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Minireview

Marine sponges and their microbial symbionts: love and other relationships

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Summary

Many marine sponges harbour dense and diverse microbial communities of considerable ecological and biotechnological importance. While the past decade has seen tremendous advances in our understanding of the phylogenetic diversity of sponge-associated microorganisms (more than 25 bacterial phyla have now been reported from sponges), it is only in the past 3–4 years that the *in situ* activity and function of these microbes has become a major research focus. Already the rewards of this new emphasis are evident, with genomics and experimental approaches yielding novel insights into symbiont function. Key steps in the nitrogen cycle [denitrification, anaerobic ammonium oxidation (Anammox)] have recently been demonstrated in sponges for the first time, with diverse bacteria – including the sponge-associated candidate phylum '*Poribacteria*' – being implicated in these processes. In this minireview we examine recent major developments in the microbiology of sponges, and identify several research areas (e.g. biology of viruses in sponges, effects of environmental stress) that we believe are deserving of increased attention.

Introduction

Marine sponges often harbour dense and diverse microbial communities, with many of the microorganisms being specific to sponge hosts. In fact, distantly related sponges from geographically disparate regions often share microbial phylotypes that have not been recorded from the surrounding seawater or any other habitat (Hentschel *et al*., 2002; Taylor *et al*., 2007a; Lafi *et al*., 2009). These microbes, which can include bacteria, archaea and single-celled eukaryotes (fungi and microalgae), comprise up to 40% of sponge volume and may have a profound impact on host biology. For example, photosynthetically fixed carbon from cyanobacterial symbionts provides > 50% of the energy requirements of certain tropical sponges (Wilkinson, 1983), while other microorganisms may contribute to host defence via the production of biologically active metabolites (Unson *et al*., 1994; Schmidt *et al*., 2000). The latter also hints at the pharmacological potential of sponge-associated microorganisms, with many 'sponge'-derived metabolites of suspected or proven symbiont origin (Hochmuth and Piel, 2009; Piel, 2009). Regardless of the nature of the specific interaction, it seems likely that microbes – both beneficial and deleterious – have played an important role in shaping sponge evolution (Taylor *et al*., 2007b).

The field of sponge symbiosis has rapidly expanded in the past decade, yet as a discipline much is owed to the pioneering work from the 1970s/1980s by researchers such as Jean Vacelet, Clive Wilkinson and Henry Reiswig (Reiswig, 1971; 1975; Vacelet, 1975; Vacelet and Donadey, 1977; Wilkinson, 1978; Wilkinson and Fay, 1979; Wilkinson and Vacelet, 1979). Many of today's major themes in sponge microbiology, such as the existence of sponge-specific microbes and the notion that bacterial symbionts may 'hide' from the sponge host within the mesohyl via the production of protective capsules, were already under investigation decades ago (Wilkinson *et al*., 1981; 1984). Since a review of sponge microbiology in 2007 (Taylor *et al*., 2007a), there have been several hundred publications about various aspects of sponge–microbe associations, and some 9374 sponge-derived microbial sequences submitted to public databases (Fig. 1). Further accelerating this rapid increase in sequences is the application of next-generation sequencing technologies (such as 454 amplicon pyrosequencing) which allows us to explore the rare biosphere of sponge microbes and which is uncovering exceptional diversity (Webster *et al*., 2010a; Lee *et al*., 2011). Not only do sponges host incredibly diverse microbial communities,

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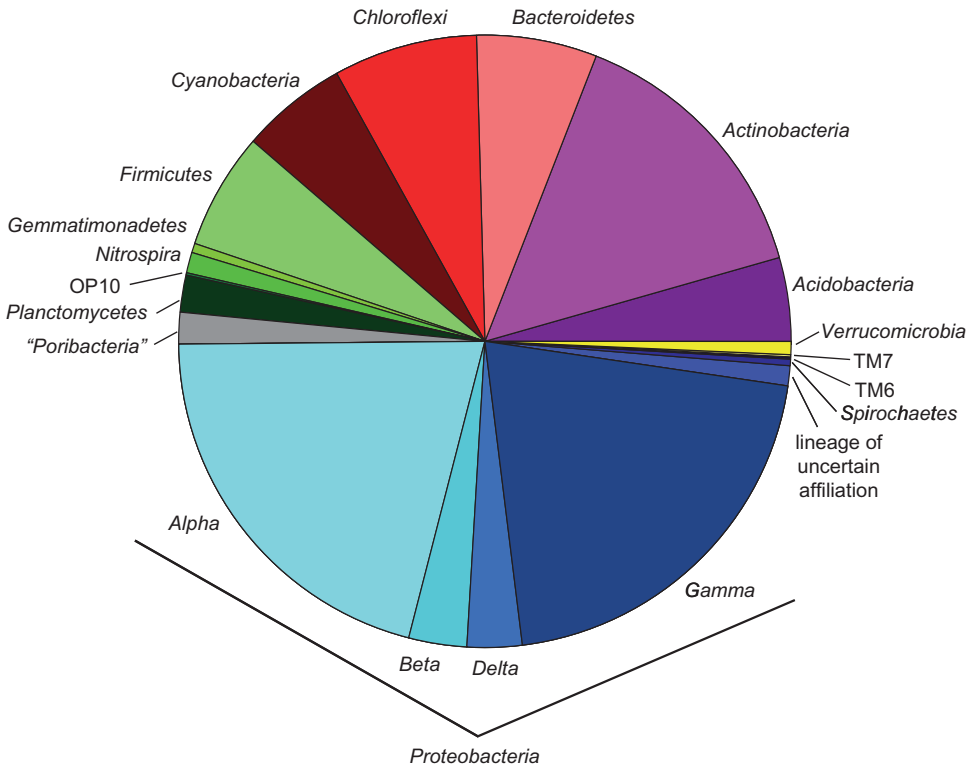

Fig. 1. Phylogenetic distribution of sponge-associated bacteria. A total of 11 284 16S rRNA gene sequences were retrieved from GenBank in September 2010 using the search string: (sponge* or porifera*) and (16S* or ssu* or rRNA*) NOT (18S* or lsu* or large subunit or mitochondri* or 23S* or 5S* or 5.8S* or 28S* or crab* or alga* or mussel* or bivalv* or crustacea*). Sequences were taxonomically assigned to a SILVA database (Version 100) using a customized Perl script; those sequences which could be unambiguously assigned at phylum level are represented here. For clarity of presentation, bacterial phyla with fewer than 10 sequences obtained from sponges were excluded; these were *Chlamydiae*, *Deferribacteres*, *Deinococcus-Thermus*, *Epsilonproteobacteria*, *Fusobacteria*, *Lentisphaerae*, OP11, *Tenericutes*, WS3. Sequences representing *Crenarchaeota* and *Euryarchaeota* (15 and 22 sequences respectively) were also present in the data set, but not included in this figure. Please note that this figure also does not include the > 250 000 sponge-derived 454 tag sequences from the recent study by Webster and colleagues (2010b).

but a recent study comparing 16S rRNA- and 16S rRNA gene-derived sequences from two sponges revealed that the majority of microbes are metabolically active within their respective hosts (Kamke *et al*., 2010). Interestingly, while the immense amount of sequence data produced by 454 amplicon pyrosequencing has altered our perception of microbial species richness in sponges, it has not changed our view of who the major microbial players are. For example, in a recent 454 tag sequencing study of Great Barrier Reef sponges (Webster *et al*., 2010a), the dominant bacterial taxa were *Chloroflexi*, *Acidobacteria*, *Actinobacteria* and *Proteobacteria* (*Alpha*, *Delta* and *Gamma*) – the same phyla that feature heavily in conventional 16S rRNA gene libraries (Taylor *et al*., 2007a). Those phyla that were newly discovered in sponges by 454 sequencing (e.g. *Deferribacteres*, *Tenericutes*) were in all cases only present at low abundance (Webster *et al*., 2010a).

The literature on sponge–microbe associations was comprehensively reviewed in 2007 (Taylor *et al*., 2007a), and it is not our intention here to re-visit this earlier research in great detail. Rather, we review recent significant developments in sponge microbiology, identifying those areas that have seen an increased research focus while highlighting several others that urgently require additional research attention.

Areas that have attracted recent research attention

Microbiology of sponges inhabiting the mesophotic (30–200 m) and deep (> 200 m) zones

The majority of sponge microbiology studies have assessed microbial associations in shallow-water temperate, tropical and Mediterranean systems. Despite the fact that sponges are highly abundant in polar regions, only two studies have utilized molecular tools to examine symbiotic associations in Arctic and Antarctic sponges. These polar sponges did not host the high diversity of sponge-specific sequence clusters (SSSC) detected in many tropical and temperate sponge species (Taylor *et al*., 2007a) but were dominated by either *Archaea* or mixed

bacterial/archaeal communities with high similarity to sea-ice and sediment-associated microbes (Webster *et al*., 2004; Pape *et al*., 2006). Compared with the underexplored polar regions, there has been a large increase in studies of sponge-associated microbes from mesophotic (30–200 m) and deep (> 200 m) zones. The depth of these habitats makes them less susceptible to natural and anthropogenic changes and considerable research is now focused on whether they may provide refugia to reef species as shallow water environments become increasingly degraded (Olson and Kellogg, 2010). Analysis of these deep-water sponges is producing some interesting insights into sponge symbiosis but unfortunately these studies are often constrained by a lack of replication, with many analysing only a single sponge individual. In contrast to the polar sponges, deep-water species tend to be more similar to shallow-water tropical sponges in that they harbour a high microbial diversity with many of the detected microbes clustering within SSSC. In the sponge *Polymastia* cf. *corticata* collected from 1127 m at the Kahouanne Basin (Caribbean Sea), 38 distinct microbial phylotypes were identified and 53% of these fell within previously described SSSC (Meyer and Kuever, 2008). The spatial distribution of the microbes within the sponge tissue was also highly specific, with distinct bacterial populations inhabiting the papillae, outer and inner cortex and the choanosome region, a very similar pattern to that described for the shallow water Mediterranean species *Tethya aurantium* (Thiel *et al*., 2007). Similarly, *Scleritoderma cyanea* collected at 242 m depth off the coast of Curacao and *Scleritoderma* sp. collected from 255 m depth off Bonaire hosted microbial communities most similar to uncultivated microbes retrieved from the shallow-water sponges *Theonella swinhoei* and *Aplysina aerophoba* (Olson and McCarthy, 2005). In contrast, three specimens of *Geodia* collected at depths of 197–304 m were analysed with group-specific fluorescence *in situ* hybridization (FISH) probes and found to host a microbial community which was highly similar to those in sediment samples from the same region, including an abundance of *Archaea*, *Gammaproteobacteria* and *Firmicutes* (Brück *et al*., 2010). However, it is difficult to directly compare this study with other analyses of deep-water sponges due to differences in the level of phylogenetic resolution between techniques. 16S rRNA gene sequencing of the microbes in *Geodia* may have revealed specific differences between the sponges and the sediments within given bacterial phyla and classes. A study of the deep-sea sponges *Characella* sp., collected at a hydrothermal vent off the coast of Japan (686 m depth), and a *Pachastrella* sp. and an unidentified Poecilosclerid sponge from an oil seep in the Gulf of Mexico (572 m depth) led to the first report of thioautotrophic symbionts in sponges (Nishijima *et al*., 2010). The thioautotroph-like microbes recovered from the Poecilosclerid sponge and *Characella* shared 99% sequence similarity to the well-studied thioautotrophic symbionts of *Bathymodiolus* mussels and were present in all tissue types throughout the sponge.

Symbiosis in aquaculture

The past few years have seen increased efforts to cultivate sponges to supply biomaterial for the pharmaceutical and cosmetics industries. The known importance of symbionts to sponge health, survival and metabolite production means that considerable research effort has also targeted the ability of sponges to maintain their symbionts during the cultivation process. The stability of microbial associations during sponge aquaculture appears to vary among different host species and under different cultivation systems. A large proportion of the bacterial communities in *Aplysina cavernicola* and *A. aerophoba* were unaffected by cultivation under different environmental conditions (Friedrich *et al*., 2001; Thoms *et al*., 2003; Gerce *et al*., 2009), yet the microbial communities of *Ircinia strobilina, Mycale laxissima* and *Clathria prolifera* undergo a distinct shift when transferred to aquaculture (Mohamed *et al*., 2008a,b; Isaacs *et al*., 2009). During *ex situ* cultivation of *A. aerophoba*, cyanobacterial density increased by 124% when sponges were grown in light conditions and decreased to 1.7% of original levels when sponges were cultivated in dark conditions (Klöppel *et al*., 2008). Despite this, metabolite concentration actually increased in the dark, implying that cyanobacteria are not involved in the production of these metabolites. The symbiont community in the sponge *Rhopaloeides odorabile* is highly stable when sponges are cultivated in the wild or in small flow-through aquaria but undergoes a large shift when sponges are cultivated in large-scale mesocosms for 12 months (Webster *et al*., 2010b). These studies have highlighted the interspecies variability in symbiont stability during cultivation and illustrate the importance of investigating host microbiology prior to adopting new models for sponge aquaculture.

Symbiont transmission

Surveys of sponge-associated microbial communities using the 16S rRNA gene have suggested that many of these microbes exclusively inhabit sponges (Hentschel *et al*., 2002; Taylor *et al*., 2007c). A fundamental question in sponge evolutionary biology has therefore been how distantly related species from geographically isolated regions could acquire shared bacterial symbionts when they were apparently absent from the seawater. Vertical transmission of sponge symbionts was originally proposed in the 1960s (Lévi and Porte, 1962) and numerous studies subsequently utilized electron microscopy to visu-

alize morphologically similar bacteria within adult sponges and their gametes/offspring (Gaino *et al*., 1987; Kaye, 1991; Sciscioli *et al*., 1991; 1994; Usher *et al*., 2001; Ereskovsky *et al*., 2005; de Caralt *et al*., 2007). In more recent years, application of molecular techniques including denaturing gradient gel electrophoresis (DGGE), 16S rRNA gene library sequencing and FISH have enabled phylogenetic identification of individual microbes present in both adult sponges and their larvae (Enticknap *et al*., 2006; Schmitt *et al*., 2007; 2008; Sharp *et al*., 2007; Steger *et al*., 2008; Lee *et al*., 2009). The apparent absence of these 'vertically transmitted' microbes from the surrounding seawater was thought to provide further evidence of their strict vertical transmission (Lee *et al*., 2009). However, deep sequencing of seawater bacteria using 16S rRNA gene-based amplicon pyrosequencing has recently suggested that the rare seawater biosphere can act as a seed bank for sponge-specific microbes and that environmental transmission may play a significant role in symbiont acquisition by juvenile sponges (Webster *et al*., 2010a). Specifically, 50% of the SSSC detected in adult and larval *R. odorabile* were also detected in the seawater, albeit in extremely low abundances (Webster *et al*., 2010a). As most species thus far examined for symbiont transmission have been brooding sponges (i.e. the adult sponge samples would have been female), it will be interesting to further explore the role of sperm in symbiont transmission and assess potential differences in sponge microbiology between male and female individuals. To date, the only published account of symbiont transmission in sponge sperm involved cyanobacteria in the Australian sponge *Chondrilla australiensis* (Usher *et al*., 2005).

Sponge symbiotic function

Our understanding of the function of sponge-associated microorganisms has traditionally lagged somewhat behind our burgeoning knowledge of their diversity (Taylor *et al*., 2007c). However, symbiont function has become a major focus of recent studies, with nitrogen metabolism in sponges coming under particularly intense scrutiny. A 2007 review by Taylor and colleagues (2007a) summarized knowledge of the nitrogen cycle in sponges, pinpointing two major processes [denitrification and anaerobic ammonium oxidation (Anammox)] which had at that time not been demonstrated in any sponge. Significant progress has been made in this area since then, with stable isotope experiments revealing denitrification in *Geodia barretti* (Hoffmann *et al*., 2009), *Dysidea avara* and *Chondrosia reniformis* (Schläppy *et al*., 2010). Analysis of NirS sequences derived from *G. barretti* indicated that various *Beta-* and *Gammaproteobacteria* were responsible for denitrification in this sponge, while genes encoding for nitrite reductase and nitric oxide reductase – key enzymes involved in denitrification – were recently identified in the genome of uncultivated '*Poribacteria*' from *A. aerophoba* (Siegl *et al*., 2011). Anammox activity and/or the presence of 16S rRNA gene sequences affiliated with Anammox planctomycetes has also now been demonstrated for sponges (Hoffmann *et al*., 2009; Mohamed *et al*., 2010). In addition, sponge-associated nitrification and nitrogen fixation have continued to receive considerable research attention (Weisz *et al*., 2007; Bayer *et al*., 2008; Steger *et al*., 2008; Mohamed *et al*., 2008c; 2010; Southwell *et al*., 2008a,b; Hoffmann *et al*., 2009; Off *et al*., 2010; Schläppy *et al*., 2010), with renewed appreciation for the potential importance of these processes to the wider reef ecosystem (Fiore *et al*., 2010).

Recent analysis of sponge–microbe associations using whole-genome amplification or metagenomics is revealing unprecedented insights into sponge symbiotic functions. Functional data on '*Poribacteria*' and a sponge-specific *Chloroflexi* clade were obtained by sequencing of a cosmid library constructed from the microbial community inhabiting *A. aerophoba* and revealed a sponge-specific polyketide synthase (PKS) from the '*Poribacteria*' (Fieseler *et al*., 2007; Siegl and Hentschel, 2010) and a non-ribosomal peptide synthetase (NRPS) from the *Chloroflexi* (Siegl and Hentschel, 2010). More recently, a single amplified genome (SAG) was recovered from an individual '*Poribacteria*' cell obtained from *A. aerophoba* by fluorescence-based cell sorting (Siegl *et al*., 2011). Genomic sequencing revealed that the '*Poribacteria*' are mixotrophs that can undertake autotrophic carbon fixation via the Wood–Ljungdahl pathway. Carbon monoxide dehydrogenase was identified in both the SAG from *Poribacteria* and the metagenome of *Cymbastela concentrica* (Siegl *et al*., 2011; Thomas *et al*., 2010). Several genomic factors were also detected in the '*Poribacteria*' that may mediate the sponge–microbe symbiosis, including adhesins, adhesion-related proteins and tetratricopeptide repeat domain-encoding proteins (TPR). Interestingly, an abundance of both ankyrin repeat proteins (ARP) and TPR was also detected in the metagenome from *C. concentrica* (Thomas *et al*., 2010) and in the genome of a novel *Deltaproteobacteria*, also from *C. concentrica* (Liu *et al*., 2011). These results are consistent with observations of intracellular symbionts in other hosts (Wu *et al*., 2004). In the metagenome of *C. concentrica* many of the ARP and TPR proteins have signal peptides for extracellular secretion in Gram-negative bacteria, suggesting that they may interact with surrounding cells and proteins. This abundance of ARP and TPR proteins may represent a mechanism which allows the host to discriminate between food and symbiont bacteria (Thomas *et al*., 2010). In the metagenome of *C. concentrica* a large number of transposable insertion elements were detected, which is pro-

posed to be critical to the evolution of bacterial genomes into symbiotic partnerships by removing non-required genes and rearranging other genes to form new pathways (Thomas *et al*., 2010). In the genome of the *Deltaproteobacteria* from *C. concentrica*, there was an abundance of orthologous proteins for translation/replication and recombination/repair (Liu *et al*., 2011). The novel deltaproteobacterium was consistently localized at the end of an unidentified cyanobacterium within the *C. concentrica* tissue, indicating a close (and possibly predatory) relationship consistent with the affiliation of this bacterium to the *Bdellovibrionales*. A potential role for *Bdellovibrio*-type bacteria in mediating microbe–microbe interactions within sponge tissue has been raised before (Wilkinson, 1979), although the importance of this phenomenon remains unknown. Interestingly, the genome contained an adventurous gliding motility protein R and Type IV pili which are both proposed to be important in cell attachment and infection. Various other functional properties of the uncultivated *Deltaproteobacteria* were described by genomic sequencing including aerobic growth, the capacity to remove toxins and antibiotics from the sponge and an apparent role in nutrient transport. Both the SAG from the '*Poribacteria*' and the metagenome from *C. concentrica* contain gene clusters associated with the metabolism of vitamin B12 (Siegl *et al*., 2011; Thomas *et al*., 2010). Not only would this provide the host with a source of vitamin B12 (eukaryotes need to acquire this vitamin via food) but vitamin B12 also serves as a cofactor for one of the key enzymes in the Wood–Ljungdahl pathway (Siegl *et al*., 2011).

While sequencing advances are facilitating rapid growth in the field of sponge symbiotic function, analyses are still somewhat constrained by the often distinct phylogenetic positioning of sponge symbionts making assignment of protein functions difficult. For example, in the genome sequence from the '*Poribacteria*', 40% of the protein-coding genes had unknown functions. Although not focusing on microorganisms *per se*, the flood of data associated with sponge genome projects [such as the recently published *Amphimedon queenslandica* genome (Srivastava *et al*., 2010)] should also provide insights into the mechanistic basis of sponge–microbe interactions and may assist interpretations of host–symbiont co-evolution. A further benefit from the *A. queenslandica* project was the incidental genome sequencing of an alphaproteobacterium that was present in the embryos from which genomic DNA was extracted.

Areas requiring additional research effort

Sponge-associated viruses and fungi

Viruses and virus-like particles (VLPs have the same morphology as viruses but their identity has not been confirmed by molecular characterization) are abundant in coral reef environments and have been found in the water column adjacent to reefs, in the coral surface microlayer, and inside the tissues of corals and their dinoflagellate endosymbionts (zooxanthellae) (Wilson *et al*., 2001; Seymour *et al*., 2005; Davy *et al*., 2006; Patten *et al*., 2006; 2008; Davy and Patten, 2007; Lohr *et al*., 2007). While VLPs have been observed in sponges by transmission electron microscopy (TEM) (Vacelet and Gallissian, 1978), to our knowledge there are no confirmed molecular reports of viruses inhabiting a marine sponge. Interestingly, a recent review on the *Mimiviridae* assessed the TEM micrographs from Vacelet's original work and predicted that these images show sponge phagocytes undergoing an infection by a giant virus related to the *Mimiviridae*, with sponge cells being referred to as 'a fully active virus factory' (Claverie *et al*., 2009). In another study, the seawater-derived bacteriophage ΦJL001 did infect an alphaproteobacterium symbiont from *Ircinia strobilina* under experimental conditions, but the occurrence of this interaction in nature has not been documented (Lohr *et al*., 2005). The lack of reports on viruses in sponges is surprising considering the large volumes of seawater filtered by sponges and the high density of microbes available for phage attack. Interestingly, recent metagenomic studies of sponge-associated microbes are revealing an elevated abundance of clustered regularly interspaced short palindromic repeats (CRISPRs) relative to seawater microbes, which may indicate high phage loads within the sponge host (Thomas *et al*., 2010). CRISPRs are sites within some bacterial genomes that contain multiple short direct repeats and function as a prokaryotic immune system. The CRISPR system confers resistance to exogenous genetic elements, such as plasmids and phage, thereby providing a form of acquired immunity against exogenous DNA.

Viruses are known to be pathogens of many marine organisms (Munn, 2006), but their role as symbionts has received very little attention. This is despite the fact that several unrelated viruses have been found to confer benefits to a suite of taxonomically diverse marine hosts (reviewed in vanOppen *et al*., 2009). Viruses can also act as agents of evolution, mainly through lateral gene transfer (Villarreal, 2005). This is a rapidly advancing field in coral microbiology but something which has not yet been addressed in sponges. Considering the large number of potential hosts for viruses in sponges (sponge, microalgae, bacteria) an increased research effort is required to explore viral biology and function as this could be of fundamental importance to understanding the ecology and evolution of sponge symbioses.

While numerous studies have cultivated fungi from sponges, molecular tools have only recently been applied. Molecular phylogenetic analysis of fungi from the marine

sponges *Suberites zeteki* and *Mycale armata* indicated that they harbour diverse fungal communities that are closely affiliated with fungi derived from other environments including seawater (Gao *et al*., 2008).

Environmental stress

In contrast to other marine symbioses (e.g. the coral-zooxanthellae model), very little research has addressed the resilience of sponge–microbe partnerships to a changing climate or other anthropogenic stressors. To date, the only studies to examine environmental stress in sponge microbiology have assessed elevated seawater temperatures (Lemoine *et al*., 2007; López-Legentil *et al*., 2008; 2010; Webster *et al*., 2008a) and heavy metals (Webster *et al*., 2001; Selvin *et al*., 2007). These studies have all detected shifts in the normally stable microbial communities due to altered environmental conditions and these shifts have simultaneously correlated with a decline in sponge health. The giant barrel sponge *Xestospongia muta* hosts *Synechococcus*-type cyanobacteria and a stable *Crenarchaeota* community that is closely related to those found in other sponges (López-Legentil *et al*., 2008; 2010). During cyclical bleaching events (from which the sponge recovers) this archaeal symbiosis is maintained, but once the sponges become fatally bleached the *Crenarchaeota* community shifts in composition to one that is more similar to that of the surrounding sediment (López-Legentil *et al*., 2010). This shift preceded an increase in *amoA* gene expression, presumably due to the elevated ammonia associated with tissue death (López-Legentil *et al*., 2010). In the Great Barrier Reef sponge *R. odorabile* exposed to thermal stress (33°C, compared with ambient of 27°C), sponges exhibited a complete loss of the primary cultivated symbiont within 24 h, a rapid increase in *Bacteroidetes* and *Firmicutes* and a community shift away from known sponge symbionts towards a microbial assemblage that was highly similar to that present in diseased corals (Webster *et al*., 2008a). In the field of coral disease there is an increasing body of literature that links stress events with disease outbreaks (Bruno *et al*., 2007; Plowright *et al*., 2008). On coral reefs in Bocas del Toro, Panama, variation in the prevalence of the sponge disease Aplysina red band syndrome (ARBS) may also be indicative of different environmental conditions and stress levels at the individual reefs (Gochfeld *et al*., 2007). To predict the consequences of these microbial shifts in response to environmental perturbations we need to understand the functional mechanisms that link symbiotic community structure and sponge health. Furthermore, additional research is required to assess the adaptive capacity of sponge symbioses and how the resilience of the partnerships is affected by possible functional redundancy provided by a rare microbial biosphere.

In 2002, Rohwer and colleagues introduced the term 'coral holobiont' to encompass the complex community that is comprised of the coral host, its eukaryotic zooxanthellae and the consortia of bacteria, archaea, fungi and viruses that also associate with the host (Rohwer *et al*., 2002). Extending this concept of 'holobiont' was the recently published 'Coral Probiotic Hypothesis' which suggests that corals form symbioses with diverse and metabolically active microorganisms (primarily bacteria) whose composition can shift in response to environmental change (Reshef *et al*., 2006). As noted by vanOppen and colleagues (2009), these symbiotic microbes contribute to the capacity of the holobiont to adapt or acclimatize to environmental stress. Considering the abundance, diversity and specificity of microbes residing within sponges, the term 'sponge holobiont' seems highly appropriate and assessment of environmental stress in sponges would need to fully explore the interactions between the different symbiotic components.

Sponge disease

Since the publication of a review of sponge diseases in 2007 (Webster, 2007) there have been numerous additional reports of disease in sponges (Gochfeld *et al*., 2007; Wulff, 2007; Webster *et al*., 2008b; Luter *et al*., 2010a,b; Maldonado *et al*., 2010). These disease events span many sponge species, occur across broad geographic areas, and sponges present with varied physiological symptoms (Fig. 2). Some disease outbreaks correlate with elevated seawater temperatures whereas others appear to be independent of prevailing environmental conditions. Despite the increasing incidence of sponge disease and realization of the importance of identifying sponge pathogens (Webster, 2007; Webster and Blackall, 2009), few studies have specifically assessed the relevant microbial communities. Of those reports that have studied the microbiology of diseased sponges, only one has managed to identify a primary pathogen as the causative agent (Webster *et al*., 2002). This sponge pathogen produces a collagenase enzyme which degrades the sponge skeletal fibres (Mukherjee *et al*., 2009), but this appears to be host species-specific and has not been identified from other diseased sponge species. Identifying the causative agents of disease is problematic in sponges due to the presence of diverse and host species-specific bacteria, archaea and other eukaryotes such as algae and fungi. Many microbiological reports of disease in sponges and corals detect a shift in the symbiotic bacterial community in affected individuals but cannot ascribe this shift to a specific pathogen (Bourne, 2005; Cervino *et al*., 2006; Olson *et al*., 2006;

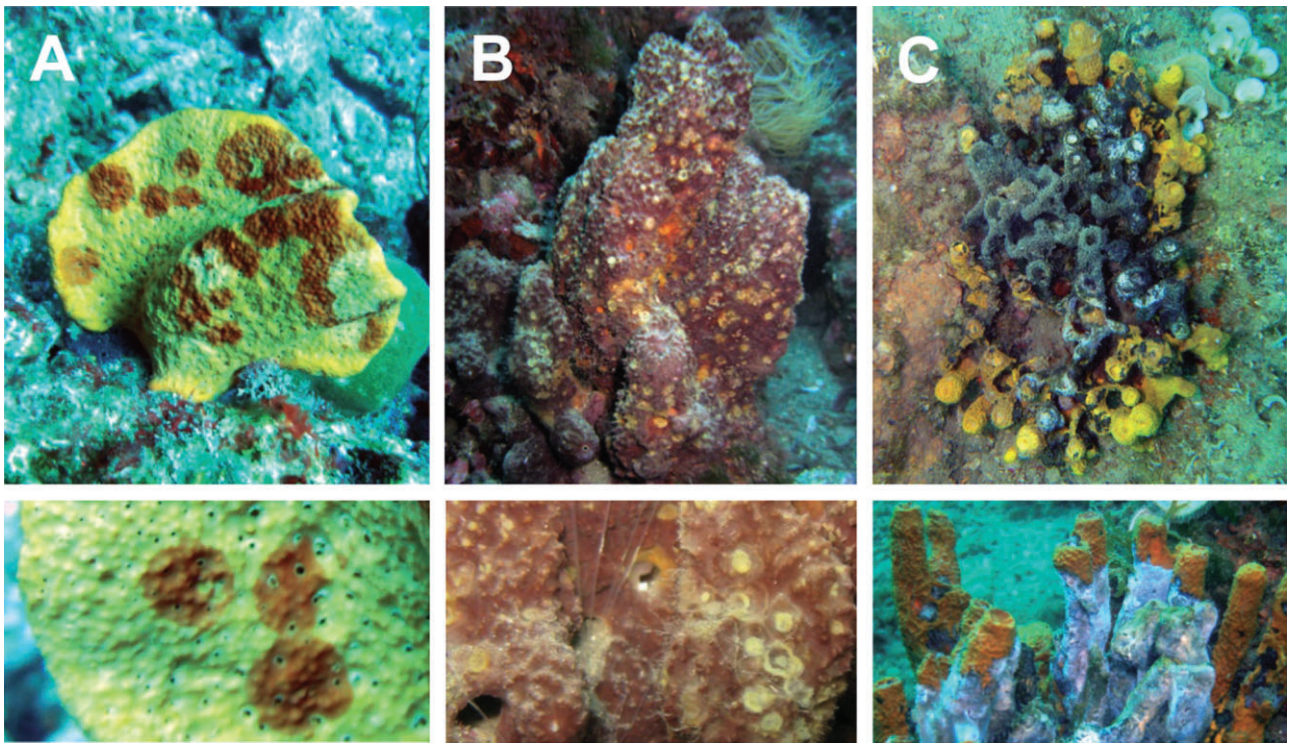

Fig. 2. Representative sponge species currently being affected by disease.
A. *Phakellia flabellata* from the Great Barrier Reef (photo courtesy of Heidi Luter).
B. *Ircinia fasciculata* from the Western Mediterranean (photo courtesy of Manuel Maldonado and Luis Sánchez-Tocino).
C. *Aplysina aerophoba* from Slovenia (photo courtesy of Joana Xavier).

Sussman *et al*., 2008; Webster *et al*., 2008b; Angermeier *et al*., 2011). In general, diseased sponges and corals tend to host a higher abundance of *Bacteroidetes, Epsilon-* and *Deltaproteobacteria* than do their healthy counterparts (Frias-Lopez *et al*., 2002; Pantos and Bythell, 2006; Webster *et al*., 2008b). In contrast, no difference was detected in the microbial communities within healthy and diseased *Ianthella basta* from Torres Strait and the diseased state could not be transmitted to healthy individuals (Luter *et al*., 2010b), suggesting that microorganisms are not responsible for the disease-like symptoms in this species.

Interestingly, apparently healthy individuals of *Agelas tubulata* and *Amphimedon compressa* from Florida reefs were recently found to harbour coral disease-associated microbes (Negandhi *et al*., 2010). Whether sponges act as a reservoir for potential coral pathogens or whether these disease-associated microbes are opportunistic bacteria that proliferate in diseased coral tissue remains to be determined. In addition, *Aspergillus sydowii*, the causative agent of catastrophic sea fan mortalities in the Caribbean, was recently isolated from healthy *Spongia obscura* in the Bahamas, providing further evidence that sponges may be a reservoir for putative marine pathogens (Ein-Gil *et al*., 2009).

Concluding remarks

During 2007–2009, numerous review articles emphasized the need to develop our understanding of sponge symbiotic function, symbiont evolution and symbiosis over wider environmental gradients (Taylor *et al*., 2007a,b,c; Vogel, 2008; Webster and Blackall, 2009). As described above, we are now seeing some major advances in these areas and no doubt the application of next-generation sequencing technologies, combined with genomic and transcriptomic approaches, will further enhance our understanding of these fields. However, even with these approaches our efforts will be hampered by the sheer number of protein-coding genes with unknown function. Despite such rapid progress in the past few years, we are yet to make significant headway in the areas of disease aetiology, viral ecology and assessing the sensitivity of sponge symbioses to environmental stress. Microbial symbionts are clearly important to sponge health, and therefore it is likely that disruptions to symbiosis as a result of climate change/environmental stress will impact upon sponge health, growth rates or their ability to protect themselves from predation, fouling and disease. By embracing the 'holobiont' concept, researchers will be better placed to consider the interactions (positive and negative) between

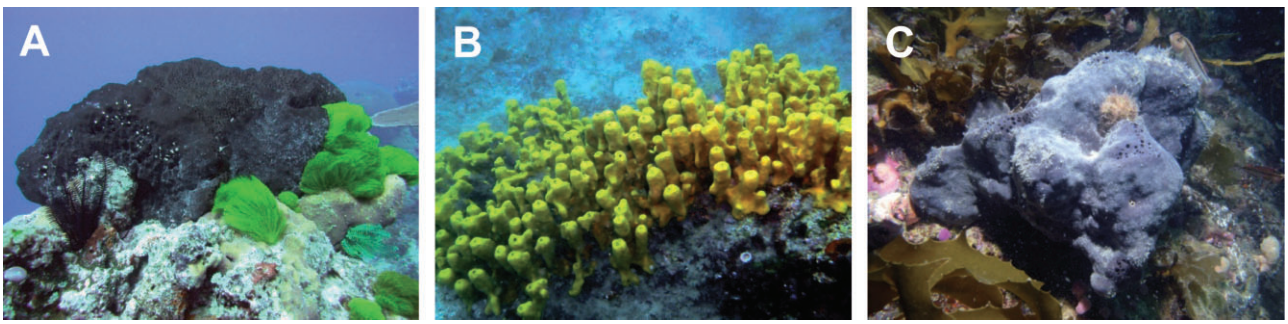

Fig. 3. Photographic examples of model sponge species currently being utilized to explore multiple aspects of microbial symbiosis.
A. *Rhopaloeides odorabile* from the Great Barrier Reef.
B. *Aplysina aerophoba* from the Mediterranean (photo courtesy of Janine Kamke).
C. *Ancorina alata* from New Zealand.
Other sponges with well-characterized microbiology include *Xestospongia muta* (Montalvo *et al*., 2005; López-Legentil *et al*., 2010), *Cymbastela concentrica* (Taylor *et al*., 2005; Longford *et al*., 2007; Liu *et al*., 2011; Thomas *et al*., 2010) and *Theonella swinhoei* (Hentschel *et al*., 2002; Piel *et al*., 2004).

all members in the symbiotic partnership. In a period of rapid environmental change and degradation of marine ecosystems, these areas constitute frontiers of science for sponge microbiology.

We conclude by highlighting a pressing need in sponge microbiology – that of identifying appropriate experimental models. Possibly due to the immense diversity of sponges and high levels of endemism, there is currently a plethora of sponge species studied by different sponge microbiology researchers (Fig. 3). While this does have the benefit of allowing results to be generalized more widely (different regions, different sponge families, etc.), it can also lead to unnecessary duplication of effort. Given the largely overlapping microbial communities of sponges, surely there is a case for limiting redundancy of research in this area. Some consolidation of global research efforts onto a few key species should enhance the discipline of sponge microbiology and allow it to develop apace.

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