Investigating the Drivers of Microbial Community Composition in Reef-building Corals

Thesis Submitted by

Hannah Elizabeth Epstein, BSc (Hons)

October 2018

For the degree of Doctor of Philosophy, Physical and Natural Sciences

The ARC Centre of Excellence for Coral Reef Studies and AIMS@JCU
at James Cook University and the Australian Institute of Marine Science
Townsville, Queensland, Australia

STATEMENT OF THE CONTRIBUTIONS OF OTHERS

Many colleagues and collaborators have contributed in a variety of ways to this thesis and the resulting manuscripts that are accepted, in-review or in preparation for publication. An overview of these contributions is outlined below.

NATURE OF CONTRIBUTION	CONTRIBUTION	CO-CONTRIBUTORS
Intellectual support	Project design	*Prof. M.J.H van Oppen (AIMS, Univ. Melbourne), Dr. G. Torda (ARC CoE, AIMS), Prof. P.L. Munday (ARC CoE), H.A. Smith (JCU), Dr. N. Cantin (AIMS) & Dr. L. Bay (AIMS)*
	Data analysis & statistical support	*K. Damjanovic (AIMS, Univ. Melbourne), H.A. Smith (JCU), A. Hernandez-Agreda (Cal. Academy), Dr. A. Kerwin (Penn State Univ.) & L. Hartman (Univ. Melbourne)*
	Artwork support	*H.A. Smith (JCU)*
	Editorial assistance	*Prof. M.J.H van Oppen (AIMS, Univ. Melbourne), Dr. G. Torda (ARC CoE, AIMS), Prof. P.L. Munday (ARC CoE), H.A. Smith (JCU) & K. Russo*
Data Collection	Fieldwork assistance	*Dr. A. Gainsford, K. Sambrook (ARC CoE), T. Nay (ARC CoE), C. Gervais (Macquarie Univ.), J. Biggs (UQ), Dr. W. Wessels (Univ. Oldenburg), L. Chakravarti (AIMS@JCU), T. Hill (ARC CoE), A. Sippel, M. Espenheira (JCU) & K. Damjanovic (Univ. Melbourne, AIMS)*
	Boating & diving support	*Orpheus Island Research Station (JCU) & Heron Island Research Station (UQ)*
	Laboratory assistance	*K. Damjanovic (Univ. Melbourne, AIMS) & L. Peplow (AIMS)*
Financial Assistance	Stipend support	*AIMS@JCU*
	Research funding	*Paul G. Allen Philanthropies, AIMS@JCU, ARC CoE & The Explorer's Club*
	Travel funding	*International Society for Microbial Ecology, AIMS@JCU & ARC CoE*

The research presented and reported in this thesis was conducted in compliance with the Great Barrier Reef Marine Park Regulations 1983 (Commonwealth) and the Marine Parks Regulation 2006 (Queensland) permitted by the Great Barrier Reef Marine Park Authority and the Queensland Parks and Wildlife Service under the permit numbers: G12/35236.1 and G17/39487.1.

I acknowledge the Wulgurukaba and Bindal peoples (Townsville region), the Manbarra people (Palm Island region) and the Gooreng Gooreng, Gurang, Bailai and Taribelang Bunda peoples (Gladstone/Bundaberg region) as the traditional owners of the land and sea country on which this research was conducted. I would like to pay my respects to Elders past, present and future.

STATEMENT OF THE CONTRIBUTIONS OF CO-AUTHORS

The following chapters have been accepted, are in review or in preparation for publication. An overview of each co-author's contributions to the publications is listed below.

CHAPTER NO.	DETAILS OF PUBLICATION(S) ON WHICH CHAPTER IS BASED	NATURE AND EXTENT OF THE INTELLECTUAL INPUT OF EACH AUTHOR, INCLUDING THE CANDIDATE
Chapter 2 (Accepted)	Epstein HE, Smith HA, Torda G & van Oppen MJH. (Accepted) Microbiome engineering: A new hope for enhancing climate resilience in coral*? Frontiers in Ecology and the Environment*.	The co-authors developed the idea. Epstein wrote the first draft, which was revised with editorial input from van Oppen, Torda and Smith. van Oppen, Smith and Epstein conceptualized the figures and Smith created them for publication.
Chapter 3 (In prep)	Epstein HE, Smith HA, Bay L, Cantin N, Mocellin V, Torda G & van Oppen MJH (In prep). Temporal variation in coral microbiomes does not reflect seasonality.	The co-authors developed the research question. Mocellin, Cantin and Bay are responsible for the original sampling design and provided the initial coral samples. Epstein performed genetic laboratory work and bioinformatics. Epstein performed statistical analyses with assistance from Smith. Epstein wrote the initial draft and all co-authors contributed, or will contribute, to editing prior to submission.
Chapter 4 (In review)	Epstein HE, Torda G & van Oppen MJH (In review). Thermal stress has little effect on the microbiome composition of the coral *Pocillopora acuta*. *Coral Reefs.*	The co-authors developed the research question. Epstein and Torda collected the samples. Epstein performed all genetic laboratory work, bioinformatics and statistical analyses. Torda provided some statistical assistance. Epstein wrote the initial draft of the manuscript with editorial support from all co-authors.
Chapter 5 (In review)	Epstein HE, Torda G, Munday PL & van Oppen MJH (In review). Evidence for mixed mode transmission of bacterial and dinoflagellate communities in a common coral. *ISMEJ*.	The co-authors developed the research question. Epstein ran the experiment, collected the samples and performed all genetic laboratory work, bioinformatics and statistical analyses. Epstein wrote the initial draft of the manuscript and received editorial support from all co-authors.

ACKNOWLEDGEMENTS

This thesis is the culmination of an unforgettable journey. I am incredibly lucky to have had such an extraordinary support network of advisors, colleagues, friends and family who have always kept me moving forward.

I would like to extend my deepest gratitude and appreciation to my advisors, Prof. Madeleine van Oppen, Prof. Philip Munday and Dr. Greg Torda, for your endless encouragement, support and patience over the past few years. To Madeleine, thank you for always keeping me focused on the end-goal and never letting me stray too far off course. To Phil, thank you for keeping the objective view on my project - it has taught me to always think outside the box. To Greg, thank you for being my biggest cheerleader and for reminding me that things are not always as bad as they seem. I would also like to give my thanks to Dr. Nicole Webster, for including me in the sponge group at AIMS, despite being a coral person, and to Prof. Mike Kingsford for your continuing support and mentorship. To Dr. Shelley Templeman, thank you for your constant guidance, encouragement, advocacy and kind words that have carried me through the tough times. I also extend my gratitude to Libby, Melissa and Lauren in the AIMS@JCU office, who have provided a great deal of assistance throughout my candidature.

I would like to thank Hillary Smith, Katarina Damjanovic and Alejandra Hernandez-Agreda, who have not only provided invaluable technical support during my PhD, but have also given me moral support when I have needed it the most. To Hillary, thank you for always listening to my complaints about codes and HPCs, about frustrating lab results and the snail-pace of journals, but also for the constant reminders that we are capable of anything when we put our minds to it. Kat, you're my partner in crime in the lab, and I could not have completed this work without your cunning ability to troubleshoot protocols, your willingness to assist in ridiculous R codes and your positive attitude – thank you for always smiling. And to Ale, all the chats about microbes, our journal club and your boundless encouragement about our place within the field have always kept me excited about whatever is coming next.

I could not have completed the work in this thesis without all of my incredible field volunteers. This includes Dr. Ashton Gainsford, Katie Sambrook, Tess Hill, Andy Sippel, Leela Chakravarti, Marta Espenheira, Dr. Weibke Wessels, Josh Biggs, Tiffany Nay and Connor Gervais. I would like to specially thank Ashton for your resourceful, creative and innovative problem-solving skills while at Heron Island, as well as for your continual support both in and out of work – you have been through thick and thin with me and I am so incredibly grateful for your friendship. I would also like to thank Katie Sambrook; your practical thinking kept me calm in the field, and your encouragement, support and general excitement for science and the oceans has always kept my spirits high.

To all my friends that have stuck with the highs and lows of the past few years, I offer a massive thank you. Specifically, I would like to thank Margaux Hein, Katharine Russo and the Darlington family, Michelle Booth, Jacob Eurich, Marnie Freckelton, Amelia Wenger, Gigi Torras, Michael Kramer, Lizzie Abbott, Mercedes Pisano and Shayle Matsuda for all of the laughs, adventures, science talk and general maintenance of my mental health. Finally, I would like to express my greatest appreciation to my family – Mom, Dad & Shaina – thank you for your unconditional support and love, for believing in my abilities even when I had doubts, and for being my rock from across oceans.

General Abstract

The abundance and diversity of microbes in corals are indicative of an intricate coexistence between the metazoan host and these unicellular partners. An increasing number of studies have identified explicit functions that some microbes perform in the coral holobiont, ranging from nutrient cycling to immunity. Rapid climate change can affect coral-associated microbial symbioses through negative shifts in microbial community structure (i.e., dysbiosis) that can lead to coral disease and mortality. Alternately, microbes may contribute to holobiont resilience by rapidly adapting to new environmental regimes and may provide the coral with an uninterrupted suite of functions. To understand whether shifts in the microbiome as a result of environmental change will be positive or negative, the drivers of natural variations in community composition must be understood. This thesis is focused on investigating and identifying some of the drivers of microbial community composition in corals, specifically of the Symbiodiniaceae and bacterial communities, to improve our ability to predict the impacts of climate change on corals, and to provide critical baseline data that may be used to inform the development of the microbial-driven restoration technique of microbiome engineering. Using both survey and experimental methods, the specific aims of this thesis were to 1) review the literature on microbiome engineering and identify new research directions that will aid the development of microbiome engineering in corals, 2) investigate the long-term seasonal variation in coral-associated microbiomes, 3) examine the effects of a temperature anomaly on the stability of the coral microbiome and 4) determine the drivers of microbial community establishment in coral offspring.

Obtaining direction from established research on microbiome engineering in biological systems such as plants, human health and waste water treatment, Chapter 2 identified three main research priorities that would not only provide a foundation of knowledge for developing coral-specific microbiome engineering tools, but also further the coral microbial ecology field in general. These research priorities were to 1) determine the variable and stable partners of the microbiome, 2) identify microbial function and 3) use experimental methods to determine key microbial players and assess the feasibility of manipulation methods. The remaining data chapters of this thesis focused on addressing some of the knowledge gaps associated with the natural variability and stability of the microbiome to identify what may be driving patterns of microbial community composition using 16S rRNA gene and ITS2 spacer metabarcoding for bacteria and Symbiodiniaceae, respectively. In Chapter 3, twelve tagged colonies each of two species of *Acropora* corals at two mid-shelf reefs on the Great Barrier Reef (GBR) were sampled over two years to examine whether temporal changes in the coral microbiome reflect cyclical seasonal cycles, and whether there is evidence for coral host-specificity or location effects on microbiome

composition. Findings from this chapter confirm that the coral microbiome is complex and dynamic, but does not reflect seasonal cycles, at least not in the species and reefs studied here. Coral microbiomes also varied within coral species according to reef, suggesting that reef environment or location further drives microbial community composition. In Chapter 4, ten tagged colonies of *Pocillopora acuta*, a comparatively bleaching resistant coral, were visually inspected and sampled during the 2016 thermal anomaly in the northern and central GBR that resulted in widespread bleaching. Despite experiencing higher than average temperatures and two-degree heating weeks, these corals exhibited no visible signs of bleaching and little variation in their bacterial and Symbiodiniaceae communities through time. Indicator analyses identified microbes that could harbor beneficial properties for thermal tolerance, but future functional studies will be necessary for validation. Finally, Chapter 5 describes the results of a manipulative experiment to determine the influence of parents and environment on the establishment of the microbiome in coral offspring of the species *Pocillopora damicornis*. Findings provided evidence for mixed mode transmission for both bacteria and Symbiodiniaceae, with offspring sharing a small number of microbial taxa with their parents and some with the water column. Microbial communities in early coral life stages were characterized by high variability and dispersion in comparison to parents, suggesting that they shape their microbial communities throughout ontogeny (i.e., “winnowing”).

This thesis has identified both host and environmental factors were crucial drivers of the coral microbiome. For some coral species in certain locations, shifts in microbial community composition may provide adaptive benefits, while for others, they may cause bleaching, disease or mortality. The effects of these shifts for the coral host, whether positive or negative, are likely host specific, reliant on their geographic location and contingent on the severity of the stress events they witness. While this may pose a challenge for implementing long-term microbial manipulations intended for reef restoration, short-term probiotic treatments for bioremediation or immediate prevention should be investigated further. Future empirical work on microbial function and the ability for the microbiome to facilitate climate resilience in corals is essential.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES & PANELS

CHAPTER 1

GENERAL INTRODUCTION

Accumulating concentrations of anthropogenic greenhouse gasses in the atmosphere are threatening coral reef ecosystems worldwide due to their effects on ocean temperature and acidity. Our capacity to protect these ecosystems now relies on our ability to reduce CO_2 emissions and, in the short term, to maintain the resilience of reefs to rebound from extreme climatic events and potentially restore those that are already damaged. Reef restoration will subsequently depend on our ability to predict the responses of coral reef organisms to such threats and how these responses can facilitate climate resilience. Reef-building corals are the foundation species of coral reefs, providing structure, food and shelter to the numerous other reef organisms that inhabit these ecosystems. The health and function of corals are supported by a healthy microbiome, consisting of a variety of microbial partners including their photosynthetic endosymbionts in the family Symbiodiniaceae, numerous prokaryotes (i.e., bacteria and archaea), single-celled and filamentous fungi and acellular viruses, that can play important functional roles (Raina et al. 2009, 2013; Krediet et al. 2013). Changes in the environment are known to affect microbial community composition (reviewed in Bourne et al. 2016), but the extent to which these changes drive negative or positive responses in the coral holobiont (the coral microbiome and the coral host, combined) has not been well characterized. As a result of our limited knowledge on what drives positive or negative shifts in the coral microbiome, contradictory ideas currently exist around the influence of the microbiome on climate resilience in corals (reviewed in Torda et al. 2017). This thesis aims to investigate and identify the drivers of coral-associated microbial community composition to facilitate our ability to predict how corals may respond to environmental change in the future, and to provide baseline knowledge intended to inform the development of microbial-driven restoration techniques.

Defining the coral microbiome

Coral reefs are among the most biodiverse ecosystems on earth. They sustain around one quarter of all marine species and greatly contribute to the economies and livelihoods of the countries that have access to them (Moberg & Folke 1999). As mass-scale bleaching events decimate coral reefs worldwide (Heron et al. 2016, Hughes et al. 2017), current research is no longer concentrated only on understanding the drivers of reef degradation, but also on determining the drivers of coral resilience and the development of progressive reef conservation and restoration tools (e.g., selective breeding, Chan et al. 2018; symbiont manipulations, Chakravarti et al. 2017, Damjanovic et al. 2017; or coral translocation and transplantation, reviewed in van Oppen et al.

2015, 2017). One driver of coral resilience is believed to be related to the coral microbiome (e.g., Reshef et al. 2006; Rosenberg et al. 2007; Peixoto et al. 2017; Torda et al. 2017).

The realization that multicellular life does not exist without associated microbial partners has uncovered a new and expanding ecological viewpoint that questions how we look at organism to ecosystem function. Host-associated microbes can play important biological roles, contributing to their host's innate immune system and metabolic function (Thaiss et al. 2016), nutrient provisioning (Flint et al. 2012), early development (Fraune & Bosch 2010), gene expression (Larsson et al. 2012) and regulation of their life span (Smith et al. 2017a). As a result, host organisms and their associated microbial counterparts are now regarded as a single ecological entity, the holobiont. Although under fierce debate (e.g., Moran & Sloan 2015), some researchers suggest that the microbial partners undergo co-evolution with their hosts, and thus act as a single entity upon which natural selection may act, as proposed by the Hologenome Theory of Evolution (Zilber-Rosenberg & Rosenberg 2008). This theory is frequently used to explain key ecological interactions between the microbiome and host as well as the holobiont and its environment (Thompson et al. 2015).

While host-associated microbial communities have been long researched in plants (reviewed in Turner et al. 2013), holobiont research has gained further momentum in recent years following the recognition of the importance of the human gut microbiome as an essential and positive health asset (O'Hara & Shanahan 2006). The breakdown of the symbiotic microbial community in the human gut is strongly associated with human disease (Turnbaugh et al. 2009, MacFabe 2012), and exemplifies the microbiome's influence on host performance. Recent advances in sequencing technology, particularly the wide implementation of next-generation sequencing (NGS), have facilitated research on the structure and function of microbial communities through their DNA and RNA. The first NGS studies on coral-associated microbiomes were conducted in the late 2000s (e.g., Wegley et al. 2007, Vega Thurber et al. 2009). Today, NGS techniques are contributing to elucidating the structure and function of the coral microbiome across host taxonomy, space and time (e.g., Vega Thurber et al. 2014, Webster et al. 2016, Neave et al. 2017a, Ziegler et al. 2017; Cai et al. 2018).

Reef-building corals have been identified as some of the most taxonomically and functionally diverse holobionts known (Rohwer et al. 2002; Blackall et al. 2015; Huggett & Apprill 2018). They act as a host to dinoflagellate algae of the family Symbiodiniaceae that aid in coral nutrition and growth (Muscatine & Porter 1977), numerous prokaryotes that contribute to immunity and nutrient cycling (e.g., Pogoreutz et al. 2017a; Neave et al. 2017a; Raina et al. 2013), and fungi whose role has yet to be characterized (Rohwer et al. 2002; Bentis et al. 2000; Amend et al. 2012). The composition of the coral microbiome can be stable or highly variable within

individuals (Sweet et al. 2011a; Apprill et al. 2016), among conspecifics (Guppy & Bythell 2006; Lee et al. 2012; Lema et al. 2014a) and across species (Ainsworth et al. 2015; Hernandez-Agreda et al. 2017). However, a small number of microbes appear to be shared by all or most conspecific, or sometimes congeneric, colonies as part of the "coral core microbiome" (reviewed in Ainsworth et al. 2015 and Hernandez-Agreda et al. 2017). In humans, it has been proposed that metabolic and functional capabilities better represent the microbial core than taxonomy alone (reviewed in Gevers et al. 2012). Although similarly suggested for corals (e.g., Krediet et al. 2013; Peixoto et al. 2017), functional capacity of the coral microbiome is still under investigation (Ainsworth & Gates 2016).

Climate change and the coral microbiome

In the last two decades, the effects of ocean warming on corals have been particularly evident, resulting in a series of global mass bleaching events in 1998, 2002, 2010 and 2016-2017, with continuous bleaching having been reported across all tropical oceans since 2014 (Eakin et al. 2016; Heron et al. 2016; Hughes et al. 2018). Coral bleaching is a stress response where algal symbionts (Symbiodiniaceae) are lost from the coral host via mechanisms such as expulsion, digestion or apoptosis (Weis 2008). This loss causes a paling of coral tissue colouration as the white skeleton becomes visible once the golden-brown algae are lost from the translucent coral tissues (Glynn 1984). Local stressors, such as eutrophication, can exacerbate the effects of global climate change (De'ath et al. 2012), where shifts in nutrient levels can increase the susceptibility of corals to bleaching (Pogoreutz et al. 2017a). Thermal stress has also been found to cause major changes in prokaryote communities (Webster et al. 2016), including increased prevalence of pathogenic bacteria (Rosenberg et al. 2007; Vega Thurber et al. 2009) and changes to bacterial functions such as a diel reversal of nitrogen fixation (Cardini et al. 2016). Local nutrient loading has been associated with increases in coral disease, and is potentially linked with the growth of pathogenic bacteria or increased pathogenicity of those already present (Vega Thurber et al. 2014).

The stress response of the entire microbiome, or particular microbial members, can vary. For instance, "shuffling" or "switching" of Symbiodiniaceae types have been found in some coral species, and result in an increase in the abundances of comparatively heat tolerant strains in response to raised ocean temperatures (Berkelmans & van Oppen 2006; Boulotte et al. 2016). However, the dynamic properties of the coral-associated prokaryotic communities in response to environmental change are rarely explored and often only semi-quantitative. Few prokaryote studies have adequately managed to incorporate large spatial or temporal scales in their research (e.g., Vega Thurber et al. 2014; Roder et al. 2014a, 2015; Ziegler et al. 2017), and even fewer have applied rigorous quantitative techniques like real-time PCR assays (e.g., Pollock et al. 2010;

Santos et al. 2014). This is likely due to timing and field logistics, safety, ethics, permits, or financial considerations that can easily limit these larger scale studies. As a result, many studies use only a single species and minimal replicates, which makes it exceedingly difficult to extrapolate changes in the prokaryotic community, particularly over long time periods. Thus, while there has been evidence for a pathogenic shift in prokaryotic communities in response to stressful environments (Rosenberg et al. 2007; Vega Thurber et al. 2009), the lack of long-term studies may have caused oversight on potentially beneficial, yet longer-term, changes in the microbiome (e.g., Ziegler et al. 2017). Importantly, microbes have short generation times in comparison with their coral host, and may have the opportunity to adapt faster to environmental changes in a way that benefits the coral host (Zilber-Rosenberg & Rosenberg 2008). As explained by the Coral Probiotic Hypothesis (Reshef et al. 2006), any adaptation occurring on the microbial level (whether this is on the community level, e.g., Reshef et al. 2006; or within an individual microbial partner, e.g., Pogoreutz et al. 2017a) could potentially become a beneficial acclimatization tool for the coral host, ultimately promoting resilience.

Environmental pressures can change the relative abundances of particular microbial partners (microbe "shuffling"), force novel associations or removal of particular microbial partners (microbe "switching"), alter microbial gene expression, and/or result in changes to microbial genomes and epigenomes through random mutations, recombination or horizontal gene transfer (Rosenberg et al. 2007; Daniels et al. 2015). Although these variations may be ecologically or evolutionarily "selfish" for the microbial partners, their flexibility in response to environmental pressures may provide an adaptive advantage to the host in the form of enhanced resilience. For example, the plant microbiome is made up largely of bacteria and fungi, where the fungi have been characterized as vital to the plant's health and function, particularly during stress events. Specifically, endophytic fungi, defined as those that reside throughout the tissue of a plant host, have been found to protect plants from salinity stress (Waller et al. 2005), heat stress (Redman et al. 2002), and drought conditions (Rodriguez & Redman 2008). In a seminal paper by Redman et al. (2002), it was found that the thermal tolerance of a grass (*Dicanthelium lanuginosum*) was dependent on the presence of a specific endophytic fungus (*Curvularis* sp.). Further research found that the ability of the fungus to confer thermal tolerance was entirely dependent on the presence of an associated mycovirus (fungal virus) (Márquez et al. 2007).

Similarly for corals, it has been hypothesized that the microbiome can allow for holobiont acclimatization to environmental stressors through changes in microbial diversity and abundances of specific members (i.e., the Coral Probiotic Hypothesis; Reshef et al. 2006). This was recently expanded into the Beneficial Microorganisms for Corals (BMC) concept (Peixoto et al. 2017), which maintains the theoretical components of the Coral Probiotic Hypothesis (Reshef et al. 2006), but further provides a framework for applying this hypothesis as a restoration tool (i.e.,

microbiome engineering; Mueller & Sachs 2015). These concepts rely on understanding how the function of the coral microbiome reflects changes in composition, as well as identifying beneficial functions of particular members of the microbiome.

Empirical evidence from manipulative experiments has confirmed that some microbial members do provide adaptive benefits to their coral host. For instance, associations with algae in the genus *Durusdinium* of Symbiodiniaceae (formerly "Clade D", LaJeunesse et al. 2018), have been suggested to increase thermal tolerance, but at an energetic cost (Jones & Berkelmans 2011), carbon translocation (Cantin et al. 2009) and growth (Little et al. 2004). However, Cunning et al. (2015) found that increases in ocean temperatures may actually eliminate the growth discrepancy between corals associated with *Durusdinium* and those associated with other more thermally sensitive Symbiodiniaceae genera as a result of species-specific growth optima of the coral host. In bacteria, the genus *Endozoicomonas* has been suggested to support thermal or bleaching tolerance due to both their close intracellular proximity to Symbiodiniaceae and an observed negative correlation with both pathogenic bacteria and bleaching (Pantos et al. 2015). Increases in nitrogen-fixing bacteria (i.e., diazotrophs) have also been observed during heat stress (e.g., Santos et al. 2014; Cardini et al. 2016), and have been suggested to be beneficial by providing additional nitrogen to the Symbiodiniaceae that can help maintain holobiont homeostasis (Lema et al. 2012; Santos et al. 2014). However, too high an increase in nitrogen fixation may instead induce the opposite effect and cause a bleaching response (Pogoreutz et al. 2017b). A firm understanding of the functional complexity of the coral microbiome will be essential for characterising the beneficial roles specific microbial members or groups of microbes play in their host's ability to adapt or acclimatize to climate stressors.

Potential for microbiome-driven reef restoration tools

With increasing knowledge of the role microbes play in the health, function and response of corals to climate stressors (e.g., Berkelmans & van Oppen 2006; Bourne & Webster 2013; Lesser et al. 2013; Webster et al. 2016; Ziegler et al. 2017), new information required for developing novel management tools for reefs is becoming available. Such tools can include microbial screenings as indicators of reef health (e.g., Glasl et al. 2017, 2018), or restoration options like phage therapy (Cohen et al. 2013) and manipulation of the microbiome to artificially enhance climate resilience (Santos et al. 2015; van Oppen et al. 2015, 2017; Chakravarti et al. 2017; Damjanovic et al. 2017; Peixoto et al. 2017).

Recently, particular focus has been directed toward theoretical and empirical research on microbial manipulations, an approach known as "microbiome engineering" (Mueller & Sachs 2015). Microbiome engineering is based around the idea that it is possible to improve host performance through employing either artificial selection on the host-microbiome association or

through targeted inoculations of the host with microbes. To date, only a few studies have used an experimental approach to manipulating coral microbiomes (e.g., Santos et al. 2015; Chakravarti et al. 2017; Damjanovic et al. 2017). However, theoretical work (e.g., Reshef et al. 2006; Peixoto et al. 2017) and empirical studies from other systems (e.g., plants, Redman et al. 2011; or humans, Gupta et al. 2016) suggest that it is possible to improve host fitness through artificial selection on the microbiome, either directly (selection on the microbiome) or indirectly (selection on a particular host phenotypic trait). Thus, microbiome engineering approaches to maintaining microbiome health and tolerance to environmental stressors may provide an avenue toward enhancing climate resilience in corals. Lessons learned from other host systems like plants, humans and even wastewater treatment can provide a foundation for developing improved experimental manipulations in corals, which, if proven successful, can be implemented as a tool for coral reef management. While taking action and developing these engineering tools is warranted at present, it does not preclude the necessity for addressing the major knowledge gaps that will help to inform and improve these methods. The efficiency, feasibility and success of manipulating the microbial community in corals will be reliant on our understanding of the function and maintenance of microbial partners, and the community as a whole, through time and when exposed to environmental stressors.

Thesis objective and specific aims

The main objective of this thesis is to explore some of the drivers of community composition of two taxonomic groups of the coral microbiome (the Symbiodiniaceae and the bacteria) in natural systems to elucidate how the coral microbiome may respond to environmental change, and to inform the development of microbiome engineering tools for corals. To place the thesis in context, Chapter 2 reviews the use of microbiome research in development of conservation or restoration tools for reefs. This literature review discusses the established use of microbiome engineering in other systems and explores how it can be applied to corals. It identifies key research goals that will help elucidate the use of microbiome engineering for enhancing coral resilience to climate change while considering the implications this may have for future reef restoration. The research goals that it addresses also identify many areas in coral microbial ecology and microbiology where research is limited or lacking. Linked to this chapter, the subsequent data chapters of this thesis strive to address some of the identified knowledge gaps around the natural variability and stability of the coral microbiome and what drives microbial community composition. Chapter 3 aims to explore the variation in the coral microbiome across two species of *Acropora* on two mid-shelf GBR reefs over a two-year time period to determine whether any changes were driven by season, host species or reef location. Chapter 4 aims to identify any microbial shifts, perhaps beneficial in nature, which may have occurred in a bleaching resilient morph of the coral *Pocillopora acuta* during the 2016 thermal anomaly that

resulted in widespread bleaching across the northern and central GBR. Finally, Chapter 5 aims to determine the influence of parents and the environment on the microbiomes of brooded offspring from the coral *Pocillopora damicornis* to elucidate the microbial transmission mode and understand what drives initial microbial establishment in early life stages.

Advances in our understanding of how the microbiome may shift or fluctuate with environmental change can provide a window into understanding the role microbes play in coral resilience to environmental stressors. Theoretically, increases in abundance or novel acquisition of better-adapted microbial taxa in response to environmental change could provide an avenue for the development of microbial engineering tools to improve coral resilience and survival.

CHAPTER 2

MICROBIOME ENGINEERING: A NEW HOPE FOR ENHANCING CLIMATE RESILIENCE IN CORAL?

ACCEPTED FOR PUBLICATION IN *FRONTIERS IN ECOLOGY AND THE ENVIRONMENT*

Epstein HE, Smith HA, Torda G & van Oppen MJH. (Accepted) Microbiome engineering: A new hope for enhancing climate resilience in coral*? Frontiers in Ecology and the Environment.*

Abstract

Climate change is placing unparalleled pressure on coral reefs worldwide, stimulating research focused on preventing further damage and loss in these ecosystems. The coral microbiome has been widely acknowledged as crucial to coral health and function, playing roles in key biological processes. Recent empirical studies suggest that microbes may contribute to coral host tolerance to thermal stress and harnessing these benefits through microbiome engineering may provide a mechanism for enhancing climate resilience in corals. While coral microbiome engineering is in its infancy, these approaches are already successful in other fields including agriculture, medicine and wastewater treatment, which can provide direction for employing and improving microbiome engineering techniques in corals. This review discusses current uses of microbiome engineering, identifies three key research priorities that will help elucidate the viability of microbiome engineering in corals, and considers the implications the use of these approaches may have for reef restoration.

In a nutshell

- The development of innovative restoration and conservation tools is urgently needed to combat the global climate change-driven decline in coral reefs
- A growing body of evidence indicates microorganisms play critical roles in coral climate resilience
- Other host systems, such as plants and humans, provide evidence for the viability of microbiome engineering as a tool to increase host resilience, as well as direction for experimentation and application of microbiome engineering in corals
- In the face of rapid climate change, microbiome engineering may provide a fast avenue for enhancing climate resilience in corals and has implications for coral reef restoration and management

Introduction

The health and function of all multicellular life on earth is reliant on the consortium of microbial partners that comprise an organism's microbiome. The microbiome includes prokaryotes (i.e., bacteria and archaea), single-celled and filamentous eukaryotes (e.g., fungi and algae), and acellular viruses, and plays a vital role in biological processes (Zilber-Rosenberg & Rosenberg 2008). Microbes that live within and on the tissues of host organisms are at the interface between a host and its environment and can contribute to immunity (Thaiss et al. 2016), nutrition (Flint et al. 2012), metabolic function (Thaiss et al. 2016), early development (Fraune & Bosch 2010), host gene expression (Larsson et al. 2012), and can even regulate life span (Smith et al. 2017a). The immense complexity of host-microbiome interactions demands an expanded ecological and evolutionary view that incorporates the entire "holobiont", that is a host, its microbiome and any other associated symbionts together.

A healthy microbiome is a diverse and highly structured community that is essential to a host's ability to survive changes in environmental conditions (Mueller & Sachs 2015). A robust microbiome is also dynamic; changes in microbiome composition may be required for a healthy holobiont under varying environmental conditions. However, when an organism endures persistent environmental perturbations, the symbiosis between host and microbiome can become compromised, leading to an unpredictable shift in microbial community structure termed dysbiosis (Roder et al. 2014b). Dysbiosis can interrupt microbiome services provided to the host, likely resulting in a loss of host resilience and the onset of disease (Teplitski et al. 2016). With environmental conditions being rapidly altered by anthropogenic climate change, dysbiosis may become more common and this will have major implications for all multicellular life.

Reef-building corals are host to one of the most taxonomically and functionally diverse microbiomes known, greatly exceeding the microbial diversity of the human gut and rivaling that of the sponge (reviewed in Blackall et al. 2015; Huggett & Apprill 2018). The success of corals in colonizing the vast majority of shallow tropical oceans is often credited to their associated microbes, specifically their endosymbiotic dinoflagellate microalgae of the family Symbiodiniacaea (Figure 2.1) and associated prokaryotes. The endosymbiotic microalgae support coral growth and health by contributing to both carbon fixation and carbon translocation to the coral host, which can meet or even exceed its respiratory requirements (Muscatine & Porter 1977). Coral-associated prokaryotes have diverse metabolic roles and contribute substantially to nitrogen (Lesser et al. 2007), carbon (Neave et al. 2017a), and

sulfur (Raina et al. 2013) cycling. Hundreds to thousands of operational taxonomic units (OTUs), or putative species, have been identified within single coral species (Hernandez-Agreda et al. 2017). The number of prokaryotic cells alone can reach more than $1x10^6$ cm^{-2} of coral surface area (Blackall et al. 2015), which is between 100 and 1000 times the number of prokaryotic cells found per square centimetre on human skin (Whitman et al. 1998). Despite the high microbial diversity and variability in corals, researchers are now working on identifying the coral core microbiome, i.e., the microbes that are commonly present among conspecifics or even across species (Hernandez-Agreda et al. 2017). Thus, it has been proposed that the coral microbiome is partitioned into a stable and persistent core component, a species or location-specific component, and a large and highly variable component that is influenced by both biotic and abiotic factors (Hernandez-Agreda et al. 2016).

Rapid climate change as a result of anthropogenic greenhouse gas emissions is putting unprecedented stress on coral reef ecosystems (Hoegh-Guldberg 1999; Hughes et al. 2018). Corals already live close to their physiological and thermal limit, and there is widespread concern that climatic changes will outpace the rate at which corals may adapt or acclimatize (van Oppen et al. 2015, 2017). While microbes have been proposed as one route for rapid acclimatization/adaptation (e.g., due to their shorter generation times; Zilber-Rosenberg & Rosenberg 2008), combined stressors including elevated seawater temperatures, ocean acidification, and eutrophication can have direct effects on coral microbial community structure (Vega Thurber et al. 2009; Ziegler et al. 2017). Anthropogenic environmental changes may drive alterations to or losses in both the Symbiodiniacaea and prokaryote communities (or their activities) that can result in coral bleaching or disease, and may lead to coral death. This process may, in part, drive reef loss on a global scale.

FIGURE 2.1: IMAGE OF A 5-DAY OLD CORAL RECRUIT SHOWING ALGAL SYMBIONTS (SYMBIODINIACAEA SPECIES) CLEARLY WITHIN ITS TISSUES.

As ocean temperatures increase and mass-scale coral bleaching events decimate coral reefs worldwide (Hughes et al. 2018), it has become necessary to focus efforts on preventing further damage and loss. While this ultimately involves tackling climate change and curbing greenhouse gas emissions, targeted efforts are being made toward applying microbiome research to inform the development of conservation tools that may buy time (Figure 2.2). One such approach is microbiome engineering (ME), which is defined here as the experimental manipulation of microbial communities or of host mechanisms of microbial recognition used to improve host or ecosystem performance and fitness. ME can be achieved through exerting artificial selection on the host-microbiome association, inoculating the host with beneficial microbes, genetically engineering specific microbial strains, or a combination of these approaches. It has been suggested that ME could be a powerful avenue for assisting coral resilience to climate change (van Oppen et al. 2015, 2017; Damjanovic et al. 2017). Industries such as agriculture and human medicine now use ME approaches regularly to improve attributes such as crop yields and human health. While only a few studies have implemented an experimental approach to shaping coral microbiomes (e.g., Santos et al. 2015; Chakravarti et al. 2017; Damjanovic et al. 2017), theoretical work (e.g., the Coral Probiotic Hypothesis; Reshef et al. 2006 and the Beneficial Microbes for Coral, or BMC, concept; Peixoto et al. 2017; Panel 2.1) and empirical studies from other systems suggest that artificial selection on a microbiome can improve host fitness over relatively short time frames. ME in corals could provide an important mechanism for disease mitigation and increasing stress tolerance or climate resilience. This review aims to outline current applications of ME, discuss how these

approaches may be applied to corals, and consider the implications they may have for coral reef restoration and conservation. For a list of terminology used in this review, see Table 2.1.

Panel 2.1: Theoretical concepts in coral microbiome engineering

1. The Coral Probiotic Hypothesis

The Coral Probiotic Hypothesis (Reshef et al. 2006) was originally proposed to help explain the evolutionary success of corals. Conceptually, this hypothesis is straightforward – the coral is host to a diverse and metabolically active population of symbiotic microorganisms (the microbiome), whose abundance and diversity can change when faced with changes in environmental conditions in a manner that may allow for holobiont acclimatization or adaptation to those new conditions. Under high selection pressure, these microbiome changes can occur within days to weeks, and may enable host adaptation at a much faster rate than natural selection on the host genome. The Coral Probiotic Hypothesis makes several predictions that can be tested. As outlined in Reshef et al. (2006), these include, but are not limited to:

1. In corals that become resistant to a particular pathogenic bacterium, there should be a visible increase in the abundance of a counteractive bacterial strain that can prevent or inhibit the pathogen.
2. In corals treated with antibiotic compounds, there should be evidence of infection and reduced metabolic activity.
3. When exposed to slowly increasing temperatures, corals should adapt or acclimatize more readily to temperature stress than when temperatures are increased at a more rapid pace.
4. A coral treated with an inoculum of bacteria taken from a stress-adapted conspecific should display an increased rate at which it adapts or acclimatizes to that stress.

2. The Beneficial Microorganisms for Corals Concept (Piexoto et al. 2017)

Piexoto et al. (2017) have recently expanded on the Coral Probiotic Hypothesis by proposing the Beneficial Microorganisms for Coral (BMC) concept. While the main theoretical components of this concept are similar to those of the Coral Probiotic Hypothesis, the BMC concept provides a method for further applying the Coral Probiotic Hypothesis to coral microbial research through two procedures:

1. First, by proposing potentially beneficial mechanisms provided by the microbiome and isolating any potentially beneficial microbial players, or BMC.
2. Second, by empirically testing these BMC for their role in coral resilience to environmental perturbations, both *in situ* and in controlled aquarium systems.

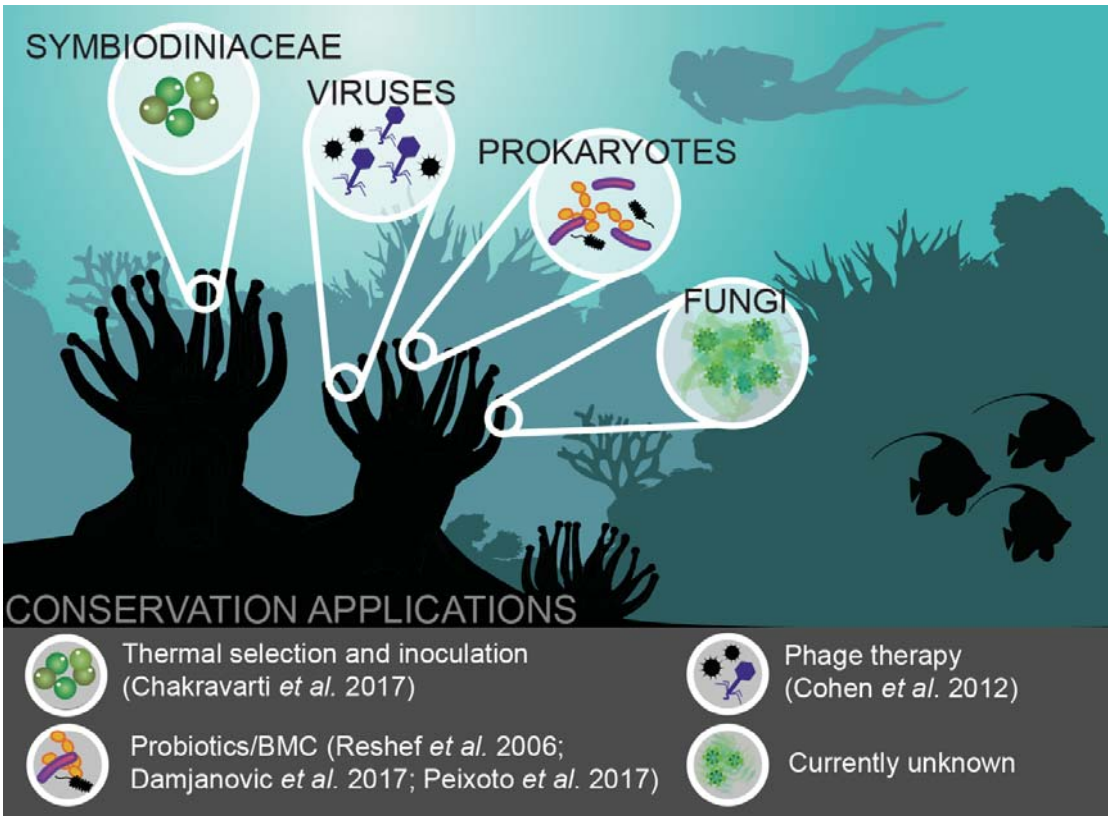

FIGURE 2.2: THE MEMBERS OF THE CORAL MICROBIOME AND POTENTIAL APPLICATIONS FOR THEIR USE IN CORAL REEF MANAGEMENT OR RESTORATION. BMC REFERS TO THE BENEFICIAL MICROBES FOR CORAL CONCEPT (PEIXOTO ET AL. 2017; PANEL 2.1).

TABLE 2.1: LIST OF TERMINOLOGY

Term	Definition
(Intragenerational) Acclimatization	A non-genetic process by which a phenotype of an organism adjusts to its environment (sometimes also called "acclimation", although this term is used by some researchers to indicate adjustment to one or a few experimental conditions)
Adaptation	A change in the mean phenotype of a population as a result of selection on genetic variation
Holobiont	A single ecological entity comprised of the host organism, its microbiome and any other associated symbionts
Horizontal Transmission	Uptake of microbial symbionts from the environment by offspring
Metagenomics	The study of genetic material (DNA) from a group of interacting organisms taken from an environmental (including a host) sample
Meta'omics	A general term that refers to all the "omics" studies including metagenomics, metatranscriptomics and metaproteomics
Metaproteomics	The study of proteins from a group of interacting organisms taken from an environmental (including a host) sample
Metatranscriptomics	The study of expressed genetic material (RNA) from a group of interacting organisms taken from an environmental (including a host) sample
Microbe	Any microorganism, including single-celled and small filamentous eukaryotes, prokaryotes (bacteria and archaea), fungi and viruses
Microbiome	A collection of microbes inhabiting an environment (including a host organism)
Microbiome engineering	Any kind of manipulation of the microbiome, including targeted inoculations or probiotics as well as genetic engineering of individual microbial organisms
Operational Taxonomic Unit (OTU)	A group of closely related individuals, clustered based on DNA sequence similarity in a certain taxonomic marker
Quorum Sensing	A form of cell-to-cell signalling in bacteria that is regulated by chemical signal molecules and used to coordinate group behavior
Rhizosphere	The narrow region of soil directly adjacent to plant roots that is influenced by root secretions and associated soil microbiota
Symbiont	A species that lives in tight association with another species
Symbiosis	The long-term intimate association of two or more species
Vertical Transmission	Direct transmission from parents to offspring of microbial partners

Current Applications of ME

ME in Plants and Soil

Plants live in a tight association with a complex and diverse microbial community that inhabits both their tissues and the soil in which they reside. Fungal communities within plant tissues provide protection from salinity (Waller et al. 2005) and heat stress (Redman et al. 2002), as well as drought conditions (Rodriguez & Redman 2008). For instance, in a seminal paper by Redman et al. (2002), the thermal tolerance of the grass species *Dicanthelium lanuginosum* was found to be dependent on the presence of a specific fungus of the genus *Curvularia*. The region of soil directly adjacent to plant roots, the rhizosphere, also hosts microbiota that play active roles in mediating nutrient cycling, pathogen resistance, host immunity, host adaptation and stress tolerance in plants (reviewed in Berendsen et al. 2012).

Exploitation of soil and plant microbiota through ME approaches has been implemented to modify specific traits to improve plant and crop quality and productivity. By using a combination of ME approaches, Panke-Buisse et al. (2015) were able to use rhizosphere bacterial inocula to modify the flowering time of plants. Over ten generations, rhizosphere bacterial communities were selected for either early or late flowering time of the plant *Arabidopsis thaliana.* These bacterial communities were then introduced to the rhizosphere of four new host plants, where they were able to reproduce a shift in their respective host's flowering time (Panke-Buisse et al. 2015). Alteration of specific host traits through microbiome manipulation provides a platform for improving agriculture and food security needs, or for increasing plant host resilience to disease and environmental stressors. Other implementations of ME approaches that utilize a plant-soil feedback include crop rotations that re-shape soil microbiota to increase the yield of subsequent crops (Deguchi et al. 2007), inoculations with specific rhizosphere bacterial strains to promote plant growth (Rojas-Solís et al. 2018), and soil inoculations with rhizosphere bacterial mixtures to promote systemic resistance against pests and disease (reviewed by Ramamoorthy et al. 2001). Further, direct wound inoculations have also been found to confer pathogen resistance. For instance, endophytic bacteria isolated from the Manuka plant, which naturally produces antimicrobial oils, have been used to treat bacterial canker in the kiwifruit (Wicaksono et al. 2018).

ME in Disease Mitigation

It has become increasingly evident that many human diseases are strongly linked to the breakdown of the symbiotic microbial community (e.g., obesity; Turnbaugh et al. 2009 or inflammatory bowel disease; Gupta et al. 2016). Mitigation of some diseases may be achieved through manipulating the human microbiome. For instance, dysbiosis of the intestinal microbial community in humans is suggested to promote inflammation and cause inflammatory bowel disease (IBD). IBD is characterized by the inflammation of the gastrointestinal tract and includes diseases such as ulcerative colitis and Crohn's disease. One potential treatment of IBD is the use of fecal microbiota transplants (FMT), which introduce fecal matter from a healthy donor to the intestinal tract of a diseased recipient to "re-set" the disrupted microbiome (reviewed in Gupta et al. 2016).

ME has been identified as a particularly important tool for mitigating outbreaks of vector-borne human diseases. Instead of the human microbiome, the microbiome of the pathogen vector (e.g., the mosquito) is targeted for manipulation. Mosquitoes can carry pathogens that cause serious human diseases such as Zika virus, yellow fever and dengue fever (Benelli 2015). Dengue fever, in particular, is one of the more common diseases affecting humans in tropical and sub-tropical areas across the globe. Traditionally, management of dengue fever has been focused on eliminating the mosquitoes themselves through insecticidal regimes, but these programs have been insufficient as evidenced by the increase in contracted cases of dengue (Schmidt et al. 2017). However, recent work has shown that inoculating dengue-carrying mosquitoes with the bacterium *Wolbachia* shortens the mosquito's lifespan and consequently reduces the potential for disease transmission to humans (McMeniman et al. 2009). *Wolbachia* has also been found to reduce the susceptibility of mosquitoes to initial dengue infection (Moreira et al. 2009) and can limit the replication of dengue virus within the mosquito (Frentiu et al. 2014). It is possible that the inoculation of *Wolbachia* into mosquitos will become an important control measure for the suppression of dengue fever in humans. Research is now focused on how to up-scale the spread of *Wolbachia*-inoculated mosquitoes to cover large urban areas (e.g., Schmidt et al. 2017; The World Mosquito Program http://www.eliminatedengue.com/program).

Inoculation with certain microbes can also prevent disease in non-human hosts. For example, bacteria recovered from coral mucus and Symbiodiniaceae symbionts can disrupt biofilm formation of the necrotizing coral pathogen *Serratia marcescens* through the inhibition of quorum sensing (Alagely et al. 2011). Quorum sensing is the cell-to-cell communication used by bacteria to control collective behavior and organize themselves spatially, particularly into biofilms. This behavior can allow these biofilms to resist anti-microbial or anti-bacterial

compounds and can also help in the regulation of pathogenicity (reviewed by Pasmore & Costerton 2003). To experimentally test the anti-microbial effect of the bacteria recovered from coral mucus and Symbiodiniaceae, Alagely et al. (2011) inoculated the anemone *Exaiptasia pallida* with the pathogen *S. marcescens*, after being introduced to a cocktail of bacteria. They found that the use of the beneficial bacteria as a probiotic inhibited the progression of the disease caused by *S. marcescens* by blocking its quorum sensing and thus interfering with biofilm formation (Alagely et al. 2011). The results of this study not only helped prove antimicrobial properties of the coral mucus and Symbiodiniaceae while identifying the success of inhibiting quorum sensing, but it was also one of the first studies to test the feasibility and viability of probiotics in a cnidarian model.

ME in enhancing stress tolerance in corals

Research and development of ME applications to corals are still in their infancy and face many challenges due to the large number of species and spatial areas that are involved. One of the first successful empirical studies of coral microbiome manipulation explored the possibility of using a microbial inoculation to increase the resistance of corals to oil pollution (Santos et al. 2015). It was found that inoculating coral with a consortium of bacteria that had been selected for its ability to degrade water-soluble oil (i.e., a BMC consortium) reduced the negative health impacts of oil exposure to the coral host in an aquarium experiment, showing 38% greater photosynthetic efficiency (Fv/Fm) than treatments without the bacterial consortium (Santos et al. 2015). Other studies have begun to investigate the possibility of inoculating corals with heat-selected members of the Symbiodiniaceae (Chakravarti et al. 2017), or communities of bacteria from “donor” heat-resistant corals (Damjanovic et al. 2017) with the aim to increase coral resilience to thermal stress. While both studies found successful inoculation, the extent to which these manipulations conferred thermal tolerance to the coral host and the long-term stability of the introduced symbiosis remain uncertain. Coral ME is clearly still in the “proof of concept” stage, but these empirical studies provide a promising foundation for future experimental work.

The way forward: research priorities for ME in corals

Like other host-microbiome systems, harnessing the benefits of the coral microbiome is a challenge due to the high diversity and both the spatial and temporal variability of microbial partners in corals. In order to progress the field of coral ME, a focus on specific research topics that will help address major knowledge gaps is required. Three major research priorities are identified here that will help elucidate the viability of using ME as a restoration tool for corals: 1) identify beneficial microbial functions, 2) identify the stability and

maintenance of microbial partners, and 3) trial experimental manipulation of the coral microbiome.

1.Identify beneficial microbial functions

Understanding and identifying the functional roles, if any, of the microbial consortia and/or individual microbes is essential in determining which services provided by the microbiome can be harnessed to increase coral host climate resilience. If specific microbes assert control over a host phenotypic trait, they will be key targets for ME (e.g., *Wolbachia* in mosquitos, McMeniman et al. 2009; *Curvularia* in grass, Redman et al. 2002). For corals, this was recently proposed as the BMC concept (Peixoto et al. 2017), and could be used to increase thermal tolerance. Functional analysis can be performed through traditional culturing methods in combination with phenotypic assays, metagenomics and metabarcoding, metatranscriptomics and metaproteomics to decipher the functional properties of either the entire microbiome or specific microbial partners within it (Marx 2017). Metabarcoding has already been widely applied to corals (see Blackall et al. 2015), and while the functional potential of the microbiome can be predicted from barcode data such as the 16S rRNA gene (e.g., through predictive functional profiling programs such as PICRUSt; Langille et al. 2013), but identifying true function requires genome sequences. Metatranscriptomics and metaproteomics reveal which microbial genes are active at a given time point. Although still facing challenges such as eukaryotic (host) contamination (e.g., Frazier et al. 2017; Meyer et al. 2017), this approach has been implemented in more recent studies, such as an examination of active microbial players in coral disease (e.g., Daniels et al. 2015), and the identification of key proteins of healthy and diseased corals providing insight into how the transition occurs between these two states (Garcia et al. 2016). Thus, ‘omics techniques, in addition to traditional culturing methods, may offer further elucidation of active and important microbial partners during climate-based stress events, such as coral bleaching, which can then be targeted for ME.

2. Identify the stability and maintenance of microbial partners

Deciphering which microbes are stably associated with corals is important for the development of microbial manipulations to enhance coral climate resilience. For instance, if probiotic inocula are comprised of microbes that form a temporally stable association, they are more likely to provide long-term benefits to the coral host. If they are not, the benefits will likely be only short-term. It is well documented that coral microbial communities are susceptible to ontogenetic shifts and changes due to environmental variation (Figure 2.3). Nevertheless, a growing body of evidence suggests that despite these temporal and spatial changes in microbial communities, a subset of taxa is stably associated with coral species or

genera (i.e., the core microbiome; reviewed in Hernandez-Agreda et al. 2017). Increasing our understanding of how and when temporal and spatial shifts occur, such as the natural winnowing of the coral microbiome (e.g., Lema et al. 2014b), and whether they represent active host-controlled processes (e.g., Sweet et al. 2011b) will allow us to determine a possible developmental stage or time at which introduced manipulations would be more likely to remain stable over time.

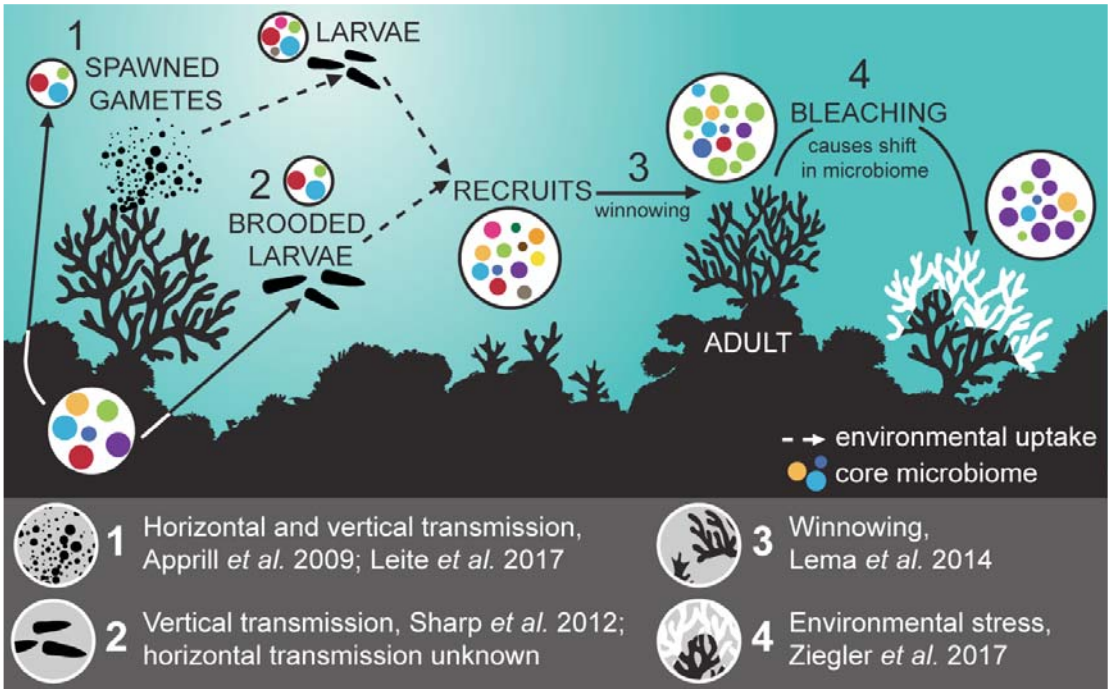

FIGURE 2.3: SCHEMATIC OF THE TRANSMISSION OF CORAL-ASSOCIATED PROKARYOTES ACROSS ONTOGENETIC STAGES AS WELL AS SHIFTS IN COMMUNITY COMPOSITION RESULTING FROM AN ENVIRONMENTAL STRESS EVENT SUCH AS BLEACHING. WHILE SOME CORE MEMBERS REMAIN THE SAME (REPRESENTED BY BLUE AND GOLD DOTS), THE PROKARYOTE COMMUNITY COMPOSITION CAN BE HIGHLY AFFECTED BY ENVIRONMENTAL VARIATION.

The stability of microbial associations with hosts has previously been suggested to relate to the mode of transmission, where maternally transmitted symbionts are typically more stably associated with their host than those transmitted from the environment (reviewed by Moran et al. 2008). However, this is not always the case. In corals, reproductive strategy is highly correlated with the mode of transmission of the dinoflagellate endosymbionts, where most broadcast spawning corals exhibit horizontal transmission (environmental transmission) and most brooders (corals that develop their larvae within the body cavity of polyps) exhibit vertical transmission (parental transmission; Lesser et al. 2013). While the long-term stability of Symbiodiniacaea symbionts in brooding corals exceeds the stability in broadcast spawners (Thornhill et al. 2006a), some brooders acquire additional symbionts from the environment, which can also be maternally transmitted to the next generation (Quigley et al. 2018). Additionally, other invertebrates have been found to form stable relationships with horizontally transmitted microbes. For example, the Hawaiian bobtail squid initially acquires

the bacterium *Vibrio fischeri* from the environment and harnesses the bacterium's bioluminescence ability for use in a bacterial light-producing organ. This symbiosis is stable throughout the life of the squid and is maintained through the expulsion of a majority of the bacteria during the day (time of quiescence), and subsequent re-growth of the bacterial population prior to night (time of activity; reviewed in Nyholm & McFall-Ngai 2004).

The mode of transmission of prokaryotes in corals appears to be less strictly correlated to reproductive strategy. For instance, horizontal transmission of prokaryotes has been detected in the broadcast spawning coral species *Pocillopora meandrina* (Apprill et al. 2009), where it was suggested that external bacteria are incorporated into the ectodermal tissues of late stage planulae via phagocytosis. Conversely, *Mussismilia hispida*, also a broadcast spawner, has been found to vertically transfer prokaryotes from parental mucus to gametes prior to spawning (Leite et al. 2017). Increasing our understanding of prokaryote transmission in a range of coral species, along with assessing the maintenance of these prokaryotic associations (e.g., whether any host or microbe-controlled cellular mechanism is at play) through time or across generations, may help to elucidate microbiome stability and identify better BMC targets for ME trials.

3. Trial experimental manipulation of the coral microbiome

Lessons learned from other host systems such as plants and humans can provide a foundation for developing improved experimental manipulations in corals that, if proven successful, can subsequently be implemented as a tool for improving the success of coral restoration initiatives. Two main experimental approaches to manipulating microbiomes, through direct and indirect selection (Mueller & Sachs 2015), may be particularly useful for structuring coral ME research (Panel 2.2). Direct selection on the microbiome can be used to identify and target specific microbes responsible for thermal or other stress tolerance (e.g., Santos et al. 2015; Chakravarti et al. 2017). Advances in coral ME for addressing climate change impacts may also focus on indirect selection. For instance, large multi-generational experiments that examine the effects of current and future climate change on corals (e.g., Evolution 21; https://www.aims.gov.au/evolution-21), provide an opportunity to obtain critical information on how microbial communities respond to climate change. As corals acclimatize to these future conditions either within or across generations, it may be possible to identify key microbial partners that aid in acclimatization of the coral holobiont to future conditions.

Experimental manipulations, whether employing direct or indirect selection, provide an important tool for addressing many of the knowledge gaps already identified. For instance, if a manipulation experiment is successful in achieving a desired trait without having prior knowledge of the underlying biological/microbial mechanisms, it is possible to work

backward to hypothesize how and why we may see shifts in the microbiome. The treatment of wastewater began in this way. Since the early 1900s, the nutrient cycling capabilities of microorganisms have been harnessed to remove excess nutrients from industrial or municipal wastewater. However, these were “black-box” treatments, where the chemical composition of influent and effluent was known, but the biochemical pathways were only hypothesized and the microbial species involved were unknown. For example, the Enhanced Biological Phosphorous Removal (EBPR) method was first employed in the 1970s (Barnard 1974), but it was not until the 1990s that polyphosphate accumulating microbes were confirmed to be responsible for phosphate removal from the wastewater (Jenkins & Tandoi 1991). EBPR works by cycling influent wastewater and its associated microbial biomass through an anaerobic zone followed by an aerobic zone in order to place selection pressure on the microbial biomass. Cycling in this way results in a selection of microorganisms with a higher capacity for intra-cellular accumulation of polyphosphates (reviewed by Blackall et al. 2002). The discovery of microorganisms as the main drivers for wastewater treatment was a revelation and modern methods are now informed by more rigorous microbiological and microbial ecological studies to enhance treatment efficiency (reviewed by Barnard et al. 2017). Although wastewater treatment is a closed system, contrary to the natural system in which corals are found, this built-environment example clearly demonstrates that working backward from experimental manipulations can result in important advances in ME techniques, and provides a unique view on using indirect selection pressure to manipulate a microbial community to select a targeted function.

Panel 2.2: Experimental Approaches to Microbiome Engineering in Corals

Two main experimental approaches, originally identified by Mueller and Sachs (2015) for plants, can be applied to structuring coral ME research: direct and indirect selection of the microbiome.

1. Direct selection (Figure 2.4a): selection of a specific beneficial microbe or community of microbes for improved host performance or fitness. **Advantages:** high control over which individual taxa or communities can be manipulated, allowing for specific beneficial roles to be targeted. **Disadvantages**: it is necessary to have prior knowledge of microbial or microbiome function through meta'omics or culturing methods, or through characterising holobiont or microbial phenotypes in order to choose the appropriate beneficial microbe or community of microbes. Further, this can only be done for culturable microbes. **Example**: Redman et al. (2011) found that inoculating rice plants with a specific endophytic fungus increased growth rate, biomass and reproductive yield while reducing water consumption by 20-30 per cent, allowing the rice plants to thrive in drought conditions. **Application to corals**: Similar to the BMC concept (Peixoto et al. 2017), direct selection on the microbiome can be performed in corals by isolating microbes with certain traits and using these isolates to create an inoculum. Experiments of this sort should be done on replicate but known coral genotypes to reveal effects of the host genotype on the microbiome.

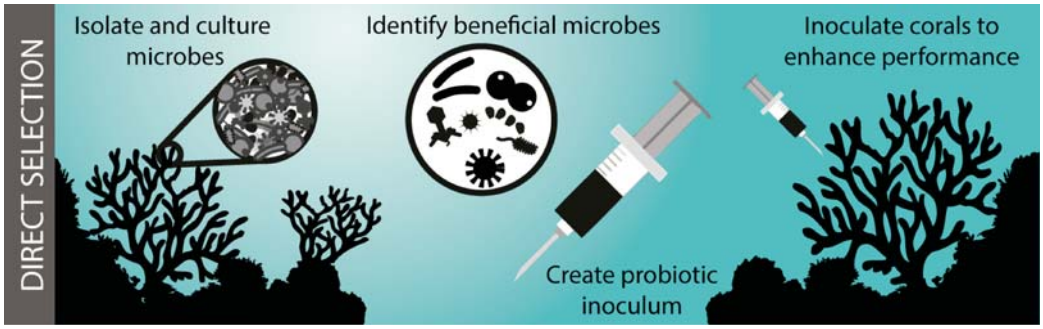

FIGURE 2.4A: IMPLEMENTATION OF DIRECT SELECTION OF BENEFICIAL MICROBES FOR INOCULATION.

2. Indirect selection (Figure 2.4b): selection of a specific host trait (phenotype), allowing for an indirect selection of microbiome function. **Advantages**: prior knowledge of microbiome function is not necessary, meaning it can be a more cost-effective method of ME. **Disadvantages**: Experiments may span multiple generations of the host and thus be time-consuming, and there is little to no control over which microbial taxa are being manipulated, making it difficult to reproduce results. It must also be demonstrated that the microbiome did in fact change and play a role in selection of the host trait. **Example:** By selectively breeding the best performing wheat plants that were growing in acidic soils, indirect selection for aluminium resistance in wheat (a process controlled by the microbiome) was achieved (de Sousa 1998). **Application to corals**: This approach, which has been previously proposed as a mechanism of coral assisted evolution (van Oppen et al. 2015), will involve the use of a selection pressure, such as elevated temperature, to select for a specific phenotypic trait (e.g., reduced susceptibility to bleaching). From here, it is possible to either continue down a selective breeding pathway of the host, or to create an inoculum with the selected host's tissue and/or mucus to inoculate other corals.

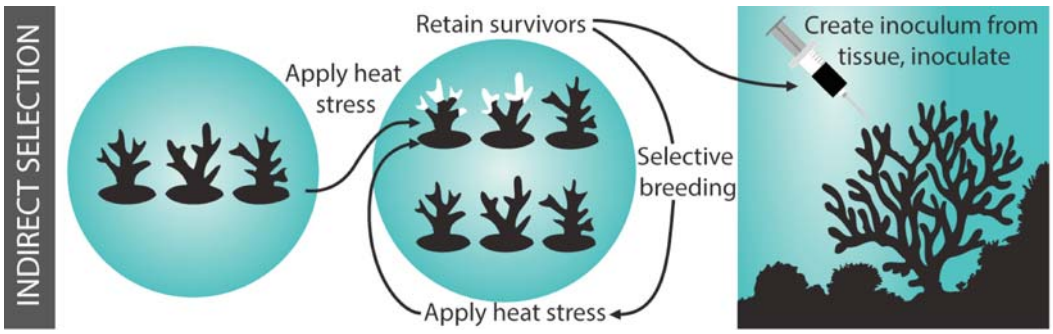

FIGURE 2.4B: IMPLEMENTATION OF A SELECTION PRESSURE ON THE CORAL HOST TO INDIRECTLY SELECT BENEFICIAL MICROBES FOR INOCULATION.

For either method to be successful, it is essential to optimize ME in a way that targets key coral host traits that will enhance climate resilience. These traits can include reduced susceptibility to bleaching, increased disease resistance, enhanced calcification or growth, or increased fecundity.

Conclusions

The broad spectrum of ME approaches provides a powerful basis for the application of ME to enhance climate resilience in coral. However, the field of coral ME is in its infancy. Current progress is directed at proof of concept, which provides the foundations for future management options, but requires further empirical and applied studies to advance toward successful implementation. Success in other fields such as agriculture, medicine and wastewater treatment can provide direction and guidance for applying ME approaches in corals to improve coral health, increase ecosystem services and enhance resilience to the stressors of climate change. In this review, three key research priorities have been identified that will foster successful development of ME approaches in corals, address crucial knowledge gaps, and provide insight into the biological challenges of implementing these approaches in a naturally complex environment and on large geographic scales. With climate stressors causing unprecedented change on coral reefs, effective conservation, restoration and management are more critical than ever. As a result, ME may become an important tool for coral reef restoration. However, this intervention should be combined with other practices to help resolve the coral reef crisis - the most important of which is the curbing of greenhouse gas emissions.

CHAPTER 3

TEMPORAL VARIATION IN CORAL MICROBIOMES DOES NOT REFLECT SEASONALITY

IN PREPARATION FOR PUBLICATION

Epstein HE, Smith HA, Bay L, Cantin N, Mocellin V, Torda G & van Oppen MJH (In prep). Temporal variation in coral microbiomes does not reflect seasonality.

Abstract

The coral microbiome is known to fluctuate in response to environmental variation and has been suggested to vary seasonally. However, most studies to date, particularly studies on bacterial communities, have examined temporal variation over a time frame of less than one-year, which is insufficient to establish if microbiome variations are indeed seasonal in nature. The present study was focused on expanding our understanding of long-term variability in microbial community composition using two common branching coral species, *Acropora hyacinthus* and *Acropora spathulata* at two mid-shelf reefs on the Great Barrier Reef. By sampling over a two-year time period, this study aimed to determine whether temporal variations reflect seasonal cycles over a two-year time period. Community composition of both bacteria and Symbiodiniaceae was characterized through 16S rRNA gene and ITS2 rDNA metabarcoding, respectively. The results confirmed that the coral microbiome is dynamic and complex, with significant variations in community composition of bacteria and Symbiodiniaceae over time for *A. hyacinthus* and *A. spathulata*. However, there was no evidence to suggest that temporal variations were cyclical in nature and represented seasonal variation. Thus, in order to identify the basis of temporal patterns in coral microbial community composition, future studies should employ longer time series of sampling at sufficient resolution to identify the environmental correlates of microbiome variation.

Introduction

Scleractinian corals are complex holobionts that host a high diversity and abundance of microbial symbionts (Blackall et al. 2015), some of which are essential to holobiont health and function. Endosymbiotic microalgae of the family Symbiodiniaceae support coral growth and health by contributing carbon and other nutrient requirements to the coral host (Lewis & Smith 1971; Muscatine & Porter 1977; Baker 2001). Similarly, prokaryotes (i.e., bacteria and archaea) play a role in nutrient cycling, nitrogen fixation and innate immunity (Rohwer & Kelley 2004; Ritchie 2006; Raina et al. 2009; Kimes et al. 2010). Other important nutrients and metabolic pathways

may also become available as a result of interactions between all members of the holobiont (Ainsworth et al. 2015), and can affect relative environmental tolerance limits (Baker 2001; Berkelmans & van Oppen 2006; Robison & Warner 2006).

The microbiome of corals is not static and its members can fluctuate as a result of changes in environmental conditions, or possibly due to host regulatory mechanisms (reviewed in Bourne et al. 2016). Symbiodiniaceae communities have been found to fluctuate according to season (e.g., Chen et al. 2005; Ulstrup et al. 2008), but can also remain stable through time (e.g., Thornhill et al. 2006a,b). Additionally, significant stress events, such as high temperatures or bleaching, can cause these microalgal symbionts to undergo shuffling (e.g., Berkelmans & van Oppen 2006) or trigger the acquisition of novel strains from the environment (i.e., "switching") (e.g., Boulotte et al. 2016). Further, the environment can modulate the initial association of Symbiodiniaceae, particularly in coral species that acquire these symbionts from the external environment during early development (LaJeunesse et al. 2004a). In prokaryote partners, variability can also occur as a result of naturally changing environmental conditions (reviewed in Thompson et al. 2015). However, some bacterial members have been found consistently within coral tissue, suggesting that there is a small number of stable members (i.e., the "coral microbial core"; Hernandez-Agreda et al. 2017). Further, coral bacterial communities can vary geographically, where the same species at different locations can harbor vastly different communities of bacterial partners (e.g., Littman et al. 2009; Lee et al. 2012; Hernandez-Agreda et al. 2016). Thus, it has been proposed that the coral bacterial community can be partitioned into a stable core component, a site-specific component, and a dynamic and variable component highly influenced by changes in abiotic and biotic factors (Hernandez-Agreda et al. 2016; Leite et al. 2018).

Recent advances in our understanding of microbial community composition and how it changes in response to environmental change have highlighted the potential role microbes play in coral host resilience to environmental stressors, such as the impacts of climate change. Environmentally driven changes in the microbiome that result in increases or incorporation of better-adapted microbial taxa could theoretically aid or improve coral survival (e.g., Reshef et al. 2006; van Oppen et al. 2015, 2017; Peixoto et al. 2017; Torda et al. 2017; Chapter 2). Understanding natural variability, particularly the potential for cyclical seasonal variation, of the coral microbiome can provide insight into how the microbiome may respond to environmental or even climatic fluctuations. Seasons can present natural changes in factors such as temperature and irradiance (Warner et al. 2002; Bahr et al. 2017), calcium carbonate levels and aragonite saturation rate (Bates et al. 2010), and nutrient content (particularly for coastal reefs influenced by run-off during rainy seasons; Costa et al. 2006). Long-term studies are available for Symbiodiniaceae communities, which have identified that some coral species exhibit seasonal variation in community composition (e.g., Chen et al. 2005; Ulstrup et al. 2008) while others remain stable

through time (e.g., Thornhill et al. 2006a,b). Seasonal changes in Symbiodiniaceae may also manifest as changes in cell density, pigmentation or photo-efficiency (Fitt et al. 2000; Warner et al. 2002; Ulstrup et al. 2008). Variations in the coral bacterial community among time points have been suggested to reflect seasonal differences in their environment (e.g., Ceh et al. 2011; Kimes et al. 2013; Li et al. 2014; Sharp et al. 2017; Cai et al. 2018); however, all of these studies have lasted less than one year, which is insufficient to test hypotheses regarding seasonality. Indeed, one longer-term study (Yang et al. 2017) found the bacterial community of the brooding coral *Stylophora pistillata* to be dynamic, but not reflective of seasonal cycles (Yang et al. 2017). Further long-term studies are needed to assess whether microbiome communities exhibit strong seasonal variation.

The present study aimed to expand our understanding of long-term fluctuations in microbial community composition and examine whether temporal variations within the coral microbiome, including both Symbiodiniaceae and bacterial communities, correlate with a seasonal cycle. The bacterial and Symbiodiniaceae community composition of two common species of branching coral, *Acropora hyacinthus* and *Acropora spathulata*, were characterized 5 times across a two-year time period from two mid-shelf reefs on the Great Barrier Reef (GBR), Australia. Specifically, seasonality in the microbiome was examined by using DNA metabarcoding to examine the community structure and taxonomic composition of both bacteria and Symbiodiniaceae, as well as the co-occurrences of these microbial taxa through time.

Methods

Sample Collection and Processing

Twelve colonies each of two species of coral, *Acropora hyacinthus* and *Acropora spathulata*, at two mid-shelf reefs in the central GBR, Rib reef (18°29’4.8” S, 146°52’13.7”E) and Davies reef (18°49’23.8”S, 147°38’56.2”E), were tagged and sampled from approximately 5 m depth over a two-year time period. Sampling took place in February/March (end of summer) and October/November (end of winter) of both 2014 and 2015, as well as an additional time point in April 2015, making a total of five time points. At each time point and each location, a small nubbin of each colony was collected and immediately snap-frozen in liquid nitrogen (LN_2). Frozen samples were then freeze-dried and crushed using a hydraulic bench top laboratory press prior to DNA extraction. Average monthly temperature data over the duration of the study period from both Rib and Davies reef were obtained from publicly available data collected by the Australian Institute of Marine Science (AIMS Historical Data Tool: http://data.aims.gov.au/aimsrtds/datatool.xhtml).

DNA was extracted using a traditional salting out method with an added lysozyme digestion and bead-beating step (Damjanovic et al. 2017). Amplification of double-stranded products from the

16S rRNA gene for bacteria and the internal transcribed spacer region 2 (ITS2) was achieved through polymerase chain reaction (PCR) using gene-specific primers. The V5-V6 region of 16S was targeted using the primers 784F 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGATTAGATACCCTGGTA-3' and 1061R 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCRRCACGAGCTGACGAC-3' (Andersson et al. 2008). ITS2 was targeted using the primers ITS2F 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGAATTGCAGAACTCCGTG-3' and ITS2R 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCTCCGCTTACTTATATGCTT-3' (Pochon et al. 2012). Both sets of primers included the Illumina adapter overhangs for illumina MiSeq sequencing, underlined in the above primer sequences.

The 16S PCR was carried out in triplicate 10 µL reaction volumes, resulting in 30 µL pooled PCR product. Each reaction consisted of: 5 µL of AmpliTaq Gold MasterMix (Applied Biosystems), 2 µL of each primer (2 µM stock), and 1 µL of DNA template. All 16S reactions were run on a Kyratec SC-200 thermal cycler (Kyratec Life Science) using the following protocol: initial denaturation at 95°C for 10 minutes, then 30 cycles of denaturation at 95°C for 30 seconds, annealing at 57°C for 60 seconds and extension at 72°C for 60 seconds, followed by a final extension at 72°C for 7 minutes. The ITS2 PCR was also carried out in triplicate 10 µL reactions. Each reaction consisted of 5 µL of Qiagen Mulitplex MasterMix (Qiagen), 3 uL Milli Q water, 0.5 µL of each primer (4uM stock), and 1 µL of DNA template. All ITS2 reactions were run on a Kyratec SC-200 thermal cycler (Kyratec Life Science) using the following protocol: 95°C for 5 minutes, then 31 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 5 minutes. All PCR products were then examined using gel electrophoresis on a 2% TBE-agarose gel stained with Ethidium Bromide (EtBr). Some ITS2 products displayed double-banding, representing both the target and a mitochondrial band. The target bands were poked using the tip of a clean pipette, introduced to clean PCR master mix, and underwent a second PCR with the same specifications but only 12 cycles of denaturation, annealing and extension. These products were again checked by gel electrophoresis to ensure no double-banding prior to sequencing. PCR clean-up, indexing and sequencing were carried out at the Ramaciotti Centre for Genomics at the University of New South Wales on a 2x300bp Illumina MiSeq run. Data was returned as de-multiplexed paired-end sequences.

Sequence Assembly, Quality Control and Taxonomic Assignment

Demultiplexed sequences for both 16S and ITS2 were assembled, checked for quality and assigned taxonomic classification using a QIIME2 v 2017.10 pipeline with additional plug-ins (Caporaso et al. 2010). The plug-in demux (Caporaso et al. 2010) was used for visualising read quality and setting quality filtering guidelines. Quality filtering, trimming of poor-quality bases, de-replication, chimera filtering, merging paired-end reads, and the identification of amplicon sequence variants (ASVs) were performed using the DADA2 plug-in (Callahan et al. 2016). For 16S, mitochondrial and chloroplast sequences were removed and taxonomy was assigned by training a naïve-Bayes classifier on the V5-V6 region of the 16S gene in the SILVA 128 database (Quast et al. 2013) using the feature-classifier plugin (Caporaso et al. 2010) to match the primers used. Due to the high number of single-variants found for ITS2 and the subsequent small taxonomic database, it was not useful to use a classifier as above because the resolution was too low. Therefore, these single-variants for ITS2 were clustered by 97% similarity using a vsearch plug-in (Rognes et al. 2016). Taxonomic assignment was done according to the database from Arif et al. (2014). This allowed assignment down to the sub-type level for Symbiodiniaceae. At the end of the pipelines for both 16S and ITS2, the taxa plug-in (Caporaso et al. 2010) was used to create a feature table (biom table) and a taxonomy table with raw sequence counts that could then be used for further downstream analyses.

Statistical Analyses

Data were read into R v. 3.5.0 (R Core Team 2018) and analysed using the package phyloseq v. 1.25 (McMurdie & Holmes 2013). Contaminants and singletons were removed from the dataset prior to further analyses. Contaminants were identified using methods outlined in Lee et al. (2015a); as contaminant taxa are expected to have high relative abundance in negatives and low relative abundance in samples, any ASVs with a ratio of relative abundance of one or above in negatives compared with samples were removed. Variations in alpha diversity (Shannon diversity index) and observed species richness of both bacterial and Symbiodiniaceae from the two coral species at both reefs and among time point were analysed by analysis of variance (ANOVA) using a linear model fit by restricted maximum likelihood (REML) for repeated measures with an added autoregressive 1^{st} order (AR1) correlation structure to account for time series autocorrelation in the R packages car v. 3.0 (Fox & Weisberg 2011) and nlme v. 3.1-137 (Pinheiro et al. 2018). Post-hoc comparisons were made using Tukey's test with the packages multcompView v. 0.1-7 (Graves et al. 2015) and lsmeans v. 2.27-62 (Lenth 2016). Differences in beta-diversity among species, reefs and time points were assessed using permutational multivariate analysis of variance (PERMANOVA) blocked by colony to account for repeated measures. Homogeneity of dispersions was assessed using PERMDISP. Both PERMANOVA and

PERMDISP were run with 999 permutations and beta-diversity was visualized using NMDS fit with environmental variables through constrained correspondence analysis (CCA) using the function envfit in vegan v. 2.5-2 (Oksanen et al. 2018). Further exploration of microbial communities included visualising relative abundances with ggplot2 v. 2.2.1 (Wickham 2009), and indicator taxa were identified for each coral species at each reef among repeated time points (i.e., February and Oct/Nov) using a multi-level pattern analysis with 999 permuations in the package indicspecies v. 1.7.6 (De Cáceres & Legendre 2009).

Co-occurrences between bacteria and Symbiodiniaceae were determined using Spearman Rank correlation coefficients on ASVs appearing at least once in 20% or more of the samples using the packages corrplot v. 0.84 (Wei & Simko 2017) and igraph v. 1.2.1 (Csardi & Nepusz 2006). Correlation matrices of each species per reef were visualized in corrplot v. 0.84 (Wei & Simko 2017). Significant correlations (> 0.6 and < -0.6, $p < 0.05$) were identified and visualized as networks for each time point using Cytoscape v. 3.6.1 (Shannon et al. 2003).

Results

Raw sequence data for this chapter will be available on the NCBI Sequence Read Archive under the accession PRJNA491379 after publication of this work.

Temperature at Rib and Davies Reef

The average monthly temperatures for both Rib and Davies reefs maintained a seasonal curve and were highly similar to each other through time (Figure 3.1). The February time points were situated at the height of the yearly temperature cycle, however February 2015 experienced approximately 0.5°C higher temperature than that of 2014 (February 2014 data are only available for Davies reef). Oct/Nov time points were situated at the end of the winter season, just after temperatures had begun to increase. In contrast, Oct/Nov 2015 experienced between 0.4 and 0.5°C cooler temperatures than Oct/Nov 2014.

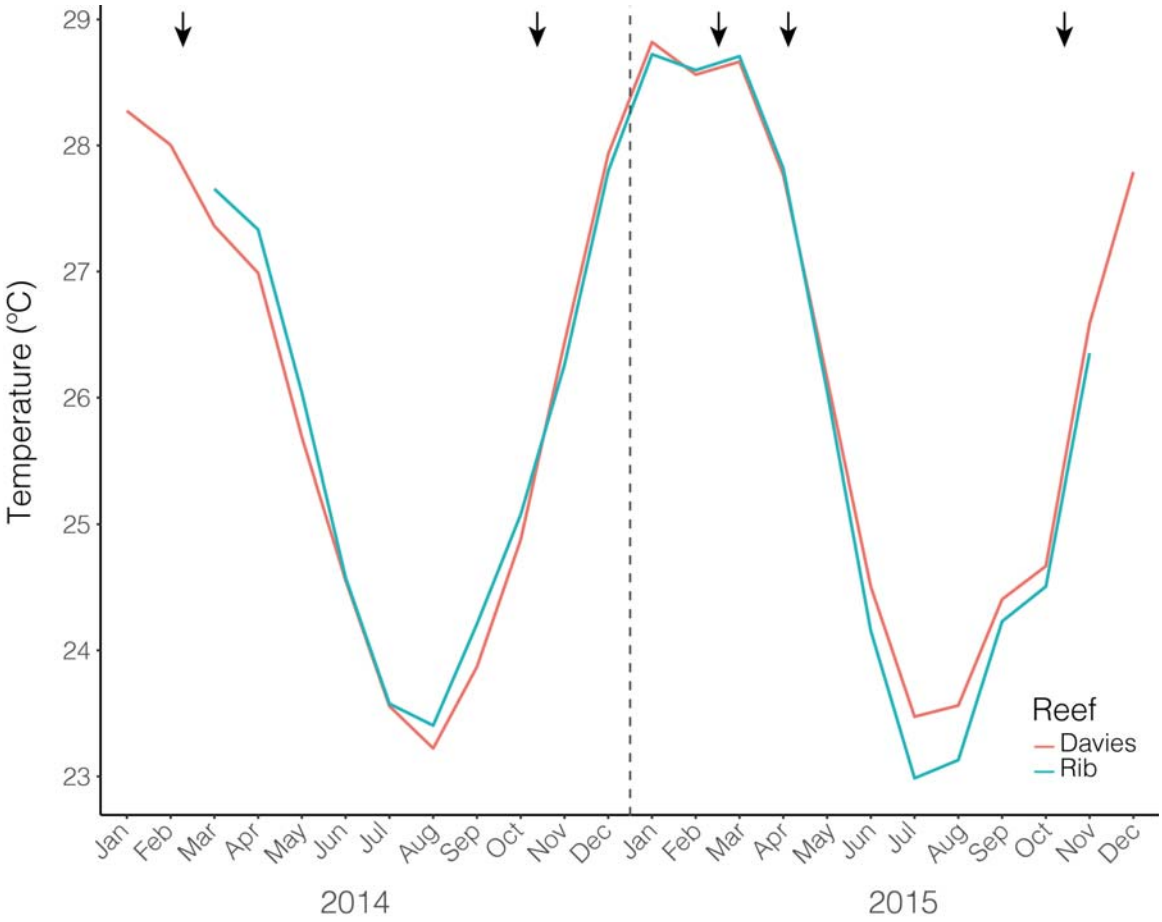

FIGURE 3.1 AVERAGE MONTHLY TEMPERATURES FROM BOTH RIB AND DAVIES REEF FROM JANUARY 2014 - DECEMBER 2015. BLACK ARROWS REPRESENT SAMPLING TIMEPOINTS.

Bacterial community characterization

A total of 5,112,489 sequences from 216 samples corresponding to 14,083 unique ASVs were recovered to characterize the bacterial communities of *A. hyacinthus* and *A. spathulata* at the two mid-shelf reefs through time. Negative controls were checked for contamination and three ASVs from the genera *Bradyrhizobium*, *Ralstonia*, and *Oxalobacteraceae* were removed from the dataset.

Alpha diversity significantly varied through time for *A. hyacinthus* at Rib reef (ANOVA: df = 4, F = 7.39, $p < 0.001$) and for *A. spathulata* at Davies reef (ANOVA: df = 4, F = 4.75, $p < 0.01$). Alpha diversity did not significantly vary over time for *A. hyacinthus* at Davies reef or *A. spathulata* at Rib reef (Figure 3.2). Observed species richness through time was also inconsistent across species and reefs. Richness significantly varied through time for *A. hyacinthus* at Rib reef (ANOVA: df = 4, F = 2.69, $p < 0.05$) and for *A. spathulata* at both Rib (ANOVA: df = 4, F = 2.79, $p < 0.05$) and Davies reefs (ANOVA: df = 4, F = 3.13, $p < 0.05$), but not for *A. hyacinthus* at Davies reef.

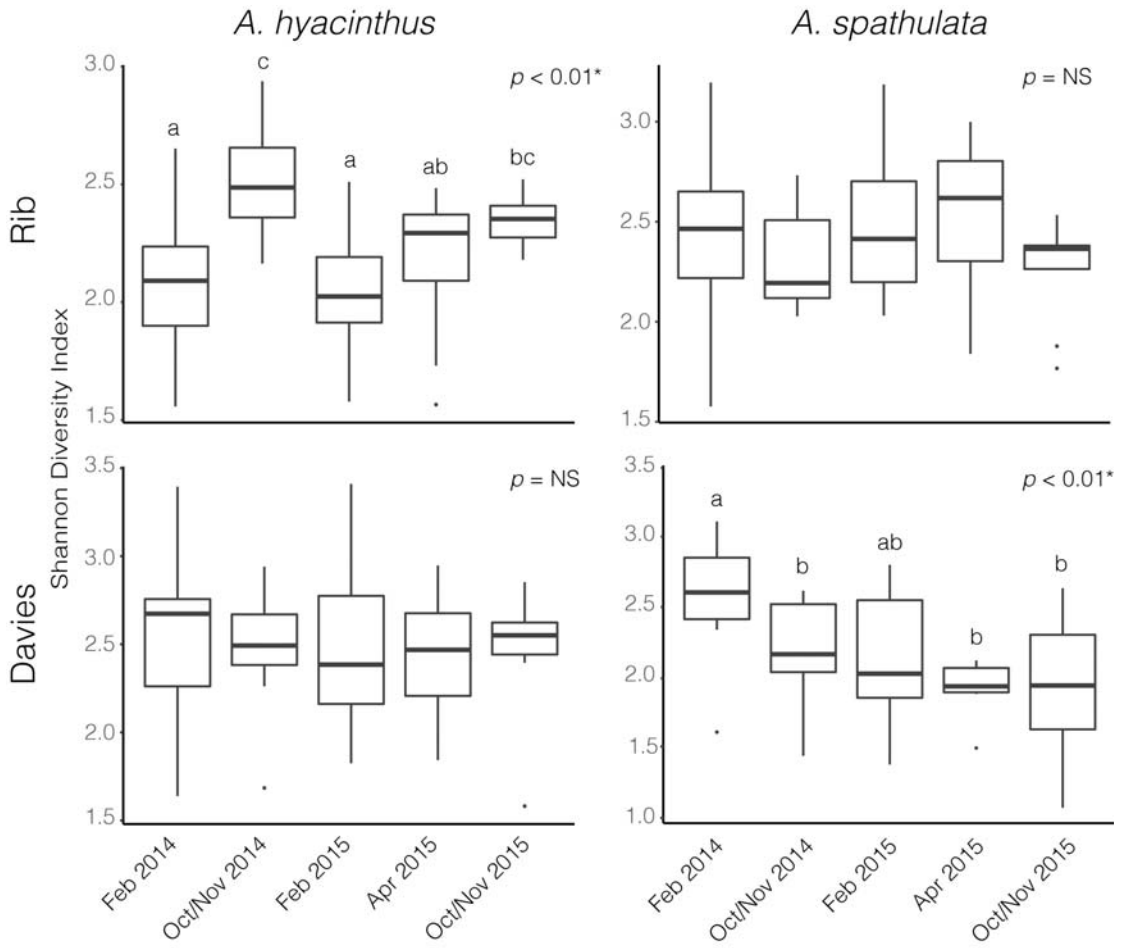

FIGURE 3.2 ALPHA DIVERSITY BASED ON THE SHANNON DIVERSITY INDEX FOR BACTERIAL COMMUNITIES THROUGH TIME FOR *A. HYACINTHUS* AND *A. SPATHULATA* AT RIB AND DAVIES REEF.

Bacterial communities of all samples were dominated by the classes Gammaproteobacteria, Betaproteobacteria, Bacilli, Alphaproteobacteria and Deltaproteobacteria (Figure 3.3). Gammaproteobacteria, which was dominated by the genus *Endozoicomonas*, made up a higher percentage of the community in samples taken from Rib reef, where it made up 90.2 ± 2.2% and 70.3 ± 2.9% (mean ± SEM) for *A. hyacinthus* and *A. spathulata*, respectively. At Davies reef, Gammaproteobacteria accounted for 60 ± 3.5% for *A. hyacinthus* and 45.4 ± 3.9% for *A. spathulata*. An opposing pattern was observed for Betaproteobacteria, where this class, which was dominated by the genus *Burkholderia-Paraburkholderia*, made up a higher percentage in samples taken at Davies reef as opposed to Rib reef. Betaproteobacteria at Davies reef accounted for 26.2 ± 2.9% and 34.3 ± 3.3% of the bacterial communities for *A. hyacinthus* and *A. spathulata*, respectively. At Rib reef, Betaproteobacteria made up only 4.54 ± 1.1% and 18.6 ± 2.02% of the communities for *A. hyacinthus* and *A. spathulata*, respectively.

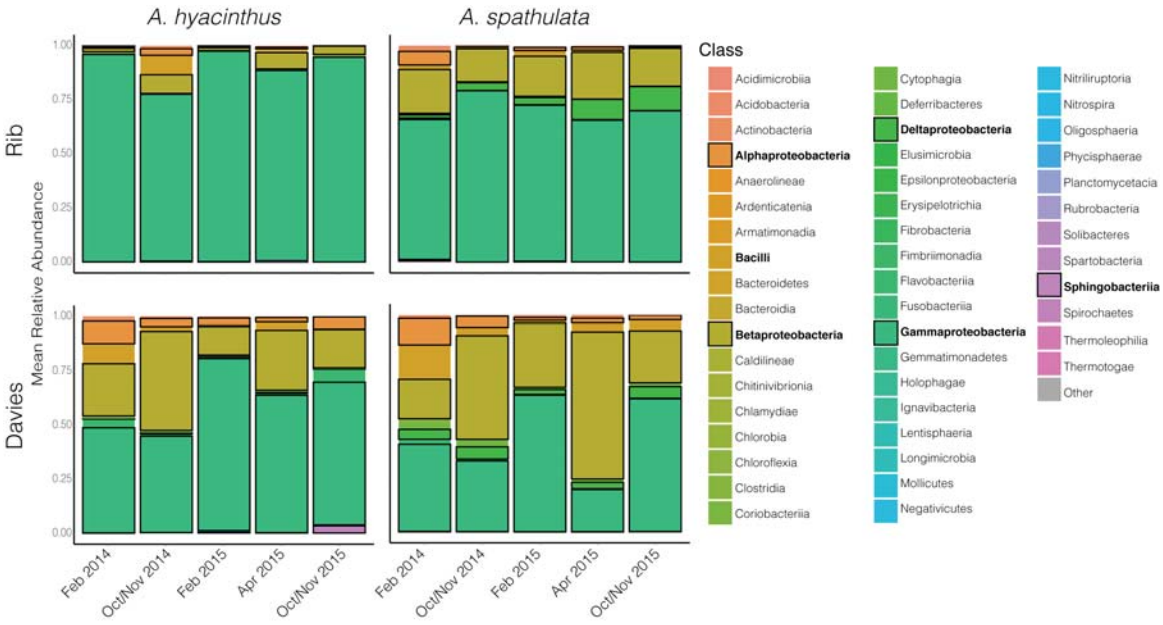

FIGURE 3.3 MEAN RELATIVE ABUNDANCE OF BACTERIAL CLASSES THROUGH TIME FOR BOTH *A. HYACINTHUS* AND *A. SPATHULATA* AT RIB AND DAVIES REEF. CLASSES IN BOLD REPRESENT THOSE THAT ARE IN HIGHEST RELATIVE ABUNDANCE.

Beta-diversity significantly varied among time points for each species at each reef; *A. hyacinthus* at Rib reef (PERMANOVA: df = 4, F = 3.97, $p < 0.01$), *A. hyacinthus* at Davies reef (PERMANOVA: df = 4, F = 3.16, $p < 0.01$), *A. spathulata* at Rib reef (PERMANOVA: df = 4, F = 1.45, $p < 0.01$) and *A. spathulata* at Davies reef (PERMANOVA: df = 4, F = 2.83, $p < 0.01$). Pairwise PERMANOVA suggested no significant differences in the bacterial communities of *A. hyacinthus* among repeated sampling time points in February, but there were significant differences between the two Oct/Nov time points (R^2 <0.05, $p < 0.05$). Pairwise PERMANOVA suggested no significant differences in bacterial communities of *A. spathulata* for all repeated sampling time points (Feb 2014 vs. Feb 2015 and Oct/Nov 2014 vs. Oct/Nov 2015) at Rib reef, but did show significant variations between repeated sampling points in February at Davies reef ($R^2 < 0.2$, $p < 0.05$). It was found for *A. hyacinthus* at both Rib and Davies reef that the two February time points did not differ significantly, but the two Oct/Nov time points did (R^2 <0.05, $p < 0.05$). These data are supported by the CCA fitted time point vectors in nMDS, where data clouds from repeated sampling time points did not pull in the same direction, except for *A. spathulata* at Rib reef, where Oct/Nov 2014 and Oct/Nov 2015 both pulled in the same direction (Figure 3.4). According to CCA, time point represented a significant proportion ($p < 0.05$) of variation for both coral species and both reefs; 34% and 42% for *A. hyacinthus* at Rib and Davies reefs, respectively, and 14% and 39% for *A. spathulata* at Rib and Davies reefs, respectively.

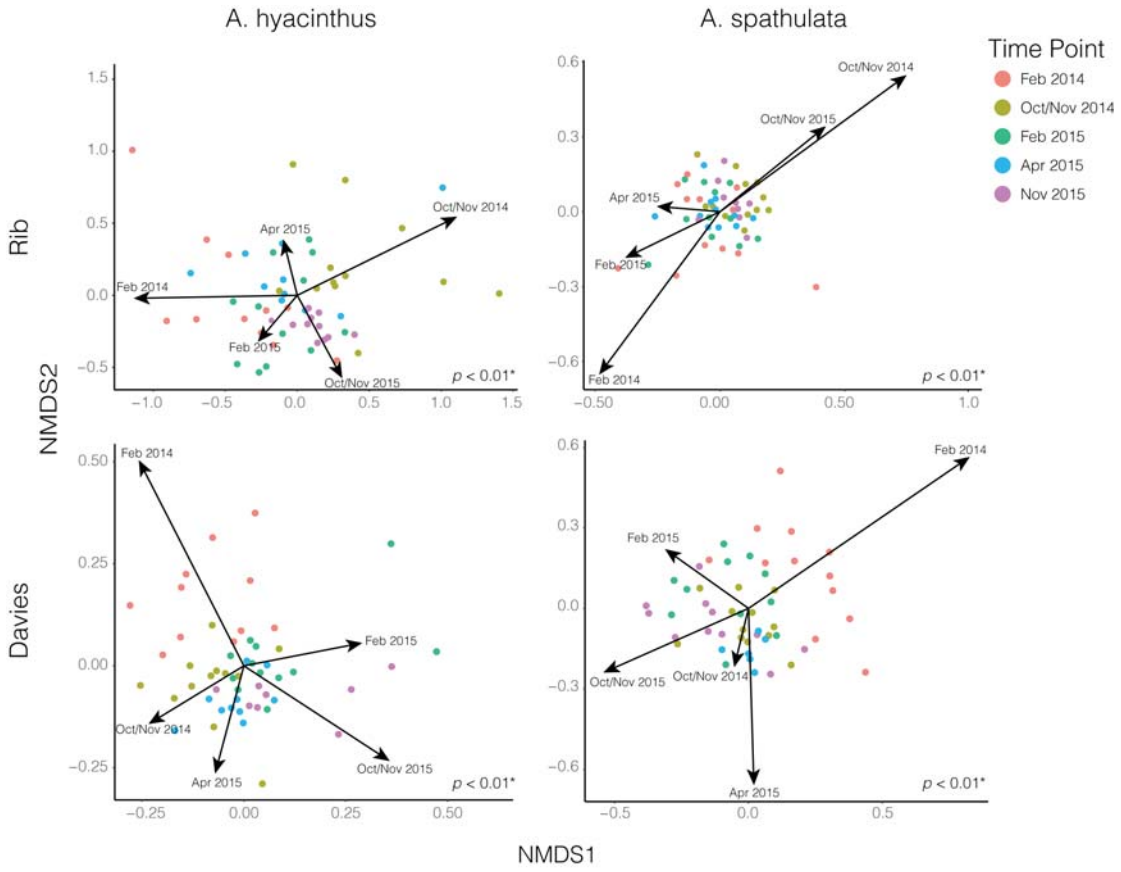

FIGURE 3.4 NMDS PLOTS OF BACTERIAL COMMUNITIES FOR BOTH *A. HYACINTHUS* AND *A. SPATHULATA* AT RIB AND DAVIES REEF. ARROWS REPRESENTING TIME POINT WERE FIT AND SCALED BY CONSTRAINED CORRESPONDENCE ANALYSIS (CCA). *P*-VALUES IN THE LOWER RIGHT HAND CORNER OF EACH NMDS PLOT REPRESENT PERMANOVA RESULTS.

Indicator taxa for repeated sampling time points

Acropora hyacinthus at Rib reef had no significant bacterial indicator taxa for February, and neither coral species at either reef showed any significant bacterial indicators for Oct/Nov time points. Two indicator taxa of the February time points were recovered for *A. hyacinthus* at Davies reef: one ASV of *Endozoicomonas* ($p < 0.01$) and one of *Pseudoalteromonas* ($p < 0.05$). Three indicator taxa of the February time points were identified for *A. spathulata* at Davies reef, including two ASVs of *Endozoicomonas* ($p < 0.05$), one of which was the same ASV as found in *A. spathulata* at Davies reef, and one of *Vibrio* ($p < 0.05$). One ASV of *Endozoicomonas* ($p < 0.05$), different from the others, was found as the single indicator taxa of the February time points for *A. spathulata* at Rib reef.

Symbiodiniaceae community characterization

A total of 6,418,776 sequences from 215 samples corresponding to 54 unique Symbiodiniaceae sequence sub-types were recovered to characterize the Symbiodiniaceae communities of both *A. hyacinthus* and *A. spathulata* at two reefs and among the five time points. Alpha diversity of Symbiodiniaceae remained stable through time for *A. hyacinthus* and *A. spathulata* at Rib reef,

but significantly varied for both species at Davies reef ($ANOVA_{hyacinthus}$: df = 4, F = 16.36, p < 0.001; $ANOVA_{spathulata}$: df = 4, F =4.07, p < 0.01; Figure 3.5). Observed species richness remained stable for *A. hyacinthus* and *A. spathulata* at both Rib and Davies reefs among time points.

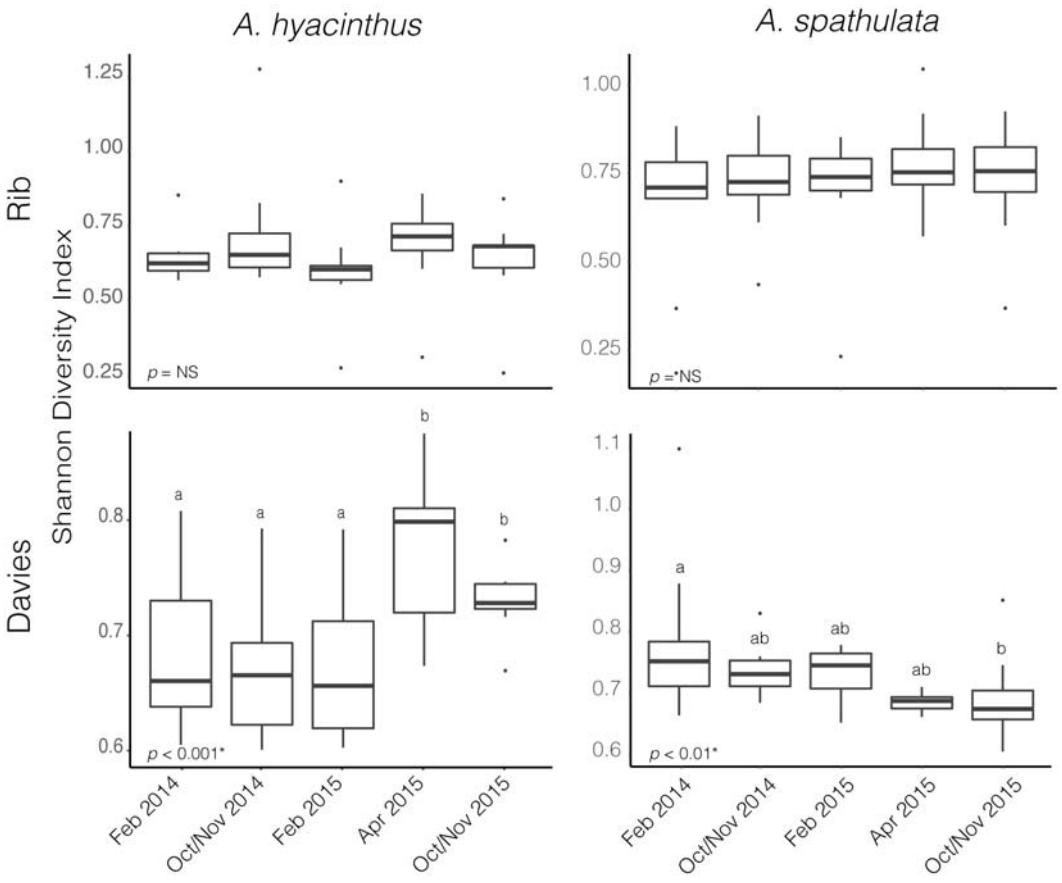

FIGURE 3.5 ALPHA DIVERSITY BASED ON THE SHANNON DIVERSITY INDEX FOR SYMBIODINIACEAE COMMUNITIES THROUGH TIME FOR BOTH *A. HYACINTHUS* AND *A. SPATHULATA* AT RIB AND DAVIES REEF.

Cladocopium C3k and Cspc sequence sub-types were the dominant Symbiodiniaceae taxa through time for both species at both reefs (Figure 3.6) making up an average of 69.3 ± 1.2% and 26.8 ± 1.2% (mean ± SEM), respectively, of the Symbiodiniaceae communities harbored by *A. hyacinthus* and *A. spathulata*. Beta-diversity also remained stable through time for *A. hyacinthus* at Rib and Davies Reef and for *A. spathulata* at Rib Reef. Beta-diversity of *A. spathulata* at Davies reef significantly varied among time point (PERMANOVA: df = 4, F = 4.86, p <0.01), driven only by community differences between the two time points February 2015 and Oct/Nov 2015. Dominant taxa remained the same between these two time points, but the colonies had incorporated a low background abundance of *Cladocopium* C1d sub-type in February 2015, while in Oct/Nov 2015 these same colonies had replaced C1d with low background abundances of *Cladocopium* C3 sub-types, including C3.10 and C3.12.

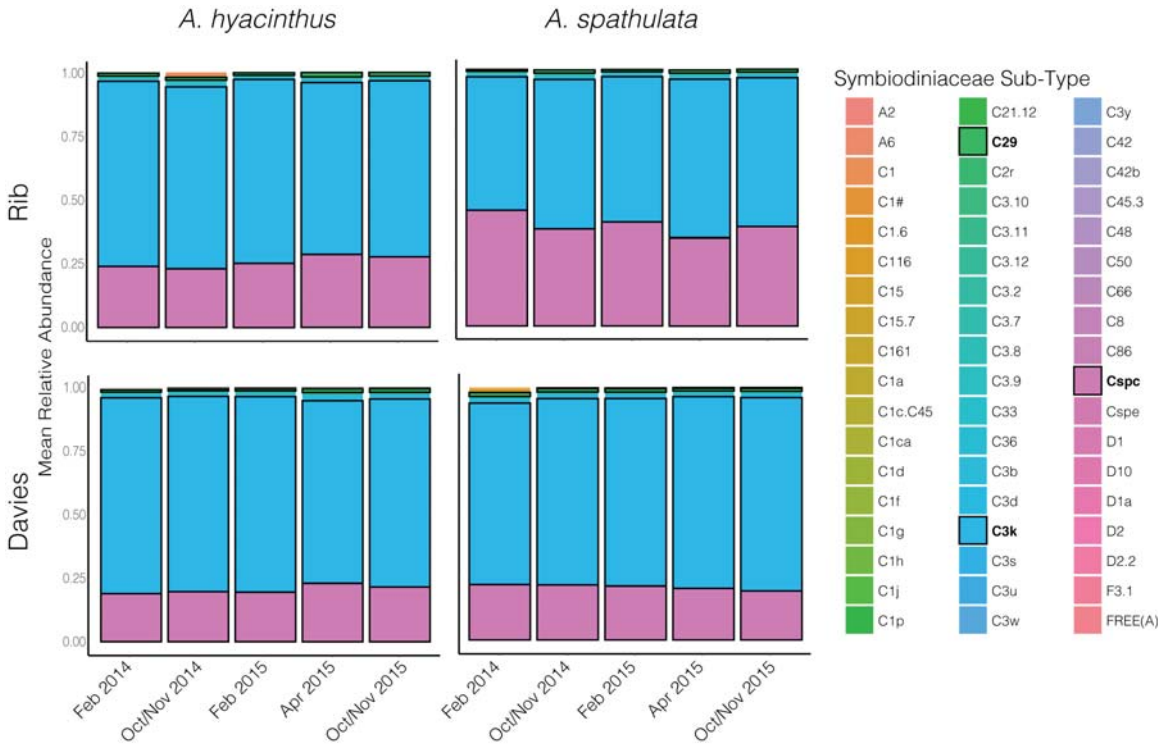

FIGURE 3.6 MEAN RELATIVE ABUNDANCE OF SYMBIODINIACEAE SEQUENCE SUB-TYPES THROUGH TIME FOR BOTH *A. HYACINTHUS* AND *A. SPATHULATA* AT RIB AND DAVIES REEF. THOSE IN BOLD REPRESENT MOST ABUNDANT SUB-TYPES.

Co-occurrences of bacterial and Symbiodiniaceae taxa

The co-occurrences and correlation strengths of bacterial and Symbiodiniaceae taxa differed among time points within species and reef (Supplementary Figure S3.1). Among time points, Symbiodiniaceae correlated both positively and negatively with a number of bacterial taxa, including *Endozoicomonas*, *Burkholderia-Paraburkholderia*, *Sphingomonas* and others. However, only a small number of taxa had significant correlations (>0.6 or < -0.6, $p < 0.05$) when all time points were considered together for each species at each reef (Figures 3.7 & 3.8). Symbiodiniaceae significantly correlated only with other Symbiodiniaceae, where the two dominant types, *Cladocopium* C3k and Cspc were negatively correlated with each other for both coral species at each reef. Most significant bacterial correlations occurred between ASVs of the same genus, for instance *Endozoicomonas* with *Endozoicomonas* and *Burkholderia-Paraburkholderia* with *Burkholderia-Paraburkholderia*. Interestingly, some *Endozoicomonas* ASVs correlated positively with each other, while others correlated negatively.

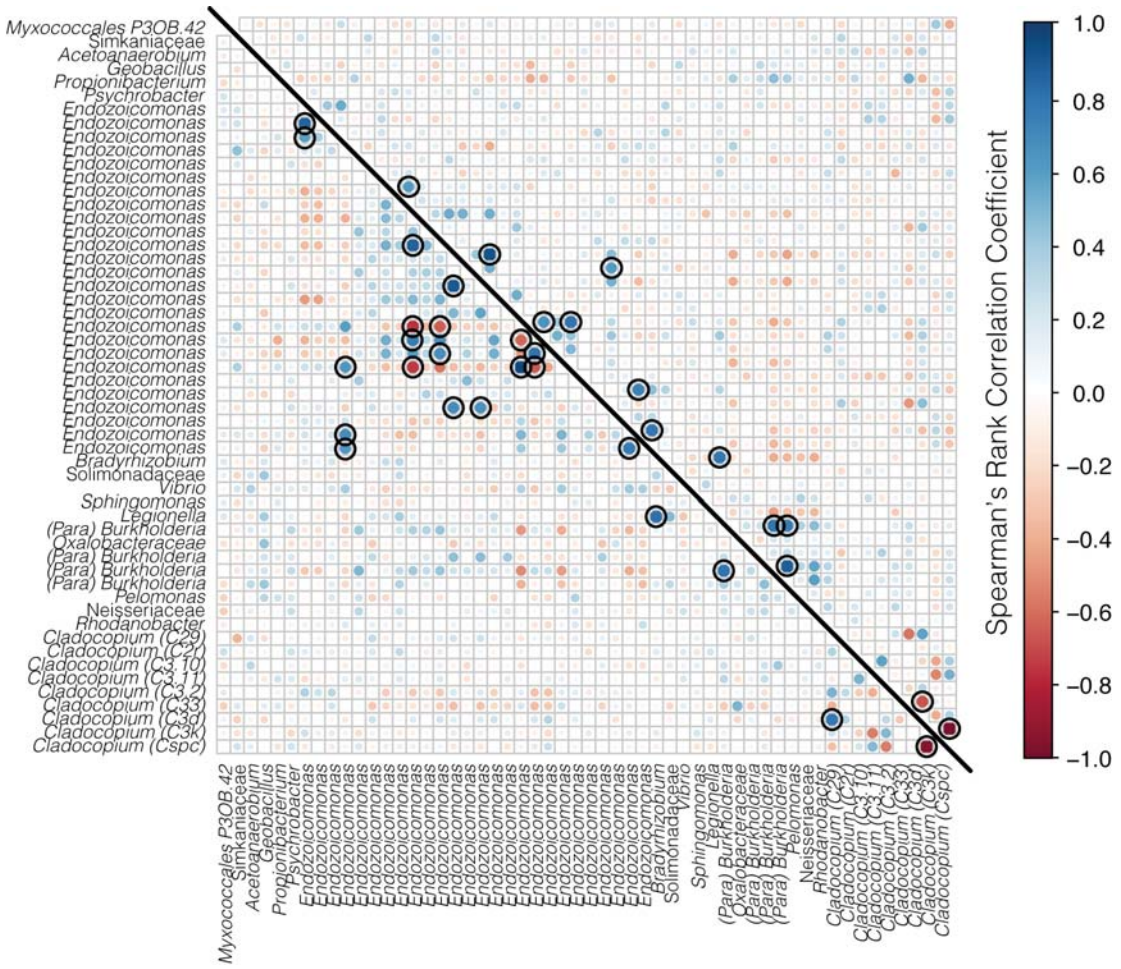

FIGURE 3.7 CORRELATION MATRIX OF BOTH BACTERIA AND SYMBIODINIACEAE FOR *A. HYACINTHUS* FROM RIB REEF (BELOW THE DIAGONAL LINE) AND DAVIES REEF (ABOVE THE DIAGONAL LINE). BACTERIAL TAXA REPRESENT AMPLICON SEQUENCE VARIANTS (ASVS) CLASSIFIED TO GENUS WHERE POSSIBLE, AND SYMBIODINIACEAE ARE REPRESENTED BY GENUS AND SUB-TYPE. POSITIVE CORRELATIONS ARE REPRESENTED IN BLUE AND NEGATIVE CORRELATIONS ARE REPRESENTED IN RED, WHERE BOTH THE SIZE AND THE COLOR OF THE DOTS REPRESENT THE STRENGTH OF THE CORRELATION. BLACK RINGS REPRESENT SIGNIFICANT POSITIVE OR NEGATIVE CORRELATIONS ($P < 0.05$).

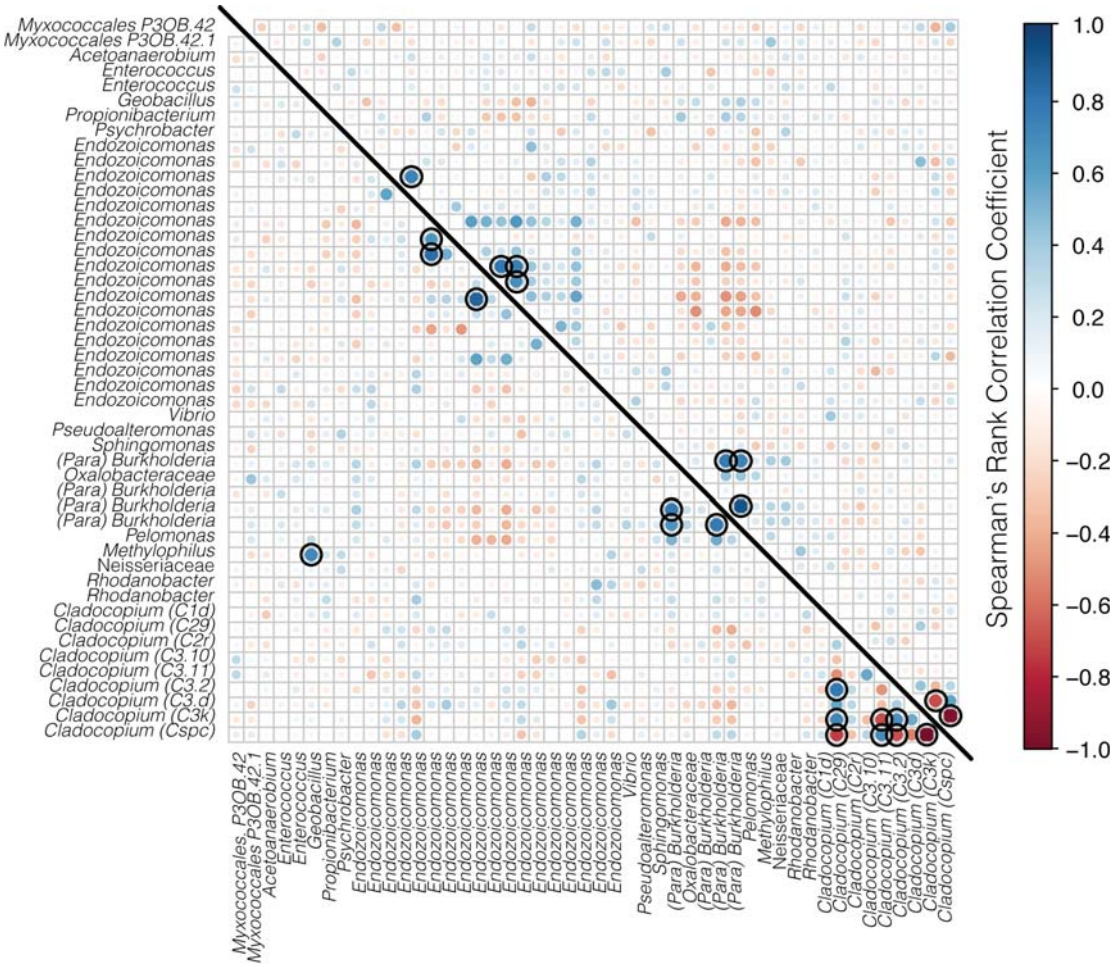

FIGURE 3.8 CORRELATION MATRIX OF BOTH BACTERIA AND SYMBIODINIACEAE FOR *A. SPATHULATA* FROM RIB (BELOW THE DIAGONAL LINE) AND DAVIES REEF (ABOVE THE DIAGONAL LINE). BACTERIAL TAXA REPRESENT AMPLICON SEQUENCE VARIANTS (ASVS) CLASSIFIED TO GENUS WHERE POSSIBLE, AND SYMBIODINIACEAE ARE REPRESENTED BY GENUS AND SUB-TYPE. POSITIVE CORRELATIONS ARE REPRESENTED IN BLUE AND NEGATIVE CORRELATIONS ARE REPRESENTED IN RED, WHERE BOTH THE SIZE AND THE COLOR OF THE DOTS REPRESENT THE STRENGTH OF THE CORRELATION. BLACK RINGS REPRESENT SIGNIFICANT POSITIVE OR NEGATIVE CORRELATIONS ($P < 0.05$).

Discussion

While there was some variation among sampling time points for *A. hyacinthus* and *A. spathulata* at both Rib and Davies reefs, there was no evidence that variation among time points has any seasonal basis. These results contradict previous studies, and could be due to specificities in the temporal variation of environmental parameters, for instance where seasonal temperatures varied slightly from year to year. Regardless, caution should be taken in suggesting observed temporal variations are a direct result of seasonal effects when examining a less than one-year time scale. Additionally, there was clear evidence for differences in the microbiome between reefs, suggesting that reef location plays a much larger role than season in driving microbial community composition in corals. However, caution is also warranted here with only two reefs, and future studies should expand the number of locations surveyed.

Microbial communities were variable, but not seasonal

The bacterial communities of both *A. hyacinthus* and *A. spathulata* were dominated by Proteobacteria, including the classes Gammaproteobacteria, Betaproteobacteria and Alphaproteobacteria, which has commonly been observed in *Acropora* species previously (e.g., Littman et al. 2011; Ceh et al. 2011; Meron et al. 2011; Ziegler et al. 2017). The overall community composition was similar between *A. hyacinthus* and *A. spathulata* at the two reefs, but there were observed consistent differences in specific taxa such as the Gamma- and Betaproteobacteria. Coral bacterial communities have previously been found to have strong location or geographic effects (e.g., Littman et al. 2009; Leite et al. 2018), and it has been proposed that the coral microbiome includes a site or location-specific component (Hernandez-Agreda et al. 2016). Rib and Davies reef are mid-shelf reefs on the GBR, both influenced by oceanic inflow (Brinkman et al. 2002). However, they are located approximately 150 km apart and could have different environmental influences, including differences in temperature or land-based influences, such as nutrient content as a result of run-off (e.g., Davies reef is much closer to the mouth of the Burdekin River than Rib reef, which can have extensive flood plumes; Wolanski & van Senden 1983). This could result in more variability in the environmental pool of microbes that may be available to the corals for acquisition.

While there were significant variations in bacterial communities through time, there was little evidence of consistent seasonal patterns within species and across reefs. Previous long-term studies on the coral microbiome have identified significant differences in the bacterial community through time (e.g., Ceh et al. 2011; Kimes et al. 2013; Li et al. 2014; Sharp et al. 2017; Cai et al. 2018). These studies were completed within a one-year time period and have

suggested the observed changes were seasonal. The pattern from the present study instead supports the findings by Yang et al. (2017), where *Stylophora pistillata* also exhibited highly dynamic temporal variations in bacterial community, but with little evidence of seasonal cycles over a two-year period. Therefore, the variations in bacterial communities among time point are likely influenced by more factors than seasonal temperature variation alone, such as differences in nutrient concentration (e.g., Costa et al. 2006). This reinforces the conclusions made by Yang et al. (2017), who recommend that greater than 1-year studies are essential for understanding the drivers of temporal change in bacterial communities. Perhaps studies that exceed even two years are necessary to find consistent cyclical patterns due to inter-annual variation in environmental parameters.

The dominant Symbiodiniaceae sequence types in *A. hyacinthus* and *A. spathulata* included *Cladocopium* C3k and *Cladocopium* Cspc. *Cladocopium* C3 has been identified as a common symbiont of acroporids from central and southern GBR reefs (LaJeunesse et al. 2003, 2004b). In the present study, *Cladocopium* Cspc also co-dominated, but was negatively correlated with C3k, perhaps representing a competitive interaction between the two dominant strains. However, these dominant strains were maintained throughout the two-year sampling period, suggesting overall community structure was stable and did not reflect seasonal variation. Although seasonal variation in the dominant Symbiodiniaceae types has been identified for some coral species (e.g., Chen et al. 2005; Ulstrup et al. 2008), such seasonal variations are often present as changes to cell density, pigment content or photosynthetic efficiency (Fitt et al. 2000; Warner et al. 2002; Ulstrup et al. 2008) while community structure remains stable (e.g., LaJeunesse et al. 2005; Thornhill et al. 2006a,b; Klepac et al. 2015; Cai et al. 2018). Seasonal variation was not evident in the data presented here, but changes among time points were reflected in background types (i.e., the rare biosphere, Quigley et al. 2014), particularly at Davies reef, where alpha diversity of both *A. hyacinthus* and *A. spathulata,* and beta-diversity of *A. spathulata* significantly varied. *Acropora spathulata* at Davies reef exhibited significant acquisition of novel background strains among time points. This symbiont switching in the rare biosphere has been found previously, but only following considerable bleaching events and has been suggested as a response mechanism to environmental change (Lewis & Coffroth 2004; Boulotte et al. 2016), which were not recorded in the present study. Further, this switching was species-specific, suggesting some level of host regulation.

The small number of significant correlations found between microbial taxa for each coral species at each reef suggests that there are few co-occurrences that are persistent through time. When time points were pooled, Symbiodiniaceae had no significant correlations with any bacterial taxa. Conversely, the microbial communities examined at each time point had both higher numbers of significant correlations and created complex networks, while

Symbiodiniaceae were interconnected with both other Symbiodiniaceae and bacterial taxa. These networks, however, were not consistent through time, and did not display any obvious seasonal patterns of microbial interactions. While some previous studies have found Symbiodiniaceae correlating only with other Symbiodiniaceae (e.g., Bonthond et al. 2018), others have found some connectivity with bacterial taxa (e.g., Bernasconi et al. 2018), but the minimal number of studies incorporating network analyses for both the Symbiodiniaceae and bacterial components of the coral microbiome has limited the ability to recognize patterns. This study suggests that not only is microbial taxonomic composition variable through time, but so are their interactions with other microbial members; few interactions remained consistent among all time points in each species at each reef. Thus, the coral microbiome could represent a rather haphazard collection of taxa with indistinct functional connections, contradicting the frequent portrayal of the microbiome as a refined functional collection of taxa important for the health of the coral (reviewed in Bourne & Webster 2013; Bourne et al. 2016). Future functional analyses will be necessary for clarification, along with a better understanding of the maintenance and regulation of microbial symbioses by the coral host. Further, these results suggest that caution should be taken when making conclusions from a network or correlation analysis that represents only a single time point.

Potential functional differentiation or redundancy in Endozoicomonas

The present study found no indicator species that were significantly associated with the Oct/Nov time points across species. However, a small number of indicator taxa were associated with the February time points for both coral species, mostly consisting of *Endozoicomonas* sequence variants. As February is the height of summer in the southern hemisphere, these may be relevant to thermal tolerance. *Endozoicomonas* is a common bacterial genus found associated with a wide range of coral species, including those from the families Acroporidae (Ziegler et al. 2016, 2017), Pocilloporidae (Bayer et al. 2013; Neave et al. 2017a; van Oppen et al. 2018), Fungiidae (Roder et al. 2015) and Poritidae (Apprill et al. 2016). This genus has been suggested to play a number of functional roles linked with coral health, such as carbohydrate cycling and protein transport (Neave et al. 2016), dimethylsufiopropionate (DMSP) degradation (Bourne et al. 2013), provision of amino acids (Neave et al. 2016), and, importantly, thermal or bleaching protection (Pantos et al. 2015). The presence of *Endozoicomonas* as an indicator of the February time points reinforces the notion that this genus could provide some thermal protection during summer, possibly as a result of host regulation to ensure this important symbiont is present under the necessary conditions.

The two coral species in the present study hosted one of the same *Endozoicomonas* ASVs as an indicator of the February time point. This could suggest some functional specificity among *Endozoicomonas* strains in relation to environmental or seasonal parameters. However, coupled with the presence of other *Endozoicomonas* ASVs as indicators in each coral species, this could suggest functional specificity, functional redundancy, or both are occurring among *Endozoicomonas* strains. Thus, while seasonal variation in taxonomy was not recovered in the results presented here, it may instead be occurring on a functional level within *Endozoicomonas*, or perhaps the entire bacterial community. Interestingly, both the correlation and network analyses found that different *Endozoicomonas* strains correlated both positively and negatively with each other. This implies functional differentiation among *Endozoicomonas* sequence variants. Recent genome sequencing of *Endozoicomonas* strains from a variety of coral species found that *Endozoicomonas* might play different roles within their coral hosts based on their specific genotype (Neave et al. 2017b). This highlights the importance of examining higher resolution taxonomic classification (e.g., ASVs) in metabarcoding studies, and future studies should incorporate functional analyses.

Conclusions

The microbiomes of *A. hyacinthus* and *A. spathulata* were found to be variable both through time and according to reef, but were not reflective of seasonality. This validates the findings of Yang et al. (2017), and reinforces their conclusion that long-term microbial surveys are essential for understanding the variable nature of the coral microbiome through time. Further, seasonal cycles may exist but may be more evident in changes in function rather than in taxonomic composition, which could be assessed using metagenomic and metatranscriptomic methods. The findings from the present study further suggest that the drivers of microbial communities are both host and environmentally regulated, particularly driven by reef location.

CHAPTER 4

THERMAL STRESS HAS LITTLE EFFECT ON THE MICROBIOME COMPOSITION OF THE CORAL *POCILLOPORA ACUTA*

IN REVIEW FOR PUBLICATION IN *CORAL REEFS*

Epstein HE, Torda G & van Oppen MJH (In review). Thermal stress has little effect on the microbiome composition of the coral *Pocillopora acuta*. *Coral Reefs.*

Abstract

Rapid climate change due to anthropogenic greenhouse gas emissions is pushing corals to their physiological limits, while their microbiome is being pressed toward dysbiosis. Microbes greatly influence the health and functioning of corals, but thermal anomalies that cause bleaching can affect certain taxa of the host-associated prokaryote and Symbiodiniaceae communities, leading corals toward a disease-prone state. Yet some coral species are more tolerant to bleaching than others, and may not bleach during thermal anomalies that cause widespread bleaching in other coral species. Whether changes in the coral microbiome occur in these resilient species during temperature anomalies is not well described. In the present study, 10 colonies of the branching coral *Pocillopora acuta* were tagged, visually assessed and sampled from a fringing reef off Orpheus Island in the central Great Barrier Reef for one year, of which the summer coincided with the 2016 mass-bleaching event. No visible signs of bleaching were observed in any of the 10 colonies throughout the study period, despite experiencing two degree heating weeks of thermal stress and observations of bleaching in other coral species on the same reef. Metabarcoding based on the Symbiodiniaceae ITS2 rDNA spacer and the bacterial 16S rRNA gene provided evidence for stability of the overall microbial community structure, although the bacterial community showed increases in a number of potentially beneficial taxa, such as diazotrophs, during the thermal stress event. These findings suggest some flexibility in the microbiome to adjust to higher than average temperatures without disrupting microbiome stability, perhaps contributing to the thermal resilience of *P. acuta*.

Introduction

Climate change is causing global coral decline as a result of increasing sea surface temperatures and changes in ocean chemistry. Specifically, mass-bleaching as a result of thermal stress has negatively affected coral reefs in the past two decades (Heron et al. 2016; Oliver et al. 2018), exemplified by the 2016 bleaching event that resulted in ~30% mortality of coral on the Great Barrier Reef (GBR) alone (Hughes et al. 2017). The health and functioning of corals are governed

in part by their associated micro-organisms. These include their photosynthetic algal endosymbionts of the family Symbiodiniaceae as well as other single-celled and filamentous eukaryotes (e.g., fungi or algae), prokaryotes (i.e., bacteria and archaea), and acellular viruses, many of which play important roles in a range of biological processes. Symbiodiniaceae contributes to both carbon fixation and translocation that meet a large portion of the coral host's respiratory requirements (Muscatine & Porter 1977). Prokaryotes contribute to cycling of various nutrients, such as nitrogen and sulfur (Raina et al. 2009; Lema et al. 2014b), and can aid in host immunity (Ritchie 2006). Thermal stress, however, can significantly alter coral-associated microbial communities. These changes may allow for rapid acclimatization or adaptation to environmental change (e.g., Reshef et al. 2006; Torda et al. 2017), but may also drive the coral holobiont toward a disease-prone state (e.g., Ben-Haim & Rosenberg 2002; Rosenberg et al. 2007; Vega Thurber et al. 2009).

Bleaching, by definition, is the loss of Symbiodiniaceae from the coral tissue (Glynn 1984), where certain Symbiodiniaceae types or sub-types may be preferentially removed by the host (Jones et al. 2008). Some corals exhibit a shift to dominance by more heat tolerant Symbiodiniaceae types following bleaching (Berkelmans & van Oppen 2006) or acquire novel strains that are more thermally tolerant from the environment (Boulotte et al. 2016). Major shifts in the composition of bacterial communities have also been observed in stressed corals (Bourne et al. 2008; Littman et al. 2011) that may include increases in pathogenic bacteria (e.g., Ben-Haim & Rosenberg 2002; Rosenberg et al. 2007; Bourne et al. 2008; Vega Thurber et al. 2009). Conversely, some coral species have been found to harbor stable communities during thermal stress (e.g., Webster et al. 2016; Hadaidi et al. 2017; Grottoli et al. 2018), and others have exhibited increases in abundances of beneficial bacteria, such as nitrogen-fixing diazotrophs (Santos et al. 2014). While these variations in response of the bacterial community to thermal stress may be specific to the coral host, characterising the response of the coral microbiome to thermal stress in resilient species may provide insight into the drivers of coral health and potential mechanisms of slowing, preventing or even reversing coral decline (i.e., "Assisted Evolution"; van Oppen et al. 2015). With the increased risk of thermal stress worldwide, investigation into the contribution of microbial communities to either coral decline or coral resilience is crucial.

The majority of literature on the effects of thermal stress on the coral microbiome is focused on changes that occur during and after bleaching. However, effects of temperature anomalies may result in changes in microbial communities that occur prior to or without visible signs of deteriorating health or bleaching. In fact, Glasl et al. (2017) proposed the use of microbial communities as a diagnostic tool, speculating that changes to coral microbial communities can provide early detection of ecosystem stress. The present study followed tagged colonies of the common branching coral *Pocillopora acuta* (chunky morphology) for one year of which the

summer spanned the 2016 mass-bleaching event on the GBR (Hughes et al. 2017). *Pocillopora acuta* has been found to show inter-colony variation in bleaching susceptibility depending on colony morphology. It is now recognized that this branching coral species appears in two morphologies; a fine-branching morph and a chunky morph, where the former tends to bleach more readily (Smith et al. 2017b). Indeed, despite experiencing heat stress, the tagged chunky-morph colonies of *P. acuta* used in this study showed no visible signs of bleaching, whereas there was widespread bleaching of other pocilloporids and acroporids. This presented an opportunity to examine the effects of a thermal stress event on the long-term stability of the coral microbiome from a thermally resilient species.

Methods

Sample Environment, Collection and Fixation

Ten colonies of *P. acuta* (chunky morphology, approximately 35cm diameter) were tagged, visually examined and sampled for one year spanning from November 2015 through November 2016 at Little Pioneer Bay (Orpheus Island) in the central Great Barrier Reef, Australia (Figure 4.1). All colonies in the present study were of the chunky morphology and were located at 2-3 m depth along a 50 m stretch of reef parallel to the shoreline. Sampling took place every two months (November, January, March (a and b), May, July, September, November). Colonies were sampled twice in March, due to the onset of the mass-bleaching event across the central and northern GBR (Hughes et al. 2017). The March (a) time point occurred when bleaching was prominent at the sampling site. At each time point, a small nubbin (2 to 5 cm in length) of each colony was collected, immediately snap-frozen in liquid nitrogen (LN_2) and stored at -80°C.

A HOBO UA-001064 Pendant temperature logger (Onset Computer Corp.) was placed at the study site at the same depth and within the vicinity of the tagged colonies throughout the study period, taking a temperature reading every 30 minutes.

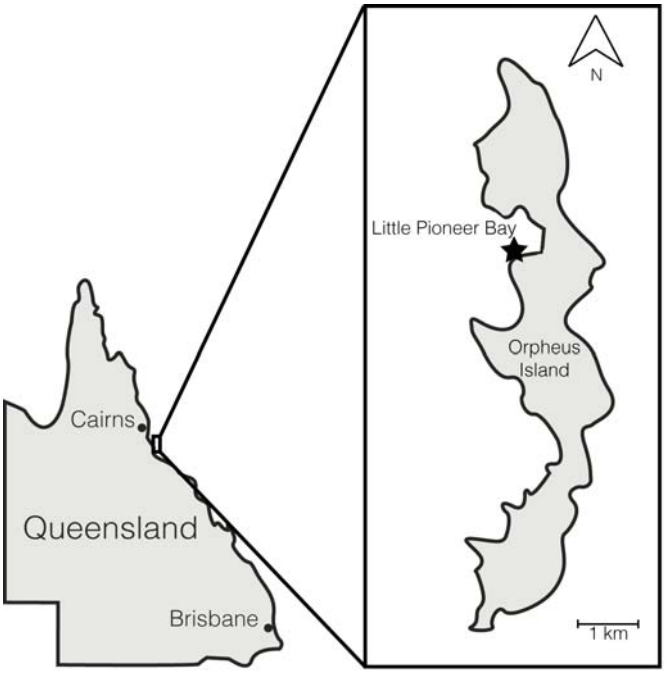

FIGURE 4.1 MAP OF STUDY SITE AT LITTLE PIONEER BAY ON ORPHEUS ISLAND IN THE CENTRAL GREAT BARRIER REEF, AUSTRALIA, LOCATED AT APPROXIMATELY 18° 36' 3.6" S 146° 29' 20.4" E.

DNA Extraction, PCR and Sequencing

Nubbins were crushed on LN_2 using a hydraulic bench top laboratory press prior to DNA extraction. DNA was extracted using a salting out method outlined in Damjanovic et al. (2017) (Appendix A), modified to include an additional lysozyme digestion and bead-beating step. Amplification of double-stranded products from the 16S rRNA gene for bacteria and the rDNA inter-transcribed spacer region 2 (ITS2) for Symbiodiniaceae was achieved through polymerase chain reaction (PCR) using gene-specific primers. The V5-V6 region of 16S was targeted using the primers 784F 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGATTAGATACCC TGGTA -3' and 1061R 5' – GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CRRCACGAGCTGACGAC -3' (Andersson et al. 2008). ITS2 was targeted using the primers ITS2F 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGAATTGCAG AACTCCGTG-3' and ITS2R 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCTCCGCTTACTTATATGCTT-3' (Pochon et al. 2012). Both sets of primers contained the Illumina adapter overhangs (underlined above). The 16S PCR was performed in triplicate 10 µL reactions. Each reaction consisted of: 5 µL of AmpliTaq Gold MasterMix (Applied Biosystems),

2 µL of each primer (2 µM stock), and 1 µL of DNA template. The following PCR protocol was used: initial denaturation at 95°C for 10 minutes, 30 cycles each of 95°C for 30 seconds, 57°C for 60 seconds and 72°C for 60 seconds, followed by a final extension at 72°C for 7 minutes. The ITS2 PCR was also performed in triplicate 10 µL reactions. Each reaction consisted of 5 µL of Qiagen Mulitplex MasterMix (Qiagen), 3 µL Milli Q water, 0.5 µL of each primer (4 µM stock), and 1 µL of DNA template. The following PCR protocol was used: 95°C for 5 minutes, 31 cycles each of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, followed by a final extension at 72°C for 5 minutes. PCR clean-up, indexing and sequencing were carried out at the Ramaciotti Centre for Genomics at the University of New South Wales on a 2x300bp Illumina MiSeq run. Negative PCR controls were also run alongside samples for sequencing to check for contamination.

Sequence Assembly, Quality Control and Taxonomic Assignment

Demultiplexed sequences for both 16S and ITS2 were assembled, checked for quality and assigned taxonomic classification using QIIME2 v 2017.10 (Caporaso et al. 2010). The plug-in demux (Caporaso et al. 2010) was used for visualising read quality and setting quality filtering guidelines. Quality filtering, trimming of poor quality bases, de-replication, chimera filtering, merging of paired-end reads and identifying fine-scale single nucleotide variation among sequences were performed using the DADA2 plug-in (Callahan et al. 2016). For 16S, mitochondrial and chloroplast sequences were removed and taxonomic assignment was done by training a naïve-Bayes classifier on the V5-V6 region of the 16S gene in the SILVA 128 database (Quast et al. 2013) to match the primers used. Due to the high number of single-variants found for ITS2 and the subsequent small taxonomic database, it was not useful to use a classifier as above due to low resolution. Therefore, these single-variants for ITS2 were clustered by 97% similarity using a vsearch plug-in (Rognes et al. 2016). Taxonomic assignment was done according to the database from Arif et al. (2014), allowing assignment down to the sub-type level for Symbiodiniaceae. At the end of the pipelines for both 16S and ITS2, the taxa plug-in (Caporaso et al. 2010) was used to create a taxonomy table and an OTU or sequence variant biom table of raw sequence counts that could then be used for further downstream analyses. Sequence variants that were present in negative controls were considered contaminants if they contained high relative abundances in negative controls and low relative abundances in samples (Lee et al. 2015a).

Statistical Analyses

All statistical analyses were performed in R v. 3.5.0 (The R Core Team 2018) and all graphics done using the R package ggplot2 v. 2.2.1 (Wickham 2009). Temperature data from the logger were collated and presented as daily average temperatures. Degree heating weeks (DHWs) were

calculated as the sum of the daily temperature anomalies (i.e., >= 1 degree higher than the maximum monthly mean) over a 90-day window, divided by 7 (Liu et al. 2013). Maximum monthly mean temperature was derived from long-term temperature data (1993-2017) at Orpheus Island that is publicly available from the Australian Institute of Marine Science (AIMS) (AIMS Historical Data Tool: http://data.aims.gov.au/aimsrtds/datatool.xhtml) and the daily temperature was taken from the HOBO logger at the study site. It was assumed there were no temperature anomalies beyond the summer maximum temperatures in the 90 days prior to the first temperature data point taken in November 2015 (see AIMS Historical Data Tool: http://data.aims.gov.au/aimsrtds/datatool.xhtml), which allowed for complete profiling of DHWs during the summer period.

Alpha diversity, richness and evenness of 16S and ITS2 sequence data were examined using the package vegan v. 2.5-2 (Oksanen et al. 2018). Significant differences in these three measures among the eight time points were assessed by analysis of variance (ANOVA) using either a linear mixed effects model fit by restricted maximum likelihood (REML) for repeated measures with an added autoregressive 1st order (AR1) correlation structure to account for the autocorrelation, or a generalized least squares model fit by REML for repeated measures using an added autoregressive moving average (ARMA) correlation structure using nlme v. 3.1-137 (Pinheiro et al. 2018) and car v. 3.0 (Fox & Weisberg 2011). The Tukey's test for pairwise comparisons was run on the model using multcompView v. 0.1-7 (Graves et al. 2015) and lsmeans v. 2.27-62 (Lenth 2016). To assess beta diversity, repeated measures permutational multivariate analyses of variance (PERMANOVA, strata = colony) were performed and homogeneity of dispersions (PERMDISP) was checked to examine variations in beta-diversity and dispersion between and among time points in vegan v. 2.5-2 (Oksanen et al. 2018); both permutational tests used 999 permutations. Post-hoc pairwise PERMANOVA was performed in pairwiseAdonis v. 0.0.1 (Arbizu 2017) to assess between time point significance. Differences among time points were visualized using nMDS, line plots and stacked bar plots. Indicator species were checked among time points using a multi-level pattern analysis with 999 permutations using indicspecies v. 1.7.6 (De Cáceres & Legendre 2009).

Using the package phyloseq v. 1.25 (McMurdie & Holmes 2013), bacterial taxa driving the temporal patterns were identified by examining the relative abundances of bacterial taxa among samples and time points as well as identifying the taxa present in 100% of the colonies at a given time point, visualized using bubble plots and heatmaps. Further exploration of individual taxon abundances were analysed by linear regression where appropriate and through time by ANOVA, again using a mixed effects model fit by REML for repeated measures with an added

autocorrelation structure in nlme v. 3.1-137 (Pinheiro et al. 2018), car v. 3.0 (Fox & Weisberg 2011), multcompView v. 0.1-7 (Graves et al. 2015) and lsmeans v. 2.27-62 (Lenth 2016).

Results

Orpheus Island seawater temperatures and thermal stress

There were no visible signs of bleaching in any of the tagged colonies throughout the study period, despite high temperatures occurring in Little Pioneer Bay during Feb-May 2016 (Figure 4.2). This time period coincided with a mass bleaching event on the Great Barrier Reef, and temperatures reached a high of 32.6°C on 20 February 2016, exceeding 32°C (daytime temperature) for 5 days in a row. February experienced a total of 8 days of temperatures reaching above 32°C, and 19 days exceeding 31°C. Little Pioneer Bay reached 2 DHWs at the end of February and remained at 2 DHWs through April (Figure 4.2). The reefs around Orpheus Island were reported between 10 and 30% bleached at this time (Hughes et al. 2017) and within the study area, many coral species, such as the less robust acroporids and pocilloporids that included other colonies of *P. acuta* of the fine branching type, were found bleached in early March (Smith et al. 2017b; Hughes et al. 2017; *pers. obsv.*).

Amplicon Sequencing

A total of 2,660,251 sequences from 79 samples were recovered to describe the Symbiodiniaceae community within *P. acuta* samples. Clustering at the 97% sequence similarity threshold identified 16 unique OTUs at the sub-type taxonomic level. Negative controls were checked visually using nMDS for overlap and removed from the dataset.

16S rRNA Bacterial Community: A total of 2,582,381 sequences from 79 samples were recovered for bacterial community analysis. Single variant methods identified 3,358 unique bacterial sequence variants (putative OTUs); no archaeal sequences were retrieved. Singletons were removed from the dataset and negative controls were checked visually using nMDS for overlap. Despite no visual overlap, negative controls contained four sequence variants of the taxa *Bradyrhizobium*, *Ralstonia*, and *Oxalobacteraceae* that followed this pattern, and were subsequently removed from all samples of the dataset for further analysis.

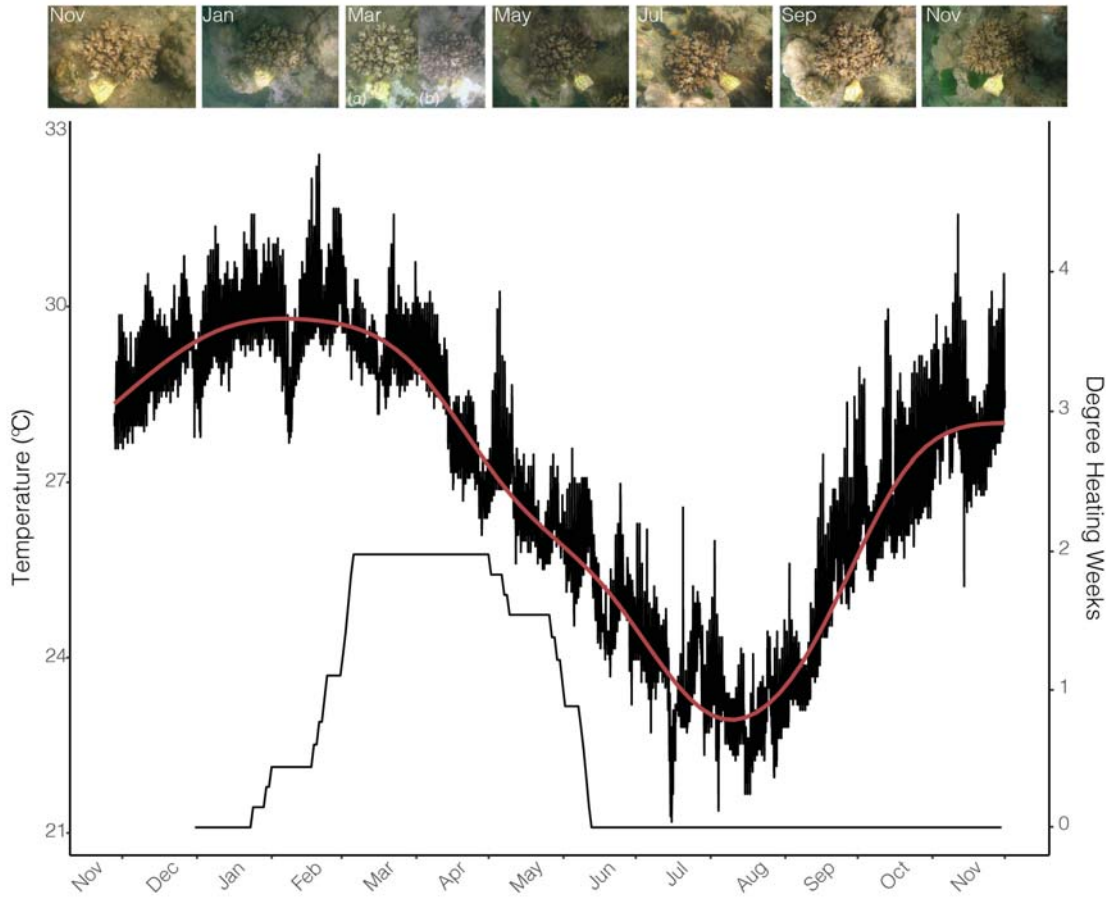

FIGURE 4.2 DAILY TEMPERATURE PROFILE (RED LINE IS THE DAILY AVERAGE; LEFT HAND Y-AXIS) AND DEGREE HEATING WEEKS (RIGHT HAND Y-AXIS) FOR LITTLE PIONEER BAY FROM NOVEMBER 2015 – NOVEMBER 2016. PHOTOGRAPHS OF A REPRESENTATIVE TAGGED COLONY (X1) SHOW NO VISIBLE SIGNS OF BLEACHING THROUGHOUT THE STUDY PERIOD.

Characterization of the Symbiodiniaceae community structure across time

The Symbiodiniaceae alpha diversity and community structure (beta diversity) within *P. acuta* did not differ significantly among time points (Figure 4.3a). At each time point, the Symbiodiniaceae communities were dominated by *Cladocopium* type C1, making up on average a relative abundance of 88.9 ± 0.3% (mean ± SE). Type C1 was largely comprised of the sub-type C1d. *Cladocopium* type C42 on average accounted for 8.2 ± 0.2% (mean ± SE) of the Symbiodiniaceae community. *Durusdinium* type D1 was present in low relative abundances (average <1%) (Figure 4.3b). However, this was entirely due to a single colony, which gained a relative abundance of 13.5% of type D1 in July, but was completely absent at all other time points and in the other nine colonies.

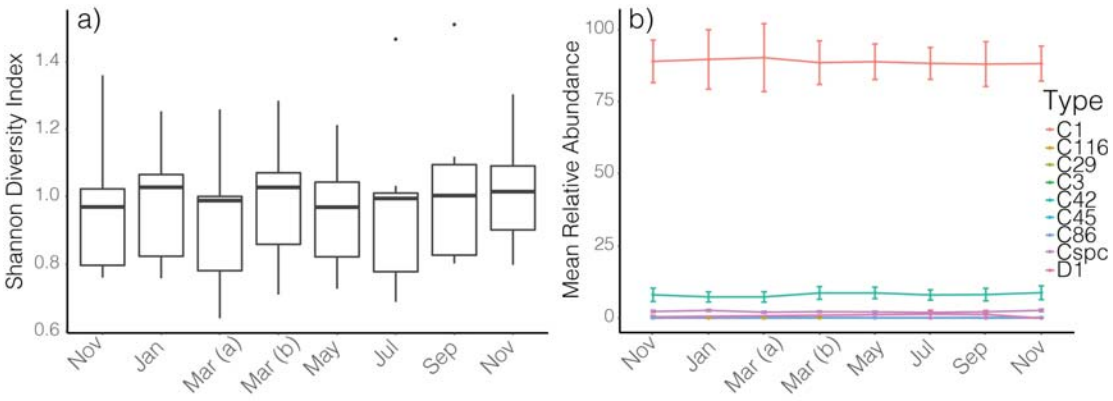

FIGURE 4.3 A) ALPHA DIVERSITY (SHANNON DIVERSITY INDEX) OF SYMBIODINIACEAE COMMUNITIES AND B) MEAN RELATIVE ABUNDANCE OF SYMBIODINIACEAE TYPES AMONG TIME POINTS RANGING FROM NOVEMBER 2015 - NOVEMBER 2016.

Characterization of the bacterial community structure over time

The most abundant bacterial phylum in *P. acuta* was Proteobacteria, with Gammaproteobacteria, Cytophagia, Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Flavobacteriia, Sphingobacteriia, Spirochaetes and Acidimicrobiia and Bacilli being the top ten most abundant classes (Supplementary Figure S4.1). The top four most abundant classes were further broken down into orders to examine the structure of the *P. acuta* microbiome among time points (Figure 4.4). The order Oceanospirillales occurred at a relative abundance between 15 and 42% among time points, where the genus *Endozoicomonas* made up over 61% of all Oceanospirillales and varied between 44.5 and 78% on average per time point (Figure 4.4a). Cytophagia was fully comprised of the order Cytophagales, in which a single candidate genus, "*Candidatus* Amoebophilus", accounted on average for over 84% of all Cytophagales (Figure 4.4b). Alphaproteobacteria was dominated by the order Rhodobacterales, which was comprised mostly of the genera *Roseovarius, Thalassobius* and other unknown or uncultured Rhodobacterales (Figure 4.4c). Betaproteobacteria consisted almost entirely of the order Burkholderiales, which was dominated by the genera *Burkholderia-Paraburkholderia* (97.8%) (Figure 4.4d).

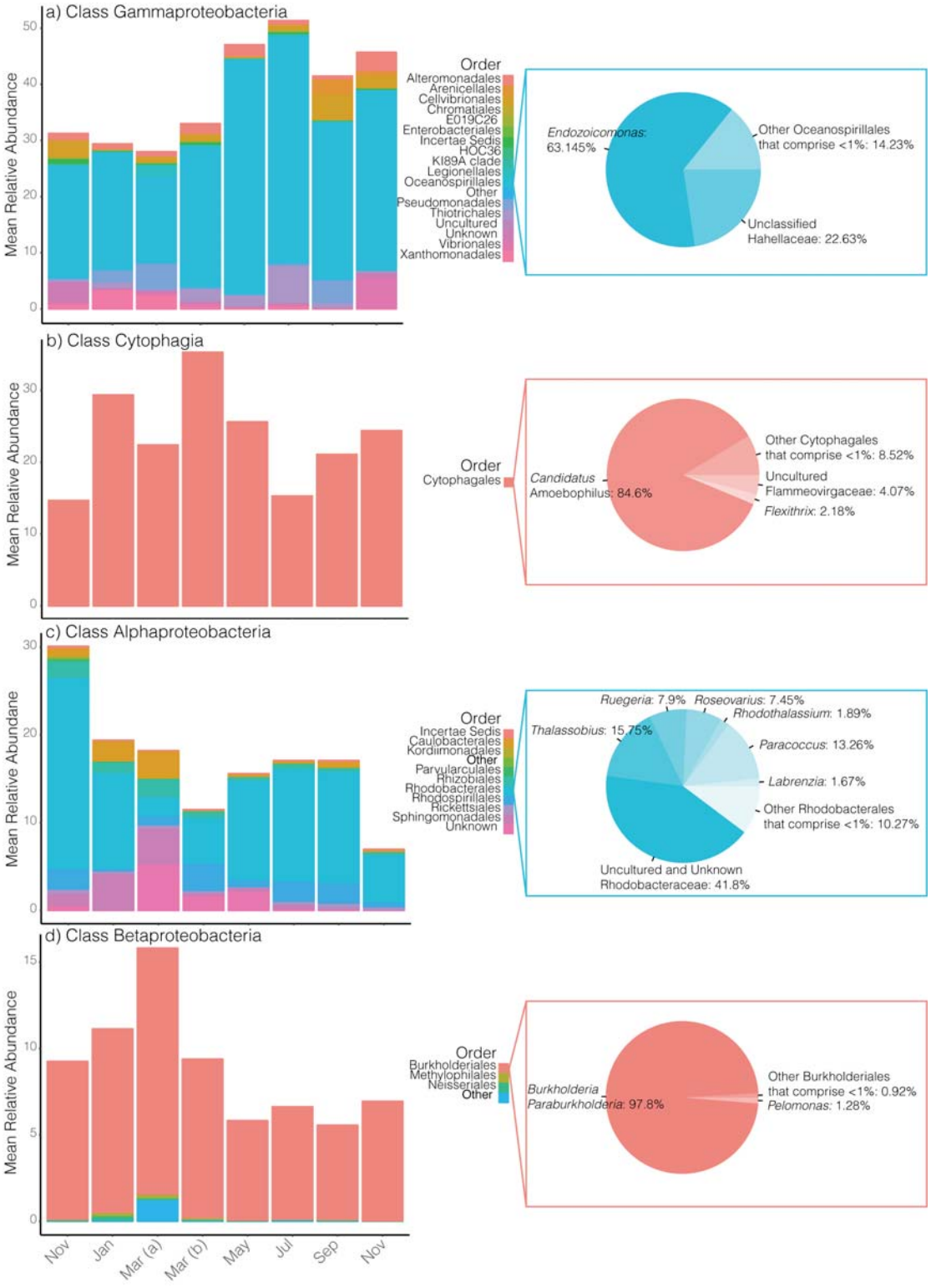

FIGURE 4.4 RELATIVE ABUNDANCES OF THE TOP FOUR MOST ABUNDANT CLASSES, A) GAMMAPROTEOBACTERIA, B) CYTOPHAGIA, C) ALPHAPROTEOBACTERIA AND D) BETAPROTEOBACTERIA, BROKEN DOWN INTO ORDER ACROSS TIME POINTS RANGING FROM NOVEMBER 2015 – NOVEMBER 2016. PIE CHARTS SHOW A BRAKDOWN OF GENERA IN THE MOST ABUNDANT ORDER FROM EACH CLASS.

Alpha diversity, richness and evenness in *P. acuta* microbial communities were variable over time (Figure 4.5a-c). At the sequence variant level, alpha diversity (Shannon-Weiner diversity index) differed significantly between time points ($ANOVA_{div}$: df = 7, F = 5.168, $p < 0.001$), as did

species richness ($ANOVA_{rich}$: df = 7, F = 3.69, p < 0.01) and evenness ($ANOVA_{ev}$: df = 7, F = 4.29, p < 0.001). Both alpha diversity and evenness showed significant variation between May 2016 and both March (a) 2016 and November 2015. Richness showed significant differences between May and November 2015 (Figure 4.5a-c). The bacterial community composition (beta-diversity) at the sequence variant level also significantly differed among time points (PERMANOVA: df = 7, MS = 0.47, p = 0.001; Figure 4.5d), with no significant variation in dispersion. The ordination plot showed some separation between January and March (a) from the rest of the time points (Figure 4.5d); however, further pairwise comparisons found no significant differences in beta-diversity between time points. Relative abundances of the top 50 families showed some individual variation but little consistency in variation among time points (Figure 4.6). Each time point contained a number of significant indicator species that ranged between 1 and 26 (Supplementary Table S4.1). March (a) 2016 had the highest number of indicators, followed by November 2015, January 2016 and March (b) 2016, respectively.

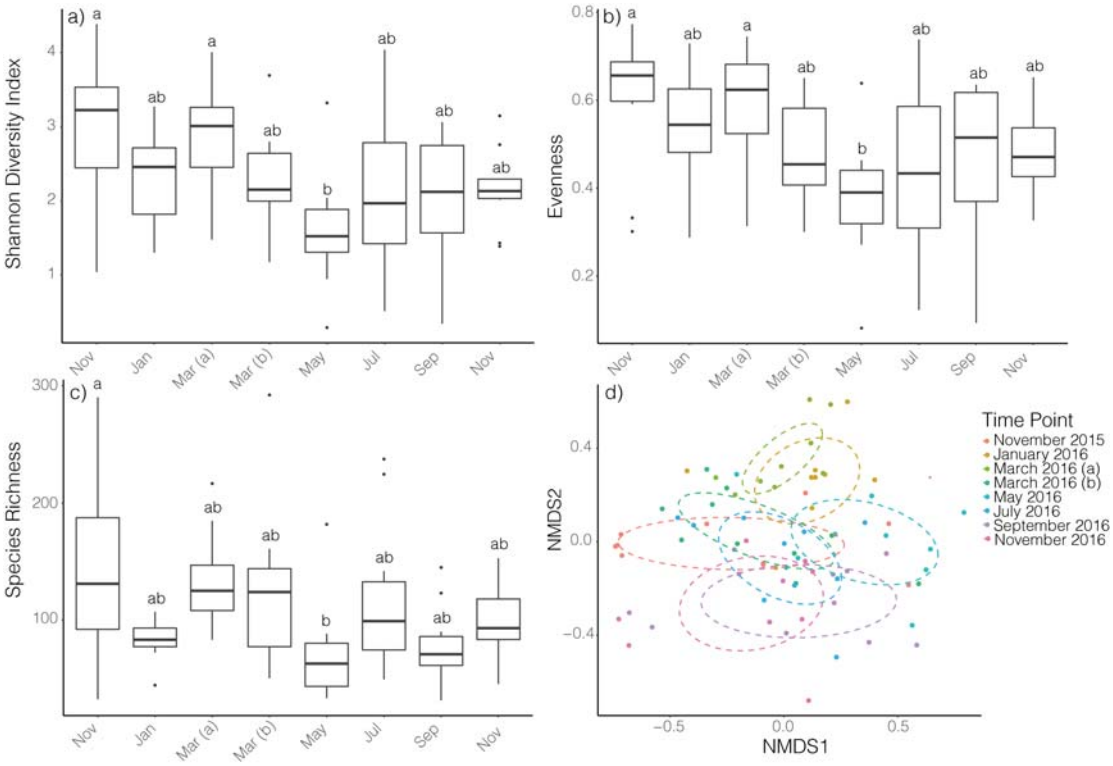

FIGURE 4.5 THE A) ALPHA DIVERSITY (SHANNON DIVERSITY INDEX), B) EVENNESS, C) SPECIES RICHNESS AND D) BETA DIVERSITY (DATA CLOUDS VISUALIZED USING NMDS) AMONG TIME POINTS, RANGING FROM NOVEMBER 2015- NOVEMBER 2016. ELLIPSES REPRESENT 95% CONFIDENCE INTERVALS.

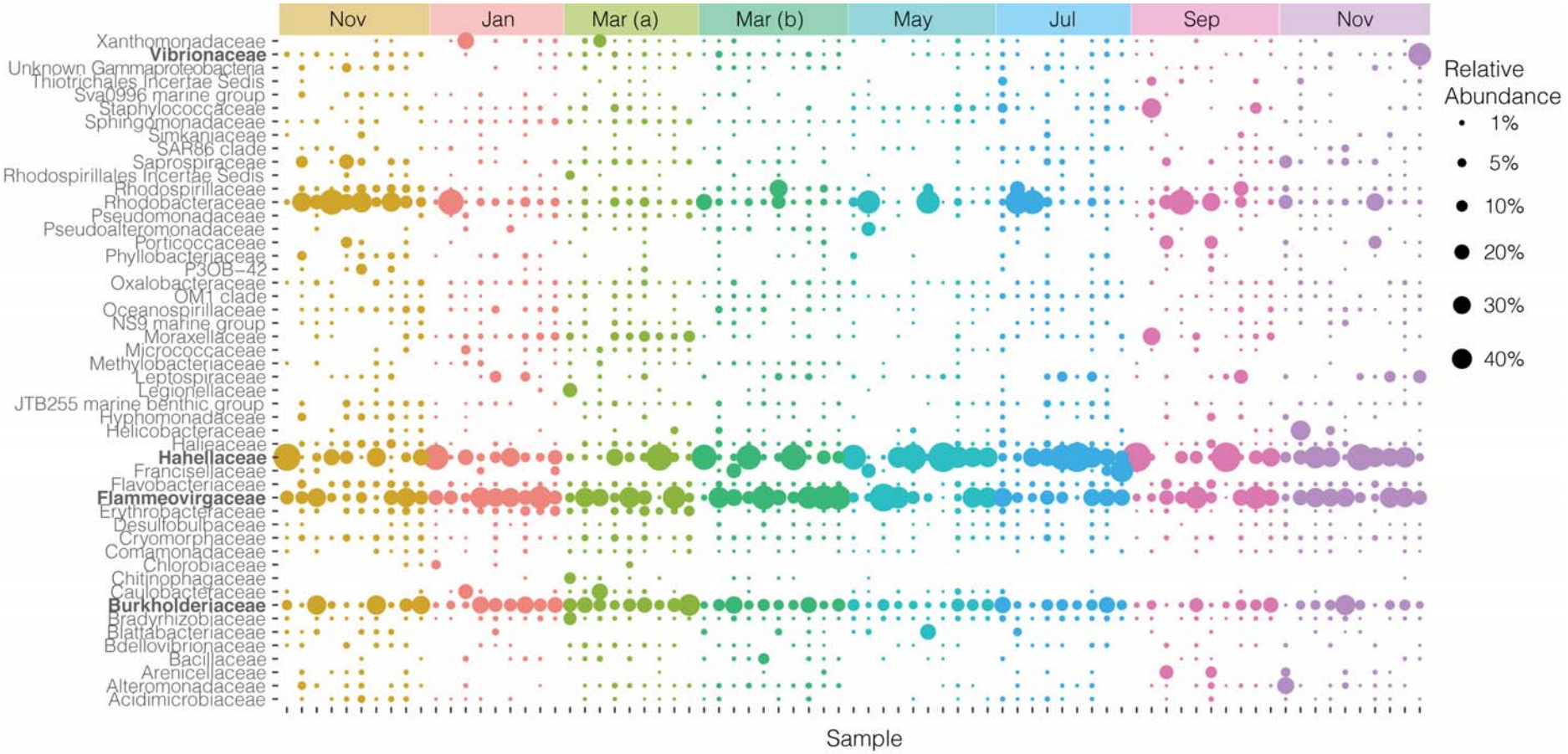

FIGURE 4.6 RELATIVE ABUNDANCE OF THE TOP 50 MOST ABUNDANT FAMILIES AMONG TIME POINTS, RANGING FROM NOVEMBER 2015 - NOVEMBER 2016. FAMILIES IN BOLD ARE MEMBERS OF THE CORAL MICROBIOME THAT ARE FURTHER DISCUSSED BELOW

Specific individual taxa were further examined across time points (Figure 4.7a). Relative abundances of the classes Gammaproteobacteria, Alphaproteobacteria and Betaproteobacteria did not vary significantly among time point. The relative abundance of Cytophagia differed significantly among time points (ANOVA: df = 7, F = 2.21, $p < 0.05$), but this was driven by significant differences only between November 2015 and November 2016. Relative abundances of the genera *Endozoicomonas* and *Vibrio* also showed no significant variation in relative abundances through time, however May and November 2016 displayed unusually high variance for *Endozoicomonas* and *Vibrio*, respectively (Figure 4.7b). There was no relationship between *Vibrio* and *Endozoicomonas* ($R^2 = 0.004$, data not shown).

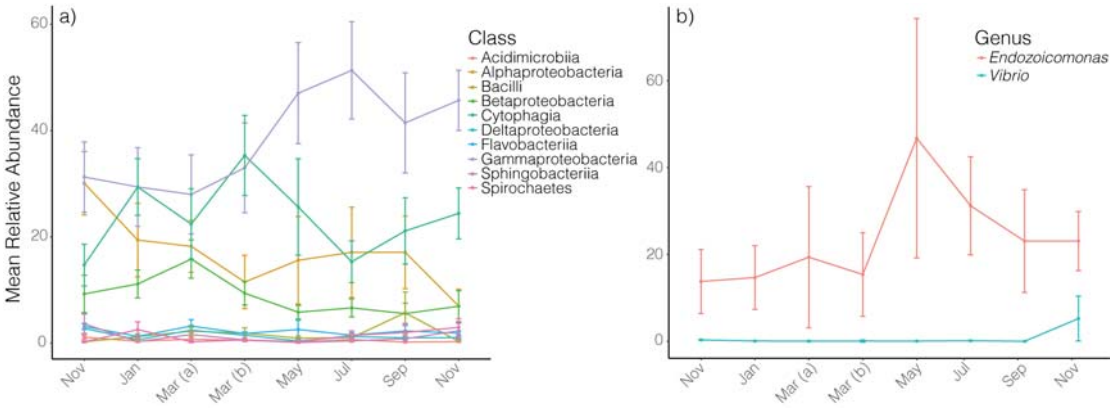

FIGURE 4.7 MEAN RELATIVE ABUNDANCE OF A) TOP MOST ABUNDANT BACTERIAL CLASSES AND B) THE TWO GENERA *ENDOZOICOMONAS* AND *VIBRIO* THROUGHOUT THE STUDY PERIOD FROM NOVEMBER 2015 - NOVEMBER 2016.

At each time point only a few members of the bacterial microbiome were present in all colonies, where *Burkholderia-Paraburkholderia* was the only taxon present in all colonies across time points. Most time points displayed differing combinations of bacteria that were present in every sample. Over 94% of samples included at least one or more sequence variants of *Endozoicomonas*, and while some colonies contained no *Endozoicomonas* at some time points, 6 of the 10 colonies had at least one or more sequence variants of *Endozoicomonas* at every time point. "*Candidatus* Amoebophilus" was also present in high abundances throughout the study period in every colony aside from one, in which it was absent during seven of the eight time points.

Discussion

This study showed that thermal stress during the 2016 summer had a minimal effect on the microbiome composition of *P. acuta* in Little Pioneer Bay. The Symbiodiniaceae community remained stable throughout the sampling period, and while the bacterial community showed some evidence of variation through time, overall stability was maintained, including during the months that coincided with the mass-bleaching event on the GBR. According to Hughes et al. (2017), 2 DHWs elicited a widespread bleaching response across the GBR at the end February 2016, and

their aerial surveys found the reefs around Orpheus Island were between 10-30% bleached during this time period. The temperature data from the present study corroborate these findings and suggest that this was a period of thermal stress for corals in Little Pioneer Bay, which experienced 2 DHWs from the end of February through April. While many corals were bleached at the study site, the targeted colonies in this study remained unbleached. Although repeated sampling could trigger increased innate immune activation as a result of injury (e.g., van de Water et al. 2015), no previous injuries or wound sites from sampling were detected at each time point as all colonies had re-grown their branchlets between sampling.

The dominant Symbiodiniaceae type was C1 and remained the same for all 10 colonies throughout the 1-year study period. Pocilloporid Symbiodiniaceae communities are commonly dominated by members in the genus *Cladocopium* (formerly Clade C, LaJeunesse et al. 2018), sometimes by type C1 (Magalon et al. 2007) and at other times types C42 and C33 (Sampayo et al. 2009; Tonk et al. 2013). Sequence types C42 and C1 are likely intragenomic variants of the same Symbiodiniaceae type (Sampayo et al. 2009). Symbiodiniaceae is a key player in the thermal acclimatization of coral hosts, and some corals have previously been found to either shuffle their dominant Symbiodiniaceae types or acquire novel types following severe bleaching (Berkelmans & van Oppen 2006; Boulotte et al. 2016; Cunning et al. 2018). However, the Symbiodiniaceae community in *P. acuta* in the present study remained unchanged throughout the entire study period. These results suggest a high degree of temporal stability in the Symbiodiniaceae community of *P. acuta* in the absence of bleaching, which is consistent with previous studies showing minimal community changes through both time (LaJeunesse et al. 2005; Thornhill et al. 2006a; Klepac et al. 2015; Cai et al. 2018) and during a similar sub-bleaching thermal stress event (Stat et al. 2009a). It is possible that sub-bleaching thermal stress does not generally affect the stability and maintenance of the Symbiodiniaceae communities. Alternatively, the ability of the coral host to shift this community in response to thermal pressure may be species-specific and reliant on the degree or severity of thermal stress (Cunning et al. 2018). Symbiodiniaceae types in the genus *Durusdinium* (formerly Clade D, LaJeunesse et al. 2018) have often been associated with high thermal tolerance of their coral host, and corals have been found to shift their dominant Symbiodiniaceae type from *Cladocopium* to *Durusdinium* following bleaching events (Berkelmans & van Oppen 2006; Jones et al. 2008). In contrast, the present study found only one colony that contained type D1, and not only was it never the dominant genus, its acquisition in the colony did not occur directly after the thermal stress event and was instead present only in July. It could be argued that this is still a delayed result of the heat stress. If some of the *Cladocopium* types were impaired in the months following the heat stress, this could have allowed the *Durusdinium* type to outcompete the C types for a short period of time. Regardless, the shifting of dominant Symbiodiniaceae from *Cladocopium* to *Durusdinium* types

was not observed within *P. acuta* during this study and suggests that the tolerance of this *P. acuta* morphology is not necessarily reliant on its association with a thermally tolerant symbiont such as those in the genus *Durusdinium*.

Gammaproteobacteria, Alphaproteobacteria, Cytophagia and Betaproteobacteria dominated the bacterial community of *P. acuta* throughout the study period. The only bacterium that was present in 100% of samples at all time points was a strain in the genus *Burkholderia-Paraburkholderia*, of the family Burkholderiaceae. *Burkholderia* spp. have previously been found to be abundant members of the early life stages of corals (Bayer et al. 2013; Leite et al. 2017) and are suggested to play a role in nitrogen fixation as has been demonstrated for plants (reviewed by Coenye & Vandamme 2003). One of the most abundant genera in this data set was the candidate genus "*Candidatus* Amoebophilus" of the family Flammeovirgaceae, and one sequence variant was present in 9 of the 10 colonies in high abundances throughout the study period. It is a recognized intracellular symbiont of amoebae (Horn et al. 2001) and has the potential for establishing a symbiotic relationship with other eukaryotes (Horn et al. 2001; Schmitz-Esser et al. 2010). Recently, it has been found to consistently associate with five species of Caribbean coral and is thought to associate with other eukaryotic hosts within the coral microbiome, such as the Symbiodiniaceae, or unidentified amoebae within coral tissue (Apprill et al. 2016). The genus *Endozoicomonas*, of the family Hahellaceae, was present in the majority of samples, likely representing an important member of the *P. acuta* microbiome. This genus has been found in a variety of coral species, including those in the families Pocilloporidae (Ziegler et al. 2016; Neave et al. 2017a), Acroporidae (Ziegler et al. 2016), Poritidae (Apprill et al. 2016), and Fungiidae (Roder et al. 2015), and their abundances are often linked with coral health (e.g., Bourne et al. 2008; Morrow et al. 2015; Ziegler et al. 2016). *Endozoicomonas* typically resides within coral tissues as aggregates and some strains have been suggested to be responsible for carbohydrate upcycling and provision of amino acids to the coral host (Neave et al. 2016, 2017a), as well as protection from thermal or bleaching stress (Pantos et al. 2015).

There was significant variation in bacterial alpha and beta diversity across the entire duration of the study. Despite the significant decrease in alpha diversity over the winter months, which could suggest some seasonal variation (e.g., Sharp et al. 2017) further statistical tests for beta diversity could not resolve which time points drove the observed significance, instead suggesting overall bacterial community stability through time and during the thermal anomaly. Multi-level pattern analysis found twenty-six significant indicator species during the March (a) 2016 time point, coinciding with the mass-bleaching event. Understanding these fine-scale microbiome changes in bleaching-resistant corals may provide an opportunity to identify beneficial microbes for coral resilience. At this time point, there were four indicator taxa of the phylum Actinobacteria, which have been found to have antimicrobial, antioxidant and antiparasitic properties (Valliappan et al.

2014). Although these bacteria may provide some level of protection to the coral host during thermal stress, other Actinobacteria species were also found as indicators during the January 2016 time point, instead suggesting that the presence of Actinobacteria may be following a seasonal pattern, as has been found previously (Cai et al. 2018). Three nitrogen-fixers (diazotrophs) from the order Rhizobiales were also found as indicators of the March (a) time point, which supports previous findings by Santos et al. (2014) who propose increases in diazotroph abundance are indicative of thermal stress on corals. This increase likely represents a beneficial mechanism for maintaining homeostasis by providing otherwise limited nitrogen to the Symbiodiniaceae (Olson et al. 2009; Lema et al. 2012; Santos et al. 2014). These fine-scale differences among time points, however, did not affect the overall stability of the microbiome, instead suggesting flexibility to adjust to environmental changes without affecting microbiome structure.

Previous studies on the coral microbiome suggest that changes in the abundances of certain taxa are common when corals are exposed to environmental stress (e.g., high temperature, Bourne et al. 2008; low pH, Webster et al. 2016; or pollution, Ziegler et al. 2016). During bleaching, the abundance of *Endozoicomonas* has been found to decrease significantly at the same time as the abundance of *Vibrio* increases (Ben-Haim & Rosenberg 2002; Bourne et al. 2008). Some suggest certain *Vibrio* species may be a causative agent of bleaching (e.g., *Vibrio shiloi* in *Oculina patagonica*, Kushmaro et al. 1996, 1997, 2001 and *Vibrio corallilyticus* in *Pocillopora damicornis*, Ben-Haim & Rosenberg 2002). Further, Bourne et al. (2008) found that the shift to a *Vibrio*-dominated microbiome occurred prior to visual signs of bleaching. In the present study, there was no evidence of significant increases in the abundance of *Vibrio* or decreases in the abundance of *Endozoicomonas* throughout the study period, nor was there any relationship between the abundances of these two genera. Similar stability in *Endozoicomonas* and *Vibrio* has been found previously under nutrient-induced bleaching conditions (Pogoreutz et al. 2018). Note that it is possible that the functional response of a microbial community could change without considerable variations in abundance of these taxa. For instance, recent metagenomic work has found that even with minimal changes in abundance of *Vibrio* species, increases in virulence genes from the *Vibrio* family were observed during bleaching, suggesting that the group may contribute disproportionately to coral microbiome function under temperature stress (Vega Thurber et al. 2009; Littman et al. 2011).

Host species is perhaps a key factor in maintaining a stable microbiome during periods of stress (e.g., Meistertzheim et al. 2016; Grottoli et al. 2018; Pogoreutz et al. 2018). Smith et al. (2017b) found that colony morphology of *P. acuta* may play a role in its bleaching tolerance where the chunky morph of *P. acuta* being more resistant to bleaching than its fine morph counterpart. It has been well documented that skeletal morphology controls intra-colonial light amplification and scattering, where fine branching can increase irradiance (Marcelino et al. 2013). While highly

beneficial for corals at depth for improving light capture, these morphologies may accelerate bleaching in shallow water corals (Marcelino et al. 2013; Swain et al. 2016). Smith et al. (2017b) therefore suggest that the closer branching of the chunky morph of *P. acuta* reduces irradiance within the coral colony and minimizes the potential for bleaching to occur, resulting in a stable Symbiodiniaceae community as seen in the present study. It can be hypothesized that this morphology may also play a role in maintaining a stable bacterial community by reducing the stress within the colony. Future experimental work on the two morphologies of genetically similar *P. acuta* could provide valuable insight into the drivers of both microbial community composition and coral resilience.

Microbiome stability could represent an adaptive advantage or disadvantage to the coral's ability to respond to environmental pressures. In an experiment by Grottoli et al. (2018), two coral species with different thermal sensitivity (one thermally sensitive and one thermally tolerant) were subjected to temperature stress. The microbiome was found to remain stable in the thermally tolerant species, but not in the sensitive species, and the authors suggest that corals with stable microbiomes are more likely to be resilient to adverse environmental conditions. Alternatively, if corals are affected by severe stress, the stability, or rather inflexibility, of the microbiome could facilitate a breakdown of the relationship between host and microbiome resulting in disease and/or mortality. For instance, Pogoreutz et al. (2018) exposed corals to extreme nutrient levels that resulted in tissue loss and mortality within 14 days. During this time, the microbiome remained stable through to coral death (Pogoreutz et al. 2018). Therefore, the ability of a stable microbiome to confer an adaptive advantage is likely highly dependent on both host factors and the severity of the stress event. In the present study, the stability of the microbiome of *P. acuta* appeared to be an advantage to host resilience during the 2016 mass-bleaching event as the corals remained pigmented and there was no evidence of increases in potentially pathogenic bacterial taxa. However, future experimental work exposing *P. acuta* to more severe stress could help identify whether this seemingly beneficial relationship between host and microbiome found in the present study is maintained or breaks down.

The present study provided a temporal view of the *P. acuta* microbiome in response to the 2016 thermal anomaly that resulted in mass-bleaching across the GBR. Despite significant thermal stress, the tagged colonies of *P. acuta* did not bleach and instead Symbiodiniaceae communities were stable through time. Small differences in relative abundance of individual bacterial taxa suggest some level of flexibility in the microbiome to respond to thermal stress, but these differences did not affect overall bacterial community composition throughout the sampling period. Further, expected changes in the taxa *Endozoicomonas* and *Vibrio*, often used as indicators of thermal stress, were not observed. Microbial community changes in response to thermal stress, and consequently their ability to confer host resilience, are likely reliant on host

species, location, and severity of the stress event, demanding caution when making generalizations across corals as a group.

Acknowledgments

I would like to thank the Coral Health Group at the Australian Institute of Marine Science for initial coral tagging, and K. Damjanovic, A. Hernandez-Agreda and H. Smith for statistical advice. This study was funded by Paul G. Allen Philanthropies and AIMS@JCU, the joint venture between James Cook University and the Australian Institute of Marine Science, as part of H.E.E's PhD research. H.E.E acknowledges receipt of an AIMS@JCU Postgraduate Scholarship. Raw sequence data for this study are available on the NCBI Sequence Read Archive under the accession SRP150755.

CHAPTER 5

EVIDENCE FOR MIXED MODE TRANSMISSION OF BACTERIAL AND DINOFLAGELLATE COMMUNITIES IN A COMMON CORAL

IN REVIEW FOR PUBLICATION IN *THE ISME JOURNAL* AS A BRIEF COMMUNICATION

Epstein HE, Torda G, Munday PL & van Oppen MJH (In review). Evidence for mixed mode transmission of bacterial and dinoflagellate communities in a common coral. *ISMEJ.*

Abstract

The establishment of coral microbial communities in early developmental stages is fundamental to coral fitness, but its drivers are largely unknown, particularly for bacteria. Using an *in situ* reciprocal transplant experiment, the influence of parents and both the planulation and early recruit environment on the microbiome of offspring in the coral *Pocillopora damicornis* were examined. ITS2 rDNA and 16S rRNA gene metabarcoding showed that bacterial and microalgal endosymbiont communities varied according to parental and planulation environments, but not with early recruit environment. Microalgal communities of recruits were highly similar to those of their respective parents, but also contained additional low abundance strains, suggesting both vertical transmission and novel ('horizontal') acquisition. Such mixed mode transmission was also suggested for bacteria, although compelling evidence for vertical transmission was found for only one strain. Altogether, recruits harbored more diverse and variable microbiomes compared to their parents, indicating winnowing occurs as corals mature.

Introduction

Corals are host to a diverse and complex consortium of microbial partners, some of which are important to holobiont health and function (reviewed in Bourne et al. 2016). This consortium includes numerous prokaryotic partners, algal endosymbionts in the family Symbiodiniaceae, as well as other protists and fungi, and acellular viruses. However, these communities are often spatially variable and host-specific. In fact, corals of the same species have been known to exhibit different microbial communities when located in different habitats (e.g., van Oppen et al. 2018), and understanding how microbial communities are initially established in corals can help elucidate why we see such spatial variability. For instance, this habitat-specific community could be achieved in each new generation of coral through the inheritance of specific microbial taxa from their parents (i.e., vertical transmission; LaJeunesse et al. 2004b; Leite et al. 2017), from chance uptake from the environment (i.e., horizontal transmission; Apprill et al. 2012; Nitschke et

al. 2016), or a combination of both (i.e., “mixed mode transmission”, Quigley et al. 2018). It could also occur through the regulation and winnowing down of a larger, more variable initial microbial community (e.g., Abrego et al. 2009). Transmission and establishment of microbial partners is key to understanding the relationships and interactions between the host coral and members of its microbiome and can provide insight into microbiome stability, variability and plasticity, which may be important in understanding the ability of corals to either resist or adjust to environmental change.

In corals, the transmission of Symbiodiniaceae symbionts is often correlated with the mode of reproduction. In many cases, brooding corals exhibit vertical transmission of these algal symbionts from parents to offspring, whereas broadcast spawning corals exhibit horizontal transmission (Lesser et al. 2013). However, the mode of transmission is not well known for microbial partners other than Symbiodiniaceae. The limited number of previous studies on bacterial transmission mode in corals has found variability within reproductive mode. Horizontal transmission of prokaryotes was reported in the broadcast spawning coral *Pocillopora meandrina* (Apprill et al. 2012) and *Montastrea, Acropora* and *Diploria* species (Sharp et al. 2010), where it was suggested that external bacteria are incorporated into the ectodermal tissues of late stage planulae via phagocytosis. Conversely, the broadcast spawners *Mussismilia hispida* (Leite et al. 2017) and *Acropora gemmifera* (Zhou et al. 2017a) and one brooding coral, *Porites astreoides* (Sharp et al. 2012), have been suggested to vertically transfer some prokaryotes from parents to gametes prior to spawning, i.e., a mixed mode transmission. Further, in a large natural transplant experiment by Ziegler et al. (2017), no bacterial taxon was associated with any particular genotype of the broadcast spawning coral *Acropora hyacinthus*, suggesting horizontal transmission due to the absence of any covariance between the host genotype and its microbiome.

While transmission of Symbiodiniaceae in corals has been well studied, the lack of pattern identified for bacterial transmission may be due to logistical difficulties in performing experiments in the corals’ natural habitat. Furthermore, laboratory studies of transmission mode can bias the results due to the artificial composition of the environmental microbial community. Using an entirely *in situ* experiment, the present study attempts to discern the transmission patterns of both Symbiodiniaceae and bacteria in the brooding coral *Pocillopora damicornis*, a cosmopolitan branching species common in multiple reef habitats. Specifically, parental corals and newly settled recruits were cross-transplanted between two adjacent reef habitat types (reef flat and reef slope). This novel approach was designed to distinguish the influence of parents and the planulation and early recruitment environments on the microbial community composition of *P. damicornis* offspring.

Methods

Field Experiment

To investigate the extent to which early life stage coral microbiomes are influenced by parents or by environment, an *in situ* double reciprocal transplant experiment on parental colonies and recruits was conducted at "Coral Canyons" on Heron Island Reef (23°27'2"S, 151°55'6"E) in the Southern Great Barrier Reef (GBR). This experiment took advantage of our ability to isolate brooding corals *in situ* during planulation and have the subsequent brooded planulae settle onto settlement tiles that were then transplanted across habitats. This experiment was timed to coincide with peak planulation of *P. damicornis* in October 2017, approximately five days after the full moon (Tanner 1996). Fully closed acrylic settlement boxes were custom made, with two sides containing 300 µm plankton mesh and the base covered in nine 11x11 cm pre-conditioned, bleached and cleaned terra cotta tiles (Figure 5.1).

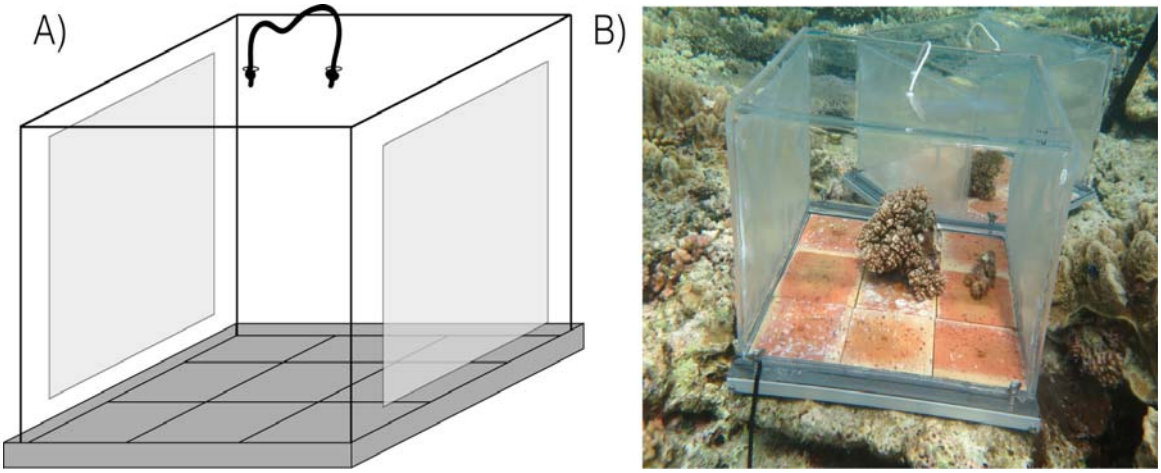

FIGURE 5.1 A) THE SETTLEMENT BOXES WERE DESIGNED TO HOUSE NINE REMOVABLE SETTLEMENT TILES IN THE BASE OF THE BOX AND TWO SIDES MESH TO ALLOW FOR WATER FLOW. B) A PHOTOGRAPH OF A SETTLEMENT BOX IN USE FOR THIS EXPERIMENT.

Adjacent reef flat and slope habitats, separated horizontally by ~30 m and vertically by 4-5 m, were used for the reciprocal transplant. Ten adult corals were carefully removed from the reef matrix in both the reef flat and slope environment and placed individually into settlement boxes. Five of the adults in boxes stayed at their home environment and five were immediately transferred in their boxes to their reciprocal environment prior to brooding to account for any microbial changes that could occur between planulae release and metamorphosis (Figure 5.2). A small nubbin was sampled from each adult prior to removal from the reef, rinsed in sterile phosphate buffered saline (PBS) and placed in liquid nitrogen (LN_2) for downstream DNA metabarcoding. Eight 500 mL water samples were taken at this time, four from the reef flat and four from the slope, filtered through 0.22 µm Sterivex^{TM} filters (Millipore). The filters were snap frozen in LN_2 for DNA metabarcoding.

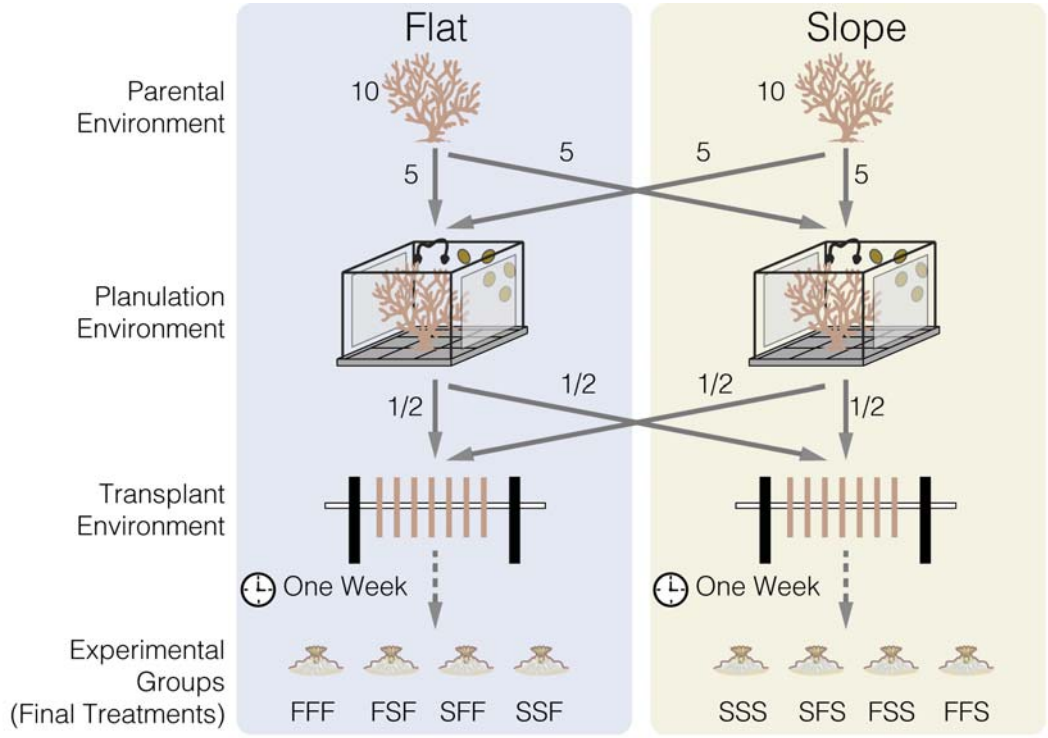

FIGURE 5.2 FULL RECIPROCAL TRANSPLANT EXPERIMENTAL DESIGN. THE EXPERIMENT BEGAN WITH 10 PARENTS FROM THE FLAT AND SLOPE REEF HABITATS (PARENTAL ENVIRONMENT). THESE WERE RECIPROCALLY TRANSPLANTED TO UNDERGO PLANULATION (PLANULATION ENVIRONMENT). SETTLEMENT TILES WITH SETTLED RECRUITS WERE AGAIN RECIPROCALLY TRANSPLANTED AND PLACED ON "SKEWERS" (TRANSPLANT ENVIRONMENT). EXPERIMENTAL GROUPS ARE LABELLED ACCORDING TO THE THREE ENVIRONMENTS CORAL OFFSPRING UNDERWENT DURING TRANSPLANTATION, WHERE "F" REFERS TO FLAT AND "S" REFERS TO SLOPE.

Once in place, the settlement boxes were cable tied to the reef matrix to ensure minimal movement over the planulation period. Parents were left to planulate overnight and the boxes were checked the following morning for signs of planulae. The released planulae were then left for one more night to ensure settlement and metamorphosis. After metamorphosis, the parents were removed from the boxes and re-attached to the reef at their collection site. The tiles with settled *P. damicornis* recruits were then split between the reef flat and reef slope environments, depending roughly on the number of tiles with recruits on them and the number of recruits on each tile to ensure there were settled recruits from each parent at both the parental and the reciprocal environment. The tiles had a hole in their centre used to skewer them on rods, and were separated by 1 cm-wide spacers (cut from polyethylene pipe) to minimize movement. These rods were attached to star pickets at the two habitats so that the tiles were vertically oriented. Experimental groups were labelled according to the parental environment, the planulation environment, and the final transplant environment. For instance, FFF refers to recruits from a parent from the flat that released planulae on the flat and tiles that were transplanted to the flat. There were eight resulting experimental groups: FFF (flat, flat, flat), FFS (flat, flat, slope), FSF (flat, slope, flat), FSS (flat, slope, slope), SSS (slope, slope, slope), SSF (slope, slope, flat), SFS (slope, flat, slope), SFF (slope, flat, flat) (Figure 5.2). The settlement tiles were left in place for

one week, after which recruits were sampled. Rods were carefully brought to the surface and sampling was done immediately on the boat. Each recruit was carefully scraped off its tile using a scalpel and washed in sterile PBS before being placed into a cryo-vial and snap-frozen in LN_2. At this one-week time point, eight 500 mL water samples were again taken, four each from the reef flat and the slope, filtered through 0.22 µm Sterivex^{TM} filters and snap frozen in LN_2.

DNA Extraction, PCR and Amplicon Sequencing

DNA extraction from parent, recruit and water samples was performed using a salting out method as described by Damjanovic et al. (2017) (Appendix A), which included a lysozyme digestion and a bead beating step prior to proteinase K digestion. The V5-V6 region of the 16S rRNA gene for bacteria and the rDNA inter-transcribed spacer region 2 (ITS2) for Symbiodiniaceae were targeted using gene-specific primers. Amplification was achieved using polymerase chain reaction (PCR) with the following primers for 16S: 784F 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGGATTAGATACCCTGGTA -3' and 1061R 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CRRCACGAGCTGACGAC-3' (Andersson et al. 2008), and ITS2: ITS2F 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGAATTGCAGAACTCCGTG -3' and ITS2R 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG CCTCCGCTTACTTATATGCTT -3' (Pochon et al. 2012), which included the underlined Illumina MiSeq 5' adapter overhangs. Both 16S and ITS2 PCRs were performed in triplicate 10 µL reactions. 16S PCR reactions were made up of 5 µL of AmpliTaq Gold MasterMix (Applied Biosystems), 2 µL of each forward and reverse primer (2µM stock), and 1 µL of DNA template and run with the following profile: initial denaturation at 95°C for 10 minutes, then 30 cycles of denaturation at 95°C for 30 seconds, annealing at 57°C for 60 seconds and extension at 72°C for 60 seconds, followed by a final extension at 72°C for 7 minutes. ITS2 PCR reactions were made up of 5 µL of Qiagen Mulitplex MasterMix (Qiagen), 3 µL MilliQ, 0.5 µL of each primer (4 µM stock), and 1 µL of DNA template and run with the following profile: 95°C for 5 minutes, then 31 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds, followed by a final extension at 72°C for 5 minutes. PCR clean-up, indexing and sequencing were carried out at the Ramaciotti Centre for Genomics at the University of New South Wales, Sydney on a 2x300bp Illumina MiSeq. Paired-end sequences were returned de-multiplexed. Both blank extractions and no template PCR controls were run alongside the samples for both 16S and ITS2 sequencing to check for possible contamination.

Assembly, Quality Control and Taxonomic Assignment of ASVs

Paired-end sequences for both 16S and ITS2 were assembled, quality checked and assigned taxonomy using a customized QIIME2 v 2017.10 (Caporaso et al. 2010) pipeline. Quality

filtering guidelines were set by visualising read quality with the plug-in demux (Caporaso et al. 2010), and subsequent quality filtering and trimming, de-replication, chimera filtering, merging of paired-end reads and identifying single nucleotide variation among sequences (i.e., amplicon sequence variants; ASVs) were performed using the DADA2 plug-in (Callahan et al. 2016). For 16S, taxonomic assignment was done by training a naïve-Bayes classifier on the V5-V6 region of the 16S gene in the SILVA 128 database (Quast et al. 2013). Due to high numbers of single nucleotide variants found for ITS2 and the small taxonomic reference database, the use of a classifier as above was inappropriate due to low taxonomic resolution. Instead, ITS2 single variants were clustered by 97% similarity using a vsearch plug-in (Rognes et al. 2016) and assigned taxonomic classification from the Arif et al. (2014) database, allowing assignment down to the sub-type level for Symbiodiniaceae. Following taxonomic assignment for both 16S and ITS2, the taxa plug-in (Caporaso et al. 2010) was used to create a sequence variant biom table of raw sequence counts (abundances) that were used for downstream statistical analyses.

Statistical Analyses

All statistical analyses were performed in R v. 3.5.0 (R Core Team 2018) using the package phyloseq v. 1.25 (McMurdie & Holmes 2013). Graphics were created using the package ggplot2 v. 2.2.1 (Wickham 2009). Alpha diversity and species richness were calculated and differences among treatment were identified by analyses of variance (ANOVA) using linear models and post-hoc comparisons using Tukey's in lsmeans v. 2.27-62 (Lenth 2016) and multcompView v. 0.1-7 (Graves et al. 2015). Beta-diversity and dispersion was assessed among treatments using permutational multivariate analysis of variance (PERMANOVA) and homogeneity of dispersions (PERMDISP), both using 999 permutations with the vegan v. 2.5-2 (Oksanen et al. 2018) plug-in for phyloseq. Post-hoc pairwise PERMANOVAs were conducted using pairwiseAdonis v. 0.0.1 (Arbizu 2017), using a Bonferroni *p* value adjustment for multiple comparisons. Differences among treatments were visualized using ordinations, specifically nonparametric multi-dimensional scaling (nMDS) and principal components analysis (PCoA), as well as bar and bubble plots. Shared taxa were visualized across treatments using Venn diagrams in the package VennDiagram v. 1.6.2 (Chen 2018) and tested and identified at the 100% level for each parent-recruit cohort using the package microbiome v. 1.3.1 (Lahti et al. 2017). Differences in specific taxa were tested among treatments using ANOVA and post-hoc comparisons as above, using an arcsine transformation on relative abundance data where necessary to validate assumptions.

Results

Planulation, Reciprocal Transplant and Sampling

There was sufficient planulation in the settlement boxes for six of the 20 adult corals. Settlement and metamorphosis of these planulae occurred within 48 hours of release with numbers of recruits on tiles varying among boxes and tiles. Due to the uneven nature of planulation, settlement, and recruit survival, the number of recruits sampled per tile and per adult varied, and only six experimental groups were recovered: FFF (n = 7), FFS (n = 5), FSF (n = 2), FSS (n = 10), SSF (n = 11), SSS (n = 15).

Amplicon Sequencing

A total of 5,468,801 sequences from 86 samples were recovered to assess the bacterial community in adults, recruits and the associated water samples. Single variant methods identified 12,860 unique amplicon sequence variants (ASVs)..Contaminant taxa would be expected to have high relative abundance in negatives and low relative abundance in samples (Lee et al. 2015a). Therefore, any ASVs with a ratio of relative abundance in negatives compared with samples that was 1 or above were removed. The following six potential contaminants were identified: 1 ASV of *Burkholderia-Paraburkholderia*, 1 ASV of *Bradyrhizobium*, 2 ASVs of *Ralstonia*, 1 ASV of *Planifilum* and 1 ASV of Oxalobacteraceae. These ASVs were removed from the dataset prior to further analysis and blanks and no-template PCR controls were also removed from the analysis (n = 8). Parents that did not provide any planulae (n = 14) were also removed from the analysis so that direct comparisons between parents and their respective recruits could be made. This resulted in a total of 64 samples including 6 adults, 42 recruits, and 16 water samples.

Symbiodiniaceae sequencing recovered a total of 3,830,902 sequences from 86 adult, recruit and water samples. Clustering at the 97% level identified 63 operational taxonomic units (OTUs) at the sub-type level. Blank extractions and negative controls were checked visually using nMDS. Again, contaminant taxa, including eight *Cladocopium* C3 sub-types and *Cladocopium* C29 and C50, were removed from the dataset as above, as well as the blanks and no-template controls (n = 8), parents that did not planulate (n = 14), and one water sample that was a clear outlier. This resulted in a total of 63 samples, which included 6 parents, 42 recruits, and 15 water samples. Analytical results did not vary with or without the inclusion of the seawater outlier; therefore, it was removed for ease of visualization. Results with the outlier included can be found in the supplementary materials (Appendix 5.1).

Patterns of bacterial communities among experimental groups

Alpha diversity and observed richness of bacterial communities were significantly different between parents, recruits and water ($ANOVA_{\alpha\text{-diversity}}$: df = 2, F = 24.6, $p < 0.001$; $ANOVA_{richness}$: df = 2, F = 13.3, $p < 0.001$), with both recruits and water having higher diversity and species richness than parents (Figure 5.3a,b). Bacterial community composition (beta-diversity) of parents, recruits and water samples was significantly different from one another (PERMANOVA: df = 2, F = 18.84, $p = 0.001$, Figure 5.3c), with significant post-hoc comparisons ($R^2 < 0.8$, $p < 0.01$). Dispersion was highest in recruits. Parents were dominated by the genus *Endozoicomonas*, of the family Hahellaceae, which made up on average 97.9 ± 0.006% (mean ± SEM) in relative abundance (Figure 5.4). Recruits also had high relative abundances of *Endozoicomonas*, as well as two genera of Rhodobacteraceae, *Ruegeria* and *Thalassobius*, and *Burkholderia-Paraburkholderia*. Water samples were dominated by "*Candidatus* Actinomarina", of the Acidimicrobiales OM1 clade, and other genera of the Oceanospirillales SAR86 clade and Flavobacteriaceae (Figure 5.4). Parents from both habitats and water across habitat and time point did not vary significantly and were pooled, respectively, for further analysis.

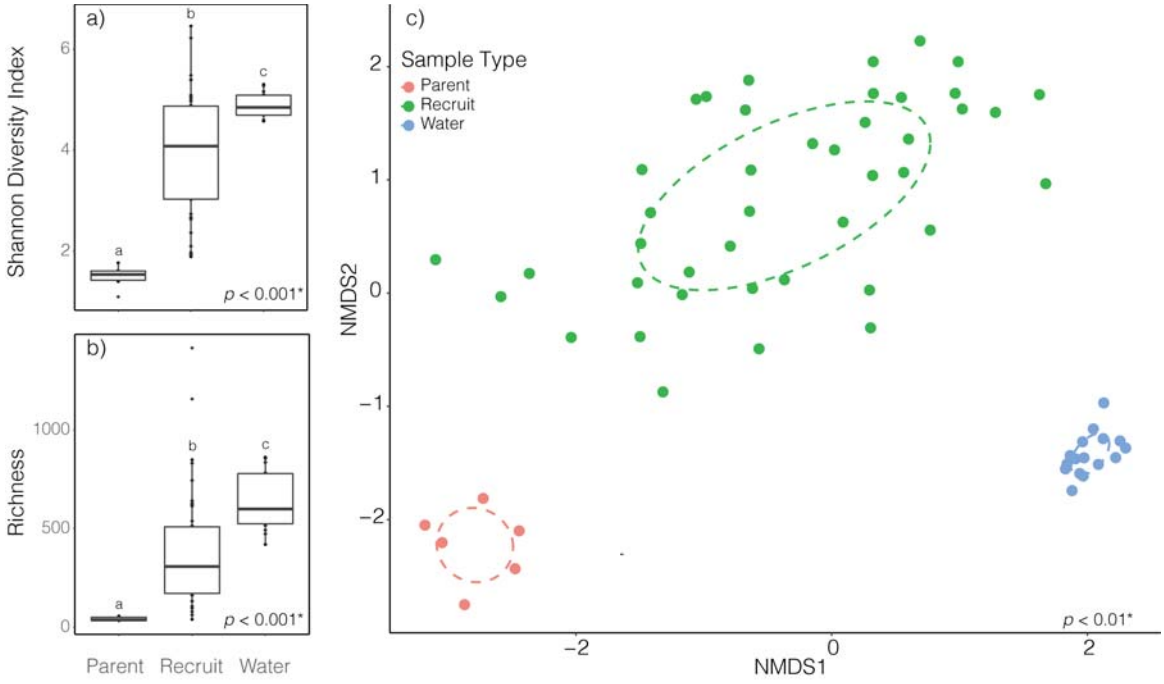

FIGURE 5.3 A) ALPHA-DIVERSITY, B) OBSERVED RICHNESS AND C) BETA-DIVERSITY BY NMDS GROUPED BY SAMPLE TYPE; PARENT, RECRUIT OR WATER.

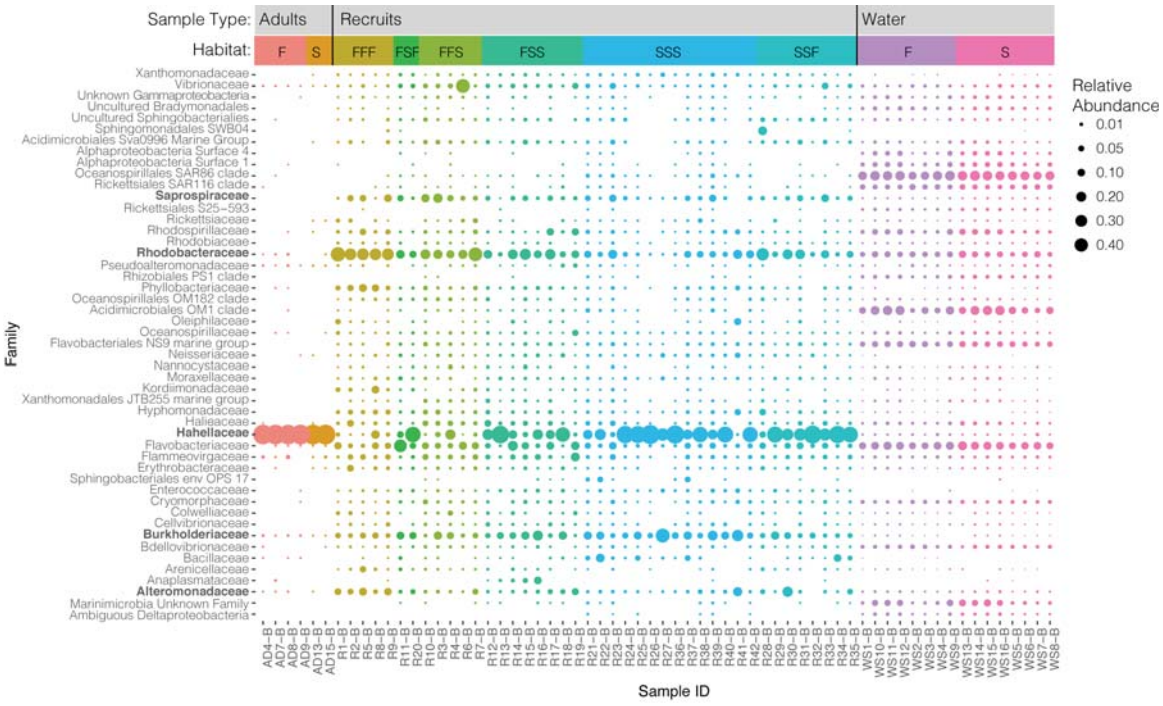

FIGURE 5.4 BUBBLE PLOT OF THE TOP 50 MOST ABUNDANT FAMILIES, WITH BUBBLE SIZE INDICATING RELATIVE ABUNDANCE. FAMILIES IN BOLD ARE THOSE THAT HAVE GENERAL HIGH RELATIVE ABUNDANCE IN RERUITS. "F" AND "S" REFER TO FLAT AND SLOPE HABITATS, RESPECTIVELY. UNDER RECRUITS, EACH LETTER COMBINATION REFERS TO THEIR EXPERIMENTAL GROUP (SEE FIGURE 5.2).

Experimental groups varied significantly from one another (PERMANOVA: df = 5, F = 2.06, p = 0.001). Pairwise comparisons only found significant differences between experimental groups that either had flat or slope as both parental and planulation environment ($R^2 < 0.21$, $p < 0.05$; Supplementary Table S5.1). No significant pairwise differences were observed between any of the other experimental groups. In support of this, the microbial communities of recruits varied significantly by parental environment (PERMANOVA: df = 1, F = 3.848, p = 0.001) and by planulation environment (PERMANOVA: df = 1, F = 4.1384, p = 0.001), but not by transplant environment (Figure 5.5a-c).

A number of ASVs were present and shared between parents, recruits and water samples (Figure 5.5d), but each parent had only between 3 and 13 ASVs that were shared with 100% of their respective offspring (Supplementary Table S5.2). Only one ASV of *Burkholderia-Paraburkholderia* was commonly shared among all coral samples. A number of *Endozoicomonas* ASVs were shared among some parents and their respective offspring, but recruits had variable relative abundance of *Endozoicomonas* depending on their treatment (ANOVA: df = 7, F = 27.49, $p < 0.001$; Supplementary Figure S5.1).

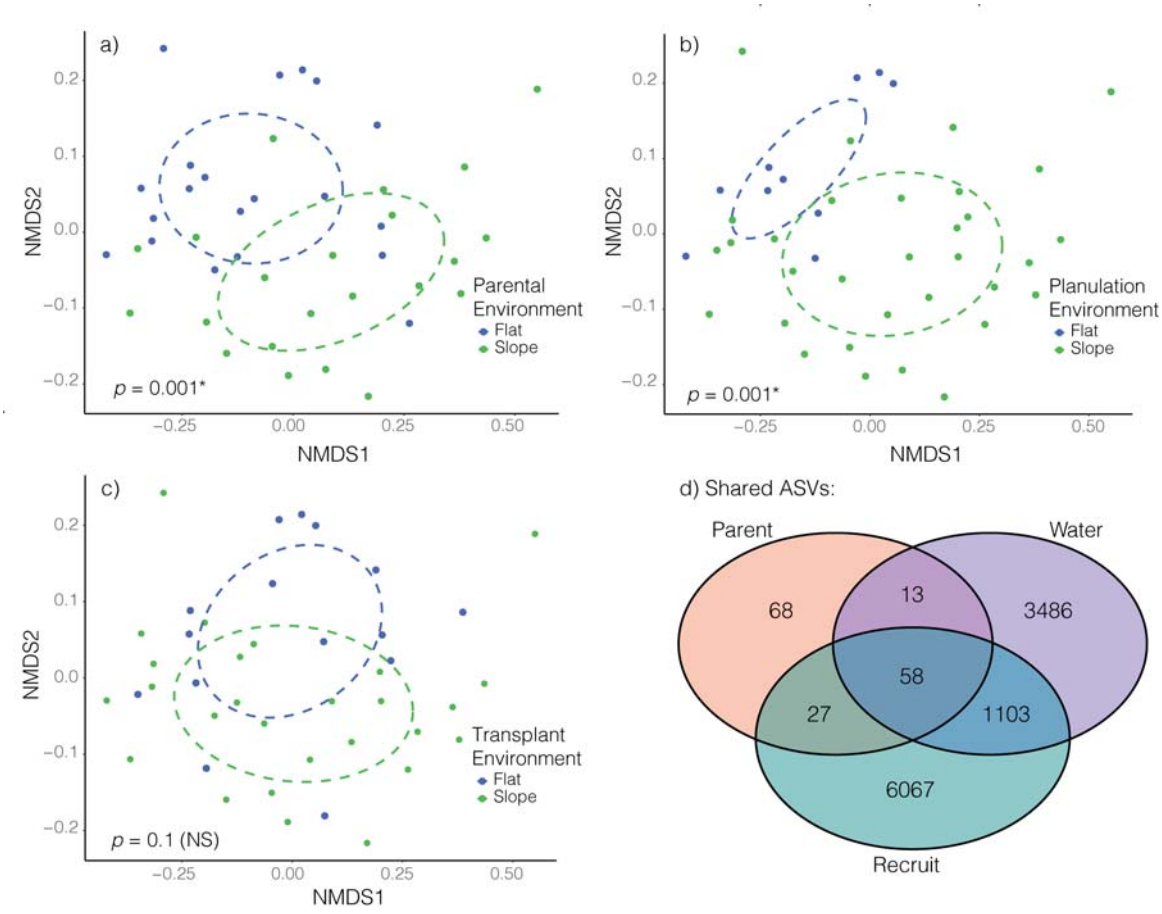

FIGURE 5.5 NMDS VISUALIZATIONS OF THE RECRUIT COMMUNITIES BY A) PARENTAL ENVIRONMENT, B) PLANULATION ENVIRONMENT AND C) TRANSPLANT ENVIRONMENT. D) VENN DIAGRAM OF ALL SHARED AMPLICON SEQUENCE VARIANTS BASED ON PRESENCE IN ONE OR MORE SAMPLE OF EACH TYPE.

Patterns of Symbiodiniaceae communities among experimental groups

Alpha diversity of Symbiodiniaceae communities was not significantly different between parents, recruits and water; however, species richness was significantly lower in the water samples compared with both recruits and parents ($ANOVA_{richness}$: df = 2, F = 11.72, $p < 0.001$, Figure 5.6a,b). The beta-diversity of Symbiodiniaceae communities varied significantly among sample type (PERMANOVA: df = 2, F = 22.2, $p = 0.001$, Figure 5.6c), but this significance was driven by differences between the coral (both parents and recruits) and water communities. Pairwise comparisons showed that Symbiodiniaceae communities did not significantly differ between parents and recruits, but both significantly differed from water ($R^2 < 0.6$, $p < 0.01$; Supplementary Table S5.3). Dispersion was also significantly different among sample types, with recruits having higher dispersion than both parents and water (PERMDISP: df = 2, F = 59.14, $p = 0.001$, Figure 5.6c). Symbiodiniaceae communities in offspring clustered with their respective parents and showed a significant difference according to both parental environment (PERMANOVA: df = 1, F = 135.94, $p = 0.001$) and planulation environment (PERMANOVA: df = 1, F = 15.2, $p = 0.001$), but not transplant environment. These differences according to environment were driven

by a single parent-offspring cohort (parental ID B13), which clustered separately from the rest of the samples (Figure 5.6c).

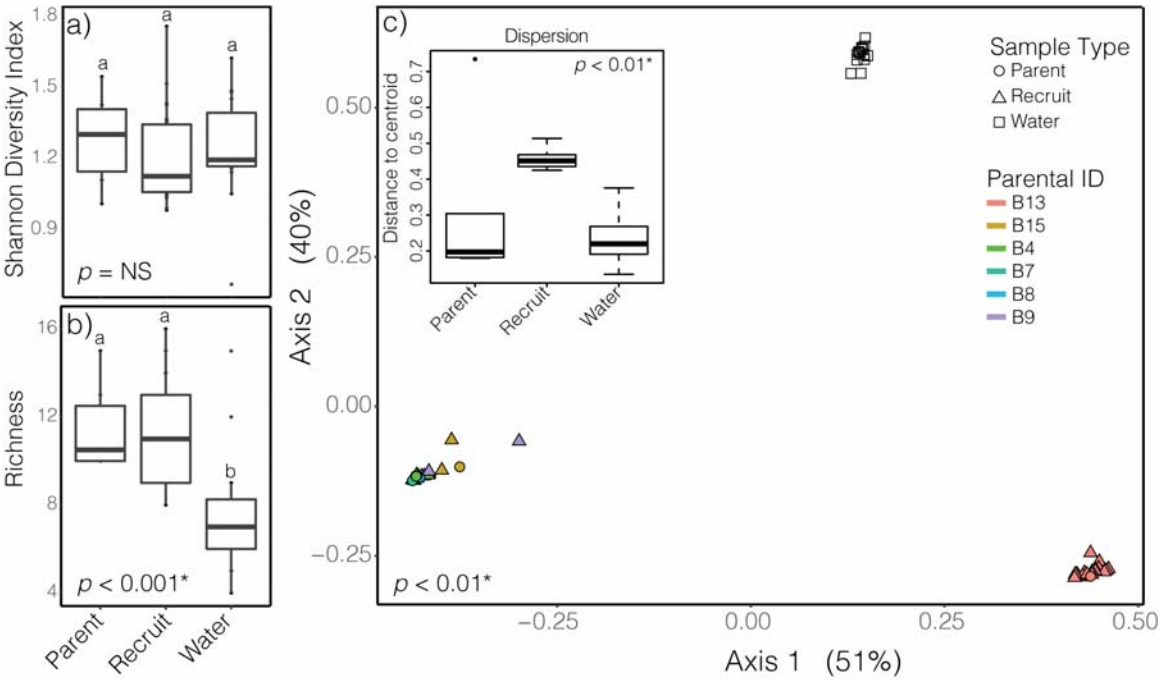

FIGURE 5.6 A) ALPHA-DIVERSITY AND B) RICHNESS BETWEEN SYMBIODINIACEAE SAMPLES. C) PRINCIPAL COMPONENT ANALYSIS (PCOA) PLOT OF PARENT AND RECRUIT SAMPLES LABELLED ACCORDING TO SAMPLE TYPE AND PARENTAL ID WHERE APPROPRIATE, SHOWING THE ONE PARENT (B13) WITH RESPECTIVE RECRUITS CLUSTERING SEPARATELY FROM THE REST OF THE CORAL SAMPLES IN THE LOWER RIGHT HAND CORNER. INSET GRAPH REPRESENTS DISPERSION BY SAMPLE TYPE.

All parents were dominated by *Cladocopium* type C42, except for the one slope parent (B13), which was dominated by sequence type C33. Each parent shared their dominant Symbiodiniaceae types with their respective offspring, but most offspring also harbored low-abundance background strains that were not present in their respective parents. These low-abundance background strains in recruits included those of genera other than *Cladocopium*, such as *Symbiodinium* (A1 & A3), *Durusdinium* (D1a & D2) and Clade F (F5.1) (Supplementary Table S5.4).

Discussion

A reciprocal transplant experiment was conducted in which both parental colonies and post-settlement recruits were cross-transplanted, to test the influence of parents and the environment on the microbial communities in coral offspring. The results suggest that both bacteria and Symbiodiniaceae likely exhibit mixed mode transmission strategies, influenced by both parents and environment. It was also evident from the high dispersion and variability of recruit microbiomes that they likely experience “winnowing” of their microbial communities throughout ontogeny.

Parents and environment exert influence on bacterial communities in offspring

The establishment of bacterial communities in offspring of *P. damicornis* was likely facilitated by a mixed mode of transmission, but with the majority of uptake occurring horizontally through chance encounter in the environment. Bacterial communities of recruits differed significantly from both parents and water, but also among experimental groups. Thus, the lack of a clear pattern indicates that the community composition in offspring was greatly influenced by the environment, particularly in their early stages of development (e.g., prior to settlement). However, the results also suggest some parental effects on the bacterial communities of offspring, such as the significant effect of parental environment on recruits as well as a small number of shared bacterial ASVs present between each cohort of recruits and respective parent.

Parents, recruits and water harbored significantly different bacterial communities. Parents were dominated by the genus *Endozoicomonas*, which made up over 97% of their community. *Endozoicomonas* is commonly found in the Pocilloporidae (e.g., Ziegler et al. 2016; Neave et al. 2017a) and is considered an important player in the coral microbiome, having been suggested as a contributor to carbohydrate upcycling (Neave et al. 2016, 2017a), dimethylsulfoniopropionate (DMSP) degradation (Raina et al. 2009) and thermal protection (Pantos et al. 2015). *Endozoicomonas* typically resides within coral tissues as aggregates, but its large genome size also suggests it has a free-living stage (reviewed in Neave et al. 2016), which casts some doubt on its role as a true coral endosymbiont (Neave et al. 2017a). Recruits also had high relative abundances of *Endozoicomonas*, but their communities were additionally co-dominated by bacteria from the families Rhodobacteraceae and Burkholderiaceae, which have previously been found associated with juvenile corals (Apprill et al. 2009; Sharp et al. 2012; Lema et al. 2014b; Williams et al. 2015; Leite et al. 2017). The water column is known to harbor vastly different bacterial communities to those within coral tissue (e.g., Apprill et al. 2016; Ziegler et al. 2016; Cai et al. 2018; Leite et al. 2018). As expected, the present study found the water samples were characterized by highly diverse and species rich bacterial communities, which differed significantly to those associated both with the parents and recruits.

The shared ASVs between parents and their respective offspring differed according to parent, perhaps representing a parental genotype effect. However, one variant of *Burkholderia-Paraburkholderia* was shared among all parents and all recruits. It is hypothesized that this single variant may be vertically transmitted due to its consistent presence among coral samples; fluorescent *in situ* hybridization with a specific probe is required to verify this conclusion. *Burkholderia* were previously suggested to be vertically transmitted in the spawning coral,

Mussimillia hispida, and were found to make up 75% of its gamete microbiome and 85% of the its planula microbiome (Leite et al. 2017). In other systems such as plants, some *Burkholderia* and *Paraburkholderia* species have been found to have key nitrogen-fixing capabilities (Coenye & Vandamme 2003; Dall'Agnol et al. 2016), and may present a similar function for early life stages of corals. The abundance of this potential nitrogen fixer could provide otherwise limited nitrogen to the Symbiodiniaceae and assist in the survival and protection of early recruits (Santos et al. 2014, 2015).

The microbial partner *Endozoicomonas* has been found to be a common and abundant member of the coral microbiome in many species. Its transmission to coral offspring could provide a clue as to its potential role as a true endosymbiont. In the present study, the relative abundance of *Endozoicomonas* was significantly less in recruits than parents, where it made up a majority (>97%) of the microbiome. Williams et al. (2015) also found that juveniles incorporated less *Endozoicomonas* into their microbiome than adults, suggesting that this proportion increases with age. Here, it was found that not only did the relative abundances differ, but the overall community of *Endozoicomonas* strains also varied between adults and recruits. This could imply either a functional shift or functional redundancy in *Endozoicomonas* through early ontogeny. There was limited evidence for vertical transmission of this microbial partner. Some, but not all, parents shared one or a few *Endozoicomonas* ASVs with all of their offspring, but these variants were not the same among each parent-offspring cohort and the relative abundances of these variants varied greatly among recruit treatments. Further, out of 66 strains of *Endozoicomonas* that were found in recruits, only 15 were also present in the parents. This suggests that *Endozoicomonas* is likely taken up through chance encounter in the water column during early development and throughout life history, although vertical transmission cannot be completely ruled out until our metabarcoding results are validated by other approaches such as fluorescent in situ hybridization.

Parents may also exert influence on the establishment of juvenile microbiomes in ways outside of vertical transmission. For instance, Ceh et al. (2013) found that adults can release bacteria into the water column at the time of planulae release, essentially seeding the water column so that the planulae can encounter and uptake key bacteria (e.g., *Roseobacter* and *Alteromonas*) that may benefit fitness in early life. Even in a study that suggests vertical transmission, it was found that vertically transmitted bacteria were not intra-planular, but rather in the mucous surrounding the planulae (Leite et al. 2017), meaning that the uptake by planulae and recruits is subject to chance. For Symbiodiniaceae partners, adult corals have been found to "seed" the sediment, providing an environmental pool of Symbiodiniaceae available for horizontal uptake by juveniles (Nitschke et al. 2016). It is possible that adult corals also seed the sediment or other environmental sources with certain bacteria that may be beneficial to offspring. Thus, parental influence may be

decoupled from vertical transmission and instead represent a pathway for facilitating horizontal transmission.

Evidence for mixed mode transmission of Symbiodiniaceae

Symbiodiniaceae communities of coral samples from both parents and offspring differed significantly from those found in the water column. Parents were dominated by the Symbiodiniaceae sequence type C42 of the genus *Cladocopium*, except for one parent from the reef slope, which was instead dominated by sequence type C33. The Symbiodiniaceae communities of *P. damicornis* that inhabit adjacent reef habitat types at Coral Canyons (Heron Island) have been found previously to house either *Cladocopium* C42 or *Cladocopium* C33 as their dominant type in the reef flat and slope, respectively (van Oppen et al. 2018). Perhaps due to the small sample size of slope parents (n = 2), the present study found only one colony that corroborates the findings of van Oppen et al. (2018). Although not characterized in this study, the one slope individual that had a Symbiodiniaceae community more similar to those from the reef flat could potentially be due to differences in microhabitat as a result of variations in light (Edmunds et al. 2014). Regardless, recruits mirrored their parents in terms of dominant Symbiodiniaceae strains, and water samples were comprised of mostly *Cladocopium* C3 sequence sub-types.

The present study found evidence for mixed mode transmission of Symbiodiniaceae communities in *P. damicornis*. The recruit communities clustered closely with their respective parents, and dominant Symbiodiniaceae strains were conserved from parents to recruits. However, the significant effect of planulation habitat found here, along with evidence of low abundance strains that were present in recruits but not parents, suggest that recruits also horizontally acquired some Symbiodiniaceae strains from their environment at this early life stage. Historically, many brooding corals have been thought to be strictly vertical transmitters, with this mechanism being well documented in the Pocilloporidae (e.g., *P. damicornis*, Tanner 1996, *Pocillopora verrucosa*, Kinzie 1993 and *Seriatopora hystrix,* Baird & Babcock 2000) and other brooding coral species (e.g., *Galaxea archella*, Baird et al. 2009a and *Goniastrea aspera*, Sakai 1997). However, with recent advances in high resolution techniques for barcoding Symbiodiniaceae communities (e.g., next generation sequencing), some brooding corals have now been found to exhibit a mixed-mode transmission strategy, where dominant Symbiodiniaceae strains are transmitted from the parents and background strains are additionally acquired from the environment (e.g., *Stylophora pistillata,* Byler et al. 2013; *Seriatopora hystrix*, Quigley et al. 2018). The present study presents another example of a brooding coral exhibiting mixed-mode transmission, which suggests that this may be a more widespread phenomenon than previously thought. In addition, this may also

suggest that even in brooding corals, the Symbiodiniaceae community may exhibit flexibility to adjust to local environmental conditions.

Recruits harbored Symbiodiniaceae communities that included some strains shared with parents and some shared with water. They also contained several strains that were seemingly unique to recruits, suggesting that there may be other environmental sources of Symbiodiniaceae aside from water that were not sampled. Quigley et al. (2017) demonstrated that acroporid juveniles selectively uptake a small proportion of their Symbiodiniaceae from the sediment, where different sediment treatments resulted in different Symbiodiniaceae strains being acquired. Thus, it is likely that horizontally acquired Symbiodiniaceae strains can come from multiple environmental sources.

In the present study, parental corals showed no significant difference in their Symbiodiniaceae communities between reef habitats. This was unexpected as previous studies have found significant effects of depth and light availability on Symbiodiniaceae communities (e.g., Rowan & Knowlton 1995; Frade et al. 2008; van Oppen et al. 2018). This could be a consequence of the small sample size, which may not have been sufficient to detect differences in the Symbiodiniaceae communities between habitats, and further studies should improve the replication of parents to ensure sufficient planulation numbers.

Microbial winnowing & host regulation

Recruits harbored a much more diverse and variable bacterial and Symbiodiniaceae communities in comparison to their parents, indicating that they obtain a considerable portion of this community from the environment. Results show that in addition to an initial uptake of some microbes from their parents, recruits must also obtain a diverse array of microbes through chance encounter in their environment. Parental microbiomes were instead much more tailored, with lower diversity and variability among samples. This suggests that recruits likely shape their microbiome into a more specific community similar to their parents as they grow into adulthood, providing evidence for ontogenetic host regulation and selectivity. Previous studies have identified the succession of the microbiome from a dynamic and diverse to a more conserved community through ontogeny, referred to as “winnowing” (Nyholm & McFall-Ngai 2004; Abrego et al. 2009; Lema et al. 2014b) where microbial assemblages are fine-tuned until a stable microbiome is achieved that is appropriate for their local environmental conditions (Lema et al. 2014b). Thus, while parents and the environment were found to influence initial establishment of the microbiome, host regulation also plays an active role in shaping this community throughout life history.

Conclusions

The results presented here suggest *P. damicornis* exhibits mixed mode transmission of both Symbiodiniaceae and bacterial communities. The dominant strains of Symbiodiniaceae remained the same for all recruits and their respective parents, but recruits also harbored additional, low-abundance strains that were likely acquired from the environment. Bacterial communities were influenced in large part by the planulation environment, suggestive of horizontal transmission in early life stages. However, at least one bacterial strain that may play important roles in the early development of corals is likely acquired via vertical transmission. Evidence was also found for host regulation of both Symbiodiniaceae and bacterial communities, where recruits begin with more diverse and species rich microbiomes that are winnowed as they grow into adulthood.

Acknowledgements

The authors would like to thank K. Sambrook and Dr. A. Gainsford for their invaluable assistance in the field at Heron Island, and the Australian Institute of Marine Science (AIMS) engineering workshop, particularly M. Kebben, for assistance with the design and construction of the planulation boxes. This study was funded by Paul G. Allen Philanthropies, AIMS@JCU (the joint venture between AIMS and James Cook University), The Explorer's Club and the ARC Centre of Excellence for Coral Reef Studies, as part of HE's PhD research. HE acknowledges receipt of an AIMS@JCU Postgraduate Scholarship. Raw sequence data for this study is available on the NCBI Sequence Read Archive under the accession PRJNA492366.

CHAPTER 6

GENERAL DISCUSSION

The aim of this thesis was to investigate the drivers of microbial community composition in corals to elucidate how the coral microbiome may respond to environmental change and to inform the development of microbiome engineering tools for restoration. The findings suggest that both environment and host-specific factors are key drivers of microbial community composition in corals, which have similarly been identified in other systems (e.g., human gut microbiome, Xie et al. 2016; plant microbiome, Schlaeppi & Bulgarelli 2015). Specifically, reef location and temperature stress were found to affect the taxonomic composition of the coral microbiome, while variations also occurred through time (Chapter 3 and 4). In addition, the coral host was found to play a valuable role in shaping its microbiome, both over time during the adult life stage (Chapters 3 and 4) and through early ontogeny (Chapter 5). This research contributes to filling key knowledge gaps about the natural variability and stability of the coral microbiome, which can also help inform the possible development of microbiome engineering as a tool to assist with coral restoration projects (Chapter 2).

Environmental effects on the microbiome

Community composition of algal endosymbionts (Symbiodiniaceae)

It has been widely acknowledged that environmental variation, both naturally occurring (e.g., seasonal; Chen et al. 2005; Ulstrup et al. 2008) and anthropogenically-induced (e.g., pollution or temperature anomalies as a result of rapid climate change; Berkelmans & van Oppen 2006; Boulotte et al. 2016), can alter the Symbiodiniaceae communities in corals. While changes in the relative abundances of Symbiodiniaceae taxa can occur seasonally (Chen et al. 2005), taxonomic composition has also been recorded to be relatively stable through time (LaJeunesse et al. 2005; Thornhill et al. 2006a,b; Klepac et al. 2015; Cai et al. 2018). This is supported by results from Chapter 3, where Symbiodiniaceae communities within the two targeted *Acropora* species maintained their dominant types throughout the two-year study, and changes that did occur were observed only in low-abundance background strains. More considerable taxonomic or compositional changes in Symbiodiniaceae communities have often been recorded during and after severe stress events, such as coral bleaching, where changes in the relative abundances of Symbiodiniaceae genera (formerly referred to as clades; LaJeuenesse et al. 2018) or types can occur (i.e., “shuffling”, Berkelmans & van Oppen 2006), as well as acquisition of exogenous strains (i.e., “switching”, Boulotte et al. 2016). This active shift in the Symbiodiniaceae

community has been found to result in increases in physiological performance and tolerance to environmental stressors (Buddemeier & Fautin 1993; Hoegh-Guldberg et al. 2002; Berkelmans & van Oppen 2006; Boulotte et al. 2016). In contrast, it was found in Chapter 4 that the Symbiodiniaceae community remained highly stable in *Pocillopora acuta* during a thermal anomaly on the Great Barrier Reef (GBR), suggesting that shuffling Symbiodiniaceae types may be species specific and/or reliant on both host factors that may affect resilience and the severity of the heat stress.

Community composition of bacteria

Similar to the Symbiodiniaceae, changes in environmental conditions have been reported to influence community composition of coral-associated bacterial communities (reviewed in Thompson et al. 2015 and Bourne et al. 2016). It is widely acknowledged that coral-associated bacterial communities are highly complex and dynamic through time. Many studies have highlighted temporal fluctuations, and in most cases have linked this to seasonal variation (e.g., Ceh et al. 2011; Kimes et al. 2013; Li et al. 2014; Sharp et al. 2017; Cai et al. 2018). However, the results from Chapter 3 suggest that while the microbiome may be dynamic and variable through time, it does not necessarily reflect cyclical or seasonal variation across repeated sampling times points. Similarly, Yang et al. (2017) found temporal variation in the coral microbiome also did not reflect season and these findings reinforce the need for studies conducted on greater than one-year time scales. Furthermore, the patterns of temporal variations may also be species-specific. For instance, *P. acuta* of the chunky morphology displayed little overall changes in community structure during a temperature anomaly (Chapter 4). These unexpected results could suggest that the chunky morph of *P. acuta* is more thermally resilient than other morphotypes and that the coral host likely plays a role in shaping, or maintaining, this stable microbiome.

Does the host have control over the composition of its microbiome?

The results from this thesis suggest that the coral host has an influence on, or plays an active role in, shaping the microbial community composition in some capacity. Chapter 3 found that changes through time were dynamic across two species of *Acropora* in both the Symbiodiniaceae and bacterial community composition, but communities of the same coral species also exhibited variations according to reef. Corals can exhibit species-specific components of their microbiome (Rohwer et al. 2002; LaJeunesse et al. 2004b; Thornhill et al. 2006a; Littman et al. 2009; Stat et al. 2009b), but these can also be strongly driven by differences in location (reviewed in Hernandez-Agreda 2016, 2017), and by variations in abiotic and biotic factors (Bourne et al. 2016; Hernandez-Agreda 2016). The microbiome changes observed in Chapter 3, particularly the "switching" of Symbiodiniaceae members of the rare biosphere and differences in species living

on the same reef provides evidence for host selectivity and specificity of microbial communities. However, the most prominent example of host regulation within this thesis comes from Chapter 5. Here, it was found that both the Symbiodiniaceae and bacterial communities were highly dispersed and variable in coral recruits in comparison to the very stable and highly specific microbial communities present in their parents. Coral offspring not only had initial uptake of some microbes from their parents, but also harbored an additional variety of environmentally sourced microbes, suggesting that they undergo "winnowing" throughout ontogeny. Winnowing refers to the succession of the microbiome from a diverse and variable microbial community to a fine-tuned and more conserved microbial community as the host matures (Nyholm & McFall-Ngai 2004; Abrego et al. 2009; Lema et al. 2014b). Thus, coral microbiomes are driven not only by what is available for acquisition from the environmental pool of microbes, but apparently also through active regulation by the host throughout their life history.

The mechanisms by which corals regulate their microbiome (e.g., select for beneficial microbes or avoid potential pathogens) represent a topic of ongoing study. Research has suggested that corals may be capable of actively shaping microbial community composition through a variety of behavioral, genetic and chemical means. For instance, some corals have been found to control the bacterial communities within their mucus through cyclical mucus shedding, where older mucous that attracts more pathogenic bacterial species can be readily shed to return the bacterial community to its original, healthier state (Glasl et al. 2016). Corals are also believed to manage or maintain their Symbiodiniaceae communities, particularly during times of heat stress, by producing fluorescent pigments (Salih et al. 2000, 2006) or acquiring mycosporine-like amino acids (Shick & Dunlap 2002) that work to reduce photo-inhibition and dissipate UV energy (reviewed in Baird et al. 2009b). Genetic mechanisms have also been found to be responsible for some microbial regulation. Cnidarian genomes (including those of some Anthozoa) have been found to code for proteins that have bacterial recognition capabilities, which could allow the host to control the composition of bacteria they associate with as well as to actively avoid potential pathogens (reviewed in Teplitski et al. 2016). For example, immune-related genes corresponding to three lectin-like molecules from the coral *Pocillopora damicornis* were found to be involved in both the recognition of and response to virulent pathogens such as *Vibrio coralliilyticus* (Vidal-Dupiol et al. 2011). Initial recognition of Symbiodiniaceae and the establishment of the symbiosis within coral have been attributed to microbe-associated molecular pattern (MAMP) - pattern recognition receptor (PRR) interactions, often involving algal-glycan and cnidarian-lectin molecules (reviewed in Davy et al. 2012). Algal-glycans and lectin-binding patterns can have high diversity and specificity depending on Symbiodiniaceae genera and types (e.g., Logan et al. 2010; Markell & Wood-Charlson 2010), but whether this affects host-specificity in the establishment of Symbiodinaceae symbioses is still under investigation (reviewed in Davy et al.

2012). More recently, it has been found that a rhamnose-binding lectin plays a role in the recognition of both a pathogenic bacteria and Symbiodiniaceae in *P. damicornis* that could result in preferential binding of one or the other depending on environmental concentration (Zhou et al. 2017b). This could have important ramifications for corals undergoing environmental stress. A better understanding of the molecular establishment of both bacteria and Symbiodiniaceae could provide key information on the response of corals to climate change.

The influence of the microbiome on climate resilience

Rapid climate change as a result of anthropogenic greenhouse gas emissions is placing unprecedented stress on coral reef ecosystems (Hoegh-Guldberg 1999, Eakin et al. 2016; Heron et al. 2016; Hughes et al. 2017, 2018), generating concern that corals may not adapt or acclimatize fast enough to keep pace (van Oppen et al. 2015, 2017). While this environmental pressure may push coral microbial communities toward dysbiosis (Roder et al. 2014a), changes in the microbiome as a result of environmental variation may have a direct impact on the ecological tolerance of the holobiont and subsequently contribute to holobiont adaptation or acclimatization to the environment (Reshef et al. 2006; Rosenberg et al. 2007; Fraune et al. 2016; Torda et al. 2017). Originally suggested by Rosenberg et al. (2007) and further expanded by Bang et al. (2018), there are essentially five ways in which the microbiome can exhibit change in response to the environment that may benefit their host: (i) the relative abundance of microorganisms associated with hosts can change, (ii) adaptive variation can occur when new microorganisms are added to, or existing ones removed from, the microbial community, (iii) changes to the microbial genomes can occur through random mutations or recombination much faster than the host and due to the short generation times of most microbes, (iv) epigenetic changes can facilitate shifts in microbial phenotypes that may affect host performance, and (v) horizontal gene transfer can occur between microbial partners and their host. While these changes in the microbiome may be evolutionarily "selfish" (reviewed in Torda et al. 2017), the plasticity or flexibility of the microbiome to adjust to changes in the environment could also provide adaptive benefits, or act as an acclimatization tool, for the host (Dunbar et al. 2007; Fraune et al. 2016). Conversely, structural inflexibility in the microbiome could have severe consequences for coral holobiont health if it cannot adapt or acclimatize to changing environmental pressures (Pogoreutz et al. 2018).

The level of flexibility of the microbiome can be related to microbial transmission strategy. Highly conserved coral-associated microbial communities, such as what may be found in strictly vertical transmitters, are suggested to be less flexible than their horizontally or mixed mode transmitting counterparts. Previously identified as an obligate vertical transmitter of Symbiodiniaceae (Tanner 1996), results from Chapter 5 suggested that *P. damicornis* exhibits a

mixed mode transmission of Symbiodiniaceae and, consequently, a higher capacity for the Symbiodiniaceae community to adjust to environmental conditions (Chapter 5). Transmission of bacterial communities in corals is less resolved; however, it is evident that coral-associated bacterial communities are often dynamic (e.g., reviewed in Bourne et al. 2016; Hernandez-Agreda et al. 2016, 2017; Chapters 3-5), exhibiting either horizontal or mixed-mode transmission (e.g., Apprill et al. 2012; Leite et al. 2017; Zhou et al. 2017a; Chapter 5). No studies to date have found any strict vertical transmission of bacterial communities in coral. The inherent flexibility of coral bacterial communities can thus provide an opportunity to adjust to changing environmental conditions at both early life stages in corals and throughout their life history.

Many microbes are now recognized as specific to their coral hosts (Huggett & Apprill 2018), and some of these have been identified as particularly beneficial to increasing heat tolerance in corals. This has been best established for Symbiodiniaceae partners. For instance, *Durusdinium*-types of Symbiodiniaceae (formerly Clade "D", LaJeunesse et al. 2018) can provide thermal tolerance to their associated coral host (Berkelmans & van Oppen 2006), but this thermal tolerance can come as a trade-off against energetic fitness and growth rates (Jones & Berkelmans 2011; Little et al. 2004). Similarly, bacteria of the genus *Endozoicomonas* have been suggested to provide thermal or bleaching tolerance due to its high abundances during and directly following bleaching events (e.g., Bourne et al. 2008; Pantos et al. 2015). Contradictory to these previous studies, there was no evidence for symbiont shuffling to *Durusdinium*-types of Symbiodiniaceae, nor increases in *Endozoicomonas* in the *P. acuta* microbiome during a thermal anomaly on the GBR (Chapter 4). The results of this chapter instead suggest that some Actinobacteria species and *Burkholderia-Paraburkholderia* may provide benefits during times of high temperatures. However, this is a hypothesis based on abundance and presence of these microbes during a thermal anomaly and must be experimentally tested to confirm their beneficial role.

Informing the development of restoration tools

The identification of beneficial microbes, including both bacteria and tolerant strains of Symbiodiniaceae, that may increase climate resilience in corals has directed some researchers toward developing innovative restoration tools. Specifically, this has included studies that artificially increase climate resilience through targeted inoculations with certain beneficial microbes (e.g., probiotics, Reshef et al. 2006; Peixoto et al. 2017; or microbiome engineering; reviewed in Chapter 2).

The dynamic nature of a large component of the coral microbiome suggests that artificially inoculated taxa may not remain host-associated through time as environments change, unless incorporated into the stable "core" (e.g., Ainsworth et al. 2015; Hernandez-Agreda et al. 2017).

Further, the location and species specificity of the coral microbiome implies there is no "one-size-fits-all" manipulation. Consequently, microbiome engineering techniques are more likely to be successful as a short-term treatment, such as through the implementation of probiotics as bioremediation or preventative tools. For instance, a probiotic treatment of beneficial microbes may be applied to corals either immediately after a bleaching event to enhance recovery, or directly prior to an expected bleaching event to boost resilience (Reshef et al. 2006; Peixoto et al. 2017). Thus, the scale at which such interventions could be attempted means they are most likely only practical for targeted, local-scale application.

There is perhaps a window of opportunity for creating successful and lasting inoculations in corals if they are implemented at very early life stages when initial establishment of the microbiome is occurring. Horizontal or mixed-mode transmitters such as *P. damicornis* (Chapter 5) may be more likely to respond positively to inoculations of beneficial microbes due to their inherent ability to uptake new microbes from the environment and maintain flexible associations (Quigley et al. 2018). Inoculating corals at very early life stages with beneficial microbes may help establish them as important members of the coral microbiome as the coral matures. Early inoculation has previously been trialled in coral larvae and recruits for bacterial (Damjanovic et al. 2017) and Symbiodiniaceae (Chakravarti et al. 2017) communities, and findings suggest that the microbiomes of early recruits can shift as a result of these inoculations. However, the extent to which these inoculations remain within the coral microbiome through time or provide tolerance to the coral host remains uncertain. Thus, there is a caveat to using horizontal or mixed-mode transmitters – the host may be just as likely to winnow out the inoculated microbes as they mature, or remove them from their microbiome when environments change. Further empirical research is necessary to determine the extent to which inoculated microbes remain, or are taken up at all, in the microbiome of horizontal, vertical and mixed-mode transmitters, and continue to provide benefits to the coral host.

Future directions

Results of this thesis challenge current perceptions of variations in the coral microbiome and highlight the necessity for future studies to further our understanding of how, when and why shifts in microbial community composition occur. At present, there is a lack of studies that are conducted on long time frames (> 1 year), yet it has been generally accepted that coral microbial community composition shifts according to season. Over a two-year time frame, results from Chapter 3 instead suggested that temporal variation does not reflect season. Further studies should consider lengthening sampling time frames, and consider other species at other reefs to gain a deeper understanding of temporal variation and to determine if any general patterns through time

and across taxa are identifiable. Additionally, reef location appeared to have an overriding effect on microbial community composition (Chapter 3). However, only two reefs were considered in this study. Thus, future studies at greater numbers of reef locations conducted across biogeographical gradients (e.g., cross-shelf and latitudinal) will be essential for establishing the relative importance and possible drivers of this variation.

Pocillopora acuta of the chunky morphology was found to remain pigmented throughout a thermal anomaly on the GBR (Chapter 4). The results from this chapter highlighted the possible beneficial microbes that could be involved in helping the chunky morphology of *P. acuta* remain unbleached. However, this study did not incorporate any samples of the fine-branching morphology of the same species, which has been found to be susceptible to bleaching under thermal stress (Smith et al. 2017b). An ideal follow-up study to this chapter would be to test the differences between these two ecomorphs (fine and chunky) when exposed to high temperatures. *Pocillopora acuta* provides the rare opportunity to identify key differences in the microbiome response to thermal anomalies in a thermally resilient versus thermally sensitive coral without the confounding effects of differences in species.

The unique experimental approach of Chapter 5 provided novel insight into the establishment and ontogenetic development of microbial communities in coral. However, as the first study to use an entirely *in situ* methodology, limitations did arise. It remained difficult to control for biological variation in this experimental design, such as the reproductive success of adults and early recruit mortality, which reduced the number of replicates that could be used. Future studies using this *in situ* method should increase the sample size of brooding adults to ensure adequate recruit sampling, not only for an initial time point, but also to follow recruits through early ontogeny and confirm microbial winnowing in the field. It was clear from the results of this study that microbial communities were established in coral offspring either before or directly following metamorphosis and settlement; however, the design of the settlement boxes did not allow for sampling in these early developmental stages. In future studies, there may be an opportunity to design the settlement boxes in a way that allows for sampling of the planulae and early metamorphosed corals, such as by adding a small plug near the base of the settlement box through which samples can be taken without the risk of losing planulae or loosely settled recruits. Sampling these stages will provide a more complete picture of the changes in microbial community composition occurring at early stages in coral development, and further our understanding of microbial transmission and establishment.

This thesis was focused entirely on taxonomic composition, yet both functional redundancy and functional differences exist within the coral microbiome (e.g., Ainsworth & Gates 2016; Neave et al. 2017b). Identifying microbial community shifts that signify changes to function will be crucial

for understanding the effects of microbiome variation on the coral holobiont. Additionally, indistinguishable or inconsistent changes in taxonomic composition may be masking discernible patterns in microbiome function. Coral microbiome studies are only beginning to incorporate functional analyses using meta'omics methods to determine functional potential (i.e., metagenomics), gene expression (i.e., metranscriptomics, metaproteomics) and metabolic pathways (i.e., metabolomics) of the entire microbiome or specific microbial partners within it. Yet, due to both operational costs and technical issues with minimising the fraction of eukaryotic reads to microbial reads within metagenomes and metatranscriptomes (e.g., Meyer et al. 2017 found over 50% eukaryotic host reads in coral metagenome assembly; Frazier et al. 2017 found over 20% eukaryotic host reads in coral metranscriptome assembly), functional complexity of the coral microbiome is not well characterized.

With a limited understanding of the functional complexity of the coral microbiome, it remains difficult to determine the implications of changes to microbial community composition for enhancing resilience in the coral holobiont. This additionally hinders the ability to identify microbes with specific beneficial functions for targeted use in microbiome engineering techniques (reviewed in Chapter 2). However, researchers across fields are already working on methods to minimize eukaryotic/host sequence "contamination" (e.g., Lim et al. 2014; Daniels et al. 2015; Thoendel et al. 2017) and improvements in sequencing platforms will continue to minimize the operational costs (reviewed in Quince et al. 2017). Thus, as meta'omics techniques improve, future studies on shifts in the coral microbiome should incorporate these methods to further elucidate functional complexity.

Concluding remarks

In this thesis, environmental and host-specific factors have been identified as important drivers of microbial community composition in reef-building corals. This research utilized both survey and experimental work to test key theories and assumptions of patterns in microbial community composition through time, across reef locations, between species and among life history stages. Findings suggest that coral microbiomes will be influenced by changing environmental conditions, but may also present an opportunity for holobiont acclimatization. This understanding of where and when changes in the microbiome occur is key for plans to develop microbiome engineering tools to assist with future coral restoration projects, which will likely be most effective when used on small-scale and short-term bases for bioremediation or immediate prevention (e.g., probiotics). However, further understanding of microbial function will be essential for identifying specific beneficial microbial partners that may aid in natural resilience or that can be targeted for microbiome engineering.

The coral microbiome is taxonomically complex, dynamic through time and variable according to species, location, and even life stage. For some coral species in certain locations, shifts in microbial communities may result in increases in potentially beneficial microbes that can contribute to climate resilience in the future. For others, they may lead to dysbiosis and higher risks of bleaching, disease and mortality. Whether the response is positive or negative will be dependent on host- and microbe-specific factors, reef or geographic location and the severity of the stress event. In the face of current reef declines, those corals that can maintain a healthy and functioning microbiome during environmental stress as a result of beneficial shifts in composition or function are likely to outcompete those that do not, ultimately affecting the overall structure of coral reefs into the future.

REFERENCES

Abrego D, van Oppen MJH, and Willis BL. 2009. Onset of algal endosymbiont specificity varies among closely related species of *Acropora* corals during early ontogeny. *Mol Ecol* **18**: 3532–43.

Ainsworth T, Krause L, Bridge T, *et al.* 2015. The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. *ISME J* **9**: 2261-74.

Ainsworth TD and Gates RD. 2016. Corals' microbial sentinels. *Science* **352**: 1518–9.

Alagely A, Krediet CJ, Ritchie KB, and Teplitski M. 2011. Signaling-mediated cross-talk modulates swarming and biofilm formation in a coral pathogen *Serratia marcescens*. *ISME J* **5**: 1609–20.

Alonso-Sáez L, Díaz-Pérez L, and Morán XAG. 2015. The hidden seasonality of the rare biosphere in coastal marine bacterioplankton. *Environ Microbiol* **17**: 3766–80.

Amend AS, Barshis DJ, and Oliver T a. 2012. Coral-associated marine fungi form novel lineages and heterogeneous assemblages. *ISME J* **6**: 1291–301.

Andersson AF, Lindberg M, Jakobsson H, *et al.* 2008. Comparative analysis of human gut microbiota by barcoded pyrosequencing. *PLoS One* **3**: e2836.

Apprill A, Marlow HQ, Martindale MQ, and Rappé MS. 2009. The onset of microbial associations in the coral *Pocillopora meandrina*. *ISME J* **3**: 685–99.

Apprill A, Marlow HQ, Martindale MQ, and Rappé MS. 2012. Specificity of associations between bacteria and the coral *Pocillopora meandrina* during early development. *Appl Environ Microbiol* **78**: 7467–75.

Apprill A, Weber LG, and Santoro AE. 2016. Distinguishing between microbial habitats unravels ecological complexity in coral microbiomes. *mSystems* **1**: e00143-16.

Arbizu PM. 2017. pairwiseAdonis: Pairwise multilevel comparison using Adonis

Arif C, Daniels C, Bayer T, *et al.* 2014. Assessing *Symbiodinium* diversity in scleractinian corals via next-generation sequencing-based genotyping of the ITS2 rDNA region. *Mol Ecol* **23**: 4418–33.

Bahr KD, Jokiel PL, and Rodgers KS. 2017. Seasonal and annual calcification rates of the Hawaiian reef coral, *Montipora capitata*, under present and future climate change scenarios. *ICES J Mar Sci* **74**: 1083–91.

Baird AH and Babcock RC. 2000. Morphological differences among three species of newly settled pocilloporid coral recruits. *Coral Reefs* **19**: 179–83.

Baird AH, Bhagooli R, Ralph PJ, and Takahashi S. 2009b. Coral bleaching: the role of the host. *Trends Ecol Evol* **24**: 16–20.

Baird AH, Guest JR, and Willis BL. 2009a. Systematic and biogeographical patterns in the reproductive biology of Scleractinian corals. *Annu Rev Ecol Evol Syst* **40**: 551–71.

Baker A. 2001. Reef corals bleach to survive change. *Nature* **411**: 765–6.

Bang C, Dagan T, Deines P, *et al.* 2018. Metaorganisms in extreme environments: do microbes play a role in organismal adaptation? *Zoology* **127**: 1–19.

Barnard J. 1974. Cut P and N without chemicals. *Water Wastes Eng* **11**: 33–44.

Barnard JL, Dunlap P, and Steichen M. 2017. Rethinking the mechanisms of biological phosphorus removal. *Water Environ Res* **89**: 2043–54.

Bates NR, Amat A, and Andersson AJ. 2010. Feedbacks and responses of coral calcification on the Bermuda reef system to seasonal changes in biological processes and ocean acidification. *Biogeosciences* **7**: 2509–30.

Bayer T, Neave MJ, Alsheikh-Hussain A, *et al.* 2013. The microbiome of the Red Sea coral *Stylophora pistillata* is dominated by tissue-associated *Endozoicomonas* bacteria. *Appl Environ Microbiol* **79**: 4759–62.

Ben-Haim Y and Rosenberg E. 2002. A novel *Vibrio* sp. pathogen of the coral *Pocillopora damicornis*. *Mar Biol* **141**: 47–55.

Benelli G. 2015. Research in mosquito control: current challenges for a brighter future. *Parasitol Res* **114**: 2801–5.

Bentis CJ, Kaufman L, and Golubic S. 2000. Endolithic fungi in reef-building corals (Order: Scleractinia) are common, cosmopolitan, and potentially pathogenic. *Biol Bull* **198**: 254–60.

Berendsen RL, Pieterse CMJ, and Bakker PAHM. 2012. The rhizosphere microbiome and plant health. *Trends Plant Sci* **17**: 478–86.

Berkelmans R and van Oppen MJH. 2006. The role of zooxanthellae in the thermal tolerance of corals: a “nugget of hope” for coral reefs in an era of climate change. *Proc R Soc B Biol Sci* **273**: 2305–12.

Bernasconi R, Stat M, Koenders A, and Huggett MJ. 2018. Global networks of *Symbiodinium* - bacteria within the coral holobiont. *Microb Ecol*. DOI: 10.1007/s00248-018-1255-4.

Blackall LL, Crocetti GR, Saunders AM, and Bond PL. 2002. A review and update of the microbiology of enhanced biological phosphorus removal in wastewater treatment plants. *Antonie Van Leeuwenhoek* **81**: 681–91.

Blackall LL, Wilson B, and van Oppen MJH. 2015. Coral-the world’s most diverse symbiotic ecosystem. *Mol Ecol* **24**: 5330–47.

Bonthond G, Merselis DG, Dougan KE, *et al.* 2018. Inter-domain microbial diversity within the coral holobiont *Siderastrea siderea* from two depth habitats. *PeerJ* **6**: e4323.

Boulotte NM, Dalton SJ, Carroll AG, *et al.* 2016. Exploring the *Symbiodinium* rare biosphere provides evidence for symbiont switching in reef-building corals. *Nature* **10**: 2693-701.

Bourne D, Iida Y, Uthicke S, and Smith-Keune C. 2008. Changes in coral-associated microbial communities during a bleaching event. *ISME J* **2**: 350–63.

Bourne DG and Webster NS. 2013. Coral reef bacterial communities. In: Rosenberg E, Delong EF, Lory S, *et al.* (Eds). The prokaryotes - Prokaryotic communities and ecophysiology. Berlin, Heidelberg: Springer Berlin Heidelberg.

Bourne DG, Dennis PG, Uthicke S, *et al.* 2013. Coral reef invertebrate microbiomes correlate with the presence of photosymbionts. *ISME J* **7**: 1452–8.

Bourne DG, Morrow KM, and Webster NS. 2016. Insights into the coral microbiome: underpinning the health and resilience of reef ecosystems. *Annu Rev Microbiol* **70**: 317–40.

Brinkman R, Wolanski E, Deleersnijder E, *et al.* 2002. Oceanic inflow from the Coral Sea into the Great Barrier Reef. *Estuar Coast Shelf Sci* **54**: 655–68.

Buddemeier RW and Fautin DG. 1993. Coral bleaching as an adaptive mechanism. *Bioscience* **43**: 320–6.

Byler KA, Carmi-Veal M, Fine M, and Goulet TL. 2013. Multiple symbiont acquisition Strategies as an adaptive mechanism in the coral *Stylophora pistillata*. *PLoS One* **8**: 1–7.

Cai L, Zhou G, Tong H, *et al.* 2018. Season structures prokaryotic partners but not algal symbionts in subtropical hard corals. *Appl Microbiol Biotechnol* **102**: 4963–73.

Callahan BJ, McMurdie PJ, Rosen MJ, *et al.* 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* **13**: 581.

Cantin NE, Oppen MJH van, Willis BL, *et al.* 2009. Juvenile corals can acquire more carbon from high-performance algal symbionts. *Coral Reefs* **28**: 405–14.

Caporaso JG, Kuczynski J, Stombaugh J, *et al.* 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* **7**: 335–6. https://qiime2.org.

Cardini U, Hoytema N van, Bednarz VN, *et al.* 2016. Microbial dinitrogen fixation in coral holobionts exposed to thermal stress and bleaching. *Environ Microbiol* **18**: 2620–33.

Cavicchioli R, Fegatella F, Ostrowski M, *et al.* 1999. Sphingomonads from marine environments. *J Ind Microbiol Biotechnol* **23**: 268–72.

Cavicchioli R, Ostrowski M, Fegatella F, *et al.* 2003. Life under nutrient limitation in oligotrophic marine environments: An eco/physiological perspective of *Sphingopyxis alaskensis* (formerly *Sphingomonas alaskensis*). *Microb Ecol* **45**: 203–17.

Ceh J, Kilburn MR, Cliff JB, *et al.* 2013. Nutrient cycling in early coral life stages: *Pocillopora damicornis* larvae provide their algal symbiont (*Symbiodinium*) with nitrogen acquired from bacterial associates. *Ecol Evol* **3**: 2393–400.

Ceh J, van Keulen M, and Bourne DG. 2011. Coral-associated bacterial communities on Ningaloo Reef, Western Australia. *FEMS Microbiol Ecol* **75**: 134–44.

Chakravarti LJ, Beltran VH, and van Oppen MJH. 2017. Rapid thermal adaptation in photosymbionts of reef-building corals. *Glob Chang Biol* **23**: 4675-88.

Chan WY, Peplow LM, Menendez P, *et al.* 2018. Interspecific hybridization provides novel opportunities for coral reef restoration. *Front Mar Sci* **5**: Article 160.

Chen CA, Wang JT, Fang LS, and Yang YW. 2005. Fluctuating algal symbiont communities in *Acropora palifera* (Scleractinia: Acroporidae) from Taiwan. *Mar Ecol Prog Ser* **295**: 113–21.

Chen H. 2018. VennDiagram: Generate high-resolution venn and euler plots.

Chiou SF, Kuo J, Wong TY, *et al.* 2010. Analysis of the coral associated bacterial community structures in healthy and diseased corals from off-shore of southern Taiwan. *J Environ Sci Heal - Part B Pestic Food Contam Agric Wastes* **45**: 408–15.

Claus D, Fahmy F, Rolf HJ, and Tosunoglu N. 1983. *Sporosarcina halophila* sp. nov., an obligate, slightly halophilic bacterium from salt marsh soils. *Syst Appl Microbiol* **4**: 496–506.

Coenye T and Vandamme P. 2003. Diversity and significance of *Burkholderia* species occupying diverse ecological niches. *Environ Microbiol* **5**: 719–29.

Cohen Y, Joseph Pollock F, Rosenberg E, and Bourne DG. 2013. Phage therapy treatment of the coral pathogen *Vibrio coralliilyticus*. *Microbiologyopen* **2**: 64–74.

Costa OS, Attrill MJ, and Nimmo M. 2006. Seasonal and spatial controls on the delivery of excess nutrients to nearshore and offshore coral reefs of Brazil. *J Mar Syst* **60**: 63–74.

Csardi G and Nepusz T. 2006. The igraph software package for complex network research.

Cunning R, Gillette P, Capo T, *et al.* 2015. Growth tradeoffs associated with thermotolerant symbionts in the coral *Pocillopora damicornis* are lost in warmer oceans. *Coral Reefs* **34**: 155–60.

Cunning R, Silverstein RN, and Baker AC. 2018. Symbiont shuffling linked to differential photochemical dynamics of *Symbiodinium* in three Caribbean reef corals. *Coral Reefs* **37**: 145–52.

Dall'Agnol RF, Plotegher F, Souza RC, *et al.* 2016. *Paraburkholderia nodosa* is the main N2-fixing species trapped by promiscuous common bean (*Phaseolus vulgaris* L.) in the Brazilian "Cerradão." *FEMS Microbiol Ecol* **92**: 1–14.

Damjanovic K, Blackall LL, Webster NS, and van Oppen MJH. 2017. The contribution of microbial biotechnology to mitigating coral reef degradation. *Microb Biotechnol* **10**: 1236-43.

Daniels CA, Baumgarten S, Yum LK, *et al.* 2015. Metatranscriptome analysis of the reef-building coral Orbicella faveolata indicates holobiont response to coral disease. *Front Mar Sci* **2**:62.

Davy SK, Allemand D, and Weis VM. 2012. Cell Biology of cnidarian-dinoflagellate symbiosis. *Microbiol Mol Biol Rev* **76**: 229–61.

De Cáceres M and Legendre P. 2009. Associations between species and groups of sites: indices and statistical inference. *Ecology* **90**: 3566–74.

de Sousa CNA. 1998. Classification of Brazilian wheat cultivars for aluminium toxicity in acid soils. *Plant Breed* **117**: 217–21.

De'ath G, Fabricius KE, Sweatman H, and Puotinen M. 2012. The 27-year decline of coral cover on the Great Barrier Reef and its causes. *Proc Natl Acad Sci* **109**: 17995–9.

Deguchi S, Shimazaki Y, Uozumi S, *et al.* 2007. White clover living mulch increases the yield of silage corn via arbuscular mycorrhizal fungus colonization. *Plant Soil* **291**: 291–9.

Dunbar HE, Wilson ACC, Ferguson NR, and Moran NA. 2007. Aphid thermal tolerance is governed by a point mutation in bacterial symbionts. *PLoS Biol* **5**: 1006–15.

Eakin C, Liu G, Gomez A, and Al. E. 2016. Global coral bleaching 2014-2017: status and an appeal for observations. *Reef Encount* **31**: 20–6.

Edmunds PJ, Nozawa Y, and Villanueva RD. 2014. Refuges modulate coral recruitment in the Caribbean and the Pacific. *J Exp Mar Bio Ecol* **454**: 78–84.

Fitt WK, Mcfarland IFK, Warner ME, and Chilcoat GC. 2000. Seasonal patterns of tissue biomass and densities of symbiotic dinoflagellates in reef corals and relation to coral bleaching. *Limnology* **45**: 677–85.

Flint HJ, Scott KP, Louis P, and Duncan SH. 2012. The role of the gut microbiota in nutrition and health. *Nat Rev Gastroenterol Hepatol* **9**: 577–89.

Fox J and Weisberg S. 2011. An R companion to applied regression, second edition.

Frade PR, Jongh F De, Vermeulen F, *et al.* 2008. Variation in symbiont distribution between closely related coral species over large depth ranges. *Mol Ecol* **17**: 691–703.

Fraune S and Bosch TCG. 2010. Why bacteria matter in animal development and evolution. *BioEssays* **32**: 571–80.

Fraune S, Forêt S, and Reitzel AM. 2016. Using *Nematostella vectensis* to study the interactions between genome, epigenome, and bacteria in a changing environment. *Front Mar Sci* **3**: 1–8.

Frazier M, Helmkampf M, Bellinger MR, *et al.* 2017. De novo metatranscriptome assembly and coral gene expression profile of *Montipora capitata* with growth anomaly. *BMC Genomics* **18**: 1–11.

Frentiu FD, Zakir T, Walker T, *et al.* 2014. Limited dengue virus replication in field-collected *Aedes aegypti* mosquitoes infected with *Wolbachia*. *PLoS Negl Trop Dis* **8**: 1–10.

Gajigan AP, Diaz LA, and Conaco C. 2017. Resilience of the prokaryotic microbial community of *Acropora digitifera* to elevated temperature. *Microbiologyopen* **6**: 1–11.

Garcia GD, Santos E de O, Sousa G V., *et al.* 2016. Metaproteomics reveals metabolic transitions between healthy and diseased stony coral *Mussismilia braziliensis*. *Mol Ecol* **25**: 4632–44.

Gevers D, Knight R, Petrosino JF, *et al.* 2012. The Human Microbiome Project: A community resource for the healthy human microbiome. *PLoS Biol* **10**: 6–10.

Gignoux-Wolfsohn SA and Vollmer S V. 2015. Identification of candidate coral pathogens on white band disease-infected staghorn coral. *PLoS One* **10**: 1–16.

Glasl B, Bourne DG, Frade PR, and Webster NS. 2018. Establishing microbial baselines to identify indicators of coral reef health. *Microbiol Aust* **39**: 42–6.

Glasl B, Herndl GJ, and Frade PR. 2016. The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. *ISME J* **10**: 2280–92.

Glasl B, Webster NS, and Bourne DG. 2017. Microbial indicators as a diagnostic tool for assessing water quality and climate stress in coral reef ecosystems. *Mar Biol* **164**: 1–18.

Glynn PW. 1984. Widespread coral mortality and the 1982–83 El Niño warming event. *Environ Conserv* **11**: 133-46.

Graves S, Piepho H-P, Selzer L, and Dorai-Raj S. 2015. multcompView: Visualizations of paired comparisons.

Grottoli AG, Martins PD, Wilkins MJ, *et al.* 2018. Coral physiology and microbiome dynamics under combined warming and ocean acidification. *PLoS One* **13**: 1–22.

Guppy R and Bythell JC. 2006. Environmental effects on bacterial diversity in the surface mucus layer of the reef coral *Montastraea faveolata*. *Mar Ecol Prog Ser* **328**: 133–42.

Gupta S, Allen-Vercoe E, and Petrof EO. 2016. Fecal microbiota transplantation: in perspective. *Therap Adv Gastroenterol* **9**: 229–39.

Hadaidi G, Röthig T, Yum LK, *et al.* 2017. Stable mucus-associated bacterial communities in bleached and healthy corals of *Porites lobata* from the Arabian Seas. *Sci Rep* **7**: 1–11.

Hernandez-Agreda A, Gates RD, and Ainsworth TD. 2017. Defining the core microbiome in corals' microbial soup. *Trends Microbiol* **25**: 125–40.

Hernandez-Agreda A, Leggat W, Bongaerts P, and Ainsworth TD. 2016. The microbial signature provides insight into the mechanistic basis of coral success across reef habitats. *MBio* **7**: 1–10.

Heron SF, Maynard JA, van Hooidonk R, and Eakin CM. 2016. Warming trends and bleaching stress of the world's coral reefs 1985–2012. *Sci Rep* **6**: 38402.

Hoegh-Guldberg O, Jones RJ, Ward S, and Loh WK. 2002. Is coral bleaching really adaptive? *Nature* **415**: 601–2.

Hoegh-Guldberg O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. *Mar Freshw Res* **50**: 839–66.

Horn M, Harzenetter MD, Linner T, *et al.* 2001. Members of the *Cytophaga-Flavobacterium-Bacteroides* phylum as intracellular bacteria of acanthamoebae: proposal of "*Candidatus* Amoebophilus asiaticus." *Environ Microbiol* **3**: 440–9.

Huggett MJ and Apprill A. 2018. Coral Microbiome Database: Integration of sequences reveals high diversity and relatedness of coral-associated microbes. *Environ Microbiol Rep*. DOI: 10.1111/1758-2229.12686.

Hughes TP, Anderson KD, Connolly SR, *et al.* 2018. Spatial and temporal patterns of mass bleaching of corals in the Anthropocene. *Science* **359**: 80–3.

Hughes TP, Kerry J, Álvarez-Noriega M, *et al.* 2017. Global warming and recurrent mass bleaching of corals. *Nature* **453**: 373–7.

Iizuka T, Jojima Y, Fudou R, and Yamanaka S. 1998. Isolation of myxobacteria from the marine environment. *FEMS Microbiol Lett* **169**: 317–22.

Ishida K, Sekizuka T, Hayashida K, *et al.* 2014. Amoebal endosymbiont *Neochlamydia* genome sequence illuminates the bacterial role in the defense of the host amoebae against *Legionella pneumophila*. *PLoS One* **9**: 1–9.

Jenkins D and Tandoi V. 1991. The applied microbiology of enhanced biological phosphate removal-accomplishments and needs. *Water Res* **25**: 1471–8.

Jones A., Berkelmans R, van Oppen MJ, *et al.* 2008. A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proc R Soc B Biol Sci* **275**: 1359–65.

Jones AM and Berkelmans R. 2011. Tradeoffs to thermal acclimation: Energetics and reproduction of a reef coral with heat tolerant *Symbiodinium* type-D. *J Mar Biol* **2011**: 1–12.

Kalimutho M, Ahmad A, and Kassim Z. 2007. Isolation, characterization and identification of coral mucus associated bacteria (*Acropora cervicornis*) from Bidong Island, Terengganu, Malaysia. *Malaysian J Sci* **26**: 27–39.

Kimes NE, Johnson WR, Torralba M, *et al.* 2013. The *Montastraea faveolata* microbiome: Ecological and temporal influences on a Caribbean reef-building coral in decline. *Environ Microbiol* **15**: 2082–94.

Kimes NE, van Nostrand JD, Weil E, *et al.* 2010. Microbial functional structure of *Montastraea faveolata*, an important Caribbean reef-building coral, differs between healthy and yellow-band diseased colonies. *Environ Microbiol* **12**: 541–56.

Kinzie R. 1993. Spawning in the reef corals *Pocillopora verrucosa* and *P. aydouxi* at Sesoko Island, Okinawa. *Galaxea* **11**: 93–105.

Klepac C, Beal J, Kenkel C, *et al.* 2015. Seasonal stability of coral-*Symbiodinium* associations in the subtropical coral habitat of St. Lucie Reef, Florida. *Mar Ecol Prog Ser* **532**: 137–51.

Kneip C, Lockhart P, Voß C, and Maier UG. 2007. Nitrogen fixation in eukaryotes - New models for symbiosis. *BMC Evol Biol* **7**: 1–12.

Krediet CJ, Ritchie KB, Paul VJ, and Teplitski M. 2013. Coral-associated micro-organisms and their roles in promoting coral health and thwarting diseases. *Proc R Soc B Biol Sci* **280**: 20122328.

Kuang W, Li J, Zhang S, and Long L. 2015. Diversity and distribution of Actinobacteria associated with reef coral *Porites lutea*. *Front Microbiol* **6**: 1–13.

Kulichevskaya IS, Suzina NE, Liesack W, and Dedysh SN. 2010. *Bryobacter aggregatus* gen. nov., sp. nov., a peat-inhabiting, aerobic chemo-organotroph from subdivision 3 of the acidobacteria. *Int J Syst Evol Microbiol* **60**: 301–6.

Kushmaro A, Banin E, Loya Y, *et al.* 2001. *Vibrio shiloi* sp. nov., the causative agent of bleaching of the coral *Oculina patagonica*. *Int J Syst Evol Microbiol* **51**: 1383–8.

Kushmaro A, Loya Y, Fine M, and Rosenberg E. 1996. Bacterial infection and coral bleaching. *Nature* **380**: 396–396.

Kushmaro A, Rosenberg E, Fine M, and Loya Y. 1997. Bleaching of the coral *Oculina patagonica* by *Vibrio* AK-1. *Mar Ecol Prog Ser* **147**: 159–65.

Lahti L, Shetty S, Blake T, and Salojarvi J. 2017. microbiome R package.

LaJeunesse TC, Bhagooli R, Hidaka M, *et al.* 2004b. Closely related *Symbiodinium* spp. differ in relative dominance in coral reef host communities across environmental, latitudinal and biogeographic gradients. *Mar Ecol Prog Ser* **284**: 147–61.

LaJeunesse TC, Lee S, Bush S, and Bruno JF. 2005. Persistence of non-Caribbean algal symbionts in Indo-Pacific mushroom corals released to Jamaica 35 years ago. *Coral Reefs* **24**: 157–9.

LaJeunesse TC, Loh WKW, van Woesik R, *et al.* 2003. Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. *Limnol Ocean* **48**: 2046–54.

LaJeunesse TC, Parkinson JE, Gabrielson PW, *et al.* 2018. Systematic revision of Symbiodiniaceae highlights the antiquity and diversity of coral endosymbionts. *Curr Biol*: 1–11.

LaJeunesse TC, Thornhill DJ, Cox EF, *et al.* 2004a. High diversity and host specificity observed among symbiotic dinoflagellates in reef coral communities from Hawaii. *Coral Reefs* **23**: 596–603.

Langille MGI, Zaneveld J, Caporaso JG *et al.* 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol* **8**: 1-10.

Larsson E, Tremaroli V, Lee YS, *et al.* 2012. Analysis of gut microbial regulation of host gene expression along the length of the gut and regulation of gut microbial ecology through MyD88. *Gut* **61**: 1124–31.

Lee MD, Walworth NG, Sylvan JB, *et al.* 2015a. Microbial communities on seafloor basalts at Dorado Outcrop reflect level of alteration and highlight global lithic clades. *Front Microbiol* **6**: 1–20.

Lee OO, Yang J, Bougouffa S, *et al.* 2012. Spatial and species variations in bacterial communities associated with corals from the Red Sea as revealed by pyrosequencing. *Appl Environ Microbiol* **78**: 7173–84.

Lee STM, Davy SK, Tang S-L, *et al.* 2015b. Successive shifts in the microbial community of the surface mucus layer and tissues of the coral *Acropora muricata* under thermal stress. *FEMS Microbiol Ecol* **91**: fiv142.

Lei X, Li Y, Wang G, *et al.* 2015. *Phaeodactylibacter luteus* sp. nov., isolated from the oleaginous microalga *picochlorum* sp. *Int J Syst Evol Microbiol* **65**: 2666–70.

Leite DCA, Leão P, Garrido AG, *et al.* 2017. Broadcast apawning coral *Mussismilia hispida* can vertically transfer its associated bacterial core. *Front Microbiol* **8**: 1–12.

Leite DCA, Salles JF, Calderon EN, *et al.* 2018. Coral bacterial-core abundance and network complexity as proxies for anthropogenic pollution. *Front Microbiol* **9**: 1–11.

Lema KA, Bourne DG, and Willis BL. 2014b. Onset and establishment of diazotrophs and other bacterial associates in the early life history stages of the coral *Acropora millepora*. *Mol Ecol* **23**: 4682–95.

Lema KA, Willis BL, and Bourne DG. 2014a. Amplicon pyrosequencing reveals spatial and temporal consistency in diazotroph assemblages of the *Acropora millepora* microbiome. *Environ Microbiol* **16**: 3345–59.

Lema KA, Willis BL, and Bourneb DG. 2012. Corals form characteristic associations with symbiotic nitrogen-fixing bacteria. *Appl Environ Microbiol* **78**: 3136–44.

Lenth R V. 2016. Least-Squares Means: The R Package lsmeans. *J Stat Softw* **69**: 1–33.

Lesser MP, Falcón LI, Rodríguez-Román A, *et al.* 2007. Nitrogen fixation by symbiotic cyanobacteria provides a source of nitrogen for the scleractinian coral *Montastraea cavernosa*. *Mar Ecol Prog Ser* **346**: 143–52.

Lesser MP, Stat M, and Gates RD. 2013. The endosymbiotic dinoflagellates (*Symbiodinium* sp.) of corals are parasites and mutualists. *Coral Reefs* **32**: 603–11.

Lewis CL and Coffroth MA. 2004. The acquisition of exogenous algal symbionts by an octocoral after bleaching. *Science* **304**: 1490–2.

Lewis DH and Smith DC. 1971. The autotrophic nutrition of symbiotic marine coelenterates with special reference to hermatypic corals. I. Movement of photosynthetic products between the symbionts. *Proc R Soc B Biol Sci* **178**: 111–29.

Li J, Chen Q, Long LJ, *et al.* 2014. Bacterial dynamics within the mucus, tissue and skeleton of the coral *Porites lutea* during different seasons. *Sci Rep* **4**: 1–8.

Lim YW, Haynes M, Furlan M, *et al.* 2014. Purifying the impure: Sequencing metagenomes and metatranscriptomes from complex animal-associated samples. *J Vis Exp*: **94**: e52117

Little AF, Oppen MJH van, and Willis BL. 2004. Flexibility in algal endosymbiosis shapes growth in reef corals. *Science* **304**: 1492–5.

Littman R, Willis BL, and Bourne DG. 2011. Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. *Environ Microbiol Rep* **3**: 651–60.

Littman RA, Willis BL, Pfeffer C, and Bourne DG. 2009. Diversities of coral-associated bacteria differ with location, but not species, for three acroporid corals on the Great Barrier Reef. *FEMS Microbiol Ecol* **68**: 152–63.

Liu G, Rauenzahn J, Heron S, *et al.* 2013. NOAA Technical Report NESDIS 143: NOAA Coral Reef Watch: 50 km satellite sea surface temperature-based decision support system for coral bleaching management. 1- 41.

Logan DDK, LaFlamme AC, Weis VM, and Davy SK. 2010. Flow-cytometric characterization of the cell-surface glycans of symbiotic dinoflagellates (symbiodinium spp.). *J Phycol* **46**: 525–33.

MacFabe DF. 2012. Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders. *Microb Ecol Heath Dis* **23**: 19260.

Magalon H, Flot J-F, and Baudry E. 2007. Molecular identification of symbiotic dinoflagellates in Pacific corals in the genus *Pocillopora*. *Coral Reefs* **26**: 551–8.

Marcelino LA, Westneat MW, Stoyneva V, *et al.* 2013. Modulation of light-enhancement to symbiotic algae by light-scattering in corals and evolutionary trends in bleaching. *PLoS One* **8**: e61492.

Markell DA and Wood-Charlson EM. 2010. Immunocytochemical evidence that symbiotic algae secrete potential recognition signal molecules *in hospite*. *Mar Biol* **157**: 1105–11.

Marquez L, Redman RS, Rodriquez RJ, and Roossinck MJ. 2007. A virus in a fungus in a plant: Three-way symbiosis required for thermal tolerance. *Science* **315**: 513–6.

Marx V. 2017. Microbiology: The return of culture. *Nat Methods* **14**: 37–40.

McKew BA, Dumbrell AJ, Daud SD, *et al.* 2012. Characterization of geographically distinct bacterial communities associated with coral mucus produced by *Acropora* spp. and *Porites* spp. *Appl Environ Microbiol* **78**: 5229–37.

McMeniman CJ, Lane R V, Cass BN, *et al.* 2009. Stable introduction of a life-shortening *Wolbachia* infection into the mosquito *Aedes aegypti*. *Science* **323**: 141–4.

McMurdie PJ and Holmes S. 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* **8**: e61217.

Meistertzheim AL, Lartaud F, Arnaud-Haond S, *et al.* 2016. Patterns of bacteria-host associations suggest different ecological strategies between two reef building cold-water coral species. *Deep Res Part I Oceanogr Res Pap* **114**: 12–22.

Meron D, Atias E, Iasur Kruh L, *et al.* 2011. The impact of reduced pH on the microbial community of the coral *Acropora eurystoma*. *ISME J* **5**: 51–60.

Meyer JL, Paul VJ, Raymundo LJ, and Teplitski M. 2017. Comparative metagenomics of the polymicrobial black band disease of corals. *Front Microbiol* **8**: 1–12.

Moberg F and Folke C. 1999. Ecological goods and services of coral reef ecosystems. *Ecol Econ* **29**: 215–33.

Moran NA and Sloan DB. 2015. The hologenome concept: Helpful or hollow? *PLOS Biol* **13**: e1002311.

Moran NA, McCutcheon JP, and Nakabachi A. 2008. Genomics and evolution of heritable bacterial symbionts. *Annu Rev Genet* **42**: 165–90.

Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, *et al.* 2009. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with Dengue, Chikungunya, and Plasmodium. *Cell* **139**: 1268–78.

Morrow KM, Bourne DG, Humphrey C, *et al.* 2015. Natural volcanic CO_2 seeps reveal future trajectories for host-microbial associations in corals and sponges. *ISME J* **9**: 894–908.

Mueller UG and Sachs JL. 2015. Engineering microbiomes to improve plant and animal health. *Trends Microbiol* **23**: 606–17.

Muscatine L and Porter JW. 1977. Reef corals : Mutualistic symbioses adapted to nutrient-poor environments. *Bioscience* **27**: 454–60.

Nakai R, Nishijima M, Tazato N, *et al.* 2014. *Oligoflexus tunisiensis* gen. nov., sp. nov., a Gram-negative, aerobic, filamentous bacterium of a novel proteobacterial lineage, and description of Oligoflexaceae fam. nov., Oligoflexales ord. nov. and Oligoflexia classis nov. *Int J Syst Evol Microbiol* **64**: 3353–9.

Neave MJ, Apprill A, Ferrier-Pagès C, and Voolstra CR. 2016. Diversity and function of prevalent symbiotic marine bacteria in the genus *Endozoicomonas*. *Appl Microbiol Biotechnol* **100**: 8315–24.

Neave MJ, Michell CT, Apprill A, and Voolstra CR. 2017a. *Endozoicomonas* genomes reveal functional adaptation and plasticity in bacterial strains symbiotically associated with diverse marine hosts. *Sci Rep* **7**: Article 40579.

Neave MJ, Rachmawati R, Xun L, *et al.* 2017b. Differential specificity between closely related corals and abundant *Endozoicomonas* endosymbionts across global scales. *ISME J* **11**: 186–200.

Nissimov J, Rosenberg E, and Munn CB. 2009. Antimicrobial properties of resident coral mucus bacteria of *Oculina patagonica*. *FEMS Microbiol Lett* **292**: 210–5.

Nitschke MR, Davy SK, and Ward S. 2016. Horizontal transmission of *Symbiodinium* cells between adult and juvenile corals is aided by benthic sediment. *Coral Reefs* **35**: 335–44.

Nyholm S V. and McFall-Ngai MJ. 2004. The winnowing: Establishing the squid - *Vibrio* symbiosis. *Nat Rev Microbiol* **2**: 632–42.

O'Hara AM and Shanahan F. 2006. The gut flora as a forgotten organ. *EMBO Rep* **7**: 688–93.

Oksanen J, Blanchet G, Friendly M, *et al.* 2018. vegan: Community ecology package.

Oliver JK, Berkelmans R, and Eakin CM. 2018. Coral bleaching in space and time. In: van Oppen MJH, Lough JM (Eds). Coral bleaching: Patterns, processes, causes and consequences. Berlin, Heidelberg: Springer-Verlag.

Olson ND, Ainsworth TD, Gates RD, and Takabayashi M. 2009. Diazotrophic bacteria associated with Hawaiian *Montipora* corals: Diversity and abundance in correlation with symbiotic dinoflagellates. *J Exp Mar Bio Ecol* **371**: 140–6.

Panke-Buisse K, Poole AC, Goodrich JK, *et al.* 2015. Selection on soil microbiomes reveals reproducible impacts on plant function. *ISME J* **9**: 980–9.

Pantos O, Bongaerts P, Dennis PG, *et al.* 2015. Habitat-specific environmental conditions primarily control the microbiomes of the coral *Seriatopora hystrix*. *ISME J* **9**: 1916–27.

Park S, Akira Y, and Kogure K. 2014. The family Rhodothermaceae. In: Rosenberg E, DeLong EF, Lory S, *et al.* (Eds). The prokaryotes. Berlin, Heidelberg: Springer.

Pasmore M and Costerton JW. 2003. Biofilms, bacterial signaling, and their ties to marine biology. *J Ind Microbiol Biotechnol* **30**: 407–13.

Peixoto RS, Rosado PM, Leite DC de A, *et al.* 2017. Beneficial microorganisms for corals (BMC): Proposed mechanisms for coral health and resilience. *Front Microbiol* **8**: Article 341.

Pinheiro J, Bates D, DebRoy S, *et al.* 2018. nlme: Linear and nonlinear mixed effects models.

Pochon X, Putnam HM, Burki F, and Gates RD. 2012. Identifying and characterizing alternative molecular markers for the symbiotic and free-living dinoflagellate genus *Symbiodinium. PLoS One* **7**: e29816.

Pogoreutz C, Rädecker N, Cárdenas A, *et al.* 2017a. Repository nitrogen fixation aligns with nifH abundance and expression in two coral trophic functional groups. *Front Microbiol* **8**: Article 1187.

Pogoreutz C, Rädecker N, Cárdenas A, *et al.* 2017b. Sugar enrichment provides evidence for a role of nitrogen fixation in coral bleaching. *Glob Chang Biol* **23**: 3838–48.

Pogoreutz C, Rädecker N, Cárdenas A, *et al.* 2018. Dominance of *Endozoicomonas* bacteria throughout coral bleaching and mortality suggests structural inflexibility of the *Pocillopora verrucosa* microbiome. *Ecol Evol* **8**: 2240–52.

Pollock FJ, Morris PJ, Willis BL, and Bourne DG. 2010. Detection and quantification of the coral pathogen *vibrio coralliilitycus* by real-time PCR with taqman fluorescent probes. *Appl Environ Microbiol* **76**: 5282–6.

Pootakham W, Mhuantong W, Putchim L, *et al.* 2018. Dynamics of coral-associated microbiomes during a thermal bleaching event. *Microbiologyopen*: e00604.

Porchas-Cornejo MA, Martínez-Porchas M, Vargas-Albores F, *et al.* 2017. High-resolution detection of bacterial profile of ocean water, before and after being used by shrimp farms. *Aquac Int* **25**: 1833–43.

Quast C, Pruesse E, Yilmaz P, *et al.* 2013. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res* **41**: 590–6.

Quigley KM, Bay LK, and Willis BL. 2017. Temperature and water quality-related patterns in sediment-associated *Symbiodinium* communities impact symbiont uptake and fitness of juveniles in the genus *Acropora*. *Front Mar Sci* **4**: 1–17.

Quigley KM, Davies SW, Kenkel CD, *et al.* 2014. Deep-sequencing method for quantifying background abundances of *Symbiodinium* types: Exploring the rare *Symbiodinium* biosphere in reef-building corals. *PLoS One* **9**: e94297.

Quigley KM, Warner PA, Bay LK, and Willis BL. 2018. Unexpected mixed-mode transmission and moderate genetic regulation of *Symbiodinium* communities in a brooding coral. *Heredity* **121**: 524-536.

Quince C, Walker AW, Simpson JT, *et al.* 2017. Shotgun metagenomics, from sampling to analysis. *Nat Biotechnol* **35**: 833–44.

R Core Team. 2018. R: A language and environmental for statistical computing. R Foundation for Statistical Computing: Vienna, Austria.

Raina JB, Tapiolas DM, Foret S, *et al.* 2013. DMSP biosynthesis by an animal and its role in coral thermal stress response. *Nature* **502**: 677–80.

Raina JB, Tapiolas D, Willis BL, and Bourne DG. 2009. Coral-associated bacteria and their role in the biogeochemical cycling of sulfur. *Appl Environ Microbiol* **75**: 3492–501.

Ramamoorthy V, Viswanathan R, Raguchander T, *et al.* 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Prot* **20**: 1–11.

Redman RS, Kim YO, Woodward CJDA, *et al.* 2011. Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: A strategy for mitigating impacts of climate change. *PLoS One* **6**: 1–10.

Redman RS, Sheehan KB, Stout RG, *et al.* 2002. Thermotolerance generated by plant/fungal symbiosis. *Science* **298**: 1581.

Reichenbach H and Dworkin M. 1992. The myxobacteria. In: Balows A, Trüper HG, Dworkin M, *et al.* (Eds). The prokaryotes. New York, NY: Springer.

Reis AMM, Araújo SD, Moura RL, *et al.* 2009. Bacterial diversity associated with the Brazilian endemic reef coral *Mussismilia braziliensis*. *J Appl Microbiol* **106**: 1378–87.

Reshef L, Koren O, Loya Y, *et al.* 2006. The coral probiotic hypothesis. *Environ Microbiol* **8**: 2068–73.

Rinke C, Schmitz-Esser S, Loy A, *et al.* 2009. High genetic similarity between two geographically distinct strains of the sulfur-oxidizing symbiont "*Candidatus* Thiobios zoothamnicoli." *FEMS Microbiol Ecol* **67**: 229–41.

Ritchie KB. 2006. Regulation of microbial population by coral surface mucus and mucus-associated bacteria. *Mar Ecol Prog Ser* **322**: 1–14.

Robison JD and Warner ME. 2006. Differential impacts of photoacclimation and thermal stress on the photobiology of four different phylotypes of *Symbiodinium* (Pyrrhophyta). *J Phycol* **42**: 568–79.

Roder C, Arif C, Bayer T, *et al.* 2014a. Bacterial profiling of White Plague Disease in a comparative coral species framework. *ISME J* **8**: 31–9.

Roder C, Arif C, Daniels C, *et al.* 2014b. Bacterial profiling of White Plague Disease across corals and oceans indicates a conserved and distinct disease microbiome. *Mol Ecol* **23**: 965–74.

Roder C, Bayer T, Aranda M, *et al.* 2015. Microbiome structure of the fungid coral *Ctenactis echinata* aligns with environmental differences. *Mol Ecol* **24**: 3501–11.

Rodriguez R and Redman R. 2008. More than 400 million years of evolution and some plants still can't make it on their own: Plant stress tolerance via fungal symbiosis. *J Exp Bot* **59**: 1109–14.

Rognes T, Flouri T, Nichols B, *et al.* 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* **4**: e2584.

Rohwer F and Kelley ST. 2004. Culture-independent analyses of coral-associated microbes. In: Rosenberg E, Loya Y (Eds). Coral health and disease. Berlin, Heidelberg: Springer Berlin Heidelberg.

Rohwer F, Seguritan V, Azam F, and Knowlton N. 2002. Diversity and distribution of coral-associated bacteria. *Mar Ecol Prog Ser* **243**: 1–10.

Rojas-Solís D, Zetter-Salmón E, Contreras-Pérez M, *et al.* 2018. *Pseudomonas stutzeri* E25 and *Stenotrophomonas maltophilia* CR71 endophytes produce antifungal volatile organic compounds and exhibit additive plant growth-promoting effects. *Biocatal Agric Biotechnol* **13**: 46–52.

Rosenberg E, Koren O, Reshef L, *et al.* 2007. The role of microorganisms in coral health, disease and evolution. *Nat Rev Microbiol* **5**: 355–62.

Rowan R and Knowlton N. 1995. Intraspecific diversity and ecological zonation in coral-algal symbiosis. *Proc Natl Acad Sci* **92**: 2850–3.

Sabdono A and Radjasa OK. 2006. Molecular characterization of bacteria associated with BBD (Black Band Disease) on coral *Acropora* sp. in Karimun J. *Ilmu Kelaut Indones J Mar Sci* **11**: 158–62.

Sabdono A, Radjasa OK, and Utomo HS. 2012. Screening of multi-metal resistances in a bacterial population isolated from coral tissues of central Java coastal waters, Indonesia. *Int J Oceanogr Mar Ecol Syst* **1**: 11–23.

Sakai K. 1997. Gametogenesis, spawning, and planula brooding by the reef coral *Goniastrea aspera* (Scleractinia) in Okinawa, Japan. *Mar Ecol Prog Ser* **151**: 67–72.

Salih A, Cox G, Szymczak R, *et al.* 2006. The role of host-based color and fluorescent pigments in photoprotection and in reducing bleaching stress in corals. *Proc 10th Int Coral Reef Symp*: 746–56.

Salih A, Larkum A, Cox G, *et al.* 2000. Fluorescent pigments in corals are photoprotective. *Nature* **408**: 850–3.

Sampayo EM, Dove S, and LaJeunesse TC. 2009. Cohesive molecular genetic data delineate species diversity in the dinoflagellate genus *Symbiodinium*. *Mol Ecol* **18**: 500–19.

Santos HF, Carmo FL, Duarte G, *et al.* 2014. Climate change affects key nitrogen-fixing bacterial populations on coral reefs. *ISME J* **8**: 2272–9.

Santos HF, Duarte GAS, Rachid CT da C, *et al.* 2015. Impact of oil spills on coral reefs can be reduced by bioremediation using probiotic microbiota. *Sci Rep* **5**: 18268.

Schlaeppi K and Bulgarelli D. 2015. The plant microbiome at work. *Mol Plant-Microbe Interact* **28**: 212–7.

Schmidt TL, Barton NH, Rašić G, *et al.* 2017. Local introduction and heterogeneous spatial spread of dengue-suppressing *Wolbachia* through an urban population of *Aedes aegypti*. *PLoS Biol* **15**: 1–28.

Schmitz-Esser S, Tischler P, Arnold R, *et al.* 2010. The genome of the amoeba symbiont "*Candidatus* Amoebophilus asiaticus" reveals common mechanisms for host cell interaction among amoeba-associated bacteria. *J Bacteriol* **192**: 1045–57.

Sekar R, Kaczmarsky LT, and Richardson LL. 2008. Microbial community composition of black band disease on the coral host *Siderastrea siderea* from three regions of the wider Caribbean. *Mar Ecol Prog Ser* **362**: 85–98.

Shannon P, Markiel A, Ozier O, *et al.* 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* **13**: 2498–504.

Sharp KH, Distel D, and Paul VJ. 2012. Diversity and dynamics of bacterial communities in early life stages of the Caribbean coral *Porites astreoides*. *ISME J* **6**: 790–801.

Sharp KH, Pratte ZA, Kerwin AH, *et al.* 2017. Season, but not symbiont state, drives microbiome structure in the temperate coral *Astrangia poculata*. *Microbiome* **5**: 120.

Sharp KH, Ritchie KB, Schupp PJ, *et al.* 2010. Bacterial acquisition in juveniles of several broadcast spawning coral species. *PLoS One* **5**: 1–6.

Sharp KH, Sneed JM, Ritchie KB, *et al.* 2015. Induction of larval settlement in the reef coral *Porites astreoides* by a cultivated marine *Roseobacter* strain. *Biol Bull* **228**: 98–107.

Shick JM and Dunlap WC. 2002. Mycosporine-like amino acids and related gadusols: biosynthesis, accumulation, and UV-protective functions in aquatic organisms. *Annu Rev Physiol* **64**: 223–62.

Smith H, Epstein H, and Torda G. 2017b. The molecular basis of differential morphology and bleaching thresholds in two morphs of the coral *Pocillopora acuta*. *Sci Rep* **7**: 1–12.

Smith P, Willemsen D, Popkes M, *et al.* 2017a. Regulation of life span by the gut microbiota in the short-lived african turquoise killifish. *Elife* **6**: 1–26.

Stat M, Loh WKW, LaJeunesse TC, *et al.* 2009a. Stability of coral–endosymbiont associations during and after a thermal stress event in the southern Great Barrier Reef. *Coral Reefs* **28**: 709–13.

Stat M, Pochon X, Cowie ROM, and Gates RD. 2009b. Specificity in communities of *Symbiodinium* in corals from Johnston Atoll. *Mar Ecol Prog Ser* **386**: 83–96.

Swain TD, DuBois E, Gomes A, *et al.* 2016. Skeletal light-scattering accelerates bleaching response in reef-building corals. *BMC Ecol* **16**: 15–9.

Sweet M and Bythell J. 2015. White syndrome in *Acropora muricata*: Nonspecific bacterial infection and ciliate histophagy. *Mol Ecol* **24**: 1150–9.

Sweet M, Burn D, Croquer A, and Leary P. 2013. Characterisation of the bacterial and fungal communities associated with different lesion sizes of dark spot syndrome occurring in the coral *Stephanocoenia intersepta*. *PLoS One* **8**: 1–9.

Sweet MJ, Croquer A, and Bythell JC. 2011a. Bacterial assemblages differ between compartments within the coral holobiont. *Coral Reefs* **30**: 39–52.

Sweet MJ, Croquer A, and Bythell JC. 2011b. Development of bacterial biofilms on artificial corals in comparison to surface-associated microbes of hard corals. *PLoS One* **6**: e21195.

Taniguchi A, Yoshida T, Hibino K, and Eguchi M. 2015. Community structures of actively growing bacteria stimulated by coral mucus. *J Exp Mar Bio Ecol* **469**: 105–12.

Tanner JE. 1996. Seasonality and lunar periodicity in the reproduction of Pocilloporid corals. *Coral Reefs* **15**: 59–66.

Teplitski M, Krediet CJ, Meyer JL, and Ritchie KB. 2016. Microbial interactions on coral surfaces and within the coral holobiont. In Goffredo S, Dubinsky Z (Eds.) The Cnidaria, Past, Present and Future. Switzerland: Springer International Publishing.

Teramoto M, Yagyu KI, and Nishijima M. 2015. *Perspicuibacter marinus* gen. nov., sp. nov., a semi-transparent bacterium isolated from surface seawater, and description of arenicellaceae fam. nov. and arenicellales ord. nov. *Int J Syst Evol Microbiol* **65**: 353–8.

Thaiss CA, Zmora N, Levy M, and Elinav E. 2016. The microbiome and innate immunity. *Nature* **535**: 65–74.

Thoendel M, Jeraldo P, Greenwood-Quaintance KE, *et al.* 2017. Impact of contaminating DNA in whole-genome amplification kits used for metagenomic shotgun sequencing for infection diagnosis. *J Clin Microbiol* **55**: 1789–801.

Thompson JR, Rivera HE, Closek CJ, and Medina M. 2015. Microbes in the coral holobiont: partners through evolution, development, and ecological interactions. *Front Cell Infect Microbiol* **4**: 1–20.

Thornhill DJ, Fitt WK, and Schmidt GW. 2006a. Highly stable symbioses among western Atlantic brooding corals. *Coral Reefs* **25**: 515–9.

Thornhill DJ, LaJeunesse TC, Kemp DW, *et al.* 2006b. Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. *Mar Biol* **148**: 711–22.

Timmis KN. 2002. *Pseudomonas putida*: A cosmopolitan opportunist par excellence. *Environ Microbiol* **4**: 779–81.

Tonk L, Bongaerts P, Sampayo EM, and Hoegh-Guldberg O. 2013. SymbioGBR: a web-based database of *Symbiodinium* associated with cnidarian hosts on the Great Barrier Reef. *BMC Ecol* **13**: 7.

Torda G, Donelson JM, Aranda M, *et al.* 2017. Rapid adaptive responses to climate change in corals. *Nat Clim Chang* **7**: 627–36.

Turnbaugh PJ, Ridaura VK, Faith JJ, *et al.* 2009. The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. *Sci Transl Med* **1**: 6ra14.

Turner TR, James EK, and Poole PS. 2013. The plant microbiome. *Genome Biol* **14**: 209.

Ulstrup KE, Hill R, van Oppen MJH, *et al.* 2008. Seasonal variation in the photo-physiology of homogeneous and heterogeneous *Symbiodinium* consortia in two scleractinian corals. *Mar Ecol Prog Ser* **361**: 139–50.

Valliappan K, Sun W, and Li Z. 2014. Marine Actinobacteria associated with marine organisms and their potentials in producing pharmaceutical natural products. *Appl Microbiol Biotechnol* **98**: 7365–77.

van de Water JAJM, Ainsworth TD, Leggat W *et al.* 2015. The coral immune response facilitates protection against microbes during tissue regeneration. *Mol Ecol* **24**: 3390-3404.

van de Water JAJM, Voolstra CR, Rottier C, *et al.* 2018. Seasonal stability in the microbiomes of temperate gorgonians and the red coral *Corallium rubrum* across the Mediterranean Sea. *Microb Ecol* **75**: 274–88.

van Empel PCM and Hafez HM. 1999. *Ornithobacterium rhinotracheale*: A review. *Avian Pathol* **28**: 217–27.

van Oppen MJH, Bongaerts P, Frade P, *et al.* 2018. Adaptation to reef habitats through selection on the coral animal and its associated microbiome. *Mol Ecol* **27**: 2956–71.

van Oppen MJH, Gates R, Blackall L, *et al.* 2017. Shifting paradigms in restoration of the world's coral reefs. *Glob Chang Biol* **23**: 3437-48.

van Oppen MJH, Oliver JK, Putnam HM, and Gates RD. 2015. Building coral reef resilience through assisted evolution. *Proc Natl Acad Sci* **112**: 2307-13.

Vega Thurber R, Willner-Hall D, Rodriguez-Mueller B, *et al.* 2009. Metagenomic analysis of stressed coral holobionts. *Environ Microbiol* **11**: 2148–63.

Vega Thurber RL, Burkepile DE, Fuchs C, *et al.* 2014. Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. *Glob Chang Biol* **20**: 544–54.

Vidal-Dupiol J, Ladriere O, Meistertzheim A-L, *et al.* 2011. Physiological responses of the scleractinian coral *Pocillopora damicornis* to bacterial stress from *Vibrio coralliilyticus*. *J Exp Biol* **214**: 1533–45.

Wade W, Thompson H, Rybalka A, and Vartoukian S. 2016. Uncultured members of the oral microbiome. *J Calif Dent Assoc* **44**: 447–56.

Waller F, Achatz B, Baltruschat H, *et al.* 2005. The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proc Natl Acad Sci* **102**: 13386–91.

Warner ME, Chilcoat GC, McFarland FK, and Fitt WK. 2002. Seasonal fluctuations in the photosynthetic capacity of photosystem II in symbiotic dinoflagellates in the Caribbean reef-building coral *Montastraea*. *Mar Biol* **141**: 31–8.

Webster NS, Negri AP, Botté ES, *et al.* 2016. Host-associated coral reef microbes respond to the cumulative pressures of ocean warming and ocean acidification. *Sci Rep* **6**: 19324.

Wegley L, Edwards R, Rodriguez-Brito B, *et al.* 2007. Metagenomic analysis of the microbial community associated with the coral *Porites astreoides*. *Environ Microbiol* **9**: 2707–19.

Wei T and Simko V. 2017. R package "corrplot": Visualization of a Correlation Matrix.

Weis VM. 2008. Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *J Exp Biol* **211**: 3059–66.

Whitman WB, Coleman DC, and Wiebe WJ. 1998. Prokaryotes: the unseen majority. *Proc Natl Acad Sci U S A* **95**: 6578–83.

Wicaksono WA, Jones EE, Casonato S, *et al.* 2018. Biological control of *Pseudomonas syringae pv. actinidiae* (Psa), the causal agent of bacterial canker of kiwifruit, using endophytic bacteria recovered from a medicinal plant. *Biol Control* **116**: 103–12.

Wickham H. 2009. ggplot2: Elegant graphics for data analysis.

Williams AD, Brown BE, Putchim L, and Sweet MJ. 2015. Age-related shifts in bacterial diversity in a reef coral. *PLoS One* **10**: 1–16.

Winkelmann N, Jaekel U, Meyer C, *et al.* 2010. Determination of the diversity of *Rhodopirellula* isolates from european seas by multilocus sequence analysis. *Appl Environ Microbiol* **76**: 776–85.

Wolanski E and van Senden D. 1983. Mixing of Burdekin river flood waters in the great barrier reef. *Mar Freshw Res* **34**: 49–63.

Xie H, Guo R, Zhong H, *et al.* 2016. Shotgun metagenomics of 250 adult twins reveals genetic and environmental impacts on the gut microbiome. *Cell Syst* **3**: 572–84.

Yang SH, Tseng CH, Huang CR, *et al.* 2017. Long-term survey is necessary to reveal various shifts of microbial composition in corals. *Front Microbiol* **8**: 1–11.

Zhang XY, He F, Wang GH, *et al.* 2013. Diversity and antibacterial activity of culturable Actinobacteria isolated from five species of the South China Sea gorgonian corals. *World J Microbiol Biotechnol* **29**: 1107–16.

Zhou G, Cai L, Yuan T, *et al.* 2017a. Microbiome dynamics in early life stages of the scleractinian coral *Acropora gemmifera* in response to elevated pCO_2. *Environ Microbiol* **19**: 3342–52.

Zhou Z, Yu X, Tang J, *et al.* 2017b. Dual recognition activity of a rhamnose-binding lectin to pathogenic bacteria and zooxanthellae in stony coral *Pocillopora damicornis*. *Dev Comp Immunol* **70**: 88–93.

Ziegler M, Roik A, Porter A, *et al.* 2016. Coral microbial community dynamics in response to anthropogenic impacts near a major city in the central Red Sea. *Mar Pollut Bull* **105**: 629–40.

Ziegler M, Seneca FO, Yum LK, *et al.* 2017. Bacterial community dynamics are linked to patterns of coral heat tolerance. *Nat Commun* **8**: 1–8.

Zilber-Rosenberg I and Rosenberg E. 2008. Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol Rev* **32**: 723–35.

APPENDICES & SUPPLEMENTARY MATERIALS

APPENDIX A

DNA Extraction Method

Original reference: Wilson KJ, Whan V, Lehnert SA, Byrne K, Moore SS, Phongsomboon S, Tassanakaion A, Rosenberg G, Ballment E, Fayazi Z, Swan J, Kenway MJ & Benzie JAH (2002). Genetic mapping of the black tiger shrimp *Penaeus monodon* with amplified fragment length polymorphisms. *Aquaculture.* 204: 297-309.

Modified for use for coral microbial DNA extractions in Damjanovic K, Blackall LL, Webster NS & van Oppen MJH (2017). The contribution of microbial biotechnology to mitigating coral reef degradation. *Microbial Biotechnology*. 10: 1236-1243.

Extraction buffer preparation:

Reagents	Stock (for 20mL)	Final Concentration	µL per reaction
Milli Q (H_2O)	11.2 mL	-	145
Tris	2 mL 1.0M	100mM pH 9	25
EDTA	4 mL 0.5M	100mM	50
NaCl	400 µL 5.0M	100mM	5
SDS	2 mL 10%	1%	25

Extraction protocol:

1. Place tissue and 0.35 mL of extraction buffer as prepared above into a sterile 1.5 mL Eppendorf tube
2. Add 7 µL of 10 mg/mL lysozyme
3. Incubate at 37°C for 30 minutes
4. Add 7 µL of 20 mg/mL Proteinase K
5. Add 30 mg of sterile glass beads and place samples into bead beater (FastPrep-24, MPBio) at 4 ms/p for 20 seconds to break apart tissue and cells
6. Incubate samples in 65°C water bath for 2 hours
7. Add 62.5 µL of 5M Potassium Acetate (KOAc) to tube (giving a final concentration of 1M)
8. Incubate on ice for 30 minutes
9. Centrifuge at max speed for 15 minutes at room temperature

10. Transfer supernatant to 1.5mL Eppendorf tubes (Note: if much floating material remains, re-spin for up to 15 minutes)
11. Add isopropanol (0.8 x the volume inside tubes) to precipitate. Mix gently by inversion and let stand for 15 minutes at room temperature
12. Centrifuge at max speed for 15 minutes at room temperature
13. Carefully remove supernatant with pipette. If the pellet comes too, re-spin briefly
14. Use 70% Ethanol to wash the pellet, them re-spin for 3 minutes at max speed. Use 50 µL of ethanol if there is no visible pellet, and up to 250 µL if a large pellet
15. Carefully remove supernatant with pipette. If pellet comes too, re-spin briefly
16. Air-dry (this can take up to 20 minutes)
17. Add 20 µL of Milli Q or TE buffer and leave overnight at 4°C to resuspend the DNA.
18. Vortex tubes and either use for PCR or place in a -20 freezer for storage (or -80°C freezer for long-term storage).

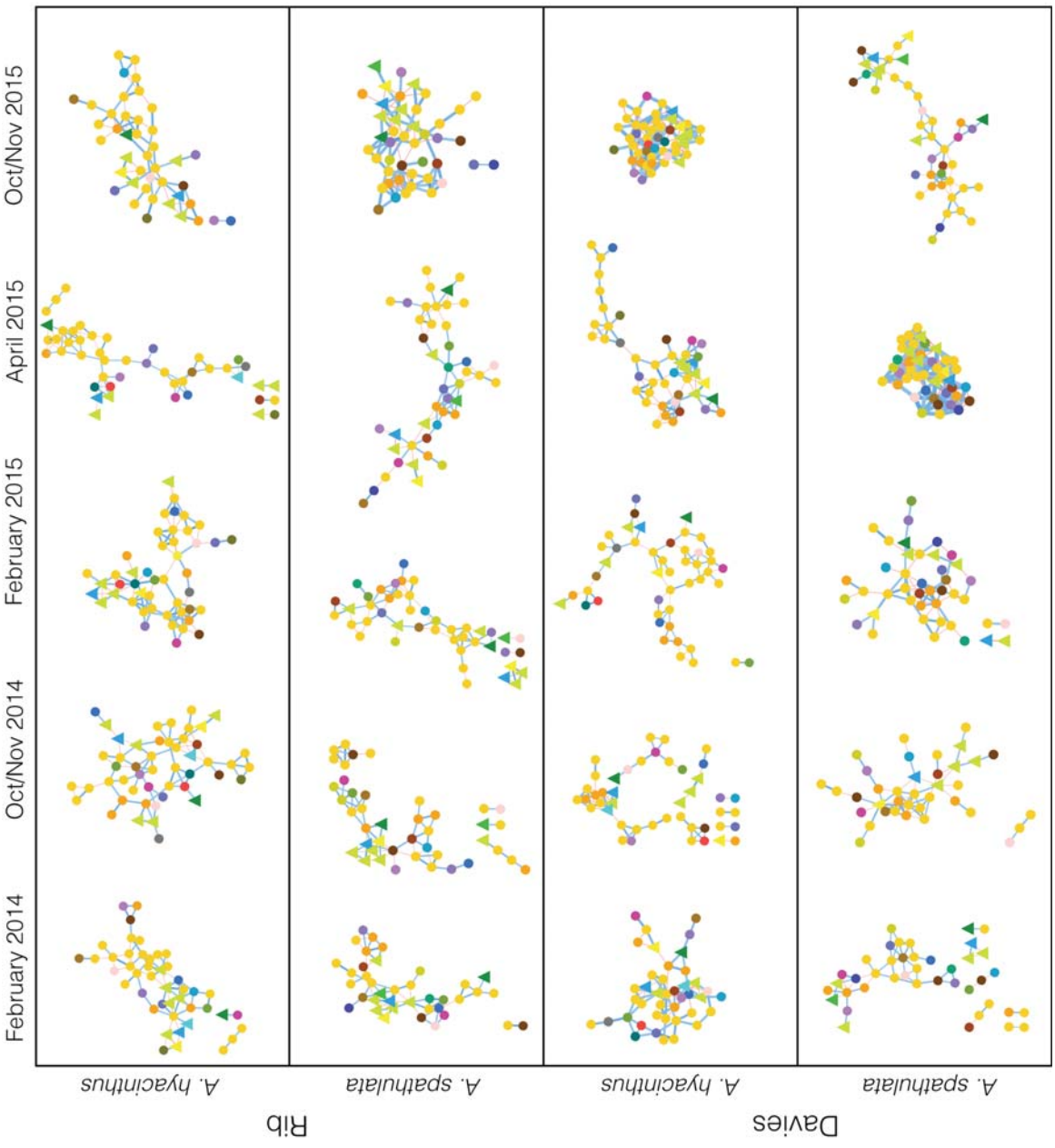

FIGURE S3.1: NETWORK ANALYSES VISUALISED PER TIME POINT FOR EACH SPECIES AT EACH REEF. DOTS REPRESENT BACTERIAL TAXA AT THE AMPLICON SEQUENCE VARIANT LEVEL,CLASSIFIED TO GENUS, AND TRIANGLES REPRESENT SYMBIODINIACEAE SEQUENCE SUB-TYPE. POSITIVE EDGES ARE ENCODED BY BLUE AND NEGATIVE EDGES ARE ENCODED BY RED.

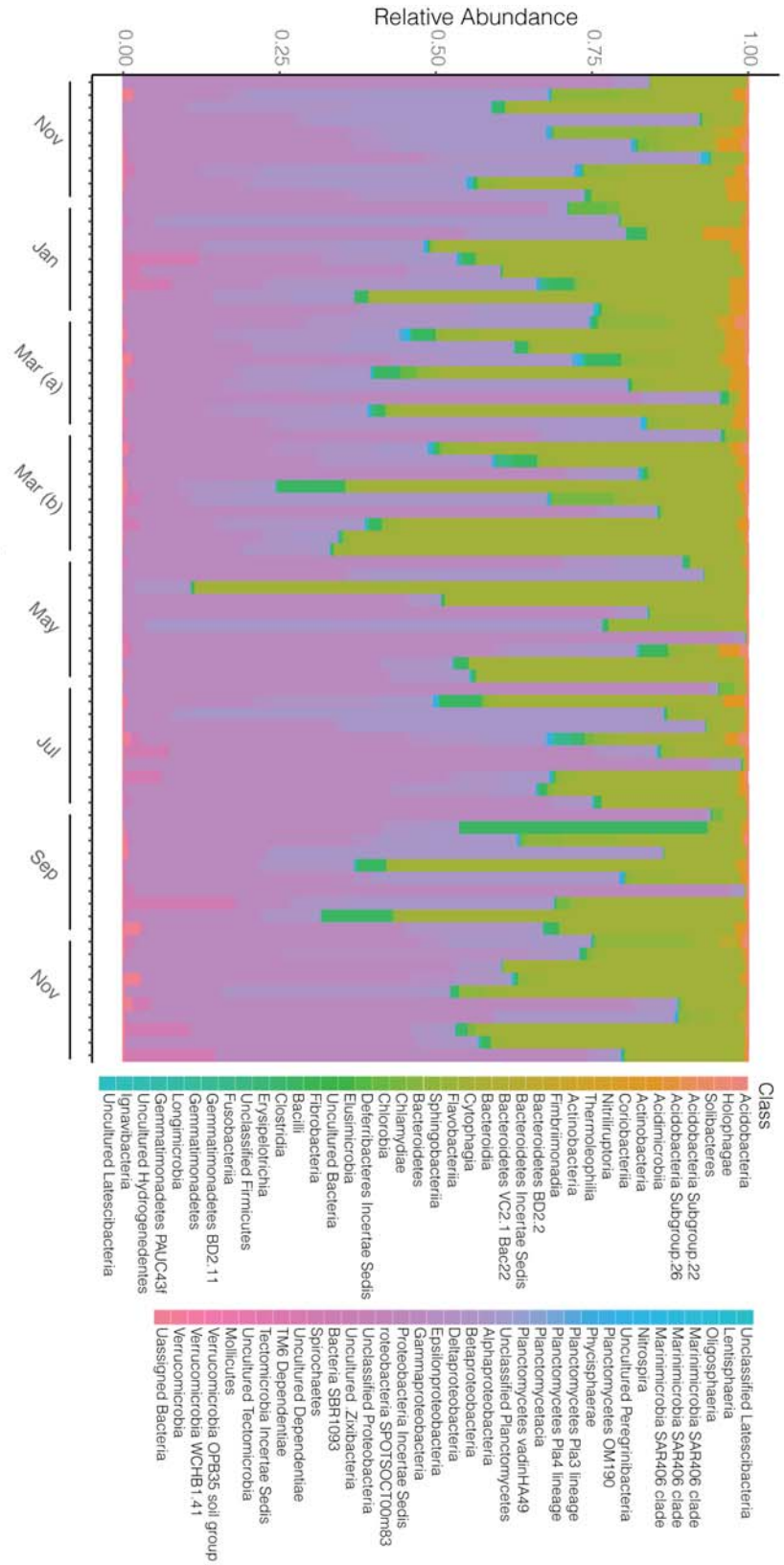

FIGURE S4.1: RELATIVE ABUNDANCE OF BACTERIAL CLASSES FOR ALL SAMPLES, ARRANGED BY TIME POINT.

TABLE S4.1 LIST OF SIGNIFICANT INDICATOR SPECIES BY TIME POINT. REFERENCES IN THE LITERATURE ARE LISTED TO PROVIDE INFORMATION ABOUT FUNCTION OR ASSOCIATIONS. FULL CITATIONS FOR SOURCES CAN BE FOUND IN THE GENERAL REFERENCES SECTION OF THIS THESIS.

Time Point	Bacterial taxa	stat	*p*-value	References in literature	Source
Nov (2015)	(p) Proteobacteria, (c) Alphaproteobacteria, (o) Rhodobacterales, (f) Rhodobacteraceae (Uncultured bacterium)	0.704	0.001	Possible coral pathogen	Roder et al. 2014a
	(p) Proteobacteria, (c) Alphaproteobacteria, (o) Rhodobacterales, (f) Rhodobacteraceae, (g) Roseivivax	0.668	0.001	Coral isolate; known to help induce larval settlement	Sharp et al. 2015
	(p) Bacteroidetes, (c) Sphingobacteriia, (o) Sphingobacteriales, (f) Saprospiraceae, (g) Phaeodactylibacter	0.617	0.019	Marine alga isolate	Lei et al. 2015
	(p) Planctomycetes, (c) Phycisphaerae, (o) Phycisphaerales, (f) Phycisphaeraceae, (g) SM1A02	0.616	0.003	Coral isolate; varied response to temperature	Webster et al. 2016
	(p) Planctomycetes, (c) Planctomycetacia, (o)Planctomycetales, (f) Planctomycetaceae. (g) Rhodopirellula	0.6	0.004	Marine isolate	Winkelmann et al. 2010
	(p) Acidobacteria, (c) Holophagae, (o) Subgroup 10, (f) CA002 (Uncultured bacterium)	0.59	0.011	Coral isolate (*Montastrea cavernosa*)	Apprill et al. 2016
	(p) Proteobacteria, (c) Alphaproteobacteria, (o) Rhodobacterales, (f) Rhodobacteraceae, (g) Marivita	0.589	0.003	Possible coral pathogen	Roder et al. 2014a
	(p) Proteobacteria, (c) Deltaproteobacteria, (o) Bdellovibrionales, (f) Bdellovibrionaceae, (g)Bdellovibrio	0.554	0.018	Coral isolate; possible microbial predator	Wegley et al. 2007
	(p) Bacteroidetes, (c) Cytophagia, (o) Cytophagales, (f) Flammeovirgaceae, (g) Flexithrix (Uncultured bacterium)	0.548	0.01	Found in healthy coral mucus	Reis et al. 2009
	(p) Chlorobi, (c) Chlorobia, (o) Chlorobiales, (f) OPB56 (Uncultured bacterium)	0.548	0.01	Possibly nitrogen fixing	Kneip et al. 2007
	(p) Proteobacteria, (c) Deltaproteobacteria, (o) Bdellovibrionales, (f) Bdellovibrionaceae, (g) Bdellovibrio	0.548	0.01	Coral isolate; possible microbial predator	Wegley et al. 2007
	(p) Proteobacteria, (c) Gammaproteobacteria, (o) Arenicellales, (f) Arenicellaceae (Uncultured bacterium)	0.548	0.012	Marine isolate	Teramoto et al. 2015
	(p) Proteobacteria, (c) Alphaproteobacteria, (o) E6aD10	0.537	0.03		
	(p) Proteobacteria, (c) Alphaproteobacteria, (o) Parvularculales, (f) Parvularculaceae (Uncultured bacterium)	0.523	0.023	Possible coral pathogen	Sweet et al. 2013
	(p) Actinobacteria, (c) Acidimicrobiia, (o) Acidimicrobiales	0.515	0.035		
Jan (2016)	(p) Proteobacteria, (c) Unknown, (o) Unknown, (f) Unknown, (g) "*Candidatus* Thobios" (Uncultured bacterium)	0.745	0.001	Sulfur-oxidizing marine isolate	Rinke et al. 2009
	(p) Proteobacteria, (c) Alphaproteobacteria, (o) Rhodobacterales, (f) Rhodobacteraceae, (g) Tropicimonas	0.577	0.001	Possible coral pathogen	Roder et al. 2014a
	(p) Proteobacteria, (c) Alphaproteobacteria, (o) Sphingomonadales, (f) Erythrobacteraceae, (g) Porphyrobacter	0.577	0.002	Marine isolate	Cavicchioli et al. 1999
	(p) Actinobacteria, (c) Actinobacteria, (o) Micrococcales, (f) Micrococcaceae, (g) Micrococcus (Ambiguous taxa)	0.502	0.007	Found in healthy coral mucus, production of bio-active compounds (e.g., antimicrobial, antioxidant, antiparasitic properties)	Kalimutho et al. 2007; Valliappan et al. 2014
	(p) Bacteroidetes, (c) Cytophagia, (o) Cytophagales, (f) Flammeovirgaceae, (g) Flexithrix (Uncultured bacterium)	0.48	0.043	Found in healthy coral mucus	Reis et al. 2009
	(p) Bacteroidetes, (c) Flavobacteriia, (o) Flavobacteriales, (f) Flavobacteriaceae, (g) Ornithobacterium	0.477	0.031	Bird isolate; Also found in shrimp farms	van Empel & Hafez 1999; Porchas-Cornejo et al. 2017
	(p) Actinobacteria, (c) Actinobacteria, (o) Micrococcales, (f) Dermacoccaceae, (g) Kytococcus (Ambiguous taxa)	0.471	0.02	Coral isolate, production of bio-active compounds (e.g., antimicrobial, antioxidant, antiparasitic properties)	Kuang et al. 2015; Valliappan et al. 2014

Mar (A) (2016)	(p) Bacteroidetes, (c) Sphingobacteriia, (o) Sphingobacteriales, (f) Saprospiraceae, (g) Aureispira	0.781	0.001	White band syndrome isolate	Sweet & Bythell 2015
	(p) Proteobacteria, (c) Gammaproteobacteria, (o) Pseudomonadales, (f) Moraxellaceae, (g) Psychrobacter	0.759	0.001	Coral mucus isolate	McKew et al. 2012
	(p) Proteobacteria, (c) Deltaproteobacteria, (o) Mycococcales, (f) Myxococcaceae, (g) Myxococcus (Ambiguous taxa)	0.745	0.001	Soil isolate; Possible marine isolate	Reichenbach & Dworkin 1992; Iizuka et al. 1998
	(p) Actinobacteria, (c) Actinobacteria, (o) Corynebacteriales, (f) Dietziaceae, (g) Dietzia (Uncultured bacterium)	0.738	0.001	Coral isolate, antibacterial	Zhang et al. 2013; Valliappan et al. 2014
	(p) Bacteroidetes, (c) Bacteroidetes Incertae Sedis, (o) Order II, (f) Rhodothermaceae (Uncultured bacterium)	0.607	0.007	Extreme environment or euphotic sea water isolate	Park et al. 2014
	(p) Proteobacteria, (c) Gammaproteobacteria, (o) Pseudomonadales, (f) Pseudomonadaceae, (g) Pseudomonas, (s) putida	0.596	0.006	Soil/ water isolate; the genus is known from coral as a DMSP metabolizer	Timmis 2002; Raina et al. 2009
	(p) Bacteroidetes, (c) Flavobacteriia, (o) Flacobacteriales, (f) NS7 Marine Group (Ambiguous taxa)	0.558	0.031	Associated with coral mucus; possibly disease-associated	Taniguchi et al. 2015; Gignoux-Wolfsohn & Vollmer 2015
	(p) Proteobacteria, (c) Alphaproteobacteria, (o) Caulobacterales, (f) Caulobacteraceae, (g) Phenylobacterium	0.554	0.007	Coral isolate (*Porites lutea*)	Pootakham et al. 2018
	(p) Proteobacteria, (c) Gammaproteobacteria, (o) Legionellales, (f) Legionellaceae, (g) Legionella (Uncultured bacterium)	0.553	0.033	Human contaminant (e.g., from water pollution)	Chiou et al. 2010
	(p) Proteobacteria, (c) Alphaproteobacteria, (o) Rhizobiales, (f) Bradyrhizobiaceae, (g) Bradyrhizobium (Ambiguous taxa)	0.552	0.011	Coral isolate, diazotroph (nitrogen fixer)	Lema et al. 2012
	(p) Proteobacteria, (c) Deltaproteobacteria, (o) Oligoflexales, (f) Oligoflexaceae (Uncultured taxa)	0.548	0.003	Desert soil isolate	Nakai et al. 2014
	(p) Actinobacteria, (c) Actinobacteria, (o) Propionibacteriales, (f) Nocardioidaceae (Ambiguous taxa)	0.54	0.009	Coral isolate, production of bio-active compounds (e.g., antimicrobial, antioxidant, antiparasitic properties)	Valliappan et al. 2014
	(p) Proteobacteria, (c) Gammaproteobacteria, (o) Cellvibrionales, (f) Cellvibrionaceae (Uncultured bacterium)	0.516	0.006	Gorgonian isolate	van de Water et al. 2018
	(p) Actinobacteria	0.471	0.026	Coral isolate, production of bio-active compounds (e.g., antimicrobial, antioxidant, antiparasitic properties)	Valliappan et al. 2014
	(p) Bacteroidetes, (c) Sphingobacteriia, (o) Sphingobacteriales, (f) Sphingobacteriaceae, (g) Sphingobacterium	0.471	0.022	Coral isolate, positively correlated with nutrient and thermal stress	Vega Thurber et al. 2009; Lee et al. 2015b
	(p) Firmicutes, (c) Bacilli, (o) Bacillales, (f) Bacillaceae, (g) Virgibacillus (Ambiguous taxa)	0.471	0.027	Coral isolate, production of bio-active compounds (e.g., thermostable proteinases and microbial osmoregulators) and inhibits surface competitors	Reviewed in Sabdono et al. 2012
	(p) Firmicutes, (c) Bacilli, (o) Bacillales, (f) Planococcaceae, (g) Sporosarcina	0.471	0.026	Halophilic isolate	Claus et al. 1983
	(p) Proteobacteria, (c) Alphaproteobacteria, (o) Rhizobiales, (f) Methylobacteriaceae (Uncultured bacterium)	0.471	0.022	Coral isolate, diazotroph (nitrogen fixer)	Lema et al. 2012
	(p) Proteobacteria, (c) Alphaproteobacteria, (o) Rhizobiales, (f) Rhizobiales Incertae Sedis, (g) Phreatobacter	0.471	0.017	Coral isolate, diazotroph (nitrogen fixer)	Lema et al. 2012
	(p) Proteobacteria, (c) Alphaproteobacteria, (o) Sphingomonadales, (f) Sphingomonadaceae, (g) Sphingopyxis	0.471	0.021	Sea water isolate	Cavicchioli et al. 2003
	(p) Proteobacteria, (c) Gammaproteobacteria, (o) Legionellales, (f) Legionellaceae, (g) Legionella (Ambiguous taxa)	0.471	0.022	Human contaminant (e.g., from water pollution)	Chiou et al. 2010
	(p) Acidobacteria, (c) Solibacteres, (o) Solibacterales, (f) Solibacteraceae (Subgroup 3), (g) Bryobacter	0.466	0.021	Soil isolate	Kulichevskaya et al. 2010
	(p) Actinobacteria, (c) Actinobacteria, (o) Propionibacteriales, (f) Propionibacteriaceae, (g) Propionibacterium	0.463	0.031	Coral isolate, positively correlated with seawater temperature, likely aids in production of bio-active	Vallippan et al. 2014; Kuang et al. 2015

				compounds like other Actinobacteria spp.	
	(p) Chlamydiae, (c) Chlamydiae, (o) Chlamydiales, (f) Parachlamydiaceae, (g) Neochlamydia (Uncultured bacterium)	0.455	0.047	Intracellular amoebal symbiont	Ishida et al. 2014
	(p) Firmicutes, (c) Clostridia, (o) Clostridiales, (f) Lachnospiraceae, (g) Lachnoanaerobaculum (Uncultured bacterium)	0.435	0.035	Human oral isolate	Wade et al. 2016
Mar (B) (2016)	(p) Proteobacteria, (c) Gammaproteobacteria, (o) Alteromonadales, (f) Alteromonadaceae, (g) Alteromonas	0.752	0.001	Coral isolate, involved in DMSP metabolism	Raina et al. 2009
	(p) Proteobacteria, (c) Gammaproteobacteria, (o) Alteromonadales, (f) Pseudoalteromonadaceae, (g) Psychrosphaera	0.707	0.001	Found in coral mucus	Gajigan et al. 2017
	(p) Proteobacteria, (c) Gammaproteobacteria, (o) Alteromonadales, (f) Pseudoalteromonadaceae, (g) Pseudoalteromonas	0.67	0.01	Coral isolate	Gajigan et al. 2017
	(p) Proteobacteria, (c) Gammaproteobacteria, (o) Alteromonadales, (f) Ferrimonadaceae, (g) Ferrimonas	0.556	0.022	Coral isolate, inhibition of pathogens	Nissimov et al. 2009
	(p) Proteobacteria, (c) Deltaproteobacteria, (o) Desulfobacterales, (f) Desulfobacteraceae	0.548	0.01	Found associated with Black Band Disease	Sekar et al. 2008
	(p) Proteobacteria, (c) Deltaproteobacteria, (o) Desulfobacterales, (f) Desulfobacteraceae (Uncultured bacterium)	0.509	0.041	Found associated with Black Band Disease	Sekar et al. 2008
	(p) Acidobacteria, (c) Subgroup 26 (Uncultured bacterium)	0.476	0.034		
May (2016)	(p) Proteobacteria, (c) Betaproteobacteria, (o) Burkholderiales, (f) Alcaligenaceae	0.632	0.001	Found associated with Black Band Disease	Sabdono & Radjasa 2006
Jul (2016)	(p) Proteobacteria, (c) Betaproteobacteria	0.548	0.018		
Sep (2016)	(p) Proteobacteria, (c) Alphaproteobacteria, (o) Rickettsiales, (f) Rickettsiaceae (Uncultured bacterium)	0.603	0.011	Possible white band disease pathogen	Gignoux-Wolfsohn & Vollmer 2015
Nov (2016)	(p) Bacteroidetes, (c) Flavobacteriia, (o) Flavobacteriales, (f) Flavobacteriaceae, (g) NS4 Marine Group	0.602	0.006	Marine isolate	Alonso-Sáez et al. 2015

SUPPLEMENTARY MATERIALS FOR CHAPTER 5

Appendix 5.1: Symbiodiniaceae community analyses with outlier water sample included

Removing the outlier did not change any of the significant results of the Symbiodiniaceae communities. With the added outlier, communities of Symbiodiniaceae remained significantly different among sample type (PERMANOVA: df = 2, F = 27.37, $p = 0.001$) and remained driven by differences in water communities. Pairwise comparisons found that Symbiodiniaceae communities did not significantly differ between parents and recruits, but both significantly differed from water ($R^2 < 0.6$, $p < 0.01$). Alpha diversity did not vary significantly different between parents, recruits and water, and observed richness remained significantly lower in water compared with both recruits and parents ($ANOVA_{richness}$: df = 2, F = 11.72, $p < 0.001$). Symbiodiniaceae communities in offspring remained significantly different according to both parental environment (PERMANOVA: df = 1, F = 135.91, $p = 0.001$) and planulation environment (PERMANOVA: df = 1, F = 15.2, $p = 0.001$), where planulation environment also displayed significant differences in dispersion (PERMDISP: df = 1, F = 90.4, $p = 0.001$).

TABLE S5.1 PAIRWISE PERMANOVA RESULTS FOR BACTERIAL BETA-DIVERSITY AMONG TREATMENTS. THOSE IN BOLD WITH "*" WERE SIGNIFICANT AT A = 0.05 ACCORDING TO THE ADJUSTED *P* –VALUE.

Pairs	Total df	F Model	R^2	*p* value	Adjusted *p* value (Bonferroni correction)
FFF vs FFS	9	1.38	0.15	0.03	0.435
FFF vs. FSF	6	1.58	0.24	0.04	0.6
FFF vs. FSS	12	2.07	0.16	0.005	0.075
FFF vs. SSS	**18**	**4.08**	**0.19**	**0.001**	**0.015***
FFF vs. SSF	**12**	**2.79**	**0.2**	**0.001**	**0.015***
FFS vs. FSF	6	1.48	0.23	0.05	0.79
FFS vs. FSS	12	1.63	0.13	0.02	0.26
FFS vs. SSS	**18**	**3.27**	**0.16**	**0.001**	**0.015***
FFS vs. SSF	**12**	**2.4**	**0.18**	**0.001**	**0.015***
FSF vs. FSS	9	1.37	0.15	0.08	1.0
FSF vs. SSS	15	2.05	0.13	0.006	0.09
FSF vs. SSF	9	1.88	0.19	0.05	0.78
FSS vs. SSS	21	1.6	0.07	0.06	0.89
FSS vs. SSF	15	1.32	0.09	0.15	1.0
SSS vs. SSF	21	1.15	0.05	0.28	1.0

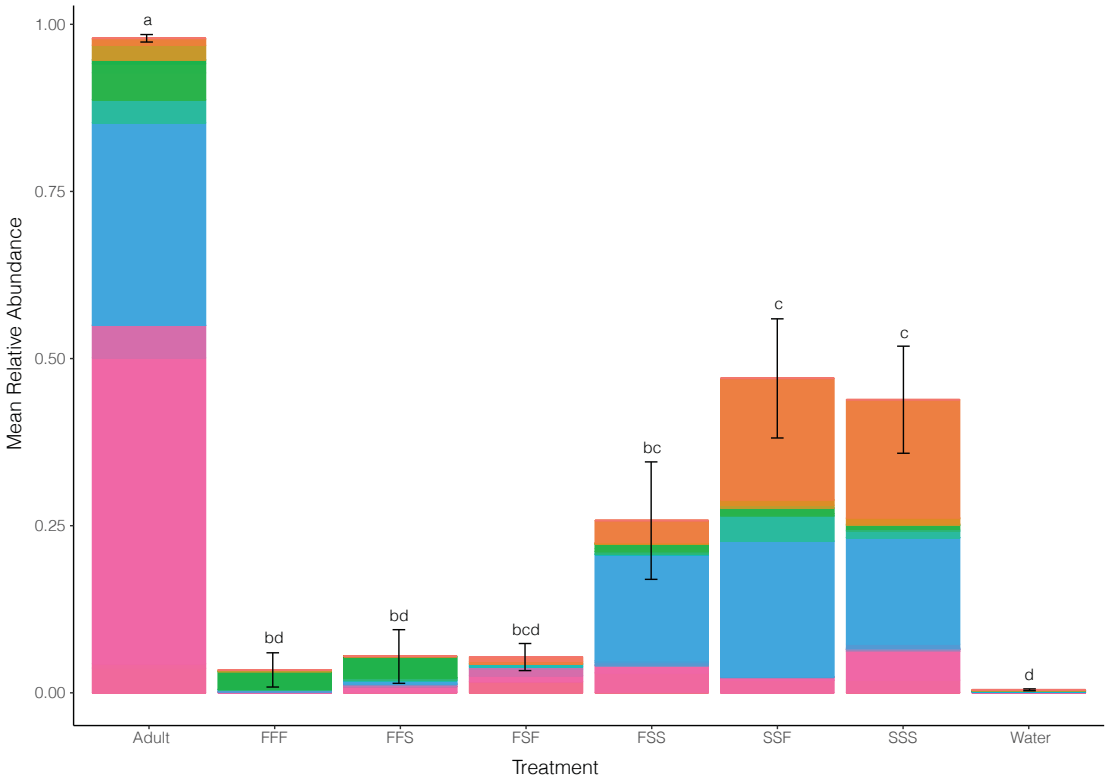

FIGURE S5.1 MEAN RELATIVE ABUNDANCE OF *ENDOZOICOMONAS* AMONG TREATMENTS. EACH COLOR IN THE BARS REPRESENTS A DIFFERENT STRAIN OF *ENDOZOICOMONAS*, AND ERROR BARS REPRESENT THE MEAN STANDARD ERROR ON TOTAL RELATIVE ABUNDANCE OF ALL STRAINS COMBINED.

TABLE S5.2: AMPLICON SEQUENCE VARIANTS (ASVS) THAT ARE PRESENT IN PARENTS AND 100% OF THEIR RESPECTIVE OFFSPRING ARRANGED BY PARENTAL IDENTIFICATION. THOSE IN BOLD ARE SHARED AMONG MORE THAN ONE PARENT-OFFSPRING COHORT. THE "*" REPRESENTS THE ONE TAXA SHARED BETWEEN ALL PARENT-OFFSPRING COHORTS.

	Shared Taxa (100%)	
Parental ID	**ASV (Feature ID)**	**Taxa**
B4	***d11575762e8afe4d96fce4fc2457dcff**	**(p) Proteobacteria; (c) Betaproteobacteria; (o) Burkholderiales; (f) Burkholderiaceae; (g) *Burkholderia-Paraburkholderia***
	1c1f840857bf9067a1fc1464f4bfa9db	**(p) Firmicutes; (c) Bacilli; (o) Lactobacillales; (f) Peptostreptococcaceae; (g) *Acetoanaerobium* (uncultured)**
B7	c9501367e416eaf52d547215cf000d1a	(p) Proteobacteria; (c) Alphaproteobacteria; (o) Rhodobacterales; (f) Rhodobacteraceae (unknown)
	d11575762e8afe4d96fce4fc2457dcff	**(p) Proteobacteria; (c) Betaproteobacteria; (o) Burkholderiales; (f) Burkholderiaceae; (g) *Burkholderia-Paraburkholderia***
	1c1f840857bf9067a1fc1464f4bfa9db	**(p) Firmicutes; (c) Bacilli; (o) Lactobacillales; (f) Peptostreptococcaceae; (g) *Acetoanaerobium* (uncultured)**
	6a9aa48199902d682f8e56c8f9b6c1f2	(p) Proteobacteria; (c) Gammaproteobacteria; (o) Vibrionales; (f) Vibrionaceae; (g) *Vibrio*
B8	00d58cb005a34a15994230604d655a9c	(p) Bacteroidetes; (c) Flavobacteriia; (o) Favobacteriales; (f) Flavobacteriaceae; (g) NS5 Marine Group
	8b2b81ac2f5a4647ff65cbea835ea0e7	(p) Proteobacteria; (c) Alphaproteobacteria; (o) Rhodobacterales; (f) Rhodobacteraceae; (g) *Ruegeria* (Ambiguous taxa)
	45f1167d09a8482ef689e112d27049fc	(p) Proteobacteria; (c) Alphaproteobacteria; (o) Rhizobiales; (f) Hyphomicrobiaceae; (g) *Pedomicrobium*
	0a6717aa74c45780487b407128fb331f	(p) Firmicutes; (c) Bacilli; (o) Bacillales; (f) Bacillaceae; (g) *Geobacillus*
	***d11575762e8afe4d96fce4fc2457dcff**	**(p) Proteobacteria; (c) Betaproteobacteria; (o) Burkholderiales; (f) Burkholderiaceae; (g) *Burkholderia-Paraburkholderia***
	cc4179b2afeb0d8c72d1e42f3c82a617	(p) Proteobacteria; (c) Betaproteobacteria; (o) Burkholderiales; (f) Burkholderiaceae; (g) *Burkholderia-Paraburkholderia*
	5f303d5361eaea06168f2dd3aae55a2f	(p) Actinobacteria; (c) Actinobacteria; (o) Propionibacteriales; (f) Propionibacteriaceae; (g) *Propionibacterium* (Uncultured)
	eee59526a11b5546724df5a9bb0e9a0e	(p) Proteobacteria; (c) Gammaproteobacteria; (o) Alteromonadales; (f) Alteromonadaceae; (g) *Alteromonas*
	a5c9c896cf829c31c15ab567d824a729	**(p) Proteobacteria; (c) Gammaproteobacteria; (o) Oceanospirillales; (f) Hahellaceae; (g) *Endozoicomonas* (Uncultured)**
	534ecb31a57d4adcec7c55a4986b71f5	(p) Proteobacteria; (c) Gammaproteobacteria; (o) Oceanospirillales; (f) Hahellaceae; (g) *Endozoicomonas* (Uncultured)
	6bbae2d1306327676ddf86b2f192d1e5	(p) Proteobacteria; (c) Gammaproteobacteria; (o) Oceanospirillales; (f) Hahellaceae; (g) *Endozoicomonas* (Uncultured)
	ed021254fb9c91dc718f25f26a361041	(p) Proteobacteria; (c) Gammaproteobacteria; (o) Oceanospirillales; (f) Hahellaceae; (g) *Endozoicomonas* (Uncultured)
	3fc2e76ea194c8add4dab2a994c90c79	(p) Actinobacteria; (c) Acidimicrobiia; (o) Acidimicrobiales; (f) OM1 Clade; (g) "*Candidatus* Actinomarina" (Uncultured)
B9	***d11575762e8afe4d96fce4fc2457dcff**	**(p) Proteobacteria; (c) Betaproteobacteria; (o) Burkholderiales; (f) Burkholderiaceae; (g) *Burkholderia-Paraburkholderia***
	5d0b711427abaec36549786873058834	(p) Proteobacteria; (c) Betaproteobacteria; (o) Burkholderiales; (f) Burkholderiaceae; (g) *Burkholderia-Paraburkholderia*
	787315948a30d013699f3bd7c9bf8e7e	(p) Proteobacteria; (c) Gammaproteobacteria; (o) Alteromonadales; (f) Pseudoalteromonadaceae; (g) *Pseudoalteromonas*
	e40f397aee41520e9425d9c0ff5e72dc	(p) Proteobacteria; (c) Gammaproteobacteria; (o) Oceanospirillales; (f) Hahellaceae; (g) *Endozoicomonas* (Uncultured)
B13	***d11575762e8afe4d96fce4fc2457dcff**	**(p) Proteobacteria; (c) Betaproteobacteria; (o) Burkholderiales; (f) Burkholderiaceae; (g) *Burkholderia-Paraburkholderia***
	a67ab1ca853dea5e0db4c60aa38b97a2	(p) Proteobacteria; (c) Betaproteobacteria; (o) Neisseriales; (f) Nesseriaceae (unknown)
	a5c9c896cf829c31c15ab567d824a729	**(p) Proteobacteria; (c) Gammaproteobacteria; (o) Oceanospirillales; (f) Hahellaceae; (g) *Endozoicomonas* (uncultured)**
B15	***d11575762e8afe4d96fce4fc2457dcff**	**(p) Proteobacteria; (c) Betaproteobacteria; (o) Burkholderiales; (f) Burkholderiaceae; (g) *Burkholderia-Paraburkholderia***
	a5c9c896cf829c31c15ab567d824a729	**(p) Proteobacteria; (c) Gammaproteobacteria; (o) Oceanospirillales; (f) Hahellaceae; (g) *Endozoicomonas* (uncultured)**

TABLE S5.3 PAIRWISE PERMANOVA RESULTS FOR SYMBIODINIACEAE BETA-DIVERSITY COMMUNITIES ACCORDING TO SAMPLE TYPE. THOSE IN BOLD WITH "*" WERE SIGNIFICANT AT A = 0.05 ACCORDING TO THE ADJUSTED *P* –VALUE.

Pairs	Total df	F Model	R^2	*p* value	Adjusted *p* value (Bonferroni correction)
Adult vs. Recruit	47	1.89	0.04	0.173	0.519
Adult vs. Water	**20**	**36.08**	**0.66**	**0.001**	**0.003***
Recruit vs. Water	**56**	**40.27**	**0.42**	**0.001**	**0.003***

TABLE S5.4: PRESENCE OF SYMBIODINIACEAE SUB-TYPES IN PARENTS AND RESPECTIVE OFFSPRING. A "✓" REPRESENTS PRESENCE OF THE SUB-TYPE IN ONE OR MORE SAMPLE. SUB-TYPES IN BOLD WERE PRESENT IN ONE OR MORE RECRUITS, BUT NOT IN PARENTS.

Parental ID	Symbiodiniaceae sub-type	Presence	
		Parent	Recruit
B4	**A1**		✓
	A3		✓
	C1	✓	✓
	C1.6	✓	✓
	C116		✓
	C1a	✓	✓
	C1c.C45	✓	✓
	C1ca	✓	✓
	C1h	✓	✓
	C1j	✓	✓
	C3.12	✓	✓
	C33 (type 2)		✓
	C33.1	✓	✓
	C3k	✓	✓
	C42 (type 1)	✓	✓
	C42 (type 2)	✓	✓
	Cspc	✓	✓
	D1a		✓
	D5	✓	✓
	C161		✓
	C86	✓	✓

Parental ID	Symbiodiniaceae sub-type	Presence	
		Parent	Recruit
B7	**A3**		✓
	C1	✓	✓
	C1.6	✓	✓
	C1a	✓	✓
	C1c.C45	✓	✓
	C1ca		✓
	C1h	✓	✓
	C1j	✓	✓
	C3.12	✓	✓
	C33 (type 2)		✓
	C33.1		✓
	C3k		✓
	C42 (type 1)	✓	✓
	C42 (type 2)	✓	✓
	Cspc	✓	✓
	C86	✓	✓
B8	C1	✓	✓
	C1.6	✓	✓
	C1a	✓	✓
	C1c.C45	✓	✓
	C1ca	✓	✓
	C1h	✓	✓
	C1j	✓	✓
	C33 (type 2)		✓
	C33.1	✓	✓
	C42 (type 1)	✓	✓
	C42 (type 2)	✓	✓
	Cspc	✓	✓
	C86	✓	✓
B9	C1	✓	✓
	C1.6	✓	✓
	Ca	✓	✓
	C1c.C45	✓	✓
	C1j	✓	✓
	C3.12	✓	✓
	C33 (type 2)		✓
	C42 (type 1)	✓	✓
	C42 (type2)	✓	✓
	Cspc	✓	✓
	C86	✓	✓

Parental ID	Symbiodiniaceae sub-type	Presence	
		Parent	Recruit
B13	**A3**		✓
	C1	✓	✓
	C125		✓
	C1ca	✓	✓
	C1d		✓
	C1h	✓	✓
	C3.2	✓	✓
	C33 (type 1)	✓	✓
	C33 (type 2)	✓	✓
	C34	✓	✓
	C3k		✓
	C42 (type 1)		✓
	C42 (type 2)		✓
	Cspc	✓	✓
	D1a	✓	✓
	D2		✓
	F5.1		✓
B15	**A1**		✓
	C1	✓	✓
	C1.6	✓	✓
	C1c.C45	✓	✓
	C1d		✓
	C1h	✓	✓
	C1j	✓	✓
	C3.12	✓	✓
	C33 (type 1)		✓
	C33 (type 2)		✓
	C3k		✓
	C42 (type 1)	✓	✓
	C42 (type 2)		✓
	C42b	✓	✓
	Cspc	✓	✓
	D1a		✓
	C86	✓	✓