

Microbial indicators as a diagnostic tool for assessing water quality and climate stress in coral reef ecosystems

Bettina Glasl^{1,2,3} · Nicole S. Webster^{1,3,4} · David G. Bourne^{1,2,3}

Received: 13 October 2016 / Accepted: 3 February 2017 / Published online: 23 March 2017
© Springer-Verlag Berlin Heidelberg 2017

Abstract Microorganisms play a fundamental role in the functioning and stability of coral reef ecosystems. However, environmental disturbances can trigger alterations to the natural microbial community composition and their functional traits with potentially detrimental consequences for host organisms, such as corals, sponges and algae and concomitant implications for the entire coral reef ecosystem. Coral reefs are increasingly affected by localized impacts such as declining water quality and global pressures derived from human-induced climate change, which severely alters the natural conditions on reefs and can push dominating benthic life forms towards the limit of their resistance and resilience. Microorganisms can respond very rapidly to these altered environmental conditions so defining their natural variability over spatial and temporal gradients is critical for early and accurate identification of environmental disturbances. The rapid response of microbes to environmental change is likely to confer significant advantages over traditional reef monitoring methods, which are based on visual signs of health deterioration in benthic coral reef macroorganisms. This review discusses the

potential of microbes as early warning indicators for environmental stress and coral reef health and proposes priorities for future research.

Introduction

Coral reefs have flourished in tropical shallow water regions for over 240 million years (Stanley and Fautin 2001). However, during the last 30 years coral reefs have faced severe threats due to anthropogenic climate change, crown of thorns starfish (COTS), disease, overfishing and pollution (Hoegh-Guldberg 2011; De'ath et al. 2012). For instance, the emission of carbon dioxide (CO₂) leads to a decrease in the ocean's pH and adversely affects calcium carbonate deposition by calcifying organisms such as environmentally susceptible coral species (Hoegh-Guldberg et al. 2007; Putnam et al. 2016). Increased ocean surface temperature as a consequence of global warming, not only result in more frequent and severe tropical storm events (reviewed by Knutson et al. 2010) but also pushes the coral-*Symbiodinium* symbiosis towards its thermal tolerance limit (Hoegh-Guldberg 1999). Once the temperature exceeds the resistance threshold of the symbiosis, the interaction between corals and their photoautotrophic *Symbiodinium* breaks down and corals bleach (Brown 1997). Mass coral bleaching events have occurred more frequently over the last decades (Donner et al. 2005; Hoegh-Guldberg et al. 2007; Baker et al. 2008). The most recent global bleaching episode in 2015–2016 was the longest bleaching event recorded in history (US National Oceanic and Atmospheric Administration) and has led to severe bleaching worldwide. In the northern sector of the Great Barrier Reef (GBR) in Australia, this bleaching event caused the mortality of up 35% of all corals (GBRMPA 2016; Normile 2016; Hughes

Responsible Editor: D. Gochfeld.

Reviewed by Undisclosed experts.

✉ Bettina Glasl
b.glasl@aims.gov.au

¹ Australian Institute of Marine Science, Townsville, Australia

² College of Science and Engineering, James Cook University, Townsville, Australia

³ AIMS@JCU, Townsville, Australia

⁴ Australian Centre for Ecogenomics, University of Queensland, Brisbane, Australia

et al. 2017). In addition to the hazards emerging from global change, localized reef-scale anthropogenic impact can further influence the ecological resilience of corals and other reef organisms to global stressors (Knowlton and Jackson 2008). For example, overfishing has led to an alteration of the trophic food web (Valentine and Heck 2005). With the removal of herbivorous fish from coral reefs, macroalgae and turf algae are less efficiently grazed. This can result in a phase shift from coral to macroalgae dominated reefs (Burkepile and Hay 2006; Vermeij et al. 2010). Changes in land-based practices can also impact reef ecosystems with land-clearing, coastal urbanization and intense agricultural practices contributing to decreased water quality on reefs, which in turn further contributes to declining reef health (De'ath and Fabricius 2010).

Monitoring programs, such as 'Reef Check' and 'The Global Coral Monitoring Network' use visual surveys of reef associated fish, invertebrates, substrate composition, reef damage and disease impacts to assess reef health (Hodgson 2001; Hill and Wilkinson 2004). Most visual cues (e.g. tissue necrosis, mortality and shifts in community composition) only become evident in the advanced stages of coral reef ecosystem stress, when ecosystem health and resistance are already compromised. At this stage the ability to recover is dependent on the resilience of the ecosystem and the success of management interventions is jeopardized. Hence, the development of a reliable

early warning system that facilitates management intervention before severe damage occurs is clearly warranted.

Microorganisms are key drivers of large-scale biogeochemical cycles in the oceans (Azam et al. 1983; Whitman et al. 1998; Falkowski et al. 2008) and also play a fundamental role in coral reef ecosystem functioning, through mediating nutrient cycling (Charpy et al. 2012; Tout et al. 2014). Furthermore, microbes live in intimate relationships with benthos-dominating life forms such as corals, sponges and macroalgae, where they have a vital role in host fitness through additional nutrient provision, removal of waste products and the exclusion of opportunistic microbial pathogens (Egan et al. 2013; Blackall et al. 2015; Bourne et al. 2016; Webster and Thomas 2016). However, disturbance events can alter the natural microbial community structure, abundance and metabolic functions either directly or indirectly (Shade et al. 2012). Disturbance-related deviations from the naturally occurring microbial communities may provide useful indications for coral reef ecosystem stability and facilitate sensitive predictions of environmental stress (Fig. 1).

This review aims to assess the utility of microorganisms as a diagnostic tool for assessing water quality and climate-driven stress in the coral reef ecosystem. Insights are provided into (1) the implementation of biological indicator approaches; (2) the potential role of microbial indicators in coral reef monitoring programs; (3) the function

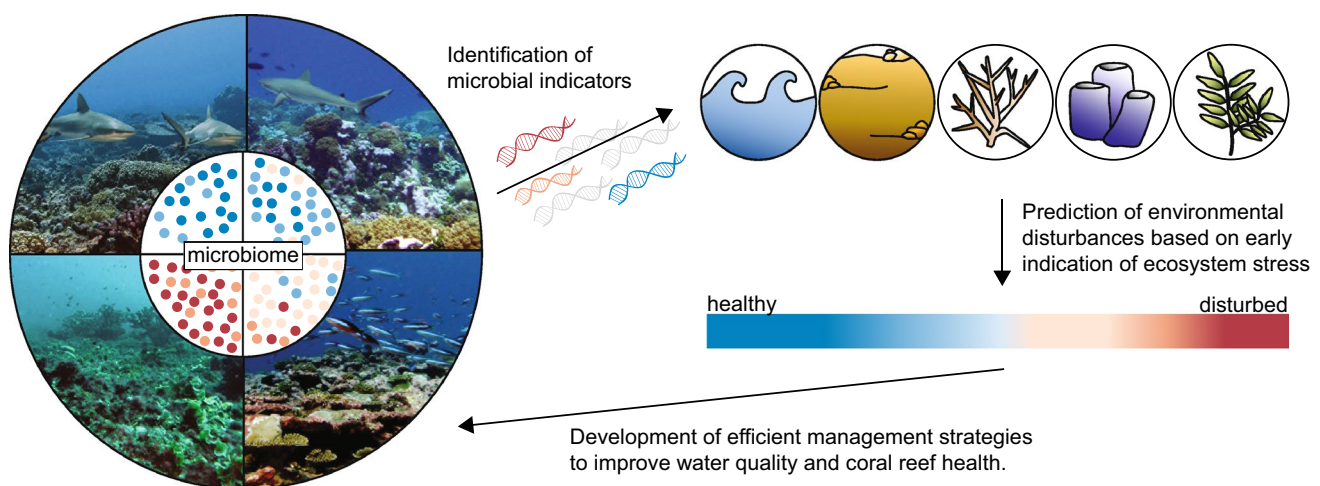


Fig. 1 Microbial indicator approach to assess coral reef health. Coral reefs are exposed to increased environmental pressures emerging from local (e.g. pollution, overfishing) and global impacts (e.g. rising sea surface temperature). This facilitates a shift from coral to algae dominated reef systems [adapted from Sandin et al. (2008)]. Accompanied by the increase in environmental pressures, the microbial community composition and function associated with coral reefs change along the gradient of disturbance from a beneficial and commensal microbiome towards microbial communities dominated by opportunists and pathogens. Overall compositional and functional changes of

microbial communities associated with seawater, sediment and habitat forming taxa (corals, sponges and macroalgae) but also the occurrence or loss of specific microbial taxa/function can provide useful indications for the prevailing environmental condition. The application of microbial indicator taxa, function and/or community assemblages will allow for a rapid prediction of environmental disturbance and the health state of a coral reef. This enhanced predictive capability is paramount to efficiently monitor coral reef health and locally manage environmental pressures such as water quality

and diversity of microbes associated with dominating benthic life forms (corals, sponges and macroalgae) and their surrounding environments (seawater and sediment) and (4) how state-of-the-art methods used to study microbial community composition and function can be implemented into diagnostic tools to assess coral reef ecosystem health.

Biological indicators: definition, function and application

Biological indicators, also referred to as indicator species, are organisms that can be used to monitor habitat conditions and environmental changes based on their niche preferences (McGeoch and Chown 1998; De Cáceres et al. 2010). The presence/absence or abundance values of selected organisms or communities at specific sampling sites are monitored, allowing conclusions to be drawn on the ecological integrity of a certain ecosystem (Carignan and Villard 2002). Depending on the response time, bioindicators can either be used to detect severe short-term stress events or to better understand the long-term effect of chronic stress exposure (Cairns and Pratt 1993). The use of taxa with moderate tolerance towards environmental variability is favored against rare or ubiquitous taxa, which respectively show high sensitivity or high tolerance towards environmental perturbations (Holt and Miller 2011). Cooper et al. (2009) identified the following five selection criteria, to be considered when choosing bioindicators: specificity, monotonicity, natural variability, practicality and ecological relevance (see Table 1).

Kolkwitz and Marsson (1908) developed one of the first indicator approaches to evaluate the degree of pollution in rivers based on the occurrence of saprobic micro- (e.g. ciliates and flagellates) and macroorganisms (e.g. insect larvae, molluscs, bivalves, annelids and crustaceans). Since then, various biomonitoring programs have been developed and successfully applied in estuarine and freshwater ecosystems by assessing indicator species within the macrobenthic invertebrate community e.g. AUSRIVAS (Australian River Assessment Scheme; Smith et al. 1999), RIVPACS (River Invertebrate Prediction and Classification System; Wright 1995), SIGNAL (Stream Invertebrate Grade Number Average Level; Chessman 1995) and WFD (European Union Water Framework Directive; European Parliament 2000). Biomonitoring approaches also find application in coral reef health monitoring (reviewed by Cooper et al. 2009). For example, the FORAM index (Hallock et al. 2003) provides insights into the water quality of coral reefs by quantifying size classes of Foraminifera in the upper sediment layer.

Although indicator approaches are frequently used in conservation biology, land management, landscape

mapping and in the design of protected areas, standardized approaches and statistical methods to identify suitable indicators are still scarce. Dufrière and Legendre (1997) developed the Indicator Value Analysis (IndVal) as a statistically valid method for determining indicator species and species assemblages. IndVal considers the specificity, which is the mean number of individuals of a species across sampling sites of a certain habitat in comparison with other habitats, and the fidelity, described as the relative frequency of occurrence of a species in the sampling sites of a specific habitat. The IndVal analysis represents an efficient method to identify indicator organisms and can provide critical information on the ecological integrity of an ecosystem (McGeoch and Chown 1998; Gardner 2010). The IndVal approach has found application in various terrestrial and aquatic studies to assess habitat quality based on both macro- (McGeoch et al. 2002; Muotka et al. 2002; Bazelet and Samways 2011) and microorganisms (Auguet et al. 2010; Fortunato et al. 2013; Glasl et al. 2016; Ziegler et al. 2017).

The integration of biological indicators into ecosystem monitoring programs can provide significant advantages. For example, by focusing on a targeted group of species, monitoring can become more cost and time efficient. Furthermore, biological indicators can provide early warning of environmental stress and might directly reveal the cause rather than simply the existence of a disturbance event. However, the choice of appropriate species or species assemblages is crucial for the effectiveness of bioindicators and hence, the selection should be based on sound quantitative approaches including consideration of the natural in situ variability (Carignan and Villard 2002).

Microorganisms as bioindicators to assess ecosystem health

The potential value of microorganisms for efficiently monitoring ecosystem health remains largely unexplored, despite the fact that microbes show fast, specific responses to environmental perturbations (Teeling et al. 2012; Wemheuer et al. 2015; Haas et al. 2016) which are the desirable characteristics of bioindicators (Table 1). One area where microorganisms have been exploited as indicators is in monitoring contamination of water supplies by coliform counts associated with feces from warm-blooded animals (Ashbolt et al. 2001). Coliform counts are now used to monitor and manage drinking water supplies and recreational water qualities worldwide (Boehm and Sassoubre 2014), successfully enabling the prevention of numerous human gastrointestinal illnesses (Wade et al. 2010). In coral reef environments, coral mucus has been shown to be more efficient in trapping fecal indicator bacteria

Table 1 Summary of criteria for the selection of microbial indicators to monitor water quality changes and predict stressors in coral reef ecosystems

Criteria	Definition	Examples	Source
Specificity	Compositional and/or functional shifts reflect specific responses to environmental stressor	Coral microbiome shifts towards an opportunistic bacterial community and shows an increase in the abundance of virulence genes in response to bleaching environmental stressors and anthropogenic impact Heavy metal pollution, increased temperature and elevated pCO ₂ can lead to changes in the composition and function of the sponge microbiome	(Bourne et al. 2008; Dinsdale et al. 2008; Vega Thurber et al. 2009; Littman et al. 2011; Kelly et al. 2014) (Webster et al. 2001; Webster et al. 2008; Selvin et al. 2009; Fan et al. 2013; Morrow et al. 2015)
Monotonicity	The magnitude of microbiome disruption reflects the intensity and duration of the stressor	The resistance and resilience of the microbial community composition and functions varies between short and long-term disturbances	(reviewed by Shade et al. 2012)
Variability	Natural variability and dynamics of the microbiome forms the baseline of a healthy microbial community. Deviations to the baseline can be used as indicators for changes in the ecosystem	Planktonic community composition and function varies between different coral reef niches and along a gradient of water quality The coral microbiome is described as host species specific, however, short and long-term temporal variation have been observed Sponge-associated microbes form a stable symbiotic relation with the host over time and seasons	(Tout et al. 2014; Angly et al. 2016) (Rohwer et al. 2002; Li et al. 2014; Glasl et al. 2016) (Erwin et al. 2012; Thomas et al. 2016)
Practicality	Prokaryotic indicator analysis should be cost and time efficient, easy to measure, non-destructive and observer independent	Technological advances allow cost and time efficient analysis of prokaryotic communities in situ (e.g. next generation sequencing techniques, meta-omic approaches)	(Schuster 2008)
Relevance	Prokaryotic shifts should be ecologically relevant and enable prediction of environmental stress and hence, ecosystem health	Prokaryotes have fundamental roles in coral reef functioning but also in coral and sponge holobiont health	(Bourne and Webster 2013)

Criteria were taken from Cooper et al. (2009) and definitions were adapted to fit the microbial indicator approach. Selected examples of studies examining microbial community responses from marine systems are provided to support how this indicator approach can satisfy the identified criteria

and human enteric viruses than the surrounding seawater; hence, coral mucus provides an improved medium to monitor sewage contamination (Lipp and Griffin 2004). Furthermore, seagrasses have recently been shown to reduce the abundance of microbial pathogens entering nearby coral reef and mitigate the disease risk in associated coral populations (Lamb et al. 2017).

Intensive research on the structural and functional capacity of the human microbiome has revealed its enormous contribution to pathogenesis and immune system modulation of the host, as well as its influence on host development and physiology (Arrieta and Finlay 2012; Eloe-Fadrosh and Rasko 2013; Kostic et al. 2015; Ghaisas et al. 2016). This knowledge has revolutionized our current understanding of host–microbe interactions and has led to the development of diagnostic and therapeutic approaches targeting the human microbiome (Reardon 2014). For example, transplantation of faecal microbiomes has successfully cured humans affected by recurrent *Clostridium difficile* infections and microbiome transplantation has been suggested as a therapeutic cure for inflammatory bowel disease and obesity (Gupta et al. 2016). In the same way, it is feasible that microbiome manipulation and probiotic treatment could be used to increase the health and tolerance of other reef based host-associated systems (Reshef et al. 2006; van Oppen et al. 2014; Webster 2017). Also, similar to the approach in humans, the identification of imbalances in the microbial communities (dysbiosis) at the ecosystem level can facilitate diagnostic interpretations of environmental health. For example, a bacterial community based index has recently been developed to assess the ecological status of estuarine and coastal environments (Aylagas et al. 2016). Another recent example applied to coral reef ecosystems has been the development of microbialization scores, which attempt to assess human impacts on coral reefs based on the metabolic rates of microbial communities and reef-associated fishes (McDole et al. 2012). While microbialization has been shown to occur on a global scale (Haas et al. 2016), additional research is needed to adopt the approach to individual reef ecosystems. Microbial monitoring has also recently been introduced to the monthly sampling program of the National Mooring Network of IMOS (Integrated Marine Observing System), which targets oceanographic phenomena in Australian coastal waters (IMOS 2016). Increased appreciation of microorganisms in host and/or ecosystem health, together with recent advances in molecular techniques, now allow for detailed in situ investigations of the microbial community structure and functions and for standardized and efficient data analysis.

Changes in microbial communities due to disturbance may directly affect ecosystem processes. Therefore, it has been suggested to include microbial community composition (e.g. 16S ribosomal RNA gene sequencing) into

process models that predict ecosystem responses to global change (Allison and Martiny 2008). Microbial functional redundancy is also of particular interest since metabolic capabilities can be decoupled from the phylogenetic position of microorganisms due to convergent evolution, gene loss or horizontal gene transfer (Ochman et al. 2000; Martiny et al. 2013). Phylogenetic diversity is thought to positively affect the stability of an ecosystem, as it increases the probability that complementary functional traits are present (Yachi and Loreau 1999). However, ecosystem processes can remain constant after disturbances even when a compositional shift has been observed (Wohl et al. 2004; Allison and Martiny 2008; Banerjee et al. 2016). Hence, functional approaches (e.g. metagenomics, metatranscriptomics) are also required for monitoring and predicting ecosystem changes. Functional and compositional changes can be assessed based on the entire community or on selected microbial indicator taxa.

Microbial life in the coral reef ecosystem

Within coral reefs, microorganisms colonize various habitats including the water column, the sediment and the benthic community, such as corals, sponges and macroalgae (Moriarty et al. 1985; Friedrich et al. 1999; Rohwer et al. 2001; Hewson and Fuhrman 2006; Barott et al. 2011; Bourne and Webster 2013). The enormous complexity of coral reefs and their associated microbial communities has resulted in studies focused on these specific compartments; however, these habitats should not be considered as isolated from each other but rather seen as parts of a single ecosystem with a strong benthic–pelagic exchange (Lesser 2006; Garren and Azam 2012). Holistic approaches that combine the different reef habitats are urgently required to better understand the function and contribution of microorganisms to reef health and resilience.

Microbial diversity and function in coral reef waters

Microorganisms are moderately abundant (average densities of between 3 and 9×10^5 cells ml^{-1}) in nutrient-poor coral reef waters, where they play diverse roles related to nutrient cycling that ultimately affect the entire reef ecosystem (Sorokin 1973, 1978; Ducklow 1990; Gast et al. 1998). For instance, photoautotrophic picophytoplankton significantly contributes to the biomass and primary productivity of oligotrophic reef waters (Stockner 1988). Additionally, as part of the marine microbial-loop, heterotrophic bacteria utilize dissolved organic matter (DOM) in the water column, establishing an important recycling step that makes energy available to higher trophic levels (Azam et al. 1983). In marine environments DOM is primarily of

phytoplankton origin, as 2–50% of the photoautotrophically fixed carbon leaks into the water column (reviewed by Thornton 2014). Coral mucus, fueled by the photosynthetic activity of the coral's algal symbionts, additionally contributes to the DOM pool of coral reef waters (Wild et al. 2004a). The fixation of inorganic nitrogen is another key microbial function in coral reef environments, where nutrients are scarce and thus limit growth (Charpy et al. 2012).

Processes shaping the microbial communities associated with coral reef waters vary over space and time. Distinct microbial communities along different niches within coral reef waters are the result of habitat structure, the presence of benthic host organisms and local biogeochemical conditions (Tout et al. 2014). Seasonal effects, such as temperature, rainfall and water quality affect microbial community composition in shallow water reef sites (Angly et al. 2016). Anthropogenic impact (e.g. land-use and fishing) also affects coral reef microorganisms with disturbed reefs possessing higher microbial abundances and a larger proportion of microbial taxa related to potential pathogens (Dinsdale et al. 2008). The replacement of macro- with microorganisms under increased human influence has been referred to as microbialization (Jackson et al. 2001). Based upon the microbialization concept, McDole et al. (2012) developed the 'microbialization score', a metric to assess the level of human impact and compare the health of coral reefs across time and space.

Microbial diversity and function in coral reef sediments

Coral reef sediments are typically dominated by calcareous sand, characterized by high permeability, porosity and surface area (Rasheed et al. 2003). This enables large numbers of microorganisms to settle and grow on the sediment grains with prokaryotic abundance estimated at between 1 and 2×10^9 cells cm^{-2} of sediment surface (Wild et al. 2006). In addition, the microbial communities associated with reef sediments are highly diverse, with vertical community stratification caused by redox gradients (Rusch et al. 2009). The oxygenated upper sediment layer is dominated by heterotrophic processes and provides an important recycling step for dissolved and particulate organic matter (Wild et al. 2004a). Wild et al. (2004b) demonstrated that sloughed coral mucus acts as an efficient particle trap in the water column and rapidly carries nutrients to reef sediments, where diverse microbial metabolisms successfully remineralize them. Fixation of inorganic nitrogen is also mediated by bacteria and archaea present within coral reef sediments and can substantially contribute to the overall coral reef nitrogen budget (Cardini et al. 2014).

Importantly, Uthicke and McGuire (2007) identified clear bacterial community differences in surface sediments collected from inshore and offshore locations on the GBR

and proposed they be used as biological indicators for water quality. Furthermore, a study conducted in the Red Sea has shown a nutritional link between seasonal dynamics and sediment-associated bacterial communities (Schöttner et al. 2011). In addition to their metabolic functions, sediment associated microbes may also act as a seed-bank for microbes associated with coral mucus (Carlos et al. 2013; Glasl et al. 2016), another example of the tight microbial connections established within the coral reef ecosystem.

Corals and their microbiomes

Coral holobionts (Rohwer et al. 2002) comprise an array of microorganisms including fungi, endolithic algae, bacteria, archaea and viruses (Ritchie and Smith 1997; Sharshar et al. 1997; Rohwer et al. 2002; Koren and Rosenberg 2006; Thurber et al. 2008). The coral host itself provides several microhabitats for its microbial associates, such as the surface mucus layer (Rohwer et al. 2002; Frade et al. 2016), the tissue (Bourne and Munn 2005), the skeleton (Sharshar et al. 1997) and the gastrovascular cavity (Herndl and Velimirov 1985). Each of the microhabitats within a coral colony is associated with a distinct microbial community (Rohwer et al. 2002; Sweet et al. 2011; Bourne et al. 2016).

Bacterial communities associated with corals are clearly distinct from the bacterial communities in the surrounding environment (Frias-Lopez et al. 2002) and bacterial cell abundance within coral mucus is also 10-fold higher than in seawater (Garren and Azam 2010). Each coral species serves as a unique habitat and is associated with a specific microbial community (Sunagawa et al. 2010), with some species maintaining stable microbiomes over large geographic scales (Rohwer et al. 2002), suggesting that the coral host plays a key role in structuring its bacterial community.

To deal with the enormous diversity of microorganisms associated with corals, and separate beneficial and opportunistic bacteria and archaea from the bulk microbial community, understanding coral holobiont stability and functionality is paramount. For instance, the mucus microbiome of healthy *Porites astreoides* colonies is dominated by *Endozoicomonadaceae*, whose loss is coupled to deterioration in holobiont health (Meyer et al. 2014; Glasl et al. 2016). *Endozoicomonas* also dominate the microbiome of *Stylophora pistillata* and *Pocillopora verrucosa* tissues; *Endozoicomonas* genotypes vary over geographic space in *S. pistillata*, whereas in *P. verrucosa* the genotype of these endosymbionts remains the same. The genotype specificity may relate to differences in the life history strategies of corals (Neave et al. 2017). Based on meta-analysis of the core microbiomes of diverse coral species, members of *Actinobacteria* were recently identified as ubiquitous symbionts

of corals (Ainsworth et al. 2015). A decrease of *Actinobacteria* in coral microbiomes during periods of temperature and algal stress further suggests their beneficial role in the coral holobiont (Zaneveld et al. 2016).

Potential functions of the coral's microbiome vary from nutrient supply and recycling (Lesser et al. 2004, 2007) to protection against pathogens (Rohwer et al. 2002; Ritchie 2006; Shnit-Orland and Kushmaro 2009; Raina et al. 2016). For example, the depletion of the coral's beneficial mucus microbiome affected holobiont health under in situ conditions (Glasl et al. 2016). This suggests that major disturbances of the surface mucus microbiome may open a niche for potentially opportunistic and/or pathogenic bacteria, which can further lead to diseases or host mortality. Recent investigation of the functional capabilities of the bacterial and archaeal community associated with corals revealed carbon fixation and degradation pathways and the presence of genes involved in sulfur and nitrogen cycling (Wegley et al. 2007; Siboni et al. 2008; Kimes et al. 2010; Yang et al. 2013; Rädercker et al. 2015). Nitrogen fixation capabilities are of particular interest as coral reefs are nitrogen-limited ecosystems (Falkowski et al. 1993). Until recently, Cyanobacteria were thought to be the primary suppliers of fixed nitrogen in the coral holobiont (Lesser et al. 2004, 2007). The discovery of the ubiquitous association with highly host specific diazotrophs (nitrogen fixing microorganisms) suggests their important functional role in the nitrogen supplementation of the coral holobiont (Siboni et al. 2008; Lema et al. 2012, 2014), especially when external nitrogen sources are limited (Cardini et al. 2015). Nitrifying, denitrifying and ammonia oxidizing microbes have also been described to be associated with corals; however, their precise functions within the holobiont remains understudied.

The microbiome of corals has been described as persistent over space and time (Rohwer et al. 2002). However, compositional and functional shifts have been observed in association with seasonal environmental variation (Li et al. 2014), host mucus-shedding dynamics (Glasl et al. 2016), thermal stress (for example coral bleaching; Bourne et al. 2008), disease (Séré et al. 2013; Meyer et al. 2014), coral's proximity to macroalgae (Barott et al. 2012; Sweet et al. 2013) and increasing environmental pollution (Garren et al. 2009; Kelly et al. 2014; Ziegler et al. 2016). For example, thermal stress leads to destabilization of coral-algae interactions (Brown 1997) and a shift towards an opportunistic and/or pathogenic microbial community (Ainsworth et al. 2008; Bourne et al. 2008; Littman et al. 2011). Water quality changes associated with increased sediment and nutrient run-off can increase microbial abundance (Dinsdale et al. 2008; D'Angelo and Wiedenmann 2014), coral disease frequency (Bruno et al. 2003; Thurber et al. 2014) and higher macroalgal abundance on coral reefs (Kline

et al. 2006). Macroalgae exudates stimulate bacterial activity and copiotrophic bacterial growth, and cause shifts in the coral microbiome attributed to increased labile DOC and toxic secondary metabolites (Morrow et al. 2011; Barott et al. 2012; Vega Thurber et al. 2012; Nelson et al. 2013; Sweet et al. 2013; Haas et al. 2016). Environmentally induced changes in the coral microbiome generally result in higher microbial abundance and a shift away from beneficial microbes towards opportunistic and/or pathogenic bacterial taxa, such as *Vibrionaceae* and *Rhodobacteraceae* (Vega Thurber et al. 2009; Bourne et al. 2016; Rothig et al. 2016; Ziegler et al. 2016). Increased nutrient run-off in combination with reduced grazers (hence higher algal abundance) destabilizes the coral microbiome with detrimental consequences for the host, particularly when exposed to additional stressors such as parrotfish bites and thermal stress (Zaneveld et al. 2016). The resulting imbalance in the holobiont composition (dysbiosis) can lead to functional changes of the microbiome and facilitate disease development or alterations in metabolism and/or immunity that lead to bleaching and/or necrosis, and ultimately coral death (reviewed by Thompson et al. 2015).

Sponges and their microbiomes

Marine sponges (phylum: Porifera) are a highly diverse component of coral reefs, usually exceeding the diversity of corals and algae (Diaz and Rützler 2001). Sponges have the ability to filter up to 50,000 times their own volume every day (Reiswig 1971a) and due to this active suspension feeding they play a key role in benthic-pelagic coupling, thus providing a vital trophic link between the benthos and the ambient seawater (Gili and Coma 1998; Southwell et al. 2008). Sponges are primarily considered to feed on picoplankton (0.2–2 µm) (Reiswig 1971b; Pile et al. 1997; Hanson et al. 2009); however, several recent studies have shown that certain sponges are also able to assimilate dissolved organic matter (DOM) from the water column (De Goeij et al. 2008; Mueller et al. 2014; Rix et al. 2016). Cryptic Caribbean sponges, for instance, transform DOM into particulate organic matter (POM) and thereby significantly contribute to the recirculation of nutrients in coral reefs, a phenomenon known as the sponge-loop (De Goeij et al. 2013), which is likely mediated by microbes living within the sponge.

Sponges generally live in close association with a wide variety of microorganisms including bacteria, archaea, unicellular algae and protists. These microorganisms are often present in high abundance, accounting for up to 40–60% of the total sponge volume and reaching cell abundances that exceed those in the surrounding seawater by several orders of magnitude (Hentschel et al. 2006). Sponges with high microbial densities are referred to as 'high microbial

abundance sponges' (Vacelet and Donadey 1977; Hentschel et al. 2003), in contrast to marine sponges that harbor only a relatively small number of microorganisms and are referred to as 'low-microbial abundance sponges' (Hentschel et al. 2003). The implications of these diversity differences for host resistance and resilience remain elusive. In addition to the importance of microorganisms as a sponge food source (Reiswig 1971b; Pile et al. 1996), microbial associates have also been reported to participate in a diverse range of interactions, including parasitism, commensalism and mutualism (reviewed by Taylor et al. 2007) with significant implications for the ecology, biology and physiology of sponges (Thacker and Freeman 2012; Bourne and Webster 2013).

Microbes are generally located in the sponge mesohyl region, a layer of connective tissue where microbial cells are either freely occurring or enclosed within specialized bacteriocyte cells (reviewed by Taylor et al. 2007). The mesohyl is also the place where phagocytosis of food particles (e.g. picoplankton) takes place; hence, host-symbiont recognition mechanisms must be in action to prevent the phagocytosis of symbiont cells (Wilkinson et al. 1984; Wehrl et al. 2007; Nguyen et al. 2014). Furthermore, archaea and Cyanobacteria have been found in the pinacoderm, the outer surface of sponges formed by epithelial cells (Wilkinson 1980; Webster et al. 2001). Spongesymbionts generally show high host species-specificity and only a few bacterial species seem to be ubiquitously present in sponges. Bacterial species associated with different sponges are more closely related to each other than to bacteria from the ambient water column and, hence, these monophyletic groupings of sponge symbionts are often referred to as 'sponge-specific sequence clusters' (SCs) (Hentschel et al. 2002; Schmitt et al. 2012). Whereas about half of the SCs have been shown to be vertically transmitted from adult sponges directly to their offspring, the other half is thought to be horizontally acquired by each generation from the surrounding seawater (Webster et al. 2010). Interestingly, the candidate phyla Poribacteria, which is highly abundant in various marine sponges over a wide geographic range (Fieseler et al. 2004), was recently described as part of the rare seawater biosphere (Webster et al. 2010). Webster et al. (2010) proposed that members of the rare seawater biosphere might act as seed organisms for widely occurring symbiont populations.

Sponge symbionts are capable of diverse metabolic functions. They play a crucial role in carbon, nitrogen, sulfur and phosphorous cycling and are also fundamental for the synthesis of essential vitamins within the sponge holobiont (reviewed by Webster and Thomas 2016). Examples of the mutualistic nature of sponge symbiont associations include the nitrifying *Thaumarchaeota* and bacteria, which gain energy through the oxidation of ammonia, a sponge waste product (Webster et al. 2001;

Bayer et al. 2008; Mohamed et al. 2010). Besides nutrient cycling capabilities within the host, symbionts are also shown to significantly contribute to host defense via the production of secondary metabolites (Hentschel et al. 2001; Kennedy et al. 2007).

Research on the resistance and resilience of the sponge microbiome to environmental stressors including local (e.g. sedimentation and nutrients) and global (e.g. elevated seawater temperature and ocean acidification) pressures has shown that responses are highly species-specific. For instance, a number of studies have revealed no compositional changes in the sponge-associated microbiome upon short-term nitrogen, temperature or sediment pulses, suggesting a highly resistant association between the sponge holobiont members (Luter et al. 2012, 2014; Simister et al. 2012). Similar observations were obtained during transplantation experiments of the sponge *Aplysina cavernicola*; light stressed sponges showed no changes in their bacterial community composition nor their production of secondary metabolites (Thoms et al. 2003). More recent research has shown that whilst the microbiome of different heterotrophic sponge species remains stable under light attenuation, the microbiome of phototrophic species can be significantly affected by light availability (Pineda et al. 2016). The symbiotic community associated with *Rhopaloeides odorabile*, a common Great Barrier Reef sponge, undergoes major changes in community structure, accompanied by host tissue necrosis, upon exposure to temperatures greater than 32 °C (Webster et al. 2008). A more recent metagenomic and metaproteomic analysis revealed that *R. odorabile* symbionts lose their metabolic functional potential during the early stages of heat stress and hence destabilize the sponge holobiont before visual signs of stress occur in the host animal (Fan et al. 2013). A comparison of healthy and diseased *Ircinia fasticulata* individuals revealed a significant shift in the microbiome prior to mass mortality attributed to high seawater temperatures. The observed shift was suggested to negatively affect host fitness and resistance to environmental stress (Blanquer et al. 2016). Numerous studies of sponge disease have reported a higher bacterial diversity in diseased tissue (Webster et al. 2008; Angermeier et al. 2011, 2012; Olson et al. 2014; Blanquer et al. 2016), suggesting that a dysbiotic microbiome rather than infection by a specific pathogen also has a role in the disease process. It is also noteworthy that some instances of 'sponge disease', do not involve detectable shifts in the composition of the microbial community (Luter et al. 2010), although no studies have yet assessed whether symbiotic function is impacted during disease which may contribute to the observed declines in host health.

Macroalgae and their microbiomes

Macroalgae have always been present in healthy coral reef ecosystems in relatively low abundance (Bruno et al. 2014). However, overfishing and eutrophication can facilitate a shift from coral dominated to algal dominated reefs (Hughes et al. 2007). The increase of macroalgal abundance on coral reefs is a threat for corals, not only because of direct competition for space but also because macroalgae have been shown to facilitate coral disease outbreaks, increase mortality and prevent larval settlement (Nugues et al. 2004; Smith et al. 2006; Sweet et al. 2013; Webster et al. 2015). Furthermore, macroalgae significantly alter their ambient environment by releasing higher amounts of labile dissolved organic carbon (DOC) compared to corals. This elevated DOC supports higher microbial growth rates, leads to a depletion of bioavailable DOC in seawater and facilitates the growth of copiotrophic and potentially pathogenic microbial taxa (Haas et al. 2011, 2016; Nelson et al. 2013). These processes have been summarized in the DDAM model (DOC, disease, algae, microorganism; Barott and Rohwer 2012), which describes the feedback of macroalgae-derived labile DOC and how it fuels the less efficient metabolism of copiotrophic and pathogenic microorganisms in coral reefs thereby contributing to increased microbial respiration and the local acidification of seawater (Sweet et al. 2013; Haas et al. 2016).

Besides their influence on the seawater microbiome, macroalgae themselves are holobionts and are associated with a highly diverse microbiota (Barott et al. 2011). Bacterial densities on the algal surface vary between 10^2 and 10^7 cells cm^2 depending on the thallus section, host species and season (Armstrong et al. 2000). The epibacterial community composition significantly differs from the community in the ambient seawater and shows high host specificity and temporal adaptation (Lachnit et al. 2009, 2011; Burke et al. 2011b; Goecke et al. 2013). In addition to the biofilm community on the surface of seaweeds, macroalgae harbor a specialized and stable endophytic bacterial community (Hollants et al. 2011a, b). The specificity of epibacterial communities associated with different macroalgae is currently under reconsideration; microbial functioning, rather than phylogeny, seems to be consistent within the holobiont (Burke et al. 2011b, a).

The algal microbiome significantly contributes to host morphogenesis, health and defense. For example, morphological abnormalities were observed in various *Ulva* species when grown under aposymbiotic conditions (Provasoli and Pintner 1980; Nakanishi et al. 1996; Singh et al. 2011). Epiphytic bacteria, such as *Vibrio sp.* and *Pseudoalteromonas sp.*, provide inhibitory properties against various biofouling organisms and hence contribute to host defense against unwanted colonization (reviewed by

Egan et al. 2013). Additionally, epiphytic bacteria supply the algal host with key nutrients. Heterotrophic bacteria provide CO_2 to the photoautotrophic host, and Cyanobacteria, dominant members of the epibacterial community, provide fixed-nitrogen (Penhale and Capone 1981; Philips and Zeman 1990; de Oliveira et al. 2012). Recent genomic and metagenomic studies of seaweed-associated bacterial communities revealed a diverse genetic repertoire including genes for phosphorous, nitrogen and iron utilization (Thomas et al. 2008; Burke et al. 2011b; Fernandes et al. 2011).

In general, little is known about the factors controlling the microbial communities in the surface biofilm of macroalgae. Bacterial richness in the biofilm of the kelp *Laminaria hyperborea* is reported to increase as the kelp ages (Bengtsson et al. 2012) and microbial community succession seems to be influenced by stochastic processes (Burke et al. 2011a; Trias et al. 2012). Furthermore, disturbances such as temperature stress are shown to disrupt algal-holobiont homeostasis, which can lead to a switch in bacterial communities from surface-associated commensals to opportunistic pathogens (Case et al. 2011).

Methods to develop novel microbial indicators for coral ecosystem health assessment

High diversity, high functional complexity and low cultivability has historically limited our ability to understand the marine microbial realm. However, advances in culture-independent techniques along with next-generation sequencing (NGS) have revolutionized the study of microbial ecology (Schuster 2008). Accompanied by meta'omic approaches, NGS provides a practical tool for the efficient analysis of microbial communities in situ which will facilitate the identification of microbial indicators.

The first step towards identification of microbial indicators to assess coral reef health is analysis of the temporal and spatial variability of microbial communities associated with certain habitats and the subsequent definition of microbial baselines. Compositional baselines can be established with high-throughput sequencing of the 16S ribosomal RNA (rRNA) taxonomic marker gene of bacteria and/or archaea associated with environmental samples. This provides taxonomic insights and, when accompanied by functional prediction tools (e.g. PICRUSt; Langille et al. 2013), may also help predict microbial functions within coral reef environments (Ainsworth et al. 2015; de Voogd et al. 2015). 16S rRNA gene sequencing is a ubiquitously applied technique in diverse fields of coral reef research (e.g. Webster et al. 2010; Bourne et al. 2013), providing an inexpensive tool to establish compositional baselines associated with coral reefs. In parallel, metagenomic

sequencing can be used to establish functional and compositional microbial baselines as this comprehensive approach uses shotgun sequencing to generate an overview of all genes present in an environmental sample (including 16S rRNA genes). Metagenomics has been successfully used to assess functional responses of the coral microbiome to various stressors (Vega Thurber et al. 2009; Littman et al. 2011; Kelly et al. 2014) and is also frequently applied to understand the fundamental basis of the sponge (Thomas et al. 2010; Fan et al. 2013), macroalgae (Burke et al. 2011a; Martin et al. 2014) and planktonic (Dinsdale et al. 2008; Tout et al. 2014; Haas et al. 2016) microbiomes.

Once baselines are established, the compositional and functional response of the microbial communities upon disturbance can be investigated. Taxonomic variation can be observed using 16S rRNA gene sequencing and compositional and functional shifts can be detected using metagenomic sequencing. However, metagenomics is limited in its ability to describe functional variations upon disturbance because of the natural discrepancy between genes that are present and genes that are actually being expressed (Wang et al. 2015). Metatranscriptomic sequencing overcomes this limitation by assessing the function encoded by mRNA sequences. Metatranscriptomics involves analyzing near-real-time gene expression patterns by sequencing mRNA molecules and has emerged as a state-of-the-art tool to study community metabolism of free-living microbes in the open ocean (Poretsky et al. 2009; Hewson et al. 2010) and to obtain insights in cell–cell signaling, development and immune response of symbiotic interactions (Chun et al. 2008; Ruby 2008; Stewart et al. 2011; Sanders et al. 2013). It has also been used to elucidate nutrient cycling and vitamin production pathways of a sponge holobiont (Fiore et al. 2015). Furthermore, responses of the coral host and its associated microbiome to coral diseases have been investigated based on mRNA sequences (Daniels et al. 2015; Arotsker et al. 2016). However, a clear limitation of this method is the short lifetime of mRNA molecules; it only takes a few minutes before mRNA molecules are degraded within the cell (Pedersen et al. 2011). Metaproteomics can characterize the protein signatures from microbial communities in situ and also provides a link between gene content and gene expression (von Bergen et al. 2013). Thus, metaproteomic studies are often complemented by metagenomic data. For example, the combination of metagenomic and metaproteomic data on the sponge microbiome provided novel insights in the activity, physiology and interactions between sponge symbionts (Liu et al. 2012) and revealed the functional role of microbes in the stability of the sponge holobiont under thermal stress (Fan et al. 2013).

Finally, a quantitative approach that establishes links between the composition / function of reef microbiomes

and environmental metadata will be required to identify microbial indicators for coral reef health and water quality. Individual microbial taxa and/or functions significantly associated with healthy versus stressed reef systems can be identified using traditional statistical approaches such as the IndVal analysis (Dufrière and Legendre 1997). An alternative approach to analyzing meta'omics data and discriminating between healthy and stressed reef ecosystems is machine learning. Instead of the identification of individual microbial indicators, the entire microbial community and its function could be used to train a model to differentiate between reef health stages or environmental stressors. Machine learning is a powerful tool and a current state-of-the-art approach to identify dysbiosis of the human microbiome and to predict human diseases (Pasolli et al. 2016).

Conclusion and future research

Microorganisms are fundamental contributors to reef ecosystem health through their biogeochemical capabilities and intimate symbiotic partnerships. Shifts in the composition or function of bacterial and archaeal communities can therefore provide crucial diagnostic information for future coral reef monitoring. Before such approaches can be developed and implemented, the following basic questions need to be resolved (see Fig. 2): (1) which taxa and functions form the microbial baseline of healthy coral reefs? (2) how does the microbial community respond to environmental change? (3) how does the microbiome influence holobiont resistance and resilience upon disturbance? and (4) are environmental disturbances predicted by compositional and functional changes in the microbial community?

Regular monitoring is a fundamental tool for conservation and resource management of marine ecosystems in both developed and developing countries. For example, in the Australian GBR extensive reef monitoring records coral abundance, disease prevalence and coral cover, with the overarching objective of determining the status of reef health and pinpointing changes in the distribution and abundance of the reef biota over large temporal and spatial scales (AIMS 2017). Extending already existing monitoring initiatives to include sample collections targeting microbial biodiversity, composition and function would provide a cost-effective strategy to establish the first microbial reference datasets for individual reef locations (Phase 1). By combining microbial community data and other environmental parameters (e.g. water quality), microbial indicators (taxa or functions) can be identified allowing for reef health diagnosis (Phase 2). Once microbial indicators have been determined, the development and testing of cost- and time-efficient microbial monitoring protocols can begin (Phase 3). The ultimate goal of

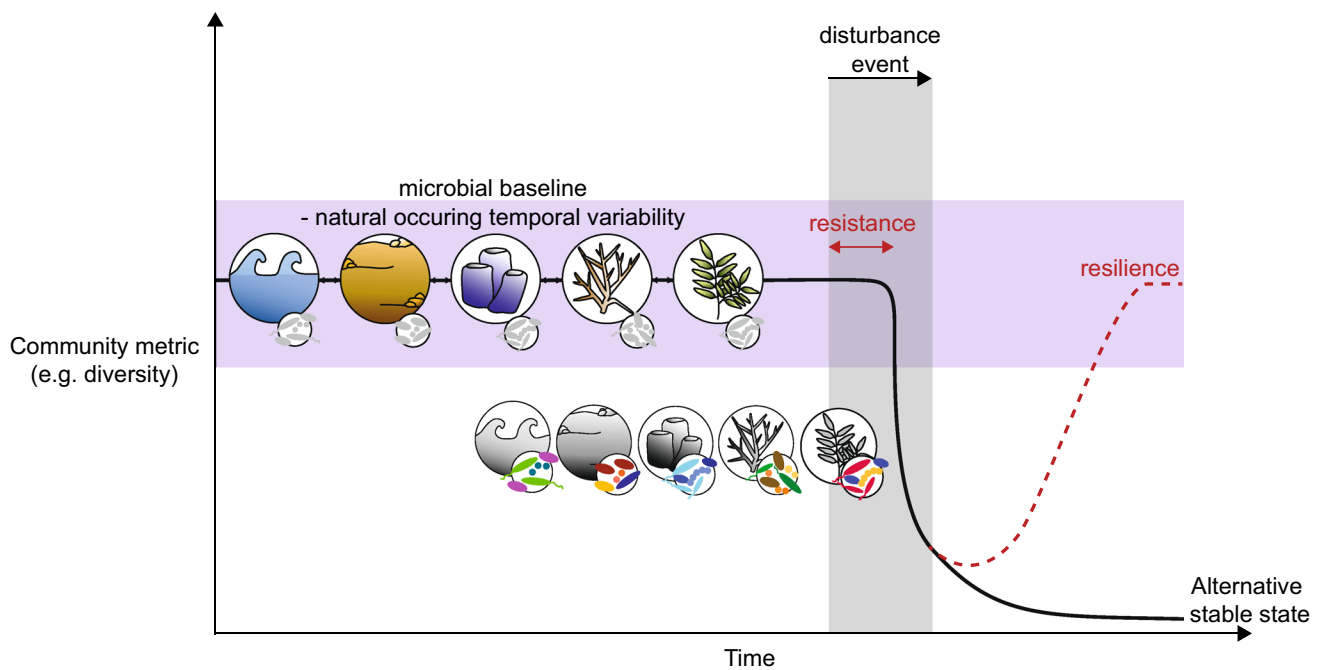


Fig. 2 Schematic representation of the outlined future research directions: The microbial baseline (*horizontal bar*) needs to be defined for a comprehensive range of coral reef habitats (including seawater, sediment, sponges, corals and macroalgae), over temporal periods for a particular microbial community parameter of interest (e.g. diversity). A disturbance event (*vertical bar*) alters the environmental conditions, and triggers a shift in the microbial community associated with each habitat (*solid black line*) leading to an alternative stable state that may significantly deviate from the natural state. The stability of a holobiont, which relates to its ability to act as a buffer to maintain

coral reef function upon environmental alteration, is here defined by the combined resistance and resilience of the microbial community. Resistance is the ability of a community to withstand a disturbance without change. Resilience is defined as the rate at which a microbial community regains its original state after a disturbance event. Combining the information on microbial community stability and variation will enable identification of microbial indicators leading to the early identification of imminent environmental stressors (e.g. water quality changes)

Phase 3 is the establishment of reliable, fast, low-cost and easy-to-use diagnostic protocols based on microbial indicators that can be integrated into current monitoring programs. Microbial monitoring protocols can be comprised of targeted PCR-based approaches (e.g. PCR screening for the occurrence of a specific microbial taxon/function, or quantification of its abundance) and/or community sequencing approaches (e.g. amplicon sequencing to track shifts in microbial community composition). The final step (Phase 4) is the integration of microbial indicators into standard reef monitoring procedures. A prospective microbial indicator tool kit will offer streamlined procedures covering sample collection and processing, an online data analysis platform, and recommended guidelines for management interventions based on the diagnosed reef conditions.

Acknowledgements We thank Pedro R. Frade for inspiring scientific discussions. This study was funded by an AIMS@JCU PhD Scholarship and the Advance Queensland PhD Scholarship to BG. NSW was funded through an Australian Research Council Future Fellowship FT120100480.

Compliance with ethical standards

Conflict of interest All authors declare they have no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

References

- AIMS (2017) Australian Institute of Marine Science. <http://www.aims.gov.au/docs/research/monitoring/reef/reef-monitoring.html>. Accessed 3 Feb 2017
- Ainsworth TD, Fine M, Roff G, Hoegh-Guldberg O (2008) Bacteria are not the primary cause of bleaching in the Mediterranean coral *Oculina patagonica*. *ISME J* 2:67–73
- Ainsworth TD, Krause L, Bridge T, Torda G, Raina J-B, Zakrzewski M, Gates RD, Padilla-Gamino 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

- Allison SD, Martiny JBH (2008) Resistance, resilience, and redundancy in microbial communities. *Proc Natl Acad Sci USA* 105:11512–11519
- Angermeier H, Glöckner V, Pawlik JR, Lindquist NL, Hentschel U (2012) Sponge white patch disease affecting the Caribbean sponge *Amphimedon compressa*. *Dis Aquat Organ* 99(2):95–102
- Angly FE, Heath C, Morgan TC, Tonin H, Rich V, Schaffelke B, Bourne DG, Tyson GW (2016) Marine microbial communities of the Great Barrier Reef lagoon are influenced by riverine floodwaters and seasonal weather events. *PeerJ* 4:e1511
- Armstrong E, Rogerson A, Leftley JW (2000) The abundance of heterotrophic protists associated with intertidal seaweeds. *Estuar Coast Shelf Sci* 50:415–424
- Arotsker L, Kramarsky-Winter E, Ben-Dov E, Kushmaro A (2016) Microbial transcriptome profiling of black band disease in a *Faviid* coral during a seasonal disease peak. *Dis Aquat Org* 118:77–89
- Arrieta MC, Finlay BB (2012) The commensal microbiota drives immune homeostasis. *Front Immunol* 3:33
- Ashbolt NJ, Grabow WOK, Snozzi M (2001) Indicators of microbial water quality. In: Fewtrell L, Bartram J (eds) *Water quality: guidelines, standards and health*. IWA Publishing, London, pp 289–316
- Auguet JC, Barberan A, Casamayor EO (2010) Global ecological patterns in uncultured Archaea. *ISME J* 4:182–190
- Aylagas E, Borja Á, Tangherlini M, Dell'Anno A, Corinaldesi C, Michell CT, Irigoien X, Danovaro R, Rodríguez-Ezpeleta N (2016) A bacterial community-based index to assess the ecological status of estuarine and coastal environments. *Mar Pollut Bull*. doi:10.1016/j.marpolbul.2016.10.050
- Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. *Mar Ecol Prog Ser* 10:257–263
- Baker AC, Glynn PW, Riegl B (2008) Climate change and coral reef bleaching: an ecological assessment of long-term impacts, recovery trends and future outlook. *Estuar Coast Shelf* 80:435–471
- Banerjee S, Kirkby CA, Schmutter D, Bissett A, Kirkegaard JA, Richardson AE (2016) Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. *Soil Biol Biochem* 97:188–198
- Barott KL, Rohwer FL (2012) Unseen players shape benthic competition on coral reefs. *Trends Microbiol* 20(12):621–628
- Barott KL, Rodriguez-Brito B, Janouskovec J, Marhaver KL, Smith JE, Keeling P, Rohwer FL (2011) Microbial diversity associated with four functional groups of benthic reef algae and the reef-building coral *Montastraea annularis*. *Environ Microbiol* 13:1192–1204
- Barott KL, Rodriguez-Mueller B, Youle M, Marhaver KL, Vermeij MJ, Smith JE, Rohwer FL (2012) Microbial to reef scale interactions between the reef-building coral *Montastraea annularis* and benthic algae. *Proc Biol Sci* 279:1655–1664
- Bayer K, Schmitt S, Hentschel U (2008) Physiology, phylogeny and *in situ* evidence for bacterial and archaeal nitrifiers in the marine sponge *Aplysina aerophoba*. *Environ Microbiol* 10:2942–2955
- Bazelet CS, Samways MJ (2011) Identifying grasshopper bioindicators for habitat quality assessment of ecological networks. *Ecol Indic* 11:1259–1269
- Bengtsson MM, Sjötn K, Lanzen A, Ovreas L (2012) Bacterial diversity in relation to secondary production and succession on surfaces of the kelp *Laminaria hyperborea*. *ISME J* 6:2188–2198
- Blackall LL, Wilson B, Van Oppen AJH (2015) Coral—the world's most diverse symbiotic ecosystem. *Mol Ecol* 24:5330–5347
- Blanquer A, Uriz MJ, Cebrian E, Galand PE (2016) Snapshot of a bacterial microbiome shift during the early symptoms of a massive sponge die-off in the western Mediterranean. *Front Microbiol* 7:752
- Boehm AB, Sassoubre LM (2014) Enterococci as indicators of environmental fecal contamination. In: Gilmore MS, Clewell DB, Ike Y, Shankar N (eds) *Enterococci: from commensals to leading causes of drug resistant infection*. Massachusetts Eye and Ear Infirmary, Boston
- Bourne DG, Munn CB (2005) Diversity of bacteria associated with the coral *Pocillopora damicornis* from the Great Barrier Reef. *Environ Microbiol* 7:1162–1174
- Bourne DG, Webster NS (2013) Coral reef bacterial communities. In: Rosenberg E, DeLong E, Lory S, Stackebrandt E, Thompson F (eds) *The Prokaryotes*. Springer Berlin, Heidelberg, pp 163–187
- Bourne DG, Iida Y, Uthicke S, Smith-Keune C (2008) Changes in coral-associated microbial communities during a bleaching event. *ISME J* 2:350–363
- Bourne DG, Dennis PG, Uthicke S, Soo RM, Tyson GW, Webster N (2013) Coral reef invertebrate microbiomes correlate with the presence of photosymbionts. *ISME J* 7:1452–1458
- Bourne DG, Morrow KM, Webster NS (2016) Coral holobionts: insights into the coral microbiome: underpinning the health and resilience of reef ecosystems. *Annu Rev Microbiol* 70:317–340
- Brown EB (1997) Coral bleaching: causes and consequences. *Coral Reefs* 16:S129–S138
- Bruno JF, Petes LE, Harvell CD, Hettinger A (2003) Nutrient enrichment can increase the severity of coral diseases. *Ecol Lett* 6:1056–1061
- Bruno JF, Precht WF, Vroom PS, Aronson RB (2014) Coral reef baselines: how much macroalgae is natural? *Mar Pollut Bull* 80:24–29
- Burke C, Steinberg P, Rusch D, Kjelleberg S, Thomas T (2011a) Bacterial community assembly based on functional genes rather than species. *Proc Natl Acad Sci USA* 108:14288–14293
- Burke C, Thomas T, Lewis M, Steinberg P, Kjelleberg S (2011b) Composition, uniqueness and variability of the epiphytic bacterial community of the green alga *Ulva australis*. *ISME J* 5:590–600
- Burkepile DE, Hay ME (2006) Herbivore vs. nutrient control of marine primary producers: context-dependent effects. *Ecology* 87:3128–3139
- Cairns J, Pratt JR (1993) A history of biological monitoring using benthic macroinvertebrates. In: Rosenberg DM, Resh VH (eds) *Freshwater biomonitoring and benthic macroinvertebrates*. Chapman & Hall, New York, pp 10–27
- 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* 282:2015257
- Carignan V, Villard M-A (2002) Selecting indicator species to monitor ecological integrity: a review. *Environ Monit Assess* 78:45–61
- Carlos C, Torres TT, Ottoboni LM (2013) Bacterial communities and species-specific associations with the mucus of Brazilian coral species. *Sci Rep* 3:1624
- Case RJ, Longford SR, Campbell AH, Low A, Tujula N, Steinberg PD, Kjelleberg S (2011) Temperature induced bacterial virulence and bleaching disease in a chemically defended marine macroalga. *Environ Microbiol* 13:529–537
- Charpy L, Casareto BE, Langlade MJ, Suzuki Y (2012) Cyanobacteria in coral reef ecosystems: a review. *J Mar Biol* 2012:259571

- Chessman BC (1995) Rapid assessment of rivers using macroinvertebrates: a procedure based on habitat-specific sampling, family-level identification and a biotic index. *Austral Ecol* 20:122–129
- Chun CK, Troll JV, Koroleva I, Brown B, Manzella L, Snir E, Almbrazi H, Scheetz TE, Bonaldo MdF, Casavant TL, Soares MB, Ruby EG, McFall-Ngai MJ (2008) Effects of colonization, luminescence, and autoinducer on host transcription during development of the squid-vibrio association. *Proc Natl Acad Sci USA* 105:11323–11328
- Cooper TF, Gilmour JP, Fabricius KE (2009) Bioindicators of changes in water quality on coral reefs: review and recommendations for monitoring programmes. *Coral Reefs* 28(3):589–606
- D'Angelo C, Wiedenmann J (2014) Impacts of nutrient enrichment on coral reefs: new perspectives and implications for coastal management and reef survival. *Curr Opin Environ Sustain* 7:82–93
- Daniels CA, Baumgarten S, Yum LK, Michell CT, Bayer T, Arif C, Roder C, Weil E, Voolstra CR (2015) Metatranscriptome analysis of the reef-building coral *Orbicella faveolata* indicates holobiont response to coral disease. *Front Mar Sci* 2:62
- De Goeij J, Van den Berg H, Van Oostveen M, Epping E, Van Duyl F (2008) Major bulk dissolved organic carbon (DOC) removal by encrusting coral reef cavity sponges. *Mar Ecol Prog Ser* 357:139–151
- De Cáceres M, Legendre P, Moretti M (2010) Improving indicator species analysis by combining groups of sites. *Oikos* 119:1674–1684
- de Oliveira LS, Gregoracci GB, Silva GGZ, Salgado LT, Filho AG, Alves-Ferreira M, Pereira RC, Thompson FL (2012) Transcriptomic analysis of the red seaweed *Laurencia dendroidea* (*Florideophyceae*, Rhodophyta) and its microbiome. *BMC Genomics* 13:487
- De Goeij JM, van Oevelen D, Vermeij MJA, Osinga R, Middelburg JJ, de Goeij AFPM, Admiraal W (2013) Surviving in a marine desert: the sponge loop retains resources within coral reefs. *Science* 342:108–110
- de Voogd NJ, Cleary DF, Polónia AR, Gomes NC (2015) Bacterial community composition and predicted functional ecology of sponges, sediment and seawater from the thousand islands reef complex, West Java, Indonesia. *FEMS Microbiol Ecol* 91(4):fiv019
- De'ath G, Fabricius KE (2010) Water quality as a regional driver of coral biodiversity and macroalgae on the Great Barrier Reef. *Ecol Appl* 20:840–850
- De'ath G, Fabricius KE, Sweatman H, Puotinen M (2012) The 27-year decline of coral cover on the Great Barrier Reef and its causes. *Proc Natl Acad Sci USA* 109:17995–17999
- Diaz MC, Rützler K (2001) Sponges: an essential component of Caribbean coral reefs. *Bull Mar Sci* 69:535–546
- Dinsdale EA, Pantos O, Smriga S, Edwards RA, Angly F, Wegley L, Hatay M, Hall D, Brown E, Haynes M, Krause L, Sala E, Sandin SA, Thurber RV, Willis BL, Azam F, Knowlton N, Rohwer F (2008) Microbial ecology of four coral atolls in the northern Line Islands. *PLoS One* 3:e1584
- Donner SD, Skirving WJ, Little CM, Oppenheimer M, Hoegh-Guldberg O (2005) Global assessment of coral bleaching and required rates of adaptation under climate change. *Glob Chang Biol* 11:2251–2265
- Ducklow H (1990) The biomass, production and fate of bacteria in coral reefs. *Ecosyst World* 25:265–289
- Dufrêne M, Legendre P (1997) Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecol Monogr* 67:345–366
- Egan S, Harder T, Burke C, Steinberg P, Kjelleberg S, Thomas T (2013) The seaweed holobiont: understanding seaweed–bacteria interactions. *FEMS Microbiol Rev* 37:462–476
- Eloe-Fadrosh EA, Rasko DA (2013) The human microbiome: from symbiosis to pathogenesis. *Annu Rev Med* 64:145–163
- Erwin PM, Pita L, López-Legentil S, Turon X (2012) Stability of sponge-associated bacteria over large seasonal shifts in temperature and irradiance. *Appl Environ Microbiol* 78:7358–7368
- European Parliament (2000) Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the Community action in the field of water policy. *Off J Eur Union* 327:1–73
- Falkowski PG, Dubinsky Z, Muscatine L, McCloskey L (1993) Population-control in symbiotic corals. *Bioscience* 43:606–611
- Falkowski PG, Fenchel T, DeLong EF (2008) The microbial engines that drive earth's biogeochemical cycles. *Science* 320:1034–1039
- Fan L, Liu M, Simister R, Webster NS, Thomas T (2013) Marine microbial symbiosis heats up: the phylogenetic and functional response of a sponge holobiont to thermal stress. *ISME J* 7:991–1002
- Fernandes N, Case RJ, Longford SR, Seyedsayamdost MR, Steinberg PD, Kjelleberg S, Thomas T (2011) Genomes and virulence factors of novel bacterial pathogens causing bleaching disease in the marine red alga *Delisea pulchra*. *Plos One* 6
- Fieseler L, Horn M, Wagner M, Hentschel U (2004) Discovery of the novel candidate phylum “Poribacteria” in marine sponges. *Appl Environ Microbiol* 70:3724–3732
- Fiore CL, Labrie M, Jarett JK, Lesser MP (2015) Transcriptional activity of the giant barrel sponge, *Xestospongia muta* Holobiont: molecular evidence for metabolic interchange. *Front Microbiol* 6:364
- Fortunato CS, Eiler A, Herfort L, Needoba JA, Peterson TD, Crump BC (2013) Determining indicator taxa across spatial and seasonal gradients in the Columbia River coastal margin. *ISME J* 7:1899–1911
- Frade PR, Roll K, Bergauer K, Herndl GJ (2016) Archaeal and bacterial communities associated with the surface mucus of Caribbean corals differ in their degree of host specificity and community turnover over reefs. *PLoS One* 11:e0144702
- Frias-Lopez J, Zerkle AL, Bonheyo GT, Fouke BW (2002) Partitioning of bacterial communities between seawater and healthy, black band diseased, and dead coral surfaces. *Appl Environ Microbiol* 68:2214–2228
- Friedrich AB, Merkert H, Fendert T, Hacker J, Proksch P, Hentschel U (1999) Microbial diversity in the marine sponge *Aplysina cavernicola* (formerly *Verongia cavernicola*) analyzed by fluorescence *in situ* hybridization (FISH). *Mar Biol* 134:461–470
- Gardner T (2010) Monitoring forest biodiversity: improving conservation through ecologically responsible management. Earthscan Publications Ltd, London
- Garren M, Azam F (2010) New method for counting bacteria associated with coral mucus. *Appl Environ Microbiol* 76:6128–6133
- Garren M, Azam F (2012) New directions in coral reef microbial ecology. *Environ Microbiol* 14:833–844
- Garren M, Raymundo L, Guest J, Harvell CD, Azam F (2009) Resilience of coral-associated bacterial communities exposed to fish farm effluent. *PLoS One* 4:e7319
- Gast GJ, Wiegman S, Wieringa E, Duyl FCV, Bak RPM (1998) Bacteria in coral reef water types: removal of cells, stimulation of growth and mineralization. *Mar Ecol Prog Ser* 167:37–45
- GBRMPA (2016) Coral bleaching. <http://www.gbrmp.gov.au/media-room/coral-bleaching>. Accessed 27 April 2016
- Ghaisas S, Maher J, Kanthasamy A (2016) Gut microbiome in health and disease: linking the microbiome-gut-brain axis and environmental factors in the pathogenesis of systemic and neurodegenerative diseases. *Pharmacol Ther* 158:52–62
- Gili J-M, Coma R (1998) Benthic suspension feeders: their paramount role in littoral marine food webs. *Trends Ecol Evol* 13:316–321

- 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
- Goecke F, Thiel V, Wiese J, Labes A, Imhoff JF (2013) Algae as an important environment for bacteria - phylogenetic relationships among new bacterial species isolated from algae. *Phycologia* 52:14–24
- Gupta S, Allen-Vercoe E, Petrof EO (2016) Fecal microbiota transplantation: in perspective. *Therap Adv Gastroenterol* 9(2):229–239
- Haas AF, Nelson CE, Kelly LW, Carlson CA, Rohwer F, Leichter JJ, Wyatt A, Smith JE (2011) Effects of coral reef benthic primary producers on dissolved organic carbon and microbial activity. *PLoS One* 6
- Haas AF, Fairoz MFM, Kelly LW, Nelson CE, Dinsdale EA, Edwards RA, Giles S, Hatay M, Hisakawa N, Knowles B, Lim YW, Maughan H, Pantos O, Roach TNF, Sanchez SE, Silveira CB, Sandin S, Smith JE, Rohwer F (2016) Global microbialization of coral reefs. *Nat Microbiol* 1:16042
- Hallock P, Lidz BH, Cockey-Burkhard EM, Donnelly KB (2003) Foraminifera as bioindicators in coral reef assessment and monitoring: the FORAM index. In: Melzian BD, Engle VMS, Eads LK (eds) *Coastal monitoring through partnerships*. Springer, pp 221–238
- Hanson CE, McLaughlin MJ, Hyndes GA, Strzelecki J (2009) Selective uptake of prokaryotic picoplankton by a marine sponge (*Callyspongia sp.*) within an oligotrophic coastal system. *Estuar Coast Shelf Sci* 84:289–297
- Hentschel U, Schmid M, Wagner M, Fieseler L, Gernert C, Hacker J (2001) Isolation and phylogenetic analysis of bacteria with antimicrobial activities from the Mediterranean sponges *Aplysina aerophoba* and *Aplysina cavernicola*. *FEMS Microbiol Ecol* 35:305–312
- Hentschel U, Hopke J, Horn M, Friedrich AB, Wagner M, Hacker J, Moore BS (2002) Molecular evidence for a uniform microbial community in sponges from different oceans. *Appl Environ Microbiol* 68:4431–4440
- Hentschel U, Fieseler L, Wehr M, Gernert C, Steinert M, Hacker J, Horn M (2003) Microbial diversity of marine sponges. In: Weg M (ed) *Molecular marine biology of sponges*. Springer Verlag, Heidelberg, pp 60–88
- Hentschel U, Usher KM, Taylor MW (2006) Marine sponges as microbial fermenters. *FEMS Microbiol Ecol* 55:167–177
- Herndl GJ, Velimirov B (1985) Bacteria in the coelenteron of Anthozoa: control of coelomic bacterial density by the coelenteric fluid. *J Exp Mar Biol Ecol* 93:115–130
- Hewson I, Fuhrman JA (2006) Spatial and vertical biogeography of coral reef sediment bacterial and diazotroph communities. *Mar Ecol Prog Ser* 306:79–86
- Hewson I, Poretsky RS, Tripp HJ, Montoya JP, Zehr JP (2010) Spatial patterns and light-driven variation of microbial population gene expression in surface waters of the oligotrophic open ocean. *Environ Microbiol* 12:1940–1956
- Hill J, Wilkinson C (2004) *Methods for ecological monitoring of coral reefs*. Australian Institute of Marine Science, Townsville
- Hodgson G (2001) Reef check: the first step in community-based management. *Bull Mar Sci* 69:861–868
- Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world's coral reefs. *Mar Freshwater Res* 50:839–866
- Hoegh-Guldberg O (2011) The impact of climate change on coral reef ecosystems. In: Dubinsky Z, Stambler N (eds) *Coral reefs: an ecosystem in transition*. Springer, pp 391–403
- Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatzioiols ME (2007) Coral reefs under rapid climate change and ocean acidification. *Science* 318:1737–1742
- Hollants J, Decleyre H, Leliaert F, De Clerck O, Willems A (2011a) Life without a cell membrane: Challenging the specificity of bacterial endophytes within Bryopsis (Bryopsidales, Chlorophyta). *BMC Microbiol* 11
- Hollants J, Leroux O, Leliaert F, Decleyre H, De Clerck O, Willems A (2011b) Who is in there? Exploration of endophytic bacteria within the siphonous green seaweed Bryopsis (Bryopsidales, Chlorophyta). *PLoS One* 6
- Holt EA, Miller SW (2011) Bioindicators: using organisms to measure environmental impacts. *Nat Educ Knowl* 3(10):8
- Hughes TP, Rodrigues MJ, Bellwood DR, Ceccarelli D, Hoegh-Guldberg O, McCook L, Moltschanowskyj N, Pratchett MS, Steneck RS, Willis B (2007) Phase shifts, herbivory, and the resilience of coral reefs to climate change. *Curr Biol* 17:360–365
- 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 JPA, Hoogenboom MO, Kennedy EV, Kuo CY, Lough CM, 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(7645):373–377
- IMOS (2016) National Mooring Network. <http://imos.org.au/national-mooringnetwork.html>. Accessed 24 Dec 2016
- Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Estes JA, Hughes TP, Kidwell S, Lange CB, Lenihan HS, Pandolfi JM, Peterson CH, Steneck RS, Tegner MJ, Warner RR (2001) Historical overfishing and the recent collapse of coastal ecosystems. *Science* 293:629–638
- Kelly LW, Williams GJ, Barott KL, Carlson CA, Dinsdale EA, Edwards RA, Haas AF, Haynes M, Lim YW, McDole T, Nelson CE, Sala E, Sandin SA, Smith JE, Vermeij MJA, Youle M, Rohwer F (2014) Local genomic adaptation of coral reef-associated microbiomes to gradients of natural variability and anthropogenic stressors. *PNAS* 111:10227–10232
- Kennedy J, Marchesi JR, Dobson ADW (2007) Metagenomic approaches to exploit the biotechnological potential of the microbial consortia of marine sponges. *Appl Microbiol Biotechnol* 75:11–20
- Kimes NE, van Nostrand JD, Weil E, Zhou J, Morris PJ (2010) Microbial functional structure of *Montastraea faveolata*, an important Caribbean reef-building coral, differs between healthy and yellow-band diseased colonies. *Environ Microbiol* 12:541–556
- Kline DI, Kuntz NM, Breitbart M, Knowlton N, Rohwer F (2006) Role of elevated organic carbon levels and microbial activity in coral mortality. *Mar Ecol Prog Ser* 314:119–125
- Knowlton N, Jackson JBC (2008) Shifting baselines, local impacts, and global change on coral reefs. *PLoS Biol* 6:e54
- Knutson TR, McBride JL, Chan J, Emanuel K, Holland G, Landsea C, Held I, Kossin JP, Srivastava AK, Sugi M (2010) Tropical cyclones and climate change. *Nat Geosci* 3:157–163
- Kolkwitz R, Marsson M (1908) *Ökologie der pflanzlichen Saprobien*. *Ber Dtsch Bot Ges* 26:505–519
- 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
- Kostic AD, Gevers D, Siljander H, Vatanen T, Hyötyläinen T, Hamalainen AM, Peet A, Tillmann V, Poho P, Mattila I,

- Lahdesmaki H, Franzosa EA, Vaarala O, de Goffau M, Harmesen H, Ilonen J, Virtanen SM, Clish CB, Oresic M, Huttenhower C, Knip M, Group DS, Xavier RJ (2015) The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host Microbe* 17:260–273
- Lachnit T, Blumel M, Imhoff JF, Wahl M (2009) Specific epibacterial communities on macroalgae: phylogeny matters more than habitat. *Aquat Biol* 5:181–186
- Lachnit T, Meske D, Wahl M, Harder T, Schmitz R (2011) Epibacterial community patterns on marine macroalgae are host-specific but temporally variable. *Environ Microbiol* 13:655–665
- Lamb JB, van de Water JAJM, Bourne DG, Altier C, Hein MY, Fiorenza EA, Abu N, Jompa J, Harvell CD (2017) Seagrass ecosystems moderate pathogens of marine animals. *Science*
- Langille MGI, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepille DE, Vega Thurber RL, Knight R, Beiko RG, Huttenhower C (2013) Predictive functional profiling of microbial communities using 16 S rRNA marker gene sequences. *Nat Biotech* 31:814–821
- Lema KA, Willis BL, Bourne DG (2012) Corals form characteristic associations with symbiotic nitrogen-fixing bacteria. *Appl Environ Microbiol* 78:3136–3144
- Lema KA, Willis BL, Bourne DG (2014) Amplicon pyrosequencing reveals spatial and temporal consistency in diazotroph assemblages of the *Acropora millepora* microbiome. *Environ Microbiol* 16:3345–3359
- Lesser MP (2006) Benthic–pelagic coupling on coral reefs: feeding and growth of Caribbean sponges. *J Exp Mar Biol Ecol* 328:277–288
- Lesser MP, Mazel C, Gorbunov M, Falkowski P (2004) Discovery of symbiotic nitrogen-fixing Cyanobacteria in corals. *Science* 305:997–1000
- 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
- Li J, Chen Q, Long L-J, Dong J-D, Yang J, Zhang S (2014) Bacterial dynamics within the mucus, tissue and skeleton of the coral *Porites lutea* during different seasons. *Sci Rep* 4
- Lipp EK, Griffin DW (2004) Analysis of coral mucus as an improved medium for detection of enteric microbes and for determining patterns of sewage contamination in reef environments. *Eco Health* 1(3):317–323
- Littman R, Willis BL, Bourne DG (2011) Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. *Environ Microbiol* 3:651–660
- Liu M, Fan L, Zhong L, Kjelleberg S, Thomas T (2012) Metaproteomic analysis of a community of sponge symbionts. *ISME J* 6:1515–1525
- Luter HM, Whalan S, Webster NS (2010) Exploring the role of microorganisms in the disease-like syndrome affecting the sponge *Ianthella basta*. *Appl Environ Microbiol* 76(17):5736–5744
- Luter HM, Whalan S, Webster NS (2012) Thermal and sedimentation stress are unlikely causes of brown spot syndrome in the coral reef sponge, *Ianthella basta*. *PLoS One* 7(6):e39779
- Luter HM, Gibb K, Webster NS (2014) Eutrophication has no short-term effect on the *Cymbastela stipitata* holobiont. *Front Microbiol* 5:216
- Martin M, Biver S, Steels S, Barbeyron T, Jam M, Portetelle D, Michel G, Vandenbol M (2014) Functional screening of a metagenomic library of seaweed-associated microbiota: identification and characterization of a halotolerant, cold-active marine endo- β -1, 4-endoglucanase. *Appl Environ Microbiol* AEM 01194–01114
- Martiny AC, Treseder K, Pusch G (2013) Phylogenetic conservatism of functional traits in microorganisms. *ISME J* 7:830–838
- McDole T, Nulton J, Barott KL, Felts B, Hand C, Hatay M, Lee H, Nadon MO, Nosrat B, Salamon P, Bailey B, Sandin SA, Vargas-Angel B, Youle M, Zgliczynski BJ, Brainard RE, Rohwer F (2012) Assessing coral reefs on a pacific-wide scale using the microbialization score. *PloS One* 7
- McGeoch MA, Chown SL (1998) Scaling up the value of bioindicators. *Trends Ecol Evol* 13:46–47
- McGeoch MA, Van Rensburg BJ, Botes A (2002) The verification and application of bioindicators: a case study of dung beetles in a savanna ecosystem. *J Appl Ecol* 39:661–672
- 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
- Mohamed NM, Saito K, Tal Y, Hill RT (2010) Diversity of aerobic and anaerobic ammonia-oxidizing bacteria in marine sponges. *ISME J* 4:38–48
- Moriarty DJW, Pollard PC, Hunt WG (1985) Temporal and spatial variation in bacterial production in the water column over a coral reef. *Mar Biol* 85:285–292
- Morrow KM, Paul VJ, Liles MR, Chadwick NE (2011) Allelochemicals produced by Caribbean macroalgae and Cyanobacteria have species-specific effects on reef coral microorganisms. *Coral Reefs* 30:309–320
- Morrow KM, Bourne DG, Humphrey C, Botte 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
- Mueller B, de Goeij JM, Vermeij MJA, Mulders Y, van der Ent E, Ribes M, van Duyl FC (2014) Natural diet of coral-excavating sponges consists mainly of dissolved organic carbon (DOC). *PLoS One* 9
- Muotka T, Paavola R, Haapala A, Novikmec M, Laasonen P (2002) Long-term recovery of stream habitat structure and benthic invertebrate communities from in-stream restoration. *Biol Conserv* 105:243–253
- Nakanishi K, Nishijima M, Nishimura M, Kuwano K, Saga N (1996) Bacteria that induce morphogenesis in *Ulva pertusa* (Chlorophyta) grown under axenic conditions. *J Phycol* 32:479–482
- Neave MJ, Rachmawati R, Xun L, Michell CT, Bourne DG, Apprill A, Voolstra CR (2017) Differential specificity between closely related corals and abundant *Endozoicomonas* endosymbionts across global scales. *ISME J* 11(1):186–200
- Nelson CE, Goldberg SJ, Kelly LW, Haas AF, Smith JE, Rohwer F, Carlson CA (2013) Coral and macroalgal exudates vary in neutral sugar composition and differentially enrich reef bacterioplankton lineages. *ISME J* 7:962–979
- Nguyen MTHD, Liu M, Thomas T (2014) Ankyrin-repeat proteins from sponge symbionts modulate amoebal phagocytosis. *Mol Ecol* 23:1635–1645
- Normile D (2016) Massive bleaching killed 35% of the coral on the northern end of the Great Barrier Reef. <http://www.sciencemag.org/news/2016/05/massive-bleaching-killed-35-coral-northern-end-great-barrier-reef>. Accessed 30 May 2016
- Nugues MM, Smith GW, Hooionk RJ, Seabra MI, Bak RPM (2004) Algal contact as a trigger for coral disease. *Ecol Lett* 7:919–923
- Ochman H, Lawrence JG, Groisman EA (2000) Lateral gene transfer and the nature of bacterial innovation. *Nature* 405:299–304
- Olson JB, Thacker RW, Gochfeld DJ (2014) Molecular community profiling reveals impacts of time, space, and disease status on the bacterial community associated with the Caribbean sponge. *FEMS Microbiol Ecol* 87(1):268–279
- Pasolli E, Truong DT, Malik F, Waldron L, Segata N (2016) Machine learning meta-analysis of large metagenomic datasets: tools and biological insights. *PLoS Comput Biol* 12:e1004977

- Pedersen M, Nissen S, Mitarai N, Lo Svenningsen S, Sneppen K, Pedersen S (2011) The functional half-life of an mRNA depends on the ribosome spacing in an early coding region. *J Mol Biol* 407(1):35–44
- Penhale PA, Capone DG (1981) Primary productivity and nitrogen fixation in two macroalgae-Cyanobacteria associations. *Bull Mar Sci* 31:164–169
- Phlips E, Zeman C (1990) Photosynthesis, growth and nitrogen fixation by epiphytic forms of filamentous Cyanobacteria from pelagic *Sargassum*. *Bull Mar Sci* 47:613–621
- Pile AJ, Patterson MR, Witman JD (1996) *In situ* grazing on plankton <10 µm by the boreal sponge *Mycale lingua*. *Mar Ecol Prog Ser* 141:95–102
- Pile AJ, Patterson MR, Savarese M, Chernykh VI, Fialkov VA (1997) Trophic effects of sponge feeding within Lake Baikal's littoral zone. 2. Sponge abundance, diet, feeding efficiency, and carbon flux. *Limnol Oceanogr* 42:178–184
- Pineda MC, Strehlow B, Duckworth A, Doyle J, Jones R, Webster NS (2016) Effects of light attenuation on the sponge holobiont-implications for dredging management. *Sci Rep* 6:39038
- Poretsky RS, Hewson I, Sun S, Allen AE, Zehr JP, Moran MA (2009) Comparative day/night metatranscriptomic analysis of microbial communities in the North Pacific subtropical gyre. *Environ Microbiol* 11:1358–1375
- Provasoli L, Pintner IJ (1980) Bacteria induced polymorphism in an axenic laboratory strain of *Ulva lactuca* (Chlorophyceae). *J Phycol* 16:196–201
- Putnam HM, Davidson JM, Gates RD (2016) Ocean acidification influences host DNA methylation and phenotypic plasticity in environmental susceptible corals. *Evol Appl* 9:1165–1178
- Rädecker N, Pogoreutz C, Voolstra CR, Wiedenmann J, Wild C (2015) Nitrogen cycling in corals: the key to understanding holobiont functioning? *Trends Microbiol* 23:490–497
- Raina J-B, Tapiolas D, Motti CA, Foret S, Seemann T, Tebben J, Willis BL, Bourne DG (2016) Isolation of an antimicrobial copound produced by bacteria associated with reef-building corals. *PeerJ* 4:e2275
- Rasheed M, Badran MI, Huettel M (2003) Influence of sediment permeability and mineral composition on organic matter degradation in three sediments from the Gulf of Aqaba, Red Sea. *Estuar Coast Shelf Sci* 57:369–384
- Reardon S (2014) Drug development microbiome therapy gains market traction. *Nature* 509:269–270
- Reiswig HM (1971a) *In situ* pumping activities of tropical Demospongiae. *Mar Biol* 9:38–50
- Reiswig HM (1971b) Particle feeding in natural populations of three marine Demosponges. *Biol Bull* 141:568–591
- Reshef L, Koren O, Lova Y, Zilber-Rosenberg I, Rosenberg E (2006) The coral probiotic hypothesis. *Environ Microbiol* 8(12):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, Smith GW (1997) Physiological comparison of bacterial communities from various species of scleractinian corals. *Proc 8th Int Coral Reef Sym* 1:521–526
- Rix L, de Goeij JM, Mueller CE, Struck U, Middelburg JJ, van Duyl FC, Al-Horani FA, Wild C, Naumann MS, van Oevelen D (2016) Coral mucus fuels the sponge loop in warm- and cold-water coral reef ecosystems. *Sci Rep* 6:18715
- Rohwer F, Breitbart M, Jara J, Azam F, Knowlton N (2001) Diversity of bacteria associated with the Caribbean coral *Montastrea franksi*. *Coral Reefs* 20:85–91
- Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. *Mar Ecol Prog Ser* 243:1–10
- Rothig T, Ochsenkuhn MA, Roik A, van der Merwe R, Voolstra CR (2016) Long-term salinity tolerance is accompanied by major restructuring of the coral bacterial microbiome. *Mol Ecol* 25:1308–1323
- Ruby EG (2008) Symbiotic conversations are revealed under genetic interrogation. *Nat Rev Micro* 6:752–762
- Rusch A, Hannides AK, Gaidos E (2009) Diverse communities of active Bacteria and Archaea along oxygen gradients in coral reef sediments. *Coral Reefs* 28:15–26
- Sanders JG, Beinart RA, Stewart FJ, Delong EF, Girguis PR (2013) Metatranscriptomics reveal differences in *in situ* energy and nitrogen metabolism among hydrothermal vent snail symbionts. *ISME J* 7:1556–1567
- Sandin SA, Smith JE, DeMartini EE, Dinsdale EA, Donner SD, Friedlander AM, Konotchick T, Malay M, Maragos JE, Obura D, Pantos O, Paulay G, Richie M, Rohwer F, Schroeder RE, Walsh S, Jackson JBC, Knowlton N, Sala E (2008) Baselines and degradation of coral reefs in the Northern Line Islands. *Plos One* 3
- Schmitt S, Tsai P, Bell J, Fromont J, Ilan M, Lindquist N, Perez T, Rodrigo A, Schupp PJ, Vacelet J, Webster N, Hentschel U, Taylor MW (2012) Assessing the complex sponge microbiota: core, variable and species-specific bacterial communities in marine sponges. *ISME J* 6:564–576
- Schöttner S, Pfitzner B, Grünke S, Rasheed M, Wild C, Ramette A (2011) Drivers of bacterial diversity dynamics in permeable carbonate and silicate coral reef sands from the Red Sea. *Environ Microbiol* 13:1815–1826
- Schuster SC (2008) Next-generation sequencing transforms today's biology. *Nat Methods* 5:16–18
- Selvin J, Shanmugha Priya S, Seghal Kiran G, Thangavelu T, Sapna Bai N (2009) Sponge-associated marine bacteria as indicators of heavy metal pollution. *Microbiol Res* 164:352–363
- Séré MG, Tortosa P, Chabanet P, Turquet J, Quod J-P, Schleyer MH (2013) Bacterial communities associated with *Porites* White Patch Syndrome (PWPS) on three Western Indian Ocean (WIO) coral reefs. *PLoS One* 8:e83746
- Shade A, Peter H, Allison SD, Baho DL, Berga M, Bürgmann H, Huber DH, Langenheder S, Lennon JT, Martiny JB, Matulich KL, Schmidt TM, Handelsman J (2012) Fundamentals of microbial community resistance and resilience. *Front Microbiol* 3:417
- Sharshar N, Banaszak AT, Lesser MP, Amrami D (1997) Coral endolithic algae: life in a protected environment. *Pac Sci* 51:167–173
- Shnit-Orland M, Kushmaro A (2009) Coral mucus-associated bacteria: a possible first line of defense. *FEMS Microbiol Ecol* 67:371–380
- 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
- Simister R, Taylor MW, Tsai P, Webster NS (2012) Sponge-microbe associations survive high nutrients and temperatures. *PLoS One* 7(12):e52220
- Singh RP, Mantri VA, Reddy CRK, Jha B (2011) Isolation of seaweed-associated bacteria and their morphogenesis-inducing capability in axenic cultures of the green alga *Ulva fasciata*. *Aquat Biol* 12:13–21
- Smith MJ, Kay WR, Edward DHD, Papas PJ, Richardson KSJ, Simpson JC, Pinder AM, Cale DJ, Horwitz PHJ, Davis JA, Yung FH, Norris RH, Halse SA (1999) AusRivAS: using macroinvertebrates to assess ecological condition of rivers in Western Australia. *Freshwat Biol* 41:269–282
- Smith JE, Shaw M, Edwards RA, Obura D, Pantos O, Sala E, Sandin SA, Smriga S, Hatay M, Rohwer FL (2006) Indirect effects of

- algae on coral: algae-mediated, microbe-induced coral mortality. *Ecol Lett* 9:835–845
- Sorokin YI (1973) Trophical role of bacteria in ecosystem of coral reef. *Nature* 242:415–417
- Sorokin YI (1978) Microbial production in the coral-reef community. *Arch Hydrobiol* 83:281–323
- Southwell MW, Weisz JB, Martens CS, Lindquist N (2008) *In situ* fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. *Limnol Oceanogr* 53:986–996
- Stanley GD, Fautin DG (2001) The origins of modern corals. *Science* 291:1913–1914
- Stewart F, Dmytrenko O, DeLong E, Cavanaugh C (2011) Metatranscriptomic analysis of sulfur oxidation genes in the endosymbiont of *Solemya velum*. *Front Microbiol* 2
- Stockner JG (1988) Phototrophic picoplankton: an overview from marine and freshwater ecosystems. *Limnol Oceanogr* 33:765–775
- Sunagawa S, Woodley CM, Medina MN (2010) Threatened corals provide underexplored microbial habitats. *PLoS One* 5:e9554
- Sweet MJ, Croquer A, Bythell JC (2011) Bacterial assemblages differ between compartments within the coral holobiont. *Coral Reefs* 30:39–52
- Sweet MJ, Bythell JC, Nugues MM (2013) Algae as reservoirs for coral pathogens. *PLoS One* 8:e69717
- Taylor MW, Radax R, Steger D, Wagner M (2007) Sponge-associated microorganisms: evolution, ecology, and biotechnological potential. *Microbiol Mol Biol Rev* 71:295–347
- Teeling H, Fuchs BM, Becher D, Klockow C, Gardebrecht A, Bennke CM, Kassabgy M, Huang S, Mann AJ, Waldmann J, Weber M, Klindworth A, Otto A, Lange J, Bernhardt J, Reinsch C, Hecker M, Peplies J, Bockelmann FD, Callies U, Gerdt G, Wichels A, Wiltshire KH, Glöckner FO, Schweder T, Amann R (2012) Populations induced by a phytoplankton bloom substrate-controlled succession of marine bacterioplankton. *Science* 336:608–611
- Thacker RW, Freeman CJ (2012) Sponge-microbe symbiosis: recent advances and new directions. *Adv Mar Biol* 62:57–111
- Thomas T, Evans FF, Schleheck D, Mai-Prochnow A, Burke C, Penesyan A, Dalisay DS, Stelzer-Braid S, Saunders N, Johnson J, Ferreira S, Kjelleberg S, Egan S (2008) Analysis of the *Pseudalteromonas tunicata* genome reveals properties of a surface-associated life style in the marine environment. *Plos One* 3
- Thomas T, Rusch D, DeMaere MZ, Yung PY, Lewis M, Halpern A, Heidelberg KB, Egan S, Steinberg PD, Kjelleberg S (2010) Functional genomic signatures of sponge bacteria reveal unique and shared features of symbiosis. *ISME J* 4:1557–1567
- Thomas T, Moitinho-Silva L, Lurgi M, Bjork JR, Easson C, Astudillo-Garcia C, Olson JB, Erwin PM, Lopez-Legentil S, Luter H, Chaves-Fonnegra A, Costa R, Schupp PJ, Steindler L, Erpenbeck D, Gilbert J, Knight R, Ackermann G, Victor Lopez J, Taylor MW, Thacker RW, Montoya JM, Hentschel U, Webster NS (2016) Diversity, structure and convergent evolution of the global sponge microbiome. *Nat Commun* 7:11870
- Thompson JR, Rivera HE, Closek CJ, Medina M (2015) Microbes in the coral holobiont: partners through evolution, development, and ecological interactions. *Front Cell Infect Microbiol* 4
- Thoms C, Horn M, Wagner M, Hentschel U, Proksch P (2003) Monitoring microbial diversity and natural product profiles of the sponge *Aplysina cavernicola* following transplantation. *Mar Biol* 142:685–692
- Thornton DCO (2014) Dissolved organic matter (DOM) release by phytoplankton in the contemporary and future ocean. *Eur J Phycol* 49:46–49
- Thurber RLV, Barott KL, Hall D, Liu H, Rodriguez-Mueller B, Desnues C, Edwards RA, Haynes M, Angly FE, Wegley L, Rohwer FL (2008) Metagenomic analysis indicates that stressors induce production of herpes-like viruses in the coral *Porites compressa*. *Proc Natl Acad Sci USA* 105:18413–18418
- Thurber RLV, Burkepile DE, Fuchs C, Shantz AA, McMinds R, Zaneveld JR (2014) Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. *Global Change Biol* 20:544–554
- Tout J, Jeffries TC, Webster NS, Stocker R, Ralph PJ, Seymour JR (2014) Variability in microbial community composition and function between different niches within a coral reef. *Microb Ecol* 67:540–552
- Trias R, Garcia-Lledo A, Sanchez N, Lopez-Jurado JL, Hallin S, Baneras L (2012) Abundance and composition of epiphytic bacterial and archaeal ammonia oxidizers of marine red and brown macroalgae. *Appl Environ Microbiol* 78:318–325
- Uthicke S, McGuire K (2007) Bacterial communities in Great Barrier Reef calcareous sediments: contrasting 16 S rDNA libraries from nearshore and outer shelf reefs. *Estuar Coast Shelf Sci* 72:188–200
- Vacelet J, Donadey C (1977) Electron microscope study of the association between some sponges and bacteria. *J Exp Mar Biol Ecol* 30:301–314
- Valentine JF, Heck KL (2005) Perspective review of the impacts of overfishing on coral reef food web linkages. *Coral Reefs* 24:209–213
- van Oppen MJH, Oliver JK, Putnam HM, Gates RD (2014) Building coral reef resilience through assisted evolution. *PNAS* 112(8):2307–2313
- Vega Thurber RL, Willner-Hall D, Rodriguez-Mueller B, Desnues C, Edwards RA, Angly F, Dinsdale EA, Kelly L, Rohwer F (2009) Metagenomic analysis of stressed coral holobionts. *Environ Microbiol* 11:2148–2163
- Vega Thurber R, Burkepile DE, Correa AM, Thurber AR, Shantz AA, Welsh R, Pritchard C, Rosales S (2012) Macroalgae decrease growth and alter microbial community structure of the reef-building coral, *Porites astreoides*. *PLoS One* 7:e44246
- Vermeij MJA, Moorselaar IV, Engelhard S, Hörnlein C, Vonk SM, Visser PM (2010) The effects of nutrient enrichment and herbivore abundance on the ability of turf algae to overgrow coral in the Caribbean. *PLoS One* 5:e14212
- von Bergen M, Jehmlich N, Taubert M, Vogt C, Bastida F, Herbst FA, Schmidt F, Richnow HH, Seifert J (2013) Insights from quantitative metaproteomics and protein-stable isotope probing into microbial ecology. *ISME J* 7:1877–1885
- Wade TJ, Sams E, Brenner KP, Haugland R, Chern E, Beach M, Wymer L, Rankin CC, Love D, Li Q, Noble R, Dufour AP (2010) Measured indicators of recreational water quality and swimming-associated illness at marine beaches: a prospective cohort study. *Environ Health* 9:66
- Wang WL, Xu SY, Ren ZG, Tao L, Jiang JW, Zheng SS (2015) Application of metagenomics in the human gut microbiome. *World J Gastroentero* 21:803–814
- Webster NS (2017) Conceptual and methodological advances for holobiont research. *Environ Microbiol Rep*. doi:10.1111/1758-2229.12500
- Webster NS, Thomas T (2016) The sponge hologenome. *mBio* 7(2):e00135–16
- Webster NS, Watts EMJ, Hill TR (2001) Detection and phylogenetic analysis of novel Crenarchaeote and Euryarchaeote 16 S ribosomal RNA gene sequences from a Great Barrier Reef sponge. *Mar Biotechnol* 3:600–608
- Webster NS, Xavier JR, Freckelton M, Motti CA, Cobb R (2008a) Shifts in microbial and chemical patterns within the marine sponge *Aplysina aerophoba* during a disease outbreak. *Environ Microbiol* 10(12):3366–3376
- Webster NS, Cobb RE, Negri AP (2008b) Temperature thresholds for bacterial symbiosis with a sponge. *ISME J* 2:830–842

- Webster NS, Taylor MW, Behnam F, Lückner S, Rattei T, Whalan S, Horn M, Wagner M (2010) Deep sequencing reveals exceptional diversity and modes of transmission for bacterial sponge symbionts. *Environ Microbiol* 12:2070–2082
- Webster FJ, Babcock RC, Van Keulen M, Loneragan NR (2015) Macroalgae inhibits larval settlement and increases recruit mortality at Ningaloo Reef, Western Australia. *Plos One* 10
- 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* 9:2807–2719
- Wehrli M, Steinert M, Hentschel U (2007) Bacterial uptake by the marine sponge *Aplysina aerophoba*. *Microb Ecol* 53:355–365
- Wemheuer B, Wemheuer F, Hollensteiner J, Meyer F-D, Voget S, Daniel R (2015) The green impact: bacterioplankton response toward a phytoplankton spring bloom in the southern North Sea assessed by comparative metagenomic and metatranscriptomic approaches. *Front Microbiol* 6:805
- Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: the unseen majority. *PNAS* 95:6578–6583
- Wild C, Huettel M, Klueter A, Kremb SG, Rasheed MYM, Jørgensen BB (2004a) Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. *Nature* 428:66–70
- Wild C, Rasheed M, Werner U, Franke U, Johnstone R, Huettel M (2004b) Degradation and mineralization of coral mucus in reef environments. *Mar Ecol Prog Ser* 267:159–171
- Wild C, Laforsch C, Huettel M (2006) Detection and enumeration of microbial cells within highly porous calcareous reef sands. *Mar Freshwater Res* 57:415–420
- Wilkinson C (1980) Cyanobacteria symbiotic in marine sponges. *Endocytobiosis Cell Res* 1:553–563
- Wilkinson CR, Garrone R, Vacelet J (1984) Marine sponges discriminate between food bacteria and bacterial symbionts: electron microscope radioautography and *in situ* evidence. *Proc R Soc Lond [Biol]* 220:519–528
- Wohl DL, Arora S, Gladstone JR (2004) Functional redundancy supports biodiversity and ecosystem function in a closed and constant environment. *Ecol* 85(6):1534–1540
- Wrede C, Dreier A, Kokoschka S, Hoppert M (2012) Archaea in symbioses. *Archaea* 2012:11
- Wright JF (1995) Development and use of a system for predicting the macroinvertebrate fauna in flowing waters. *Aust J Ecol* 20:181–197
- Yachi S, Loreau M (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *PNAS* 96:1463–1468
- Yang S, Sun W, Zhang FL, Li ZY (2013) Phylogenetically diverse denitrifying and ammonia-oxidizing bacteria in corals *Alcyonium gracillimum* and *Tubastraea coccinea*. *Mar Biotechnol* 15:540–551
- Zaneveld JR, Burkepille DE, Shantz AA, Pritchard CE, McMinds R, Payet JP, RoryWelsh, Correa AMS, Lemoine NP, Rosales S, Fuchs C, Maynard JA, Thurber RV (2016) Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. *Nat Commun* 7:11833
- 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 Commun* 8:14213