Influence of Environment and Climate Change on Coral-associated Microbial Communities and Trophic Strategies

Dissertation

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Abstract

Global increases in atmospheric CO_2 are leading to ocean warming and acidification, causing more frequent occurrences of coral bleaching, outbreaks of disease, and as a result, widespread coral mortality. Yet, some corals appear to be more tolerant of the effects of a changing climate than others. This has been attributed to several parameters of coral physiology, including greater levels of energy reserves and the ability to incorporate more heterotrophic resources, or hosting more thermally tolerant lineages of endosymbiotic algae (i.e., Symbiodiniaceae). The bacteria and archaea associated with a coral, hereafter referred to as microbial communities, are also thought to support corals by changing in response to environmental conditions, potentially providing a first line of defense as corals attempt to acclimatize. However, it is unclear whether most corals will be able to adapt or acclimatize to ocean warming and acidification expected by the end of this century. Further, little is known about potential connections between parameters of coral physiology and their associated microbial communities, and how they may affect the ability of a coral to persist in the face of global climate change. To explore this, a two-pronged approach was used: (1) a natural survey of corals around the island of O‘ahu, Hawai‘i, with corals collected from several sites across a gradient of ocean conditions to assess natural variability in the coral-associated microbial community composition and coral trophic strategies, and (2) a 22-month mesocosm experiment,

where corals were exposed to chronic temperature and pH stress to explore potential relationships between coral-associated microbial communities and the ability of corals to persist in end-of-century ocean conditions. The natural survey revealed a diversity of microbial associates and trophic strategies among the Hawaiian corals, with the greatest differences often occurring among species rather than among locations. For the first time, the microbial community diversity was also found to correlate with the relative contribution of heterotrophy among corals, suggesting that resource use by Hawaiian corals and the structure of their microbial communities are intertwined. The 22-month mesocosm experiment revealed connections between coral-associated microbial community composition and coral mortality under predicted end-of-century ocean conditions. Specifically, two patterns were found, where *Porites compressa* and *Porites lobata* had lower mortality and their microbial communities changed in response to experimental heat and acidity stress, while *Montipora capitata* and *Pocillopora acuta* had greater mortality and their microbial communities appeared generally inflexible.

Overall, the findings of this dissertation research suggest that Hawaiian corals host a diverse range of microbial communities and employ a variety of trophic strategies, such that *Porites compressa* and *Porites lobata* corals are likely to be more tolerant of stress than others and more likely to persist through this century. The coral trophic strategies were also related to the composition of the microbial communities, supporting past hypotheses of close connections between coral health and the microbiome. This was confirmed in the mesocosm experiment, as *Porites compressa* and *Porites lobata* hosted flexible microbial communities and had lower mortality in predicted end-of-century

conditions than *Montipora capitata* and *Pocillopora acuta*, suggesting that those corals which are more plastic in their response will likely be more tolerant of changing ocean conditions. However, some species and populations of coral remain susceptible to the stresses expected with global climate change by the end of this century and are less likely to persist.

Dedication

This dissertation is dedicated to Montana, Mom, Dad, and Josh for the years you have spent supporting me.

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Fields of Study

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Chapter 1. Introduction

1.1 Background

1.1.1 Coral biology

Corals are sessile marine invertebrates belonging to the Class Anthozoa within the phylum Cnidaria. They exist as either single polyps or colonial organisms consisting of up to thousands of polyps. Each polyp is symmetrical and has a ring of tentacles that can capture and move prey to a gastrovascular cavity. Corals grow via the budding of an already existing polyp, and their reproduction is accomplished either asexually through fragmentation or sexually via synchronous spawning events. Shallow tropical corals are largely divided in to two groups, 'hard' and 'soft' corals. The hard corals, or scleractinian corals, build reefs through the deposition of calcium carbonate material that constitutes their skeleton. This process of skeletal growth is termed calcification and occurs at a rate of between 0.3 and 2 cm per year in mounding corals, and up to 10 cm per year in branching corals (e.g., Shinn 1966; Baker and Weber 1975; Lough and Barnes 2000).

Shallow tropical reef-building corals are dependent on a symbiotic relationship with dinoflagellates of the family Symbiodiniaceae to support the majority of their daily energetic requirements through the translocation of photosynthetically fixed

carbon (e.g., Muscatine and Cernichiari 1969; Muscatine et al. 1981; Tanaka et al. 2018). These endosymbiontic algae are small, (~10μm in diameter) but typically exist at densities of 1 x 10^5 to 5 x 10^6 cells per cm^{-2} within coral tissues (e.g., Drew 1972; Porter et al. 1984; Schoepf et al. 2013). The endosymbiontic algae can support more than 100% of a coral's daily energetic requirements through the translocation of photosynthetically fixed carbon to the coral host (e.g., Muscatine et al. 1981; Anthony and Fabricius 2000; Grottoli et al. 2006, 2014). The disruption of this symbiosis due to heat stress (or other stressors) causes the endosymbiont to be expelled by the coral, known as coral bleaching (Glynn 1983; Glynn 1993; Brown 1997).

Corals can also capture zooplankton, bacteria, and other organic matter from the water column, accounting for up to 50% of the daily energetic requirement of the coral (e.g., Grottoli et al. 2006, 2014; Palardy et al. 2008; Houlbreque and Ferrier-Pages 2009; Levas et al. 2016). However, estimates of heterotrophic carbon incorporation in bleached corals can sometimes exceed 100% of metabolic demand (e.g., Grottoli et al. 2006; Palardy et al. 2008; Levas et al. 2016). Many of the nutrients necessary for tissue growth (i.e. nitrogen, phosphorous, and others) are also assimilated during the uptake of heterotrophic carbon (e.g., Piniak et al. 2003; Hughes et al. 2010; Baumann et al. 2014). These photoautotrophic and heterotrophic sources combine to provide a complete balance of fixed carbon and nutrients that provides for metabolic processes, including respiration, calcification, and mucus production (e.g., Muscatine and Porter 1977; Grottoli et al. 2006; Palardy et al. 2008; Houlbreque and Ferrier-Pages 2009; Levas et al. 2016).

The proportionate contribution of photoautotrophically-fixed carbon relative to heterotrophic carbon can vary between species (e.g., Rodrigues and Grottoli 2006; Palardy et al. 2008; Houlbreque and Ferrier-Pages 2009; Grottoli et al. 2014), locations (Alamaru et al. 2009; Fox et al. 2018; Wall et al. 2019), seasons (Tremblay et al. 2011; Nahon et al. 2013), and differing environmental conditions (e.g., Alamaru et al. 2009; Williams et al. 2018; Fox et al. 2018). Yet, unless one can repeatedly measure photosynthesis, respiration, and feeding rates of a coral, directly evaluating the proportionate contribution of different carbon sources to a coral's metabolic demands over time is not possible. Stable carbon isotopes, however, are reflective of this underlying coral biology.

The stable carbon isotopes ($\delta^{13}C$ = the ratio of ^{13}C:^{12}C relative to Vienna Peedee Belemnite Limestone Standard, or vPDB) of the coral host and endosymbiotic algae change as a function of the proportionate contribution of photoautotrophy and heterotrophy to a coral's tissues (e.g., Muscatine et al. 1989; Rodrigues and Grottoli 2006; Grottoli et al. 2017; Fox et al. 2018). During photosynthesis, $\delta^{13}C$ is influenced by metabolic fractionation, which is the preference for isotopically lighter molecules in a given biological or chemical reaction (e.g., Park and Epstein 1960; Wong et al. 1979). Thus, $\delta^{13}C$ of coral and algal endosymbiont tissues typically increases as photosynthesis increases and fractionation decreases (Wong et al. 1979; Muscatine et al. 1989; Swart et al. 2005). However, interpreting the $\delta^{13}C$ signal can also be more complicated in corals, as both seawater dissolved inorganic carbon (DIC) and CO_2 respired by the coral itself are used for photosynthesis, meaning that multiple DIC

sources with different $\delta^{13}C$ signals are incorporated into coral tissues. The DIC from the water column is enriched ($\delta^{13}C$ = –7‰ after converting from HCO_3^- to CO_2) relative to respired CO_2, which likely resembles the $\delta^{13}C$ of coral host tissue ($\delta^{13}C$ = –9‰ to –18‰, depending on the species and habitat) (Muscatine et al. 1989; Swart et al. 2005). Conversely with heterotrophy, the ingestion of organic matter such as zooplankton ($\delta^{13}C$ = –14 to –25‰, Rau et al. 1989, 1990, Grottoli-Everett 1998), DOC ($\delta^{13}C$ = ~-20 to -22‰, Druffel et al. 1992, Benner et al. 1997), and POC ($\delta^{13}C$ = –23 to –25‰, Benner et al. 1997), provides a source of carbon that is depleted relative to DIC used for photosynthesis. Therefore, variation in photosynthesis rates and in naturally available carbon sources (i.e. DOC, POC, and zooplankton) can influence the isotopic composition of a coral tissues (e.g., Rodrigues and Grottoli 2006; Grottoli et al. 2017; Fox et al. 2018, 2019; Wall et al. 2019). The difference between $\delta^{13}C_h$ and $\delta^{13}C_e$ can be used as a proxy for the proportionate contribution of photosynthesis and heterotrophy to coral tissues (e.g., Muscatine et al. 1984; Rodrigues and Grottoli 2006; Fox et al. 2018). For instance, Grottoli et al. (2017) used $\delta^{13}C_{h\text{-}e}$ to identify that *Stylophora pistillata* and *Pocillopora damicornis* in the northern Red Sea relied less on heterotrophic carbon incorporation during thermal stress than *Favia favus*.

The stable nitrogen isotopes ($\delta^{15}N$ = the ratio of ^{15}N:^{14}N relative to air) of the coral host and endosymbiotic algae can also change as a function of the proportionate contribution of photoautotrophy and heterotrophy (e.g., Rodrigues and Grottoli 2006; Ferrier-Pagès et al. 2011; Conti-Jerpe et al. 2020). However, using the same approach as carbon (i.e., $\delta^{15}N_{h\text{-}e}$) provides mixed results, possibly due to the long turnover time

(>1 year) of nitrogen in coral tissues relative to carbon (<40 days) (Tanaka et al. 2018). For example, when assessing natural variability in heterotrophic contribution among corals from reefs near Hong Kong, $\delta^{15}N_{h\text{-}e}$ increased with coral heterotrophy and polyp size (Conti-Jerpe et al. 2020). Conversely, corals along a primary productivity gradient in the Southern Line Islands showed no change in $\delta^{15}N_{h\text{-}e}$ with greater heterotrophic contribution (Fox et al. 2018). The difference in $\delta^{15}N_{h\text{-}e}$ patterns between studies may also be related to the amount of nitrogen recycling between the host and algal endosymbiont (e.g., Reynaud et al. 2009; Tanaka et al. 2015, 2018), which can reduce the typical $\delta^{15}N$ enrichment of ~3.4‰ expected with each trophic level of heterotrophic resource use (Post 2002; Newsome et al. 2010) to 0‰.

1.1.2 Coral Microbiome

Corals host a diverse set of microorganisms, including protists, fungi, bacteria, archaea, and their viruses (e.g., Ainsworth et al. 2015; Bourne et al. 2016; Hernandez-Agreda et al. 2017). The whole coral host, its endosymbiotic algae, and the community of other microorganisms comprise what is referred to as the 'coral holobiont'. While the connections between the coral host and their endosymbiotic algae are well established, the relationships between the coral and the rest of its microbiome are somewhat more unclear (Bourne et al. 2016; van Oppen and Blackall 2019). However, our understanding of the coral microbiome has been advancing rapidly, and new putative functions, roles, and relationships are being frequently described (e.g., Ziegler et al. 2017; Peixoto et al. 2017; Grottoli et al. 2018; Voolstra and Ziegler 2020).

Coral-associated microbial communities, particularly the bacteria and archaea, help maintain the overall health of a host through supporting immune response, biosynthetic pathways, and nutrient recycling (e.g., Thurber et al. 2009; Bourne et al. 2016; Neave et al. 2016). The density of coral-associated microorganisms ranges from 1×10^6 to $>1 \times 10^8$ cells/cm^2 (Garren and Azam 2010). However, these communities can also shift toward antagonistic roles when stressed (Rohwer et al. 2002; Rosenberg and Falkovitz 2004; Bourne et al. 2016). The microbial communities are thought to be regulated and maintained by the coral host itself. However, recent studies have also suggested that certain bacterial endosymbionts (Neave et al. 2016) or environmental conditions could contribute to community assembly (Ainsworth et al. 2010; Lee et al. 2017; Grottoli et al. 2018; Wang et al. 2018). Therefore, it is likely that changes in the microbial community of a coral are linked to the overall health of the coral holobiont (Thurber et al. 2009; Grottoli et al. 2018; Wang et al. 2018; Rosales et al. 2019).

Bacterial groups are the most often studied coral-associated microbial community member (excluding the Symbiodinaceae), and the most commonly identified bacteria are affiliated with the Proteobacteria, Actinobacteria, and Bacteroidetes (e.g., Ainsworth et al. 2015; Bourne et al. 2016; Hernandez-Agreda et al. 2017; Grottoli et al. 2018). For example, the genus *Endozoicomonas* within the Proteobacteria is highly abundant in many coral microbiomes, comprising up to 90% of the community in some corals (e.g., Bayer et al. 2013; Pogoreutz et al. 2018) and are thought to be one of the key bacterial endosymbionts in tropical corals (Neave et al. 2016). Bacteria of the genus *Candidatus* Amoebophilus are also commonly found in

coral-associated microbial communities but their roles are less clear. As the name suggests, these bacteria were first identified as parasites in amoebae, but are so often found in corals that they are hypothesized to associate with other members of the holobiont, such as the Symbiodiniaceae (Epstein et al. 2019; Huggett and Apprill 2019). Several others, including bacteria of the family Rhodobacteraceae and the genera *Burkholderia*, *Pseudomonas*, and *Acinetobacter* are posited as members of the core coral microbiome, and these community members specifically can be passed on during spawning through contact with the surface mucus layer (Leite et al. 2017; Bernasconi et al. 2019).

Coral-associated microbial communities are further divided into microhabitats, with different microorganisms populating the coral mucus, epithelial tissues, and the coral skeleton. The mucus, rich in discarded organic matter, often supports larger communities of microorganisms (Garren and Azam 2010). These exterior bacteria can assist with nutrient cycling and form a protective layer against external pathogens (Brown and Bythell 2005). The microbes within the epithelial and gastrodermal tissues typically form more specific symbioses (e.g. the genus *Endozoicomonas* is regularly found within these tissues), but often exist at lower abundances (Garren and Azam 2010). Finally, endolithic microorganisms within the skeleton may play a role in translocating photosynthetic products to the host (Fine and Loya 2002) and act as a microbial reservoir in instances when microbial communities in the coral tissues are disrupted (Ricci et al. 2019). The skeleton also creates unique 'micro-niches', where anaerobic photoautotrophic bacteria are often abundant, potentially supporting pH

regulation within the coral skeleton (Yang et al. 2019; Ricci et al. 2019).

Still, the coral microbial community is not entirely symbionts and commensal organisms. For instance, sulfur-reducing bacteria within the coral skeleton have been indicated as causal agents of black band disease (Yuen et al. 2013). Other bacteria, such as *Vibrio sp.*, can target the coral mucus layer via chemotaxis and destabilize the structure of the microbiome (Littman et al. 2011; Garren et al. 2014), an activity that has been linked to coral bleaching (Rosenberg and Falkovitz 2004). However, few have been able to identify specific mechanisms by which these diseased-state microbial communities negatively affect the coral itself (Wright et al. 2017).

1.1.3 Corals in a changing climate

Increasing concentrations of atmospheric CO_2 are leading to global warming and ocean acidification (OA), which threatens the long-term survival of corals and the persistence of coral reef ecosystems (e.g., Hoegh-Guldberg et al. 2007; Eakin et al. 2009; Veron et al. 2009). By the year 2100 tropical waters are projected to be 1 – 3°C warmer than today with a parallel increase in acidity up to 150% by the year 2100 (IPCC 2019). The response by corals to these relatively rapid changes in ocean conditions can range from a loss of their endosymbiotic algae (i.e., coral bleaching), to reduced skeletal growth, to more frequent outbreaks of disease (e.g., Bruno et al. 2007; Maynard et al. 2015) and death (e.g., Hoegh-Guldberg et al. 2007; Cooper et al. 2008; Anthony et al. 2008; Carroll et al. 2017). Specifically, rising seawater temperatures have been linked to massive tropical coral bleaching and mortality events in 1998,

2010, and 2015/2016 (Hughes et al. 2018). With an expected warming rate of 0.2 °C per decade, widespread coral bleaching events are predicted to occur even more frequently (Donner 2009) with bleaching events occurring annually as early as 2030 (van Hooidonk et al. 2016). This will have increasingly damaging consequences for the survival and proliferation of corals, as the cumulative effects of repeat bleaching events can be detrimental to the health of corals (Hughes and Grottoli 2013; Grottoli et al. 2014; Levas et al. 2016). While occurrences of coral bleaching become more common, the severity of these events may also become more extreme. For example, greater than 90% of sites surveyed along the Great Barrier Reef in Australia had a visible bleaching response during the 2015/2016 bleaching event (Hughes et al. 2017). Severe bleaching was also documented throughout the shallow reefs of the Hawaiian Islands in 2014, with several reefs experiencing bleaching in >50% of corals (Bahr et al. 2015; Rodgers et al. 2017; Couch et al. 2017).

As coral bleaching and mortality events increase in parallel with ocean warming and acidification, there are also observations of corals with greater tolerance and/or resistance to those environmental stressors. The ability for some corals to resist bleaching and maintain their health indicates that select individuals and species may be less susceptible to changing ocean conditions (Loya et al. 2001; Fabricius et al. 2011; Mayfield et al. 2013; Palumbi et al. 2014). Indeed, in experiments conducted nearly five decades apart (1970 vs. 2017), present day Hawaiian corals experienced only 22% mortality when exposed to the same elevated temperature profile that led to 85% mortality in 1970, suggesting that corals may be able to adapt or acclimatize to rising

temperatures given an appropriate amount of time (Coles et al. 2018). Resistance by corals to these phenomena has been linked to numerous possible factors, such as endosymbiont type (Stimson et al. 2002; Rowan 2004; Ziegler et al. 2017b), coral morphology (Loya et al. 2001), levels of energy reserves (Rodrigues and Grottoli 2007; Anthony et al. 2009; Schoepf et al. 2013), and heterotrophic capacity (e.g., Grottoli et al. 2006, 2014; Levas et al. 2013; Schoepf et al. 2015). For example, resistance can be conferred by the algal endosymbiont, as there are several lineages of Symbiodiniaceae that encompass a diverse array of photochemical productivity and tolerance to stress. The genus *Durusdinium* can confer bleaching resistance to corals up to 1–2 °C beyond other endosymbiont types, and this tradeoff between different Symbiodiniaceae can lead some corals to change their dominant endosymbiont after bleaching events (e.g., Baker 2001; Grottoli et al. 2014; Cunning et al. 2018). In addition, multiple parameters of coral physiology, including the ability to maintain energy reserves and increase heterotrophic capacity, are linked to a faster recovery from bleaching in Hawaiian corals (e.g., Grottoli et al. 2006; Rodrigues and Grottoli 2007; Hughes et al. 2010; Hughes and Grottoli 2013; Levas et al. 2013).

Recent literature also suggests that coral-associated microbial communities play a vital role in the susceptibility or resistance of coral to elevated seawater temperature and/or ocean acidification (Webster et al. 2016; Ziegler et al. 2017a; Peixoto et al. 2017; Grottoli et al. 2018; Voolstra and Ziegler 2020). Like Symbiodiniaceae shuffling, the potential plasticity of coral-associated microbial communities provides a relatively rapid path for corals to acclimatize to shifting

environmental baselines (Peixoto et al. 2017; Voolstra and Ziegler 2020). Other evidence suggests that microbial community stability may be related to thermal tolerance in some corals (Grottoli et al. 2018; Pogoreutz et al. 2018; Epstein et al. 2019). Multiple patterns of microbial community maintenance clearly exist in corals, but the roles of these communities in coral acclimatization and long-term thermal tolerance is only beginning to be understood. Indeed, bleaching and mortality events are still occurring at increasing frequencies, requiring further exploration of the complex relationships between coral physiology, the microbiome, and coral persistence in a changing climate.

1.2 Dissertation Outline

The goals of this dissertation research were to characterize the microbial communities and evaluate trophic strategies of Hawaiian corals across a range of natural environmental conditions, and to experimentally investigate the influence of climate change (i.e., elevated temperature and ocean acidification) on the microbial communities and survivorship of corals. Specifically, I investigated the microbial community composition and trophic strategies of corals collected from six sites around the island of O‘ahu, Hawai‘i (HI), across a natural physicochemical environmental gradient. Further, I characterized coral-associated microbial communities following a controlled 22-month mesocosm experiment to explore potential relationships between microbial communities and coral mortality under ocean warming and acidification conditions expected by the end of this century.

The results of this research are presented in three chapters:

Chapter 2: Isotopic approaches to estimating the contribution of heterotrophic sources to Hawaiian corals

Chapter 3: Effect of species, provenance, and coral physiology on the composition of Hawaiian coral-associated microbial communities

Chapter 4: Long-term coral microbial community acclimatization is associated with coral survival in a changing climate

1.2.2 Chapter 2: Isotopic approaches to estimating the contribution of heterotrophic sources to Hawaiian corals

Corals can obtain more than 100% of their daily energetic demands from their photosynthetic endosymbionts, Symbiodiniaceae, but the heterotrophic capacity of a coral can also be a key contributor to their resistance to bleaching and resilience following bleaching events (e.g., Grottoli et al. 2006; Rodrigues and Grottoli 2006; Anthony et al. 2009; Hughes and Grottoli 2013; Conti-Jerpe et al. 2020). However, determining the proportionate contribution of photoautotrophic and heterotrophic sources to coral diets is complicated, as resources are constantly recycled between the coral host and endosymbionts.

Stable carbon and nitrogen isotopes have commonly been used to broadly estimate the proportionate contribution of photoautotrophy and heterotrophy to coral tissues. Here, three approaches with stable carbon and nitrogen isotopes were used to

determine the proportionate contribution of various nutritional sources to the tissues of seven species of Hawaiian corals. I 1) calculated the difference between both the carbon and nitrogen isotope values of the coral host and algal endosymbiont, 2) estimated the contribution of heterotrophy to coral tissues by calculating the overlap between the isotopic composition (carbon and nitrogen together) of the coral host and algal endosymbiont, and 3) estimated the proportionate contribution of different nutritional sources to corals surrounding the island of O‘ahu using a Bayesian mixing model. To assess the possible effects of variable environmental conditions on the contribution of photoautotrophic and heterotrophic sources to these corals, results were also compared among the collection sites within each species around O‘ahu.

1.2.3 Chapter 3: Effect of species, provenance, and coral physiology on the composition of Hawaiian coral-associated microbial communities

The microbial communities associated with corals often vary among species, locations, and environmental conditions. Indeed, corals which are exposed to warmer or more variable ocean conditions often host distinct microbial communities (Ziegler et al. 2017a), suggesting those communities may confer thermal tolerance to the whole coral holobiont. However, we are only beginning to explore the relationships between the physiology of a coral and the composition of its microbial communities (Grottoli et al. 2018). Yet these relationships may be fundamentally important to the health, productivity, and persistence of coral reefs in the face of a changing climate.

Here, the microbial communities associated with four Hawaiian coral species (*Porites compressa*, *Porites lobata*, *Pocillopora acuta*, and *Pocillopora meandrina*) were characterized at six sites surrounding the island of O‘ahu, HI. The sites occupied a gradient of environmental conditions, providing a natural laboratory for evaluating the potential differences in microbial community composition over a small geographic area. Relationships between the composition of the microbial communities and several physiological parameters of the coral holobiont (tissue biomass, total chlorophyll [chlorophyll *a* and c_2], total soluble lipid concentration, total soluble protein concentration, and estimates of relative heterotrophic contributions to coral tissues measured via stable carbon and nitrogen isotopes of the coral host and its endosymbiotic algae, Symbiodiniaceae) were also explored. Finally, an ecological modeling approach was used to investigate both coral physiology and environmental parameters as potential drivers of coral-associated microbial community composition.

1.2.4 Chapter 4: Long-term coral microbial community acclimatization is associated with coral survival in a changing climate

An increasing concentration of atmospheric CO_2 is leading to ocean warming and acidifications, such that tropical ocean temperatures are expected to rise 1 – 3 °C with a parallel increase in acidity by 150% (approximately 0.2 pH units), threatening the persistence of coral reef ecosystems (IPCC 2019). However, the potential for some corals to acclimatize to ocean warming and acidification may help support the survival of coral reefs as we know them today. Microbial communities are often sensitive to

environmental conditions, shifting the community composition when exposed to warmer (Littman et al. 2011; Ziegler et al. 2017a; Lee et al. 2017) (e.g., Littman et al. 2011; Ziegler et al. 2017; Lee et al. 2017; Grottoli et al. 2018), or more acidic waters (Meron et al. 2011), or both (Webster et al. 2016; Grottoli et al. 2018), suggesting that the plasticity of these communities may play a role in their tolerance to stress. However, most studies of the effect of stress on coral-associated microbial communities have durations of days to weeks, and it is unknown how these microbial communities respond to chronic stress over annual scales.

To better understand how the pressures associated with global climate change affect the microbial communities of coral, microbial communities of four Hawaiian coral species (*Porites compressa*, *Porites lobata*, *Montipora capitata*, and *Pocillopora acuta*) were characterized following a 22-month mesocosm experiment, where corals were exposed to ocean acidification, ocean warming, and a combined dual stress treatment representing conditions expected by the end of this century. The microbial communities were compared among treatments and among individuals to assess whether their composition changed throughout the experiment, if those changes related to the mortality of the corals, and to determine if the microbial communities supported coral acclimatization to future ocean conditions. We hypothesized that the microbial community composition of all four Hawaiian coral species would shift in response to treatment, with the greatest shifts in composition and the greatest mortality expected in the ocean warming and dual stress treatments. This is the first study to experimentally characterize the response of tropical coral-associated microbial communities to ocean

warming and acidification over a multi-year time frame, providing insight into the potential roles of these microbial communities in the acclimatization of corals.

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Chapter 2: Isotopic approaches to estimating the contribution of heterotrophic sources to Hawaiian corals

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2.1 Abstract

1. Corals obtain nutrition from both the photosynthetic products of their endosymbiotic algae and the ingestion of organic material and zooplankton from the water column.

2. Here, we use stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes to assess the proportionate contribution of photoautotrophic and heterotrophic sources to seven Hawaiian coral species collected from six locations around the island of O‘ahu, Hawai‘i. We analyzed the $\delta^{13}C$ and $\delta^{15}N$ of coral tissues and their algal endosymbionts, as well as that of dissolved inorganic matter, particulate organic matter, and zooplankton from four sites around O‘ahu, Hawai‘i.

3. Estimates of heterotrophic contribution varied among coral species and sites. Bayesian mixing models revealed that heterotrophic sources (particulate organic material and zooplankton together) contributed the most to *Pocillopora acuta* and *Montipora patula* corals at 49.5% and 47.3%, respectively, and the least to *Porites lobata* at 24.3%.

4. Estimates of heterotrophic contribution based on the difference between the $\delta^{13}C$ of the host and symbiont ($\delta^{13}C_{h\text{-}e}$) often differed, while estimates based on the $\delta^{15}N_{h\text{-}e}$ and isotopic niche overlap approach were slightly more aligned with the estimates produced using Bayesian mixing models. These findings suggest that the utility of each approach may vary with coral health status, and among regions and coral species.

5. Overall, we find that the heterotrophic contribution to Hawaiian coral tissues ranges from 20-50%, suggesting a range of trophic strategies. However, these findings did not always match past direct measurements of heterotrophy, indicating that heterotrophically acquired nutrition does not necessarily get incorporated into tissues but can be respired or exuded in mucus.

2.2 Introduction

The function, growth, and overall health of most shallow-water reef-building corals is dependent on a fundamental relationship with their endosymbiotic algae, Symbiodiniaceae (Muscatine and Porter 1977). The coral host benefits from the translocation of photoautotrophically derived organic carbon and organic nitrogen by their algal endosymbionts, while the algal endosymbionts benefit from heterotrophically derived carbon (C) and nitrogen (N) and respired C from the coral host to support growth and photosynthesis (e.g., Muscatine 1990; Piniak et al. 2003; Hughes et al. 2010; Tanaka et al. 2015, 2018). Although the translocation of carbon from the endosymbiotic algae can satisfy greater than 100% of coral daily metabolic demands (e.g., Muscatine et al. 1984; Edmunds and Davies 1989; Grottoli et al. 2006), healthy corals can also fulfill between 5% – 50% of their daily metabolic demands through the capture and assimilation of organic matter and plankton from the water column (e.g., Palardy et al. 2008; Houlbreque and Ferrier-Pages 2009; Tremblay et al. 2011; Grottoli et al. 2014; Levas et al. 2016). Indeed, heterotrophy is a vital component of coral trophic strategies, as the heterotrophic capacity of a coral is a key contributor to their resistance to bleaching and resilience following bleaching events (e.g., Grottoli et al. 2006; Rodrigues and Grottoli 2007; Anthony et al. 2009; Hughes and Grottoli 2013; Conti-Jerpe et al. 2020). However, determining the proportionate contribution of photoautotrophic and heterotrophic sources to coral diets is complicated as heterotrophic effort and/or the nutritional sources available to corals in the marine environment can vary with upwelling (Palardy et al. 2005; Radice et al. 2019), turbidity (e.g., Anthony 1999; Anthony and Fabricius 2000;

Fabricius 2005), and primary productivity (Fox et al. 2018), lunar cycle (Palardy et al. 2006), coral surface to volume ratio (Palardy et al. 2005), and water flow rates (Ribes and Atkinson 2007; Wijgerde et al. 2012). The recycling of C, N, and phosphorous between the coral host and its algal endosymbiont (e.g., Hughes et al. 2010; Hughes and Grottoli 2013; Gustafsson et al. 2013; Tanaka et al. 2015, 2018) further complicates interpretations of trophic strategies among corals.

Ideally, direct measurements of photosynthesis, respiration, and feeding rates are used to assess the contributions of photoautotrophy and heterotrophy to the daily metabolic demands of corals (e.g, Anthony and Fabricius 2000; Grottoli et al. 2006, 2014; Palardy et al. 2008; Tanaka et al. 2015). However, these methods can be costly, labor-intensive, and destructive. Further, direct measurements of photosynthesis and feeding rate may not directly relate to the ultimate incorporation of those nutritional resources into coral tissue. For example, there is preferential allocation of autotrophic and heterotrophic sources to host and algal endosymbiont tissues, which can vary with prior thermal and nutritional regimes (Piniak et al. 2003; Hughes et al. 2010; Hughes and Grottoli 2013; Baumann et al. 2014; Krueger et al. 2018).

Alternatively, natural abundance stable carbon and nitrogen isotopes of the coral host and endosymbiotic algae can be used to broadly estimate the proportionate contribution of photoautotrophy and heterotrophy to coral tissues under natural and experimental conditions (e.g., Muscatine et al. 1989; Rodrigues and Grottoli 2006; Ferrier-Pagès et al. 2011; Levas et al. 2013; Nahon et al. 2013; Wall et al. 2019). Trophic strategies of corals have also been identified using tissue stable isotopes in corals from

Hong Kong (Conti-Jerpe et al. 2020), the South China Sea (Xu et al. 2020), and Maldives (Radice et al. 2019), and shown to vary along a natural gradient of primary productivity among the Southern Line Islands in the Central Pacific (Fox et al. 2018). However, unlike other ecological disciplines, isotope mixing model approaches have not been widely adapted in coral research to evaluate the proportionate contribution of sources to coral diets.

Heterotrophic plasticity and/or high baseline heterotrophic capacity have been associated with lower susceptibility to, and faster recovery from, heat stress (e.g., Grottoli et al. 2006; Hughes et al. 2010; Hughes and Grottoli 2013; Levas et al. 2013, 2016; Conti-Jerpe et al. 2020). Knowing baseline heterotrophic contributions to coral tissues could be important when determining which species are more likely to survive climate change and are better candidates for coral restoration and conservation efforts. Here, we use three approaches with stable carbon and nitrogen isotopes to determine the proportionate contribution of various nutritional sources to the tissues of seven species of Hawaiian corals as follows: 1) we calculated the difference between both the carbon and nitrogen isotope values of both the coral host and algal endosymbiont (*sensu* Muscatine et al 1989), 2) estimated the contribution of heterotrophy to coral tissues by calculating the overlap between the isotopic composition of the coral host and algal endosymbiont (*sensu* Conti-Jerpe et al. 2020), and 3) estimated the proportionate contribution of different nutritional resources to corals surrounding the island of O‘ahu using a Bayesian mixing model (developed in this manuscript). To assess the possible effect of variable environmental conditions on the contribution of photoautotrophic and heterotrophic

resources to these corals, we also compared the mean estimated contribution of each source to each coral species among the collection sites around O‘ahu.

2.3 Methods

2.3.1 Coral Sampling

Corals were collected between 17 August and 13 November 2015 from six sites (Electric Beach, Hale‘iwa, Hawai‘i Institute of Marine Biology [HIMB], Magic Island, Sampan Channel, and Waimānalo) surrounding the island of O‘ahu, Hawai‘i (HI), USA (Fig. 2.7). Typical environmental conditions at each collection site are summarized in Table 2.2. Methods used to collect the environmental data are summarized in the Supporting Information. Ramets of seven coral species (*Montipora capitata*, *Montipora patula*, *Pocillopora acuta*, *Pocillopora meandrina*, *Porites compressa*, *Porites evermanni*, and *Porites lobata*) were collected at a depth of 0.5 – 5 m. A 5 – 10 cm coral ramet (branch or mound) was removed underwater via hammer and chisel from healthy parent colonies separated by at least 5 m on the reef to minimize the possibility of selecting corals of the same genet (Baums et al. 2019). Corals were only sampled from sites where they were relatively abundant, and therefore not all coral species were sampled at every site (see Table 2.3 for the number of samples collected per site and species). The coral ramets were subsequently frozen at –20 ˚C at HIMB and later shipped to The Ohio State University where they were stored at –80 ˚C.

Stable isotope analysis of sources

Sampling of potential sources of carbon and nitrogen to corals was not performed at the time of the coral collection due to logistical constraints. Between 6 – 12 December 2017, water and zooplankton (150 – 800 µm) samples were collected at 0.5 – 1.0 m depth, close to corals from all sites. Up to 8 L of seawater was collected in pre-acidified 2 L brown Nalgene bottles during the day (1200 – 1400 hrs) and night (1800 – 2000 hrs) and placed on ice in a cooler. Seawater was subsampled from the Nalgene bottles, filtered and preserved for isotopic analysis of dissolved inorganic carbon (DIC), particulate organic carbon (POC) and particulate organic nitrogen (PON) according to established methods (e.g., Moyer et al. 2013; Kelsey et al. 2020). In brief, 20 ml seawater was filtered (0.45 µm pore size) and preserved in a glass crimp-top bottle with 200 µl of dried mercuric chloride for subsequent DIC analysis. The remaining seawater was pre-filtered through 55 µm nylon mesh to remove large particulates and zooplankton. Next, 3 – 4 L of seawater was filtered through a QM-A filter until the filter became light brown in color and was immediately stored at –20 °C for subsequent POC and PON analysis. We did not analyze samples for DIN due to the difficulty in making these analyses and lack of resources for these analyses. Instead, we used published $\delta^{15}N$ values for DIN (Table 2.1). Zooplankton samples were only collected during the nighttime sampling at four of the six sites, as surf conditions at Hale‘iwa and Electric Beach were unsafe on the day of sampling. To collect zooplankton, a bucket with an illuminated dive torch affixed to the bottom was placed on the seafloor near the reef at 1m depth for 5 min. The zooplankton were separated into 400 – 800 µm and 150 – 400 µm size fractions in the field, stored on

ice, and then isolated onto a glass fiber filter and stored at –20 °C upon return to the lab the same day.

2.3.2 Sample processing for isotopic analyses

Detailed methods for the processing and separating coral host tissue and algal endosymbionts tissues for isotopic analyses is described in Price et al. (2020). Briefly, a small subsample (approximately 4 – 6 cm^2) of each collected coral ramet was removed via hammer and a sterile chisel, the bulk coral tissue removed from the skeleton by airbrushing, and the resulting slurry was homogenized. A 0.5 ml sub-sample was removed, dried into silver capsules, and acidified via fumigation with 1N HCl for whole coral isotopic analysis. The remaining slurry was separated into animal host and endosymbiotic algal fractions via centrifugation and filtering steps. Overall, this process resulted in a whole coral sample, a host tissue sample, and an isolated algal endosymbiont sample for each coral collected (Table 2.3). All samples were combusted using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the University of California (UC) Davis Stable Isotope Facility. The carbon isotopic signature of the animal host ($\delta^{13}C_h$), algal endosymbiont ($\delta^{13}C_e$), and whole coral ($\delta^{13}C_w$) are reported as the per mil deviation of the stable isotopes ^{13}C:^{12}C relative to Vienna Peedee Belemnite Limestone Standard (v-PDB). Repeated measures of internal standards had a standard deviation of ± 0.2‰ for $\delta^{13}C$. The nitrogen isotopic signature of the animal host ($\delta^{15}N_h$), algal endosymbiont ($\delta^{15}N_e$), and whole coral ($\delta^{15}N_w$) are reported as the per mil deviation of the stable

isotopes ^{15}N:^{14}N relative to air. Repeated measures of internal standard had a standard deviation of ± 0.2‰ for $\delta^{15}N$. At least 10% of all coral measurements were made in duplicate. The standard deviation of duplicate sample analyses was ± 0.14‰ for $\delta^{13}C_h$, ± 0.26‰ for $\delta^{13}C_e$, ± 0.12‰ for the $\delta^{13}C_w$, ± 0.07‰ for $\delta^{15}N_h$, ± 0.22‰ for $\delta^{15}N_e$, and ± 0.06‰ for the $\delta^{15}N_w$.

The $\delta^{13}C$ of DIC samples was analyzed at the Duke University Environmental Isotope Laboratory using a Thermo Finnigan Delta Plus XL continuous flow mass spectrometer via a ThermoFinnigan GasBench II and reported in permil relative to V-PDB. Repeated measurements of internal standards had a standard deviation of ± 0.2‰. The filters with the POM and zooplankton samples were fumigated with 12 M hydrochloric acid to remove carbonates (Moyer et al. 2013) and analyzed for both $\delta^{13}C$ and $\delta^{15}N$ at the UC Davis Stable Isotope Facility and reported in permil relative to V-PDB and air, respectively. Due to the possibility of acid fumigation affecting the $\delta^{15}N$ of zooplankton values, separate zooplankton subsamples were prepared without acid treatment (Schlacher and Connolly 2014). The standard deviation of replicate analyses of these samples was ± 0.42‰ for $\delta^{13}C$ POM, and ± 0.55‰ for $\delta^{15}N$ of zooplankton. The standard deviations of replicate analyses for $\delta^{15}N$ of POM and $\delta^{13}C$ of zooplankton were not able to be calculated due lack of additional sample material. Although the analysis of the POM and zooplankton samples were less precise than expected due to low sample amounts, all $\delta^{13}C$ measurements were included and $\delta^{15}N$ measurements were only excluded if they contained less than 10 µg N, as these were below the typical detection limit. Further, the $\delta^{13}C$ and $\delta^{15}N$ of zooplankton measured in this study were similar to

values from other studies (Grottoli-Everett 1998; Rodrigues and Grottoli, unpubl.) from Hawaii, while the $\delta^{13}C$ and $\delta^{15}N$ of POM measured here was similar to previously reported values for Palmyra Atoll (Fox et al. 2019), relative to the isotope values of the corals.

2.3.3 Statistical analyses

All analyses were performed using R software package version 3.5.0 (R Core Team 2015) and PRIMER v6 (Clarke and Gorley 2006). Statistical significance was defined as $\alpha \leq 0.05$.

2.3.4 Approach 1: $\delta^{13}C$ and $\delta^{15}N$ of the host minus symbiont

The difference between the $\delta^{13}C$ of the host tissue ($\delta^{13}C_h$) and the endosymbiotic algae ($\delta^{13}C_e$) (henceforth referred to as $\delta^{13}C_{h\text{-}e}$) values were computed to assess the relative contribution of photosynthesis and heterotrophy in corals (e.g., Muscatine et al. 1989; Rodrigues and Grottoli 2006; Grottoli et al. 2017; Wall et al. 2019; Xu et al. 2020). Higher (lower) $\delta^{13}C_{h\text{-}e}$ values typically indicate that photosynthesis (heterotrophy) contributes a larger proportion of fixed carbon to coral tissues than heterotrophy (photosynthesis). Recent evidence suggests that $\delta^{15}N_h - \delta^{15}N_e$ (henceforth referred to as $\delta^{15}N_{h\text{-}e}$) is also informative of the source contributions to coral tissues (e.g., Reynaud et al. 2009; Nahon et al. 2013; Conti-Jerpe et al. 2020), although the patterns among studies are less clear. Here, $\delta^{13}C_{h\text{-}e}$ and $\delta^{15}N_{h\text{-}e}$ were each compared among coral species using a

nonparametric Kruskal-Wallis test followed by a post hoc Dunn's test, as normality and homoscedasticity of variance could not be achieved.

2.3.5 Approach 2: Stable Isotope Bayesian Ellipses

Using the R package SIBER (Stable Isotope Bayesian Ellipses in R) (Jackson et al. 2011), ellipses encompassing 40% of the variation in the overall isotopic signature of the host and algal endosymbiont tissue were fitted and centroid means for those ellipses were calculated. The trophic strategy of each coral species was then quantified based on the amount of overlap between host and algal endosymbiont standard ellipse areas corrected for sample size (SEA_C) following methods described by Conte-Jerpe et al. (2020). PERMANOVA was used to determine whether the centroid (mean) isotopic composition of the host and algal endosymbiont differed.

2.3.6 Approach 3: Bayesian Mixing Models

Proportionate contribution of DIM, POM, and zooplankton to whole coral tissues was estimated using the Bayesian isotope mixing models via the R package, MixSIAR (Stock et al. 2018). Since C and N recycling is rapid and continuous between the host and algal endosymbiont in corals (e.g., Hughes et al. 2010; Tremblay et al. 2012; Tanaka et al. 2015, 2018; Rangel et al. 2019), the mixing models were performed using whole coral samples as the consumer. Because potential C and N sources were only sampled once around O'ahu or were derived from the literature (Table 2.1), DIM, POM, and

zooplankton isotopic values were averaged across sites. For DIN, only the $\delta^{15}N$ of Kāne‘ohe Bay nitrate was used in the model, as these were the only published nitrate values found for the island of O‘ahu (Wall et al. 2019). While ammonium can be a source of DIN to corals, $\delta^{15}N$ of ammonium can be highly variable and $\delta^{15}N$ of nearby ammonium has not been measured. Additional details about the mixing model procedures, source values, and information about fractionation and trophic discrimination factors (TDF) are described in the Supplemental Methods. Pearson's correlations were used to test for relationships between the mean estimated percent contribution of DIM (photoautotrophy) and POM + Zooplankton (heterotrophy) with $\delta^{13}C_{h\text{-}e}$, $\delta^{15}N_{h\text{-}e}$, and the percent overlap of SEA_C among all coral species, with the mean values for each species used to build the correlations.

2.4 Results

All corals used in this study appeared healthy at the time of collection. The mean isotopic composition (i.e., $\delta^{13}C$ and $\delta^{15}N$) of whole coral, host tissue, and algal endosymbiont values for each species and site are presented in Table 2.4 & 2.5. The average $\delta^{13}C$ and $\delta^{15}N$ values of POM and zooplankton are shown in Table 2.1.

2.4.1 Approach 1: $\delta^{13}C$ and $\delta^{15}N$ of the host minus symbiont

The mean $\delta^{13}C_{h\text{-}e}$ ranged from -0.34 ± 0.63 to 2.23 ± 0.81 across all species and sites (Fig. 2.1A–G, Table 2.4). The $\delta^{13}C_{h\text{-}e}$ differed significantly among coral species of

different genera, but not always among species within the same genus (Table 2.6A). Of the seven species, *M. capitata* and *M. patula* had the greatest mean $\delta^{13}C_{h\text{-}e}$ values of 1.53 ± 0.66‰ and 1.35 ± 0.66‰, respectively, while *P. meandrina* and all three *Porites* species had the lowest mean values near zero. The mean $\delta^{15}N_{h\text{-}e}$ ranged from -1.92 ± 0.55 to 2.29 ± 0.55 across all species and sites (Fig. 2.1H–N, Table 2.5). The $\delta^{15}N_{h\text{-}e}$ also differed among species, but these differences were not always delineated by genera (Table 2.6B). *M. patula* and *P. acuta* corals had the greatest mean $\delta^{15}N_{h\text{-}e}$ values at 1.23 ± 0.66‰ and 1.42 ± 0.95‰, respectively, while *P. evermanni* had the lowest mean value of -1.79 ± 0.68‰.

2.4.2 Approach 2: Stable Isotope Bayesian Ellipses

Overlap in isotopic composition of the host and endosymbiotic algae ranged from 0.0% – 60.4% across all species (Fig. 2.2), with lower SEA_C overlap values indicating relatively low amounts of resource sharing between the coral and algal endosymbiont partners, and therefore a higher estimated contribution of heterotrophic resources to coral tissues. Overlap patterns were not always conserved within a coral genus, and the two species with the greatest overlap were *P. meandrina* (60.4%) and *P. lobata* (51.6%), while *M. patula* and *P. evermanni* had 0% overlap between host and algal endosymbiont tissues. Interestingly, these latter two species displayed opposite patterns in their measured isotopic values, as the mean $\delta^{15}N$ of the host was enriched over the algal endosymbiont in *M. patula*, but the host was depleted relative to the symbiont in *P. evemanni*. In addition, the overall isotopic composition of the host and algal

endosymbiont tissues differed significantly within all coral species except for except *P. meandrina* whose SEA_C values overlapped the most (Table 2.7).

2.4.3 Approach 3: Bayesian Mixing Models

The proportionate contribution of each source to whole coral tissue was estimated with MixSIAR. When considering all corals together, heterotrophy (POM + zooplankton) contributed a mean of 34.8%, while DIM contributed an estimated mean of 65.2% to whole coral tissue (Table 2.8, Fig. 2.3). However, when each species was considered separately, heterotrophy had the highest estimated contribution to *P. acuta* of 49.5% and the lowest contribution to *P. lobata* of 24.3% (Figs. 2.4 & 2.5, Table 2.8). POM was estimated to be the most consistently incorporated heterotrophic source in healthy corals at 15.3 – 46.5%, rather than zooplankton (1.0 – 9.0%). To account for the possibility that there was minimal trophic enrichment of $\delta^{13}C$ and $\delta^{15}N$ in the available heterotrophic sources due to the recycling of C and N between the host and algal endosymbiont, mixing models were also produced using TDF values of zero for POM and zooplankton. With TDF values of zero, mean estimated contribution of heterotrophy as a whole only increased by 2.6 ± 2.6 %, but zooplankton increased by an average of 17.9 ± 11.8% across all species (Table 2.9).

Within each species, site-specific differences in the estimated proportionate contribution of each source was observed (Fig. 2.4). The mean estimated proportionate contribution of heterotrophy to *M. capitata* from Hale‘iwa was 12.8% vs. 39.0% at the other collection sites, resulting in a higher proportionate contribution of DIM to those

corals at Hale‘iwa than their conspecifics around O‘ahu (Fig. 2.4A). Magic Island corals had the lowest estimated mean contribution of heterotrophy to their tissues in four of the five species collected there (*P. meandrina*, *P. compressa*, *P. lobata*, and *P. evermanni*, but not *P. acuta*, see Table 2.8). The mean estimated proportionate contribution of heterotrophy to *P. evermanni* from Sampan was 51% vs. 28.4% at the other collection sites together, resulting in a lower proportionate contribution of DIM to those corals at Sampan than their conspecifics around O‘ahu. Corals from HIMB (*M. capitata*, *P. acuta*, *P. compressa*) had consistently high proportionate contributions of heterotrophy relative to most other sites, however only three species were collected there (Table 2.3). The patterns among collection sites were generally not consistent among coral species, and most differences in photoautotrophic vs. heterotrophic contribution were species-specific.

2.5 Discussion

Here we assessed the proportionate contribution of organic sources (derived through heterotrophy by the coral animal host) and inorganic sources (derived primarily through photosynthesis and inorganic uptake by the endosymbiotic algae) to the tissue of seven Hawaiian coral species from six sites surrounding O‘ahu, HI. We compared four isotopic approaches (Fig. 2.6) and found that overall, healthy coral tissues are 4.3 – 57.0% derived from heterotrophic sources depending on the coral species, location, and modeling approach used.

2.5.1 Approach 1: $\delta^{13}C$ and $\delta^{15}N$ of the host minus symbiont

The utility of this approach for estimating the proportionate contribution of heterotrophic and photoautotrophic carbon to coral tissues is well established, as the $\delta^{13}C_{h\text{-}e}$ values of a coral often decrease in deeper waters (e.g., Muscatine et al. 1989; Alamaru et al. 2009; Williams et al. 2018), more productive waters (Fox et al. 2018), or following bleaching (e.g., Rodrigues and Grottoli 2006; Schoepf et al. 2015; Wall et al. 2019) as a function of increases in the proportionate contribution in heterotrophic carbon to coral tissues. Among the seven species, average $\delta^{13}C_{h\text{-}e}$ values were highest in the two *Montipora* species, intermediate in the two *Pocillopora* species, and lowest in the *Porites* corals (Fig. 2.1, Fig. 2.6A). Low (high) $\delta^{13}C_{h\text{-}e}$ values indicate the highest (lowest) proportionate contribution of heterotrophically derived carbon to coral tissues (Fig. 2.6A). In the Montiporids, the $\delta^{13}C_{h\text{-}e}$ values were 1 – 2‰ higher than previously reported for nonbleached Hawaiian *M. capitata* (Rodrigues & Grottoli 2006), but consistent with past studies showing that baseline feeding rates are low and the contribution of heterotrophic carbon relative to daily respiratory demand (CHAR, Grottoli et al 2006) is only 18% in healthy *M. capitata* (Grottoli et al 2006; Palardy et al 2008). In the *Porites* species, the low $\delta^{13}C_{h\text{-}e}$ values were similar to those previously reported for healthy *P. lobata, P. compressa, and Porites astreoides* (Rodrigues and Grottoli 2006; Levas et al. 2013, 2018), and consistent with past studies showing that baseline feeding rates were moderate and zooplankton CHAR values were 30 – 70% in healthy *P. compressa, P. lobata,* and *P. astreoides* corals (Palardy et al. 2008; Levas et al. 2016). However, $\delta^{13}C_{h\text{-}e}$ alone may be indicative of the proportion of resources shared

rather than a measure of heterotrophic contribution to tissues. For example, *P. evermanni* from Sampan were depleted by 2‰ in both $\delta^{13}C_h$ and $\delta^{13}C_e$ relative to conspecifics from Waimānalo, but the $\delta^{13}C_{h\text{-}e}$ values at both sites were near 0‰, possibly obscuring a difference in heterotrophic contribution between these sites.

The patterns found using $\delta^{15}N_{h\text{-}e}$ did not match those observed using $\delta^{13}C_{h\text{-}e}$ (Fig. 2.1H–N, Fig. 2.6B). However, patterns of $\delta^{15}N_{h\text{-}e}$ in response to the proportionate contribution of heterotrophy and photoautotrophy are less established than $\delta^{13}C_{h\text{-}e}$. Based on $\delta^{15}N_{h\text{-}e}$, the Montiporid corals appeared more heterotrophic and the Poritid corals less heterotrophic than indicated by the $\delta^{13}C_{h\text{-}e}$ results, and species of the same genus were often separated by more than 1‰. The $\delta^{15}N_{h\text{-}e}$ was lower in *M. capitata* than *M. patula*, but the $\delta^{15}N_{h\text{-}e}$ of approximately 0‰ for *M. capitata* is similar to previously reported values for that species (Rodrigues and Grottoli 2006), consistent again with low baseline heterotrophy in healthy *M. capitata* (Grottoli et al. 2006; Palardy et al. 2008). In the *Porites* corals, the slightly negative $\delta^{15}N_{h\text{-}e}$ values of *P. compressa* and *P. lobata* are approximately 0.5 – 1.0‰ lower than previously measured values in Hawaiian *P. compressa* (Rodrigues and Grottoli 2006), but are similar to values measured in Caribbean *P. astreoides* (Levas et al. 2018), suggesting that $\delta^{15}N_{h\text{-}e}$ is generally negative or near zero in *Porites* corals and may not be reflective of their previously reported moderate feeding rates and CHAR (Rodrigues and Grottoli 2006; Palardy et al. 2008; Grottoli et al. 2018). Indeed, some studies have found that the $\delta^{15}N_{h\text{-}e}$ values likely show the opposite relationship to $\delta^{13}C_{h\text{-}e}$, such that $\delta^{15}N_{h\text{-}e}$ increases with greater proportionate contribution of heterotrophy, due to trophic enrichment of the host tissues (~3.4‰ $\delta^{15}N$)

expected with the assimilation of DON, PON, and zooplankton (Ferrier-Pagès et al. 2011; Conti-Jerpe et al. 2020). In another study, increased feeding did not result in any changes in nitrogen isotopes of the host tissue (Reynaud et al. 2009). A potential reason for variability in $\delta^{15}N_{h\text{-}e}$ is the relatively slow turnover time (> 1 year) of N in coral tissues (Tanaka et al. 2018). Because the endosymbiont cells are typically expelled during bleaching, one might expect a significant shift in the $\delta^{15}N_{h\text{-}e}$ of corals immediately following a bleaching event, whereas shifts in $\delta^{15}N_{h\text{-}e}$ of healthy corals may be less pronounced (Reynaud et al. 2009; Ferrier-Pagès et al. 2011; Radice et al. 2019). Further, heterotrophic nutrition may not be completely incorporated into coral and endosymbiont tissues, being respired or exuded in mucus instead (e.g., Hughes et al. 2010; Levas et al. 2016; Tanaka et al. 2018). This should be considered among different coral species or systems (e.g., how feeding capacity by be affected by experimental stress), to accurately interpret the isotopic data. Nonetheless, the variable results of past studies and the findings here certainly suggest that the values of $\delta^{13}C_{h\text{-}e}$ and $\delta^{15}N_{h\text{-}e}$ may not always follow the same pattern, and that each proxy may be better suited for different applications.

2.5.2 Approach 2: Stable Isotope Bayesian Ellipses

The overlap between the host and algal endosymbiont overall isotopic composition calculated via SIBER suggests that corals utilize a variety of strategies (Fig. 2.2 & 2.6C). High degrees of overlap in *M. capitata*, *P. meandrina*, and *P. lobata* indicate a relatively low proportionate contribution of heterotrophy and high degree of

resource sharing between the host and endosymbiotic algae while the absence of any overlap in *M. patula* and *P. evermanni* suggests a high proportionate contribution of heterotrophy and an apparent disconnect between the host tissue and algal endosymbiont. Unlike the findings by Conti-Jerpe et al. (2020) where variability between the host and endosymbiotic algae was restricted to $\delta^{15}N$, here variability was observed in both the $\delta^{13}C$ and $\delta^{15}N$ isotope space (Fig. 2.2). For example, the $\delta^{15}N$ of the host tissue in the *Porites* corals was often depleted relative to the algal endosymbiont but both fractions have similar $\delta^{13}C$ values (Fig. 2.1), which resulted in low $\delta^{15}N_{h\text{-}e}$ values and $\delta^{13}C_{h\text{-}e}$ near zero (Figs. 2.1, 2.6A&B). In the most extreme case with *P. evermanni,* this produced a 0% SEA_C between the host and algal endosymbiont (Fig. 2.2G, 2.6C). In contrast, *M. patula* coral had high $\delta^{13}C_{h\text{-}e}$ and $\delta^{15}N_{h\text{-}e}$ values (Fig. 2.6A, B) that also resulted in a 0% SEA_C between the host and algal endosymbiont (Fig. 2.2B, 2.6C) but in a completely different way than for *P. evermanni*. The consistently low host $\delta^{15}N$ values (compared to endosymbiotic algae) in *P. evermanni,* and to a lesser extent in the other two Poritid species, may indicate a higher reliance of feeding (Reynaud et al 2009) and possible incorporation of $\delta^{15}N$ depleted mucous-associated bacteria (Montoya et al. 2002). The contribution of diazotrophs to coral tissue is variable among species and locations (Shashar et al. 1994; Lesser et al. 2007; Alamaru et al. 2009; Radice et al. 2019), but could be a source of depleted $\delta^{15}N$ of the host tissue in the *Porites* corals. Together, these findings suggest that the proportionate contribution of heterotrophic sources to the tissues of healthy Poritids is probably higher than suggested by the $\delta^{15}N_{h\text{-}e}$, and more consistent with the predictions from $\delta^{13}C_{h\text{-}e}$ and possibly SIBER. The opposite pattern was observed

in the $\delta^{13}C$ and $\delta^{15}N$ values of *M. patula* (Fig 2B, 6A&B), but the enriched $\delta^{15}N$ of the host tissues relative to the endosymbiont suggests that the contribution of heterotrophic sources to tissues in this coral is relatively high.

2.5.3 Approach 3: Bayesian Mixing Models

Bayesian mixing models via MixSIAR were used to specifically estimate the contribution of DIM and heterotrophic sources (POM and zooplankton) to whole coral tissue (host and endosymbiotic algae). With the incorporation of uncertainty in both source values and trophic enrichment factors, mixing models present a conservative estimate of trophic strategies among Hawaiian corals. Overall, when considering models with the typical consumer trophic enrichment of 1‰ for $\delta^{13}C$ and 3.4‰ for $\delta^{15}N$, corals were estimated to derive 24.3% – 49.5% (median of 32.7%) of their tissues from heterotrophic sources (Fig. 2.5 & 2.6D). This is consistent with previous findings that corals typically meet 5 – 50% of metabolic demand heterotrophically (e.g., Grottoli et al. 2006, 2014; Palardy et al. 2008; Tremblay et al. 2011; Levas et al. 2016) and require heterotrophically derived C for tissue and lipid synthesis, especially following bleaching (Hughes et al. 2010; Hughes and Grottoli 2013; Baumann et al. 2014).

The two coral species estimated to rely most on heterotrophy were *P. acuta* and *M. patula* at approximately 50% (Figs. 2.4B, 2.4C, 2.5B, 2.5C, & 2.6D). Although heterotrophy in these two coral species has not previously been studied, research on corals of the *Pocillopora* and *Montipora* genera show that both can effectively feed on zooplankton and capture POM, the proportions varies among locations, local

environmental conditions, and health status (Moberg et al. 1997; Anthony 1999; Palardy et al. 2005, 2006, 2008). Interestingly, *P. acuta* and *M. patula* were estimated to incorporate approximately 15% more heterotrophically derived sources into their tissues than their congeners, *P. meandrina* and *M. capitata*. Both *P. meandrina* and *M. capitata* are flexible in their incorporation of heterotrophically derived organic matter depending on environmental conditions (Fox et al. 2018) and health status (Grottoli et al. 2006; Palardy et al. 2008), respectively. It is unclear what might drive these intrageneric differences in heterotrophy, but increased heterotrophic capacity may contribute to the rapid recruitment and growth of *P. acuta* colonies in warmer ocean conditions (Bahr et al. 2020), or aid encrusting *M. patula* corals to compete for space in turbid environments (Brown and Friedlander 2007).

P. lobata had the lowest proportionate contribution of heterotrophically derived organic matter in its tissues at 24.7% (Figs. 2.3F, 2.4F, 2.5D). This appears contradictory to past evidence showing that this species has an elevated baseline feeding capacity relative to *P. compressa* and *M. capitata* (Palardy et al. 2008) and the capacity to take up DOC as a nutritional source (Levas et al. 2013). However, the sources allocated for tissue building and energy storage, which drives the tissue isotopic composition, may be different than what is used for fulfilling daily energetic demands. For example, healthy *P. compressa* incorporates similar levels of heterotrophically derived C into its tissues as *M. capitata* (Hughes et al. 2010; Baumann et al. 2014) even though its feeding capacity is higher than that of *M. capitata* (Palardy et al. 2008). The low heterotrophic contribution to tissues in *P. lobata* suggests that heterotrophically acquired organic matter contributes

more to meeting metabolic demand than to tissue building. This is plausible, given that nonbleached Hawaiian *P. lobata* can meet 45% of metabolic demand from heterotrophy alone and that Caribbean *P. astreoides* can meet up to 70% this way (Palardy et al. 2008; Levas et al. 2016). Heterotrophically acquired nutrition has also been observed to meet all of metabolic demand in bleached *M. capitata* and bleached *P. astreoides*, providing a vital strategy for resilience post-bleaching (Grottoli et al. 2006; Hughes et al. 2010; Levas et al. 2016). Our findings provide further evidence that healthy *P. lobata* utilizes heterotrophically acquired organic matter for meeting metabolic demand and relies primarily on photoautotrophically derived organic matter for tissue building, possibly accounting for its resilience to bleaching compared to many other Hawaiian coral species (e.g., Hueerkamp et al. 2001; Kenyon et al. 2006).

Due to intense recycling of organic matter between the coral host and its endosymbiotic algae, it is possible that there is minimal fractionation offset in $\delta^{13}C$ and $\delta^{15}N$ between the two symbiotic partners. When TDF in both $\delta^{13}C$ and $\delta^{15}N$ for all heterotrophic sources was reduced to 0.0‰ in the second model scenario, the proportionate contribution of zooplankton increased substantially from 2.5% to 17.9% (Tables 2.8 & 2.9), suggesting that zooplankton may be more valuable to coral tissues than estimated by the primary mixing models in this study. Nevertheless, with either model scenario DIM still comprises two-thirds of the estimated contribution to coral tissues on average (Tables 2.8 & 2.9).

Finally, the contribution of heterotrophic sources to coral tissues varied among sites for some coral species (Table 2.8, Fig. 2.4). For example, over 50% of *M. capitata*

tissues are derived from heterotrophic sources at HIMB, but only 13% are heterotrophically derived in Hale‘iwa (Table 2.8). However the contribution of heterotrophic sources to *M. patula* tissues was over 50% at both HIMB and Hale‘iwa (Tables 2.8). Thus, the proportionate contribution of heterotrophic sources to healthy coral tissues was not dependent on site, but on the interaction between coral species and site effects.

2.5.4 Comparing Approaches

While each of the four tools (i.e., $\delta^{13}C_{h\text{-}e}$, $\delta^{15}N_{h\text{-}e}$, SIBER, and MixSIAR) have provided a useful measure of photoautotrophic and heterotrophic contributions to coral tissues in past studies, our direct comparison of these approaches reveals previously unrecognized differences in their interpretations (Figs. 2.5 & 2.6). Though not statistically significant, both the mean $\delta^{13}C_{h\text{-}e}$ and $\delta^{15}N_{h\text{-}e}$ tended to increase as the proportionate contribution from heterotrophic sources derived from MixSIAR increased (Fig 2.2A&B), while the reverse was true for SIBER (Fig. 2.2C). The positive relationship between MixSIAR and $\delta^{13}C_{h\text{-}e}$ contradicts past evidence that $\delta^{13}C_{h\text{-}e}$ typically decreases with greater heterotrophic contributions (e.g., Rodrigues and Grottoli 2006; Fox et al. 2018), which suggests that $\delta^{13}C_{h\text{-}e}$ in healthy Hawaiian corals may not be comparable among species. Here, $\delta^{13}C_{h\text{-}e}$ appears most useful for comparisons within species, such as heterotrophic contribution across depth gradients (e.g., Muscatine et al. 1989; Alamaru et al. 2009; Williams et al. 2018) or following bleaching events (e.g., Rodrigues and Grottoli 2006; Wall et al. 2019). Interestingly, the correlation between the

mixing model output and the percent overlap metric calculated via SIBER was similar to that found with $\delta^{15}N_{h\text{-}e}$ (Fig. 2.2), even though the patterns in Fig. 2.6 show few obvious similarities. This suggests that if measurements for sources like DIM, POM, and zooplankton were not available, $\delta^{15}N_{h\text{-}e}$ and SIBER could potentially provide some measure of the relative contribution of heterotrophy to healthy Hawaiian corals. The mixing models also require some caution in their interpretation, as changes in the fractionation assumptions and the possible contribution of unmeasured sources (e.g., diazotrophically fixed nitrogen) could lead to different conclusions.

Overall, the contribution of heterotrophically derived organic matter to healthy coral tissues varied among species and collection sites. There were no consistent patterns among the seven coral species, suggesting that environmental influences on the proportionate contribution of sources used by these corals is species-specific. Heterotrophy is considered a key trait in resistance and resilience to changing ocean conditions expected with climate change because it provides essential nutrients for tissue building and an alternate source of food that does not depend on the endosymbiotic algae (e.g., Grottoli et al. 2006; Palardy et al. 2008; Houlbreque and Ferrier-Pages 2009; Hughes and Grottoli 2013; Levas et al. 2016; Conti-Jerpe et al. 2020). All coral species in this study have a heterotrophic contribution to their tissues of at least 24.7% – 49.5%, suggesting that Hawaiian corals use a range of trophic strategies for growth. However, for some resilient species like *P. lobata*, the heterotrophic contribution to coral tissues does not always align with known feeding capacity and resilience on the reef, indicating that heterotrophic contribution to coral tissues may not always be an effective measure of

their potential tolerance to changing ocean conditions associated with global climate change.

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Table 2.1 Summary of mean $\delta^{13}C$ and $\delta^{15}N$ values and fractionation or trophic discrimination factors (TDF) for each of the sources surrounding O‘ahu.

	Dissolved Inorganic Matter		Particulate Organic Matter		Zooplankton (150 - 800 µm)	
	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Measured or Estimated Value	0.54 ± 0.14[d]	4.30 ± 1.00[a]	-21.17 ± 0.85[d]	3.02 ± 1.39[d]	-18.42 ± 1.46[d]	6.45 ± 0.59[d]
Trophic Discrimination Factor	-12.10 ± 3.00[b]	0.00 ± 0.00[c]	1.00 ± 1.00	3.4 ± 1.00	1.00 ± 1.00	3.4 ± 1.00

[a] $\delta^{15}N$ of DIN for O‘ahu from nitrate collected in Kāne‘ohe Bay by Wall et al. (2019)
[b] Estimated fractionation value of $\delta^{13}C$-DIC incorporated into whole coral and endosymbiont tissue from Swart et al. (2005)
[c] Absence of a $\delta^{15}N$ TDF for DIN incorporation into whole coral and algal endosymbionts from Muscatine and Kaplan (1994)
[d] this study

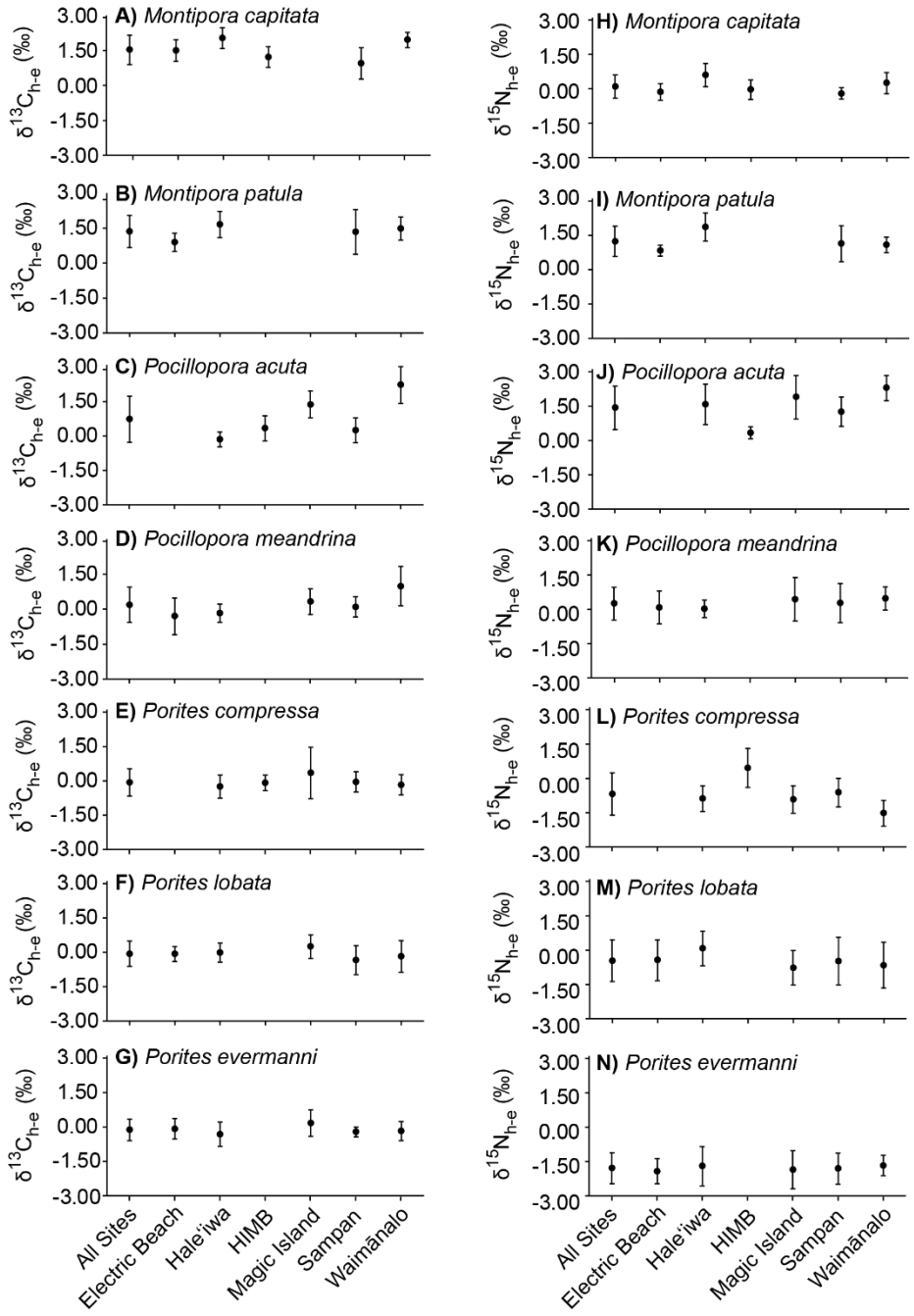

Figure 2.1. Mean (± SD) $\delta^{13}C$ of the coral host – $\delta^{13}C$ of the endosymbiont ($\delta^{13}C_{h\text{-}e}$) and and $\delta^{15}N$ of the coral host – $\delta^{15}N$ of the endosymbiont ($\delta^{15}N_{h\text{-}e}$) from all collection sites in O'ahu, HI (approach 1).For $\delta^{13}C_{h\text{-}e}$, plots correspond to A) *Montipora capitata*, B) *Montipora patula*, C) *Pocillopora acuta*, D) *Pocillopora meandrina*, E) *Porites compressa*, F) *Porites lobata*, and G) *Porites evermanni*. For $\delta^{15}N_{h\text{-}e}$, plots correspond to H) *Montipora capitata*, I) *Montipora patula*, J) *Pocillopora acuta*, K) *Pocillopora meandrina*, L) *Porites compressa*, M) *Porites lobata*, and N) *Porites evermanni*.

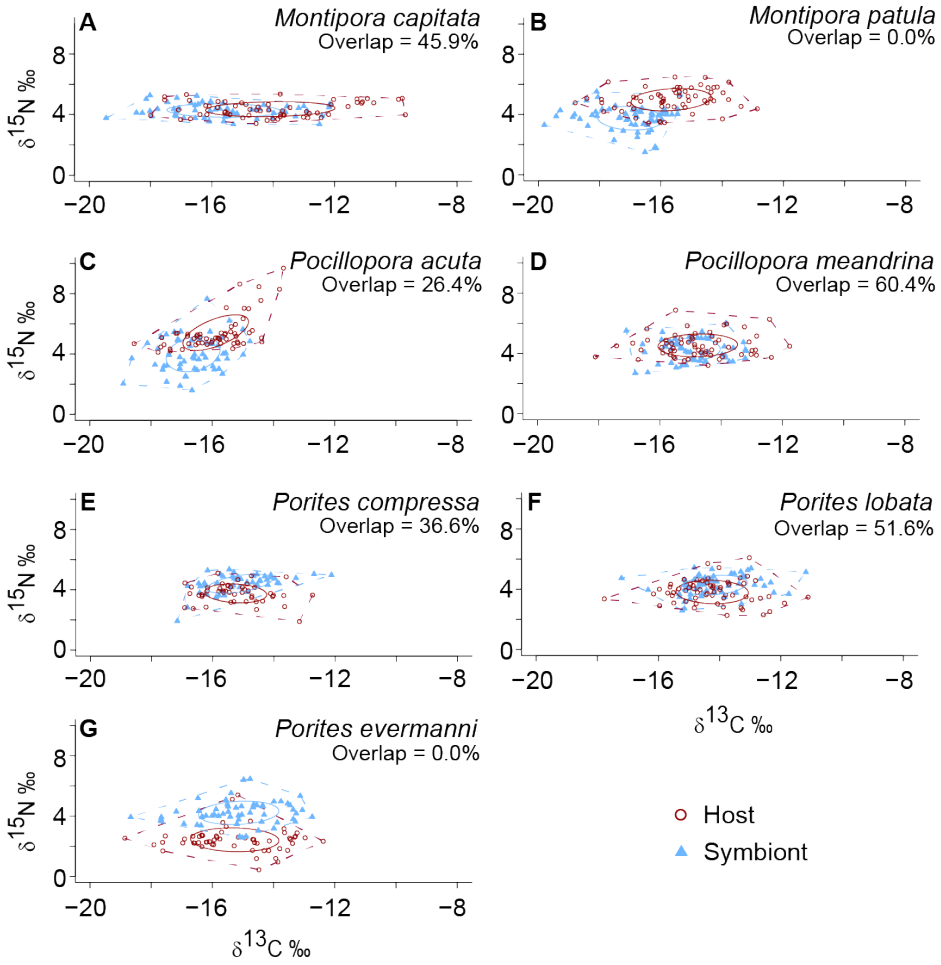

Figure 2.2. Results of SIBER (approach 2) analysis showing biplots of δ13C and δ15N with SIBER analysis for seven coral species collected surrounding O‘ahu, HI.Degree of overlap calculated from standard ellipse area (SEA_C) suggests the potential for resource sharing between the coral host and endosymbiont, such that greater overlap represents relatively high sharing of dietary resources incorporated into tissues between the host and its endosymbiotic algae, while lower overlap represents relatively low sharing of dietary resources between the symbiotic partners. The solid ellipses encompass 40% of variability in the host and endosymbiotic algal groups, while dotted lines encompass 100% of the variability in each group.

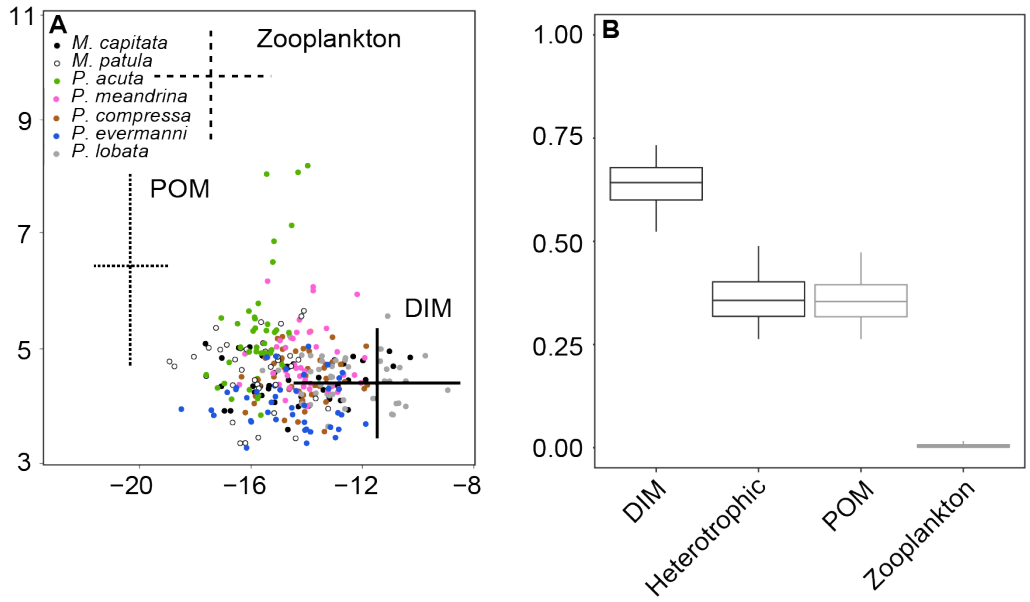

Figure 2.3. A) Isospace plot for the seven sampled species in O‘ahu, HI and B) Posterior probabilities of the proportionate contribution of each source as determined by MixSIAR for all species combined (approach 3). Each source is plotted with trophic discrimination factors considered (see Table 2.1). The total heterotrophic contribution is the sum of the POM and zooplankton contributions. The line at the center of each box is the median, with boxes extending to 25% and 75% credible intervals and whiskers representing the 5% and 95% credible intervals. The corresponding MixSIAR output is listed in Table 2.8A.

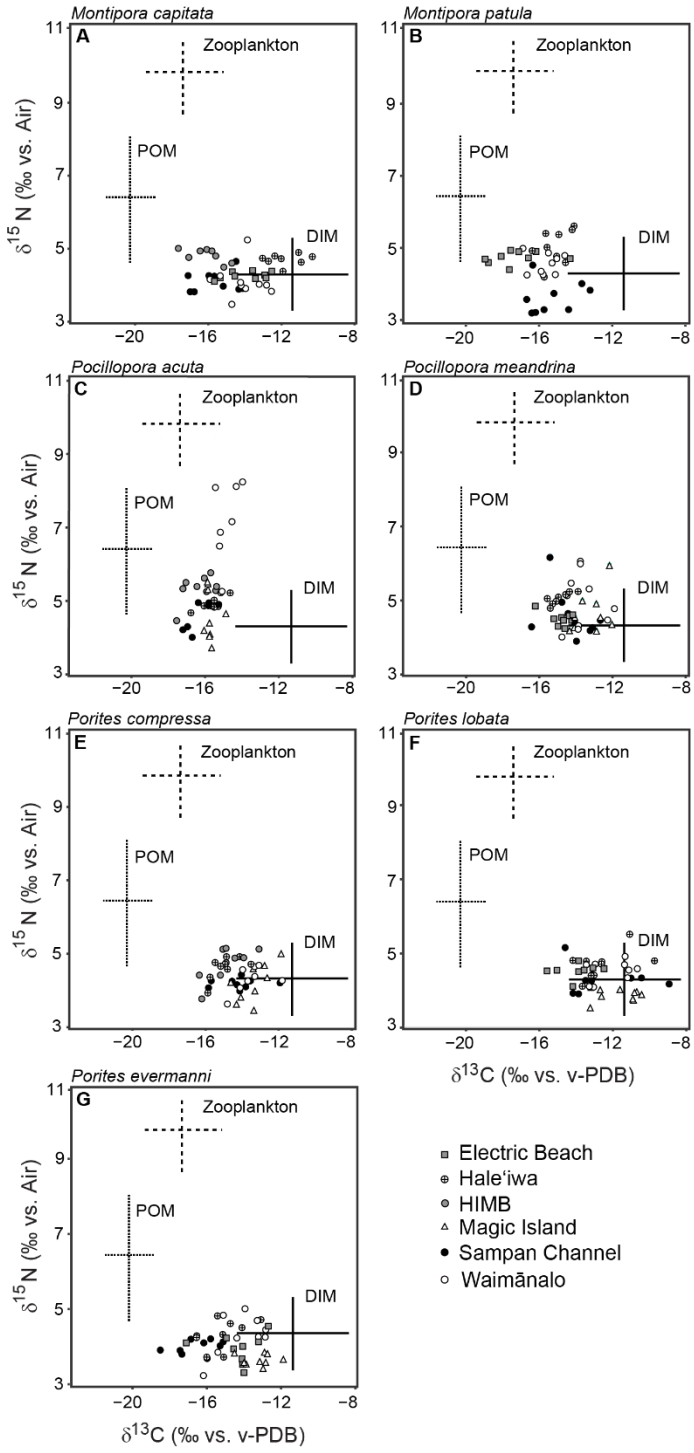

Figure 2.4. Isospace plot for each coral species from each site in O'ahu (approach 3). Each source is plotted with trophic discrimination factors considered (see Table 2.1). The corresponding MixSIAR output is listed in Table 2.8.

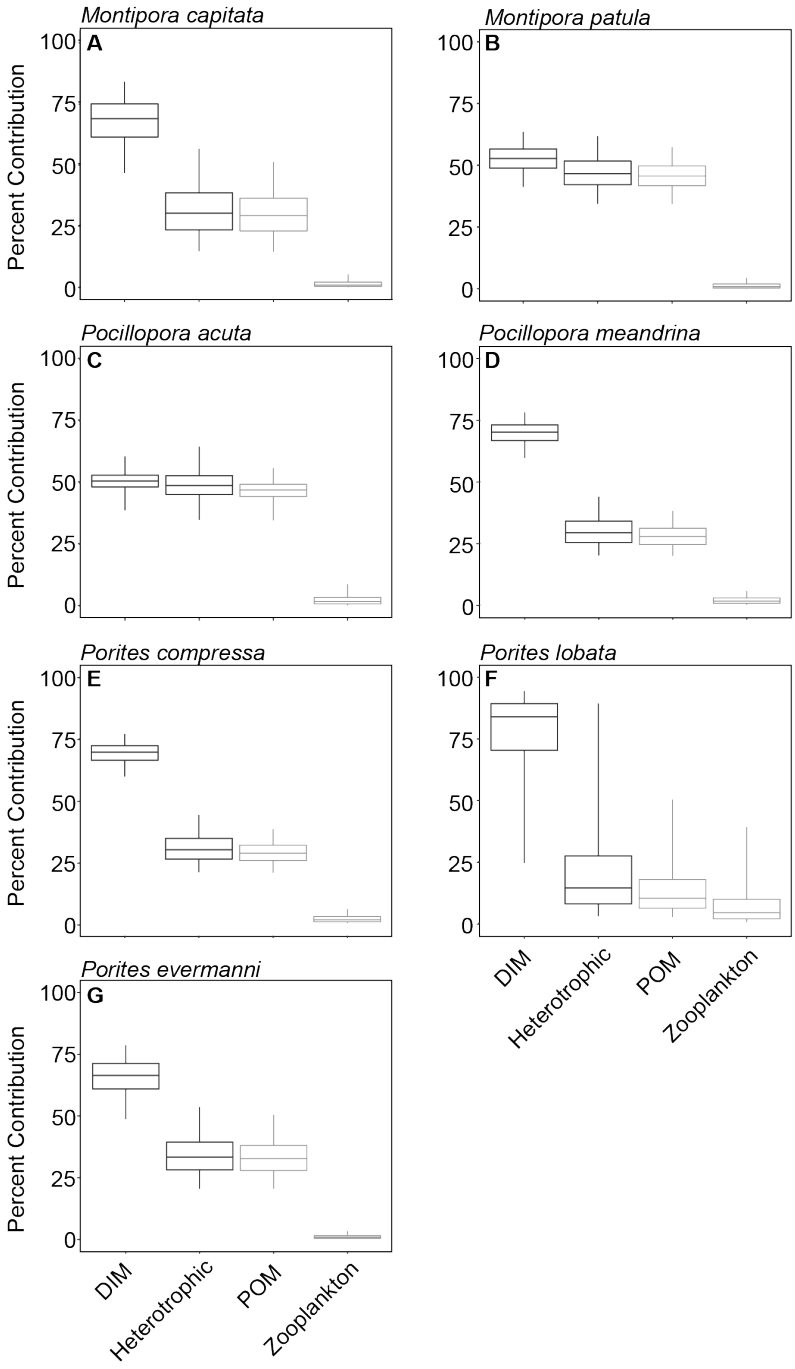

Figure 2.5. Posterior probabilities of the proportionate contribution of each source (heterotrophic = POM + Zooplankton) as determined by MixSIAR (approach 3) for each coral species in O‘ahu, HI. The line at the center of each box is the median, with boxes extending to 25% and 75% credible intervals and whiskers representing the 5% and 95% credible intervals. The corresponding MixSIAR output is listed in Table 2.8.

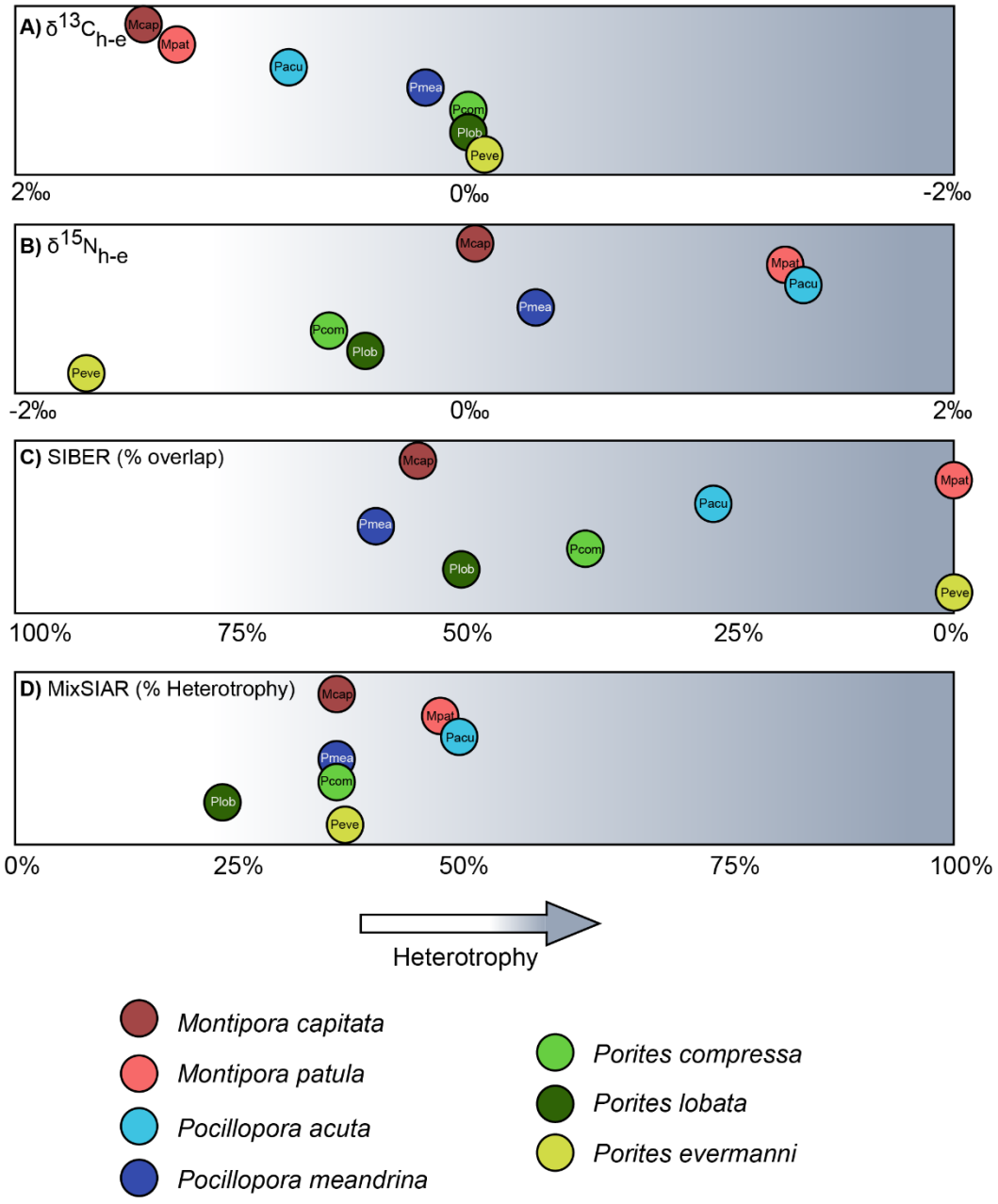

Figure 2.6. A gradient showing patterns in the four methods used to estimate the proportionate contribution of heterotrophy to Hawaiian coral tissue. A gradient showing patterns in the four methods used to estimate the proportionate contribution of heterotrophy to Hawaiian coral tissue. The mean value for each coral species using each approach, A)$\delta^{13}C_{h-e}$ (Table 2.4), B) $\delta^{15}N_{h-e}$ (Table 2.5), C) SIBER (Fig. 2.2), and D) MixSIAR (Table 2.8), is presented on the x-axis and values are ordered on the y-axis by species. The relative heterotrophic contribution of each approach increases from left to right following the arrow.

2.8 Supporting Information

2.8.1 Environmental Data

Mean annual sea surface temperature (SST), sea surface chlorophyll *a* concentration, and significant wave height were determined for each collection site for the collection year of 2015. To calculate mean annual SST, mean daily SST values were first calculated from measured seawater temperature via buoys near each of the six collection sites. Mean SST was also calculated for the warmest period of the year (20 June – 10 October), hereafter referred to as mean summertime SST. Quality-controlled buoy data was downloaded from NOAA's National Data Buoy Center and the National Center for Environmental Information. Two adjustments to the buoy data were necessary to have complete datasets for 2015: (1) SST values for Hawai‘i Institute of Marine Biology (HIMB) were also used for the Sampan Channel, as these sites are both within Kāne‘ohe Bay and seawater temperature data for the Sampan Channel was missing for large periods of the year, and (2) mean daily SST values calculated for Electric Beach between 28 February and 17 April were also used for Magic Island, as seawater temperature at Magic Island was unavailable on those dates. Any other unavailable mean daily SST values were interpolated by averaging the next available previous and subsequent mean daily SST. However, this approach was only needed for three or fewer days at each collection site. Weekly spatiotemporally composited chlorophyll *a* data were retrieved from NOAA Coral Reef Watch's Ocean Color at a resolution of 750 m via the Virtual Infrared Imaging Radiometer Suite (VIIRS) aboard the Suomi National Polar-

orbiting Partnership (S-NPP) satellite. Any unavailable weekly chlorophyll *a* data were interpolated by averaging the next available previous and subsequent weekly composited measurements. Significant wave height was collected at weekly intervals from a Simulated Waves Nearshore (SWAN) hindcast regional model for O‘ahu (Arinaga & Cheung 2012), to be used as a proxy for near-bed shear stress on the seafloor and averaged to produce an annual average wave height value.

2.8.2 Bayesian Mixing Models

The Bayesian mixing models produced for each coral species via MixSIAR were run until they reached convergence, such that the Markov Chain Monte Carlo parameters used were at least “normal” (chain length=100000, burn=50000, thin=50, chains=3). The models produced for all species together (Tables 2.8A and 2.9A) were run using the “long” parameters (chain length=300000, burn=200000, thin=100, chains=3), and reached convergence according to the Gelman diagnostic, but not the Geweke diagnostic.

Dissolved inorganic matter (DIM) fractionation: The $\delta^{13}C$ fractionation of DIC for photosynthesis was based on Swart et al. (2005), such that the net fractionation value from DIC in seawater through the fixation of carbon by photosynthesis is 12.1‰ ± 3.0‰. The first process in this fractionation is the conversion of HCO_3^- to CO_2, which is approximately –7‰ in tropical seawater (Deuser and Degens 1967), either via carbonic anhydrase or via equilibrium effects from decreases in seawater pH near the boundary layers of the coral tissue. The remaining fractionation processes are less defined,

including diffusion of CO_2 into coral tissues and the potential fractionation during photosynthesis, but are estimated to be approximately 5.1‰ ± 3.0‰ (Swart et al. 2005). Therefore, the fractionation value used for δ^{13}C-DIC in these mixing models was 12.1 ± 3.0‰. We also assumed that fractionation of δ^{13}C-DIC associated with photosynthesis was similar among all algal endosymbiont Symbiodiniaceae types. However, Hawaiian corals in this study associate with several different Symbiodiniaceae types (LaJeunesse et al. 2004), and it is possible that the δ^{13}C-DIC fractionation does differ among them. The δ^{15}N of internal DIN is likely similar to DIN of the water column, as fractionation is minimal when photosynthesis rates are high, due to low concentrations of inorganic nitrogen surrounding oligotrophic reefs (Hattori and Wada 1974, Muscatine and Kaplan 1994). Therefore, a fractionation value of 0.00 was used for the DIN source, which was an average of δ^{15}N-NO_3^- in Kāneʻohe Bay, Oahu (Wall et al. 2019).

Heterotrophic sources trophic discrimination factor (TDF): The mean TDF for δ^{13}C of all heterotrophic sources (i.e., POC and zooplankton) was set to 1.0‰ ± 1.0‰, based on measurements for marine predators (Newsome et al. 2010), due to the lack of published TDF estimates for coral heterotrophic sources. The TDF of 3.4‰ ± 1.0‰ for δ^{15}N of the same heterotrophic sources was chosen based on numerous past measurements of enrichment in nitrogen isotopes with trophic level (Post 2002, Newsome et al. 2010). Due to the uncertainty of TDF values for heterotrophic source use in symbiotic corals, a second mixing model approach was included in this study. The TDF values for heterotrophic sources were reduced to 0.0‰ ± 0.0‰, accounting for the

possibility that the recycling of carbon and nitrogen between the host and the symbiont eliminates or reduces any trophic enrichment of these isotopes. The TDF for DIM (i.e., DIC + DIN) source values remained unchanged in this second model iteration.

In addition, the $\delta^{13}C$ of dissolved organic carbon (DOC) was measured at the six collection sites around O‘ahu. The $\delta^{15}N$ of DON (approximately 4‰) was estimated via literature values from the Bermuda Atlantic Ocean Time Series (Knapp et al. 2005). However, the isotopic signatures of DOM (DOC + DON) and POM (POC + PON) were similar and covaried based on diagnostic plots provided in the MixSIAR model outputs. Given the strong covariance, and given that $\delta^{15}N$ values of DON were estimated from Atlantic Ocean sources and may not be representative of the values near O‘ahu, DOM was excluded as a source in the mixing models. However, it is important to note that the proportionate contribution of POM to Hawaiian coral tissues likely includes contributions of DOM.

Table 2.2. Summary of environmental conditions at the six coral collection sites surrounding Oahu, HI. Means are shown ± 1 SD. HIMB = Hawai‘i Institute of Marine Biology

Collection Site	Coordinates	Mean Annual SST[a] (˚C)	Mean Summertime SST (˚C)	Mean Annual Chl a[b] (mg m^{-3})	Mean Annual Significant Wave Height[c] (m)
HIMB	21° 26' 3.35" N, 157° 47' 12.53" W	26.14 ± 2.00	28.44 ± 1.04	4.05 ± 2.77	0.18 ± 0.07
Sampan	21° 27' 3.6" N, 157° 47' 45.71" W	26.14 ± 2.00	28.44 ± 1.04	3.31 ± 0.91	0.42 ± 0.10
Magic Island	21° 17' 10.00" N, 157° 51' 2.00" W	26.19 ± 1.35	27.68 ± 0.75	0.50 ± 0.28	0.80 ± 0.30
Electric Beach	21° 21' 16.33" N, 158° 6' 15.37" W	26.34 ± 1.40	27.85 ± 0.75	0.37 ± 0.21	0.65 ± 0.30
Hale‘iwa	21° 35' 39.09" N, 158° 6' 38.69" W	25.80 ± 1.19	27.06 ± 0.79	1.83 ± 1.77	0.98 ± 0.40
Waimānalo	21° 19' 42.00" N, 157° 40' 59.00" W	26.00 ± 1.41	27.55 ± 0.89	1.44 ± 0.51	0.86 ± 0.17

[a]SST values were calculated using quality-controlled buoy data available from NOAA’s National Data Buoy Center and the National Center for Environmental Information

[b]Chlorophyll *a* measurements were composited at a 750-m resolution from the VIIRS instrument by NOAA Coral Reef Watch and NOAA/NESDIS Ocean Color Team

[c]Significant wave heights were extracted from the SWAN hindcast model (Arinaga & Cheung 2012)

Table 2.3. Number of each sample type (whole, host, and algal endosymbiont) analyzed for $\delta^{13}C$ and $\delta^{15}N$ for each coral species and collection site.

	Montipora capitata			*Montipora patula*		
Sites	Whole	Host	Endosymbiont	Whole	Host	Endosymbiont
Electric Beach	9	12	12	9	14	14
Hale‘iwa	8	12	12	9	15	15
HIMB	9	12	12	-	-	-
Magic Island	-	-	-	-	-	-
Sampan	9	12	12	9	16	16
Waimānalo	10	12	12	9	15	15

	Pocillopora acuta			*Pocillopora meandrina*		
Sites	Whole	Host	Endosymbiont	Whole	Host	Endosymbiont
Electric Beach	-	-	-	9	12	12
Hale‘iwa	9	12	12	9	12	12
HIMB	9	12	12	-	-	-
Magic Island	9	12	12	9	12	12
Sampan	7	12	12	9	12	12
Waimānalo	7	9	9	9	12	12

	Porites compressa			*Porites lobata*		
Sites	Whole	Host	Endosymbiont	Whole	Host	Endosymbiont
Electric Beach	-	-	-	9	12	12
Hale‘iwa	9	12	12	9	12	12
HIMB	9	12	12	-	-	-
Magic Island	8	8	8	9	12	12
Sampan	9	12	12	9	12	12
Waimānalo	9	12	12	9	12	12

	Porites evermanni		
Sites	Whole	Host	Endosymbiont
Electric Beach	8	10	10
Hale‘iwa	9	12	12
HIMB	-	-	-
Magic Island	9	12	12
Sampan	9	12	12
Waimānalo	9	12	12

Table 2.4. The mean (± SD) $\delta^{13}C$ values of each species and their respective collection sites for samples of whole coral ($\delta^{13}C_w$), isolated coral tissue ($\delta^{13}C_h$), isolated algal endosymbionts ($\delta^{13}C_e$), and coral – algal endosymbionts ($\delta^{13}C_{h-e}$). Samples sizes for each mean is 7 – 16 (see Table 2.3).

Species	Site	$\delta^{13}C_w$	$\delta^{13}C_h$	$\delta^{13}C_e$	$\delta^{13}C_{h-e}$
Montipora capitata	All Sites	-14.32 ± 1.76	-14.09 ± 2.09	-15.62 ± 1.83	1.53 ± 0.63
	Electric Beach	-13.95 ± 1.15	-13.99 ± 1.34	-15.49 ± 1.56	1.50 ± 0.46
	Hale‘iwa	-11.80 ± 0.94	-10.95 ± 0.81	-12.99 ± 0.75	2.03 ± 0.44
	HIMB	-15.91 ± 1.01	-15.58 ± 1.40	-16.79 ± 1.06	1.21 ± 0.45
	Sampan	-15.62 ± 1.17	-15.80 ± 1.27	-16.75 ± 1.39	0.95 ± 0.67
	Waimānalo	-14.08 ± 1.06	-14.14 ± 1.05	-16.09 ± 1.09	1.95 ± 0.33
Montipora patula	All Sites	-15.82 ± 1.34	-15.66 ± 1.29	-17.01 ± 1.05	1.35 ± 0.69
	Electric Beach	-17.22 ± 1.43	-16.65 ± 1.38	-17.55 ± 1.30	0.90 ± 0.39
	Hale‘iwa	-15.13 ± 0.76	-14.89 ± 0.69	-16.54 ± 0.68	1.65 ± 0.57
	Sampan	-15.31 ± 1.27	-15.73 ± 1.36	-17.07 ± 1.02	1.34 ± 0.96
	Waimānalo	-15.63 ± 0.75	-15.45 ± 1.07	-16.93 ± 1.00	1.48 ± 0.49
Pocillopora acuta	All Sites	-15.77 ± 0.80	-15.89 ± 1.06	-16.63 ± 0.87	0.74 ± 1.00
	Hale‘iwa	-15.56 ± 0.60	-16.00 ± 0.75	-15.86 ± 0.59	-0.14 ± 0.32
	HIMB	-16.33 ± 0.81	-16.31 ± 1.11	-16.66 ± 0.94	0.34 ± 0.55
	Magic Island	-15.72 ± 0.33	-15.32 ± 0.60	-16.70 ± 0.55	1.38 ± 0.58
	Sampan	-16.30 ± 0.71	-16.84 ± 0.72	-17.09 ± 0.86	0.25 ± 0.54
	Waimānalo	-14.82 ± 0.55	-14.67 ± 0.64	-16.90 ± 0.94	2.23 ± 0.81
Pocillopora meandrina	All Sites	-14.13 ± 1.06	-14.77 ± 1.29	-14.95 ± 0.91	0.19 ± 0.76
	Electric Beach	-14.81 ± 0.62	-15.57 ± 1.01	-15.27 ± 0.62	-0.30 ± 0.79

Continued

Table 2.4 continued

	Hale‘iwa	-14.85 ± 0.55	-15.64 ± 0.78	-15.47 ± 0.55	-0.18 ± 0.39
	Magic Island	-13.21 ± 0.86	-13.81 ± 1.15	-14.15 ± 0.75	0.33 ± 0.55
	Sampan	-14.23 ± 1.19	-14.95 ± 1.22	-15.05 ± 1.20	0.10 ± 0.43
	Waimānalo	-13.54 ± 0.91	-13.85 ± 0.96	-14.84 ± 0.80	0.99 ± 0.85
Porites compressa	All Sites	-14.18 ± 1.13	-15.09 ± 1.07	-15.02 ± 1.04	-0.07 ± 0.59
	Hale‘iwa	-14.95 ± 0.76	-15.7 ± 0.51	-15.45 ± 0.62	-0.25 ± 0.50
	HIMB	-14.88 ± 1.03	-15.42 ± 0.88	-15.33 ± 1.00	-0.09 ± 0.35
	Magic Island	-13.25 ± 0.86	-14.14 ± 0.96	-14.48 ± 0.71	0.34 ± 1.12
	Sampan	-14.20 ± 1.16	-15.62 ± 0.97	-15.58 ± 0.98	-0.05 ± 0.44
	Waimānalo	-13.52 ± 0.82	-14.26 ± 0.94	-14.09 ± 0.96	-0.17 ± 0.45
Porites lobata	All Sites	-12.53 ± 1.50	-14.24 ± 1.17	-14.17 ± 1.07	-0.07 ± 0.55
	Electric Beach	-13.88 ± 0.97	-15.00 ± 0.51	-14.93 ± 0.55	-0.07 ± 0.32
	Hale‘iwa	-12.62 ± 1.39	-13.75 ± 0.65	-13.73 ± 0.70	-0.02 ± 0.42
	Magic Island	-11.55 ± 1.04	-13.11 ± 0.97	-13.36 ± 0.97	0.25 ± 0.51
	Sampan	-12.54 ± 1.94	-15.03 ± 1.17	-14.69 ± 0.99	-0.34 ± 0.63
	Waimānalo	-12.05 ± 1.11	-14.34 ± 1.17	-14.17 ± 1.26	-0.18 ± 0.68
Porites evermanni	All Sites	-14.61 ± 1.51	-15.27 ± 1.43	-15.14 ± 1.30	-0.13 ± 0.47
	Electric Beach	-13.96 ± 0.71	-14.81 ± 1.02	-14.73 ± 0.75	-0.08 ± 0.44
	Hale‘iwa	-15.19 ± 1.13	-15.78 ± 1.34	-15.45 ± 1.26	-0.32 ± 0.53
	Magic Island	-13.21 ± 0.79	-14.03 ± 0.74	-14.19 ± 0.86	0.16 ± 0.58
	Sampan	-16.49 ± 1.12	-16.82 ± 0.96	-16.61 ± 1.06	-0.21 ± 0.22
	Waimānalo	-14.13 ± 1.21	-14.83 ± 1.22	-14.65 ± 1.00	-0.18 ± 0.42

Table 2.5. The mean (± SD) $\delta^{15}N$ values of each species and their respective collection sites for samples of whole coral ($\delta^{15}N_w$), isolated coral tissue ($\delta^{15}N_h$), isolated algal endosymbionts ($\delta^{15}N_e$), and coral – algal endosymbionts ($\delta^{15}N_{h-e}$). Samples sizes for each mean is 7 – 16 (see Table 2.3).

Species	Site	$\delta^{15}N_w$	$\delta^{15}N_h$	$\delta^{15}N_e$	$\delta^{15}N_{h-e}$
Montipora capitata	All Sites	4.38 ± 0.40	4.36 ± 0.49	4.27 ± 0.44	0.09 ± 0.50
	Electric Beach	4.26 ± 0.10	4.11 ± 0.26	4.25 ± 0.20	-0.15 ± 0.36
	Haleʻiwa	4.70 ± 0.15	4.79 ± 0.30	4.19 ± 0.40	0.60 ± 0.50
	HIMB	4.79 ± 0.19	4.69 ± 0.42	4.73 ± 0.46	-0.04 ± 0.42
	Sampan	4.09 ± 0.28	4.02 ± 0.37	4.23 ± 0.32	-0.21 ± 0.24
	Waimānalo	4.10 ± 0.45	4.19 ± 0.49	3.94 ± 0.41	0.25 ± 0.46
Montipora patula	All Sites	4.50 ± 0.64	4.99 ± 0.73	3.76 ± 0.78	1.23 ± 0.66
	Electric Beach	4.75 ± 0.16	4.94 ± 0.21	4.12 ± 0.18	0.83 ± 0.23
	Haleʻiwa	5.05 ± 0.47	5.69 ± 0.23	3.83 ± 0.51	1.86 ± 0.62
	Sampan	3.64 ± 0.45	4.10 ± 0.47	2.97 ± 0.78	1.13 ± 0.78
	Waimānalo	4.57 ± 0.29	5.28 ± 0.65	4.19 ± 0.74	1.09 ± 0.34
Pocillopora acuta	All Sites	5.27 ± 1.08	5.42 ± 1.17	4.00 ± 1.16	1.42 ± 0.95
	Haleʻiwa	4.94 ± 0.18	5.20 ± 0.17	3.64 ± 0.85	1.57 ± 0.89
	HIMB	5.32 ± 0.36	5.21 ± 0.31	4.88 ± 0.24	0.33 ± 0.26
	Magic Island	4.59 ± 0.68	5.04 ± 0.60	3.15 ± 0.85	1.89 ± 0.95
	Sampan	4.60 ± 0.41	4.55 ± 0.25	3.30 ± 0.72	1.25 ± 0.64
	Waimānalo	7.18 ± 1.09	7.68 ± 1.26	5.40 ± 1.10	2.29 ± 0.55
Pocillopora meandrina	All Sites	4.73 ± 0.56	4.53 ± 0.75	4.28 ± 0.74	0.25 ± 0.71
	Electric Beach	4.48 ± 0.18	3.98 ± 0.30	3.91 ± 0.57	0.08 ± 0.71

Continued

Table 2.5 continued

	Haleʻiwa	5.05 ± 0.15	4.69 ± 0.22	4.68 ± 0.25	0.02 ± 0.38
	Magic Island	4.62 ± 0.57	4.39 ± 0.77	3.94 ± 0.52	0.44 ± 0.95
	Sampan	4.57 ± 0.67	4.61 ± 1.00	4.34 ± 0.89	0.27 ± 0.85
	Waimānalo	4.94 ± 0.78	5.00 ± 0.82	4.53 ± 0.99	0.47 ± 0.51
Porites compressa	All Sites	4.42 ± 0.41	3.66 ± 0.62	4.35 ± 0.63	-0.69 ± 0.92
	Haleʻiwa	4.62 ± 0.31	3.94 ± 0.21	4.83 ± 0.45	-0.88 ± 0.56
	HIMB	4.72 ± 0.45	4.36 ± 0.51	3.92 ± 0.78	0.45 ± 0.85
	Magic Island	4.23 ± 0.54	3.14 ± 0.90	3.06 ± 0.29	-0.92 ± 0.59
	Sampan	4.19 ± 0.13	3.53 ± 0.43	4.16 ± 0.48	-0.62 ± 0.62
	Waimānalo	4.31 ± 0.32	3.17 ± 0.54	4.69 ± 0.25	-1.52 ± 0.56
Porites lobata	All Sites	4.37 ± 0.41	3.81 ± 0.77	4.27 ± 0.64	-0.46 ± 0.91
	Electric Beach	4.48 ± 0.24	3.74 ± 0.47	4.17 ± 0.68	-0.44 ± 0.90
	Haleʻiwa	4.70 ± 0.39	4.79 ± 0.64	4.72 ± 0.46	0.07 ± 0.76
	Magic Island	3.84 ± 0.16	3.03 ± 0.53	3.80 ± 0.40	-0.77 ± 0.75
	Sampan	4.31 ± 0.37	3.76 ± 0.53	4.24 ± 0.79	-0.48 ± 1.04
	Waimānalo	4.52 ± 0.28	3.76 ± 0.54	4.42 ± 0.54	-0.66 ± 1.00
Porites evermanni	All Sites	3.97 ± 0.45	2.43 ± 0.78	4.22 ± 0.77	-1.79 ± 0.68
	Electric Beach	3.84 ± 0.41	2.48 ± 0.27	4.39 ± 0.44	-1.92 ± 0.55
	Haleʻiwa	4.26 ± 0.41	2.47 ± 0.47	4.17 ± 0.57	-1.70 ± 0.86
	Magic Island	3.55 ± 0.16	1.59 ± 0.55	3.44 ± 0.62	-1.86 ± 0.83
	Sampan	3.92 ± 0.19	2.43 ± 0.23	4.24 ± 0.56	-1.81 ± 0.68
	Waimānalo	4.25 ± 0.56	3.19 ± 1.06	4.87 ± 0.86	-1.67 ± 0.44

Table 2.6. Summary of Kruskal-Wallis and post hoc Dunn's Test comparing the A) $\delta^{13}C_{h-e}$ and B) $\delta^{15}N_{h-e}$ among coral species. Significant differences ($p < 0.05$) are noted in bold.

A) $\delta^{13}C_{h-e}$

Factor	df	Chi-Squared	P-value
Species	6	204.09	**0.0001**

Species comparisons	Chi-Squared	P-value
M. capitata – P. compressa	9.231	**0.0001**
M. capitata – P. lobata	9.181	**0.0001**
M. capitata – P. meandrina	7.519	**0.0001**
M. capitata – M. patula	0.826	0.2044
M. capitata – P. acuta	4.521	**0.0001**
M. capitata – P. evermanni	9.763	**0.0001**
M. patula – P. acuta	3.706	**0.0001**
M. patula – P. evermanni	8.944	**0.0001**
P. acuta – P. evermanni	5.155	**0.0001**
P. meandrina – M. patula	6.693	**0.0001**
P. meandrina – P. acuta	2.9	**0.0019**
P. meandrina – P. evermanni	-2.309	**0.0105**
P. compressa – P. lobata	-0.21	0.4168
P. compressa – P. meandrina	-1.843	**0.0326**
P. compressa – M. patula	8.42	**0.0001**
P. compressa – P. acuta	4.672	**0.0001**
P. compressa – P. evermanni	0.44	0.3297
P. lobata – P. meandrina	-1.662	**0.0482**
P. lobata – M. patula	8.355	**0.0001**
P. lobata – P. acuta	4.541	**0.0001**
P. lobata – P. evermanni	-0.66	0.2545

B) $\delta^{15}N_{h-e}$

Factor	df	Chi-Squared	P-value
Species	6	268.92	**0.0001**

Species comparisons	Chi-Squared	P-value
M. capitata – P. compressa	3.604	**0.0002**
M. capitata – P. lobata	2.502	**0.0062**
M. capitata - P. meandrina	-0.695	0.2436

Continued

Table 2.6 continued

M. capitata - M. patula	-5.516	**0.0001**
M. capitata - P. acuta	-5.47	**0.0001**
M. capitata - P. evermanni	7.549	**0.0001**
M. patula - P. acuta	-0.025	0.4901
M. patula - P. evermanni	13.018	**0.0001**
P. acuta - P. evermanni	12.878	**0.0001**
P. meandrina - M. patula	4.821	**0.0001**
P. meandrina - P. acuta	4.784	**0.0001**
P. meandrina - P. evermanni	-8.287	**0.0001**
P. compressa - P. lobata	3.845	**0.0001**
P. compressa - P. meandrina	-4.287	**0.0001**
P. compressa - M. patula	9.025	**0.0001**
P. compressa - P. acuta	8.936	**0.0001**
P. compressa - P. evermanni	3.845	**0.0001**
P. lobata - P. meandrina	-3.197	**0.0001**
P. lobata - M. patula	8.018	**0.0001**
P. lobata - P. acuta	7.94	**0.0001**
P. lobata - P. evermanni	-5.068	**0.0001**

Table 2.7. Summary of PERMANOVA model comparing the overall isotopic signature of host tissue vs. algal endosymbionts within each coral species. Significant differences ($p < 0.05$) are noted in bold.

Factor	df	Pseudo-F	P(perm)	Unique Permutations
Type (by Species)	7	31.879	**0.0001**	9931
Species		t-value	P-value	Unique Permutations
Montipora capitata		3.9648	**0.0002**	9878
Montipora patula		7.8199	**0.0001**	9897
Pocillopora acuta		6.126	**0.0001**	9901
Pocillopora meandrina		1.5347	0.0929	9891
Porites compressa		3.6639	**0.0001**	9855
Porites lobata		2.587	**0.0015**	9869
Porites evermanni		8.643	**0.0001**	9930

Table 2.8. Summary statistics for the Bayesian mixing models for A) all coral species, and B–H) each coral species produced via MixSIAR with trophic enrichment for heterotrophic resources as shown in Table 2.1. Percentages represent the upper and lower credible intervals for the contribution of each source to the consumer, such that between 2.5% – 97.5% represent 95% of the variability in estimated source contribution to the consumer and 50% represents the median estimate. DIM = dissolved inorganic matter, POM = particulate organic matter, Zoop = Zooplankton, ELEB = Electric Beach, HALE = Hale‘iwa, HIMB = Hawai‘i Institute of Marine Biology, MAGI = Magic Island, SAMP = Sampan, WAI = Waimānalo.

A) All Species	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Epsilon.1	0.420	0.037	0.355	0.364	0.393	0.418	0.443	0.483	0.497
Epsilon.2	0.357	0.065	0.243	0.260	0.311	0.353	0.398	0.469	0.492
Species.SD	0.562	0.232	0.290	0.313	0.412	0.508	0.655	0.966	1.127
Global.DIM	0.652	0.071	0.495	0.537	0.616	0.657	0.695	0.753	0.771
Global.POM	0.341	0.070	0.224	0.243	0.298	0.335	0.376	0.457	0.496
Global.Zooplankton	0.007	0.007	0.000	0.000	0.002	0.005	0.010	0.022	0.027
M_capitata.DIM	0.711	0.026	0.661	0.669	0.694	0.710	0.728	0.754	0.761
M_patula.DIM	0.532	0.028	0.477	0.485	0.514	0.531	0.550	0.577	0.586
P_acuta.DIM	0.493	0.027	0.436	0.447	0.474	0.493	0.512	0.537	0.546
P_compressa.DIM	0.689	0.026	0.640	0.647	0.672	0.689	0.707	0.730	0.737
P_evermanni.DIM	0.672	0.026	0.622	0.630	0.655	0.672	0.690	0.715	0.722
P_lobata.DIM	0.864	0.028	0.811	0.821	0.845	0.863	0.883	0.910	0.919
P_meandrina.DIM	0.686	0.026	0.637	0.644	0.669	0.686	0.703	0.730	0.740
M_capitata.POM	0.283	0.026	0.230	0.239	0.265	0.283	0.300	0.324	0.333
M_patula.POM	0.461	0.028	0.407	0.415	0.443	0.462	0.480	0.508	0.518
P_acuta.POM	0.498	0.029	0.439	0.450	0.479	0.498	0.517	0.544	0.554
P_compressa.POM	0.303	0.026	0.252	0.260	0.285	0.304	0.321	0.346	0.354
P_evermanni.POM	0.321	0.026	0.270	0.280	0.304	0.322	0.338	0.364	0.372
P_lobata.POM	0.130	0.028	0.073	0.084	0.112	0.131	0.150	0.174	0.183

Continued

Table 2.8 continued

P_meandrina.POM	0.305	0.027	0.248	0.259	0.288	0.306	0.324	0.350	0.356
M_capitata.Zoop	0.007	0.008	0.000	0.000	0.001	0.004	0.009	0.022	0.030
M_patula.Zoop	0.007	0.008	0.000	0.000	0.002	0.004	0.009	0.024	0.030
P_acuta.Zoop	0.009	0.012	0.000	0.000	0.002	0.005	0.011	0.031	0.047
P_compressa.Zoop	0.008	0.010	0.000	0.000	0.002	0.004	0.010	0.026	0.034
P_evermanni.Zoop	0.006	0.007	0.000	0.000	0.001	0.004	0.009	0.020	0.026
P_lobata.Zoop	0.006	0.008	0.000	0.000	0.001	0.003	0.007	0.020	0.028
P_meandrina.Zoop	0.008	0.011	0.000	0.000	0.002	0.004	0.010	0.029	0.038

B) *Montipora capitata*	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Epsilon.1	0.332	0.081	0.211	0.225	0.275	0.320	0.376	0.480	0.520
Epsilon.2	0.072	0.031	0.029	0.032	0.050	0.066	0.087	0.130	0.147
Region.SD	0.999	0.666	0.347	0.391	0.592	0.813	1.179	2.211	2.786
Global.DIM	0.676	0.115	0.406	0.468	0.615	0.690	0.751	0.842	0.870
Global.POM	0.306	0.113	0.116	0.147	0.231	0.294	0.365	0.513	0.576
Global.Zoop	0.017	0.022	0.000	0.001	0.004	0.010	0.022	0.054	0.072
M_capitata.ELEB.DIM	0.745	0.051	0.650	0.665	0.709	0.742	0.777	0.831	0.852
M_capitata.HALE.DIM	0.900	0.050	0.801	0.817	0.866	0.902	0.936	0.984	0.991
M_capitata.HIMB.DIM	0.517	0.041	0.439	0.451	0.489	0.515	0.544	0.589	0.602
M_capitata.SAMP.DIM	0.566	0.044	0.484	0.497	0.536	0.564	0.593	0.640	0.656
M_capitata.WAI.DIM	0.722	0.048	0.635	0.646	0.689	0.719	0.752	0.804	0.824
M_capitata.ELEB.POM	0.239	0.053	0.121	0.148	0.206	0.242	0.276	0.320	0.333
M_capitata.HALE.POM	0.087	0.049	0.004	0.009	0.050	0.085	0.121	0.169	0.188
M_capitata.HIMB.POM	0.467	0.045	0.373	0.391	0.439	0.468	0.498	0.538	0.549
M_capitata.SAMP.POM	0.423	0.046	0.326	0.345	0.393	0.424	0.453	0.496	0.508

Continued

Table 2.8 continued

M_capitata.WAI.POM	0.264	0.050	0.156	0.177	0.234	0.267	0.297	0.341	0.354
M_capitata.ELEB.Zoop	0.017	0.023	0.000	0.000	0.002	0.008	0.021	0.064	0.085
M_capitata.HALE.Zoop	0.012	0.019	0.000	0.000	0.002	0.006	0.015	0.049	0.068
M_capitata.HIMB.Zoop	0.016	0.020	0.000	0.000	0.003	0.009	0.021	0.059	0.077
M_capitata.SAMP.Zoop	0.012	0.015	0.000	0.000	0.002	0.007	0.016	0.040	0.051
M_capitata.WAI.Zoop	0.014	0.019	0.000	0.000	0.002	0.008	0.018	0.053	0.069

C) *Montipora patula*	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Epsilon.1	0.542	0.166	0.297	0.323	0.427	0.519	0.630	0.832	0.937
Epsilon.2	0.148	0.069	0.052	0.062	0.098	0.135	0.181	0.279	0.318
Region.SD	0.372	0.282	0.035	0.069	0.194	0.302	0.467	0.934	1.123
Global.DIM	0.527	0.069	0.374	0.413	0.489	0.528	0.567	0.636	0.667
Global.POM	0.458	0.070	0.318	0.344	0.418	0.457	0.498	0.574	0.608
Global.Zoop	0.015	0.016	0.001	0.001	0.004	0.010	0.020	0.045	0.057
M_patula.ELEB.DIM	0.431	0.059	0.323	0.339	0.388	0.428	0.469	0.531	0.550
M_patula.HALE.DIM	0.555	0.046	0.468	0.481	0.523	0.554	0.584	0.634	0.650
M_patula.SAMP.DIM	0.590	0.054	0.496	0.509	0.552	0.587	0.625	0.683	0.704
M_patula.WAI.DIM	0.531	0.045	0.448	0.460	0.500	0.531	0.561	0.608	0.623
M_patula.ELEB.POM	0.556	0.061	0.437	0.454	0.515	0.558	0.599	0.650	0.668
M_patula.HALE.POM	0.430	0.048	0.329	0.346	0.400	0.432	0.463	0.507	0.521
M_patula.SAMP.POM	0.397	0.055	0.280	0.301	0.363	0.401	0.435	0.479	0.495
M_patula.WAI.POM	0.454	0.047	0.359	0.375	0.424	0.456	0.486	0.528	0.540
M_patula.ELEB.Zoop	0.014	0.014	0.000	0.001	0.004	0.009	0.018	0.042	0.052
M_patula.HALE.Zoop	0.015	0.017	0.000	0.001	0.004	0.010	0.021	0.049	0.065

Continued

Table 2.8 continued

M_patula.SAMP.Zoop	0.013	0.013	0.000	0.001	0.004	0.009	0.018	0.038	0.046
M_patula.WAI.Zoop	0.014	0.016	0.000	0.001	0.004	0.009	0.020	0.046	0.056

D) *Pocillopora acuta*	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Epsilon.1	0.177	0.050	0.104	0.112	0.141	0.169	0.202	0.270	0.298
Epsilon.2	0.346	0.156	0.147	0.168	0.238	0.313	0.416	0.639	0.740
Region.SD	0.477	0.827	0.023	0.040	0.115	0.191	0.346	2.175	3.007
Global.DIM	0.505	0.075	0.324	0.388	0.483	0.507	0.531	0.607	0.664
Global.POM	0.465	0.075	0.257	0.347	0.444	0.471	0.494	0.559	0.610
Global.Zoop	0.030	0.048	0.001	0.002	0.008	0.018	0.035	0.088	0.132
P_acuta.HALE.DIM	0.530	0.031	0.472	0.481	0.510	0.530	0.550	0.583	0.594
P_acuta.HIMB.DIM	0.468	0.032	0.404	0.414	0.447	0.470	0.491	0.519	0.530
P_acuta.MAGI.DIM	0.510	0.029	0.451	0.463	0.491	0.509	0.529	0.561	0.569
P_acuta.SAMP.DIM	0.473	0.035	0.403	0.414	0.451	0.475	0.496	0.527	0.537
P_acuta.WAI.DIM	0.546	0.052	0.417	0.453	0.518	0.550	0.580	0.621	0.635
P_acuta.HALE.POM	0.448	0.035	0.373	0.387	0.426	0.450	0.472	0.501	0.510
P_acuta.HIMB.POM	0.509	0.035	0.440	0.452	0.486	0.509	0.532	0.568	0.578
P_acuta.MAGI.POM	0.471	0.032	0.406	0.417	0.451	0.472	0.491	0.521	0.531
P_acuta.SAMP.POM	0.508	0.037	0.438	0.449	0.483	0.507	0.532	0.569	0.583
P_acuta.WAI.POM	0.370	0.127	0.011	0.037	0.369	0.412	0.444	0.481	0.491
P_acuta.HALE.Zoop	0.022	0.021	0.000	0.001	0.006	0.016	0.030	0.064	0.076
P_acuta.HIMB.Zoop	0.022	0.022	0.000	0.001	0.007	0.016	0.030	0.067	0.082
P_acuta.MAGI.Zoop	0.019	0.018	0.000	0.001	0.006	0.015	0.028	0.055	0.066
P_acuta.SAMP.Zoop	0.019	0.018	0.000	0.001	0.006	0.015	0.028	0.055	0.067
P_acuta.WAI.Zoop	0.084	0.154	0.001	0.002	0.008	0.020	0.046	0.491	0.532

Continued

Table 2.8 continued

E) *Pocillopora meandrina*	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Epsilon.1	0.175	0.044	0.111	0.117	0.143	0.168	0.199	0.256	0.280
Epsilon.2	0.279	0.117	0.110	0.128	0.195	0.260	0.341	0.490	0.574
Region.SD	0.404	0.252	0.121	0.146	0.244	0.340	0.489	0.871	1.076
Global.DIM	0.697	0.058	0.566	0.597	0.668	0.702	0.732	0.782	0.799
Global.POM	0.282	0.058	0.183	0.200	0.246	0.278	0.312	0.383	0.413
Global.Zoop	0.020	0.019	0.000	0.001	0.007	0.016	0.029	0.057	0.069
P_meandrina.ELEB.DIM	0.642	0.036	0.574	0.586	0.618	0.639	0.665	0.702	0.716
P_meandrina.HALE.DIM	0.628	0.036	0.558	0.570	0.603	0.626	0.651	0.690	0.702
P_meandrina.MAGI.DIM	0.786	0.038	0.715	0.726	0.761	0.785	0.811	0.850	0.865
P_meandrina.SAMP.DIM	0.707	0.035	0.637	0.650	0.684	0.707	0.729	0.765	0.778
P_meandrina.WAI.DIM	0.756	0.037	0.686	0.698	0.731	0.755	0.780	0.818	0.830
P_meandrina.ELEB.POM	0.339	0.039	0.256	0.272	0.314	0.341	0.365	0.397	0.409
P_meandrina.HALE.POM	0.348	0.040	0.266	0.280	0.323	0.349	0.375	0.412	0.424
P_meandrina.MAGI.POM	0.196	0.039	0.113	0.129	0.171	0.197	0.222	0.258	0.270
P_meandrina.SAMP.POM	0.274	0.037	0.198	0.211	0.250	0.274	0.299	0.334	0.348
P_meandrina.WAI.POM	0.224	0.039	0.145	0.157	0.199	0.226	0.250	0.285	0.298
P_meandrina.ELEB.Zoop	0.020	0.018	0.000	0.001	0.006	0.015	0.028	0.057	0.067
P_meandrina.HALE.Zoop	0.024	0.024	0.000	0.001	0.007	0.017	0.033	0.070	0.089
P_meandrina.MAGI.Zoop	0.018	0.017	0.000	0.001	0.005	0.013	0.025	0.052	0.064
P_meandrina.SAMP.Zoop	0.019	0.018	0.000	0.001	0.006	0.014	0.027	0.054	0.066
P_meandrina.WAI.Zoop	0.020	0.021	0.000	0.001	0.006	0.014	0.027	0.063	0.074

Continued

Table 2.8 continued

F) *Porites compressa*	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Epsilon.1	0.221	0.054	0.139	0.149	0.183	0.213	0.252	0.319	0.347
Epsilon.2	0.200	0.086	0.079	0.088	0.138	0.186	0.244	0.360	0.402
Region.SD	0.377	0.228	0.106	0.135	0.230	0.324	0.462	0.796	0.965
Global.DIM	0.691	0.054	0.561	0.597	0.663	0.696	0.723	0.770	0.787
Global.POM	0.289	0.056	0.192	0.206	0.255	0.285	0.318	0.383	0.420
Global.Zoop	0.020	0.019	0.000	0.001	0.006	0.014	0.028	0.058	0.070
P_compressa.HALE.DIM	0.623	0.039	0.550	0.561	0.596	0.621	0.649	0.687	0.699
P_compressa.HIMB.DIM	0.632	0.039	0.557	0.569	0.605	0.631	0.658	0.697	0.709
P_compressa.MAGI.DIM	0.768	0.041	0.693	0.705	0.739	0.768	0.796	0.833	0.850
P_compressa.SAMP.DIM	0.717	0.038	0.647	0.657	0.691	0.716	0.742	0.781	0.792
P_compressa.WAI.DIM	0.753	0.039	0.679	0.690	0.726	0.751	0.777	0.819	0.834
P_compressa.HALE.POM	0.356	0.042	0.268	0.286	0.329	0.358	0.384	0.421	0.434
P_compressa.HIMB.POM	0.347	0.042	0.264	0.279	0.320	0.348	0.376	0.413	0.426
P_compressa.MAGI.POM	0.214	0.041	0.129	0.144	0.187	0.215	0.243	0.278	0.290
P_compressa.SAMP.POM	0.263	0.040	0.183	0.197	0.236	0.264	0.290	0.326	0.335
P_compressa.WAI.POM	0.228	0.040	0.147	0.159	0.203	0.229	0.256	0.294	0.306
P_compressa.HALE.Zoop	0.022	0.022	0.000	0.001	0.006	0.015	0.029	0.066	0.080
P_compressa.HIMB.Zoop	0.021	0.021	0.000	0.001	0.006	0.014	0.029	0.061	0.077
P_compressa.MAGI.Zoop	0.018	0.019	0.000	0.001	0.005	0.012	0.025	0.055	0.069
P_compressa.SAMP.Zoop	0.020	0.020	0.000	0.001	0.005	0.013	0.027	0.060	0.073
P_compressa.WAI.Zoop	0.019	0.020	0.000	0.001	0.005	0.013	0.025	0.057	0.072

Continued

Table 2.8 continued

G) *Porites lobata*	Mean	SD	2.50%	5%	25%	50%	75%	95%	97.50%
Epsilon.1	0.275	0.071	0.170	0.181	0.226	0.265	0.312	0.401	0.443
Epsilon.2	0.190	0.093	0.065	0.077	0.124	0.173	0.235	0.358	0.420
Region.SD	2.762	3.662	0.267	0.343	0.694	1.278	2.913	11.771	14.803
Global.DIM	0.757	0.211	0.147	0.245	0.703	0.840	0.894	0.945	0.957
Global.POM	0.153	0.150	0.015	0.025	0.061	0.101	0.177	0.503	0.603
Global.Zoop	0.090	0.130	0.002	0.003	0.017	0.042	0.097	0.391	0.493
P_lobata.ELEB.DIM	0.746	0.054	0.654	0.667	0.709	0.742	0.779	0.845	0.870
P_lobata.HALE.DIM	0.891	0.059	0.778	0.794	0.850	0.889	0.933	0.999	1.000
P_lobata.MAGI.DIM	0.957	0.038	0.871	0.886	0.931	0.964	0.991	1.000	1.000
P_lobata.SAMP.DIM	0.924	0.052	0.820	0.835	0.888	0.924	0.970	1.000	1.000
P_lobata.WAI.DIM	0.929	0.051	0.826	0.842	0.894	0.932	0.973	1.000	1.000
P_lobata.ELEB.POM	0.213	0.070	0.041	0.076	0.173	0.222	0.262	0.312	0.325
P_lobata.HALE.POM	0.070	0.054	0.000	0.000	0.022	0.066	0.109	0.165	0.182
P_lobata.MAGI.POM	0.028	0.029	0.000	0.000	0.003	0.020	0.044	0.085	0.099
P_lobata.SAMP.POM	0.051	0.045	0.000	0.000	0.010	0.044	0.082	0.137	0.152
P_lobata.WAI.POM	0.044	0.040	0.000	0.000	0.008	0.036	0.069	0.122	0.140
P_lobata.ELEB.Zoop	0.041	0.052	0.000	0.000	0.004	0.021	0.060	0.149	0.189
P_lobata.HALE.Zoop	0.039	0.047	0.000	0.000	0.004	0.020	0.057	0.139	0.168
P_lobata.MAGI.Zoop	0.016	0.021	0.000	0.000	0.001	0.007	0.023	0.061	0.073
P_lobata.SAMP.Zoop	0.024	0.031	0.000	0.000	0.002	0.012	0.035	0.090	0.111
P_lobata.WAI.Zoop	0.027	0.034	0.000	0.000	0.002	0.013	0.040	0.100	0.123

Continued

Table 2.8 continued

H) *Porites evermanni*	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Epsilon.1	0.318	0.082	0.196	0.209	0.261	0.306	0.360	0.465	0.503
Epsilon.2	0.170	0.079	0.061	0.071	0.112	0.155	0.213	0.319	0.367
Region.SD	0.641	0.388	0.229	0.262	0.402	0.542	0.770	1.341	1.615
Global.DIM	0.654	0.093	0.426	0.486	0.609	0.664	0.713	0.786	0.813
Global.POM	0.336	0.093	0.178	0.204	0.278	0.326	0.380	0.504	0.565
Global.Zoop	0.010	0.013	0.000	0.000	0.002	0.006	0.013	0.031	0.041
P_evermanni.ELEB.DIM	0.725	0.049	0.634	0.648	0.691	0.724	0.758	0.808	0.825
P_evermanni.HALE.DIM	0.605	0.044	0.524	0.535	0.575	0.604	0.634	0.679	0.698
P_evermanni.MAGI.DIM	0.811	0.049	0.720	0.734	0.776	0.809	0.842	0.891	0.909
P_evermanni.SAMP.DIM	0.490	0.046	0.405	0.420	0.458	0.488	0.519	0.568	0.588
P_evermanni.WAI.DIM	0.715	0.048	0.629	0.640	0.682	0.713	0.746	0.795	0.812
P_evermanni.ELEB.POM	0.265	0.050	0.162	0.179	0.231	0.267	0.299	0.345	0.357
P_evermanni.HALE.POM	0.385	0.045	0.292	0.309	0.357	0.388	0.415	0.455	0.468
P_evermanni.MAGI.POM	0.181	0.050	0.080	0.095	0.149	0.183	0.215	0.259	0.273
P_evermanni.SAMP.POM	0.501	0.046	0.404	0.421	0.472	0.503	0.534	0.572	0.585
P_evermanni.WAI.POM	0.276	0.049	0.176	0.192	0.243	0.278	0.309	0.351	0.362
P_evermanni.ELEB.Zoop	0.010	0.013	0.000	0.000	0.002	0.005	0.013	0.034	0.047
P_evermanni.HALE.Zoop	0.010	0.012	0.000	0.000	0.002	0.006	0.013	0.034	0.044
P_evermanni.MAGI.Zoop	0.009	0.013	0.000	0.000	0.002	0.005	0.011	0.031	0.043
P_evermanni.SAMP.Zoop	0.009	0.010	0.000	0.000	0.002	0.005	0.012	0.029	0.038
P_evermanni.WAI.Zoop	0.010	0.014	0.000	0.000	0.002	0.005	0.012	0.034	0.047

Table 2.9. Summary statistics for the Bayesian mixing models for each coral species produced via MixSIAR without trophic enrichment for heterotrophic sources (POM and Zooplankton). Percentages represent the upper and lower credible intervals for the contribution of each source to the consumer, such that between 2.5% – 97.5% represent 95% of the variability in estimated source contribution to the consumer and 50% represents the median estimate. DIM = dissolved inorganic matter, POM = particulate organic matter, Zoop = Zooplankton, ELEB = Electric Beach, HALE = Haleʻiwa, HIMB = Hawaiʻi Institute of Marine Biology, MAGI = Magic Island, SAMP = Sampan, WAI = Waimānalo.

A) All Species	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Epsilon.1	0.456	0.044	0.380	0.390	0.424	0.454	0.483	0.533	0.548
Epsilon.2	0.555	0.120	0.350	0.383	0.470	0.544	0.629	0.770	0.823
Region.SD	0.672	0.244	0.345	0.374	0.506	0.626	0.788	1.137	1.285
Global.DIM	0.628	0.071	0.475	0.505	0.587	0.634	0.675	0.735	0.756
Global.POM	0.165	0.061	0.074	0.083	0.124	0.157	0.196	0.273	0.314
Global.Zoop	0.206	0.063	0.102	0.115	0.162	0.199	0.244	0.322	0.348
M_capitata.DIM	0.690	0.034	0.616	0.633	0.670	0.692	0.713	0.742	0.753
M_patula.DIM	0.520	0.035	0.444	0.461	0.498	0.522	0.544	0.575	0.585
P_acuta.DIM	0.422	0.052	0.309	0.329	0.389	0.424	0.459	0.504	0.514
P_compressa.DIM	0.655	0.035	0.583	0.596	0.632	0.656	0.678	0.710	0.719
P_evermanni.DIM	0.660	0.029	0.598	0.610	0.641	0.660	0.680	0.706	0.715
P_lobata.DIM	0.846	0.032	0.780	0.791	0.826	0.847	0.868	0.898	0.911
P_meandrina.DIM	0.645	0.040	0.562	0.575	0.621	0.647	0.672	0.707	0.719
M_capitata.POM	0.154	0.051	0.052	0.066	0.119	0.153	0.191	0.234	0.248
M_patula.POM	0.269	0.071	0.128	0.150	0.221	0.269	0.319	0.383	0.400
P_acuta.POM	0.142	0.067	0.029	0.041	0.092	0.137	0.185	0.263	0.289
P_compressa.POM	0.145	0.049	0.054	0.067	0.111	0.144	0.179	0.228	0.244
P_evermanni.POM	0.216	0.048	0.117	0.134	0.185	0.216	0.249	0.290	0.301
P_lobata.POM	0.061	0.026	0.014	0.020	0.042	0.060	0.079	0.108	0.116

Continued

Table 2.9 continued

P_meandrina.POM	0.117	0.050	0.028	0.039	0.082	0.116	0.152	0.202	0.217
M_capitata.Zoop	0.156	0.067	0.036	0.051	0.107	0.155	0.200	0.269	0.298
M_patula.Zoop	0.211	0.088	0.050	0.067	0.148	0.208	0.272	0.358	0.385
P_acuta.Zoop	0.437	0.101	0.224	0.260	0.370	0.442	0.508	0.595	0.625
P_compressa.Zoop	0.200	0.067	0.069	0.086	0.154	0.200	0.245	0.310	0.328
P_evermanni.Zoop	0.125	0.058	0.024	0.034	0.084	0.121	0.163	0.223	0.250
P_lobata.Zoop	0.092	0.038	0.026	0.034	0.065	0.090	0.118	0.159	0.171
P_meandrina.Zoop	0.238	0.073	0.098	0.116	0.187	0.237	0.289	0.354	0.375

B) *Montipora capitata*	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Epsilon.1	0.352	0.092	0.212	0.228	0.287	0.340	0.401	0.518	0.568
Epsilon.2	0.123	0.058	0.043	0.052	0.083	0.112	0.150	0.232	0.264
Region.SD	1.016	0.637	0.377	0.419	0.636	0.847	1.187	2.162	2.617
Global.DIM	0.655	0.118	0.371	0.436	0.595	0.671	0.734	0.819	0.843
Global.POM	0.206	0.113	0.058	0.073	0.132	0.187	0.251	0.410	0.504
Global.Zoop	0.139	0.091	0.018	0.029	0.074	0.118	0.183	0.310	0.363
M_capitata.ELEB.DIM	0.713	0.056	0.601	0.622	0.676	0.713	0.750	0.805	0.824
M_capitata.HALE.DIM	0.893	0.055	0.778	0.798	0.857	0.896	0.932	0.979	0.990
M_capitata.HIMB.DIM	0.486	0.060	0.367	0.387	0.447	0.485	0.526	0.583	0.601
M_capitata.SAMP.DIM	0.558	0.047	0.466	0.481	0.528	0.559	0.588	0.635	0.651
M_capitata.WAI.DIM	0.705	0.050	0.608	0.625	0.671	0.705	0.737	0.789	0.806
M_capitata.ELEB.POM	0.151	0.065	0.028	0.044	0.105	0.150	0.198	0.256	0.271
M_capitata.HALE.POM	0.053	0.036	0.002	0.004	0.025	0.049	0.076	0.119	0.136
M_capitata.HIMB.POM	0.247	0.098	0.057	0.085	0.178	0.246	0.317	0.410	0.434
M_capitata.SAMPOM	0.321	0.074	0.157	0.189	0.274	0.329	0.375	0.429	0.443
M_capitata.WAI.POM	0.183	0.070	0.037	0.062	0.137	0.188	0.233	0.289	0.304

Continued

Table 2.9 continued

M_capitata.ELEB.Zoop	0.136	0.083	0.006	0.014	0.072	0.126	0.192	0.288	0.317
M_capitata.HALE.Zoop	0.054	0.043	0.001	0.003	0.020	0.044	0.078	0.137	0.161
M_capitata.HIMB.Zoop	0.267	0.135	0.020	0.037	0.167	0.274	0.367	0.483	0.513
M_capitata.SAMP.Zoop	0.121	0.088	0.004	0.009	0.050	0.104	0.176	0.288	0.323
M_capitata.WAI.Zoop	0.111	0.081	0.003	0.008	0.046	0.097	0.162	0.265	0.296

C) *Montipora patula*	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Epsilon.1	0.628	0.220	0.301	0.339	0.477	0.588	0.739	1.032	1.196
Epsilon.2	0.374	0.185	0.125	0.151	0.244	0.339	0.463	0.717	0.833
Region.SD	0.530	0.404	0.065	0.109	0.267	0.428	0.664	1.309	1.588
Global.DIM	0.472	0.092	0.269	0.321	0.425	0.474	0.521	0.623	0.664
Global.POM	0.247	0.087	0.104	0.126	0.193	0.239	0.291	0.391	0.455
Global.Zoop	0.280	0.093	0.105	0.131	0.220	0.278	0.335	0.434	0.477
M_patula.ELEB.DIM	0.363	0.068	0.231	0.250	0.316	0.362	0.409	0.473	0.495
M_patula.HALE.DIM	0.511	0.062	0.394	0.412	0.469	0.509	0.550	0.612	0.638
M_patula.SAMP.DIM	0.550	0.055	0.449	0.465	0.513	0.548	0.585	0.641	0.662
M_patula.WAI.DIM	0.477	0.053	0.372	0.390	0.442	0.477	0.510	0.561	0.584
M_patula.ELEB.POM	0.269	0.080	0.126	0.146	0.214	0.263	0.321	0.408	0.441
M_patula.HALE.POM	0.187	0.075	0.042	0.059	0.137	0.188	0.239	0.307	0.333
M_patula.SAMPOM	0.268	0.070	0.135	0.154	0.220	0.266	0.317	0.386	0.405
M_patula.WAI.POM	0.235	0.070	0.103	0.125	0.187	0.231	0.279	0.356	0.379
M_patula.ELEB.Zoop	0.368	0.110	0.134	0.175	0.293	0.376	0.447	0.537	0.564
M_patula.HALE.Zoop	0.302	0.103	0.102	0.137	0.232	0.298	0.370	0.474	0.504
M_patula.SAMP.Zoop	0.181	0.082	0.023	0.044	0.123	0.183	0.238	0.316	0.338
M_patula.WAI.Zoop	0.289	0.092	0.098	0.130	0.230	0.290	0.352	0.437	0.461

Continued

Table 2.9 continued

D) *Pocillopora acuta*	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Epsilon.1	0.184	0.055	0.104	0.113	0.146	0.175	0.213	0.284	0.306
Epsilon.2	0.889	0.384	0.371	0.417	0.621	0.813	1.068	1.611	1.822
Region.SD	0.322	0.225	0.023	0.048	0.172	0.279	0.420	0.728	0.888
Global.DIM	0.452	0.058	0.340	0.364	0.421	0.450	0.481	0.546	0.576
Global.POM	0.146	0.046	0.064	0.077	0.116	0.144	0.174	0.224	0.247
Global.Zoop	0.402	0.068	0.268	0.294	0.358	0.401	0.444	0.513	0.538
P_acuta.HALE.DIM	0.459	0.042	0.376	0.388	0.431	0.459	0.487	0.526	0.539
P_acuta.HIMB.DIM	0.390	0.051	0.281	0.299	0.360	0.393	0.425	0.467	0.475
P_acuta.MAGI.DIM	0.456	0.041	0.373	0.389	0.428	0.457	0.483	0.522	0.534
P_acuta.SAMP.DIM	0.421	0.042	0.330	0.348	0.395	0.423	0.450	0.484	0.495
P_acuta.WAI.DIM	0.517	0.062	0.398	0.418	0.477	0.521	0.559	0.610	0.629
P_acuta.HALE.POM	0.139	0.045	0.056	0.070	0.109	0.138	0.167	0.216	0.233
P_acuta.HIMB.POM	0.134	0.047	0.042	0.057	0.104	0.133	0.165	0.213	0.231
P_acuta.MAGI.POM	0.172	0.059	0.069	0.083	0.131	0.168	0.208	0.275	0.301
P_acuta.SAMPOM	0.186	0.064	0.072	0.089	0.142	0.182	0.226	0.295	0.317
P_acuta.WAI.POM	0.110	0.044	0.023	0.034	0.080	0.110	0.139	0.184	0.196
P_acuta.HALE.Zoop	0.402	0.064	0.272	0.298	0.360	0.402	0.444	0.507	0.529
P_acuta.HIMB.Zoop	0.476	0.075	0.334	0.358	0.427	0.473	0.522	0.607	0.631
P_acuta.MAGI.Zoop	0.372	0.077	0.202	0.238	0.323	0.379	0.424	0.490	0.508
P_acuta.SAMP.Zoop	0.393	0.076	0.237	0.263	0.347	0.395	0.442	0.510	0.531
P_acuta.WAI.Zoop	0.373	0.081	0.222	0.246	0.319	0.370	0.423	0.506	0.543

E) *Pocillopora meandrina*	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Epsilon.1	0.186	0.046	0.115	0.123	0.153	0.179	0.214	0.271	0.291
Epsilon.2	0.391	0.166	0.155	0.179	0.270	0.363	0.480	0.706	0.794

Continued

Table 2.9 continued

Region.SD	0.484	0.284	0.163	0.195	0.303	0.421	0.584	0.974	1.191
Global.DIM	0.653	0.071	0.485	0.530	0.620	0.658	0.697	0.753	0.773
Global.POM	0.098	0.047	0.025	0.034	0.066	0.092	0.121	0.177	0.205
Global.Zoop	0.250	0.072	0.124	0.144	0.201	0.245	0.291	0.372	0.405
P_meandrina.ELEB.DIM	0.591	0.042	0.508	0.521	0.565	0.591	0.619	0.660	0.673
P_meandrina.HALE.DIM	0.554	0.050	0.452	0.472	0.522	0.554	0.588	0.637	0.651
P_meandrina.MAGI.DIM	0.760	0.046	0.670	0.685	0.730	0.761	0.790	0.835	0.850
P_meandrina.SAMP.DIM	0.680	0.043	0.593	0.609	0.652	0.680	0.708	0.749	0.764
P_meandrina.WAI.DIM	0.730	0.046	0.639	0.652	0.699	0.730	0.760	0.806	0.817
P_meandrina.ELEB.POM	0.130	0.057	0.028	0.042	0.089	0.126	0.167	0.229	0.253
P_meandrina.HALE.POM	0.094	0.045	0.020	0.026	0.061	0.091	0.122	0.173	0.190
P_meandrina.MAGI.POM	0.069	0.034	0.013	0.019	0.044	0.065	0.090	0.133	0.145
P_meandrina.SAMPOM	0.101	0.049	0.020	0.029	0.065	0.097	0.130	0.190	0.211
P_meandrina.WAI.POM	0.072	0.036	0.012	0.018	0.045	0.068	0.096	0.136	0.149
P_meandrina.ELEB.Zoop	0.279	0.072	0.128	0.156	0.230	0.281	0.327	0.392	0.414
P_meandrina.HALE.Zoop	0.352	0.074	0.199	0.229	0.303	0.355	0.400	0.468	0.495
P_meandrina.MAGI.Zoop	0.171	0.056	0.060	0.079	0.133	0.170	0.208	0.262	0.279
P_meandrina.SAMP.Zoop	0.219	0.068	0.075	0.099	0.177	0.222	0.264	0.326	0.346
P_meandrina.WAI.Zoop	0.198	0.060	0.080	0.100	0.158	0.197	0.239	0.296	0.317

F) *Porites compressa*	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Epsilon.1	0.238	0.062	0.145	0.156	0.195	0.228	0.269	0.351	0.383
Epsilon.2	0.333	0.161	0.114	0.133	0.220	0.304	0.408	0.640	0.734
Region.SD	0.385	0.226	0.091	0.121	0.240	0.335	0.475	0.814	0.937
Global.DIM	0.670	0.057	0.547	0.575	0.638	0.672	0.707	0.755	0.773
Global.POM	0.150	0.060	0.046	0.061	0.107	0.145	0.188	0.255	0.283

Continued

Table 2.9 continued

Global.Zoop	0.181	0.078	0.040	0.057	0.124	0.178	0.234	0.313	0.342
P_compressa.HALE.DIM	0.599	0.050	0.501	0.514	0.566	0.601	0.633	0.678	0.693
P_compressa.HIMB.DIM	0.617	0.048	0.516	0.536	0.586	0.619	0.650	0.690	0.704
P_compressa.MAGI.DIM	0.747	0.048	0.652	0.667	0.715	0.748	0.780	0.824	0.838
P_compressa.SAMP.DIM	0.685	0.045	0.597	0.609	0.656	0.685	0.714	0.756	0.773
P_compressa.WAI.DIM	0.730	0.048	0.639	0.654	0.699	0.728	0.763	0.811	0.825
P_compressa.HALE.POM	0.178	0.073	0.046	0.063	0.125	0.175	0.231	0.302	0.325
P_compressa.HIMB.POM	0.172	0.072	0.043	0.056	0.118	0.170	0.222	0.294	0.315
P_compressa.MAGI.POM	0.115	0.047	0.030	0.041	0.081	0.113	0.147	0.197	0.212
P_compressa.SAMPOM	0.144	0.057	0.040	0.053	0.103	0.142	0.183	0.241	0.258
P_compressa.WAI.POM	0.121	0.049	0.031	0.043	0.087	0.120	0.152	0.205	0.222
P_compressa.HALE.Zoop	0.223	0.098	0.044	0.062	0.148	0.223	0.292	0.385	0.411
P_compressa.HIMB.Zoop	0.211	0.096	0.035	0.059	0.140	0.208	0.277	0.370	0.405
P_compressa.MAGI.Zoop	0.138	0.064	0.025	0.037	0.091	0.134	0.181	0.250	0.275
P_compressa.SAMP.Zoop	0.171	0.076	0.036	0.049	0.113	0.170	0.227	0.298	0.321
P_compressa.WAI.Zoop	0.149	0.067	0.026	0.040	0.100	0.147	0.196	0.260	0.282

G) *Porites lobata*	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Epsilon.1	0.282	0.071	0.175	0.188	0.231	0.271	0.320	0.413	0.454
Epsilon.2	0.215	0.105	0.068	0.083	0.141	0.197	0.267	0.407	0.468
Region.SD	2.466	3.172	0.233	0.322	0.673	1.204	2.676	9.694	12.637
Global.DIM	0.767	0.192	0.211	0.320	0.721	0.840	0.890	0.940	0.950
Global.POM	0.106	0.135	0.003	0.005	0.028	0.059	0.117	0.402	0.548
Global.Zooplankton	0.127	0.138	0.004	0.008	0.043	0.085	0.152	0.422	0.577
P_lobata.ELEB.DIM	0.727	0.065	0.599	0.619	0.684	0.728	0.770	0.836	0.859
P_lobata.HALE.DIM	0.890	0.061	0.773	0.792	0.848	0.888	0.931	0.998	1.000

Continued

Table 2.9 continued

P_lobata.MAGI.DIM	0.948	0.043	0.853	0.871	0.921	0.955	0.986	1.000	1.000
P_lobata.SAMP.DIM	0.920	0.054	0.810	0.828	0.883	0.921	0.963	1.000	1.000
P_lobata.WAI.DIM	0.927	0.053	0.814	0.834	0.892	0.932	0.971	1.000	1.000
P_lobata.ELEB.POM	0.110	0.087	0.000	0.000	0.029	0.098	0.184	0.256	0.274
P_lobata.HALE.POM	0.042	0.041	0.000	0.000	0.007	0.031	0.067	0.123	0.140
P_lobata.MAGI.POM	0.021	0.024	0.000	0.000	0.002	0.013	0.031	0.069	0.084
P_lobata.SAMPOM	0.035	0.036	0.000	0.000	0.005	0.023	0.055	0.107	0.124
P_lobata.WAI.POM	0.028	0.030	0.000	0.000	0.003	0.018	0.044	0.089	0.104
P_lobata.ELEB.Zoop	0.163	0.119	0.000	0.001	0.054	0.157	0.262	0.354	0.381
P_lobata.HALE.Zoop	0.068	0.061	0.000	0.000	0.013	0.055	0.108	0.183	0.206
P_lobata.MAGI.Zoop	0.031	0.034	0.000	0.000	0.003	0.020	0.049	0.100	0.118
P_lobata.SAMP.Zoop	0.045	0.047	0.000	0.000	0.005	0.031	0.073	0.140	0.162
P_lobata.WAI.Zoop	0.045	0.046	0.000	0.000	0.005	0.030	0.069	0.138	0.160

H) *Porites evermanni*	**Mean**	**SD**	**2.50%**	**5%**	**25%**	**50%**	**75%**	**95%**	**97.50%**
Epsilon.1	0.333	0.088	0.203	0.220	0.272	0.322	0.378	0.489	0.532
Epsilon.2	0.230	0.112	0.078	0.094	0.152	0.210	0.282	0.448	0.511
Region.SD	0.570	0.286	0.230	0.263	0.382	0.509	0.689	1.080	1.257
Global.DIM	0.657	0.073	0.500	0.527	0.614	0.660	0.702	0.773	0.796
Global.POM	0.269	0.076	0.134	0.154	0.217	0.263	0.316	0.406	0.439
Global.Zoop	0.074	0.054	0.005	0.008	0.033	0.064	0.104	0.177	0.207
P_evermanni.ELEB.DIM	0.714	0.049	0.620	0.635	0.681	0.713	0.747	0.793	0.813
P_evermanni.HALE.DIM	0.608	0.044	0.520	0.537	0.580	0.608	0.637	0.680	0.693
P_evermanni.MAGI.DIM	0.783	0.050	0.687	0.704	0.750	0.783	0.815	0.865	0.884
P_evermanni.SAMP.DIM	0.488	0.043	0.405	0.420	0.460	0.487	0.516	0.561	0.577
P_evermanni.WAI.DIM	0.711	0.047	0.621	0.634	0.680	0.711	0.742	0.787	0.805

Continued

Table 2.9 continued

P_evermanni.ELEB.POM	0.220	0.055	0.106	0.125	0.185	0.222	0.260	0.306	0.319
P_evermanni.HALE.POM	0.307	0.062	0.169	0.195	0.269	0.315	0.352	0.397	0.411
P_evermanni.MAGI.POM	0.161	0.050	0.062	0.078	0.127	0.161	0.195	0.242	0.254
P_evermanni.SAMPOM	0.422	0.063	0.285	0.307	0.384	0.428	0.466	0.513	0.527
P_evermanni.WAI.POM	0.218	0.056	0.099	0.121	0.182	0.222	0.256	0.302	0.316
P_evermanni.ELEB.Zoop	0.066	0.053	0.003	0.005	0.024	0.054	0.093	0.175	0.200
P_evermanni.HALE.Zoop	0.085	0.070	0.003	0.006	0.030	0.068	0.121	0.221	0.262
P_evermanni.MAGI.Zoop	0.056	0.044	0.002	0.005	0.022	0.047	0.080	0.143	0.167
P_evermanni.SAMP.Zoop	0.090	0.072	0.004	0.007	0.032	0.072	0.132	0.228	0.259
P_evermanni.WAI.Zoop	0.071	0.058	0.003	0.006	0.027	0.058	0.100	0.189	0.224

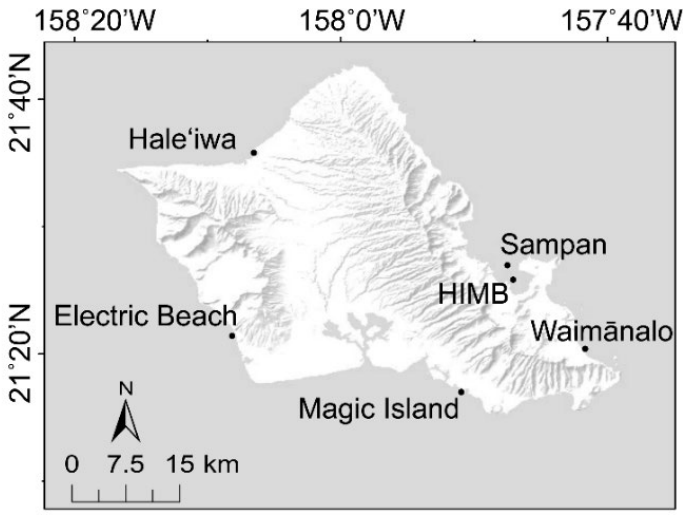

Figure 2.7. Coral collection sites surrounding O'ahu, HI. HIMB = Hawai'i Institute of Marine Biology. Specific coordinates of each site are listed in Table 2.2.

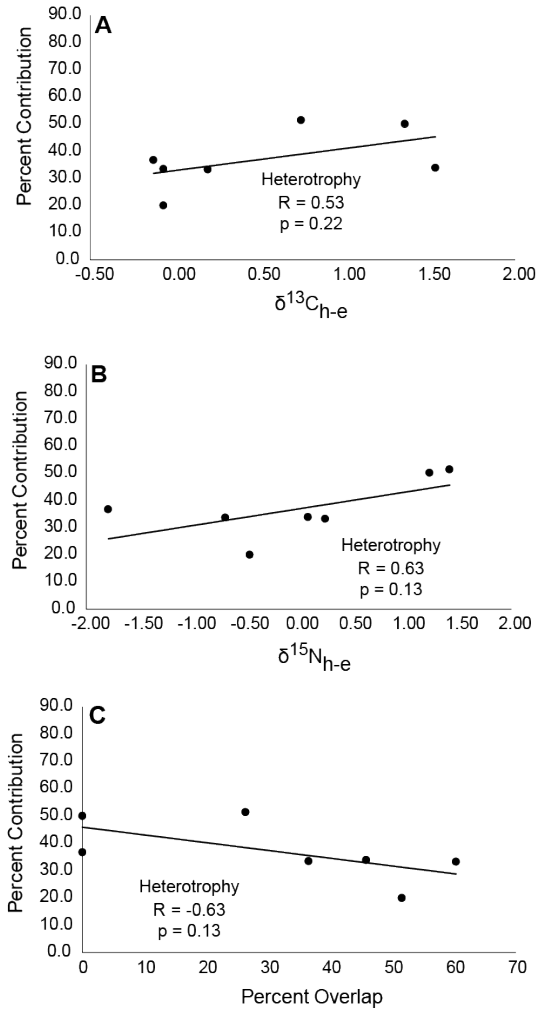

Figure 2.8. Pearson's correlations between the percent contribution of heterotrophic sources (POM + zooplankton) from Table 2.8 and species overall A) mean $\delta^{13}C_{h-e}$, B) mean $\delta^{15}N_{h-e}$, and C) SEA_C (percent overlap) calculated via SIBER. Each value represents the overall average for each species in the study.

Chapter 3: Effect of species, provenance, and coral physiology on the composition of Hawaiian coral-associated microbial communities

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3.1 Abstract

Increasing concentrations of atmospheric CO_2 are leading to elevated seawater temperatures and ocean acidification, which threatens the health and long-term survival of corals and the persistence of functional coral reef ecosystems. The resistance of corals to a rapidly changing climate has been linked to physiological parameters including heterotrophic capacity, levels of energy reserves, and shuffling of their endosymbiotic algal partners. Recently, the potential flexibility and diversity of coral-associated microbial communities has also been connected with coral health and resistance to environmental stress. This study uses the island of O‘ahu in Hawai‘i, USA, as a natural laboratory to explore variability in the microbial community composition of four coral species (*Porites compressa*, *Porites lobata*, *Pocillopora acuta*, *Pocillopora meandrina*) across a gradient of natural ocean conditions. In addition, we assessed potential relationships between the composition of the coral-associated microbial communities with coral physiology. We found that the microbial community composition differed greatly among all four coral species, as well as among several of the collection sites. However, microbial community assembly appeared to be governed by a combination of stochastic processes and coral physiology, rather than measured environmental conditions among the collection sites. Specifically, microbial community diversity decreased as the proportionate contribution of heterotrophy relative to photoautotrophy in coral tissues increased. These correlations provide a novel connection between measured coral physiology and the composition of the microbiome. Overall, our study suggests that an increase in coral heterotrophy expected with changing ocean conditions may co-occur

with a decrease in microbial community diversity in some coral species, possibly due to lower production of photosynthates. Such a relationship suggests that for some species, there may be a trade-off between heterotrophic capacity and microbial diversity – both of which are generally viewed as traits that confer resilience to climate change. Thus, for at least these coral species, microbial diversity and heterotrophic plasticity are not a consistent indicator of coral health in the face of climate change.

3.2 Introduction

Increasing concentrations of atmospheric CO_2 are leading to elevated seawater temperatures and ocean acidification, which threatens the health and long-term survival of corals and the persistence of functional coral reef ecosystems (e.g., Brown 1997; Hoegh-Guldberg et al. 2007). At the current rate of CO_2 emissions, models predict average temperature increases in tropical waters of 3°C, with a parallel increase in acidity of up to 150% by the year 2100 (IPCC 2019). The resistance of corals to stress associated with rapidly changing ocean conditions likely depends on numerous physiological factors, including levels of energy reserves (e.g., Grottoli et al. 2006; Rodrigues and Grottoli 2007; Anthony et al. 2009; Schoepf et al. 2013), heterotrophic capacity (e.g., Grottoli et al. 2006, 2017; Palardy et al. 2008; Houlbreque and Ferrier-Pages 2009; Hughes and Grottoli 2013; Levas et al. 2013), and shuffling of algal endosymbiont types (e.g., Abrego et al. 2008; Putnam et al. 2012; Grottoli et al. 2014). More recently, resistance to environmental stress in corals, particularly thermal stress, has also been linked to the structure and function of their microbiome (e.g., Bourne et al. 2016; Peixoto et al. 2017; Grottoli et al. 2018). Indeed, the composition of the coral-associated bacterial and archaeal communities (hereafter referred to collectively as microbial communities) has been connected to disease resistance (e.g., Ritchie 2006; Rosales et al. 2019), nutrient cycling (e.g., Lesser et al. 2007; Thurber et al. 2009; Littman et al. 2011; Rädecker et al. 2015), and potentially to the physiology of the coral host (Glasl et al. 2016; Grottoli et al. 2018). Understanding these connections between a coral and its microbial communities is of increasing importance, as these relationships may have fundamental roles in the health,

productivity, and persistence of coral reefs in the face of a changing climate (Bourne et al. 2016; Torda et al. 2017; Webster and Reusch 2017).

The coral holobiont (i.e. the coral host and its associated microbial communities) is diverse, and the composition of coral-associated microbial communities is often species-specific (Stat et al. 2012; Bourne et al. 2016; Grottoli et al. 2018) and variable across spatial and temporal scales (e.g., Rohwer et al. 2002; Salerno et al. 2016; Epstein et al. 2019; Wainwright et al. 2019). Specifically, there can be variability in the coral-associated microbial communities among reefs with disparate environmental conditions including naturally elevated temperatures (e.g., Ziegler et al. 2017; van Oppen et al. 2018), lower pH (Morrow et al. 2015), or increased water flow (Lee et al. 2017). For example, the coral *Porites lobata* in the Hawaiian archipelago is known to host microbial communities that are increasingly dissimilar as geographic separation increases, possibly relating to differences in temperatures among the sampled reefs (Salerno et al. 2016). It has also been suggested that the microbial communities associated with corals from sites with variable temperature conditions may confer thermal tolerance as seawater temperatures continue to increase (Ziegler et al. 2017; van Oppen et al. 2018). This mirrors studies of coral phenotype, which also show that corals from sites with variable temperature environments have improved potential to tolerate heat stress (e.g., Barshis et al. 2013; Kenkel and Matz 2017; Jury et al. 2019). However, the relationships between coral physiology and microbial community composition are poorly understood, particularly in the context of a changing climate.

To examine the relationships between coral-associated microbial communities with different coral species across a range of naturally occurring environmental conditions, we characterized the microbial communities associated with four species of Hawaiian corals at six sites surrounding the island of O‘ahu, Hawai‘i (HI). The sites varied in their environmental conditions providing a natural laboratory for evaluating the potential coral microbial community responses over a small geographic area. We hypothesized that coral-associated microbial communities differ among coral species and collection sites, and that the composition of those communities correlates with environmental conditions at each site.

To examine potential relationships between coral-associated microbes and coral physiology, we coupled the microbial community analyses with physiological measurements of the same corals, reported separately in McLachlan et al. (in prep). Previous work by Grottoli et al. (2018) showed that corals with stable microbial communities are also more physiologically resilient to experimentally induced temperature and pH stress, suggesting potential connections between the health of a coral and the composition of its microbiome. Therefore, we hypothesized that changes in coral-associated microbial community composition would correlate with specific coral physiology parameters. Although confirming connections between coral physiology and the microbiome may be key to improving our understanding of coral health future ocean conditions, this study is only the second to directly assess those potential relationships to date (Grottoli et al. 2018), and the first on naturally occurring corals that appear to be adapted to their respective environments (Jury and Toonen 2019). Further, this study

introduces ecological modeling as an approach that can be used to investigate both coral physiology and environmental parameters as potential drivers of coral-associated microbial community composition.

3.3 Methods

3.3.1 Study Sites

Corals were collected between 17 August and 13 November 2015 from six sites (Electric Beach, Haleʻiwa, Hawaiʻi Institute of Marine Biology [HIMB], Magic Island, Sampan Channel, and Waimānalo) surrounding the island of Oʻahu, HI (Fig. 3.3.1). The collection sites were chosen to represent the range of environmental conditions around Oʻahu, serving as a natural laboratory (Table 3.1). Mean annual sea surface temperature (SST), sea surface chlorophyll *a* concentration, and significant wave height were determined for each collection site throughout 2015. To calculate mean annual SST, mean daily SST values were first calculated from measured seawater temperature via buoys near each of the six collection sites. Mean SST was also calculated for the warmest period of the year (20 June – 10 October), hereafter referred to as mean summertime SST. Quality-controlled buoy data was downloaded from NOAA's National Data Buoy Center and the National Center for Environmental Information. Two adjustments to the buoy data were necessary to have complete datasets for 2015: (1) SST values for HIMB were also used for the Sampan Channel, as these sites are both within Kāneʻohe Bay and seawater temperature data for the Sampan Channel had inconsistent availability

throughout the year, and (2) mean daily SST values calculated for Electric Beach between 28 February and 17 April were also used for Magic Island, as seawater temperature at Magic Island was unavailable on those dates. Any other unavailable mean daily SST values were interpolated by averaging the next available previous and subsequent mean daily SST, however this approach was only needed for three or fewer days at each collection site. Weekly spatiotemporally composited chlorophyll *a* data were retrieved from NOAA Coral Reef Watch's Ocean Color at a resolution of 750 m via the Virtual Infrared Imaging Radiometer Suite (VIIRS) aboard the Suomi National Polar-orbiting Partnership (S-NPP) satellite. Any unavailable weekly chlorophyll *a* data were interpolated by averaging the next available previous and subsequent weekly composited measurements. Significant wave height was collected at weekly intervals from a Simulated Waves Nearshore (SWAN) hindcast regional model for O'ahu (Arinaga & Cheung 2012), to be used as a proxy for near-bed shear stress on the seafloor and averaged to produce an annual average wave height value.

3.3.2 Coral Collection

Samples of four coral species (*Porites compressa*, *Porites lobata*, *Pocillopora acuta*, and *Pocillopora meandrina*) were collected at a depth of 0.5 – 5 m (Table 3.2). A 5 – 10 cm ramet (branch or mound) was removed underwater via hammer and chisel from healthy parent colonies separated by at least 5 m to minimize the possibility of selecting corals of the same genet. Corals were only sampled from sites where they were relatively abundant, and therefore not all coral species were sampled at every site. The

coral ramets were subsequently frozen at the HIMB and later shipped to The Ohio State University (OSU) where they were stored at -80˚C.

3.3.3 Sample Processing for Microbial Analyses

In the lab at OSU, a small subsample (approximately 1 – 2 cm) of the collected coral ramet was removed via hammer and a sterile chisel for microbial community characterization. Bulk coral tissue for each subsample was removed from the skeleton by airbrushing with autoclaved ultrapure 0.22 µm filtered fresh water. DNA was extracted from the resulting slurry using PowerSoil DNA Isolation kits (Qiagen, Hilden, Germany) following the manufacturer protocol. Successful extraction of genomic DNA was confirmed using a Qubit fluorometer prior to amplification of the V5-V6 region of the 16S rRNA gene using the primers CS1_784F and CS2_1061R (forward: 5'-AGGATTAGATACCCTGGTA-3'; reverse: 5'-CRRCACGAGCTGACGAC-3'). These primers included CS1 and CS2 linkers to allow the downstream application of adapter sequences and sample-specific barcodes. Polymerase chain reaction (PCR) was completed in two stages. Stage one PCR used Amplitaq Gold 360 DNA polymerase (Thermo Fisher Scientific, Waltham, Massachusetts, USA) in 25 µl reaction volumes. Stage one PCR cycling conditions were as follows: 15 min at 95 °C, followed by 28 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, with a final extension time of 10 min. Successful amplification was visualized via gel electrophoresis. Stage two PCR used MyTaq HS mastermix (Bioline, Memphis, Tennessee, USA) in 20 µl reaction volumes and cycling conditions were as follows: 95 °C for 5 minutes, followed by 8

cycles of 95 °C for 30 seconds, 60 °C for 30 seconds, and 68 °C for 30 seconds. A final elongation period was performed at 68 °C for 7 minutes. These amplicons were subsequently prepared for multiplexed sequencing on an Illumina MiniSeq system (2 x 151 base pairs, mid-output). The second stage of the PCR process and the Illumina sequencing were completed by the DNA Services Facility at the University of Illinois at Chicago.

Reads produced by Illumina sequencing were processed using the QIIME software package version 1.9 (Caporaso et al. 2010). Within QIIME, forward and reverse reads were joined, while chimeras were removed via USEARCH and a quality threshold of 20 was set to filter remaining sequences. Operational taxonomic units (OTUs) were assigned based on release 132 of the Silva ribosomal database and were clustered at 97% similarity via UCLUST. These OTUs were retained only if present in greater than 10% of samples. Any OTUs which were identified as chloroplast, mitochondria, or eukaryotic in origin were removed from further analyses, in addition to laboratory contaminants confirmed via sequenced negative PCR controls. Prior to diversity analyses, three samples with final read counts below 1,000 were also removed to limit the consideration of samples with low sequencing depth. All raw unprocessed reads are available on NCBI's Sequence Read Archive under accession number PRJNA645694.

3.3.4 Physiological Analyses

A suite of physiological analyses were conducted as part of a separate publication (McLachlan et al. 20xx): tissue biomass, total chlorophyll (chlorophyll *a* and c_2), total

soluble lipid concentration, total soluble protein concentration, and the stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) of both the coral host and its endosymbiotic algae, Symbiodiniaceae. The difference between both $\delta^{13}C$ and $\delta^{15}N$ of the coral host and its endosymbiotic algae (i.e., $\delta^{13}C_{h\text{-}e}$ and $\delta^{15}N_{h\text{-}e}$), was calculated to assess the relative contribution of heterotrophically and photosynthetically acquired carbon and nitrogen (Muscatine et al. 1989; Rodrigues and Grottoli 2006; Nahon et al. 2013). The complete details of the analytical methods are in Price et al. (2020).

3.3.5 Statistical Analyses

All analyses were performed using R software package version 3.5.0 (R Core Team 2015) and PRIMER v6 (Clarke and Gorley 2006). Statistical significance was defined as $\alpha = 0.05$. Alpha diversity of microbial communities among coral species and collection sites was measured via the number of observed OTUs, Shannon's Diversity Index, Pielou's evenness (Pielou 1966), and Faith's phylogenetic diversity (Faith 1992). Normality and homoscedasticity assumptions for parametric analyses were unable to be met, therefore all alpha diversity metrics were compared via a Kruskal-Wallis one-way analysis of variance with a Dunn's post hoc test. Beta diversity was visualized using weighted non-metric multidimensional scaling (NMDS) plots calculated with a Bray-Curtis dissimilarity. Beta diversity data were compared among coral species and collection sites via an analysis of similarities (ANOSIM). The ANOSIM produces both a p-value for testing statistical significance and an R-value between 0 and 1, where a higher R-value indicates a more dissimilar microbial community composition between groups.

Similarity percentage analyses (SIMPER) were used to identify the microbial OTUs that differed most in relative abundance among coral species and collection sites.

Null models using OTU data were performed to assess relationships between phylogeny of the coral microbiome and potential controls on that microbial community composition (i.e., environmental conditions and coral physiology). As outlined in Stegen et al. (2015) and Danczak et al. (2016) the β-mean nearest taxon distance (βMNTD) was calculated for each possible pairwise comparison between samples of the same coral species in order to better understand the potential controls on microbial community composition. Using 999 community randomizations to create null models, β-nearest taxon index (βNTI) was calculated to determine the deviation of the observed βMNTD from the null βMNTD. The resulting βNTI values were then used to predict whether deterministic (i.e., selection) or stochastic (i.e., random) processes shape the community. If the resulting βNTI value is > 2 or is < -2, a deterministic process is most likely responsible for differences between microbial communities in two samples. Conversely, if a βNTI value is between 2 and –2, a stochastic process better explains observed differences in microbial community composition between two samples.

Stochastic processes can be further classified as either dispersal limitation, homogenizing dispersal, or ecological drift using the Raup-Crick metric with a Bray-Curtis dissimilarity matrix (RC_{BC}) (Stegen et al. 2015). The RC_{BC} metric probabilistically assembles 999 iterations of microbial communities from each of the sampled communities, providing a null distribution of Bray–Curtis values to assess compositional turnover. The deviation between the observed Bray–Curtis and the null distribution was

then standardized to provide a value between 1 and –1. Values > 0.95 and < –0.95 are interpreted as statistically significant departures from drift (i.e. chance events), such that values > 0.95 suggest dispersal limitation between sampled communities supported by drift (i.e. spatial turnover between these communities is greater than expected by chance alone) and values < –0.95 suggest homogenizing dispersal between sampled communities (i.e. communities are homogenized and turnover is lower than by drift alone). Since RC_{BC} values did not satisfy the ANOVA assumption of normality, non-parametric Kruskal-Wallis one-way analysis of variance and post hoc Dunn's tests were used to test for differences in the RC_{BC} metric among coral species. Coral physiological parameters and environmental parameters at each site were then tested for correlations with βNTI values to elucidate which factors may have been responsible for observed differences in microbial community structures across coral ramets and collection sites in O'ahu, HI. Distance matrices were calculated for each physiological and environmental variable, and then correlations were performed using Spearman's rank-order correlation specification in Mantel tests (*mantel*, ecodist package v2.0.1). Spearman's rank-order correlations were also used to test for relationships between the previously described alpha diversity metrics and the physiological and environmental measurements associated with each coral ramet.

3.4 Results

Overall, there were 981 OTUs across the 1,674,981 reads included in this analysis

of microbial communities from the four coral species collected around O‘ahu. OTUs affiliated with the orders Oceanospirillales and Rhodobacterales were the most abundant among all corals, but their relative abundance varied among coral species (Fig. 3.2). The order Oceanospirillales were found to be most abundant among *Porites compressa* and *Porites lobata* corals (63.0% and 31.7%, respectively), with the genus *Endozoicomonas* comprising approximately 97% of those observations within the Oceanospirillales. Among *Pocillopora acuta* corals, OTUs affiliated with the order Propionibacteriales had the highest relative abundance (21.3%) followed by Lactobacillales (18.4%). Sequences matching taxa within the Rhodobacterales were most the most abundant order among *Pocillopora meandrina* corals (27.0%), followed by Propionibacteriales (15.1%).

Closer examination at the OTU level revealed that all four coral species hosted significantly different microbial communities from each other, though greater dissimilarity in community composition was found between the *Porites* and *Pocillopora* genera (Fig. 3.3, Table 3.4). Further, SIMPER analyses revealed that bacteria from the genus *Endozoicomonas* were the primary contributor to differences among all coral species’ bacterial communities, but the genera *Streptococcus* and *Propionibacterium* contributed most to differences between the two *Pocillopora* corals (Table 3.5). Both *Pocillopora* corals were also found to have significantly lower numbers of observed OTUs and Faith’s PD than *Porites* corals, but *Porites compressa* had lower mean evenness than the other coral species due to the high relative abundances of the bacterial genus *Endozoicomonas* (Table 3.3). There were no differences in Shannon’s Diversity Index among the coral species (Table 3.3).

Considering each species individually, *Porites compressa*, *Porites lobata*, and *Pocillopora acuta* all hosted microbial communities that differed between at least two collection sites (Fig. 3.4 and Table 3.6A–C). For example, microbial communities associated with *Porites compressa* collected from HIMB differed from those at all other sites except the neighboring Sampan Channel, and those from Magic Island differed from all other sites. *Porites lobata* corals collected from Haleʻiwa hosted microbial communities that differed significantly in composition from all sites except the Sampan Channel. *Pocillopora acuta* also hosted distinct microbial communities in Haleʻiwa, differing significantly from all collection sites except HIMB. Finally, *Pocillopora meandrina* microbial communities did not differ among any sites.

Ecological null modeling using βNTI revealed that the microbial communities of corals surrounding Oʻahu were largely controlled by stochastic processes (70.6% of all pairwise comparisons had a βNTI between 2 and –2) and variable selection to a lesser extent (29.4% of all pairwise comparisons had a βNTI > 2). Although all coral species had some microbial community comparisons with βNTI values above the variable selection threshold of 2, the median βNTI values were between 2 and –2 for all coral species and collection sites (Fig. 3.5). The Raup-Crick Bray-Curtis (RC_{BC}) values differed between *Porites lobata* and the other three coral species (Kruskal-Wallis, $p < 0.001$). *Porites lobata* had a mean value of 0.53 ± 0.58, suggesting that microbial communities associated with that coral were often controlled by a combination of random ecological drift and limited dispersal potential (Fig. 3.4B). Conversely, mean RC_{BC} values for *Porites compressa*, *Pocillopora acuta*, and *Pocillopora meandrina* were lower,

at 0.17 ± 0.64, 0.09 ± 0.66, and 0.06 ± 0.71, respectively, suggesting the microbial communities associated with these corals were governed primarily by ecological drift (Fig. 3.4 A, C, D).

Although the mean βNTI values suggest that stochastic processes are the dominant controller of microbial community assembly, there were several significant relationships between coral physiology and microbial community diversity (Table 3.7 & 3.8). Indeed, the $\delta^{13}C_{h\text{-}e}$ values of both *Porites compressa* and *Pocillopora acuta* were significantly related to the βNTI values, such that pairs of corals with more dissimilar $\delta^{13}C_{h\text{-}e}$ values have higher βNTI values (Table 3.7A&C). Similarly, the number of observed OTUs, Shannon's diversity, and Faith's PD increased with $\delta^{13}C_{h\text{-}e}$ values for *Pocillopora acuta*, while the microbial communities of *Porites compressa* show a trend of increasing Shannon's diversity and evenness with increasing $\delta^{13}C_{h\text{-}e}$values (Table 3.8A&C). The $\delta^{15}N_{h\text{-}e}$ of *Porites lobata* also had a significant positive correlation with βNTI, such that pairs of corals with more dissimilar $\delta^{15}N_{h\text{-}e}$ values have higher βNTI values (Table 3.7B). Similarly, the number of observed OTUs and Faith's PD increased with higher $\delta^{15}N_{h\text{-}e}$ for *Porites lobata* (Table 3.8B). Pielou's evenness also increased in *Porites lobata* with higher lipid levels, while Faith's PD decreased with higher lipid levels. Finally, only biomass of *Pocillopora meandrina* corals correlated positively with βNTI, such that pairs of corals with more dissimilar biomass had greater βNTI values (Table 3.7). There was no relationship between the alpha diversity metrics and biomass for *Pocillopora meandrina*, but Shannon's Diversity and Pielou's evenness did increase with greater chlorophyll and protein levels, respectively (Table 3.8). Interestingly, unlike

Porites lobata, Shannon's Diversity significantly decreased with greater $\delta^{15}N_{h\text{-}e}$ values for *Pocillopora meandrina* (Table 3.8).

Among the environmental parameters, mean significant wave height was found to have a significant negative relationship with the βNTI values only for microbial communities of *Porites compressa* (Table 3.7A). No measures of alpha diversity were found to correlate significantly with any environmental parameters (Table 3.8).

3.5 Discussion

Here, we find that the microbial community composition differed greatly among all four coral species and among some sites within species (Fig. 3.3 & 3.4). Further, several parameters of coral physiology related to observed differences in microbial community composition, rather than environmental conditions alone.

The microbial community composition of the four coral species differed primarily in their relative abundance of the bacterial genus, *Endozoicomonas*. The very low relative abundance of *Endozoicomonas* sp. in both *Pocillopora* corals (Fig. 3.2) is surprising, given the dominance of *Endozoicomonas* sp. in many other tropical corals, including other Pocilloporids (Bayer et al. 2013; Pogoreutz et al. 2018; Wainwright et al. 2019). However, the most abundant bacterial group in *Pocillopora acuta* was found to be *Propionibacterium* sp. (Fig. 3.2). While not typically found in the high abundances seen in this study (21.2% and 15.1% for *Pocillopora acuta* and *Pocillopora meandrina*, respectively, see Fig. 3.2), the *Propionibacterium sp.* are generally widespread in the

coral microbiome, found consistently as members of the core microbiome across large geographic scales (Ainsworth et al. 2015; Sweet et al. 2017). It is possible that the proximity of the collection sites to community beaches and terrestrial runoff around O‘ahu could affect the two *Pocillopora* corals differently the *Porites* corals, leading to a higher relative abundance of these Firmicute bacteria in the two *Pocillopora* corals and low relative abundance of *Endozoicomonas sp.* (Yang et al. 2017). Conversely, the high relative abundance of *Endozoicomonas sp.* in *Porites* corals and the Rhodobacterales in all four coral species is congruent with numerous past studies of the coral microbiome (Bayer et al. 2013; Glasl et al. 2016; Morrow et al. 2018; Pogoreutz et al. 2018). The *Endozoicomonas sp.* in particular, are thought to assist with nutrient acquisition via nitrogen and carbon cycling, as well as structuring of the microbiome through regulation of bacterial colonization (Neave et al. 2016). While the exact roles of these bacteria are unclear and may vary among hosts, their high abundance is generally linked with healthy corals (Bayer et al. 2013; Neave et al. 2016; Pogoreutz et al. 2018). Though corals need not have these bacteria to be healthy (Grottoli et al. 2018), corals with lesions, disease, or under environmental stress often exhibit low abundances of *Endozoicomonas* (Vezzulli et al. 2013; Meyer et al. 2014; Ziegler et al. 2016), suggesting that the *Pocillopora* corals in O‘ahu may be more susceptible to future changes in ocean conditions than the *Porites* corals.

While we found the greatest variability in microbial community composition to exist among coral species, differences also existed across some of the collection sites within three of the four coral species (Fig. 3.4, Table 3.6.B). For instance, in both *Porites*

species and *Pocillopora acuta*, corals collected from Hale‘iwa often hosted microbial communities that differed from the other sites (Table 3.6.B), suggesting that either spatial separation between sites or environmental conditions, such as the high significant wave height at Hale‘iwa, could be influencing microbial community composition. However, the absence of correlations between microbial alpha diversity and environmental parameters measured in this study suggest that environmental filtering likely plays a minor role in microbial community assembly around O‘ahu (Table 3.8). Furthermore, the βNTI null modeling also revealed a lack of correlations between microbial community assembly and environmental conditions, with the exception of a negative correlation between the βNTI of communities associated with *Porites compressa* and significant wave height (i.e., the microbial communities are more *dissimilar* as significant wave heights are more *similar*, possibly resulting from variability of the coral microbiome within each site). Overall, these results may suggest that either: (1) the measured environmental parameters surrounding O‘ahu are measured at too low of a resolution to effectively detect effects on the composition of the coral-associated microbial communities, (2) the community assembly is controlled by other environmental factors not included in this study, (3) there are species-specific effects, or 4) some combination of all three.

Indeed, the lack of consistent differences in microbial community composition among collection sites is likely driven by multiple variables, including stochastic processes. We found that stochastic processes control most variability in microbial community composition within each coral species, and that the dominant type of

stochastic process is species-specific. Mean RC_{BC} values for *Porites lobata* were positive, trending towards greater than expected microbial community turnover and a more divergent composition among these sampled communities, driven by a combination of ecological drift and dispersal limitation (Fig. 3.6). Conversely, the mean RC_{BC} values for the microbial communities associated with *Porites compressa* and the two *Pocillopora* corals were closer to zero, suggesting that turnover in these communities is no more or less random than expected on average and are controlled primarily by random ecological drift. While stochastic processes are known to drive some variability in the stressed coral microbiome (Adair and Douglas 2017; Zaneveld et al. 2017), our study found that this is also the case in corals which appear otherwise healthy. Therefore, ecological drift over time in these coral-associated microbial communities could explain some of the divergence among sites, rather than any specific environmental variable. While this may limit our ability to predict the performance of specific coral-associated microbial communities in the face of a changing climate, there are clear differences among the four coral species in this study that may still confer benefits, such as the high relative abundance of *Endozoicomonas sp.* in the two *Porites* corals.

However, stochastic processes are unlikely to be the sole governing factor leading to differences in microbial communities among these corals. In the absence of weak environmental controls on coral microbiome assembly, we instead identified associations between coral-associated microbial communities and coral physiological traits. For *Porites compressa*, *Pocillopora acuta*, and *Pocillopora meandrina* we found consistent significant relationships between microbial community composition and $\delta^{13}C_{h\text{-}e}$ or $\delta^{15}N_{h\text{-}}$

$_{e}$, which are proxies for the proportionate contribution of heterotrophically and photoautotrophically derived organic matter to coral tissues (Muscatine et al. 1989; Grottoli et al. 2006; Nahon et al. 2013; Conti-Jerpe et al. 2020). $\delta^{13}C_{h\text{-}e}$ positively correlated with βNTI and several alpha diversity metrics of the microbial communities associated with *Porites compressa* and *Pocillopora acuta*, although these relationships were weaker in *Porites compressa* (Tables 3.7 & 3.8). Specifically, this suggests that microbial diversity decreased with a greater proportionate contribution of heterotrophy to coral tissues (e.g., Muscatine et al. 1989; Grottoli et al. 2006). The $\delta^{15}N_{h\text{-}e}$ correlated negatively with both the Shannon's diversity and Faith's PD of the microbial communities associated with *Pocillopora meandrina*, suggesting a similar decrease in microbial diversity with greater heterotrophic contribution (Table 3.8). These relationships with $\delta^{13}C_{h\text{-}e}$ or $\delta^{15}N_{h\text{-}e}$ suggest that nutritional sources to the coral and nutrient cycling within the coral are potentially controlling factors in the composition of the microbiome. *Porites compressa* is known to have a moderate baseline heterotrophic contribution from feeding on zooplankton, amounting to approximately 25% of daily metabolic demand (Grottoli et al. 2006; Palardy et al. 2008). Although greater microbial diversity is not necessarily a universal sign of good health in the coral holobiont (Pratte et al. 2018), our results suggest that all species, except *Porites lobata,* host more diverse microbial communities as the relative contribution of heterotrophically derived organic matter to coral tissues decreases (i.e. higher $\delta^{13}C_{h\text{-}e}$ and lower $\delta^{15}N_{h\text{-}e}$ values). This could be due to greater compensation with photosynthesis at lower heterotrophic rates, leading to a greater release of photosynthates by the endosymbiotic algae, which are known to be

metabolized by members of the coral microbiome (e.g. *Endozoicomonas sp.*, *Alteromonas sp.*, etc.) (Bourne et al. 2013; Neave et al. 2016).

In contrast to *Pocillopora meandrina*, alpha diversity of the microbial communities of *Porites lobata* actually increased with greater $\delta^{15}N_{h\text{-}e}$ values (i.e. higher proportionate contribution of nitrogen to tissues from heterotrophic sources, see Conti-Jerpe et al. 2020). Interestingly, *Pocillopora meandrina* and *Porites lobata* both hosted the greatest relative abundances of bacteria from the family Rhodobacteraceae, which are often associated with nitrogen fixation in corals (Lesser et al. 2018). These opposing relationships suggest that nitrogen incorporation by corals affects microbial diversity, but the pattern is not consistent among coral species. Therefore, contrary to the other three coral species, *Porites lobata* could exhibit a concomitant increase in microbial diversity with increased heterotrophy. Although increased microbial diversity with a greater proportionate contribution of heterotrophy may relate to the resistance of *Porites lobata* to the stresses of a changing climate (Rodgers et al. 2017), it is unclear what factors might be responsible for this increase in diversity while the other three coral species showed the opposite relationship.

Overall, we found that the four Hawaiian corals in this study host distinct microbial communities, with even greater separation among coral genera. While the coral-associated microbial communities also differed among collection sites around the island of O‘ahu, it was likely not because of local environmental conditions but rather due to the isolation of each population from each other and ecological drift or other stochastic processes. Within each coral species, we further discovered that the nutritional

sources accessed by a coral (i.e., photoautotrophic vs heterotrophic) may partially govern the structure of its microbial community. This structuring has important implications for corals in a changing climate, as heterotrophic capacity and plasticity are posited as key factors in the potential resilience of corals to rising seawater temperatures (Grottoli et al. 2006, 2017; Palardy et al. 2008). As corals increase heterotrophy to support reduced photosynthesis rates or higher energy demands in times of thermal stress, our study suggests that the decrease in observed microbial diversity may simply be an artifact of that shift in resource use. Therefore, decreases in microbial diversity are not necessarily an indication that the coral is stressed, but instead a consequence of the attempt to acclimate. However, not all corals shared the same relationship between microbial community composition, environmental conditions, and coral physiology, indicating a high degree of species specificity in how coral-associated microbial communities respond to the chronic pressures of climate change.

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Table 3.1. Summary of environmental conditions at the six coral collection sites surrounding O‘ahu, HI. Means are shown ± 1 SD. HIMB = Hawai‘i Institute of Marine Biology

Collection Site	Coordinates	Mean Annual SST[a] (˚C)	Mean Summertime SST (˚C)	Mean Annual Chl a[b] (mg m-3)	Mean Annual Significant Wave Height[c] (m)
HIMB	21° 26' 3.35" N, 157° 47' 12.53" W	26.14 ± 2.00	28.44 ± 1.04	4.05 ± 2.77	0.18 ± 0.07
Sampan	21° 27' 3.60" N, 157° 47' 45.71" W	26.14 ± 2.00	28.44 ± 1.04	3.31 ± 0.91	0.42 ± 0.10
Magic Island	21° 17' 10.00" N, 157° 51' 2.00" W	26.19 ± 1.35	27.68 ± 0.75	0.50 ± 0.28	0.80 ± 0.30
Electric Beach	21° 21' 16.33" N, 158° 6' 15.37" W	26.34 ± 1.40	27.85 ± 0.75	0.37 ± 0.21	0.65 ± 0.30
Hale‘iwa	21° 35' 39.09" N, 158° 6' 38.69" W	25.80 ± 1.19	27.06 ± 0.79	1.83 ± 1.77	0.98 ± 0.40
Waimānalo	21° 19' 42.00" N, 157° 40' 59.00" W	26.00 ± 1.41	27.55 ± 0.89	1.44 ± 0.51	0.86 ± 0.17

[a]SST values were calculated using quality-controlled buoy data available from NOAA’s National Data Buoy Center and the National Center for Environmental Information

[b]Chlorophyll *a* measurements were composited at a 750-m resolution from the VIIRS instrument by NOAA Coral Reef Watch and NOAA/NESDIS Ocean Color Team

[c]Significant wave heights were extracted from the SWAN hindcast model (Arinaga & Cheung 2012)

Table 3.2. Number of coral ramets analyzed for microbial community composition from each collection site surrounding O‘ahu, HI. Corals were not collected from sites where they were not sufficiently abundant. HIMB = Hawai‘i Institute of Marine Biology,

Collection Sites	***P. compressa***	***P. lobata***	***P. acuta***	***P. meandrina***
HIMB	6	-	4	-
Sampan	6	6	5	6
Magic Island	4	6	5	4
Electric Beach	-	6	-	-
Hale‘iwa	6	6	6	2
Waimānalo	6	6	6	6
Total	28	30	26	18

Table 3.3. Summary of coral-associated microbial community alpha-diversity metrics. Significant statistical differences ($p < 0.05$) among groups indicated by letters.

Species	Observed OTUs	Shannon's Diversity	Pielou's Evenness	Faith's PD
Porites compressa	454.63 ± 177.20^{a}	2.80 ± 1.32^{a}	0.46 ± 0.19^{a}	16.39 ± 4.72^{a}
Porites lobata	279.70 ± 180.17^{b}	3.26 ± 1.14^{a}	0.61 ± 0.20^{b}	10.60 ± 5.32^{b}
Pocillopora acuta	66.23 ± 56.26^{c}	2.68 ± 0.98^{a}	0.68 ± 0.16^{b}	3.68 ± 2.08^{c}
Pocillopora meandrina	84.00 ± 96.30^{c}	2.67 ± 0.59^{a}	0.69 ± 0.10^{b}	4.53 ± 3.40^{c}

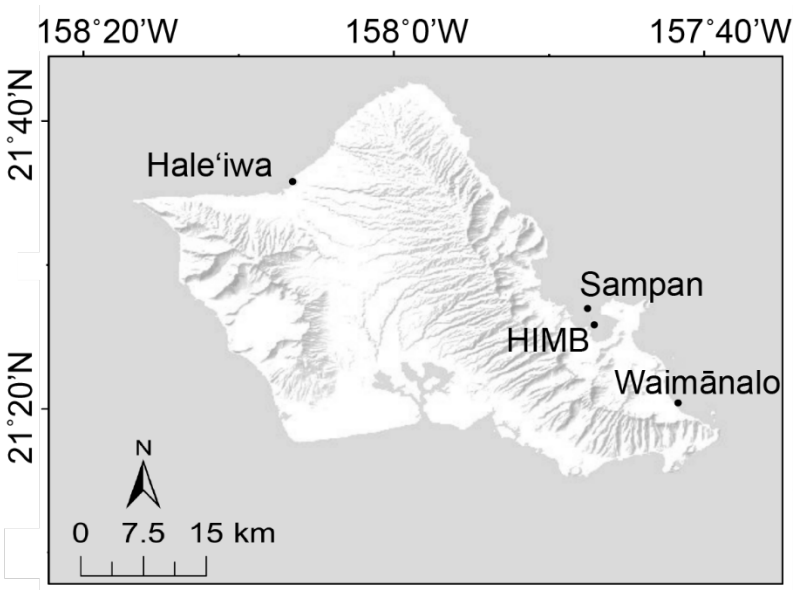

Figure 3.1. Coral collection sites surrounding O'ahu, Hawai'i. HIMB = Hawai'i Institute of Marine Biology. Specific coordinates of each site are listed in Table 3.1.

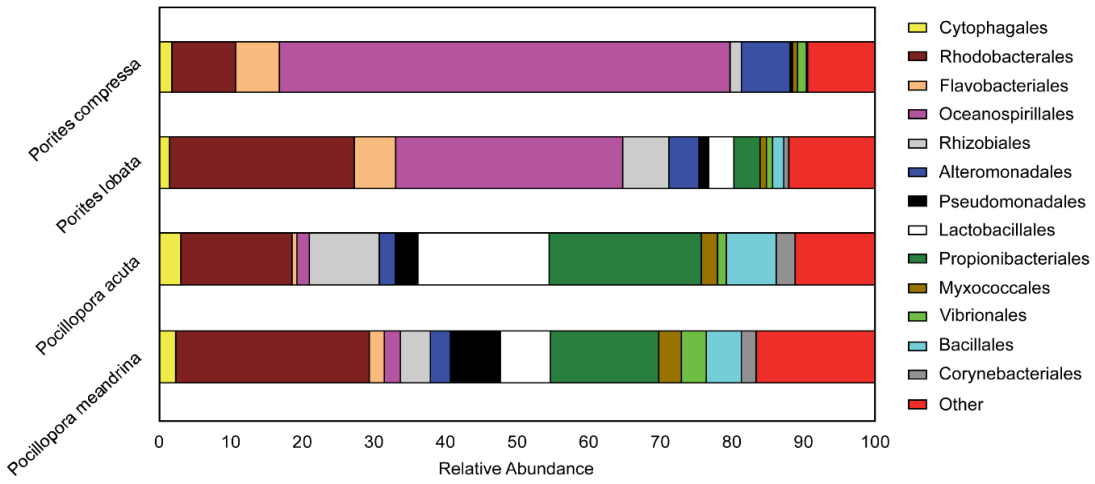

Figure 3.2. Relative abundances of microbial community members by coral species. Microbial Orders with less than 2.5% mean relative abundance in at least one coral species are excluded from this plot.

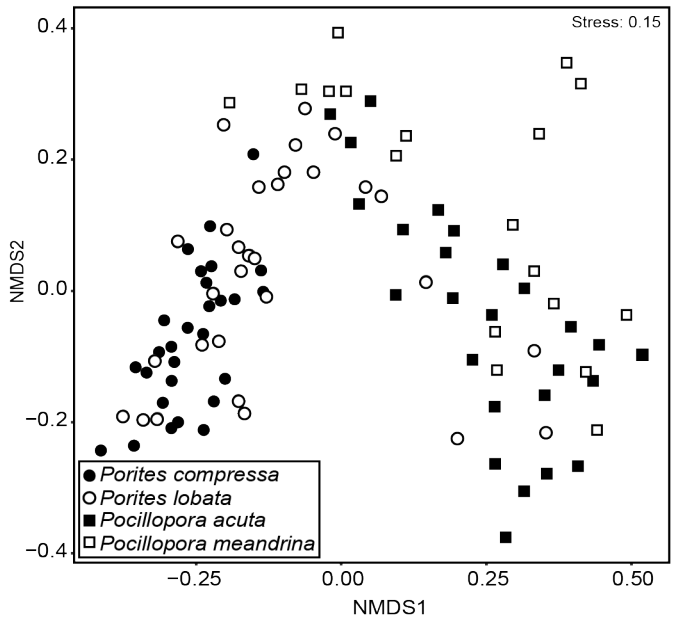

Figure 3.3. Non-metric multidimensional scaling (NMDS) plot of microbial community composition of *Porites compressa* (closed circle), *Porites lobata* (open circle), *Pocillopora acuta* (black square), and *Pocillopora meandrina* (open square) coral collected from sites surrounding O‘ahu, HI. Species significantly differed from each other, and the *Pocillopora* differed from the *Porites* more than from each other and vice versa (see ANOSIM results in Table 3.4).

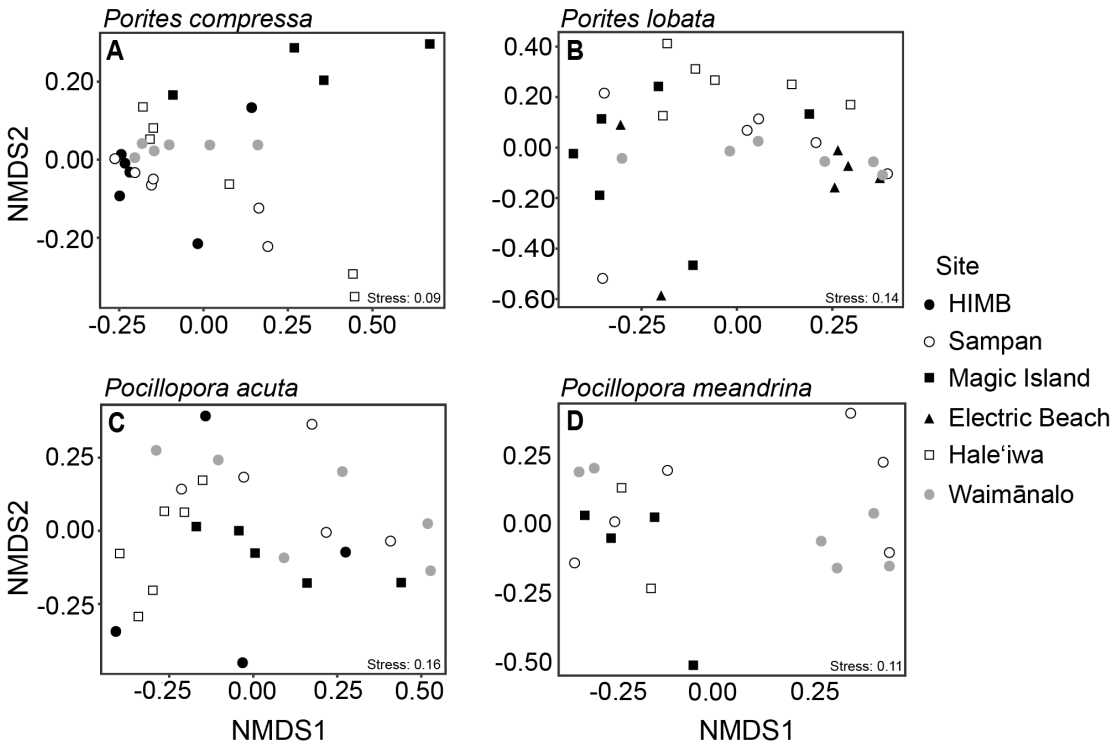

Figure 3.4. Non-metric multidimensional scaling (NMDS) plot of microbial community composition of (A) *Porites compressa*, (B) *Porites lobata*, (C) *Pocillopora acuta*, and (D) *Pocillopora meandrina* coral collected from sites surrounding O'ahu, HI. See full ANOSIM results in Table 3.6.

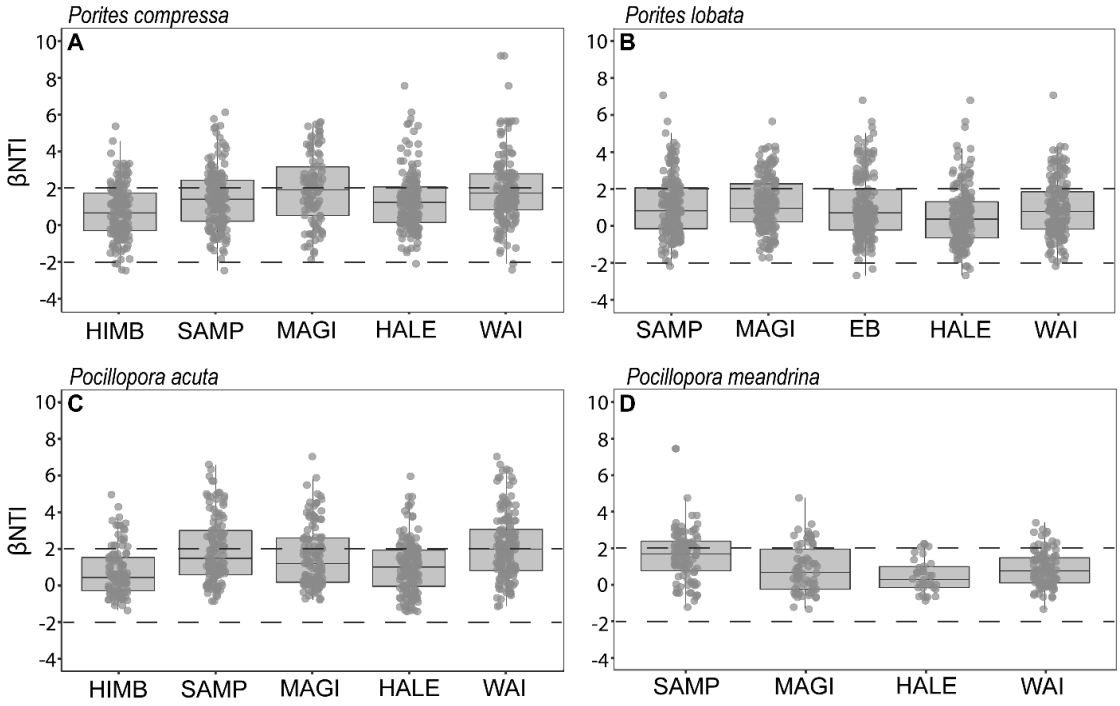

Figure 3.5. β-nearest taxon index (βNTI) of microbial communities for each collection site for A) *Porites compressa*, B) *Porites lobata*, C) *Pocillopora acuta*, and D) *Pocillopora meandrina*. Collection sites are abbreviated as follows: HIMB = Hawai‘i Institute of Marine Biology, SAMP = Sampan Channel, MAGI = Magic Island, EB = Electric Beach, HALE = Hale‘iwa, and WAI = Waimānalo.

3.8 Supporting Information

Table 3.4. Summary of pairwise one-way ANOSIM statistics among coral species across all collection sites.

Pairwise Group Comparison	**R- Statistic**	**P-value**
Porites compressa – Porites lobata	0.182	**0.001**
Porites compressa – Pocillopora acuta	0.876	**0.001**
Porites compressa – Pocillopora meandrina	0.777	**0.001**
Porites lobata – Pocillopora acuta	0.495	**0.001**
Porites lobata – Pocillopora meandrina	0.439	**0.001**
Pocillopora acuta – Pocillopora meandrina	0.152	**0.005**

Table 3.5. Similarity Percentage (SIMPER) analysis output for coral-associated microbial communities. Operational taxonomic units (OTUs) included up to 15% cutoff for cumulative contribution to dissimilarity between coral species. UG = Unclassified Genus

A) *Porites compressa* & *Porites lobata*

Average dissimilarity = 69.74

Bacterial OTU	*P. compressa* Average Abundance (%)	*P. lobata* Average Abundance (%)	Average Dissimilarity (%)	Cumulative Contribution to Dissimilarity (%)
Endozoicomonas sp.	5.98	2.81	2.25	3.23
Endozoicomonas sp.	4.09	3.14	1.51	5.39
Rhodobacteraceae;__	1.00	1.71	0.64	6.32
Streptococcus sp.	0.21	0.67	0.48	7.00
Propionibacterium sp.	0.20	0.67	0.46	7.67
Rhodobacteraceae;__	0.49	1.02	0.45	8.32
Roseobacter sp.	0.60	0.96	0.43	8.94
Pseudoalteromonadaceae;__	0.79	0.28	0.40	9.51
Propionibacterium sp.	0.28	0.65	0.39	10.07
Rhodobacteraceae;__	0.64	0.66	0.37	10.59
Rhodobacteraceae;__	0.39	0.81	0.36	11.10
Photobacterium sp.	0.74	0.54	0.35	11.60
Staphylococcus sp.	0.11	0.45	0.33	12.08
Rugeria sp.	0.62	0.81	0.33	12.54
Tenacibaculum sp.	0.58	0.41	0.32	13.01
Rhodobacteraceae;__	0.27	0.59	0.31	13.46
Phyllobacteriaceae;__	0.21	0.62	0.30	13.88

Continued

Table 3.5 continued

Thalassomonas sp.	0.52	0.34	0.29	14.30
Tenacibaculum sp.	0.52	0.36	0.29	14.71
Tenacibaculum sp.	0.41	0.37	0.28	15.12

B) *Porites compressa* & *Pocillopora acuta*

Average dissimilarity = 90.84

Bacterial OTU	***P.compressa* Average Abundance (%)**	***P. acuta* Average Abundance (%)**	**Average Dissimilarity (%)**	**Cumulative Contribution to Dissimilarity (%)**
Endozoicomonas sp.	5.98	0.35	4.41	4.85
Endozoicomonas sp.	4.09	0.12	3.05	8.21
Propionibacterium sp.	0.28	2.91	1.99	10.40
Streptococcus sp.	0.21	2.42	1.82	12.40
Propionibacterium sp.	0.20	2.27	1.64	14.21
Staphylococcus sp.	0.10	1.45	1.02	15.34

C) *Porites lobata* & *Pocillopora acuta*

Average dissimilarity = 84.80

Bacterial OTU	***P. lobata* Average Abundance (%)**	***P. acuta* Average Abundance (%)**	**Average Dissimilarity (%)**	**Cumulative Contribution to Dissimilarity (%)**
Endozoicomonas sp.	3.14	0.12	2.50	2.95
Endozoicomonas sp.	2.81	0.35	2.18	5.52
Streptococcus sp.	0.67	2.42	2.05	7.93
Propionibacterium sp.	0.65	2.91	1.90	10.17

Continued

Table 3.5 continued

Propionibacterium sp.	0.67	2.27	1.74	12.23
Rhodobacteraceae;__	1.71	1.23	1.10	13.53
Staphylococcus sp.	0.31	1.45	1.08	14.80
Staphylococcus sp.	0.45	0.95	0.91	15.87

D) *Porites compressa* & *Pocillopora meandrina*

Average dissimilarity = 88.95

Bacterial OTU	***P. compressa* Average Abundance (%)**	***P. meandrina* Average Abundance (%)**	**Average Dissimilarity (%)**	**Cumulative Contribution to Dissimilarity (%)**
Endozoicomonas sp.	5.98	0.10	4.54	5.10
Endozoicomonas sp.	4.09	0.12	3.02	8.49
Propionibacterium sp.	0.28	2.76	1.94	10.68
Streptococcus sp.	0.21	1.51	1.12	11.93
Rhodobacteraceae;__	0.27	1.50	1.00	13.06
Pseudomonas sp.	0.03	1.08	0.88	14.05
Staphylococcus sp.	0.10	1.15	0.87	15.03

E) *Porites lobata* & *Pocillopora meandrina*

Average dissimilarity = 85.46

Bacterial OTU	***P. lobata* Average Abundance (%)**	***P. meandrina* Average Abundance (%)**	**Average Dissimilarity (%)**	**Cumulative Contribution to Dissimilarity (%)**
Endozoicomonas sp.	3.14	0.12	2.46	2.88
Endozoicomonas sp.	2.81	0.10	2.24	5.51

Continued

Table 3.5 continued

Propionibacterium sp.	0.65	2.76	1.90	7.73
Streptococcus sp.	0.67	1.51	1.40	9.37
Rhodobacteraceae;__	0.59	1.50	1.13	10.70
Propionibacterium sp.	0.67	0.90	1.04	11.91
Rhodobacteraceae;__	1.71	1.33	1.03	13.11
Staphylococcus sp.	0.31	1.15	0.97	14.25
Pseudomonas sp.	0.10	1.08	0.96	15.38

F) *Pocillopora acuta* & *Pocillopora meandrina*

Average dissimilarity = 79.49

Bacterial OTU	***P. acuta* Average Abundance (%)**	***P. meandrina* Average Abundance (%)**	**Average Dissimilarity (%)**	**Cumulative Contribution to Dissimilarity (%)**
Streptococcus sp.	2.42	1.51	2.64	3.33
Propionibacterium sp.	2.27	0.90	2.28	6.19
Propionibacterium sp.	2.91	2.76	2.03	8.74
Staphylococcus sp.	1.45	1.15	1.71	10.89
Rhodobacteraceae;__	0.24	1.50	1.43	12.69
Pseudomonas sp.	0.33	1.08	1.43	14.48
Rhodobacteraceae;__	1.23	1.33	1.39	16.23

Table 3.6. Summary of pairwise one-way ANOSIM statistics among collection sites for microbial communities associated with A) *Porites compressa*, B) *Porites lobata*, C) *Pocillopora acuta*, and D) *Pocillopora meandrina*. Collection sites shown in Fig. 3.1.

Variable		R- Statistic	P-value
A) *Porites compressa*	HIMB – Magic Island	0.361	**0.048**
	HIMB – Sampan	0.098	0.190
	HIMB – Haleʻiwa	0.376	**0.017**
	HIMB – Waimānalo	0.370	**0.011**
	Magic Island – Sampan	0.389	**0.029**
	Magic Island – Haleʻiwa	0.508	**0.010**
	Magic Island – Waimānalo	0.480	**0.014**
	Sampan – Haleʻiwa	-0.020	0.429
	Sampan – Waimānalo	0.272	**0.030**
	Haleʻiwa – Waimānalo	0.252	0.058
B) *Porites lobata*	Magic Island – Sampan	0.059	0.229
	Magic Island – Electric Beach	0.143	0.143
	Magic Island – Haleʻiwa	0.274	**0.013**
	Magic Island – Waimānalo	0.289	**0.024**
	Sampan – Electric Beach	0.030	0.381
	Sampan – Haleʻiwa	0.085	0.121
	Sampan – Waimānalo	0.017	0.364
	Electric Beach – Haleʻiwa	0.344	**0.006**
	Electric Beach – Waimānalo	0.006	0.379
	Haleʻiwa – Waimānalo	0.407	**0.002**
C) *Pocillopora acuta*	HIMB – Magic Island	0.100	0.254
	HIMB – Sampan	0.125	0.206
	HIMB – Haleʻiwa	0.246	0.062
	HIMB – Waimānalo	0.111	0.210
	Magic Island – Sampan	-0.048	0.587
	Magic Island – Haleʻiwa	0.411	**0.006**
	Magic Island – Waimānalo	0.027	0.364
	Sampan – Haleʻiwa	0.480	**0.006**
	Sampan – Waimānalo	-0.189	0.985
	Haleʻiwa – Waimānalo	0.528	**0.002**
D) *Pocillopora meandrina*	Magic Island – Sampan	0.052	0.281
	Magic Island – Haleʻiwa	-0.286	0.867
	Magic Island – Waimānalo	0.246	0.095
	Sampan – Haleʻiwa	-0.125	0.786
	Sampan – Waimānalo	-0.033	0.468
	Haleʻiwa – Waimānalo	0.188	0.179

Table 3.7. Mantel correlations between Beta-nearest taxonomic index (βNTI) values and Euclidean distance matrices of coral physiology and environmental parameters. One-tailed P-values test null hypothesis of either a negative test statistic (R-value).

Variable	R-value	P-value (one-tailed, negative R)	P-value (one-tailed, positive R)	P-value (two-tailed)
A) *Porites compressa*				
Coral Chlorophyll	0.041	0.330	0.670	0.654
Coral Lipid	-0.062	0.750	0.251	0.505
Coral Protein	0.081	0.168	0.832	0.332
Coral Biomass	0.068	0.237	0.763	0.484
Coral $\delta^{13}C_{host\text{-}symbiont}$	0.238	**0.004**	0.996	**0.009**
Coral $\delta^{15}N_{host\text{-}symbiont}$	0.021	0.399	0.602	0.797
Mean Summer Sea Surface Temperature	-0.065	0.907	0.093	0.186
Mean Annual Significant Wave Height	-0.188	0.998	**0.003**	**0.003**
Mean Annual Chlorophyll	-0.089	0.932	0.068	0.133
B) *Porites lobata*				
Coral Chlorophyll	-0.063	0.765	0.236	0.476
Coral Lipid	-0.075	0.852	0.149	0.282
Coral Protein	-0.031	0.644	0.357	0.717
Coral Biomass	-0.054	0.730	0.270	0.539
Coral $\delta^{13}C_{host\text{-}symbiont}$	0.038	0.350	0.650	0.693
Coral $\delta^{15}N_{host\text{-}symbiont}$	0.130	**0.031**	0.969	0.069
Mean Summer Sea Surface Temperature	-0.123	0.947	0.053	0.094
Mean Annual Significant Wave Height	-0.071	0.841	0.160	0.317
Mean Annual Chlorophyll	-0.043	0.730	0.270	0.555

Continued

Table 3.7 Continued

C) *Pocillopora acuta*				
Coral Chlorophyll	0.122	0.099	0.901	0.193
Coral Lipid	-0.179	0.954	**0.046**	0.100
Coral Protein	0.073	0.166	0.834	0.334
Coral Biomass	0.081	0.197	0.803	0.400
Coral $\delta^{13}C_{host\text{-}symbiont}$	0.258	**0.007**	0.993	**0.010**
Coral $\delta^{15}N_{host\text{-}symbiont}$	0.073	0.175	0.825	0.372
Mean Summer Sea Surface Temperature	-0.018	0.621	0.379	0.763
Mean Annual Significant Wave Height	-0.123	0.927	0.073	0.155
Mean Annual Chlorophyll	-0.049	0.740	0.260	0.521
D) *Pocillopora meandrina*				
Coral Chlorophyll	-0.156	0.874	0.126	0.254
Coral Lipid	0.081	0.221	0.779	0.444
Coral Protein	-0.229	0.937	0.063	0.128
Coral Biomass	0.264	**0.018**	0.983	**0.032**
Coral $\delta^{13}C_{host\text{-}symbiont}$	0.014	0.531	0.469	0.919
Coral $\delta^{15}N_{host\text{-}symbiont}$	-0.082	0.729	0.271	0.536
Mean Summer Sea Surface Temperature	0.075	0.195	0.805	0.400
Mean Annual Significant Wave Height	0.075	0.203	0.203	0.394
Mean Annual Chlorophyll	0.091	0.138	0.863	0.278

Table 3.8. Spearman's rank-order correlations between coral-associated microbial community alpha diversity metrics and both coral physiology and environmental parameters.

	Observed OTUs		Sh	's H'	Pielou's Evenness		Faith's PD	
Variable	**R-value**	**P-value**	**R-value**	**P-value**	**R-value**	**P-value**	**R-value**	**P-value**
A) *Porites compressa*								
Coral Chlorophyll	-0.07	0.713	-0.26	0.183	-0.26	0.185	0.00	0.998
Coral Lipid	-0.25	0.206	-0.13	0.577	-0.11	0.577	-0.21	0.286
Coral Protein	0.00	0.989	0.07	0.742	0.05	0.789	0.06	0.761
Coral Biomass	-0.06	0.771	-0.07	0.740	-0.05	0.782	-0.05	0.797
Coral $\delta^{13}C_{h\text{-}e}$	0.24	0.210	0.35	0.065	0.34	0.080	0.27	0.166
Coral $\delta^{15}N_{h\text{-}e}$	-0.02	0.917	-0.02	0.931	-0.01	0.979	0.01	0.970
Mean Summer Sea Surface Temperature	-0.18	0.361	-0.10	0.622	-0.07	0.715	-0.16	0.427
Mean Annual Significant Wave Height	0.2	0.306	0.11	0.562	0.08	0.674	0.17	0.397
Mean Annual Chlorophyll	-0.26	0.182	-0.36	0.057	-0.35	0.068	-0.19	0.327
B) *Porites lobata*								
Coral Chlorophyll	0.07	0.694	0.11	0.569	0.08	0.673	0.00	0.981
Coral Lipid	-0.29	0.117	0.25	0.190	**0.43**	**0.018**	**-0.37**	**0.043**
Coral Protein	-0.09	0.621	-0.13	0.504	-0.09	0.634	-0.07	0.730
Coral Biomass	0.32	0.086	0.15	0.433	-0.02	0.934	0.28	0.133
Coral $\delta^{13}C_{h\text{-}e}$	-0.08	0.691	0.15	0.428	0.16	0.410	-0.06	0.740
Coral $\delta^{15}N_{h\text{-}e}$	**0.49**	**0.007**	0.2	0.282	-0.15	0.433	**0.51**	**0.004**
Mean Summer Sea Surface Temperature	-0.35	0.055	-0.26	0.168	-0.05	0.808	-0.31	0.095
Mean Annual Significant Wave Height	0.35	0.055	0.26	0.168	0.05	0.808	0.31	0.095
Mean Annual Chlorophyll	0.27	0.145	0.11	0.567	-0.06	0.742	0.23	0.224

Continued

Table 3.8 continued

C) *Pocillopora acuta*								
Coral Chlorophyll	-0.35	0.076	-0.2	0.331	0.02	0.941	**-0.40**	**0.045**
Coral Lipid	-0.30	0.130	-0.39	0.051	-0.38	0.059	-0.30	0.142
Coral Protein	0.10	0.644	0.17	0.403	0.23	0.252	0.10	0.612
Coral Biomass	-0.27	0.187	-0.15	0.475	-0.09	0.677	-0.27	0.150
Coral $\delta^{13}C_{h\text{-}e}$	**0.5**	**0.010**	**0.42**	**0.034**	0.21	0.298	**0.49**	**0.011**
Coral $\delta^{15}N_{h\text{-}e}$	0.29	0.144	0.37	0.061	0.26	0.196	0.32	0.112
Mean Summer Sea Surface Temperature	0.28	0.169	0.22	0.283	0.14	0.486	0.28	0.173
Mean Annual Significant Wave Height	-0.26	0.201	-0.16	0.428	-0.07	0.719	-0.24	0.230
Mean Annual Chlorophyll	-0.26	0.197	-0.31	0.118	-0.20	0.332	-0.27	0.181
D) *Pocillopora meandrina*								
Coral Chlorophyll	0.44	0.069	**0.67**	**0.002**	0.07	0.779	0.43	0.078
Coral Lipid	-0.02	0.935	0.16	0.526	0.04	0.874	0.05	0.829
Coral Protein	-0.31	0.207	0.17	0.489	**0.60**	**0.009**	-0.20	0.428
Coral Biomass	-0.11	0.677	0.08	0.760	0.11	0.675	-0.09	0.723
Coral $\delta^{13}C_{h\text{-}e}$	0.19	0.454	-0.17	0.488	-0.44	0.067	0.07	0.791
Coral $\delta^{15}N_{h\text{-}e}$	-0.44	0.068	**-0.59**	**0.011**	-0.02	0.932	**-0.59**	**0.001**
Mean Summer Sea Surface Temperature	0.19	0.452	0.26	0.300	-0.06	0.799	0.32	0.191
Mean Annual Significant Wave Height	-0.19	0.452	-0.26	0.300	0.06	0.799	-0.32	0.191
Mean Annual Chlorophyll	0.14	0.575	0.25	0.326	-0.01	0.959	0.22	0.381

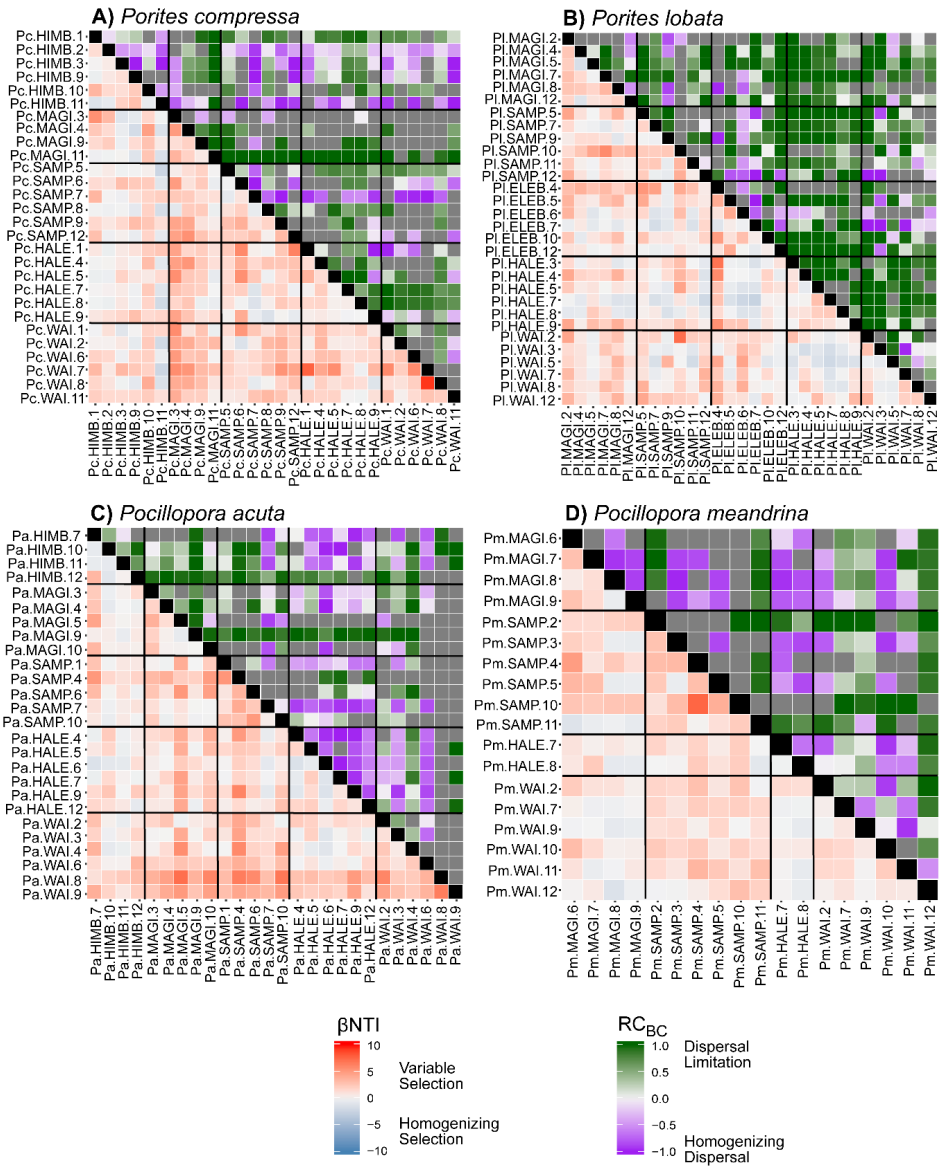

Figure 3.6. Heat maps showing Beta-nearest taxonomic index (βNTI) values and Raup-Crick (RC_{BC}) values for paired comparisons of each coral ramets' microbial communities within each coral species. Labels on the x- and y-axis correspond to individual coral ramets, such that A) Pc = *Porites compressa*, B) Pl = *Porites lobata*, C) Pa = *Pocillopora acuta*, and D) Pm = *Pocillopora meandrina*. Collection sites are abbreviated as HIMB = Hawai'i Institute of Marine Biology, MAGI = Magic Island, SAMP = Sampan Channel, ELEB = Electric Beach, HALE = Hale'iwa, WAI = Waimānalo. Each coral ramet is then identified with a number that was assigned during collection. The color of each box corresponds with the continuous scales for βNTI values (bottom portion of each heatmap) and RC_{BC} values (top portion of each heatmap).

Chapter 4: Long-term coral microbial community acclimatization is associated with coral survival in a changing climate

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4.1 Abstract

Changes in coral-associated microbial community composition are often linked to stressed, bleached, or otherwise unhealthy corals. However, experimental investigations of microbial responses to thermal stress have typically lasted days to weeks. It is unknown how coral-associated microbial communities respond to long-term chronic ocean warming and acidification expected with global climate change. The plasticity of microbial communities suggests that they may play an important role in potential acclimatization of the coral holobiont to future ocean conditions. Here, coral-associated microbial communities were characterized after a 22-month mesocosm experiment where four Hawaiian coral species (*Porites compressa*, *Porites lobata*, *Montipora capitata*, and *Pocillopora acuta*) were exposed to a fully factorial design with two pH levels (present day: pH of 8.1 – 8.2 vs. ocean acidification:–0.2 pH units) and two temperature levels (present day: 23.5 – 27.5 °C annually vs. ocean warming: +2.0 °C), representing conditions expected by the end of this century. Mortality was the highest in the ocean warming and dual stress treatments. Under ocean warming and dual stress, mortality was at least 33.0%, 33%, 58.3%, and 66.7% for *P. compressa*, *P. lobata, M. capitata*, and *P. acuta*, respectively. Microbial community composition associated with surviving ramets of *Porites compressa* and *Porites lobata* differed between the control and dual stress treatment, while no significant change in alpha or beta diversity was found for the microbial communities of *Montipora capitata* and *Pocillopora acuta*. However, the communities associated with the latter two species differed among ramets in the control based on the survival of their genetic counterparts in the dual stress treatment. These two

patterns in microbial community composition aligned closely with mortality, such that the *Porites* corals had the lowest mortality and microbial communities that shifted by the end of the experiment, whereas *Montipora capitata* and *Pocillopora acuta* had high mortality and their microbial communities did not change. This suggests that the microbial communities of *P. compressa* and *P. lobata* acclimatized to climate change conditions and conferred some resilience to the corals, whereas the microbial communities of *M. capitata* and *P. acuta* did not change over time and corals that survived were likely pre-adapted to tolerate the dual stress. While there were tolerant individuals within all four species that may be capable of surviving on Hawaiian reefs of the future, species with flexible microbial communities had higher survivorship and could become the dominant species on future reefs.

4.2 Introduction

Increasing concentrations of atmospheric CO_2 are leading to global warming and ocean acidification, threatening the long-term survival of corals and the persistence of coral reef ecosystems. By the year 2100, tropical ocean temperatures are expected to rise 1 – 3 °C with a parallel increase in acidity by 150% (approximately 0.2 pH units) (IPCC 2019). These changing conditions will lead to reduced skeletal growth (e.g., De'ath et al. 2009; Schoepf et al. 2013; Dove et al. 2013), coral bleaching (e.g., Hughes et al. 2017, 2018; Couch et al. 2017), outbreaks of disease (e.g., Brown 1997; Sokolow 2009; Maynard et al. 2015), and increased coral mortality (e.g., Loya et al. 2001; Grottoli et al. 2006; Rodgers et al. 2017; Hughes et al. 2018). However, the potential for some corals to acclimatize to ocean warming and acidification may help support the survival of coral reefs as we know them today.

Several studies have found evidence of acclimatization by corals to rising ocean temperature and/or acidification conditions (e.g., Maynard et al. 2008; Oliver and Palumbi 2011; Grottoli et al. 2014; Coles et al. 2018). Corals can acclimatize through several mechanisms, including shuffling to more thermally tolerant Symbiodiniaceae (Baker 2001; Baker et al. 2004; Grottoli et al. 2014), maintaining or increasing their energy reserves (Rodrigues and Grottoli 2007; Grottoli et al. 2014, 2017; Schoepf et al. 2015; Levas et al. 2018), and increasing the contribution of heterotrophy (Palardy et al. 2008; Hughes et al. 2010; Hughes and Grottoli 2013; Levas et al. 2016; Conti-Jerpe et al. 2020), changing gene expression (Oliver and Palumbi 2011; Palumbi et al. 2014). The bacterial and archaeal communities of a coral, hereafter referred to as microbial

communities, have also been posited as a potential source of coral resistance and/or resilience in a changing climate (e.g., Bourne et al. 2016; Ziegler et al. 2017; Peixoto et al. 2017; Grottoli et al. 2018).

Coral-associated microbial communities play important roles within the coral holobiont (i.e., the coral and microbiome together), including nutrient cycling and immune response (e.g., Lesser et al. 2007; Bourne et al. 2013; Krediet et al. 2013; Peixoto et al. 2017). These microbial communities can be sensitive to environmental conditions, with some coral species shifting community composition when exposed to warmer (e.g., Littman et al. 2011; Ziegler et al. 2017; Lee et al. 2017; Grottoli et al. 2018), or more acidic waters (Meron et al. 2011), or both (Webster et al. 2016; Grottoli et al. 2018). These changes in microbial community composition are often linked to stressed, bleached, or otherwise unhealthy corals. But experimental investigations of the microbial responses to thermal stress have typically lasted only days to weeks (e.g., Ziegler et al. 2017; Grottoli et al. 2018; Pratte and Richardson 2018; Pootakham et al. 2019), with the longest lasting two months (Webster et al. 2016). These studies provide important information on short to moderate-term microbial responses to heat stress or coral bleaching, but it remains unclear if or how microbial responses persist over multi-year periods of stress such as those expected later this century, and whether these community shifts confer resistant characteristics to the coral (i.e., similar to shuffling Symbiodiniaceae types) or are simply a sign of degrading health (Peixoto et al. 2017).

To better understand how the pressures associated with global climate change might affect the microbial communities of coral, we characterized microbial community

composition of four Hawaiian coral species following a 22-month mesocosm experiment. Corals were exposed to ocean acidification, ocean warming, and a combined dual stress treatment representing conditions expected by the end of this century. This is the first study to experimentally characterize the response of tropical coral-associated microbial communities to ocean warming and acidification over a multi-year time frame, providing insight into the potential roles of these microbial communities in the acclimatization of corals. Given the changes seen in shorter term experiments, we hypothesized that the microbial community composition of all four Hawaiian coral species would shift in response to treatment, with the greatest shifts in composition expected in the ocean warming and dual stress treatments. Further, we used an ecological null modeling approach to characterize the potential controls on coral-associated microbial community composition changes due to warming, ocean acidification, and both stresses combined.

4.3 Methods

4.3.1 Experimental design and coral collection

This study was conducted between February 2016 – December 2017 in a mesocosm setup at the Hawai‘i Institute of Marine Biology (HIMB) on Moku O Lo‘e Island (24.43413 °N, 157.78802 °W), adjacent to the island of O‘ahu, Hawai‘i (HI), USA. Forty flow-through mesocosms (0.5 m x 0.5 m x 0.3 m, ~70 L) were divided into a fully factorial design with two pH levels (present day pH of 8.1 – 8.2 vs. ocean acidification with pH at –0.2 relative to present day levels) and two temperature levels

(present day daily average of 23.5 – 27.5 °C over the 22 months vs. ocean warming of +2.0 °C above present day), resulting in four treatments (n = 10 mesocosms per treatment) that ran for 22 months. The full design of the mesocosms and maintenance of the treatment conditions is described by Bahr et al. (2020) and Jury et al. (in prep). Briefly, the mesocosms were originally stocked with approximately a 2 cm layer of carbonate reef sand and gravels from an adjacent backreef, three replicate 10 – 20 cm pieces of reef rubble, a juvenile convict surgeonfish (*Acanthurus triostegas*), a threadfin butterfly fish (*Chaetodon auriga*, a generalist grazer of non-coral invertebrates), and ramets from eight common reef-building coral species from around O‘ahu as part of a broader study on the effects of ocean warming and acidification on coral reefs. Seawater was unfiltered and any organisms that entered the mesocosms through the inflow were allowed to remain, resulting in slight community differences among individual mesocosms.

The four species included in this study were *Montipora capitata*, *Porites compressa*, *Porites lobata*, and *Pocillopora acuta*. Six parent colonies (i.e., genets) from each species were collected between 17 August and 13 November 2015 from a depth of 0.5 – 5 m at each of four sites (Hale‘iwa, HIMB, Sampan, and Waimānalo) surrounding the island of O‘ahu, Hawai‘i (HI), (Fig. 4.6, Table 4.1). *Porites lobata* was unable to be sampled from HIMB due to low abundance at that site. A 5 – 10 cm ramet (branch or mound) was removed underwater via hammer and chisel from visually healthy parent colonies separated by at least 5 m to minimize the possibility of selecting corals of the same genotype. Following collection, each parent colony was confirmed to be genetically

distinct. Each genet was then fragmented into four ramets, attached to a ceramic plug, and randomly assigned to one of the 40 mesocosms, such that each genet was represented in each treatment. Coral ramets acclimated to the mesocosms for at least 30 days under present-day Hawaiian seawater conditions (i.e., control conditions) prior to the commencement of the experiment. On 01 February 2016 the experiment began with a gradual increase of +0.5 °C and a decrease of 0.05 pH units every 10 days for 40 days to avoid shocking the mesocosm communities. The final treatment conditions were reached on 15 February 2016 and were as follows: (1) control treatment (mean present day temperature and pH), (2) ocean acidification treatment (present day temperature and –0.2 pH units), (3) ocean warming treatment (+2.0 °C and present day pH), and (4) dual stress treatment (+2.0 °C and –0.2 pH units). These corals were maintained in mesocosm conditions for 22 months until sampling between 25 November and 04 December 2017, and the full profile of temperature and pH conditions are presented in Bahr et al. (2020). Coral ramets or other recruited invertebrates were not permanently removed from the mesocosms if they had died during the study to most closely mimic reef conditions. Fish were removed from the mesocosms if they died and replaced with new ones as much as possible.

4.3.2 Coral Mortality

All coral ramets sampled in this study were photographed between 25 November and 10 December 2017. Coral mortality at the end of the 22-month experiment was assessed visually through estimations of live tissue coverage from photographs of each

coral ramet. Ramets with an estimated live coral surface coverage of >25% of the skeletal surface were considered alive. The live tissue was sampled for microbial community without incorporating marginal or dead tissue. Ramets with <25% live tissue coverage were considered dead and no further analyses were conducted on these corals.

4.3.3 Coral-associated microbial community sampling

Subsamples (1 – 3 cm^2) were collected from the growing tip of each surviving ramet of the four coral species across the four treatments using sterile bone cutters and wearing gloves. For *Porites lobata*, a small sterile cork borer was used instead due to the mounding morphology of that coral species, and these sub-samples were collected near the center of healthy tissue on the ramet. Once the sub-sample was removed from the ramet, it was immediately placed into a 5 ml Eppendorf tube (Hamburg, Germany) and stored in the dark at room temperature in a preservative of 20% DMSO-0.5 M EDTA saline saturated solution (pH = 8.0). Only ramets with sufficient live tissue were sampled for microbial community analyses (see Table 4.1 for full list of samples collected)

After shipment to Ohio State University, each sample was rinsed lightly with autoclaved ultrapure 0.22 µm filtered artificial saltwater (3.5% NaCl) to remove residual preservation buffer. The coral tissue was then removed from the skeleton by airbrushing with the same sterile artificial seawater. DNA was extracted from the resulting slurry using PowerSoil DNA Isolation kits (Qiagen, Hilden, Germany) following the manufacturer protocol. Successful extraction of genomic DNA was confirmed using a Qubit fluorometer prior to amplification of the V5-V6 region of the 16S rRNA gene

using the primers CS1_784F and CS2_1061R (forward: 5'-AGGATTAGATACCCTGGTA-3'; reverse: 5'-CRRCACGAGCTGACGAC-3'). These primers included CS1 and CS2 linkers to allow the downstream application of adapter sequences and sample-specific barcodes. Polymerase chain reaction (PCR) was completed in two stages. Stage one PCR used Amplitaq Gold 360 DNA polymerase (Thermo Fisher Scientific, Waltham, Massachusetts, USA) in 25 µl reaction volumes. Stage one PCR cycling conditions were as follows: 15 min at 95 °C, followed by 28 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, with a final extension time of 10 min. Successful amplification was visualized via gel electrophoresis. Stage two PCR used MyTaq HS mastermix (Bioline, Memphis, Tennessee, USA) in 20 µl reaction volumes and cycling conditions were as follows: 95 °C for 5 minutes, followed by 8 cycles of 95 °C for 30 seconds, 60 °C for 30 seconds, and 68 °C for 30 seconds. A final elongation period was performed at 68 °C for 7 minutes. These amplicons were subsequently prepared for multiplexed sequencing on an Illumina MiniSeq sequencer (2 x 151 base pairs, mid-output). The second stage of the PCR process and the Illumina sequencing were completed by the DNA Services Facility at the University of Illinois at Chicago.

Reads produced by Illumina sequencing were processed using the QIIME software package version 1.9 (Caporaso et al. 2010). Within QIIME, forward and reverse reads were joined using default parameters of fastq-join, while chimeras were removed via USEARCH and a quality threshold of 20 was set to filter remaining sequences. Operational taxonomic units (OTUs) were assigned based on the Silva v132 ribosomal

database and were clustered at 97% similarity. These OTUs were retained only if 10 reads or greater were present across all samples to avoid including sequencing errors. Any OTUs which were identified as chloroplast, mitochondria, or eukaryotic in origin were removed from further analyses, in addition to laboratory contaminants confirmed via sequenced negative PCR controls. Prior to diversity analyses, two samples with final read counts below 500 were also removed to limit the consideration of samples with extremely low sequencing depth. All raw unprocessed are available on NCBI's Sequence Read Archive under accession number PRJNA645714.

4.3.4 Statistical Analyses

All analyses were performed using R software package version 3.5.0 (R Core Team 2015) and PRIMER v6 (Clarke and Gorley 2006). Statistical significance was defined as $\alpha \leq 0.05$. First, to assess whether microbial community composition differed among coral species and collection sites at the end of the experiment, both alpha diversity and beta diversity were compared among microbial communities associated with corals in the control. Alpha diversity of microbial communities among coral species and collection sites was measured using all reads via the number of observed OTUs, Chao1 (estimated species richness) (Chao 1984), Shannon's Diversity Index (Shannon 1948), and Faith's phylogenetic diversity (Faith's PD) (Faith 1992). Alpha diversity values were compared using a Kruskal-Wallis one-way analysis of variance and a post hoc Dunn's Test. Next, beta diversity was calculated with a Bray-Curtis dissimilarity matrix (Bray and Curtis 1957) and then compared using an analysis of similarities (ANOSIM). The

ANOSIM produces both a p-value for testing statistical significance and an R-value between -1 and 1, where a higher R-value indicates a more dissimilar microbial community composition between groups. Similarity percentage analyses (SIMPER) were used to identify the microbial OTUs that differed most in relative abundance among coral species and collection sites and thus were the greatest contributors to dissimilarity between sample groupings.

Next, to assess whether coral-associated microbial communities differed between the control and treatments after 22 months, alpha and beta diversity of each coral species were compared between ramets of genets that survived in both the control and each treatment condition (Fig. 4.1A). Further, to determine what processes potentially control community composition between the control and treatments, null models using OTU data were performed to assess relationships between phylogeny of the coral microbiome and potential controls on that microbial community composition (i.e., environmental conditions). As outlined in Stegen et al. (2015) and Danczak et al. (2016), the β-nearest taxon index (βNTI) was calculated using 999 community randomizations for each possible pairwise comparison between samples of the same coral species in order to better understand the potential controls on microbial community composition. The resulting βNTI values were then used to predict whether deterministic (i.e., selection) or stochastic (i.e., random) processes shaped the microbial community assemblies. If the resulting βNTI value is > 2 or is < -2, a deterministic process is responsible for differences between microbial communities in two samples. Conversely, if a βNTI value is between 2 and –2, a stochastic process explains observed differences in microbial

community composition between two samples.

Stochastic processes can be further classified as either dispersal limitation, homogenizing dispersal, or ecological drift using the Raup-Crick metric with a Bray-Curtis dissimilarity matrix (RC_{BC}) (Stegen et al. 2015). The RC_{BC} metric probabilistically assembles 999 iterations of microbial communities from each of the sampled communities, providing a null distribution of Bray–Curtis values to assess compositional turnover. The deviation between the observed Bray–Curtis and the null distribution was then standardized to provide a value between 1 and –1. Values > 0.95 and < –0.95 are interpreted as statistically significant departures from drift (i.e. chance events), such that values > 0.95 suggest dispersal limitation between sampled communities supported by drift (i.e. spatial turnover between these communities is greater than expected by chance alone) and values < –0.95 suggest homogenizing dispersal between sampled communities (i.e. communities are homogenized and turnover is lower than by drift alone).

Finally, to determine whether coral mortality in the dual stress treatment was related to coral-associated microbial composition of control corals, alpha and beta diversity were compared between coral ramets in the control whose ramets of the same genet survived versus those that died in the dual stress treatment (Fig. 4.1B).

4.4 Results

Across the species considered in this study, average coral mortality was minimal in the control (7.64%), and the ocean acidification treatment (7.29%), and highest in the

ocean warming (47.9%) and dual stress treatments (53.1%). Within species, mortality in the ocean warming and dual stress treatments was lowest in *P. compressa* (33.3% and 33.3%, respectively) and *P. lobata* (33.3% and 50.0%, respectively), followed by *Montipora capitata* (62.5% and 58.3%, respectively), and *Pocillopora acuta* (66.6% and 75%, respectively) (Fig. 4.4.2).

Overall, there were 15,947 OTUs across the 5,994,595 sequences included in the analysis of microbial communities associated with the four Hawaiian coral species after 22 months in the mesocosm experiment. When considering all four coral species together, the most abundant OTUs were associated with the orders Oceanospirillales and Cytophagales, primarily in the genera *Endozoicomonas* and *Candidatus* Amoebophilus, respectively (Fig. 4.3).

In the controls, alpha diversity of the microbial communities did not differ among coral species (Table 4.2), but beta diversity differed significantly among all four coral species, though the two *Porites* species were the least dissimilar (Fig. 4.3A, Table 4.3A). *M. capitata* had the greatest relative abundance of Oceanospirillales (26.7%), followed by *Porites lobata*, *Porites compressa*, and *Pocillopora acuta* with averages of 19.3%, 15.8%, and 11.5%, respectively (Fig. 4.3B). *Pocillopora acuta* hosted the greatest relative abundance of bacteria in the order Cytophagales at 25.4%, followed closely by *Porites lobata* with 21.1%, while *Porites compressa* and *M. capitata* hosted a much lower abundance with only 9.4% and 2.6%, respectively (Fig. 4.3B). Within each species, coral-associated microbial communities did not differ based on the provenance of the ramets in *P. compressa* and *P. lobata* but did differ based on provenance of the ramets in

M. capitata and *P. acuta* (Table 4.3 & 4.4). Specifically, *M. capitata* and *Pocillopora acuta* corals originally collected from Hale‘iwa hosted distinct microbial communities from those originally collected from HIMB and Sampan (Table 4.3D&E), including reduced richness in *M. capitata* corals from HIMB (Table 4.4). The microbial communities associated with *M. capitata* corals from Hale‘iwa had a lower abundance of the order Oceanospirillales in comparison with conspecifics collected from other sites, primarily due to a reduced abundance of one OTU (DQ917863) from the genus *Endozoicomonas* (Table 4.5A). The microbial communities associated with *P. acuta* corals from Hale‘iwa had lower relative abundances of OTUs within the order Myxococcales and a high relative abundance one OTU within *Vibrio sp.* (11.54%) compared to conspecifics at other sites (Table 4.5B).

Among genets that survived both the control and ocean acidification treatments, there were no differences in either the alpha diversity metrics of the microbial community composition for any coral species (Table 4.6A). Similarly, the ocean warming treatment did not significantly affect the alpha diversity of microbial communities associated with any coral species (Table 4.4B). In the dual stress treatment, all four metrics of alpha diversity increased in microbial communities associated with *Porites lobata* relative to the control treatment but did not significantly differ in any other coral species (Table 4.6C).

There were no differences in the beta diversity of microbial communities associated with any coral species between the genets that survived both the control and ocean acidification treatment (Table 4.7). Microbial communities associated with *P.*

compressa, *P. lobata*, and *M. capitata* all differed or nearly differed between the control and the ocean warming treatment (Table 4.7B). In the dual stress treatment, only communities associated with *P. compressa* and *P. lobata* differed from the control (Table 4.7C, Fig. 4.7) due to a nearly complete loss of one specific OTU in the order Oceanospirillales (New.ReferenceOTU57; *Kistimonas sp.*) (Table 4.8A&B). Interestingly, *Porites compressa* in the dual stress treatment had an almost three-fold relative abundance increase in bacteria of the order Cytophagales, from 5.7% in the control to 14.2% in the dual stress on average, while *Porites lobata* actually had a slight decrease from 22.0% to 17.5% in the same bacteria (Fig. 4.7). Changes in relative abundance of Cytophagales for both *Porites* corals was driven primarily by one OTU of the bacterial genus *Candidatus* Amoebophilus (GU119061) (Table 4.8A&B).

Between the control and treatment conditions, βNTI comparisons above the variable selection threshold of 2 accounted for 11.4% – 33.2% of pairwise comparisons (Table 4.9), indicating that a combination of predominantly stochastic processes and to a lesser extent variable selection likely influenced the composition of the coral-associated microbial communities among treatments for all coral species. RC_{BC} values, which are used to identify the type of stochastic processes acting on a community, displayed similarly mixed patterns where ecological drift was the primary stochastic process in all species except *Porites compressa* (Table 4.9). Dispersal limitation (an RC_{BC} value > 0.95) was the dominant stochastic process in more than half of comparisons between *Porites compressa* ramets in the control and both the ocean warming and dual stress treatments. Together, these metrics indicate that differences in microbial community

composition of each coral species between the control and treatment conditions is largely driven by a combination of dispersal limitation, ecological drift, and variable selection, but the dominance of each process can vary among species.

Finally, coral ramets within the control were compared based on the survival of their corresponding ramet from the same genet in the dual stress treatment (illustrated in Fig. 4.1B). Beta diversity of the microbial communities associated with *Porites compressa* and *Porites lobata* did not have significant differences in either alpha or beta diversity, but *M. capitata* trended towards differences ($p = 0.057$), and *P. acuta* significantly differed ($p = 0.033$) (Tables 4.10 & 4.11). In *M. capitata*, genets in the control whose ramets survived the dual stress treatment had a more than three-fold higher relative abundance of bacteria in the order Oceanospirillales (45.4%) than genets whose ramets did not survive the dual stress treatment (14.2%) (Table 4.12A). This difference in relative abundance of Oceanospirillales was largely due to one OTU of *Endozoicomonas sp.* (DQ917863), which made up more than 25.1% of the microbial community composition of the *M. capitata* genets that survived vs. 0.88% in those that died. In *Pocillopora acuta*, the microbial community composition of ramets from genets that survived the dual stress treatment had a greater relative abundance of an OTU in the order Myxococcales (15.0% in surviving genets vs. 0.03% in dead genets) and the order Rhodovibrionales (14.3% in surviving genets vs. 0.02% in dead genets), while the ramets from genets that died in the dual stress had a high relative abundance of the same OTU (GU119061) of *Candidatus* Ameobophilus that was common in both *Porites* coral species (Table 4.12B).

4.5 Discussion

Here, four species of Hawaiian coral were exposed to temperature and pH conditions expected by the end of this century for 22 months in outdoor mesocosms. The corals were found to have species-specific patterns in mortality and microbial community composition, linking the potential tolerance of Hawaiian corals with their microbiome.

4.5.1 *Porites compressa* and *Porites lobata*

Overall, our findings suggest that lower mortality in *P compressa* and *P. lobata* under future ocean conditions is associated with acclimatization of their microbial communities. Multiple lines of evidence lead us to this conclusion. 1) On the reef, both coral species host different microbial communities that differ based on their provenance (Price et al. in prep, Ch3). In the experimental control, no provenance differences were detected within each species (Table 4.3B&C), suggesting that the microbial communities had changed in response to their environment. 2) The microbial communities of *P. compressa* and *P. lobata* in the control differed from those in the ocean warming and dual stress treatments (Fig. 4.4A&B, Table 4.7A&B), also suggesting that these microbial communities changed in response to their new environmental situation. Short-term studies find that shifts in microbial community composition can be accompanied by a decline in overall coral health (e.g., Lee et al. 2017; Grottoli et al. 2018; Pootakham et al. 2019). However, after nearly two years of chronic future ocean conditions, the shifts in microbial

community composition observed here may represent acclimatization to future ocean conditions by these corals, given that these two species also had the lowest mortality rates among the four species in the experiment (Fig. 4.2). Indeed, *Porites* corals are among the most resistant to bleaching and mortality in the Hawaiian Islands (Jokiel and Coles 1990; Kenyon et al. 2006), although this can vary among locations (Rodgers et al. 2017).

The difference in the microbial community composition between the control and the dual stress treatment in both *Porites* corals was largely attributed to a reduction in the relative abundance of the bacterial genus, *Kistimonas sp.*, in the order Oceanospirillales (Table 4.8, Fig. 4.7). Given that bacteria of the order Oceanospirillales are known to persist in coral-associated microbial communities at elevated temperatures (Webster et al. 2016), it is unclear why the *Porites* corals had such a marked decrease in relative abundance of *Kistimonas sp.* in the dual stress treatment. The relatively low mortality of *Porites* corals and the minimal changes in abundance of known pathogenic bacteria (i.e., *Vibrio sp.*) (Lee et al. 2015) suggests that *Kistimonas sp.* losses were replaced with other more tolerant community members.

Changes in microbial community composition were primarily governed by a combination of stochastic dispersal limitation (i.e., high community turnover due to an inability to mix) and ecological drift, and secondarily governed by selective pressures of the environmental conditions (Table 4.9A&B). These findings are consistent with previous observations that stochastic processes play a more significant role in microbial community assembly in stressed corals due to the corals having reduced resources for controlling these processes (Zaneveld et al. 2017; Ahmed et al. 2019). Therefore, there

may be multiple possible explanations for the divergence of microbial communities between treatments for the *Porites* corals: (1) *P. compressa* and *P. lobata* have acclimatized to the ocean warming and dual stress treatment conditions, but the corals had less control during the initial months of the treatment conditions, allowing microbial communities to diverge or (2) *P. compressa* and *P. lobata* have not acclimatized to the treatment conditions, and the divergence between treatments represents 22 months of stochastic processes slowly driving microbial communities in each treatment further apart. The first scenario may be more likely, given that corals which do not acclimatize would have been stressed for nearly two years, and would likely show greater mortality than what was observed. However, it is also possible that these corals sacrifice control over their microbiome as a tradeoff that allows the coral to continue growing and to maintain critical metabolic processes (Zaneveld et al. 2017).

4.5.2 *Montipora capitata* and *Pocillopora acuta*

Overall, our findings suggest that higher mortality in *M. capitata* and *P. acuta* under future ocean conditions is associated with a lack of any changes in their microbial communities. Multiple lines of evidence lead us to this conclusion. 1) Both *M. capitata* and *P. acuta* ramets within the control continued to host distinct microbial communities based on their provenance after almost two years in the mesocosm (Table 4.3D&E). This stability, even under common environmental conditions of the control mesocosm, suggests that the microbial communities of these corals are relatively unresponsive to environmental changes. 2) When considering ramets of genets that survived both the

control and dual stress treatment, the microbial community compositions did not differ in either species (Fig. 4.4C&D, Table 4.7C&D) and mortality was higher (Fig. 4.2), suggesting that the lack of responsiveness by the microbial communities to environmental conditions are associated with the higher mortality rate in these corals compared to the *Porites*. This contrasts with findings showing stable microbial community composition is associated with better physiological health in *Turbinaria reniformis* (Grottoli et al 2018). 3) Ramets in the control whose genets survived in the dual stress treatment had distinctly different or nearly different microbial communities than ramets whose genets died in the dual stress treatment (Fig. 4.5, Table 4.11), suggesting that individual *M. capitata* and *P. acuta* corals either have microbial communities associated with tolerance of future ocean conditions or they do not. Nearly all *M. capitata* and *P. acuta* corals that survived the dual-stress treatment were originally collected from HIMB or Sampan – two of the warmest sites surrounding O‘ahu (Jury and Toonen 2019, Price et al. in prep, Chapter 3) – suggesting these corals may have been pre-adapted to better manage the long-term exposure to the dual stress treatment (Ziegler et al. 2017; Coles et al. 2018) and/or that any potential for acclimation of microbial communities in these corals takes longer than two years. Due to the apparent stability of microbial communities associated with these two coral species, one may be able to predict which genets would perform better under expected future ocean conditions and could be targeted for restoration efforts. Indeed, *M. capitata* ramets in the control whose counterparts survived the dual stress treatment hosted a greater relative abundance of bacteria in the genus *Endozoicomonas* than those ramets whose counterparts died, largely

driven by one specific OTU (DQ917863) that was 25 times more abundant in survivors (Table 4.12A). Greater relative abundances of *Endozoicomonas* bacteria may be key to the survival of individual *M. capitata* coral genets, as these bacteria have important roles in nutrient cycling, host health, and control over the microbial community composition (Neave et al. 2016). Microbial communities associated with ramets of *P. acuta* in the control whose genetic counterparts survived in the dual stress had high relative abundances of bacteria in the orders Myxococcales (Table 4.12B), which have been associated with disease resistance in *Acropora* corals (Rosales et al. 2019). Interestingly, surviving *P. acuta* ramets hosted lower abundances of *Endozoicomonas* bacteria than those ramets that died (Table 4.12). This is contrary to other studies that find *Endozoicomonas* bacteria to be associated with coral health and/or resilience (e.g., Meyer et al. 2014; Lee et al. 2015; Epstein et al. 2019), and indicates that there is not a single universal microbial group that can serve as an indicator for coral tolerance and resilience to predicted future ocean conditions that applies to all coral species. Nevertheless, *M. capitata* and *P. acuta* corals from naturally warmer sites appear to already host communities of microbes that are associated with increased coral tolerance and survivorship of conditions expected with climate change.

4.5.3 Implications

All four species of Hawaiian corals had surviving genets after 22 months of chronic environmental stress, indicating that future Hawaiian reefs may still include each of these coral species, but with *Porites* corals being proportionately more abundant. Our

findings suggest that corals with microbial communities that can shift and potentially acclimatize to future ocean conditions may be more resilient and become more abundant on the coral reefs of the future. In addition, species-specific patterns in the responses of coral microbial communities to future ocean conditions may provide some strategies for coral restoration. For example, microbial community composition would not be an important factor in the selection of *Porites* corals from O‘ahu for transplantation to other sites needed to be restored. Whereas, *M. capitata* and *P. acuta* ramets harvested from warmer sites are more likely to have microbial communities that are pre-selected for survival under future ocean conditions and may be better targets for restoration long-term than ramets from genets harvested elsewhere in O‘ahu.

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Table 4.1. Total number of coral genets in each treatment at the beginning of the mesocosm experiment, followed by the number of genets sampled for microbial community analysis at the end of the 22-month experiment in parentheses. Each unique genet was represented once in each treatment at the start of the experiment.

Collection Site	Treatment	*Porites compressa*	*Porites lobata*	*Montipora capitata*	*Pocillopora acuta*
HIMB	Control	6 (6)		6 (6)	6 (4)
(21.43426° N,	Acidification	6 (3)		6 (5)	6 (6)
157.78680° W)	Warming	6 (4)		6 (2)	6 (3)
	Dual Stress	6 (3)		6 (2)	6 (3)
Sampan Channel	Control	6 (5)	6 (6)	6 (5)	6 (6)
(21.45100° N,	Acidification	6 (6)	6 (6)	6 (5)	6 (5)
157.79600° W)	Warming	6 (4)	6 (3)	6 (5)	6 (4)
	Dual Stress	6 (4)	6 (3)	6 (5)	6 (3)
Haleʻiwa	Control	6 (5)	6 (5)	6 (3)	6 (6)
(21.59419° N,	Acidification	6 (5)	6 (6)	6 (4)	6 (6)
158.11070° W)	Warming	6 (1)	6 (4)	6 (0)	6 (1)
	Dual Stress	6 (4)	6 (3)	6 (0)	6 (0)
Waimānalo	Control	6 (5)	6 (6)	6 (6)	6 (6)
(21.32833° N,	Acidification	6 (6)	6 (6)	6 (6)	6 (5)
157.68310° W)	Warming	6 (3)	6 (4)	6 (1)	6 (0)
	Dual Stress	6 (4)	6 (3)	6 (1)	6 (0)

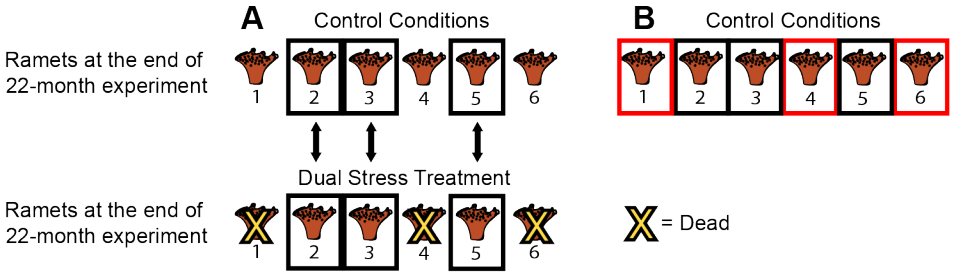

Figure 4.1. Chart showing how coral-associated microbial communities were compared within and among treatments, using the dual stress treatment as an example. (A) To evaluate if the microbial community composition changed in response to the dual stress treatment, comparisons were restricted to genets that survived and were able to be sampled in both the control and the dual stress treatment. (B) To evaluate if the baseline microbial community composition differed among genets that survived or did not survive the dual stress treatment, comparisons were restricted to genets in the control whose lineages survived or did not survive the 22 months in the dual stress treatment.

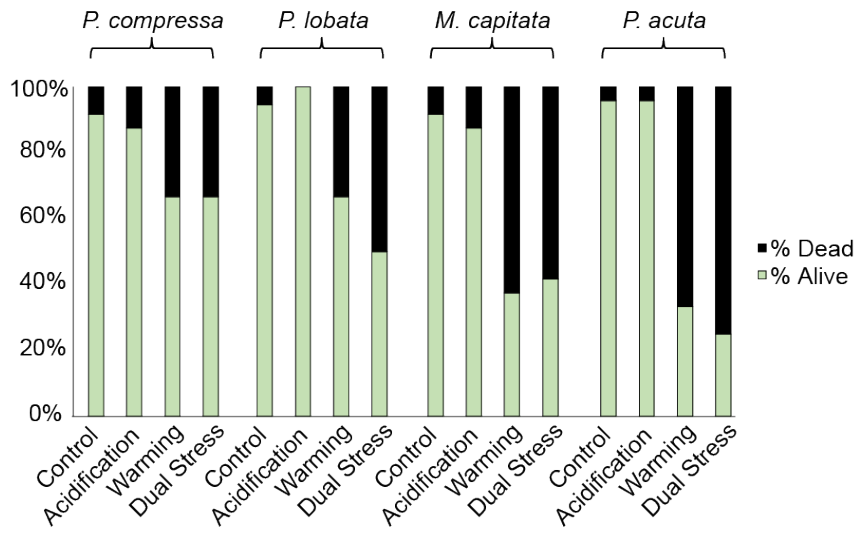

Figure 4.2. Mortality of each coral species in each treatment at the end of the 22-month mesocosm experiment.

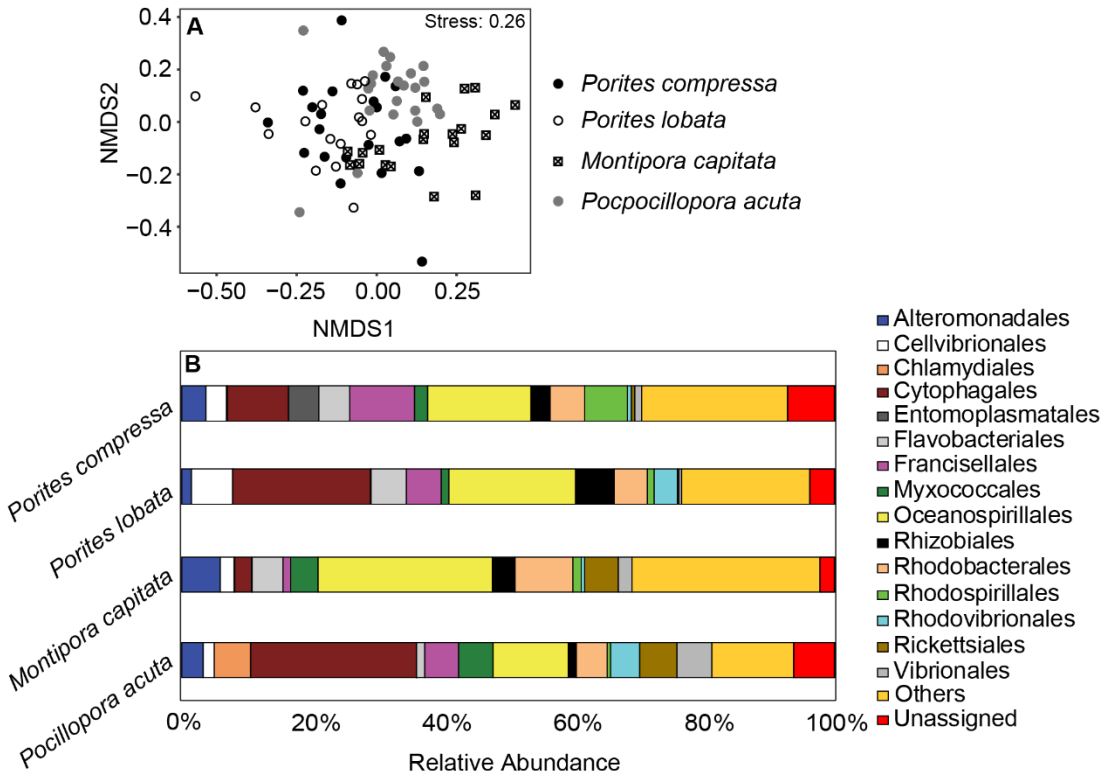

Figure 4.3. Microbial communities associated with corals in the control. (A) NMDS plot of microbial communities associated with each coral species in the control. (B) Mean Relative abundances of the most common microbial Orders associated with each coral species in the control.

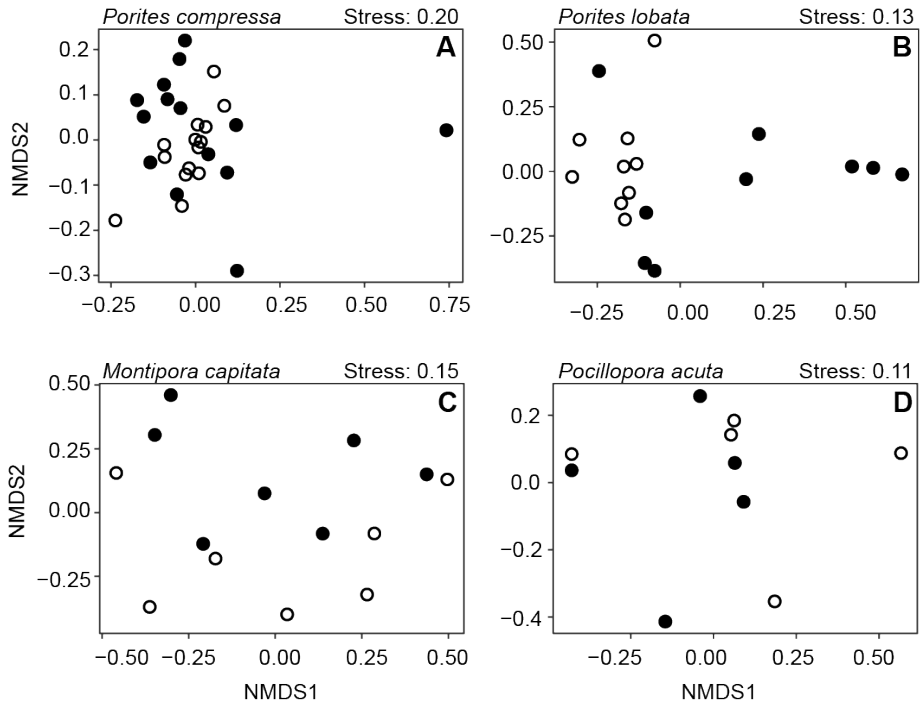

Figure 4.4. NMDS plot of coral-associated microbial community composition between the control condition (closed circles) and the dual stress treatment (open circles) for genets of each coral species that survived the dual stress treatment (illustrated in Fig. 4.1A). Corresponding statistical comparisons are in Table 4.7.

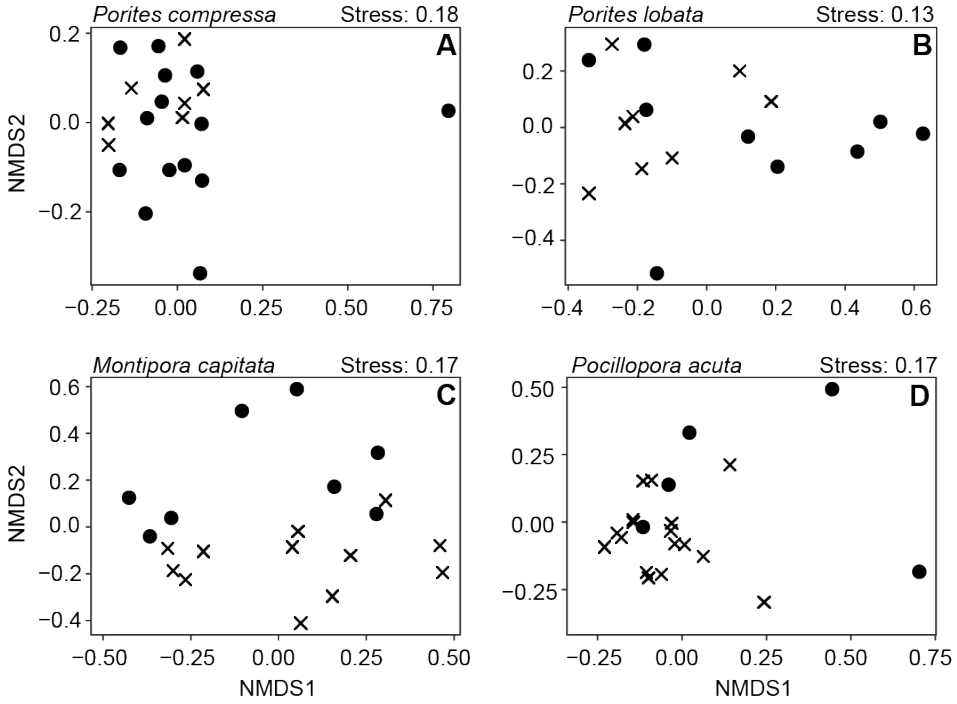

Figure 4.5. NMDS plot of coral-associated microbial community composition of A) *Porites compressa,* B) *Porites lobata,* C) *Montipora capitata,* and D) *Pocillopora acuta* in the control categorized by whether each corresponding genet survived (closed circles) or died (X) in the dual stress treatment (Illustrated in Fig. 4.1B). Corresponding statistical comparisons are in Table 4.11.

4.8 Supporting Information

Table 4.2. Alpha diversity metrics for the microbial communities associated with each coral species in the control. No significant differences ($p < 0.05$) were found among species for any alpha diversity metric.

Species	Observed OTUs	Chao1	Shannon's Diversity	Faith's PD
P. compressa (n = 21)	646.00 ± 748.53	901.65 ± 1057.83	3.35 ± 1.45	25.46 ± 18.68
P. lobata (n = 17)	1157.41 ± 1345.31	1714.65 ± 1977.38	3.55 ± 1.82	37.06 ± 33.00
M. capitata (n = 20)	680.90 ± 808.65	903.33 ± 1107.75	3.65 ± 1.54	22.89 ± 21.24
P. acuta (n = 22)	371.68 ± 376.32	523.41 ± 552.89	2.66 ± 1.19	16.90 ± 10.56

Table 4.3. ANOSIM statistics comparing A) the coral-associated microbial communities of each species in the control. ANOSIM statistics for comparisons among corals within species in the control based on their provenance in (B) *Porites compressa*, (C) *Porites lobata*, (D) *Montipora capitata*, and (E) *Pocillopora acuta*. Significant differences are noted in bold ($p < 0.05$).

A) By Species			
Groups	**R-value**	**P-value**	**Permutations**
Porites compressa – Porites lobata	0.08	**0.04**	999
Porites compressa – Montipora capitata	0.26	**<0.01**	999
Porites compressa – Pocillopora acuta	0.33	**<0.01**	999
Porites lobata – Montipora capitata	0.39	**<0.01**	999
Porites lobata – Pocillopora acuta	0.33	**<0.01**	999
Montipora capitata – Pocillopora acuta	0.35	**<0.01**	999

B) *Porites compressa*			
Groups	**R-value**	**P-value**	**Permutations**
Haleʻiwa – HIMB	-0.08	0.78	462
Haleʻiwa – Sampan	0.02	0.35	126
Haleʻiwa – Waimānalo	0.12	0.19	126
HIMB – Sampan	-0.02	0.53	462
HIMB – Waimānalo	-0.12	0.84	462
Sampan – Waimānalo	0.20	0.09	126

C) *Porites lobata*			
Groups	**R-value**	**P-value**	**Permutations**
Haleʻiwa – Sampan	-0.06	0.58	462
Haleʻiwa – Waimānalo	-0.11	0.79	462
Sampan – Waimānalo	-0.05	0.58	462

D) *Montipora capitata*			
Groups	**R-value**	**P-value**	**Permutations**
Haleʻiwa – HIMB	0.54	**0.03**	84
Haleʻiwa – Sampan	0.60	**0.04**	56
Haleʻiwa – Waimānalo	0.31	0.06	84
HIMB – Sampan	0.09	0.25	462
HIMB – Waimānalo	0.24	**0.05**	462
Sampan – Waimānalo	0.11	0.22	462

Continued

Table 4.3 continued

E) *Pocillopora acuta*

Groups	R-value	P-value	Permutations
Haleʻiwa – HIMB	0.29	**0.05**	210
Haleʻiwa – Sampan	0.15	**0.04**	462
Haleʻiwa – Waimānalo	-0.02	0.44	462
HIMB – Sampan	-0.01	0.47	210
HIMB – Waimānalo	0.20	0.10	210
Sampan – Waimānalo	0.02	0.34	462

Table 4.4. Alpha diversity metrics for the microbial communities associated with each the collection sites for each coral species. Letters indicate significant differences ($p < 0.05$) among the alpha diversity metrics within each species.

Species	Observed OTUs	Chao1	Shannon	Faith's PD
Porites compressa				
Hale'iwa	738.00 ± 783.40	1030.78 ± 1135.83	3.96 ± 1.30	27.80 ± 17.70
HIMB	483.00 ± 279.00	662.06 ± 335.50	3.44 ± 1.24	23.49 ± 10.57
Sampan	1127.80 ± 1244.86	1595.56 ± 1752.64	3.28 ± 2.38	36.43 ± 30.63
Waimānalo	267.80 ± 146.30	366.12 ± 158.51	2.70 ± 0.49	14.51 ± 6.43
Porites lobata				
Hale'iwa	1044.80 ± 1467.81	1594.67 ± 2180.02	3.30 ± 2.03	35.42 ± 36.51
Sampan	1342.33 ± 1437.75	1923.95 ± 2075.26	3.65 ± 1.81	43.29 ± 33.76
Waimānalo	1255.60 ± 1467.24	1889.42 ± 2190.32	3.85 ± 2.15	37.27 ± 36.17
Montipora capitata				
Hale'iwa	1487.67 ± 725.16[a]	2123.13 ± 1289.32[a]	5.33 ± 0.33	48.13 ± 16.26
HIMB	200.50 ± 239.87[b]	243.56 ± 279.35[b]	2.72 ± 1.29	9.23 ± 6.91
Sampan	505.00 ± 651.53[a,b]	618.03 ± 722.49[a,b]	3.08 ± 1.48	17.64 ± 18.71
Waimānalo	904.50 ± 1057.82[a]	1190.97 ± 1380.82[a]	4.23 ± 1.45	28.31 ± 24.73
Pocllopora acuta				
Hale'iwa	214.17 ± 99.05	282.46 ± 119.30	2.51 ± 0.79	12.53 ± 3.65
HIMB	173.00 ± 32.09	250.36 ± 62.09	2.22 ± 1.01	10.70 ± 1.21
Sampan	464.33 ± 548.72	708.75 ± 871.71	2.92 ± 1.73	19.94 ± 14.19
Waimānalo	569 ± 406.09	761.06 ± 518.82	2.84 ± 1.19	22.38 ± 12.34

Table 4.5. SIMPER statistics of the microbial communities associated with corals in the control based on their provenance. Only species with significantly different microbial communities are included, as assessed via ANOSIM (see Table 4.3B–E). Results shown up to 25% cumulative contribution.

A) *Montipora capitata*

Order	Genus	OTU	Average Abundance (%)	Average Abundance (%)	Average Dissimilarity (%)	Cumulative Contribution (%)
			Hale'iwa	HIMB		
Oceanospirillales	*Endozoicomonas sp.*	DQ917863	0.00	14.95	7.48	7.76
Oceanospirillales	*Endozoicomonas sp.*	New.ReferenceOTU733	0.00	11.00	5.50	13.46
Rickettsiales	Midichloriaceae_MD3-55	AY942762	0.01	8.51	4.26	17.88
Rhodospirillales	*Roseospira sp.*	AM282560	6.11	0.00	3.05	21.05
Flavobacterales	*Aquimarina sp.*	JN233118	5.10	0.00	2.55	23.69
Rhizobiales	*Methylobacterium radiotolerans*	AB518685	0.08	4.81	2.37	26.15
			Hale'iwa	Sampan		
Oceanospirillales	*Endozoicomonas sp.*	DQ917863	0.00	22.45	11.22	11.67
Oceanospirillales	*Endozoicomonas sp.*	New.ReferenceOTU733	0.00	17.01	8.50	20.51
Rhodospirillales	*Roseospira sp.*	AM282560	6.11	0.00	3.05	23.68
Flavobacterales	*Aquimarina sp.*	JN233118	5.10	0.02	2.55	26.33
			Hale'iwa	Waimānalo		
Oceanospirillales	*Endozoicomonas sp.*	New.ReferenceOTU733	0.00	8.59	4.29	4.79
Sphingomonadales	Sphingobium sp.	AB453306	0.00	6.16	3.08	8.23
Rhodospirillales	*Roseospira sp.*	AM282560	6.11	0.01	3.06	11.64
Desulfovibrionales	Desulfovibrionales_Unclassified	DQ517281	0.00	5.92	2.96	14.95
Myxococcales	Myxococcales_P3OB-42	GU118271	0.00	5.75	2.88	18.16
Flavobacterales	*Aquimarina sp.*	JN233118	5.10	0.09	2.55	21.01
Rickettsiales	Midichloriaceae_MD3-55	AY942762	0.01	3.11	1.55	22.74

Continued

Table 4.5 continued

Oceanospirillales	Halomonas sp.	GU001884	0.01	2.72	1.35	24.25
Rhodobacterales	Rhodobacteraceae_Unclassified	AB470948	1.57	1.77	1.31	25.71
			HIMB	Waimānalo		
Oceanospirillales	*Endozoicomonas sp.*	DQ917863	14.95	1.59	7.98	8.65
Oceanospirillales	*Endozoicomonas sp.*	New.ReferenceOTU733	11.00	8.59	7.44	16.72
Rickettsiales	Midichloriaceae_MD3-55	AY942762	8.51	3.11	4.71	21.83
Myxococcales	Myxococcales_P3OB-42	GU118271	1.73	5.75	3.43	15.54
			Sampan	Waimānalo		
Oceanospirillales	*Endozoicomonas sp.*	DQ917863	22.45	1.59	11.15	12.66
Oceanospirillales	*Endozoicomonas sp.*	New.ReferenceOTU733	17.01	8.59	8.36	22.16
Myxococcales	Myxococcales_P3OB-42	GU118271	1.69	5.75	3.41	26.03
			HIMB	Sampan		
Oceanospirillales	*Endozoicomonas sp.*	DQ917863	14.95	22.45	14.82	16.57
Oceanospirillales	*Endozoicomonas sp.*	New.ReferenceOTU733	11.00	17.01	10.25	28.04

B) *Pocillopora acuta*

	Genus		**Average Abundance (%)**	**Average Abundance (%)**	**Average Dissimilarity (%)**	**Cumulative Contribution (%)**
			Hale'iwa	HIMB		
Myxococcales	Myxococcales_Unclassified	New.ReferenceOTU1442	0.01	22.48	11.24	12.63
Cytophagales	*Candidatus* Amoebophilus sp.	GU119061	24.54	7.00	10.47	24.39
Vibrionales	*Vibrio sp.*	AB000390	11.54	6.45	7.80	33.15

Continued

Table 4.5 continued

			Hale‘iwa	Sampan		
Cytophagales	*Candidatus* Amoebophilus sp.	GU119061	24.54	24.07	14.36	16.56
Kiloniellales	Fodinicurvataceae_Unclassified	AY654833	0.00	14.24	7.12	24.77
Vibrionales	*Vibrio sp.*	AB000390	11.54	0.53	5.85	31.52
			Hale‘iwa	Waimānalo		
Cytophagales	*Candidatus* Amoebophilus sp.	GU119061	24.54	23.37	9.86	12.28
Oceanospirillales	*Endozoicomonas sp.*	New.ReferenceOTU733	6.30	17.33	9.09	23.60
Vibrionales	*Vibrio sp.*	AB000390	11.54	0.29	5.75	30.76
			HIMB	Waimānalo		
Myxococcales	Myxococcales_Unclassified	New.ReferenceOTU1442	22.48	0.06	11.26	12.99
Cytophagales	*Candidatus* Amoebophilus sp.	GU119061	7.00	23.37	9.20	23.61
Oceanospirillales	*Endozoicomonas sp.*	New.ReferenceOTU733	0.59	17.33	8.62	33.56
			Sampan	Waimānalo		
Cytophagales	*Candidatus* Amoebophilus sp.	GU119061	24.07	23.37	14.01	16.57
Oceanospirillales	*Endozoicomonas sp.*	New.ReferenceOTU733	0.09	17.33	8.63	26.78
			HIMB	Sampan		
Cytophagales	*Candidatus* Amoebophilus sp.	GU119061	7.00	24.07	12.12	13.71
Myxococcales	Myxococcales_Unclassified	New.ReferenceOTU1442	22.48	0.00	11.24	26.43

Table 4.6. Alpha diversity metrics for the microbial communities associated with the surviving ramets of each coral species in the (A) ocean acidification treatment, (B) ocean warming treatment, and (C) dual stress treatment compared to the ramets of the same genets in the control (illustrated in Fig. 4.1A). Letters indicate significant differences ($p < 0.05$) among the alpha diversity metrics for each comparison within each species.

A) Control and Acidification Treatment				
Factor	Observed OTUs	Chao1	Shannon	Faith's PD
P. compressa				
Control (n = 17)	620.29 ± 733.74	854.91 ± 1033.07	3.37 ± 1.44	24.89 ± 18.71
Acidification (n = 17)	808.94 ± 814.52	1128.05 ± 1127.01	3.77 ± 1.56	29.41 ± 21.69
P. lobata				
Control (n = 17)	1157.41 ± 1345.31	1714.65 ± 1977.38	3.55 ± 1.82	37.06 ± 33.00
Acidification (n = 17)	1194.06 ± 1309.43	1754.61 ± 1951.52	3.98 ± 1.99	38.00 ± 32.26
M. capitata				
Control (n = 19)	709.47 ± 820.37	942.55 ± 1123.75	3.63 ± 1.58	23.64 ± 21.55
Acidification (n = 19)	474.42 ± 632.22	628.05 ± 855.76	3.06 ± 1.79	17.87 ± 17.63
P. acuta				
Control (n = 19)	297.68 ± 268.91	408.94 ± 347.87	2.38 ± 0.95	14.71 ± 7.71
Acidification (n = 19)	298.37 ± 207.34	400.28 ± 269.14	2.87 ± 0.98	14.40 ± 6.89

B) Control and Warming Treatment				
Factor	Observed OTUs	Chao1	Shannon	Faith's PD
P. compressa				
Control (n = 12)	679.00 ± 860.36	959.03 ± 1209.57	3.13 ± 1.63	25.95 ± 21.39
Warming (n = 12)	935.44 ± 905.49	1270.13 ± 1209.20	3.9 ± 1.63	32.77 ± 23.79
P. lobata				
Control (n = 12)	1211.75 ± 1510.17	1806.42 ± 2210.55	3.41 ± 1.95	37.01 ± 37.74
Warming (n = 12)	2023.58 ± 1602.94	2981.53 ± 2397.99	4.65 ± 2.11	58.70 ± 39.00

Continued

Table 4.6 continued

M. capitata				
Control (n = 8)	616.50 ± 635.07	751.36 ± 727.84	3.24 ± 1.74	20.69 ± 17.33
Warming (n = 8)	279.00 ± 340.14	361.24 ± 378.60	1.90 ± 0.92	12.43 ± 10.13
P. acuta				
Control (n = 7)	212.43 ± 59.49	305.48 ± 101.29	2.53 ± 1.02	13.44 ± 1.69
Warming (n = 7)	409.43 ± 454.03	567.04 ± 613.40	2.07 ± 1.22	17.47 ± 14.99

C) Control and Dual Stress Treatment

Factor	Observed OTUs	Chao1	Shannon	Faith's PD
P. compressa				
Control (n = 14)	663.86 ± 803.93	925.17 ± 1129.45	3.15 ± 1.58	25.99 ± 20.54
Dual Stress (n = 14)	1460.64 ± 1084.93	2179.84 ± 1691.58	4.45 ± 1.47	46.45 ± 25.27
P. lobata				
Control (n = 9)	860.44 ± 1324.27[a]	1271.71 ± 1887.78[a]	2.94 ± 1.68[a]	28.42 ± 33.12[a]
Dual Stress (n = 9)	3074.56 ± 1275.09[b]	4425.23 ± 1721.36[b]	5.61 ± 1.11[b]	83.10 ± 27.03[b]
M. capitata				
Control (n = 7)	470.29 ± 520.58	591.62 ± 616.36	2.92 ± 1.60	16.55 ± 13.81
Dual Stress (n = 7)	179.29 ± 107.73	245.84 ± 121.50	2.20 ± 1.61	9.47 ± 4.32
P. acuta				
Control (n = 6)	209.17 ± 34.71	293.72 ± 73.67	2.29 ± 1.33	12.45 ± 2.25
Dual Stress (n = 6)	168.33 ± 52.47	258.00 ± 93.85	1.35 ± 0.55	10.95 ± 3.06

Table 4.7. ANOSIM statistics comparing the microbial communities associated with the surviving ramets of each species in the treatments compared to the ramets of the same genets in the control for (A) *Porites compressa* (B) *Porites lobata*, (C) *Montipora capitata*, (D) *Pocillopora acuta* (illustrated in Fig 1A). Significant differences are noted in bold ($p < 0.05$).

A) *Porites comressa*			
Groups	**R-value**	**P-value**	**Permutations**
Control – Acidification	-0.02	0.69	999
Control – Warming	0.08	**0.05**	999
Control – Dual Stress	0.17	**<0.01**	999
B) *Porites lobata*			
Groups	**R-value**	**P-value**	**Permutations**
Control – Acidification	-0.03	0.66	999
Control – Warming	0.17	**0.02**	999
Control – Dual Stress	0.34	**<0.01**	999
C) *Montipora capitata*			
Groups	**R-value**	**P-value**	**Permutations**
Control – Acidification	0.03	0.19	999
Control – Warming	0.21	**0.03**	999
Control – Dual Stress	0.03	0.34	999
D) *Pocillopora acuta*			
Groups	**R-value**	**P-value**	**Permutations**
Control – Acidification	-0.01	0.53	999
Control – Warming	0.02	0.25	999
Control – Dual Stress	-0.18	0.95	126

Table 4.8. SIMPER statistics for the microbial communities associated with the surviving ramets within the dual stress treatment compared to the ramets of the same genets in the control (illustrated in Fig. 4.1A). Only species with significantly different microbial communities between treatments are included, as assessed via ANOSIM (see Table 4.7). Results shown up to 25% cumulative contribution.

A) *Porites compressa*

Average dissimilarity = 94.69

Order	Genus	OTU	Control Average Abundance (%)	Dual Stress Average Abundance (%)	Average Dissimilarity (%)	Cumulative Contribution (%)
Rhodospirillales	*Roseospira sp.*	AM282560	7.47	0.00	3.74	3.94
Thiotrichales	*Caedibacter sp.*	FJ202708	7.36	0.31	3.66	7.81
Oceanospirillales	Kistimonas sp.	New.ReferenceOTU57	7.02	0.00	3.51	11.52
Entomoplasmatales	*Spiroplasma sp.*	New.ReferenceOTU480	6.95	0.00	3.47	15.19
Cytophagales	*Candidatus* Amoebophilus sp.	GU119061	1.65	5.56	3.22	18.59
Oceanospirillales	*Endozoicomonas sp.*	FJ930289	3.56	1.01	2.18	20.89
Oceanospirillales	*Endozoicomonas sp.*	New.ReferenceOTU733	3.91	0.65	2.10	23.11
Unassigned	Unassigned	New.ReferenceOTU1706	3.73	0.29	1.96	25.18

B) *Porites lobata*

Average dissimilarity = 89.90

Order	Genus	OTU	Control Average Abundance (%)	Dual Stress Average Abundance (%)	Average Dissimilarity (%)	Cumulative Contribution (%)
Oceanospirillales	Kistimonas sp.	New.ReferenceOTU57	23.51	0.03	11.75	13.07
Cytophagales	*Candidatus* Amoebophilus sp.	GU119061	15.86	7.97	9.24	23.36
Rhodobacterales	Rhodobacteraceae_Unclassified	FJ202414	0.00	5.71	2.85	26.53
Oceanospirillales	*Kistimonas sp.*	FN562810	3.52	0.01	1.76	28.49
Rhodospirillales	*Tistlia sp.*	New.ReferenceOTU85	2.64	1.06	1.73	30.41

Table 4.9. The proportion of comparisons in each category for βNTI (variable selection, stochastic processes, or homogenous selection) and RC_{BC} (dispersal limitation, ecological drift, or homogenizing dispersal), based on comparisons between the microbial communities associated with surviving ramets in each treatment compared with ramets of the same genets in the control (illustrated Fig. 4.1A). Instances where a majority of comparisons are significantly different from the null communities are noted in bold.

A) *Porites compressa*				
Treatment	βNTI		RC_{BC}	
Control – Acidification				
	Variable Selection	18.3%	Dispersal Limitation	37.2%
	Stochastic Processes	81.7%	Ecological Drift	61.8%
	Homogenous Selection	0.0%	Homogenizing Dispersal	0.0%
Control – Warming				
	Variable Selection	19.4%	**Dispersal Limitation**	**52.6%**
	Stochastic Processes	80.6%	Ecological Drift	46.6%
	Homogenous Selection	0.0%	Homogenizing Dispersal	0.8%
Control – Dual Stress				
	Variable Selection	28.6%	**Dispersal Limitation**	**63.6%**
	Stochastic Processes	71.4%	Ecological Drift	36.4%
	Homogenous Selection	0.0%	Homogenizing Dispersal	0.0%
B) *Porites lobata*				
Treatment	βNTI		RC_{BC}	
Control – Acidification				
	Variable Selection	11.4%	Dispersal Limitation	21.1%
	Stochastic Processes	88.6%	Ecological Drift	70.3%
	Homogenous Selection	0.0%	Homogenizing Dispersal	8.6%
Control – Warming				
	Variable Selection	24.8%	Dispersal Limitation	35.2%
	Stochastic Processes	75.2%	Ecological Drift	57.1%
	Homogenous Selection	0.0%	Homogenizing Dispersal	7.7%
Control – Dual Stress				
	Variable Selection	21.0%	Dispersal Limitation	43.8%
	Stochastic Processes	79.0%	Ecological Drift	53.1%
	Homogenous Selection	0.0%	Homogenizing Dispersal	3.1%

Continued

Table 4.9 continued

C) *Montipora capitata*

Treatment	βNTI		RC_{BC}	
Control – Acidification				
	Variable Selection	33.2%	Dispersal Limitation	23.8%
	Stochastic Processes	66.2%	Ecological Drift	75.7%
	Homogenous Selection	0.6%	Homogenizing Dispersal	0.4%
Control – Warming				
	Variable Selection	28.1%	Dispersal Limitation	19.6%
	Stochastic Processes	71.9%	Ecological Drift	73.9%
	Homogenous Selection	0.0%	Homogenizing Dispersal	6.5%
Control – Dual Stress				
	Variable Selection	20.4%	Dispersal Limitation	2.6%
	Stochastic Processes	77.6%	Ecological Drift	89.5%
	Homogenous Selection	2.0%	Homogenizing Dispersal	7.9%

D) *Pocillopora acuta*

Treatment	βNTI		RC_{BC}	
Control – Acidification				
	Variable Selection	12.5%	Dispersal Limitation	7.0%
	Stochastic Processes	87.5%	Ecological Drift	92.7%
	Homogenous Selection	0.0%	Homogenizing Dispersal	0.3%
Control – Warming				
	Variable Selection	26.5%	Dispersal Limitation	16.7%
	Stochastic Processes	73.4%	Ecological Drift	80.6%
	Homogenous Selection	0.0%	Homogenizing Dispersal	2.7%
Control – Dual Stress				
	Variable Selection	12.0%	Dispersal Limitation	18.2%
	Stochastic Processes	88.0%	Ecological Drift	77.3%
	Homogenous Selection	0.0%	Homogenizing Dispersal	0.5%

Table 4.10. Alpha diversity metrics for the microbial communities associated with coral ramets in the control whose ramets from the same genet in the dual stress treatment survived or died (Illustrated in Fig 4.1B). Letters indicate significant differences ($p < 0.05$) among the alpha diversity metrics for each comparison within each species.

A) Control and Acidification Treatment				
Factor	Observed OTUs	Chao1	Shannon	Faith's PD
P. compressa				
Survived (n = 15)	761 ± 861.22	1066.55 ± 1218.34	3.31 ± 1.64	28.16 ± 21.51
Died (n = 6)	358.5 ± 159.93	489.39 ± 185.14	3.46 ± 0.95	18.7 ± 4.85
P. lobata				
Survived (n = 9)	860.44 ± 1324.27	1271.71 ± 1887.78	2.94 ± 1.68	28.42 ± 33.12
Died (n = 8)	1491.5 ± 1375.37	2212.97 ± 2080.48	4.23 ± 1.82	46.78 ± 32.12
M. capitata				
Survived (n = 8)	616.5 ± 635.07	751.36 ± 727.84	3.24 ± 1.74	20.69 ± 17.33
Died (n = 12)	723.83 ± 931.55	1004.65 ± 1324.55	3.93 ± 1.4	24.36 ± 24.13
P. damicornis				
Survived (n = 5)	198.6 ± 25.85	278.85 ± 71.58	1.88 ± 0.97	12.1 ± 2.33
Died (n = 17)	422.59 ± 416.64	595.34 ± 612.99	2.89 ± 1.18	18.32 ± 11.65

Table 4.11. ANOSIM statistics comparing the microbial communities associated with coral ramets in the control whose ramets from the same genet in the dual stress treatment survived or died (Illustrated in Fig. 4.1B). Significant differences are noted in bold ($p < 0.05$).

Survived vs. Died			
Species	R-value	P-value	Permutations
Porites compressa	-0.03	0.54	999
Porites lobata	0.09	0.12	999
Montipora capitata	0.16	0.05	999
Pocillopora acuta	**0.35**	**0.03**	999

Table 4.12. Summary of SIMPER statistics between the microbial communities associated with corals genets in the control condition, based on whether each genet survived or died in the dual stress treatment. Only species with significantly different microbial communities are included, as assessed via ANOSIM (see Table 4.11). Results shown up to 25% cumulative contribution.

A) *Montipora capitata*

Average dissimilarity = 92.07

Order	Genus	OTU	Alive: Average Abundance (%)	Dead: Average Abundance (%)	Average Dissimilarity (%)	Cumulative Contribution (%)
Oceanospirillales	*Endozoicomonas sp.*	DQ917863	25.11	0.88	12.57	13.65
Oceanospirillales	*Endozoicomonas sp.*	New.ReferenceOTU733	10.72	9.74	7.64	21.95
Rickettsiales	Midichloriaceae_MD3-55	AY942762	0.01	5.81	2.90	25.11

B) *Pocillopora acuta*

Average dissimilarity = 88.74

Order	Genus	OTU	Alive: Average Abundance (%)	Dead: Average Abundance (%)	Average Dissimilarity (%)	Cumulative Contribution (%)
Cytophagales	*Candidatus* Amoebophilus sp.	GU119061	12.42	24.09	12.55	14.14
Myxococcales	Myxococcales_Unclassified	New.ReferenceOTU1442	14.99	0.03	7.50	22.60

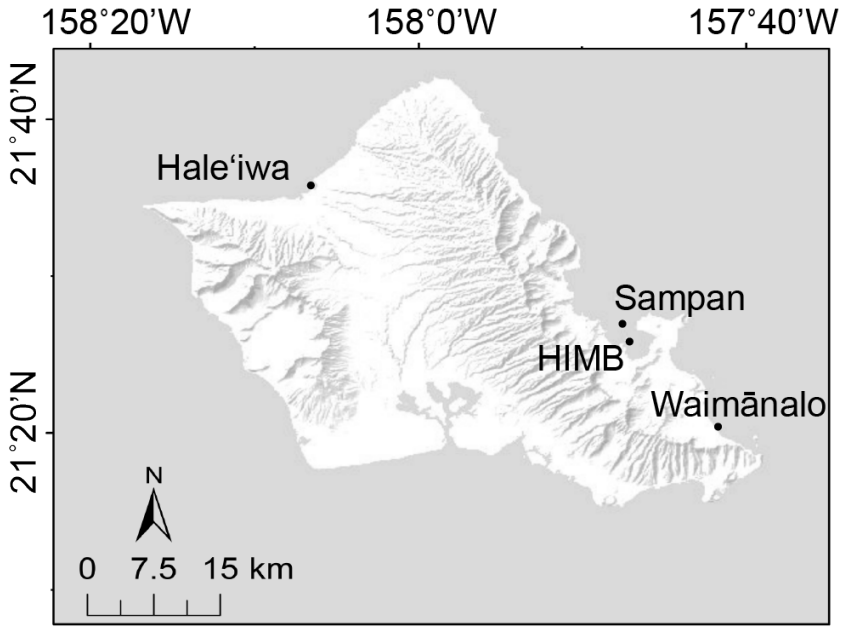

Figure 4.6. Coral collection sites surrounding O'ahu, HI. HIMB = Hawai'i Institute of Marine Biology. Specific coordinates of each site are listed in Table 2.1.

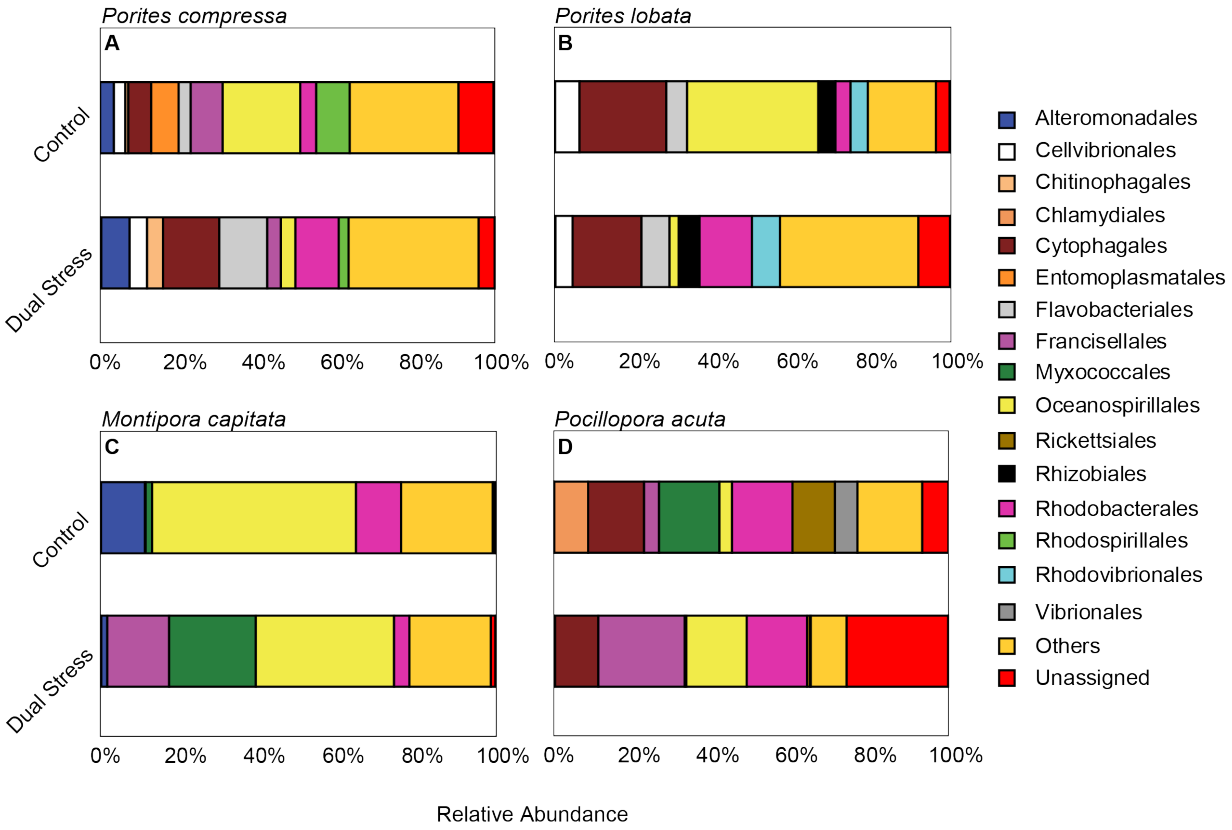

Figure 4.7. Mean relative abundances of the most common microbial Orders associated with surviving genets of each coral species in the control condition and the dual stress treatment. (A) *Porites compressa*, (B) *Porites lobata*, (C) *Montipora capitata*, and (D) *Pocillopora acuta*.

Chapter 5: Summary and Future Research

The survival of corals and the persistence of coral reef ecosystems are threatened by ocean warming and acidification associated with global climate change. Some coral species are more tolerant of the effects of a changing climate than others, but it is unclear whether most corals will be able to adapt or acclimatize to ocean warming and acidification expected by the end of the 21st century. While several physiological parameters are associated with resilience in rising seawater temperatures and more acidic waters, little is known about the potential connections between coral physiology and their associated microbial communities, and how these connections may affect the ability of a coral to persist in the future. Therefore, the goals of this dissertation were as follows:

1. Evaluate the trophic strategies of seven Hawaiian coral species across a natural physicochemical environmental gradient.
2. Characterize the microbial communities associated with four Hawaiian coral species across the same environmental gradient and assess potential relationships between the microbiome and coral physiology.
3. Assess the effects of long-term ocean warming and/or acidification on the microbial communities associated with four Hawaiian coral species.

5.1 Summary

1. Hawaiian corals utilize a variety of trophic strategies, such that the estimated contribution to coral tissues from heterotrophic sources is 20 – 50%. However, the estimates of heterotrophy among the seven corals (*Montipora capitata*, *Montipora patula*, *Pocillopora acuta*, *Pocillopora meandrina*, *Porites compressa*, *Porites lobata*, and *Porites evermanni*) varied greatly depending on the isotopic approach used, and these estimates did not always match past direct measurements of heterotrophy. Bayesian mixing models revealed that heterotrophic sources contributed the most to *P. acuta* and *M. patula* at 49.5% and 47.3%, respectively, and the least to *Porites lobata* at 24.3%. Estimates of heterotrophic contribution based on the $\delta^{15}N$ of the host and algal endosymbiont, as well as the isotopic niche overlap approach, were slightly more aligned with the estimates produced using Bayesian mixing models than $\delta^{13}C$ of the host and algal endosymbiont. These findings suggest that estimates of heterotrophic contribution to coral tissues do not always match known feeding capacity and resilience on the reef, and that the utility of each approach may vary with coral health status, and among regions and coral species.
2. The microbial community composition of Hawaiian corals varied among species, provenance, and is related to the estimated proportionate contribution of heterotrophy to coral tissues. While microbial communities differed greatly among the four coral species (*P. compressa*, *P. lobata*, *P. acuta*, and *P. meandrina*), differences among the collection sites were likely due to stochastic

processes among the separated sites, rather than the local environmental conditions. Within each coral species, the nutritional sources accessed by a coral (i.e., heterotrophic vs. photoautotrophic) may partially govern the structure of its microbial community. This structuring has important implications for corals in a changing climate, as heterotrophic capacity and plasticity are posited as key factors in the potential resilience of corals to rising seawater temperatures. However, not all corals shared the same relationship between microbial community composition, environmental conditions, and coral physiology, indicating a high degree of species specificity in how coral-associated microbial communities may respond to the chronic pressures of climate change.

3. Corals had species-specific patterns in mortality and associated microbial community composition, linking the microbiome to the potential acclimatization of Hawaiian corals to end-of-century ocean conditions. The associated microbial community composition of surviving *P. compressa* and *P. lobata* differed between the control and predicted end-of-century ocean conditions (+2.0 °C and/or -0.2 pH units relative to present day), while those of *M. capitata* and *P. acuta* did not. However, the communities associated with the latter two species differed among ramets within the control based on the survival of their genetic counterparts in the end-of-century conditions. These two patterns in microbial community composition aligned closely with mortality, such that *Porites* corals had the lowest mortality and microbial communities that shifted by the end of the experiment, whereas *Montipora capitata* and *Pocillopora acuta* had high

mortality and their microbial communities did not change. This suggests that corals with microbial communities that can shift and potentially acclimatize to future ocean conditions may be more resilient and become more abundant on the coral reefs of the future.

Overall, these findings suggest that Hawaiian corals employ a variety of trophic strategies and host a diverse range of associated microbial communities, which will likely support the persistence of some of these corals in a changing climate. Corals like *P. compressa* and *P. lobata,* which can use a variety of nutritional sources and have flexible associated microbial communities, appear to be the most tolerant of conditions expected by the end of this century. However, the great diversity of individual corals and associated microbial communities around O‘ahu suggests that even coral species that appear most susceptible could still persist through a few resilient individuals. Yet, climate change is rapidly advancing, and ocean warming and acidification will continue to place increasingly severe stress on corals and the organisms they support. Although some coral species are more tolerant of changing ocean conditions, the health of reefs will likely still decline and it is unclear how a loss of diversity may affect the function of these ecosystems. Without a concerted effort to lessen anthropogenic effects on corals, the persistence of future coral reefs is unknown.

5.2 Future Research

The findings presented in this dissertation lead to the following questions and lines of possible future research:

1. Among the several isotopic approaches used to estimate the proportionate contribution of heterotrophy to coral tissues, none showed a strong relationship with another. In addition, corals can incorporate and allocate nutritional resources differently depending on the species, location, and health status. For example, carbon and nitrogen are constantly recycled between the host and algal endosymbiont, but it is unknown how this internal recycling between different coral species and endosymbiont types may affect the fractionation of $\delta^{13}C$ and $\delta^{15}N$, and whether this is consistent among populations of the same species. Therefore, further investigation is needed to better understand how the different isotopic approaches used to assess trophic strategies can be best employed and what factors affect interpretations using each approach.
2. We showed for the first time that nutritional resource use by a coral is related to the associated microbial community composition, linking coral physiology with the coral microbiome. However, further research is needed to assess whether changes in coral resource use (or any other physiological parameter) relate to the composition of associated microbial communities over time, seasonally, or with experimental stress. This study provides an initial link between natural variation in physiology and coral-associated microbial communities at one time point, but

assessing whether these change together would help to define potential roles of the coral microbiome in a changing climate.

3. The 22-month mesocosm experiment was the first characterization of coral-associated microbial community responses to stress on a multi-annual scale, providing insight into relationships between coral resilience and their microbiome. Characterizing the microbial community at several points throughout a long-term ocean warming and acidification experiment would help highlight how the microbial community is able to respond to both the chronic stress of the experiment, as well as the short-term stress of seasonal changes and potential bleaching events.
4. Further characterization of the functional potential of coral-associated microbial communities is necessary to begin exploring the roles of these microorganisms. The 16S rRNA approach used here provides important information about the composition of coral-associated microbial communities in response to a changing climate, but the interpretations are limited to a broad taxonomic scale. Metagenomic, metatranscriptomic, and other fine-scale molecular approaches would provide an in-depth characterization of the microbial community dynamics and potential changes in the functional roles of microoganisms in response to stress associated with global climate change.

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Appendix A. Chapter 2 coral stable carbon and nitrogen isotope data

Table A.1 Chapter 2 raw stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope data for host tissue ($\delta^{13}C_h$, $\delta^{15}N_h$), algal endosymbiont ($\delta^{13}C_h$, $\delta^{15}N_h$), host minus algal endosymbiont ($\delta^{13}C_{h\text{-}e}$, $\delta^{15}N_{h\text{-}e}$), and whole coral tissue ($\delta^{13}C_w$, $\delta^{15}N_w$). MC = *Montipora capitata*, MP = *Montipora patula*, PA = *Pocillopora acuta*, PM = *Pocillopora meandrina*, PC = *Porites compressa*, PL = *Porites lobata*, and PE = *Porites evermanni*

ID	Sp	Site	$\delta^{13}C_h$	$\delta^{15}N_h$	$\delta^{13}C_e$	$\delta^{15}N_e$	$\delta^{13}C_{h\text{-}e}$	$\delta^{15}N_{h\text{-}e}$	$\delta^{13}C_w$	$\delta^{15}N_w$
MC-EB-1	MC	ELEB	-12.87	3.78	-13.55	4.55	0.68	-0.77		
MC-EB-2	MC	ELEB	-15.41	4.01	-17.24	4.37	1.83	-0.36	-14.55	4.24
MC-EB-3	MC	ELEB	-16.39	4.01	-18.27	4.11	1.88	-0.10	-15.65	4.10
MC-EB-4	MC	ELEB	-13.68	3.98	-15.50	4.40	1.81	-0.42	-13.44	4.20
MC-EB-5	MC	ELEB	-13.45	4.47	-15.32	4.20	1.87	0.27	-13.57	4.40
MC-EB-6	MC	ELEB	-12.92	4.19	-14.70	4.31	1.77	-0.12	-12.92	4.27
MC-EB-7	MC	ELEB	-15.49	4.09	-17.16	4.23	1.66	-0.14	-15.37	4.20
MC-EB-8	MC	ELEB	-14.54	4.29	-16.54	3.76	2.00	0.52	-14.68	4.37
MC-EB-9	MC	ELEB	-12.43	3.80	-13.60	4.17	1.17	-0.37	-12.84	4.20
MC-EB-10	MC	ELEB	-13.64	3.96	-14.57	4.32	0.93	-0.37		
MC-EB-11	MC	ELEB	-14.89	4.67	-15.74	4.39	0.85	0.29		
MC-EB-12	MC	ELEB	-12.19	4.01	-13.71	4.19	1.52	-0.18	-12.54	4.37
MC-H-1	MC	HALE	-11.27	5.03	-13.00	4.55	1.73	0.48	-12.02	4.73
MC-H-2	MC	HALE	-11.26	4.80	-12.49	3.36	1.23	1.44		
MC-H-3	MC	HALE	-9.91	5.01	-12.16	4.67	2.25	0.34	-11.07	4.90
MC-H-4	MC	HALE	-10.72	4.78	-13.27	3.87	2.55	0.92	-11.92	4.38
MC-H-5	MC	HALE	-11.56	4.53	-14.02	3.77	2.47	0.77		
MC-H-6	MC	HALE	-12.03	5.00	-13.98	4.09	1.95	0.92	-13.03	4.74
MC-H-7	MC	HALE	-9.79	5.07	-12.18	4.48	2.39	0.58	-10.92	4.63
MC-H-8	MC	HALE	-9.69	3.99	-12.13	4.65	2.44	-0.65		
MC-H-9	MC	HALE	-12.11	4.66	-14.12	4.30	2.01	0.37	-12.71	4.67
MC-H-10	MC	HALE	-10.93	5.06	-13.17	4.43	2.24	0.63	-12.37	4.80
MC-H-11	MC	HALE	-11.12	4.75	-12.89	4.24	1.77	0.51		
MC-H-12	MC	HALE	-11.09	4.84	-12.41	3.92	1.33	0.92	-10.32	4.79
MC-KB-1	MC	HIMB	-16.09	4.56	-17.14	5.10	1.05	-0.55	-15.80	4.95
MC-KB-2	MC	HIMB	-17.54	4.97	-18.46	4.13	0.92	0.84	-17.05	4.76
MC-KB-3	MC	HIMB	-13.77	3.86	-15.48	3.88	1.71	-0.03		
MC-KB-4	MC	HIMB	-13.70	4.25	-15.18	4.07	1.48	0.18		
MC-KB-5	MC	HIMB	-15.57	4.85	-16.68	4.76	1.11	0.09	-15.59	4.80
MC-KB-6	MC	HIMB	-14.49	4.50	-16.31	4.86	1.82	-0.36	-15.15	4.49
MC-KB-7	MC	HIMB	-14.76	4.34	-16.04	4.87	1.29	-0.52	-14.70	4.61
MC-KB-8	MC	HIMB	-15.80	4.87	-16.49	5.23	0.69	-0.36	-16.08	4.97
MC-KB-9	MC	HIMB	-16.73	4.94	-18.02	5.27	1.29	-0.33	-16.44	4.94
MC-KB-10	MC	HIMB	-14.16	4.67	-16.05	4.79	1.89	-0.12	-14.69	4.60
MC-KB-11	MC	HIMB	-17.54	5.18	-18.14	5.03	0.60	0.16	-17.64	5.02
MC-KB-12	MC	HIMB	-16.83	5.32	-17.45	4.82	0.62	0.49		

MC-S-1	MC	SAMP	-16.70	3.72	-17.96	3.92	1.26	-0.20	-16.77	3.82
MC-S-2	MC	SAMP	-17.02	3.67	-17.95	3.87	0.93	-0.20	-16.99	3.82
MC-S-3	MC	SAMP	-14.99	3.78	-16.06	4.24	1.07	-0.45	-15.18	3.97
MC-S-4	MC	SAMP	-13.85	3.70	-15.57	3.86	1.72	-0.16	-14.13	3.91
MC-S-5	MC	SAMP	-14.79	3.95	-16.55	4.27	1.76	-0.32		
MC-S-6	MC	SAMP	-15.58	4.14	-16.45	4.68	0.88	-0.54	-15.67	4.25
MC-S-7	MC	SAMP	-14.20	3.62	-15.33	4.12	1.13	-0.50	-14.33	3.89
MC-S-8	MC	SAMP	-16.16	4.00	-17.07	4.41	0.91	-0.41	-15.96	4.26
MC-S-9	MC	SAMP	-17.09	4.31	-17.77	4.37	0.67	-0.05	-17.10	4.26
MC-S-10	MC	SAMP	-18.00	3.94	-19.45	3.77	1.45	0.17		
MC-S-11	MC	SAMP	-15.03	4.78	-14.31	4.63	-0.72	0.15		
MC-S-12	MC	SAMP	-16.20	4.59	-16.55	4.61	0.34	-0.03	-14.48	4.65
MC-W-1	MC	WAI	-13.01	3.83	-14.45	4.36	1.45	-0.53	-13.22	4.03
MC-W-2	MC	WAI	-14.15	4.30	-16.25	3.76	2.10	0.54	-14.23	4.07
MC-W-3	MC	WAI	-14.42	4.25	-16.91	3.76	2.49	0.49		
MC-W-4	MC	WAI	-15.56	4.18	-17.20	3.96	1.64	0.23	-15.90	4.15
MC-W-5	MC	WAI	-14.01	3.70	-15.88	4.33	1.88	-0.63	-14.21	3.98
MC-W-6	MC	WAI	-14.56	3.41	-16.09	3.59	1.53	-0.18	-14.71	3.48
MC-W-7	MC	WAI	-13.75	4.00	-15.61	3.55	1.86	0.45	-13.96	3.91
MC-W-8	MC	WAI	-15.23	4.57	-17.16	3.89	1.93	0.67	-15.35	4.25
MC-W-9	MC	WAI	-12.56	4.11	-14.53	3.70	1.97	0.41	-12.54	3.83
MC-W-10	MC	WAI	-12.82	4.08	-15.30	3.35	2.47	0.74	-12.81	4.00
MC-W-11	MC	WAI	-15.82	4.46	-18.02	4.24	2.20	0.22		
MC-W-12	MC	WAI	-13.77	5.35	-15.64	4.76	1.88	0.59	-13.86	5.24
MP-EB-1	MP	ELEB	-17.52	4.44	-18.25	3.96	0.73	0.48	-17.61	4.43
MP-EB-2	MP	ELEB	-17.94	5.00	-18.88	3.84	0.94	1.15	-18.05	4.78
MP-EB-3	MP	ELEB	-18.80	4.78	-19.29	4.28	0.49	0.50	-18.75	4.60
MP-EB-4	MP	ELEB	-18.42	5.07	-19.42	3.88	1.00	1.19	-18.93	4.70
MP-EB-5	MP	ELEB	-16.66	4.88	-16.69	4.23	0.03	0.65	-16.58	4.73
MP-EB-6	MP	ELEB	-16.81	5.10	-18.13	4.18	1.32	0.92	-17.10	4.91
MP-EB-7	MP	ELEB	-14.27	4.91	-15.46	4.04	1.19	0.86	-14.30	4.73
MP-EB-8	MP	ELEB	-15.40	5.20	-16.62	4.42	1.23	0.78	-17.51	4.94
MP-EB-9	MP	ELEB	-16.02	5.22	-16.84	4.03	0.81	1.19	-16.16	4.91
MP-EB-10	MP	ELEB	-17.93	4.79	-18.82	4.01	0.88	0.78		
MP-EB-11	MP	ELEB	-15.92	4.90	-17.15	4.05	1.23	0.85		
MP-EB-12	MP	ELEB	-15.94	4.85	-16.28	4.04	0.34	0.81		
MP-EB-13	MP	ELEB	-14.57	4.92	-15.90	4.34	1.33	0.58		
MP-EB-15	MP	ELEB	-16.89	5.17	-17.92	4.35	1.03	0.82		
MP-H-1	MP	HALE	-13.97	5.44	-16.27	3.55	2.31	1.89	-14.56	4.61
MP-H-2	MP	HALE	-13.78	6.15	-15.97	3.66	2.19	2.49	-14.19	5.51
MP-H-3	MP	HALE	-14.46	5.78	-15.98	4.07	1.52	1.71	-15.05	4.78
MP-H-4	MP	HALE	-15.01	5.77	-16.42	2.81	1.41	2.96	-14.92	5.38
MP-H-5	MP	HALE	-15.57	5.64	-17.07	3.69	1.50	1.95	-15.57	5.03

MP-H-6	MP	HALE	-16.08	5.33	-16.92	4.31	0.84	1.02	-16.38	4.93
MP-H-7	MP	HALE	-15.38	5.79	-17.62	2.98	2.25	2.81	-15.64	5.41
MP-H-8	MP	HALE	-14.16	5.82	-16.83	3.31	2.67	2.51	-15.72	4.21
MP-H-9	MP	HALE	-14.36	5.48	-15.64	4.01	1.29	1.47	-14.10	5.61
MP-H-10	MP	HALE	-15.21	5.54	-17.49	4.58	2.28	0.96		
MP-H-11	MP	HALE	-15.46	5.81	-17.34	3.80	1.88	2.02		
MP-H-12	MP	HALE	-14.27	5.79	-15.45	4.36	1.19	1.43		
MP-H-13	MP	HALE	-15.40	5.50	-16.30	3.92	0.90	1.58		
MP-H-14	MP	HALE	-14.70	5.99	-15.97	4.04	1.27	1.94		
MP-H-15	MP	HALE	-15.51	5.45	-16.83	4.31	1.32	1.14		
MP-S-1	MP	SAMP	-16.01	3.47	-17.53	2.30	1.52	1.16	-16.21	3.22
MP-S-2	MP	SAMP	-13.99	3.71	-16.15	1.76	2.16	1.95	-14.40	3.30
MP-S-3	MP	SAMP	-16.41	3.43	-17.23	2.92	0.82	0.52	-16.39	3.21
MP-S-4	MP	SAMP	-15.88	3.47	-16.31	2.95	0.43	0.51	-15.74	3.31
MP-S-5	MP	SAMP	-16.82	3.76	-16.97	3.35	0.15	0.41	-16.67	3.59
MP-S-6	MP	SAMP	-14.97	4.03	-16.77	2.50	1.81	1.53	-15.17	3.76
MP-S-7	MP	SAMP	-12.85	4.38	-16.19	1.85	3.34	2.53	-13.23	3.84
MP-S-8	MP	SAMP	-16.04	3.55	-16.76	2.80	0.72	0.75		
MP-S-9	MP	SAMP	-13.38	4.54	-16.52	1.51	3.14	3.02	-13.68	4.03
MP-S-10	MP	SAMP	-15.98	4.80	-17.13	3.63	1.15	1.17	-16.33	4.54
MP-S-11	MP	SAMP	-15.97	4.54	-16.34	3.65	0.37	0.89		
MP-S-12	MP	SAMP	-16.89	4.39	-18.65	3.71	1.76	0.68		
MP-S-13	MP	SAMP	-15.57	4.51	-16.08	3.94	0.51	0.57		
MP-S-15	MP	SAMP	-17.87	4.19	-19.81	3.31	1.94	0.88		
MP-S-16	MP	SAMP	-15.77	4.57	-16.56	3.41	0.79	1.16		
MP-S-17	MP	SAMP	-17.28	4.21	-18.09	3.90	0.81	0.30		
MP-W-1	MP	WAI	-14.87	5.24	-15.84	4.20	0.97	1.04	-15.03	4.72
MP-W-2	MP	WAI	-14.49	4.78	-16.13	3.57	1.64	1.21	-15.12	4.27
MP-W-3	MP	WAI	-15.09	4.92	-16.57	3.36	1.49	1.56	-15.29	4.59
MP-W-4	MP	WAI	-15.92	5.04	-17.37	3.76	1.45	1.28	-16.65	4.26
MP-W-5	MP	WAI	-15.28	4.67	-16.31	4.09	1.03	0.57	-15.78	4.28
MP-W-6	MP	WAI	-14.37	5.04	-16.44	3.29	2.07	1.75	-14.60	4.80
MP-W-7	MP	WAI	-15.21	4.48	-16.79	3.85	1.58	0.63	-15.87	4.36
MP-W-8	MP	WAI	-15.11	5.21	-16.65	4.17	1.54	1.04	-15.48	4.88
MP-W-9	MP	WAI	-16.31	5.13	-17.69	4.35	1.38	0.77	-16.87	5.00
MP-W-10	MP	WAI	-13.79	4.99	-15.82	4.00	2.03	0.98		
MP-W-11	MP	WAI	-17.04	4.68	-18.39	3.25	1.35	1.43		
MP-W-12	MP	WAI	-17.72	6.17	-18.43	4.94	0.70	1.23		
MP-W-13	MP	WAI	-16.51	5.86	-18.06	5.16	1.55	0.70		
MP-W-14	MP	WAI	-15.54	6.49	-18.10	5.52	2.56	0.98		
MP-W-15	MP	WAI	-14.55	6.46	-15.37	5.32	0.82	1.14		
PA-H-1	PA	HALE	-16.51	5.20	-16.27	3.03	-0.24	2.17	-16.07	4.87
PA-H-2	PA	HALE	-15.89	5.07	-15.67	2.69	-0.22	2.38	-15.51	4.85

PA-H-3	PA	HALE	-15.78	5.04	-15.78	3.02	0.00	2.02	-15.47	4.87
PA-H-4	PA	HALE	-15.40	5.48	-15.51	3.69	0.11	1.79	-15.15	5.22
PA-H-5	PA	HALE	-16.40	5.12	-15.98	3.18	-0.41	1.94	-15.64	4.85
PA-H-6	PA	HALE	-15.82	5.07	-16.00	4.69	0.18	0.37	-15.52	5.02
PA-H-7	PA	HALE	-14.79	5.41	-14.99	3.96	0.21	1.44	-14.64	5.22
PA-H-8	PA	HALE	-17.65	5.09	-17.06	3.04	-0.59	2.05	-16.78	4.68
PA-H-9	PA	HALE	-15.59	5.30	-15.64	4.02	0.06	1.28	-15.30	4.85
PA-H-10	PA	HALE	-16.80	5.33	-16.58	3.04	-0.22	2.29		
PA-H-11	PA	HALE	-15.76	4.98	-15.00	5.64	-0.75	-0.66		
PA-H-12	PA	HALE	-15.57	5.37	-15.82	3.65	0.24	1.72		
PA-KB-1	PA	HIMB	-16.81	5.19	-16.67	4.91	-0.14	0.28	-16.49	5.38
PA-KB-2	PA	HIMB	-18.55	4.69	-17.78	4.36	-0.77	0.33	-17.61	4.45
PA-KB-3	PA	HIMB	-15.22	5.18	-16.04	4.67	0.81	0.50	-15.45	5.26
PA-KB-4	PA	HIMB	-15.54	5.00	-15.70	5.10	0.16	-0.10	-15.40	5.37
PA-KB-5	PA	HIMB	-16.19	5.02	-16.18	4.74	-0.01	0.28	-15.93	5.25
PA-KB-6	PA	HIMB	-17.19	5.38	-17.50	5.30	0.31	0.08	-17.07	5.48
PA-KB-7	PA	HIMB	-16.40	5.32	-16.41	4.96	0.01	0.36	-16.08	5.61
PA-KB-8	PA	HIMB	-15.24	5.97	-16.48	5.10	1.24	0.87		
PA-KB-9	PA	HIMB	-16.48	5.29	-17.09	4.90	0.60	0.39	-15.74	5.75
PA-KB-10	PA	HIMB	-17.14	5.39	-18.12	4.72	0.97	0.68	-17.26	5.30
PA-KB-11	PA	HIMB	-14.39	5.07	-14.77	4.84	0.38	0.22		
PA-KB-12	PA	HIMB	-16.59	5.04	-17.13	4.97	0.54	0.07		
PA-MI-1	PA	MAGI	-15.16	4.72	-15.82	4.55	0.65	0.17		
PA-MI-2	PA	MAGI	-15.30	4.70	-16.88	2.51	1.58	2.19	-16.06	4.18
PA-MI-3	PA	MAGI	-15.38	5.82	-16.58	3.96	1.20	1.86	-15.87	5.50
PA-MI-4	PA	MAGI	-14.98	4.70	-16.66	1.60	1.68	3.10	-15.65	3.72
PA-MI-5	PA	MAGI	-14.99	6.35	-17.58	3.20	2.59	3.15	-15.85	5.47
PA-MI-6	PA	MAGI	-15.71	5.40	-17.05	3.33	1.34	2.07	-15.85	5.30
PA-MI-7	PA	MAGI	-15.33	4.36	-16.50	2.94	1.17	1.42	-15.76	4.02
PA-MI-8	PA	MAGI	-14.69	5.56	-15.93	2.98	1.24	2.57	-15.77	4.38
PA-MI-9	PA	MAGI	-16.54	4.61	-17.48	3.24	0.93	1.37		
PA-MI-10	PA	MAGI	-16.17	4.64	-16.63	3.80	0.47	0.84		
PA-MI-11	PA	MAGI	-15.18	4.80	-17.05	1.89	1.87	2.91	-15.74	4.08
PA-MI-12	PA	MAGI	-14.38	4.81	-16.22	3.76	1.84	1.05	-14.89	4.63
PA-S-1	PA	SAMP	-17.37	4.21	-17.87	3.29	0.49	0.91	-17.21	4.22
PA-S-2	PA	SAMP	-17.38	4.34	-17.62	2.97	0.24	1.37	-16.96	4.30
PA-S-3	PA	SAMP	-17.68	4.47	-18.91	2.03	1.23	2.44		
PA-S-4	PA	SAMP	-16.77	4.69	-17.49	2.16	0.73	2.53		
PA-S-5	PA	SAMP	-16.98	4.30	-16.92	3.51	-0.06	0.79		
PA-S-6	PA	SAMP	-16.46	4.73	-17.06	3.79	0.61	0.95	-16.37	4.95
PA-S-7	PA	SAMP	-16.00	4.88	-16.31	4.00	0.30	0.88	-15.80	4.95
PA-S-8	PA	SAMP	-16.02	4.77	-16.44	3.64	0.43	1.12	-15.81	4.88
PA-S-9	PA	SAMP	-16.33	4.74	-16.59	3.70	0.26	1.04		

PA-S-10	PA	SAMP	-17.58	4.62	-17.41	3.93	-0.17	0.69		
PA-S-11	PA	SAMP	-17.77	4.12	-16.86	2.51	-0.91	1.61	-16.70	4.01
PA-S-12	PA	SAMP	-15.72	4.71	-15.56	4.07	-0.16	0.64	-15.26	4.90
PA-W-1	PA	WAI	-14.96	7.04	-17.13	4.89	2.17	2.15	-15.18	6.87
PA-W-2	PA	WAI	-15.54	7.08	-16.72	4.53	1.18	2.54	-15.22	6.50
PA-W-3	PA	WAI	-13.80	8.30	-15.44	6.20	1.64	2.10	-13.97	8.24
PA-W-4	PA	WAI	-14.81	7.01	-16.07	5.49	1.26	1.51		
PA-W-5	PA	WAI	-15.26	5.36	-18.63	3.72	3.36	1.65	-15.09	5.26
PA-W-6	PA	WAI	-14.47	8.47	-17.24	5.47	2.77	2.99	-14.31	8.12
PA-W-7	PA	WAI	-14.41	7.56	-17.68	5.12	3.26	2.44	-14.54	7.16
PA-W-8	PA	WAI	-13.67	9.70	-16.17	7.66	2.50	2.04		
PA-W-9	PA	WAI	-15.10	8.64	-17.03	5.50	1.93	3.14	-15.44	8.09
PM-EB-1	PM	ELEB	-15.57	4.34	-15.47	3.07	-0.10	1.26	-14.38	4.57
PM-EB-2	PM	ELEB	-15.91	4.03	-15.14	3.21	-0.77	0.82	-14.99	4.28
PM-EB-3	PM	ELEB	-15.55	3.75	-15.16	4.14	-0.40	-0.39	-14.76	4.53
PM-EB-4	PM	ELEB	-16.71	3.59	-15.85	4.45	-0.87	-0.86	-15.21	4.48
PM-EB-5	PM	ELEB	-15.51	3.67	-15.34	4.33	-0.17	-0.66	-14.70	4.44
PM-EB-6	PM	ELEB	-18.11	3.77	-16.50	4.08	-1.62	-0.31		
PM-EB-7	PM	ELEB	-15.65	4.20	-14.42	4.97	-1.23	-0.76		
PM-EB-8	PM	ELEB	-14.66	4.07	-16.06	3.26	1.40	0.80		
PM-EB-9	PM	ELEB	-14.74	4.22	-14.45	4.09	-0.29	0.12	-14.22	4.58
PM-EB-10	PM	ELEB	-14.86	4.43	-14.95	3.64	0.08	0.79	-14.24	4.43
PM-EB-11	PM	ELEB	-15.08	3.59	-14.89	3.54	-0.19	0.06	-14.61	4.22
PM-EB-12	PM	ELEB	-14.50	4.13	-15.00	4.09	0.49	0.04	-16.21	4.83
PM-H-1	PM	HALE	-15.30	4.69	-15.02	4.82	-0.28	-0.13	-14.50	5.11
PM-H-2	PM	HALE	-14.93	4.97	-15.29	4.32	0.36	0.65	-14.57	5.13
PM-H-3	PM	HALE	-16.62	4.23	-15.67	4.75	-0.96	-0.51	-15.40	4.77
PM-H-4	PM	HALE	-16.41	4.88	-15.81	4.44	-0.60	0.43	-15.57	5.03
PM-H-5	PM	HALE	-15.94	4.40	-15.45	4.72	-0.49	-0.32	-14.96	5.07
PM-H-6	PM	HALE	-14.19	4.70	-14.51	5.01	0.32	-0.31	-13.91	5.22
PM-H-7	PM	HALE	-15.32	4.97	-15.48	4.59	0.16	0.38	-15.08	4.96
PM-H-8	PM	HALE	-15.79	4.67	-15.63	4.29	-0.15	0.38	-15.28	4.89
PM-H-9	PM	HALE	-14.87	4.58	-14.77	5.05	-0.10	-0.47	-14.36	5.22
PM-H-10	PM	HALE	-16.89	4.68	-16.57	4.61	-0.32	0.07		
PM-H-11	PM	HALE	-15.81	4.88	-15.95	4.90	0.15	-0.02		
PM-H-12	PM	HALE	-15.62	4.65	-15.42	4.61	-0.20	0.04		
PM-MI-1	PM	MAGI	-14.08	4.89	-14.50	3.46	0.42	1.43	-13.66	4.96
PM-MI-2	PM	MAGI	-15.20	3.52	-14.69	3.50	-0.51	0.01	-14.36	4.15
PM-MI-3	PM	MAGI	-12.85	3.88	-13.62	3.46	0.76	0.42	-12.89	4.14
PM-MI-4	PM	MAGI	-12.36	3.74	-13.21	3.68	0.85	0.06	-12.05	4.31
PM-MI-5	PM	MAGI	-12.42	6.27	-13.74	3.45	1.32	2.82	-12.19	5.91
PM-MI-6	PM	MAGI	-14.52	3.99	-14.65	3.74	0.13	0.25	-14.11	4.24
PM-MI-7	PM	MAGI	-12.99	4.19	-13.18	4.17	0.19	0.02	-12.68	4.47

PM-MI-8	PM	MAGI	-14.61	4.46	-15.08	4.06	0.47	0.41	-14.08	4.52
PM-MI-9	PM	MAGI	-13.09	4.76	-13.87	3.73	0.78	1.03	-12.90	4.87
PM-MI-10	PM	MAGI	-14.96	5.07	-14.65	5.01	-0.30	0.05		
PM-MI-11	PM	MAGI	-13.02	3.94	-13.27	4.73	0.25	-0.78		
PM-MI-12	PM	MAGI	-15.66	3.92	-15.28	4.33	-0.37	-0.41		
PM-S-1	PM	SAMP	-13.96	3.78	-13.61	4.36	-0.35	-0.58	-13.08	4.25
PM-S-2	PM	SAMP	-14.43	3.21	-14.53	3.44	0.10	-0.23	-13.99	3.88
PM-S-3	PM	SAMP	-12.88	4.22	-13.21	4.23	0.33	-0.01	-12.68	4.45
PM-S-4	PM	SAMP	-14.31	4.23	-14.64	4.07	0.33	0.16	-14.06	4.35
PM-S-5	PM	SAMP	-14.06	3.61	-13.87	3.86	-0.19	-0.26	-13.24	4.16
PM-S-6	PM	SAMP	-17.42	4.51	-16.81	2.69	-0.61	1.82	-16.42	4.27
PM-S-7	PM	SAMP	-15.53	4.17	-15.04	5.11	-0.49	-0.94	-14.44	4.63
PM-S-8	PM	SAMP	-15.49	6.87	-15.92	5.42	0.43	1.45	-15.41	6.15
PM-S-9	PM	SAMP	-15.07	4.74	-15.63	3.37	0.56	1.37	-14.76	4.95
PM-S-10	PM	SAMP	-16.43	5.62	-17.10	5.51	0.66	0.12		
PM-S-11	PM	SAMP	-14.30	5.13	-14.78	5.02	0.47	0.10		
PM-S-12	PM	SAMP	-15.51	5.21	-15.50	4.97	-0.02	0.24		
PM-W-1	PM	WAI	-14.34	3.87	-14.86	3.56	0.52	0.31	-13.86	4.30
PM-W-2	PM	WAI	-12.84	5.15	-15.71	4.74	2.87	0.41	-13.29	5.30
PM-W-3	PM	WAI	-14.88	3.95	-16.40	2.73	1.52	1.22	-14.77	3.99
PM-W-4	PM	WAI	-13.34	3.92	-13.97	3.92	0.63	0.00	-12.29	4.45
PM-W-5	PM	WAI	-11.76	4.48	-13.90	4.46	2.14	0.02	-11.92	4.76
PM-W-6	PM	WAI	-13.76	4.41	-15.29	3.19	1.53	1.22	-13.90	4.19
PM-W-7	PM	WAI	-14.85	5.33	-14.95	5.01	0.10	0.32	-14.27	5.45
PM-W-8	PM	WAI	-13.82	5.95	-14.63	5.43	0.81	0.52	-13.77	6.05
PM-W-9	PM	WAI	-13.91	5.89	-14.32	5.16	0.41	0.73	-13.77	5.98
PM-W-10	PM	WAI	-15.11	5.83	-15.62	4.71	0.51	1.12		
PM-W-11	PM	WAI	-14.37	5.41	-14.62	5.42	0.25	-0.01		
PM-W-12	PM	WAI	-13.24	5.76	-13.84	6.01	0.60	-0.25		
PC-H-1	PC	HALE	-16.18	3.92	-15.67	4.89	-0.51	-0.97	-15.18	4.66
PC-H-2	PC	HALE	-15.50	4.29	-16.19	3.67	0.69	0.62		
PC-H-3	PC	HALE	-16.17	4.13	-15.21	4.65	-0.96	-0.52	-15.75	4.36
PC-H-4	PC	HALE	-15.77	4.07	-15.46	5.34	-0.31	-1.27	-14.86	4.92
PC-H-5	PC	HALE	-15.58	3.90	-15.18	4.84	-0.40	-0.94	-14.81	4.58
PC-H-6	PC	HALE	-15.80	3.89	-15.30	4.73	-0.51	-0.84	-13.52	4.72
PC-H-7	PC	HALE	-16.35	3.55	-16.17	5.27	-0.18	-1.73	-15.49	4.76
PC-H-8	PC	HALE	-15.65	3.91	-15.31	5.15	-0.34	-1.24	-14.92	4.72
PC-H-9	PC	HALE	-15.03	4.15	-14.56	5.02	-0.47	-0.88	-14.12	4.93
PC-H-10	PC	HALE	-14.96	4.01	-14.30	4.89	-0.66	-0.88		
PC-H-11	PC	HALE	-15.04	3.82	-15.75	5.01	0.71	-1.19		
PC-H-12	PC	HALE	-16.36	3.67	-16.27	4.44	-0.09	-0.77	-15.89	3.93
PC-KB-1	PC	HIMB	-15.18	4.66	-14.87	3.85	-0.31	0.81	-14.40	4.88
PC-KB-2	PC	HIMB	-15.83	5.09	-15.80	3.61	-0.03	1.48	-14.87	5.14

PC-KB-3	PC	HIMB	-14.88	4.37	-15.39	4.15	0.51	0.21	-13.91	4.88
PC-KB-4	PC	HIMB	-15.42	4.17	-15.62	3.61	0.20	0.56	-15.18	4.42
PC-KB-5	PC	HIMB	-14.91	3.21	-14.54	4.25	-0.37	-1.04		
PC-KB-6	PC	HIMB	-16.70	4.17	-17.16	1.91	0.46	2.26	-16.20	3.77
PC-KB-7	PC	HIMB	-15.51	4.26	-15.12	4.15	-0.39	0.11	-14.89	4.76
PC-KB-8	PC	HIMB	-13.60	4.85	-13.28	4.77	-0.32	0.08	-13.08	5.13
PC-KB-9	PC	HIMB	-16.91	4.44	-16.43	3.79	-0.48	0.64	-16.34	4.42
PC-KB-10	PC	HIMB	-15.65	3.82	-15.89	3.49	0.24	0.33		
PC-KB-11	PC	HIMB	-15.69	4.39	-15.36	4.86	-0.33	-0.47	-15.05	5.11
PC-KB-12	PC	HIMB	-14.74	4.92	-14.48	4.56	-0.26	0.36		
PC-MI-3	PC	MAGI	-13.64	3.38	-14.85	3.13	1.20	0.25	-13.38	4.58
PC-MI-4	PC	MAGI	-14.40	3.30	-14.08	4.70	-0.32	-1.40	-13.31	3.96
PC-MI-5	PC	MAGI	-13.26	2.92	-14.86	3.54	1.60	-0.62	-14.07	3.78
PC-MI-7	PC	MAGI	-15.82	3.02	-14.24	3.84	-1.58	-0.81	-11.87	4.97
PC-MI-8	PC	MAGI	-15.17	2.99	-15.41	3.82	0.24	-0.83	-14.63	4.19
PC-MI-9	PC	MAGI	-13.48	3.42	-15.17	4.29	1.69	-0.87	-12.78	4.64
PC-MI-10	PC	MAGI	-14.25	2.88	-13.94	4.30	-0.31	-1.42	-13.36	3.42
PC-MI-11	PC	MAGI	-13.10	3.24	-13.30	4.88	0.20	-1.64	-12.63	4.33
PC-S-1	PC	SAMP	-16.75	3.74	-16.46	4.41	-0.29	-0.67	-15.69	4.26
PC-S-2	PC	SAMP	-14.72	3.58	-14.87	3.84	0.15	-0.25	-13.82	4.10
PC-S-3	PC	SAMP	-14.63	4.15	-15.33	4.27	0.70	-0.12	-13.54	4.25
PC-S-4	PC	SAMP	-16.45	3.62	-16.82	4.23	0.37	-0.61	-15.84	4.07
PC-S-5	PC	SAMP	-15.41	3.16	-15.71	3.89	0.30	-0.73	-14.15	3.99
PC-S-6	PC	SAMP	-14.84	3.92	-14.55	4.32	-0.28	-0.40	-14.28	4.15
PC-S-7	PC	SAMP	-16.64	2.74	-16.41	4.22	-0.24	-1.48		
PC-S-8	PC	SAMP	-14.04	3.54	-13.89	4.44	-0.15	-0.90	-11.93	4.20
PC-S-9	PC	SAMP	-16.34	3.65	-16.83	2.82	0.50	0.83		
PC-S-10	PC	SAMP	-15.46	3.82	-15.08	4.68	-0.38	-0.86	-14.55	4.23
PC-S-11	PC	SAMP	-15.29	3.69	-14.70	4.49	-0.59	-0.80	-14.04	4.43
PC-S-12	PC	SAMP	-16.92	2.80	-16.26	4.27	-0.66	-1.47		
PC-W-1	PC	WAI	-14.11	3.75	-13.97	4.73	-0.14	-0.98	-13.08	4.68
PC-W-2	PC	WAI	-14.02	3.62	-14.10	4.81	0.08	-1.19	-13.55	4.29
PC-W-3	PC	WAI	-13.68	3.61	-14.10	4.97	0.42	-1.36	-13.30	4.39
PC-W-4	PC	WAI	-12.74	3.64	-12.69	5.03	-0.04	-1.39		
PC-W-5	PC	WAI	-14.58	3.20	-14.67	4.90	0.08	-1.69	-14.12	4.08
PC-W-6	PC	WAI	-14.73	3.47	-14.45	4.60	-0.28	-1.12	-13.96	4.56
PC-W-7	PC	WAI	-13.65	2.88	-14.23	4.65	0.58	-1.77	-11.83	4.27
PC-W-8	PC	WAI	-15.31	3.20	-14.88	4.49	-0.42	-1.29	-13.32	4.64
PC-W-9	PC	WAI	-14.37	3.24	-14.20	4.31	-0.16	-1.07	-13.70	4.26
PC-W-10	PC	WAI	-13.17	1.88	-12.12	4.97	-1.05	-3.10		
PC-W-11	PC	WAI	-16.22	2.84	-15.86	4.43	-0.36	-1.59	-14.81	3.63
PC-W-12	PC	WAI	-14.57	2.68	-13.84	4.41	-0.73	-1.73		
PL-EB-1	PL	ELEB	-15.60	4.32	-15.15	4.63	-0.45	-0.31	-15.05	4.55

PL-EB-2	PL	ELEB	-15.10	3.19	-14.74	4.58	-0.35	-1.38	-14.19	4.10
PL-EB-3	PL	ELEB	-14.95	4.34	-14.71	4.12	-0.24	0.22	-12.50	4.60
PL-EB-4	PL	ELEB	-14.40	3.72	-14.23	4.56	-0.18	-0.84	-13.10	4.61
PL-EB-5	PL	ELEB	-15.51	3.77	-15.15	2.71	-0.36	1.05		
PL-EB-6	PL	ELEB	-14.18	3.82	-14.36	4.69	0.18	-0.87	-13.86	4.51
PL-EB-7	PL	ELEB	-14.64	3.97	-14.97	3.36	0.34	0.61		
PL-EB-8	PL	ELEB	-15.96	2.90	-16.34	3.95	0.38	-1.05	-15.59	4.53
PL-EB-9	PL	ELEB	-14.73	3.78	-14.83	4.62	0.10	-0.84	-13.86	4.80
PL-EB-10	PL	ELEB	-15.11	3.90	-14.65	4.43	-0.46	-0.53		
PL-EB-11	PL	ELEB	-14.77	3.04	-14.67	4.97	-0.10	-1.93	-13.19	4.08
PL-EB-12	PL	ELEB	-15.00	4.10	-15.31	3.48	0.31	0.62	-13.56	4.55
PL-H-1	PL	HALE	-13.97	4.12	-13.62	5.04	-0.35	-0.92	-13.67	4.12
PL-H-2	PL	HALE	-13.33	4.75	-12.86	4.28	-0.47	0.47	-13.09	4.41
PL-H-3	PL	HALE	-14.21	4.22	-14.10	4.51	-0.11	-0.29	-12.62	4.72
PL-H-4	PL	HALE	-14.87	4.25	-14.53	4.30	-0.34	-0.05	-14.19	4.81
PL-H-5	PL	HALE	-14.38	4.73	-14.07	4.07	-0.31	0.67	-9.74	4.80
PL-H-6	PL	HALE	-13.70	4.32	-13.52	5.03	-0.17	-0.71	-13.15	4.40
PL-H-7	PL	HALE	-13.03	6.09	-12.97	5.38	-0.06	0.72	-11.09	5.52
PL-H-8	PL	HALE	-13.09	4.86	-13.08	5.27	-0.01	-0.41	-12.61	4.76
PL-H-9	PL	HALE	-14.39	5.70	-14.42	4.41	0.02	1.29	-13.45	4.79
PL-H-10	PL	HALE	-12.83	4.57	-13.31	5.16	0.48	-0.59		
PL-H-11	PL	HALE	-14.02	5.43	-15.06	4.27	1.04	1.16		
PL-H-12	PL	HALE	-13.21	4.38	-13.23	4.88	0.02	-0.50		
PL-MI-1	PL	MAGI	-11.11	3.47	-11.74	3.68	0.63	-0.22	-10.90	3.73
PL-MI-2	PL	MAGI	-13.25	2.63	-13.46	3.76	0.21	-1.13	-12.63	3.83
PL-MI-3	PL	MAGI	-12.36	2.51	-12.23	3.77	-0.13	-1.26	-10.44	3.88
PL-MI-4	PL	MAGI	-13.58	3.60	-13.37	4.35	-0.21	-0.75	-12.68	4.00
PL-MI-5	PL	MAGI	-13.30	2.76	-14.60	3.52	1.31	-0.76	-11.62	4.02
PL-MI-6	PL	MAGI	-12.57	2.30	-12.33	4.44	-0.24	-2.14	-10.78	3.93
PL-MI-7	PL	MAGI	-13.14	3.49	-13.29	3.02	0.15	0.46	-10.70	3.94
PL-MI-8	PL	MAGI	-14.07	3.03	-14.56	3.67	0.49	-0.64	-13.23	3.52
PL-MI-9	PL	MAGI	-11.88	3.07	-12.54	4.22	0.67	-1.15	-10.94	3.74
PL-MI-10	PL	MAGI	-13.76	2.26	-14.43	3.91	0.66	-1.65		
PL-MI-11	PL	MAGI	-13.81	3.62	-13.83	3.47	0.02	0.15		
PL-MI-12	PL	MAGI	-14.44	3.63	-13.93	3.81	-0.51	-0.18		
PL-S-1	PL	SAMP	-14.24	3.59	-14.48	4.77	0.24	-1.18	-13.87	3.91
PL-S-2	PL	SAMP	-15.62	4.15	-14.83	5.43	-0.79	-1.28	-14.62	5.16
PL-S-3	PL	SAMP	-13.88	2.82	-14.20	4.86	0.32	-2.04	-11.02	4.34
PL-S-4	PL	SAMP	-14.79	3.41	-14.63	4.29	-0.15	-0.88	-14.18	3.93
PL-S-5	PL	SAMP	-15.70	3.75	-15.14	3.98	-0.57	-0.23	-10.44	4.34
PL-S-6	PL	SAMP	-17.77	3.36	-17.21	4.73	-0.56	-1.37		
PL-S-7	PL	SAMP	-16.16	3.69	-15.15	3.15	-1.01	0.54	-13.17	4.26
PL-S-8	PL	SAMP	-13.73	4.48	-13.69	4.39	-0.04	0.10	-13.14	4.43

PL-S-9	PL	SAMP	-14.29	4.23	-14.25	4.12	-0.04	0.11		
PL-S-10	PL	SAMP	-14.41	4.43	-15.22	2.58	0.81	1.85		
PL-S-11	PL	SAMP	-14.23	4.11	-13.29	4.76	-0.94	-0.65	-13.50	4.27
PL-S-12	PL	SAMP	-15.50	3.08	-14.18	3.80	-1.32	-0.72	-8.94	4.18
PL-W-1	PL	WAI	-15.34	4.02	-14.95	3.65	-0.39	0.37		
PL-W-2	PL	WAI	-13.99	3.98	-15.16	4.28	1.17	-0.30	-13.07	4.10
PL-W-3	PL	WAI	-15.97	4.00	-15.32	3.68	-0.65	0.32	-13.27	4.11
PL-W-4	PL	WAI	-15.55	3.58	-14.72	4.85	-0.82	-1.27	-12.96	4.71
PL-W-5	PL	WAI	-13.87	3.41	-13.71	4.22	-0.16	-0.81	-11.13	4.56
PL-W-6	PL	WAI	-12.39	3.51	-12.66	4.68	0.26	-1.17	-10.66	4.58
PL-W-7	PL	WAI	-14.56	4.56	-15.01	4.06	0.45	0.50	-13.45	4.70
PL-W-8	PL	WAI	-14.84	4.02	-14.77	4.01	-0.07	0.01	-11.34	4.92
PL-W-9	PL	WAI	-12.67	3.40	-11.18	5.13	-1.49	-1.73	-11.20	4.34
PL-W-10	PL	WAI	-14.83	2.76	-14.25	5.33	-0.58	-2.57		
PL-W-11	PL	WAI	-12.95	3.29	-13.17	4.82	0.22	-1.52	-11.36	4.70
PL-W-12	PL	WAI	-15.16	4.65	-15.09	4.34	-0.07	0.31		
PE-EB-1	PE	ELEB	-13.31	2.60	-13.54	4.83	0.23	-2.23	-12.71	4.49
PE-EB-2	PE	ELEB	-15.70	2.93	-15.05	4.65	-0.65	-1.72	-14.96	4.18
PE-EB-3	PE	ELEB	-14.54	2.20	-14.65	4.77	0.10	-2.57	-14.07	3.45
PE-EB-4	PE	ELEB	-14.93	2.55	-14.67	4.61	-0.26	-2.06	-14.54	3.85
PE-EB-5	PE	ELEB	-16.45	2.24	-16.34	3.95	-0.11	-1.72		
PE-EB-7	PE	ELEB	-16.10	2.30	-15.42	3.96	-0.68	-1.65		
PE-EB-8	PE	ELEB	-14.62	2.24	-14.55	4.72	-0.07	-2.48	-14.09	3.90
PE-EB-10	PE	ELEB	-14.68	2.45	-14.31	4.09	-0.37	-1.64	-14.08	3.59
PE-EB-11	PE	ELEB	-13.64	2.90	-14.39	3.62	0.76	-0.72	-13.21	4.06
PE-EB-12	PE	ELEB	-14.14	2.35	-14.36	4.71	0.22	-2.36	-14.00	3.21
PE-H-1	PE	HALE	-16.35	2.74	-16.01	4.02	-0.34	-1.28	-15.43	4.77
PE-H-2	PE	HALE	-15.96	2.10	-15.15	4.03	-0.80	-1.94	-15.10	3.64
PE-H-3	PE	HALE	-17.62	1.69	-17.18	4.30	-0.44	-2.61	-16.53	4.21
PE-H-4	PE	HALE	-16.34	1.97	-15.23	4.55	-1.11	-2.58	-15.15	4.26
PE-H-5	PE	HALE	-16.58	2.21	-16.34	3.94	-0.24	-1.73	-16.01	3.62
PE-H-6	PE	HALE	-16.91	2.48	-16.93	3.49	0.03	-1.01	-16.54	4.19
PE-H-7	PE	HALE	-14.65	2.44	-14.67	4.58	0.02	-2.14	-14.11	4.45
PE-H-8	PE	HALE	-15.87	2.68	-15.20	3.48	-0.67	-0.80	-14.74	4.56
PE-H-9	PE	HALE	-13.45	2.45	-13.46	4.72	0.02	-2.27	-13.09	4.67
PE-H-10	PE	HALE	-13.29	2.66	-13.13	5.34	-0.16	-2.68		
PE-H-11	PE	HALE	-15.66	3.57	-16.50	3.41	0.83	0.16		
PE-H-12	PE	HALE	-16.64	2.67	-15.65	4.15	-0.99	-1.48		
PE-MI-1	PE	MAGI	-13.38	2.19	-13.36	3.80	-0.02	-1.62	-12.75	3.73
PE-MI-2	PE	MAGI	-13.93	1.19	-13.29	3.63	-0.64	-2.44	-12.85	3.46
PE-MI-3	PE	MAGI	-13.86	0.96	-13.99	3.18	0.13	-2.22	-12.88	3.76
PE-MI-4	PE	MAGI	-12.38	2.33	-12.74	3.95	0.36	-1.61	-11.88	3.56
PE-MI-5	PE	MAGI	-14.31	1.71	-15.14	3.76	0.83	-2.05	-13.86	3.42

PE-MI-6	PE	MAGI	-13.80	1.84	-13.36	4.42	-0.44	-2.58	-13.14	3.50
PE-MI-7	PE	MAGI	-15.29	2.03	-14.90	2.65	-0.39	-0.62	-14.51	3.73
PE-MI-8	PE	MAGI	-14.56	1.18	-15.04	4.09	0.49	-2.92	-14.01	3.46
PE-MI-9	PE	MAGI	-13.54	1.73	-14.50	3.04	0.96	-1.31	-12.97	3.31
PE-MI-10	PE	MAGI	-14.16	1.71	-15.02	2.57	0.87	-0.85		
PE-MI-11	PE	MAGI	-14.69	1.73	-15.11	2.64	0.42	-0.91		
PE-MI-12	PE	MAGI	-14.48	0.44	-13.88	3.57	-0.60	-3.13		
PE-S-1	PE	SAMP	-16.32	2.71	-16.37	3.37	0.04	-0.67	-15.99	3.60
PE-S-2	PE	SAMP	-17.63	2.26	-17.67	3.93	0.04	-1.68	-17.43	3.81
PE-S-3	PE	SAMP	-15.94	2.42	-15.56	5.08	-0.38	-2.66		
PE-S-4	PE	SAMP	-16.01	2.13	-15.51	4.78	-0.50	-2.65		
PE-S-5	PE	SAMP	-16.34	2.70	-15.94	4.10	-0.40	-1.40	-15.79	4.14
PE-S-6	PE	SAMP	-16.97	2.22	-16.47	5.19	-0.50	-2.97		
PE-S-7	PE	SAMP	-17.91	2.10	-17.67	3.67	-0.24	-1.58	-17.34	3.72
PE-S-8	PE	SAMP	-16.18	2.28	-15.88	4.45	-0.30	-2.17	-15.12	4.06
PE-S-9	PE	SAMP	-15.76	2.48	-15.48	4.29	-0.27	-1.81	-15.27	3.95
PE-S-10	PE	SAMP	-16.43	2.65	-16.44	4.34	0.01	-1.69	-16.15	4.03
PE-S-11	PE	SAMP	-17.54	2.68	-17.68	3.77	0.14	-1.08	-16.85	4.13
PE-S-12	PE	SAMP	-18.86	2.53	-18.67	3.93	-0.19	-1.40	-18.50	3.83
PE-W-1	PE	WAI	-15.16	2.60	-14.77	4.22	-0.39	-1.61	-14.38	4.16
PE-W-2	PE	WAI	-12.97	2.56	-13.99	4.96	1.03	-2.41	-13.25	4.64
PE-W-3	PE	WAI	-16.20	2.27	-15.80	4.01	-0.40	-1.74	-15.40	3.77
PE-W-4	PE	WAI	-13.48	2.54	-13.26	4.17	-0.22	-1.62	-12.83	4.39
PE-W-5	PE	WAI	-16.61	2.22	-16.21	4.02	-0.40	-1.80	-16.16	3.13
PE-W-6	PE	WAI	-14.76	3.67	-14.35	4.64	-0.41	-0.97	-13.94	4.97
PE-W-7	PE	WAI	-15.55	3.31	-15.39	5.52	-0.15	-2.21	-15.10	4.79
PE-W-8	PE	WAI	-13.66	3.16	-13.76	4.97	0.10	-1.82	-13.22	4.20
PE-W-9	PE	WAI	-13.23	2.89	-13.06	4.40	-0.17	-1.50	-12.85	4.19
PE-W-10	PE	WAI	-15.85	2.58	-15.49	4.64	-0.36	-2.06		
PE-W-11	PE	WAI	-15.35	5.12	-14.77	6.46	-0.58	-1.34		
PE-W-12	PE	WAI	-15.17	5.40	-14.98	6.41	-0.19	-1.01		

Appendix B. Chapter 3 alpha diversity of coral-associated microbial communities

Table B.1 Chapter 3 coral associated microbial community alpha diversity data. PC = *Porites compressa*, PL = *Porites lobata*, PA = *Pocillopora acuta*, PM = *Pocillopora meandrina*

ID	Species	Site	Shannon Diversity	Pielous's Equitability	Observed OTUs	Faith's PD
Pc.H.1	PC	HALE	1.66	0.29	299	12.00
Pc.H.4	PC	HALE	3.79	0.59	623	20.40
Pc.H.5	PC	HALE	1.83	0.29	588	19.84
Pc.H.7	PC	HALE	4.09	0.65	522	18.11
Pc.H.8	PC	HALE	3.99	0.62	595	19.97
Pc.H.9	PC	HALE	1.34	0.22	393	15.87
Pc.KB.1	PC	HIMB	4.36	0.68	634	20.81
Pc.KB.10	PC	HIMB	3.70	0.58	567	19.70
Pc.KB.11	PC	HIMB	1.10	0.23	132	8.98
Pc.KB.2	PC	HIMB	2.33	0.43	228	10.95
Pc.KB.3	PC	HIMB	1.24	0.22	312	14.31
Pc.KB.9	PC	HIMB	1.61	0.27	355	14.59
Pc.MI.11	PC	MAGI	5.38	0.82	682	22.07
Pc.MI.3	PC	MAGI	2.51	0.50	154	7.40
Pc.MI.4	PC	MAGI	4.75	0.75	586	19.10
Pc.MI.9	PC	MAGI	4.83	0.73	726	23.47
Pc.S.12	PC	SAMP	2.00	0.34	331	12.54
Pc.S.5	PC	SAMP	4.06	0.63	649	22.18
Pc.S.6	PC	SAMP	1.80	0.31	343	14.05
Pc.S.7	PC	SAMP	0.82	0.20	63	4.28
Pc.S.8	PC	SAMP	3.80	0.60	574	19.46
Pc.S.9	PC	SAMP	2.36	0.37	564	19.58
Pc.W.1	PC	WAI	1.60	0.25	542	18.71
Pc.W.11	PC	WAI	1.61	0.28	315	12.67
Pc.W.2	PC	WAI	3.45	0.55	563	18.59
Pc.W.6	PC	WAI	2.37	0.39	462	16.58
Pc.W.7	PC	WAI	1.95	0.32	435	16.26
Pc.W.8	PC	WAI	3.96	0.64	493	16.48
Pl.EB.10	PL	ELEB	4.75	0.85	276	10.97
Pl.EB.12	PL	ELEB	2.86	0.46	475	16.48
Pl.EB.4	PL	ELEB	2.23	0.70	24	2.27
Pl.EB.5	PL	ELEB	1.97	0.34	348	13.87
Pl.EB.6	PL	ELEB	2.10	0.51	63	4.66
Pl.EB.7	PL	ELEB	1.06	0.22	126	8.56
Pl.H.3	PL	HALE	3.53	0.58	420	15.00
Pl.H.4	PL	HALE	3.93	0.61	605	20.05

Pl.H.5	PL	HALE	4.09	0.64	593	18.58
Pl.H.7	PL	HALE	4.12	0.65	538	17.19
Pl.H.8	PL	HALE	3.09	0.59	192	7.67
Pl.H.9	PL	HALE	4.76	0.80	380	12.67
Pl.MI.12	PL	MAGI	3.85	0.80	123	4.75
Pl.MI.2	PL	MAGI	3.79	0.81	107	5.20
Pl.MI.4	PL	MAGI	4.42	0.85	178	7.37
Pl.MI.5	PL	MAGI	4.45	0.78	311	10.52
Pl.MI.7	PL	MAGI	4.28	0.70	469	16.06
Pl.MI.8	PL	MAGI	2.49	0.68	40	2.88
Pl.S.10	PL	SAMP	4.14	0.82	154	7.39
Pl.S.11	PL	SAMP	3.74	0.66	289	10.61
Pl.S.12	PL	SAMP	0.96	0.20	128	7.23
Pl.S.5	PL	SAMP	3.52	0.55	594	20.05
Pl.S.7	PL	SAMP	4.04	0.70	334	12.92
Pl.S.9	PL	SAMP	2.14	0.64	29	2.39
Pl.W.12	PL	WAI	4.06	0.71	309	10.03
Pl.W.2	PL	WAI	1.76	0.28	487	18.07
Pl.W.3	PL	WAI	1.09	0.22	148	8.59
Pl.W.5	PL	WAI	3.96	0.83	117	5.15
Pl.W.7	PL	WAI	2.67	0.47	312	11.57
Pl.W.8	PL	WAI	4.01	0.74	222	9.32
Pa.H.12	PA	HALE	2.42	0.70	32	2.86
Pa.H.4	PA	HALE	1.42	0.41	31	2.13
Pa.H.5	PA	HALE	2.78	0.83	28	2.10
Pa.H.6	PA	HALE	1.15	0.48	11	1.13
Pa.H.7	PA	HALE	1.51	0.45	28	2.10
Pa.H.9	PA	HALE	2.36	0.73	25	2.27
Pa.KB.10	PA	HIMB	1.17	0.32	39	3.38
Pa.KB.11	PA	HIMB	1.95	0.57	30	2.02
Pa.KB.12	PA	HIMB	4.07	0.80	163	6.84
Pa.KB.7	PA	HIMB	0.99	0.31	24	1.42
Pa.MI.10	PA	MAGI	3.09	0.79	51	3.43
Pa.MI.3	PA	MAGI	3.14	0.79	52	3.41
Pa.MI.4	PA	MAGI	3.37	0.73	98	5.41
Pa.MI.5	PA	MAGI	2.81	0.73	48	3.79
Pa.MI.9	PA	MAGI	3.50	0.75	106	5.20
Pa.S.1	PA	SAMP	2.66	0.74	36	2.31
Pa.S.10	PA	SAMP	2.99	0.79	45	3.33
Pa.S.4	PA	SAMP	4.50	0.82	241	9.82
Pa.S.6	PA	SAMP	3.50	0.79	82	4.22
Pa.S.7	PA	SAMP	2.16	0.70	22	2.22
Pa.W.2	PA	WAI	3.76	0.77	134	6.55

Pa.W.3	PA	WAI	2.39	0.68	34	2.17
Pa.W.4	PA	WAI	2.42	0.52	107	5.10
Pa.W.6	PA	WAI	1.87	0.78	11	1.26
Pa.W.8	PA	WAI	3.84	0.85	92	4.83
Pa.W.9	PA	WAI	3.89	0.77	152	6.27
Pm.H.7	PM	HALE	2.26	0.70	25	2.19
Pm.H.8	PM	HALE	2.47	0.84	19	2.05
Pm.MI.6	PM	MAGI	2.41	0.75	25	2.28
Pm.MI.7	PM	MAGI	3.11	0.70	87	6.74
Pm.MI.8	PM	MAGI	2.16	0.67	25	2.48
Pm.MI.9	PM	MAGI	2.44	0.78	23	1.90
Pm.S.10	PM	SAMP	4.07	0.68	390	14.17
Pm.S.11	PM	SAMP	2.53	0.50	153	6.79
Pm.S.2	PM	SAMP	3.17	0.65	129	7.86
Pm.S.3	PM	SAMP	2.44	0.79	22	2.33
Pm.S.4	PM	SAMP	2.68	0.76	34	2.72
Pm.S.5	PM	SAMP	2.30	0.73	23	2.08
Pm.W.10	PM	WAI	1.87	0.69	15	1.40
Pm.W.11	PM	WAI	3.69	0.80	99	5.60
Pm.W.12	PM	WAI	2.84	0.52	228	9.28
Pm.W.2	PM	WAI	1.95	0.55	35	2.18
Pm.W.7	PM	WAI	3.24	0.69	107	5.48
Pm.W.9	PM	WAI	2.35	0.55	73	3.91

Appendix C. Chapter 4 coral-associated microbial community alpha diversity data

Table C.1 Chapter 4 coral-associated microbial community alpha diversity. MC = *Montipora capitata*, PA = *Pocillopora acuta*, PC = *Porites compressa*, and PL = *Porites lobata*. Treat = Treatments, LL = present day seawater temperature and pH (control), LH = present day seawater temperature and -0.2 pH units, HL = +2.0 °C seawater temperature and present day pH, HH = +2.0 °C seawater temperature and -0.2 pH units

ID	Species	Site	Treat	Tank	Observed OTUs	Chao1	Shannon's Diversity	Faith's PD
Mc.H.1.3.LL	MC	HALE	LL	9	1073	1336.13	5.14	38.63
Mc.H.1.5.LH	MC	HALE	LH	14	259	369.55	1.54	15.23
Mc.H.2.1.LH	MC	HALE	LH	28	1723	2168.84	6.04	48.40
Mc.H.2.4.LL	MC	HALE	LL	35	1065	1422.18	5.15	38.85
Mc.H.3.3.LH	MC	HALE	LH	15	1470	1743.22	5.53	46.33
Mc.H.5.1.LL	MC	HALE	LL	11	2325	3611.09	5.71	66.90
Mc.H.5.3.LH	MC	HALE	LH	1	166	201.29	2.45	9.85
Mc.H.6.1.LH	MC	HALE	LH	30	1799	2312.84	6.00	54.79
Mc.KB.1.2.LH	MC	HIMB	LH	12	136	166.88	1.19	6.46
Mc.KB.1.7.LL	MC	HIMB	LL	2	72	85.00	2.34	4.81
Mc.KB.2.10.LH	MC	HIMB	LH	39	66	92.00	2.17	4.53
Mc.KB.2.13.HL	MC	HIMB	HL	34	138	236.08	3.05	8.96
Mc.KB.2.17.LL	MC	HIMB	LL	35	680	798.10	4.16	22.29
Mc.KB.2.6.HH	MC	HIMB	HH	24	234	287.00	2.21	13.85
Mc.KB.3.1.LH	MC	HIMB	LH	12	332	412.16	3.85	14.84
Mc.KB.3.4.LL	MC	HIMB	LL	2	42	57.00	2.78	4.08
Mc.KB.4.4.LH	MC	HIMB	LH	30	139	170.17	2.28	7.75
Mc.KB.4.6.LL	MC	HIMB	LL	37	92	119.27	2.14	4.82
Mc.KB.5.1.HH	MC	HIMB	HH	6	154	223.79	1.21	9.61
Mc.KB.5.2.LH	MC	HIMB	LH	1	112	135.40	0.54	7.79
Mc.KB.5.6.LL	MC	HIMB	LL	11	179	243.69	0.77	10.79
Mc.KB.5.7.HL	MC	HIMB	HL	19	175	284.50	1.52	10.13
Mc.KB.6.6.LL	MC	HIMB	LL	23	138	158.31	4.13	8.58
Mc.S.1.1.LL	MC	SAMP	LL	17	1640	1869.51	5.50	49.65
Mc.S.1.2.HL	MC	SAMP	HL	3	246	326.50	2.14	10.30
Mc.S.1.5.LH	MC	SAMP	LH	7	88	119.63	3.39	5.69
Mc.S.2.2.HL	MC	SAMP	HL	31	766	1680.00	1.41	35.87
Mc.S.2.6.HH	MC	SAMP	HH	21	48	70.67	0.30	2.93
Mc.S.3.2.HL	MC	SAMP	HL	8	95	110.87	0.60	5.89
Mc.S.3.4.LL	MC	SAMP	LL	9	79	133.38	3.14	3.39
Mc.S.3.5.HH	MC	SAMP	HH	20	391	497.89	5.04	15.36
Mc.S.3.6.LH	MC	SAMP	LH	14	69	146.50	2.87	4.73
Mc.S.4.4.LL	MC	SAMP	LL	35	469	608.28	2.35	18.30
Mc.S.4.5.HL	MC	SAMP	HL	40	196	270.39	1.14	10.29
Mc.S.4.6.HH	MC	SAMP	HH	29	64	134.00	0.40	2.98

Mc.S.4.7.LH	MC	SAMP	LH	28	146	196.27	1.47	6.91
Mc.S.5.3.LL	MC	SAMP	LL	10	153	249.25	2.89	9.49
Mc.S.5.4.HL	MC	SAMP	HL	18	130	174.40	2.67	9.12
Mc.S.5.5.LH	MC	SAMP	LH	15	173	251.69	2.47	6.61
Mc.S.5.7.HH	MC	SAMP	HH	4	178	209.95	3.59	10.65
Mc.S.6.2.HL	MC	SAMP	HL	22	139	204.00	1.16	7.55
Mc.S.6.3.HH	MC	SAMP	HH	33	109	157.24	1.15	6.03
Mc.S.6.4.LH	MC	SAMP	LH	30	297	375.62	1.00	15.03
Mc.S.6.7.LL	MC	SAMP	LL	37	184	229.72	1.54	7.37
Mc.W.1.10.LL	MC	WAI	LL	10	231	403.13	2.71	11.97
Mc.W.1.3.LH	MC	WAI	LH	7	177	291.88	1.62	9.23
Mc.W.2.1.LH	MC	WAI	LH	28	176	214.00	3.92	10.77
Mc.W.2.11.HL	MC	WAI	HL	34	1113	1283.20	2.89	37.22
Mc.W.2.2.HH	MC	WAI	HH	29	125	211.00	1.80	7.82
Mc.W.2.3.LL	MC	WAI	LL	35	1548	1878.96	5.56	44.25
Mc.W.3.1.LL	MC	WAI	LL	2	93	133.62	2.98	5.59
Mc.W.3.5.LH	MC	WAI	LH	12	2352	3351.38	6.27	67.58
Mc.W.4.3.LH	MC	WAI	LH	25	1266	1562.73	5.90	45.22
Mc.W.4.6.LL	MC	WAI	LL	32	2772	3705.00	6.36	70.57
Mc.W.5.2.LH	MC	WAI	LH	7	666	825.97	4.05	25.50
Mc.W.5.5.LL	MC	WAI	LL	17	226	312.00	3.80	13.62
Mc.W.6.2.LL	MC	WAI	LL	37	557	713.10	3.97	23.84
Mc.W.6.7.LH	MC	WAI	LH	30	671	881.00	5.19	27.36
Pc.H.1.1.LL	PC	HALE	LL	11	306	387.89	3.82	21.22
Pc.H.1.3.LH	PC	HALE	LH	1	278	448.63	3.72	13.80
Pc.H.2.2.LH	PC	HALE	LH	36	2689	3699.42	6.41	74.69
Pc.H.2.5.LL	PC	HALE	LL	23	607	724.07	4.87	25.73
Pc.H.2.6.HH	PC	HALE	HH	38	2623	3957.53	5.38	72.90
Pc.H.3.1.LL	PC	HALE	LL	2	361	609.11	2.11	19.20
Pc.H.3.2.HL	PC	HALE	HL	5	424	587.77	1.79	18.21
Pc.H.3.3.LH	PC	HALE	LH	12	167	302.00	2.78	11.10
Pc.H.3.5.HH	PC	HALE	HH	16	2490	3994.94	5.63	71.88
Pc.H.4.2.LL	PC	HALE	LL	32	2121	3045.90	5.47	58.58
Pc.H.5.3.LL	PC	HALE	LL	9	295	386.94	3.51	14.25
Pc.H.5.5.LH	PC	HALE	LH	14	1743	2115.01	5.65	49.93
Pc.H.5.6.HH	PC	HALE	HH	20	1115	1463.52	3.91	42.39
Pc.H.6.5.HH	PC	HALE	HH	29	168	235.57	1.49	11.23
Pc.H.6.6.LH	PC	HALE	LH	28	468	613.41	4.46	22.73
Pc.KB.1.10.LL	PC	HIMB	LL	17	416	552.98	3.83	19.85
Pc.KB.1.2.LH	PC	HIMB	LH	14	1068	1407.89	2.97	36.79
Pc.KB.1.4.HL	PC	HIMB	HL	8	264	343.44	3.47	13.79
Pc.KB.2.12.HH	PC	HIMB	HH	29	581	751.08	3.56	28.60
Pc.KB.2.16.LL	PC	HIMB	LL	32	183	377.13	2.03	12.64

Pc.KB.2.9.HL	PC	HIMB	HL	40	2358	3167.89	6.29	68.89
Pc.KB.3.1.LL	PC	HIMB	LL	10	471	653.18	1.83	25.31
Pc.KB.3.2.HL	PC	HIMB	HL	18	415	602.61	3.51	18.33
Pc.KB.3.5.HH	PC	HIMB	HH	4	2661	4178.95	5.41	74.67
Pc.KB.4.1.LL	PC	HIMB	LL	23	246	324.96	3.71	15.05
Pc.KB.5.1.LL	PC	HIMB	LL	11	635	829.26	4.70	26.02
Pc.KB.5.5.LH	PC	HIMB	LH	1	65	89.43	0.81	4.36
Pc.KB.5.6.HL	PC	HIMB	HL	19	454	573.39	3.20	21.18
Pc.KB.6.1.LH	PC	HIMB	LH	36	805	1148.07	4.07	34.75
Pc.KB.6.2.HH	PC	HIMB	HH	38	1325	1618.00	5.03	42.25
Pc.KB.6.3.LL	PC	HIMB	LL	32	947	1234.81	4.57	42.10
Pc.S.1.1.LL	PC	SAMP	LL	9	1099	1404.71	4.27	35.13
Pc.S.1.2.HL	PC	SAMP	HL	8	2162	2647.10	5.35	58.78
Pc.S.1.3.LH	PC	SAMP	LH	14	233	304.29	2.83	14.08
Pc.S.1.4.HH	PC	SAMP	HH	20	3577	5343.72	6.64	89.20
Pc.S.2.1.LH	PC	SAMP	LH	28	680	986.28	4.65	30.12
Pc.S.2.2.HH	PC	SAMP	HH	29	2129	2697.17	6.46	61.04
Pc.S.2.3.LL	PC	SAMP	LL	35	1122	1588.39	3.34	45.65
Pc.S.2.4.HL	PC	SAMP	HL	40	234	340.11	2.90	14.41
Pc.S.3.2.LH	PC	SAMP	LH	15	682	1120.29	1.82	29.96
Pc.S.3.4.LL	PC	SAMP	LL	10	178	370.50	2.04	12.20
Pc.S.4.1.LH	PC	SAMP	LH	30	185	224.81	2.24	10.55
Pc.S.4.2.HH	PC	SAMP	HH	33	290	395.00	3.26	20.02
Pc.S.4.3.LL	PC	SAMP	LL	37	3171	4513.59	6.56	82.95
Pc.S.4.6.HL	PC	SAMP	HL	22	249	384.03	2.46	16.50
Pc.S.5.2.LH	PC	SAMP	LH	7	131	162.50	2.21	11.19
Pc.S.6.1.LH	PC	SAMP	LH	39	2468	3625.73	5.91	71.35
Pc.S.6.3.HH	PC	SAMP	HH	21	148	197.00	2.71	8.33
Pc.S.6.4.LL	PC	SAMP	LL	26	69	100.63	0.21	6.20
Pc.S.6.6.HL	PC	SAMP	HL	31	1859	2784.82	6.13	64.85
Pc.W.1.1.HL	PC	WAI	HL	5	929	1148.12	4.49	35.13
Pc.W.1.2.HH	PC	WAI	HH	4	1396	2877.26	5.35	48.15
Pc.W.1.3.LH	PC	WAI	LH	15	995	1340.09	4.34	38.13
Pc.W.1.5.LL	PC	WAI	LL	10	92	166.38	2.44	7.15
Pc.W.2.1.LL	PC	WAI	LL	37	348	480.45	2.23	16.22
Pc.W.2.3.HH	PC	WAI	HH	33	152	201.79	1.92	9.85
Pc.W.2.5.LH	PC	WAI	LH	30	291	379.14	3.53	16.89
Pc.W.3.4.LL	PC	WAI	LL	11	370	470.74	2.66	17.84
Pc.W.3.6.LH	PC	WAI	LH	1	92	117.30	2.76	6.43
Pc.W.4.1.LL	PC	WAI	LL	23	127	221.60	3.52	8.81
Pc.W.4.3.HH	PC	WAI	HH	38	567	738.59	2.78	30.60
Pc.W.4.5.LH	PC	WAI	LH	36	144	234.46	3.79	10.45
Pc.W.4.6.HL	PC	WAI	HL	27	229	341.89	4.52	12.21

Pc.W.5.3.LH	PC	WAI	LH	7	1524	1947.10	6.23	51.87
Pc.W.6.2.HL	PC	WAI	HL	31	837	1096.67	5.12	35.24
Pc.W.6.3.LL	PC	WAI	LL	26	402	491.41	2.67	22.55
Pc.W.6.5.HH	PC	WAI	HH	21	1395	2103.13	4.24	50.44
Pc.W.6.6.LH	PC	WAI	LH	39	1167	1634.06	5.79	46.54
Pa.H.2.1.LH	PA	HALE	LH	25	119	185.60	2.74	8.04
Pa.H.2.2.LL	PA	HALE	LL	32	211	316.00	3.19	12.33
Pa.H.3.2.LH	PA	HALE	LH	14	110	184.10	2.54	8.09
Pa.H.3.3.LL	PA	HALE	LL	9	69	97.11	1.87	6.28
Pa.H.4.3.LH	PA	HALE	LH	28	676	883.02	3.28	26.25
Pa.H.4.4.LL	PA	HALE	LL	35	189	271.27	2.25	11.71
Pa.H.5.3.LL	PA	HALE	LL	10	310	389.02	3.69	16.70
Pa.H.5.5.LH	PA	HALE	LH	15	103	121.07	0.85	8.42
Pa.H.6.1.LH	PA	HALE	LH	30	567	699.22	2.44	24.08
Pa.H.6.5.LL	PA	HALE	LL	37	340	417.00	1.64	15.60
Pa.H.8.2.HL	PA	HALE	HL	27	182	270.50	1.77	10.95
Pa.H.8.4.LL	PA	HALE	LL	23	166	204.33	2.43	12.56
Pa.H.8.6.LH	PA	HALE	LH	36	160	236.56	1.24	10.16
Pa.KB.1.17.LL	PA	HIMB	LL	7	211	300.44	2.84	9.83
Pa.KB.1.3.LH	PA	HIMB	LH	20	126	175.58	3.95	9.41
Pa.KB.1.8.HH	PA	HIMB	HH	2	88	139.67	0.92	5.36
Pa.KB.2.1.LL	PA	HIMB	LL	32	187	304.60	2.58	12.33
Pa.KB.2.5.HL	PA	HIMB	HL	40	1306	1752.07	3.65	46.63
Pa.KB.2.6.LH	PA	HIMB	LH	25	766	1008.22	3.11	29.98
Pa.KB.4.3.LH	PA	HIMB	LH	39	232	309.29	2.74	12.42
Pa.KB.4.5.HL	PA	HIMB	HL	31	739	1060.76	3.54	28.84
Pa.KB.4.7.LL	PA	HIMB	LL	26	140	216.56	2.73	10.92
Pa.KB.5.2.HL	PA	HIMB	HL	19	320	441.92	0.93	19.72
Pa.KB.5.3.LH	PA	HIMB	LH	1	1545	1878.88	5.41	47.02
Pa.KB.6.2.LH	PA	HIMB	LH	36	193	292.75	2.92	11.00
Pa.KB.6.5.HH	PA	HIMB	HH	38	1142	1310.07	5.79	40.41
Pa.KB.7.1.LL	PA	HIMB	LL	17	154	179.83	0.71	9.73
Pa.KB.7.2.LH	PA	HIMB	LH	7	151	203.93	3.19	8.34
Pa.KB.7.8.HH	PA	HIMB	HH	13	144	227.25	1.78	10.72
Pa.S.1.16.HL	PA	SAMP	HL	2	161	247.13	1.02	10.53
Pa.S.1.17.LH	PA	SAMP	LH	16	386	485.74	4.28	19.31
Pa.S.1.3.LL	PA	SAMP	LL	18	218	320.24	1.07	13.21
Pa.S.1.5.HH	PA	SAMP	HH	12	186	229.13	0.87	11.53
Pa.S.2.11.LH	PA	SAMP	LH	35	148	218.91	1.76	11.01
Pa.S.2.2.LL	PA	SAMP	LL	40	315	490.38	1.73	15.71
Pa.S.2.4.HL	PA	SAMP	HL	28	268	369.94	2.45	12.66
Pa.S.5.2.LL	PA	SAMP	LL	17	1581	2480.07	5.61	48.81
Pa.S.6.1.LH	PA	SAMP	LH	36	168	193.14	2.44	12.37

Pa.S.6.2.HH	PA	SAMP	HH	38	200	422.60	1.25	13.03
Pa.S.6.3.LL	PA	SAMP	LL	23	211	359.57	1.98	12.55
Pa.S.7.5.LL	PA	SAMP	LL	2	262	368.11	4.36	14.20
Pa.S.7.6.HL	PA	SAMP	HL	5	158	189.71	1.64	8.41
Pa.S.8.1.LH	PA	SAMP	LH	39	243	318.14	3.08	11.52
Pa.S.8.2.HH	PA	SAMP	HH	21	240	287.90	1.02	14.26
Pa.S.8.5.LL	PA	SAMP	LL	26	199	234.15	2.78	15.18
Pa.S.8.8.HL	PA	SAMP	HL	31	52	79.20	0.42	4.32
Pa.W.1.6.LL	PA	WAI	LL	9	678	897.06	3.43	29.37
Pa.W.10.1.LH	PA	WAI	LH	25	304	437.00	3.96	14.14
Pa.W.10.5.LL	PA	WAI	LL	26	333	448.50	3.02	14.78
Pa.W.2.4.LH	PA	WAI	LH	10	558	797.57	4.42	19.70
Pa.W.2.6.LL	PA	WAI	LL	15	647	1077.18	3.46	25.65
Pa.W.5.2.LL	PA	WAI	LL	28	411	488.03	1.65	17.45
Pa.W.5.4.LH	PA	WAI	LH	35	151	209.57	2.22	7.37
Pa.W.6.1.LH	PA	WAI	LH	30	436	574.24	3.84	21.15
Pa.W.6.3.LL	PA	WAI	LL	37	79	104.50	1.19	5.93
Pa.W.8.1.LH	PA	WAI	LH	36	265	364.40	2.41	11.84
Pa.W.8.5.LL	PA	WAI	LL	23	1266	1551.07	4.32	41.07
Pl.H.1.1.HL	Pl	HALE	HL	3	238	331.84	1.66	16.01
Pl.H.1.2.LH	Pl	HALE	LH	7	178	250.77	2.85	9.26
Pl.H.1.6.LL	Pl	HALE	LL	17	3594	5397.21	5.54	96.70
Pl.H.2.2.HH	Pl	HALE	HH	29	3152	4442.35	5.44	86.30
Pl.H.2.3.LL	Pl	HALE	LL	35	52	133.20	0.52	4.78
Pl.H.2.4.LH	Pl	HALE	LH	28	3146	4384.42	6.43	81.14
Pl.H.2.6.HL	Pl	HALE	HL	40	2584	3979.23	6.43	72.81
Pl.H.3.1.HH	Pl	HALE	HH	20	2856	4181.70	5.99	76.97
Pl.H.3.2.LH	Pl	HALE	LH	14	2565	4231.85	6.55	74.48
Pl.H.3.3.HL	Pl	HALE	HL	8	3413	5178.95	6.68	87.95
Pl.H.3.5.LL	Pl	HALE	LL	9	234	391.50	2.10	17.77
Pl.H.4.1.HL	Pl	HALE	HL	22	1176	1585.79	4.69	41.73
Pl.H.4.2.HH	Pl	HALE	HH	33	3191	4729.45	6.68	81.93
Pl.H.4.3.LH	Pl	HALE	LH	30	479	781.04	3.83	23.49
Pl.H.4.4.LL	Pl	HALE	LL	37	357	625.93	3.55	17.92
Pl.H.5.3.LH	Pl	HALE	LH	15	125	177.50	0.75	7.59
Pl.H.5.4.LL	Pl	HALE	LL	10	987	1425.49	4.78	39.94
Pl.H.6.3.LH	Pl	HALE	LH	36	3178	4987.37	5.47	88.34
Pl.S.1.1.LH	Pl	SAMP	LH	14	98	136.15	1.18	8.21
Pl.S.1.12.HL	Pl	SAMP	HL	8	4895	7031.36	6.25	122.34
Pl.S.1.15.HH	Pl	SAMP	HH	4	3847	5647.30	6.32	100.31
Pl.S.1.6.LL	Pl	SAMP	LL	9	230	330.63	1.70	13.37
Pl.S.2.1.LL	Pl	SAMP	LL	35	3209	4576.91	5.44	85.23
Pl.S.2.15.HH	Pl	SAMP	HH	27	3143	4586.86	5.48	86.47

Pl.S.2.5.LH	Pl	SAMP	LH	33	2123	3242.42	5.07	69.64
Pl.S.2.7.HL	Pl	SAMP	HL	36	2034	2740.55	5.97	64.01
Pl.S.3.3.LL	Pl	SAMP	LL	17	560	829.18	1.33	25.66
Pl.S.3.5.LH	Pl	SAMP	LH	7	106	159.13	3.44	9.35
Pl.S.4.1.LL	Pl	SAMP	LL	26	3174	4607.96	5.63	87.44
Pl.S.4.2.HL	Pl	SAMP	HL	31	2343	3338.64	5.69	70.82
Pl.S.4.5.LH	Pl	SAMP	LH	39	889	1148.22	3.56	34.08
Pl.S.4.6.HH	Pl	SAMP	HH	21	5517	7505.55	6.33	132.08
Pl.S.5.4.LL	Pl	SAMP	LL	2	541	751.02	3.94	28.66
Pl.S.5.6.LH	Pl	SAMP	LH	12	150	226.56	3.91	11.28
Pl.S.6.4.LL	Pl	SAMP	LL	32	340	448.00	3.83	19.39
Pl.S.6.5.LH	Pl	SAMP	LH	25	206	261.00	2.48	13.05
Pl.W.1.1.LH	Pl	WAI	LH	1	451	631.62	4.17	25.46
Pl.W.1.5.HL	Pl	WAI	HL	18	226	296.83	2.87	10.50
Pl.W.1.6.LL	Pl	WAI	LL	9	193	292.53	2.11	10.43
Pl.W.2.11.LH	Pl	WAI	LH	28	2862	4173.47	6.46	75.62
Pl.W.2.16.LL	Pl	WAI	LL	23	3010	4541.99	6.75	81.26
Pl.W.2.18.HL	Pl	WAI	HL	22	115	183.33	0.41	8.93
Pl.W.3.1.LL	Pl	WAI	LL	17	205	281.00	2.56	11.48
Pl.W.3.2.HH	Pl	WAI	HH	13	2904	3994.98	5.82	84.00
Pl.W.3.3.HL	Pl	WAI	HL	3	3481	5313.18	5.79	96.10
Pl.W.3.6.LH	Pl	WAI	LH	7	2595	3627.23	5.79	75.20
Pl.W.4.1.HH	Pl	WAI	HH	21	643	971.76	2.87	28.88
Pl.W.4.3.LH	Pl	WAI	LH	39	200	303.31	1.37	13.51
Pl.W.4.5.HL	Pl	WAI	HL	31	3427	5285.37	6.25	96.15
Pl.W.4.6.LL	Pl	WAI	LL	26	163	313.23	2.27	10.97
Pl.W.6.1.LL	Pl	WAI	LL	32	2707	4018.36	5.57	72.20
Pl.W.6.2.LH	Pl	WAI	LH	25	3774	5674.47	7.06	96.91
Pl.W.7.1.LL	Pl	WAI	LL	2	120	185.00	2.74	6.83
Pl.W.7.2.HL	Pl	WAI	HL	5	351	513.30	3.08	16.98
Pl.W.7.3.LH	Pl	WAI	LH	12	352	419.16	2.83	17.81
Pl.W.7.5.HH	Pl	WAI	HH	16	2418	3767.16	5.57	70.92