AN ABSTRACT OF THE THESIS OF

Erica Ewton for the degree of Master of Science in Microbiology presented on June 17, 2021.

Title: Species- and Temporal-Specific Coral Microbiome Fluctuations in Response to a Natural Bleaching Event

Abstract approved:

_____

Andrew Thurber

Corals provide a diversity of ecosystem services, are among the most biologically diverse ecosystems on Earth, and directly support ~500 million people globally; however, corals are increasingly experiencing significant threats and are undergoing severe bleaching events as the result of the warming climate. Using a two-year data set surrounding a massive bleaching event around the island of Mo'orea, French Polynesia, this study examines a vital determiner of coral health: its microbiome. We hypothesized that the microbiomes of the dominant corals *Acropora hyacinthus*, *Pocillopora verrucosa*, and *Porites lobata* would show stochastic responses to bleaching, yet degrees of resistance to bleaching would be coral species specific. Among all coral microbiomes, certain dominant families shifted in response to bleaching; however, coral species-specific responses were seen regarding fluctuations of Shannon diversity and richness, in addition to shifts in the relative abundance of the highly abundant family *Endozoicomonas*. *Acropora hyacinthus* bleached most readily. *Pocillopora verrucosa* had the lowest percent abundance of *Endozoicomonas* months before and during bleaching. In addition, it was the only coral species to have its microbiome increase in diversity and decrease in *Endozoicomonas* percent abundance before bleaching; however, its health trajectory followed that of *Porites lobata* relatively closely. Trends in the microbial community inhabiting *Porites lobata* including family level richness and diversity and *Endozoicomonas* levels followed those of *Acropora hyacinthus* closely.

Species- and Temporal-Specific Coral Microbiome Fluctuations in Response to a Natural Bleaching Event

by
Erica Ewton

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented June 17, 2021
Commencement June 2022

Master of Science thesis of Erica Ewton presented on June 17, 2021.

APPROVED:

Major Professor, representing Microbiology

Head of the of Department of Microbiology

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Erica Ewton, Author

ACKNOWLEDGEMENTS

I would like to express sincere appreciation for a number of people who expanded my scientific and computational understanding, encouraged me to swim – not sink – amidst a sea of data, and immersed me in the down and dirty of 'scientist'. Andrew, thank you for propelling me through such a foundational part of my career. You stuck with me through highs and lows and believed in my abilities before I even knew I had them. You taught me to assert rather than question my theories while still contemplating alternate views – this is a lesson I will carry ceaselessly. Thank you to my thesis committee for your flexibility and support through this unusual accelerated masters experience; your constructive feedback and seasoned points of view kept my project within reasonable bounds while expanding my view of possibilities.

Thank you to my undergraduate and graduate professors who have broadened my understanding and self-confidence in microbiology. You taught me the material, but also how to conduct myself in scientific and professional settings through stellar examples that I strive to embody throughout my career. Thank you to the GeoMicrobiology group for all your support and enthusiasm for learning. You encouraged me to share my science in a welcoming atmosphere that boosted my presentation and discussion skills like no other environment. Thank you to the coding geniuses out there – those of you who stayed up late helping trouble shoot have taught me not only where the parentheses go but how far camaraderie does as well. Thank you to my close friends and fiancé for lifting my spirits during stressful times and reminding me that we are all simply human.

Thank you to my undergraduate mentee Heather Hirsch for asking amazing questions and volunteering countless hours towards sample preparation. Thank you to the Thurber, Vega-Thurber, and Correa labs for collecting samples, processing some data, and sharing knowledge of protocols and the Mo'orea reef system. This research was supported in part by National Science Foundation, Biological Oceanography Grants: 1635913, 1933165, and 1635798.

TABLE OF CONTENTS

Page

LIST OF FIGURES

LIST OF TABLES

LIST OF SUPPLEMENTARY MATERIALS

DEDICATION

I would like to dedicate my thesis to my family: my mom, Stacey Schultz and my sister, Jessica Ewton. You both have always supported me in everything I did and dreamed of. Thank you.

Introduction

Though corals are estimated to cover only 0.1-0.5% of the ocean floor (Moberg & Folke, 1999), they provide a suite of services to life on Earth. Almost a third of the world's marine fish species are found on coral reefs, which contribute 10% of fish consumed by humans (Lough & van Oppen, 2009). Their topography and primary production support ~25% of all described marine species (Knowlton, 2001). Coral reefs also supply people with other ecosystem services such as coastal protection, recreation, and cultural and historical context (Lough & van Oppen, 2009). Coral reefs act as carbon dioxide sinks over geological time scales (Hallock, 2001) and provide ~½ of the calcium delivered to the sea each year (Smith, 1978).

A scleractinian coral is composed of three distinct sets of organisms that interact with each other to make up the holobiont: eukaryotic coral, dinoflagellate photosynthetic symbiont, and microbiome. In fact, there is evidence of cophylogeny and phylosymbiosis between the coral and its symbiont, indicating an intricate relationship between these members of the coral holobiont (Pollock et al., 2018). Further, the microbiome of a reef-forming coral determines the health of a reef (Bourne et al., 2016). Corals host a metabolically diverse yet taxonomically restricted set of vital microorganisms in their tissues, skeleton, and mucus that together define the coral phenotype. A key example of the importance of a coral's microbiome occurs when the coral is exposed to stressful conditions including increased temperature (Lesser et al., 1990; Maher et al., 2020; McGinty et al., 2012), nitrogen (Burkepile et al., 2020; Vega-Thurber et al., 2014), phosphate (Rosset et al., 2017; Vega-Thurber et al., 2014), urea (Burkepile et al., 2020), sugars (Pogoreutz et al., 2017), irradiance

(Hawkins et al., 2015), and ultraviolet light (Lesser et al., 1990) or lack thereof (Hawkins et al., 2015). Temperature is the best studied of these stressors. It has been shown that during temperature stress the symbiotic dinoflagellates express decreased photosystem health, resulting in the formation of reactive oxygen species (ROS); this erodes the mutualistic symbiosis, resulting in loss of the symbiont (Nielsen et al., 2018). This process is termed bleaching due to a corresponding loss of color that leaves the coral bleach-white. If the stressor is ameliorated, the coral host can reacquire its symbiont and persist; however, if the coral is unable to, it will die (Ainsworth et al., 2016). In some cases, stressors may lead to a shift in coral microbiome structure without resulting in visible bleaching (Glasl et al., 2019).

Microbiome structure of corals is species-specific (Gardner et al., 2019; Maher et al., 2020) and has been hypothesized to be a major factor influencing holobiont bleaching susceptibility (Krueger et al., 2015). For example, following a bleaching-inducing temperature stress around the island of Seychelles, *Coelastrea aspera* corals bleached less than *Acropora gemmifera* and *Porites lutea* species, corals that had lower bacterial diversity, taxonomic family richness, and community evenness than *C. aspera* (Gardner et al., 2019). Also in support of this is a coral species-specific variation between *Porites compressa*, *Porites lobata*, *Montipora capitata*, *Pocillopora damicornis*, and *Fungia scutaria* in superoxide levels following a 2014 bleaching event in the Hawaiian Islands (Diaz et al., 2016).

A number of studies have noted underlying aspects that may contribute to a coral's ability to resist bleaching. In 1998, a severe coral bleaching event in the Australian Great Barrier Reef reduced coral cover by 75%; however, by the time of

resampling in 2010 cover had been restored (Done et al., 2015). *Acropora*, a broadcast spawning genus, was severely impacted by the bleaching; however, its cover returned to higher than in pre-stress conditions due to its ability to disperse broadly. Vegetative growth allowed regenerating corals, such as some species of *Porites*, to avoid death. Brooding corals such as some *Isopora*, *Seriatopora*, and *Stylophora* were among the most susceptible to bleaching, and their brooding method of reproduction prevented a rebound as extensive as other reproduction types. Golbuu et al. (2007) examined recovery following a 1998 temperature-induced bleaching event in Micronesia and discovered that recovery rates were initially higher at 10 m compared to 3 m when sampling in 2001. Cover increased the most in sheltered bays despite low recruitment rates; recovery was attributed to buffering by remnant survival and recruitment from less impacted sites. Thermal bleaching thresholds may be determined by which type of *Symbiodinium* symbiont a coral houses, which varies by coral species (Krueger et al., 2015). An experimental nutrient-induced bleaching led to conjecture that a cause of bleaching is phosphorus starvation of symbionts caused by skewed N:P ratios instead of the common theory of nutrient enrichment (Rosset et al., 2017). Instead of bleaching being triggered by the symbiont, it may instead be induced by host mitochondria, which were seen to morphologically change and degrade when exposed to thermal stress due to inhibition of protein synthesis, electron transport, and light energy harvesting (Dunn et al., 2012).

Significant advances have been made in identifying strain-level variation between corals' dinoflagellate symbionts and bleaching susceptibility (Sampayo et al., 2008); however, increasingly the roles of Bacteria and Archaea in coral health and

bleaching resistance is being appreciated (e.g. Garren & Azam, 2012; Krediet et al., 2013; Rosenberg et al., 2007). For example, *Endozoicomonas*, an often dominant bacteria within the coral microbiome, is beginning to be linked to why certain coral species are less susceptible to bleaching (Neave et al., 2017). Pogoreutz (2017) noted that bacterial assemblages remained 'remarkably stable' throughout carbon- or nitrogen-induced bleaching and that two *Endozoicomonas* phylotypes dominated the microbiome at over 90% of 16S rRNA gene sequences. They concluded that the bacterial community of *Pocillopora verrucosa* is inflexible and therefore unable to acclimatize to rapid environmental changes. A number of publications have suggested a contrasting view that stressed corals exhibit changes in bacterial community structure (e.g. Jessen et al., 2013; Maher et al., 2020; Röthig et al., 2016; Zaneveld et al., 2016; Ziegler et al., 2017). For example, Zaneveld et al. (2016) report an increase in microbial beta diversity following a 3-year field experiment that applied stressors to corals in the Florida Keys, USA. When simulating overfishing and nutrient pollution, they observed increased dispersion of microbial taxa as compared to pre-stress states. The findings of Zaneveld et al. and others across a wide biological scope are discussed in a review article (Zaneveld et al., 2017) that terms this effect of stress-induced microbiome stochasticity the 'Anna Karenina Principle' (AKP). The AKP (termed so to reflect a Tolstoy dictum) suggests that organisms in an imbalanced state vary in microbiome composition more than healthy individuals. Whereas previous studies tested whether stressed microbiomes remained stable or shifted to a dysbiotic alternate stable state, the AKP suggests instead that stressed individuals may showcase many alternate states that were historically labeled as statistical anomalies

and removed from datasets. This new scope of community composition analysis provides a more complete picture of responses to stress and praises an investigative look into how each individual may react when attempting to survive in adverse conditions.

The objective of this study was to establish the relationship of a coral's microbiome with its ability to resist and recover from bleaching. To accomplish this, we sampled three of the dominant coral species - *Pocillopora verrucosa, Acropora hyacinthus,* and *Porites lobata* - around the island of Mo'orea, French Polynesia, four times in two years surrounding a massive bleaching event that occurred from March - May 2019. 16S rRNA gene amplification and sequencing of coral samples was conducted and diversity measures and shifts in key dominant families were examined in addition to richness, diversity, and health of the sampled coral. We hypothesized that during a bleaching event, a coral's microbiome shifts in a stochastic manner as quantified via dispersion and following a bleaching event a coral's microbiome returns to the pre-bleaching microbial community composition. We expected the microbiome of three coral species (*Acropora hyacinthus*, *Pocillopora verrucosa*, and *Porites lobata*) to respond differently to this bleaching event and that there would be a clear shift in the percent abundance of *Endozoicomonas*.

Materials and Methods

Site Description:

The three sides of the island of Mo'orea, French Polynesia are exposed variably to the open ocean, as Tahiti is upcurrent to the North side of the island due to the South Equatorial Current whereas the West and East sides of Mo'orea are exposed to swell from the Southern Ocean (Leichter et al., 2013). Additionally, due to a lagoon structure that acts as a buffer to the open ocean (Leichter et al., 2013), the island houses three distinct reef types, two of which were included in this study. The back reefs are more proximal to the island than fore reefs, which are situated approximately 0.5 - 1.5 km from the shore. Back reefs are characterized by shallow water and incline to a crest, which then slopes downward as the fore reef. Fore reefs protect back reefs by acting as barriers to the ocean; they are the outer sloping edge that connects the lagoon structure to the outer ocean.

In addition to its unique physical characteristics, Mo'orea also has a history of environmental disturbances including a cyclone in 1991 (Adjeroud et al., 2009), a number of temperature-induced bleaching events (Adjeroud et al., 2009), and a crown of thorns outbreak in the 2000's (Holbrook et al., 2018). These events have caused major disturbances to Mo'orea's ecosystem structure, destroying aspects of the reef structure due to physical destruction (in the case of the cyclone), overgrazing (in the case of the crown of thorns starfish outbreak), and bleaching and death of corals due to abnormal ecosystem fluctuations (in the case of temperature spikes). Though in some cases coral cover was reduced by approximately 30%, in all cases the coral coverage rebounded to pre-disturbance levels within a decade (Adjeroud et al., 2009).

In May of 2019 a significant temperature increase induced a massive bleaching event (Burgess et al., 2021) wherein greater than 70% of fore reef corals bleached (Thurber pers. obs.) (Figure 1), which forms the basis of this study.

Figure 1: Bleached corals following the 2019 massive bleaching event around Mo'orea.

Sample Recovery and Data Gathering:

Coral samples were collected in September 2017, March 2018, May 2019, and August 2019 (Figure 2) including opportunistic sampling during a massive bleaching event in May 2019. Sampling was conducted for three prominent coral species (*Acropora hyacinthus, Pocillopora verrucosa,* and *Porites lobata*) on all sides of the

island and throughout all reef types (Figure 2A). Here we focused on the Fore reefs and Back reefs, where sampling of all three species was possible; *Acropora hyacinthus* is not found on fringing reefs in sufficient abundance to sample on Mo'orea.

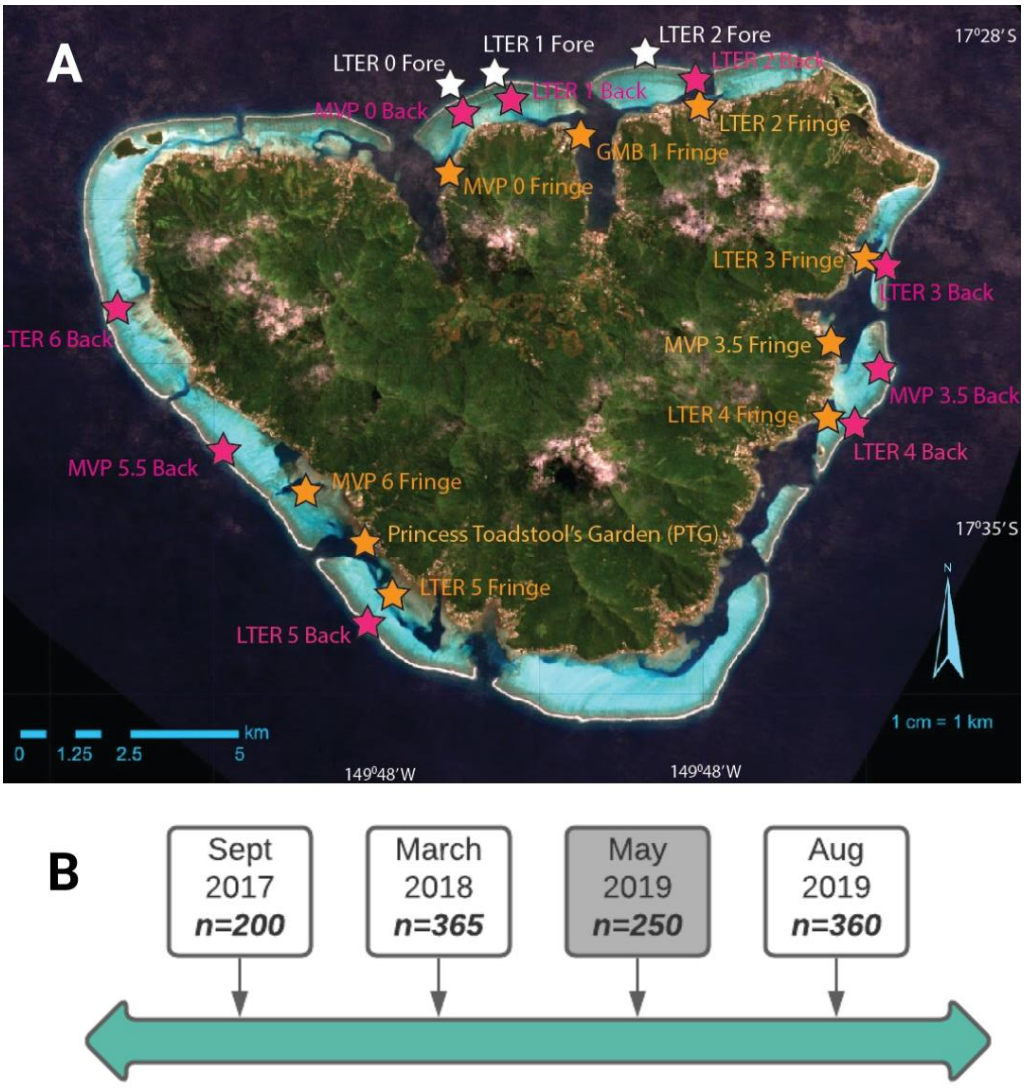

Figure 2: Spatial and temporal sampling distribution of corals around Mo'orea, French Polynesia. A. Map of the island with colored stars indicating sampling locations. White and pink starred locations were included in this study. Base map was

provided by B. Black, OSU. B. Timeline of sampling collection dates and sample number; the grey box indicates the bleaching time point.

To collect each coral sample, snorkelers or SCUBA divers wore nitrile gloves and collected small (1-2 cm^2) pieces using bone cutters. Each individual was tagged during the first sampling period to ensure future sampling of the same individuals. For back reefs, individuals were chosen within a 100 m^2 area, and fore reef corals were selected along a 50 m semi-permanent transect. Logistical constraints prevented sampling of corals on the west and east fore reefs. Sampled material was immediately deposited into 15 mL falcon tubes containing DNA/RNA Shield (Zymo Research, Irvine, CA, USA) and transported at -40ºC to GUMP field station on the island. Upon arrival, samples were vortexed in bead beat tubes for 20 minutes, which transformed the material into a slurry that was preserved and transported to laboratories in the United States for further processing.

Water temperature data was accessed from HOBO thermistors and the MCR LTER website (Moorea Coral Reef LTER & Aldridge et al., 2019).

Coral bleaching severity of each individual was documented by field observations during sampling using the CoralWatch Coral Health Chart (http://coralwatch.org).

<u>DNA Extraction, Purification, Amplification, and Sequencing:</u>

DNA from each sample was extracted using a ZymoBIOMICS DNA Miniprep Kit following manufacturer's instructions (Zymo Research), except two enzyme

digestions were performed on the preserved coral slurry to increase the relative proportion of 16S:12S rRNA genes. First, 30 uL of 10 mg/uL chicken lysozyme, 1.8 uL 50 KU/mL mutanolysin, and 1.8 uL 4 KU/mL lysostaphin were added to 300 uL of the coral slurry and incubated at 37°C for 1 hour. Second, 15 uL of Proteinase K and 30 uL Proteinase K digestion buffer were added to the mixture and incubated at 50°C for 1 hour. The Proteinase K digestion buffer consisted of 0.1M NaCl, 10mM Tris pH 9, 1mM EDTA, 0.5% SDS, and ddH_20. Periodically, negative controls using DNase-free water were subjected to gel electrophoresis for verification, and no bands were seen.

The V4 hypervariable regions of bacterial and archaeal 16S rRNA genes were then amplified following a modified version of the Earth Microbiome Protocol (see Supplementary Material 2), using 515-forward and 806-reverse primers (Caporaso et al., 2011) that contained dual-indexed barcodes in addition to the Illumina sequencing adaptors (Kozich et al., 2013). Amplicons were purified using a QIAQuick PCR purification kit according to manufacturer's instructions (QIAGEN Inc.), then verified via gel electrophoresis. BluePippin was used as a size selection tool to remove contaminant 12S eukaryotic rRNA genes. Illumina MiSeq V2 paired-end 250 bp sequencing was conducted at the Center for Genome Research and Biocomputing at Oregon State University. For sequencing runs conducted in July 2017, September 2017, August 2018, April 2019, and November 2020 the following parameters were used: VDF2010, cassette definition 2%, DF marker V1, range of 430 bp start and 650 bp end, target product size 540 bp, product contamination to avoid is 350 bp. The range was slightly modified part-way through this study to minimize loss of 16S

rRNA genes during size selection. For sequencing runs conducted in January 2021 and May 2021 the range was shifted to 360 bp start and 490 bp end.

Data Analysis:

16S rRNA gene sequence data were processed in Qiime2 version 2019.7.0 (Bolyen et al., 2019) using DADA2 version 2019.7.0 (Callahan et al., 2016) to filter sequences and rarify to 998 reads minimum per sample, define amplicon sequence variants (ASVs), and remove chimeras. See Supplementary Methods for a full sequence analysis pipeline. A pre-trained classifier from the SILVA 16S reference database was used to assign taxonomy to ASVs (Bokulich et al., 2018; Nicholas Bokulich et al., 2021). Statistics were conducted and visualizations created using RStudio version 1.3.1093 and PRIMER7 version 7.0.17 with PERMANOVA+ add-on. A square root transformation was applied to lower non-metric multidimensional scaling plot stress from 0.21 to 0.15. Water temperature data was analyzed and visualized in RStudio version 1.3.1093.

Results

Thermal Stress Event:

In April of 2019, a statistically significant temperature increase was observed ($p < 0.001$), where temperatures averaged 30.28°C and peaked at 32.5°C (see Figure 3). Temperatures in May 2019, the sampling period in which bleached corals were sampled, averaged 28.87°C with a maximum temperature 30.36°C. Average temperatures for 2018 were 29.16°C in April and in 28.72°C in May. Bleaching was

observed on all reefs around the island during the May 2019 sampling date (Thurber pers. obs.).

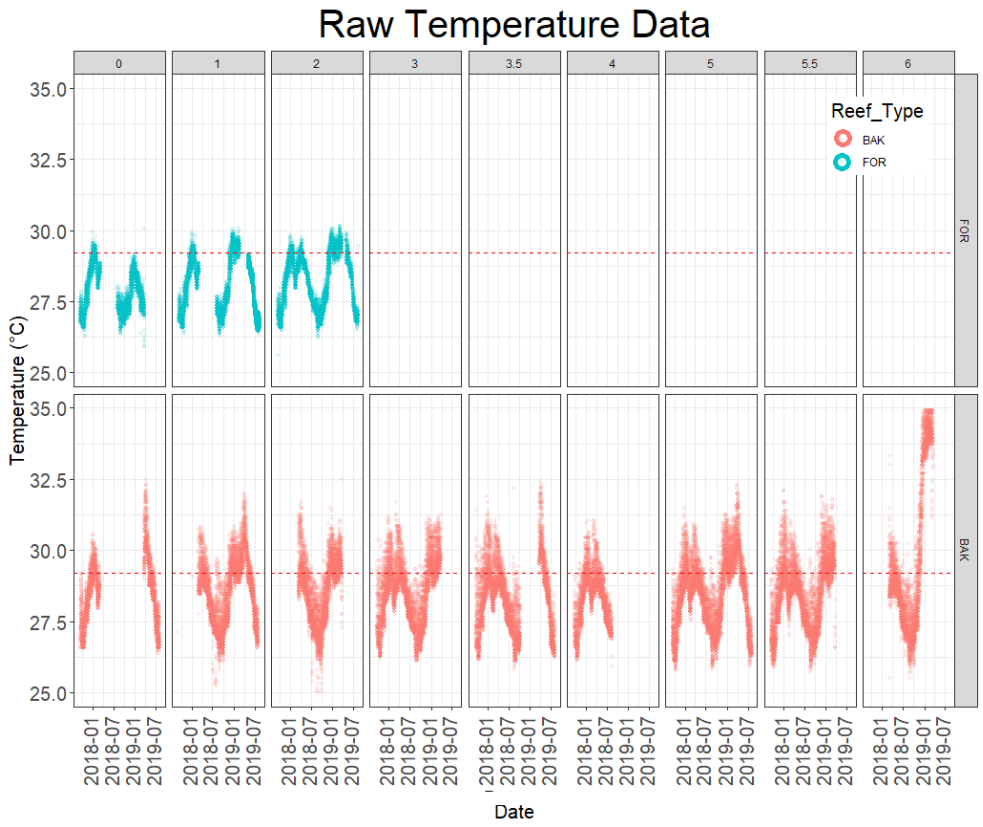

Figure 3: Temperature at all reefs in Mo'orea from 2017-2019. Reef types back and fore are denoted by red and blue, respectively. The typical bleaching threshold for Mo'orean corals (29.2°C; Adjeroud et al., 2009) is marked by a red dashed line.

16S Community Structure:

After quality control, 2,0606,846 reads were present in 605 samples of corals across the time period and range; we restricted our analysis to samples that were longitudinally sampled across this study. The mean sequencing depth was 34,061 ± 1718 (SE) reads per sample, and sequences were rarified to 998 reads minimum due

to low sampling depth at certain time points. We identified a total of 53,392 ASVs within these corals, and the mean family richness was 88.5 $\pm$ 3.6 (SE) ASVs per sample.

Corals bleached to varying degrees, as quantified by comparison to the CoralWatch Coral Health chart, influenced by time point, coral species, species within shore, species within time point, shore within time point, and species within shore within time point (all 3-way ANOVA $p \leq 0.01$; Table 1). The CoralWatch Coral Health chart uses a color guide to assign a number to each individual, where higher numbers are darker colors. So, the closer to 1 an individual's assigned number is, the more severe its bleaching is. Coral health of *Acropora hyacinthus* and *Pocillopora verrucosa* varied significantly at all shores throughout time (all 3-way ANOVA $p < 0.001$, ≤ 0.04, respectively; Table 2). Baseline pre-bleaching average health was 3.0 and reached a low in May 2019 (average 1.8). *Porites lobata* varied among reef types within the North shore (3-way ANOVA $p \leq 0.001$). *Acropora hyacinthus* was most susceptible to bleaching (average 1.1 in May 2019); however, all coral species showed increased health post-beaching in August 2019 when compared to during bleaching (average 2.3). Individual variability was observed across all coral species; some corals died completely while others showed no visible signs of bleaching. *Acropora hyacinthus* individuals died as a result of bleaching most frequently and on the north shore there was near 100% mortality for many *Acropora hyacinthus* individuals.

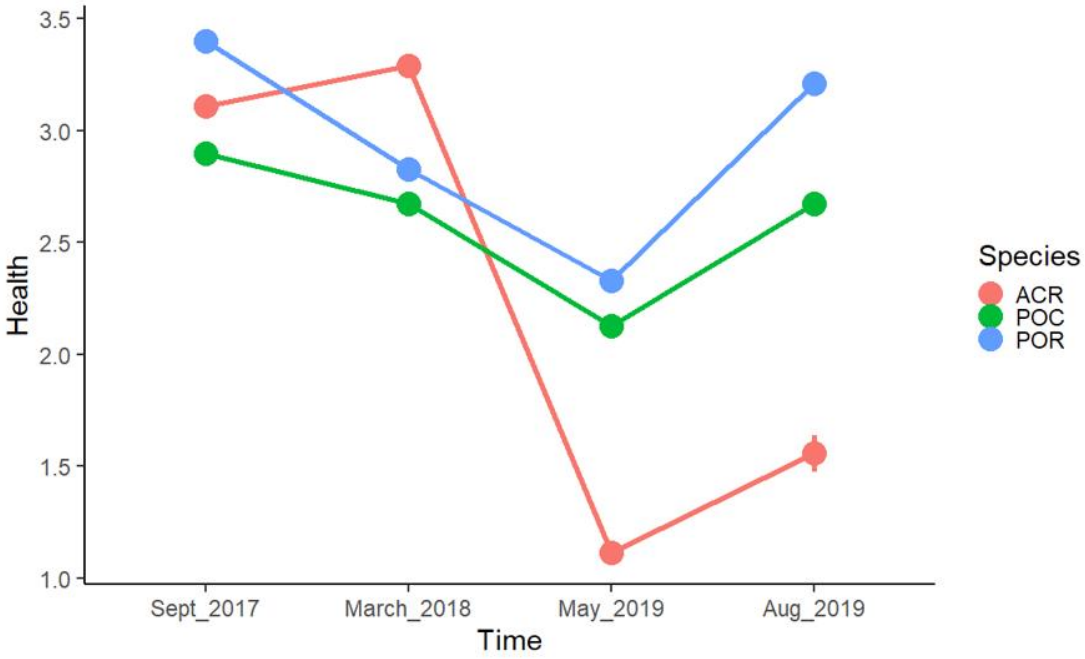

Figure 4: Average coral health for all samples from which corals were taken as quantified by comparison to the CoralWatch Coral Health Chart. Health is estimated on a numeric scale, with darker corals given higher numbers, suggesting that they have not lost symbionts and bleached. Bars are standard error. Species *Acropora hyacinthus*, *Pocillopora verrucosa*, and *Porites lobata* are denoted by colors red, green, and blue, respectively.

Table 1: 3-way ANOVA statistics for coral health as quantified by the CoralWatch Coral Health Chart for all time points. Only statistically significant values at an alpha of 0.05 are shown.

Group	Degrees Freedom (n)	Degrees Freedom (d)	F Statistic	P value
Species	2	597	27.2	<0.001
Time point	4	597	58.2	<0.001
Species w/in Shore	6	597	2.7	0.01
Species w/in Time point	8	597	14.2	<0.001
Shore w/in Time point	9	597	3.2	<0.001
Species w/in Shore w/in Time point	15	597	4.1	<0.001

Table 2: Simple-simple main effect statistics of 3-way ANOVA of time point for coral health as quantified by the CoralWatch Coral Health Chart. Only statistically significant values at an alpha of 0.05 are shown. df(n) is degrees freedom of the factor and df(d) is degrees freedom of the error, here and throughout.

	Acropora hyacinthus	*Pocillopora verrucosa*	*Porites lobata*
North Shore (FOR)	$F = 49.7$, $p < 0.001$, $df(n) = 2$, $df(d) = 597$	$F = 3.1$, $p = 0.03$, $df(n) = 2$, $df(d) = 597$	$F = 11.9$, $p < 0.001$, $df(n) = 3$, $df(d) = 597$
North Shore (BAK)	$F = 10.1$, $p < 0.001$, $df(n) = 3$, $df(d) = 597$	$F = 7.9$, $p < 0.001$, $df(n) = 3$, $df(d) = 597$	$F = 4.5$, $p = 0.001$, $df(n) = 4$, $df(d) = 597$
West Shore (BAK)	$F = 36.8$, $p < 0.001$, $df(n) = 3$, $df(d) = 597$	$F = 3.2$, $p = 0.04$, $df(n) = 2$, $df(d) = 597$	
East Shore (BAK)	$F = 21.9$, $p < 0.001$, $df(n) = 4$, $df(d) = 597$	$F = 2.8$, $p = 0.04$, $df(n) = 4$, $df(d) = 597$	

Overall microbial community structure was significantly different among coral species (1-way PERMANOVA global and pairwise $p = 0.001$, F statistic = 49.6; Figure 5). Time point was the next greatest influencer in microbial community structure (1-way PERMANOVA global $p = 0.001$, F statistic = 10.3). Pairwise tests between time points were also all statistically significant from each other; however, pre-bleaching time points were least significantly different (1-way PERMANOVA $p = 0.017$, all other $p = 0.001$). Reef types back and fore were also statistically significant in microbiome structure ($p = 0.001$); however, were the least influential grouping (1-way PERMANOVA F statistic = 5.1).

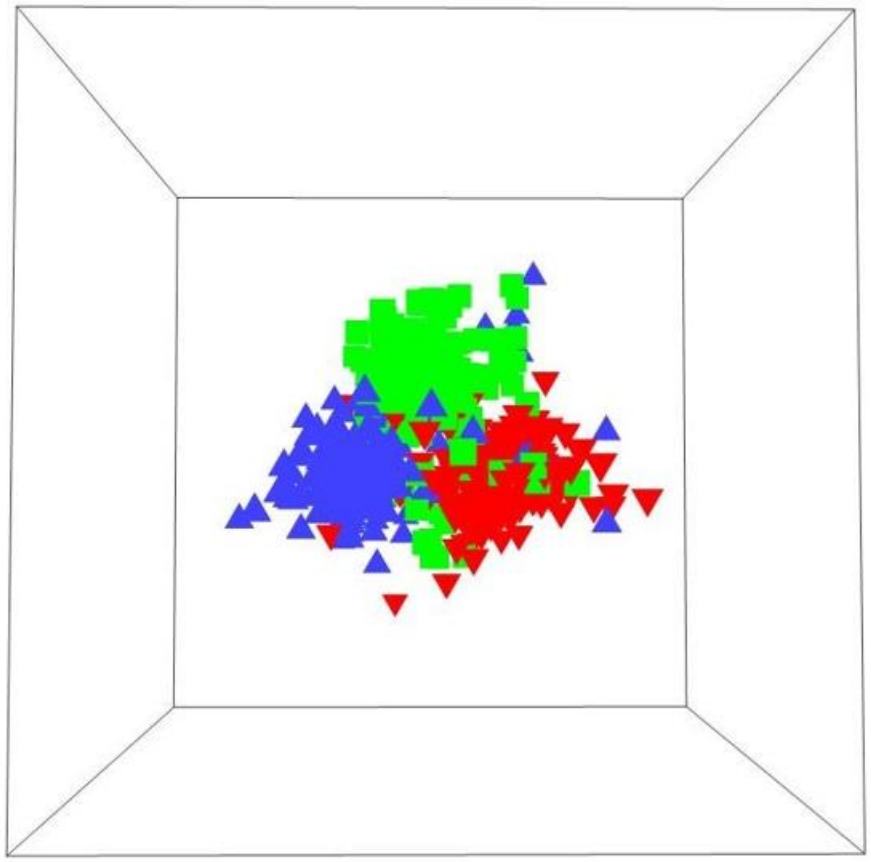

Figure 5: 3-dimensional non-metric multidimensional scaling plot of the microbiome of *Pocillopora verrucosa* (POC), *Porites lobata* (POR), and *Acropora hyacinthus* (ACR). Species microbiomes are indicated by the colors blue, red, and green, respectively. Stress in this 3D plot is 0.15 and most clear axis orientation is presented.

Microbiome family richness differed by time point and shore within each time point (Table 3). Richness was not differed among the pre-bleaching time periods (1-way ANOVA September 2017 $p = 0.55$, March 2018 $p = 0.47$) with an average of 83.8 ASVs. During and after bleaching, richness changed significantly (1-way ANOVA May 2019 $p < 0.001$, August 2019 $p = 0.001$), increasing to an average

111.4 ASVs during the bleaching period and decreasing to an average of 69.28 ASVs in August 2019 (Figure 6). Richness was rarely significantly different when examining samples grouped by coral species among shores by time point; however, the North shore fore reef was different between *Pocillopora verrucosa* and *Porites lobata* microbiome samples (3-way ANOVA p = 0.002, 0.05, respectively; Table 4) and between *Pocillopora verrucosa* East shore and back reef samples (3-way ANOVA p = 0.01; Table 4).

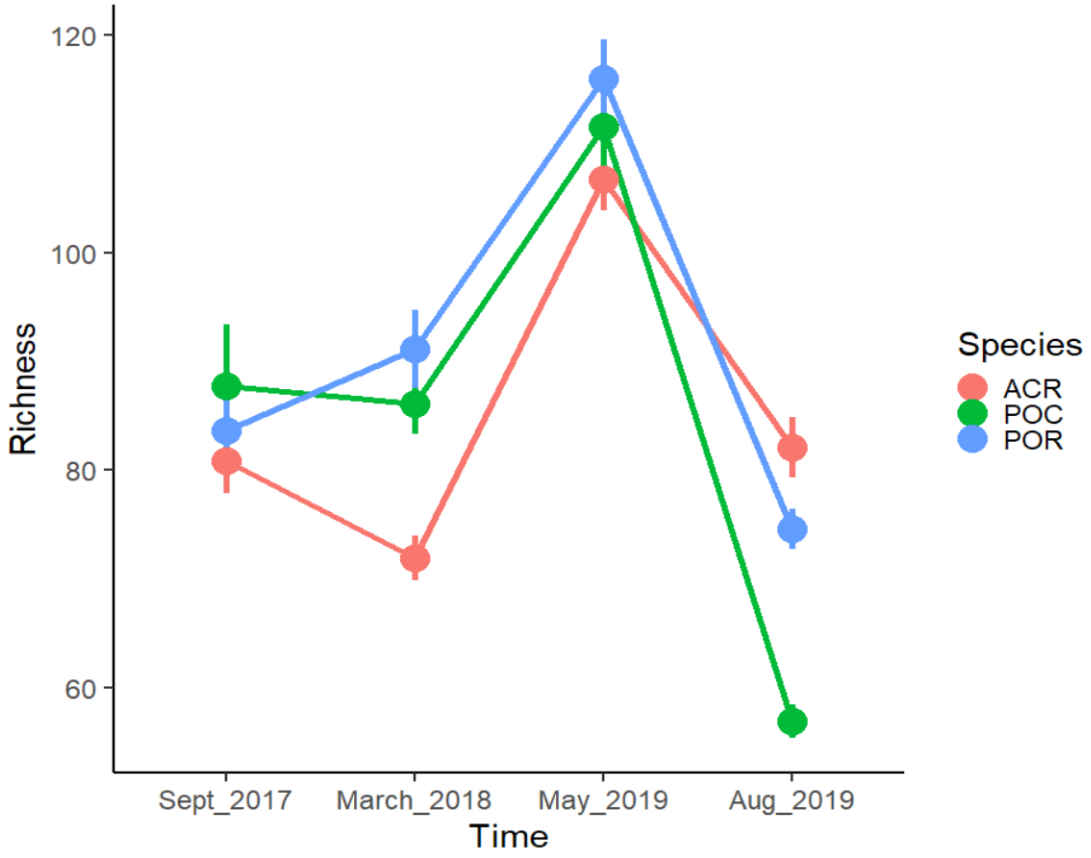

Figure 6: Average richness of coral samples through time among all samples. Bars are standard error. Species *Acropora hyacinthus*, *Pocillopora verrucosa*, and *Porites lobata* are denoted by colors red, green, and blue, respectively.

Table 3: 3-way ANOVA statistics for coral microbiome sample richness across time. Only statistically significant values at an alpha of 0.05 are shown.

Group	Degrees Freedom (n)	Degrees Freedom (d)	F Statistic	P value
Time point	3	557	7.9	<0.001
Shore w/in Time point	9	557	2.7	<0.001

Table 4: Simple-simple main effect statistics of 3-way ANOVA of time point for coral sample microbiome richness. Only statistically significant values at an alpha of 0.05 are shown.

	Pocillopora verrucosa	*Porites lobata*
North Shore (FOR)	F = 3.5, p = 0.002, df(n) = 3, df(d) = 557	F = 2.6, p = 0.05, df(n) = 3, df(d) = 557
East Shore (BAK)	F = 4.9, p = 0.002, df(n) = 3, df(d) = 557	

Overall diversity of samples, as quantified using the Shannon diversity index on family level data, did not vary significantly over time nor space, however, some species-within-shore interactions throughout time were observed (Figure 7). *Acropora hyacinthus* and *Pocillopora verrucosa* microbiomes varied among all shores and reef types throughout time (all 3-way ANOVA $p < 0.001$; Table 5). *Porites lobata* North shore fore and back reef coral microbiomes were different from other shores throughout time (3-way ANOVA $p \leq 0.001$; Table 5). Overall no significant difference was seen among samples grouped by coral species, shore, time point, species within shore, species within time point, and species within shore within time point.

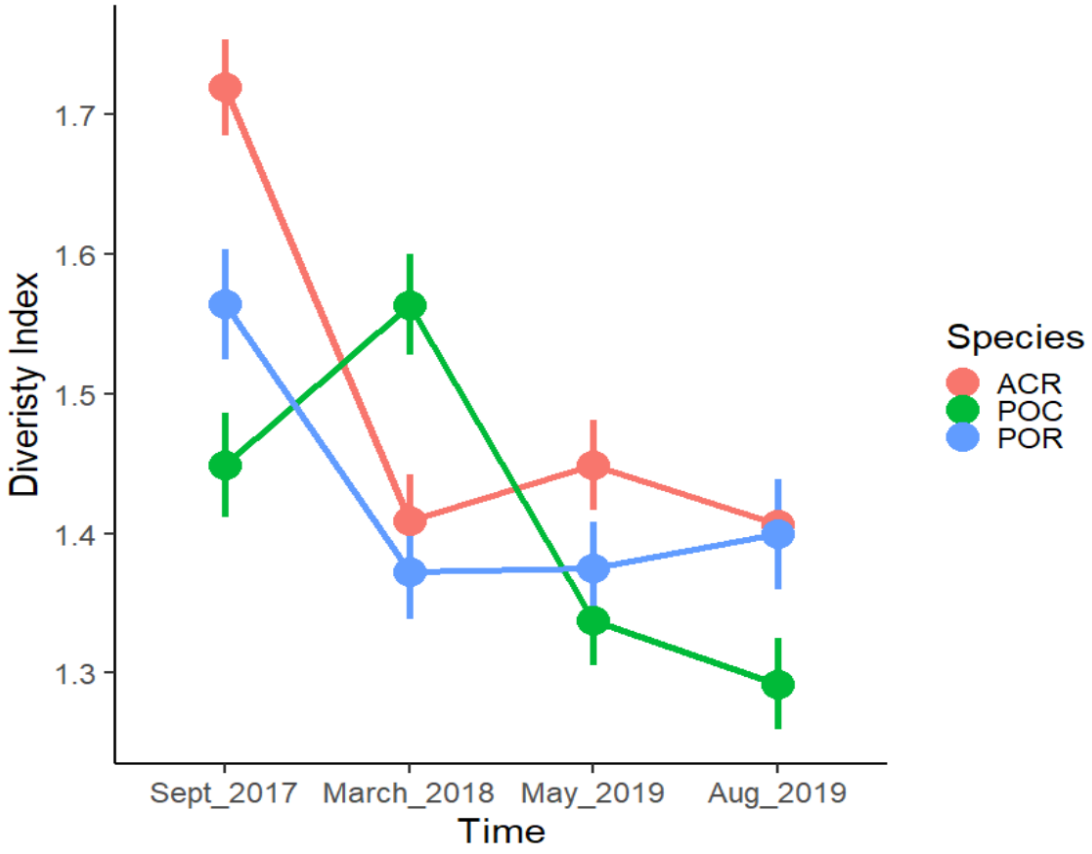

Figure 7: Mean Shannon diversity of the microbiome of *Acropora hyacinthus (ACR)*, *Pocillopora verrucosa (POC)* , and *Porites lobata (POR)*. Bars are standard error. are denoted by colors red, green, and blue, respectively.

Table 5: Simple-simple main effect statistics of 3-way ANOVA of time point for Shannon diversity of samples as quantified by the CoralWatch Coral Health Chart. Only statistically significant values at an alpha of 0.05 are shown.

	Acropora hyacinthus	*Pocillopora verrucosa*	*Porites lobata*
North Shore (FOR)	F = 49.7, p < 0.001, df(n) = 2, df(d) = 557	F = 3.1, p = 0.03, df(n) = 2, df(d) = 557	F = 11.9, p < 0.001, df(n) = 3, df(d) = 557
North Shore (BAK)	F = 10.1, p < 0.001, df(n) = 3, df(d) = 557	F = 7.9, p < 0.001, df(n) = 3, df(d) = 557	F = 4.5, p = 0.001, df(n) = 4, df(d) = 557
West Shore (BAK)	F = 36.8, p < 0.001, df(n) = 3, df(d) = 557	F = 3.2, p = 0.04, df(n) = 2, df(d) = 557	
East Shore (BAK)	F = 21.9, p < 0.001, df(n) = 4, df(d) = 557	F = 2.8, p = 0.04, df(n) = 4, df(d) = 557	

The top 10 most abundant families from all samples largely drive the community structures explained above. *Endozoicomonas* was the most abundant taxonomic group within all three coral species' microbiomes(Figure 8), making up an average 31.0% of the microbiome with a maximum relative abundance of 97.6%. Other highly abundant families are *Amoebophilaceae*, *Beijerinckiaceae*, *Burkholderiaceae*, *Nostocaceae*, *Ostreobium queketti* and sp. HV05042, *Rhodobacteraceae*, *Simkaniaceae*, and an uncultured division of CAB-1 bacterium.

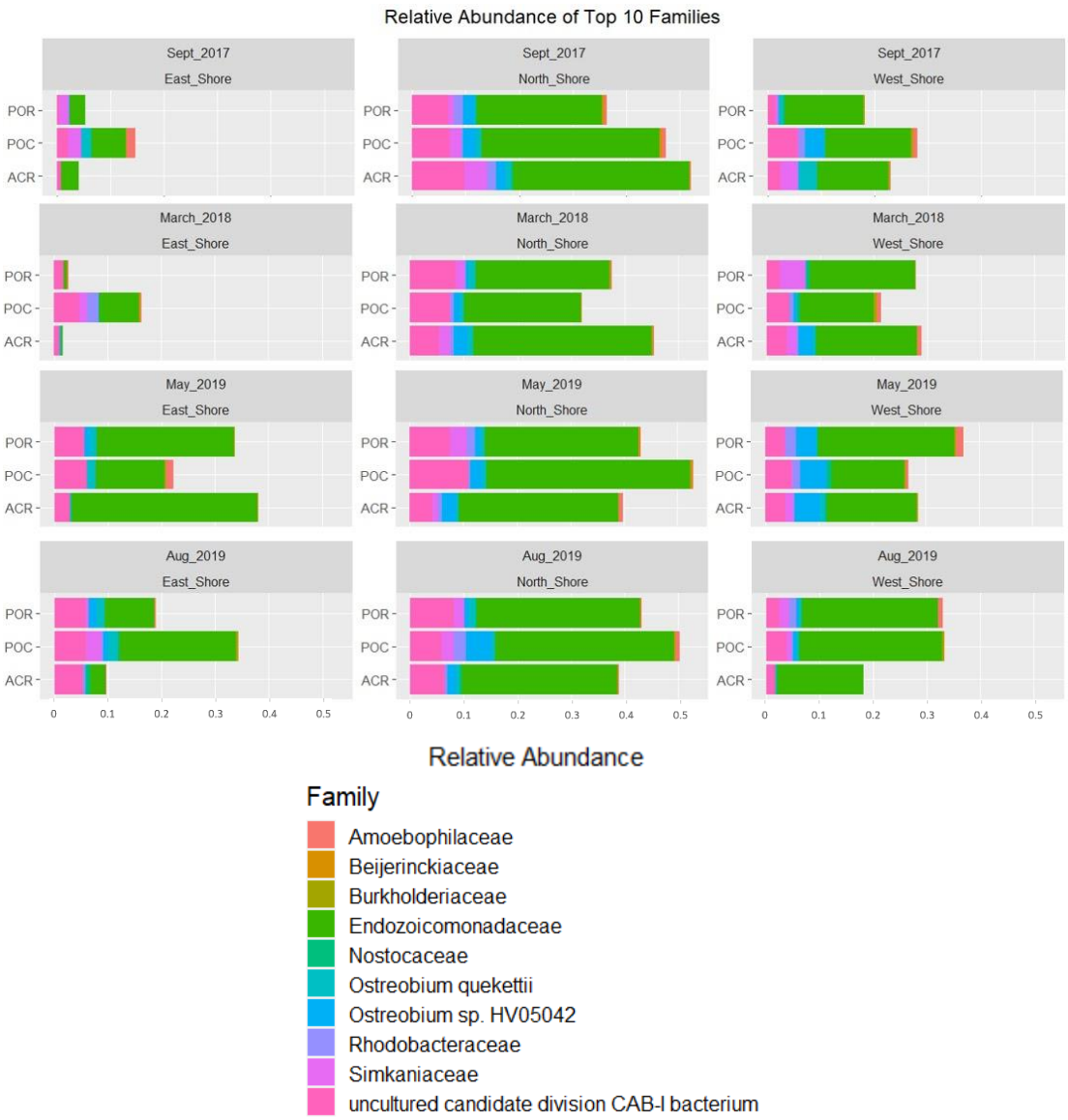

Figure 8: Bar plot of the top 10 most abundant families of bacteria and archaea in all corals sampled split up by time, island side and coral species. Microbial family is indicated by color as specified in the legend at the bottom of the figure.

Endozoicomonas percent abundance was divergent among samples grouped by coral species, shore, and time point. *Pocillopora verrucosa's* microbiome had the lowest percent abundance of *Endozoicomonas* directly before and during bleaching

(1-way ANOVA $p < 0.001$), similar to the microbiomes of the other two species included in this study (*Acropora* 1-way ANOVA $p < 0.001$ and F statistic = 58.76, *Porites* $p < 0.001$ and F statistic = 64.89; Figure 9). Following bleaching, *Endozoicomonas* levels did not differ overall from other time points ($p = 0.9$); however, it did shift differentially across the species. *Endozoicomonas* relative abundance in samples from *Porites lobata* corals increased (1-way ANOVA $p < 0.001$) whereas in *Acropora hyacinthus* and *Pocillopora verrucosa* they decreased ($p = 0.02, < 0.001$, respectively). To better identify the pattern driven by bleaching we then focused on within coral species and across-island patterns. Coral species and shore were the most significant influencers of *Endozoicomonas* percent abundance (3-way ANOVA $p < 0.001$; Table 6), followed by time point (3-way ANOVA $p = 0.02$; Table 6). Samples from coral species within shore and within time point as well as shore within time point also differed significantly in *Endozoicomonas* abundance (3-way ANOVA $p < 0.001, = 0.01, 0.02$, respectively; Table 6). Independent of time point, *Acropora hyacinthus* corals in the North shore fore and West shore back reefs differed in *Endozoicomonas* abundance from other reefs (3-way ANOVA $p = 0.02$, 0.01, respectively; Table 7). *Porites lobata* coral samples also varied in percent abundance of *Endozoicomonas* among the North shore fore reef (3-way ANOVA $p = 0.01$), and among the East shore back reef (3-way ANOVA $p = 0.01$). *Pocillopora verrucosa* samples varied within the East shore back reef (3-way ANOVA $p = 0.03$; Table 7).

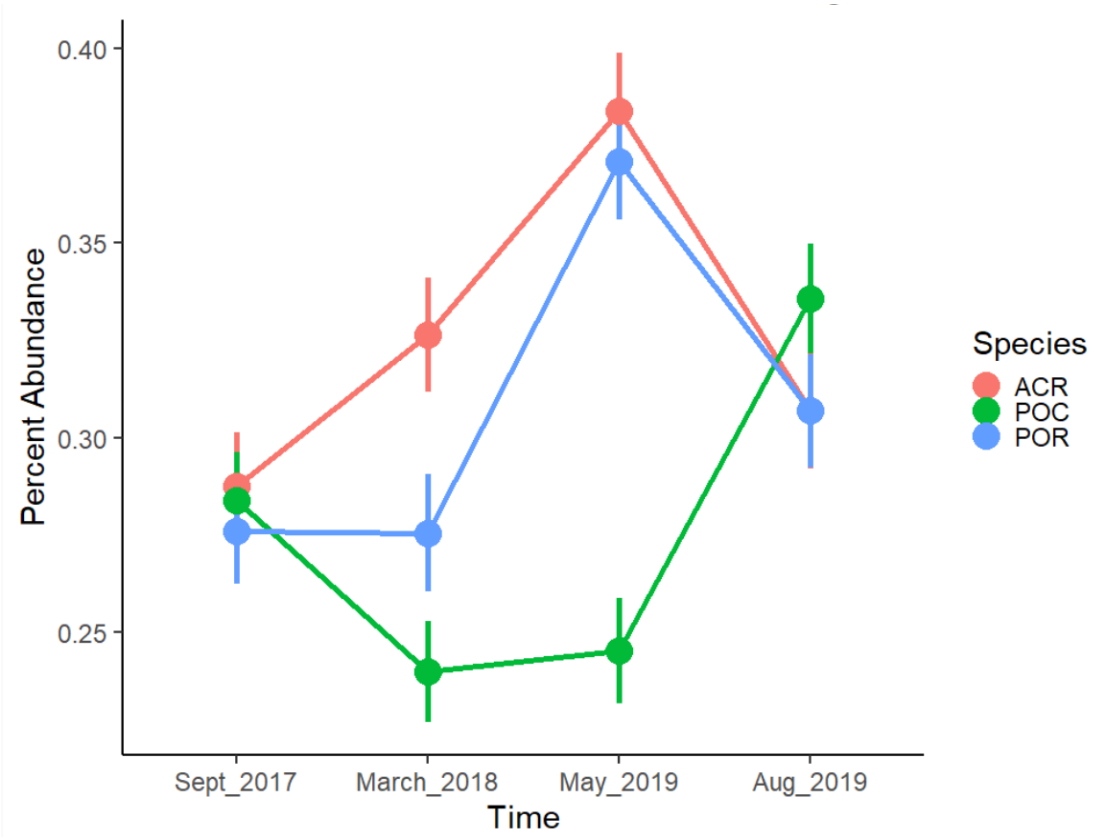

Figure 9: *Endozoicomonas* average percent abundance within the microbiome of *Acropora hyacinthus (ACR)*, *Pocillopora verrucosa (POC)*, and *Porites lobata (POR)* through time. Bars are standard error.

Table 6: *Endozoicomonas* percent abundance 3-way ANOVA statistics for all time points. Only statistically significant values at an alpha of 0.05 are shown.

Group	Degrees Freedom (n)	Degrees Freedom (d)	F Statistic	P value
Species	2	532	205.6	<0.001
Shore	3	532	14.7	<0.001
Time point	3	532	3.4	0.02
Species w/in Shore	6	532	22.1	<0.001
Species w/in Time point	6	532	2.8	0.01
Shore w/in Time point	9	532	2.2	0.02

Table 7: *Endozoicomonas* simple-simple 3-way ANOVA statistics of time point with samples grouped by coral species. Only statistically significant values at an alpha of 0.05 are shown.

	Acropora hyacinthus	*Pocillopora verrucosa*	*Porites lobata*
North Shore (FOR)	F = 3.1, p = 0.02, df(n) = 2, df(d) = 532		F = 3.6, p = 0.01, df(n) = 3, df(d) = 532
North Shore (BAK)			
West Shore (BAK)	F = 3.8, p = 0.01, df(n) = 3, df(d) = 532		
East Shore (BAK)		F = 3.1, p = 0.03, df(n) = 3, df(d) = 532	F = 3.6, p = 0.01, df(n) = 3, df(d) = 532

The overall stochasticity of microbiome structures was driven by time point and host species. Microbial dispersion was not statistically significant throughout time when grouping all coral species together (global 1-way ANOVA p = 0.29, F statistic = 1.41). Significant pairwise differences were observed. Dispersion was highest among May 2019 samples (average distance from centroid 50.2 ± 1.1; Table 8) and lowest in August 2019 (average distance from centroid 46.9 ± 1.0; Table 8), which marked the only statistically significant pairwise differences between time points (p = 0.04; Table 9). Dispersion was different between May and August 2019 within *Pocillopora verrucosa* coral samples (1-way ANOVA p = 0.03, see Table 9) and when comparing August 2019 to March 2018 and September 2017 (1-way

ANOVA p = 0.02, 0.03, respectively; Table 9). *Acropora hyacinthus* corals' microbiome dispersion differed between September 2017 and March 2018 (1-way ANOVA p = 0.03; Table 9).

Table 8: PERMDISP dispersion statistics grouped by time point among samples that were consistently sampled among all time points. Dispersion was calculated using PERMDISP in Primer7.

	All Species	*Acropora hyacinthus*	*Pocillopora verrucosa*	*Porites lobata*
September 2017	49.0± 1.2	43.0± 3.0	43.1± 2.6	43.1± 2.8
March 2018	48.2± 1.0	33.4± 2.3	40.7± 1.8	46.2± 1.8
May 2019	50.2± 1.1	39.7± 2.6	43.6± 2.9	49.9± 1.9
August 2019	46.9± 1.0	39.9± 2.0	32.2± 1.9	43.1± 2.5

Table 9: Pairwise dispersion statistics grouped by time point among samples that were consistently sampled among all time points. Dispersion was calculated using PERMDISP in Primer7. Only statistically significant values at an alpha of 0.05 are shown.

Species	Time points	T Statistic	P value
All species	(Aug_2019, May_2019)	2.18	0.04
Acropora hyacinthus	(March_2018, Sept_2017)	2.5	0.03
Pocillopora verrucosa	(Aug_2019, March_2018)	2.5	0.03
Pocillopora verrucosa	(Aug_2019, May_2019)	2.7	0.03
Pocillopora verrucosa	(Aug_2019, Sept_2017)	2.8	0.02

Discussion

Corals influence all domains of life, as they are involved in biogeochemical cycling and provide many ecosystem services. Due to temperature increases and other effects of climate change, corals are increasingly experiencing bleaching-inducing stress. The degree to which a coral bleaches and is able to recover from bleaching is greatly determined by its microbiome (Kreuger et al., 2015). This study characterizes shifts in aspects of corals' microbiomes including abundant families, sample richness, and sample diversity and compares them to trends in coral health throughout a massive bleaching event in Mo'orea, French Polynesia.

Coral species-specificity was the most significant grouping factor in microbial community, likely driven by particulars of each species. Stony corals reproduce via

broadcast spawning, regeneration, and brooding, which vary within genera but are species-specific (Neave et al., 2017; Quigley et al., 2017). These reproduction approaches can influence microbiome acquisition: both vertical via parent and horizontal via environment are practiced, and degrees of selectivity vary between species and environment (Quigley et al., 2017). Symbionts and bacteria are passed both horizontally and vertically with species-specific relative influence; broadcast spawners tend to acquire more microbes horizontally than vertically (Epstein et al., 2019). Species in this study reproduce via broadcast spawning and regeneration/fragmentation, however, regeneration is very limited in *Acropora hyacinthus* (Glynn et al., 1994; Hall & Hughes, 1996).

Coral holobionts were structured by shore and time point. Different sides of the island are differentially exposed to a number of factors including open ocean water (Leichter et al., 2013) and different anthropogenic activities are conducted on different parts of the island, exposing corals to varied nutrient concentrations. Nutrient exposure can dictate a coral's microbial community structure (Kelly et al., 2014). Sampled time points occurred during periods with different mean and peak temperatures. Though communities from all time points were significantly different from each other, corals that had not undergone thermal stress were more similar than those that had undergone stress (pre-bleach $p = 0.017$, post-bleach $p = 0.001$). This indicates that bleaching due to thermal stress affected microbial community structure.

Our mostly consistent diversity measures of the coral's microbiomes align with the findings of Pogoreutz et al. (2017) who concluded that bacterial diversity was rigid, however, we also found differences in the microbiome structure of coral

species throughout time. After enriching mesocosm tanks of *Pocillopora verrucosa* coral with excess labile dissolved organic carbon and nitrogen, Pogoreutz et al. (2017) observed severe bleaching phenotypes and corresponding changes in symbiont clades yet no significant changes in bacterial diversity. In contrast, we found *Acropora hyacinthus* and *Pocillopora verrucosa* differed throughout time among all reef types, and between North shore reef types within *Porites lobata* corals. Perhaps this is an effect of *in situ* versus experimental bleaching, as many variables changed in our study whereas all conditions save for dissolved carbon and nitrogen were stable throughout the experiment of Pogoreutz et al. (2017). Additionally, Pogoreutz et al. observed corals for 14 days during which they induced bleaching, whereas our samples spread a longer time period that included months of pre- and post-bleaching time for microbiome structure to possibly change and settle to new levels. The long term exposure in contrast to rapid bleaching may have led to the divergence in our observed microbiomes trajectories in these two students. Our significant differences in diversity between shores and reef types also likely reflect the varying oceanographic conditions around the island (Leichter et al., 2013).

We observed a non-constant microbiome structure, however, a few commonly abundant taxonomic families were present and in particular all holobionts were dominated by *Endozoicomonas* with a few other families common. *Endozoicomonas* has been observed as a highly abundant families among many studies focusing on the coral microbiome (Epstein et al., 2019; Glasl et al., 2019; Maher et al., 2020; Pogoreutz et al., 2017). Some studies mention high abundances of higher classifications of bacteria, namely *Proteobacteria* (Zhou et al., 2017) and

Gammaproteobacteria (Glasl et al., 2019; Pogoreutz et al., 2017) that may also obscure an abundance of *Endozoicomonas,* as it falls within the Gammaproteobacterial Class. *Endozoicomonas* has been identified as a likely key player in carbon cycling and protein provisioning due to sugar transport and protein secretion genes (Neave et al., 2017). *Rhodobacteriacea* is another group that is commonly reported as part of the coral holobiont (Glasl et al., 2019; Pootakham et al., 2019). Analogous to *Endozoicomonas*, as many studies noted high abundances of a higher classification of *Rhodobacteriacea* or *Alphaproteobacteria* which may have indeed been *Rhodobacteriacea* (Glasl et al., 2019; Pogoreutz et al., 2017; Zhou et al., 2017). Members of *Alphaproteobacteria* have been associated with diseased corals or those with lowered immune systems resulting from repeated stress (Ziegler et al., 2016). *Amoebophilaecae,* another observed taxa within this study, was also highly abundant in a corals that experienced a natural bleaching event in 2016 on the fore reef of the same reef system (Maher et al., 2020) as well as in Great Barrier Reef corals not during a bleaching event (Pollock et al., 2018). Also annotated as *Amoebophilus*, this family of bacteria has been proposed as an additional possible symbiont that likely interacts with *Endozoicomonas*.

Though some families were similarly abundant across many studies, families identified as particularly abundant in this study but not others included *Ostreobium* and *Simkaniacaea*. *Ostreobium* has been referred to as an additional mutualistic symbiont of corals, as it is involved in nitrogen and carbon exchange and provision of nutrients and compounds produced by photosynthesis (del Campo et al., 2017). *Simkaniaceae* is commonly associated with coral microbiomes (Bernasconi et al.,

2019). *Vibrio*, mentioned by Glasl et al. (2019) and Maher et al. (2020) both as a highly abundant family in bleached corals, was not abundant within corals in this study. While *Vibrio* is a diverse group of bacteria, certain members are opportunistic pathogens of corals (Maher et al., 2020) and may indicated a diseased corals or those with lowered immune systems. Perhaps discrepancies in top microbial abundance reflects varying sampling techniques between studies, as there are 3 components of the a coral holobiont that are difficult to parse yet each has different characteristics including richness and composition (Pollock et al., 2018). In this context, the unified sampling approach embraced within this study further indicates the robustness of the differences observed in those studies as well as across the reefscape of Moorea.

Our results indicate that *Endozoicomonas* relative abundance levels are coral species-specific and provocatively are correlated with a coral host's bleaching resistance and recovery. *Pocillopora verrucosa* corals had the lowest percent abundance directly before and during bleaching. *Acropora hyacinthus* had the highest abundance of *Endozoicomonas* during bleaching, however bleached and died most commonly. Maher et al. (2020) reported an increase in *Endozoicomonas* and decrease in diversity in post-stressed coral microbiomes, which aligns with our *Pocillopora verrucosa* results. This may indicate that although *Endozoicomonas* is vital to a coral's health during steady-state, it may be highly susceptible to stressors or its regulation of carbon cycling may be decoupled from ROS levels. Dissolved organic carbon has a tendency to produce ROS (Johannsson et al., 2017), which is a common theory of the cause of bleaching. Higher levels of *Endozoicomonas*, if producing high levels of DOC, may result in pushing corals past the bleaching threshold through

ROS production. After bleaching has ceased, high levels of *Endozoicomonas* would then aid in rebounding coral health as it is vital for holobiont function and the overall C flow throughout the coral holobiont (Neave et al., 2017). The drop in levels among *Pocillopora verrucosa* and *Acropora hyacinthus* post-bleaching likely indicate a return to stable state or leveling off to an alternate stable-state. Interestingly, although *Pocillopora verrucosa* corals had lowest *Endozoicomonas* percent abundance *Porites lobata* showed a similar health projection. This inconsistent tie between *Endozoicomonas* levels and bleaching susceptibility and recovery may indicate that there are other mechanisms involved in recovery from bleaching stress, such as reproduction and symbiont type (Quigley et al., 2017). Additionally, the microbiome of a coral shifts throughout a coral's lifetime, so perhaps that is reflected in these comparisons (Zhou et al., 2017).

Dispersion analysis indicated that the May 2019 bleached time point samples had the greatest variance of a coral individuals' microbiome (average distance from centroid 50.2 ± 1.10 (SE)) when compared to other time points sampled. Corals not yet exposed to thermal stress had similar dispersion (September 2017 distance to centroid 49.0 ± 1.2 (SE), March 2018 48.2 ± 1.0 (SE)). These results support the AKP, in which organisms undergoing stressful events showcase increased stochasticity (as quantified through dispersion) within their microbiome structure (Zaneveld et al., 2017). Following bleaching, dispersion levels dropped to their lowest (46.9 ± 0.96 (SE)) and corresponded with increased dominance of *Endozoicomonas* in *Porites lobata* and *Acropora hyacinthus* corals, providing an indicator of the cessation of thermal stress resulting in a rebound in the microbiome to

a level of lower dispersion. This, in line with the AKP (Zaneveld et al., 2017), was also observed by Maher et al. (2020) as they documented an increased proportion of *Endozoicomonas* after bleaching had ceased. *Pocillopora verrucosa* was the only coral to show significant pairwise differences in dispersion when comparing post-bleaching with pre- and current bleaching time points in this study. This is another indicator of coral species-specific host selectivity of microbiome structure, in this case possibly linked with an increase in *Endozoicomonas* to its highest abundance while dispersion dropped to its lowest, suggesting a stress-induced selection event.

While variable, many of the top most abundant taxonomic families present within the corals around the island of Mo'orea coincided with those found in other studies throughout the world and across coral species. It appears that increased levels of *Endozoicomonas* may induce bleaching more readily because they produce more ROS as a result of dissolved organic carbon cycling. During bleaching, *Pocillopora verrucosa* had the lowest percent abundance of *Endozoicomonas*. Diversity within the *Pocillopora verrucosa* microbiome increased in March 2018 as temperatures began to rise above typical levels then decreased during bleaching, possibly showcasing a coral species-specific response wherein *Endozoicomonas* was shunted and other more tolerant microbes were replaced or pathogenic microbes proliferated. Following bleaching, diversity and *Endozoicomonas* percent abundance increased again, which could indicate that the coral experienced a decrease in average temperature so was reacquired or allowed *Endozoicomonas* to proliferate. Dispersion was only significantly different between May and August 2019 wherein the largest and smallest average distances from centroid were seen, respectively. This may suggest

that bleaching induced selectivity of microbiomes to reflect more bleaching-tolerant individuals, as those who bleached most heavily were not able to recover as frequently. Future research should establish the relationship between temperature and *Endozoicomonas* levels in *Pocillopora verrucosa* in comparison to other coral species, and how the diversity and dispersion changes surrounding thermal stress may affect a coral's ability to resist and recover from bleaching. The disparity between coral species responses to bleaching regarding diversity and *Endozoicomonas* levels indicates the complexity of coral species-specific bleaching tolerance and that there are likely underlying mechanisms that contribute to coral health through stressful conditions.

Conclusion

Temperature increases have increasingly led to coral bleaching events and begun displacing reefs from their natural habitats towards more poleward latitudes (Yamano et al., 2011), affecting organisms across all domains of life. Corals provide almost a third of the world's marine fish species and supply people with many ecosystem services including jobs and cultural context (Lough & van Oppen, 2009). Reduction in coral cover due to bleaching drastically hurts these vital services, with impacts that range far past those services directly supported by corals. Corals contribute vastly to biogeochemical cycling including calcium distribution within the oceans (Smith, 1978).

It is vital that research in slowing coral bleaching rates be continued to reduce loss of reef habitats. Better quantifying the relationship between *Endozoicomonas*

percent abundances and bleaching from thermal stress will help inform scientists which species and individuals may be more susceptible to extinction. Modification of a coral's microbiome to reduce the relative percent abundance of *Endozoicomonas* at the beginning of bleaching may reduce its bleaching susceptibility due to less ROS production, and returning this vital family to the coral directly after the thermal stress is gone may aid in its recovery. This preventative strategy may reduce coral deaths as a result of bleaching as part of a holistic approach inclusive of fundamental anthropogenic changes to reduce the temperature anomalies that frequently cause coral bleaching events.

Bibliography

Alldredge, A and C. Carlson of Moorea Coral Reef LTER. 2019. MCR LTER: Coral Reef: Water Column: Nearshore Water Profiles, CTD, Primary Production, and Chemistry ongoing since 2005. knb-lter-mcr.10.36 doi:10.6073/pasta/6908c811232b1bf27f384bb1c58837a2

Adjeroud, M., Michonneau, F., Edmunds, P. J., Chancerelle, Y., de Loma, T. L., Penin, L., Thibaut, L., Vidal-Dupiol, J., Salvat, B., & Galzin, R. (2009). Recurrent disturbances, recovery trajectories, and resilience of coral assemblages on a South Central Pacific reef. *Coral Reefs*, *28*(3), 775–780. https://doi.org/10.1007/s00338-009-0515-7

Ainsworth, T. D., Heron, S. F., Ortiz, J. C., Mumby, P. J., Grech, A., Ogawa, D., Eakin, C. M., & Leggat, W. (2016). Climate change disables coral bleaching protection on the Great Barrier Reef. *Science*, *352*(6283), 338–342. https://doi.org/10.1126/science.aac7125

Bernasconi, R., Stat, M., Koenders, A., Paparini, A., Bunce, M., & Huggett, M. J. (2019). Establishment of Coral-Bacteria Symbioses Reveal Changes in the Core Bacterial Community With Host Ontogeny. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.01529

Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A., & Gregory Caporaso, J. (2018). Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome*, *6*(1), 90. https://doi.org/10.1186/s40168-018-0470-z

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E.,

Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., … Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology*, *37*(8), 852–857. https://doi.org/10.1038/s41587-019-0209-9

Bourne, D. G., Morrow, K. M., & Webster, N. S. (2016). Insights into the Coral Microbiome: Underpinning the Health and Resilience of Reef Ecosystems. *Annual Review of Microbiology*, *70*(1), 317–340. https://doi.org/10.1146/annurev-micro-102215-095440

Burgess, S. C., Johnston, E. C., Wyatt, A. S. J., Leichter, J. J., & Edmunds, P. J. (2021). Response diversity in corals: Hidden differences in bleaching mortality among cryptic Pocillopora species. *Ecology*, *n/a*(n/a), e03324. https://doi.org/10.1002/ecy.3324

Burkepile, D. E., Shantz, A. A., Adam, T. C., Munsterman, K. S., Speare, K. E., Ladd, M. C., Rice, M. M., Ezzat, L., McIlroy, S., Wong, J. C. Y., Baker, D. M., Brooks, A. J., Schmitt, R. J., & Holbrook, S. J. (2020). Nitrogen Identity Drives Differential Impacts of Nutrients on Coral Bleaching and Mortality. *Ecosystems*, *23*(4), 798–811. https://doi.org/10.1007/s10021-019-00433-2

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National*

Academy of Sciences, *108*(Supplement 1), 4516–4522. https://doi.org/10.1073/pnas.1000080107

del Campo, J., Pombert, J.-F., Šlapeta, J., Larkum, A., & Keeling, P. J. (2017). The 'other' coral symbiont: Ostreobium diversity and distribution. *The ISME Journal*, *11*(1), 296–299. https://doi.org/10.1038/ismej.2016.101

Diaz, J. M., Hansel, C. M., Apprill, A., Brighi, C., Zhang, T., Weber, L., McNally, S., & Xun, L. (2016). Species-specific control of external superoxide levels by the coral holobiont during a natural bleaching event. *Nature Communications*, *7*(1), 13801. https://doi.org/10.1038/ncomms13801

Done, T., Gilmour, J., & Fisher, R. (2015). Distance decay among coral assemblages during a cycle of disturbance and recovery. *Coral Reefs*, *34*(3), 727–738. https://doi.org/10.1007/s00338-015-1302-2

Dunn, S. R., Pernice, M., Green, K., Hoegh-Guldberg, O., & Dove, S. G. (2012). Thermal Stress Promotes Host Mitochondrial Degradation in Symbiotic Cnidarians: Are the Batteries of the Reef Going to Run Out? *PLOS ONE*, *7*(7), e39024. https://doi.org/10.1371/journal.pone.0039024

Epstein, H. E., Torda, G., Munday, P. L., & van Oppen, M. J. H. (2019). Parental and early life stage environments drive establishment of bacterial and dinoflagellate communities in a common coral. *The ISME Journal*, *13*(6), 1635–1638. https://doi.org/10.1038/s41396-019-0358-3

Gardner, S. G., Camp, E. F., Smith, D. J., Kahlke, T., Osman, E. O., Gendron, G., Hume, B. C. C., Pogoreutz, C., Voolstra, C. R., & Suggett, D. J. (2019). Coral microbiome

diversity reflects mass coral bleaching susceptibility during the 2016 El Niño heat wave. *Ecology and Evolution*, *9*(3), 938–956. https://doi.org/10.1002/ece3.4662

Garren, M., & Azam, F. (2012). New directions in coral reef microbial ecology. *Environmental Microbiology*, *14*(4), 833–844. https://doi.org/10.1111/j.1462-2920.2011.02597.x

Glasl, B., Smith, C. E., Bourne, D. G., & Webster, N. S. (2019). Disentangling the effect of host-genotype and environment on the microbiome of the coral Acropora tenuis. *PeerJ*, *7*, e6377. https://doi.org/10.7717/peerj.6377

Golbuu, Y., Victor, S., Penland, L., Idip, D., Emaurois, C., Okaji, K., Yukihira, H., Iwase, A., & van Woesik, R. (2007). Palau's coral reefs show differential habitat recovery following the 1998-bleaching event. *Coral Reefs*, *26*(2), 319–332. https://doi.org/10.1007/s00338-007-0200-7

Hallock, P. (2001). Coral Reefs, Carbonate Sediments, Nutrients, and Global Change. In G. D. Stanley (Ed.), *The History and Sedimentology of Ancient Reef Systems* (pp. 387–427). Springer US. https://doi.org/10.1007/978-1-4615-1219-6_11

Hawkins, T., Krueger, T., Wilkinson, S., Fisher, P., & Davy, S. (2015). Antioxidant responses to heat and light stress differ with habitat in a common reef coral. *Coral Reefs*. https://doi.org/10.1007/s00338-015-1345-4

Holbrook, S. J., Adam, T. C., Edmunds, P. J., Schmitt, R. J., Carpenter, R. C., Brooks, A. J., Lenihan, H. S., & Briggs, C. J. (2018). Recruitment Drives Spatial Variation in Recovery Rates of Resilient Coral Reefs. *Scientific Reports*, *8*(1), 7338. https://doi.org/10.1038/s41598-018-25414-8

Jessen, C., Lizcano, J. F. V., Bayer, T., Roder, C., Aranda, M., Wild, C., & Voolstra, C. R. (2013). In-situ Effects of Eutrophication and Overfishing on Physiology and Bacterial Diversity of the Red Sea Coral Acropora hemprichii. *PLOS ONE*, *8*(4), e62091. https://doi.org/10.1371/journal.pone.0062091

Johannsson, O. E., Smith, D. S., Sadauskas-Henrique, H., Cimprich, G., Wood, C. M., & Val, A. L. (2017). Photo-oxidation processes, properties of DOC, reactive oxygen species (ROS), and their potential impacts on native biota and carbon cycling in the Rio Negro (Amazonia, Brazil). *Hydrobiologia*, *789*(1), 7–29. https://doi.org/10.1007/s10750-016-2687-9

Kelly, L. W., Williams, G. J., Barott, K. L., Carlson, C. A., Dinsdale, E. A., Edwards, R. A., Haas, A. F., Haynes, M., Lim, Y. W., McDole, T., Nelson, C. E., Sala, E., Sandin, S. A., Smith, J. E., Vermeij, M. J. A., Youle, M., & Rohwer, F. (2014). Local genomic adaptation of coral reef-associated microbiomes to gradients of natural variability and anthropogenic stressors. *Proceedings of the National Academy of Sciences*, *111*(28), 10227–10232. https://doi.org/10.1073/pnas.1403319111

Knowlton, N. (2001). The future of coral reefs. *Proceedings of the National Academy of Sciences*, *98*(10), 5419–5425. https://doi.org/10.1073/pnas.091092998

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. *Applied and Environmental Microbiology*, *79*(17), 5112–5120. https://doi.org/10.1128/AEM.01043-13

Krediet, C. J., Ritchie, K. B., Paul, V. J., & Teplitski, M. (2013). Coral-associated micro-organisms and their roles in promoting coral health and thwarting diseases. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1755), 20122328. https://doi.org/10.1098/rspb.2012.2328

Krueger, T., Hawkins, T., Becker, S., Pontasch, S., Dove, S., Hoegh-Guldberg, O., Leggat, W., Fisher, P., & Davy, S. (2015). Differential coral bleaching—Contrasting the activity and response of enzymatic antioxidants in symbiotic partners under thermal stress. *Comparative Biochemistry and Physiology - Part A Molecular & Integrative Physiology*, *190*, 15–25. https://doi.org/10.1016/j.cbpa.2015.08.012

Leichter, J. J., Alldredge, A. L., Bernardi, G., Brooks, A. J., Carlson, C. A., Carpenter, R. C., Edmunds, P. J., Fewings, M. R., Hanson, K. M., Hench, J. L., Holbrook, S. J., Nelson, C. E., Schmitt, R. J., Toonen, R. J., Washburn, L., & Wyatt, A. S. J. (2013). Biological and physical interactions on a tropical island coral reef: Transport and retention processes on moorea, French Polynesia. *Oceanography*, *26*(3), 52–63. https://doi.org/10.5670/oceanog.2013.45

Lesser, M. P., Stochaj, W. R., Tapley, D. W., & Shick, J. M. (1990). Bleaching in coral reef anthozoans: Effects of irradiance, ultraviolet radiation, and temperature on the activities of protective enzymes against active oxygen. *Coral Reefs*, *8*(4), 225–232. https://doi.org/10.1007/BF00265015

Lough, J. M., & van Oppen, M. J. H. (2009). Introduction: Coral Bleaching — Patterns, Processes, Causes and Consequences. In M. J. H. van Oppen & J. M. Lough (Eds.), *Coral Bleaching: Patterns, Processes, Causes and Consequences* (pp. 1–5). Springer. https://doi.org/10.1007/978-3-540-69775-6_1

Maher, R. L., Schmeltzer, E. R., Meiling, S., McMinds, R., Ezzat, L., Shantz, A. A., Adam, T. C., Schmitt, R. J., Holbrook, S. J., Burkepile, D. E., & Vega Thurber, R. (2020). Coral Microbiomes Demonstrate Flexibility and Resilience Through a Reduction in Community Diversity Following a Thermal Stress Event. *Frontiers in Ecology and Evolution*, *8*. https://doi.org/10.3389/fevo.2020.555698

McGinty, E. S., Pieczonka, J., & Mydlarz, L. D. (2012). Variations in reactive oxygen release and antioxidant activity in multiple Symbiodinium types in response to elevated temperature. *Microbial Ecology*, *64*(4), 1000–1007. https://doi.org/10.1007/s00248-012-0085-z

Moberg, F., & Folke, C. (1999). Ecological goods and services of coral reef ecosystems. *Ecological Economics*, *29*(2), 215–233. https://doi.org/10.1016/S0921-8009(99)00009-9

Neave, M. J., Michell, C. T., Apprill, A., & Voolstra, C. R. (2017). *Endozoicomonas* genomes reveal functional adaptation and plasticity in bacterial strains symbiotically associated with diverse marine hosts. *Scientific Reports*, *7*(1), 40579. https://doi.org/10.1038/srep40579

Neave, M. J., Rachmawati, R., Xun, L., Michell, C. T., Bourne, D. G., Apprill, A., & Voolstra, C. R. (2017). Differential specificity between closely related corals and abundant *Endozoicomonas* endosymbionts across global scales. *The ISME Journal*, *11*(1), 186–200. https://doi.org/10.1038/ismej.2016.95

Nicholas Bokulich, Mike Robeson, Matthew Dillon, Michal Ziemski, Ben Kaehler, & Devon O'Rourke. (2021). *bokulich-lab/RESCRIPt: 2021.4.0.dev0*. Zenodo. https://doi.org/10.5281/zenodo.4629996

Nielsen, D. A., Petrou, K., & Gates, R. D. (2018). Coral bleaching from a single cell perspective. *The ISME Journal*, *12*(6), 1558–1567. https://doi.org/10.1038/s41396-018-0080-6

Pogoreutz, C., Rädecker, N., Cárdenas, A., Gärdes, A., Voolstra, C. R., & Wild, C. (2017). Sugar enrichment provides evidence for a role of nitrogen fixation in coral bleaching. *Global Change Biology*, *23*(9), 3838–3848. https://doi.org/10.1111/gcb.13695

Pollock, F. J., McMinds, R., Smith, S., Bourne, D. G., Willis, B. L., Medina, M., Thurber, R. V., & Zaneveld, J. R. (2018). Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. *Nature Communications*, *9*(1), 4921. https://doi.org/10.1038/s41467-018-07275-x

Pootakham, W., Mhuantong, W., Yoocha, T., Putchim, L., Jomchai, N., Sonthirod, C., Naktang, C., Kongkachana, W., & Tangphatsornruang, S. (2019). Heat-induced shift in coral microbiome reveals several members of the Rhodobacteraceae family as indicator species for thermal stress in Porites lutea. *MicrobiologyOpen*, *8*(12), e935. https://doi.org/10.1002/mbo3.935

Quigley, K. M., Willis, B. L., & Bay, L. K. (2017). Heritability of the Symbiodinium community in vertically- and horizontally-transmitting broadcast spawning corals. *Scientific Reports*, *7*(1), 8219. https://doi.org/10.1038/s41598-017-08179-4

Rosenberg, E., Koren, O., Reshef, L., Efrony, R., & Zilber-Rosenberg, I. (2007). The role of microorganisms in coral health, disease and evolution. *Nature Reviews Microbiology*, *5*(5), 355–362. https://doi.org/10.1038/nrmicro1635

Rosset, S., Wiedenmann, J., Reed, A., & D'Angelo, C. (2017). Phosphate deficiency promotes coral bleaching and is reflected by the ultrastructure of symbiotic

dinoflagellates. *Marine Pollution Bulletin*, *118*. https://doi.org/10.1016/j.marpolbul.2017.02.044

Röthig, T., Ochsenkühn, M. A., Roik, A., Merwe, R. van der, & Voolstra, C. R. (2016). Long-term salinity tolerance is accompanied by major restructuring of the coral bacterial microbiome. *Molecular Ecology*, *25*(6), 1308–1323. https://doi.org/10.1111/mec.13567

Sampayo, E. M., Ridgway, T., Bongaerts, P., & Hoegh-Guldberg, O. (2008). Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. *Proceedings of the National Academy of Sciences*, *105*(30), 10444–10449. https://doi.org/10.1073/pnas.0708049105

Smith, S. V. (1978). Coral-reef area and the contributions of reefs to processes and resources of the world's oceans. *Nature*, *273*(5659), 225–226. https://doi.org/10.1038/273225a0

Thompson, L. R., Sanders, J. G., McDonald, D., Amir, A., Ladau, J., Locey, K. J., Prill, R. J., Tripathi, A., Gibbons, S. M., Ackermann, G., Navas-Molina, J. A., Janssen, S., Kopylova, E., Vázquez-Baeza, Y., González, A., Morton, J. T., Mirarab, S., Zech Xu, Z., Jiang, L., … Knight, R. (2017). A communal catalogue reveals Earth's multiscale microbial diversity. *Nature*, *551*(7681), 457–463. https://doi.org/10.1038/nature24621

Vega-Thurber, R. L. V., Burkepile, D. E., Fuchs, C., Shantz, A. A., McMinds, R., & Zaneveld, J. R. (2014). Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. *Global Change Biology*, 20(2), 544–554. https://doi.org/10.1111/gcb.12450

Yamano, H., Sugihara, K., & Nomura, K. (2011). Rapid poleward range expansion of tropical reef corals in response to rising sea surface temperatures. *Geophysical Research Letters*, *38*(4). https://doi.org/10.1029/2010GL046474

Zaneveld, J. R., Burkepile, D. E., Shantz, A. A., Pritchard, C. E., McMinds, R., Payet, J. P., Welsh, R., Correa, A. M. S., Lemoine, N. P., Rosales, S., Fuchs, C., Maynard, J. A., & Thurber, R. V. (2016). Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. *Nature Communications*, *7*(1), 11833. https://doi.org/10.1038/ncomms11833

Zaneveld, J. R., McMinds, R., & Vega Thurber, R. (2017). Stress and stability: Applying the Anna Karenina principle to animal microbiomes. *Nature Microbiology*, *2*(9), 1–8. https://doi.org/10.1038/nmicrobiol.2017.121

Zhou, G., Cai, L., Yuan, T., Tian, R., Tong, H., Zhang, W., Jiang, L., Guo, M., Liu, S., Qian, P.-Y., & Huang, H. (2017). Microbiome dynamics in early life stages of the scleractinian coral Acropora gemmifera in response to elevated pCO2. *Environmental Microbiology*, *19*(8), 3342–3352. https://doi.org/10.1111/1462-2920.13840

Ziegler, M., Arif, C., Burt, J. A., Dobretsov, S., Roder, C., LaJeunesse, T. C., & Voolstra, C. R. (2017). Biogeography and molecular diversity of coral symbionts in the genus Symbiodinium around the Arabian Peninsula. *Journal of Biogeography*, *44*(3), 674–686. https://doi.org/10.1111/jbi.12913

Ziegler, M., Roik, A., Porter, A., Zubier, K., Mudarris, M. S., Ormond, R., & Voolstra, C. R. (2016). Coral microbial community dynamics in response to anthropogenic impacts near a major city in the central Red Sea. *Marine Pollution Bulletin*, *105*(2), 629–640. https://doi.org/10.1016/j.marpolbul.2015.12.045

Supplementary Materials:

Supplementary Material 1: Complete Data Analysis Pipeline used to annotate ASVs via Qiime2.

```
qiime tools import \
--type 'SampleData[SequencesWithQuality]' \
--input-path raw-reads/ \
--input-format CasavaOneEightSingleLanePerSampleDirFmt \
--output-path demux-single-end.qza

qiime demux summarize \
--i-data demux-single-end.qza \
--o-visualization demux.qzv

qiime dada2 denoise-single \
--i-demultiplexed-seqs demux-single-end.qza \
--p-trim-left 13 \
--p-trunc-len 195 \
--o-representative-sequences unfiltered-rep-seqs.qza \
--o-table unfiltered-table.qza \
--o-denoising-stats unfilter-denoise-stats.qza

qiime metadata tabulate \
--m-input-file unfilter-denoise-stats.qza \
--o-visualization unfilter-denoise-stats.qzv
```

```
qiime feature-table filter-samples \
--i-table unfiltered-table.qza \
--p-min-frequency 998 \
--o-filtered-table filtered-table.qza

qiime feature-table filter-seqs \
--i-data unfiltered-rep-seqs.qza \
--i-table filtered-table.qza \
--o-filtered-data filtered-rep-seqs.qza

qiime feature-table summarize \
--i-table filtered-table.qza \
--m-sample-metadata-file map_withmeta.tsv \
--o-visualization filtered-table.qzv

qiime feature-table tabulate-seqs \
--i-data filtered-rep-seqs.qza \
--o-visualization filtered-rep-seqs.qzv

qiime feature-classifier classify-sklearn \
--i-classifier silva-classifier.qza \
--i-reads filtered-rep-seqs.qza \
--o-classification taxonomy-silva.qza
```

qiime metadata tabulate

--m-input-file taxonomy-silva.qza \

--o-visualization taxonomy-silva.qzv

qiime taxa barplot \

--i-table filtered-table.qza \

--i-taxonomy taxonomy-silva.qza \

--m-metadata-file map_withmeta.tsv \

--o-visualization taxa-bar-plots-silva.qzv

Supplementary Material 2: Complete 16S rRNA gene amplification protocol used for all samples. This PCR protocol follows the Earth Microbiome Project (Thompson et al., 2017) with modifications.

Stage 1: 95°C for 5 minutes, 1 cycle

Stage 2: 94°C for 45 seconds then 50°C for 1 minute then 72°C for 1.5 minutes, 30 cycles

Stage 3: 72°C for 10 minutes then 4°C holding temperature, 1 cycle