spatial genetic structure corresponded to the combination between four genetic clusters concordant with the four regions and isolation by distance between populations. We took advantage of this dataset to test the feasibility of assignment tests in *Corallium rubrum.*

We conducted three different tests using STRUCTURE (Prichard et al. 2000) one of the most commonly used software for clustering and assignment tests. In the first test, our objective was to evaluate the ability of STRUCTURE to identify the region of origin of unknown individuals. We considered 1105 individuals from 35 populations shared among Provençe-Liguria, North-Western Corsica and Catalonia. We selected randomly 50 individuals from these populations as individuals of unknown origin. The second and third tests were conducted at lower spatial scale to test whether the population of origin of unknown individuals can be identified. These two tests were conducted considering a reduced dataset of 20 populations from the Liguro-Provençal cluster. For the second test, we considered each population as a putative origin whereas the third test was based on the result of the clustering analyses identifying seven different genetic groups within the Liguro-Provençal cluster. For these two tests, we selected randomly 50 individuals coming from the 20 populations. The three different tests were conducted based 10 different runs with 150,000 burn-in followed by 500,000 iterations and using the POPINFO model with correlated allele frequencies. Regarding the first test, 92 % of individuals (46) were correctly assigned to their region of origin. Among these, 46 individuals the mean probability of assignment to the correct region of origin over the ten runs ranged from 0.58 to 0.97 (mean = 0.91). Thirty-two (69 %) individuals were assigned to their region of origin with a probability higher than 0.9 and

(continued)

Box 45.5 (continued)
42 individuals (92 %) when considering a probability of 0.75. When focusing on the Liguro-Provencal region and considering the sample sites as the population of origin, 54 % of individuals (27) were correctly assigned to their population of origin with a mean probability of assignment ranging from 0.23 to 0.90. Only one (2 %) and 10 (20 %) individuals were assigned with a probability higher than 0.9 and 0.75 respectively. When considering the seven clusters identified by STRUCTURE in the Liguro-Provençal region, the number of individuals correctly assigned to the cluster of origin was 42 (84 %) with a probability of assignment ranging from 0.37 to 0.97. Fifteen (30 %) and 29 (58 %) individuals were assigned with a probability higher than 0.9 and 0.75 respectively. These results are summarized in Figure bV-1. They represent preliminary data and complementary analyses including different assignment methods are needed to formally validate these microsatellites and dataset for forensics purposes.

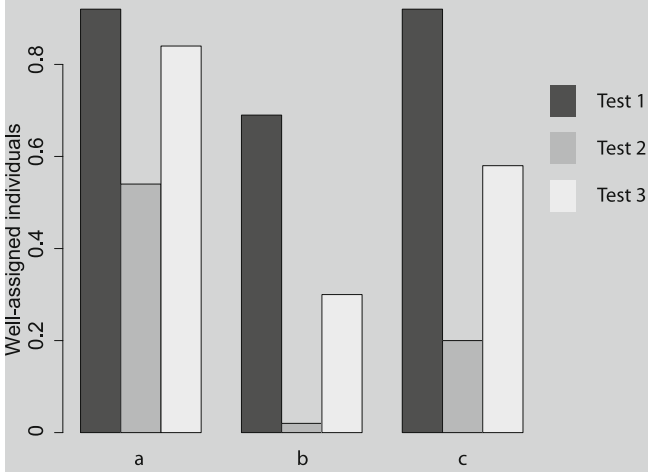

Figure bV-1 Assignment tests in the red coral, *Corallium rubrum*. Three different tests were conducted. Test 1: colonies were assigned to one of the three regional clusters; Test 2: focusing on the Liguro-Provençal cluster, colonies were assigned to one of the 20 sampled populations; Test 3: focusing on the Liguro-Provençal cluster, colonies were assigned to one of the seven identified subclusters. (a) proportion of colonies successfully assigned; (b) proportion of colonies successfully assigned with a assignment probability > 0.9; (c) proportion of colonies successfully assigned with a assignment probability > 0.75. (Data from Ledoux et al. 2010)

were genotyped with three microsatellites (Baco and Shank 2005). Therefore, direct applications for forensics seem currently limited and complementary works to increase the number of molecular markers and surveyed populations are needed. For the remaining precious coral species, development of markers and reference database should be the next priority for managers and conservation biologists. Nevertheless, due to the habitat depth of most of these species (below 100 m), this would likely be achieved through an active cooperation between fishermen and forensics scientists. As for species identification, Next Generation Sequencing technologies and the decrease in the cost of genetic analyses should open new perspectives in assignment tests applied to precious corals.

45.4 Molecular Forensics and Next Generation Sequencing

Over the last decades, struggle against poaching and illegal trade of wildlife resources was influenced by the development of molecular forensics analyses. These developments were particularly significant for species identification and for assignment tests. Nevertheless, to date the development of molecular forensics remains marginal in precious corals. Improvements in molecular analyses and particularly in –*omic*s technologies should give a new twist to precious coral molecular forensics. More particularly, Next Generation Sequencing (NGS), which allows the cheap and reliable sequencing of large amount of DNA are promising in various ways. The availability of large sequence dataset will induce significant progress in the phylogenetic of Anthozoans, which may lead to the identification of efficient molecular barcodes. These technologies, by producing more data at a lower cost will also increase the resolving power of forensics analyses. For instance, the use of NGS may lead to the detection of new microsatellite markers or to the development of large array of Single Nucleotide Polymorphism markers (SNPs). SNPs are nucleotide sites in a DNA sequence where more than one nucleotide (A, T, C or G) is present in the population (Morin et al. 2004). They represent a relevant alternative to microsatellites in molecular forensics since, they can be easily discovered and genotyped also in non-model organisms using NGS approaches. Various reviews addressed the way to isolate, genotype and analyze SNPs (e.g. Budowle 2004) as well as their benefits and caveats compared to microsatellites (e.g. Morin et al. 2004). SNPs

target multiple region of the genome and they are more suited than microsatellites for automated allele calling. They are more prone for data comparisons among laboratories and can be amplified using highly degraded DNA.

These molecular markers may be used for species identification or assignment tests (Alacs et al. 2010). It is noteworthy that SNPs allow the identification of species from a restricted pool of possible taxa (Ogden 2011). Ogden et al. (2013) illustrated how SNPs combined with NGS may be used both for species identification and population assignment in sturgeons. Nielsen et al. (2012) also demonstrated how large array of SNPs may be used to identify illegal fishing and false eco-certification. These approaches remain to be tested in precious corals. Nevertheless, the decrease in costs associated with NGS represents an opportunity that should be seized by manager and scientific communities to develop efficient management tools and to enforce precious corals conservation policies.

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Advances in Management of Precious Corals to Address Unsustainable and Destructive Harvest Techniques

46

Andrew W. Bruckner

Abstract

Precious corals have been used in jewelry, decorative arts, religious artifacts, and for medicinal purposes for over 5,000 years. Traditional harvest of precious corals involves the use of non-selective coral dredges and trawls. In many fisheries, these destructive fishing gears are still legally used due to the logistical challenges and high costs associated with extraction of these sessile organisms from great depths. The high value of these resources have triggered exploitation patterns that are similar to strip mining. This has resulted in unstable yields and frequent population collapses, and fishers have been forced to search for new stocks as local areas were depleted. Tangle net dredges have been slowly eliminated from some fisheries, beginning in the 1980s, with harvest undertaken using conventional and mixed-gas SCUBA, ROVs and submersibles. These newer techniques have allowed fishermen to harvest black coral, Mediterranean red coral and certain Pacific species of red coral more selectively and efficiently. While SCUBA fisheries cause less damage to the habitat, targeted species are being extracted from refuges that were previously inaccessible to traditional coral dredges and trawls. This practice has helped maintain landings at artificially high levels, providing a false sense that the fishery is sustainable. Nevertheless, in the same manner as non-selective trawl fisheries, SCUBA fishers have been forced to expand their search into new areas and deeper waters as shallow coral beds are depleted, contributing to further decline of precious coral stocks.

The majority of the research conducted worldwide has concluded that most historically harvested precious coral beds in the Mediterranean and Pacific are depleted, and populations that are being targeted today are experiencing rapid declines. Although several countries have taken steps to conserve precious coral resources, effective management of precious coral stocks is lacking in many countries and poaching is increasing. Measures to restrict international trade have been proposed, yet a select few of these have been implemented. Recent management interventions are certainly a step forward, but they fail to achieve the intended goals of sustainable fisheries because (1) management has traditionally focused on single species instead of a more holistic ecosystem management approach; (2) the biology and life history of these species are not adequately considered; (3) known coral beds continue to be intensively fished and new areas are exploited without first acquiring scientific knowledge of the status of the resource and levels of harvest they can support; and (4) the escalating value and demand for precious corals in concert with short-term economic gains is driving unsustainable exploitation and increased poaching.

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Keywords
Precious corals • Antipatharians • Black coral • Bamboo coral • Red coral • Gold coral • Coral fishery • Coral jewelry

46.1 Introduction

Precious corals have been collected for over 5,000 years for use as jewelry, homeopathic medicines, religious and cultural artifacts, and ornamentation using a variety of selective and non-selective fishing methods and destructive fishing gear. These corals remain highly vulnerable to depletion because of their biology and life history, combined with a boom and bust strategy of most commercial fisheries that is characterized by the removal of all accumulated standing stock from one bed before fishermen move on to explore new areas and exploit new stocks. In addition to their slow growth, late reproductive maturity, low rates of natural mortality and other k-selective strategies, these sessile corals exhibit a patchy and limited distribution. Precious coral fisheries have followed a historic tradition centered on an artisanal industry specializing in highly ornate carvings and extensive trade routes. Throughout the history of this industry, the value of raw materials have fluctuated based on the supply, quality and demand. An increasing consumption and demand for coral products since 2009 has caused prices to escalate to such high levels that some species are now more valuable than gold, fueling excessive exploitation and illegal poaching (Tsounis et al. 2010, 2013; Chang 2015). Globally, most precious coral stocks are overharvested and the industry faces a growing threat of localized depletion and even economic extinction, sparking debates about the adequacy of management measures and enforcement. This review summarizes biological attributes of precious corals and how this affects their potential for sustainable harvest. A historical overview of the industry is presented, focusing on the socio-economic patterns of use, historical and present day fisheries and the supply chain, and traditional approaches to manage these resources. Recent efforts undertaken to improve the management and conservation of these species are discussed, along with some of the limitations of these approaches. By analyzing all data, the review illustrates how modern day fishing practices along with newly emerging technology, a paucity of suitable management interventions, limited enforcement capacity, and escalating poaching continue to drive an unsustainable fishery, to the extent that these resources are quickly becoming commercially extinct. The chapter concludes with a discussion of the need for a holistic ecosystem-based approach to avoid the eventual collapse of the industry and to achieve a sustainable fishery.

46.1.1 Biology and Ecology of Precious Corals

Precious corals belong to three taxonomically distinct orders of cnidarians in the class *Anthozoa*: Zoanthidae, Antipatharia, and Gorgonacea. The most valuable of these are the pink and red corals in the family Corallidae (order Gorgonacea), with 2 genera (*Corallium* and *Paracorallium*) and 33 described species, 8 of which are commercially harvested: Mediterranean red coral (*Corallium rubrum)* and 7 Pacific species [*Paracorallium japonicum*, *C. elatius*, *C. konojoi*, *C. secundum, C. lauuense (C. regale), C. sulcatum* and *Corallium* sp. nov.] (Bayer and Cairns 2003; Simpson and Watling 2011; Tu et al. 2012). Next in importance are the black corals in the order Antipatharia, which includes seven families (Antipathidae, Aphanipathidea, Cladopathidae, Leiopathidae, Myriopathidae, Schizopathidae and Stylopathidae) and over 200 known species, with at least 13 species in 11 genera used in the jewelry trade (Opresko 2001; WCMC 2008). Other precious corals fabricated into jewelry include two types of gold corals, zoanthids in the family Gerardidae (*Gerardia* spp,) and gorgonians in the family

Table 46.1 Precious and semi-precious corals that are commercially harvested

Order	Family	Genus most common in commerce	Common name	Total # taxa	# Harvested taxa
Gorgonacea	*Corallidae*	*Corallium*, *Paracorallium*	Pink and red corals	33 species	8
Gorgonacea	*Isididae*	*Keratoisis, Acanella, Lepidisis*	Bamboo Coral	38 genera, 138 species	4
	Antipathidae	*Antipathes, Cirrhipathes, Leiopathes*	Black coral	200+ species	11 genera, 13 species
Zoanthidae	*Gerardidae*	*Gerardia*	Gold Coral	3 species	1
Gorgonacea	*Primnoidae*	*Primnoa, Narella, Callogorgia*	Gold Coral	>200 species	3
Gorgonacea	*Melithaeidae*	*Melithaea*	Sponge coral	5 genera	3+
Gorgonacea	*Helioporacea* and gorgonians in the family	*Heliopora*	Blue coral	1 species	1
Gorgonacea	*Tubiporidae*	*Tubipora*	Organ pipe coral	1 species	1

Primnoidae (*Primnoa*, *Narella* and *Callogorgia*), and bamboo corals (family Isididae: *Acanella*, *Keratoisis*, and *Lepidisis*) (Table 46.1).

Semi-precious species, including several hydrozoan corals, blue coral (*Heliopora coerolea*), organ pipe coral (*Tubipora musica*), numerous scleractinian corals, and gorgonians in the family Melithaeidae (sponge or apple corals) are used primarily for curios and in aquarium displays, while some (blue coral and sponge coral) are also made into jewelry (Wells 1981, 1983). Sponge corals have a porous skeleton, full of holes and irregular surfaces. The most notable feature of the skeleton is that it is segmented, consisting of a series of nodes that contain sclerites embedded in a gorgonian matrix and internodes formed from sclerites infused with calcite. They are found in shallow, tropical and subtropical waters and tend to be relatively short-lived compared to precious corals. They include hundreds of species with a variety of colors, but *Melithaea ochracea* is the only species reported to be currently used for jewelry. *Melithaea* forms fan-shaped colonies with a skeleton that is red with yellow-orange-brown streaks. Most of the harvest occurs in the South China Sea, from Taiwan to Indonesia. It is typically infiltrated with resin to harden and stabilize the skeleton. Blue coral (*Heliopora coerulea*) forms branching, submassive and erect plate-like colonies on shallow tropical reefs throughout much of the Indo-Pacific. Its common name stems from the coral's ability to extract iron from seawater that it forms into a blue salt which is incorporated into its skeleton, turning it a sky blue color. Like red sponge coral, the skeleton is stabilized with resin when used for jewelry.

Precious corals are widely distributed but most populations tend to be small. They occur from the temperate waters of New Zealand, through the tropical Indo-Pacific, the Red Sea, the Caribbean, tropical and temperate Atlantic Ocean, the Mediterranean and the north Pacific. Precious corals are most abundant below 100 m depth, but some can be found within SCUBA diving depths. Antipatharians inhabit the shallowest depths, typically occurring from 10 to 100 m depth, with large populations in the New Zealand fjords, the temperate and tropical Pacific, the Caribbean, and the Mediterranean; commercial fisheries for these species are primarily in the tropical Pacific, Caribbean, Red Sea and Mediterranean. Species in the family Corallidae inhabit tropical, subtropical and temperate oceans, but only two areas historically had large populations and are commercially exploited: the Mediterranean Sea and the northern Pacific Ocean. The Mediterranean red coral, *Corallium rubrum* is distributed throughout the Mediterranean and neighboring Atlantic coasts, with commercial beds known from Sardinia, Corsica, Elba, southern Italy, Croatia, the Greek islands, Turkey, Mallorca, Alboran Sea, Costa Brava, southern France and the northern African coast (Fig. 46.1). Small colonies of red coral can be found in dimly lit submarine caves and under ledges from 3 to 10 m depths (Tescione 1973; Tsounis et al. 2006b), while larger, commercially valuable colonies occur on banks, boulders and other hard substrates to 800 m depth (Costanti et al. 2010), being most abundant from 30 to 200 m (Rossi et al. 2008). Other species in the family Corallidae occur below the euphotic zone. Pacific red and pink corals are mostly found within two

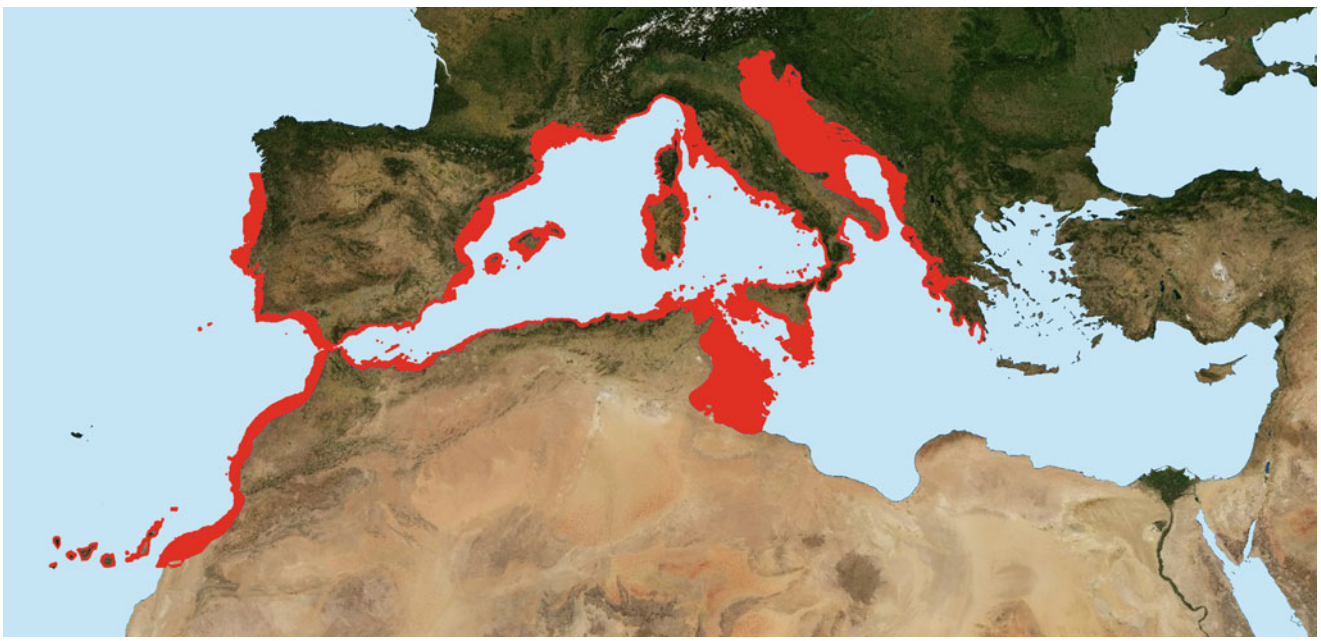

Fig. 46.1 Known range of *Corallium rubrum*. The coral occurs along the central and western basin of the Mediterranean and the neighboring eastern Atlantic along the coast of Africa and the Canary Islands, southern Portugal and Cape Verde. It occurs in shallow water (<10 m) in dimly lit caves and can be found on deeper banks at 800 m depth, being most abundant from 30 to 200 m

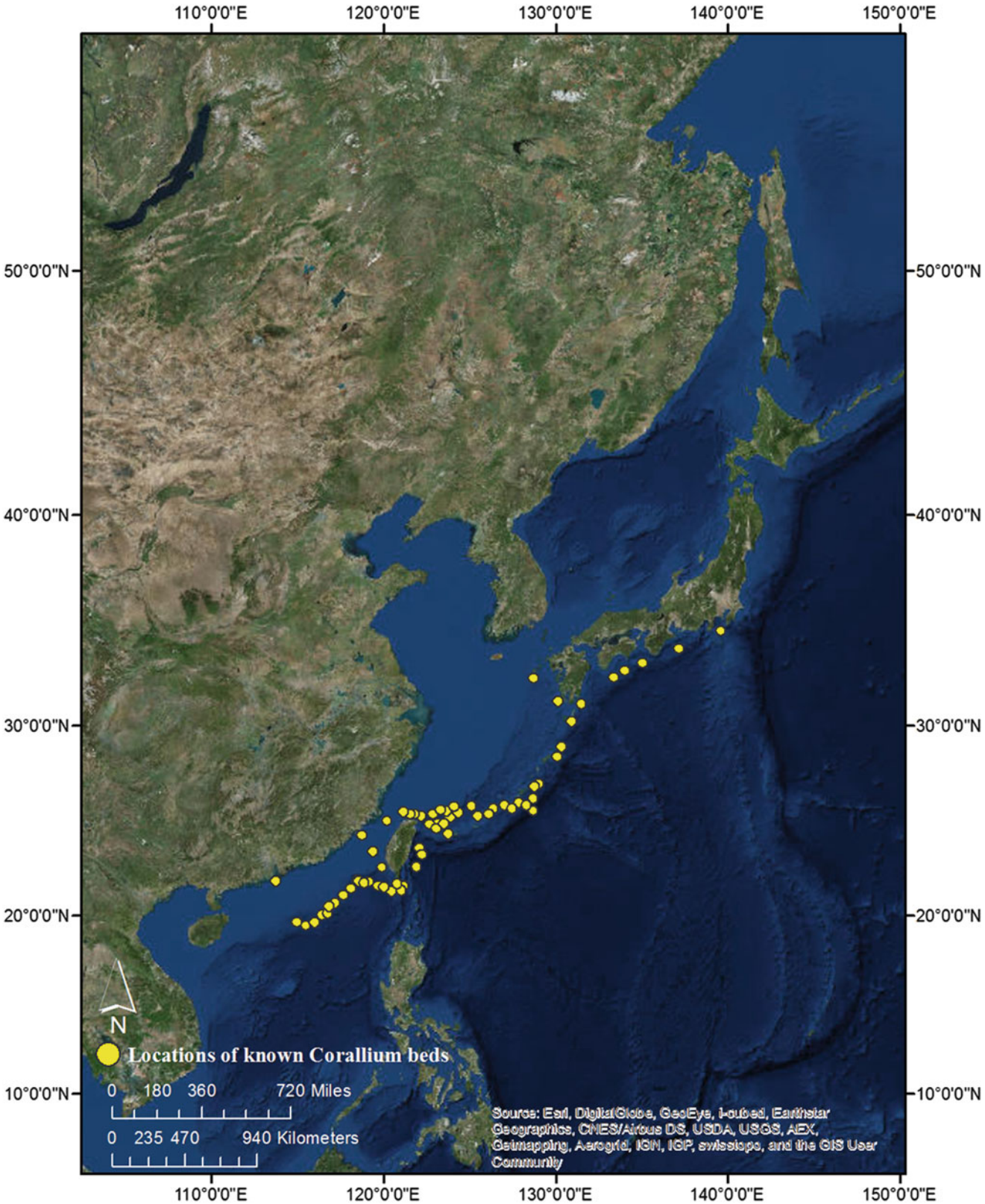

Fig. 46.2 Location of inshore North Pacific coral beds. Red coral *Paracorallium japonicum*, pink coral is found around Japan, Okinawa and Bonin Islands from 80 to 300 m. *Corallium elatius* is reported from the northern Philippines to Japan, around the island of Taiwan, and Mauritius and Palau from 150 to 330 m. White coral *C. konojoi* occurs from Japan to northern Philippines, around Palau and the Chinese islands of Hainan from 50 to 200 m. A fourth coral, *Corallium sulcatum* is reported to have been intensively harvested around Japan and Taiwan between the 1970s–1990s (Tu et al. 2012), but this is not reported in the FAO dataset and is likely pooled with other species

depth zones, 50–400 m and 1,000–1,500 m. Three commercially valuable species inhabit waters around Japan, the Philippines and Taiwan: *C. konojoi* is found from 50 to 150 m and *Paracorallium japonicum* and *Corallium elatius* occur from 75 to 330 m (Fig. 46.2). *Corallium* sp. nov. is found on the Emperor Seamounts at depths of 1,000–1,500 m, while *C. secundum* is found on the Emperor seamounts and also throughout the Hawaiian archipelago, in shallower water (350–475 m). Hawaiian gold coral (*Gerardia*), bamboo coral (*Lepidisis olapa*) and angel skin coral (*Corallium secundum*) coexist in the same depth zone (350–600 m) and habitat, with the largest known populations found in Makapu'u bed off Oahu and smaller populations identified in at least 16 other locations (Fig. 46.3; Grigg 1984; Baco 2007; Parrish and Baco 2007). Bamboo corals are also found in the Gulf of Mexico, Southeast USA, the northeast Pacific and Indo-Pacific, with most harvest currently occurring in waters surrounding Indonesia (Fabricious and Aldersdale 2001; Etnoyer and Morgan 2003). The primnoid gold corals have one of the broadest bathymetric ranges of all precious corals, occupying depths of 25–2,600 m, while the commercially valuable *P. resedaeformis* is found in Alaska between 91 and 548 m (Cairns and Bayer 2005) (Table 46.2). Ecological requirements of precious corals include the presence of a hard substratum, strong bottom currents, and an absence of significant sources of sediment.

All precious corals are ahermatypic species – meaning they lack symbiotic algae and do not form reefs. They have a hard axial skeleton composed of a complex of proteins and/or calcium carbonate and typically exhibit an arborescent (branched, bushy or fan-like) or an unbranched whip-shape, or spiral growth form. These sessile invertebrates are considered habitat engineering species because they provide structural complexity that fish and mobile invertebrates use as feeding, spawning, and resting grounds, and colonies provide valuable habitat for other sessile invertebrates by protecting them from strong currents and predators (Fig. 46.4; Gili and Coma 1998; Husebo et al. 2002). Precious corals create some of the most complex communities in the deep ocean through their trophic activity and biogenic structure, hosting a wide variety of suspension feeders and a high species richness and functional diversity of motile invertebrates, fishes, turtles, and marine mammals (Parrish 2006a). Furthermore, due to their slow growth and long life spans, the skeletons of precious corals and other deep corals provide geochemical and isotopic data that can serve as a proxy for reconstructing past changes in ocean climate and oceanographic conditions (Smith et al. 2002; Adkins et al. 1998; Weinbauer et al. 2000; Risk et al. 2002; Frank et al. 2004; Thresher et al. 2004; Williams et al. 2009).

Because of their sessile life history and k-selected biological traits, precious corals are particularly vulnerable to physical damage from fishing gear such as bottom trawls and long lines. Furthermore, populations under pressure from harvest often exhibit dramatic shifts in age and size structure, and recovery may require decades (Andrews et al. 2002).

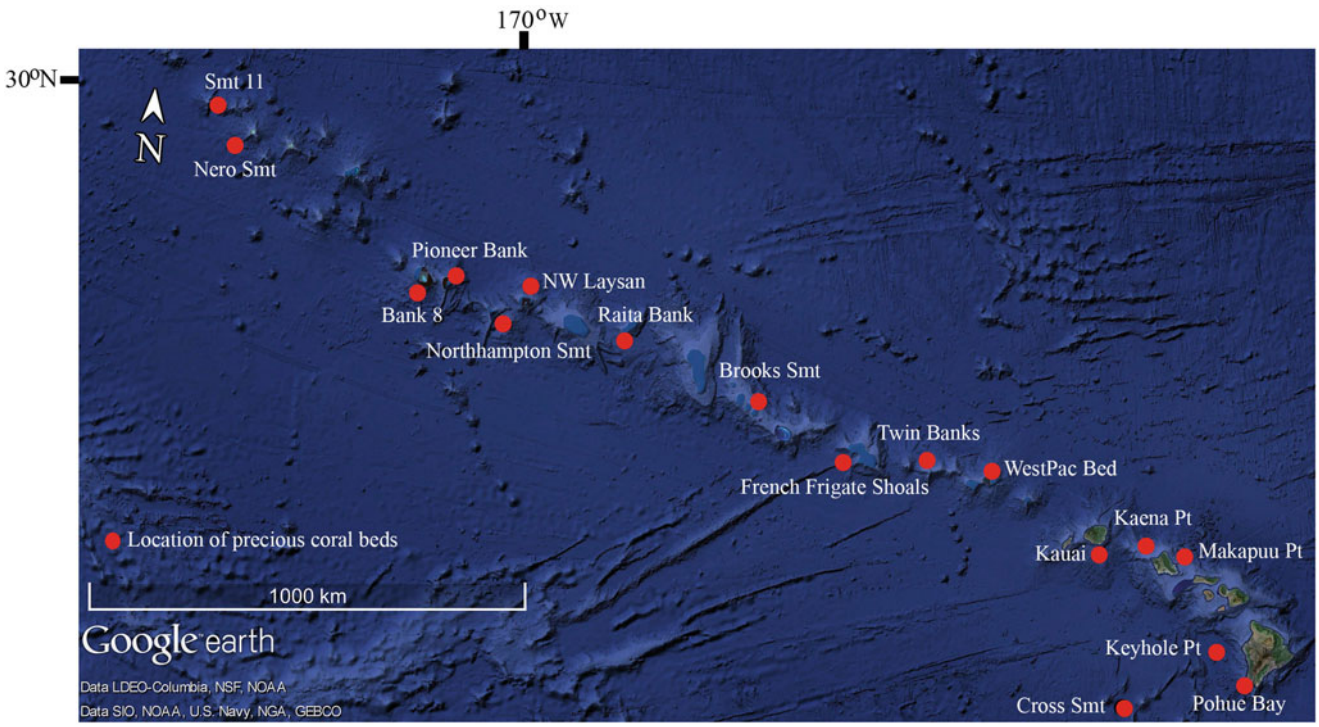

Fig. 46.3 Known locations of precious coral beds (*Corallium* and *Gerardia*) along the Hawaiian Archipelago to the Emperor Seamount chain. Coral is found at depths of 350–600 m

Table 46.2 Distribution of commercially valuable species of precious corals

Species	Common name	Distribution	Depth (m)	Reference
Corallium rubrum	Red coral, chichuukai sango, kowatari sango, Sciacca coral	Mediterranean Sea, primarily coasts of Sardinia, Corsica, southern Italy, Sicily and northern Tunisia	7.5–800; most from 30 to 200	Tsounis et al. (2007) and Costanti et al. (2010)
Corallium secundum	Angel skin coral, pelle d'ange, boke, mittdo sango, Midway coral	Hawaiian archipelago to the Milwaukee Banks; (20–36° N)	350–475	Grigg (1974b)
Corallium sp. nov.	Midway deep-sea coral	Midway Island to Emperor Seamounts, 28–36° N	1,000–1,500	Grigg (1982)
Paracorallium japonicum	Deep red coral, Aka-sango, tiaka	Ishigaki Islands, Sagami Bay on Japan's Pacific coast, between the Ogasawara Islands and Taiwan, and near the Goto Islands, Nagasaki (24–36° N)	76–310	Kitahara (1904), Grigg (1982), Nonaka and Musik (2009), Iwasaki and Suzuki (2010)
Corallium konojoi	White coral, Siro sango	Southern Kagoshima region of Ryukyu Archipelago, Japan to northern Philippines, 19–36° N	50–250	Kitahara (1904), Grigg (1982), Nonaka and Musik (2009)
Corallium elatius	Pink coral, Momoiro sango, momo	Pacific coast near Wakayama, from the Ogasawara islands to the northern China Sea, off the Goto Islands and Northern Philippines to Japan, 19–36° N	100–330	Kitahara (1904), Grigg (1982), Iwasaki and Suzuki (2010)
Corallium lauuense (C. regale)	Garnet coral	Hawaii to north of Midway Island, 20–32° N	400–700	Grigg (1974b)
Corallium sulcatum	Miss coral, Pink coral	Japan (Boso peninsula) and Taiwan	180–550	Tu et al. (2012)
Primnoa resedaeformis, Primnoa willeyi	Alaskan gold coral	Southeastern Alaska (Dixon Entrance) to Amchitaka, Aleutian Islands	10–800	Cairns and Bayer (2005)
Gerardia sp.	Hawaiian gold coral	Hawaiian archipelago & Emperor seamounts	300–400	Grigg (1974b)
Antipathes griggi (dichotoma)	black coral	Main Hawaiian Islands, Indo-West Pacific region	30–110	Grigg (1976)
Antipathes grandis	black coral, pine or umimatsu	Main Hawaiian Islands (Hawaii to Niihau)	45–146	Grigg (1976)
Antipathes spp.	Black coral	Caribbean Sea, Hawaii,		
Antipathes ulex	Fern black coral	Hawaii	40–100	Grigg (1993)
Cirrhipathes anguina	Wire coral	Taiwan, Philippine Sea	10–100	Wells (1983) and Bruckner et al. (2008)
Leiopathes glaberimma	Black coral	Mediterranean, Malta	500–600	Deidun et al. (2010)
Lepidisis olapa	Unbranched bamboo coral	Hawaiian Archipelago	375–400	Grigg (1984) and Tsounis et al. (2010)
Acanella spp.	Branched bamboo coral	Hawaiian Archipelago to Indo West Pacific	375–450	Grigg (1984) and Tsounis et al. (2010)
Isis hippuris	Jointed coral	Australia, India, Indonesia, Japan, Palau, Papua New Guinea, Philippines, Taiwan	1–40	Cooper et al. (2011)

First, all species are long-lived, reaching an age from upwards of a century to several millennia, depending on the taxa (Roark et al. 2006). Second, they tend to achieve a very large size if undisturbed, yet their growth is extremely slow. Most precious corals are characterized by low fecundity and low rates of recruitment, and first reproduction is reached at an age of more than a decade. *Corallium rubrum* is unusual in that shallow populations exhibit high recruitment and a small size and early age at first reproduction. Yet, comparable to other precious corals, *C. rubrum* is gonochoric with discrete annual reproduction, a low fecundity, slow growth rate and a long life span (Santangelo et al. 2003, 2012; Tsounis et al. 2006a; Bramanti et al. 2007; Torrents and Garrabou 2011). In all precious corals, including *C. rubrum*, fecundity is positively related to both colony size and age, with smaller colonies being less fecund and damaged or

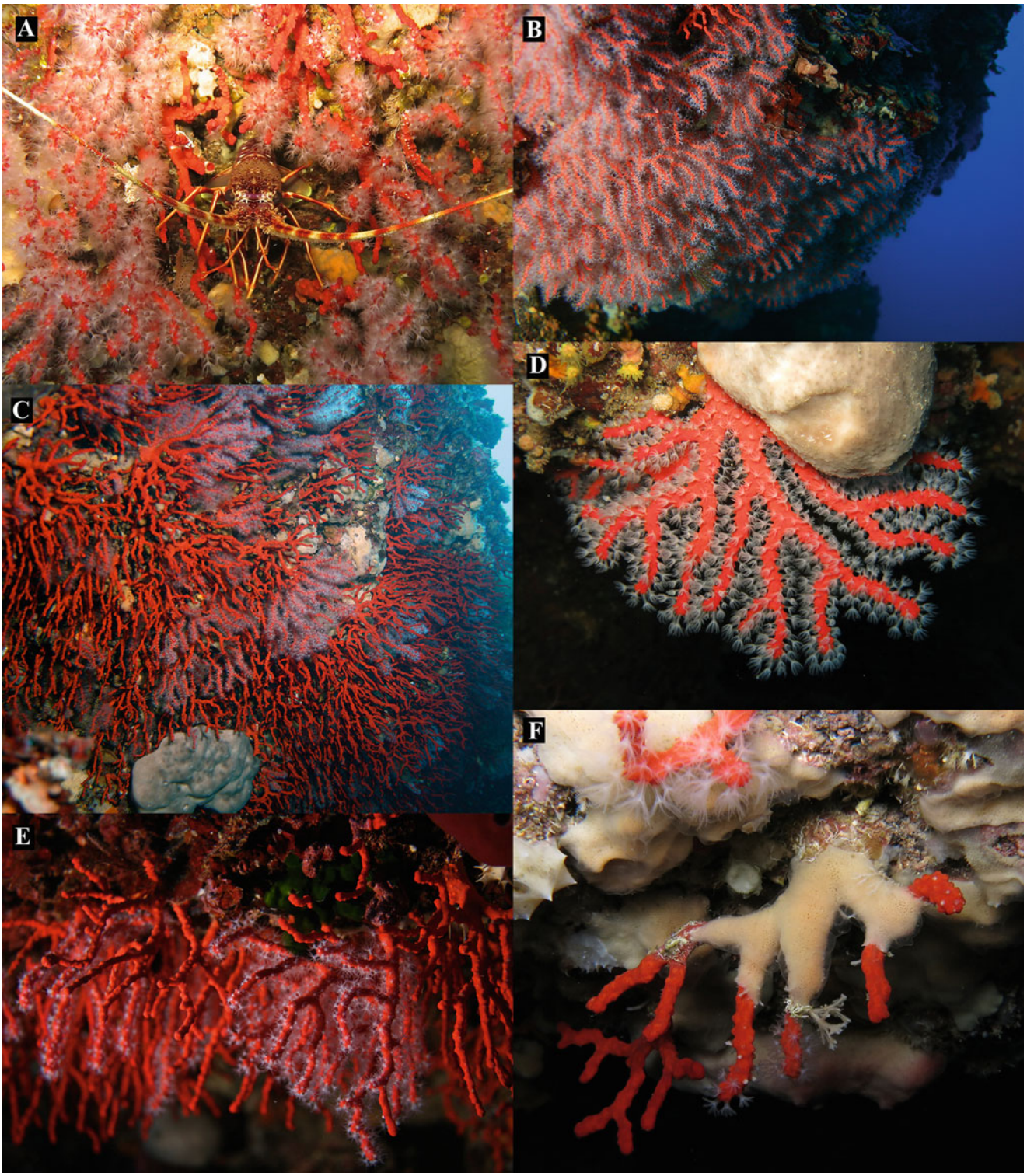

Fig. 46.4 Living colonies of *Corallium rubrum*. (**a**) A Mediterranean lobster hiding in a bed of *C. rubrum*. (**b**) A dense population of *C. rubrum* in shallow water under a ledge. Most colonies have their polyps extended. (**c**) In dimly lit shallow water habitats, *C. rubrum* can form dense assemblages, but most colonies tend to be small (<5 cm) and have few primary and secondary branches. (**d**) Larger colonies tend to occur at very low densities primarily at mid depths (below 30 m) and in deep water. (**e**) A close-up of several colonies of *C. rubrum*, some with polyps retracted and others with expanded polyps. (**f**) Because of the sessile nature of *C. rubrum* and high competition for space, colonies are often overgrown by other encrusting organisms such as sponges (Photos by Betta Nelblu)

injured colonies showing reduced or no reproduction (Abbiati et al. 1991; Weinbauer and Velimirov 1996; Grigg 1974a, 1976).

Precious corals tend to grow much more slowly than shallow water gorgonian and scleractinian corals, and generally have lower rates of recruitment and longer lifespans. The largest precious coral is *Primnoa*, which can reach a height of 7 m (Krieger 2001); nonetheless, annual growth rates of *Primnoa resedaeformis* are only 1.6–2.32 cm in height and 0.36 mm in diameter (Andrews et al. 2002). Both antipatharian black corals and gold coral (*Gerardia*) form dendritic colonies with heights of up to 4 m or more, and trunks that are 1–15 cm diameter. Antipatharians exhibit a diametric increase that ranges from 0.26 to 2.28 mm per year, and linear growth rates of up to 6.4 mm per year (Grigg 1976), while gold coral (*Gerardia*) has the slowest growth of all precious corals (0.03 mm per year) and colonies can live for thousands of years (Roark et al. 2006). Growth rates of bamboo coral is equally slow: *Lepidisis sp*. colonies from New Zealand increase in diameter only 0.05–0.117 mm per year and colonies are estimated to live from 40 to 150 years (Roark et al. 2005; Tracy et al. 2007).

Precious corals in the family Corallidae will form large bushes when undisturbed for long periods, and all species exhibit slow growth. *Corallium elatius* is one the largest species in the family, achieving a maximum size of 1.1 m in height and 1.7 m in width and a weight of 67 kg (Iwasaki and Suzuki 2010). *Corallium konojoi* is one of the smallest commercially harvested coral in this family, with a maximum height of only about 30 cm. *Corallium rubrum* historically formed bushes up to about 75 cm height with a basal diameter of 1–5 cm, while shallow-water populations (<50 m depth) today consist predominantly of 2–5 cm tall, unbranched colonies (Fig. 46.4; Santangelo et al. 2007; Rossi et al. 2008; Tsounis et al. 2006b). *Corallium secundum* was estimated to increase in height 9 mm per year using growth ring analysis (Grigg 1976), whereas more accurate radiometric studies of Roark et al. (2006) have demonstrated much slower growth rates (0.11 mm per year). Linear growth rates of Japanese red coral ranges from 2.22 to 6.66 mm per year (Luan et al. 2013). Growth of *C. rubrum* is slower (0.2–1.83 mm per year), with growth rates declining with age. The average increase in diameter of *Corallium* ranges from 0.19 mm per year (*C. elatius)* to 0.58 mm per year (*C. konojoi*) for Pacific species (Luan et al. 2013), and 0.20–0.62 mm per year for *C. rubrum* (Bramanti et al. 2005; Garrabou and Harmelin 2002).

Due to their sessile nature, precious corals are very sensitive to perturbations. Precious corals are vulnerable to smothering by movement of sand along the bottom, toppling caused by organisms that bore into their skeletons and weaken the site of basal attachment, and encrustation and overgrowth by other invertebrates including invasive species (Fig. 46.4; Grigg 2004; Corriero et al. 1997; Calcinai et al. 2008; Tsounis et al. 2010). These corals are also affected by diseases and several corallivores, such as predatory gastropods (Omi 2007; Priori et al. 2015). Climate change is an emerging threat, especially for shallow species such as *C. rubrum*, as this coral is particularly vulnerable to temperature shifts, and possibly acidification of the oceans as pH decreases and the global-mean depth of calcite and aragonite saturation horizon changes (Cerrano et al. 2013). Mass mortalities of *C. rubrum* have already been reported during abnormally warm summers (Cerrano et al. 2000; Garrabou et al. 2001; Bramanti et al. 2005, 2007). Direct human impacts associated with bottom trawling, long lines and other types of bottom fishing are extremely harmful to sessile precious corals, as these gear types scour the bottom, alter bottom features, re-suspend sediment, dislodge and entangle corals, and cause incidental damage to corals through removal as bycatch.

For most commercially valuable precious corals, unsustainable harvest is the primary threat, as it has triggered large shifts in population dynamics and abundance. Dredges are the most widely used gear for harvest, whereby the coral is entangled in nets and pulled to the surface. This practice is a destructive and wasteful process that breaks and dislodges coral, with 60–90 % detached and lost during collection, much of which consequently dies (GFCM 1984; CITES 2007). Dredging operations dislodge and remove non-target sessile invertebrates, including undersized precious corals of low value that are subsequently discarded. Dredges also destabilize the bottom, reducing availability of hard substrates for future settlement of larvae. The use of dredges to harvest *Corallium* is known to have caused extensive habitat impacts to the coralligenous zone in the Mediterranean (Chessa and Cudoni 1988). Furthermore, the combined use of coral dredges and intensive harvest on SCUBA has degraded the three-dimensional "forest-like" structure created by large, highly branched *C. rubrum* colonies to a "grass-plain-like" structure dominated by unbranched colonies 10–50 mm in height (Garcia-Rodríguez and Massò 1986; Tsounis et al. 2006a, b; Rossi et al. 2008).

46.2 Value as a Raw Material

The skeletons of precious corals provide the raw materials used in jewelry, sculptures, figurines, amulets, clothing ornamentation, and a variety of art objects, and are also reported to have medicinal and mystical powers. Red and pink corals have very hard, magnesium-rich calcitic skeletons, instead of aragonitic skeleton found in scleractinian corals (Dauphin 2006). Antipatharians have a skeleton made of a horn-like protein called gorgonin that is typically dark

brown to black and of a consistence resembling hard wood. Gold corals of the genus *Gerardia* secrete a golden keratin protein, but lack calcium carbonate found in other precious corals. Gorgonin is also an important component of the skeletons of *Primnoa* gold corals, but the protein is infused with calcite ($CaCO_3$) spicules. Skeletons of bamboo corals are constructed of alternating sections of calcium carbonate and protein.

The value of precious corals varies depending on the color, luster and hardness, condition, and size of the skeleton (Torntore 2002). The color tends to differ among species and locality where collected, and the popularity of colors and value follows fashion trends. Of all the precious corals, *Corallium* skeletons have the widest spectrum of colors, ranging from pure white through shades of pink, salmon, and orange to a very dark blood red, and less commonly a bluish, violet or yellow. These colors, originally attributed to the incorporation of iron oxide (Ranson and Durivault 1937), were later confirmed to be due to a specific carotenoid pigment, canthaxanthin (Cvejic et al. 2007). Individual colonies often lack a uniform coloration throughout the skeleton, and presence of striations of different colors may lower the value. For example, many of the Pacific species of *Corallium* are pink to red with a white core, white spots, or veins of different shades of red and pink. Red coral is composed primarily of calcium carbonate (82–87 %) and magnesium carbonate (7 %), and has a hardness of about 3.5–3. 75 on Mohs' scale, which means the skeleton is easily broken but hard enough to be polished (Liverino 1989; Webster 1975). Black coral can also be polished to an onyx-like luster. Because of its horn-like protein skeleton, it can also be bent and molded while being heated. Natural bamboo coral is a creamy white to grey in color, with a discernible dark core, however it is often dyed pink or red to imitate Mediterranean coral. Small coral branches (less than 7 mm in diameter) used to be discarded due to their low value. The introduction of composite coral manufacture, where small branches and fragments are ground into powder and formed into larger blocks and beads with plastic epoxy, has changed this component of the industry (Tsounis et al. 2010). Specimens of *Gerardia* and *Primnoa* exhibit growth rings and have a similar texture and consistency. However, *Gerardia* skeletons are more golden in color and have a smooth surface texture that is finely dimpled, rather than the striated surface of the skeleton of *Primnoa.* The condition of the skeleton refers to the state of the precious coral when it was collected, and the extent of bioerosion. Japanese fishermen distinguish four conditions: colonies harvested when attached and living (seiki), dead but still attached (ichi-kari), dead and toppled (ni-kari), and long-dead unattached colonies that are bioeroded by worms and other invertebrates (san-kari) (Grigg 1971; Cooper et al. 2011; Fig. 46.5). One additional consideration for black, gold, and bamboo corals is the degree to which dead portions of the skeleton are encrusted by *Parazoanthus* and other invertebrates (Grigg 1971, 2004).

46.2.1 *Corallium* and *Paracorallium*

46.2.1.1 Historical Significance

Red coral has been used for rituals, ornaments, talismans, medicines, aphrodisiacs and currency since Paleolithic times. Some of the earliest remains are perforated beads of *Corallium rubrum* uncovered in Germany from the Aurignacian Period (Tescione 1965) and in the Chamblandes settlement on Lake Lemano in Switzerland, dating from roughly 30,000 years ago (Ascione 1993). In the alpine region of Italy and around the island of Sardinia, beads, pendants and suspended ornaments from the Neolithic and Copper Age (4200–3400 B.C.) were found among human remains (Skeates 1993). One of the oldest pieces of engraved red coral, believed to be a talisman, was found in Neolithic tombs of Grotta dei Piccioni in Bolognano, Italy, providing some of the earliest evidence that magical powers have been attributed to red corals since ancient times.

Red coral formed an important component of trade routes throughout Africa, Asia and Europe since pre-Christian times. The presence of red coral in ancient Mesopotamia was linked with the fortunes of the empires and the city-states along the Tigres-Euphrates river system, corresponding to modern day Iraq, Kuwait, Palestine, Turkey and Iran (Ascione 1993). During the Bronze Age, ornate gold cups, helmets, crowns, bracelets, and other artifacts were inlaid with precious stones and red coral by Sumerian craftsmen. Precious corals became one of the most sought after exchange products on the oriental markets of the Phoenicians and Egyptians up to fifteenth century B.C., following the shipping routes used to transport incense across the Mediterranean and into Asia (Liverino 1989). The Phoenicians obtained large quantities of coral from Spain, Sardinia, Sicily and North Africa which they used to make highly ornate jewelry and amulets (Ascione 1993).

In the Middle Ages, coral products could be found in most Mediterranean countries and red coral became an important commodity in the great Eurasian caravan routes. In the tenth and eleventh century, Marsa'el Karez on the northern African coast became the largest coral port and trade center. During the Classical Period, Rome, India, Persia, and China carried on an extensive international trade in coral and other luxury goods, with much of the trade conducted by water via the Mediterranean and the Indian Ocean (Warmington 1974; Francis 1989). One of the main areas of coral fishing at this time was the Greek Islands (the Hellenic regions of the Cyclades between Europe and Asia Minor), with the main center of production of coral goods centered around Izmar, Turkey (Ascione 1993). Trade routes also expanded across

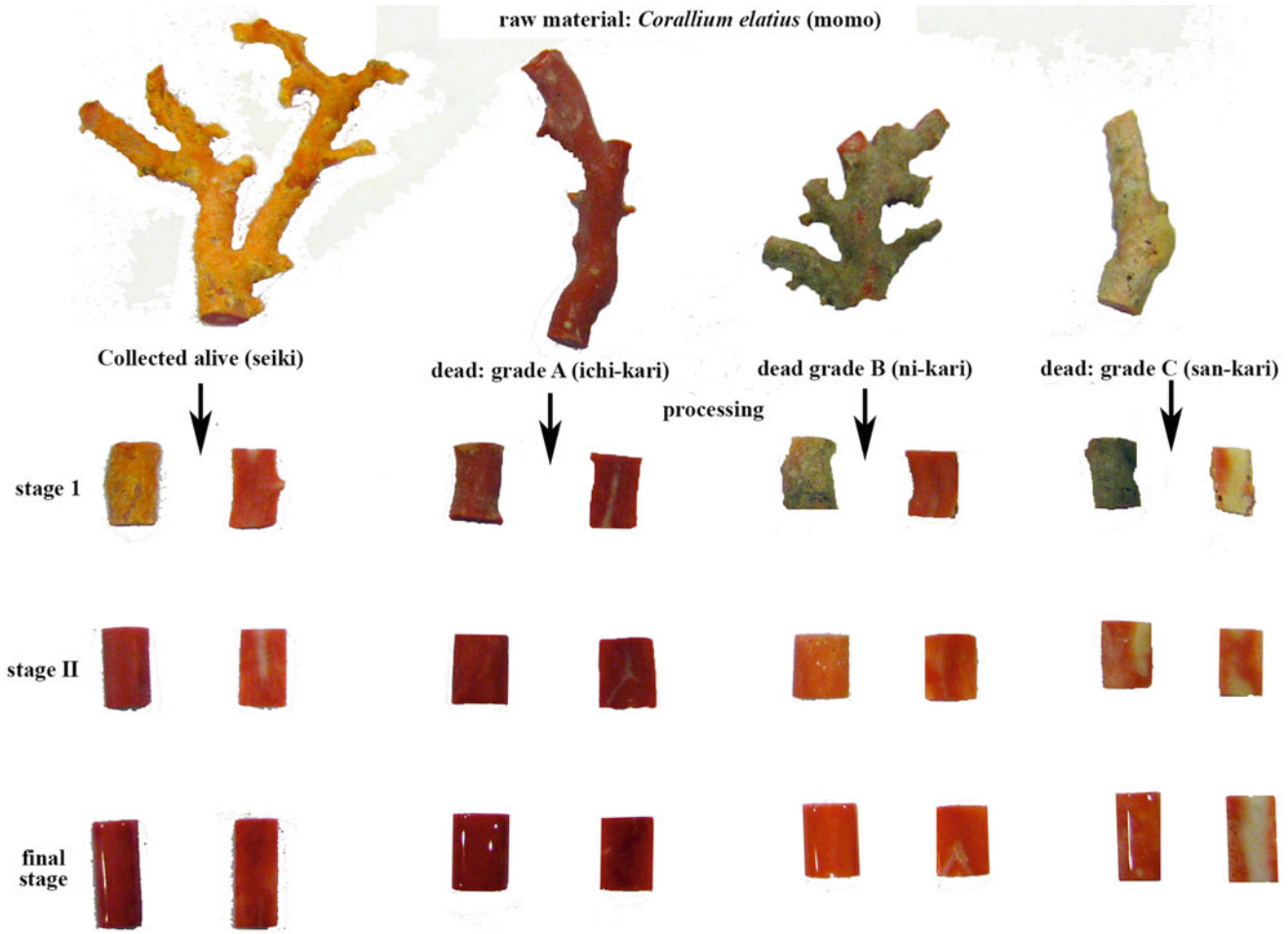

Fig. 46.5 A comparison of pink coral (*Corallium elatius*, momo coral) specimens graded following the Japanese system of classification: (**a**) collected live (grade A; seiki). (**b**) Harvested dead but still standing in growth position with minimal deterioration (grade B; ichi-kari). (**c**) Harvested dead after falling to the ocean floor (grade C; ni-kari). (**d**) Harvested dead with advanced bioerosion and deterioration (grade D; san-kari). Specimens are shown after collection and three subsequent stages of polishing

the Red Sea into the southern end of the Arabian Peninsula and into southern coast of India. The peoples of Arabia, Turkey, Turkmenistan, Uzbekistan, Tibet, Mongolia and India all became captivated by the beauty and allure of red coral. Coral was worn as jewelry by women in Morocco and Arabia. In Turkey, both men and women wore coral jewelry, and inlays of coral were used to create ornate knife handles, mural decorations, ornamentation of pipes and weapons, and harnesses for horses (Liverino 1989). The largest demand for red coral at this time was in India, where it was worn as bracelets, necklaces, anklets, used for prayer beads and amulets, and buried or burned with the dead under the presumption that it had the power of preventing evil spirits (Busuttil 1971). African kingdoms became important importers and consumers of coral beginning around the seventh century (Schick 1995). East Africa was connected to India and the Arabian Peninsula through the Indian Ocean merchant trade, and North Africa was connected through Mediterranean ports in Egypt and Tunisia. Red coral was also known from the far corners of Asia, where they were it was used in jewelry, ornaments, and depicted as a symbol of wealth and treasure (Tescione 1973). The use of red coral as a decoration in Chinese clothes dates back several thousand years (Knuth 1999). In Japan, coral beads were used to ornament Kimono belts, a tradition that survived into modern times (Liverino 1983). *Corallium rubrum* was first introduced to the New World by the Spaniards some 300 years ago, and it still is commonplace in Native American jewelry today.

Ancient writings on the mythology, origins, and magical properties of corals appeared in Greece in fourth century B.C. and during the first century A.D. in Italy. Theoprastus wrote a monograph titled *Concerning Stones* (Greece, 315 B.C.) where he described coral as a stone, red in color, which grows in the sea and is rounded like a root (Caley and Richards 1956). Pliny (79 A.D.), in his treatise *Naturalis Historia*, presented the first account of known areas of coral fishing, types of harvested coral, and trade with India. The

origin of red coral and its mystical powers are also explained in Greek mythology by the story of Perseus and the Gorgon Medusa. According to legend, Perseus used the head of Medusa to petrify Cetus, the sea monster threatening Andromeda. Wanting to wash his hands of the sea monster's blood, Perseus placed the Medusa's head on a bed of leaves and seaweed on a riverbank. When he picked the head back up, he saw that the touch of the severed head of Medusa had petrified the seaweed and stained it red by its blood. The legend also states that Perseus gave Medusa's head to the goddess Athena, who used it as a shield against her enemies, possibly explaining why red coral talismans were used for protection (Tsounis et al. 2010). The Greek word for coral, "Gorgeia", originated from this legend, as Medusa was one of the three Gorgons.

Throughout its history, red coral has served as a symbol of life, good health and fortune, a protectorate, and remedy for a host of maladies. The Romans used powdered *Corallium* skeleton in liquid tonics, granules and pills as an antidote for poisons, and also as an antacid, astringent, emmenagogue, nervine tonic, laxative, diuretic, emetic and antibilious agent (Wells 1981). It was used to treat snake bites and insect and scorpion stings. Red coral was believed to have many other healing powers, such as comfort for fainting spirits, to counteract fascinations, to purify the blood, to ward off epilepsy, to cure imbecility of the soul, melancholy, mania, and to eliminate hatred from the home (Busuttil 1971). *Corallium* was thought to protect against Satan in Christian cultures and was worn as a protector against the evil eye and other misfortunes in other religions (Wells 1983; Tescione 1973). The writings of Pliny first described the superstitious belief in coral's alleged power against the evil eye and its ability to protect children from diseases. According to Pliny, red coral was used to predict the onset of illness and it was also a cure for kidney stones, ulcers, and hemorrhages. Belief of its magical and protective powers were commonplace in Italy and parts of Northern Europe throughout the Middle Ages, and it also had a number of other religious and cultural uses. Because of its luster and blood red color, it symbolized Christ's blood shed to redeem humankind, and its hardness and tree-like shape made it a symbol of life (e.g. tree of life), rebirth and immortality. When mixed with grains of corn during planting, red coral was believed to protect crops from drought. In Iranian beliefs, amulets and ornaments made of red coral served to defend ships and homes against lightning and storms. In Buddhism, precious corals were relished as treasures from paradise and used to decorate Buddha statues (Kosuge 1993).

Red coral has continued to play an important role in religious art, jewelry, sculptures, ornaments and other artifacts into the modern age, although market saturation, political and economic pressures, and changes in demand have affected the style, quality, and levels of production (Fig. 46.6; Ascione 1993). Beginning in the fourteenth century, coral necklaces and coral branches appeared in works of spiritual and religious art by Renaissance artists, such as the paintings of the Madonna and Child by Piero della Francesca and Andrea Mantegna (Ascione 1993). In Italy, the art of working coral was introduced in the 1400s by Jewish craftsmen. Jewelry production started in Trapani, a tuna and coral fishing village on the northwest coast of Sicily and then spread to Naples, Liverno, Genoa and Torre de Greco (Fig. 46.7). Much of the jewelry from the early 1800s consisted of uncarved beads (Fig. 46.8), often faceted into large pieces, while the Renaissance-style corals involved new designs that included allegoric and mythological compositions, cherubs and racemes, and many pieces had a "flower and leaves" style (Ascione 1993). With the introduction of Pacific *Corallium* in the early twentieth century, larger sculptures, inlays and statues were created, due to the larger sizes and different colors of these species (Fig. 46.6). Newly emerging fisheries and discoveries of unusually large precious coral beds in the 1960s–1980s inundated markets with Pacific *Corallium*; new production centers emerged, and the coral trade sky rocketed. Periodic gluts of coral, as well as an abundance of low-quality raw material caused prices to tumult, and fishing effort temporarily declined, but these trends were short lived. Since 2008, the value and demand for precious coral has achieved new record levels, leading to excessive exploitation and illegal poaching (Chang 2015). Coral artisans from Torre del Greco continue to produce some of the most intricate and valuable designs, but international markets are flooded with pink and red coral jewelry, sculptures and other decorations from India, Taiwan and Japan, Native American tribes of Arizona (Ascione 1993) and new markets have emerged in China (Chang 2015).

46.2.1.2 Mediterranean *Corallium* Fisheries

Mediterranean red coral was first collected in the form of fragments and large branches that washed up on the shore after storms, possibly as early as the Aurigacian Period, Late Paleolithic (Tescione 1973). The first recorded evidence of fisheries for *Corallium* date from about 5,000 years ago, with skin divers removing colonies from shallow caves (Grigg 1984). Harvesting of coral in ancient times is assumed to have been undertaken by free divers, initially without goggles, while at a later stage breath-hold divers were equipped with Japanese goggles. These early coral free-divers were likely quite proficient at breath-holding, as demonstrated in other free-diving fisheries in the region (Tsounis et al. 2010). Greek sponge divers capable of free diving to over 80 m depth fabricated iron hooks called "kouralio" and other primitive tools to dislodge the corals (Mayol 2000). These

Fig. 46.6 Examples of carvings, inlays and sculptures made from *Corallium* and *Paracorallium*

Fig. 46.7 Processing of *Corallium* in Torro de Greco, Italy. Common lapidary techniques are used to cut, grind and polish precious corals. In some cases the carves pieces and beads are soaked in a solution of hot water with hydrogen peroxide and muriatic acid and then buffed to obtain a high gloss finish

Fig. 46.8 The principle products made from pink and red corals are beads which are strung into necklaces, bracelets and earings. (**a**–**f**) Several different sizes and shapes of beads. (**g**) The production process involves at least 12 different stages including washing, cutting, shaping, cleansing, polishing and finally stringing of red coral colonies. (**h**) Typical necklace, bracelet and earings made from beads of *Corallium rubrum*

tools were first used between first and fourth century B.C. by Greek divers and by Roman divers into the first century A.D.

During the Neolithic, a larger-scale boat-based fishery was established following the development of nets used to drag for the coral (Grigg 1974b). The Greeks are credited with inventing the first dredging devices capable of being towed behind small row boats between the fourth and third century B.C. (the Hellenistic, Roman-Carthaginian, and late Etruscan ages) (Galasso 1998, 2000; Galili and Rosen 2008). Dredges of a similar style, varying mainly in size, weight and shape of materials used as ballast, and number of attached nets, gradually spread throughout the region. When Arab fishermen began harvesting coral in the tenth century, they developed a wooden dredge which became known as the "ingegno", or "Saint Andrews Cross". The ingegno consisted of a wooden cross with a central ballast of stone or lead and nets attached along the beams that was towed by a boat and dragged along the bottom, detaching and entangling red coral. These early dredges were used by Spanish, Sardinian, and Catalonian fishers into the Middle Ages, eventually being replaced with a larger and heavier metal dredge, the "barra italiana" during the industrial age (Galasso 2000). The barra italiana was made of a heavy iron bar (>1 t) with chains and nets attached along its length. Although the barra italiana could be dragged in deeper water and was considerably larger, it still was not very efficient: it is estimated that only about 40 % of the coral broken off the substrate was entangled and retrieved (GFCM 1984).

The coral fishery in the Mediterranean has fluctuated between periods of prosperity and decline, depending on supply (new discovery of *Corallium* beds) and demand, political regime changes, wars, restrictions on fishing, and various political struggles for supremacy over the fishing grounds. Introduction of large wooden and metal dredges allowed the fishery to shift from one based on individual divers and small row boats to industrial-scale operations, as fleets of boats could be deployed and multiple dredges used simultaneously. With the emergence of industrial fisheries, securing of the right to the coral fisheries off the African coasts became the object of considerable rivalry among the Mediterranean communities of Europe, and processing centers and fisheries moved around the region. For example, in the twelfth and thirteenth century most of the exploited coral banks were located in Tunisia and Algeria with fishermen from Catalonia, Genoa and Marseille (Tescione 1973). In the mid-1500s, one coral harvesting company in Marseille had a monopoly of fishing along a 250 km stretch of the Northern African coast, and also collected coral off Corsica, Sardinia, Tunisia and Karpathos (a Greek Island in the south Aegean Sea). During the sixteenth and seventeenth centuries, most coral fishing boats were based out of Genoa and Naples, with harvest occurring off the North African Coast. By the late seventeenth century, the majority of the coral fishermen were based out of Lisbon and Marseille, while Livorno and Torre del Greco emerged in the eighteenth century as the major production centers (Francis 1994; Tescione 1973; GFCM 1989). For a short period, Tunisian fisheries were secured by Charles V for Spain. The monopoly soon fell into the hands of the French, who held the right to the fishery until the end of the French Revolution when it shifted from Marseille to Naples, Rome and Genoa.

In 1806, the British government controlled the fisheries, but this quickly returned to the French and Italians. The Italian fishery achieved an extended period of prosperity in the mid to late 1800s, with thousands of small sail and row-boats, tens of thousands of workers, and dozens of processing centers. In 1862, there were 347 registered coral fishing boats. By 1865 this increased to 1,200 vessels, with 24 Italian factories processing coral, employing about 17,000 fishermen and jewelers (Tescione 1965, 1973). Most coral fishing in Italy had shifted to Torre del Greco by 1870, with smaller fisheries based out of Livorno, Genoa and Corsica (Torntore 2002). Almost 250 vessels were dredging for coral off Algeria in the late 1870s, with landings off the east coast of approximately 250–300 metric tons per vessel each year. In the 1880s, following discoveries of three large beds of dead, subfossil red coral on Sciacca banks, between Sicily (Italy) and Tunis (Tunisia), a coral rush began. At its peak, over 2,000 boats worked these banks, levels of harvest quadrupled, and numbers of processing factories increased from 40 to over 80. Intense fishing quickly depleted these grounds, and they were abandoned around 1915, but the glut of low quality coral simultaneously lowered prices and reduced fishing in other areas (Geronimo et al. 1993; Tsounis et al. 2010). Ironically, Sciacca coral is some of the most valuable found in the market today.

Coral fishing in the Mediterranean stopped completely between 1914 and 1918. At about the same time, imports of Japanese pink and red coral started to reduce demand of Mediterranean red coral (Tescione 1973). The Italian coral industry adapted to market demand and crafted large coral pieces of the popular pale pink coral, but coral artisans struggled to survive. Demand for Mediterranean coral increased once again in the 1930s, followed by another decline during the early 1940s. In 1941, there were only 5 active coral fishing boats; this increased to 31 in 1947 and industrial scale exploitation began once again (Liverino 1983).

Shortly after the invention of the Cousteau/Gagnan Aqualung, SCUBA diving emerged as a new, highly profitable method for the harvest of red coral, as it allowed divers to selectively remove large corals in protected crevices that were previously inaccessible to dredges. Leonardo Fusco is credited as being the pioneer SCUBA red coral fisher, harvesting 250 kg in his first season (Liverino 1983). Several other professional divers from Italy also began harvesting coral off banks in Sardinia, Elba and Corsica in the mid-1950s

(Roghi 1966). In 1956, divers were harvesting coral from a cavern off Sardinia 30–35 m depth, but these resources were quickly depleted. To continue finding coral, divers had to descend to 40–45 m by 1958, followed by 70–90 m in the mid-1960s, and 100 m in the early 1970s as shallow water resources became scarce (Liverino 1983). Similar trends for the dive fishery, with divers working in shallow water and descending into progressively deeper water, were documented from other coral beds in Italy, France and Spain (Liverino 1983; Galasso 2000; Tsounis et al. 2010). Helium-based mixed gas diving technologies began to be used by coral divers in 1974, permitting them to work between 80 and 150 m for longer periods (Liverino 1983). Discovery of large resources along the 14 mile Scherchi Channel (located between Sicily and Tunisia) led to a short-lived coral rush beginning in 1978, with 80 divers from Italy, France and Spain working these beds. The divers initially harvested coral from 60 m depth, and gradually worked their way down to 130 m, with 366 boats and 150 divers extracting coral from Scherchi beds by 1979 (Liverino 1983).

From the advent of SCUBA fisheries and continuing until the mid-1980s, coral dredges continued to play a major role in the Mediterranean fishery, as they could be used to drag for coral in deeper areas that could not be exploited by divers. Small boats were replaced by larger motorized vessels capable of dragging immense (7 m long, 800 kg) metal dredges at much greater depths (180 m) in the 1970s (GFCM 1989). Tunisia allowed Italian dredging boats and French divers until it developed its own dredge and dive fishery in 1974. Both dive and dredge fisheries continued to expand throughout the region; by 1982, there were 150 factories, employing 4,000 workers and 1,600 fishermen (Tsounis et al. 2010).

As research began to expand in Mediterranean waters, scientists began to document immense damage to the coralligenous habitat in the Mediterranean caused by the coral dredge (Thrush and Dayton 2002). As a protective measure, Algeria was the first country to ban its use in 1977, followed by Morocco, Spain, Tunisia and Croatia in the mid-1980s. By 1994, the barra italiana and other types of coral dredges were completely phased out throughout European Union waters. SCUBA diving remains the dominant method of exploitation today, with traditional diving from 30 to 80 m depth and technical (mixed-gas) SCUBA used between 80 and 150 m depth. There are an estimated 350 licensed SCUBA divers collecting coral from the Mediterranean, and numerous more that are reported to be fishing illegally (Tsounis et al. 2010). In some areas fishermen have begun to rely on remote operated vehicles (ROV) to locate colonies, but the use of manipulator arms to remove the corals is currently illegal throughout the region, and some countries even prohibit its use for exploration. The use of rebreather technology is being considered in some areas, as most of the shallow traditional SCUBA-accessible beds are overexploited, and regulations have been implemented in some countries to close fisheries above 60 m depth (Tsounis et al. 2013). There is a single area off the Spanish coast (Islan de Alboran) where submarines have been used to collect corals (Paracuellos et al. 2006). There were two authorized submarines, but only one was ever used. It began operating in 1989 and worked until 1997 harvesting corals from 120 to 180 m depth. Landings were approximately 500 kg/year, which was substantially less than the 1500 kg quota.

46.2.1.3 Pacific *Corallium* Fisheries

The first record of precious coral harvest in Japan was in 1812, when a fisherman found a precious coral entangled in his net off Muroto, Kochi Prefecture (Suzuki 1999). For the next several decades, coral fishing was very limited because coral dredging was prohibited under Tokugawa Shogunate law, and the local rulers (Tosa Clan) did not permit the collection and trading in coral that was collected by other fisheries within their domain (Kosuge 1993). Furthermore, any coral that was landed could not be sold on the open market, but was required to be given to the regional rulers (the Shoguns). A document published in 1838 stated that anyone harvesting precious coral was prohibited from selling the coral freely, but they could offer it to the government (Kosuge 2010). In 1868, during the Meiji Reform, the Tokugawa feudal system was abolished and along with it the Tosa Clan laws banning coral fisheries. By 1871, precious coral fisheries began operating in the Muroto region of Tosa Bay (Kosuge 2010). The fisheries quickly expanded into western and southern Japan (Okinawa and the Bonin Islands), with about 100 boats gathering coral from 100 to 400 m depth (Kitahara 1904). Initially, rectangular nets attached to bamboo sticks were dragged across the bottom, but in 1890 this was modified by Konojoi Ebisuya to include additional netting at the rear of the dredge, thereby increased the efficiency of harvest (Kosuge 1993). Landings in coastal waters were sporadic, with several pulses as new beds were discovered and quickly exploited, and abandonment of most beds after a few years of intense fishing (Table 46.3). During World War II, fishing effort dropped by about 80% (Grigg 1971a; Kosuge 2010). After World War II, Japanese coral fisheries began to spread to more distant locations. From 1950 to 1951 most of the harvest by Japanese fishermen consisted red coral *Paracorallium japonicum* and pink coral *Corallium elatius* from Hachijo (150 miles off Tokyo). Shortly thereafter, fishermen began harvesting coral from inshore areas near Arnani, Shikoku, Kyushu, and Goto islands and Tosa Bay. These areas supported unusually large populations of *P. japonicum* and *C. elatius* and a third species, white coral *C. konojoi* was discovered (Liverino 1983). By 1960, the fishery had expanded to more distant Ryu-Kiu Islands, south of Kagoshima (Okinawa, Amani, Miyado), and southeast of

Table 46.3 Changes in the fishing ground for precious coral and duration of fisheries in Japan. Only two areas are currently active (Liverino 1983; Kosuge 2010)

Location	Period of activity
Tosa Bay	1800s–present day
Hachijo (150 miles off Tokyo)	1950–1951
Goto and Danjyo Islands	1900–1936, 1952–1995
Amani and Goto Islands	1952–1953
Nomozaki, Nagasaki Prefecture	1911–1915, 1935–1938
Koshiki Islands	1886
Okinawa Islands	1926, 1960
Sumizu, off Okinawa and Hachijo	1961–1963
Izu Islands	1939–1960
Bonin Islands	1918–1955
Southern Kagoshima Islands	1954–present day
Miyako Island	1959–1973
South China Sea	1965

Osaka and Yokohama (Ogosawara, Hachijo, Sumisu) (Ogi 2010). In 1965, fishermen began targeting beds in the South China Sea, with harvest of *C. elatius* and a new species, *C. secundum* (Liverino 1983).

In inshore areas of Japan, most coral fishing vessels were small (5 t). To accommodate the greater distance to fishing grounds, 2–5 month fishing trips became common, with larger vessels (150–180 t), more crew (25–30 men) and up to 18 mini-dredges used simultaneously (Liverino 1983). These dredges consisted of a round, 10 kg stone with five attached nets, allowing harvest by individual boats of up to 80 kg of coral per day. Japanese fisheries and subsequent commerce were locally managed by the three fishery associations: All Japan Sango Fishing Association, the Sukomo Kyodo Kumiai, and the Goto Sango Kogei Kyodo Kumiai (Iwasaki et al. 2012).

Taiwanese coral fisheries commenced in 1924 in offshore regions of Taiwan and south of Japan, shortly after discovery of precious coral by Japanese shark and porgy longline fisheries (Chang et al. 2013). The precious coral fishery in Taiwan prospered during the Japanese Colonial Period between 1930 and 1940, with up to 180 vessels fishing at one time for coral (Huang and Ou 2010). During this first phase of the fishery, landings peaked at 10–20 t per year. Each peak corresponded to the discovery of new fishing grounds and a dramatic, rapid increase in the number of fishing vessels shortly thereafter. These rich periods were followed by sharp declines within a few years, because the coral beds were small and easily overexploited. In 1934, the Penghu government supported research on coral beds, and placed limits on the number of coral fishing boats (20–40 vessels) in attempt to promote sustainable harvest (Liverino 1983). Nevertheless, "boom and bust" trends repeated four to five times until World War II, when the fishery went dormant (Huang and Ou 2010). Coral fishing was briefly revived for one season in 1954. AT this time, the Taiwanese Fisheries Research Institute and the Taiwanese Coral Industry cooperated in efforts to assess the economic feasibility of coral dredging. During these surveys only 19 % of the dredge hauls were successful and costs greatly exceeded the value of landings. As a result, it was recommended that new grounds be searched for coral, but the fishery was not revived until 1962 (Huang and Ou 2010).

Since the inception of the precious coral fishery in Taiwan, Taiwanese fishermen have used non-selective fishing gear that is similar to the gear used by the Japanese, and they continue to use this type of gear today (Fig. 46.9). Their coral tangle net dredges are comprised of a cobblestone weighing approximately 30 kg tied with steel wire in a crisscross fashion, with four to five nets attached to the steel wire around the cobblestone. Individual boats may deploy several dozen of these dredges simultaneously, depending on the number of winches and the size of the boat. Precious corals harvested in Taiwanese waters include four species, *P. japonicum, C. konojoi, C. secundum* and *C. elatius* (Lai 2006), although most of what is landed today is *C. elatius*.

Beginning in the 1960s, Japanese and Taiwanese fishermen expanded their search for precious coral into international waters, as most beds in their own waters were no longer productive. Taiwanese fishermen discovered coral banks off Hong Kong in 1962 in 70–100 m depth, in 1963 on the Oza Banks, 100 miles south of Okinawa, and then in 1968 off the Pratas Islands (Liverino 1983). In 1965, an unusually large population of *Corallium secundum* was found at Koko Seamount in the Milwaukee Banks, on Mellish Bank, and in surrounding seamounts in the Emperor Seamount Chain, all of which were located in international waters near the Hawaiian Archipelago (Grigg 1974b). The coral occurred much deeper than most of the coral harvested from inshore waters (375–450 m), and it required much larger fishing boats.

Over the next 20 years, most of the world's harvest of *Corallium* came from the Milwaukee Bank and the surrounding Emperor Seamounts. About 200 vessels from Taiwan and Japan made up to seven trips per year during the first peak, with 150 tons landed in 1969 (CITES 2007). After a few years stocks became depleted, and yield remained low until discovery of new beds in at depths of 900–1,500 m 5 years later. These beds were dominated by an undescribed deep water species (Midway coral, *Corallium sp. nov.*). This discovery led to another coral rush, with over 100 boats from Japan and Taiwan involved in the harvesting. Landings increased substantially between 1978 and 1981, declined briefly, and then reached a second peak in 1985. This was short-lived, with virtually no coral landed from these beds after 1989.

Three separate groups of coral fishermen have operated out of Japan: the All Japan Coral Fisheries Association

Fig. 46.9 Taiwanese fisheries. (**a**) Tangle nets are still the only fishing tackle used to collected precious corals in Taiwan. Each tangle nets is composed of one big stone (**e**) used to sink the net and up to seven pieces of net. Windlasses are used to release and manipulate tangle nets. (**b**) Precious coral fishing vessel. Most of the fishing vessels are smaller than 50 tons, but vessels of up to 100 tons can be used for coral fishing. All vessels must be equipped with VMS (Fishing Vessels Monitoring System). (**c**) Each fishing vessel has four workers, one captain, and one chief mate. All tangle nets are equipped in the head of the vessel. The tail of the vessel is living space. The fishing vessel usually spend 2–3 weeks at a time collecting precious corals. (**d**) Fisherman works on the raw material on boat, cutting off and disposing of the thin branches at sea because each vessel is only allowed a total harvest of 200 kg. Most harvest are corals that have been for a long time and covered by sediments. (**e**) The cobblestone used to sink the net is about 30 kg tied with steel wire and attached to four to five nets (Images by Professor Tzu-Hsuan Tu)

(JCFA) operating out of Kochi City, Kochi Prefecture; coastal fishermen using tangle net dredges; and submarine and robot harvesters. The JCFA was comprised of "Midway Deep Sea Coral" fishermen who operated 17 vessels in 1981, but due to the collapse of the resource no vessels operated in 1987 and 1988, and 1 vessel fished these offshore beds in 1989; JCFA also harvested *Corallium* near the Bonin Islands off Tokyo (Grigg 2003). The coastal and shallow-sea coral fishermen operated out of Sukumo City, Kochi Prefecture with 100 small vessels (3–10 tons) in 1981, but this declined to 60 vessels in 1989. Each was manned by one fisherman for day-fishing off Tosa City, Kochi Prefecture (eastern Shikoku). Four species were harvested: *Paracorallium japonicum* (200–300 m depths); *C. elatius* (250–400 m); *C. nobile* (150–300 m); and *C. konojo* (100–200 m) using dredges consisting of 20 kg concrete weights with embedded rings attached to netting and hauling lines. Coral fisheries around the Amami Islands started in 1910 and continued intermittently until the late 1970s using tangle nets (Ogi 2010). Dredges were replaced by a small two-man submarine and a robot from Tokyo 1978, and then in 1987 by ROVs (Iwasaki et al. 2012).

Although Japan continued to harvest *Corallium* during the 1980s and 1990s, many of the historic fishing grounds were depleted and landings remained at historic low levels. Areas that were no longer productive included: (1) the Peng-hu Islands near Taiwan; (2) continental slope areas near the southern tip of Taiwan; (3) Oza bank near Miyako Island; (4) the Danjo Islands near Kyushu; (5) Japan Banks of Tosu; and (6) Shikoku, Smith Bank, Tourishima Island and Sofu Island located between Japan and the Bonin Islands (Grigg 1971). Japanese fishermen also abandoned coral beds around the Emperor Seamounts in the late 1980s, and many inshore fisheries began to focus more on food fishes due to the higher value of food fishes, with coral harvest occurring secondarily (Chen 2012; Chang et al. 2013).

As the value of precious corals rebounded, harvest resumed and continues to the present day in two regions of Japan: the Kochi Prefecture and Kagoshima and Okinawa Prefectures. Of 24 areas that were traditionally harvested only three are open. In Kochi, two areas are fished using traditional stone-weighted non-selective tangle nets. The number of vessels that are active in these areas ranges from 120 to 230, with each fishing for approximately 2 weeks per year. In Kagoshima and Okinawa coral is selectively harvested using manned and unmanned submersibles (Iwasaki and Suzuki 2010).

During the coral boom in the Midway Islands, the number of Taiwanese fishing vessels was greater than the Japanese vessels, and they continued to target these grounds for a longer duration (until 1992). The larger fishery was partially attributed to government fishery enhancement programs and bank loans designed to promote and modernize the precious coral fisheries industry. Initially, inshore fisheries expanded, but numerous longline vessels were remodeled to allow coral fishing in more distant locations. The project was terminated in 1979, and regulations were subsequently implemented to reduce the number of vessels in the coral fishery because the government realized that the coral fishing grounds were not large enough to support the expanded fishing fleet and coral fishermen had started to illegally enter and fish the EEZ of Japan and the USA (Chen 2012; Chang 2015). To address these concerns, Taiwan first began requiring licenses for small (less than 50 tons) coral fishing vessels, expanding the licensing program to all vessels in 1983, and also implementing a maximum number of allowable vessels (150). The combined impact of the new regulations, along with a substantial drop in the value of *Corallium* (partially attributed to a glut of coral and the low quality of the harvested material), caused a sharp decline in landings and effort during 1982. Shortly thereafter there was a renewed influx of fishing effort that lasted until 1985, followed by a rapid collapse of the Midway coral fishing grounds and abandonment of the fishing grounds beginning in 1989.

Contrary to the expected impacts of the collapse of the Emperor Seamount fisheries, Taiwanese coral fisheries continued to survive. Many of the longline vessels were subsequently abandoned or re-outfitted for dolphinfish fisheries, while smaller vessels focused their effort in inshore waters for *C. elatius* and *P. japonicum*, the dominant species reported in landings since 1991 (Chang 2015). The fishery continued to operate through illegal, unregulated and unreported (IUU) fishing with an estimated 50 vessels in the Nan-Fan-Ao area of Yilan County and an increase to close to 100 vessels following discovery of *C. secundum* south of Lanyu Island in 2001 (Huang and Ou 2010). Although only three coral fishing boats were licensed in 2007, an investigation carried out by Taiwan Fisheries Agency identified 96 unregulated vessels equipped with coral fishing gear that were illegally fishing and not reporting their catch (Huang and Ou 2010). To address IUU fishing, the government issued new management standard for Taiwan's precious coral fisheries. This included a maximum of 60 licensed fishing boats and five designated fishing grounds (Huang and Ou 2010; Chen 2012; Fig. 46.10).

46.2.1.4 Hawaii *Corallium* Fisheries

Collections containing *Corallium* were first made during the Albatross Expedition of 1900, but it was not until 1966 that a small fishery was initiated, following discovery of a large bed (Makapu'u) of *C. secundum* approximately 9 km off Oahu in the Molokai Channel (Grigg 1974b). Using heavy stone or iron bars with attached netting (tangle net dredges), approximately 1,800 kg of *Corallium* was harvested from this bed between 1966 and 1969. Maui Divers of Hawaii, Inc., the leading manufacturer and retailer of precious coral

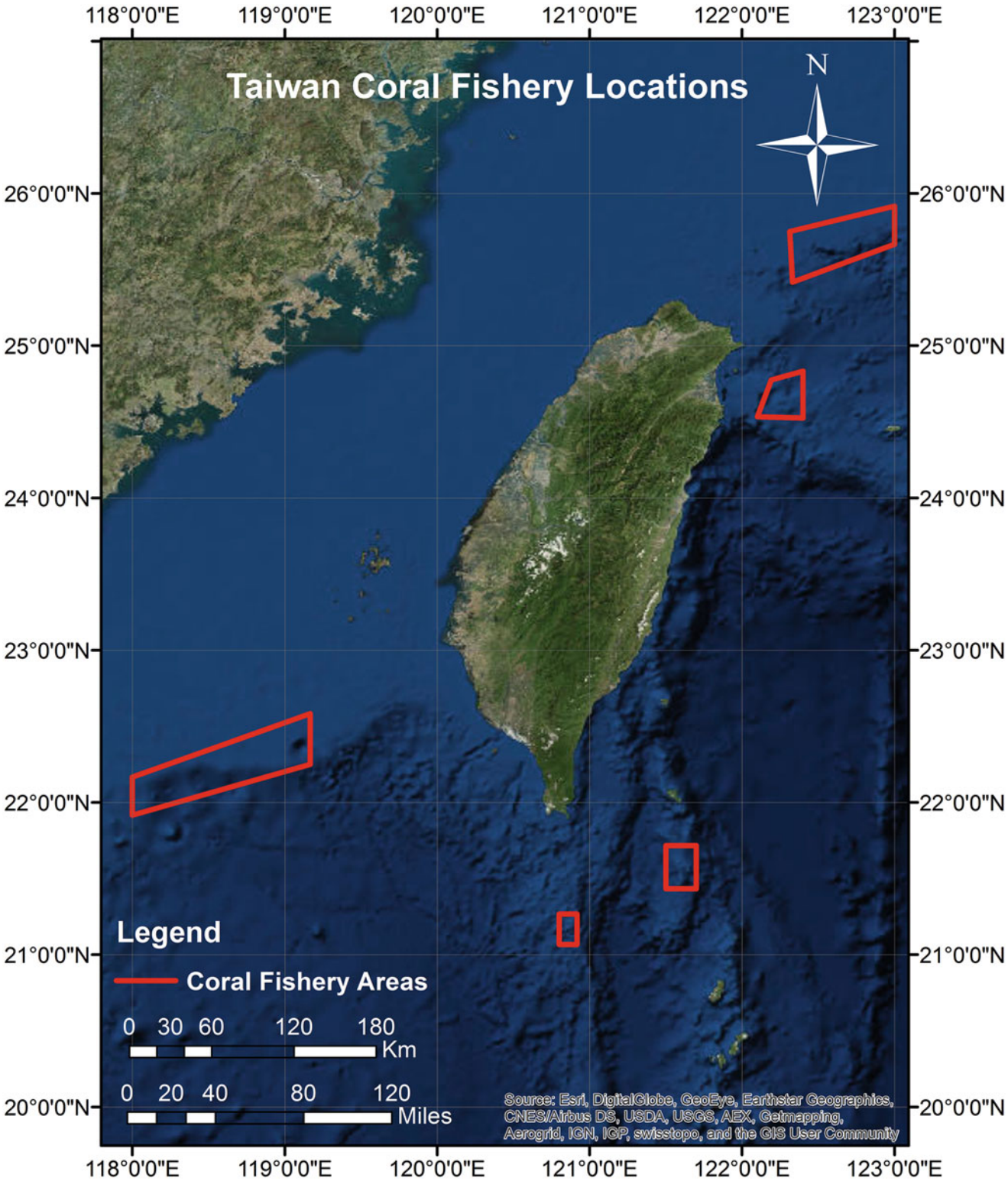

Fig. 46.10 Location of the boundaries of the five legal coral fishing areas off Taiwan (*red polygons*)

jewelry in Hawaii, harvested an additional 6,400 kg of coral from Makapu'u between 1972 and 1978 using a manned submersible (Grigg 2010). Harvest was discontinued in 1978 due to high operating costs and a diving accident (Grigg 2002). In 1988, the domestic fishing vessel Kilauea used a tangle-net dredge to harvest precious coral at Hancock

Seamount, landing approximately 500 kg of coral. Because of the high costs and landings that consisted mostly of dead or low-quality pink coral, the operation was discontinued. A *Corallium* fishery was revived briefly by American Deepwater Engineering between 1999 and 2001, using a one-person submersible. A total of 1,216 kg *C. secundum* was collected from the Makapu'u Bed, along with 61 kg of *C. lauuense* (*C. regale)* from exploratory areas off Kailua, Kona (Grigg 2002). No harvest of *Corallium* has occurred since 2002.

46.2.1.5 Landings of *Corallium*

By piecing together available *Corallium* literature and official catch records compiled by FAO available since the late 1970s a consistent boom and bust cycle becomes apparent. Throughout the history of coral fisheries, both effort and landings increased dramatically following the discovery of a precious coral bed, with sharp declines occurring within a few years, as the resource is depleted (Grigg 2010; Tsounis et al. 2010; Bruckner 2014). Precious coral fisheries are similar to strip mining and clear cutting of forests, as the sessile nature of the corals makes them vulnerable to depletion, and use of destructive fishing gear (trawls and dredges) dislodges and removes all corals from the fished area while simultaneously destroying the habitat (Bruckner 2009).

In addition to the depletion of the resource, economics have contributed to the observed changes in landings for both Mediterranean and Pacific fisheries. In situations where harvest levels were so high that the volume exceeded demand and/or the market was flooded with low quality coral, the resulting surfeit of coral has caused a drop in market value. The wholesale value of the coral in some instances fell to such low levels that the operating costs of the vessels exceeded the value of the commodity, and fishing boats were abandoned or re-outfitted for food fisheries. One of the best examples of this is from the Mediterranean following the discovery of large coral beds off Sciacca (southwest coast of Sicily). The exploitation of the Sciacca's coral cays had two fundamental repercussions on the coral market. First, the surge in fishing effort led to overproduction of low-quality raw coral, followed by a surplus of *Corallium* and a steady fall in price. The drop in prices, compounded by the emergence of new sources of Pacific coral from Japan caused a serious crisis that led to a reduction in fishing effort and landings, with repercussions felt by Italy's coral artisans. A second example is the overharvest of Pacific *Corallium* around Emperor Seamounts between the 1960s and 1980s. Over this period there were several sharp increases in landings and rapid declines, and eventual abandonment of the fishing grounds in the late 1980s. These dramatic cycles can be attributed to the discovery of new beds followed by an influx of coral fishing boats, with a subsequent reduction in fishing fleet due to the commercial extinction of individual coral beds, elimination of government subsidies and bank loans to Taiwanese fishers, and most importantly, declines in market value due to market gluts and increased landings of low quality, dead coral (Bruckner 2009, 2014; Chang 2012).

46.2.1.6 Mediterranean Landings of *Corallium*

In the Mediterranean, 12 countries have reported landings (yield) data to FAO since 1978 (FAO 2015; Fig. 46.11). These data were highest during the first years of reporting, and show a sharp decline in over 20 years, from a maximum of 98 tons in 1978 to 18.9 tons in 1998. The largest declines over this period were reported by Italy and Tunisia, while yield from African countries (Morocco and Algeria) increased substantially in the mid-1980s. The declines observed in the 1980s can be partially attributed to a phase-out of the use of non-selective dredges, as well as a reduction overall in effort and a change in management schemes. However, these changes are not the only reason for the decline. SCUBA fisheries were first introduced in the 1950s, while the use of the coral dredge was not banned until 1989 (in Sardinia only) or 1994 (throughout the rest of European waters). Landings were already less than 50 % of 1978 levels (42 tons) in 1984, and they remained at these low levels (32–48 tons) for the next 5 years, all prior to the ban on dredging. Even in Italy, where the coral dredge was banned 5 years earlier than in other locations, landings had already declined from a peak of over 72 tons in 1978 to 40 tons in 1980 and 19.3 tons in 1985, with less than 10 tons landed each year up to 1988. Landings from Italian fisheries declined further following the ban by close to 50 %, yet this reduction is minimal when compared to changes observed during years when the ingegno was still legally used (Fig. 46.11).

Despite a dramatic change in fishing techniques with the introduction of SCUBA diving, and the enhanced efficiency and specialization of the fishery, landings increased but never returned to levels reported in the 1970s. Mediterranean-wide landings have steadily increased over the last 20 years, reaching a second peak of 54.11 tons in 2010, but have since begun to decline slightly (Bruckner 2014). Furthermore, landings reported by individual countries show sharp swings in quantity since inception of SCUBA fishing, which are suggestive of the discovery of large aggregations of coral in a particular area, followed by rapid overexploitation of these populations.

An examination of catch data by country reveals relatively stable landings in some countries, while others show a slow but progressive increase, giving a false impression that the fishery has been sustainable. Part of this increase is due to the expansion of fisheries outside the European Mediterranean to the North African countries, the Atlantic coastline, Turkey, and the Eastern Adriatic Sea. Simultaneously, as shallow beds (30–50 m) have been progressively overfished, divers in the European Mediterranean

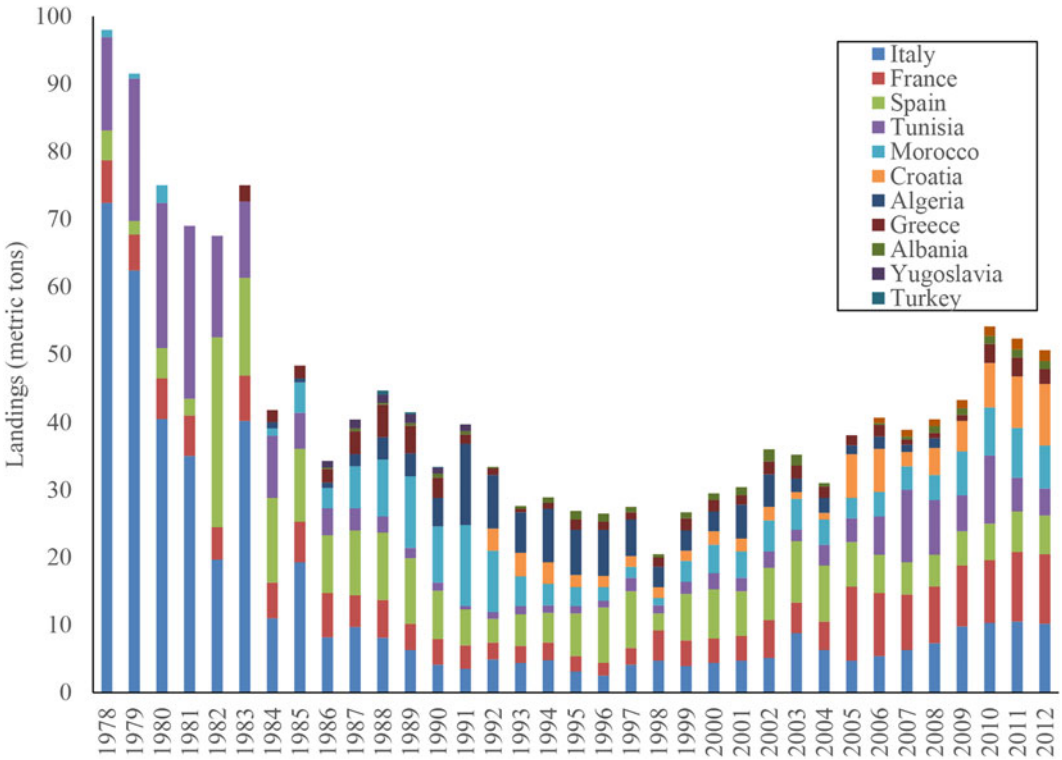

Fig. 46.11 Global catch data for the Mediterranean between 1963 and 2012 broken down by individual country

have begun to exploit unfished areas in deeper water (80–130 m). Individual peaks in landings during a single year reflect a pulse mode of fishing associated with SCUBA fisheries, whereby all of the large colonies are cleared from an individual bed, and then divers move on to new areas. Furthermore, in some countries there are reports that SCUBA divers are harvesting higher numbers of smaller corals in areas where the large size classes have been depleted (Tsounis et al. 2010). The recent use of ROVs to search for colonies, and in some cases illegally harvest corals, is an important factor in the ever increasing landings being reported from some countries (Tsounis et al. 2010, 2013). Proposals to list corals in CITES, as well as new measures being developed and implemented through FAO, may have pushed divers to harvest as much coral as possible before new regulations on trade and harvest are implemented (Tsounis et al. 2013). This is especially true around Sardinia, where landings of red coral doubled from 4.7 tons in 2005 to 10 tons per year since 2009. France also shows large increases in landings between 2004 and 2010 and landings from Croatia and Morocco both increased since 2009. Data from Tunisia shows substantial increases from 2006 to 2010 but landings have since begun to decline (Fig. 46.11).

46.2.1.7 Pacific Landings of *Corallium*

In the North Pacific, precious corals has been harvested at low levels since the 1870s, primarily from inshore waters around Japan and Taiwan (Fig. 46.2). The annual Japanese catch from the inception of the fishery until several decades after World War II has varied from about 2–20 metric tons (Wells 1981; Grigg 2010). Production records Japanese fishermen operating near Okinawa in the 1960s were similar, reaching a peak of 14.78 tons in 1963 and dropping to less than 1 ton by 1969 as the resource was entirely wiped out (Grigg 1971). In Taiwan, during the early days of the fishery (1925–1940), catch ranged from 10 to 20 tons per year during peak periods with declines to 3–5 tons in low periods (Fig. 46.12).

Pacific landings greatly expanded following discovery of a large bed of *C. secundum* in Midway Islands in 1965, and again in the mid-1970s when a second (undescribed) species was found around Emperor Seamounts. Reconstructed FAO Global Capture Production data (FAO 2015) show five major peaks over this period (Fig. 46.13), followed by a progressive decline between 1984 and 1989. Landings reported by Japan exceeded 300 tons per year between 1965 and 1967, sharply declined to 52 tons by 1968, and reached a second peak of 113 tons in 1969 before declining once more to less than 50 tons until 1975 (Fujioka 2008). Annual yield from Japan was the greatest from 1965 to 1969 and 1975–1980 (63–103 t/year), with 70–90 % of the harvest in later years consisting of Midway deep-sea coral (*Corallium* sp. nov.). Landings from these beds reported by Taiwan were similar to Japan between 1968 and 1970, but they then dropped off

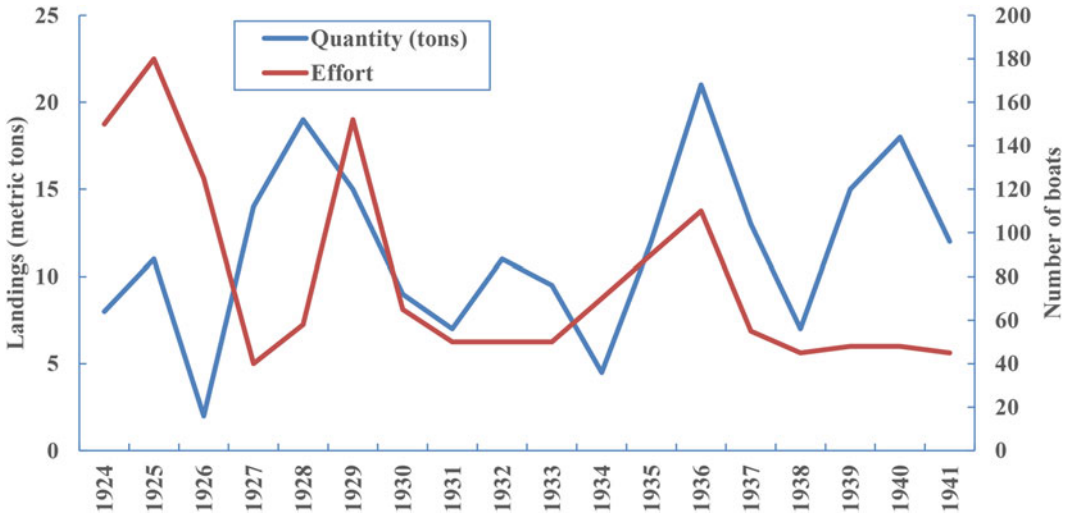

Fig. 46.12 Reported landings of precious coral (*C. konojoi, C. elatius* and *P. japonicum* are pooled) from inshore waters of Taiwan and approximate number of boats fishing for coral between 1924 and 1940 (Data extrapolated from Grigg 1971)

until 1976 when they climbed to values that were two to three times greater than that reported by Japan (Fig. 46.13a) with peaks in landings of *C. secundum* in 1969 (112 tons), 1976 (102 tons), 1981 (270 tons) and 1984 (226 tons), and even higher amounts of *Corallium* sp. nov. from 1983 to 1986 (564 t), most of which was harvested off Midway Island (Bruckner 2014).

The cause of the decline of the Emperor Seamount fisheries has been widely debated. Several reports from Japan and Taiwan indicate the precipitous declines are due solely to a decrease in effort but not the depletion of the resource (Iwasaki and Suzuki 2010; Chang et al. 2013). Firstly, excessive harvest in the 1970s resulted in a glut in the market which caused the wholesale value to drop below harvest costs. Secondly, the fishery was reported to have taken off shortly after discovery of these resources primarily because of incentives provided by the government to promote the fishery, which were terminated in 1979. A limit on the total number of coral fishing vessels in the 1980s led to the pulling out of Japanese fishing vessels, and later Taiwanese fishing vessels (Iwasaki and Suzuki 2010). To date, a coral fishery never resumed in these areas, largely because (1) these corals are less valuable than other species; (2) there are reports of remaining stockpiles; and (3) the costs of fuel and operations associated with offshore fisheries exceeds the value of the coral (Chang et al. 2013).

While economics and government incentives certainly affected landings around Emperor Seamounts, it is reasonable to assume the fishery caused the depletion of most of the coral from these depths relatively quickly, based on the biological attributes of these corals and the large scale nature of this fishery in comparison to the overall amount of accessible coral habitat (Bruckner 2009). With such a large number of fishing vessels, individual areas would have been dragged repeatedly; fishers are also likely to have continuously spread into new fishing grounds in search of new stocks, as indicated by reports of illegal poaching outside of international waters, especially in US waters around Hawaii (Grigg 1993). The presence of a large proportion dead, poor quality corals in landings is further evidence that live colonies were severely depleted, and much of what was landed in later years was coral that had been broken off by dredges during earlier efforts. Although few biological data exist from these areas, submersible surveys conducted by Japan in 2008 on Emperor Seamounts included areas targeted by coral draggers in the 1960s–1980s. These surveys identified isolated live colonies and dead and broken colonies, but they failed to identify a single large patch of *Corallium* (Fisheries Agency of Japan 2008). This further suggests the resource was overexploited beyond limits of recovery, and even in absence of fishing for more than 20 years populations have not rebounded.

Since the late 1980s, following the reduction and eventual termination of coral fisheries on the Emperor Seamounts, only a fraction of the historic harvest has been landed. The JCFA estimates that the total Japanese harvest of precious corals decreased from over 55 metric tons (t) in 1982 to only 3 t in 1988 and have remained at levels that are 10–20 % of that reported in the late 1980s (Carleton and Philipson 1987; Grigg 1989). Reported landings (compiled by FAO) of all species from inshore waters around Japan have varied from 7 to 8 tons/year in the early 1980s, dropping to 1–3 tons in the 1990s and stabilizing at about 5 tons/year over the last decade (Fig. 46.14). The coral consists predominantly of *C. elatius* (mean = 1.97 tons/year) and *P. japonicum* (1.2 tons/year), with minimal amounts of *C. konojoi* and *Corallium* sp. nov. (0.15 tons/year) reported in the catch statistics (Fig. 46.14a). Interestingly, vessels using non-selective tangle net dredgesPacific coral was in the

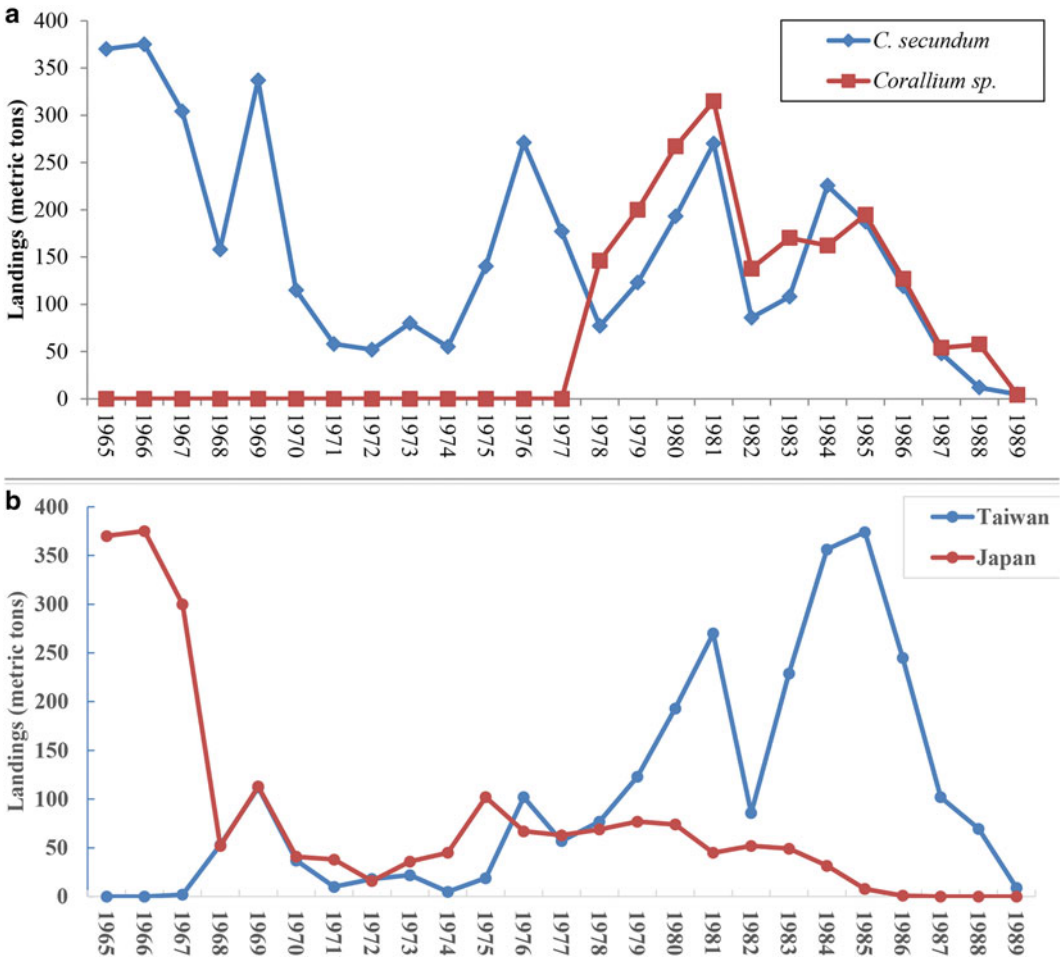

Fig. 46.13 Global capture production for precious corals harvested off Midway Islands and Seamounts extrapolated from FAO Fishery and Aquaculture Global Statistics (FAO 2015). (**a**) Landings of angel skin (*C. secundum*) and Midway deep sea coral (*Corallium* sp.) pooled for both countries. (**b**) Landings for all species for Taiwan (*blue*) and Japan (*red*). *Corallium secundum* was the only species harvested between 1965-approx. 1978, while Midway deep sea coral was first discovered around 1978 and harvested until 1989

Kochi Prefecture operate for only about 2 weeks per year, with each of the 120–230 boats landing approximately 12 kg per year (Tsounis et al. 2010).

Landings by Taiwanese coral fisheries have shown much more fluctuation. Landings between 1990 and 2008 were higher than Japan (mean = 7.1 ton/year), but they are still a fraction of the coral harvested in the 1970s and 1980s. The highest landings occurred in 1996 (12.6 ton), 2002–2004 (35 ton), and 2008 (11.95 ton); most of this was *C. elatius* harvested from 300 to 500 m between Taiwan and the Philippines. Since 2009, when Taiwan introduced new regulations, landings have been considerably less (2.9–4.8 ton/year), and appear to show a downward trend. A surge in Pacific coral was reported in FAO statistics in 2012 (21 ton) consisting mostly of *C. konojoi* harvested by Chinese fishing vessels operating illegally in waters around the Diaoyu islands and Miyako islands and off Taiwan (Chang 2015; Fig. 46.14b).

46.2.2 Black Coral

46.2.2.1 Historical Significance

Like *Corallium*, similar mystical powers and medicinal properties have been attributed to black coral. The skeleton of the larger black coral species has been used for jewelry and religious articles from at least the time of the ancient Greeks. In Indonesia, black coral branches are boiled in oil and when softened are bent to form arm bangles. Indonesian folklore suggests that a black coral bracelet worn on the right arm increases virility, while one worn on the left arm cures rheumatism (Wells 1981). In the Red Sea, black coral was used as an aphrodisiac and to cure eye diseases (Castorena and Metaca 1979). The name "*Antipathes*" translated from Latin means "against disease or suffering". It is thought to have originated in North Africa, where black coral was prized because it "neutralized" the magic of the dreaded "evil eye" (Hansen 1981). Black coral branches are also

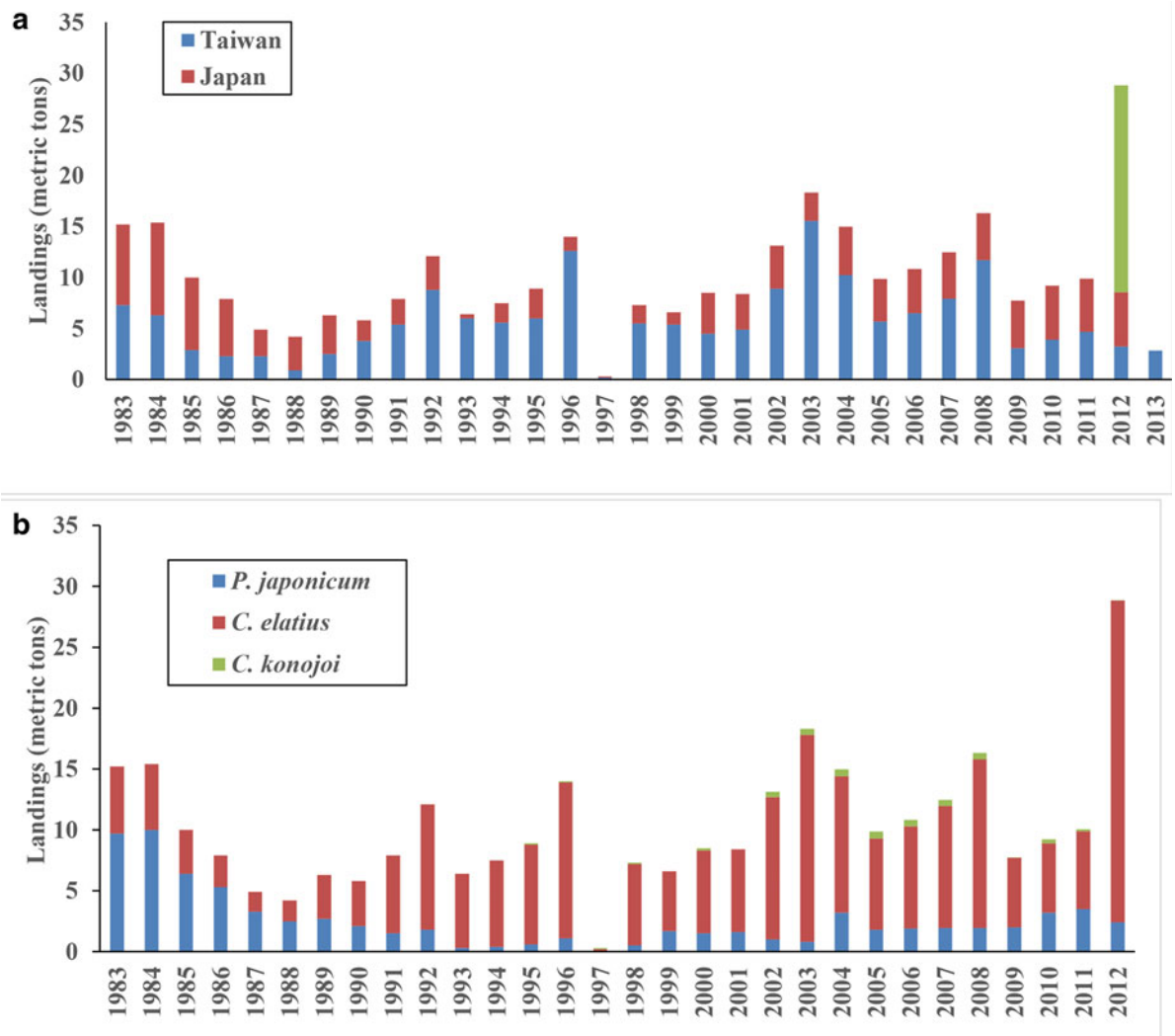

Fig. 46.14 Global production statistics for inshore precious coral fisheries of Japan, Taiwan and China between 1983 and 2013. (**a**) Landings pooled for all species by country. China first reported landings of *Corallium* in 2012 (green bar). Landings data for 2013 were only available from Taiwan. (**b**) Landings data pooled for the three countries for each species. *P. japonicum* made up the bulk of the landings in the early 1980s, while most coral landed since 1990 consists of *C. elatius*. The large spike reported for 2012 are due to landings of *C. elatius* from China

ground into a medicinal powder and used in Chinese traditional medicine; black corals are said to relieve pain, reduce fever, stop bleeding, and soften hard masses (Qi et al. 2009). In ancient Greco-Roman culture, black coral powder was applied to wounds before and after cranial surgery (Mariani-Costantini et al. 2000). In Hawaiian culture, black coral powder was mixed with other natural ingredients and used to treat mouth sores and lung diseases (Nagata 1971; Chun 1994). The high cultural importance of black coral is still apparent today, as it is the official state gemstone of the State of Hawaii (USA). In many tropical nations, especially Hawaii and the Cayman Islands, high end jewelry and carvings of black coral are commonplace.

46.2.2.2 Black Coral Fisheries

The earliest known fisheries for black coral were in the Red Sea. One of the largest fisheries in this time was based out of Jeddah, Saudi Arabia, where corals were harvested along a 100 mile stretch of coastline. Much of the coral from Saudi Arabia was carved into beads and mouthpieces for cigar holders (Tressler and Lemon 1951). Black coral was also marketed in the Far East for use as scepters, divining rods and amulets. Black coral (*Antipathes ternatensis* and *Cirrhipathes* spp.) has also been extensively harvested off the Vietnamese coast and Sumatra Malaysia region, while *A. arborea* and *Isidis plecarus* were historically harvested off Sri Lanka.

Commercial beds of black coral were discovered in 1958 in two locations off Hawaii (Auau channel off Maui and the southwestern coast of Kauai) at 30–90 m depth and have been fished almost continuously for over 50 years (Grigg 2001). Maui Divers first established a small black coral jewelry industry in 1960, employing 10–12 divers who removed corals with axes, hammers and saws (Grigg 1993). Between 1963 and 1970, these divers harvested over 23,000 kg from the two locations, most of which was *Antipathes dichotoma* (90 %) with lesser amounts of *A. grandis* (10 %) and *A. ulex* (1 %) (Grigg 1971; NOAA 2002; Bruckner et al. 2008). During the 1970s and 1980s, consumer demand for black coral was greatly reduced, and the market shifted to *Corallium* and *Gerardia* (Grigg 1993, 2001), although landings still remained quite high. Grigg (2010) estimated that approximately 8,000 kg was harvested annually from the Maui Bed and 4,000 kg from the Kauai bed over this period. Technological advances in coral processing in Hawaii in the 1980s led to a dramatic decrease of the amount of coral needed to produce the same value of finished product, and many of the items are smaller in size and of a higher quality (Grigg 1993). The import of cut and polished black coral from Taiwan further affected the volume of harvest of coral by Hawaiian coral fishers, as the industry consumed less than 2 tons per year on average during the 1980s (Oishi 1990). Hawaii also imported roughly 70 t of *Cirrhipathes anguina* (whip coral) that had been harvested in the Philippines and processed in Taiwan into beads, rings, bracelets and necklaces (Carleton 1987). Lesser amounts of black coral were imported from Tonga (Harper 1988).

Since 1980, virtually all black coral harvested in the Hawaiian Islands has been taken from the Au'Au Channel Bed (total size of about 1.7 km^2) with a lesser harvest from the smaller (0.4 km^2) Kauai Bed. Black coral landings in Hawaii increased considerably during the 1990s and early 2000s (WPRFMC 2006). From 1981 to 1990 the state of Hawaii reported that landings of black coral amounted to 6,200 kg, with an annual take of 72–1,977 kg (Oishi 1990). The total black coral landings increased to over 9,000 kg over the next 7 years (1992–1998) and they continued to increase between 1999 and 2005, comprising 58 % of the total harvest since 1985 (Parrish 2006b). Currently, only about five coral divers are active in this region.

Although black coral was fished commercially from the 1960s to the 1980s in many other Pacific locations, such as Ecuador, Indonesia, and Palau, the quantities taken were generally small, and landings were rarely reported (Wells 1983; Grigg 1993). For example, black corals were intensively collected around the Galapagos and Ecuador in the 1980s, and these species have completely disappeared from several sites (Martinez and Robinson 1983).

In the Caribbean, most of the harvest has been *A. pennacea*, *A. dichotoma*, *Leiopathes glaberrima*, and *Cirrhipathes lutkeni*, primarily for use in the tourist jewelry trade and not for export. In general, most Caribbean fisheries were short-lived and collection was sporadic as the resource was patchy and shallow water populations were easily overexploited. By the early 1980s, depletion of local populations as a result of unsustainable harvest had been reported in St. Lucia, Barbados, Netherlands Antilles, Bahamas, and British Virgin Islands (Anon 1979a, b; Wells 1981). Much like the red coral fishery, divers depleted resources from shallow water and progressively expanded their search into deeper water. Following adoption of international regulations on trade through CITES, legislation curtailing or prohibiting collection and trade was implemented in Antigua, Belize, Trinidad and Tobago, the Bahamas, the Netherlands Antilles, the US Virgin Islands, and British Virgin Islands (Wells 1981).

Large populations of black coral (*Antipathes caribbeana*) were first discovered in Cuba in the 1960s and collection began in the 1970s. The fishery expanded substantially in the mid-1980s in the Cazones Gulf, with 1,355 kg of black coral harvested between 1987 and 1993. Black coral was also extracted from Pinar del Rio, with a maximum of 301 kg taken in 1992 (Guitart et al. 1997). The fishery was unregulated until 1990, when recently minimum size limits and on boat inspections began. The most recent landings data for Cuba are from 1998, when 1,468.6 kg of coral were landed from depths of 20–55 m by four enterprises (Alcolado et al. 2003).

In 1984, a government-owned company, the Mediterranean Coral Fishing Company, began to conduct surveys to identify and harvest precious corals in Maltese waters. These fisheries relied on SCUBA divers using helium-based breathing gas mixtures, an ROV, and a manned submersible. In addition to *Corallium*, 250 tons of black coral were harvested from 500 to 600 m between 1984 and 1987. The black coral fishery in the Maltese Islands eventually came to the attention of the International Union for the Conservation of Nature (IUCN) which, in 1987, solicited the Maltese government to sustainably manage and regulate these slow-growing species. They based their concerns on scientific studies, highlighting the fact that this was the only known black coral fishery in the Mediterranean (Deidun et al. 2010).

Southeast Asia and the South Pacific islands continue to be an important source of black coral for international markets, however very limited information is available on the status of the industry or the amount of harvest. In the 1980s, an estimated 60–100 tons of black coral were harvested from the Philippines each year, with most of the remainder (about 8 % of all world harvest) collected from South Pacific countries, principally Tonga, Fiji and Papua New Guinea (JP Parish SPC/Inshore Fish. Res./WP.2). According to the CITES database, a total of 72 metric tons

and 7,400,000 pieces of black coral were recorded as being traded between 1982 and 1998, with most exported from Taiwan, the Philippines, and the Dominican Republic.

46.2.3 Other Precious Corals

Other types of precious corals, including gold corals and bamboo corals have made up a small fraction of the industry and very few data are available on locations of fisheries and landings. It is likely that most fisheries have been active for short periods in more of an exploratory manner, and quickly terminated due to the scarcity of these resources. This presumption is further supported by the limited amount of bamboo coral and gold coral jewelry that has appeared in the marketplace. During the 1970s, small quantities of four species of bamboo corals, *Acanella eburnea, Keratoisis flexibilis, K.ornata* and *Lepidisis caryophyllia* were collected commercially from the Gulf of Mexico and used to produce beads (Wells 1981). Bamboo coral harvest in Bone Bay, Sulawesi (Indonesia), appears to have increased significantly in recent years with exports of more than 100 tons reported in 2005 (Department of Fishery and Marine Affairs [Dinas Perikanan dan Kelautan Bone sudah tercatat]).

Hawaiian Gold Coral was first discovered in Makapu'u Bed in 1971 and later found in small quantities in 16 other locations throughout the Hawaiian Archipelago (Fig. 46.3). It was first introduced to the jewelry industry in 1974, and quickly gained popularity due to its interesting color patterns. Unlike other precious corals, its color ranged from a sandy beige to almost black. It has a special characteristic called "Chatoyance," from the French word for "cats eye," which describes a mysterious moving inner light. The only commercial fishery established to date is in Hawaii, with selective harvest using a small submersible from approximately 400 m depth. The initial landings from Makapu'u Bed in 1974 amounted to 734 kg (Grigg 1993). *Gerardia* was harvested from the same beds between 1975 and June 1978, with reported landings of 621 kg in 1975, decreasing each year to 363, 329 and 50 kg respectively, after which the operation was discontinued. Hawaii placed a 5-year moratorium on gold coral harvest in 2008 and this was recently extended for another 5 years. A limited harvest of *Gerardia* spp. was reported from waters surrounding Turkey (Deudin et al. 2010).

Primnoa gold corals from Australia were never commercially harvested, but they did appear as bycatch of halibut fisheries in the 1980s. These were sold to jewelers for approx. US $20–$25/lb (Cairns and Bayer 2005). Cimberg et al. (1981) reported two species of *Primnoa* in trade in the 1970s: *P. resedaeformis* and *P. willeyi*. Between 1997 and 2002, less than 200 kg of *Primnoa* was harvested per year for the jewelry trade (Krieger and Wing 2002).

46.2.4 Management of Precious Coral Fisheries

Throughout the history of precious coral fisheries, there have been numerous attempts to sustainably manage the harvest. The most common measures applied to these fisheries have focused on licensing schemes, limited entry, quotas and minimum sizes, gear restrictions, area closures, and rotation of harvest, as well as proposed restrictions on international trade through the Convention on the Trade in Endangered Species (CITES). Some of the earliest measures date from the tenth century when Arabs rotated fishing effort within *Corallium rubrum* beds located off Tunis. The French controlled the trade in the eighteenth century and also recommended rotation of fishing grounds, advising the Algerians to fish their coral beds only once every 5 years (Mcintosh 1910; Douglas 1947; WPFMC 1980). Historic measures applied to Pacific fishery resources by Japan and Taiwan have also focused on limiting effort through licensing schemes, limited entry, allowable sizes of fishing vessels, quotas, and area closures. For instance, the Penghu government attempted to promote longevity for the emerging fishery in Taiwan by limiting the fishery to 20–40 vessels (Liverno 1983).

The success of these measures has been extremely limited and efforts continue to be plagued with problems including frequent incursions by foreign fishermen into the territorial limits of other countries, lack of oversight, and inadequate enforcement (Grigg 2010). Besides a growing need to address poaching, successful management requires a better scientific understanding of the biology and ecology of these species, more thorough stock assessments, and application of sustainable yield methods similar to those applied to fisheries of other long-lived species (Grigg 2010). In the past, this would have been virtually impossible to achieve due to the type of fishing gear most commonly employed (destructive tangle nets) and the damage it causes to the habitat, combined with the sessile nature of these organisms that exposes all size classes to harvesting at the same time. Furthermore, the difficulty of conducting scientific research at these depths has limited our understanding of the locations and sizes of coral beds, the size/age structure of colonies within those beds, and other biological attributes such as natural mortality and rates of replenishment.

46.2.4.1 Management of Mediterranean Fisheries

Since the earliest days of the red coral fishery, harvest has followed a practice similar to strip mining, where one coral bed was depleted before fishermen began exploring and harvesting coral in new areas (Tsounis et al. 2010). Several centuries of this type of intense commercial harvest led to sharp declines in landings, causing scientists and conservation

groups to question the sustainability of the fishery and adequacy of management measures. Scientific data collected over four decades provided further evidence of the depleted states of these resources, and debates about the potential commercial extinction of these species have steadily increased (Tsounis et al. 2013; Bruckner 2014; FAO 2015).

Management of red coral fisheries since the 1970s has included a variety of national measures within territorial waters (12 miles from the coast), EU-wide directives and international legal instruments. These range from broad directives to conserve the coral and protect the coralligenous habitat to very specific measures directed towards harvest in individual countries (Table 46.4). In the 1980s, the UN Food and Agriculture Organization (FAO) General Fisheries Commission for the Mediterranean (GFCM) convened a series of technical consultation meetings to develop a management framework for a sustainable coral fishery. The key recommendations from these consultations included (1) bans on the use of the coral dredge; (2) adoption of quotas; and (3) recommendations of a voluntary minimum size for harvest for SCUBA fishers of 7 mm (GFCM 1984, 1988; Council for the European Union 1994). Other actions that have been discussed and in some cases adopted include rotation of fishing grounds and area closures, as well as licensing and reporting requirements (Caddy 1993). Several of these measures have been incorporated into National Management Plans, along with a handful of other measures (Table 46.4).

The proposed listing of the family Corallidae in CITES in 2007 and 2009 (CITES-USA 2007; CITES-Sweden-USA 2010; see below) brought these corals back to the forefront. Although they were not listed, several ad hoc workshops held in response to these listing proposals, along with Scientific Advisory Committee meetings convened FAO GFCM has led to the development of an adaptive management plans for red coral fisheries in the Mediterranean (Bruckner and Roberts 2009; Bussoletti et al. 2010; FAO 2007, GFCM 2010, 2011, 2013). The current draft management plan for the Mediterranean region includes three key provisions: (1) a ban on harvest above 60 m depth; (2) a minimum size of 7 mm basal diameter; (3) allowable harvest using only SCUBA and hand tools and a ban on the use of ROVs with manipulator arms for collection.

46.2.4.2 Management of Japanese Inshore Fisheries

The harvest of precious coral in Japan is regulated by the prefectural governors (Kochi, Okinawa, Kagoshima, Nagasaki), according to the fishery rule for adjustment under the *Fishery Law and Conservation Policy for Marine Resource*. Both fishermen and vessels are licensed and three legal harvest zones are designated. No specific harvest season or quotas exist. New management measures were introduced in 2012, including gear restrictions (e.g. non-motorized tangle net dredges of a certain size or submersibles, depending on location), maximum total number of licenses, specific fishing grounds and depths, seasonal closures, maximum annual harvest (in some areas) and a minimum size.

From a biological perspective, the benefits of several of these measures are unclear. First, the 4 month closure (January, February, June and July) is unlikely to be a long enough duration to promote recovery of fished areas, given the slow growth rates of these species. There is also a requirement that undersized corals (<3 cm high or 7 mm in diameter) are "released", but these are unlikely to survive as they have been detached from the bottom.

A recent fishery-independent study comparing population structure inside and outside fished areas found alarming trends of decline in fished areas. Coral colonies in both areas had a similar density but dramatically different size structure: 10–20 year old colonies in fished areas and 20–60 year old colonies in no fished areas (Iwasaki et al. 2012). Since commercially collected species are ordinarily 30–40 years old and these are now rare in fished areas (Iwasaki et al. 2012), the resource must be reaching an overfished status. Furthermore, the finding of a similarly (very low) density in fished areas may result in reproductive failure of the populations. Firstly, because fertilization of broadcast spawners occurs externally, the number of gametes are greatly diluted because colonies are widely spaced; secondly, smaller sized corals are capable of producing fewer larvae (Beiring and Lasker 2000; Marschal et al. 2004; Bruckner 2009). Pacific corals are also characterized by much slower growth rates than *C. rubrum* (Iwasaki et al. 2012) and hence recovery may be significantly delayed.

46.2.4.3 Management of Taiwanese Inshore Fisheries

Fishing grounds from inshore areas around Taiwan have been historically managed through limits on the number of vessels, largely because of the tendency for newly discovered fishing grounds to be quickly inundated with fishing boats. Even with the licensing of vessels, one of the main issues in Taiwan has been illegal fishing and poaching. For instance, in 2007 there were three legally licensed vessels in Taiwan, yet investigations by the Taiwan Fishery Agency identified 90 vessels with coral fishing gear. Amended regulations adopted by Taiwan in 2009 include licensing of vessels (approximately 60 currently fish), an annual quota (200 kg *Corallium* and 120 g *Paracorallium* per vessel), five designated fishing grounds, and requirements of a Vessel Monitoring System (VMS), daily logbooks, designated landing ports, centralized auction markets, and an observer program (Huang and Ou 2010; Chen 2012).

These measures appear to have been effective in regulating the amount and spatial distribution of effort. Landing data are quite concerning, however, as they indicate the

Table 46.4 Summary of National Legislation for Mediterranean *Corallium rubrum* fisheries

Country	Gear types	Licenses	Closure	Quota	Size limit
Albania	Coral fishing is banned, yet landings reported	10 divers (estimated and unlicensed)			
Algeria	Dredge banned in 1977; no fishing from 1977 to 1982	1995: 8 areas divided among 100 divers	5 year harvest followed by 15 year closure; fishery closed in 2001	Max quota 850–1,200 kg/year per area, 8.9 tons total	8 mm min basal diameter, colony removed 3 cm from base
Croatia	Dredge banned in 1985	15 divers	Seasonal closure (Dec–March)	200 kg/year/diver, 3 tons total	No size restriction
France	No use of ROV (2012)	2011: 21 permits	No harvest above 50 m	50 kg/divers	8 mm min diameter
France, Corsica	1994	2006: 10 licenses	Voluntary min depth of 50 m	No limit	No size restriction
Greece	1994	Licensing introduced in 1987; 10 divers; no licenses granted in 2011	1994: Rotation of harvest over 5 areas, 50–110 m Harvest allowed for 5 years followed by a 20 year closure	No limit	No size restriction
Sardinia, Italy	Dredge banned in 1989	2012: 25 Regional permit issued annually; fines and confiscation of gear for harvest without permit and collection in prohibited areas	seasonal fishery (May-Nov); 7 MPAs	Max 2.5 kg/diver/day, 2 divers per boat, 13.5 tons total; Landings in 8 designated ports	2012: 10 mm minimal basal diameter (20 % tolerance)
	NO ROV, except for scientific observers		2008: All harvest must be below 80 m		
			7 protected areas		
Tuscany, Italy	Fishery closed July 2012-Dec 2013		2014: Harvest below 60 m		2014: 8 mm min diameter (5 % tolerance)
	No ROV allowed				
Malta	No exploitation allowed				
Monaco	*Corallium* habitat protected no exploitation allowed				
Montenegro	No data on regulations	Estimated 10 divers			
Morocco, Atlantic	Dredge Banned in the 1980s	10 boats/area, 3 divers/boat	2011: 40–80 m only from Larache to Cap Spartela	600 kg/boat, 6 tons total	No size limit
Morocco, Mediterranean	Dredge banned in the 1980s	Mediterranean: In 2006, Tofino (Al Hoceima) had a limit of 10 boats smaller than 50 t	Tofina area closed for 10 years in Aug. 2010	500 kg quota per boat; 6 tons total	No size limit

(continued)

Table 46.4 (continued)

Country	Gear types	Licenses	Closure	Quota	Size limit
Spain, external waters	Dredge banned 1983–1985; submarine allowed until 1997 off Isla de Alboran	47 authorized divers	5 areas: Catalonya, Illes Balears/Mallorca, Illes Balears/Menorca, Almería, South Atlantic Region from the border with Portugal to Punta Tarifa.	400 kg/fishermen/year, 10 % variance; quota of the submarine was 1,500 kg/year	7 mm diameter
Spain interior waters	Dredge banned 1983–1985	16 divers: Catalunya, 10; Balears, 6	4 MPAs, Cap de Creus, El Montgri, las Illes Medes, El Baix Ter	400 kg/diver	2012: 7 mm, 5 % tolerance
Tunisia	Dredge allowed below 100 m from 1982 to 1985; Banned in 1985	Divers licensed; estimated 30 divers	Closed areas off Bizerte, from Cap Blanc to Cap Zebit; fishing banned above 50 m at Cani Island	None	Catch weighed and reported
Turkey	Corallium harvest prohibited in 1990				

resource is at the limit of exploitation. The designated fishing grounds were fished intensively between 2009 and 2010, but most of the coral consists of long dead "fossilized" colonies and the proportion of live coral collected in the catch declined from 5 % to a low of 3 % over 2 years (Chen 2012).

46.2.4.4 Management of U.S. *Corallium* and *Gerardia* Fisheries

The Western Pacific Fishery Management Council's (WPFMC) Precious Corals Fisheries Management Plan (FMP) has regulated the harvest of precious corals since 1983 (NOAA 2002). The FMP imposes permit requirements valid for specific locations, harvest quotas for precious coral beds, a minimum size for pink coral, gear restrictions, area restrictions, and fishing seasons. The initial plan created four categories of coral beds: established beds, conditional beds, exploratory areas and refugia. At this time, a single bed, the Makapu'u Bed off Oahu Hawaii, was defined as an Established Bed for pink coral; four beds were established as Conditional Beds: Keahole Point, Kaena Point, Brooks Bank, and 180 Fathom Bank; and Westpac Bed was established as a refugium where coral harvest was not allowed (NOAA 2002; Grigg 2010). Specific quotas and size limits were established for precious coral beds in Hawaii based on the size of these beds, the standing stock and estimates of maximum sustainable yields and optimum yields. For Makapu'u bed, a 2-year harvest quota using selective gear was set at 2,000 kg for *C secundum*, 600 kg for *Gerardia* sp. and 500 kg for *L. olapa* and a minimum size (10 in. height) was set for *C. secundum*. The quotas for the conditional beds were directly related to their size, assuming the same optimal yield as Makapu'u. Because these beds are substantially smaller, the quotas for pink coral ranged from 67 to 444 kg and gold and bamboo corals were a fraction of these amounts (NOAA 2002). Because of the absence of data on the size of the bed and the population structure of corals within the EEZ exploratory areas, quotas of 1,000 kg were established for all species of precious coral combined, with the intent of modifying these based on future harvest records and research. Exploratory areas open to commercial coral harvesting at this time included the Main Hawaiian Islands, American Samoa, Guam, Rota, Tinian, and Saipan, while coral harvest was prohibited within the Northwest Hawaiian Islands (NWHI) National Monument.

The Fishery Management Plan has been amended eight times since its inception. These amendments reclassified eight of the exploratory areas as a single large area, defined overfishing of precious corals, established a framework to adjust management measures if necessary in the future, and designated the only established bed, Makapu'u, as a Habitat Area of Particular Concern because of its ecological function, rarity of the habitat and sensitivity to human perturbations. In late 2000, a Coral Reef Ecosystem Reserve was established in the Northwestern Hawaiian Islands (NWHI), protecting an estimated two thirds of the potential deep water habitat for precious corals from exploration and harvest (Grigg 2002). This measure placed a permanent zero-harvest cap on harvestable NWHI beds, including Brooks Banks and 180 Fathom Banks. Subsequent amendments to the fishery have included bans on the use of non-selective gear. In 2008, a 5 year moratorium was placed on the harvest of gold coral, which was extended indefinitely in 2014. Although earlier measures included new Experimental Fishing Permits to stimulate exploration, the *Corallium* fishery had only a brief revival between 1999 and 2001, and has remained dormant since 2002 (Grigg 2010).

46.2.4.5 Management of Black Coral Fisheries

Black coral beds in Hawaii are found in both Federal and state waters, with most of the fishing effort located in state waters, within 3 miles of islands. Similar to other precious coral fisheries in the U.S., regulations include specific provisions for harvest within designated known beds of precious corals, maximum sustainable yield (MSY), size restrictions and gear restrictions. State management involves a system of licensing and reporting requirements, as well as maximum sustainable yields, and minimum size limits. Black coral beds in Federal waters are classified as established beds, conditional beds, refugia beds and exploratory permit areas (Grigg 1994). The Makapu'u Bed and Au'Au channel bed are currently the only established beds. The established beds can only be harvested using selective gear. The state first established a minimum size requirement for black corals in 1998, limiting harvest to specimens with a minimum base diameter of 1.91 cm (0.75 in.). A minimum size of 122 cm (48 in.) height or 2.54 cm (1 in.) diameter became effective in Federal waters on April 17, 2002, but a grandfathering scheme was introduced, allowing veteran divers to harvest smaller size classes (Bruckner et al. 2008). This exemption was removed in 2007; current fishing regulations prohibit harvesting of colonies <90 cm in height in state waters, and colonies <120 cm in height in Federal waters.

Since inception of the Hawaii fishery, most of the harvest has occurred within the two major commercial beds, one situated off Maui (Au'au Channel) and the other off Kauai (Makawaena Point) (Grigg 1993). Black coral is selectively harvested by divers using SCUBA, which limits harvest to depths above 246 ft (Kahng 2006). Because there are significant amounts of black coral that are below the limit using standard SCUBA equipment, these colonies were thought to serve as a refuge for shallower populations. However, surveys from 2001 to 2004 on reefs below 70 m showed that over 50 % of the colonies were overgrown by a species of invasive coral, *Carijoa riisei* (Grigg 2003, 2004; Kahng 2006).

Biological surveys conducted between 2002 and 2004 illustrate a downward shift in the age structure of colonies as well as a reduction in biomass of about 25 %, and a decline in both recruitment and abundance of legal-sized black coral colonies (Grigg 2004). This was compounded by continued spread of the invasive coral (Kahng and Grigg 2005) and a large increase (25–50 %) in demand for black coral since 1998, leading to increased harvest pressure. Based on recommendations of a downward adjustment of MSY, state and federal authorities recently amended quotas for harvest within Au'au channel. The quota, previously set at 5,000 kg annually in Federal waters, was reduced to 5,000 kg biannually for both state and Federal waters (Bruckner et al. 2008).

While this fishery represents the first precious coral fishery that was managed using biological attributes of the coral along with detailed population surveys (Grigg 2001), it still requires further modification. In order to ensure that harvesting is sustainable and does not significantly limit recruitment, optimal harvest yields must be determined using measures of abundance, growth, natural mortality and recruitment, and not necessarily maximum profit. A minimum allowable size of harvest should provide sufficient time between age (size) at first reproduction and age (size) at first capture. The age at maximum yield per recruit for the dominant species in the Hawaiian black coral fishery (*A. dichotoma)* was estimated to be 22–40 years, corresponding to corals that measure 1.7 and 3.2 m in height. This is much less than the maximum size of these corals but much more than the minimum allowable size of harvest. The allowable minimum size has been developed based on presumed optimal yield whereby profit is maximized at disproportionately less effort (Tsounis et al. 2010).

Size limits have been applied to black corals in other countries, but in absence of other controls they appear to have been ineffective. For instance, Cuba established a minimum size of 1.2 m height and 2.5 cm diameter for *Antipathes caribbeana* in the 1990s. Nevertheless, black coral populations have been depleted along the Pinar del Rio Province, in Matanzas Bay (northeast Cuba), Puerto de Sagua (north-central Cuba) and Cazones Gulf (Alcolado et al. 2003).

46.3 International Regulations

Since the 1980s, several proposals have been submitted to include precious corals in Appendix II of the Convention on the International Trade in Endangered Species of Flora and Fauna (CITES). CITES, an agreement between 175 governments, was created in 1975 to ensure that international trade of wild animals does not threaten their survival. CITES actively controls trade in Appendix II listed species by requiring export permits from the country of origin for listed species. These permits are supposed to be issued if there is a finding that the harvest of a listed species does not affect the survival of that species in the wild or its role in the ecosystem. The determination of allowable harvest is supposed to be based on the biology of the species and status of the stocks. Stony corals, including Coenothecalia (blue corals), Tubiporidae (organ-pipe corals), Stylasteridae (lace coral) and Scleractinia (stony coral) have been listed for several decades mainly because of concerns of the curio trade (and more recently the aquarium trade).

Black corals were the first precious corals to be added to Appendix II of CITES, following a proposal at COP III (1981) by the United Kingdom on behalf of the Virgin Islands. Black coral began to be heavily collected in tropical countries in the 1970s for tourist souvenirs, and the Virgin Islands were concerned that Caribbean populations were

being overexploited (Anon 1979b). At the time of listing, available data did not fulfill the criteria required to list a species today, mainly due to a lack of biological and population data, however concern about depletion of Caribbean stocks led to the international support for its listing (Wells and Bardzo 1991).

Following successful listings of stony corals and black corals, Spain proposed to include *Corallium rubrum* on Appendix II of CITES in 1986. The proposal was based on the conclusions that known coral beds in Spanish waters were overexploited – control of illegal collecting was necessary, and the use of non-selective dredges was causing considerable habitat damage. Spain also noted that there was uncontrollable poaching of *Corallium* by Sicilian fleets at Alboran. The proposal was overturned because of a suggested lack of data that demonstrated that these species were threatened due to unsustainable trade. After the proposal was rejected, GFCM convened three Technical Consultations on Red Coral (Spain 1983, Italy 1988 and Algeria 1989). These consultations were intended to provide helpful guidelines for a more effective management of the resource, but only a handful of the suggested measures were adopted (Table 46.5).

As new information on declines in yield, mounting evidence of overexploitation of coral beds, and additional supporting scientific information on the vulnerability of *Corallium* populations has accumulated, international conservation organizations began evaluating the need for new measures to protect these species. Since precious coral fisheries are largely driven by international demand, the U.S. proposed the genus *Corallium* for listing in Appendix II of the Convention on the Trade in Endangered Species (CITES) in 2007 (CITES-USA 2007). The 2007 proposal was adopted in Committee One and then subsequently overturned through a secret ballet during deliberations in Plenary on the final day. The main justification against a listing centered on concerns regarding the implementation of the listing (Table 46.5; Morell 2007). FAO, in a consulting role to CITES, reviewed the proposal and concluded that there were uncertainties whether the criterion of biomass decline was met for all species and noted that a deficiency of data was identified for several species (FAO 2007). Two ad hoc international workshops were held to clarify these issues and discuss whether a CITES listing could be effectively implemented (Bruckner and Roberts 2009; Bussoletti et al. 2010). The United States and Sweden submitted an updated proposal at the Conference of Parties 15 in 2010 (CITES-Sweden-USA 2010). While this clarified many of the CITES implementation issues and substantial new information was included on the extent of depletion of these species, the listing was not adopted.

The FAO review once again found that the data do not meet the decline criteria, highlighted some of the implementation concerns surrounding a CITES listing, and suggested revised local and regional management as a more effective mechanism to prevent unsustainable harvesting (FAO 2010). Furthermore, consensus from the second *ad hoc* workshop in Italy was that the FAO GFCM (General Fisheries Commission of the Mediterranean) was the appropriate organization to effectively manage *Corallium rubrum* (Bussoletti et al. 2010).

One of the main arguments against the CITES listing proposal is that declines in landings may not necessarily reflect biomass trends, unless effort is considered. A decrease in

Table 46.5 Implementation challenges associated with a CITES Appendix II listing for Corallidae

Issue	Concern	Solution and rationale
Identification of species in trade	Worked specimens (jewelry, carvings) of *Corallium* cannot be distinguished to species	Report only to family Corallidae. Some taxa of other listed corals are recorded on CITES documents to Genus
	Challenges in identifying raw coral to species	An identification manual for *Corallium* in trade was recently developed (Cooper et al. 2011)
Stockpiles of raw and semi-worked pieces of *Corallium*	Pre-existing corals that were collected prior to the listing could not be exported without permits	Delayed implementation would allow invoicing of stockpiles. Trade could occur under a CITES-preconvention certificate as done with other listed species
Legal acquisition findings	The high value of precious corals would encourage illegal harvest and trade	This is already a large problem; a listing would provide a mechanism for cooperation to eliminate poaching
Paperwork to track trade	Significant paperwork requirements and costs associated with international trade in listed species	CITES allows exemptions for personal use which would eliminate paperwork by tourists travelling abroad and can limit permitting requirements to commercial trade
Making non detriment findings	Countries lack the capacity and data to make a non-detriment finding, as required to export the coral	The listing would promote more research to determine the status and sustainable levels of harvest. Improvements to national and regional management plans could help ensure sustainable harvest

fishing intensity due to market forces that render fishing economically infeasible may result in declines in landings not necessarily due to a decline in the abundance of the resource. An overabundance of coral, and a glut of poor quality coral, drove prices to such low levels twice in the history of *Corallium* fisheries: (1) the discovery and excessive harvesting of poor quality fossilized Sciacca coral in the 1880s, and (2) the overharvesting of low quality Midway corals in 1982 (Tsounis et al. 2010). The declines in yield of Midway corals in the later years of the fishery as well as the declines in the Mediterranean landings prior to elimination of the dredge in the early 1980s reflect biomass declines – in both cases prices increased.

While countries have not adopted international trade restrictions through CITES for *Corallium* as of 2015, China listed four species of Coralliidae on Appendix-III of CITES *(P. japonicum, C. elatius, C. konojoi, C. secundum)* in 2008 due to concerns of illegal harvest and trade. This listing places a requirement on countries to identify worked and raw *Corallium* in trade (Cooper et al. 2011), thus they already have to deal with some of the implementation issues identified as a hindrance to the precious coral industry. Proponents of a CITES listing recognize that trade restrictions are not a substitute for local management, but existing efforts to manage these resources have failed to protect the resource largely because the necessary scientific information is unavailable. Because countries are required to assess the population status and the fisheries prior to the export of raw *Corallium* and *Corallium* products, a listing would stimulate the research and management necessary to make a non-detriment finding (Bruckner 2009). Furthermore, poaching has become one of the main problems plaguing the industry. A CITES listing may lead to more severe penalties for illegal fishing and export, thereby enhancing local management.

46.4 A Paradigm Shift for Precious Coral Fisheries

Management of precious coral fisheries has been hampered by complications associated with enforcement and jurisdiction, illegal and unregulated fisheries, and organized poaching that authorities are unable to control. Challenges are also due to the multinational character of the fishery and the presence of precious coral beds outside territorial limits in international waters. Because of the general nature of precious coral fisheries, fisheries are likely to continue to face growing problems as stocks are driven to commercial extinction. In all regions, throughout history of these fisheries, landings have shown large peaks shortly after the discovery of a coral bed followed by rapid and precipitous declines in yield within a few years, with continued expansion into new areas as known beds become overexploited (Tsounis et al. 2013; Bruckner 2014). Throughout the Mediterranean, large shifts in population structure are documented within fished areas, and all shallow *C. rubrum* beds (above 60 m) are now considered overexploited (Bussoletti et al. 2010). These areas have shown reductions in colony size from historic averages of 30–50 cm height (10–30 mm diameter) to populations dominated by small colonies (3–5 cm height and 5–7 mm diameter) (Garrabou and Harmelin 2002; Tsounis et al. 2006b, 2007). Defined areas where fishing is allowed is now established in both Japan and Taiwan, but landings from both regions consist predominantly of dead colonies (Kosuge 2010; Chen 2012). Even in Hawaii, which supports the only precious coral fishery in the world with defined quotas and size limits derived from models of maximum sustainable yield determined using information on the biology of the target species and detailed assessments of population size and structure, resources are in decline (Grigg 2001, 2003, 2004; WPFMC 2007). Over a decade ago, scientists observed a 25 % decline in biomass and shifts in the size structure of harvested black coral populations, which was attributed to a combination of fishing pressure and increased mortality associated with an invasive species (Grigg 2004, 2010). The precarious state of these precious coral fisheries suggests that existing management measures have not been adequate to conserve precious coral populations (Bruckner 2014).

46.4.1 Gear Types

The use of non-selective dredges in Mediterranean coral fisheries was first banned in Albania in 1977, in Sardinia and Spain in 1986, and finally Mediterranean-wide in 1994. In Hawaii and some areas of Japan, non-selective gear is also prohibited, with harvest being undertaken by SCUBA divers (Hawaii) or submersibles (Japan). The elimination of the tangle net dredge was a major step forward for conservation, as this gear type caused considerable damage to the habitat and it was non-selective and wasteful, with much of the detached coral remaining on the bottom. Nevertheless, it is still used in Taiwan and parts of Japan, mainly because the coral beds are too deep for harvest with SCUBA, currents are too strong for use of a submersible, and more advanced technologies are cost prohibitive (Kosuge 2010; Grigg 2010). Conventional and mixed gas SCUBA diving is one of the best options to ensure selective harvest, but there are potential problems with SCUBA fisheries (see below). Furthermore, SCUBA fisheries are only possible to depths of about 130 m. Submersibles have been used in Hawaii and in the Mediterranean, but they are currently used only in a single fishery in Japan, mainly due to the high cost of operations. Robotic harvesting using remote operated vehicles (ROVs) has been proposed in the Mediterranean, but these have the potential to cause habitat damage and they are likely

to be less efficient than harvest on SCUBA. Furthermore, SCUBA divers are limited by time and maximum depth. The use of an ROV provides access to corals without depth restrictions, allowing harvest of deeper resources that currently serve as a refuge. If adopted, ROVs have the capability to exploit over 99 % of the world's *C. rubrum* populations, potentially harvesting the last remaining viable stocks (Tsounis et al. 2013).

46.4.2 Size Limits

One of the key strategies to help maintain precious coral populations is a minimum size of harvest. This approach has been applied to *Corallium* and black coral fisheries in Hawaii and to *C. rubrum* fisheries in certain parts of the Mediterranean, but this measure is only possible when the corals are selectively harvested. SCUBA fishing has the advantage of allowing a more selective harvest of only the largest colonies, leaving smaller colonies in place. There are reports, however, that divers operated in a pulse fishing mode, removing all colonies above a certain threshold size from individual patches, and returning to harvest smaller size classes once the larger colonies were depleted (Barletta et al. 1968; Santangelo and Abbiati 2001). To maintain similar levels of catch thereby necessitated collection of increasing numbers of colonies of a smaller size which has resulted in the progressive harvest of stocks down to very young age classes throughout depths accessible to SCUBA (Tsounis et al. 2010). Unless the sizes of individual colonies that are removed are verified, the removal of undersized specimens is unlikely to be noticed by management agencies since landings data are typically recorded by weight and not size.

Protection of precious corals from harvest until they reach a large size is likely the most important measures for conservation of coral resources, as fertility and reproductive success increases exponentially as colonies get larger and develop more branches (Bruckner 2009). Recognizing this life-history attribute, FAO first recommended a minimum basal diameter of 7 mm for red coral in 1983 (GFCM 1984), but this was not adopted through the region. As of 2013, only three countries (Spain, Italy and Algeria) have included a minimum size in their management plan. In an early demographic study of *C. rubrum*, García-Rodríguez and Massò (1986) documented the harvest of colonies that were 5–14 years in age which they concluded were well below MSY. Tsounis et al. (2007) estimated a MSY of 98 years, whereas the current practice of harvesting colonies once they achieve a 7 mm basal diameter (11 year old colonies) results in only 6 % of the potential yield. Based on recent age studies, *Corallium* colonies are two to four times older and growth rates are 2.6–4.5 times lower than previously thought (Marschal et al. 2004; Roark et al. 2006). For a modular organism like *C. rubrum* that characteristically forms highly complex, branched colonies, and can reach sizes of 30–50 cm, a mean height of 3–5 cm is equivalent to a loss of 80–90 % of the reproductive modules (polyps) of individual colonies, due to the absence of second, third and fourth order branches (Bruckner 2009). These small colonies can become sexually mature at a young age (2–3 cm height), although they don't achieve 100 % fertility until about 6 cm height and 10–20 years in age or older. The spawning potential in *C. rubrum* (and other gorgonians) increases exponentially with size, with larger arborescent colonies producing up to 90 % of the recruits. Given settlement rates of no more than 5 % of the total larval production, and continued removal of colonies by fisheries after they have reproduced no more than one or two times, typical shallow population today may produce 80–90 % fewer recruits than in the 1960s, and about half of that produced by populations that have been protected from fishing for 15–20 years and contain colonies twice as large.

Recognizing that colonies of *C. rubrum* achieve full sexual maturity only after approximately 10 years, but at least 20 years or more is needed to reach a size able to ensure a higher reproductive potential, scientists at recent international workshops have recommended that the proposed minimum size of 7 mm is not adequate and it should be increased to 10 mm (Bussoletti et al. 2010). To date, a 10 mm diameter has been implemented only in Sardinia, but collectors are allowed a 20 % variance (in effect, pushing the minimum size back to 8 mm), and in fact fisheries landings data show that a large proportion of the colonies (>50 %) are undersized (Chessa and Scardi 2010). Furthermore, contrary to scientific consensus, and recommendations at two GFCM workshops (GFCM 2010; 2013) the draft 2014 management plan developed by GFCM is recommending adoption of only the 7 mm minimum diameter (GFCM 2013), and the agreement to increase in the minimum size to 8 mm or 10 mm remains largely ignored (Bussoletti et al. 2010; Tsounis et al. 2013).

46.4.3 Biology, Life History and Population Status

For most precious coral fisheries around the world, exploitation of the resource has been undertaken without preexisting knowledge of the size or condition of a precious coral bed. One of the most critical steps in the development of a sustainable fishery is the completion of detailed baseline studies on population dynamics before a new area is open to fishing. Conservative estimates of landings from these areas should be adopted based on the size of the precious coral bed, and the biomass (abundance and size structure) of the resource, using a similar approach to that applied to food fisheries. Collection areas must be monitored to assess collection impacts and to modify measures as needed to prevent undesirable shifts in species assemblages. Furthermore, better

reporting and monitoring of landings is necessary, including data on locations of harvest and numbers and sizes of colonies removed, and the condition of these colonies.

Using only colony abundance and density as an indication of population size and viability can be misleading, as the only precious coral known to occur in dense populations is *C. rubrum* colonies found in shallow water, and these tend to be dominated by small, reproductively immature colonies. For colonial organisms, change in population structure (size frequency distribution) is a more suitable measure of decline than changes in the absolute numbers of colonies (Bruckner 2009). Commercial extraction primarily eliminates the largest corals, followed by smaller colonies over time, but it is the largest, oldest colonies that contribute most to the replenishment of the population (Tsounis et al. 2006a). Furthermore, shifts in the size structure of populations due to fishing pressure can be directly compared, while density and abundance cannot. Even in the case of *C. rubrum*, colony density measured over the entire suitable habitat is likely to be much less than the density of small patches occupied by the coral within this habitat. In fact, a less-dense population is likely to represent an older, more stable and viable population as open substrates of suitable habitat can support high numbers of recruits, but these exhibit size-related survival that increases as the colonies get larger. Thus, populations with a high abundance and density, such as *C. rubrum* found in shallow water, are an indication of frequent and continuing perturbations responsible for rapid turnover of populations and a persistent state of early-stage recovery. This is similar to observations of other corals that brood their larvae; however, most corals that are brooders are considered early colonizing, "weedy" species, while *C. rubrum* is a long-lived species that may be attempting to adapt to increasing localized (direct human impacts) and global stressors (climate change). These types of populations are much less resilient to other stressors and are more likely to exhibit localized extirpations when compounded by fishing pressure than populations that contain a mix of small (10–50 mm tall), medium (60–140 mm), and large (150–500 mm) colonies, like that formerly observed in the Mediterranean and still present in some deep-water areas that have not been targeted by fisheries for several decades.

46.4.4 Protected Areas

Marine protected areas provide an efficient tool to maintain and manage fisheries and enhance biodiversity conservation. The success of an area closed to fishing depends on the size of the protected area, linkages with surrounding areas, and the duration of closure, with the life history of a species being a key determinant of these variables (Halpern 2003). Recovery of a target species within a MPA is also affected by the degree of exploitation and status of the species before the creation of the marine reserve. In the case of a long-lived, slow growing precious coral with limited capacity for dispersal, recovery of populations is likely to be slow, as coral harvest principally threatens the largest colonies.

Currently, less than 1 % of the coralligenous habitat in the Mediterranean is protected from harvest of red coral. The existing no take areas (protected areas) for *C. rubrum* are relatively small and it is unclear whether they are sited such that they would replenish fished areas, given the high degree of genetic structure and evidence that populations are self-seeding (Costantini et al. 2007). Within the protected areas colonies are substantially larger than in surrounding fished areas, yet still smaller than that observed at similar depths in the 1960s even after 22–30 years of protection (Garribou and Harmelin 2002; Linares et al. 2010). These observations illustrate the importance of MPAs, but they also emphasize the fact that full recovery from fishing can require many decades (Marschal et al. 2004; Torrents 2007).

An alternate approach to rebuild precious coral populations after intensive fishing involves a rotating harvest (Caddy 1993), which has been attempted and is currently used in parts of the Mediterranean and North Pacific (Table 46.4; Chen 2012; Iwasaki et al. 2012). One problem associated with this idea is the long recovery times due to slow growth rates and low recruitment success of these corals. If a minimum harvesting diameter of 7 mm for *C. rubrum* is considered, this would imply a recovery time of at least 30 or 40 years (Tsounis et al. 2006a, b). Time needed to reestablish populations to pre-harvest levels is probably considerably longer, largely because coral growth rates have been found to be much slower than previously estimated and some sites in the Mediterranean have not achieved the sizes observed in the 1960s, even after 30 years of protection (Caddy 1993; Francour et al. 2001; Tsounis et al. 2006a). The time for recovery may be further compounded in sites with non-selective fisheries, as tangle-net dredges remove all corals from an area. Unless adjacent upstream areas are left unfished, their recovery may be hampered because of lack of local sources of larvae. Thus, a rotating harvesting model would have to deal with long periods of closure for each area and also maintenance of neighboring unfished areas. This would restrict harvest into smaller defined areas, which is unlikely to sustain a profitable fishery as is becoming apparent in existing defined fishing grounds off Taiwan (Huang and Ou 2010).

One of the most important accomplishments in the conservation of Mediterranean red coral, was a recommended ban on shallow water fishing that was adopted in response to a proposed CITES listing. The consensus at one of the ad hoc workshops was the protection of corals shallower than 80 m depth to allow these populations to recover from past fishing impacts (Bussoletti et al. 2010). Unfortunately, this

recommendation was ignored and the recent Mediterranean GFMC draft management recommends full protection for shallow resources, allowing harvest only below 60 m (GFCM 2013). In addition, proposals to limit collection to 60–130 m (mixed gas diving) appear to be short-sighted at this time because we know too little about these populations. This move to deeper water may be a reflection of the depleted nature of the resource – all acceptable (large) colonies have been removed from shallow water and collectors are now targeting areas that have not been fished for 20 years or more (e.g. since elimination of the dredge). First, genetic studies from the Mediterranean also suggest local recruitment, very limited dispersal of gametes and limited connectivity between deep populations (Costantini et al. 2007), which is where the bulk of the fisheries are now located. Secondly, the available information from these deeper areas indicates that populations occur at a lower density than in shallow water. Removal of colonies from these areas would further reduce density, possibly hampering potential for successful fertilization due to allee effects. Recent studies from Sardinia suggest deep water populations (60–150 m) are in better shape than shallow areas (Cannas et al. 2010), based on the indication that populations are dominated by larger sized corals. Nevertheless, this study acknowledges that colonies are much sparser and the data show that more than one third of all colonies examined in transects were still <5 cm and only 23 % are above 10 cm height. Even though there are larger colonies in deep water off Sardinia, it is unclear whether these are abundant enough to support a sustainable fishery, as biometrical analyses done by Chessa and Scardi (2010) showed that a very large percentage of the corals harvested by fishermen in deep water off Sardinia were well under the minimum allowable size of 10 mm (basal diameter).

46.5 Conclusion

Effective conservation of precious corals, with the intention of rebuilding of overexploited stocks and sustaining continued fisheries, will require stronger management measures, better enforcement and possible supplementary protection through trade restrictions in CITES. From a habitat protection standpoint, non-selective gear such as the tangle net dredges need to be completely eliminated from the fishery. From a biological perspective, the minimum size of allowable harvest must be further increased so that colonies can develop complex branching patterns to ensure a reproductive output that can compensate for fishing pressure. Rotation of fishing grounds is unlikely to be economically sustainable or effective as recovery of fished areas would require many decades due to the slow growth of precious corals and their limited recruitment. As an alternative to rotational harvest, the size and locations of marine protected areas that are completely closed to fishing need to be expanded, especially in shallow water where other stressors (e.g. climate change) are having compounding impacts. New approaches for the precious coral management should be based on current knowledge of the biology of the species and models considering maximum sustainable yield (MSY) must incorporate information on the size of the targeted coral bed and the abundance, density and size of the colonies within the bed, with fishing occurring only after the resource has been properly assessed. Further, these plans need to be adaptive, responding to changes associated with fishing and other natural and anthropogenic stressors.

While trade restrictions alone may be insufficient to fully protect these resources, they can complement local management by promoting stronger local measures, necessary research and data acquisition. China took an ambitious step to list four Pacific species of *Corallium* on Appendix III in 2008, but the protective measures need to extend to other countries and all species. Through a CITES Appendix II listing, commercial trade would still be allowed, provided that exporting countries demonstrate the established level of harvest and trade is not detrimental to the survival of the species. Thus a listing would give both exporters and consumers joint responsibility for management and enforcement, and it would require annual monitoring and reporting of fisheries and trade. A listing would also promote research on the status and trends of listed precious coral populations, the impacts of fisheries, and the development of sustainable harvest guidelines. A CITES listing could also reduce illegal trade and fishing, and promulgate stronger local and regional management and enforcement.

Precious coral fisheries are at a crossroads. The global rate of depletion of these resources and continued damage to habitats has made these long lived species particularly vulnerable to extirpation, seriously hampering their potential for recovery. Action must be taken now, if these fisheries are to survive into the future. It is possible to sustainably harvest precious coral resources, provided that management agencies completely eliminate the use of non-selective gear, new harvest guidelines are developed that fully consider the life history attributes of these species, and coral beds are not fished until a thorough assessment is undertaken to characterize the size of the bed, the abundance of the resource and the population structure of the corals. Areas that are off limits to fishing must be established, and these must be of adequate size and be connected to fished populations so they can serve as a refugia and seed stock to replenish fished areas. Furthermore, enforcement to eliminate poaching is critical. As stronger management measures are developed, considerations must be given to the local communities and fishers dependent on these resources. Through an expanded education program and involvement of the precious coral fishers and local communities in decision-making, it may be possible to halt the decline of these precious resources and work towards sustainable fisheries.

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Conservation and Restoration of Coral Reefs Under Climate Change: Strategies and Practice

47

Suchana Chavanich and Voranop Viyakarn

Abstract

Coral reefs can be found in both tropical and subtropical oceans. They provide valuable and vital ecosystem services. Coral reefs also serve as habitat, shelter, and food sources for variety of organisms. Yet some estimated put the total diversity of life found in and on coral reefs at up to two million species. This biodiversity can be transformed directly into food security, income, and other benefits to people. Unfortunately, at present, coral reefs have been declined due to anthropogenic activities, natural phenomena, and poor management practices. More than 75 % of coral reefs around the world are already threaten by a combination of local and global stressors, and scientists have predicted that by 2050, more than 75 % of world reefs will be in critical threat level if the stressors are not lower. This chapter will provide insights on the current strategies and practices of coral reefs conservation and restoration under the climate change. The need for a strategic approach to conservation and restoration to maximize the effectiveness of conservation and restoration efforts has become an urgent issue, particular under the climate change. To conserve coral reefs, multiple stressors threaten reefs need to be identified and reduced. In addition, a combination of reef restoration techniques and effective management should be implemented to ensure the protection of coral reef ecosystems.

Keywords

Coral reef • Asexual propagation • Sexual propagation • Management • Conservation

47.1 Introduction

Coral reefs can be found in both tropical and subtropical oceans. These reefs provide valuable and vital ecosystem services. Coral reefs also serve as habitat, shelter, and food sources for various of organisms (Sheppard et al. 2009). Yet some estimated put the total diversity of life found in and on coral reefs at up to two million species or more (Norse 1993). This biodiversity can be directly transformed into food security, income, and other benefits to humans (Wilkinson 2008; Burke et al. 2011). Some reef organisms represent important sources for medical treatments and cosmetics (Rocha et al. 2011). These organisms produce chemical compounds that can be utilized as sources for new medicines to treat cancers, human bacterial infections, virus infection, heart disease, arthritis, and other diseases (Amador et al. 2003; Jain et al. 2008; Fenical et al. 2009; Rocha et al. 2011) (Figs. 47.1, 47.2, and 47.3).

Unfortunately, at present, coral reefs have been declined due to anthropogenic activities, natural phenomena, and poor management practices (Norse 1993; Cote and Reynolds 2006; Burke et al. 2011). Many factors including coastal development, overfishing, destructive fishing, marine pollution, sedimentation, unsustainable tourism, introduction of non-indigenous species, climate change, ocean acidification, and other natural phenomena such as tsunami are responsible for current reef degradation (Norse 1993; Brown 1987;

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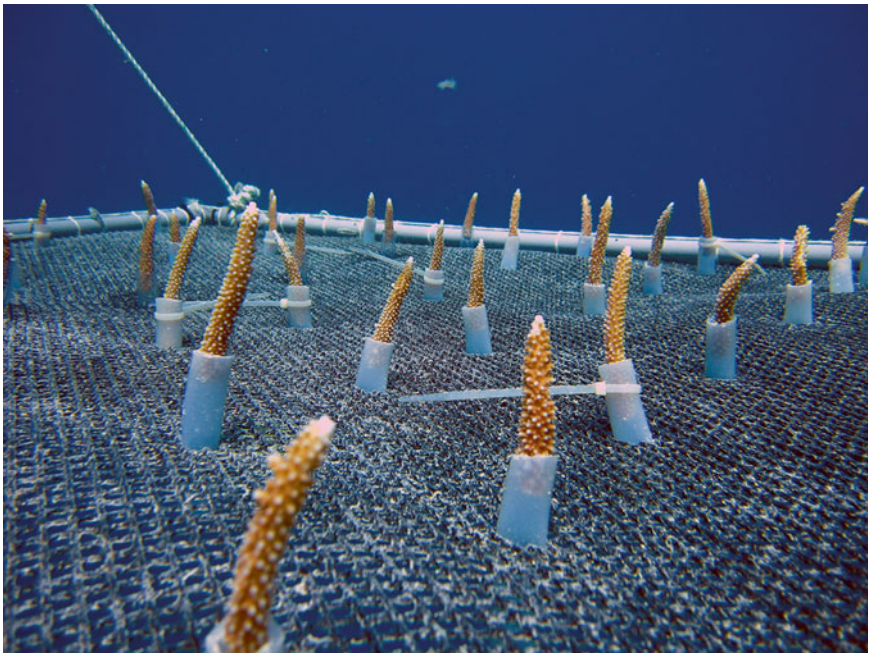

Fig. 47.1 Asexual propagation: coral fragments

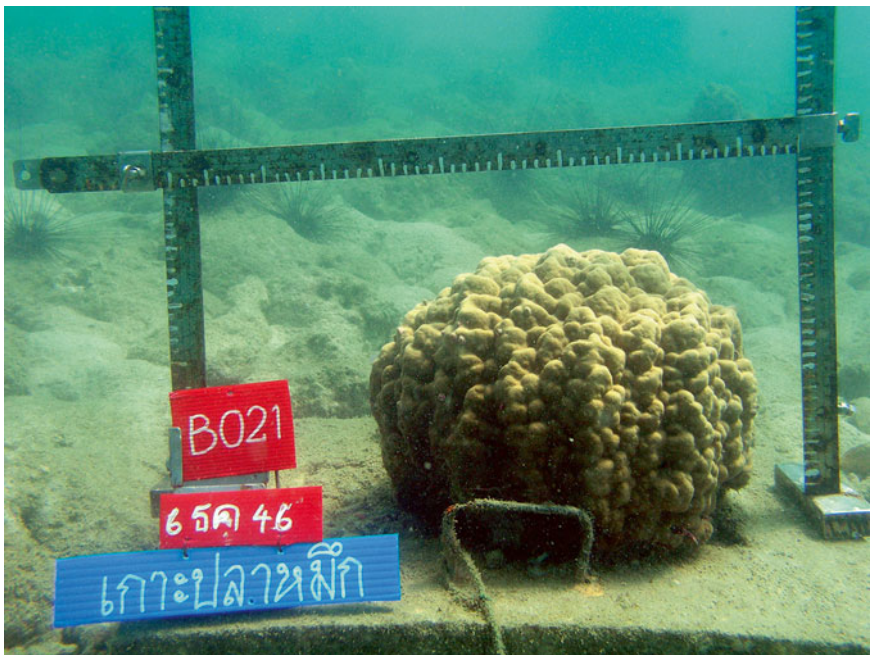

Fig. 47.2 Transplantation of whole coral colony

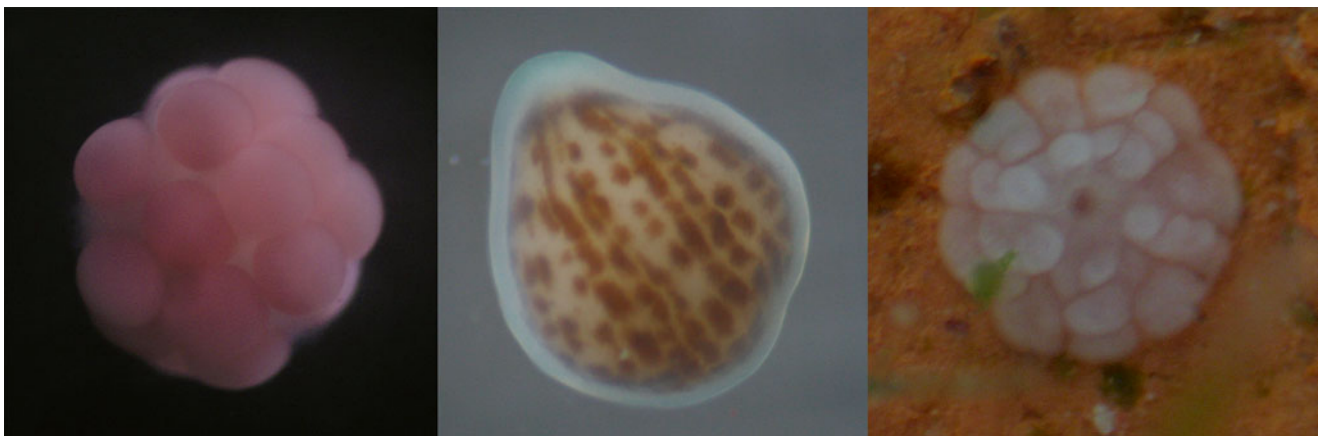

Fig. 47.3 Sexual propagation technique from egg bundle to juvenile coral

Bellwood et al. 2004; Chavanich et al. 2005, 2009, 2010; Cote and Reynolds 2006; Hoegh-Guldberg et al. 2007; Wilkinson 2008; Burke et al. 2011). More than 75 % of coral reefs worldwide are already threaten by a combination of local and global stressors, and scientists have predicted that by 2050, approximately 75 % of world's reefs will be in critical threat level if stressors are not reduced (Burke et al. 2011). Burke et al. (2011) reported that of the six coral reef regions (Atlantic, Australia, Indian Ocean, Middle East, Pacific, and Southeast Asia), local pressure on coral reefs is highest in Southeast Asia where 95 % of reefs are threatened. Threats and stress to coral reefs will result in varying degrees of damage to specific coral reefs. For example, pollution is a great threat in reef areas in close proximity to human populations whereas global climate change poses a major threat in remote reefs.

Over the past years, the major emerging threat to coral reefs worldwide has been coral bleaching and mortality associated with global climate change. The effects from coral bleaching are increasing in frequency and intensity, and becoming global in scale (Marshall and Schuttenberg 2006; Burke et al. 2011). The first global bleaching event was occurred in 1998, followed by 2010, and 2015 is the latest third event (Hoegh-Guldberg 1999; Eakin et al. 2009; Chavanich et al. 2012; Phongsuwan and Chansang 2012; Alemu and Clement 2014; www.noaa.gov).

Pressures on reefs will continue to increase as human populations, economies, and synergies between stressors increase (Birkeland 2004; Burke et al. 2011). This chapter will provide insight on the current strategies and practices of coral reef conservation and restoration under the climate change. The need for a strategic approach to conservation and restoration to maximize the effectiveness of conservation and restoration efforts has become an urgent issue in particular under the climate change. To conserve coral reefs, multiple stressors threaten reefs need to be identified and reduced. In addition, a combination of reef restoration techniques and effective management should be implemented to ensure the protection of coral reef ecosystems.

47.2 Coral Reef Restoration

In recognition of the consequences of coral degradation, many nations have recently made major commitments to reduce the anthropogenic factors that affect reefs and to improve coral reef conditions. Of these, coral restoration and rehabilitation have attracted attention, and their importance has been recognized as one of the management tools. Many reefs are severely affected by multiple local stressors, and can hardly be recovered naturally through coral larval recruitment. Thus, restoration and rehabilitation can help to regenerate coral communities where natural recovery is limited. Even though current technologies cannot build a functioning coral reef in a few years, many useful lessons have been provided to set a guideline for restoration projects.

Based on Edwards and Gomez (2007), Edwards (2010), and Society for Ecological Restoration Science and Policy Working Group (2002), definitions of coral restoration and related terminologies can be defined below:

Restoration
: The act of bring a degraded ecosystems back into, as nearly as possible, its original condition.

Rehabilitation
: The act of partially or fully replacing structural or functional characteristics of an ecosystem that have been diminished or lost, or the substitution of alternative qualities or characteristics of those originally present with the proviso that they have more social, economic or ecological value than existed in the disturbed or degraded state.

Remediation
: The act or process of remedying or repairing damage to an ecosystem.

Mitigation
: The reduction or control of the adverse environmental effects of a project, including restitution for any damage to the environment through replacement, restoration, or creation of habitat in one area to compensate for loss in another.

Ecological restoration
: The process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed.

Recruitment
: The successful establishment of viable individuals into a population or a habitat.

Techniques for reef restoration and rehabilitation include both physical and biological restorations (Edwards and Gomez 2007). Physical restoration is defined as the repair of reef environment from an engineering approach as opposed to the restoration of the biological process (Edwards and Gomez 2007). The goal is to mimic nature as closely as possible. Physical restoration includes artificial reef creation, which is a common and popular technique for reef aggregation. Several artificial structures including rock, concrete (e.g. ReefBallls™), ceramic (e.g. EcoReefs™), and carbon steel structure (e.g. BioRock™) have been used in physical restoration (Edwards and Gomez 2007; Goreau et al. 2004; Chavanich et al. 2014).

An advanced reef restoration, carbon steel structure and mineral accretion, has recently been introduced to several countries (Schumacher et al. 2000; Goreau et al. 2004; Chavanich et al. 2014). This technique applies low voltage electric energy to a structure to form a carbonate substrate (Goreau et al. 2004; www.biorock.net). Several studies have reported that the mineral accretion technology induced set-

tlement of marine organisms including corals and other sessile reef organisms, and can enhance the survival and growth of corals on the carbonate substrate (Schuhmacher et al. 2000; Goreau et al. 2004; Borell et al. 2010; Chavanich et al. 2013). However, more studies need to be conducted to understand the mechanisms between corals and mineral accretion.

Artificial reefs not only increase the reef areas, but can also serve as potential new sites for recreational divers (Jaap et al. 2006; Edwards 2010). However, the key criterion to their success is that they should be located in suitable sites that fit the designed purposes. This scenario will result in improved biogenic construction, increased three-dimensional structure leading to an increase in the diversity of fish and other reef organisms (Jaap et al. 2006).

Biological restoration involves sexual and asexual propagations. Asexual propagation is a popular technique in the past several years because it is an easy and simple technique, and requires no complicated procedures. Asexual propagation is the creation of new individuals from a single coral parent colony by means of fragmentation (Edwards and Gomez 2007). Fragments less than 5 mm comprising a few coral polyps are usually referred to as nubbins (Edwards and Gomez 2007; Rinkevich 2014). In a restoration exercise, no more than 10 % of a parent colony may be broken into numerous fragments, and the parent colony should be left to recover naturally (Edwards 2010; Chavanich et al. 2014). Fragments can be then reattached or transplanted onto suitable substrates such as PVC pipes, golf tees, pins, tiles, and concrete (Chavanich et al. 2014). To reattached coral fragments, many restoration projects have succeeded in using hydraulic cement or epoxy (Neely 1988; Jaap and Morelock 1997). For attaching small coral fragments, epoxy seems to work better despite of higher cost compared to the cement (Jaap et al. 2006). In case of nubbins which coral fragments are less than 5 mm, a nursery, either land based or ocean based, is required to increase the growth of fragments before a competent size can be outplanted, which usually take months (Edwards and Gomez 2007; Chavanich et al. 2014). In some places, whole coral transplantation may be conducted in where the reef may be completely lost due to the consequence of habitat or harbor development. However, as a general rule, whole coral colonies should not be harvested and transplanted for restoration purposes (Edwards and Gomez 2007; Chavanich et al. 2014).

For sexual propagation, this technique includes broadcasting and brooding corals. The method requires the accurate prediction of spawning nights and the species identification in order to collect gametes. The process of sexual reproduction is an important source of genetic variation and genetic diversity. The genetic diversity of sexually-produced offspring is thought to give species a better chance of surviving in a changing environment (Cote and Reynolds 2006). An important caveat for managers to consider this technique is the need for land-based facilities for producing larvae and rearing juvenile coral before outplanting. So far, few studies have successfully showed rearing broadcasting corals from eggs to spawning adults (Iwao et al. 2010; Guest et al. 2014). However, this method is currently limited in both scale of application and the diversity of species being used (Edwards 2010; Iwao et al. 2010; Guest et al. 2014).

Asexual propagation is a quick and simple technique for rapid restoration of degraded reefs with limited impact to the parent corals. It is a suitable technique when donor sources are readily available and when resources are limited and higher level techniques are unavailable. In contrast, sexual propagation is rather complicated technique, but can generate high genetic diversity and resilient species to cope with environmental challenges and global changes of the future.

Coral reef restoration can be a limited and expensive toolbox, and thus, careful planning is important for any restoration (Jaap 2000). Other issues such as scaling, monitoring the results to assess success, and natural recruitment should also be considered (Jaap et al. 2006). It is clear that some areas of reefs can recover naturally from devastating natural disturbances or human activities, and there are no methods for creating a functioning coral reef (Cote and Reynolds 2006). Nevertheless, when passive management measures have failed to achieve recovery, active restoration can play a crucial role (Edwards 2010). The decision whether this technique should be applied, depends on the project planning, risks, and costs and benefits of different management approaches (Edwards 2010).

47.3 Conservation and Management of Coral Reefs

Management and conservation of coral reefs have been improved in the past years in several regions around the world (Wilkinson 2008). Effective management can minimize threats to coral reefs. Numerous local organizations have been established to address the importance and needs of coral reef conservation and management (Wilkinson 2008). In addition, several regional programs have been created and implemented on reef conservation. The example is a Green Fins Program in Southeast Asia region organized by the Coordinating Body for the Seas of East Asia (COBSEA). The program addresses the impact of diving and snorkeling industry on coral reefs, and establishes environmentally friendly guidelines to promote a sustainable diving tourism industry (Wilkinson 2008). Other programs and projects including coral reef conservation projects under UNEP and UNESCO IOC-WESTPAC are also initiated to protect and improve the reef conditions in different regions.

Ideally, protected areas should shelter the full range of species and ecosystem diversity to safeguard important ecosystem functions. Thus, the protected areas remain a major

hope for coral reef conservation. One of the ideal and hopeful solutions for protecting coral reefs is that management is targeted and enforcement is effective. At present, there are many marine parks and protected areas in existence, but very few are effective. Twenty-seven percent of all reefs around the world are located inside marine protected areas (MPAs). Yet, only 6 % are in the effective MPAs (Côté and Reynolds 2006; Burke et al. 2011). For effectiveness, MPAs should take local factors into account. Monitoring of reef ecosystems, enforcement of use restrictions, and education of local residents and visitors should also be considered as parts of the success of MPAs. Usually, unsuccessful MPAs are attributed to a lack of staff, budget, poor enforcement, and absence of community involvement (Cote and Reynolds 2006; Burke et al. 2011; Chavanich et al. 2015). To make MPAs becoming effective management tools, those issues should be addressed. In addition, it is important that new protected areas are identified correctly for optimal conservation outcomes. Although large protected areas are clearly often required, there is no consensus on the minimum reserve sizes needed in marine ecosystem.

A comprehensive coastal management plan that includes entire watersheds as well as the human uses of coastal areas is necessary for effective coral reef conservation. The framework for a comprehensive coastal management plan should be made at both local and national levels. In addition, understanding of ecological functions and community structures including identification of the effects of disturbance is a must (Chavanich et al. 2015). To make a better management decisions, understanding of the total value of the goods and services of reefs is necessary (Burke et al. 2011; Chavanich et al. 2015).

For effective management of coral reef ecosystems, good governance is essential. Strong collaborations among local, national, regional, and international stakeholders is a good environmental governance. Local and national non-governmental organizations can also be used to evaluate the adequacy of reef protection and degradation. So far, inadequate governance regarding the problems of coral reefs can be seen in many developing countries (Burke et al. 2011). Therefore, mechanisms for implementing governance including environmental policies and regulations for effective coral reef management are needed (Burke et al. 2011; Chavanich et al. 2015).

At present, there is still a need to convey effectively the importance and complexity of healthy, and functioning reef ecosystems to general publics. Studies show that people who have personal connections with natural areas are more motivated to protect natural resources (Schultz 2000). Thus, reconnecting people with nature should be a challenge (Balmford and Cowling 2006). Better conservation education can be achieved through inter-disciplinary approaches, and conservation education should target all sectors of society from children to adults (Balmford and Cowling 2006).

To conserve and manage coral reefs in the next 50 years under the changes of climate and environment, multiple stressors and their interactions that threaten coral reefs will likely pose a great challenge. Future needs for reef conservation and management will include model projections, identification of scientific gaps, and strategies for cost-effectiveness (Chavanich et al. 2015). Those components should be developed and measured at both national and international levels to ensure the success of reef conservation in the future (Burke et al. 2011; Chavanich et al. 2015).

47.4 Conclusions

In conclusion, there are feasible ways to conserve at least some coral reef areas. Clearly, the successful reef conservation means maintaining reef resilience in the face of global climate change. Key solutions should include enhancing public environmental awareness and good governance. Technological advances for coral restoration and rehabilitation are also needed to increase the efficient restoration. In addition, success of any management effort is dependent on the support of all involved partners and stakeholders and cost-effectiveness and model projections of future reef distribution.

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Part XI

In Myth and Art

Medusa of Caravaggio, 1597

The Myth of the Lernaean Hydra

48

Carl A.P. Ruck

Abstract

The Hydra monster lurking beneath the waters of the Halcyon Lake of ancient Lerna, which figured as one of the heroic exploits of Hercules in Greek mythology, was credited with attributes of toxicity and curative regenerative abilities, offering a remarkable prototype for the freshwater invertebrate polyp that resembles its appearance and bears its name in scientific nomenclature. The resemblance, however, is fortuitous since the animal's characteristics are not observable to the unaided eye and it was unknown to the Classical world. The mythical Hydra was a zoomorphism of a psychoactive drug that figured in the very ancient Mystery rites that were still being enacted at the sacred lake well into Roman times, when the original offering of human victims was replaced by initiatory experiences of spiritual transcendence.

Keywords

Mythical Hydra • Hercules labors • Mystery religion • Arrow poison • Gorgon Medusa

Although there was no fixed canon for the sequence of 12 labors or tasks (the *dodécathon*) performed by the Greek hero Heracles (Herakles, Latin Hercules), the contest with the female 'water creature' or Hydra was often cited as the second. It was thus presented in the sequence of metopes in the decoration of the fifth-century BCE Temple of Zeus at the sanctuary of Olympia in the western Peloponnesus, the original site of the sporting event restored and inaugurated for modern times at the end of the nineteenth century as the International Olympic Games. The Nemean Lion was generally accorded precedence as the first of the labors since the hero was always distinguished in his iconographic portrayal as wearing its magical pelt, or at least its head, and both the Hydra and the Lion were located topographically near Mycenae, the citadel of Eurystheus, in whose servitude the hero was obliged to perform the tasks. The Nemean Lion was located toward the west in the mountain pass out of the Mycenaean plain, and the Hydra was south along the shore of the plain, and hence both could be considered early in the heroic career of Heracles, as he expanded his scope to more distant sites that would take him as far as Gibraltar and into Africa and also into the northern reaches of Siberia. Just as the Lion provided him with the iconographic emblem of its impervious pelt, the Hydra by all accounts supplied the deadly toxin with which he anointed his arrows. The word 'toxin' is derived from the Greek word for the archer's bow (*tóxon*), and the words for arrow and poison (*iós*) are homophonous in Greek.

Heracles was imagined as belonging to the generation of heroes before the Trojan War, a contemporary of Jason, who sought the Golden Fleece at the far end of the Black Sea, and of Theseus, who was credited with establishing patriarchal descent in the city of Athens and who encountered the Minotaur in the labyrinth of Cretan Knossos. It was Heracles who had placed Priam upon the throne of Troy, and Priam's son Paris who occasioned the War by abducting Helen from

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Sparta. Heracles was supposedly the son of Zeus, the head of the Olympian family of twelve deities, and all the heroes were engaged in tasks that symbolized the conversion of the world to the mode of male dominant culture brought into the Mediterranean lands by the migration of the Indo-European peoples from their hypothesized homeland in the central Asiatic plateau. In the Indus valley of modern northeast Afghanistan and Pakistan, their language developed into Sanskrit, and in the Balkans, it became Greek. A Celtic branch of this same migration developed into Latin in the Italian peninsula. The establishment of the sanctuary of Olympia was the crowning achievement of Heracles' heroism in that he reassigned it from its previous existence as a place of worship in honor of a goddess to its role as the central shrine to his Olympian father, whose rise to dominance was commemorated in the quadrennial celebration of the Olympian athletic contests. Similarly, the conquest of the Nemean Lion was the etiology for the inauguration of the Nemean Games. Theseus' defeat of Sinis was commemorated by the Isthmian Games, in his battle with Cercyon at Eleusis, he supposedly invented the sport of wrestling.

The particular supposedly insurmountable attribute of the Hydra that threatened the hero was its ability to regenerate its multiple heads when severed, 7, 9, or 50, and by some accounts as many as 100, each doubling whenever decapitated. Only one of them was mortal, indistinguishable and protected by the others. Heracles could defeat the Hydra only with the help of his nephew Iolaus (Greek Ioläos) by cauterizing the wounds with a torch before each head could regenerate or multiply.

The Hydra was imagined as a gigantic serpent with branching heads, resembling the stem or trunk of some plant with its spreading branches. It also breathed out so foul a sulfurous stench and expelled excrement so noisome that the smell alone would inflict a writhing fit of lethal torment, probably associating it with chthonic subterranean volcanic activity (Jayne 2003). The sulfurous toxic fumes could kill all the birds within a 20-mile radius and all the fish swimming in the surrounding waters.

The venom of the Hydra essentially caused a burning sensation. As a dermal agent, it induced an extreme fiery irritation. It could be likened, moreover, to a kind of wine, and hence an intoxicant. This in itself was not unusual since oral toxins and medicinal drugs were generally administered in wine, and wine ordinarily was drunk diluted with three or four parts water, greatly reducing its alcoholic content, but its intoxicating potency was greatly fortified by the addition of variable toxins derived from animal and botanical poisons, including deadly toxins like hemlock in sub-lethal dosages. Some of these were identical with the substances employed as arrow venoms (Hillman 2008).

When a tuft of wool imbued with the Hydra venom fell by chance into the sunlight on the ground, Heracles' wife Dejanira, who intended to employ it as a love philter to regain her husband's affection, was aghast to notice, as Sophocles in the *Trachiniae* said, that 'it grew warm, melted, and crumbled into pieces, like sawdust from a leveled tree, and from the ground where it lay, a clotted foam seethed up, like a libation of the rich potion from the Bacchic vine.' She unwittingly had anointed a robe with the toxin and sent it ahead to her returning mate, and when he appears onstage, the robe clings to his sides, like a deadly net, eating away his flesh, like a miasma suffocating his breath, and causing burning pangs of delusional convulsions, like a flower of madness (*manías ánthos*). 'Madness' or mania does not mean insanity, but inspired ecstatic frenzy, often seen as communion with divine forces or superior modes of cognition and knowledge. It was this deadly burning that would culminate in a terminal fiery immolation as spiritual transcendence, burning off his mortal physical remains when Heracles' spirit ascended at the end of his life to the celestial realm through the funeral pyre lit on Mount Oeta, above the thermal volcanic springs of Thermopylae at the base of the mountain. That is the event that awaits him as he is carried away at the end of Sophocles' *Trachiniae* tragedy.

Thereupon, the toxic bow passed into the possession of the tormented hero Philoctetes, whose noisome stench was characterized as the odor of rotting flesh from his gangrenous foot, whose toxins were further intensified by the venomous bite of a sacred serpent on the volcanic isle of Lemnos. His fellow Greeks had abandoned him there as they sailed toward Troy since his fits of madness and noisome stench rendered him a disruptive member of their communal drinking parties. It is this toxic bow with the Hydra's venom that is destined to end the long Trojan War by an arrow delivered to the heel of Paris.

The mountaintop of Heracles' immolation and the holy fire at the core of the volcano on Lemnos establish the axis for spiritual ascendance from the chthonic to the empyreal realms. As the fit of madness overtakes him in Sophocles' tragedy, Philoctetes begs, 'O burn me, please, take me and burn me in this fire of the volcano that I summon. Do for me what I did once for the son of Zeus in exchange for this poisoned bow.' It is thematically significant that the isle of Lemnos was the site of a Mystery sanctuary of the chthonic little Kabeiroi creatures and that the caldera of the volcano there was the forge of the smithy god Hephaestus (Hephaistos), whose name itself means 'volcano' in Greek. In Latin, he is called Vulcanus (Anglicized as Vulcan).

All the monsters encountered by the heroes in their exploits represent zoomorphic materializations for the toxic indwelling spirits of entheogens (plants 'imbued with deity'), which is to say, magical psychoactive flowers of ecstatic madness or shamanic mania involved as sacraments affording visionary communion with the divine (Ruck et al. 1979). As with the sanctuary of Olympia, moreover, all the particular

sites represent religious shrines whose sanctity the hero's exploit will reorient toward the honor of the emerging dominance of the Olympian family. The rites enacted at these sites originally required the offering of human sacrifices in which the entheogen played an essential role in preparing the victims to acquiesce in accepting their fate, both as sedatives and also affording a vision of the coming paradise. The hero's successful completion of his ordeal is signified by his acquiring mastery over the psychoactive agent and by supplanting it with either a symbolic placebo or one associated with the Indo-European tradition, and by the pacification of the ritual sacrifice, often converting it into something mimetic like athletic contests.

All the materialized monsters were toxic, some more identifiably so than others as particular plants. Thus the implausible weapon that Heracles uses to confront the Hydra is a pruning hook, more appropriate for harvesting a botanical item than a proper weapon of warfare. It was such a pruning hook that was also the weapon of choice for the hero Perseus in his decapitation of the Gorgon Medusa, and he placed the plucked head in a *kibisis*, which was a sack slung gaping open upon the arm, recognizable as a harvester's bag as still employed today for the picking of fruits like the apple. Some ancient accounts and depictions of the scene of decapitation with the pruning hook placed Perseus at the magical Tree of the Golden Apples and equated the plucked Medusa head as both one of those apples and also a psychoactive mushroom, of the type traditionally viewed as a golden red fruit of its host tree, found growing at its base lying on the ground (Ruck et al. 2011). The mythic paradigm of the heroic quest generally requires the hero not simply to confront the monstrous materialization, but actually to fetch and bring it back to the opponent who had assigned the task. Just as Heracles anointed his arrows with the Hydra's venom, Perseus employed the severed Gorgon head as his weapon, displaying his mastery of its toxicity by simply removing it from his harvester's sack and showing it to his enemies.

Heracles sometimes was depicted wielding a club against the Hydra, instead of the harvest implement, or even shooting arrows at it, which some accounts considered the fatal blow. The symbolism, however, is the same, since a club is also a bizarre weapon of warfare, but the particular club of Heracles was pruned from an olive tree on the instructions of the goddess Athena, and hence it was a plant brought against a botanical enemy, on the basis of the common homeopathic belief that the only cure for intoxicated madness was the same toxin that induced the madness. Hellebore, which induces delusionary visions, sometimes interpreted as artistic inspiration, was generally cited as a cure for madness. Molière's doctor who cures madness in *Le médecin malgré lui* (1666) is named Hellebore. In the same way, even the poisoned arrows of Heracles present the same pattern, apart from the logical problem, which is not applicable to the non-linear chronological symbolism of myth, that the hero could not have anointed his arrows with the Hydra's toxins until he had defeated the monster. In the case of the hero Theseus, his weapon of choice was a magical club, made of bronze in the alchemical caldera of a magical volcano like forge of Hephaestus on Lemnos, but so like the real thing that it was indistinguishable from its botanical model, the pruned olive.

The role of the olive in this regard is also traditional and significant, since it was a common motif in the depictions of the various heroes' exploits to include an olive tree at the exact spot where the monster was encountered. Heracles signified the rededication of the Olympian sanctuary by planting the first sacred grove of the tree at the site, and its branches were used to crown the victorious athletes. The olive was emblematic of toxic wild botanical growths that could be pruned from their natural state into a fruiting and productive cultivated transmutation. Olive trees, in fact, require annual pruning to maintain the potential to fruit. The olive was particularly sacred to Athena, which explains her role in providing Heracles with his defining club. The club of Theseus was even more magical since the plant had undergone alchemical transmutation in the caldera of the volcano.

As depicted in vase paintings and art, the Hydra bears a remarkable similarity to the tiny hydra, a multi-cellular freshwater invertebrate polyp, which the Dutch natural scientist Antonie van Leeuwenhoek was the first to name after the mythical prototype in 1702 (Leeuwenhoek 1702–1703). The miniscule animal is now recognized as potentially immortal, avoiding senescence by continual regeneration, able to grow anew from its severed parts. The Hydrozoa are only a few centimeters in length (roughly 10–15 cm, 0.6 in.) and just barely visible to the naked eye, but with the aid of the microscope, Leeuwenhoek noted and drew several of its fundamental characteristics. It is unlikely, however, that the actual *Hydra* animal was ever seen in antiquity and served as the model for the mythical monster, nor do any ancient sources notice its existence. The Swiss naturalist Abraham Trembley, unaware of Leeuwenhoek's precedent, nearly half a century later published his own observations on the freshwater polyp, which he discovered with his two young pupils while acting as tutor for the Earl of Portland's children (Trembley 1791). In the interim, the Swedish devisor of the system of binomial nomenclature Carl Linnaeus, who made little use of the microscope (Ford 2009), in 1735 had debunked the famous taxidermied remains of the quite sizeable seven-headed monster preserved in Hamburg as a pious fraud, assembled from weasel jaws and feet and the skins of serpents, probably the work of monks attempting to create the beast of *Revelation* (Linnaeus 1735). Leeuwenhoek's name for the polyp entered the Linnaean system of nomenclature in 1758.

Nevertheless, in addition to the remarkable coincidence of the *Hydra*'s appearance and regenerative ability, it presents

another fortuitous similarity to the mythical Hydra, with regard to the envenomed arrows of Heracles. The *Hydra* polyp is assigned in the Linnaean scientific nomenclature to the phylum of the Cnidaria, named from the Greek *knízein*, 'to nettle or sting,' which includes the jellyfish or 'medusa,' so called in English as early as 1758, and the stinging corals, which according to myth first came into existence from the spilled blood from the severed head of the Medusa. Before submerging into the sea, coral was soft and spongy like a fungus (Ruck and Hoffman 2012). The Greek verb equally means 'to provoke, tickle, eroticize.' This is another fortuitous coincidence with Dejanira's employment of the Hydra toxin as a love philter or aphrodisiac.

The *Hydra* and other Cnidaria actually shoot barbs infected with a paralyzing toxin at their prey. This would certainly have been unobservable in antiquity, at least in the case of the miniscule *Hydra*, and would appear to be an accidental similarity as the model for the poisoned arrows of Heracles. Since the animal is so tiny, it can cause no harm to humans, although its jellyfish relatives can deliver a lethal sting. With modern technology, the hydralysin toxins can be concentrated by ultra-filtration and demonstrate paralytic and other effects similar to certain mushroom and toad toxins (Sher et al. 2005).

Although these barbs offer a perfect analogue for an arrow, the *Hydra* obviously is not lethal enough to serve as the model for the hero's arrows anointed with the toxin of the mythical Hydra. Nor does the Mediterranean area even provide a suitably deadly water snake to fulfill the role of the Hydra as a freshwater serpent. Even the bite of the moray eel *Murena helena*, which generally is native to brackish rather than freshwaters, is not a good candidate. Cooking destroys its toxins and it was considered edible in antiquity. Its deadliness resides in its potential feeding upon human flesh, biting with a clamp-like jaw and draining the blood. Vedius Pollio, a friend of the Emperor Augustus, was said to have fed a slave to his pool of culinary eels as punishment for breaking a crystal cup, and then eaten the eel to taste his revenge. The anecdote was often cited in antiquity as an exemplar of exorbitant luxury and cruelty. The eel's fresh blood, however, remains toxic, and as little as one-tenth of a milliliter per kilogram of prey is sufficient to kill small mammals. These toxins are produced in the serpent's mucous tissues and skin and may aggravate the effect of its bite, but the animal lacks fangs for delivering its toxins effectively.

Inasmuch as the mythical Hydra, however, was a serpent, as such it provides the rationale for its role as the source of Heracles' toxic arrows. The Medusa similarly had vipers for chevelure. Serpents were milked to access their venom as psychoactive toxins, both to serve as arrow poisons, but also as unguents in sub-lethal dosages to access sacred states of ecstasy. The latter is what is implicated in the numerous depictions of women or priestesses handling serpents, dating from the pre-Greek Minoan period of the second millennium BCE through to late Roman times (Hillman 2013). As a weapon of combat, the arrow's toxin would have to be fast acting (Mayor 2009). Certain serpents, like the cobra, as well as toads spit their venom at their prey and offer a more suitable paradigm for a poisoned arrow. Arrows were also dipped in excrement to assure that the wound would fester. Heracles' Hydra toxin, however, is a mythical construct, implying the de-fleshing of material mortality through fiery transcendence to spiritual existence, and it was employed in the transmutation of the world into Olympian ascendancy, and was finally so used in ending the conflict of the Trojan War, whose ultimate agendum was the restoration of Helen to the dominance of her wedded husband Menelaus.

The Hydra monster, however, although a serpent, was a zoomorphism like the Gorgon Medusa of a botanical original. The *knída* after which the Cnidaria are named is the common stinging nettle, *Urtica dioica*. The fifth-century CE lexicographer Hesychius of Alexandria speculated that the verb *knízein* meant that the herb's effect was like a 'bite.' Its many hollow stinging hairs on its leaves and stems offer another intriguing analogue of the toxic arrow and act like hypodermic needles, injecting chemicals that produce a stinging burning sensation, hence its common names as burn nettle, burn weed, and burn hazel. The genus *Urtica* is so named from the Latin verb *urere*, 'to burn,' suggestive of the fiery immolation of Heracles induced by the cloak that Dejanira imbued with the blood of the dying centaur contaminated with the lethal Hydra toxin. The chemicals include serotonin and methyltryptamine hallucinogens, found also in certain mushrooms and toad venoms, and nettles are brewed into alcoholic cordials and country beers to access their psychoactive potential. In all likelihood, however, the nettle's burning sting is another fortuitous coincidence with the Hydra's venom, since the burning induced by the dermal application of its toxin is probably purely symbolic of the transcendence through immolation to the fiery realm of the empyreal paradise of the Olympian deities.

The *knída* was also identified as the sea nettle (*Actinia equina*) or anemone, which has the same stinging barbs as the *Hydra*, but is large enough to present a likeness to the unaided eye to the multiple heads of the mythical Hydra. Its polypeptide toxins are a possible source for a psychedelic agent similar to LSD. If there was an actual animal after which the Hydra was modeled, it offers an intriguing candidacy.

As Heracles struggled with the mythical Hydra, however, Hera sent a crab to aid the monster. This is a bizarre element in the tale since in no other heroic exploit does the monster have an ally. It is this crab (*kárkinos*) that became the constellation of Cancer. Vase paintings sometimes include the crab in the depictions of the hero's encounter with the Hydra, or even other crustaceans, hence linking the freshwater

Hydra with marine creatures like the anemone and corals, and probably also with the metaphor of the Medusa's blood transformed into sponges and coral. It was thought in antiquity that sea crabs turned into scorpions if their claws were removed, and then planted in the ground, from which it sprouted like a plant, with only a single venomous tail in place of its claws. Scorpion venom also offers hallucinatory and lethal potential.

The crab probably figures, however, as an element of the mythical paradigm of single-footed creatures, like autochthonous beings born from the earth (Lévi-Strauss 1963). Such autochthony implies a botanical identity, sprouted from the earth like a plant, and traditionally such creatures initially have a difficulty in walking (Lévi-Strauss 1955). It bit Heracles on his foot, which is a motif in a series of maimed, often toxic feet that includes Philoctetes, bitten by the serpent, and Achilles, who will be shot in his heel with an arrow delivered by Paris, who in turn would be shoot in the same way by Heracles' toxic arrow delivered by Philoctetes as the final settlement of the Trojan War. The best know exemplar of such a maimed hero is Oedipus, who is named as the 'swollen foot,' with implications of a phallic identity, found abandoned lying on the mountainside. Mythology offers examples of fungal anthropomorphisms of the single-footed creature as the fantastical tribe of the 'Shade-foots,' who have only a single vigorous leg, with a broad foot, which they use as a parasol when they tire and rest upon their backs, (Ruck 1986) and similarly the 'Cover-mushrooms,' who were little mushroom warriors, using their stipes a spears as they fight beneath the cover of their caps (Ruck and Staples 1994).

In all probability, however, the vaunted toxin of Heracles' bow is a purely mythical construct derived from the nature of the sacred place where the Hydra was supposedly encountered. Nothing remains today of the Lernaean Alcyonian Lake except some wetlands just south of the town of Argos along the northeastern coast of the Peloponnesus, near the modern village of Mili (*Myloi*). Although in antiquity its depth was without limit and swimmers were dragged down, never to reappear, it silted up into a malarial swamp and was finally drained away in the nineteenth century, although the powerful karstic springs remain, which implies that there were once also accessible subterranean caverns. A karst spring is the termination of a cave system formed where a subterranean river reaches the surface, usually affording access to a vast system of underground chambers. The limestone terrain is similar to the Mexican Yucatán with its sacred *cenotes* or sinkholes, and the tale of the disappeared swimmers probably indicates that the Lernaean Lake was once used for similar rites of human victims. The modern village of Mili (the 'Mills') is named for these springs. Heracles, had he failed, was intended to become one of these sacrificial victims.

The lake and its springs were associated with the myth of the Danaïds, and the waters welling up from its purported hundred wells, represented the futile attempt of the maiden daughters of Danäus to gather water for the nuptials that they had desecrated by murdering their husbands on their marriage night, 50 brides and 50 husbands, equal in number to the supposedly hundred springs at Mili, one for each head of the Hydra by some accounts. The Danaïds had supposedly arrived from Egypt with their father six generations before Heracles, which would be seven generations before the Trojan War and hence in the mid-second millennium BCE, and the controversy that had occasioned the murder of the husbands and the subsequent execution of the maidens involved the essential role of marriage in subjugating the female as subservient to her husband. Thus in fetching the toxin of the female Hydra, Heracles was performing his paradigmatic role in suppressing the power of the goddess and reassigning the cosmos to his Olympian father. Perseus was descended from these Danaïds three generations later and his fetching of the toxic female Medusa head had a similar symbolism, indicated by his subduing into marriage a transmutation of that Medusa in the form of his bride Andromeda.

He sought the advice of the water nymphs as he set out on his quest, which probably implies the Danaïds and that the pathway to the Gorgons was through the karst caverns of the Lerna Lake. Medusa means merely 'queen or female sovereign' of the sisterhood of Gorgons, and the same verbal formation underlies Andromeda as a 'man-sovereign,' descriptive of the Medusa's male or hermaphroditic attribute as bearded, as well as her sovereignty over men. In the underworld, the waters of Lake Lerna welled upwards, either from the sieves that the Danaïds used to gather it or from the leaking urn into which they poured it. All 100 of the corpses lay down there just beyond the Hydra that was the guardian of the gateway to the chthonic realm of the dead. The maidens were supposedly gathering the water for their prenuptial bath as eternal penance for the matrimonial rite that they had refused, a rite that would have signified their acceptance of a husband's dominance over his wife.

One of the sisters, Hypermnestra, the only daughter of Danäus to survive, accepted her husband, the great-grandparents of Danaë, who was the mother of Perseus as the son of Zeus. The same motif of transition from matriarchy to marriage and patriarchy is indicated in the tradition that the river that fed the karst springs of the Lernaean Lake first flowed from the Danaïd Amymonë, who was abducted, without benefit of matrimony, by the god Poseidon. Although by Classical times, Poseidon was the lord of the oceans, his more ancient persona associated him with sacred springs and freshwater fountains, inasmuch as he was counted an Olympian deity who migrated with Zeus from the Indo-European homeland where there was no ocean, but only

rivers and springs. The probable etymology of his Latin equivalent as Neptune associates him with such springs.

The lake's name is a corruption of Halcyon, which despite its pleasant connotations of the 'halcyon days,' which are the brief period of calm seas after the first storms of winter, masks the sinister reality that the halcyon bird supposedly lost her mate in such a storm at sea and the grieving little sparrow was granted respite of a few days, just long enough to lay her eggs safely on the lakeshore, while she lamented her dead husband. She was the mother of the chicks, born with no living father, and hence they were counted as her offspring in matriarchal fashion. Her mate could be considered as one of the victims sacrificed to the waters of the lake.

The Alcyonian Lake of Lerna was once one of the entrances to the underworld, where the Lernaean Mystery rites, sacred in later times to the Olympian Demeter, were performed. This was the route that Dionysus used when he descended to resurrect his dead mother Semele for her Ascension to Olympus, a myth similar to the Christian Feast of the Ascension or the Assumption of the Virgin or the Orthodox Dormition. The mythic paradigm represents the elevation of the unwedded virginal mother to the celestial realm as the Queen of Heaven (*Regina Coeli*), the bride of her son, who was her former abducting inseminator, as the supreme self-begotten god (Ruck and Hoffman 2013). This pattern is another instance of the reconciliation with female primacy as subservient to the ultimate dominance of the masculine principle presiding over the universe. The rescue of Semele is also the same celestial transcendence that was afforded by Heracles' mastery over the Hydra's venom as the toxin for his arrows.

The boatman who rowed Dionysus to the middle of the lake on the occasion of his descent to retrieve his dead mother had demanded that the god make love to him as recompense for facilitating his journey, but Dionysus cheated the boatman of his payment by returning by a different route. When the god discovered that in the interim the boatman had died, he ritually fulfilled his promise by fashioning a phallus out of fig wood, which he applied to himself while sitting, grieving like the little halcyon bird, upon the boatman's tomb. The death of the boatman, who was called Polymnos (the theme of 'Many Hymns'), masks the reality of his persona as one of these praiseworthy much hymned victims tossed into the waters, like the poor little halcyon's mate. The eroticism is an indication of the motif of shamanic rapture, which is commonly experienced as cosmic orgasm (Ruck and Hoffman 2012), similarly implied by the flight of the amorous water birds. The murdered husbands, whom the executed Danaïds joined in death, belong to the tradition of human victims of pubescent males offered to the waters of the Hydra's lake.

At the time of the boatman's death, probably dateable to the mid-second millennium BCE, Dionysus, however, had not yet achieved his divine persona as represented among the assemblage of the Olympian family, to which he originally did not belong, and even in later times he was considered somewhat marginal and an outsider, more at home in the chthonic realm of the netherworld. Dionysus, in general, appears to be a latecomer to the Greek pantheon, and although wine drinking is a common theme in the Homeric poems (recorded in writing at the end of the eighth century BCE, but narrating events of the early twelfth century), the god plays no role in the action. His greater antiquity associates him with wild intoxicants, which in the course of his evolution will be transmuted into the inebriating potion manufactured by the subduing of fungal growths into the controlled and civilizing fermentation of yeasts grown on the juice of the grape. This primordial persona, balanced with his patronage of the wine, was essential to his dual symbolism and was repeatedly enacted in the rituals performed in all aspects of his worship. The plant-gathering revels it is generally assumed once involved the sacrificial death of a young male, like the rejected suitors of the Danaïds. This tradition underlies the role of Pentheus in Euripides *Bacchae* tragedy.

The myth of the boatman was the etiology for the wooden phallus as a cultic item in the Mystery enacted at Lake Lerna, and the god's employment of it as a dildo to fulfill his promise to the sacrificed boatman, as he sat on the tombstone mourning, suggests the anal application of the shamanic agent. Since tombstones are not suitable for use as seats, in all probability the tombstone was in the shape of a phallus, or was the dildo itself. The Halcyon myth suggests the same phallic sacred dildo, inasmuch as the erect penis was commonly a metaphoric bird, as amply elaborated in Aristophanes' *Birds* comedy (414 BCE). The dildo was still employed well into the late Roman period for the sacred application of ecstasy inducing toxins, both vaginally and rectally (Hillman 2013).

Such phallic tombstones were often in the shape more correctly recognized as a mushroom (Kurtz and Boardman 1971). In fact, 'mushroom' appears to have been a metaphor for the burial coffin or for the tomb itself. "Mushroom' (*mykes*), moreover, was a common term for an erect penis. In addition, rock-cut monuments as mushroom markers for organized necropolises or cemeteries occur throughout northeastern Greece (Thrace, Macedonia, Bulgaria, Anatolia), mostly in the Late Bronze Age and Early Iron Age (second millennium BCE), with some continuing to operate through the Classical, Hellenistic, and Roman eras. The mushroom marker is often a natural rock formation, sometimes showing signs of human intervention to improve the likeness to the fungus. Further carving in some instances depicts a doorway on the stipe, imparting the sexual metaphor of the vulva as the entrance to the world beyond and the bisexual or hermaphroditic symbolism of the mushroom.

Dionysus even up into the Roman period was summoned from the Lenaean Lake's depths as *Bougenes*, 'bull-begotten,'

with an archaic trumpet, and a lamb was tossed into its waters as an offering to the Keeper of the Gate. The Gorgons where probably also lurking down there along with the Hydra beneath the calm halcyonic watery surface, since they, too, were counted among the other traditional monsters as guardians of the gate to Hades. The site was occupied as early as the fifth millennium BCE. Almost nothing is known about the Mystery, but as a secret mystical rite, it undoubtedly involved a psychoactive sacrament represented as the Hydra zoomorphism. No remains of the Mystery sanctuary survive, probably because the religion was practiced within the system of subterranean caverns.

Dionysus, in fact, was not 'begotten of a bull.' He did, however, materialize in taurine form among the ecstatic revels of his female devotees in mountain rituals of herb gathering, in which the 'bull' was identified as a zoomorphism of the sacred mushroom. As Euripides wrote in his *Bacchae* tragedy: "You seem to be a bull now leading me, and you've grown horns on your head. Were you always a beast, because you certainly have become a bull now?"

The emblem of the thyrsus/narthex, which characterized these revels, was like the *kibisis* of Perseus an implement of herb cutters, so identified by Theophrastus. Mushrooms were thought to bellow like bulls as they burst like autochthonous creatures from the ground. It should be noted also that the Gorgons, who are anthropomorphized zoomorphic visualized versions of these same mushrooms, also 'mooed' as the cows that are the mate of the bull, and that this same bellowing was the sound of the thunder whose accompanying lightning flash was the commonly accepted origin for the fruiting mushrooms (Wasson 1986).

Another mythical tradition about the famous lake of the Hydra at Lerna tends toward this same conclusion and that the visionary sacrament or entheogen of the Mystery rite of the Halcyon Lake partook of the symbolism of wild and cultivated fungal growths. This involves the story about the Proïtids, who by one account were the half-sisters of Perseus. In this version, his father was not Zeus, but Proïtos (Latin Proëtus), after whom the girls were named as his offspring by their mother Stheneboea (Greek Sthenéboia) of the citadel of Tiryns on the shore of the Mycenaean plain.

These maiden half-sisters of Perseus became afflicted with a bizarre mania, which would cause the death of the hero. In matrilineal tradition they might be seen as the daughters, not of their father, but of their mother Stheneboea, who is named for the 'Strength of the Cow.' She was also known for her uncontrollable sexual lust, which would eventually cause the death of Bellerophon, who is a double for Perseus from a parallel version of the Medusa tale (Ruck and Staples 1994). The maidens developed an extreme dermatological deformity, with their skin growing red and scabby characterized with white patches, which was accompanied by the delusion that they were metamorphosing into cows driven madly ecstatic with their sexual lusting for the bull, wandering the mountainside in uncontrollable estrus like maenads or bacchants, and when they came upon Perseus, they tore him to pieces.

Scholars have assumed that the affliction was scabies, psoriasis, leprosy, or elephantiasis, all of which are purely wild guesses and take no account of the delusion that they were becoming cows in estrus lowing like Gorgons for the erotic communion with the bull and the fact that they were maenads or bacchants, involved in the plant-gathering rites of the god's mountain revels. It is ludicrous to imagine the poor girls lugging their deformed leprous or elephantine limbs about the mountainside in a bacchanalian frenzy, or to divorce their bovine delusion from their sexual longing for the bull-begotten god. The nettlesome itching of their psoriasis required sexual gratification.

The girls were metamorphosing into a metaphor of the sacred mushroom, of the Amanita species, characterized by its red cap splotched with white scabs. They were becoming Gorgons. This is borne out exactly by a parallel version of the hero's death which claims that Perseus, thinking that the Gorgon head had lost its power, turned and looked at it (Ogden 2008). This would mean that the hero who had harvested and tamed the Medusa zoomorphism and its fungal analogue finally succumbed to its deadly potential. Such is the common motif in the tales of the hero and his liminal or dichotomous potential or dual personae, analogous to the fatal recycling of the Hydra toxins back to Heracles, as the hero who had formerly mastered them (Ruck and Hoffman 2013).

The seer Melampous is credited with curing the maidens by using the waters of the Lerna Lake polluted with the noisome sulfurous stench of the Hydra as a homeopathic agent. The mushroom complex cured the mushroom complex. The Hydra's toxin is the Hydra's medicinal drug.

Melampous has the same name as Molière's Doctor Hellebore. Melam-pous is named as 'Black-foot,' which is descriptive of the symbolic homeopathic agent that also caused the delusions, since hellebore was also called *melampodion*. Like Oidipous (Oedipus, 'Swollen-foot' or 'Know-foot'), his name suspiciously sounds as though he has but a single foot, like the 'black-foot' herb *melampodion*.

'Black Foot' is the common name for some mushrooms, such as the *Polyporus elegans*, and characteristically, the psychoactive *Amanita muscaria* (often called the 'fairytale mushroom') is pulled from the earth with some black soil still adhering to its bulbous base, yielding a dirty foot. In the folklore of mushrooms, this becomes the black boots of the anthropomorphized little creature. Exemplars range from the popular Santa Claus, now commonly recognized as derived ultimately from the traditions of Siberian reindeer shamanism, to *nain rouge* ("red-dwarf"), a *lutin*, imported from Normandy, that has haunted the region around Detroit,

Michigan ever since the early eighteenth century, and the redcap *powrie*, that inhabits the ruined castles along the border between Scotland and England. All gnomes are said to wear black boots. Fairies, who are now widely recognized as fungal anthropomorphisms, similarly have dirty feet. The lore of the fairies is not confined to the Celtic traditions of the British Isles, but amply documented in Classical antiquity (Ruck and Larner 2013).

Some claimed that the cure was accomplished at Sicyon, near Corinth, where according to Ovid the aboriginal people first sprouted from the earth as mushrooms that turned into men. Since the delusion that visited the daughters of Proïtos was characterized as the lowing of cows for the bull, we should not inquire too closely into the method of administering the gnomish misshapen phallic black-footed antidote.

Dr. Black-foot, however, was no stranger to the sacred dildo. Herodotus credited him with introducing the phallic procession of Dionysus from the Egyptians. In these parades, women carried dolls or puppets about a foot and a half tall, fitted with a penis of about the same length and hinged to bob up and down by the mechanism of attached strings. The Egyptians had an etiological myth for this oversized bobbing member, but the historian, to our loss, declined to convey it.

We should recognize that Melampous never existed, except as a figure in myth, or as a title for a tradition of shamanic priests. There is still another tale about the famous Black-foot seer that suggests that the Mystery of the Hydra's Halcyon Lake partook of the same pattern that is discernable in the Great Eleusinian Mysteries, namely of a transition from the wild mushroom and the offering of human victims to a communal experience of initiation into a shared vision of spiritual transcendence, such as is indicated by the apotheosis of Heracles and by the resurrection of Semele to the celestial empyrean (Ruck 2006). The tradition of human offerings at Eleusis underlies the plot of Euripides' *Suppliants* tragedy. At Eleusis, the potion for the initiation contained an entheogen derived from ergot, which yields a visionary substance similar to LSD (Wasson et al. 1978). The participants in the ancient secret rites practiced in the cavern of the Lernean Hydra well into Roman time obviously did not go there to be offered as victims, but to experience the Herculean transcendence.

Melampous supposedly ruled as king in Argos, which neighbors the nearby Halcyon Lake of Lerna. There are two traditions that connect the famous supposed seer with ergot. In one, he cured a prince of impotence. Seeing his father about to geld a sacrificial offering, the boy ran away, suspecting that he was the intended victim, pursued by his father with the still bloody knife (Frazer 1922). A gelding knife is usually curved, resembling a pruning hook. Hence it is not difficult to imagine that this is not an historical event, but a sacred etiology with botanical implications. In fact, by one account, the father wasn't gelding rams, but using the knife to prune trees. The father, to calm his son's suspicion, tossed the bloody knife aside, by chance lodging it in a tree, which according to some accounts, was no ordinary tree, but a sacred tree, whose indwelling deity in consequence had cursed the boy. It might have been the same sacred oak that stood in front of the house of Melampous and harbored a brood of serpents, from whom the seer was said to have received his shamanic powers. The serpents imply something toxic and psychoactive associated with the tree, since it empowered the shaman, and the species as an oak obviously partakes of the sanctity of that tree in Druidic traditions. The episode of the gelding-pruning knife and the boy's anxiety probably masks the fact that he was indeed the intended victim, not for castration, but as a human offering.

As a shaman, Melampous could speak with birds. A vulture told him of the incident with the gelding-pruning knife, and when it approached the tree, it told the black-footed hellebore shaman the cure. He was to remove the knife from the tree's trunk, boil it, and give the boy the rusty water in wine to drink, wine being the customary mode of administering drugs. The sacrificial knife that rusted in the oak represents the transition from the Druidic sacred tree that is host to the parasitic mistletoe and the *Amanita muscaria*, linked in symbolism and sanctity, to the cultivated psychoactive agent derived from rust.

One of the common names for ergot in Greek, as in English, was 'rust' (*erysíbe*). It served also as an epithet of the goddess of the Eleusinian Mystery. It is descriptive of the color, but implies as well the metaphor of the rusting of iron, which signifies the reversion of the manufactured metal back to the primitive ore from which it was extracted. Hence it is a symbolic mediation between the dichotomy between the wild and the cultivated antitheses. The ergot-infested kernels of barley, in fact, appear to provide a seed for the otherwise seedless growth of mushrooms, since as with all fungi, the mycelium under suitable conditions of moisture sprouts into the fruiting bodies, recognizable as mushrooms to the unaided eye.

A similar tale of rust as a cure was told about Telephus, a son of Heracles. The story of his infancy displays all the mythical motifs that would identify him with the attributes of fungal consubstantiality. He had received a wound from the spear of Achilles that would not heal, and the oracle at Delphic advised that what had caused the wound would be its cure. The cure for the incurable wound of Telephus was the rust scraped from the blade of the magical spear of Achilles. This weapon, like the club of Theseus, was no ordinary blade, but had been forged in the volcanic caldera of the god Hephaestus. The tale was the subject of tragedies by both Sophocles and Euripides. Pliny interpreted the cure not as sympathetic magic, but assumed that there was some kind of homeopathic remedy involved. Scholars have commented on his naïveté, but in reality we might have assumed that Achilles would not let his blade rust, or that the workman-

ship of the volcanic smithy might have produced a weapon not susceptible to corruption.

The other tradition involves Melampous with King Midas. The ergot kernels suggest another metaphoric description as 'ass's ears' (Ruck et al. 2011). As king of Phrygia, Midas wore a Phrygian cap, which is perhaps the most ubiquitous representation of the *pileus* or 'cap' of the *Amanita muscaria* and its involvement in the myth of Perseus and in the symbolism of Mithraism and the secret initiatory societies derived from it. Midas judged the famous musical contest between Apollo and the satyr Marsyas. The presence of the satyr obviously implies the theme of a bacchanalian revel. When the king judged the satyr victor, Apollo called him an ass and gave him ass's ears, which the ashamed king kept hidden beneath his Phrygian cap, which is o say, his Amanita hat. Only the barber knew, since the king would have to remove his cap for his haircut, but he was sworn to secrecy.

The secret proved more than the barber could bear. As Ovid told the story, he dug a hole by the banks of the Pactolus River and shouted his secret into the ground and then buried it. Unfortunately reeds grew from the secret burial site and as the wind blew through them rustling, they whispered that the king had ass's ears. The rustling reeds, of course, are like the sheaves of grain bearing their infestation of ergot. This was the same river, moreover, where the king washed away his notorious affliction that turned all that he touched to gold. It is another case of homeopathic therapy, and the golden touch is probably metaphoric for alchemical transmutation and the primordial analogue of the ergot fungus.

Although the Hydra provided a very fortuitous name for the *Hydra* Hydrozoa, its mythical construct in antiquity derives from the same sacred botanical lore that surrounded the other stinging Cnidaria.

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The Myth of Medusa: Benvenuto Cellini and the "Loggia de' Lanzi" in Florence

49

Ugo Bardi

Abstract

The statuary group of Perseus and Medusa by Benvenuto Cellini, kept in the "*Loggia de' Lanzi*" in Florence, Italy, is a major artistic and metallurgical feat. The present paper will outline the several links between the monumental casting work and the political and cultural situation of the time. In particular, it will describe the symbolism of the myth of Medusa as it would have been interpreted at the time and will cast some light of the different interpretations that the person who commissioned the piece (Duke Cosimo 1st) and the artist (Benvenuto Cellini) had of the meaning of the myth.

Keywords

Medusa • Perseus • Cellini • Renaissance art • Florence • Piazza della Signoria

The statuary groups of *Piazza della Signoria,* in Florence, offer to us a fascinating insight on the way of thinking of the people who commissioned and created them during the Renaissance, and also how this way of thinking evolved over more than a century of time. Among these pieces, the "Perseus and Medusa" by Cellini (Fig. 49.1) is perhaps the one which brings the greatest wealth of symbols and the deepest significance. Still today, Benvenuto Cellini's statuary group remains a major feat of artistic and metallurgic prowess. Even more so it must be appeared to Cellini's contemporaries, when it was unveiled, in 1554, as one of the most impressive pieces of statuary to be placed in the central "*Piazza della Signoria,*" in Florence.

The artistic features of Cellini's statuary group have been described in detail in Cole (1999) and the main purpose of the present paper will be to put the piece in the cultural and political context of its times and how the characteristics of the very ancient Medusa myth had been interpreted at that time. In particular, the present paper will examine the question of what exactly led Duke Cosimo the first to invest considerable financial resources to commission this particular piece. We will see that the "Medusa" group had a clear political meaning and that it was supposed to outline the political ideas of the newly established Medici rulers in Tuscany. In this sense, however, Cellini's interpretation of the myth may have turned out to be disappointing for the Duke Cosimo.

The "Perseus and Medusa" is just one of the several statuary groups visible today in the central square of Florence. These pieces were placed there over a period of approximately two centuries, starting in the early 1400s. The aspect of the square was largely completed at the times of Grand Duke Ferdinando 1st (who ruled from 1597 to 1609), although the position of some of the statuary pieces in the square changed in later ages and, at present, most of them are copies of the originals. The pieces can be divided in two groups: those in the square itself, or facing it directly from the nearby *Loggia de Lanzi*, and those inside the *Loggia;* less visible and less prominent. We will consider here mainly the first group of statues, the most important and the best known. Here is a list of the pieces, arranged as a function of the year in which they were erected in the square.

1. The *Marzocco Lion*, by Donatello (1420) (Wirtz 1998)
2. *Judith and Olophernes* by Donatello (placed in the square in 1494, actually created in 1453–1457) (Wirtz 1998)

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Fig. 49.1 Perseus and Medusa; the statuary group created by Benvenuto Cellini in Firenze (Image from http://commons.wikimedia.org/wiki/File:Firenze.Loggia.Perseus02.JPG (PUBLIC DOMAIN))

3. *David* by Michelangelo Buonarroti (1504) (Baldini 1973)
4. *Hercules and Cacus*, by Baccio Bandinelli (1534) (Bush 1980)
5. *Medusa and Perseus* by Benvenuto Cellini (1554) (Venturi 1939)
6. *The Fountain of Neptune*, by Bartolomeo Ammannati (1565) (Heikamp 1973)
7. *The equestrian statue of Cosimo I*, by Giambologna (1594)

The covered space of the "Loggia de Lanzi", hosts antique pieces going back to Roman times and two pieces by Giambologna: *The Rape of the Sabine Women* (1583) and *Hercules beating the Centaur Nessus,* created in 1599 but placed in the Loggia only in 1841.

The statuary pieces of the Signoria Square were never intended simply as decorative elements. They were an integral part of the area where the "*Palazzo Vecchio*" stands, the seat of the city government (and later of the government of Tuscany). On the one hand, these pieces were supposed to inspire the rulers of the state by providing them with visible examples of good behavior and wise government. On the other hand, they also carried a message to the people of Florence, embodying the power and the ideas of the rulers of the city. They were, in short, an early version of what we call today "consensus building" (also known as "propaganda" up to no long ago).

The messages carried by the statuary in *Piazza Signoria* evolved in time, changing with the changes in the government of the city. The age when the most important pieces were created, the sixteenth century, was an age of great cultural and political turmoil. A profound switch in taste and in sources of artistic inspiration was taking place during that period. Biblical subjects for art were being abandoned; replaced by subjects inspired by classical mythology (as discussed, for instance, in the 1932 book by Amy Warburg (1999)). The reasons for the return of classical pagan symbolism and ideas are complex and are discussed, for instance, by Betz (1998). In Tuscany, we can see a fundamental turning point with the foundation of the Neoplatonic Academy in Florence in 1474 (Kristeller 1961), but we can follow the trend visually in the succession of statuary pieces in the *Signoria* Square. Apart from the Marzocco Lion, which is a Medieval symbol, the earliest statuary pieces, Judith and Olophernes, as well as Michelangelo's David, are related to Biblical myths. After the David, all the later pieces, instead, are related to Classical myths.

The switch from Biblical to Classical mythology as a theme for art pieces carried a deep political meaning. The waning of the Middle Ages and the arrival of Renaissance saw all over Europe the emergence of a new class of nobles who were finding the roots of their power in their military force and in their financial assets, while the power and the influence of the Catholic Church was declining. Tuscany underwent this transformation just as the rest of Europe did, and saw the gradual decline, and eventually the disappearance, of the Florentine Republic in 1530, when Florence capitulated to the imperial armies of Charles V. The Medicis then ruled Florence and Tuscany until 1737, first as dukes and then as grand-dukes. This political transformation was far from unopposed and we may still see its evolution reflected in the statuary groups of the *Signoria* Square.

The Marzocco lion was the first piece placed in the square, but it does not have a strong political significance. The subsequent piece, instead, the "Judith and Holofernes" by Donatello, was heavily charged in political terms. It shows the Jewish heroine Judith and the Assyrian general Holofernes, killed by Judith in his sleep, as described in the Bible. This piece was originally commissioned as a decorative element for one of the gardens of the Medici Family and only later moved to the square. We don't know what those who commissioned this piece had in mind, but we cannot miss its political significance when it was publicly shown in the Signoria square. The inscription placed on the basement, which still stands there today, says in Latin, "*exemplum . sal[utis] . pub[licae] . cives . pos(uerunt).*" That is, "The

citizens posed as an example of public virtue." The "Judith and Holophernes" was a powerful moral message which could be interpreted as the triumph of humility against arrogance and it was seen as such in Medieval and early Renaissance times. But, more than that, the figure of Judith could be seen (and was seen in Florence) as a political message representing the people killing a tyrant in the name of justice (McHam 2001). On the subject of the importance of this piece, we should not be misled by its relatively small size in comparison with the other pieces in the square. For its times, the "Judith" was a major achievement, both in artistic and in technological terms. It was one of the first freestanding Italian Renaissance statues in bronze that was conceived "in the round". And it was among the largest bronze pieces ever cast up to then. When it was exposed in the square, it was also completely gilded and its golden color must have made it even more impressive. This statue was a powerful assertion of the power of the Florentine republic.

Continuing the time sequence, we can now consider Michelangelo's David, today likely the best known piece in the *Signoria* square. When it was erected, in 1504, Donatello's Judith was inside the lateral loggia and therefore the David was dominating the visual space of the square. The political message carried by the David was similar to that of the Judith group. Both the Jewish hero, David, and the Jewish heroine, Judith, could be seen as saviors of the Jewish people from the oppression of foreign tyrants (McHam 2001) and, therefore, saviors of all people oppressed by tyrants. Note also that Donatello had sculpted at least two all round Davids as an indication of his interest in the theme. Michelangelo had also painted Judith in a fresco of the Sistine Chapel in Rome. Clearly, the related meaning of the two Biblical myths was familiar to these artists. Indeed, the image of strength and virility of Michelangelo's piece carries a strong message, even though its meaning as a republican icon is not so obvious as in the case of Donatello's Judith and Holophernes.

Then, we arrive at a turning point: some 30 years after that Michelangelo's David had appeared in the *Signoria* Square, the Florentine Republic fell after a dramatic siege, in 1530, where Michelangelo himself fought on the side of the Republic. The arrival of the new Medici rulers, first Alessandro (reigning 1532–1537) and then Cosimo I (reigning 1537–1574) was to be soon felt in many places in Florence and the *Signoria* Square was not to escape the influence of the new government. Indeed, the new addition to the square (1534), Baccio Bandinelli's "Hercules and Cacus" (Baldini 1973) had style and meaning completely different from those of the earlier pieces, even though it had been commissioned in Republican times. First, it portrayed a theme from classic antiquity, rather than a Biblical one. Then, it could not be possibly interpreted as representing the defeat of a tyrant at the hands of the people. In the original myth, Cacus can be seen as an ancient cattle thief, transmogrified into a monster; a worthy adversary for Heracles/Hercules. The triumph of the hero, in this case, seems to represent justice meted out by a legitimate lord over a criminal (Sutton 2009). This piece could be interpreted as a complete reversal of the message carried by the David and by the Judith. The defeat of Cacus represented the defeat of the riotous Florentine republic at the hands of the legitimate rulers of the city, Hercules; that is, the dukes of the Medici family. A telltale element of this message is how the defeated Cacus turns his head in the direction of the *Palazzo Vecchio*, the residence of the Duke, as if asking for the clemency of the ruler (Bush 1980).

Bandinelli's "Hercules and Cacus" succeeded only in part in counteracting the effect of Michelangelo's David. If its political message was clear, it was a failure as a work of art. Perhaps some of the criticism the piece received was unjust, and many commentators missed the elements of novelty and originality of this statuary group. Nevertheless, the piece by Bandinelli was comparable to the "David" only in size, but it was clearly inferior to it in all other respects: the figures are stiff and unnatural, the composition is static, the structure of the bodies is unrealistic, and the facial expressions of the two figures verge on the ridiculous rather than on the dramatic. As a consequence, this piece was strongly criticized by Bandinelli's contemporaries, in particular by Cellini himself who, later on, would learn the lesson and strive to avoid Bandinelli's mistakes (Waldman 1994).

So, when Cosimo I became the duke of Florence, in 1937, after the assassination of his relative and predecessor, Alessandro, he must have found that the *Signoria* Square needed some political balance. Despite the recent addition of the "Hercules and Cacus," the Republican component of the statuary in the square was still largely predominant in terms of both quality and numbers. Hence, the need to redress the balance with a new piece that would lean on the side of the duchy. In looking for the appropriate symbolism for this new piece, Duke Cosimo had ample choice in the corpus of classical mythology. There, he could find several examples of shiny heroes killing ugly monsters and any of these could be interpreted as the triumph of dictatorship against democracy. Among these myths, the story of Perseus and Medusa, was, as for many Greek myths, a very ancient story which had evolved and changed over time. In its earliest form, Medusa may have been a moon goddess who underwent the sad destiny of other female goddesses overwhelmed, and usually killed, by male gods. It is a theme that goes all the way back to the slaying of Tiamat at the hands of Marduk in the Babylonian myth (Jacobsen 1968), and to even earlier times, as for the slaying of Huwawa at the hands of Enkidu and Gilgamesh in the Sumerian epics (we cannot exclude that Huwawa was seen as a female). In the early Greek version of the myth, Medusa was described as one of the three Gorgon sisters, monstrous creatures said to be living in

Hades, as mentioned, for instance, in Hesiod's *Theogony* (ll 270–294). In time, the myth evolved into a somewhat gentler story, with Medusa described as a maiden famed for her beauty and the envy of other girls. She was violated by Poseidon in Athena's temple and, as a punishment for having defiled the sacredness of the temple, the goddess transformed Medusa's beautiful hair into serpents and made her face so horrid that just the sight of it would turn people to stone. However, the fact that Medusa had started her career as a normal human being didn't change her destiny: all the versions of the story had her killed by Perseus who used his shield as a mirror to avoid to have to look at her in the face and be turned into stone as a result.

Duke Cosimo I was surely acquainted with the myth of Medusa. For instance, we know that he used to wear a medallion with the face of Medusa on it and we can still see it depicted on a bust that Benvenuto Cellini made for him 1548 (Today at the Bargello Museum in Florence). Duke Cosimo may have known about the myth from various sources. It is likely that he could read Latin and therefore he had access to the treatise on ancient deities (*Genealogia deorum gentilium libri*) that Giovanni Boccaccio (1313–1375) wrote in 1360 and which describes in some detail the story of Perseus and Medusa. From the viewpoint of the duke, this story had an evident advantage over similar myths: a happy ending. On the contrary, in some classical stories involving a shiny hero killing a monster, the hero ends up being punished by the Gods or by Fate. This was the case, for instance, of Bellerophon killing the Chimera and of Oedipus killing the Sphinx. They both ended their careers blind and accursed after various misadventures. We may find the same sad destiny for the hero in an early version of the same myth, when Enkidu is struck by sickness and dies as a punishment for having killed Huwawa. There is a logic in this pattern: ancient mythology follows rather strict rules (Campbell 1959), one of them is that a divine being cannot really be killed, only sent back to the divine realm. And when a mortal manages to do something that resembles killing a divine being, then he must suffer a punishment. But, in the case of Medusa, nothing bad happens to Perseus and that makes sense according to the rules of ancient mythology because we are explicitly told by Hesiod that she was mortal; unlike her two sisters of the Gorgon trio. We don't know why Medusa was so different from her two sisters, most likely it was a post-hoc explanation to explain the lack of retribution for Perseus' deed. In any case Perseus later married the beautiful princess Andromeda after having saved her from another ugly monster (Cetus) to became king of Mycenae. It seems that the royal couple lived happily ever after.

Duke Cosimo may also have been attracted to the myth of Medusa because of the spectacular characteristics of Perseus; in particular the variety of gadgets that the hero was said to be able to muster. Among these, winged sandals that enabled him to fly, a cap of invisibility, a special curved sword called *harpe*, and a magic bag called *kibisis*. He also rode the flying horse Pegasus which sprang forth from the neck of Medusa when he beheaded her. As noted, for instance, by Wilk (2000), Perseus looks like a modern superhero and perhaps he exerted the same kind of fascination on young men that today's superheroes do. On this point, it is worth noting that Cosimo became Duke of Florence when he was only 18 years old (in 1537) and that he was immediately involved in a major battle against Republican exiles in the same year (whom he defeated at *Montemurlo*). So, we may imagine how he would see himself as a monster slaughterer and identify himself with the shiny hero Perseus. As a result, it made sense for him to try to create for the *Signoria* square an image of the slaying of Medusa that would represent and proclaim the death of the republic at the hands of the triumphant Medici rulers.

We can now consider how Benvenuto Cellini was charged with the task of creating the Perseus and Medusa piece. Born in Florence in 1500, Cellini was around 50 when the duke commissioned to him the piece. In his "Memories" Cellini doesn't tell us much about what pushed the Duke to choose this particular subject and what he wanted to express with it, only that the Duke was very keen to have a piece representing the myth of Perseus and Medusa. When Cellini showed him a model of the statue he planned to create, the duke told him that for the finished statue he could "ask him whatever he wanted" (a promise that was not kept). It was an exceptional, and perhaps unexpected, chance for someone who so far had not been especially renown as a master sculptor. Over his career, Cellini had created some large pieces such as a Mars for a fountain at Fontainebleau and a life-sized silver Jupiter (both lost today), but he had not been very prolific in this field. Cellini was better known for smaller pieces, such as the famous "*saliera*" (salt cellar) that he made for King Francis I of France in 1543 (presently at the Kunsthistorisches Museum, Vienna). The *saliera* is a spectacular piece which clearly reflects the tendencies of the time in terms of the interest in classical myths. But this piece is only 26 cm tall, and that hardly prefigures the gigantic figure of Perseus that Cellini would create some years later. Nevertheless, Duke Cosimo decided to gamble on Cellini's ability in entrusting to him the manufacture of such a large and difficult piece. If that was a gamble, it paid handsomely with the creation of a true masterpiece.

In facing the task of representing the myth of Perseus and Medusa, Benvenuto Cellini could not invent everything from scratch; he needed some kind of models. One possibility for him was to look to ancient images of the myth. Obviously, he couldn't have access to the same wealth of ancient artifacts we have today, but, nevertheless, the Renaissance was an age in which a keen interest in ancient art had developed and ancient artifacts were actively sought and unearthed

Fig. 49.2 An example of an archaic image of Medusa's head, shown as a monster. Greek, South Italy, 500–480 B.C. Bronze (Courtesy of the J. Paul Getty Museum. http://www.getty.edu/art/gettyguide/artObjectDetails?artobj=35494)

Fig. 49.3 An example of a late classical image of Medusa's head, with gentler features than earlier ones. Roman, circa 100–125 CE, (Courtesy of the J. Paul Getty Museum. http://www.getty.edu/art/gettyguide/artObjectDetails?artobj=35503)

everywhere. An example is the discovery of the bronze piece known as the Chimera of Arezzo, unearthed in 1553 and which Cellini surely saw as part of Duke Cosimo's collection of antique objects (Bardi 2008). The story of Perseus and Medusa, as we said, is extremely ancient and today we have images that we can relate to the myth as early as in the seventh century BCE (Wilson 1920). Up to ca. the fourth century BCE, these images show a round head, a bloated face, protruding tongue, and fixed eyes (Fig. 49.2). This kind of representation could be seen as the representation of a corpse (Wilk 2000), in tune with the origins of Medusa, ancient underworld demoness. When the whole body of Medusa was shown, she was usually endowed with wings. Often, these archaic images show the scene of the fight of Perseus and Medusa, with Perseus represented in the act of beheading her. In time, these representations evolved into something gentler, with the head of Medusa not anymore so grotesquely deformed; although there remained the characteristic snakes around her head (Fig. 49.3). Good examples of this kind of images are the one on the back of the "Tazza Farnese" (the Farnese Cup) which goes back to the second century BCE (Frothingham 1911) as well as the Rondanini Medusa, a marble head which is believed to have been created in the fifth century BCE but which is probably a later piece (Belson 1980). In time, the representations of Medusa evolved into something even gentler, a good example was a fresco of the Roman Villa of Stabia (Fig. 49.4); an area that was buried during the eruption of the Vesuvius in 79 A.D. (Barbet and Miniero 1999). Here, Medusa's head has human features, even juvenile ones, although she maintains the detail of snakes as hair. It is not the only one of this kind: similar images are observable in private auctions, even though they have never been described in the scientific literature. An ancient Roman cameo (25 B.C.–A.D. 25) showing a similar composition of Perseus and Medusa in the J. Paul Getty collection.

It appears that the myth of Medusa was not very popular during the Middle Ages, even though we may perhaps identify some of its elements in Romanesque art. But the myth enjoyed a return of interest with the Renaissance, together with a general return of interest in anything that had to do with ancient mythology. Renaissance artists took up with great enthusiasm the task of reviving ancient myths in their work and they soon found themselves confronting the myth of Perseus and Medusa. Perhaps the first to try this subject was Leonardo Da Vinci (1452–1519) who is reported by Vasari (1991) to have created a Medusa head in his youth. This head is lost today, but we have this description of it by Vasari (as translated from Italian by E. L. Seeley).

> *….he began to think what he could paint upon it, that might be able to terrify all who should come upon it, producing the same effect as once did the head of Medusa. For this purpose, then, Leonardo carried to a room of his own into which no one entered*

Fig. 49.4 The Perseus and Medusa Fresco found in the Roman Villa of Stabia, which was buried by the Vesuvio eruption of 79 CE. The composition of this scene is very similar to the statuary group created by Cellini, although Cellini could not possibly have seen it, since the villa was excavated for the first time in 1745. However, this and other similar images (see text) indicate that the myth was represented in this form in late Roman times (Photo from: http://blog.rome101.com/2014/02/12/perseus-and-pals/ – (Permission to reproduce obtained by owner, Mr. Bill Storage))

> *save himself alone, lizards great and small, crickets, serpents, butterflies, grasshoppers, bats, and other strange kinds of such-like animals (some of these animals he dissected), out of the number of which, variously put together, he formed a great ugly creature, most horrible and terrifying, which emitted a poisonous breath and turned the air to flame; and he made it coming out of a dark and jagged rock, belching forth venom from its open throat, fire from its eyes, and smoke from its nostrils, in so strange a fashion that it appeared altogether a monstrous and horrible thing.*

Clearly, Leonardo was thinking of Medusa as a horrible monster, although we cannot say if his inspiration came from ancient pieces. Later on, Leonardo painted another Medusa head. This piece is also lost today, although a Medusa head present at the Uffizi museum is often – but wrongly – attributed to Leonardo. Vasari also tells us how Baldassare Peruzzi (1481–1536) painted for pope Giulio 2nd in Rome a "*loggia sul giardino… con le istorie di Medusa quando ella converte gli uomini in sasso e quando Perseo le taglia la testa,*" (A loggia on the garden, with the stories of Medusa when she turns people into stone and when Perseus cuts her head.).

From these data, we see that when Cellini approached the subject of Perseus and Medusa, he had probably no significant contemporary or recent model to follow. It is likely, however, that he had seen at least some ancient pieces representing the myth. A likely one is the Tazza Farnese, which was purchased by Lorenzo de' Medici (1449–1492) (see e.g. Frothingham 1911). We know that it remained in possession of the Medici Family up to the death of Duke Alessandro, in 1537, when it was taken by his widow, Margaret of Parma, who gave it to her second husband, Ottavio Farnese. Cellini returned to Florence from France in 1545, so at that time the cup was not there any longer to serve for him as a direct source of inspiration. However, it is perfectly possible, actually likely, that he saw it in Florence in earlier times and we may imagine that it suggested to him that Medusa was not necessarily an ugly monster. Another representation of Medusa that Cellini is likely to have seen is the Rondanini Medusa in Rome, another piece which shows a relatively gentle Medusa face. Neither of these two pieces, however, show Perseus slaying Medusa, which was what Cellini had to show if the piece was to be the counterpart of Donatello's Judith and Holofernes piece. Here, it is interesting to note how the fresco of the Villa of Stabia (Fig. 49.4) shows Perseus and Medusa almost exactly in the positions that Cellini would give them in his piece, with a naked Perseus holding high Medusa's head in his left hand and the sword in his right hand. Cellini couldn't possibly have seen this piece, since the remains of the Roman villa at Stabia were discovered only in 1745. Nevertheless, this piece gives us an indication of where Cellini could have found inspiration for the composition of his piece: it was from the line of representation of the myth that became popular with the first and second century CE, an example can be found in the J. Paul Getty museum collection (Anon 2016).

These considerations tell us something of the sources of inspiration Benvenuto Cellini for his "Perseus and Medusa." Clearly, he was not interested in showing Medusa as an ugly monster: that simply was not a choice for an artist like him who had been living during the full blossoming of the artistic movement we call "mannerism" and which was concerned mainly with the representation of perfect and elegant human bodies. So, Cellini built on the late Roman tradition of the way to represent the myth, to create an interpretation which was largely original and steeped in the way of thinking of Renaissance artists. He may have been also inspired, or at least challenged, by Michelangelo's David, which was a piece the Perseus had to compete with. And he had to be careful to avoid the mistakes that others had done, such as in

Fig. 49.5 Detail of Medusa's body in Cellini's piece (Original photo by the author)

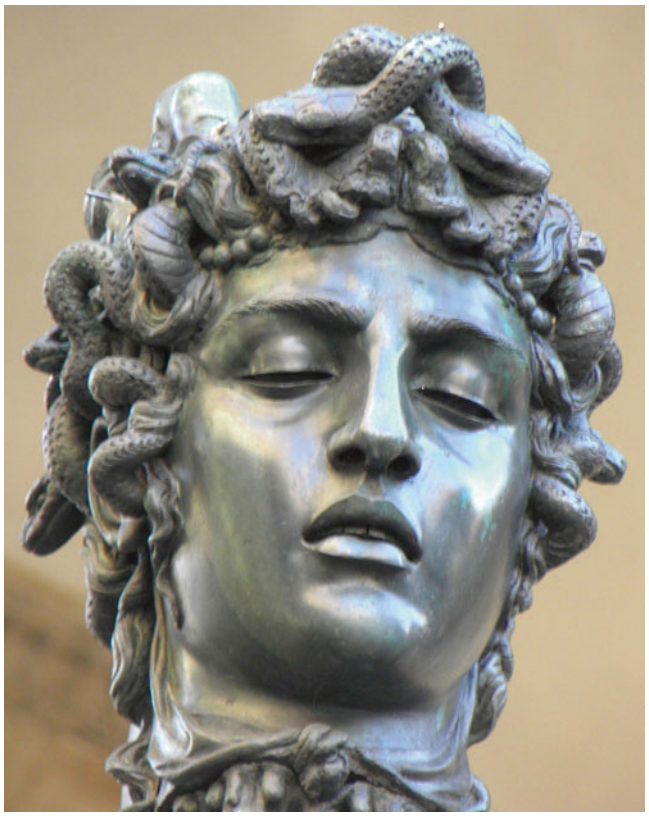

Fig. 49.6 Detail of Medusa's head in Cellini's piece (Original photo by the author)

the case of the "Hercules and Cacus" group by Baccio Bandinelli. As a result, Cellini created the bodies of Perseus and Medusa from real life models. For Perseus, he tells us in his "Life" that his model was "the son of Gambetta, the prostitute", a boy whom he later calls "*Cencio*" and who was probably Cellini's lover. For Medusa we know that the model was a 16 years old Florentine girl named *Dorotea,* whom we know was the daughter of Giovanni di Matto da Carota (Haskins 2001) and maybe a lover of Cellini as well. The boy named "Cencio" may also have been the model of Medusa's face which, when carefully examined, can be seen as somewhat masculine. Instead, Perseus's face is almost identical to that of Donatello's marble David, an evident indication that Cellini here was following a canonical Renaissance way to interpret the concept of a beautiful face.

The idea of using real life models for the creation of this piece surely avoided the mistakes that Bandinelli had made but it created other kinds of problems inherent in the idea of representing semi-divine creatures with bodily features of Florentine boys and girls. The final result was a fascinating mix of realistic and idealized characteristics not all of which are perfectly attuned with each other. Cellini tells us in his "Life" that a piece of statuary "must have eight views, and it must be that all of them be of the same quality". Indeed, you may look at the Perseus from any of its "eight sides" and from each one you'll notice new details and different qualities. The majestic hero is what catches the attention first, but this hero is at the same time a demi-god and a very realistically cast human being. For instance, Perseus's belly is not as flat as it would be fitting for such a radiant hero, actually it can be defined as something of a paunch. Then, Perseus' head is certainly handsome but in its abstract perfection it hardly fits with the rest of the body which, as we said, is that of a real person.

The body of Medusa, trampled under Perseus' feet, is less impressive at first sight, but it is sculpted with no less loving care than the male figure. She is not a monster but a woman, headless of course, but a beautiful woman. She lies on the pedestal, her right hand abandoned on a side, her left hand clasping with great force her right ankle. Medusa is dying, but she is not completely defeated yet. Perseus foot presses against her belly as if to keep her down in a pose which has a strong misogynistic aspect, a typical feature of several of the statues of the *Loggia* (Even 1991). The way Medusa's body lies has a strongly sensual character and this sensuality is enhanced by the thick flow of sprouting blood which is more of a coral-like intriguing substance rather than a repulsive one (Fig. 49.5). And then, Medusa's head, held so high and in such prominent position is not a monster's head, this much is obvious even when observing it from the ground level. But from close range pictures (Fig. 49.6) its sensual beauty is stunning: eyes closed, mouth half open, a hint of teeth, the oval of the face framed in a mesh of snakes above and the folds of the skin at the neck wound, where neither the snakes nor the blood pouring out are shocking or repulsive but instead carnally sensuous. Perhaps the most peculiar fea-

ture of the statuary group is how he two faces, Perseus and Medusa, look similar to each other. Not in all details, of course, but the similarity is evident in elements such as the mouth and the nose. In addition, Medusa's hair, made out of snakes, is nearly identical to Perseus curly hair. The similarity of the two heads is just one of a series of questions that we may ask about the Perseus and Medusa. What did Cellini wanted to express with this piece?

We don't know if Cellini understood the symbolic relations duke/Perseus and republic/Medusa; that is, if Cellini understood that he was being asked to represent in bronze the slaughter of the Florentine republic; in his "Life", he does not tell us anything on this matter. And, we may ask at this point: if the political position of the Duke is obvious, how about Cellini's one? We have no evidence that Cellini ever took a public stance on political matters or, at least, we find nothing of that sort described in his "Life." This is understandable, since when he wrote his memories he was an old man, not especially wealthy, living in Florence under what we would call today a dictatorship. Duke Cosimo I liked to appear as a benevolent ruler, but when it was question of showing his teeth, he was ruthless and heads rolled not just in a figurative sense. The same is true for Cosimo's son, Francesco, who took over from his father as regent in 1564. So, Cellini's "life" is outspoken and detailed on many things but silent about others. For instance, Cellini tells us at length how he had fought as a gunner against the Imperial Spanish army at the siege of Rome in 1526 and how he performed a number of spectacular (and exaggerated) deeds. But when it was Florence's turn to be besieged by the Spanish troops, in 1530, Cellini only tells us that he was in Florence and that he was recalled to Rome by the Pope and that later on he moved to France to work for king Francis I. Apparently, he had no wish to repeat in Florence the performance he had given in Rome 4 years before (or, at least, didn't want to write about it in his memories). If we consider that it was the Spanish army that had reinstated the Medici family in Florence and hence, indirectly, Cosimo I, we may understand that Cellini may have been reticent in telling us anything about what he had done in Florence during the turbulent year 1530. The only mention in terms of political opinions we find in the "life" in something that happened when he was in Rome, after the Medici restoration in Florence. In the community of Florentine Republican exiles there came news that Duke Alessandro had been assassinated in Florence, in 1537. Cellini tells us that exiles rejoiced; believing that the republic would soon be reinstated, but Cellini told them "*isciocconi*" ("fools"), "in three days we'll have another duke", which is exactly what came to pass. Surely, this statement by Cellini cannot be taken as an expression of Republican sympathies, although the very fact that Cellini frequented such a group may tell us something.

So, we can hardly think of Cellini as a republican at heart but, still, he was a man of his times. Cellini's life was a series of fights and battles. A man of such independent spirit hardly submits to the power of an absolute ruler, be him a Duke or an Emperor. Yet, even independent spirits must at some point come to compromises and in the "life" many times we find a Cellini eager to please the despot Cosimo; even too much, we are tempted to say. Given this mix of contrasting feeling and the fact that the creative process of an artist can hardly be controlled, it is not surprising that what came out of Cellini's work on the Perseus and Medusa was not exactly what the Duke – or Cellini himself – had intended.

The Perseus and Medusa statue that we can still see today is hardly a triumph of dictatorship over democracy or, at least, if it is a triumph it is a cruel and brutal triumph. Cellini's Perseus has nothing of the moral righteousness that pervades Donatello's Judith. It has nothing of the virile strength of Michelangelo's David, and nothing of the stiff righteousness of Bandinelli's Hercules. What we see in this piece is a man who has surprised and beheaded a young woman in her sleep. There is no glory in having killed a woman and there is no glory in pinning her body to the ground as she withers in death's pains. If we look at the faces, we may think we are seeing demigods engaged in a mythical battle. But, if we look at the bodies we are seeing real human bodies, and we are seeing Cencio killing Dorotea with a butcher's knife. And, even as a demigod, Perseus does not seem to be so proud of what he has done: he is looking pensive, somewhat subdued, or maybe he is even ashamed. Then, the face of Medusa is so similar to Perseus' face that we are tempted to conclude that they might be relatives. In killing Medusa, Perseus has not only killed a helpless woman but he also betrayed and killed one of his own kin. It was exactly what had happened to the Florentine Republic in 1530, when it was betrayed by the *condottiero*, Malatesta Baglioni, who had been hired to defend it with his mercenary troops and who then defeated to the enemy. Then, if Perseus is a representation of Duke Cosimo, Duke Cosimo, too, is a murderer and, worse, a traitor.

This is, or course, just a possible interpretation of the meaning of Cellini's piece, but it may have been not an uncommon interpretation after the piece was unveiled, as we can read in Cellini's memories. He tells us that when the Duke saw the statue for the first time, he was enthusiastic. But as time went by, it something changed. The description of the effect of the unveiling of the Perseus is better left to Cellini's own words in the "life" (translation by Anne MacDonnel, Everyman Libray ed. 1968):

> *Now, as it pleased my glorious Lord, the immortal God, I brought the thing at last to its end, and one Thursday morning I showed it openly to the whole city. No sooner I had removed the screen, though the sun was barely risen, than a great multitude of people gathered round – it would be impossible to say how*

many – and all with one voice strove who should laud it highest. The Duke stood at one of the lower windows of the Palace, just above the door; and there, half hidden in the embrasure, he heard every word that was said about the statue.

Even though Cellini reports us that, later on, the Duke was still very pleased with his "Perseus", we can't avoid to think that these several hours of listening to comments on the statue changed something. And indeed, the about-face of the Duke with Cellini was evident. There had been ups and downs in their relation, despots are known to be capricious, but always the Duke had appreciated Cellini's work. But, from then on, Cellini was cut off with all contacts with the court and never made anything again for the Duke. Not much remained of the Duke initial promise, "that Cellini could have asked him anything he wanted". Cellini had to content himself with a modest sum, paid in installments (and not always in time).

Of course, we cannot say with any certainty that the loss of interest of the Duke in Benvenuto Cellini was due to the Duke's disappointment with the popular reaction to the Perseus. There may have been plenty of different reasons why the Duke got tired of the aging sculptor who was a troublemaker and, on top of that, an expensive one. Cellini himself tells us in his memories that in more than an occasion he had annoyed the Duke's wife, Eleonora of Toledo, who had resented his presence in her rooms. And, of course, the Duke never said explicitly what he had found wrong with Cellini's work or with Cellini himself. Whatever the case, the Duke was surely sufficiently intelligent to understand that even if the piece he had commissioned was not as good as he had hoped it to be as a piece of political propaganda, it was still a masterpiece. So, the Perseus and Medusa remained where it was, where we can still admire it.

But, with the "Perseus and Medusa" Cellini didn't just leave to us an ill-conceived piece of political propaganda. Cellini was a true genius and his interpretation of the myth went well beyond the mere purposes of the political situation of his times; he went to the very roots of the ancient myth. As we said at the beginning, the ultimate origin of Medusa are those of an ancient Moon Goddess. No matter how much later writers and artists attempted to diminish her role to that of a mere monster, her links to the divine realm remained there, hidden but impossible to miss for those who took the time to look. For instance, the wings so often shown in archaic representations are a telltale sign of Medusa's divine origin. So, the mellowing of Medusa's features in ancient art was simply a return in sight of the original meaning of the myth. This return has been ongoing for millennia and Cellini's piece is one more step in that direction. Cellini managed to reproduce in his piece some deep roots existing in many religious rituals. In many mythological stories, we are told how divine beings may appear to humans not in their real forms, but as "avatars", a term taken from Hinduism that has become common in modern times (Campbell 1959). Avatars are manifestations of the deity, not the real deity, and as such cannot be killed, only sent back to the realms they came from. This is the essence of many religious rituals, some still alive in relatively recent times, such as the Ainu rituals described by Fosco Maraini (Delaby and Maraini 1981) (see also Kindaichi and Yoshida 1949). In the Ainu ritual, a young bear is given the role of the avatar of the divinity and then ritually killed in order to send the divine being back to its proper realms. In this ritual, killing the bear implies no malevolence; on the contrary, it is seen as a service done to the divine creature incarnated in the bear. So, the killing of Medusa in Cellini's piece can be seen as something very different than the depiction of a murder but, rather, of a sacrificial ritual. An astonishing result for someone who, probably, knew nothing about Moon Goddesses and his knowledge of ancient religious rituals was, most likely, limited or non existent. In particular, the position of the head, kept so high in Perseus' hand (Fig. 49.6) seem to indicate the road to heaven that the divine essence of Medusa is taking. The separation of the body and of the head of Medusa represents the separation of her mortal and divine essences. Indeed, Cellini managed to build up his "Perseus and Medusa" as a piece of powerful and intricate symbolism and meaning; as it is proper for an art masterpiece.

Benvenuto Cellini lived to a relatively old age and died in 1571 at 70 in Florence. He never had a chance again to make anything comparable to the Perseus and his last work of art of large size is a marble crucifix, at present at the at El Escorial Monastery in Spain. It is a piece not without merits, but it is nothing comparable to the "Perseus and Medusa". Duke Cosimo died at 55, 3 years after Cellini. With the Perseus, the Duke may not have been able to erase completely the Republican dreams of his subjects, but for sure he didn't give them any chance to putting them into practice. The Duchy of Tuscany, later to become Grand-Duchy, was to last until 1861, when it peacefully merged with the newly created Italian state.

Benvenuto Cellini's "Perseus and Medusa" crowns an age and in some ways ends it. In the turmoil of the sixteenth century, Florence, Tuscany, and all Europe were in the midst of a profound transformation. The discovery of new worlds beyond the Atlantic ocean had changed everything for the Italian states, which were rapidly catapulted from the center stage to the periphery of Europe, not anymore players in continental politics but a battleground for France and Spain to fight out their dreams of domination. Eventually Spain emerged as the winner in Italy, but it took a century of struggle, and in this struggle something perished: it was the intellectual freedom of the Renaissance. Things such as democracy and free speech were lost, and not only these: the great human achievements of Renaissance in all fields of art and science were going to wither and disappear. Today we see these events as an unavoidable progression, but the people

of the time were fighting their personal battles, some uphill, some downhill, most of them could not see as clearly as we do that an age was closing with them. Some tried to resist, some fought back, some let themselves be carried by the events. In the end it didn't matter, it was a battle that could not be won. But against this dark background some figures stand out in their struggle. Many left us poignant stories of their times, one of them is, no doubt, Benvenuto Cellini, master goldsmith and perhaps the last person of his time who had the chance to prove himself a sculptor on a par with Michelangelo. He could create something that would transcend the limits of his time and become a universal masterpiece: his "Perseus and Medusa".

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Medusa and Perseus, and the Relationship Between Myth and Science

50

Margaret M. Toscano

Abstract

This essay examines the Greek myth of Perseus killing the Gorgon Medusa as emblematic of the relationship between science and myth, and between culture and nature. While most people in the contemporary world dismiss myth as the product of a primitive imagination that has been superseded by scientific knowledge, scholars view myths as stories that are told and transmitted because they have social significance. Myths change to meet the interests and needs of their narrators and audiences. This will be demonstrated by examining three visual representations of the Medusa myth, as seen on three Greek vases (from 525 to 400 BC). In each case, the goddess Athena is a central figure for understanding how Perseus relates to Medusa. Athena not only gives him knowledge to defeat the monster, but she holds up her shield as a mirror through which he can reflect on his relationship to the Gorgon head. If Athena, the goddess of wisdom and technical skill, represents scientific knowledge and tools, what is the price a human like Perseus pays to conquer the sea goddess through Athena's power? This essay juxtaposes myth and science as compatible but different discourses that in dialogue can suggest productive ways for human cultures to rethink and restructure themselves to live harmoniously with the physical world rather than to destroy it.

Keywords

Medusa • Gorgons • Perseus • Athena • Hermes • Myth theory • Ancient Greek vases • Myth vs. science

50.1 Introduction

Myth is most often viewed as the opposite or precursor to science, especially on a popular level in our contemporary world. Most people, even those with college degrees, see myth only as the product of a primitive imagination that lacked the tools and knowledge to give an adequate explanation of the real scientific facts of the natural world and its origins.[1] For this reason ancient societies came up with fanciful stories to explain how the world works, such as the ancient Greek explanation for the advent of the seasons when Persephone, the goddess of springtime, was forced to spend part of the year in the underworld after Hades tricked her into eating pomegranate seeds in his realm.

This chapter will propose, in contrast, myth and science as coextensive, if very different, discourses and methodologies that both struggle to deal with the relationship between humans and nature, among other things. The myth of Medusa and Perseus can be seen as emblematic of this struggle. Medusa and her sisters, the Gorgons, were sea goddesses,

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[1]This observation reflects my experience in teaching college students over the past 30 years, but it is also evident in introductions to the topic of myth, such as Armstrong's.

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ancient and sometimes monstrous creatures, born from Phorcys and Ceto, two other sea gods.[2] Perseus fits into the pattern of the celebrated Greek male, the cultural hero who must slay a monster to rescue himself and others from the powers of destruction, represented by the chaotic forces of nature.[3] Though he is helped by many gods, it is Athena, the goddess of wisdom and culture, who gives him the knowledge and aid he needs to cut off Medusa's serpent-covered head, which he will later use as a weapon to kill his foes, including another sea monster, Cetus, thus freeing Andromeda from the clutches of this other watery serpent to become the bride of the hero so he can return home in triumph. The story seems to carry with it a straightforward moral for human societies: bring nature under submission before it destroys you. But the loose ends of the ancient Greek myth, like the flowing hair of Medusa, suggest something more subtle, with multiple possibilities and perspectives.

50.2 Theories of Myth

Neither of the above propositions is new, whether it is questioning the validity of myth or proposing myth and science as compatible discourses.[4] The last 150 years has witnessed heated debates among scholars about the origin, meaning, and function of myth, including the meaning of the term "myth" itself.[5] Edward (E.B.) Tylor (1832–1917) is a pivotal figure because he was one of the first to pit science against myth, arguing that science makes myth not merely irrelevant but an obstacle to modern scientific thinking. And modern religion too, he asserted, should eschew mythical thinking that tries to explain the physical world; rather, it should concern itself with ethics and meaning alone, the realm of the immaterial. On a popular level, Tylor's view has dominated the last century in such a way that most people still think of myth as simply a false story. But other twentieth-century scholars quickly responded to Tylor to explain why myths are so tenacious and influential and how they function on both a societal and individual level. The prevailing belief now is that myth has a different function than science. If it did not, science would have done away with the need for myth in the modern world; but it has not.

50.3 Psychological and Sociological Approaches to Myth

Still, opinions have varied as to what the function and content of myth is. Sigmund Freud and Bronislaw Malinowski, who gave early counter-arguments to Tylor, went a long way toward redeeming myth for moderns, and in the process they also shaped their scholarly disciplines as well. Their importance for redefining the value of myth for moderns cannot be overstated, for both argued that myth has something other than an immaterial or symbolic function. Myths can work toward the healthy functioning of individuals and communities.

For Freud (1856–1939), myths and dreams were the form in which the subconscious reveals itself—dreams for the individual, myths for the collective. They are the sign of a repressed libido trying to express itself through symbols in a very indirect way that can work toward overcoming neurosis. Carl Jung (1875–1961), the student and opponent of Freud, saw an understanding of mythic archetypes as the key for unlocking the unconscious and healing the individual from alienation and dysfunction. While Freudian and Jungian analysts still argue that therapy is indispensible for psychological healing, others who have followed the psychological mode of interpretation see myths as a crucial way to understand human personality, to construct individual identity, and to work through personal issues.[6]

Bronislaw Malinowski (1884–1942), in contrast to Tylor, saw myths not simply as a sign of primitive understanding, though that was his starting point. But in the process, he came to see myth as an active force in all societies because they work on an everyday level. Myths help societies form and understand their social organizations and act as charters to give legitimacy to social practices. Myth serves "an indispensable function: it expresses, enhances, and codifies belief; it safeguards and enforces morality; it vouches for the efficiency of ritual and contains practical rules for the guidance

[2] Hesiod (7th c. BC) gives the earliest account of Medusa's genealogy. See Trzaskoma et al. (2004, 139–40).

[3] Mills explores this pattern for Achilles and Scamander, and Odysseus and Poseidon (2002, 55–133). Beal connects the Mesopotamian chaos goddesses with the sea monsters of the Hebrew bible (2002, 16–30).

[4] With the rise of ancient science and philosophy from the sixth century BC on, the Greeks themselves began to see their myths as problematic from a literal point of view; and thus they began to develop theories about the nature and function of myth, though the general populace most likely continued to see the stories as literally true. Xenophanes (570–475 BC) criticized religious myths, seeing the stories of gods as mere projections of human characteristics and values. Euhemerus (4th c. BC) saw myths as exaggerated human history; the gods and heroes of myth were actually rulers whose stories were embellished. And Plato (427–347 BC) read Homer as allegory and made up his own myths to replace those he did not like in traditional religion. Segal notes that Marcel Detienne and others have emphasized this old debate (1999, 19).

[5] Four excellent books that give a thorough and critical overview of all the major myth theories are: Csapo (2005), Scarborough (1994), and Segal (1999, 2004).

[6] See Segal's discussion of the influence of Joseph Campbell, who popularized the notion of myth as a mechanism for personal healing, transformation, and identity formation in the acclaimed 1987 TV series *The Power of Myth* (1999, 135–41).

of man" (1954, 101). As Robert Segal points out, Malinowski believed that myths not only persuade people to accept social phenomena, but also help them to be reconciled to physical phenomena, such as natural catastrophes, aging and death, that cannot be controlled or resisted (2004, 28).

50.4 Structuralism

Structuralism provides another major mechanism for understanding the meaning and function of myth. The key figure here is cultural anthropologist Claude Lévi-Strauss (1908–2009). For him, myths are connected with language and reveal the structure of the human mind, which is built on a binary system, pairs of contradictions or opposites. And it is the function of myth to mediate between these opposing extremes: raw/cooked, life/death, hunter/hunted, nature/culture, and so on. Lévi-Strauss believed myths are a concrete, rather than an abstract way of dealing with human problems. The ancients were not inferior in intellect; they thought differently, but still intelligently.[7]

Roland Barthes (1915–1980), who was both a premier literary critic and social theorist, focused on myth as ideology from a Marxist perspective. In his famous *Mythologies* (1957), he argued that myth cannot be defined by content, but rather it is a type of speech or mode of signification. Myth is an attempt to create a grand narrative, to conflate culture with nature, and to depoliticize when it is really all about constructing and stabilizing power regimes. For Barthes, myth is primarily an ideology of the right that tries to hide the fact that its power base is socially and linguistically constructed, rather than how things are naturally; if the left uses myth, it does so clumsily. "The bourgeoisie hides the fact that it is the bourgeoisie and thereby produces myth; revolution announces itself openly as revolution and thereby abolishes myth" (1972, 146). In this way Barthes moved theory toward poststructuralism.

50.5 Poststructuralism

More recent poststructural theorists, such as Bruce Lincoln and Eric Csapo, also argue that myth is ideology, but in a more even-handed way than Barthes.[8] Lincoln calls myth "ideology in narrative form" (1999, 207). But these scholars do not see myth as inherently or simply a conscious tool of those in power to maintain control, though it can be that. Rather, myth is at play on every level of culture, high and low, used both consciously and unconsciously. Csapo argues for a broad conception of myth "as anything which is told, received, and transmitted in the conviction of its social importance … the question of whether or not something is true or false is largely irrelevant to the question of whether or not it is a myth. … It is much more relevant to consider the motive behind its transmission and reception" (2005, 279). For both Lincoln and Csapo, the theorist's job is to deconstruct mythic narratives to show the political/social ideological frameworks at play by putting myths in specific historical contexts. As Csapo explains, "ideological analysis regards language and semiotic systems as practical, not abstract, as social, not autonomous, and as conflicted, not homogenous" (299). And such an approach is free to adapt the methods of other myth theories for this end.[9]

50.6 The Perseus and Medusa Myth in Art

With these theoretical frames in mind, I want to return to the myth of Perseus killing Medusa. It is easy for moderns to think of the story as having a single version and purpose because in anthologies or textbooks about classical mythology the story is told as a continuous narrative from the birth of Perseus to his return to his homeland and ascent to kingship. However, the ancient sources themselves each give us different parts of the whole (sometimes because the author is simply referencing a part of the story, and sometimes because the source itself is incomplete).[10] Moreover, each author has a different purpose and approach. Every telling of the myth has a different historical context and therefore is part of a different ideological framework. When looked at as a whole, the different ancient versions of the Perseus and Medusa story are, as Csapo explains, "conflicted, not homogenous," though there are threads of continuity that show obvious responses and reactions to earlier patterns and motifs.

To illustrate the complexity of this myth, I will focus not on the literary tellings of the story but on three artistic images, which each show a different depiction of the mythic characters involved. The Medusa figure especially was very popular in ancient Greek art; she appeared on pots, shields, and coins, on freestanding and relief sculptures, in mosaics and wall paintings, on bronze mirrors, and jewelry engravings. Surely the visual images outnumber the literary ones.[11] My purpose is not to give an overview here. Rather I have selected three vase paintings that span the period 525–400

[7] See Segal's discussion of Lévi-Strauss (2004, 29–30).

[8] Still, both Lincoln and Csapo emphasize the political nature of the ideology of myth and admit their own leanings.

[9] Csapo asserts: "Psychoanalysis and structuralism have proven particularly adaptable to the ends of ideological analysis" (2005, 297).

[10] *Anthology of Classical Myth* (Trzaskoma et al. 2004) and *The Medusa Reader* (Garber and Vickers 2003) are excellent sourcebooks of ancient authors.

[11] I am speculating since I have not counted them. Wilk includes a chapter on the "Gorgon in Art" (2000, 31–54), and the majority show the Medusa head as monstrous.

BC in order to show three very different responses to the killing of Medusa. I will treat them chronologically in terms of vase production rather than in their narrative order as portrayed in the composite myth.

One black-figure Greek vase (Fig. 50.1), c. 525–470 BC, shows Perseus fleeing from the two Gorgon sisters of Medusa, who remains headless but standing.[12] Perseus is accompanied and assisted by Athena and Hermes. This vase painting maintains a tension between movement and stasis: the figures are depicted in action, but the nature of visual art keeps the figures fixed in one stance. The six major figures move around the circumference with legs stretched out running. But who is chasing whom? The configuration almost looks like a round dance without any forward movement, so there is no beginning to the chase. The headless Medusa is clearly recognizable. She is flanked on either side by the winged horse Pegasus and the golden boy Chrysaor, her children conceived in her union with Poseidon but not released from her body until she is decapitated. One looks one way, and the other the other way. But they do not seem part of the chase since their diminutive size puts them in the background.

Straight across from the headless Medusa is Perseus. Wearing his winged boots and cap, he carries two hunting spears and the *kibisis* bag that apparently contains Medusa's head, as literary sources indicate. He looks back at Athena, who watches and follows him with her spear stretched out. Her aegis is spread out like a single wing, making her resemble the Gorgons somewhat. Her head is also quite similar to the two Gorgon sisters who still have theirs. They are at either side of their headless sister. All three Gorgons have two outspread wings and a pair of snakes sprouting from each side of their waists. In front of Perseus is Hermes, his second divine helper. The two males also resemble each other; their double spears cross; and their winged boots give flight to their gait. Hermes' cap is flatter than Perseus'; and he wears his traditional traveler's cloak. He looks toward the Gorgon in front of him, and she looks back at him, while her other sister faces Medusa.

Though literary versions of the myth depict a victorious Perseus who evades his Gorgon pursuers, this vase painting keeps the question of triumph open. The two Gorgon sisters of Medusa are an ongoing reminder of the power of the sea monsters, who are also gods, like Athena and Hermes, but of an older generation. While Medusa can be slain, she is the only mortal among the three Gorgon sisters. Though the other two usually stand in the shadow of their more famous sister, their immortal presence in this painting represents an ongoing threat to Perseus and other mortals.

[12]This black-figure pyxis, attributed to the Haimon Painter, originates from Athens and currently is held in the Louvre in Paris (Ca2588; Beazley 352011).

Fig. 50.1 Perseus, Medusa, and the Gorgons, c. 525–470 BC, black figure, Haimon Painter, Musée du Louvre, Paris, Ca2588 (Photo by Herve Lewandowski. ©RMN-Grand Palais, Art Resource, New York)

The second image (Fig. 50.2) comes from an Attic red-figure amphora, dated to the same period, about 525 BC.[13] It does not depict the Perseus myth. Rather it illustrates Heracles fighting Apollo for the god's sacred tripod. Athena stands by Heracles; Artemis stands by Apollo.[14] I have chosen to focus on the detail of the Athena figure on the left because of the large Medusa symbol on the right side of her aegis. Sometimes Athena sports the Medusa head on her shield, but more often it adorns her aegis. And often Athena displays the Medusa figure on her aegis while she is assisting a hero: here Heracles; elsewhere it is Jason or Theseus, or one of the Trojan heroes. In this picture Athena has thoroughly incorporated the power of the Medusa into her own person. She has conquered the monster and uses her increased power to promote the welfare of the Greek male heroes and the Athenian state. She stands in full armor as the warrior goddess. The monstrous and frightening Medusa head portrayed here is the most famous and recognizable of her artistic representations.

The third image (Fig. 50.3), on a red-figure vase, c. 400–385 BC, depicts Perseus standing across from his patroness, Athena.[15] She holds the Medusa head aloft. Neither one gazes at the woeful head that still has the power to turn all to stone. Instead, they both look down at the image of the

[13]This vase painting is attributed to the Andokides Painter. The pot is held by the Antikenmuseen in Berlin (Berlin F2159; Beazley 200001).

[14]Some identify the female figure on the right as the Phthia of Delphi.

[15]The red-figure bell krater is from Apulia, South Italian, and attributed to the Tarporley Painter. It is currently held by the Boston MFA (1970.237).

Fig. 50.2 Detail of Athena with Medusa head, c. 525 BC, Attic red-figure amphora, Andokides painter, Antikensammlung, Berlin (F2159) (Photo by Ingrid Geske-Heiden. Art Resource, New York)

Fig. 50.3 Perseus and Athena with the head of Medusa, c. 400–385 BC, Apulian red-figure krater, Tarporley painter, Museum of Fine Arts, Boston (1970.237) (Photograph © 2015 Museum of Fine Arts, Boston)

Medusa head reflected in the shield that stands between them, upside down to represent the mirror image. Though there are four snakes coming from the top and bottom of Medusa's head, she does not look monstrous, but sad. Her eyes are cast down, though in her image on the shield they are open. And the snakes are not visible on the shield, blending in with her hair tendrils. The Athena figure on this vase is very different from the armed warrior of Fig. 50.2. She wears an embroidered and belted chiton, with a necklace, earrings, and a hair band (*sphendone*). The only sign of her role as warrior goddess is her spear, pointed downward, and the shield that will bear the Medusa image. Hermes stands behind Athena, with his traveler's cloak over his shoulder and his caduceus as the only sign of his identity. He too looks down, avoiding the gaze of the Medusa head.

50.7 Comparing the Three Images

All three Athena figures are very different. Only Fig. 50.2 shows the most obvious emblems of her power and identity. Here she stands in her iconic pose as warrior goddess and protector of heroes, wearing her full armor with helmet, spear, and shield, with the Medusa head prominently displayed facing outward with its grimace underneath Athena's face, which is turned sideways.[16] Four large snakes protrude from Athena's aegis, overpowering the smaller snakes sprouting from the Medusa head. More snakes protrude from Athena's hips. Though it is common for Athena to be associated with snakes apart from the Medusa insignia (her aegis often bears a snake-like fringe even when she is not wearing a Medusa head on her person), the snakes coming from her hips are unusual.[17] And this repeats the pattern found in Fig. 50.1, where the Gorgons bear similar snakes at their hips. In contrast to Athena triumphant in Fig. 50.2, who stands watching at the sideline, Athena in Fig. 50.1 is an active participant who interacts with the rest of the actors: the Gorgons, Perseus, and Hermes. Figure 50.3 reveals the feminine side of Athena, which is rare in both literary and visual depictions of her. In general she sides with male gods and heroes, though she is the goddess of weaving and womanly skills. If it weren't for the Medusa head and Perseus's presence, she might be unrecognizable. Although she is carrying a spear, it could pass as a staff if the viewer does not look down at the point that is almost hidden in the folds of her chiton. Though the shield in this picture must belong to Athena since it is leaning toward her and the Medusa head is on it, still Perseus, as the only warrior in the frame, could make claim to it.

[16]The common gaze of figures on Greek vases is to look sideways toward the scene depicted. Since the Medusa head looks at the viewer of the vase, this makes her unusual and more terrifying.

[17]Burkert explores Athena as a snake goddess (1985, 140, 229).

All three Medusa figures are very different as well. Only Fig. 50.2 is monstrous. Because the Gorgon sisters in Fig. 50.1 appear as winged goddesses with beautiful heads like Athena's, Medusa too appears more sympathetic, especially since her head is not visible. But it is the Medusa in Fig. 50.3 who draws our pity. Not only is she more human (which of course makes us relate to her), but she appears beautiful and sad as well, a victim of sorts.[18]

Perseus and Hermes both appear in Figs. 50.1 and 50.3, but not Fig. 50.2. In Fig. 50.1, the two males seem to be almost a reflection of each other. They both wear winged boots and caps, and carry double spears. Only the cloak and *kibisis* pouch differentiate them. In Fig. 50.3, Perseus wears the winged boots, which in literary versions he must return to Hermes. He appears as a Greek warrior coming home from battle. Perseus' hat is a helmet rather than his usual Hermes-like cap.

These three pictures show very different attitudes toward the conquering of Medusa. In Fig. 50.1, there is a more symbiotic relationship among all of the characters. If, as is often true in Greek literature and art, Athena and Perseus and Hermes represent the triumph of male power, reason, and Greek civilization over irrationality, the female, and chaotic nature (also seen as the triumph of the Olympians over the older generations of gods), then this vase painting fails to capture that ideology.[19] Figure 50.1, in contrast, shows a dynamic relationship among the forces of nature and culture, male and female. Spatially, the female Gorgons dominate the picture. Figure 50.2 more clearly represents the triumph of culture (represented by Athena) over nature (represented by the Medusa head). And finally, Fig. 50.3 takes the story in a very different direction. Here the focus seems to be on the question of representation itself. Athena's shield acts as a mirror. But only the Medusa head is seen in the mirror. Neither Perseus nor Athena can see themselves, nor can they look at nature (reality?) straight on. They must see it reflected. And through the reflected image of Medusa, they also see themselves indirectly. Hermes standing apart is unobserved but observing. He seems to stand in for the viewer who becomes the interpreter. The central role of Hermes in classical Greece is the god of borders. He has the power to cross realms and boundaries, in other words to see things from different perspectives. This was the source of his talent and role as a messenger, for the messenger must interpret and go from one realm and one person to another to convey knowledge.[20] Figure 50.3 takes the viewer to a meta-level of interpretation. It asks us to be self-reflective about the ways we interpret myths and use their power to represent the world.

50.8 Systems of Representation, and the Future of Myth and Science

Images, like stories, are about the power to define and categorize human experience and knowledge. It was the eighteenth-century Swedish scientist Carl Linnaeus who coined the term *medusa* to describe and categorize the non-polyp forms of the phylum cnidaria, which are marine animals with gelatinous umbrella-shaped bodies from which tentacles resembling the streaming hair of the Greek Medusa flow. Old and enduring like the Gorgons, jellyfish are both threatening and beneficial to humans. And they are beautiful. Medusa, too, was once beautiful until Athena turned her hair to serpents, which are not only symbols of monstrosity but of regeneration.

I want to return to my assertion at the beginning of this chapter that the story of Perseus killing Medusa can be seen as a metaphor for the relationship between myth and science in the modern world. But I want to challenge the simple interpretation I proposed there that the story shows the need for humans to conquer the natural world in order to survive. Myth is indeed connected with ideology, as contemporary theorists assert. But no mythic story or image has a single meaning, as I have tried to show in my examination of the three vase paintings. There are always many ways to represent and interpret any story or image.[21] Is it Medusa who is equated with nature?[22] And who represents culture? Perseus or Athena? Though I did not include the goddess in my title, her dominant presence in the vases complicates the role of Perseus.[23] In each case, the goddess Athena is a central figure for understanding how Perseus relates to Medusa. Athena not only gives him the knowledge to defeat the monster, but

[18] The Roman poet Ovid picks up on the theme of Medusa as beautiful victim. In Book 4 of his *Metamorphoses*, he describes the unjust rage of Athena (Minerva), who makes Medusa a victim twice over by turning her hair to serpents after Poseidon rapes Medusa in Minerva's temple. See Garber and Vickers (2003, 35–36).

[19] Hurwit (2004) discusses the depictions of battles on the Parthenon that connect cosmic battles with the power of the Greeks to destroy their enemies, who also can be mythic powers: Olympians vs. Giants, Greeks vs. Amazons and Centaurs, etc. Classical scholars, such as G.S. Kirk, have often used structuralism to examine Greek myths since they seem to employ oppositional pairs, such as nature vs. culture (1970, 132–171).

[20] Much later the mythic figure of Hermes gets conflated with Hermes Trismegistus, the source of hermetic or secret knowledge.

[21] In Leeming's (2013) examination of the Medusa figure, he explores the multitude of ways the image has been interpreted, from the ancient Greeks, through the Middle Ages, Renaissance, Romantic period, into our contemporary world, including feminist interpretations and contemporary popular culture, including the Versace icon.

[22] Though Medusa's origins connect her to the sea gods, her wings on vases make her bird-like. duBois argues that when Medusa is turned to stone, she becomes connected to the earth since the Greeks saw stones as the bones of Mother Earth, Gaia (1988, 87–92). And perhaps her association with serpents makes her amphibious, of the earth and water.

[23] For a comparative approach to the complex ways Perseus appears in myth, see Ogden (2008).

she holds up her shield as a mirror through which he can reflect on his relationship to the Gorgon head. In Fig. 50.3, the viewer is even asked to consider whether Medusa and her head are monstrous. If Athena, the goddess of wisdom and technical skill, represents scientific knowledge and tools, what is the price a human like Perseus pays to conquer the sea goddess through Athena's power? And should we connect science with nature or culture? The associations are not straightforward or easy to equate on a one-to-one basis. Science attempts to understand the properties of the physical world, but it uses tools and methodologies developed through culture.

Robert Segal asserts: "Whether myth has a future depends on its capacity to meet the challenge posed by modern science" (1999, 19), which revolves around myth's capacity to self-reflectively examine its relationship to the natural world. Is myth mere fantasy, or does it offer practical tools for the ways humans live in the world? This is the central question of myth's role in society today, as Karen Armstrong also acknowledges in her *A Short History of Myth*:

> The imagination is the faculty that produces religion and mythology. Today mythical thinking has fallen into disrepute; we often dismiss it as irrational and self-indulgent. But the imagination is also the faculty that has enabled scientists to bring new knowledge to light and to invent technology that has made us immeasurably more effective. The imagination of scientists has enabled us to travel through outer space and walk on the moon, feats that were once only possible in the realm of myth. Mythology and science both extend the scope of human beings. Like science and technology, mythology … is not about opting out of this world, but about enabling us to live more intensely within it. (2005, 2–3)

In turn, whether science has a future depends on whether it can meet the challenge of using scientific knowledge to live harmoniously with the physical world rather than destroy it. What can Greek myths offer us as we think about the ways human cultures must evolve to live more confluently with the natural world? As Armstrong argues, the human imagination is central to both myth and science; it is the imagination that allows us to think of new ways, new technologies, which are not simply tools to conquer nature but to live dynamically within it. The best myths explore fundamental human dilemmas about the possibility of right ways to live in the world, which always involve tensions or competing values that must be resolved or at least balanced to create justice among humans and a symbiotic relationship with nature. These are ethical questions that myth is equipped to address, when it is self-reflective and not obsessed with justifying systems of power. Science, too, cannot avoid ethical questions; and, certainly, it should not use the construct of objectivity as an excuse to avoid them. Athena, who represents the best of immortal wisdom and human inventiveness, and is thus the proponent of a rational and scientific approach, is also a warrior goddess. She can both destroy and create; she can dominate or share power. We have seen both modes in the three Greek vases in this chapter. Both myth and science have the capability for these human impulses: creation and destruction, equality and domination. To return to Armstrong again: We "need myths that help us to venerate the earth as sacred once again … because unless there is some kind of spiritual revolution that is able to keep abreast of our technological genius, we will not save our planet" (2005, 137). When myth and science are seen as coextensive discourses that have something to say to each other, we will be better equipped to think about the crucial ecological questions that should engage every citizen of our world today. I applaud the editors of this volume for their decision to include a section on the myths behind scientific classification. This kind of interdisciplinary discourse is a beginning place for a fruitful dialogue about the most pressing of human problems.

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Beheading the Gorgon: Myth, Symbolism and Appropriation

51

Susan Deacy, Pauline Hanesworth, Greta Hawes, and Daniel Ogden

Abstract

Medusae Jellyfish were named as such by Linnaeus because of their intriguing similarity to a particular monster of classical mythology, Medusa (also known as the Gorgon). Medusa's disembodied head with hissing snakes for hair, together with a deadly gaze that could literally petrify, made her the most horrible of mythological monsters. This chapter explores how Medusa came to be beheaded, and what this episode has signified both in antiquity and subsequently, where it has had an afterlife as among the most powerful and contested of mythological symbols. We consider how the ancient myth might have come about, what it meant to the ancients, what its value is as a symbol and how and why it has such a rich tradition of appropriation by particular users, each of whom creates a new beheading myth while engaging with various earlier adaptations.

Keywords

Gorgon • Medusa • Gorgoneion • Perseus • Athena

Abbreviations

LIMC Kahil et al. 1981–1999.
TrGF Snell, Kannicht and Radt (1971–2004).

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51.1 Introduction

When Linneaus chose the name Medusae for a form of the Cnidarians, he joined a long line of users of one of the most popular, flexible and meaningful of classical myths. This chapter explores an aspect of this myth that has generated many interpretations: the beheading of Medusa, also known as the Gorgon. Cellini's 1545 *Perseus with the head of Medusa* (Fig. 51.1) is but one of the many representations of the beheading that precede Linnaeus, and - as we will show - many more would follow.[1] Among the most enduring of mythological moments, it has been used to support conceptions of what it is to be male or female, what it means to experience fear, or war, or natural disaster, and what it is to

[1]We are indebted to Evelien Bracke (Swansea) for her comments on a draft of this chapter. For the ancient myth of Perseus see Robert (1920): 222–45, Woodward (1937), Jameson (1990), Gantz (1993): 300–11, Roccos (1994), Ogden (2008), Fowler (2000–2014): ii, 248–59. For *gorgoneia*, Gorgons and Medusa more specifically see Will (1947), Phinney (1971), Floren (1977), Halm-Tisserant (1986), Krauskopf and Dahlinger (1988), Vernant (1991), Wilk (2000) (with caution), Vernant (1991), Ogden (2013a:92–8, b:82–97).

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Fig. 51.1 Benevento Cellini, *Perseus with the head of Medusa*, 1545. Bronze sculpture, Florence, Piazza della Signoria. B. ©Dragoneye Dreamstime.com

have consciousness. It has been interpreted by psychoanalysts and film theorists as well as specialists in fields including art theory, monster theory, feminism and disability studies – often because of its perceived potential to get to the core of what particular commentators are seeking to accomplish. We explore its use in ancient Greek and Roman literature and art as well as assorted meanings created by postclassical users. We also consider the tricky issue of the possible origins of the beheading motif, survey the 'back story' of Medusa and discuss how various interpreters, both classical and postclassical, have depicted the adventures of her disembodied head: the gorgoneion.

51.2 The Myth -- or Myths -- of Medusa

Greek myths were subject to infinite variation. They knew little orthodoxy, save when strongly tied to cults. Ancient literary authors, Greek and Roman, upon whom we principally depend for our own access to the myths, were particularly inclined to renew them in creative ways. This was as true of the myth of the Gorgon Medusa as it was of any other. We will begin by outlining its main variants.

51.2.1 Ancient Traditions

A useful starting point is the account of the broader myth of Perseus formulated by Pherecydes of Athens (his traditional floruit is 454 BC). The original version of this account, which was evidently highly influential throughout antiquity, is lost to us, but it is reflected in the works of later writers. We can reconstruct it as follows.

Acrisius, king of Argos, was told by the Delphic oracle that his only daughter, Danae, was destined to have a son that would kill him. Accordingly, he took the precaution of confining her within a bronze-bound chamber sunk into the courtyard of his palace, so that no man could gain access to her. But Zeus fell in love with her, and penetrated the chamber's skylight in the form of a shower of gold, impregnating her. The child she bore she named Perseus, and she reared him secretly until discovered by her father. Thereupon Acrisius enclosed mother and child in a great chest and dumped them in the sea: whether to kill them, or to have them carried away to a land from which they could not harm him, remains unclear. The chest washed ashore on the island of Seriphos, where the pair were rescued and maintained by the simple fisherman Dictys (Pherecydes F10 Fowler).

When Perseus had come to manhood Polydectes, the wicked king of Seriphos, fell in love with Danae and so schemed to remove her protective son. He invited him to a contribution-feast but demanded that he should bring as his contribution the head of the Gorgon – a mission from which he trusted he would never return. The gods Hermes and Athena took pity on Perseus and offered him guidance. First, he tracked down the terrible sisters, the Graeae or 'Grey Ones', who shared a single eye and a single tooth between them. By seizing the eye as they passed it between themselves he compelled them to tell him where he could find the Nymphs that were to give him a special set of equipment with which to face the Gorgons, namely the Cap of Hades, which conferred invisibility upon its wearer, winged sandals that would enable him to fly to the impossible land that was the Gorgon's home and a special pouch, the *kibisis*, a sort of toxic container that alone could hold the Gorgon's head without being turned to stone. Duly equipped, he flew to the edge of Ocean where the Gorgons lived, finding them asleep. He decapitated Medusa with a sickle, whilst turning away from her to avoid petrifaction, but observing her image in a mirror held for him by the two gods. He fled the scene pursued by Medusa's two Gorgon sisters, but evaded them

with the help of his flying sandals and his cap of invisibility (Pherecydes F11).

As Perseus continued to flee, he passed over Ethiopia, where he saw the beautiful princess Andromeda pinned out by the sea for a marauding sea-monster to devour. This was the dreadful price her father King Cepheus had had to pay because his wife Cassiepeia had boasted that she herself was more beautiful than the Nereids, the Sea-Nymphs. Perseus fell in love with her and quickly made a bargain with her father to the effect that he could take her for his bride if he killed the monster, which he duly did (Apollodorus *Bibliotheca* 2.4.3, first or second century AD, after Pherecydes). Returning to Seriphos, Perseus used the gorgoneion to petrify Polydectes and his men, giving the throne to Dictys. He then gave the head to Athena, who henceforth wore it on her breast, mounted upon a goatskin, the 'aegis.' Perseus tracked his grandfather down to Larissa in Thessaly, where he reconciled with him, but then killed him accidentally with a discus, the Delphic prophecy thus being fulfilled (Pherecydes FF11-12).

51.2.2 Origins, Innovations and Transformations in Antiquity

Let us look now at some of the more significant Medusa-related variants, embellishments and additions to this core. The origins of the myth of Medusa are complex and controversial. The iconographies (as opposed to the mythologies) of Near-Eastern cultures seem to have been part of the mix, as do architectural *gorgoneia*, the hideous, leering, fanged faces that the Greeks mounted around their temple roofs to repel evil spirits and the so-called evil eye from the buildings. These are found from ca. 675 BC onwards (*LIMC* Gorgo 1–79). There may have been a sense in which Medusa had a detached head before she ever had a body, or, indeed, a story.

However, two images made within 25 years of the first known *gorgoneia* illustrate different stages of the now established decapitation episode. One of them, on a Proto-Attic amphora, is noteworthy for being the first to attach to the Gorgons' heads the snakes that were to become so central to their identity (LIMC Perseus 151). The other, on a Boeotian relief jar, uniquely and remarkably depicts Medusa as a female centaur (Fig. 51.2). However, traces of equine affinities may be found for her elsewhere in her tradition. When Polydectes demanded that Perseus bring the head of the Gorgon to his contribution feast, he had demanded of other Seriphans merely that they should bring a horse (Pherecydes F11). Medusa, as we will see, comes to be loved by Poseidon, the patron god of horses. And when she was decapitated one of the children born from her severed neck was the winged horse Pegasus (Hesiod *Theogony* 270–83, seventh century BC), the horse upon which Bellerophon was to defeat another partially serpentine monster, the Chimaera (Pindar *Olympians* 13.63–6 and 84–90; cf. *Isthmians* 7.44–7). The notion that Perseus himself should have ridden Pegasus to fight Andromeda's sea-monster is an erroneous modern one.

A detail the tradition could never settle upon is the mechanism of the Gorgons' petrifaction. On the one hand, Perseus' averting of his own gaze as he attacks Medusa (first found on the centaur vase) and then his use of a mirror to track her (first in Pherecydes) imply that petrifaction occurred when their victim fixed his eyes (directly) upon the Gorgons. On the other hand, Perseus' use of the invisibility-conferring Cap of Hades (first attested in the sixth-century BC Hesiodic *Shield* 216–37), and his attack upon Medusa whilst she was asleep (first in Aeschylus *Phorcides* FF261-2 *TrGF*, earlier fifth-century BC) imply rather that petrification occurred when the Gorgons fixed their gaze upon their victim. There were different conceptualisations too of the way in which a victim's body became petrified. Vase images of the mid-fifth century BC show Polydectes being encased in a sheath of rough rock that grows over him from the ground upwards (*LIMC* Polydektes 7–8). But for Ovid the Gorgon's victims were instantaneously frozen into perfect statues of themselves (*Metamorphoses* 4.780–91, 8 AD). A notion that only becomes explicit late in the tradition is that one of Medusa's immortal Gorgon sisters at any rate, Euryale (the other was Sthenno), could kill with her bellowing voice as well as with her gaze (Nonnus *Dionysiaca* 30.264–7, ca. 400 AD).

One of the most striking innovations in the tradition is the development of the notion that Medusa's face was in itself, far from being hideously ugly, a beautiful one. Pindar is the first to tell us that Medusa was 'fair-cheeked' (*Pythians* 12.6–26, 490 BC), but we must wait until Ovid's *Metamorphoses* for a back-story. Here we learn that she had once been a beautiful human girl with particularly attractive hair. This had attracted the attention of Poseidon, who, in the form of a bird, had seduced or raped her in the temple of Athena. Angry at the desecration of her temple, the goddess had transformed that beautiful hair into a mass of writhing snakes (4.794–803, 6.119–20). Later the Virgilian commentator Servius gave this episode a different spin: Medusa's head was turned by Poseidon's flattering attentions, and so she boasted that her hair was fairer than Athena's, whereupon the goddess transformed it in resentment (on *Aeneid* 6.289, fourth-century AD); there are flavours here of the episode of Cassiepeia and the Nereids.

Finally in this section, let us consider some more specific traditions about the transformations Perseus effected with Medusa's decapitated head. Already from the fifth century BC the ever firmer tendency was developing to locate the home of the Gorgons in North Africa (e.g. Herodotus 2.91). One consequence of this was the association of Perseus with the creation of Mount Atlas (in modern Morocco). Although there are indications that some version of this tale too existed

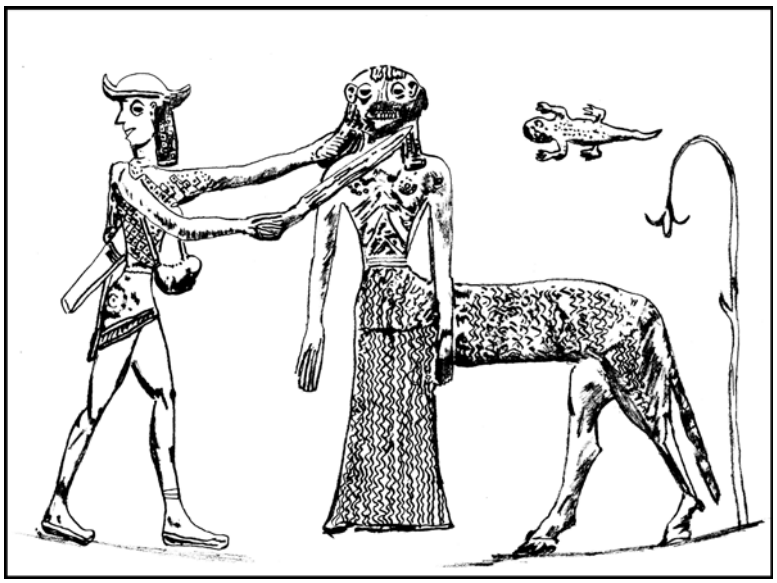

Fig. 51.2 Perseus decapitates a centaur-bodied Medusa. Cycladic relief pithos, ca. 675–50 BC. Paris, Louvre CA 795. *LIMC* Perseus no. 117. Redrawn by Eriko Ogden

already from the fifth century BC, we once again depend upon Ovid for our earliest proper account of it. According to this the giant Atlas was king of the world's western extremity. After killing Medusa Perseus came to him for shelter, declaring his credentials as a son of Zeus. This affiliation misled Atlas into identifying him as Heracles, who, a prophecy had told him, would one day steal his golden apples, and so he drove Perseus off with violence. In revenge Perseus showed him the Medusa-head, thereby transforming the giant into a mountain of commensurate size (*Metamorphoses* 4.621–62). The Medusa-head was also used to explain another of North Africa's distinctive phenomena: its plagues of terrible snakes. These were held to have been generated from the drips of blood that fell from Medusa's freshly decapitated head as Perseus first escaped aloft with it (Apollonius *Argonautica* 4.1513–17, ca. 270–40 BC).

The earliest (iconographic) source for Perseus' battle with Andromeda's sea-monster has him fighting it with rocks even as he has Medusa's head slung over his arm in its *kibisis* (*LIMC* Andromeda 1). We have to wait until the fourth century BC for the obvious to occur. Here at last a tiny but eloquent fragment of an Etruscan vase shows Perseus attacking the sea-monster with his trusty sickle in one hand and the unsheathed Medusa-head in the other (*LIMC* Perseus 192). Only in the imperial period does the notion emerge in the literary sources (e.g. in Antiphilus at *Greek Anthology* 16.147).

We hear surprisingly little in the ancient sources about Perseus' death, but one late tradition stands out here. The chronographer John Malalas tells that Perseus eventually found himself at war with his now elderly father-in-law Cepheus and carried Medusa's head into battle against him. However, Cepheus was blind and so the head had no effect upon him (Malalas evidently subscribes to the man-sees-Gorgon model of petrifaction). Perseus, mistakenly thinking the head was defunct, turned it to his own face and was instantaneously petrified (*Chronicle* 38–9 Dindorf, sixth-century AD).

51.3 The Ancient Story: Whose Story?

As we have emphasised, in common with all classical myths, there is no one ancient version of the beheading myth. However, there are some recurrent themes which particular tellers emphasise or develop. This section considers the interplay between variation and consistency by looking at the beheading from three different perspectives: as part of the story of Perseus, as part of the story of Athena, and as part of the story of Medusa herself.

51.3.1 Perseus' Story

The beheading of Medusa is the main accomplishment of Perseus who, like many Greek heroes, goes on a quest. His destination is a site on the borders of the known world – the land of the Gorgons, the literal 'Medusa and her sisters' of this book's title. As we outlined above, Perseus is aided by various helpers and gifts until, reaching this land, he kills Medusa. A black-figured jug by the Amasis Painter dating to ca. 550–530 BC (Fig. 51.3) shows the hero grabbing his

Fig. 51.3 Perseus slaying Medusa in the presence of Hermes. Black-figured olpe by the Amasis Painter, ca. 550–530 BC. London, British Museum

victim around the neck with one hand while thrusting his sword into her neck with the other. After Perseus decapitates Medusa, he puts the head in a bag, and flies away both from the faraway land and from the grieving and furious sisters, thanks to the winged sandals of Hermes and to the cap of Hades.

Although Medusa is described as the sole mortal sister (e.g. at Hesiod, *Theogony* 277, seventh-century BC; Apollodorus 2.4.3, first or second-century AD), the head retains some kind of living force, and Perseus utilises this on several of his subsequent adventures until he performs two acts, each of which rounds off the story in particular ways. First, he uses it to petrify Polydectes, the 'evil guardian' who sent him on the quest in the first place (while also effecting yet another rescue of a woman in distress, his mother Danae). Second, he presents it to Athena and thus resolves another aspect of the story: Athena, who had turned Medusa into the Gorgon, now gains the head of this monster she had created as a trophy.

51.3.2 Athena's Story

The story is that of Athena as well as that of Perseus. Indeed, as Athena's story it is potentially more multi-layered. The goddess sets the story in motion when, in anger at some perceived offence by Medusa, she turns the young female into a monster, either because Medusa had sex in her sanctuary, or because, according to Apollodorus, 'the Gorgon had claimed to rival the goddess in beauty' (2.4.3). Indeed, according to Apollodorus, **'**There are some who say that Medusa was beheaded because of Athena' (ref. cit.). Athena's reaction to Medusa's actions bears comparison with other furious responses by goddesses to perceived offences by mythological females, including one by another victim of a sexual offence in a temple of Athena: Auge (who - like Perseus - is, in some versions, cast adrift at sea along with her child.[2]) Once Perseus has left Polydectes, Athena gives hands-on help to her protégé by equipping him for his quest.

The beheading also has the aetiological (explanatory) function of showing the origin of the Gorgon's head on Athena's aegis, itself a snake-fringed object which protects the wearer by inducing fear in others. According to some ancient sources, the aegis was originally a monster or giant whom Athena killed and whose flayed body she transformed into the object. At Euripides *Ion* 987–997 this monster is actually the Gorgon. Here, the goddess is not just responsible for the killing of the monster, but is herself the Gorgon-slayer (*gorgophona*: Euripides, *Ion* 1478). When roused to anger, Athena could become *gorgopis*, 'Gorgon-eyed' or 'Gorgon-faced,' as when she unleashes her anger against Little Ajax for attacking both Cassandra and the image of the goddess in her temple at Troy (see esp. Alcaeus fr. 298). Thanks to Athena, the Gorgon becomes a disembodied, terrible face, but that face is also the face the goddess displays when her fury is unleashed against an assailant.

51.3.3 Medusa's Story

As well as being the victim of Athena, and the object of the quest of Perseus, Medusa has her own story. As described in the earliest source for this story (Hesiod, *Theogony* 270–281) she is the offspring of Ceto and Phorcys and the sister of Sthenno and Euryale who 'suffered banefully' – as she could because she was mortal and because she had sex in an improper place, in this source not a sanctuary of Athena but a spring meadow, a location where gods elsewhere encounter mortal women, but where they do not actually deflower(!) them. Intercourse is supposed to take place somewhere else, the place where the woman's offspring will be born. Conversely, Medusa's children remain stuck inside her body. Thus, as well as an act of revenge by Athena, and an act of heroic achievement by Perseus, the beheading is an act of release. Perseus and Athena act as midwives, rather like

[2]On Auge and related stories, see McHardy (2008).

Hephaistos when he releases Athena from the head of Zeus.[3] By severing Medusa's head, Perseus enables her children to be born out of her neck – children that are adult born like Athena was. One is a winged horse, the other a warrior named 'Golden-Sword' (Chrysaor). These are at once denied a relationship with their mother and depicted along with her. For example, Medusa is shown fully bodied on the pediment of the temple of Artemis on Corfu (ca. 580 BC), alongside the smaller, but adult-proportioned Chrysaor. Her other son, Pegasus, was originally on the viewer's left.

The beheading of Medusa is part of the quest of Perseus, part of the myth of Athena, and also part of the story of a violated woman whose birth pangs resulted in the birth of children from her neck while her severed head remained in a permanent, agonised scream.

51.4 Interpretations from Antiquity to the Renaissance

Complex myths give rise to complex traditions of interpretation. Attempts to make sense of Medusa appeared even in antiquity, and continue to this day. What is notable about such attempts is their plurality: just as mythic narratives appear in any number of forms, so too the meaning of a myth is never singular or stable. We should not think of myths as having essential significances, just as they seldom have canonical forms. The significance that each listener, reader, or commentator finds in a story is always partial. It highlights particular aspects of the myth, and ties it to particular ideas. In this way, interpretations of myth typically reflect the interpreter's own interests – and the prevailing interests of his or her culture – played out with the myth as an illustrative paradigm. The kinds of mythical exegesis which we outline below served in part to keep Greek mythology relevant to new audiences by constantly 'updating' the meanings of these stories to fit changed social, literary and religious contexts. These exegetes found useful new morals and knowledge in traditional stories; they also showed off their hermeneutic prowess by presenting to the reader a familiar story in a surprising new light.

This section traces some particular modes of interpretation apparent in antiquity, the Byzantine and medieval periods, and the Renaissance.[4] Although attempts to explain the myth of Medusa proliferate in the literature of these periods, we focus on two main themes: firstly, commentary on the effects of petrification as a reaction to an alluring sight or experience (a reading common in allegorical approaches); and secondly, rationalistic readings of the story of Medusa as an actual historical event.

51.4.1 Allegories of Beauty and Lust: From the Italian Renaissance to Antiquity

As we have seen, there was an ancient story which told of Medusa's original transformation from a beautiful woman to a hideous monster. This facet of the story proved highly productive for later interpretative retellings. The *Cité des Dames* (1405) of the Venetian writer Christine de Pizan is a description of famous women of the classical and Christian traditions which highlights their virtues and achievements. In it, we see mythmaking and mythography harnessed in parallel (Blumenfeld-Kosinski 1997: 205–6). Christine's Medusa is the daughter of King Phorcys and is so renowned for her mesmerising beauty – including long golden tresses – that she was said to make people incapable of moving as she gazed on them. Christine's reading of this myth foreshadows in spirit – but certainly not in form – the feminist approaches which sought to bring a new perspective to the story in the twentieth and twenty-first centuries, and which we explore below. Her insistence on the power of Medusa's *beauty* is not the surprising element here (as we have seen, that has clear classical antecedents). Rather, what is striking is her ability to make this figure a wholly positive paradigm of femininity.

In medieval and Renaissance allegorical traditions, Medusa was more commonly held up as a symbol of moral danger to men. So, Isidore of Seville (d. 636) branded the Gorgons 'harlots' (*Etymologiae* 11.3.29). From this association, more complex allegories formed. Pierre Bersuire (ca. 1290–1362) made them 'bad and beautiful women' (*Ovidius Moralizatus* cap. 5 [Ghisalberti p. 118]). Christoforo Landino (1424–1498) argued that Medusa's head represents 'the allurements of passion' which capture the foolish, but which the prudent overcome 'with Pallas' shield and Mercury's sword' (*Disputationes Camaldulenses* 235–6). In his encyclopedic *Mythologiae*, Natale Conti (1520–1582) made the story of Perseus' defeat of the Gorgon an allegory for the defeat of lust by reason (7.11). Such allegorical readings, which found philosophical truths in stories of the ancients, did not function merely as impositions onto the myths they explained. Rather, they became inextricable parts of these stories. In this way, Medusa was surrounded by an ever more complex set of associations.

The conventions of courtly love poetry, which depicted a man in the thrall of a beautiful woman, found an obvious paradigm in the story of Medusa which provided a classical parallel for the 'petrifying' effects of love on men, and a female figure which typified threat, attraction, and metamorphic power. Given the allegorical associations of Medusa in the late antique and medieval commentary tradition,

[3] On the possible connections between rape and difficult labour, see Bachvarova (2013). On the birth of Athena, see Deacy (2008): 17–32.

[4] For post-antique receptions of Medusa, see the material collected in Garber and Vickers (2003). Some general commentary on medieval and Renaissance treatments can be found in Ogden (2008): 131–8, Leeming (2013): 30–43, Pàmias (2014): 52–62

however, Medusa's appearance in love poetry can as easily undercut the conventions of amorous discourse as support them. We see this clearly in the *Rime Sparse* ['Scattered poems', also known as *Canzoniere* ('Songbook')] of Petrarch (1304–1374), a collection of lyric poetry in the Italian vernacular. Petrarch four times attributes to the object of his love, Laura, the powers of Medusa (51.12–14, 179.9–11, 197.5–6, 366.111–12). On one level, this is a comment on the psychosomatic effects of love. Laura's extraordinary beauty captivates Petrarch just as Medusa's monstrous appearance had turned to stone anyone who gazed on her. The paradox of beauty and monstrosity effecting similar ends is not lost in these complex lyrics. Indeed, Petrarch's final canzone offers a prayer to the Virgin Mary which confesses that 'Medusa e l'error mio m'an fatto un sasso d'umor vano stillante' ('Medusa and my mistake transformed me into a stone dripping with vain moisture' – 366.111–12). Here Petrarch's susceptibility to Laura's sensual charms (l'error mio) is finally repented as an obstacle to spiritual progress.[5]

Petrarch's immediate model in invoking this problematic figure of Medusa is Dante, another poet who pointed to the disjunction between the secular pleasures of love poetry and the ethical and philosophical demands of Christianity. In the ninth canto of his *Inferno*, Dante and his guide Virgil cannot proceed within the gates of the city of Dis. They encounter three snake-haired Furies, who command Medusa to appear; Virgil protects his charge by covering Dante's eyes with his hands. At this point, the author addresses his readers directly: 'O you who have sound intellects, gaze on the teaching that is hidden beneath the veil of the strange verses' (*Inf*. 9.61–3 trans. R. M. Durling).

The ironical nature of this command is striking: Virgil had just warned Dante not to look; now Dante advises his readers to 'look harder'. What the reader is to find 'beneath the veil of the strange verses' has been a matter for great debate.[6] Dante's *Commedia* is one of the most complex texts of the medieval period. Through the narrative of a journey through hell, purgatory and paradise, Dante creates an ambitious allegory whose polysemic dynamics repay close study. Among the various interpretations of the 'hidden teaching' referenced by Dante in this passage, John Freccero's classic article ties Medusa's threatened appearance into the nexus of associations that we have been examining (Freccero 1972). He argues that Dante's message here is that only the wise man who averts his gaze from Medusa can indeed 'see' the teachings which underpin true spiritual conversion. Medusa embodies the sensory temptations of erotic attraction. In his youthful Rime petrose, Dante had written of his obsession with the Donna Pietra ('Stony Lady') and her petrifying effect on him. The passage in the *Inferno*, then, functions in part as Dante's recantation of that kind of love in favour of the divine love represented by Beatrice.

This allegorical tradition assumes a physically beautiful Medusa, whose outer appearance is alluring but whose inner nature is dangerously monstrous in that it harms the moral fabric of the spiritual being. The allegorical argument that Medusa's ability to petrify illustrates the ethics of attraction and desire takes its particular dynamic from the context of Christian doctrine; the broader idea that the experience of petrification is a reaction to beauty rather than monstrousness has, however, a much longer history. A number of Greek authors from the Roman Imperial period riff playfully on this theme. The satirist Lucian (ca. AD 120–180) enjoys surprising his readers by turning myths on their heads. In *The Hall* he points out that Medusa's beauty was so stunning that it rendered men speechless. In another work, *Portraits*, one of his characters alludes to a more specific effect of female beauty on the male body: the sight of a beautiful woman is 'petrifying' as it gives him an erection (1). The exegete Heraclitus (first or second century AD) makes Medusa a prostitute: again, her beauty was such that it rendered men incapable of escaping her. But at this point Heraclitus adds something new: she fell in love with one of her clients, Perseus, and was ultimately 'destroyed' by her affection for him (*On Unbelievable Tales* 1). Here then, we have an ancient interpretative tradition which also highlights Medusa's physical allure, and which explains her metamorphic powers in psychosomatic terms, but which lacks entirely the ethical and religious dimensions of the later readings.

51.4.2 Medusa as Historical Queen: From Antiquity to Renaissance England

Heraclitus' interpretation is best understood not as part of the ancient allegorical tradition, but as an example of rationalisation, since it seeks to uncover in the language of the myth a record of some historical event.[7] We see a similar attempt at this in the work of the travel writer Pausanias (second half of the second century AD). Pausanias describes a mound at Argos where, it is said, the head of Medusa was buried. He does not believe that this could be the case: Medusa, he says was in fact the leader of a Libyan tribe. She was killed by Perseus' troops, and he cut off her head and took it back to Greece to show others her beauty (2.21.5). The historian Diodorus also places the Gorgons (he describes them as a

[5] Sturm-Maddox (1985): 32–4, 66–7, 152–4, Sturm-Maddox (1992): 62, 119–20.

[6] On the hermeneutic dynamics of this passage, see Franke (1996): 82–113.

[7] On ancient traditions of rationalisation and allegory, see Hawes (2014): esp. 23–36.

race of female warriors) in Libya, alongside the Amazons (3.52.4). The activity of manipulating myths to make them seem historically plausible was especially associated with a mythographer of the 4th c BC called Palaephatus. In his *On Unbelievable Stories* he includes a detailed reworking of the story of the Gorgons. These three sisters, he says, each ruled over their own island. They all used the services of a single attendant. Because this man's name was 'Eye', the story arose that they shared a single eye between them. Perseus kidnapped this man, and killed one of the sisters, Medusa, when she refused to give up a statue called 'Gorgon'. After finally getting his hands on this 'Gorgon', Perseus attached its head to the front of his ship and continued his piratical raids. When some villagers set up stones in their marketplace to cover their escape, Perseus turned this to his advantage, warning others he came across of his powers of petrification.

We can see in Palaephatus' account the bare bones of the Medusa narrative now re-cast in an innovative light. Although the ancient rationalisations of the story of Medusa differ greatly, most agree on certain basic premises: they make Medusa a powerful ruler, and they make Perseus' defeat of her an act of military or piratical aggression. In this way, the story seems to allude to a shift in political power. This implication comes to the fore in the *Chronicles*, an attempt at world history written by the Byzantine scholar John Malalas in the middle of the sixth century. Malalas recasts the heroes and gods of Greece as kings and adventurers: Medusa, he says, was merely a Libyan girl 'with wild hair and eyes' that Perseus encountered on his way to conquering Libya. He beheaded her, performed magical rites over the head, and carried it with him to secure victory (2.14, 16). Malalas' bloodthirsty Perseus is, however, unusual. John Harington's historicisation of the episode (in the preface to his translation of *Orlando Furioso* [1591]) has him again as the hero: his slaying of Medusa represents the warranted slaying of a tyrant. We might mention one further use of the myth of Medusa in this context. The English statesman Francis Bacon used Perseus' defeat of Medusa in his *Wisdom of the Ancients* (1609) to illustrate some principles of judicious warfare: most notable for our purposes is his argument that war must be justified, as indeed the defeat of a tyrannical ruler whose people have been subjugated to the point of – metaphorical – petrification would be. Similarly, he continues, one must 'pick one's battles': when Perseus chose to attack the only mortal Gorgon, he taught us that one should only engage in battles that one has a chance of winning.

Just as we can trace elements of later allegorical readings back to ancient antecedents, so too can we see that some of the rationalistic readings offered by Greek writers provided a paradigm for the treatment of Perseus as a victorious leader in Renaissance texts. Myths are not only stories; they serve also as a shared store of common knowledge: Bacon can use the story of Perseus to illustrate ideal military tactics of his own time in part because the continued popularity of this ancient story was assured through an unbroken tradition of interpretation and storytelling. This tradition did not transmit Greek myths frozen in time, however. It lent them meanings that were both pluralistic and continually being updated to suit new contexts. And it is to the pluralistic capacity of the myth in more recent times that we will now turn.

51.5 Medusa and Her Beheading in the Modern Worlds

Medusa, her metamorphosis to monster, her murder by Perseus and her post-mortem weaponisation have found countless recastings and resurrections not only in those ancient, medieval and Renaissance worlds we have explored but also in more recent eras. Indeed, modern manipulations of her myth are too numerous and varied to recount in full here. More detailed explorations can be found in, *inter alia*, Wilk (2000), Garber and Vickers (2003), Ogden (2008: 131–143), and Leeming (2013). For the purposes of this piece, we will explore three thematic areas of utilisation: Medusa as other, Medusa and the gaze, and reclaiming Medusa.

51.5.1 Medusa as Other

In Cohen's (1996) theory on monsters and culture, emphasis is placed on the alterity for which the monster stands. The monster, he claims, 'dwells at the gates of difference' (7), representing an other often based on cultural, political, racial, economic and sexual norms. So it is with Medusa. In her simplest guise, she is made to stand for that ultimate other: death. Whether placing her geographically in the underworld (as in Milton's *Paradise Lost*; cf. also Neumann (1954: 213–219)), whether claiming an historical basis for her in pre-classical death goddesses (Gimbutas 1989: 205–208), in apotropaic rituals featuring masks of underworld demons or deities (Harrison 1903: 187–196; Croon 1955) or in decomposing corpses (Wilk 2000: 183–192), or whether rationalising her as a symbol for natural disasters such as volcanoes and storm-clouds (Roscher 1879; Kaplan 2012: 65), she embodies that which we fear most. However, it is not only in the alterity of death that Medusa emerges. We find her as political other when she is made to symbolise the dangerous anarchism of revolution (Hertz 1983), as racial other in historicist readings that claim her origins to be found in

Moroccan Amazons or African queens (Sjöö and Mor 1987: 209–210; Goodrich 1989: 179; Wilk 2000: 220), as religious other in the anthropological writings of Obeyesekere (1981) and as economical other when she is made to stand for the hidden evils of capitalism (Marx 1867: X).

It is perhaps, though, as sexual other that Medusa has most commonly been put to use. We have already seen such a reading in Renaissance and earlier receptions of the myth. In more recent times, we find the most famous example in Freud's theory of the Medusa head (Freud 1940, originally written in 1922). According to the psychoanalyst, the myth of Perseus beheading Medusa symbolises the castration complex present in young boys. 'To decapitate,' Freud tells us, '= to castrate.' Further, Medusa's head, with its gaping mouth and serpentine (read pubic) hair, stands for the female genitalia; that is, for Freud, the castrated male. Arousing castration anxiety, the sight of Medusa terrorises; it also, however, reassures: the hair of snakes is made to symbolise multiple phalluses whilst the petrifying gaze of this female monster is said to remind the viewer of the male erection. In short, Medusa represents both the fear of the female and, in her defeat, the reassurance of the masculinity of the male. Such a reading has proved immensely popular over the years (see Ogden 2008: 135 and 165n3 for a summary), and has inspired such artwork as Marcel Duchamp's arresting *Étant doneés* (1946–1966; perhaps also inspired by Gustave Courbet's 1866 *L'Origine du monde*).

Medusa symbolising fear of the female other is not, however, limited to the psychoanalysts and their adherents in the modern worlds. Consider Goethe's *Faust* of 1808. Here we find Mephistopheles warning Faust away from Gretchen, a girl of whom he is enamoured. The beauty of Gretchen, Mephistopheles claims, is an illusion. The reality is a Medusa, a dangerous entity who leads men to their doom. Here female is both beauteous and monstrous: beauty, though, is the illusion; the monster, the dangerous destroyer of man, is the reality. Similar is Percy Bysshe Shelley's 1819 *On the Medusa of Leonardo da Vinci* which talks of the 'tempestuous loveliness of terror,' the aspect of Medusa that attracts men like moths to a flame; that is like men to their death. Goethe and Shelley are but two of many (see further examples referenced in Conley 1996: 110; Wilk 2000: 200; Rowe 2001 as well as the ancient and medieval examples explored above) who articulate woman as a dangerous attraction, a siren figure to be either avoided or destroyed, the fear of whom is articulated through monsterisation.[8]

For Cohen this is the precise purpose of monsterising the other: by demonising that which is not of the norm, that which trespasses the boundaries of 'I' or 'us', we invite action (1996: 13), action – here the beheading by Perseus – which minimises the threat the other poses – here the threats of death, of revolution, of capitalism, or of the sexualised woman.

51.5.2 Medusa and the Gaze

Medusa should not, however, be read simply – or rather only – as a generalised, monsterised other. A key theme can be seen to run throughout the othering activities noted above: that of seeing and miss-seeing. So it is that Medusa is connected to the mask in the othering of death: what we see is not the figure underneath. So it is also that she features as the hidden dangers of capitalism: we have to penetrate the illusion to see the reality. And so it is that she is the dangerous reality unseen or hidden behind the beauty of woman. It takes a Perseus to see true.

It should come as no surprise that a myth itself comprised of multiple layers of sight and illusion (the Graeae's one eye which Perseus steals, the cap of invisibility which he wears, the mirror by which he views the Gorgon and, of course, the petrifying gaze of Medusa which he wins) finds echoes in theories bound up in seeing and understanding, and in concepts of the gaze. Let us take Sartre's influential *Being and Nothingness* (1943) as our first example. In this philosophical work we find Sartre using the Medusa myth to explain consciousness of being.[9] In our non-conscious state, Sartre argues, we exist as pure subject to whom all around – being and non-being – are objects. It is the gaze of another upon us, and our awareness of that gaze, that brings us to realise that we exist as objects for other subjects. That is to say that brings us self-consciousness of our existence in a wider scheme of reality. Here Medusa's gaze is made to symbolise that moment of self-consciousness, that feeling of another's gaze, and our awareness/meeting of that gaze, that immobilises us in our moment of understanding.

This importance of the gaze has been taken up especially in art and film theory. Consider Marin's exploration of Caravaggio's *Medusa* (1977). Clearly inspired by Sartre, Marin details the arresting effect of full-face portraits (119–122). He argues that in meeting the gaze of the figure represented we become entrapped – frozen – in an inescapable war for clarification of subject and object: is 'I' the viewer perceiving the portrait or is 'I' the figure portrayed perceiving the viewer? This is a war avoided in Caravaggio's 1590–1600 *The Head of Medusa*, and in many other portrayals of the monster, owing to the misdirected gaze of the painting's subject: she tends to be portrayed looking beyond or away

[8] So it is that we later find Versace co-opting the head of Medusa for his line in order to signify 'dangerous attraction' (Garber and Vickers 2003: 276) or Damien Hirst turning the singer Rihanna into Medusa for the 25th anniversary cover of *GQ* (December 2013) to signify her as a 'badass': here there is no fear, only celebration.

[9] For an excellent exploration of Sartre's theory see Barnes (1974).

from the viewer. Contrast this with Fernand Khnopff's 1898 *Blood of Medusa* in the Bibliotheque Royale Albert 1er, Brussels: full frontal, Khnopff's Medusa captures our gaze, ensnaring us with her petrifying eyes.

How then can we safely gaze at Medusa? Kracauer (1960: 305–306) explores such a question in his theory of film. For Kracauer, Medusa represents the horrors of reality, the terrors that on viewing would 'paralyze us with blinding fear' (305). The greatest achievement of Perseus, he argues, is not beheading Medusa but in looking at her, a feat he achieves through Athena's shield. Like Perseus, we too are able to confront terrors through a mirror that deflects the petrifying gaze of reality. For us, this mirror is the cinematic screen. By looking through cinema, Kracauer argues, we can defeat: we can behead and exorcise that which terrorises.

A different approach to Medusa and the gaze can be found in disability studies. Combining Sartre with Lacan, disability studies explores the petrifying nature of the gaze in relation to Lacan's fragmented and unified sense of self. Let us take Davis (1995: 126–157) as our example. Moving away from the deficit model of disability, Davis underscores its cultural context, that is to say the model whereby disability exists not in the body but in the disabling gaze. Here Medusa is that which we perceive as disabled: her body is disfigured and fragmented in relation to the ideal body's supposed (and false Lacan argues) unity. The unified body (here 'I') perceives the fragmented body (here 'You') and is frozen, turned to stone by the visual interaction. The petrification here is not simply in the war between subject and object, but in the power of the fragmented other, a power that 'I' must decapitate and contain through rationalising as 'disabled'.

51.5.3 Reclaiming Medusa

Key to both the articulation of Medusa as other and the use of Medusa in the question of the gaze is the presumption of an 'I' or 'us' against whom the other or 'You' is extrapolated. In both sections this 'I' is often white, Western, non-disabled and male: the 'I' is Perseus. What happens when 'I' is Medusa herself? It is this question that has driven many feminist uses of the Medusa myth. Consider De Lauretis' feminist exploration of cinema (1984: 134–136). Continuing Kracauer's theory of Athena's shield standing for the silver screen, De Lauretis asks us how Medusa, that is the female viewer, feels viewing herself on screen considering the vision portrayed is one alien to such a viewer constructed as it is by a patriarchal medium. Developing this, Rowe (2001) advocates the creation of Medusan films that allow for female autonomous subjectivity. This theorist sees Medusa as the 'unruly woman' free from a patriarchal society's constructs.

This aligning of Medusa-female-matriarchy against Perseus-male-patriarchy has precedence in the field of Classics and Ancient Religion in the historicist reading of the myth as a narration of the destruction of a supposed pre-Hellenic matriarchy by the Greek patriarchal system (Graves 1955: 244) or as originating in initiatory cults (in which, based on readings of the Artemis temple in Corfu, Medusa is made to figure as a goddess alongside Artemis) that were orientated around Medusa's supposed role as both danger to and protector of men (Marinatos 2000). Perhaps though, the strongest influence on such feminist readings of cinema/Medusa derives from Hélène Cixous' 1976 *The Laugh of the Medusa*. Influenced by Freud, Cixous reclaims Medusa from the psychoanalyst proposing that it is the male who is the deformed to the female viewer and that the female (Medusa) is only deadly because Perseus refuses to meet her gaze. She has been robbed of her voice and subjectivity – that is decapitated – by men who refuse to see that she is not monstrous but beautiful: she is laughing.[10]

Laughing she may be, but this is not how many women have taken Medusa on. Rather, it is Medusa's rage, her monstrosity rather than her beauty, that we find adopted. So in poetry we find May Sarton in her 1971 *The Muse as Medusa* looking at the monster, turning the face around and seeing that 'It is my face. That frozen rage is what I must explore' or we see Carol Ann Duffy in her 1999 poem *Medusa* portraying her protagonist becoming a Gorgon, owning the Medusa's monstrous visage and petrifying abilities as jealousy consumes her. In art we find Christina Biaggi's 1998 *Raging Medusa* (Fig. 51.4), a sculptural depiction of the monster in all her anger representing, Biaggi tells us (2010), 'the rage of women duped and enslaved by patriarchy.' Similarly, we find Medusa featuring on the front covers of such publications as Valentis and Devane's (1994) *Female Rage* and Dykewoman's (1976) *They Will Know Me by My Teeth*. It is this rage and teeth that we find carefully articulated in Emily Culpepper's narration of Medusa's rage (1986), a narrative that tells of her own metamorphosis into a gorgonic figure on the occasion of an attack at home. No longer the female other, the dangerous other, the monstrous other: these women have reclaimed Medusa in all her monstrous guises as representing themselves, their own rage and their own power. Indeed, as Wilk (2000: 219) argues, it is not general female rage that Medusa is made to represent here but 'empowered feminine rage': rage that can act and assert subjectivity and dominance over that which they believe to have been subjected, rage that reverses their decapitation.

These are, of course, just a few examples of the ways in which Medusa has been used, adapted and reclaimed by

[10]The influence of this theory can be seen in the deliberate naming of a volume on feminism and the classics after Cixous' work (Zajko and Leonard 2006).

Fig. 51.4 Cristina Biaggi. *Raging Medusa*, 1998. 5 ft diameter, fiberglass with patina finish. CristinaBiaggi.com. Photograph, Eileen Brady Nelson. We are grateful to Cristina Biaggi for permission to reproduce her work and to Robin Mooring for arranging the permission and photograph

artists, scholars and theorists over the centuries and much more could be said for example of black and minority ethnic or lesbian reclaimings of the monster or of the highlighting of the rape of Medusa (Garber and Vickers 2003: 135–136, 232–237, 272–273), her doubling with Athena and Perseus (Siebers 1983), her recasting into Christian guises (Ogden 2008: 136–138), or even her portrayal on the cinematic screen (Wilk 2000: 202–214). Beyond narrative and iconography, Medusa, her monsterisation and her beheading has once again become a mask, a veneer waiting to be inscribed and adorned and made to represent whatever the wearer desires.

51.6 Summary

Our exploration of the beheading of the Gorgon has taken us from the earliest ancient Greek sources, and further back still to their possible Near Eastern antecedents. From here we have moved through evidence from ancient Rome into the myth's rich postclassical afterlife. We have shown that it has always been a complex myth, and we have traced the many ways in which it has been used, transformed, appropriated and hybridised. We have considered its role as a stand-alone motif – indeed, we have noted that Medusa's detached head might even predate her body or her story. We have also considered how it interrelates with other stories including those associated with Perseus and Athena. As we have shown, its appeal for the ancients included its aetiological potential, for instance to explain how and why a disembodied head was displayed on temples and on the aegis of Athena. Since antiquity, it has served numerous functions culminating in an array of modern appropriations, sometimes contrary, often inventive – for instance it has exemplified female rage and it has stood for 'badass' femininity. This single motif has come to connote such diverse things as patriarchal power, the disabled body, the violated female body and the powerful, laughing female self. It can be a symbol of revenge, of ugliness, of fear of the other, of fear of ourselves, of monstrosity and of the human condition, and more. Its power to inspire varied interpretations and appropriations shows no sign of diminishing.

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