UNIVERSITY OF CALIFORNIA SAN DIEGO

The Stability of the Porites Lobata Microbiome and the Local Adaptation of Its Competition

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Marine Biology

by

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2023

The Thesis of Sabrina Aileen Sauri is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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ABSTRACT OF THE THESIS

The Stability of the Porites Lobata Microbiome and the Local Adaptation of Its Competition

by

Sabrina Aileen Sauri

Master of Science in Marine Biology

University of California San Diego, 2023

Professor Linda Wegley Kelly, Chair

In recent years Hawaiian corals have been exposed to the increasing frequency and intensity of annual warming events, nutrient pollution and overfish which has negatively impacted these holobionts through bleaching, microbial infection, and direct competition. This study focuses on reefs at two sites in Maui, Olowalu and Wahikuli. The Wahikuli site experiences elevated nutrient runoff from the nearby Lahaina Wastewater Treatment Plant. Using rRNA gene biomarkers, this study shows that the microbial communities from partially

bleached *Porites lobata* have relatively similar compositions to their healthy counter parts regardless of site or proximity to turf overgrowth. Further analysis of the turf algae assemblages overgrowing the *P. lobata* colonies reveal a higher abundance of ammonia-oxidizing archaea from the family *Nitrosopumilaceae* in Wahikuli compared to Olowalu which indicate site-specific differences in the microbial community compositions. Nutrient pollution from land-based activities is a cause for concern because it causes shifts in the coral microbiome, reducing host fitness, while simultaneously stimulating the growth of macroalgal competitors. Combined with the increasing frequency and intensity of warming events, additional nutrient inputs diminish coral competition leading to succession from coral to algae dominated habitats.

INTRODUCTION

The Coral Microbiome

Corals maintain symbiotic relationships with a multitude of microbiota including Zooxanthellae (Symbiodiniaceae), fungi, protozoa, viruses, Bacteria and Archaea, which function together as a unit coined the coral holobiont (Knowlton & Rohwer, 2003; Rohwer et al., 2001, 2002). Each symbiont provides services to the coral host that maintain physiological processes, support the immune system, and contribute to nutrient cycling (McFall-Ngai et al., 2013; Wegley et al., 2007). Viruses, for example, contribute to the high diversity of the coral holobiont and provide the host with innate and specific immunity against pathogenic bacteria that interact with the coral surface mucus layer (Barr et al. 2013). Coral associated bacteria contribute to the coral immune system function by occupying niche space in the surface mucus layer (SML), tissues, and skeletal interface making it more difficult for opportunistic pathogenic species to invade the holobiont (Sweet et al., 2011; Glasl et al., 2016). Coral associated bacteria have also been found to produce antimicrobial metabolites, which provides the coral host with adaptive immunity against potentially pathogenic bacterial strains endemic to a specific location (Ritchie 2006; Karim et al., 2022; Raina et al., 2016). Although the benefits of the coral holobiont's microbial consortia are extensive, holobiont functioning is negatively impacted by external stressors (Muller et al., 2018).

Coral Bleaching and Infectious Diseases

Coral bleaching is a disease in which the coral host expel their symbiotic zooxanthellae (Muscatine 1990), resulting in a loss of their primary energy source and can become more susceptible to pathogenic infections and invasions by benthic competitors (Boilard et al, 2020).

This bleaching response is caused by a variety of factors such as disease, sedimentation, elevated UV radiation and pollution, but bleaching is most commonly associated with periods of increased seawater temperature referred to as marine heatwaves (Hughes et al., 2018; Lamb et al., 2014). The breakdown in the relationship between the coral host and its symbionts causes the coral to bleach, which alters the metabolic functions carried out by the microbiome (e.g., nutrient cycling), and significant decreases in metabolic activity. This loss in energy source is debilitating to most coral species when the coral is not able to obtain sufficient energy from its Symbiodiniaceae. For example, mucus production is reduced.

Coral mucus is vital for the cultivation of beneficial microbes, but also functions in trapping sediments and pathogens (Shnit-Orland and Kushmaro, 2009; Glasl et al., 2016). Further, the ability of the bacteria in the surface mucus layer to produce antimicrobial metabolites is reduced by marine heatwaves, which decreases the capacity of bacteria associated with a healthy microbiome to compete with pathogenic competitors to occupy niche space when seawater temperatures rise, making the coral more susceptible to coral disease (Krediet et al., 2013; Lokmer and Wegner, 2015; Maher et al., 2019). Coral disease has been detrimental to coral reefs by inducing coral tissue bleaching and causing drastic increases in corals' mortality rate (Pollack et al., 2011; Miller et al., 2009). This decline in coral density and diversity on reefs due to diseases such as white plague, black-band disease, and yellow band disease has been linked to architectural loss and consequently decreases in overall reef biodiversity and ecosystem functioning (Alvarez-Filip et al., 2009; Graham and Nash, 2013).

Competitive Interactions with Benthic Algal Holobionts

While the mode of infection for certain coral diseases is not well understood, coral competitors such as fleshy algae have been shown to promote the growth of opportunistic

pathogens and weaken the defenses of their coral neighbors against (Morrow et al., 2011; Vega Thurber et al., 2012; Pratte et al., 2018).

Tissue necrosis through disease or physical attacks (e.g., shading, abrasion, and allelochemicals) allow other benthic species vying for reef space and resources to reduced competition through weakened coral defense mechanisms (Slattery & Lesser, 2014). Once the coral is damaged, uninhibited growth of coral competitors is promoted by anthropogenic factors that lead to conditions that create a positive feedback loop of algal growth, such as increasing water temperature, decreasing ocean pH, and nutrient enrichment. As proposed in the DDAM model (DOM, disease, algae and microbes), algae produce DOM which promotes microbial growth, especially of pathogenic microbes that promote coral mortality (Barott & Rohwer 2012; Smith et al., 2006). Increased microbial respiration causes hypoxia, leading to coral and decreased coral recruitment, affecting coral resiliency and recovery on reefs following environmental disturbances (Hughes and Tanner, 2000).

Turf algae is an assemblage of filamentous algae, macroalgae, and cyanobacteria that hosts a distinct microbial community (Barott et al. 2011; Fricke et al. 2011). These assemblages are strong competitors on reefs due to their ability to inhibit coral growth, cause coral tissue necrosis through shading and abrasions, and induce hypoxia (Smith et al., 2006). Certain coral species, such as *Porites lobata* along the Great Barrier Reef, have the ability to successfully compete with algae by affecting their growth (McCook 2001). Other corals, such as *Cladocora caespitosa* in the Mediterranean Sea, exhibit lethal activity towards two specific invasive algae, *Lophocladia lallemandii* and *Caulerpa racemose*, stunting algal growth near their colonies (Kersting et al. 2014). However, this ecological dominance over algae is diminished when environmental conditions trigger coral bleaching.

Turf Algae Enrichment on Coral Reefs

The environmental conditions that negatively affect coral growth and resilience are the same conditions that allow algae to proliferate. The restricted ability of the coral to recover after bleaching combined with increased turf algae growth results in phase shifts from reef-building coral to fleshy algae dominated states. Increased seawater temperature and higher pCO2 has been found to increase the photosynthetic and epilithic growth rate of turf algae (Johnson et al. 2017). Even within turf assemblages, certain species of cyanobacteria, such as *Lynbya* on the Great Barrier Reef, become more abundant under conditions of higher temperatures and lower pH (Bender et al. 2014). Certain cyanobacteria produce toxins that reduce its palpability for herbivore communities, which is a major control of algal growth on reefs, and thus increases in abundance of turf associated cyanobacteria are a cause for concern. Nutrient enrichment in reefs is another factor that promotes algal growth. Turf algae found in the gut contents of *Stegastes nigricans* show that the microbiomes from nutrient enriched turf have higher alpha diversity and different microbial compositions compared to turf from unenriched areas (Degregori et al. 2021), meaning that the increase in rare microbes increases the ecological functions available for the holobiont to survive environmental disturbances (Coveley et al., 2015; Aanderud et al., 2015).

In this study, we measured how microbiome composition and diversity changes (i) across the coral-algae interface on partially bleached coral experiencing turf-algae overgrowth, (ii) between partially bleached and healthy coral colonies, (iii) and how the compositions change when one site is experiencing nutrient enrichment. The model coral species in this study is *Porites lobata*, which is known for its large bouldering morphology and relatively high resilience to marine heatwaves compared to other Hawaiian coral. Results suggest that the microbial community in *P. lobata* remains relatively constant in composition regardless of coral status

(healthy, bleached, partially bleached, turf overgrown) and exposure to nutrient runoff. The microbiome of the turf community, however, does experience a site-based change in community composition when in closer proximity to a source of nutrient pollution. This study highlights the bottom-up effect of localized anthropogenic nutrient enrichment on *P. lobata* microbiome and reveals implications of future regime changes as the occurrence of marine heat waves continue to rise.

CHAPTER 1

Materials and Methods

Location and sample collection

Porites lobata and turf algae samples were collected from two coral reef sites near Olowalu and Wahikuli at Maui, Hawai'i, USA. The site at Olowalu is located 0.23 miles offshore between 5 and 8 meters in depth. The site at Wahikuli is 0.10 miles offshore between 4 and 5 meters in depth. Both sites are on the lee-ward side of West Maui near the Lahaina Water Treatment facility (Figure 1). The reef site at Olowalu is farther from the treatment plant (6.56 miles) compared to Wahikuli (2.54 miles). Sites in Maui and around the Lahaina Water Treatment facility have been previously surveyed to identify areas and sources of anthropogenic nutrient enrichment (Dailer et al. 2010). Based on δ^{15}N values of surveys conducted around the world, background levels of nitrogen loading from natural sources was able to be determined and compared to values derived from areas near possible anthropogenic sources. The work done by Dailer et al. (2010) found that areas along the Maui coastline like Waiehu, Wahikuli and Maui Meadows have high δ^{15}N values which suggest leakages from sewage based sources such as septic tanks and cesspools.

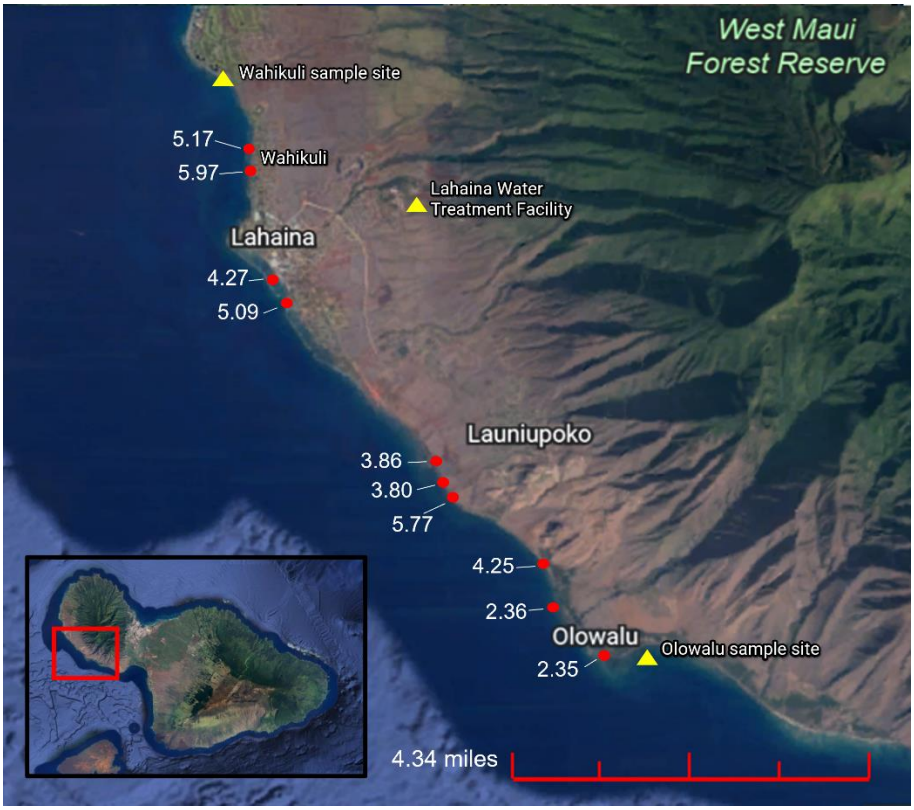


Figure 1. Map of Maui Sites. View of Western Maui with position of Lahaina Water Treatment Facility and off-shore Wahikuli and Olowalu sample sites. Red circles signify average δ15N values from intertidal survey to identify sources of nitrogen enrichment (Figure adapted from Dailer et al. 2010)

Tissue samples from the massive boulder coral, *P. lobata* were collected on SCUBA with ¼ inch diameter punches and immediately stored in RNALater (Life Technologies, Carlsbad, USA), transported on dry ice and stored at -20˚ C until nucleic acid extraction. Four partially bleached and four unbleached *P. lobata* colonies were sampled at Olowalu and three partially bleached and three unbleached colonies (i.e., not experiencing visible bleaching or algae overgrowth) were sampled at Wahikuli. The partially bleached punch samples were collected along a transect spanning across the interface of coral colonies being overgrown with turf algae (n=5 punches per colony, Figure 2B).

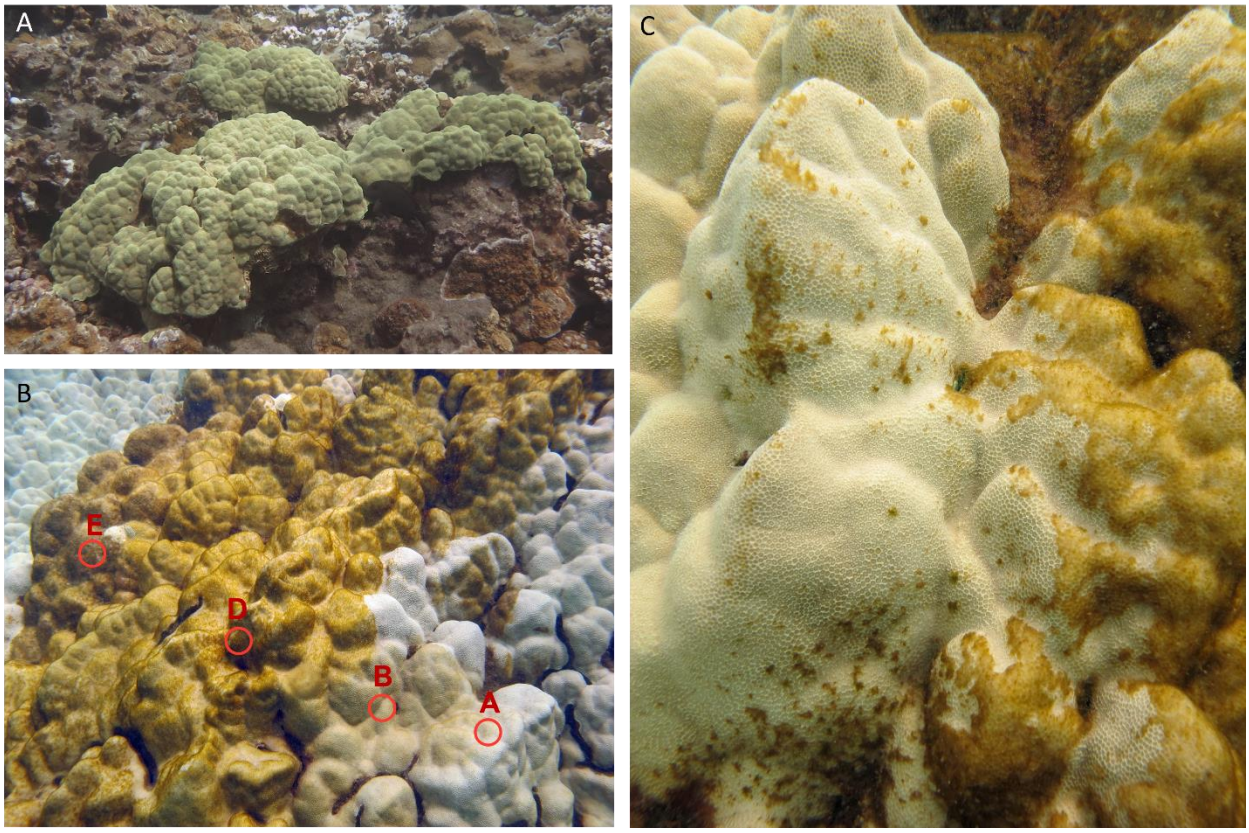


Figure 2. Photographs of Porites lobata colonies and sample design. Unbleached P. lobata colony (a). Partially bleached P. lobata colony and turf algae overgrowth (b). Red circles indicate positions of tissue punches taken in a transect A-E across the coral-algae interface. A & B samples on coral-side of the transect, C samples on interface, D & E samples on algae-side of the transect. Closer view of the coral-algae interface (c). Photographer: Darla White

Nucleic acid extractions

Coral tissue samples were extracted for nucleic acids at Scripps Institution of Oceanography following procedures from ZymoBIOMICS. First, the storage buffer was removed by centrifuging the tissue punches at 4°C at 6000rpm and decanting the supernatant. To homogenize the remaining tissue pellets and lyse microbial cell walls DNA/RNA Shield and ZR bashing beads were added to each sample then vortexed. The resulting supernatant was mixed with DNA/RNA lysis buffer then extracted using the ZymoBIOMICS DNA/RNA Miniprep kit

and protocol for downstream DNA analysis. Punch samples traversing the coral-algae interface were omitted during extractions.

16S and 18S amplicon sequencing

Extracted DNA amplicon sequencing was conducted at the University of Hawai'i at Mānoa Advanced Studies in Genomics, Proteomics and Bioinformatics facility amplifying the V4 region of the 16S rRNA gene using polymerase chain reaction following protocols of (Kozich et al., 2013) and the 515F and 806R primers recommended by (Caporaso et al., 2011; Parada et al., 2016; Apprill et al., 2015; and Walters et al. 2015). The V9 region of the 18S rRNA gene was amplified following the protocol of (Caporaso et al., 2012) and using the 1391f and EukBr primers recommended by (Amaral-Zettler et al., 2009); Stoek et al., 2010). The amplicons were created using dual index primers that include index sequences, Illumina spacers, Illumina adapters and 16S & 18S rRNA gene template region. Illumina MiSeq V3 600 paired-end cycle was used to pool and sequence the 16S amplicons (Arisdakessian et al., 2020) and Illumina MiSeq V9 150 paired-end cycle was used to pool and sequence the 18S amplicons (Caporaso et al., 2012). Subsequent sequences were merged and trimmed, then OTUs were defined as unique "amplicon sequence variants" by the dada2 R package (Callahan et al., 2016). Mothur (Schloss et al., 2009) aligned and annotated sequences using the Silva database (release 132; Quast et al., 2012). Operational Taxonomic Units (OTUs) were clustered at 97% sequence variance.

Data analysis

All statistical analyses were done in R Studio (Version 4.1.2) using the Tidyverse (Wickham 2022), randomForest (Breiman 2001), Vegan (Oksanen et al., 2022), wesanderson (Karthik et al., 2018), ape (Paradis et al. 2022), car (Fox and Weisberg, 2019), ggplot2 (Wickham et al., 2022), broom (Robinson et al., 2022) and lmPerm (Wheeler and Torchiano, 2016) packages; all code and data is available on Github (github.com/Ssauri/Olowalu). Microbial 16S and 18S rRNA gene data was imported into Rstudio and OTU reads were relativized to sample abundance (relative abundance: RA) and the 16S data filtered to only those which were abundant (defined as RA greater than 0.05 in at least one sample) which reduced the number of OTUs from 9058 to 700.

Z-score was calculated for the average relative abundance of each sample type (A, B, D, E, and Control) for both sites and used to create hierarchal clustering to visualize the difference in communities in a dendrogram heatmap for both 16S and 18S data sets.

To test which 16S OTUs were significantly different between geographic location and transect position, a two-way permutation ANOVA was run (aovp(RA ~ location*transect position; Wheeler and Torchiano, 2016)). All reported p-values were Benjamini-Hocheberg false-discovery rate corrected (Benjamini and Hochberg, 1995). Principal coordinate analysis was built using Bray Curtis dissimilarities and a PERMANOVA was used to determine if visual groupings of sample types were significantly different. Shannon sample diversity was calculated using the Vegan R package. A Mann-Whitney U test was used to compare OTU distributions of transect positions by site and difference in Shannon Diversity.

Results

Identification of Taxonomic Units in Tissue Samples

The dendrogram heatmap using z-scores calculated for the average of each sample type (A, B, E, D and Control) across locations used hierarchal clustering to identify outgroups in the data. For 18s sequencing data, the dendrogram group by site rather than transect position (D or E). In Olowalu the algal communities have more red algae from the order Gelidiales and unidentified order from the class Rhodymeniophycidae. The algal communities in Wahikuli however have a larger presence of red algae from the Ceramiales, Peyssoneliales, and Corallinales orders (Figure 3).

For the 16s OTU data, algae samples grouped together based on site and apart from coral samples. Z-score values of each 16s OTU per site and transect position reveal that partially bleached coral transect positions (A & B) and unbleached coral (Control) are dominated by Endozoicomonas (OTU 0006) from the Endozoicomonadaceae family ranging between 16-95% of the entire microbial composition in coral tissue samples across both sampling locations (Figure 4). There is also positive z-scores for coral transect position A and control coral samples for the Burkholderiaceae family while only transect B samples across both sites have higher abundances of microbes from the Vibrionaceae, Fusobacteriaceae, and Xenococcaceae families (Figure 4).

Statistical Analysis of 16s OTUs

The two-way Permutation ANOVA identified OTUs that were significant to specific sampling locations and transect positions as well as to determine differences between each group. A principal coordinate analysis (PCoA) was used to visualize how OTUs from the

ANOVA differ within each sample. Axis 1 describes OTU variability which separates coral samples (A, B, & Control) from the turf-algae samples (D & E). Axis 2 of the PCoA describes the OTU variability which separates turf-algae samples by the location they were collected from (Figure 5). PERMANOVA results confirm visual separation of coral and turf-algae samples (P-value = 0.0009, R^2= 0.406) as well as turf-algae samples by site (P-value = 0.0009, R^2= 0.196). Sequence data suggests no variation between partially bleached and unbleached coral, with no microbial clustering resulting from proximity to the coral-algae interface on the transect.

No significant difference (Mann Whitney U-test p-value < 0.05) in microbial compositions were found between the partially bleached coral transect positions (A & B) and Control samples (healthy coral). There was no significant difference in microbial composition across transect positions (A vs B vs D vs E vs Control). However, there are differences between OTU relative abundances in turf-algae samples by site. Wahikuli coral-turf (D, E) samples were found to have significantly higher abundance of Alphaproteobacteria, Deltaproteobacteria**,** Gammaproteobacteria and Nitrososphaeria. Turf samples taken from Olowalu contained significantly higher abundances of Tenacibaculum from the Bacterodia class (2.7%) while Wahikuli has more unclassified Cyclobacteriacea also from the Bacterodia class (0.2%) (Figure 6).

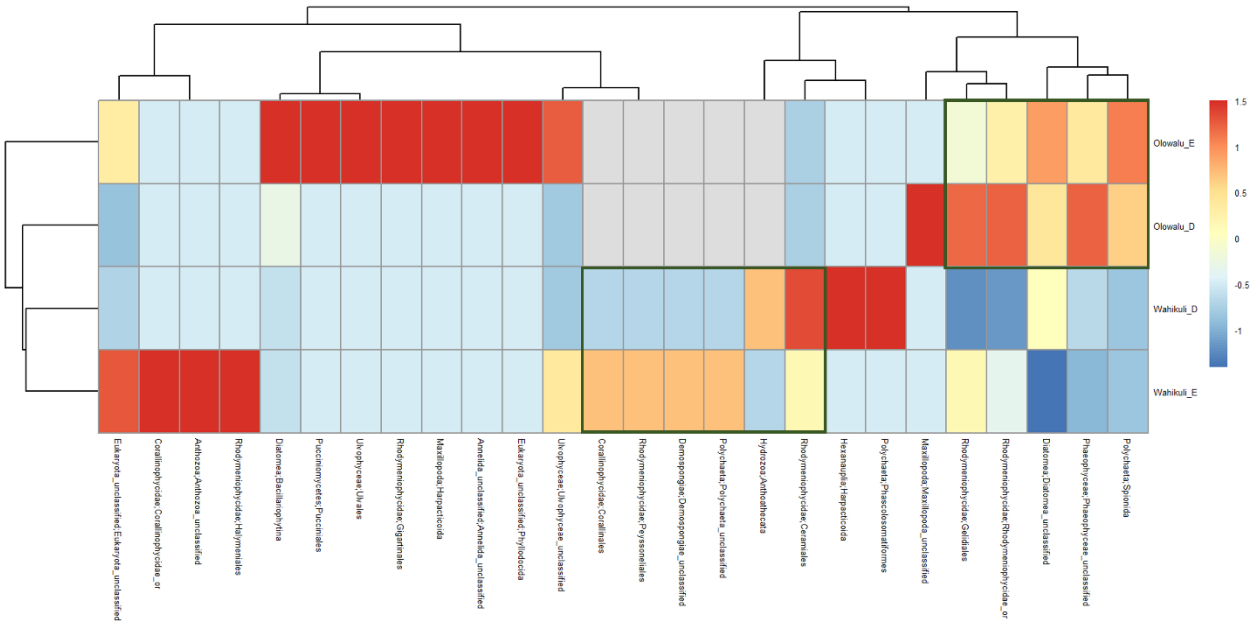


Figure 3. Dendrogram of 18s OTUs Clustered by Order and Transect Position by Site The dendrogram above shows the z-score values of 18s OTU data. Green boxes indicate differences in order clusters by site.

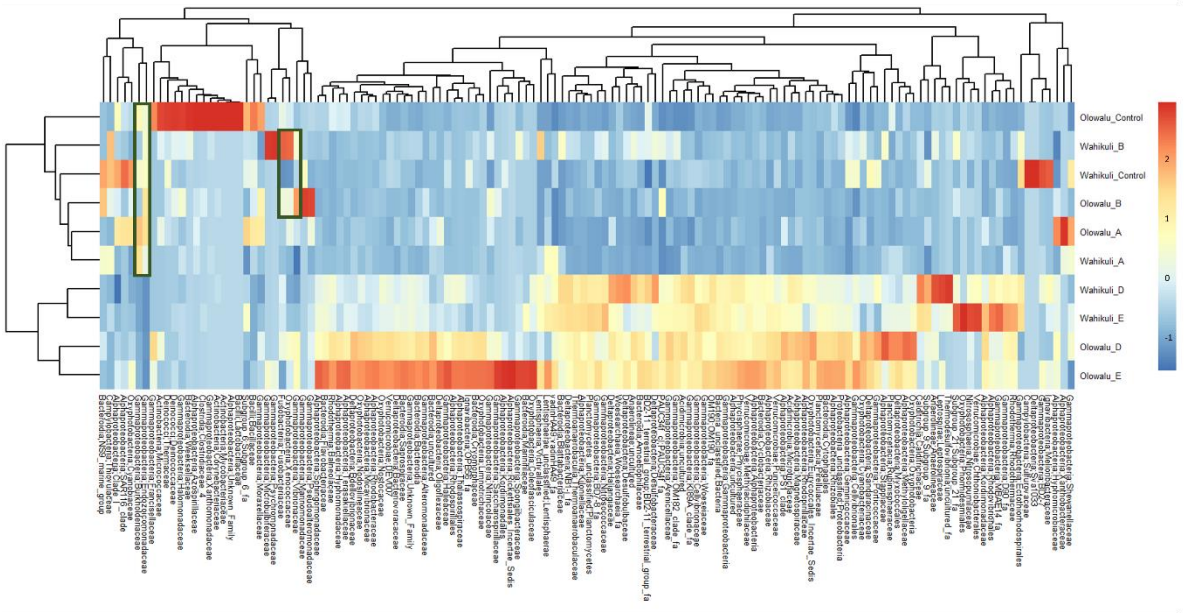


Figure 4. Dendrogram of 16S OTUs Clustered by Family and Transect Position by Site The dendrogram above shows the z-score values of the 16s OTU data filtered to only show class and families with a relative abundance of 0.5% or greater. Green boxes indicate differences in family clusters by transect position.

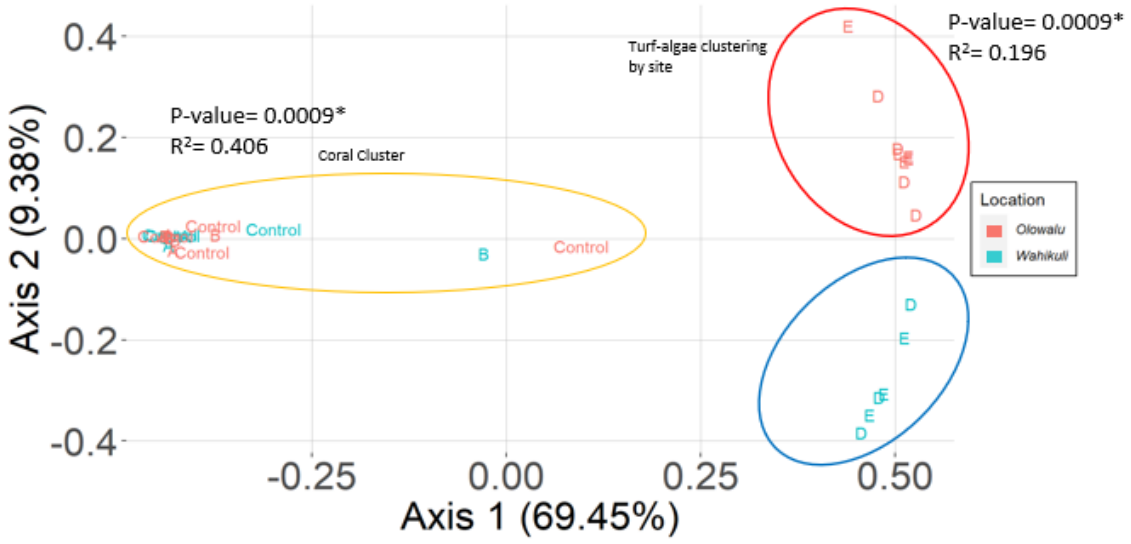


Figure 5. Principal Coordinate Analysis Visualization of variability in sample type (Transect position A-E and control) of OTUs after conducting a two-way permutation ANOVA. Axis 1 describes 69.45% of the OTU variability which separates coral samples (A, B, & Control) and turf-algae samples (D & E). Axis 2 describes 9.38% of the OTU variability which separates turf-algae samples by the location they were collected from. PERMANOVA results signify differential clustering of coral vs turf-algae (P-value < 0.005) and turf-algae sample by site.

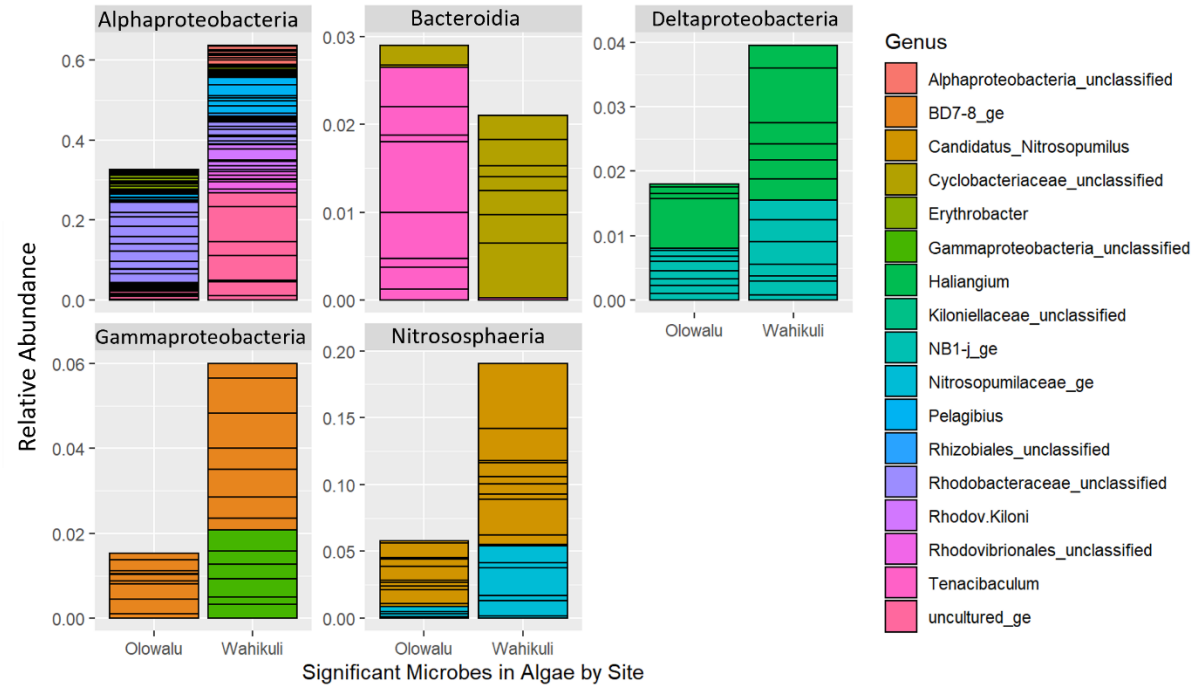


Figure 6. Relative Abundance of 16s OTUs in Turf-algae samples OTUs noted as significant (p-value < 0.05) from the two-way permutation ANOVA were run through a Mann-Whitney U test to determine how OTUs varied by site. Resulting OTUs from Mann-Whitney U test are grouped by class. False discovery rate corrections were applied (FDR) then filtered out any OTU with p-values < 0.05. Black lines signify various OTUs within Genus.

Diversity and Evenness

Evenness was calculated and plotted against Shannon diversity scores for each sample. Turf-algae samples (D & E) have highest Shannon diversity and Evenness followed by unbleached coral without turf overgrowth (Control) then bleached coral samples (A & B) follow last with the lowest evenness and microbial diversity (Figure 7).

Shannon diversity and Evenness is not different (Mann Whitney U-test p-value < 0.05) when comparing corresponding transect positions and control samples between both sites. Comparisons of diversity in transect positions within a single site (ANOVA p-value < 0.05) indicate that Olowalu transect positions A & B and control samples (unbleached coral) have lower diversity values compared to transect positions D & E (Figure 8). In Wahikuli there is a

similar trend of high Shannon diversity in turf-algae samples compared to coral samples however only transect position A has significantly lower (ANOVA p-value < 0.05) Shannon diversity values (Figure 8).

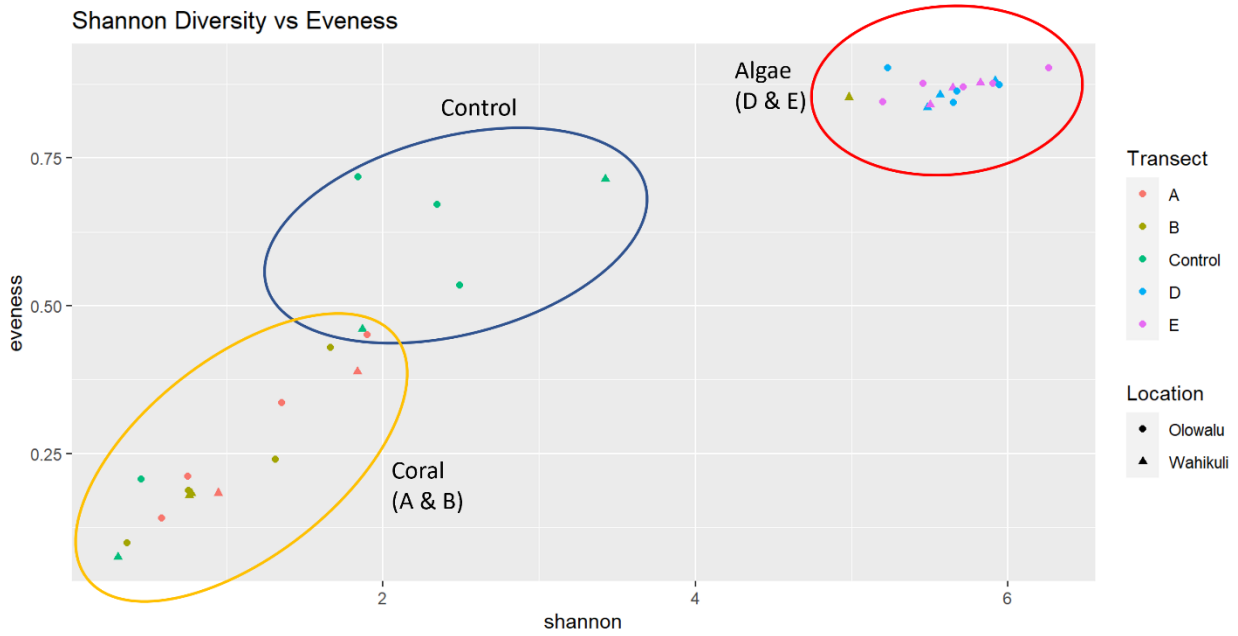
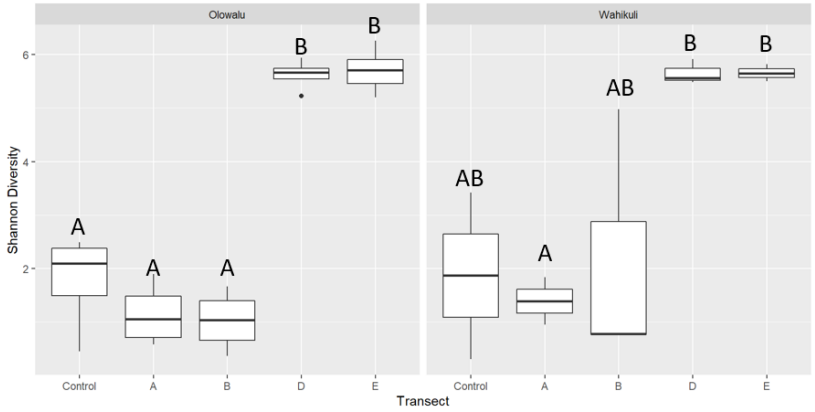


Figure 7. Comparison of Shannon Diversity & Evenness Scores of evenness are plotted against Shannon diversity scores for each sample. Each circle represents main grouping of turf-algae (D & E), control coral, and bleached coral (A & B).



	Wahikuli	Olowalu
Coral A-Control	0.995	0.589
Coral B- Control	0.998	0.427
Coral A- Coral B	0.968	0.998
Algae D- Control	0.051	1.897e-06
Algae E- Control	0.051	1.704e-06
Algae D- Algae E	1.000	0.999
Coral A- Algae E	0.049	2.36e-07
Coral B- Algae D	0.075	1.81e-07
Coral B- Algae E	0.075	1.65e-07
Coral A- Algae D	0.049	2.59e-07

Figure 8. Boxplot of Shannon Diversity of Sample Type Shannon diversity of each position on transect and controls is grouped by location. Letters above boxplots indicate similarity of Shannon diversity based on results of Post-hoc test based on P-values from ANOVA analysis (p-value < 0.05).

CHAPTER 2

Discussion

Microbiome of punches across transect

There are various conditions that elicit dynamic shifts in the coral microbiome, namely changes in the local environment that facilitate growth of interspecific competition. Our results reveal there is a higher-than-average presence of Vibrionaceae, Fusobacteriaceae, Xenococcocaceae in coral punches taken closest to the coral-algal interface (Figure 4). These taxons have been previously noted to pathogenic species or associated with cyanobacterial mats, however further analysis reveals that there are no specific OTU signals or significant differences in OTU relative abundance that indicate a change in microbiome across the sampling transect. This demonstrates that the microbial composition of *P. lobata* does not undergo significant changes when in close proximity with turf algae. The only significant difference in composition across the transect is between coral and algae tissue samples (Figure 4). These results are contrary to the findings of other studies which found that the microbiome of massive Porites spp. did change during the 2016 El Nino to be more similar to the microbiome of turf algae within 5cm of the coral-algae interface (Pratte et al., 2018). Other scleractinian coral such as Pocillopora species were also found to have higher relative abundances of Saprospiraceae, Rhodobacteraceae, and Alteromonadaceae in tissue near algae overgrowth (Lu et al., 2022). It is possible that we did not see this major shift in the microbial communities of our Porites samples because environmental conditions were not extreme enough to observe the same changes as Pratte et al. (2018) and because massive *P. lobata* colonies have a resilient nature that allow them to appear ultimately untouched when exposed to conditions that would normally cause damage or mortality in other coral species (Marcelino et al., 2017).

Microbiome composition between healthy and partially bleached coral

Environmental stressors such as marine heatwaves or decreases in ocean pH, cause the coral microbial community to undergo taxonomic shifts that can reduce host fitness (Bourne et al., 2008). However, when examining the microbial composition between healthy and partially bleached coral we found little difference between healthy and partially bleached groups (Figure 4, 5). This finding is similar to Hadaidi et al. (2017) who found that the microbiomes in the SML of *P. lobata* colonies in the Red Sea and Persian Arabian Gulf did not change whether or not the colony experienced bleaching. Consequently, other research has found the microbiome within the coral tissue to change between bleached and unbleached colonies, usually with an increase in Vibrio spp. in bleached tissue (Bourne et al., 2008), which was a trend we did observe in coral tissue nearest to the coral-algae interface but was not statistically supported (Figure 4). A possible reason for the lack of differentiation between bleaching groups is that the microbiome of the *P. lobata* samples in this study are mainly comprised of the gammaproteobacterial, Endozoicomonas which form large aggregates near the Symbiodiniaceae within the coral tissue. Other studies have found that certain coral species remain maintain their high abundance of Endozoicomonas whether or not the colony was experiencing bleaching (Pogoreutz et al., 2018; Gardener et al., 2019). It is thought that some Endozoicomonas species are able to dominate the microbiome after environmental stresses or marine heatwaves because they are able to metabolize DMSP, a chemical upregulated by coral during oxidative stresses, to promote growth (Broadbent and Jones, 2004; Deschaseaux et al., 2014; Tandon et al., 2020). The ability of Endozoicomonas to survive and continue nutrient cycling in the holobiont may be contributing to the stability of the coral microbiome and the resilience of the coral hosts during bleaching events.

Microbiome of turf-algae between sites

As we know from previous work (Quinlan et al., 2018; Nelson et al., 2014; Hass et al., 2016; Wear et al., 2015; Wegley Kelly et al., 2022) large differences in benthic megafauna will result in differences between organic compounds produced which will have variable downstream impacts on the microbial community. While there is a difference in red algae found between each sampling location, there is still high variability of the algal communities in each sample (Figure 3). The composition of the turf algae microbiome is significantly different between sample sites with a notably higher abundance of ammonia-oxidizing species in Wahikuli. The enrichment of these taxa in Wahikuli may be due to a few reasons. Because ammonia-oxidizers are believed to be geographically dependent rather than host specific, it is likely that the nutrient regime in Wahikuli is enriched in ammonia due to run-off by the nearby Lahaina Water Treatment Facility (Siboni et al., 2012). The heightened levels of nitrogen in the water could be driving the growth of certain microbial species in the turf algae (Kelly et al., 2014). Another possibility is that the ammonia oxidizers themselves are being leaked into the nearby reefs because of run off from the Lahaina Water Treatment Facility. Ammonia oxidizing bacteria (AOB) are used in wastewater treatment facilities to convert the ammonia to nitrite and remove nitrogen from the water (Radniecki and Lauchnor, 2011).

Conclusions

The reef building coral *Porites lobata* is a coral that is known for its large bouldering formations and resilience to bleaching during marine heatwaves. There are several theories for this resilience to environmental changes however the high abundance of Endozoicomonas in all coral samples regardless of bleaching status along with the overall lack of variability in the microbiome between visually afflicted and unafflicted coral colonies suggest that there are unknown interactions occurring between bacterial symbionts and the Symbiodiniaceae, abiotic environmental cues, and even the coral host itself that contribute to maintaining the physiological processes that are normally disrupted during bleaching events.

On the other hand, the microbiome of turf algae overgrowing the coral colonies in this study reveal community responses to local conditions, which further support the idea that certain reef associated microbes are dependent on nutrient regimes rather than their hosts. While the scope of this study does not investigate exactly what conditions are leading to these changes in the turf microbiome, the δN^{15} values reported in Dailer et al. (2010) indicate a trend of higher δN^{15} in sites closer to Wahikuli rather than Olowalu which may be due to the land-based runoff from local tourism and proximity to the Lahaina Water Treatment facility. In any case, the presence of certain members in the microbial community of turf-algae assemblages are indicators of the abiotic conditions affecting the local reefs especially because of how variable these communities tend to be especially when compared to the microbiome of *P. lobata*.

Further testing with consideration for the degree of bleaching experienced by the tissue could help distinguish more subtle variations in the coral microbiome and possibly even allow us to explore how the functions of the holobiont change. Understanding the interactions of the

microbial communities in coral reefs could be used in future monitoring systems to evaluate the health of the reefs.

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