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What factors structure Anthozoan microbial communities?

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Abstract

The coral holobiont is a complex and diverse composition of organisms including algae, bacteria and viruses. A number of factors suggested as vital in shaping these communities are considered here. The anthozoan host generates great diversity within its microbiota via spatial and metabolic structuring at both macro- and micro-scales; coral communities appear to be somewhat species-specific, yet spatial and temporal variation in coral microbiota suggests the significance of environmental agents. Increasing sea temperatures may cause fatal dissolution of the coral-algal partnership (coral bleaching), and although some adaptation to elevated temperature appears possible, anthropogenic stressors such as reduced pH and sedimentation have been implicated in microbial community shifts, coral disease, and decreasing holobiont resilience. Holobiont interactions appear vital in maintaining coral health, thus disturbance of any community members may cause problematic indirect effects. Enhanced understanding of holobiont health and function is therefore crucial for coral reef conservation.

Keywords: Scleractinian; zooxanthellae; antimicrobials; eutrophication; climate change.

Introduction

The study of anthozoans, such as tropical scleractinian corals, has been fundamental in generating the holobiont concept: anthozoans do not exist as isolated individuals. Associated microbes include *Bacteria*, *Archaea*, Fungi, protozoa, algae and viruses (Rohwer *et al.,* 2002; Marhaver *et al*., 2008). Coral microbiota is known to be tremendously diverse and abundant (Rohwer *et al.*, 2002; Chen *et al.*, 2011); microbial partners range from obligate dinoflagellate symbionts vital to host functioning (e.g. *Symbiodinium*), through to bacterial associates that provide protection via antibiotic production (Castillo *et al.*, 2001). Bacteria inhabit the surface mucus (Ritchie, 2006), epidermis and gastrodermis (Koren & Rosenberg, 2006; Rosenberg *et al.*, 2009), and skeleton (Shashar *et al.*, 1994) of tropical corals. Evidence that these microenvironments support different microbial communities (Rohwer *et al.*, 2002; Bourne & Munn, 2005) suggests that this assortment of habitats, and thus the host itself, is crucial to microbiota diversity.

Despite such observations, the influence of host physiology is not always clear: species-specificity in coral microbial communities has been observed (Kvennefors *et al.*, 2010), as has variation of coral species microbiota in different geographical locations (Littman *et al.*, 2009a). Thus environmental factors are implicated in shaping coral microbiota. Conditions known to cause shifts in community structure include raised temperatures (Bourne *et al.*, 2008) and water quality changes, e.g. anthropogenic pollution (Sutherland *et al.*, 2010). Investigations into the influence of host or environment on microbiota structure are further complicated by holobiont interactions and interactions with external microbes. Pathogenic and antagonistic activity of bacteria is thought to be important (Ritchie, 2006; Bourne *et al.*, 2009), and the abundance of viruses recorded, over 10^8 virus-like particles (VLPs) per cm^2 in apparently healthy coral (Marhaver *et al.*, 2008), has been proposed to reflect the potential role of viruses in microbiota organisation (van Oppen *et al.*, 2009).

The aim of this review is to consider evidence for the capacity of the host, the environment, and microbial interaction to shape anthozoan microbiota. Understanding what drives microbial community composition is crucial: evidence revealing the importance of anthozoan microbiota to host health and function is plentiful and varied (see: Castillo *et al.*, 2001; Bourne *et al.,* 2008; van Oppen *et al.*, 2009). With increasing incidence and severity of coral disease, and corals in ocean-wide decline (Mumby & Steneck, 2008), an understanding of what factors structure the microbiota of the coral holobiont is essential.

Microbial diversity and the role of the host

The diversity of microbes in coral communities is well documented. In 2002, Rohwer *et al.* recorded 430 distinct bacterial ribotypes (i.e. 16S rDNA sequences that were <97% identical) in 14 coral samples obtained from three tropical species; statistical analysis suggested additional sequencing would have resulted in a total of 6000 ribotypes (Rohwer *et al.*, 2002). However, recent evidence suggests even greater coral-bacteria diversity. Using massively parallel pyrosequencing (MPP), Chen *et al.* (2011) reported over 11000 operational taxonomic units (OTUs: <97% similar V6 hypervariable regions of bacterial 16S rRNA genes) in 21 samples from just one tropical coral species (obtained over seven months). What evidence exists for host facilitation of this microbial diversity?

The anthozoan host can structure associated microbial communities at different scales, from the characteristics of microenvironments within the host, to differences between species' physiologies and morphological structure. At macro scale, the structural complexity and morphological plasticity of corals causes an environmental gradient across coral structures, e.g. UV level and water flow in branching coral bases compared to tips (Ainsworth *et al.*, 2010). Environmental variation enhances coral heterogeneity, promoting microbial differentiation: low *Symbiodinium* densities in branch tips compared to branch bases (Jones & Yellowlees, 1997), and observation of a specific bacterial ribotype in *Porites furcata* branch tips (not mid-sections) (Rohwer *et al*., 2002) illustrate such spatial structuring.

At micro scale, anthozoan hosts provide a variety of metabolic substrates in a range of microhabitats, dictating the types of microbes able to survive. Microenvironment partitioning has been shown to influence anthozoan-bacterial diversity and abundance in various studies. Sweet *et al.* (2010) observed that bacterial 16S rRNA gene diversity was significantly different between the coral mucus (an important carbon source, Allers *et al*., 2008), tissue and skeleton. Compartmentalisation has even been recorded in the seldom-sampled cold water corals, where differences in mucus and coral-surface microbiota were observed by Schöttner *et al.* (2012). Earlier work by Bourne & Munn (2005) identified γ-proteobacteria as dominant within coral tissue, whereas α-proteobacteria were found to dominate the coral mucus. Sweet *et al*. (2010) reported similar findings, but additionally recorded members of the *Chloroflexi*, *Flavobacteria* and *Cyanobacteria* in coral mucus.

Some nitrogen-fixing (diazotrophic) bacteria are known to associate with the skeleton of tropical corals, producing nitrogenous compounds valuable to the host in its oligotrophic environment. Energy for dinitrogen reduction is thought to originate from organic photosynthates produced by *Symbiodinium* in host tissues, which are then transported to the coral skeleton (Shashar *et al.*, 1994). Evidence of host influence on coral-community structure at both microenvironmental and species scales comes from Lema *et al.* (2012): diazotrophic communities were found to differ between tissue and mucus samples from the same species, and microbial profiles of any one species collected in different sites were more alike than profiles from different species in the same location.

Some investigations have reported species-specificity in coral communities across both temporal and spatial scales (Rohwer *et al.*, 2002). This specificity may be related to inherent differences between the host species, e.g. amino acid and monosaccharide composition (Klaus *et al.*, 2007). However, evidence of distinct differences between the microbiota of juvenile *Acropora* sp. compared to adult colonies (Littman *et al.*, 2009b), and of increased bacterial diversity after spawning events (Ceh *et al.*, 2012), serves to illustrate the importance of temporal structuring effects related to the host. Yet, despite numerous examples of species-specificity in anthozoan microbial communities (e.g. Fraune & Bosch, 2007; Lema *et al.*, 2012), others have found no convincing evidence for species-specificity in anthozoan microbiota (Klaus et al, 2004; Littman et al, 2009a).

Potential reasons for such discrepancies include selection of: molecular methods, taxonomic resolution, cut-off level for OTU classification and coral sampling method (Mouchka *et al*., 2010; Sweet *et al*., 2010). Sampling methods used include swabbed- and milked-mucus, and whole tissue coral crushing, yet different sampling

techniques produce significantly different results in bacterial diversity (Sweet *et al*., 2010), illustrating the dangers inherent in comparing studies which have used different techniques. Furthermore, Kvennefors *et al*. (2010) report that a minimum of six replicates (from different colonies) are required to describe natural variation in coral microbiota; few studies have included this level of replication. Table 1 incorporates the results and method discrepancies of various species-microbiota comparisons.

Studies that have shown spatial and/or temporal variation in anthozoan microbiota (Koren & Rosenberg, 2006; Schöttner *et al.*, 2012; Morrow *et al.*, 2012), have lead to some debate regarding the spatial and temporal stability of such associations (Mouchka *et al.*, 2010). Any survey where samples are collected in one time or place will constitute only a snapshot of the anthozoan microbial community; the impact of environmental factors on anthozoan microbiota structure must also be considered.

Environmental Influences

Environmental factors have been shown as important structuring agents for anthozoan microbial communities in many investigations (Bally & Garrabou, 2007; Klaus *et al.*, 2007; Thurber *et al.*, 2009). For example, the effect of season (thus water temperature) on coral microbiota was investigated by Koren & Rosenberg (2006). The authors recorded marked differences between the bacterial communities associated with *Oculina patagonica* in winter (17^{o}C) and summer (27^{o}C). Moreover, raised Sea Surface Temperature (SST) and its effect on the relationship between scleractinian corals and their pigmented algal partners (*Symbiodinium*) is perhaps the best recognised environmental stressor of corals (i.e. coral bleaching). It is thought that increased SST (or solar irradiance) causes photoinhibition of *Symbiodinium* photosystem two (PSII) and thus excess production of reactive oxygen species (ROS), triggering eviction of *Symbiodinium* by the coral host (Dove & Hoegh-Guldberg, 2006). *Symbiodinium* provide vital energy for scleractinian corals. Short-term bleaching arrests reef construction and lowers coral resistance to disease; long-term bleaching causes coral death (Phinney & Veron, 2006). Temperatures elevated by just $1\text{-}2^{0}$C above summer maximum can produce mass bleaching (Vernon *et al.*, 2009), as observed in El Nino–Southern Oscillation (ENSO) events (Hughes *et al.*, 2003).

Corals that survive bleaching events may have markedly altered microbial communities. On the Great Barrier Reef (GBR), Jones *et al.* (2008) used single-stranded conformation polymorphism (SSCP) analysis to record changes in *Symbiodinium* communities of *Acropora millepora*, from temperature sensitive clades before a bleaching event, to heat tolerant clades afterwards. Some have suggested that endosymbiont exchanges such as this are adaptive and evidence of coral acclimatisation to increased SST (the Adaptive Bleaching Hypothesis (ABH), Buddmeier & Fautin, 1993). Increased ambient temperature causes physiological changes to the host (that may vary between species, e.g. reduced mucus production, Fitt *et al*., 2009), and also affects the physiology of the different microbial partners (Ainsworth *et al*., 2010). Therefore, microbial community structure might be expected to alter due to the differential reactions of various community members, both to elevated temperature itself (direct effects), and to modified host physiology (indirect effects). The former is illustrated by season-dependant domination of

Table 1: Evidence for species-specificity in studies of anthozoan microbiota. Indications of spatial and/or temporal stability are included. Different methods employed are listed: replication level, molecular approach/s (note the following abbreviations: Restriction/Terminal-Restriction Fragment Length Polymorphism: RFLP/T-RFLP; Denaturing Gradient Gel Electrophoresis: DGGE (which has inherent limitations in revealing ribotype dominance (Sweet *et al*., 2010)); conserved subunit of a gene encoding for the dinitrogenase iron protein: *fnifH*; Automated Ribosomal Intergenic Spacer Analysis: ARISA; cold water corals: CWC; Great Barrier Reef: GBR); taxonomic resolution (the taxonomic difference between anthozoans in comparisons, i.e. species from the same genus or different genera), sample method (swabbed mucus; milked mucus; blasted tissue; whole crushed coral), and cut-off level used to define OTUs (for sequence comparisons). Recent applications of metagenomic analysis and pyrosequencing may facilitate adequate replication: pyrosequencing is faster and more cost effective than Sanger (Shen & Qin, 2012), and provides more information on rare community members (Mouchka *et al.*, 2010), facilitating large-scale microbial diversity investigations (Ceh *et al.*, 2012).

Anthozoan species; Location; no. sites; [no. replicates]	Anthozoan Taxonomic Resolution;	Sample Method	Approach/s (OTU 'cut-off'/inclusion level)	Evidence of Species-Specificity (SS)? Evidence SS over space and/or time?	Study
Montastrea annularis; Montastrea cavernosa; Diploria strigosa Curacao, Netherlands Antilles; 2 sites; [1]	Between genera and within genus	Whole coral crushed	16s rDNA sequence clone libraries (compared at division level)	**Yes:** SS observed in all species (healthy coral); Over space: N/A Over time: N/A	Frias-Lopez & Zerkle, 2002
Montastraea franksi; Diploria strigosa; Porites astreoides Panama; Bermuda; 3 sites; [1]	Between genera	Coral tissue blasted (airbrush)	16s rDNA sequence clone libraries (97% seq. match)	**Yes**: SS observed in all species; Over space: Yes Over time: Yes	Rohwer *et al*., 2002
Diploria strigosa Montastraea annularis Curacao, Netherlands Antilles; 5 sites; [4]	Between genera	Coral tissue blasted (microdrill)	T-RFLP analysis of 16S rDNA (peaks above 50 U above background)	**No**: SS not observed	Klaus *et al*., 2004
Hydra oligactis; Hydra vulgaris Germany; 2 Lakes; [1]	Within genus	Whole animal crushed	RFLP analysis of 16S rRNA (97% seq. match)	**Yes:** SS observed in both wild and lab-cultured polyps Over space: N/A Over time: Yes	Fraune & Bosch, 2007
Fungia scutaria ; *Platygyra lamellina* Red sea; 1 site; [1]	Between genera	Milked mucus; Swabbed mucus	16s rDNA sequence clone libraries (83% seq. match)	**Yes:** SS observed Over space: N/A Over time: N/A	Lampert *et al*., 2008
Acropora millepora; Acropora tenuis; Acropora valida GBR; 2 sites; [3]	Within genus	Coral tissue blasted (airbrush)	16s rDNA sequence clone libraries (>97% seq. match) DGGE analysis T-RFLP analysis	**No:** SS not observed	Littman *et al*., 2009a
Acropora hyacinthus; Stylophora pistillata GBR; 3 sites; [8]	Between genera	Coral tissue blasted (airbrush)	DGGE analysis of the 16S rDNA V3 region (presence/absence of bands)	**Yes:** SS observed Over space: yes, with some site specific variation Over time: N/A	Kvennefors *et al*., 2010
Acropora millepora; Acropora muricata; Pocillopora damicormis GBR; 3 sites; [pooled:1]	Between genera and within genus (mucus & tissue sampled)	Coral tissue blasted (airbrush)	*fnifH* gene sequence clone library (diazotrophic phylotypes cut-off at 0.1)	**Yes:** SS observed in all species in coral tissue Over space: Yes Over time: N/A	Lema *et al*., 2012
Lophelia pertusa; Madrepora oculata, Norwegian CWC reefs; 4 sites (2 offshore, 2 inshore); [1]	Between genera (surface & mucus sampled)	Coral scraped (scalpel)	ARISA based on 3 PCR replicates per sample	**Yes:** SS observed on coral surface Over space: No Over time: N/A	Schöttner *et al*., 2012
Montastraea faveolata; *Porites astreoides* Caribbean; 4 sites (various distances offshore); [3]	Between genera	Mucus syringed	DGGE fingerprinting 454 bar-coded pyrosequencing (<97% seq. match)	**Yes**: SS observed Over Space: Yes, but with some site specific variation Over time: N/A	Morrow *et al*., 2012

vibrios with different temperature optima in coastal environments (Thompson *et al.*, 2004).

Littman *et al.* (2010) report evidence linking *Symbiodinium*-based heat tolerance with decreased bacterial community alteration. After exposure to elevated temperature (32^{o}C), juveniles of *Acropora tenuis* with clade D *Symbiodinium* underwent a 44% decline in photochemical efficiency, plus an increase in *Vibrio coralliilyticus* abundance. In contrast, Littman *et al.* (2010) observed that juveniles associated with clade C *Symbiodinium* exhibited only a 10% decline in photochemical efficiency, with no major shift in microbiota structure. Temperature induced bleaching was also linked to *Vibrio* domination in coral microbiota by Bourne *et al.* (2008). A shift to *Vibrio* domination was observed before any reduction in *Symbiodinium*, suggesting that bacterial changes are linked to early stages of the partnership collapse, although whether via infection or opportunistic response is unknown. Significantly, microbiota metabolism shifts in elevated temperatures have also been recorded. Thurber *et al.* (2009) observed greater expression of virulence and motility genes by vibrios of heat stressed *Porites compressa* (although vibrio abundance was unaffected).

Other environmental factors believed to cause shifts in anthozoan microbiota structure include reduced pH (Thurber *et al.,* 2009), eutrophication/sedimentation (Klaus *et al*., 2004;), and increased levels of Dissolved Organic Carbon (DOC) (Dinsdale *et al.*, 2008). In laboratory experiments, microbial communities of *Porites compressa* shifted to a more disease-associated state (i.e. more bacterial and fungal members previously found in diseased corals) on exposure to each environmental factor above (Thurber *et al.*, 2009). It has been shown that the same factors cause abundant induction of herpes-like viruses (Thurber *et al.*, 2008) (note: DOC not investigated). Field studies by Dinsdale *et al*. (2008) and Morrow *et al.* (2012) also support the correlation between eutrophication and increased numbers of pathogenic or disease-associated bacteria.

The actual mechanisms driving such shifts in anthozoan-microbial community structure are largely unknown. Direct and indirect effects of different host and environmental factors, and complex interactions between all members of the coral holobiont are suspected (Mouchka *et al.*, 2010). Furthermore, the prevalence and severity of coral disease (Bourne *et al.*, 2009), and abundance of anthozoan-associated fungi and viruses (Marharver *et al.*, 2008; Thurber *et al.* 2009) highlights the necessity of exploring the role of holobiont interactions in shaping microbiota structure.

Holobiont Interactions

Before interactions between members of the anthozoan microbial community can be addressed, it is necessary to review which groups are thought to be prominent, and what the functional roles of these groups have been predicted to include (shown in table 2).

Table 2: Prominent groups of anthozoan microbial communities with functional roles, or predicted functional roles, of the group. The increasing application of metagenomic analysis in coral-microbial surveys is expected to help clarify functional roles in the near future (Ainsworth *et al*., 2010). Note that only symbiotic members are included; for information on potentially pathogenic microbiota members, who have a role in structuring the microbial community, see text.

Type of Anthozoan Associate	Groups (Most Important/Abundant)	Functional/ Hypothesised Role	Studies/Sources
Symbiodinium	8 phylogenetic clades: A–H; (A and B: Caribbean corals) (C and D: Indo-W and E. Pacific corals) (F, G and H: foraminifera) (E: anemones)	Photosynthetic symbionts supply 90-99% of photosynthate (all clades), and are essential for reef-building and coral growth (all clades except E). Heat tolerance/sensitivity important to coral resilience to thermal bleaching.	Knowlton & Rohwer, 2003; Jones *et al.,* 2008; Chen *et al.*, 2011; Rohwer, 2010; Tonk, 2010
Bacteria	γ-Proteobacteria; α Proteobacteria; β-Proteobacteria; *Firmicutes;* *Cyanobacteria;* *Actinobacteria*	Heterotrophs: acquire C (complex compounds, e.g. proteins, polysaccharides), S and N from anthozoan. C and N fixation (Cyanobacteria). Stress response (DNA repair); antibiotic resistance, virulence.	Rohwer *et al.*, 2002; Lesser *et al.*, 2004; Ritchie, 2006; Wegley *et al.* 2007; Thurber *et al*., 2009
Archaea	*Crenarchaeota*; *Euryarchaeota*	N recycling: nitrification and denitrification processes; nutritional sink for excess ammonium (in mucus layer).	Siboni *et al.*, 2008; Thurber *et al*., 2009
Endolithic Fungi	*Ascomycota; Basidiomycota*; *Chytridiomycota*	N cycling (inc. ammonification and assimilation of ammonia for use in biosynthesis)	Wegley *et al.* 2007; Thurber *et al*., 2009
Bacteriophage	*Microviridae*; *Myoviridae*; *Siphoviridae;*	Regulation of bacterial populations, including cyanobacteria and vibrios; Horizontal gene transfer (e.g. antibiotic resistance, virulence factors)	Wegley *et al.,* 2007; Marhaver *et al.,* 2008; Bourne *et al*., 2009; van Oppen *et al*., 2009

Bacterial community changes have been observed in corals with diseases such as Black Band (Frias-Lopez *et* al, 2002), White Pox (Sutherland *et al.*, 2010) and Aspergillosis (Gil-Agudelo *et al.*, 2006). Yet, despite efforts to understand the dynamics of coral infection, it is still unknown whether specific community changes are causative of diseases, or are a symptom of disease, or both (e.g. a pathogen inducing deleterious changes to other community members) (Mydlarz *et al*., 2010). However, outbreaks of coral disease have often been linked to thermal stress and coral bleaching (Mao-Jones *et al*., 2010), and pathogens are just one aspect of the three-way interaction between environment, agent and host, that results in coral disease (Bourne *et al.*, 2009).

Coral immunology includes cellular processes and physiochemical barriers. Production of melanin bands (via the prophenoloxidase cascade) and induction of phagocytic amoebocytes are two such defences, as observed in fungi-infected and/or heat-stressed Caribbean sea fan corals (Mydlarz *et al.,* 2008). Coral mucus (produced by coral mucocytes utilizing *Symbiodinium* photosynthate) provides multifunctional defence, from environmental protection (e.g. sediment removal) and mechanical exclusion of exogenous microbes (Brown & Bythell, 2005), to inhibition of potential pathogens and selection for antibiotic-producing bacteria (Ritchie, 2006). Evidence of coral mucus antimicrobial properties (Ritchie, 2006) has lead to some debate as to the source/s of antimicrobial compounds (Geffen *et al.*, 2009; Teplitski & Ritchie, 2009).

Many anthozoan-associated bacteria demonstrate antibacterial activity against putative coral pathogens (Ritchie, 2006; Nissimov *et al*., 2009; Rypien *et al*., 2010), potentially providing protection against disease. Castillo *et al*. (2001) determined that around one third of isolated coral bacteria were capable of producing antibiotics. It should be noted however that many coral-associated bacteria are currently non-culturable. Culturable members tend to be α-proteobacteria such as *Pseudoalteromonads* and *Vibrios* (Rower *et al.,* 2002), suggesting that the percentage of antagonistic bacteria reported by Castillo *et al*. (2001) may not be representative of the full bacterial community. Recently, compelling evidence of *in situ* protection by bacterial associates was observed in *Aptasia pallida* anemones. Alagely *et al*. (2011) recorded reduced White-Pox disease symptoms in individuals previously inoculated with a cocktail of bacterial associates.

Antibiotic production by holobiont bacteria is probably just one facet of many antagonistic and cooperative interactions within the microbiota (Teplitski & Ritchie, 2009). The mechanics of such interactions are believed to include exchanges of small diffusible molecules between bacterial cells, linked to increasing population density: Quorum Sensing (QS) (Golberg *et al.*, 2011). QS alters gene expression, bringing about phenotypic changes such as antibiotic production, swarming, and biofilm formation (Alagely *et al*., 2011). Various coral-associated bacteria have been shown to disrupt both swarming and/or biofilm formation behaviours in the coral pathogen *S. marcescens* (Alagely *et al*., 2011), and 30% of coral isolates tested by Goldberg *et al*., (2011) produced QS signals (N-acyl homoserine lactones: AHLs). Therefore, QS appears to be one mechanism controlling the structure of coral-associated bacteria.

Despite the evidence above, the bacterial focus of many holobiont investigations may be responsible for serious underestimations of the diversity and possible structuring roles of other groups, such as fungi and viruses. The first metagenomic analysis of microbes associated with tropical reef-building coral revealed that an unexpectedly large proportion of known sequences, 38%, were fungal (bacteria constituted 7%; bacteriophages 3%; eukaryotic viruses 2%; Archaeal sequences < 1%; mitochondrial sequences 49%; coral and *Symbiodinium* cells were previously removed) (Wegley *et al*., 2007). A large number of the fungal genes observed were involved in carbon and nitrogen metabolism, thus potentially important to holobiont function (Wegley *et al*., 2007). Marhaver *et al.* (2008) theorise that the abundance of viruses observed in healthy corals indicates a diversity of function within this group, as opposed to a severity of infection. VLPs have been detected in the mucus layer, epidermis, gastrodermis and *Symbiodinium* of scleractinian corals (Thurber & Correa, 2011), leading to some conjecture as to the possible structuring roles of viruses in anthozoan microbiota. Although there are no confirmed viral pathogens of corals (Bourne *et al*., 2009), a potential link to coral bleaching has been reported: filamentous VLPs were induced from *Symbiodinium* on exposure to UV, causing *Symbiodinium* lysis (Lohr *et al.*, 2007).

There is also potential for viruses to aid holobiont photosynthesis. Bacteriophage of marine cyanobacterium *Synechococcus* carry genes for PSII repair, aiding prevention of photo-inhibition by horizontal gene transfer between infected cells (van Oppen *et al*., 2009). Cyanobacteria are an important holobiont group (Lesser *et al.*, 2004), so the significance of this kind of viral activity is clear. Moreover, horizontal gene transfer has been linked to both acquired antibiotic resistance (Dang *et al*.,

2007) and virulence factors (Thurber *et al*., 2009), and thus has important structural implications for the entire coral holobiont. Additionally, phages may influence microbiota structure as top-down controllers of coral-associated bacteria (Marhaver *et al*., 2008). Bourne *et al*. (2009) propose that phage may play an important role in holobiont homeostasis. Controlling the abundance of the most successful bacterial groups ensures that no one group is able to totally outcompete any other, the 'Kill the Winner' hypothesis (Thingstad, 2000). The presence of cyanophages and vibriophages within the holobiont suggests that phage contribute to the structuring of both mutualistic and potentially pathogenic bacteria community members, thus affecting holobiont function as a whole (Marhaver *et al*., 2008).

Discussion

The factors thought to shape anthozoan microbiota (as shown in figure 1) provide sources of both optimism and pessimism regarding the future of coral reefs. Support for the ABH from cold-to-warm water transplantation experiments (Berkelmans & van Oppen, 2006; Jones *et al.*, 2008) suggests that some corals are able to adapt temperature thresholds and withstand some increase in SST. Yet, the link between community shifts, coral disease, and anthropogenic disturbances of the coral environment such as eutrophication, acidification, overfishing, increased DOC, and elevated temperatures (Klaus *et al.*, 2007; Thurber *et al.*, 2009) does not bode well for coral resilience. Changes to any members of the community can compromise the health of the entire holobiont (Bourne *et al.*, 2009). The priorities for future research must therefore be centred on gaining the necessary understanding to protect the holobiont health.

Comprehension of the healthy baseline is vital. elucidating the functional roles of, and interactions between, all members of a healthy holobiont (including host immunology, fungi and viruses) is necessary to assist in our understanding of the changes which occur under environmental stress, and any subsequent transition into a diseased state (Bourne *et al.*, 2009). Simultaneously, investigations into disease management strategies (e.g. phage therapy (Efrony *et al.*, 2006 and 2009); antagonistic mutualists (Teplitski & Ritchie, 2009)) are needed for potential amelioration of severe outbreaks. However, care must be taken with any human intervention in a natural system, and issues such as acquired bacterial resistance and toxin release from bacterial lysis need to be resolved before any treatments are undertaken (Marhaver *et al.*, 2008; Efrony *et al.*, 2009).

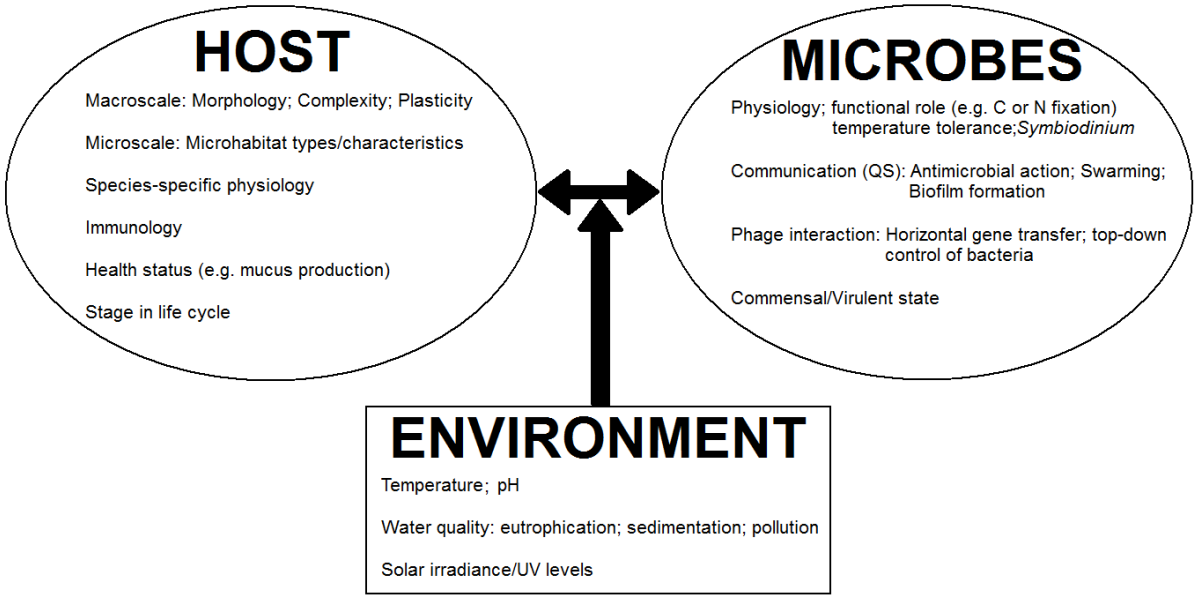

Figure 1: Summary of the factors thought to structure anthozoan microbial communities. Coral microbiota is shaped by interactions between the coral host, its associated microbes, and the external environment. Exogenous organisms are also thought to have some importance (e.g. herbivorous fish which control macroalgae growth, Rohwer, 2010). (figure by author).

In summary, although some progress has been made in understanding the roles of the host, the environment, and holobiont interactions in structuring coral microbial communities, predictions of increasing in SST (1.8-4.0^{o}C) in the 21st century (IPCC, 2007), necessitates a comprehensive understanding of anthozoan holobiont composition and function, in order to aid the conservation of tropical coral reefs.

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