Interactions Between Corals and Their Symbiotic Algae

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Abstract

The mutualistic relationship between corals and their dinoflagellate endosymbionts is a key factor in the evolutionary success of hermatypic (reef building) corals. The endosymbiotic algae benefit corals in numerous ways that have contributed to the long term persistence of coral reefs over geologic time. In this chapter we review ecological and physiological aspects of the interactions between corals and their symbiotic algae in light of recent advances in our knowledge of the diversity of these symbionts. While the role of symbiont diversity in promoting coral survival during environmental bleaching events has been a major focus of recent research, its importance in other physiological and ecological contexts such as inorganic nutrient dynamics and photosynthetic carbon fluxes has received much less attention. We suggest that cost-benefit analysis is a useful approach to examine these symbioses in the context of environmental change and human impacts upon corals and coral reefs. Weighing the costs versus the benefits of the symbiotic association under specific environmental perturbations has potential for use as an indicator of the health of not only corals but indeed the whole coral reef ecosystem. Drastic changes in the stability of the symbiosis, evidenced by changes in the ratio of zooxanthellae to animal biomass in corals, may turn out to be a useful diagnostic indicator of stresses to coral reefs. By using new tools developed to assess the stability of the symbiosis, we may be better able to understand and predict the effects of future stressors and perturbations that threaten these beautiful reef ecosystems.

Keywords

Cost-benefit • Stability • Holobiont • Bleaching • Calcification

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5.1 Introduction

The mutualistic relationship between corals and their dinoflagellate endosymbionts is a key factor in the evolutionary success of **hermatypic** (reef-building) corals. In the two decades since the original publication of this chapter, there has been an explosion of research on *Symbiodinium*

We dedicate this chapter to our late mentor and friend, Len Muscatine, who was a leading figure in research on algal-invertebrate symbiosis, and who played a major role in our professional development.

(the genus of coral endosymbionts), fueled by advances in molecular genetics. These advances have the potential to better understand the adaptive capabilities of the symbiotic partners that contribute to this success. Different types or species of zooxanthellae are likely to have differing adaptive capabilities and tolerances to environmental extremes (Chap. 7) that will impact the survival of corals. Changes in the composition of symbiont populations may result in an increased ability to survive future environmental stresses. Although the increasing importance of other microbial associates in the coral **holobiont** (i.e., the coral host and all symbionts; Knowlton and Rohwer 2004) is recognized, our focus here is on corals and their dinoflagellate symbionts, generally referred to as **zooxanthellae**.

It has long been known that these endosymbiotic algae are beneficial to their coral hosts. These include faster rates of calcification, photoautotrophy as a source of nutrition, the ability to acquire inorganic nutrients, and many other attributes that are believed to contribute to the persistence of coral reefs in geologic time. Consequently, environmental and physiological conditions that result in changes in the relationship between the animal host and its symbiotic algae may have profound ecological effects. The genetic diversity of the endosymbionts that is now known to exist contributes to the coral holobiont's ability to cope with changing environmental conditions (Stat et al. 2006).

This chapter discusses the coral-zooxanthella symbiosis from the perspective of the nutrient dynamics and energetics of the association and in the context of the stability and adaptability of the symbiosis in the coral-reef ecosystem. We examine aspects of the structure and function of the symbiosis that contribute to the high rates of calcification and productivity exhibited by reef corals. We discuss some of the factors that influence the density of symbionts and hence the physiological balance between the symbiotic partners. We consider the effects of both natural and anthropogenic events on coral reef ecosystems with respect to how they might affect the stability and survival of the symbiosis. We also consider the possible costs and benefits associated with the symbiotic condition. While we refer to relevant advances in our understanding of the genetic diversity of the endosymbionts, a more detailed discussion is found in other chapters (Chap. 6). It is becoming increasingly clear that specific host-symbiont combinations play vital roles in the physiological and ecological fitness of corals. We conclude our discussion by speculating about the value of using the coral symbiotic association as a measure of the "health" of coral reef ecosystems. Other aspects of the symbiosis are reviewed elsewhere (cell biology: Davy et al. 2012; ecological diversity of *Symbiodinium*: Finney et al. 2010; infection by *Symbiodinium*: Fransolet et al. 2012).

5.2 Description of the Symbiosis

5.2.1 Coral Anatomy and Location of Zooxanthellae

For our purposes here, a brief review of coral morphological features will suffice; a more complete description of coral morphology can be found in other publications (e.g., Veron 2000; Borneman 2001). Scleractinian corals are typically colonies of polyps linked by a common gastrovascular system, although some solitary, single-polyped forms exist (e.g., *Fungia* spp.). Polyps are small, fleshy extensions of the live coral tissue covering a non-living calcareous structure of the colony, referred to as the **corallum**. Although the living tissue is normally a veneer of just a few millimeters in thickness, the calcium carbonate it has deposited over time can be meters in height or diameter (Fig. 5.1). Irrespective of the size colonies may attain, the thin layer of coral tissue itself is simply composed of two cell layers: the epidermis (sometimes referred to as ectodermis) and the gastrodermis (sometimes referred to as endodermis; Fig. 5.1). A thin connective-tissue layer, the **mesoglea**, composed of collagen, mucopolysaccharides, and some cells, separates these two cell layers. The lower epidermal layer, the **calicoblastic epidermis**, secretes the calcareous external skeleton. The upper layer of epidermis (oral, or free epidermis) is in contact with seawater (Fig. 5.1). The individual polyps form **corallites**, i.e., skeletal tubes of deposited calcium carbonate that may be connected and fused in brain corals or are interconnected by what is referred to as the **coenenchyme**. The tissues of **imperforate corals** are restricted to the surface of the skeleton. In **perforate corals**, the skeleton is penetrated by pores containing live coral tissue, but even in this case living tissues do not extend deeply into the corallum, and diffusive exchange of oxygen and other metabolites appears to occur quite readily, aided by many cilia in the coral's epidermal layers. Coral colonies grow by depositing new skeleton and budding additional polyps as the surface area of the tissues increases with size.

Many coral polyps are biradially symmetrical, with the central gut cavity lined by gastrodermis (Fig. 5.1). Tentacles, typically in multiples of six, surround the mouth and are used for capture of particulate food, including zooplankton. Food consumed by one polyp is shared with neighboring polyps via the gastrovascular system that functions in circulation and digestion of food particles. Polyp mouths also provide direct exchange of water and particulate food and wastes between the gastrovascular system and the external seawater.

The arrangement, density and size of polyps are characteristic for each coral species. However, coral species may

Fig. 5.1 The coral symbiosis. A cross-section of a single coral polyp from a coral colony is shown in the upper left-hand boxed *inset*. The *arrow* leading from the coral polyp points to a section through the two tissue layers of the oral surface of the polyp (see also Fig. 5.2). The epidermis is the upper tissue layer in contact with the seawater, and the gastrodermis is the lower tissue layer in contact with the gastrovascular cavity. The gastrodermis contains zooxanthellae. The *arrow* from a zooxanthella points to a cross-section of the alga. The alga is enclosed in a perivacuolar animal membrane. Internal features shown in the cross-section include the nucleus with its permanently condensed chromosomes, sections of the chloroplast with banded photosynthetic membranes, a large (*white*) vacuole, and starch and lipid inclusions
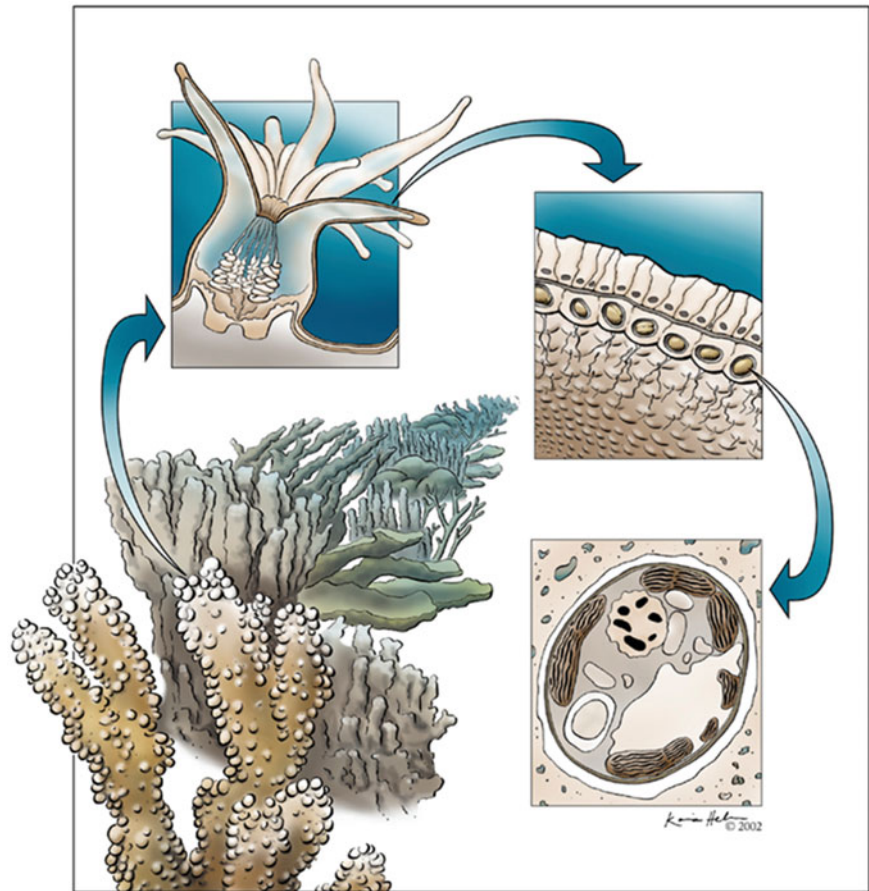

exhibit different morphologies depending on environmental conditions such as water motion and light, and this causes problems in taxonomy of corals (Veron and Pichon 1976; Veron 2000). Molecular approaches to coral taxonomy have been useful in circumventing these problems (Stat et al. 2012).

5.2.2 Zooxanthellae

"**Zooxanthella**" is a general descriptive term for all symbiotic golden-colored algae that live in animals, including corals, sea anemones, molluscs and other taxa. Although the term has no taxonomic meaning, "zooxanthellae" is used primarily to refer to dinoflagellate symbionts, a group of diverse algae. It continues to be a useful label, given the current state of uncertainty in the taxonomy of coral symbionts.

Zooxanthellae found in corals are typically 8–12 μm diameter cells that reside exclusively in membrane-bound vacuoles in the gastrodermal cells (Fig. 5.1). Their areal density normally ranges from 1×10^6 cm^{-2} to 6×10^6 cells cm^{-2} of coral surface, although this may be highly variable on both temporal and spatial scales (e.g., Fitt

et al. 2000). Seasonal differences exist in the density of zooxanthellae in corals; tropical corals during low light ("winter") months have greater numbers of zooxanthellae (Fagoonee et al. 1999; Fitt et al. 2000). More information about the variability of zooxanthellae densities within colonies and among coral species is needed, especially given the critical role of zooxanthellae in coral nutrition, and the underlying responses to coral bleaching, a phenomenon in which the host loses its symbionts under conditions of stress.

Based on early morphological studies, zooxanthellae in corals and other cnidarians were originally believed to belong to one cosmopolitan species, *Symbiodinium microadriaticum*. The genetic diversity of zooxanthellae was first discovered by comparing the morphology and growth of symbionts isolated from different hosts in culture, resulting in the formal description of four species and the realization that different zooxanthellae were found in different animal hosts (reviewed by Trench 1993; Rowan 1991; Rowan and Powers 1992). This led to the concept of symbiont specificity, with one host selecting and maintaining one type of zooxanthella (its specific symbiont) over all other zooxanthellae. This concept, too, has changed. Our ability to distinguish among zooxanthellae through genetic analyses

of molecular sequences (Chap. 6) has resulted in an explosion of different zooxanthella "taxa" (with some named as species, LaJeunesse et al. 2012) and the discovery that different taxa may inhabit the same host species and even the same host organism. With the use of next-gen and other sequencing approaches, it is becoming increasingly apparent that individual hosts may harbor multiple genotypes, with some persisting at low frequencies (Fay and Weber 2012), indicating that the symbiotic relationship is much more flexible than previously thought (Baker 2003), including the possibility that a given coral may acquire different types under different conditions. As the potential diversity of symbionts within a species or individual coral is limited, the significance of symbiont specificity with respect to the stability and ecological persistence of the coral symbiosis is a major research question.

Based on their genetic relatedness, zooxanthellae of corals and other invertebrate hosts are currently placed in nine major groups (= clades; designated A through I), and others are likely to be identified. Although members of a clade are more closely related to each other than to members of other clades, individual taxa within each clade exhibit broad genetic diversity. Zooxanthellae belonging to five of the clades (A–D and G) occur in corals. The individual genotypes of most zooxanthellae identified by their DNA sequences have yet to be formally described as new species. Those coral symbionts that have been given species designations have been assigned to the genus *Symbiodinium*.

The biogeographic distribution of members of the different clades of zooxanthellae in corals indicates the diversity of zooxanthellae is higher in the Caribbean than in the Indo-Pacific, where the vast majority of corals contain only members of clades C and D. The reasons for these ocean-basin differences are, as yet, unresolved. In the Caribbean, clade A zooxanthellae are found in high light shallow water corals, and members of clade C are found in deeper corals (LaJeunesse 2002), in the same coral or among different species. However, interpretation of ecological distribution patterns is complicated by the observation that some zooxanthellae are specialists, maintaining specific associations with only one host, while other generalist zooxanthellae associate with many hosts and have a wide biogeographic distribution. No strong relationship is evident between the types of zooxanthellae in closely related hosts, nor among the types of hosts inhabited by closely related zooxanthellae (Baker 2003; LaJeunesse et al. 2010).

The dinoflagellates comprise a diverse group of mostly planktonic, free-swimming single-celled microscopic algae that exhibit a variety of feeding modes ranging from **photoautotrophy** (photosynthetic carbon fixation) to **heterotrophy** (dissolved organic carbon uptake or feeding on particulate food). Zooxanthellae are photosynthetic and contain characteristic dinoflagellate pigments (diadinoxanthin,

peridinin) in addition to chlorophylls *a* and *c*. They are brown or yellow-brown in color. Although zooxanthellae may be heterotrophic in corals as they have been shown to take up and assimilate dissolved organic carbon from host sea anemones, their own photosynthesis seems to contribute most to their energetic needs.

Zooxanthellae can live independently of their animal host. Free-living zooxanthellae have been found in sediment and water column samples (Takabayashi et al. 2012). Many strains of *Symbiodinium* isolated from host tissues have been cultured in inorganic algal media, although the inability to culture some strains is of great potential interest. These uncultivable strains may have specific requirements for some set of as yet undefined host or other microbial symbiont conditions (including nutritional requirements) that warrant further study. Cultured zooxanthellae have been used to study growth rates and compare genetic and physiological characteristics of symbionts from different hosts. Zooxanthellae living in animal cells are usually found in the coccoid stage (non-motile, lacking flagella); this differs from the free-living motile (dinomastigote) stage that possesses two flagella (Fig. 5.2) and exhibits a characteristic swimming pattern. In culture, zooxanthellae alternate between the coccoid and dinomastigote stages, often on a diurnal pattern. The dominant dinoflagellate feature evident on the ultrastructural level is the nucleus with permanently condensed chromosomes (**dinokaryon**; Fig. 5.1).

Santos and Coffroth (2003) showed that the life cycle of zooxanthellae, like that of most dinoflagellates, is dominated by asexual reproduction of haploid vegetative cells. The high genetic diversity of zooxanthellae suggests extensive recombination, although sexual reproduction has not been documented for these algae. As these authors point out, "questions pertaining to recombination in these enigmatic dinoflagellates, such as the factors that induce it and whether it occurs inside or outside a host, remain to be answered" (Santos and Coffroth 2003).

5.2.3 Acquisition of Zooxanthellae by Corals

Young corals derived from both asexual and sexual reproduction typically contain zooxanthellae. In asexually produced (clonal) coral colonies, zooxanthellae are directly transmitted in the coral buds or fragments that form new colonies. In sexually produced corals, acquisition of zooxanthellae is either directly from the parent (vertical), or indirectly from the environment (horizontal). The method of symbiont acquisition and whether or not the eggs contain zooxanthellae are characteristics of each coral species. A confounding factor is the frequency of sexual versus asexual reproduction in each coral. A coral that relies almost exclusively on asexual reproduction (i.e., budding or
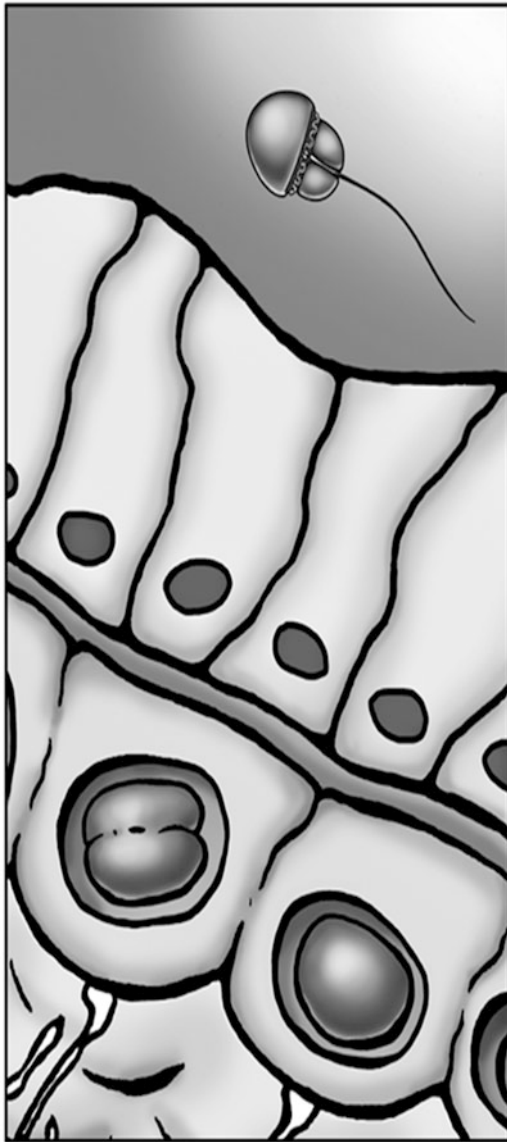
Fig. 5.2 A section through the two tissue layers of the oral surface of the polyp showing the different life forms of zooxanthellae. A free-living motile cell (dinomastigote) is shown swimming in the seawater above the layer of nucleated oral epidermal cells. The two gastrodermal cells each contain one coccoid zooxanthella; the cell on the left is in the process of dividing. The actual sizes of zooxanthellae range from 5 to 20 um, depending on species and life form

fragmentation) for propagation, where direct transmission of zooxanthellae is guaranteed, may not exhibit highly developed mechanisms for transmission of these algae during sexual reproduction.

During direct transmission via sexual reproduction, zooxanthellae are transferred to the eggs or to larvae brooded by the parent. The eggs of most species of corals do not contain zooxanthellae. For those that do, zooxanthellae free in the gastrovascular cavity may be ingested by gastrodermal follicle cells and expelled near the oocytes by passing through

temporary gaps in the mesoglea, where they are phagocytosed by the mature oocyte (Hirose et al. 2001). Alternatively, cytoplasmic extensions of the gastrodermal cells that contain zooxanthellae may invade the egg plasm, as has been described for marine hydroids (Trench 1987). These eggs may be released and fertilized in the water, or the larvae may develop from eggs fertilized and retained within the parent coral. If fertilized eggs do not contain zooxanthellae, larvae brooded by the parent through the early stages of development may take up zooxanthellae at any time prior to release. The presence or absence of zooxanthellae in eggs and planula larvae may affect their ability to persist in the plankton, as the photoautotrophic contributions of the zooxanthellae are potentially important factors in explaining the large distance of dispersion of some species (Richmond and Hunter 1990).

Corals that do not inherit parental zooxanthellae must obtain them from seawater. The concentration of zooxanthellae in seawater over the reefs is likely to be quite low under normal conditions; free-living zooxanthellae also occur in sediments (Takabayashi et al. 2012). Positive chemotaxis of motile zooxanthellae towards the coral animal increases the probability of contact between appropriate partners (Fitt 1984; Hollingsworth et al. 2005). Free-living zooxanthellae may show preferential chemotaxis towards newly settled nonsymbiotic coral polyps. In experiments with the soft coral *Heteroxenia fuscescens*, motile zooxanthellae were attracted to animal extracts of juvenile nonsymbiotic polyps but not to extracts of adult symbiotic polyps and seawater controls (Pasternak et al. 2004). Zooxanthellae also may be supplied indirectly to the coral by ingestion of fecal material released by corallivores and of zooplankton prey containing zooxanthellae. Regardless of the mechanism, indirect acquisition of zooxanthellae provides the potential for colonization by zooxanthellae that are genetically distinct from parental symbionts. Whether or not this actually occurs depends on host coral recognition and acceptance of a symbiont, and the chance encounter of the appropriate partners. There is no indication that host corals with direct, vertically transmitted zooxanthellae contain fewer zooxanthellae types than corals that acquire their symbionts horizontally by open acquisition (Baker 2003). Together with recent evidence that newly settled polyps of some species contain different zooxanthellae than their respective adult colonies (Coffroth et al. 2001; Cumbo et al. 2013); corals are likely to be able to acquire different zooxanthellae throughout their lives.

Coral "bleaching" also provides the potential for establishment of a new population of zooxanthellae in adult corals. Buddemeier and Fautin (1993) originally proposed the "Adaptive Bleaching Hypothesis" as a possible explanation for the ability of corals to change the composition of their zooxanthellae symbionts from one clade to another.

Corals lose coloration (become bleached) when they lose most of their zooxanthellae or when stressed zooxanthellae lose their photosynthetic pigments. Corals that survive a bleaching event involving the loss of zooxanthellae eventually regain normal densities of zooxanthellae (they "re-brown") when environmental conditions improve. The source of zooxanthellae for the recovery and re-browning of a bleached coral is unknown. Free-living zooxanthellae may invade corals after a bleaching event, residual zooxanthellae (of the former dominant type or of a cryptic resident type) may re-populate their bleached host coral, or both may occur.

Field studies have shown that the re-population of bleached tissues by free-living or residual zooxanthellae may change the genetic composition of the population of symbiotic algae within a coral (Baker et al. 2004; Kemp et al. 2014). Although changes in the composition of symbiont populations are hypothesized to result in an increased ability to survive future environmental stresses (Buddemeier and Fautin 1993), these changes may be temporary, with subsequent reversions to original symbiont complements (Thornhill et al. 2006). Some evidence indicates that resistance to bleaching increases after symbiont populations change (Baker et al. 2004), and that thermally resistant symbionts may represent a temporary stopgap solution to surviving periods of heat stress. However, these newly acquired symbiont strains may confer lowered fitness upon hosts compared to the normal symbiont complement (Little et al. 2004; Mieog et al. 2009), and may be opportunistic "weed" species that confer short term advantages only during stressful periods.

5.3 Nutrition and Adaptations to Environmental Factors

5.3.1 Coral Nutrition

The success of corals in low-nutrient tropical waters is due largely to the variety of modes that corals utilize to obtain nutrition (Fig. 5.3). The animal has two primary nutritional modes: capture of particulate food by polyps and input of translocated photosynthetic products from its zooxanthellae. The amount of photosynthetic carbon translocated to the animal host is often sufficient to meet its metabolic respiratory requirements. Corals may also take up dissolved organic compounds from seawater, a process that is aided by the extremely high surface area to tissue volume ratio of corals and the presence of cilia on their epidermal cells. However, the nutritional importance of this uptake, and of that of other food sources such as microplankton and bacteria (either free-living or associated with mucus and particulate debris) is uncertain. Animal metabolic waste products derived from
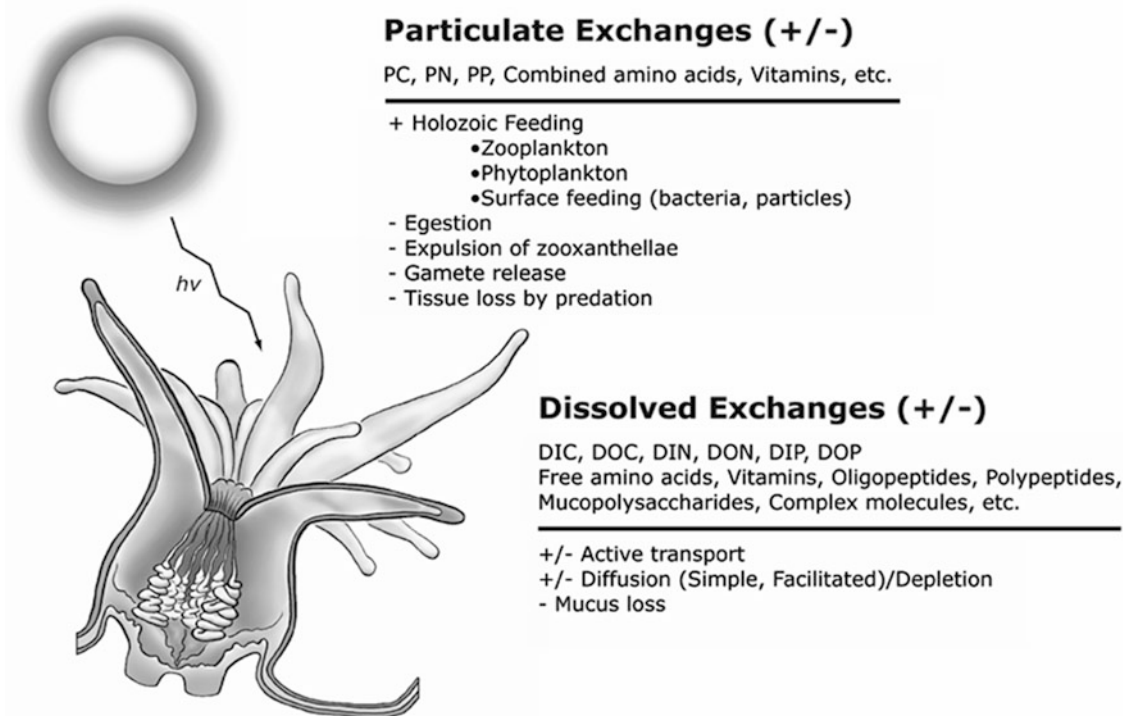
feeding may be retained within the coral, as they are a source of the inorganic nutrient elements (e.g., N, P) required by the zooxanthellae.

Zooxanthellae are photoautotrophs and thus require only inorganic nutrients, carbon dioxide, and light for photosynthetic carbon fixation. Inorganic nutrients may be acquired from coral animal waste metabolites, or from seawater after passage through animal tissues, or from nitrogen fixation by symbiotic cyanobacteria. Host feeding is probably more important as a source of N for corals and zooxanthellae at depth (Muscatine and Kaplan 1994; Heikoop et al. 1998). Zooxanthellae may also obtain organic nutrients from the animal, although the extent to which this occurs, and its significance, is not well understood. The extent of symbiont heterotrophy may depend on environmental considerations (light, depth) and the particular strain of symbiont involved.

The variety of coral nutritional modes suggests that corals are adaptively polytrophic and opportunistic feeders. This polytrophism seems to account for corals' ability to thrive in low-nutrient water (Muscatine and Porter 1977). However, environmental constraints and energetic costs associated with the maintenance of symbiotic algae, as discussed below, may under certain circumstances favor holozoic modes of nutrition, e.g., at depth (Muscatine et al. 1989) or following bleaching events (Grottoli et al. 2007).

Uptake of nutrient elements in an inorganic form from seawater by zooxanthellate corals was first observed by Yonge and Nicholls (1931) working with phosphate (PO_4^{3-}). Subsequently, such uptake has been observed by many authors for a variety of inorganic nutrient forms of nitrogen and phosphorus (PO_4^{3-}, NO_3^-, NH_4^+), and it has been firmly established that net uptake of inorganic nutrients occurs even at the characteristically low environmental concentrations observed in tropical seas. This mode of nutrient acquisition, once considered a curiosity, is now believed by many researchers to constitute a significant source of N and P to the symbiotic association (Fig. 5.3). However, the physiological mechanism by which this uptake occurs has been the subject of considerable debate.

Two alternatives exist to explain the uptake of dissolved inorganic nutrients even at low ambient concentrations, algal-mediated assimilation and host-mediated assimilation. This phenomenon has been investigated most extensively for ammonium. With respect to algal mediation of assimilation, D'Elia et al. (1983) proposed the "depletion-diffusion" hypothesis, suggesting that zooxanthellae reduce the coral intracellular concentration of inorganic metabolites such as NH_4^+ to such low levels that a concentration gradient is established resulting in an inward (perhaps enzymatically facilitated) diffusion of dissolved inorganic nutrient from the external seawater. In contrast, proponents of the host-mediated assimilation model (Rees 1987; Miller and Yellowlees 1989) suggest that the uptake of NH_4^+ (and by

Particulate Exchanges (+/-)

PC, PN, PP, Combined amino acids, Vitamins, etc.

+ Holozoic Feeding
- Zooplankton
- Phytoplankton
- Surface feeding (bacteria, particles)
- Egestion
- Expulsion of zooxanthellae
- Gamete release
- Tissue loss by predation

Dissolved Exchanges (+/-)

DIC, DOC, DIN, DON, DIP, DOP
Free amino acids, Vitamins, Oligopeptides, Polypeptides,
Mucopolysaccharides, Complex molecules, etc.

+/- Active transport
+/- Diffusion (Simple, Facilitated)/Depletion
- Mucus loss

Fig. 5.3 Particulate (P) and dissolved (D) exchanges of inorganic (I) and organic (O) carbon (C), nitrogen (N), and phosphorus (P) between a coral polyp and the seawater environment. "+" exchanges represent inputs from the environment to the coral, and "−" exchanges represent losses from the coral to the environment. Internal exchanges (not shown) include uptake by symbiotic algae and translocation between the algae and the host

extension, other nutrient ions) is more likely mediated by assimilatory enzymes in animal tissues, and then transported in an organic form to the zooxanthellae.

For ammonium uptake and assimilation, at least, several strong lines of evidence suggest that the former mechanism is the more likely of the two alternatives. Kawaguti (1953) and Muscatine and D'Elia (1978) found that zooxanthellate, but not azooxanthellate corals take up ammonium. D'Elia et al. (1983) found that isolated zooxanthellae take up ammonium, and that uptake kinetics of isolated algae closely approximate those of intact symbioses. These authors found differences in uptake kinetics between isolates of zooxanthellae, but the genetic basis of these differences is not known. Given the genetic tools now available for *Symbiodinium*, this is a question that should be addressed.

D'Elia and Cook (1988) provided indirect evidence that ammonium concentrations in host cytoplasm are indeed low enough to create a concentration gradient from seawater to the cytoplasm, and Szmant et al. (1990) showed that corals conserve N, which is consistent with establishing a low internal ammonium concentration. Most significantly, ^{15}N studies show that ammonium is initially assimilated by the zooxanthellae, and that some of this assimilated N is transferred to the host (Swanson and Hoegh-Guldberg 1998; Kopp et al. 2013). However, there is some evidence that

host tissue may also assimilate ammonium (Lipschultz and Cook 2002).

The ramifications of the algal-mediated nutrient uptake mechanism are significant: zooxanthellae **in hospite** (living within host cells) are likely to be nutrient-limited, as hypothesized by Cook and D'Elia (1987), who provided a list of criteria for establishing nutrient limitation. Accordingly, increases in nutrient concentrations in ambient seawater that corals are exposed to, should, in fact, enhance cell division, growth rates and biomass of zooxanthellae, as observed by numerous authors (e.g., Hoegh-Guldberg 1994), affecting the stability and physiological balance of the symbiotic association.

5.3.2 Productivity of Corals and Role of Zooxanthellae in Calcification

Photosynthetic carbon fixation by zooxanthellae (P_{zx}) accounts for the high productivity of corals (Hatcher 1988, 1997). Any carbon fixed by zooxanthellae in excess of their own respiratory (R_{zx}) and growth requirements is potentially available to the host coral as a carbon and energy source. If the carbon fixed by zooxanthellae meets or exceeds the combined respiratory carbon requirement of the coral and

zooxanthellae ($P_{zx} > R_{zx + animal}$; ratio of P:R > 1), the coral is potentially photoautotrophic with respect to carbon and does not require external carbon sources to obtain energy. When P:R is less than one, carbon must be supplied from other nutritional sources, either particulate or dissolved (Fig. 5.3) for the coral to satisfy energetic needs. P:R ratios derived from oxygen measurements generally show that shallow water corals have a P:R > 1, while the P:R of deep water corals is less than one. Therefore, deep water corals are more likely to require external subsidies of organic carbon for maintenance and growth than are shallow water corals.

Zooxanthellae enhance coral calcification and thus are responsible for much of the formation of the massive coral reef framework. The direct relationship between coral calcification rate and light indicates the fundamental importance of symbiont photosynthesis (Barnes and Chalker 1990), although how photosynthesis enhances calcification is still unresolved. Several models have been proposed for the mechanism of enhancement of calcification by zooxanthellae. One is the contribution photosynthetic products of zooxanthellae to the process of calcification. These products could enhance calcification by providing energy for aragonite deposition or for the active transport of calcium ions across the calicoblastic epidermis for calcification. These products could also provide precursors for the synthesis of the organic matrix upon which $CaCO_3$ is deposited. Another possibility is that symbiont photosynthesis alters the physico-chemical environment to provide favorable conditions for calcification (Gattuso et al. 1999). Photosynthesis removes CO_2, raising the pH and the aragonite saturation state, which favors carbonate deposition. In turn, calcification generates protons (H^+) that are transported back into the coral tissues. The decrease in pH from the extra protons results in an increase in carbon dioxide, supplying the primary substrate needed for photosynthesis by zooxanthellae in the light (Al-Horani et al. 2003). A third possibility is that the removal of phosphate by zooxanthellae enhances calcification since phosphate is an inhibitor of $CaCO_3$ crystal formation (Simkiss 1964). Despite the abundance of models, the significance of each one with respect to the link between calcification and symbiosis with zooxanthellae has not been determined.

The following section describes the factors that influence the productivity of zooxanthellae and hence the amount of carbon potentially available to the coral. The balance between primary production and respiration (P:R) for a coral with a stable population density of zooxanthellae depends on environmental factors that affect both photosynthesis of the zooxanthellae and respiration rates of both partners. The most important factors are light and temperature.

5.3.3 Effect of Light and Temperature on Productivity of Zooxanthellae

The reliance of corals on phototrophic nutrition and photosynthetically enhanced calcification favors the proliferation of corals in shallow, clear waters. Total daily production depends on the amount of light received by zooxanthellae, which is related to the light penetrating to the seawater depth where the coral is located and to the light transmitted through the coral animal tissues and reflected by the coral skeleton. Photosynthetic rates increase directly in response to increase in light intensity up to a certain light intensity, after which they are either independent of further increases in light or inhibited by high light (Hatcher 1988). Consequently, mechanisms for regulating light capture and penetration through the coral are advantageous and are evident in both the zooxanthellae and the host coral.

Some corals grow well in the presence of high levels of ultraviolet (UV) radiation, while others are killed by exposure to high UV. The former include corals that are normally found in high light (in shallow water), while the latter include corals that live at greater depths or that are "shade-loving" species. Corals have a suite of molecules that protect against UV-induced reactive oxygen species (Lesser 1997; Mazel et al. 2003) as well as UV-absorbing "sunscreen" compounds that protect against UV damage (Dunlap and Chalker 1986). The quantity of their UV-absorbing sunscreens is related to the incident UV energy, and declines in corals with depth. These sunscreens include mycosporine-like amino acids (MAAs), and appear to be located primarily in the animal tissues and afford protection to symbiotic zooxanthellae that do not make MAAs (e.g., members of Clade B; Banaszak et al. (2000)) or that have low concentrations of these compounds.

Animal tissue pigments, contributed by four or more groups of color types of GFP-like proteins (GFP, green fluorescent protein), may serve to regulate the light received by zooxanthellae. In high light-adapted corals, fluorescent proteins are concentrated in the epidermis above the algae and may serve as a protective screen to scatter the light and remove excess light energy by fluorescence; the same proteins located below the algae in the gastrodermis in shade-adapted corals might help collect light under low light conditions by back-scattering and transformation of light to photosynthetically-active wavelengths (Salih et al. 2000). However, Mazel et al. (2003) propose that the physical absorption, emission and reflection properties of GFPs do not function in optimizing light conditions for photosynthesis; instead, GFPs may help remove potentially damaging reactive oxygen species (ROS) produced during photosynthesis. Given the role of ROS in coral bleaching (Lesser 1997), GFPs may serve as a "bleaching monitor" (Roth and Deheyn 2013).

By their location in a thin layer of photoprotective living tissue, supported by a strong skeleton formed by their animal partner (Fig. 5.1), zooxanthellae maximize light capture and are highly productive. This light capture is enhanced by the physical properties of the skeleton (Enríguez et al. 2005). Although they are essentially "imprisoned phytoplankton," they enjoy many of the benefits of a macroscopic benthic lifestyle such as that of a seaweed. Such advantages include maintenance in the photic zone with good water exchange. In addition, individual polyps exhibit behaviors (contraction and expansion) that help regulate and optimize the light environment for their symbiotic algae. The coral also provides protection against herbivores that feed on planktonic algae. As in trees, upper layers of the coral canopy receive more light than the understory surfaces of coral branches, and zooxanthellae in shaded and unshaded portions of the colony may exhibit different degrees of photoadaptation and exist at different population densities in coral tissues.

Individual zooxanthellae acclimate to changes in light by changes in their photosynthetic systems, including the light-harvesting ability of photosynthetic units (amount of pigment) and the rate of carbon fixation (enzymatic adaptations). Zooxanthellae in corals from shaded habitats usually contain more chlorophyll and accessory light-harvesting pigments, held in larger chloroplasts with a greater number of chloroplast membranes, and thus are more efficient at light capture under low light conditions. The size of their light harvesting units is large. Zooxanthellae in corals in high light environments contain less photosynthetic pigment, in smaller light harvesting units in fewer chloroplast membranes, and sustain high rates of carbon fixation under high light conditions by containing more photosynthetic units. As light intensity also varies on a daily and seasonal basis, zooxanthellae are also likely to acclimate to these temporal changes in light conditions.

The differential ability of coral species to photoacclimate to prevailing light regimes via these mechanisms may limit their distribution in different light environments. Genetic differences in the photophysiology of different species or taxa of zooxanthellae are also likely to have a large effect on the optimal light regimes of individual coral species, and on the distribution and ecological role of these corals under changing environmental conditions. In some cases, a species may exhibit changes in symbiont composition with depth (Rowan and Knowlton 1995), while other coral species do not (Iglesias-Prieto et al. 2004). Clearly, proper quality and quantity of light are essential at the coral holobiont level for (1) the overall stability of the association; (2) the ability of the symbiosis to exhibit net production (P > R); (3) the expression of photosynthetic pigments and the density of the zooxanthellae; and (4) diel behavioral aspects such as polyp expansion and contraction.

Temperature also affects metabolic rates of corals and their symbionts. The influence of temperature on productivity depends on how photosynthesis of the algae and respiration of both the algae and coral animal respond to changes in temperature. In general, corals are adapted to their ambient temperature conditions. Shallow corals may tolerate a wider range of temperatures than deep water corals, and corals (and their zooxanthellae) may show latitudinal differences in their temperature responses. For example, P:R ratios for the same species of corals in Hawaii and Enewetak across a temperature range (18–31 °C) showed that Enewetak corals were adapted to their higher ambient temperatures (Coles and Jokiel 1977). As was discussed for light, temperature tolerances may depend on the individual coral species and the particular genetic strains of zooxanthellae involved. See Chap. 7 by Barshis for further discussion.

High temperature is known to cause coral bleaching, but there is no given temperature that causes coral bleaching. Instead, prolonged exposure to increases of 1–3 °C above long-term annual maximum temperatures (ranging from 25 to 35–36 °C) are likely to induce bleaching (Coles and Brown 2003). High light and ultraviolet light exposure may exacerbate the effects of high temperature, and other stressors may also initiate this phenomenon, which argues for caution in interpreting every bleaching event as the result of a temperature anomaly. Clearly, exposure to temperatures exceeding the tolerance range of the symbiosis affects its stability, generally resulting in the loss of zooxanthellae and possibly in the death of the host. The loss may be due to effects on the zooxanthellae (e.g. Tchernov et al. 2004), on the host (e.g. Fitt et al. 2009), or on both partners. Both the rate of the temperature change and the duration of the temperature anomaly can effect bleaching. As with changes in light, the ability of the coral to adapt to change in temperature or in other environmental factors depends on the acclimatory capability of one or more of the following: the animal, the zooxanthellae, and the symbiotic association as a whole.

5.3.4 Effect of Nutrient Supply on Zooxanthellae in Corals

Corals thrive in seawater where the concentrations (standing stock) of the major growth-limiting nutrient elements, nitrogen and phosphorus, are typically very low. A tenet of algal-animal symbiosis is that it evolved in response to relatively low ambient nutrient concentrations, and that accordingly, such conditions provide corals a competitive advantage over other benthic species. Corals conserve nitrogen by having low rates of protein catabolism and catabolizing translocated lipids and carbohydrates (Szmant et al. 1990).

Various sources of N and P exist for symbiotic zooxanthellae. The algae can obtain inorganic nutrients via recycling of waste products from their animal host, and "new" nutrients from the uptake of dissolved inorganic compounds from seawater and zooplankton capture by the coral (Fig. 5.3). In fact, early investigations of the role of symbiotic algae in corals suggested that they served as the kidneys for the animal (Yonge and Nicholls 1931), although this is now considered highly unnecessary because of the proximity of the tissues to seawater and the relatively small thickness of the coral tissue layer. Although dissolved concentrations of nutrients are very low in most tropical waters, mass transport of nutrients via diffusion or transport across coral surfaces may be sufficient, when assimilated, to supply the nutrient requirements of the algae and its host (Hoegh-Guldberg and Williamson 1999). Such transport is enhanced by water movement on reefs (Thomas and Atkinson 1997).

Although corals are adapted to waters containing very low levels of nutrients, corals can persist when nutrient levels around reefs become periodically elevated due to increased run-off from adjacent land, point source inputs (from sewage and industrial effluents), or periodic upwelling. Most corals are unable to adapt to acute, high level nutrient enrichments and generally compete poorly with benthic macroalgae under such conditions. Excess nutrients may decrease calcification rates, as described earlier phosphate interferes with aragonite crystal formation during calcification. Elevated nitrate inhibits coral calcification (Marubini and Davies 1996). However, given the differences in uptake kinetics between cultured isolates of zooxanthellae (D'Elia et al. 1983), it is possible that symbiont strain is important in those corals that exist in environments with reduced nutrients. For example, Godinot et al. (2013) found differences in alkaline phosphatase activity between zooxanthellae from different clades, although it is not clear how these differences relate to environmental concentrations.

Elevated phosphate levels (exceeding about 1 μM) and dissolved inorganic nitrogen levels (exceeding about 6 μM) destabilize the symbiosis by enhancing growth rates of the zooxanthellae, but it is unclear how often reef seawater concentrations exceed these values. Recent studies have shown that higher symbiont densities increase the likelihood that elevated temperatures will trigger bleaching events (Wooldridge 2009; Wiedenmann et al. 2012; Cunning and Baker 2013). Under less stressful conditions, elevated growth rates of the zooxanthellae relative to the host may simply result in the expulsion of extra algae, The expulsion of zooxanthellae may also represent a unique detoxification mechanism for the coral. For example, zooxanthellae have a high tolerance for heavy metals and accumulate them from seawater. Periodic expulsion of zooxanthellae could reduce the "body burden" of heavy metals (and nutrients) in the coral animal, as demonstrated for temperate sea anemones (Harland and Nganro 1990).

Corals that survive direct effects of added nutrients may succumb to indirect effects such as reduction of light by increased phytoplankton biomass in the water column and overgrowth by fleshy seaweeds.

5.4 Stability of the Symbiosis

A stable symbiosis is defined as one in which the density of zooxanthellae in corals remains relatively constant under a given set of environmental conditions and the symbiotic partners do not change over time. This constancy may be important in balancing the benefits and the costs of the symbiosis (Table 5.1). Thus, the growth of zooxanthellae in corals must be regulated somehow relative to the growth of the host. Either the growth rates of the zooxanthellae and the animal cells are comparable, or, if different, excess zooxanthellae are expelled from the coral.

Direct measurements of growth rates of zooxanthellae in coral tissue are difficult to make. Doubling times have been estimated from diel measurements of the mitotic index (= the percent of dividing cells, Fig. 5.2) of zooxanthellae. However, this approach makes assumptions about the duration and phasing of cell cycle stages that may be difficult to validate. Doubling times for symbiotic zooxanthellae calculated by this approach show them to be at least an order of magnitude lower than for cultured zooxanthellae maintained in nutrient-enriched seawater.

Zooxanthellae growth rates and densities may be regulated by the availability of nutrients. Resource limitation may help maintain the balance between zooxanthellae and animal biomass and growth rates. Upper density limits are likely controlled indirectly by the animal cell habitat, slowing zooxanthella division rates by limitations on space or diffusion of gases (CO_2, O_2) through animal tissue. It has been suggested that the host may produce a compound that inhibits algal growth once a certain density has been reached, but this has yet to be demonstrated. Corals are known to expel zooxanthellae, and this is believed to be an important mechanism for controlling densities. As discussed above, corals with elevated symbiont densities following elevated nutrients may be more susceptible to bleaching events. Furthermore, as numbers of zooxanthellae increase in coral tissue, self-shading of cells will reduce available light and there will be intense competition for limited resources, CO_2 and nutrients, potentially reducing net production and growth. In certain rapidly growing areas of the coral, animal growth rates may exceed those of the zooxanthellae, and populations of zooxanthellae are thus "diluted." For example, tips of branches of rapidly growing species often appear white due to reduced algal densities.

Table 5.1 Putative benefits and costs of the symbiotic relationship for the coral animal, for zooxanthellae, and for the holobiont

Benefits	Costs	Indirect (+/− effects)
A. Animal		
Supply of reduced carbon, offsetting respiration costs and conserving metabolic reserves	Regulation of algal growth and production of peri-algal vacuoles	High surface area-to-volume ratio favors both light capture and prey capture
Increased growth and reproduction	Defenses against high oxygen tension, high light, and UV	Restriction to the photic zone
Increased calcification rate	Mechanisms for rejection of non-beneficial types or excess algae	
Conservation of nutrients	Vulnerability to environmental stresses that affect plants	
Sequestration of toxic compounds by algae		
B. Zooxanthellae		
Supply of CO_2 and nutrients from host	Translocation of a significant fraction of photosynthetic carbon to animal; growth rate is regulated	Nutrient supply is regulated
Maintenance in photic zone	Regulation of growth rate; growth slower in coral than in free-living state	Protection from grazers
Protection from UV damage by animal tissues	Expulsion from host	Dispersal by predators on animal tissue
Maintenance of a high population density of a single or few genotypes by host under uniform environmental conditions	Supply of CO_2 and nutrients limited by host	
C. Coral symbiotic association		
Increased growth, more competitive for space on reef	Compounded sensitivity to environmental stresses that affect algae, animals, or both	
Increased ability to partition resources of food and space	Restricted tolerance range of light, temperature, and sedimentation conditions for growth	
Increased resistance to water motion due to high calcification		

Notes: Factors which are not direct benefits or costs are listed as indirect effects. The relative contribution of each factor to maintaining the balance between benefit and cost of the symbiotic association is unknown, as is the synergistic interaction among these factors. Also, not all of these putative benefits have been documented in corals, such as sequestration of toxic compounds by algae

Environmental factors that are likely to affect both animal and algal growth include physical conditions and the availability of prey. Light directly affects photosynthetic productivity, while prey capture directly affects animal tissue growth and indirectly affects growth of zooxanthellae by its potential supply of nutrients and creation of new animal tissues that provide habitat for more zooxanthellae. Although the intracellular habitat of zooxanthellae has been considered a nutrient-rich environment (Fig. 5.2), these algae display characteristics that suggest that their growth is normally nutrient-limited (Cook and D'Elia 1987). It has been frequently observed that the addition of dissolved inorganic nitrogen to seawater causes an increase in the growth rate of zooxanthellae (e.g., Hoegh-Guldberg 1994) and in their nitrogen to phosphorus ratio (Muller-Parker et al. 1994). The opposite trends occur when symbiotic associations are maintained with no particulate food resources in low nutrient seawater. It is unknown if the animal withholds nutrients from its algae, or if the supply is limited by the availability of nutrients (including animal prey and seawater as sources), or both. Nutrient limitation of the growth of zooxanthellae may favor the coral animal by creating an excess of photosynthetic carbon products that cannot be used for production of new cells and is therefore translocated to the animal host (Falkowski et al. 1993). Conversely, the addition of nutrients may trigger the diversion of photosynthate to fuel symbiont growth. Wooldridge (2010) has characterized the situation as one of "controlled parasitism", in which the host may regulate its symbionts via a number of mechanisms.

The review of Fay and Weber (2012) and more recent work on the diversity of zooxanthellae taxa in corals make it clear that apparently stable populations may exhibit profound changes in the genetic composition of the zooxanthellae inhabiting corals over time. We may expect more examples of this as more sensitive next-gen sequencing techniques are used to study these associations. As noted above, the extent of these changes depends on the specific combinations of host and symbiont. Furthermore, symbiont densities may follow predictable seasonal cycles, with highest densities of symbionts during low light winter

months, highest animal tissue biomass in spring, and lowest population densities of zooxanthellae during late summer and fall months (Fitt et al. 2000). These seasonal cycles may represent fine-tuning of the symbiosis in response to seasonal changes in light and temperature, with resultant effects on coral growth. In some cases these seasonal patterns may include periodic changes in the relative frequencies of symbionts, with more heat-resistant strains becoming more frequent during warmer months (Chen et al. 2005). As opposed to these normal cycles in zooxanthellae population dynamics, symbiont population densities may be disrupted by stressors that result in the mass expulsion of zooxanthellae, resulting in visibly bleached corals. Environmental stresses such as extreme temperatures, high light, air exposure, or rapid change in salinity cause coral bleaching. These large-scale disruptions in the symbiosis may produce positive or negative shifts in the balance between benefits and costs of the symbiosis (Table 5.1). If the coral survives the stress and regains a normal population density of zooxanthellae, there may be a period during the re-population phase when algal growth rates exceed those of the animal tissue.

5.5 Cost-Benefit Analysis of the Symbiosis

Table 5.1 presents features that we consider to represent significant benefits and costs of the symbiotic relationship between zooxanthellae and their coral animal hosts. These features are presented from the perspectives of each partner and of the complete association. We suggest that this approach, albeit somewhat anthropomorphic, is a useful way to consider the symbiosis and may be helpful in framing future research directions. Other perspectives may be found in Wooldridge (2010) and Lesser et al. (2013).

From the animal's perspective, "sufficient" numbers of zooxanthellae (partially determined by strain or type of zooxanthella) must provide some input of energy towards offsetting its respiratory requirements. A balance must exist between photosynthetic production and the metabolic cost of maintaining the algae. The costs include mechanisms to cope with high oxygen tension (activation and increase in levels of antioxidant enzymes) and possible regulation of the growth rate of zooxanthellae (Table 5.1). Since most corals contain 1×10^6 cm^{-2} to 6×10^6 zooxanthellae cm^{-2}, it is likely that this range represents optimal algal densities that balance the benefits and costs of the symbiosis. Rapid changes in densities of zooxanthellae in corals due to environmental perturbations, for example coral bleaching in response to high temperature and algal growth in response to increase in seawater nutrients, will upset this balance and may stress the coral by uncoupling algal and animal growth. From the alga's perspective, the coral must provide a good habitat. The "economic" benefit of the partnership may be viewed as the net return based on the relative costs of the symbiosis between zooxanthellae and the coral animal (see also Wooldridge 2010).

Although it is often difficult to evaluate benefits and costs, obviously when benefits exceed costs there is a net benefit to sustaining the symbiosis, and the association might be expected to persist in a stable state. Conversely, when costs exceed benefits, the net costs could result in the end of the association. Thus, the persistence and stability of the symbiotic relationship at both ecological and evolutionary scales must depend on the net benefit of the symbiosis over relevant time scales with respect to its ability to withstand environmental stresses and to compete for space and other resources with other benthic organisms.

In some cases, benefits or costs of the partnership have been experimentally verified. For example, the enhancement of coral calcification by zooxanthellae is documented, both from comparison of calcification rates of symbiotic and non-zooxanthellate corals and by the light-enhanced calcification rates of symbiotic corals. In other cases, the relationships are less obvious. One must recognize that our knowledge of the costs and benefits of the relationship is limited. Subtle yet crucial benefits and costs may exist that we cannot yet identify or quantify: the use of transcriptomic, proteomic and metabolomic approaches will likely identify more of these in the future. Moreover, the cumulative effect of different costs and benefits may not be simply additive. The interactive and synergistic effects between factors are not likely to be easily quantified.

The diversity and number of the entries in Table 5.1 suggest that the balance between benefit and cost for the relationship is highly dynamic and varies according to both previous and current conditions, as well as the particular combinations of host and symbiont. Organisms have a physiological minimum and maximum tolerance to, and an optimum value for, any given factor. Within limits, such ranges of tolerance are useful constructs for the consideration of the environmental conditions both necessary and sufficient for survival. We can only speculate whether the susceptibility of a coral to given stressors will be increased, decreased or modulated when compared to the susceptibility of the individual partners to the same stressors.

When a coral is stressed to a point where the relationship is disrupted, the costs of maintaining a symbiosis have clearly exceeded the benefits. This may provide for the short-term survival of the coral and of the zooxanthellae, and involve changes in symbiont populations, as noted above during bleaching events. When favorable conditions return, the original symbiosis would be expected to be re-established because the benefits to the coral are required in the long-term.

Disruption of the association by stressors may, in turn, have major consequences not only for the individual corals

but also for the coral reef ecosystem. A particularly good example of this can be seen in the effect of temperature-induced coral bleaching on community structure in the Eastern Tropical Pacific (Glynn 1991). In 1982–1983, a very strong El Nino-Southern Ocean oscillation (ENSO) event resulted in severe warming and severe bleaching of corals in Costa Rica, Panama, Colombia and Ecuador. Mass mortalities of corals occurred and reef structure changed substantially. The mass bleaching events of 1999 caused widespread mortality of corals in the Indo-Pacific, but many of these reefs have shown signs of recovery (Wilkinson 2008). Such severe effects notwithstanding, disruption of the symbiosis by stressors may also provide the opportunity not just to "weather a storm" but to "change partners" to other zooxanthella taxa or species that can provide better benefits and lower costs for particular environmental conditions (Buddemeier and Fautin 2004a). For example, if zooxanthellae with high thermal tolerance successfully populate bleached corals, the new combination may be more resistant to subsequent high temperature stresses.

As we consider the factors affecting the costs and benefits of maintaining the symbiosis, it is appropriate to consider three questions: (1) Is viewing symbiosis in terms of benefits and costs a useful way of assessing the ability of a symbiosis to persist? (2) What are the factors that shift the balance from benefit (+) to cost (−) to the symbiotic association? (3) Are such factors interrelated? Since we are only capable of making crude determinations of relative cost or benefit of a given factor, we cannot realistically provide numbers (limits) for the quantification of benefits and costs. Although this means that the answer to the first question is "no" in most cases, consideration of the relative benefits and costs does facilitate our ability to conceptualize the response of the symbiotic association to changes in any factor.

Exposure to extremes in temperature, oxygen, salinity and nutrient supply are all known to destabilize the symbiosis and result in the loss of zooxanthellae (coral bleaching). Each of these stressors is likely to exact costs of sustaining the zooxanthellae that are too great, so that either the host actively expels them or the zooxanthellae leave on their own accord. The stressor may damage the zooxanthellae, creating a liability for the host if the cells were to remain in their tissues. For example, disruption of photosynthetic membranes by high temperature leads to the uncoupling of photosynthetic energy transduction and the production of damaging reactive oxygen species, killing the zooxanthellae and damaging host cells (Tchernov et al. 2004). As discussed above, nutrients may also result in the active expulsion of zooxanthellae by the host following periods of overgrowth. In this case, symbionts divert carbon from translocate to growth under conditions of nutrient repletion, and algal expulsion serves to keep the host from being overgrown by

its less beneficial endosymbionts. A disruption of the balance between the animal host and its zooxanthellae may result in reductions in productivity and coral growth, leading to possible overgrowth by faster-growing organisms, especially seaweeds. Below we discuss some practical examples of how natural and anthropogenic stresses to corals affect the stability of the symbiosis.

The third question was whether factors that affect the net benefit of the symbiosis interrelate with one another. It is possible to define a set of conditions under which a symbiosis will persist, and conversely, under which it will not. Nonetheless, we presently have almost no information regarding synergistic interactions and the effects of multiple stress factors on the net benefits to maintaining the symbiosis.

5.6 Environmental Effects on the Symbiosis

Other chapters in this book review general ecological features relating to corals and coral reefs. Here, we consider the stability of coral/zooxanthellae symbioses with respect to environmental stresses. We approach this topic first from the perspective of local and regional effects, and then from the perspective of global environmental changes and effects through the alteration of the essential factors of sedimentation, light, nutrients, temperature and pH.

5.6.1 Local and Regional Stresses to Symbiotic Corals

In coastal areas, human population densities are increasing at an alarming rate, as people are migrating to within a few hundred kilometers of coasts as was dramatically illustrated by the tsunami tragedy of 2004. This demographic factor is having substantial environmental effects in all coastal areas in temperate and tropical regions, but to date most attention has been paid to temperate areas. That situation is beginning to change (cf Knowlton and Jackson 2008). Numerous meetings of international authorities on coral reefs over the last two decades have concluded that the cumulative effects of local coastal development are presenting more immediate problems than any present global effect such as ozone depletion or enhanced greenhouse effect due to the anthropogenic release of carbon dioxide. This is particularly important because much international policy concern has been focused on controlling greenhouse gases and climate change, while equivalent concern about recognizing on an international level the cumulative threat local stressors has only recently developed (see below).

Human coastal development brings with it increased inputs of nutrients from sewage and runoff. The process of

over-enrichment by anthropogenic nutrient inputs ("cultural eutrophication") is widely recognized and is becoming better understood in tropical environments where coral reefs are found. The classic example of this is Kaneohe Bay, Hawaii, where coral reefs suffered greatly from increases in nutrient inputs and sedimentation (Smith 1981). Without recognition or fanfare in the formal scientific literature, remarkable advances in understanding of coral nutrition and maintenance of stable associations has occurred through the work of aquarium hobbyists. Building on the work of scientists seeking to improve public aquarium exhibits, they have revolutionized the ability to maintain and grow corals in their own homes (Borneman 2001). The work of Adey (1983), who recognized the importance of macroalgae in "scrubbing" excess nutrients from aquaria, and Jaubert and Gattuso (1989), who recognized the value of enhancing coupled nitrification and denitrification to maintain a low-N environment, have stimulated the adoption of devices such as the "Jaubert plenum," which is an undergravel biological filter that maintains low N levels in aquaria. The success of this system illustrates the value of controlling serious destabilizing effects of high N levels on zooxanthellate corals. The aquarium hobbyists have also learned how to maintain proper seawater chemistry to maintain and promote the growth (calcification) of corals.

Increased sedimentation and runoff are two of the most pronounced early effects of coastal development, and it has been recognized for some time that global sediment fluxes from land to sea are increasing (Milliman and Meade 1983). In mountainous high precipitation areas especially, clearcutting of forests and development of agrarian economies result in increased levels of water-borne sediments and nutrients (Sect. 9.2.1), and decreases (or increases in the seasonal variation in) salinity. These activities have been associated with a reduction in coral cover and diversity (Bartley et al. 2014). At the same time, symbiotic corals can provide a useful temporal record of environmental changes within a reef ecosystem because of the dependence of calcification on zooxanthellae; changes in calcification rate due to variation in parameters such as temperature, salinity, turbidity, and pollution are recorded in the density banding patterns of the coral skeletons.

Studies of terrestrial runoff in Kaneohe Bay, Hawaii, and other places suggest that suspended sediment in the water is one of the foremost enemies of reef corals (Chaps. 9 and 11). Although the major effect of sediment on corals is the accumulation of particles on coral surfaces that interferes with feeding, turbidity affects the quantity and quality of light available for photosynthesis, a matter of clear bearing on the persistence of the coral-zooxanthellae symbiosis, and on coral calcification. Alteration of light quality and quantity results both from sediment-related turbidity and indirectly to phytoplankton growth stimulated by increased nutrient

loadings (Sect. 9.2) associated with sedimentation and agricultural land practices (increased fertilizer and pesticide application, slash and burn and deep tillage agriculture) (see Sect. 5.2.2 on coral nutrition and calcification).

In addition to the effects on corals, these factors can also affect the trophic structure of the water column overlying reefs. This in turn may affect the nutrition and stability of the symbiosis. The predominant effect of elevated nutrient levels on corals and coral reefs seems to result from altered trophic structure resulting from overgrowth of corals by fleshy seaweeds, high bacterial biomass, increased disease (Chaps. 8, 9, and 11), etc., which are beyond the scope of this chapter.

5.6.2 Global Stresses to Symbiotic Corals

Scientists are increasingly concerned about the effects of global stresses and global change on corals and coral reefs (Hoegh-Guldberg et al. 2007). With respect to the coral-zooxanthellae symbiosis, global stresses of particular relevance include: (1) increased UV irradiation due to a reduction in the ozone layer; (2) temperature increases due to global warming and related changes in oceanic circulation patterns leading to variation in temperature and nutrient inputs; (3) cumulative increases in nutrients and turbidity due to industrial and agricultural development; and (4) reduction in world ocean pH due to CO_2-derived acidification.

The effect of chlorofluorocarbons (CFCs) on the depletion of the ozone layer and the subsequent increase in the flux of ultraviolet (UV) light to the Earth's surface have received substantial attention with respect to coral reefs. Conditions that favor photosynthesis by zooxanthellae expose corals to UV damage. Although corals contain pigments and anti-oxidant defenses that may afford considerable protection from UV, the effective metabolic cost of UV protection for the animal and zooxanthellae with respect to the symbiosis is unknown. If the cost to the symbiosis is greater than the benefit of light-driven photosynthesis, then the symbiosis becomes a liability.

Temperature is a crucial factor affecting the stability of the coral/zooxanthellae symbiosis at the individual level, and certainly, in a larger sense, of coral reefs (Hoegh-Guldberg 1999). Limits of temperature tolerance for corals and well-developed coral reefs are considered to range from a winter minimum of approximately 18 °C to a summer maximum of approximately 30 °C, although to be sure, thriving reefs are found at either extreme that appear to be uniquely adapted to such conditions. Early studies on the effects of temperature stress on photosynthesis and respiration in corals and coral reefs developed from concerns about the thermal effects of power plant effluents on local biota

(e.g., Coles and Jokiel 1977). However, over the past 30 years it has become clear that that increases in the frequency of worldwide bleaching events are related to rising global temperatures, due to the enhanced greenhouse effect resulting from anthropogenic emissions of infrared-absorbing greenhouse gases (e.g., Hoegh-Guldberg et al. 2007).

Exposure to temperature extremes may or may not affect the stability of the symbiosis. Both the length of exposure to and the severity of a given temperature stress or anomaly are important factors, as is the particular combination of host and symbiont. As an example, probably the best-known response that indicates a destabilization of the coral/zooxanthellae symbiosis, "bleaching," depends on all three of these factors (Fitt et al. 2001). Corals bleach, or actively expel their zooxanthellae most typically when temperatures increase sharply for a short period of time (+3-4 °C, several days) or increase moderately for a longer period of time (+0.5-1.5 °C, several weeks) (Glynn and D'Croz 1990; Jokiel and Coles 1990). Since coral calcification, and therefore reef growth, depends on the presence of zooxanthellae, a gradual rise in sea level with global warming might result in the demise of coral reefs at low latitudes and a shift to higher latitudes. The effects of low light and increased nutrient inputs from global changes in atmospheric deposition and oceanic circulation patterns factors are discussed in the previous section.

Whether the global extent of disruption of the coral symbiotic association with zooxanthellae will provide an accurate "barometer" of coral reef degradation due to global climate change remains an issue of debate and uncertainty. Secular increases in ocean temperatures are very likely to increase the number of coral bleaching episodes and the combination of global climate change and local effects is a serious threat to coral reefs (Buddemeier et al. 2004b). Bleaching may simply represent a temporary disruption of the symbiosis that allows each partner to survive the stress on its own. As Baker (2004) states, "...in an era of climate change and global warming, the continued success of [coral reef] ecosystems is dependent on the stable association of these symbionts with the reef-building organisms which depend upon them." The potential for new and more tolerant combinations of partners after bleaching makes this issue more complicated, but does offer some hope that successful combinations may ensue. The recent finding that corals and their zooxanthellae may have greater acclimatory ability than previously thought (Palumbi et al. 2014; see also Chap. 7 by Barshis) adds hope.

A concern now looming on the horizon is the prospect of the "titration" of oceanic pH as a direct effect of anthropogenic CO_2 inputs. Hoegh-Guldberg et al. (2007) used conservative IPCC models to estimate that by the end of this century atmospheric CO_2-driven ocean pH reductions could reach approximately 0.4 units. This change in the ocean's carbonate buffer system could lead to an enhancement of photosynthesis in marine algae by increasing the pCO_2, but it may also decrease carbonate saturation and thereby decrease calcification (Takahashi 2004). The combination of these two effects would have profound effects on the distribution of symbiotic corals by reducing the areal extent of the ocean suitable to sustain coral growth and survival.

5.7 Summary

The symbiotic association with zooxanthellae is clearly beneficial to corals. Increasing evidence has shown that the symbiotic state is accompanied by sensitivity to environmental stress, since a common response to a stress is the disruption of the symbiosis, resulting in coral bleaching. The response is complex, since zooxanthella taxa (or species) and different species or genotypes of coral animals may have different adaptive capabilities and tolerances to environmental extremes, and we are just beginning to understand the role that host-symbiont diversity plays in this process. As the host animal depends on its complement of zooxanthellae for reduced carbon compounds, coral death will ensue if stresses persist for long periods of time or if they are at levels outside of the tolerance range of the coral and of the zooxanthellae. Factors that induce a stress response include: light (quantity and UV), temperature, sewage and run-off inputs (high nutrients, increased turbidity), salinity (freshwater run-off from land due to deforestation and other land-use practices), pH, and physical damage.

Disruption of the symbiotic association, in turn, has potential for use as an indicator of the health of the coral reef ecosystem. Drastic changes in the stability of the symbiosis, as evidenced by changes in the ratio of zooxanthellae to animal biomass in corals, may turn out to be a useful diagnostic indicator of stresses to coral reefs. Present research is leading to improved understanding of how and when this can occur, especially as techniques are being refined to assess relative algal densities non-invasively by in situ fluorescence readings and reef color assessed by satellites.

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