

7 The coral holobiont highlights the dependence of cnidarian animal hosts on their associated microbes

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Contents

7.1	Introduction - The coral holobiont as an ecosystem engineer and its reliance on associated microbes.....	XX
7.2	The coral-Symbiodiniaceae relationship.....	XX
7.2.1	Symbiodiniaceae - micro-algal engines of the coral holobiont machinery.....	XX
7.2.2	Innate immunity, symbiosis sensing, and cell signaling.....	XX
7.2.3	Coral Bleaching - The breakdown of the coral-Symbiodiniaceae relationship.....	XX
7.3	Symbiodiniaceae-bacteria relationships.....	XX
7.4	Diversity and function of microbes associated with the coral host.....	XX
7.4.1	The host as a habitat.....	XX

7.4.2	Diversity of coral-associated bacteria and interspecies interactions.....	XX
7.4.3	Acquisition of bacterial associates and their roles in early coral life-stages.....	XX
7.4.4	Coral probiotics.....	XX
7.4.5	Contribution of bacteria to holobiont nutrient cycling.....	XX
7.4.6	Archaea associated with the coral holobiont.....	XX
7.4.7	Protists and fungi associated with the coral holobiont.....	XX
7.5	Summary and Outlook.....	XX
	References.....	XX

7.1 Introduction - The coral holobiont as an ecosystem engineer and its reliance on associated microbes

The productivity and biodiversity of coral reefs are unmatched in the marine environment (Hatcher 1990). Surrounded by oligotrophic oceans, coral reefs are buzzing oases of life in a marine desert (Darwin 1842). Tropical coral reefs cover just 0.1% of the seafloor but provide habitat for ~32% of all marine multicellular species (Fisher et al. 2015) and contribute to the livelihoods of more than 600 million people (Moberg and Folke 1999; Wilkinson 2008; Spalding et al. 2017). This entire ecosystem is supported by its foundation species: reef-building corals (Figure 1). The calcareous skeleton of corals forms the structural basis of the reef framework. Furthermore, corals are also an important component of the coral reef food web and support the immense productivity of these reefs (Wild et al. 2004).

[Insert Figure 7.1 here]

As early as in the 19th century, scientists recognized that the productivity of corals and other benthic cnidarians derives from a symbiosis with intracellular photosynthetic dinoflagellates in the family Symbiodiniaceae (LaJeunesse et al. 2018) (Figure 2). Building on more than a century of research, the coral-dinoflagellate symbiosis is one of the best characterized eukaryotic-eukaryotic endosymbioses and a powerful model to understand the functioning of symbioses in general. Corals also harbor a diverse array of other microbes comprised of protists, fungi, bacteria, archaea, and viruses (Figure 3). This collective is called the coral holobiont (Rohwer et al. 2002). The coral metaorganism, by comparison, is a more restricted definition and typically only describes the coral host and associated microbes for which a function has been proposed or is known (Jaspers et al. 2019). This suite of organisms forms a complex network of symbiotic interactions that extend the metabolic repertoire, immunity, and environmental adaptation of the coral host (Muscatine 1990; Rosenberg et al. 2007; Ritchie 2012; Räddecker et al. 2015; Ziegler et al. 2017, 2019; Robbins et al. 2019). Microbes can therefore be considered fundamental to the ecological success of corals and the reefs they build (Bang et al. 2018).

Reef ecosystems have existed for ~240 million years, but face unprecedented and accelerating decline: the 2015-2017 global coral bleaching event affected 74% of reefs worldwide, and up to half of the coral cover was lost on the Great Barrier Reef alone, the largest reef system in the world (Hughes et al. 2018b). Future predictions for coral reefs are dramatic: even under a 1.5°C warming scenario, it is expected that coral reefs will decline by a further 70-90 %, with larger losses of up to 99% projected to be highly likely under a 2.0°C warming scenario (IPCC 2018). Such trajectories make the understanding of coral holobiont functioning and the contribution of

its various microbes critical, not only to comprehend how symbiotic interactions have shaped the most biodiverse marine ecosystem on Earth, but also to help conserve and protect corals and the reefs they build for future generations. In this chapter, we discuss the state of knowledge of coral-microbe interactions and how they shape the ecology, resilience, and adaptation of the coral holobiont.

[Insert Figure 7.2 here]

7.2 The coral-Symbiodiniaceae relationship

7.2.1 Symbiodiniaceae - micro-algal engines of the coral holobiont machinery

The ecological success of the coral-Symbiodiniaceae symbiosis is based on efficient nutrient recycling between host and symbiont (Figure 3). The driving force of this bidirectional nutrient exchange ultimately lies in the complementary nutrient limitations of the two symbiotic partners (Shantz et al. 2016; Bang et al. 2018). While the heterotrophic coral host is limited by organic nutrient availability (e.g., glucose), the autotrophic intracellular dinoflagellates are limited by inorganic nutrients (e.g., carbon dioxide or ammonium) (Muscatine et al. 1989; Falkowski et al. 1993; Rädicker et al. 2015). These reciprocal metabolic exchanges are governed by the take-up of limiting nutrients and release of excess nutrients by each symbiotic partner (Muscatine 1990; Cunning et al. 2017). Symbiodiniaceae translocate high rates of excess photosynthetically fixed carbon to the coral host. The host metabolism, in turn, produces waste products such as carbon dioxide through respiration available to the Symbiodiniaceae (Falkowski et al. 1993). This nutrient exchange is so efficient that the translocation of

photosynthates can fully meet or even exceed the respiratory requirements of the coral host (Muscatine and Porter 1977; Muscatine 1990; Falkowski et al. 1993), and hence constitute its primary energy source (Tremblay et al. 2012). As a consequence, symbiotic coral hosts may overcome their carbon limitation and shift instead towards a nitrogen-limited state (Cunning et al. 2017; Rådecker et al. 2018).

In a stable symbiosis, host and symbionts are nitrogen-limited and compete for available environmental ammonium (Pernice et al. 2012). Increasing evidence suggests that this resource competition is critical for maintaining the functioning of the coral-Symbiodiniaceae symbiosis (Cui et al. 2019). Coral hosts use the translocated carbon for ammonium assimilation required for amino acid synthesis (Cui et al. 2019). Consequently, carbon translocation reduces nitrogen availability for the dinoflagellates. The nutrient cycling in the intact symbiosis is thus stabilized by a positive feedback loop: because symbionts translocate carbon, they are nitrogen-limited; and because they are nitrogen-limited, a substantial fraction of photosynthetically-fixed carbon cannot be channeled towards their biomass and growth, and is hence released to the host.

While this 'selfish' interaction between host and symbiont is central to the ecological success of the coral-Symbiodiniaceae relationship, it also renders the symbiosis highly susceptible to environmental disturbance.

Initially considered to be a single species, *Symbiodinium microadriaticum* (Freudenthal 1962), the recently established family Symbiodiniaceae currently encompasses seven distinct genera (*Symbiodinium* – formerly Clade A, *Breviolum* (B), *Cladocopium* (C), *Durusdinium* (D), *Effrenium*

(E), *Fugacium* (F), *Gerakladium* (G)) that have originated and diversified alongside reef-building corals approximately 160 mya ago (LaJeunesse et al. 2018). Coral reefs of the Indo-Pacific are almost exclusively dominated by *Cladocopium* and *Durusdinium* (LaJeunesse et al. 2003; 2004), with at least 50-100 possible “species” currently identified around Australia alone (LaJeunesse et al. 2003; Silverstein et al., 2011). The remarkable diversity of this family influences their host’s susceptibility to environmental fluctuations, such as thermal stress and salinity (Berkelmans and van Oppen 2006; Sampayo et al. 2008). In the world’s warmest reefs of the Persian/Arabian Gulf, corals predominantly harbor *Cladocopium thermophilum*, an association that is central to the thermo-tolerance of these coral communities (Hume et al. 2016). Similarly, the stress tolerant *D. trenchii* has rapidly taken over Caribbean corals following repeated anthropogenic disturbances and increasing seawater temperatures (Pettay et al. 2015). However, thermal adaptation of reef communities represents a significant physiological tradeoff: corals harboring *Durusdinium trenchii* typically grow slower (Little et al. 2004) and incorporate half the amount of photosynthates compared to those associated with *Cladocopium* (Cantin et al. 2009). Recently, multiple Symbiodiniaceae genomes have become available (Shoguchi et al. 2013; Lin et al. 2015; Aranda et al. 2016), and comparative analyses have revealed that these organisms possess an extensive transporter repertoire for carbon and nitrogen metabolites, which is unique among dinoflagellates and likely underpin their symbiotic lifestyle (Aranda et al. 2016).

7.2.2 Innate immunity, symbiosis sensing, and cell signaling

The regulation of the coral-algal partnership is complex and only partially understood. There is strong evidence that the host innate immune system plays a big role in mechanisms of recognition, maintenance, and dysbiosis of the association (Weis 2008). In the majority of coral species, the symbiosis is established anew with each host generation. The algae are acquired via phagocytosis by nutritive phagocytes that comprise the host gastrodermal tissue (endoderm) (Fadlallah 1983). However instead of being digested, the algae specific to a particular host, persist and proliferate within host vacuoles (termed symbiosomes) (Colley and Trench 1983; Schwarz et al. 1999). This process is mediated by the host innate immune system (Palmer 2018). The process of phagocytosis is a complex part of innate immunity that is highly conserved across the Metazoa (Underhill and Ozinsky 2002).

Microbes, including Symbiodiniaceae, are arrayed with a variety of microbe-associated molecular patterns (MAMPs), including glycans, that are recognized by host pattern recognition receptors (PRRs) on phagocyte cell surfaces (Weis 2008). These MAMP-PRR interactions launch a variety of signaling cascades that determine the fate of the phagocytized microbe. The model in corals is that tolerogenic pathways allow for the persistence and proliferation of symbionts inside symbiosomes, while resistant pathways are launched during dysbiosis and bleaching (see next section) that reject and remove the symbiont. There is overwhelming evidence for the presence of MAMP-PRR interactions and downstream innate immune signaling in corals. The majority of evidence comes from now extensive omics studies that repeatedly point to elaboration, enhancement, and over-expression of innate immunity genes in corals and other symbiotic cnidarians (Rodriguez-Lanetty et al. 2004; Shinzato et al. 2011; Baumgarten et al.

2015; Mohamed et al. 2016; Voolstra et al. 2017; Cunning et al. 2018; Shumaker et al. 2019).

There are also a variety of studies, often in sea anemone model systems, that provide evidence of innate immune pathway function including: host lectin-symbiont glycan interactions (Wood-Charlson et al. 2006; Bay et al. 2011; Parkinson et al. 2018), scavenger receptors (Neubauer et al. 2016), thrombospondin type 1 repeat proteins (Wolfowicz et al. 2016; Neubauer et al. 2017), complement system (Poole et al. 2016), the master immunity gatekeeper NF κ B (Mansfield et al. 2017), tolerogenic TGF β pathway (Detournay et al. 2012; Berthelie et al. 2017), sphingolipid signaling (Kitchen and Weis 2017; Kitchen et al. 2017), and Rab protein signaling and other evidence of endosomal trafficking (Chen et al. 2003, 2004).

Also critical to symbiosis regulation is the maintenance of host and symbiont biomass ratios through cell cycle regulation. Host and symbiont biomass ratios reach a dynamic homeostasis after symbionts fully colonize a host (Davy et al. 2012). Symbiont populations in hospite grow much more slowly than those in culture and are arrested at the G1/S transition (Smith and Muscatine 1999). Mechanisms that coordinate the two cell cycles and an understanding of how this co-regulation becomes decoupled during dysbiosis have yet to be revealed.

7.2.3 Coral Bleaching - The breakdown of the coral-Symbiodiniaceae relationship

Stressful environmental conditions such as temperature anomalies, nutrient enrichment, or pollution can result in so-called coral bleaching, a (general) stress response characterized by whitening of the coral tissue caused primarily by the loss of Symbiodiniaceae endosymbionts from the coral tissue via expulsion, host cell apoptosis/detachment, digestion, or exocytosis of

the symbiont cells (Gates et al. 1992; Douglas 2003; Dunn et al. 2007; Davy et al. 2012). Coral bleaching can also occur via the loss of photosynthetic pigment from the symbionts *in hospite* (Jones et al. 1998). Several triggers that induce the coral bleaching cascade have been proposed, including oxidative stress (Lesser 1997) and changes in nutrient stoichiometry (Wiedenmann et al. 2012; Morris et al. 2019). However, the underlying cellular mechanisms are still not fully understood.

Corals may recover from a bleaching event by repopulating their tissues with Symbiodiniaceae (Jones et al. 2008; Silverstein et al. 2015). However, as bleaching effectively results in the disruption of carbon fixation by Symbiodiniaceae and subsequent loss of photosynthate translocation to the coral host (Ezzat et al. 2015), coral host starvation may eventually result in mortality as a consequence of prolonged bleaching. The availability of heterotrophic food sources and the heterotrophic capacity of the host, thus, determine the resilience of corals during heat stress (Grottoli et al. 2006). Mass coral bleaching events at the ecosystem scale have resulted in the loss of entire reefs, or reef systems (Hoegh-Guldberg 2011; Hughes et al. 2018a) and are projected to increase in the future due to climate change driving ocean warming (IPCC 2018).

The majority of contemporary observations of ecosystem-scale coral bleaching coincide with high-temperature anomalies, such as the El Niño Southern Oscillation (ENSO), or marine heatwaves, which can push corals beyond their thermal limits (Hoegh-Guldberg 1999; Hughes et al. 2003, 2017, 2018a). The first observations of coral bleaching were made in 1983 in the

Eastern Pacific near the Panama-Costa Rica border (Glynn 1983). Due to the increasing severity and frequency of high-temperature anomalies attributed to global warming, coral bleaching events have become more common in the past decades. Since then, three mass bleaching events have occurred at the global scale, subsequently named the First, Second, and Third Global Bleaching Event, recorded in 1997/1998, 2009/2010, and 2015-2016, respectively (Hughes et al. 2017). A recent analysis of bleaching records of 100 reefs from 1980 - 2016 has shown that the average turnaround time between bleaching events has halved in the past thirty years and is now only 6 years (Hughes et al. 2018a). This concerning trend increases the likelihood of annual mass bleaching events in the coming decade, with recovery windows too short for full reef recovery in between bleaching events (Hughes et al. 2018a).

7.3 Symbiodiniaceae-bacteria relationships

Interactions between bacteria and Symbiodiniaceae are challenging to study *in hospite* because of the complex nature of the coral holobiont. The physiology, functional diversity, and stress tolerance of Symbiodiniaceae have been studied in cultured strains for decades in order to disentangle their contribution to the health and functioning of the holobiont. Symbiodiniaceae cultures harbor very diverse bacterial communities with abundances exceeding those of the algal cells by almost two orders of magnitude (Ritchie 2012; Lawson et al. 2018). Recurring bacterial taxa such as *Marinobacter* (Gammaproteobacteria), *Labrenzia* and other *Roseobacter* (Alphaproteobacteria), have been identified in association with a wide diversity of Symbiodiniaceae genera (Ritchie 2012; Lawson et al. 2018). Strikingly, the same bacterial taxa are also known to positively influence the growth of many phytoplankton species (Seymour et

al. 2017). Future studies on Symbiodiniaceae-bacteria interactions should use axenic cultures to characterize the effect of specific bacteria on the growth and physiology of the dinoflagellates. Protocols to set up axenic microalgal cultures exist and have successfully been applied to multiple phytoplankton species (Shishlyannikov et al. 2011; Cho et al. 2013). Establishing axenic Symbiodiniaceae cultures is an important first step towards identifying the functional roles and reciprocal exchanges occurring between Symbiodiniaceae and their bacterial partners.

7.4 Diversity and function of microbes associated with the coral host

7.4.1 The host as a habitat

Coral holobionts associate with a range of bacteria, archaea, and viruses, among other microorganisms (Rohwer et al. 2002; Wegley et al. 2007) (Figure 3). Coral holobionts can be separated into three distinct compartments: the surface mucus layer (SML), the coral tissue, and the underlying aragonite skeleton. The three compartments are governed by distinct physicochemical properties and environmental gradients (Ferrer and Szmant 1988; Wangpraseurt et al. 2016; Pernice et al. 2019) as well as distinct associated microbial communities (Sweet et al. 2011; Pollock et al. 2018). Most coral-associated prokaryotes have not yet been cultivated and the functional contributions of specific taxa are largely unknown. Nonetheless, prokaryotic functions are crucial to understanding microbial contribution to coral health and adaptation. Molecular tools have allowed for the in-depth characterization of bacterial communities associated with corals and revealed specific assemblages of bacteria differing between the SML, tissue, and skeleton. SML-associated bacteria are largely dominated

by Gammaproteobacteria, commonly represented by members of the genus *Vibrio* (Koren and Rosenberg 2006) and by a broad range of taxa presumed to be commensals that can inhibit the growth of pathogens (Ritchie 2006). Shifts in SML-associated bacteria communities towards a dominance of Alphaproteobacteria, Verrucomicrobia, and Cyanobacteria have been reported in mucus sheets of *Porites* corals, in particular related to aging mucus (Glasl et al. 2016), variation in nutrient composition, and heat stress (Lee et al. 2015, 2016), although studies from bleached and healthy coral mucus from the Persian/Arabian Gulf showed no difference between bacterial communities (Hadaidi et al. 2017).

Bacterial assemblages associated with coral tissues have been widely studied. Tissue-associated bacterial identities are strongly driven by coral and Symbiodiniaceae genotypes (Rohwer et al. 2002). *Endozoicomonas*, Alteromonadaceae, and *Ralstonia* are examples of tissue-associated taxa ubiquitously found across many coral species from widely separated locations (Bayer et al. 2013; Ainsworth et al. 2015; Roder et al. 2015; Hernandez-Agreda et al. 2016; Neave et al. 2016b, 2016a; Certner and Vollmer 2018). Endosymbiotic and episymbiotic tissue-associated bacterial communities have been characterized by coupling laser microdissection and next-generation sequencing. The orders Rhizobiales, Caulobacteriales, and Burkholderiales are identified in association with Symbiodiniaceae, while members of the family Endozoicomonadaceae, and orders Rickettsiales and Rhodobacteriales are identified in association with coral host cells (Ainsworth et al. 2015; Neave et al. 2016b, 2017). To date, several genomes of coral-associated *Endozoicomonas* are available (Neave et al. 2014, 2017; Ding et al. 2016), as well as protocols for isolation and culture (Pogoreutz and Voolstra 2018).

The coral skeleton contains a rich microbial community, which is often reported to be dominated by a diversity of cyanobacteria (Yamazaki et al. 2008). Some of these cyanobacteria have been cultured and characterized, such as *Plectonema terebrans*, *Mastigocoleus testarum*, and *Halomicronema excentricum*. Recent studies revealed that while skeleton-associated cyanobacteria occur at low relative abundances, they can be highly diverse (Marcelino and Verbruggen 2016). Other bacterial functional groups, such as anoxygenic phototrophic bacteria and anaerobic green sulphur bacteria, have also been identified in coral skeletons by spectral signatures of bacteriochlorophylls and via next-generation sequencing (Magnusson et al. 2007; Ralph et al. 2007; Yang et al. 2016).

Besides such broad compartmentalization, localization of microbes within a host can provide information on putative functions. Fluorescence *in situ* hybridization (FISH) approaches have shown that in healthy hosts, bacteria tend to form aggregates (Ainsworth et al. 2006; Bayer et al. 2013; Wada et al. 2016, 2019; Neave et al. 2017), which can contain one or more cell morphologies (Wada et al. 2019). In contrast, diseased and lesioned coral tissues appear 'overgrown' and crowded by bacteria (Ainsworth et al. 2006; Wada et al. 2016). Two bacterial genera previously identified in coral tissues are particularly worthwhile mentioning: (1) the abundant *Endozoicomonas*, which aggregate in the gastroderm of *Stylophora pistillata* (Bayer et al. 2013; Neave et al. 2017) and *Pocillopora verrucosa* (Neave et al. 2017); and (2) the putative parasite *Candidatus Aquarickettsia rohweri*, which is present in the mucocytes of the coral ectoderm (Klinges et al. 2019). This genus lacks genes for nitrogen metabolism and the

synthesis of most sugars but maintains the genetic machinery for sensing and responding to extracellular nitrogen, as well as a complete type IV secretion system, collectively suggesting that *Ca. A. rohweri* may be a true coral parasite (Klinges et al. 2019).

[Insert Figure 7.3 here]

7.4.2 Diversity of coral-associated bacteria and interspecies interactions

Studies on coral-associated microbes have mostly focused on bacterial diversity associated with coral tissues. Coral bacterial communities are typically diverse, uneven, and consist of hundreds to thousands of bacterial taxa (Rohwer et al. 2002; Bourne et al. 2008; Ziegler et al. 2016).

Structure and composition of bacterial communities readily respond to the host's environment, i.e. reflect environmental gradients, fluctuations, and habitat suitability (Thurber et al. 2009; Morrow et al. 2015; Roder et al. 2015; Ziegler et al. 2017). In adult corals, bacterial community shifts due to environmental changes can occur within a few days (Thurber et al. 2009) and in some cases within hours (Ziegler et al. 2017), with older colonies exhibiting delayed microbiome shifts, possibly due to differences in microbial composition, bacteria-bacteria interactions, or host energetics (Sweet et al. 2017).

The tissue-associated *Endozoicomonas* commonly display substantial changes in relative abundance depending on coral or reef health (Bayer et al. 2013; Roder et al. 2015; Neave et al. 2016a). The relative abundance of this genus is usually high in healthy corals and on reefs with high coral cover, but low on degraded reefs and in stressed, bleached, or diseased corals (Bourne et al. 2008; Meyer et al. 2014; Morrow et al. 2015). It was hence suggested that

Endozoicomonas may be important for coral holobiont health, but its potential roles have yet to be identified. Increases in abundance of opportunistic bacteria and putative pathogens, such as Vibrionaceae, Rhodobacteraceae, or Flavobacteriaceae, often occur in chronically or severely stressed corals (Cárdenas et al. 2012; Roder et al. 2014a; 2014b; Ziegler et al. 2016; Gignoux-Wolfsohn et al. 2017; Certner and Vollmer 2018). Stress-associated bacterial community shifts in corals are also reflected in the functional gene repertoire of the microbiome, showing increases in abundance of genes involved in virulence, stress resistance, or sulfur and nitrogen metabolism (Thurber et al. 2009).

Interactions between bacteria exert essential selective forces that sculpt coral microbial assemblages. For instance, the production of antibacterial molecules is one of the most important mechanisms used by native commensal bacteria for shaping the diversity of coral-associated prokaryotes and controlling the presence of pathogens (Sweet et al. 2011). Antibacterial activity has been demonstrated in numerous bacterial taxa (Ritchie 2006; Nissimov et al. 2009; Rypien et al. 2010; Kvennefors et al. 2012; Pereira et al. 2017) and coral extracts (Kelman et al. 2006; Gochfeld and Aeby 2008), but only a few compounds have been identified to date (Raina et al. 2016). Other bacteria-bacteria interactions also occur through quorum sensing (QS), which allows cells to communicate and synchronize gene expression in a concerted density-dependent manner to coordinate population behaviors. A broad spectrum of coral-associated bacteria, including some known coral pathogens, rely on QS to control colonization, virulence and extracellular enzyme production (Tait et al. 2010; Alagely et al. 2011; De O Santos et al. 2011; Golberg et al. 2011). Given the importance of QS in the induction

of virulence-related traits in opportunistic and pathogenic bacteria, many coral-associated commensal bacteria are capable of disrupting QS circuits as a strategy to prevent and mitigate pathogen invasion (Golberg et al. 2013) and some of these taxa might be critical for our understanding of QS in the context of coral diseases (Zimmer et al. 2014; Meyer et al. 2016; Certner and Vollmer 2018).

7.4.3 Acquisition of bacterial associates and their roles in early coral life-stages

Coral sexual reproduction occurs either through spawning where gametes are released into the water column, or through internally fertilizing gametes and brooding larvae inside the coral polyp (Fadlallah 1983). In spawning corals, the establishment of coral-bacteria symbioses is widely believed to happen horizontally (i.e., through acquisition of symbionts from the environment) during the coral pelagic larval phase or even after settlement and metamorphosis (Aprill et al. 2009; Sharp et al. 2010). Conversely, in brooders, bacteria can be vertically transmitted (i.e., directly passed from the parent colony to the planula before its release). Bacterial communities exhibit dynamic changes between the different ontogenetic stages of coral development (Damjanovic et al. 2019; Epstein et al. 2019), likely reflecting a succession of microbial functions relevant for the holobiont (Bernasconi et al. 2019) and potentially following a winnowing process (Epstein et al. 2019).

A broad phylogenetic range of bacteria can be vertically or horizontally acquired. Most common associates of gametes, embryos, and larvae of brooders and spawners include the genera *Roseobacter*, *Marinobacter*, *Alteromonas*, *Vibrio*, *Bradyrhizobia* and *Endozoicomonas*.

Roseobacter- and *Alteromonas*-affiliated sequences are consistently found in very early developmental stages of several coral species (Apprill et al. 2009; Ceh et al. 2012, 2013; Sharp et al. 2012). These taxa are metabolically diverse and have been linked with the production of antibiotic compounds to counter coral pathogens (Piekarski et al. 2009). Furthermore, *Alteromonas* spp., *Vibrio* spp., and the diazotroph *Bradyrhizobia* might provide biologically available nitrogen to coral larvae (Ceh et al. 2013; Lema et al. 2014). The transmission of potentially beneficial bacteria from parent coral colonies to gametes or early ontogenetic stages might be a mechanism to ensure microbial inheritance across generations. In addition, bacterial chemotaxis is likely to play a role in microbiome establishment. Indeed, many environmentally acquired bacterial symbionts use chemotaxis - the ability to direct movement towards or away from specific chemicals - to locate their hosts (Raina et al. 2019). Chemotaxis may be a particularly prevalent mechanism employed on reefs because the coral surface is characterized by strong gradients of organic compounds that can act as cues for microorganisms (Ochsenkühn et al. 2018). Several coral-associated bacterial families, such as Endozoicomonadaceae, Rhodobacteraceae, or Oceanospirillaceae, exhibit chemoattraction towards constituents of coral mucus (Tout et al. 2015). Chemotaxis and motility however can also enable coral pathogens, such as *Vibrio shiloi* and *V. coralliilyticus*, to locate and infect their hosts (Banin et al. 2001; Meron et al. 2009) using chemical cues such as dimethylsulfoniopropionate (DMSP) present in coral mucus (Garren et al. 2014).

Bacteria are also directly involved in the transition between pelagic and benthic life stages in the coral life cycle. Indeed, after a pelagic phase (typically ranging from a week to more than

100 days), coral larvae must attach to a suitable reef structure to metamorphose into colony-forming juveniles (Connolly and Baird 2010). Habitat-specific environmental cues, mainly produced by specific bacteria associated with crustose coralline algae (CCA), are responsible for coral larval recruitment and metamorphosis (Harrington et al. 2004; Webster et al. 2004; Tebben et al. 2015). Clear shifts in the bacterial community structure of CCA occur following thermal stress, resulting in significant reduction of coral larvae recruitment (Webster et al. 2011). Similarly, antibiotic treatment of larval cultures reduced settlement rates, suggesting that the presence of certain bacterial is essential for settlement induction (Vermeij et al. 2009).

7.4.4 Coral probiotics

Related to studying the setup, maintenance, and inheritance of microbial relationships is understanding the dynamic interplay of coral-associated microbes in relation to the prevailing environment. The underlying premise is that microbial associates can adapt quickly to the surrounding environment and contribute functions that support coral holobiont health and resilience (Reshef et al. 2006; Ziegler et al. 2017, 2019; Bang et al. 2018). In recent years, efforts have been channeled into coral ‘probiotics’ applications. Their ultimate goal is to fast-track ecological adaptation to global climate change by designing physiologically augmented coral holobionts (Peixoto et al. 2017). The research field of coral probiotics includes the isolation and screening of native bacterial associates for functional genes beneficial to coral health, and subsequent physiological assays to determine holobiont performance after inoculation with putatively beneficial bacterial isolates (Rosado et al. 2018). Experimental inoculation with mixed consortia of native coral bacterial isolates harboring N₂ fixation (*nifH*), denitrification

(*nirK*), and DMSP-degrading (*dmdA*) genes resulted in partial mitigation of coral bleaching compared to controls or corals challenged with the temperature-dependent pathogen *Vibrio coralliilyticus* (Rosado et al. 2018). Open questions to this line of research are the temporal stability of the observed beneficial effects, the underlying mechanistic nature, and the potential for application of coral probiotics at the reef scale.

7.4.5 Contribution of bacteria to holobiont nutrient cycling

The ubiquitous coral symbionts *Endozoicomonas* harbor large numbers of genes involved in amino acid synthesis and carbohydrate cycling, suggesting its involvement in holobiont nutrient cycling (Neave et al. 2017), with different strains potentially exhibiting a different genetic and metabolic makeup (Neave et al. 2017; Pogoreutz et al. 2018). Taxonomy-based functional inference was used recently and suggested a role of Endozoicomonadaceae in processes related to nitrate reduction in giant clams (Rossbach et al. 2019). As such, Endozoicomonadaceae may provide otherwise inaccessible nitrogen sources, including ammonia, to the coral host.

Nitrogen (N) cycling is a critical component of holobiont health (Cardini et al. 2014; Rådecker et al. 2015; Pogoreutz et al. 2017a). Most research on N cycling in the coral holobiont has focused on prokaryotic dinitrogen (N₂) fixation (Shashar et al. 1994; Lesser et al. 2007; Rådecker et al. 2014; Bednarz et al. 2017), while the assessment of other major N cycling pathways such as nitrification, denitrification, and ANAMMOX has only received marginal attention to date (Wafar et al. 1990; Tilstra et al. 2019), and is limited to describing the presence of functional

genes in sequencing datasets (Wegley et al. 2007; Siboni et al. 2008; Neave et al. 2017).

Prokaryotic N₂ fixation commonly occurs in reef-building corals, helping supply the holobiont with 'new' bioavailable N (Lesser et al. 2007; Cardini et al. 2015; Benavides et al. 2017). The biologically fixed N is then assimilated by both, coral (Benavides et al. 2016; Bednarz et al. 2017) and Symbiodiniaceae (Lesser et al. 2007; Cardini et al. 2015; Pogoreutz et al. 2017a). N assimilation rates, however, appear to depend on environmental N availability, highlighting the importance of integrating environmental context into the study of coral holobiont function. The particular role of N₂ fixation to coral heat stress (bleaching) remains to be determined. Elevated temperatures rapidly cause an increase in the relative abundance and activity of coral-associated N₂ fixers (Santos et al. 2014; Cardini et al. 2016). It was previously concluded that excess N supply has the potential to ameliorate the effects of heat stress caused by global warming in corals (Santos et al. 2014; Cardini et al. 2016). An increase in holobiont N₂ fixation however can shift the N:P ratio of dinoflagellate symbionts (Pogoreutz et al. 2017a), thereby destabilizing the coral-algae symbiosis, resulting in coral bleaching (Wiedenmann et al. 2012). Ultimately, whether increases in N₂ fixation during heat stress have beneficial or detrimental effects on coral holobiont health may likely be determined by the environmental context (e.g., ambient nutrient regime), host nutritional state, or heterotrophic capacity (Bednarz et al. 2017; Pogoreutz et al. 2017b), and will require further mechanistic studies considering all major nitrogen cycling pathways.

In addition, symbiotic interactions between corals and bacteria might involve the cycling of essential compounds, e.g. vitamins. Indeed, the cnidarian host may rely on bacterial symbionts

for the provision of cobalamin, which is required for methionine synthesis by both corals and Symbiodiniaceae (Robbins et al. 2019). Concentrations of cobalamin in the coral gastrovascular cavity (coelenteron) are up to 35 times higher than in surrounding reef waters (Agostini et al. 2009), strongly suggesting that the dense bacterial communities harbored in the gastrovascular cavity are producing this essential molecule. In addition, some species from the genus *Acropora* are lacking an essential enzyme to synthesize the amino acid cysteine (Shinzato et al. 2011) and likely rely on their associated microbes for its provision.

7.4.6 Archaea associated with the coral holobiont

Corals associate with a diversity of archaea including representatives from the Crenarchaeota and Euryarchaeota. Crenarchaeota of the class Thermoprotei often dominate the archaeal community, while most abundant euryarchaeal members are affiliated to the Marine Group II and Thermoplasma (Kellogg 2004; Siboni et al. 2008). Interestingly, SML-associated archaeal sequences are most similar to obligate and facultative anaerobic and uncultivated archaea from anoxic environments, suggesting anaerobic microniches within the SML (Kellogg 2004). In terms of absolute abundance, archaeal cells can comprise up to half of the prokaryotic community with an average of $>10^7$ cells/cm² on the surface of *Porites astreoides* colonies (Wegley et al. 2004).

The diversity of ammonia-oxidizing archaea (AOA) has also been evaluated in coral tissues by amplifying the *amoA* gene encoding the alpha-subunit of the ammonia monooxygenase (Beman et al. 2007; Siboni et al. 2008). In addition, AOA are suggested to be less host-specific and more

geographically dependent (Siboni et al. 2012). The presence of *amo* genes in coral-associated archaea was also supported by an integrated genomic approach of Thaumarchaeota genomes assembled from *Porites lutea* metagenomes (Robbins et al. 2019). This study revealed the presence of other relevant key metabolic pathways, including the reductive tricarboxylic acid cycle, cobalamin synthesis, and taurine dioxygenase in the Thaumarchaeota genomes, suggesting these symbionts may contribute to the host's demand for essential vitamins and carbon metabolism (Robbins et al. 2019).

7.4.7 Protists and fungi associated with the coral holobiont

Two photosynthetic alveolates, *Chromera velia* (Moore et al. 2008) and *Vitrella brassicaformis* (Oborník et al. 2012), which are the closest free-living relatives of the large parasitic phylum Apicomplexa, are protists commonly associated with corals worldwide (Janoušek et al. 2013). A recent transcriptomic study revealed that the coral host response to *C. velia* inoculation was similar to that of a parasite or pathogen infection in vertebrates (Mohamed et al. 2018). This suggests that *C. velia*, despite its photoautotrophic capabilities, is not involved in mutualistic interactions with corals, but rather parasitic or commensal (Mohamed et al. 2018). In addition to these two alveolates, the presence of apicomplexans in coral tissues has been reported for the past 30 years (Upton and Peters 1986; Toller et al. 2002; Šlapeta and Linares 2013; Clerissi et al. 2018) and it was recently revealed that a single apicomplexan lineage is ubiquitously associated with corals and might be the second most abundant microeukaryote group (after Symbiodiniaceae) associated with coral tissues (Janoušek et al. 2012; Kwong et al. 2019). Although the nature of the association between these “corallicolids” and the coral

host remains unknown, their genomes lack all genes for photosystem proteins, but retained the four ancestral genes involved in chlorophyll biosynthesis (Kwong et al. 2019).

Endolithic protist algae can form dense bands visible to the unaided eye in the skeleton of many coral species and are often dominated by the filamentous green algae *Ostreobium* spp. (Siphonales, Chlorophyta) (Kornmann and Sahling 1980). Recent molecular studies have revealed the astonishing genetic diversity of this group, with up to 80 taxonomic units at the near-species level (Marcelino and Verbruggen 2016; Marcelino et al. 2017, 2018; Verbruggen et al. 2017). These filamentous algae colonize the skeleton of coral juveniles early in their development (Massé et al. 2018) and can interact with the coral tissue through transfers of photosynthates (Schlichter et al. 1995; Fine and Loya 2002; Pernice et al. 2019). High-throughput amplicon sequencing has also revealed the presence of other, less abundant, boring green microalgae closely related to *Phaeophila*, *Bryopsis*, *Chlorodesmis*, *Cladophora*, *Pseudulvella*, and red algae from the Bangiales order in coral skeletons (Marcelino and Verbruggen 2016).

Fungi are prevalent in corals and have been well studied in the coral skeleton where they penetrate the calcium carbonate microstructures and ultimately interact with *Ostreobium* cells (Le Campion-Alsumard et al. 1995). Along with endolithic algae, fungi are present in the newly deposited coral skeleton (Bentis et al. 2000; Golubic et al. 2005), where they exhibit rapid growth to match skeletal accretion (Le Campion-Alsumard et al. 1995). Fungi were the most abundant microorganisms in the *Porites astreoides* metagenome, contributing to 38% of the

microbial sequences (Wegley et al. 2007). These fungi belonged mainly to the phylum Ascomycota, but also comprised Basidiomycota and Chytridiomycota. Based on their genomic potential, these organisms might play a role in nitrogen recycling through the reduction of nitrate and nitrite to ammonia and subsequent ammonia assimilation (Wegley et al. 2007).

7.5 Summary and Outlook

The coral-dinoflagellate symbiosis is a well-studied system with high potential for understanding mechanisms of host-microbe interactions, controls, and co-evolution. The functional importance of bacteria, archaea, and other protists in this complex system has only recently started to emerge and the resulting picture is that of a holobiont where all partners depend on and interact with each other. These complex inter-domain relationships are orchestrated and regulated by immune systems. It is therefore important to adopt a two-pronged approach, elucidating the immune responses of each individual partner (i.e., coral host, Symbiodiniaceae, bacteria, and other microbes), but also gaining a greater understanding of immunity at the holobiont scale. To elucidate this complex network of interactions, the Aiptasia model system may help to functionally interrogate the mechanistic underpinnings shaping the ecology, stress resilience, and environmental adaptation of cnidarian holobionts. For instance, the ability of genetic and microbiome manipulation of Aiptasia allows for detailed studies into the contribution of specific genes or microbes.

It is understood that productivity, structural complexity, and biodiversity of coral reef ecosystems critically depend on healthy coral holobionts, which in turn are linked to the diversity and identity of the associated microbes. This implies the need for a radically different

approach to address the key question of coral health and resilience in the face of climate change: one must examine the complexity of the coral holobiont in its entirety, i.e. considering the diversity and function of all associated organisms. Gaining a holistic understanding of the biology and functioning of coral holobionts is of prime importance, given that coral reefs are at the brink of ecological collapse due to climate change and local stressors. New strategies to mitigate reef loss are now more important than ever. One such strategy may lie in the manipulation of holobiont immunity or associated microbes to enable ecological adaptation at a rate and scale that matches the pace of environmental change.

Figure legends

Figure 7.1. Scleractinian corals are the foundation species of coral reef ecosystems. Complex symbiotic interactions facilitate nutrient uptake and recycling, thereby enabling corals to thrive in highly oligotrophic environments and build the structural framework of coral reefs.

Figure 7.2. Overview and diversity of cnidarian-dinoflagellate symbioses. a) Reef-building or stony coral (Anthozoa, Scleractinia: *Acropora humilis*); b) Fire coral, a calcifying hydrocoral (Hydrozoa: *Millepora platyphylla*); c) Blue coral, a calcifying 'soft' coral (Anthozoa, Octocorallia: *Heliopora coerulea*); d) upside-down jellyfish (Scyphozoa: *Cassiopea* sp.). All of these marine cnidarian holobiont systems are intimately associated in a mutualistic relationship with dinoflagellates of the family Symbiodiniaceae.

Figure 7.3. The coral holobiont consists of the animal host and a wide diversity of eukaryotic and prokaryotic microorganisms, and their potentially numerous metabolic interactions. Microbial community composition and structure within the holobiont readily respond to environmental fluctuations (e.g., of temperature, nutrients, or light availability). The eukaryotic **Symbiodiniaceae** are intracellular tissue-associated microalgal symbionts that engage in an intimate and efficient nutrient-exchange mutualism with their coral host. Through their high carbon acquisition, fixation, and translocation rates to the host, Symbiodiniaceae can be considered true unicellular engines of the coral holobiont, which are able to provide organic carbon at rates that can fully meet or even exceed the respiratory demands of the coral animal host. Among the prokaryotic associates of the coral holobiont, **bacteria** are the best studied members. Coral-associated bacterial communities are highly diverse, uneven, and exhibit distinct compositions in the coral surface mucus layer, the coral tissues, and the coral skeleton. In the coral skeleton, a diversity of **endolithic algae** (including the abundant and diverse *Ostreobium* spp.) and **fungi** form dense bands and are hypothesized to metabolically interact with the coral host tissue. The metabolic functions of prokaryotes and endolithic algae and fungi are potentially highly diverse; whether and how they may contribute to holobiont functioning is an area of active exploration.

References

- Agostini S, Suzuki Y, Casareto BE, Nakano Y, Michio H, Badrun N (2009) Coral symbiotic complex: Hypothesis through vitamin B12 for a new evaluation. *Galaxea, J Coral Reef Stud* 11:1–11
- Ainsworth T, Krause L, Bridge T, Torda G, Raina J-B, Zakrzewski M, Gates RD, Padilla-Gamiño JL, Spalding HL, Smith C, Woolsey ES, Bourne DG, Bongaerts P, Hoegh-Guldberg O, Leggat W (2015) The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. *ISME J* 9:2261–2274
- Ainsworth TD, Fine M, Blackall LL, Hoegh-Guldberg O (2006) Fluorescence *In Situ* Hybridization

- and spectral imaging of coral-associated bacterial communities. *Appl Environ Microbiol* 72:3016–3020
- Alagely A, Krediet CJ, Ritchie KB, Teplitski M (2011) Signaling-mediated cross-talk modulates swarming and biofilm formation in a coral pathogen *Serratia marcescens*. *ISME J* 5:1609–1620
- Aprill A, Marlow HQ, Martindale MQ, Rappé MS (2009) The onset of microbial associations in the coral *Pocillopora meandrina*. *ISME J* 3:685–699
- Aranda M, Li Y, Liew YJ, Baumgarten S, Simakov O, Wilson M, Piel J, Ashoor H, Bougouffa S, Bajic V, Ryu T, Ravasi T, Bayer T, Micklem G, Kim H, Bhak J, LaGeunesse TC, Voolstra CR (2016) Genomes of coral dinoflagellate symbionts highlight evolutionary adaptations conducive to a symbiotic lifestyle. *Sci Rep* 6:39734
- Bang C, Dagan T, Deines P, Dubilier N, Duschl WJ, Fraune S, Hentschel U, Hirt H, Hülter N, Lachnit T, Picazo D, Pita L, Pogoreutz C, Rädicker N, Saad MM, Schmitz RA, Schulenburg H, Voolstra CR, Weiland-Bräuer N, Ziegler M, Bosch TCG (2018) Metaorganisms in extreme environments: do microbes play a role in organismal adaptation? *Zoology* 127:1–19
- Banin E, Israely T, Fine M, Loya Y, Rosenberg E (2001) Role of endosymbiotic zooxanthellae and coral mucus in the adhesion of the coral-bleaching pathogen *Vibrio shiloi* to its host. *FEMS Microbiol Lett* 199:33–37
- Baumgarten S, Simakov O, Esherick LY, Liew YJ, Lehnert EM, Michell CT, Li Y, Hambleton EA, Guse A, Oates ME, Gough J, Weis VM, Aranda M, Pringle JR, Voolstra CR (2015) The genome of *Aiptasia*, a sea anemone model for coral biology. *Proc Natl Acad Sci* 112: 11893–11898
- Bay LK, Cumbo VR, Abrego D, Kool JT, Ainsworth TD, Willis BL (2011) Infection dynamics vary between *Symbiodinium* Types and cell surface treatments during establishment of endosymbiosis with coral larvae. *Diversity* 3:356–374
- Bayer T, Neave MJ, Alsheikh-Hussain A, Aranda M, Yum LK, Mincer T, Huguen K, Aprill A, Voolstra CR (2013) The microbiome of the Red Sea coral *Stylophora pistillata* is dominated by tissue-associated *Endozoicomonas* bacteria. *Appl Environ Microbiol* 79:4759–4762
- Bednarz VN, Grover R, Maguer JF, Fine M, Ferrier-Pagès C (2017) The assimilation of diazotroph-derived nitrogen by scleractinian corals depends on their metabolic status. *MBio* 8:1–14
- Beman JM, Roberts KJ, Wegley L, Rohwer F, Francis CA (2007) Distribution and diversity of archaeal ammonia monooxygenase genes associated with corals. *Appl Environ Microbiol* 73:5642–5647
- Benavides M, Bednarz VN, Ferrier-Pagès C (2017) Diazotrophs: Overlooked key players within the coral symbiosis and tropical reef ecosystems? *Front Mar Sci* 4:10
- Benavides M, Houlbreque F, Camps M, Lorrain A, Grosso O, Bonnet S (2016) Diazotrophs: A non-negligible source of nitrogen for the tropical coral *Stylophora pistillata*. *J Exp Biol* 219:2608–2612
- Bentis CJ, Kaufman L, Golubic S (2000) Endolithic fungi in reef-building corals (Order: Scleractinia) are common, cosmopolitan, and potentially pathogenic. *Biol Bull* 198:254–260
- Berkelmans R, Van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: A ‘nugget of hope’ for coral reefs in an era of climate change. *Proc R Soc B Biol Sci* 273:2305–2312
- Bernasconi R, Stat M, Koenders A, Papparini A, Bunce M, Huggett MJ (2019) Establishment of coral-bacteria symbioses reveal changes in the core bacterial community with host ontogeny.

Front Microbiol 10:1–16

- Berthelie J, Schnitzler CE, Wood-Charlson EM, Poole AZ, Weis VM, Detournay O (2017) Implication of the host TGF β pathway in the onset of symbiosis between larvae of the coral *Fungia scutaria* and the dinoflagellate *Symbiodinium* sp. (clade C1f). *Coral Reefs* 36:1263–1268
- Bourne D, Iida Y, Uthicke S, Smith-Keune C (2008) Changes in coral-associated microbial communities during a bleaching event. *ISME J* 2:350–363
- Le Campion-Alsumard T, Golubic S, Priess K (1995) Fungi in corals: symbiosis or disease? Interaction between polyps and fungi causes pearl-like skeleton biomineralization. *Mar Ecol Prog Ser* 117:137–147
- Cantin NE, Van Oppen MJH, Willis BL, Mieog JC, Negri AP (2009) Juvenile corals can acquire more carbon from high-performance algal symbionts. *Coral Reefs* 28:405–414
- Cárdenas A, Rodríguez-R LM, Pizarro V, Cadavid LF, Arévalo-Ferro C (2012) Shifts in bacterial communities of two Caribbean reef-building coral species affected by white plague disease. *ISME J* 6:502–512
- Cardini U, Bednarz VN, Foster RA, Wild C (2014) Benthic N₂ fixation in coral reefs and the potential effects of human-induced environmental change. *Ecol Evol* 4:1706–1727
- Cardini U, Bednarz VN, Naumann MS, van Hoytema N, Rix L, Foster RA, Al-Rshaidat MMD, Wild C (2015) Functional significance of dinitrogen fixation in sustaining coral productivity under oligotrophic conditions. *Proc R Soc B Biol Sci* 282:20152257
- Cardini U, van Hoytema N, Bednarz VN, Rix L, Foster RA, Al-Rshaidat MMD, Wild C (2016) Microbial dinitrogen fixation in coral holobionts exposed to thermal stress and bleaching. *Environ Microbiol* 18:2620–2633
- Ceh J, van Keulen M, Bourne DG (2013) Intergenerational transfer of specific bacteria in corals and possible implications for offspring fitness. *Microb Ecol* 65:227–231
- Ceh J, Raina JB, Soo RM, van Keulen M, Bourne DG (2012) Coral-bacterial communities before and after a coral mass spawning event on Ningaloo Reef. *PLoS One* 7:3–9
- Certner RH, Vollmer SV (2018) Inhibiting bacterial quorum sensing arrests coral disease development and disease-associated microbes. *Environ Microbiol* 20:645–657
- Chen M, Cheng Y, Hong M, Fang L (2004) Molecular cloning of Rab5 (ApRab5) in *Aiptasia pulchella* and its retention in phagosomes harboring live zooxanthellae. *Biochem Biophys Res Comm* 324:1024–1033
- Chen M, Cheng Y, Sung P, Kuo C, Fang L (2003) Molecular identification of Rab7 (ApRab7) in *Aiptasia pulchella* and its exclusion from phagosomes harboring zooxanthellae. *Biochem Biophys Res Comm* 308:586–595
- Cho DH, Ramanan R, Kim BH, Lee J, Kim S, Yoo C, Choi GG, Oh HM, Kim HS (2013) Novel approach for the development of axenic microalgal cultures from environmental samples. *J Phycol* 49:802–810
- Clerissi C, Brunet S, Vidal-Dupiol J, Adjeroud M, Lepage P, Guillou L, Escoubas JM, Toulza E (2018) Protists within corals: The hidden diversity. *Front Microbiol* 9:1–13
- Colley N, Trench R (1983) Selectivity in phagocytosis and persistence of symbiotic algae by the scyphistoma stage of the jellyfish *Cassiopeia xamachana*. *Proc R Soc B Biol Sci* 219d:61–82
- Connolly SR, Baird AH (2010) Estimating dispersal potential for marine larvae: Dynamic models applied to scleractinian corals. *Ecology* 91:3572–3583

- Cui G, Liew YJ, Li Y, Kharbatia N, Zahran NI, Emwas A-H, Eguiluz VM, Aranda M (2019) Host-dependent nitrogen recycling as a mechanism of symbiont control in *Aiptasia*. *PLOS Genet* 15:e1008189
- Cunning R, Bay RA, Gillette P, Baker AC, Traylor-Knowles N (2018) Comparative analysis of the *Pocillopora damicornis* genome highlights role of immune system in coral evolution. *Sci Rep* 8:1–10
- Cunning R, Muller EB, Gates RD, Nisbet RM (2017) A dynamic bioenergetic model for coral-*Symbiodinium* symbioses and coral bleaching as an alternate stable state. *J Theor Biol* 431:49–62
- Damjanovic K, van Oppen MJH, Menéndez P, Blackall LL (2019) Experimental inoculation of coral recruits with marine bacteria indicates scope for microbiome manipulation in *Acropora tenuis* and *Platygyra daedalea*. *Front Microbiol* 10:1–18
- Darwin C (1842) The structure and distribution of coral reefs. Smith, Elder and Co.
- Davy SK, Allemand D, Weis VM (2012) Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiol Mol Biol Rev* 76:229–261
- Detournay O, Schnitzler CE, Poole A, Weis VM (2012) Regulation of cnidarian-dinoflagellate mutualisms: Evidence that activation of a host TGF β innate immune pathway promotes tolerance of the symbiont. *Dev Comp Immunol* 38:525–537
- Ding JY, Shiu JH, Chen WM, Chiang YR, Tang SL (2016) Genomic insight into the host-endosymbiont relationship of *Endozoicomonas montiporae* CL-33T with its coral host. *Front Microbiol* 7:251
- Douglas AE (2003) Coral bleaching - How and why? *Mar Pollut Bull* 46:385–392
- Dunn SR, Schnitzler CE, Weis VM (2007) Apoptosis and autophagy as mechanisms of dinoflagellate symbiont release during cnidarian bleaching: Every which way you lose. *Proc R Soc B Biol Sci* 274:3079–3085
- Epstein HE, Torda G, Munday PL, Oppen MJH Van (2019) Parental and early life stage environments drive establishment of bacterial and dinoflagellate communities in a common coral. *ISME J* 13:1635-1638
- Ezzat L, Maguer J-FJ-F, Grover R, Ferrier-Pagès C (2015) New insights into carbon acquisition and exchanges within the coral – dinoflagellate symbiosis under NH_4^+ and NO_3^- supply. *Proc R Soc B Biol Sci* 282:20150610
- Fadlallah YH (1983) Coral reefs sexual reproduction, development and larval biology in scleractinian corals: A review. *Coral Reefs* 2:129-150
- Falkowski PG, Dubinsky Z, Muscatine L, McCloskey L (1993) Population control in symbiotic corals. *Bioscience* 43:606–611
- Ferrer LM, Szmant AM (1988) Nutrient regeneration by the endolithic community in coral skeletons. *Proceedings of the 6th International Coral Reef Symposium* 2:1–4
- Fine M, Loya Y (2002) Endolithic algae: an alternative source of photoassimilates during coral bleaching. *Proc R Soc B Biol Sci* 269:1205–1210
- Fisher R, O’Leary RA, Low-Choy S, Mengersen K, Knowlton N, Brainard RE, Caley MJ (2015) Species richness on coral reefs and the pursuit of convergent global estimates. *Curr Biol* 25:500–505
- Freudenthal HD (1962) *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov., a zooxanthella: Taxonomy, life cycle, and morphology. *J Protozool* 9:45–52
- Garren M, Son K, Raina JB, Rusconi R, Menolascina F, Shapiro OH, Tout J, Bourne DG, Seymour

- JR, Stocker R (2014) A bacterial pathogen uses dimethylsulfoniopropionate as a cue to target heat-stressed corals. *ISME J* 8:999–1007
- Gates RD, Baghdasarian G, Muscatine L (1992) Temperature stress causes host cell detachment in symbiotic cnidarians: implications for coral bleaching. *Biol Bull* 182:324–332
- Gignoux-Wolfsohn SA, Aronson FM, Vollmer SV (2017) Complex interactions between potentially pathogenic, opportunistic, and resident bacteria emerge during infection on a reef-building coral. *FEMS Microbiol Ecol*
- Glasl B, Herndl GJ, Frade PR (2016) The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. *ISME J* 10:2280–2292
- Glynn PW (1983) Extensive “bleaching” and death of reef corals on the Pacific coast of Panama. *Environ Conserv* 10:149–154
- Gochfeld DJ, Aeby GS (2008) Antibacterial chemical defenses in Hawaiian corals provide possible protection from disease. *Mar Ecol Prog Ser* 362:119–128
- Golberg K, Eltzov E, Shnit-Orland M, Marks RS, Kushmaro A (2011) Characterization of quorum sensing signals in coral-associated bacteria. *Microb Ecol* 61:783–792
- Golberg K, Pavlov V, Marks RS, Kushmaro A (2013) Coral-associated bacteria, quorum sensing disruptors, and the regulation of biofouling. *Biofouling* 29:669–682
- Golubic S, Radtke G, Le Campion-Alsumard T (2005) Endolithic fungi in marine ecosystems. *Trends Microbiol* 13:229–235
- Grottoli AG, Rodrigues LJ, Palardy JE (2006) Heterotrophic plasticity and resilience in bleached corals. *Nature* 440:1186–1189
- Hadaidi G, Röthig T, Yum LK, Ziegler M, Arif C, Roder C, Burt J, Voolstra CR (2017) Stable mucus-associated bacterial communities in bleached and healthy corals of *Porites lobata* from the Arabian Seas. *Sci Rep* 7:1–11
- Harrington L, Fabricius K, De’Ath G, Negri A (2004) Recognition and selection of settlement substrata determine post-settlement survival in corals. *Ecology* 85:3428–3437
- Hatcher BG (1990) Coral reef primary productivity. A hierarchy of pattern and process. *Trends Ecol Evol* 5:149–55
- Hernandez-Agreda A, Leggat W, Bongaerts P, Ainsworth T (2016) The microbial signature provides insight into the mechanistic basis of coral success across reef habitats. *MBio* 7:1–10
- Hoegh-Guldberg O (1999) Climate Change, coral bleaching and the future of the world’s coral reefs. Ove Hoegh-Guldberg. *Mar Freshw Res* 50:839–866
- Hoegh-Guldberg O (2011) Coral reef ecosystems and anthropogenic climate change. *Reg Environ Chang* 11:215–227
- Hughes TP, Anderson KD, Connolly SR, Heron SF, Kerry JT, Lough JM, Baird AH, Baum JK, Berumen ML, Bridge TC, Claar DC, Eakin CM, Gilmour JP, Graham NAJ, Harrison H, Hobbs JPA, Hoey AS, Hoogenboom M, Lowe RJ, McCulloch MT, Pandolfi JM, Pratchett M, Schoepf V, Torda G, Wilson SK (2018a) Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. *Science* 359:80–83
- Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Hoegh-Guldberg O, Jackson JBC, Kleypas J, Lough JM, Marshall P, Nyström M, Palumbi SJ, Pandolfi JM, Rosen B, Roughgarden J (2003) Climate change, human impacts, and the resilience of coral reefs. *Science* 301:929–933

- Hughes TP, Kerry JT, Álvarez-Noriega M, Álvarez-Romero JG, Anderson KD, Baird AH, Babcock RC, Beger M, Bellwood DR, Berkelmans R, Bridge TC, Butler IR, Byrne M, Cantin NE, Comeau S, Connolly SR, Cumming GS, Dalton SJ, Diaz-Pulido G, Eakin CM, Figueira WF, Gilmour JP, Harrison HB, Heron SF, Hoey AS, Hobbs J-PA, Hoogenboom MO, Kennedy E V., Kuo C, Lough JM, Lowe RJ, Liu G, McCulloch MT, Malcolm HA, McWilliam MJ, Pandolfi JM, Pears RJ, Pratchett MS, Schoepf V, Simpson T, Skirving WJ, Sommer B, Torda G, Wachenfeld DR, Willis BL, Wilson SK (2017) Global warming and recurrent mass bleaching of corals. *Nature* 543:373–377
- Hughes TP, Kerry JT, Simpson T (2018b) Large-scale bleaching of corals on the Great Barrier Reef. *Ecology* 99:501
- Hume BCC, Voolstra CR, Arif C, D'Angelo C, Burt JA, Eyal G, Loya Y, Wiedenmann J (2016) Ancestral genetic diversity associated with the rapid spread of stress-tolerant coral symbionts in response to holocene climate change. *Proc Natl Acad Sci* 113:4416–4421
- IPCC (2018) Summary for Policymakers. In: *Global warming of 1.5°C*. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. <https://www.ipcc.ch/sr15/>. Accessed 10/2019
- Janouškovec J, Horák A, Barott KL, Rohwer FL, Keeling PJ (2012) Global analysis of plastid diversity reveals apicomplexan-related lineages in coral reefs. *Curr Biol* 22:R518–R519
- Janouškovec J, Horák A, Barott KL, Rohwer FL, Keeling PJ (2013) Environmental distribution of coral-associated relatives of apicomplexan parasites. *ISME J* 7:444–447
- Jaspers C, Fraune S, Arnold AE, Miller DJ, Bosch TCG, Voolstra CR (2019) Resolving structure and function of metaorganisms through a holistic framework combining reductionist and integrative approaches. *Zoology* 133:81–87
- Jones AM, Berkelmans R, Van Oppen MJH, Mieog JC, Sinclair W (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: Field evidence of acclimatization. *Proc R Soc B Biol Sci* 275:1359–1365
- Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U (1998) Temperature-induced bleaching of corals begins with impairment of the CO₂ fixation mechanism in zooxanthellae. *Plant, Cell Environ* 21:1219–1230
- Kellogg CA (2004) Tropical Archaea: Diversity associated with the surface microlayer of corals. *Mar Ecol Prog Ser* 273:81–88
- Kelman D, Kashman Y, Rosenberg E, Kushmaro A, Loya Y (2006) Antimicrobial activity of Red Sea corals. *Mar Biol* 149:357–363
- Kitchen SA, Poole AZ, Weis VM (2017) Sphingolipid metabolism of a sea anemone is altered by the presence of dinoflagellate symbionts. *Biol Bull* 233:242–254
- Kitchen SA, Weis VM (2017) The sphingosine rheostat is involved in the cnidarian heat stress response but not necessarily in bleaching. *J Exp Biol* 220:1709–1720
- Klinges JG, Rosales SM, McMinds R, Shaver EC, Shantz AA, Peters EC, Eitel M, Wörheide G, Sharp KH, Burkepille DE, Silliman BR, Thurber RLV (2019) Phylogenetic, genomic, and biogeographic characterization of a novel and ubiquitous marine invertebrate-associated Rickettsiales parasite, *Candidatus Aquarickettsia rohweri*, gen. nov., sp. nov. *ISME J* 5:1-16
- Koren O, Rosenberg E (2006) Bacteria associated with mucus and tissues of the coral *Oculina*

- patagonica* in summer and winter. Appl Environ Microbiol 72:5254–5259
- Kornmann P, Sahling P (1980) *Ostreobium quekettii* (Codiales, Chlorophyta). Helgoländer Meeresuntersuchungen 34:115–122
- Kvennefors ECE, Sampayo E, Kerr C, Vieira G, Roff G, Barnes AC (2012) Regulation of bacterial communities through antimicrobial activity by the coral holobiont. Microb Ecol 63:605–618
- Kwong WK, del Campo J, Mathur V, Vermeij MJA, Keeling PJ (2019) A widespread coral-infecting apicomplexan with chlorophyll biosynthesis genes. Nature 568:103–107
- LaJeunesse TC, Bhagooli R, Hidaka M, DeVantier L, Done T, Schmidt GW, Fitt WK, Hoegh-Guldberg O (2004) Closely related *Symbiodinium* spp. differ in relative dominance in coral reef host communities across environmental, latitudinal and biogeographic gradients. Mar Ecol Prog Ser 284:147–161
- LaJeunesse TC, Loh WKW, Van Woesik R, Hoegh-Guldberg O, Schmidt GW, Fitt WK (2003) Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. Limnol Oceanogr 48:2046–2054
- LaJeunesse TC, Parkinson JE, Gabrielson PW, Jeong HJ, Reimer JD, Voolstra CR, Santos SR (2018) Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. Curr Biol 28:2570–2580
- Lawson CA, Raina JB, Kahlke T, Seymour JR, Suggett DJ (2018) Defining the core microbiome of the symbiotic dinoflagellate, *Symbiodinium*. Environ Microbiol Rep 10:7–11
- Lee STM, Davy SK, Tang SL, Fan TY, Kench PS (2015) Successive shifts in the microbial community of the surface mucus layer and tissues of the coral *Acropora muricata* under thermal stress. FEMS Microbiol Ecol 91:1–11
- Lee STM, Davy SK, Tang SL, Kench PS (2016) Mucus sugar content shapes the bacterial community structure in thermally stressed *Acropora muricata*. Front Microbiol 7:1–11
- Lema KA, Bourne DG, Willis BL (2014) Onset and establishment of diazotrophs and other bacterial associates in the early life history stages of the coral *Acropora millepora*. Mol Ecol 23:4682–4695
- Lesser MP (1997) Oxidative stress causes coral bleaching during exposure to elevated temperatures. Coral Reefs 16:187–192
- Lesser MP, Falcón LI, Rodríguez-Román A, Enríquez S, Hoegh-Guldberg O, Iglesias-Prieto R (2007) Nitrogen fixation by symbiotic cyanobacteria provides a source of nitrogen for the scleractinian coral *Montastraea cavernosa*. Mar Ecol Prog Ser 346:143–152
- Lin S, Cheng S, Song B, Zhong X, Lin X, Li W, Li L, Zhang Y, Zhang H, Ji Z, Cai M, Zhuang Y, Shi X, Lin L, Wang L, Wang Z, Liu X, Yu S, Zeng P, Hao H, Zou Q, Chen C, Li Y, Wang Y, Xu C, Meng S, Xu X, Wang J, Yang H, Campbell DA, Sturm NR, Dagenais-Bellefeuille S, Morse D (2015) The *Symbiodinium kawagutii* genome illuminates dinoflagellate gene expression and coral symbiosis. Science 350:691–694
- Little AF, Van Oppen MJH, Willis BL (2004) Flexibility in algal endosymbioses shapes growth in reef corals. Science 304:1492–1494
- Magnusson SH, Fine M, Kühl M (2007) Light microclimate of endolithic phototrophs in the scleractinian corals *Montipora monasteriata* and *Porites cylindrica*. Mar Ecol Prog Ser 332:119–128
- Mansfield KM, Carter NM, Nguyen L, Cleves PA, Alshanbayeva A, Williams LM, Crowder C, Penvose AR, Finnerty JR, Weis VM, Siggers TW, Gilmore TD (2017) Transcription factor NF-

- kB is modulated by symbiotic status in a sea anemone model of cnidarian bleaching. *Sci Rep* 7:1–14
- Marcelino VR, Morrow KM, van Oppen MJH, Bourne DG, Verbruggen H (2017) Diversity and stability of coral endolithic microbial communities at a naturally high pCO₂ reef. *Mol Ecol* 26:5344–5357
- Marcelino VR, Van Oppen MJH, Verbruggen H (2018) Highly structured prokaryote communities exist within the skeleton of coral colonies. *ISME J* 12:300–303
- Marcelino VR, Verbruggen H (2016) Multi-marker metabarcoding of coral skeletons reveals a rich microbiome and diverse evolutionary origins of endolithic algae. *Sci Rep* 6:1–9
- Massé A, Domart-Coulon I, Golubic S, Duché D, Tribollet A (2018) Early skeletal colonization of the coral holobiont by the microboring Ulvophyceae *Ostreobium* sp. *Sci Rep* 8:1–11
- Meron D, Efrony R, Johnson WR, Schaefer AL, Morris PJ, Rosenberg E, Greenberg EP, Banin E (2009) Role of flagella in virulence of the coral pathogen *Vibrio coralliilyticus*. *Appl Environ Microbiol* 75:5704–5707
- Meyer JL, Gunasekera SP, Scott RM, Paul VJ, Teplitski M (2016) Microbiome shifts and the inhibition of quorum sensing by Black Band Disease cyanobacteria. *ISME J* 10:1204–1216
- Meyer JL, Paul VJ, Teplitski M (2014) Community shifts in the surface microbiomes of the coral *Porites astreoides* with unusual lesions. *PLoS One* 9: e100316
- Moberg F, Folke C (1999) Ecological goods and services of coral reef ecosystems. *Ecol Econ* 29:215–233
- Mohamed AR, Cumbo V, Harii S, Shinzato C, Chan CX, Ragan MA, Miller DJ (2016) The transcriptomic response of the coral *Acropora digitifera* to a competent *Symbiodinium* strain: the symbiosome as an arrested early phagosome. *Mol Ecol* 5:3127–3141
- Mohamed AR, Cumbo VR, Harii S, Shinzato C, Chan CX, Ragan MA, Satoh N, Ball EE, Miller DJ (2018) Deciphering the nature of the coral-*Chromera* association. *ISME J* 12:776–790
- Moore RB, Oborník M, Janouškovec J, Chrudimský T, Vancová M, Green DH, Wright SW, Davies NW, Bolch CJS, Heimann K, Šlapeta J, Hoegh-Guldberg O, Logsdon JM, Carter DA (2008) A photosynthetic alveolate closely related to apicomplexan parasites. *Nature* 451:959–963
- Morris LA, Voolstra CR, Quigley KM, Bourne DG, Bay LK (2019) Nutrient availability and metabolism affect the stability of coral-Symbiodiniaceae symbioses. *Trends Microbiol* 27:678–689
- Morrow KM, Bourne DG, Humphrey C, Botté ES, Laffy P, Zaneveld J, Uthicke S, Fabricius KE, Webster NS (2015) Natural volcanic CO₂ seeps reveal future trajectories for host-microbial associations in corals and sponges. *ISME J* 9:894–908
- Muscatine L (1990) The role of symbiotic algae in carbon and energy flux in reef corals. In: Dubinsky Z. (eds) *Ecosystems of the World: Coral Reefs*. Elsevier, Amsterdam, pp 75–87
- Muscatine L, Falkowski PG, Dubinsky PA, Cook CA, McCloskey LRR (1989) The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. *Proc R Soc London Ser B Biol Sci* 236:311–324
- Muscatine L, Porter JW (1977) Reef corals: Mutualistic symbioses adapted to nutrient-poor environments. *Bioscience* 27:454–460
- Neave MJ, Apprill A, Ferrier-Pagès C, Voolstra CR (2016a) Diversity and function of prevalent symbiotic marine bacteria in the genus *Endozoicomonas*. *Appl Microbiol Biotechnol* 100: 8315–8324

- Neave MJ, Michell CT, Apprill A, Voolstra CR (2014) Whole-genome sequences of three symbiotic *Endozoicomonas* strains. *Genome Announc* 2:e00802-14
- Neave MJ, Michell CT, Apprill A, Voolstra CR (2017) *Endozoicomonas* genomes reveal functional adaptation and plasticity in bacterial strains symbiotically associated with diverse marine hosts. *Sci Rep* 7:40579
- Neave MJ, Rachmawati R, Xun L, Michell CT, Bourne DG, Apprill A, Voolstra CR (2016b) Differential specificity between closely related corals and abundant *Endozoicomonas* endosymbionts across global scales. *ISME J* 11:186-200
- Neubauer E-F, Poole AZ, Neubauer P, Detournay O, Tan K, Davy SK, Weis VM (2017) A diverse host thrombospondin-type-1 repeat protein repertoire promotes symbiont colonization during establishment of cnidarian-dinoflagellate symbiosis. *Elife* 6:1–26
- Neubauer EF, Poole AZ, Detournay O, Weis VM, Davy SK (2016) The scavenger receptor repertoire in six cnidarian species and its putative role in cnidarian-dinoflagellate symbiosis. *PeerJ* 4:e2692
- Nissimov J, Rosenberg E, Munn CB (2009) Antimicrobial properties of resident coral mucus bacteria of *Oculina patagonica*. *FEMS Microbiol Lett* 292:210–215
- de O Santos E, Alves N Jr, Dias GM, Mazotto AM, Vermelho A, Vora GJ, Wilson B, Beltran VH, Bourne DG, Le Roux F, Thompson FL (2011) Genomic and proteomic analyses of the coral pathogen *Vibrio coralliilyticus* reveal a diverse virulence repertoire. *ISME J* 5:1471–1483
- Oborník M, Modrý D, Lukeš M, Černotíková-Stříbrná E, Cihlář J, Tesařová M, Kotabová E, Vancová M, Prášil O, Lukeš J (2012) Morphology, ultrastructure and life cycle of *Vitrella brassicaformis* n. sp., n. gen., a novel chromerid from the Great Barrier Reef. *Protist* 163:306–323
- Ochsenkühn MA, Schmitt-Kopplin P, Harir M, Amin SA (2018) Coral metabolite gradients affect microbial community structures and act as a disease cue. *Commun Biol* 1:184
- Palmer C V (2018) Immunity and the coral crisis. *Commun Biol* 1:1–7
- Parkinson JE, Tivey TR, Mandelare PE, Adpressa DA, Loesgen S, Weis VM (2018) Subtle differences in symbiont cell surface glycan profiles do not explain species-specific colonization rates in a model cnidarian-algal symbiosis. *Front Microbiol* 9:1–12
- Peixoto RS, Rosado PM, Leite DC de A, Rosado AS, Bourne DG (2017) Beneficial microorganisms for corals (BMC): Proposed mechanisms for coral health and resilience. *Front Microbiol* 8:1–16
- Pereira LB, Palermo BRZ, Carlos C, Ottoboni LMM (2017) Diversity and antimicrobial activity of bacteria isolated from different Brazilian coral species. *FEMS Microbiol Lett* 364:1–8
- Pernice M, Meibom A, Van Den Heuvel A, Kopp C, Domart-Coulon I, Hoegh-Guldberg O, Dove S (2012) A single-cell view of ammonium assimilation in coral–dinoflagellate symbiosis. *ISME J* 6:1314–1324
- Pernice M, Raina J-B, Rådecker N, Cardénas A, Pogoreutz C, Voolstra CR (2019) Down to the Bone: the role of overlooked endolithic microbiomes in reef coral health. *ISME J* pp. 1-10; doi: <https://doi.org/10.1038/s41396-019-0548-z>
- Pettay DT, Wham DC, Smith RT, Iglesias-Prieto R, LaJeunesse TC (2015) Microbial invasion of the Caribbean by an Indo-Pacific coral zooxanthella. *Proc Natl Acad Sci* 112:7513–7518
- Piekarski T, Buchholz I, Drepper T, Schobert M, Wagner-Doebler I, Tielen P, Jahn D (2009) Genetic tools for the investigation of *Roseobacter* clade bacteria. *BMC Microbiol* 9:265
- Pogoreutz C, Rådecker N, Cárdenas A, Gärdes A, Voolstra CR, Wild C (2017a) Sugar enrichment

- provides evidence for a role of nitrogen fixation in coral bleaching. *Glob Chang Biol* 23:3838–3848
- Pogoreutz C, Rådecker N, Cárdenas A, Gärdes A, Wild C, Voolstra CR (2017b) Nitrogen fixation aligns with *nifH* abundance and expression in two coral trophic functional groups. *Front Microbiol* 8:1187
- Pogoreutz C, Rådecker N, Cárdenas A, Gärdes A, Wild C, Voolstra CR (2018) Dominance of *Endozoicomonas* bacteria throughout coral bleaching and mortality suggests structural inflexibility of the *Pocillopora verrucosa* microbiome. *Ecol Evol* 8:2240–2252
- Pogoreutz C, Voolstra CR (2018) Isolation, culturing, and cryopreservation of *Endozoicomonas* (Gammaproteobacteria: Oceanospirillales: Endozoicomonadaceae) from reef-building corals. *Protocols.io*. doi: [dx.doi.org/10.17504/protocols.io.t2aeqae](https://doi.org/10.17504/protocols.io.t2aeqae)
- Pollock FJ, McMinds R, Smith S, Bourne DG, Willis BL, Medina M, Thurber RV, Zaneveld JR (2018) Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. *Nat Commun* 9:1–13
- Poole AZ, Kitchen SA, Weis VM (2016) The role of complement in cnidarian-dinoflagellate symbiosis and immune challenge in the sea anemone *Aiptasia pallida*. *Front Microbiol* 7:519
- Rådecker N, Meyer FW, Bednarz VN, Cardini U, Wild C (2014) Ocean acidification rapidly reduces dinitrogen fixation associated with the hermatypic coral *Seriatopora hystrix*. *Mar Ecol Prog Ser* 511:297–302
- Rådecker N, Pogoreutz C, Voolstra CR, Wiedenmann J, Wild C (2015) Nitrogen cycling in corals: the key to understanding holobiont functioning? *Trends Microbiol* 1–8
- Rådecker N, Raina J, Pernice M, Perna G, Guagliardo P, Kilburn MR, Aranda M, Voolstra CR (2018) Using *Aiptasia* as a model to study metabolic interactions in cnidarian-*Symbiodinium* symbioses. *Front Physiol* 9:214
- Raina J-B, Fernandez V, Lambert B, Stocker R, Seymour JR (2019) The role of microbial motility and chemotaxis in symbiosis. *Nat Rev Microbiol* 17:284–294
- Raina J-B, Tapiolas D, Motti CA, Foret S, Seemann T, Tebben J, Willis BL, Bourne DG (2016) Isolation of an antimicrobial compound produced by bacteria associated with reef-building corals. *PeerJ* 4:e2275
- Ralph PJ, Larkum AWD, Kühl M (2007) Photobiology of endolithic microorganisms in living coral skeletons: 1. Pigmentation, spectral reflectance and variable chlorophyll fluorescence analysis of endoliths in the massive corals *Cyphastrea serailia*, *Porites lutea* and *Goniastrea australensis*. *Mar Biol* 152:395–404
- Reshef L, Koren O, Loya Y, Zilber-Rosenberg I, Rosenberg E (2006) The Coral Probiotic Hypothesis. *Environ Microbiol* 8:2068–2073
- Ritchie KB (2006) Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. *Mar Ecol Prog Ser* 322:1–14
- Ritchie KB (2012) Bacterial symbionts of corals and *Symbiodinium*. In: Rosenberg E., Gophna U. (eds) *Beneficial Microorganisms in Multicellular Life Forms*. Springer, Berlin, Heidelberg, pp 139–150
- Robbins SJ, Singleton CM, Chan CX, Messer LF, Geers AU, Ying H, Baker A, Bell SC, Morrow KM, Ragan MA, Miller DJ, Forêt S, ReFuGE2020 Consortium, Voolstra CR, Tyson GW, Bourne DG (2019) A genomic view of the reef-building coral *Porites lutea* and its microbial symbionts. *Nat Microbiol* 1-11

- Roder C, Arif C, Daniels C, Weil E, Voolstra CR (2014a) Bacterial profiling of White Plague Disease across corals and oceans indicates a conserved and distinct disease microbiome. *Mol Ecol* 23:965–974
- Roder C, Arif C, Bayer T, Aranda M, Daniels C, Shibl A, Chavanich S, Voolstra CR (2014b) Bacterial profiling of White Plague Disease in a comparative coral species framework. *ISME J* 8:31–39
- Roder C, Bayer T, Aranda M, Kruse M, Voolstra CR (2015) Microbiome structure of the fungid coral *Ctenactis echinata* aligns with environmental differences. *Mol Ecol* 24:3501–3511
- Rodriguez-Lanetty M, Krupp DA, Weis VM (2004) Distinct ITS types of *Symbiodinium* in Clade C correlate with cnidarian/dinoflagellate specificity during onset of symbiosis. *Mar Ecol Prog Ser* 275:97–102
- Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. *Mar Ecol Prog Ser* 243:1–10
- Rosado PM, Leite DCA, Duarte GAS, Chaloub RM, Jospin G, Nunes da Rocha U, P. Saraiva J, Dini-Andreote F, Eisen JA, Bourne DG, Peixoto RS (2018) Marine probiotics: increasing coral resistance to bleaching through microbiome manipulation. *ISME J* 921–936
- Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. *Nat Rev Microbiol* 5:355–362
- Rosbach S, Cárdenas A, Perna GH, Duarte CM, Voolstra CR (2019) Tissue specific microbiomes of the Red Sea giant clam *Tridacna maxima* highlight differential abundance of Endozoicomonadaceae. *Front Microbiol* 10:2661
- Rypien KL, Ward JR, Azam F (2010) Antagonistic interactions among coral-associated bacteria. *Environ Microbiol* 12:28–39
- Sampayo EM, Ridgway T, Bongaerts P, Hoegh-Guldberg O (2008) Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. *Proc Natl Acad Sci* 105:10444–10449
- Santos HF, Carmo FL, Duarte G, Dini-Andreote F, Castro CB, Rosado AS, van Elsas JD, Peixoto RS (2014) Climate change affects key nitrogen-fixing bacterial populations on coral reefs. *ISME J* 8:2272–2279
- Schlichter D, Zscharnack B, Krisch H (1995) Transfer of photoassimilates from endolithic algae to coral tissue. *Naturwissenschaften* 82:561–564
- Schwarz J, Krupp D, Weis V (1999) Late larval development and onset of symbiosis in the scleractinian coral *Fungia scutaria*. *Biol Bull* 196:70–79
- Seymour JR, Amin SA, Raina JB, Stocker R (2017) Zooming in on the phycosphere: The ecological interface for phytoplankton-bacteria relationships. *Nat Microbiol* 2:17065
- Shantz AA, Lemoine NP, Burkepille DE (2016) Nutrient loading alters the performance of key nutrient exchange mutualisms. *Ecol Lett* 19:20–28
- Sharp KH, Distel D, Paul VJ (2012) Diversity and dynamics of bacterial communities in early life stages of the Caribbean coral *Porites astreoides*. *ISME J* 6:790–801
- Sharp KH, Ritchie KB, Schupp PJ, Ritson-Williams R, Paul VJ (2010) Bacterial acquisition in juveniles of several broadcast spawning coral species. *PLoS One* 5:1–6
- Shashar N, Cohen Y, Loya Y, Sar N (1994) Nitrogen fixation (acetylene reduction) in stony corals: evidence for coral-bacteria interactions. *Mar Ecol Prog Ser* 111:259–264
- Shinzato C, Shoguchi E, Kawashima T, Hamada M, Hisata K, Tanaka M, Fujie M, Fujiwara M, Koyanagi R, Ikuta T, Fujiyama A, Miller DJ, Satoh N (2011) Using the *Acropora digitifera*

- genome to understand coral responses to environmental change. *Nature* 476:320–323
- Shishlyannikov SM, Zakharova YR, Volokitina NA, Mikhailov IS, Petrova DP, Likhoshway Y V. (2011) A procedure for establishing an axenic culture of the diatom *Synedra acus* subsp. *radians* (Kütz.) Skabibitsch from Lake Baikal. *Limnol Oceanogr Methods* 9:478–484
- Shoguchi E, Shinzato C, Kawashima T, Gyoja F, Mungpakdee S, Koyanagi R, Takeuchi T, Hisata K, Tanaka M, Fujiwara M, Hamada M, Seidi A, Fujie M, Usami T, Goto H, Yamasaki S, Arakaki N, Suzuki Y, Sugano S, Toyoda A, Kuroki Y, Fujiyama A, Medina M, Coffroth MA, Bhattacharya D, Satoh N (2013) Draft assembly of the *Symbiodinium minutum* nuclear genome reveals dinoflagellate gene structure. *Curr Biol* 23:1399–1408
- Shumaker A, Putnam HM, Qiu H, Price DC, Zelzion E, Harel A, Wagner NE, Gates RD, Yoon HS, Bhattacharya D (2019) Genome analysis of the rice coral *Montipora capitata*. *Sci Rep* 9:1–16
- Siboni N, Ben-Dov E, Kushmaro A (2012) Geographic specific coral-associated ammonia-oxidizing archaea in the northern Gulf of Eilat (Red Sea). *Microb Ecol* 64:18–24
- Siboni N, Ben-Dov E, Sivan A, Kushmaro A (2008) Global distribution and diversity of coral-associated Archaea and their possible role in the coral holobiont nitrogen cycle. *Environ Microbiol* 10:2979–2990
- Silverstein RN, Correa AMS, LaJeunesse TC, Baker AC (2011) Novel algal symbiont (*Symbiodinium* spp.) diversity in reef corals of Western Australia. *Mar Ecol Prog Ser* 422:63–75
- Silverstein RN, Cuning R, Baker AC (2015) Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. *Glob Chang Biol* 21:236–249
- Šlapeta J, Linares MC (2013) Combined amplicon pyrosequencing assays reveal presence of the apicomplexan “type-N” (cf. *Gemmocystis cylindrus*) and *Chromera velia* on the Great Barrier Reef, Australia. *PLoS One* 8:1–9
- Smith GJ, Muscatine L (1999) Cell cycle of symbiotic dinoflagellates: Variation in G1 phase-duration with anemone nutritional status and macronutrient supply in the *Aiptasia pulchella-Symbiodinium pulchrorum* symbiosis. *Mar Biol* 134:405–418
- Spalding M, Burke L, Wood SA, Ashpole J, Hutchison J, zu Ermgassen P (2017) Mapping the global value and distribution of coral reef tourism. *Mar Policy* 82:104–113
- Sweet MJ, Brown BE, Dunne RP, Singleton I, Bulling M (2017) Evidence for rapid, tide-related shifts in the microbiome of the coral *Coelastrea aspera*. *Coral Reefs* 36:815–828
- Sweet MJ, Croquer A, Bythell JC (2011) Bacterial assemblages differ between compartments within the coral holobiont. *Coral Reefs* 30:39–52
- Tait K, Hutchison Z, Thompson FL, Munn CB (2010) Quorum sensing signal production and inhibition by coral-associated *Vibrios*. *Environ Microbiol Rep* 2:145–150
- Tebben J, Motti CA, Siboni N, Tapiolas DM, Negri AP, Schupp PJ, Kitamura M, Hatta M, Steinberg PD, Harder T (2015) Chemical mediation of coral larval settlement by crustose coralline algae. *Sci Rep* 5:10803
- Thurber RV, Willner-Hall D, Rodriguez-Mueller B, Desnues C, Edwards RA, Angly F, Dinsdale E, Kelly L, Rohwer F (2009) Metagenomic analysis of stressed coral holobionts. *Environ Microbiol* 11:2148–2163
- Tilstra A, El-Khaled Y, Roth F, Rädercker N, Pogoreutz C, Voolstra CR, Wild C (2019) Denitrification aligns with N₂ fixation in Red Sea corals. *Sci Rep* 9:19460

- Toller WW, Rowan R, Knowlton N (2002) Genetic evidence for a protozoan (phylum Apicomplexa) associated with corals of the *Montastraea annularis* species complex. *Coral Reefs* 21:143–146
- Tout J, Jeffries TC, Petrou K, Tyson GW, Webster NS, Garren M, Stocker R, Ralph PJ, Seymour JR (2015) Chemotaxis by natural populations of coral reef bacteria. *ISME J* 9:1764–1777
- Tremblay P, Grover R, Maguer JF, Legendre L, Ferrier-Pages C (2012) Autotrophic carbon budget in coral tissue: a new ¹³C-based model of photosynthate translocation. *J Exp Biol* 215:1384–1393
- Underhill DM, Ozinsky A (2002) Phagocytosis of microbes: complexity in action. *Annu Rev Immunol* 20:825–852
- Upton SJ, Peters EC (1986) A new and unusual species of *Coccidium* (Apicomplexa: Agamococcidiorida) from Caribbean scleractinian corals. *J Invertebr Pathol* 47:184–193
- Verbruggen H, Marcelino VR, Guiry MD, Cremen MCM, Jackson CJ (2017) Phylogenetic position of the coral symbiont *Ostreobium* (Ulvophyceae) inferred from chloroplast genome data. *J Phycol* 53:790–803
- Vermeij MJA, Smith JE, Smith CM, Vega Thurber R, Sandin SA (2009) Survival and settlement success of coral planulae: Independent and synergistic effects of macroalgae and microbes. *Oecologia* 159:325–336
- Voolstra CR, Li Y, Liew YJ, Baumgarten S, Zoccola D, Flot JF, Tambutté S, Allemand D, Aranda M (2017) Comparative analysis of the genomes of *Stylophora pistillata* and *Acropora digitifera* provides evidence for extensive differences between species of corals. *Sci Rep* 7:1–14
- Wada N, Ishimochi M, Matsui T, Pollock FJ, Tang S-L, Ainsworth TD, Willis BL, Mano N, Bourne DG (2019) Characterization of coral-associated microbial aggregates (CAMAs) within tissues of the coral *Acropora hyacinthus*. *Sci Rep* 9:14662
- Wada N, Pollock FJ, Willis BL, Ainsworth TD, Mano N, Bourne DG (2016) *In situ* visualization of bacterial populations in coral tissues: pitfalls and solutions. *PeerJ* 4:e2424
- Wafar MM, Wafar S, David JJ (1990) Nitrification in reef corals. *Limnol Oceanogr* 35:725–730
- Wangpraseurt D, Pernice M, Guagliardo P, Kilburn MR, Clode PL, Polerecky L, Kühl M (2016) Light microenvironment and single-cell gradients of carbon fixation in tissues of symbiont-bearing corals. *ISME J* 10:788–792
- Webster NS, Smith LD, Heyward AJ, Watts EM, Webb RI, Blackall LL, Negri P, Watts JEM, Negri AP (2004) Metamorphosis of a scleractinian coral in response to microbial biofilms. *Appl Environ Microbiol* 70:1213–1221
- Webster NS, Soo R, Cobb R, Negri AP (2011) Elevated seawater temperature causes a microbial shift on crustose coralline algae with implications for the recruitment of coral larvae. *ISME J* 5:759–770
- Wegley L, Edwards R, Rodriguez-Brito B, Liu H, Rohwer F (2007) Metagenomic analysis of the microbial community associated with the coral *Porites astreoides*. *Environ Microbiol* 9:2707–2719
- Wegley L, Yu Y, Breitbart M, Casas V, Kline DI, Rohwer F (2004) Coral-associated archaea. 273:89–96
- Weis VM (2008) Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *J Exp Biol* 211:3059–3066
- Wiedenmann J, D'Angelo C, Smith EG, Hunt AN, Legiret F-E, Postle AD, Achterberg EP (2012)

- Nutrient enrichment can increase the susceptibility of reef corals to bleaching. *Nat Clim Chang* 3:160–164
- Wild C, Huettel M, Klueber A, Kremb SG, Rasheed MYM, Jørgensen BB (2004) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. *Nature* 428:66–70
- Wilkinson C (2008) Status of coral reefs of the world: Global Coral Reef Monitoring Network and Reef and Rainforest Research Centre, Townsville, Australia. 296 pp.
- Wolfowicz I, Baumgarten S, Voss PA, Hambleton EA, Voolstra CR, Hatta M, Guse A (2016) *Aiptasia* sp. larvae as a model to reveal mechanisms of symbiont selection in cnidarians. *Sci Rep* 6:1–12
- Wood-Charlson EM, Hollingsworth LL, Krupp DA, Weis VM (2006) Lectin/glycan interactions play a role in recognition in a coral/dinoflagellate symbiosis. *Cell Microbiol* 8:1985–1993
- Yamazaki SS, Nakamura T, Yuen YS, Yamasaki H (2008) Reef-building coral *Goniastrea aspera* harbor a novel filamentous cyanobacterium in their skeleton. *Proceedings of the 11th International Coral Reef Symposium* 1:265–268
- Yang SH, Lee STM, Huang CR, Tseng CH, Chiang PW, Chen CP, Chen HJ, Tang SL (2016) Prevalence of potential nitrogen-fixing, green sulfur bacteria in the skeleton of reef-building coral *Isopora palifera*. *Limnol Oceanogr* 61:1078–1086
- Ziegler M, Grupstra CGB, Barreto MM, Eaton M, BaOmar J, Zubier K, Al-Sofyani A, Turki AJ, Ormond R, Voolstra CR (2019) Coral bacterial community structure responds to environmental change in a host-specific manner. *Nat Comm* 10:1-11
- Ziegler M, Roik A, Porter A, Zubier K, Mudarris MS, Ormond R, Voolstra CR (2016) Coral microbial community dynamics in response to anthropogenic impacts near a major city in the central Red Sea. *Mar Pollut Bull* 105:629–640
- Ziegler M, Seneca FO, Yum LK, Palumbi SR, Voolstra CR (2017) Bacterial community dynamics are linked to patterns of coral heat tolerance. *Nat Comm* 8:14213
- Zimmer BL, May AL, Bhedi CD, Dearth SP, Prevatte CW, Pratte Z, Campagna SR, Richardson LL (2014) Quorum sensing signal production and microbial interactions in a polymicrobial disease of corals and the coral surface mucopolysaccharide layer. *PLoS One* 9: e108541