Impact of parrotfish predation on coral health: changes in microbiome and pathogen defense

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Impact of parrotfish predation on coral health: changes in microbiome and pathogen defense

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Table of Contents

Page

Abstract …… 4

Introduction …… 5

Literature Review ……6

Methods ……9

Results ……12

Discussion …… 16

Conclusion ……17

Acknowledgements ……18

References ……19

Appendix ……24

Abstract

Coral reefs are in rapid decline, and it is imperative to study reef community interactions in order to mitigate and reverse this trajectory. This study explores the relationship between corals and parrotfish, investigating how parrotfish bites on coral impact the composition of the coral's microbiome and the corals suppression of a common bacterial pathogen. Fragments of *Porites lobata* coral colonies that were heavily predated by parrotfish or that showed no signs of parrotfish predation were shaken in seawater, and this seawater was bioassayed against the common coral pathogen *Vibrio coralliilyticus* to assess the effects of previous predation on the coral's ability to suppress this ecologically relevant pathogen. Additionally, we sequenced the 16S rRNA gene from each coral sample to investigate possible alterations of the coral's microbiome due to predation. Neither alpha diversity nor beta diversity of the microbiome was impacted by parrotfish predation. However, some bacteria were differentially abundant, such as those of the genus *Endozoicomonas*. Bioassays of water in which coral fragments were agitated detected no impact of previous parrotfish attack on the coral's suppression of the pathogen *Vibrio coralliilyticus*. Overall, this speaks to the resistance and strength that corals demonstrate in the face of parrotfish predation.

Introduction

Coral reefs are rapidly declining worldwide. Major factors associated with their decline are increasing ocean temperatures and disease outbreaks (Harvell et al., 2007; Hughes et al., 2017). Both of these factors are linked, as increased temperatures induce heightened pathogen virulence and can lead to opportunistic pathogen takeover (Harvell et al., 2007). Corals also have constituents of their microbiome that appear to suppress pathogenic microbes (Ritchie, 2006). Dependent on the environment, the potency of these protective microbes or the potency of protective compounds produced by the coral or its associated microbes can fluctuate (Beatty et al., 2019). A healthy microbiome may be critical to coral survival, and ultimately, reef health (Barott and Rohwer 2012, Krediet et al. 2013).

Parrotfish play an important role in regulating algae that compete with corals within the reef community (Mumby and Steneck, 2008; Shantz et al., 2020). However, some species of parrotfishes also bite into and consume adult corals; such feeding can shift the microbial communities of the corals they bite (Ezzat et al., 2020). Currently, it is unknown how parrotfish predation impacts the antipathogen capacity of the coral holobiont on which they feed. In this study, we investigated the antipathogen properties of water collected from fragments of 15 corals heavily predated by parrotfish, as compared to 15 healthy, non-predated coral colonies of the same species. Additionally, sequencing of the 16S rRNA gene from each coral sample was performed to allow a comparative assessment of coral microbiomes near sites of parrotfish scars and unbitten corals.

Literature Review

Coral reefs are in rapid decline, producing a noticeable shift from coral dominated to algal dominated reef communities (Mumby & Steneck, 2008). Drivers of this decline include overfishing of herbivorous fishes, increasing water temperatures, coral disease, corallivore outbreaks, nutrient pollution, increasing turbidity and sedimentation, and potentially other factors (De'ath, et al, 2012; Harvell et al., 2007; Hughes et al., 2020; Hughes et al., 2003; Pandolfi et al., 2003; Mumby & Steneck, 2008). Research exploring the best options for local reef management to protect reefs suggests the implementation of no-take-areas (NTAs), although this solution is not perfect as their effectiveness is limited (Hughes et al., 2003; Hughes, et al., 2010; Mumby & Steneck, 2008). It is evident that without investigation into the underlying causes of coral disease and death, coral reef management will not be maximally effective.

Impacts of parrotfish on the reef

Parrotfish play an important role in regulating algae that compete with corals within the reef community (Mumby and Steneck 2008, Shantz et al., 2020). In addition to consuming macroalgae, they are corallivores, directly impacting coral recruitment and reef growth (Mumby et al., 2007). In a study by Mumby et al. (2007), coral recruitment increased parrotfish density, demonstrating the net beneficial role that parrotfish serve in aiding coral and controlling macroalgal growth (2007). However, some species of parrotfishes also bite into and consume adult corals; such feeding can shift the microbial communities of the corals they bite (Ezzat et al., 2020).

Coral-associated antibiotic properties

Some corals contain antibiotic compounds thought to regulate the coral's microbial community (Ritchie, 2006). In an experiment conducted by Ritchie (2006), coral mucus was

found to lose its antibiotic properties after the reef experienced a temperature induced bleaching event. This is notable, as it suggests that the capacity for corals to protect themselves is impared when under stress.

In similar work, Beatty et al. (2019) found that antibacterial properties for *Acropora millepora* differed when the corals were found in healthy, coral-dominated reefs (marine protected areas) as opposed to fished macroalgae-dominated reefs. Washes collected from *A. millepora* on healthy reefs were found to be 75%-154% more effective at suppressing pathogen growth of a common coral pathogen, *Vibrio coralliilyticus,* than washes collected from *A. millepora* located in a macroalgae-dominated reef (Beatty et al., 2019). From both of these studies, it is evident that coral-associated antibacterial compounds may regulate coral microbiomes and susceptibility to coral pathogens, thus directly affecting the heath and resilience of the reef community.

Coral microbiome composition

Coral microbiome composition is directly linked to colony health and has been found to fluctuate with environmental conditions and disease state (Bourne et al., 2016, Bourne et al., 2008; Glasl et al., 2016; Thurber et al., 2012; Thurber et al., 2009). In work conducted by Glasl et al., (2016), aging mucus was collected from corals through time allowing for comparison between new and aged mucus, as well as surrounding sediment. The older mucus contained a bacterial community representative of a combination of the microbial communities from the new mucus, as well as the sediment. As the coral secreted new mucus, the microbial population was effectively reset.

Additionally, in work conducted by Thurber et al. (2012), microbial variance of sampled corals increased due to macroalgal contact. In the case of increased nutrient load, dissolved

organic carbon, temperature and acidified sea water, coral microbiomes also fluctuated (Thurber et al., 2009). As demonstrated by this previous research, a coral's health can be closely linked to its microbial composition. Thus, when studying potential stressors for corals, it is imperative to investigate potential changes to the microbial community.

Summary

Coral reefs are complex ecological systems experiencing global decline due to many factors, such as disease outbreaks, increased ocean temperatures and overfishing leading to macro-algal takeover (Harvell et al., 2007; Hughes et al., 2017; Jackson et al., 2001) . In order to slow or halt reef deterioration, it is important to understand how factors influencing coral health impact these threatened ecosystems. Specifically, we wish to explore the effect that parrotfish predation has on the coral's microbiome, potentially though the spread of pathogens, as well as the impact that predation has on the coral's capacity to chemically defend itself.

Methods

Coral selection

Thirty *Porites lobata* colonies (15 with clear signs of predation by parrotfish and 15 unbitten, healthy controls) were randomly selected for DNA sequencing and antipathogen bioassays within the back reef of Moorea, French Polynesia. From each colony we collected small chips of coral tissue (~2 ml volume) of the bitten area on attacked colonies and from similar (but unbitten) locations on control colonies lacking signs of parrotfish damage. For samples used to assess the coral microbiome, these small coral fragments were collected from each colony, immediately immersed in the nucleic acid preservative RNAlater, placed on ice in the field, and frozen at -20°C upon return to shore. Procedures for samples used to assess antipathogen activity are described below.

DNA extraction and 16S rRNA gene sequencing

DNA extractions were performed using Qiagen's DNeasy PowerSoil Pro Kit, adding approximately 250mg of coral for each extraction. All steps for DNA extractions were conducted according to the manufacturer's guidelines. Following DNA extraction, a two-step PCR was performed. The first PCR amplified the 16S rRNA gene using modified 16S rRNA gene primers 16S-515FB (5′- GTGYCAGCMGCCGCGGTAA-3′) and 16S-806RB (5′-GGACTACNVGGGTWTCTAAT-3′) (Caporaso et al, 2011). The second PCR appended dual indices to each sample. PCR cycling conditions were as follows: initial denaturation at 95°C (3 min) followed by 30 cycles of denaturation also at 95°C (30s), annealing of primers at 62°C (30s), and then primer extension at 72°C (30s). Lastly, final extension at 72'C (4 min). Samples were sequenced using Illumina MiSeq.

Antipathogen bioassays

Using methods similar to Beatty et al. (2019), ~2 mL of coral fragments were taken from the perimeter line of the parrotfish bite, or control coral surface, and placed in 5 mL tubes, displaced in a 1:1 ratio with seawater and shaken for 20 seconds. The resulting solution, hereafter called "coral water", was decanted into a secondary 5 mL tube, and contains all chemicals from internal and external tissues released by the coral holobiont. Paired seawater was also collected within 1m of the coral water collection site. This seawater served to control for any anti-pathogen activity in the seawater that was not associated with the coral. All samples were stored on ice in the field, immediately frozen upon return to shore, and remained frozen until processed for antipathogen activity.

To assay for anti-pathogen activity, coral water samples were thawed over ice and 100 µL of coral water and paired seawater were aliquoted separately into a 96 well plate and then lyophilized overnight. Following lyophilization, plates were UV radiated for 90 seconds to kill microbes or viruses that may have survived lyophilization (Vaidya et al. 2018). 100 µL of *Vibrio coralliilyticus* suspended in marine broth (Zobell) at a concentration of 100 cells/mL was added to each well. 100 µL of marine broth was added to blank wells and 20 µL of tetrazolium chloride (TTC) (0.3mg/mL) was added to all wells. The addition of tetrazolium chloride allows for visualization of bacterial growth as the compound is taken in by bacteria and metabolized, causing the solution to turn pink. Optical density readings at 490 nm wavelength are indicative of bacterial growth. Plates were incubated at 28°C for 16 and 24 hours, at which time plate optical density readings were taken (490 nm wavelength).

Data analysis

16S sequencing data were analyzed using DADA2 within QIIME2 (Bolyen et al., 2018; Callahan et al., 2016). For taxonomic assignments, comparisons were conducted against the

SILVA ribosomal RNA database (Quast et al., 2012). RStudio was used to perform statistics for alpha (t-test and Wilcoxon signed-rank test) and beta (permanova using distance matrixes) diversity analysis, as well as differential species abundance using DEseq2 (RStudio Team., 2020; Love et al., 2014). Coral water data was also analyzed within RStudio (Wilcoxon signed-rank test), using the package Coin and graphs were generated using ggplot2 (RStudio Team., 2020; Hothorn et al., 2006; Wickham, 2016).

Results

16S rRNA gene sequencing

There were no significant differences in alpha diversity, using multiple metrics (figure 1). Additionally, investigation into beta diversity also revealed no differences in the microbial taxonomic composition between samples (figure 2). However, some bacteria sequence variants were found to be differentially present within corals predated by parrotfish versus those serving as healthy controls.

Figure 1 shows the three different alpha diversity metrics of Chao1, Shannon, and Simpson. There were no differences between the parrotfish treatment and healthy control corals (t-test for Chao1, p = 0.623; Wilcoxon signed-rank test used for Shannon and Simpson with p = 0.984 and p = 0.886, respectively).

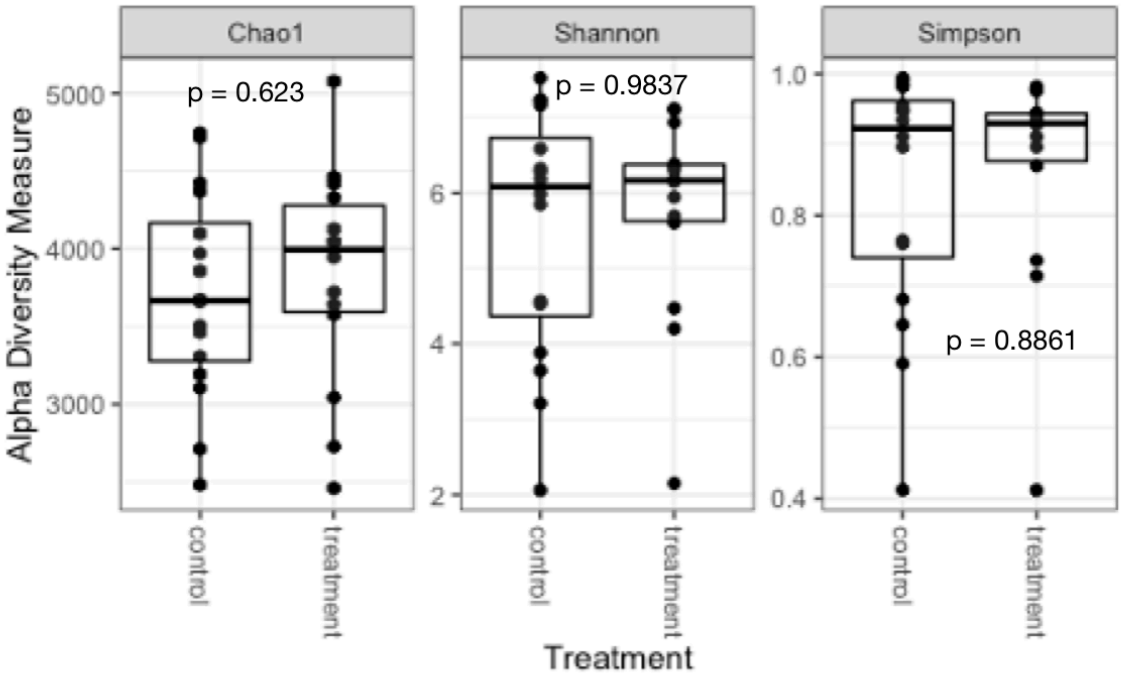

Figure 1. Chao1, Shannon and Simpson alpha diversity metrics are shown above. The measures for treatment and control samples are plotted for each metric, showing no differences in alpha diversity between parrotfish treatment and healthy control.

Metrics were calculated using Phyloseq within RStudio. (t-test used for Chao1, Wilcoxon signed-rank test used for Shannon and Simpson data, p = 0.623, p = 0.9837 and p = 0.8861 respectively)

Beta diversity was also analyzed using a Bray Curtis distance matrix. A PERMANOVA indicated no significant difference in microbial community composition (p = 0.680), as can be visualized using non-metric multidimensional scaling (figure 2).

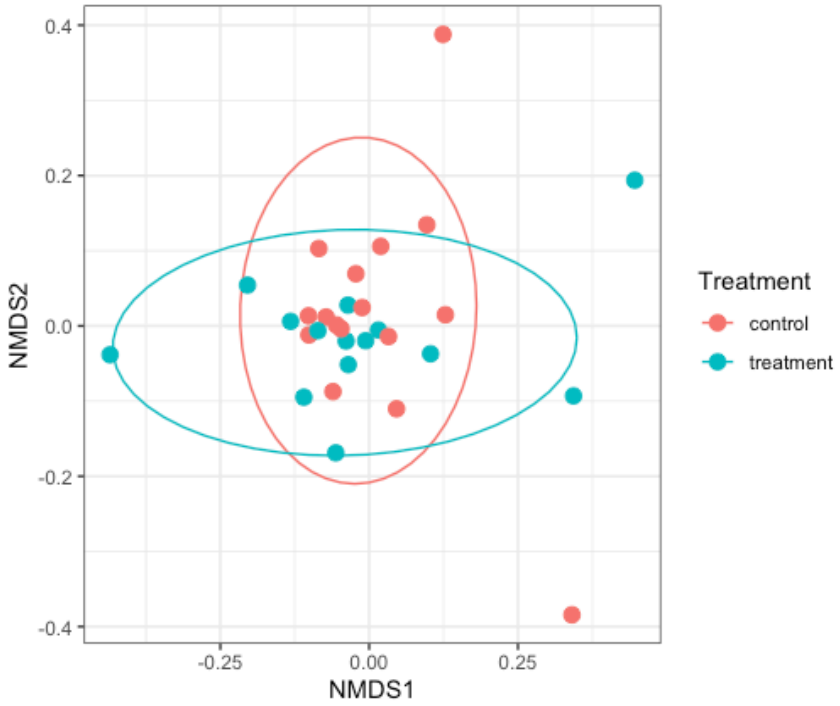

Figure 2. Beta diversity visualization performed using non-metric multidimensional scaling conducted within RStudio. Using a square root transformed sequence variant table, PERMANOVA indicated no differences in beta diversity between parrotfish predated treatment corals and healthy controls. (PERMANOVA using Bray Curtis distance matrix, p = 0.6796, see appendix 1 for PERMANOVA table).

In addition to alpha and beta diversity analysis, differential sequence variant analysis was performed using DESeq2, revealing that certain bacterial variants were differentially abundant in corals heavily predated by parrotfish versus their healthy controls as shown in (table 1).

log2FoldChange	pvalue	padj	kingdom	phylum	class	order	Family	genus
-23.94889961	1.88E-17	3.23E-13	d__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Anaerolineales	f__Anaerolineaceae	g__uncultured
-23.49829677	4.67E-17	3.23E-13	d__Bacteria	p__Chloroflexi	c__Anaerolineae	o__uncultured	f__uncultured	g__uncultured
-23.41501781	3.59E-17	3.23E-13	d__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Thiomicrospirales	f__Thiomicrospiraceae	g__endosymbionts
-22.29607217	3.71E-14	1.1E-10	d__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Anaerolineales	f__Anaerolineaceae	g__RBG-16-58-14
-22.1606882	5.31E-14	1.25E-10	d__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Oceanospirillales	f__Endozoicomonadaceae	g__Endozoicomonas
21.3881742	3.45E-13	7.16E-10	d__Bacteria	p__Chloroflexi	c__Anaerolineae	o__Anaerolineales	f__Anaerolineaceae	g__uncultured
22.09793276	5.42E-14	1.25E-10	d__Bacteria	p__Chloroflexi	c__Anaerolineae	o__SBR1031	f__SBR1031	g__SBR1031
22.48669341	1.95E-14	6.74E-11	d__Bacteria	p__Chloroflexi	c__Anaerolineae	o__SBR1031	f__SBR1031	g__SBR1031
22.61151636	1.4E-14	5.79E-11	d__Bacteria	p__Proteobacteria	c__Gammaproteobacteria	o__Burkholderiales	f__SC-I-84	g__SC-I-84
22.68741351	1.14E-14	5.79E-11	d__Bacteria	p__Chloroflexi	c__Anaerolineae	o__SBR1031	f__SBR1031	g__SBR1031

Table1. Use of DEseq2 allowed for bacterial sequence variants to be identified as differentially abundant between treatment and control groups. Sequence variants found in blue are more prominent within healthy control corals, while those in red are more abundant in parrotfish predated treatment corals (see p-adjusted and log fold change columns).

Coral water

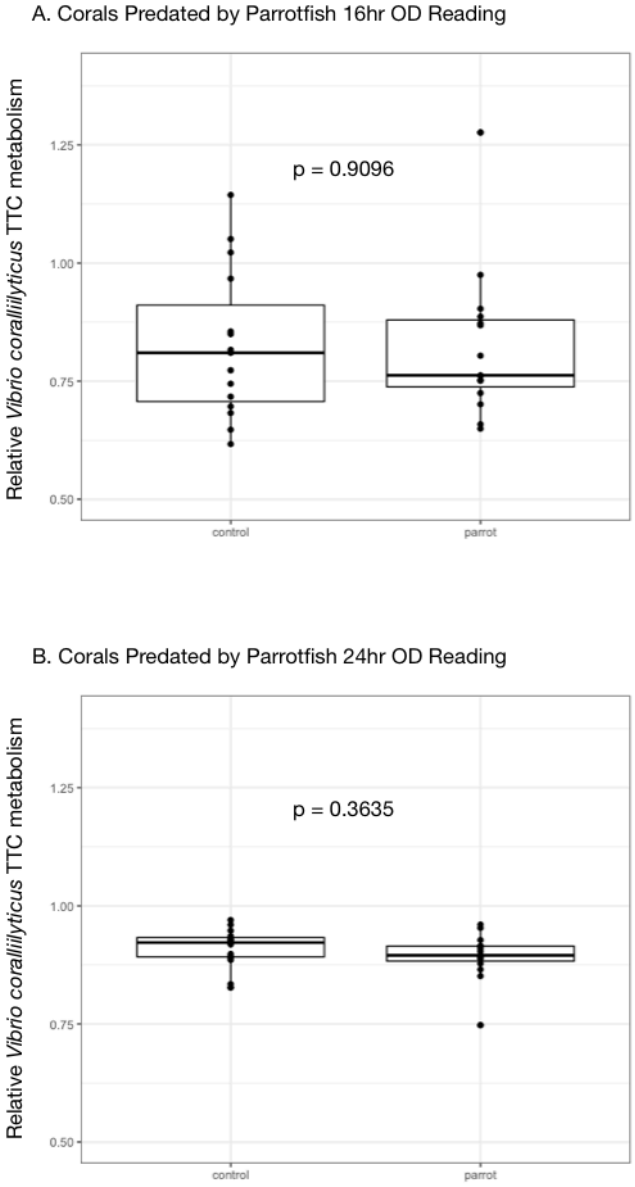

Figure 3A and 3B. Coral water collected from parrotfish bite locations vs. healthy coral tissue demonstrates no significant differences in the ability to suppress the common coral pathogen *Vibrio coralliilyticus* at either 16 or 24 h (p=0.910 and 0.364, respectively; Wilcoxon signed-rank test, n = 15)

Coral water collected from bitten areas of predated corals versus healthy areas of unbitten corals did not differ in potency against the coral pathogen *Vibrio coralliilyticus* (figure 1A&B).

Discussion

Overall, these findings were surprising, as we expected to see a change in chemical suppression, as well as differences in alpha diversity between predated and control samples (Ezzat et al., 2020). However, we observed no differences in alpha and beta diversity, as well as no differences in pathogen suppression. In the interest of the coral, this is beneficial and indicative that the coral's defenses are not dramatically hindered by parrotfish predation on the reef.

There were, however, specific bacterial 16S rRNA gene sequence variants that were found to be differentially abundant between the control and treatment samples. Most fascinating are variants identified to the genus *Endozoicomona*, which are known to be abundant in healthy corals for their role in metabolizing dimethylsulfoniopropionate (Tandon et al., 2020) and are found to be dominant within our healthy control samples. Additionally, variants identified to the order Burkholderiales were more abundant within the predated corals. The family, genus or species of our variant was uncertain, but other bacteria within this order are pathogenic (Voronina et al., 2018).

There are limitations to our experimental approach. Parrotfish bites were identified, with no known timeline as to when they were inflicted, introducing potential variability toward potency of coral water collected, as well as potential for microbiome communities to shift with time between bites and sampling. Additionally, the sample size was small (n=15) and in the future more robust sampling would be beneficial in gathering a larger picture for parrotfish dynamics on the reef.

Conclusion

As suggested by the findings of this study, the coral *Porites lobata* may be relatively resilient to parrotfish bites, proving themselves resistant to dramatic shifts in microbial community composition and the capacity to fight pathogenic bacteria (maintaining constant suppression). Overall, this work provides important insight into the impact that parrotfish-coral-microbiome-pathogen interactions have on coral reefs, ultimately increasing our understanding of the complex biological interactions affecting reef health and resilience.

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Appendix

	Df	SumsOfSqs	MeanSqs	F. Model	R2	Pr(>F)
Treatment	1	0.1442	0.14422	0.7949	0.02761	0.6796
Residuals	28	5.0799	0.18143		0.97239	
Total	29	5.2241			1.00000	

Appendix 1. Permanova table calculated for beta diversity metric analysis