Chapter 5
Molecular and Functional Ecology of Sponges and Their Microbial Symbionts

Malcolm S. Hill and Oriol Sacristán-Soriano

Abstract Sponge-microbe symbiotic interactions are important features of modern marine ecosystems. It is likely that these ancient partnerships are as old as the phylum Porifera. Powerful new tools have exposed remarkable microbial diversity within sponge tissues. We are now able to study the composition and structure of the microbial communities at unprecedented levels of resolution. We also recognize that the partnership cannot be disaggregated and should be considered as an integrated holobiont. New hypotheses (e.g., the sponge loop hypothesis) have opened exciting avenues for future experimental work that link holobiont performance from micro- to macro-perspectives. This type of research has taken on added significance given that our planet is experiencing accelerating rates of ocean warming and ocean acidification. It is essential that we examine how sponges respond to environmental stressors that are increasing in intensity and frequency. This review focuses on the molecular and functional ecology of sponge-based microbial symbioses. We discuss the coevolutionary processes that operate to generate partner specificity or to maintain promiscuous partnerships and consider reciprocal selective forces that shape the material exchanges that occur between the partners. We focus attention on the functional ecological role the holobiont plays in marine habitats. The role that the symbionts play in host physiology, and ultimately in the function of sponges on marine ecosystems, is also discussed. We stand to gain important basic information about symbiotic interactions through the detailed study of sponge-microbe interactions, but important practical lessons will be afforded to resource managers who are looking for strategies to protect aquatic habitats worldwide.

Keywords Symbiosis • Bacteria • Holobiont • Porifera • Coevolution

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5.1 Introduction

Symbiotic interactions between sponges and microbes date back to the Precambrian era and represent one of the keys to the evolutionary success of the Porifera (Wilkinson 1984; Jackson et al. 2010; Uriz et al. 2012). Our understanding of the ecological and evolutionary consequences of sponge-microbe relationships is emerging, and several reports and recent comprehensive reviews have evaluated the scope of work that has been done while highlighting the work that remains to be done (Taylor et al. 2007a, b; Hentschel et al. 2012; Thacker and Freeman 2012; Webster and Taylor 2012; Webster and Thomas 2016; Thomas et al. 2016; Sacristán-Soriano and Becerro 2016). This chapter, rather than iterate upon these contributions, presents distinctive perspectives on the molecular and functional ecology of the microbial symbionts that reside in sponges, the specificity of partners, and interspecific interdependencies specifically within the context of climate change. Since the first report of bacteria living in the tissues of sponges, biologists have made important strides in understanding the nature of the association. With new tools, we are entering a period that will allow even greater understanding of these important symbioses. However, we are also entering a period of significant environmental challenges. Ocean habitats are changing due to human-induced modification to Earth's atmosphere. The consequences of these changes are not fully understood, and it is more important than ever that we continue to explore interspecific associations involving sponge hosts.

This chapter will explore sponge microbial symbioses from five interrelated perspectives. The first perspective focuses on microbial and host diversity. From this vantage point, we will examine the types of microbes that have been discovered in sponges and how that diversity is distributed among extant sponge taxa. The second perspective focuses on host-symbiont interactions at molecular and chemical levels. We will examine what is known about the genetic and cellular means of communication between partners and look for evidence of genomic interactions. We will also examine how the metabolic characteristics of the symbionts (individually and collectively) interact with the physiological capabilities of the host to create an "extended phenotype" of the holobiont. The third perspective explores how the actions of the holobiont influence whole community processes. Given the importance of sponges in many benthic habitats around the world, we examine how the integration of host and symbiont behaviors infiltrates different levels in marine food webs. The fourth perspective focuses on symbiont community structure as an independent line of inquiry. Are sponge symbiont ecological communities purely neutral, weakly niche structured, or strongly niche structured? Sponge symbiont communities may provide important opportunities to compare the relative importance of niche structuring and species-specific interactions in shaping ecological communities. While of fundamental intellectual interest, the answer to this question is also important for predicting how symbiont communities might respond to human-induced changes to marine ecosystems. The final perspective will examine sponge-microbe associations through the lens of climate change. We will examine recent data that investigate ocean acidification and thermal stressors on symbiotic systems involving sponges. While necessarily incomplete due to the scope of the topic, the ultimate goal of this chapter is to investigate the relationship between community structure and community function in the symbiotic assemblages associated with sponges and to point toward future work in these systems.

5.2 The Partners: A Holobiont Perspective

5.2.1 Defining Terms: "Symbiosis"

Symbiosis is a term often used in ordinary scientific language, but generates confusion unless semantic intent is identified and ascribed (see Saffo 1992). One of the challenges of a term as flexible as "symbiosis" is that it includes ecological interactions of high and low levels of intimacy, with different outcomes for the partners, and that can span temporal and spatial scales that range across orders of magnitude (e.g., subcellular to biome level and seconds to decades). de Bary's (1879) original sense for the word was outcome neutral—it only required the living together ("zusammenlebens") of two dissimilarly named organisms. Thus, mutualism (both partners benefit, +,+), parasitism (one partner benefits and the other is harmed, +,−), inquilinism (neither partner may benefit from the association, 0,0), and commensalism (one partner benefits while the other is unaffected, +,0) could be included under the umbrella term symbiosis. More often, however, symbiosis is used to indicate positive outcomes, usually for both partners, which distinguish the term from parasitism. If we focus only on "positive" associations, however, the term becomes static in that the ecological outcomes for both partners are assumed to be constant.

Throughout this chapter, we adopt a definition of symbiosis that includes intimate ecological interactions, and as in the manner of de Bary, our definition allows for flexibility in sign of the interaction (i.e., a positive interaction might change to neutral or even negative depending upon context). We also use the convention of naming the host as the larger of the interacting species. Finally, we apply an explicit evolutionary perspective so that we may employ the greatest diversity of comparative tools (e.g., phylogenetic, physiological, ecological). In this context, sponges offer an extraordinary opportunity to learn about the forces that shape interspecific interactions between multicellular hosts and microbial partners.

5.2.2 Everything Is Everywhere: Probably More so for Sponges

For symbionts looking for potential hosts, sponges represent a diverse and ancient group to colonize. A substantial diversity of potential host partners can be found in marine and freshwater ecosystems. As of early 2016, 8730 valid sponge species have been identified, with many other extant species yet to be validated (World Porifera Database: http://www.marinespecies.org/porifera/porifera.php?p=stats). These modern-day descendants of the earliest multicellular metazoans have an evolutionary history that extends for more than 500 million years (Hooper and van Soest 2000). The four poriferan classes include the Demospongiae (7287 valid species), Calcarea (720 valid species), Hexactinellida (618 valid species), and Homoscleromorpha (104 valid species). Recent progress in demosponge systematics (see Morrow and Cárdenas 2015) has identified 22 orders within the three subclasses (Verongimorpha, Keratosa, and Heteroscleromorpha). The new demosponge classification scheme provides a framework to test ideas about the evolution of symbiotic interactions because comparisons of various symbiont strategies among and within families become possible. For example, despite the diversity of potential hosts, we have a limited understanding of the features of sponge biology that make one host a preferred habitat for dense populations of microbes while other potential hosts are minimally infected by bacteria (e.g., Gloeckner et al. 2014; Poppell et al. 2014).

Sponge-microbe symbiotic partnerships are ubiquitous in marine and freshwater habitats from the poles to the tropics (Vacelet and Donadey 1977; Wilkinson 1978a, b, c; Taylor et al. 2007a, b, 2013; Thomas et al. 2016). Intra- and intercellular associations can co-occur in the same host, and these interactions range from persistent to facultative. Next generation sequencing has revealed impressive bacterial diversity that had previously been hidden from view (e.g., Caporaso et al. 2011; Thomas et al. 2016). Given that the host represents habitat for the symbionts, it is perhaps unsurprising that the composition of the microbiota is often specific to the host (i.e., not a random sampling from the environment) and that some degree of specialization is observed (e.g., Ochman et al. 2010; Sipkema et al. 2015; Steinert et al. 2016). The reciprocal nature of the selective forces that act on the host and symbiont partners is one of the more fascinating aspects of this type of ecological interaction. The host may provide selective nutrients to favor growth of one type of microbe, or it might produce discriminating antimicrobials ("the potter's wheel" of Bevins and Salzman 2011). Microbial symbionts can also exert selective pressure on hosts and can direct host evolution to increase the likelihood of continued occupation of a particular host (e.g., the well-known sharing of amino acid biosynthesis pathways between the aphid and *Buchnera* (The International Aphid Genomics Consortium 2010)). Recent work has called into question the degree of specialization among hosts and symbionts (Taylor et al. 2013), and this represents an active area of research, which we discuss in more detail later.

A thorough exploration of the uneven taxonomic distribution of symbioses across the poriferan phylogeny would be fruitful. Not all types of symbioses are found in all sponge groups. For example, *Symbiodinium* species can be found in the vast majority of cnidarian hosts on tropical reefs, but the distribution of this dinoflagellate in sponges is limited primarily to the members of the Clionaidae (Schönberg and Loh 2005; Schönberg and Suwa 2007; Hill et al. 2011; Hill and Hill 2012; Hill 2014). One of the most notable aspects of sponge-microbe symbioses involves differences in density of bacteria among sponge hosts. Some sponge species harbor extraordinarily dense communities of microbes, and these sponges are referred to as high microbial abundance (HMA) sponges (Fig. 5.1; Hentschel et al. 2006; Taylor et al. 2007a; Schmitt et al. 2012; Gloeckner et al. 2014; Poppell et al. 2014). Other sponge hosts harbor bacterial communities at very low levels (low microbial abundance sponges (LMA)). Some of the differences between LMA and HMA sponges will be discussed later, but evidence indicates that the ecological structure of HMA and LMA communities appears to differ in terms of species richness and evenness (e.g., Poppell et al. 2014). Furthermore, HMA symbiont communities appear to play a role in nitrogen cycling on coral reefs (Fiore et al. 2010), but microbial communities play important nutrient cycling roles *in hospite* in a number of settings regardless of HMA or LMA status (e.g., Schläppy et al. 2010; Thacker and Freeman 2012).

The characteristics of hosts that permit residency of high or low concentrations of bacteria, or the preferences of the symbionts to colonize particular hosts at high or low abundances, are poorly understood but offer a promising line of inquiry. For example, Gloeckner et al. (2014) found 29 HMA and 28 LMA species in sponges from the Caribbean, Mediterranean, and Red Sea waters. The taxonomic distribution of HMA and LMA sponges indicated some degree of convergence in that symbiont status was independent of the evolutionary history of the host. All of the sponges in the Verongida were HMA species, and all sponges in the Poecilosclerida were LMA. However, several orders contained sponge taxa that were either LMA or HMA, often in the same family (e.g., *Spheciospongia* (HMA) and *Cliona* (LMA); *Svenzea* (HMA) and *Scopalina* (LMA)). Poppell et al. (2014) found that the community characteristics (e.g., species richness, evenness) differed between HMA and LMA sponges.

The underlying cause of this pattern is unknown, but it is clear that much additional work is required to determine which factors create conditions favoring HMA or LMA symbioses (e.g., Blanquer et al. 2013).

While the density of the symbiont communities is an important factor to consider, equally, perhaps more, important is the diversity of microbes that associate with sponges, and there has been considerable effort focused on this aspect of the symbiosis (Hentschel et al. 2002, 2012; Usher et al. 2004; Hill et al. 2006; Webster et al. 2010; Erwin et al. 2012a, b; Schmitt et al. 2012; Simister et al. 2012a, b, c; Thacker and Freeman 2012; Fan et al. 2012; Gloeckner et al. 2013; Schöttner et al. 2013; Taylor et al. 2013; Easson and Thacker 2014; Moitinho-Silva et al. 2014; Reveillaud et al. 2014). The advent of deep-sequencing technologies has opened opportunities to study the microbial biosphere far beyond the detection limit of conventional molecular approaches. We are now able to establish whether bacteria are specific to particular hosts or are distributed in other habitats throughout the environment. A number of studies have described the existence of sponge-specific 16S ribosomal RNA (rRNA) gene sequence clusters, but 77 of the 173 previously described "sponge-specific" clusters have been detected in seawater or other non-sponge samples, albeit generally at extremely low abundances (Taylor et al. 2013). For example, the candidate phylum "*Poribacteria*" (so named because it was presumed to be restricted to sponge habitats) was recently detected in >11% of seawa-

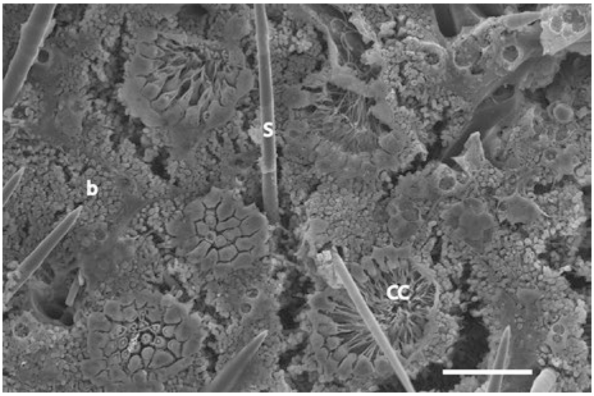

Fig. 5.1 Scanning electron micrograph of freeze-fractured tissue from the HMA sponge, *Calyx podatypa*. Choanocyte chambers (*CC*) and spicules (*S*) of the host sponge are obvious. The dense extracellular microbial community (*b*) can be seen throughout internal regions of the mesohyl. Scale bar = 20 μm (Image taken by E. Poppell)

ter samples and nearly 32% of sediment samples. These data highlight how little we understand about the mode of transmission between generations of sponge (Sipkema et al. 2015) or the degree of the intimacy between partners in sponge-microbe associations.

The most recent survey uncovered "exceptional microbial diversity" in sponges compared to microbial communities associated with sediment and water samples (Thomas et al. 2016). This was the most comprehensive analysis of symbiont microbial communities in sponges and involved a survey of 81 species of poriferan host sampled from habitats in the Mediterranean, Red Sea, Atlantic, Indian Ocean, and Pacific (Thomas et al. 2016). Multiple individuals were sampled from each species of sponge host (from 3 to 133 individuals per host), which allowed for intraspecific as well as interspecific comparisons of microbial community structure. This extensive survey used the Earth Microbiome Project's procedures and focused on the V4 region of the 16S rRNA gene. While this study did not consider the intrahost microbial load (see below), the authors did use network analyses to infer important ecological interactions among symbiont compartments. Representatives from each of the three domains of life (Archaea, Bacteria, and Eukarya) have been observed as sponge symbionts. Attempts to estimate the potential diversity of prokaryotes in sponges are difficult given that a unified classification system and nomenclatural framework for all bacteria and archaea do not exist (Yarza et al. 2014). Nonetheless, many of the major prokaryotic groups were identified (over 40 phyla), though sponges seem to have a tendency toward harboring *Proteobacteria*, especially *Gammaproteobacteria* and *Alphaproteobacteria* (Thomas et al. 2016). Richness of the symbiont communities varies widely among hosts, with estimates of anywhere from 50 to over 3000 distinct symbionts per host (Thomas et al. 2016). In aggregate, Thomas et al. (2016) estimated 40,000 operational taxonomic units (i.e., putative microbial species) in the 81 sponge species they examined. Given that this number of hosts represents almost 1% of the total global diversity of sponges, the phylum Porifera is undoubtedly a major source of unique microbial diversity.

One of the intriguing findings of Thomas et al. (2016) was the substantial variability that exists among sponge hosts in terms of the core and variable microbial communities. Hentschel et al. (2002) were the first to call attention to the possibility that the sponge microbiome might consist of core, variable, and species-specific components (Schmitt et al. 2012). Polónia et al. (2016) found that the biotope could explain over 50% of the variation in archaeal and bacterial communities and that two congeneric sponge species hosted communities of distinct biodiversities. Thomas et al. (2016) found that the core microbiome for a given species includes a relatively small number of generalist microbes (from 7 to 20 OTUs) and some of these are truly cosmopolitan (found in several species of host sponge). The majority of OTUs in a given species, however, seem to be unique to that host species. This level of specificity indicates that community assembly (through ecological interactions or evolutionary specialization) is occurring in sponge-microbe associations. Reconciling these data with the realization that the bacteria can persist in non-sponge habitats (e.g., Taylor et al. 2013) should be a goal of future research.

Thomas et al. (2016) did not consider the density of microbes that were harbored by a particular host species. The ecological behavior of particular microbiomes might differ depending on the nature of resource limitation, quorum sensing, or any of a number of other density-dependent factors that differentiate HMA from LMA microbiomes. In fact, the effects of nutrient limitation might change interactions from competitive to mutualistic (e.g., Le Chevanton et al. 2016). Thus, the networks that were constructed by Thomas et al. (2016), with predicted interspecific interactions, will likely require much more detailed work to determine how members of the core microbiome interact with one another. Furthermore, differences in community structure appear to define HMA and LMA sponges (Weisz et al. 2007; Erwin et al. 2011; Schmitt et al. 2012; Giles et al. 2013; Moitinho-Silva et al. 2014; Poppell et al. 2014). Moitinho-Silva et al. (2014) examined the microbiota associated with the HMA sponge *Xestospongia testudinaria* and the LMA sponge *Stylissa carteri*. While both sponges harbored specific microbiota, the latter was found to harbor a microbiome more similar to that found in seawater (24% of the sequences examined matched those found in seawater), while the former shared only about 6% of its sequences with seawater. Poppell et al. (2014) found that the microbiomes in HMA sponges were more diverse and even, while LMA sponge microbial communities were dominated by one or a few OTUs. The ecological processes that would generate those patterns are decidedly different, which indicates that density is an important factor in any consideration of the interspecific interactions that shape the microbiome.

5.3 Partner Interactions and Host-Symbiont Phenotypic Synergisms

We are entering an exciting era of research in sponge symbiosis that involves a paradigm shift in the way biologists approach the concept of "phenotype." We now recognize that any multicellular organism must be considered a metaorganism or holobiont and that we must adopt a holistic perspective on ecological performance that accounts for the host and its associated symbionts (Bosch and McFall-Ngai 2011; McFall-Ngai et al. 2013). Gene expression must now be considered from the perspective of multiple genomes (the hologenome) of the holobiont (Webster and Thomas 2016). The balance between health and disease, proper development and nutrition, of most host organisms relies on the activities of members of complex microbial symbiont communities (e.g., Mazmanian et al. 2008). The synergistic products of holobiont activities shape the functional ecology, and ultimately the reciprocal evolution, of all members of the consortium (Hill 2014). Thus, holobiont characteristics affect fitness of all participants, and the evolutionary consequences of this phenomenon are just now being considered (Kau et al. 2011; Hacquard et al. 2015).

Identifying the specific forms of interaction that occur between a host and a symbiont at a physiological, cellular, and genetic level is a major objective of current research. New tools are opening lines of research that had been impossible in the past, and new insights into genetic integration, trophic interrelationships, and secondary metabolite production are elucidating ways that sponge hosts and their associated microbial communities are linked together in complex networks. Clear evidence is emerging that host sponges and their microbial communities are involved in highly intimate, ancient relationships and that these interactions have far-reaching effects on marine communities (Thomas et al. 2016; Webster and Thomas 2016).

5.3.1 Genomic Interactions Among Partners

Genomic adaptations for symbiotic lifestyles provide compelling evidence for the degree of intimacy and length of time partners have been interacting. Evidence for metabolic interchange is accumulating (e.g., Fiore et al. 2015). Compelling evidence for the selective forces that shape evolutionary outcomes for sponge symbionts comes from two recent studies (Gao et al. 2014a; Burgsdorf et al. 2015) that evaluated genome structure for the cyanobacterial "*Candidatus* Synechococcus spongiarum" group (Erwin and Thacker 2008; see also Liu et al. 2011). Gao et al. (2014b) examined sponge-symbiont strain SH4 obtained from the Red Sea sponge *Carteriospongia foliascens*. When compared to free-living cyanobacterial strains, they found that SH4 had a reduced genome size, a lack of methionine precursor biosynthesis genes, and appeared to have lost genes involved in cell wall formation. Intriguingly, Gao et al. (2014b) also found in the SH4 genome (1) an enrichment of eukaryotic-type domains that appear to be involved in sponge-symbiont interaction and (2) the loss of genes that encode low-molecular-weight peptides of photosystem II. These last two observations are significant in that the former may represent a strategy to avoid digestion by the sponge and the latter indicates that photosynthetic efficiency of SH4 may rely on host participation. They hypothesized that the genome streamlining of the sponge symbiont was a product of adapting to low-light environments of the "sponge's mild intercellular environment" (Gao et al. 2014a).

Burgsdorf et al. (2015) expanded the coverage of the diverse clade VI group of cyanobacteria by examining the genomes of three additional lineages of cyanobacteria collected from *Theonella swinhoei*, *Ircinia variabilis*, and *Aplysina aerophoba*. All four of the symbiont genomes that were compared appeared to be specialized for living in sponge hosts. For example, each of the four lineages examined had elevated numbers of DNA modification and recombination genes, but reduced numbers of genes and genetic pathways involved in inorganic ion transport and metabolism, antioxidant enzymes, cell wall biogenesis, and signal transduction (Burgsdorf et al. 2015). The loss of low-weight peptides of photosystem II was observed in all four lineages. An intriguing observation was the large degree of variability within the "*Candidatus* Synechococcus spongiarum" group often related to immune system features and siderophore-mediated metal transport. The former is likely important in mediating the interactions between host and symbiont and might afford some level of protection from the phagocytotic capabilities of the host. The latter may represent a mechanism the host can employ to control the growth of phototrophic symbiont populations (e.g., limiting availability of metals used in processes such as electron transport).

Work with the heterotrophic microbial (Bacteria and Archaea) populations has also pointed to genomic coupling between sponges and their symbionts. Hallam et al. (2006) showed that the *Cenarchaeum symbiosum* genome was distinct from those of other Archaea, shared many core metabolic features with free-living planktonic relatives, but also had some signature sequences that may be involved in genetic interactions between host and symbiont. Using single-cell genomics, Kamke et al. (2013); see also Siegl et al. (2011), examined individual cells representing three distinct phylogenetic groups within *Poribacteria* to explore the metabolic potential of this important group of sponge symbionts. The genomes contained genes and pathways likely involved in carbohydrate metabolism, uronic acid degradation, and sulfatases, which indicates that the symbiont may be involved in the breakdown of the proteoglycan host matrix. Thomas et al. (2010) found genomic signatures in sponge bacteria that pointed to host-symbiont interaction. These included mechanisms to protect against foreign DNA, vitamin-linked metabolic interactions between the host and symbiont, nutrient transport and utilization pathways, and ankyrin and tetratricopeptide repeat-mediated protein interactions. Nguyen et al. (2014) also found that ankyrin repeats might be important targets of host-symbiont genomic interface when they identified ankyrin repeats from an uncultured putative sponge symbiont that modulated phagocytotic behavior of heterotrophs (see below).

5.3.2 Trophic Interactions Among Partners

Linking the exceptional pumping and filtering characteristics of sponges (Fig. 5.2; Vogel 1977; Reiswig 1971a, b, 1974, 1975, 1981; Riisgård et al. 1993; Pile et al. 1996, 1997; Turon et al. 1997; Lynch and Phlips 2000) with the microbiome is an important step toward understanding sponge metabolic processes. Many, if not most, of the cells in a sponge can capture material phagocytotically (Fig. 5.2; information from Simpson 1984). Particles of the largest-size classes (>50 μm in diameter or with a diameter larger than the incurrent pores) that cannot enter the aquiferous system can be phagocytosed by exopinacocytes on the surface of the sponge (see 1 in Fig. 5.2). Particles with a diameter greater than the choanocyte collar (>2 μm depending on species) can be digested in one of four ways. Roaming archaeocytes can move through the canals to phagocytose particles that stick to the channel walls (see 2 in Fig. 5.2). Particles can pass through holes in the canal system where they are ingested by archaeocytes (aka amebocytes) in the mesohyl (3 in Fig. 5.2). Endopinacocytes can phagocytose particles. These pinacocyte cells can then migrate into the mesohyl and undergo “cellular transformations” to become archaeocytes, or they can pass their contents to roaming archaeocytes (4 in Fig. 5.2). Prey items can pass through openings in the choanocyte chamber where they are phagocytosed by archaeocytes within the mesohyl (5 in Fig. 5.2). Finally, the smallest prey items (<2 μm) are phagocytosed by choanocytes.

Capture may occur within the collar as well as at the interface between two cells. Choanocytes pass their captured particles to archaeocytes (6 in Fig. 5.2). At each of these stages, dissolved organic matter (DOM) can pass into the sponge though the precise mechanism of this transfer is poorly understood but likely involves pinocytosis and adsorptive processes.

Reiswig (1971a, b) conducted some of the first work to understand how sponges feed by examining incurrent (i.e., ambient) and excurrent water samples from sponges in Jamaica. While Reiswig could not identify the major source of food for sponges using the tools available to him, others (e.g., Pile 1997; Pile et al. 1997; Turon et al. 1997) used flow cytometry to determine that sponges feed on nanoplankton and picoplankton. But the role of the microbial symbionts (phototrophic and heterotrophic) in processing other material in the water column remains obscure (Hentschel et al. 2003, 2006). A diverse range of microbially mediated processes undoubtedly affect the processing of carbon, nitrogen, and other elements necessary for sponge growth. For example, Reiswig (1981) provided evidence that the HMA sponge *Aplysina fistularis* required DOM to meet its carbon and energy needs—particulate organic matter (POM; like pico- and nanoplankton) could not sate the sponge’s energetic needs. This was among the first pieces of evidence that a sponge’s microbial community influenced the trophic status of its host. Refining Reiswig 1981 study, two studies highlight some of the ways that the microbiome influences host feeding. Yahel et al. (2003) demonstrated that dissolved organic carbon (DOC) provided the majority of carbon taken up by the HMA sponge *Theonella swinhoei*. Subsequently, Diaz et al. (2004) showed that nitrifying bacteria harbored by another

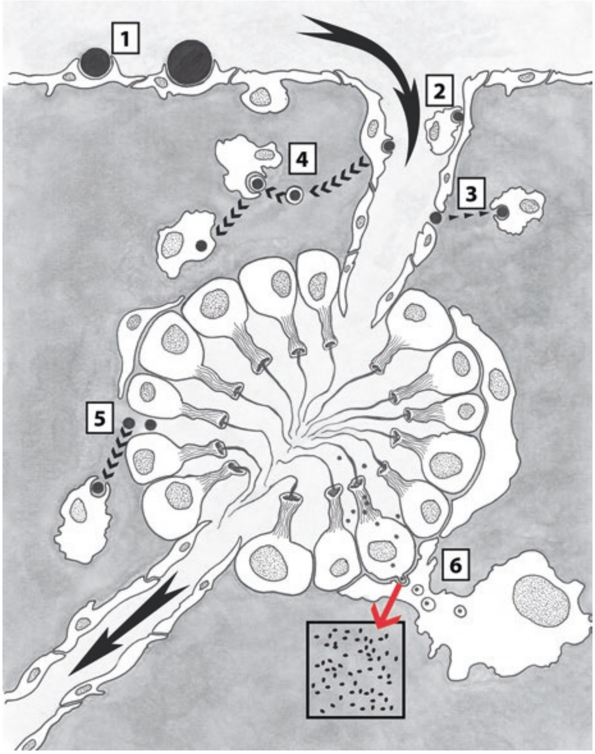

Fig. 5.2 Schematic representation of various phagocytotic pathways of POM (i.e., prey) and potential DOM capture in sponges (see also Simpson 1984). The pathways by which bacteria enter the mesohyl and gain residency within the host sponge remain obscure. One possibility involves symbiont escape from endomembranous compartments in sponge cells after phagocytotic capture (*red arrow* and inset *black box*). Drawing courtesy of S. Friday

HMA sponge (i.e., *Chondrilla*) oxidize the host-generated ammonia and the nitrate released from the sponge can be used to support primary productivity on a reef (see also Hoffmann et al. 2009; Radax et al. 2012a). This opens significant possibilities to study the integration of host and symbiont populations in terms of movement of material and energy between partners.

Stable isotopes of C ($^{13}C/^{12}C$) and N ($^{15}N/^{14}N$) offer the ability to trace movement of material through food webs (e.g., Fogel and Cifuentes 1993; Turner et al. 2010; Layman et al. 2012) and, in the case of sponges, through the microbiome (e.g., Weisz et al. 2010; Van Duyl et al. 2011; Radax et al. 2012b; Fiore et al. 2013a, b, 2015; Simister et al. 2013). Weisz (2006) was among the first to apply this technology to sponges, and in one of the largest comparisons to date (nearly 50 sponge species; Fig. 5.3), he showed that each occupied a distinct isotopic niche space (INS). This provided clear evidence that different species of sponge perform very different roles in terms of processing material from the water column. In fact, despite the fact that all sponges are bacterivorous filter feeders (capturing POM >0.1 µm from seawater

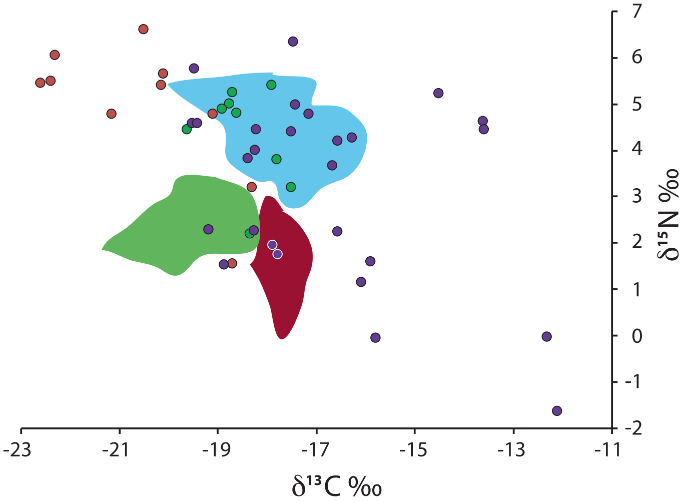

Fig. 5.3 Average stable isotope values for Atlantic, Caribbean, and Indo-Pacific sponges. Points represent data from Weisz (2006) for 48 sponges from the Florida Keys (*purple*), North Carolina (*red*), and Papua New Guinea (*green*). Clouds represent estimates of Freeman et al. (2014) clusters of isotope niche space (i.e., convex hulls) obtained from sponges collected in the Miskito Cays, Honduras. The *green cloud* is an HMA cluster of three sponges with high and low chlorophyll content, and the *red cloud* contains two HMA sponges with high chlorophyll content. The *blue cloud* represents a broad assortment of high chlorophyll content HMA sponges ($n = 2$), low chlorophyll content HMA ($n = 3$), and LMA ($n = 9$) sponges

(Riisgård and Larsen 2010)), it is clear that different species, even sympatric sponges coexisting in close proximity on the same reef, appear to occupy distinctive niches based on the characteristics of their feeding behavior—that is, these sponges appear to exist in different levels within a food web (Fig. 5.3). Freeman et al. (2014) compared INS for sponges from the Miskito Cays, Honduras. It is important to note that the Weisz (2006) and Freeman et al. (2014) datasets overlap in species composition and thus provide an opportunity to evaluate the potential influence of environmental drivers on isotopic signatures. For example, differences in δ^{15}N for *Aplysina* and *Ectyoplasia* are minor between Weisz et al. (2007) and Freeman et al. (2014), while sponges like *Ircinia campana* and *Callyspongia* differ more significantly. It is also important to note that all habitats in each of these studies contained both HMA and LMA sponges.

It is likely that the metabolic properties of the microbiome directly influence the trophic behavior of the host sponge in terms of INS (Weisz et al. 2007; Freeman et al. 2014). Weisz (2006) proposed that "the strong association between low δ^{15}N tissue values and the presence of abundant microbes in sponges suggest that internal microbial activity can exert significant control on the N isotopic composition of HMA sponges." Viewing sponge behavior through their INS raises important questions. The first is: why do sponges have such disparate INS within and among species? In reality, we know very little about the metabolic processes that operate within a sponge, nor the nuances of a sponge's heterotrophic feeding. Our ignorance of the role that microbial symbionts play in those processes is deeper. Do patterns exist in what niche space is available to sponges, and if so, what role do microbial symbionts play in expanding (or contracting) INS? Are some transformations improbable given the metabolic pathways available to microbial communities (e.g., combinations of lighter C and N (δ^{13}C <−19; δ^{15}N <1))? Freeman et al. (2014) have pointed out that variation in biogeochemical cycling of C and N observed among species is complex and suggested some level of niche partitioning may be made possible by the microbial symbionts. They suggest that variation in INS may be driven more by the presence or absence of particular microbial taxa than by overall symbiont or photosymbiont abundance (see also Freeman et al. 2013) while recognizing that symbionts within HMA species increase the metabolic capacity of the holobiont. They argue that the presence or absence of productive photosymbiont taxa has a large effect on the INS available to host sponges (see also Weisz et al. 2010).

Despite recognizing their importance, the reality is that we know relatively little about the energetic transformations that occur within individual sponges, though that situation is improving (e.g., Yahel et al. 2005; Duckworth et al. 2006; Weisz et al. 2008; Hanson et al. 2009; Topçu et al. 2010; Radax et al. 2012b). We are learning that sponges demonstrate some selectivity for particular prey items. For example, Perea-Blázquez et al. (2013) found that retention efficiencies changed for three species of sponge in response to picoplanktonic species composition and abundance in temperate habitats around New Zealand. In this study, sponges appeared to obtain 20–40 times more C from heterotrophic bacteria than from the consumption of cyanobacteria like *Synechococcus* or *Prochlorococcus*. However, the sponges studied appeared to have fixed feeding strategies that did not respond to changes in ambient bacterioplankton concentrations (Perea-Blázquez et al. 2013). In contrast, Topçu et al. (2010) found that the Mediterranean *Spongia officinalis* had similar levels of capture efficiency for the major picoplanktonic groups, but lower efficiencies for the nanoplankton.

Despite the abundance of picoplankton, *S. officinalis* appeared to convert a larger percentage of nanoeukaryotic cells into sponge biomass as indicated by δ^{13}C INS. As in Weisz (2006), Topçu et al. (2010) argued that the low δ^{15}N signature was due to microbial symbionts (see also Freeman et al. 2014).

But what precise role do the symbionts play in holobiont trophic behavior? It is clear that a variety of experimental approaches are required including pulse-chase analyses. Using stable isotope tracers, Weisz et al. (2010) were able to demonstrate movement of C between *Symbiodinium* and *Cliona*, but the story with N was more subtle and complex. Are some metabolic processes linked among the members of the microbiome such that only some chemical transformations are possible (e.g., rules of community function deriving from rules of community assembly)? This is an area of research that deserves greater attention. We clearly have much to learn about sponge trophic ecology to understand how the host and microbial symbiont communities interact.

5.3.3 Natural Products: Another Level of Host-Symbiont Integration

Natural product chemistry is an area where clear phenotypic synergisms can be identified because large amounts of data are becoming more widely available (e.g., Hill 2004; Müller et al. 2004; Sipkema et al. 2005; Proksch et al. 2010; Sacristán-Soriano et al. 2012). Although Porifera has been the preferred phylum of the marine natural product community for the last 50 years (Blunt et al. 2015), the origin of many compounds remains controversial (König et al. 2006; Wang 2006; Hentschel et al. 2012). Many sponge-derived metabolites are suspected to be produced by bacteria, since they resemble bacterial compounds or belong to substance-type characteristic for these microorganisms. Vacelet (1975) was among the first to implicate bacteria in the origin of sponge natural products given the huge numbers of bacteria that inhabited sponge tissues. The classical strategy to isolate the putative microbial producer was to apply culture-dependent methods (Piel 2004 and references therein). However, the complexity and the difficulties in cultivation of associated microbial communities represented new challenges to explore secondary metabolite production.

Culture-independent methods have opened new paths to identify the true producer of sponge-derived compounds. In situ localization of a compound would indicate the origin of production, as is the case of the sponge *Dysidea herbacea* and its associated cyanobacterium *Oscillatoria spongeliae* that accumulates brominated and chlorinated metabolites (Unson and Faulkner 1993; Unson et al. 1994). Other examples of a suggested bacterial origin of sponge-derived compounds have been described in the sponge *Theonella swinhoei* (Bewley et al. 1996; Schmidt et al. 2000). Additional studies on other species reported instead a localization of natural products in sponge cells, suggesting a sponge origin (Uriz et al. 1996; Turon et al. 2000). However, localization studies have a major problem that the presence of a compound in a cell type does not undoubtedly prove the location of biosynthesis since organisms producing bioactive compounds frequently excrete them in the surrounding environment (Méndez and Salas 2001). Thus, one cell type could produce the metabolite that may be accumulated in a second.

Several researchers have attempted to use observational studies to link secondary metabolite production to the microbial consortia. Recent work in *Aplysina* using DNA-based molecular approaches highlights close associations between some bacterial phylotypes and major brominated compounds sharing spatial variation patterns (Sacristán-Soriano et al. 2011a, b). These correlative studies are promising and require further exploration given that the microorganisms could be directly producing or halogenating such compounds (van Pée et al. 2006) or contributing to their catabolism (Ahn et al. 2003). These results underline the complex control of the production of secondary metabolites with a potential environmental modulation. Experimentally modifying the concentrations of natural products or the abundance of endobiotic bacteria through alterations of environmental parameters might reveal their true association and infer functional relationships. Several studies successfully attempted to culture specimens of different sponge species to monitor both bacterial communities and chemical profiles, mimicking wild conditions (Klöppel et al. 2008; Mohamed et al. 2008) or altering a particular environmental factor (Friedrich et al. 2001; Klöppel et al. 2008; Sacristán-Soriano et al. 2016). All of those studies concluded major stability of chemical fingerprints and prevalence of microbial assemblages upon culture. Where some degree of variability in associated bacteria was detected, those changes were not linked with compound abundances. So, the possible involvement of symbionts in secondary metabolism could not be drawn using the molecular tools available to date.

The application of cutting-edge molecular techniques has transformed the field of sponge symbiosis. Next-generation sequencing offers the ability to uncover the diversity of the sponge microbiome (Webster et al. 2010). Besides the use of the 16S rRNA gene as a phylogenetic marker to characterize associated microbial communities, other genes have been selected to disclose putative functions from sponge symbionts, for example, secondary metabolite biosynthetic gene clusters. Different molecular approaches (e.g., gene-derived PCR amplicons, metagenomics, single-cell genomics) have led to the identification of several polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS) in microbes from marine sponges, both of which represent prominent enzyme classes that are responsible for the synthesis of bioactive compounds (Schirmer et al. 2005; Piel 2009; Siegl and Hentschel 2010; Hochmuth et al. 2010; Hardoim and Costa 2014). Other target markers are those genes that encode for halogenase enzymes, which are responsible for the halogenation process of secondary metabolites. Bayer et al. (2013) identified three different halogenase gene clusters present in three symbiotic bacterial phyla from *A. aerophoba*. Culture-dependent methods also allowed detection of halogenase genes but with the limitations of the culture of sponge symbionts (Öztürk et al. 2013).

The ecological and metabolic significance of the expressed natural product biosynthetic genes still remains unclear. Once those gene clusters have been identified, the heterologous expression of the biosynthetic genes in culturable bacteria may prove the function of these genes in their original host, assess their role in symbiont physiology, and determine host-microbe integration. This knowledge will help to address a major challenge of human population growth, to make the marine drugs discovered marketable (see Indraningrat et al. 2016).

5.3.4 Establishing Residency: Phagotrophic Escape?

While many bacteria are consumed by the sponge, many are not and persist in the mesohyl indefinitely. The pathways by which bacteria enter the mesohyl and gain residency within the host sponge remain obscure. Nguyen et al. (2014) found eukaryotic-like, ankyrin repeat proteins in an uncultured gammaproteobacterial sponge symbiont that modulated the phagocytosis behavior of amoebal cells. Specifically, when *Escherichia coli* expressed the four symbiont-derived genes, the bacteria accumulated in a phagosome of the amoeba. Furthermore, phagosome development was disturbed in a way that reduced vacuole acidification because lysosomal fusion was blocked. The authors postulated that this might be an escape mechanism by which potential symbionts could make their way into the mesohyl of the sponge after phagocytosis (see red arrow in Fig. 5.2). If this is a strategy employed by extracellular symbionts, then the pathways that are usurped for exocytosis post-capture require elucidation. Hill and Hill (2012) proposed that *Symbiodinium* employ a mimicry strategy to avoid detection by the host. Their arrested phagosome hypothesis suggested that translocation of photosynthates might mimic digesting prey, thus providing a mechanism to avoid digestion by the host. The molecular mechanisms by which such arrest occurs are unknown, but might involve pathways similar to the one proposed by Nguyen et al. (2014). If the process of transfer into the mesohyl involves transport via endomembranous compartments, it is possible that vertically transmitted bacterial symbionts engage similar genetic pathways to gain entry to eggs or sperm (e.g., Sciscioli et al. 1994; Usher et al. 2001). The ontogenetic changes in symbiont communities (from larvae to adult) also deserve attention. For many sponge species, larvae are nonfeeding during the planktonic larval stage. Broadcast spawning species would have to pass symbionts to the larvae through the eggs, while brooders could transmit bacteria from mother to larvae (Kaye 1991). At metamorphosis, however, the sponge will shift from nonfeeding to filter feeding, which would potentially precipitate a rapid mixing of environmental populations of bacteria with resident symbionts. We require detailed study of the transition from one life stage to another in the context of the composition, structure, and stability of symbiotic communities in sponges and how the host distinguishes food from symbiont.

5.4 Community-Wide Consequences of Holobiont Behavior

Consideration of the holobiont is of paramount importance when one examines ecosystem-level metabolic processes involving sponges. Sponges are abundant and integral members of many marine communities from the poles to the tropics (e.g., Díaz and Rützler 2001; McClintock et al. 2005; Ribes et al. 2012). On Caribbean reefs, sponges occupy about the same amount of benthic cover as corals, but provide more biomass to these habitats. While the amount of sponge biomass matters, it is the behavior of sponges that can have profound effects on the entire community. Sponges are becoming the dominant faunal component of many reefs (McMurray et al. 2010; Colvard and Edmunds 2011; Bell et al. 2013), and their ecological significance to these communities is well established (Wulff 1997, 2006, 2008a, b, 2012; Hill 1998). However, biologists are beginning to focus attention on the role sponges play in creating bioavailable material for the entire ecosystem through their trophic behavior.

As efficient filterers that spend the majority of their days capturing prey items and processing and modifying large volumes of water (Fig. 5.2), sponges provide a locus for the movement of autochthonous and allochthonous carbon and nitrogen (among other nutrients) through marine communities. Sponge-mediated benthic:pelagic coupling influences properties of marine habitats in profound ways (Reiswig 1971a, b, 1974, 1981; Corredor et al. 1988; Riisgård et al. 1993; Pile et al. 1996, 1997; Diaz and Ward 1997; Savarese et al. 1997; Turon et al. 1997; Ribes et al. 1999; Lynch and Phlips 2000; Yahel et al. 2003; Lesser 2006; Peterson et al. 2006; Perea-Blázquez et al. 2012; Lesser and Slattery 2013). Sponges appear to contribute to the population dynamics of phytoplankton and benthic algae (e.g., Peterson et al. 2006; Lesser 2006; Lesser and Slattery 2013), sponges are dominant competitors for space (Rützler 1970; Hill 1998), and sponges serve as prey and habitat for other organisms (Westinga and Hoetjes 1981; Duffy 1996; Hill 1998; Ávila et al. 2007; Azzini et al. 2008; Ramsby et al. 2012). Thus, in marine habitats, sponges perform ecosystem services with far-reaching effects on community structure. These keystone ecological outcomes are made possible by the ability of sponges to convert POM and DOM into sponge biomass (Reiswig 1981; Pile et al. 1996, 1997; Pile 1997; Yahel et al. 2003, 2005; Hadas et al. 2009 Maldonado et al. 2012). Modes of action in converting matter from one form to another involve synergistic and interactive properties of the symbiont and host compartments (e.g., Erwin and Thacker 2007; Southwell et al. 2008; Gibson 2011), and teasing apart the contributions of microbial symbionts (e.g., HMA vs. LMA strategies) remains an important goal (e.g., Reiswig 1981; Weisz et al. 2008).

5.4.1 Sponge Loops and Detrital Linkages

The oligotrophic waters of tropical coral reefs support some of the most diverse and productive ecosystems on the planet. This enigmatic situation, known as Darwin's paradox, may have an explanation via the recently proposed sponge loop hypothesis (SLH—de Goeij et al. 2013). The "sponge loop" considers the trophic behavior of sponges as efficient filter feeders and the rapid cell turnover found in sponges, to posit a detrital-based component to coral reef food webs. That is, sponges represent sinks for POM and DOM, produce POM of their own through release of spent sponge cells, and mediate biogeochemical fluxes through their consumption and release of nutrients (e.g., nitrates, nitrites, phosphates, etc.; Bell 2008; Southwell et al. 2008; Fiore et al. 2010; Maldonado et al. 2012; Zhang et al. 2015). This bottom-up hypothesis provides a mechanism by which a large proportion of POM is released by sponges and then enters the food web through detritivorous benthic reef fauna. For example, Silveira et al. (2015) demonstrated that the SLH may help explain changes in fish biomass on coral reefs. According to the SLH, the loop begins with DOM, principally DOC, that is released by autotrophic organisms (e.g., seaweeds and *Symbiodinium*-bearing corals), taken up by the sponge, and converted to biomass through holobiont metabolic processes.

Thus, the SLH predicts that sponges perform vital roles in retaining biomass in these marine habitats. de Goeij et al. (2013) suggested that the conversion of DOC to POC by all the sponges on a reef is on par with the daily gross primary production of the entire reef ecosystem. The imagined steps in the trophic chain, that is, movement of sponge material through detritivores into higher trophic levels, are still hypothetical, and empirical work demonstrating these linkages is required.

While the SLH is an important contribution, several questions remain. What role do symbionts play in the SLH? Symbionts contribute to the sponge's diet. For example, Erwin and Thacker (2008) demonstrated that cyanobacterial symbionts contribute significantly to the energetic budgets of two Caribbean sponges, and Weisz et al. (2010) demonstrated that carbon is translocated from *Symbiodinium* to *Cliona varians*. Autotrophic contributions are easier to follow than the subtle chemical and energetic transformations that involve heterotrophic bacteria and archaea. Nitrogen translocation, or at least manipulation of the N profiles of the host sponge, has been more difficult to assess (Zhang et al. 2013). Weisz et al. (2010) found little evidence that N was transferred to *Symbiodinium* populations via phagotrophic capture of N-labeled bacteria in *C. varians*. Freeman and Thacker (2011) showed that the translocation pathways may be species specific. They found that symbionts transfer C but not N to *Neopetrosia subtriangularis*, N but not C to *Aplysina fulva*, and both C and N to *A. cauliformis*. While it is highly likely that the non-photosynthetic component of the microbial community plays important metabolic roles in determining the fraction of a sponge's diet that is comprised of DOM versus POM, the reasons for the types of discrepancies among species observed by Freeman and Thacker (2011) remain obscure. It is clear that many sponge-based anabolic and catabolic processes that shape the conversion of DOM/DOC to POM/POC are poorly understood, and this represents an important area of future research.

Some have questioned whether the bioavailability of DOC is broad enough to support the trophic cascades implied by the SLH (e.g., Pawlik et al. 2015). Labile DOM includes organic compounds that are present in systems from hours to days (e.g., sugars, lipids). Semi-labile and semi-refractory DOM are turned over on time scales of years and decades, but refractory DOM can last 104 years. Refractory DOM is the most ubiquitous fraction of DOM in the world's oceans and is not subject to cellular metabolism (Osterholz et al. 2015) though microbial community structure influences DOM composition (Kujawinski et al. 2016). The question sponge biologists are now facing is whether and how much the microbial symbiont communities affect the consumption and production of labile and refractory DOM. There are abundant opportunities to contrast HMA and LMA sponges to determine the extent of contributions of symbiont communities to holobiont behavior. It is likely that different sponge species, especially those with different symbiont communities, consume DOM and POM differently (e.g., Freeman and Thacker 2011). A common perception among sponge biologists is that HMA sponges pump at slower rates compared to LMA species and that HMA sponges obtain most of the dietary carbon from DOC, while LMA sponges, as true heterotrophs, use POC to fuel growth. This simplified view of HMA/LMA consumptive behavior is likely false in that feeding behavior is likely species specific and context dependent (see e.g., Topçu et al. 2010; Freeman and Thacker 2011; Perea-Blázquez et al. 2013). The HMA/LMA distinction is also likely a false dichotomy (Gloeckner et al. 2014; Poppell et al. 2014). For a few sponge species, an intermediate microbial load is harbored by the host sponge (Gloeckner et al. 2014). Mueller et al. (2014)

also provided data indicating that LMA sponges use DOC in a manner similar to HMA sponges. What is becoming clear is that trait-mediated ecosystem impacts caused by sponge feeding behavior (e.g., McMurray et al. 2014) must take into consideration characteristics of the holobiont. In the coral literature, an emerging perspective is that partner identity *and* density matter in determining holobiont performance (e.g., Cunning and Baker 2014). Thus, while density of microbial communities is important (e.g., Poppell et al. 2014), species richness—more importantly the type of species in the community—likely influences the trophic characteristics of the holobiont's extended phenotype.

5.5 Structure of Microbial Communities

Sponges offer ecologists incredible opportunities to test emerging ideas about community structure. This phylum has the opportunity to contribute to broader ecological issues as suggested in the analyses of the publication impact in sponge chemical and microbial ecology (Sacristán-Soriano and Becerro 2016). As with systems like gut microbiota, sponge microbial communities are island-like in that they occupy clearly delimited habitats. There is also an influx and efflux of microbes associated with the host, though the mechanisms that govern movement between host occupancy and the environment are obscure. At the simplest level, island biogeography theory may be a useful heuristic to determine whether a dynamic and equilibrial microbial species richness exists within sponges. In this regard, HMA sponges are probably the most appropriate study organisms. Nonetheless, understanding metacommunity dynamics is essential for sponge symbioses, and this is a wide-open area of study. We know very little about the effects of dispersal among connected communities. Furthermore, competition-colonization models may explain the coexistence of superior and inferior competitors as the result of trade-offs in colonization and competitive ability. Superior competitors may have a high probability of taking over a particular host, but may have reduced capacities for moving among hosts. On the other hand, superior colonizers may be inferior competitors despite their ability to more easily move between sponges. Dynamics such as these may explain the core, variable, and species-specific components that have been identified in sponge symbiont communities (Schmitt et al. 2012).

5.5.1 Neutral or Not: Networks and Rules of Community Assembly

Of the major processes (e.g., ecological drift, selection, dispersal, and mutation/speciation) that govern the assembly of ecological communities, our goal should be to determine under which conditions each has the strongest influence on sponge holobiont systems. While ecologists are largely agreed that neutral theory has provided valuable null models for community ecology (e.g., Gotelli and McGill 2006), particularly for species-rich systems, which sponge microbial communities may represent, there is much debate about the importance of interspecific interactions. Indeed, a central debate right now is over the relative significance of stochastic ("ecologically neutral") processes versus deterministic selection by specific environmental factors, which is especially important when considering the development of the host (e.g., Stegen et al. 2012; Yan et al. 2012). While selection is deterministic and drift stochastic (and speciation and dispersal can contribute to both deterministic and stochastic processes—Zhou et al. 2014), we do not know the relative contributions these processes make to sponge microbial communities. Indeed, we do not know the effect of these processes, in most cases, on any microbial community. Sponges offer the opportunity to couple spatiotemporal data on microbial symbiont communities from different sponge (i.e., environmental) habitats with metrics and null models of within and between community phylogenetic composition. If community assembly is controlled predominantly by stochastic processes, we would expect a high degree of site-to-site variation in composition regardless of respective niches in that habitat (Hubbell 2001; Purves and Turnbull 2010; Rosindell et al. 2012). Deterministic processes, on the other hand, would likely generate similar communities in similar hosts and would imply some rules for community assembly (Diamond 1975; Gotelli and McCabe 2002).

Debates about which ecological process dominates the development and maintenance of microbial communities continue (e.g., Erwin et al. 2012b, Fan et al. 2012). For example, Zhou et al. (2013) found that microbial communities in bioreactors were mainly shaped by stochastic processes, while Wang et al. (2013) argued that many aquatic bacterial communities were dominated by deterministic processes. The holobiont perspective adds another layer of complexity given that reciprocal evolutionary responses (i.e., coevolution) can further modify the nature of the interaction. If sponges are like biological fermenters (Hentschel et al. 2006), we might expect stochastic processes to dominate. However, the relative contributions of deterministic and stochastic processes to the assembly of microbial communities may vary across hosts. The relative importance of these processes may also shift within a specific host, especially when environmental disturbances occur (Ferrenberg et al. 2013; Zhou et al. 2014). The influence of host physiology is another factor that complicates aspects of community assembly in sponge symbiont microbiota given that environmental stressors and host performance influence the microbes' environment (Benson et al. 2010; Wong and Rawls 2012; Bolnick et al. 2014).

Questions about rules of assembly of complex microbial communities through space and time may be especially tractable using sponge models (e.g., Liu et al. 2012). In this regard, the use of ecological networks makes it possible to analyze and partition the effects of community properties and their effect on the stability of an ecosystem. Thomas et al. (2016) began to estimate the positive and negative effects, both direct and indirect, that species within sponge microbial communities might have on each other. Their approach is promising and demonstrates how large datasets can be employed to tease apart interspecific interactions. For example, the role of ecosystem complexity on community stability is an area of exploration that may benefit from the symbioses we see in sponges. Network structure may reduce the spread of indirect effects and thus enhance ecosystem stability. Sponges afford many important tests of ecological theory in this time of climate change-related environmental stress and disturbance.

Given that the sponge host develops from larvae and sponges have broad biogeographic distributions, we need to know if taxonomic composition and phylogenetic diversity change through community turnover on temporal (seasonal, life history) and spatial (disturbed vs. undisturbed habitats) scales (e.g., Easson and Thacker 2014; Luter et al. 2015). Several studies have shown that bacterial symbionts exhibit high levels of temporal stability in community composition despite large fluctuations in temperature (Erwin et al. 2012a, b). Other sponges that occur across sharp environmental gradients (e.g., subtidal and intertidal *Hymeniacidon heliophila*) have symbiont communities that differ in overall diversity, structure, and composition, and this appears to be driven by abiotic features of the different habitats (Weigel and Erwin 2015).

Processes that drive interindividual variation, if this is a major feature of sponge symbioses, are poorly understood, and this is an area that deserves greater attention. Burns et al. (2016) found that during host development, the fit of microbial communities to a neutral model of assembly decreased in later developmental stages, which points to deterministic factors such as microbe-microbe interactions, active dispersal, and selection by the host as driving the ultimate shape of the microbial community. If the evolutionary history of the symbiont communities matters, more closely related taxa are likely to have more similar habitat associations, which would be apparent in testing for phylogenetic signal. As has been found in subsurface microbial communities (Stegen et al. 2012), we should examine whether sponge microbial symbiont communities composed of ecologically similar taxa coexist to a greater degree than expected by chance (see Thomas et al. 2016).

5.5.2 *Horizontal or Vertical, High or Low?*

What features of HMA and LMA sponges are responsible for the obvious differences we see in microbial densities among species? One possibility is that HMA sponges actively recruit high densities of microbes. Conversely, HMA sponges may lack a particular type of immunological response, which LMA sponges have, that prevents clearing of resident microbes from their tissues once the symbiont establishes residency. Another possibility is that LMA sponges encourage recruitment of a competitive bacterial taxon that excludes other microbes from colonizing the host. Each of these hypotheses points to the key fact that we have very limited understanding of ecological, evolutionary, physiological, and developmental factors that produce the patterns we see among sponge hosts. Kaye (1991) provided the first clear evidence that brooding sponges had mechanisms to inoculate larvae with microbial consortia.

More recently, Schmitt et al. (2008; see also Lee et al. 2009, 2011) compared the microbial communities found in adult and reproductive material from five species of sponge. They identified 28 microbial clusters that co-occurred in the adult and larval tissue, which they deemed as strong candidates for vertically transmitted symbionts.

However, the lack of co-speciation between host and symbiont, and the clear evidence for some degree of horizontal transmission of symbionts between generations (Schmitt et al. 2008), indicates that we still have an incomplete understanding of the mechanism(s) by which sponge-symbiont associations develop. If sponge symbiont communities are influenced by competition-colonization trade-offs among their members, then we may be able to make some predictions about community structure in HMA and LMA sponges. For example, Orrock and Watling (2010) showed that the size of communities in a metacommunity framework dictated whether community dynamics were niche structured or neutral. Large communities, perhaps analogous to HMA sponges, would be expected to fit classic competition-colonization models where niche-based interactions (i.e., competitive hierarchies) drive overall outcomes (Orrock and Watling 2010). Small communities, like the symbiont communities found in LMA sponges, might be expected to experience greater levels of ecological drift because competitive ability between microbial species would be effectively neutralized given the infrequency of interactions and the lack of a correlation of species abundance to competitive ability. An interesting consequence of Orrock and Watling (2010) model is that poor competitors with good colonizing capabilities may find refuges in small communities, so sponge "specialists" might appear even if those species are merely a part of the larger metacommunity.

5.6 Sponge Symbioses in a Changing Ocean

The world's oceans are changing, though attention to this issue has lagged behind that for land-based change (Allison and Bassett 2015; Bennett et al. 2017). Part of the reason for this is that the effects of climate change on marine systems are invisible to many humans (Weber 2013). Nonetheless, compelling evidence indicates that human activities are modifying the physical characteristics of the atmosphere and the oceans (IPCC 2013, 2014). The biological consequences of these changes are difficult to predict, but scientists are united in their concern that the rapidity of change will have spreading detrimental effects on marine communities. Subtle shifts in currents, temperature regimes, primary productivity, pH of the water, etc., all lead to modifications of the interspecific interactions that have shaped ecosystems for millennia (Bell et al. 2013).

With a holobiont perspective, it is now recognized that we must understand how these changing conditions will affect not just the host but the plethora of organisms that reside within and on that host (e.g., Selvin et al. 2009; Cárdenas et al. 2014). The reasons for adopting this perspective are practical. Conservation of marine habitats and their organisms relies on healthy specimens, healthy organisms have healthy symbiont communities (Mazmanian et al. 2008), and healthy individuals contribute to robust marine benthic communities. In fact, theory indicates that if sponge symbiont communities are more strongly niche structured, which some evidence indicates they may be (Thomas et al. 2016), then they will be more stable than neutral communities in a static environment. These communities will, however, show more pronounced, predictable, and directional responses to environmental change.

Strongly niche-structured communities will also more tightly regulate the biogeochemical functioning of the ecosystem (Beare et al. 1995; Hector and Bagchi 2007), but they are more sensitive, in terms of biogeochemical functioning, to the loss of a particular species. Thus, a holobiont perspective is required as we think of ways to protect marine environments from the consequences of climate change: the unpredictable outcomes of climate-related modifications to the holobionts that reside in marine environments are cause for great concern.

5.6.1 Heat and pH

Early work explored the disruptive effects of stressful temperatures on the structure of sponge-associated microbial communities (Lemoine et al. 2007; Webster et al. 2008a) or on host responses to stress (López-Legentil et al. 2008). Despite some evidence indicating thermal tolerance (Schönberg et al. 2008; Webster et al. 2011; Duckworth et al. 2012; Luter et al. 2012; Simister et al. 2012c), small changes in temperature have been shown to cause shifts in the composition and behavior of sponges and their microbial communities (Lemoine et al. 2007; Webster et al. 2008a; Pantile and Webster 2011; Massaro et al. 2012; Simister et al. 2012a; Fan et al. 2013; but see Pita et al. 2013). Webster et al. (2008a) demonstrated a clear shift in community composition through the loss of symbiont species for the tropical sponge *Rhopaloeides odorabile* when temperatures reached those that induce coral bleaching. The loss of the symbionts from *R. odorabile* had several undesirable effects including the establishment of alien microbial populations that included potential pathogens and significant changes in sponge feeding behavior (Massaro et al. 2012). Most recently, Bennett et al. (2017) demonstrated that temperature and pH have complicated effects on sponges that harbor distinct symbiont communities, and these effects depend upon the developmental stage of the host.

The effects of warmer water extend to intracellular eukaryotic sponge symbionts. A mass bleaching event involving clionaid sponges, coincident with a mass coral bleaching event, was observed in the Florida Keys in 2015 (Fig. 5.4; Hill et al. 2016). Events like the 2015 sponge bleaching raise significant concern about the future health of marine sponges and the broader effects that destabilizing symbiont communities might have on the health of the entire ecosystem. Specifically, if the sponge loop relies on holobiont performance and if symbiont communities are disturbed by stressful environmental conditions, we may see unwanted and far-reaching changes to coral reef and other marine ecosystems. A more complete understanding of sponge impacts on coral reef biogeochemistry is important given that coastal eutrophication, climate warming, and overfishing create conditions detrimental to corals while favoring increases (Zea 1994; Rützler 2004) or, in some instances, decreases (Butler et al. 1995) in sponge abundance. The challenge now facing the scientific community is to develop tools that allow for nuanced examination of the subtle shifts in microbial

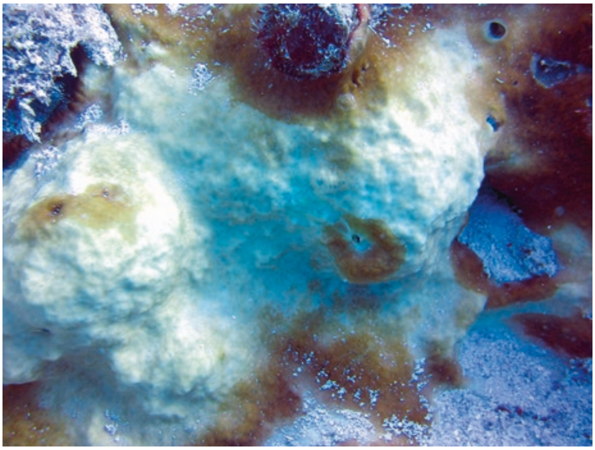

Fig. 5.4 Bleached *Cliona varians* from the Florida Keys observed during a mass bleaching event involving sponges in October, 2015 (Hill et al. 2016). Notice the unbleached circular region around the osculum located to the right of center of the image. This pattern was not uncommon; more extensive bleaching was observed in regions of the sponge some distance from an osculum, while resident *Symbiodinium* often remained in clearly delineated circular patches around oscula. Photo courtesy of C. Walter

community function and physiology and to correlate those changes with host behavior and host gene expression (e.g., Webster et al. 2013; Bennett et al. 2017).

Ocean acidification (OA) is another growing threat in marine habitats caused by human-induced changes to the atmosphere (Gattuso et al. 2015). Among sponges, the Calcarea may face challenges building calcitic skeletons under future OA projections. The majority of focus, however, has been on the effects of shifting pH (and temperature) on bioeroding sponges (Wisshak et al. 2012, 2013; Stubler et al. 2014). The chemical etching component of clionaid bioerosion was elevated under several OA scenarios (Wisshak et al. 2012; Bell et al. 2013). If clionaids experience faster growth with concomitant increases in bioerosion when temperatures and OA increase, then we can expect further declines in the development of coral reef habitats.

Stressors like temperature and ocean pH do not act in isolation (Bennett et al. 2017). Lesser et al. (2016) recently found that the microbiome of *Xestospongia muta* responded to OA and elevated seawater temperature, but did so in complicated, interactive, and indirect ways. They found that the microbiome (*sensu lato*) was generally resistant to temperature and OA stress. These results support those found in Bennett et al. (2017) where interactive effects of ocean warming and OA occurred and were species specific. Indeed, the fact that the sponges that relied on phototrophic symbionts were less susceptible to elevated $p\mathrm{CO}_2$ and temperature stress than those sponges that relied heavily on heterotrophic feeding deserves much greater attention (Bennett et al. 2017).

However, Lesser et al. (2016) demonstrated a decline in the productivity of symbiotic cyanobacteria, the effects of which manifested themselves to the entire microbiome through changes in nutrient translocation dynamics between symbiont and host. Lesser et al.'s (2016) observation that the stability, if not the species composition, of the microbiome was affected most by OA is important. This has not been a major line of inquiry and indicates that we need more research in this area.

5.6.2 Disease

Mass mortality events, and the incidence of unhealthy sponge hosts, appear to have increased in frequency, and disease events are positively correlated with anomalously high seawater temperatures (Olson et al. 2006; Webster 2007; Webster et al. 2008b; Angermeier et al. 2011; Uriz et al. 2016). In one of the better studied systems, a mass mortality event involving sponges from the Mediterranean affected over 90% of the *Ircinia fasciculata* observed in some localities during the warmest months of the year (Maldonado et al. 2010; Cebrian et al. 2011). Other die-offs of *Ircinia* populations have been recorded from the Adriatic and Ionian Seas (Stabili et al. 2012; Di Camillo et al. 2013). The causative agent is unknown, with some authors believing the disease is caused by a twisted rod bacterium, *Vibrio rotiferianus*, or thermally stressed cyanobacteria (Maldonado et al. 2010; Cebrian et al. 2011; Stabili et al. 2012). Interestingly, Blanquer et al. (2016) recently suggested that sponge disease was caused by a shift in the relative abundance of bacterial OTUs, which lead to community dysfunction (see also, Uriz et al. 2016). Thus, disease in this case would not be caused by the presence of a single pathogen, but rather a subtle shift in the microbiome may have resulted in host sickness, which is reminiscent of the balance between health and disease for hosts in gut symbioses relying on the activities of members of the entire microbial symbiont community (e.g., Mazmanian et al. 2008). Indeed, Gao et al. (2015) recently demonstrated shifts of prokaryotic communities between healthy and disease-like sponge tissues. Thus, Koch's postulates may not apply to some climate change-instigated diseases of the future, and results like those described above highlight the concerns that many have about a warming ocean that is experiencing shifts in general chemistry (e.g., Luter et al. 2010; Olson et al. 2014; Sweet et al. 2015). Stable microbial communities have been built, evolutionarily speaking, upon rates of material flux and strengths of interspecific interactions, and climate change-mediated fluctuations in environmental conditions may move sponge holobionts away from "equilibrial" conditions that support the health of the host.

5.7 Conclusions

It is well documented that the environment is changing due to our alteration of the earth's atmosphere and the world's oceans. Marine biologists face the daunting task of estimating and predicting the consequences, in terms of the structure and function of marine ecosystems, of those environmental changes. Given their essential ecological roles, we must increase attention on the effects that climate change will have on sponges and their microbial symbionts. Several recent studies highlight how urgent the problem has become given clear evidence that sponges are responding to environmental stressors that are becoming more frequent and more intense. The scale and scope of future studies must extend from the most nuanced aspects of integrated gene interactions to community-wide ramifications of holobiont behavior. Fortunately, we have entered a technological period where powerful tools are opening lines of investigation that previously have been difficult or impossible to pursue. The timing could not be any more propitious. If we can provide resource managers with guidelines about expected responses of sponge communities, we may be able to craft policies that protect marine habitats to a greater extent than is the case currently.

Furthermore, the sponge holobiont may provide a perspective on the way that symbiotic interactions can be harnessed to help address major challenges of human population growth (e.g., the manufacture of new drugs, sustainable production of energy, conservation of marine environments). The holobiont perspective of symbioses advocated here and by many authors promises to shed light on important questions of broad interest to the scientific community.

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