EFFECTS OF MACROALGAL VERSUS CORAL REEF DOMINANCE ON CORAL SURVIVAL, CHEMICAL DEFENSE, AND MICROBIOMES

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EFFECTS OF MACROALGAL VERSUS CORAL REEF DOMINANCE ON CORAL SURVIVAL, CHEMICAL DEFENSE, AND MICROBIOMES

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I would like to dedicate my dissertation to my loving and supportive parents, Mary L. Beatty and Michael D. Beatty.

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LIST OF ABBREVIATIONS

ANOVA	analysis of variance
AIC	Akaike Information Criterion
bp	base pair
CCA	crustose coralline algae
cm	centimeter
df	degrees of freedom
DNA	deoxyribonucleic acid
E	east
F515	forward primer nucleotide position 515 of the 16S gene
FSW	filter-sterilized seawater
g	grams
km	kilometers
m	meters
min	minute
mL	milliliters
mm	millimeters
MPA	marine protected area
NCBI	National Center for Biotechnology Information
no-take	fishing prohibited
nt	nucleotide
OTU	operational taxonomic unit
PhiX	balanced base pair composition of GCAT from bacteriophage PhiX
rpm	rotations per minute
rRNA	ribosomal ribonucleic acid

R806	reverse primer nucleotide position 806 of the 16S gene
R926	reverse primer nucleotide position 806 of the 16S gene
S	south
SE	standard error
sec	second
spp.	species
TTC	tetrazolium chloride
µL	microliter
µm	micrometer
v.	version
vs.	versus
16S	component of prokaryotic ribosome

LIST OF SYMBOLS

>	greater than
≥	greater than or equal to
<	less than
≤	less than or equal to
°	degrees
%	percentage
±	plus or minus
~	approximately

SUMMARY

Coral reefs are among the earth's most biodiverse and productive ecosystems, but are undergoing precipitous decline due to coral bleaching and disease following thermal stress events, which are increasing in frequency and spatial scale. These effects are exacerbated by local stressors such as overfishing and pollution, collectively causing an increasing number of reefs to shift from coral to macroalgal dominance. These stressors can harm or kill corals through diverse mechanisms, including alterations in how corals interact with microorganisms. By employing a variety of field sampling and field experimental approaches, I investigated consequences of local protection from fishing and coral versus macroalgal dominance of the benthos on coral survival, chemical defense, and microbiomes within paired algal dominated fished areas and coral dominated marine protected areas (MPAs) in Fiji. I demonstrate that *i*) coral larvae from a macroalgal dominated area exhibited higher pre-settlement mortality and reduced settlement compared to those from a coral dominated area, *ii*) juveniles planted into a coral dominated MPA survived better than those planted into a macroalgal dominated fished area and differential survival depended on whether macroalgae were immediately adjacent to juvenile coral, *iii*) corals possess chemical defenses toward the thermally-regulated coral bleaching pathogen *Vibrio coralliilyticus*, but this defense is compromised by elevated temperature, *iv*) for a bleaching susceptible but ecologically important acroporid coral, anti-pathogen chemical defense is compromised when coral resides within macroalgal dominated reefs and this effect can be influenced by both the current and historic state of the reef. Effects on coral survival and chemical defense for individuals residing within coral versus macroalgal dominated areas largely coincided with nuanced differences in coral microbiomes (e.g., in microbiome variability and specific indicator bacterial taxa) but not with major shifts in microbiome composition.

These findings have implications for reef conservation and for understanding how coral-microbe interactions will respond to the pressures of global change.

CHAPTER 1

INTERGENERATIONAL EFFECTS OF MACROALGAE ON A REEF CORAL: MAJOR DECLINES IN LARVAL SURVIVAL BUT SUBTLE CHANGES IN MICROBIOMES

Abstract

Tropical reefs are shifting from coral to macroalgal dominance, with macroalgae suppressing coral recovery, potentially via effects on coral microbiomes. Understanding how macroalgae affect corals and their microbiomes requires comparing algae- versus coral-dominated reefs without confounding aspects of time and geography. We compared survival, settlement, and post-settlement survival of larvae, as well as the microbiomes of larvae and adults, of the Pacific coral *Pocillopora damicornis* between an Marine Protected Area (MPA) dominated by corals versus an adjacent fished area dominated by macroalgae. Microbiome composition in adult coral, larval coral, and seawater did not differ between the MPA and fished area. However, microbiomes of adult coral were more variable in the fished area and Vibrionaceae bacteria, including strains most closely related to the pathogen *Vibrio shilonii*, were significantly enriched, but rare, in adult and larval coral from the fished area. Larvae from the macroalgae-dominated area exhibited higher pre-settlement mortality and reduced settlement compared to those from the coral-dominated area. Juveniles planted into a coral-dominated area survived better than those placed into a fished area dominated by macroalgae. Differential survival depended on whether macroalgae were immediately adjacent to juvenile coral rather than on traits of the areas *per se*. Contrary to our expectations, coral microbiomes were relatively uniform at the community level despite dramatic differences in macroalgal cover between the MPA (~2% cover) and fished (~90%) area. Reducing macroalgae may elicit declines in rare but potentially harmful

microbes in coral and their larvae, as well as positive intergenerational effects on offspring survival.

Introduction

Coral reefs support great biodiversity and provide critical ecosystem services (Cesar et al. 2003). They buffer coastal populations from storms, provide a primary source of protein for many island and coastal nations (Dalzell et al. 1996, Cesar et al. 2003), and generate billions of dollars annually in tourism-related income (Cesar et al. 2003). However, coral reefs are in rapid global decline, with coral cover decreasing by 80% in the Caribbean since the 1970's (Gardner et al. 2003, Jackson et al. 2014) and by >50% in the Pacific since the 1980's (Bruno & Selig 2007, De'ath et al. 2012). Threats to reefs include overfishing, pollution, disease (Bellwood et al. 2004), thermal stress, and ocean acidification (Hoegh-Guldberg et al. 2007, Hughes et al. 2017). These stressors may directly harm corals, but may also indirectly lower coral health by allowing proliferation of competitive macroalgae (Hughes et al. 2010). Contact with algae has been linked to bleaching, disease, and tissue death in adult corals (Nugues et al. 2004, Rasher and Hay 2010), potentially harming coral through diverse mechanisms, including allelopathy, oxygen depletion, and destabilization of coral-associated microbial communities (microbiomes) (Rasher & Hay 2010, Barott & Rohwer 2012, Zaneveld et al. 2016, Morrow et al. 2017). Furthermore, macroalgae can directly inhibit both settlement and survivorship of coral larvae (Kuffner et al. 2006, Hughes et al. 2007, Dixson et al. 2014, Webster et al. 2015), often in a species-specific manner (Vermeij et al. 2009). Macroalgae may also disrupt microbiomes of coral larvae, but to our knowledge effects of macroalgae- versus coral-dominance of reefs on larval microbiomes or pre-settlement survival has not been investigated. Identifying the mechanisms and consequences of

coral-algae interactions is vital for understanding coral resilience under changing ocean conditions, as well as for creating effective conservation strategies.

Coral microbiomes may play important roles in coral acclimation to variable ocean environments (Rosenberg et al. 2007, Krediet et al. 2013, Peixoto et al. 2017). For example, corals that maintain or acquire thermotolerant strains of the symbiotic alga *Symbiodinium* have a lower risk of bleaching and mortality in response to fluctuating water temperatures (Pettay et al. 2015), and disrupting coral microbiomes with antibiotics can increase tissue loss in response to temperature stress (Gilbert et al. 2012). It is thus worrisome that microbial dysbiosis (i.e., a shift to higher abundances of harmful microbes or lower abundances of beneficial microbes) is becoming more common on degraded reefs (Dinsdale et al. 2008, Dinsdale & Rohwer 2011) and may render corals more susceptible to bleaching and mortality (Ritchie 2006, Harvell et al. 2007, Rosenberg et al. 2007).

Coral-macroalgae interactions on degraded reefs may drive dysbiosis, shifting the coral microbiome to an alternative state via mechanisms such as the production of algal allelochemicals (Morrow et al. 2012, Morrow et al. 2017), release of dissolved organic matter (Dinsdale & Rohwer 2011, Barott & Rohwer 2012, Haas et al. 2016), or transfer of harmful bacteria to corals interacting with algae (Nugues et al. 2004, Sweet et al. 2013, Pratte et al. 2017). Alternatively, changes in coral microbiomes in response to increasing algal cover could be a mechanism by which corals cope with algal competition or other biotic and abiotic stressors (Rosenberg et al. 2007).

Comparisons of adjacent reef areas that vary in algal cover due to protection status provide unique opportunities to explore coral-algae-microbiome interactions *in situ* without the confounding effects of contrasts across large spatial or temporal scales. Marine Protected Areas (MPAs) that prohibit fishing are valuable conservation tools for maintaining or restoring reef health. Corals in “no take” MPAs benefit from enhanced

herbivore grazing that removes competing seaweeds (Mumby et al. 2007, Rasher et al. 2013) or via reduced fishing-associated damage to corals that increases coral susceptibility to disease (Lamb et al. 2015, 2016). Healthy MPA corals may serve as a source of coral larvae to "rescue" degraded areas beyond reserve boundaries (Almany et al. 2009, McCook et al. 2010, Selig & Bruno 2010), but this rescue will depend upon survival of exported larvae during dispersal and on post-settlement survival if the larvae recruit to degraded reefs. Furthermore, ecosystem processes within MPAs, such as predation or herbivory, might also aid in conservation of microbiota required for coral health (Krediet et al. 2013), development (Vermeij et al. 2009, Tran & Hadfield 2011, Sneed et al. 2014), and ecosystem function (Ainsworth et al. 2010). By comparing islands that span ~2,000 km in the Pacific, reefs from populated islands were found to differ in reef fish biomass, abundances of fleshy algae, and benthic reef water microbiomes compared to reefs on unpopulated islands, suggesting that human use alters reef microbiomes (Dinsdale et al. 2008, Sandin et al. 2008, Kelly et al. 2014). Haas et al. (2016) also found positive correlations between fleshy algal cover and microbial abundance and community composition in benthic water across 60 reef sites spanning three ocean systems, while Zaneveld et al. (2016) found that herbivore exclusion plots had higher algal abundances and more variable coral microbiomes compared to plots with herbivores. However, to our knowledge, no studies have compared coral microbiomes in MPAs versus fished areas or investigated how microbiome composition may relate to survival of larvae produced from these areas. Such comparisons would help determine the extent to which coral microbiomes change across reefs dominated by corals versus macroalgae when not confounded by time or large distances.

We evaluated the effects of differing macroalgal abundance (resulting from reef protection status) on coral microbiomes using reef areas separated by only 100 – 500

meters. We conducted experiments in long-term (>10 yr) MPAs and adjacent fished areas (two MPAs and two fished areas – one pair of sites for pre-settlement experiments and one pair of sites for post-settlement experiments) along the southwest coast of Viti Levu, Fiji. Corals within the fished areas experience 5 to 15-fold more frequent and 23 to 67-fold more extensive algal contact (measured by proportion of colony perimeter in contact with macroalgae) than those in adjacent MPAs (Bonaldo & Hay 2014), allowing us to investigate how chronic interactions with macroalgae affect microbiomes of adult coral and their offspring under natural conditions and how this relates to juvenile coral survivorship. Specifically, we asked whether: 1) coral and seawater microbiome composition differed between a coral-dominated MPA and an adjacent fished area, 2) potentially harmful microbial taxa were less abundant in coral from the MPA compared to the fished area, 3) larvae from the MPA experience higher survivorship prior to settlement compared to larvae from the fished area, 4) post-settlement juvenile coral experience higher survivorship in an MPA compared to a fished area, and 5) higher juvenile survivorship depends on settlement on substrate free of macroalgae.

Materials and Methods

Study sites and focal coral

We focused on the coral *Pocillopora damicornis* because it occurs commonly in both MPA and fished areas and produces brooded larvae that could be obtained easily. Our study sites were shallow back-reef lagoons of 1-3 m water depth within two, small (0.5 – 0.8 km^2), locally managed MPAs and their adjacent fished areas at Vatu-o-lalai (18°12.26’ S, 177°41.26’ E) and Votua villages (18°13.08’ S, 177°42.59’ E) along the southwest coast of Viti Levu, Fiji. The MPA was established in 2002 at Vatu-o-lalai and in 2003 at Votua. These sites are approximately three kilometers apart and the MPA and fished area at each site experience similar physical regimes as judged by algal and coral growth rates when relieved of biotic pressures (Rasher et al. 2012, Dell et al. 2016,

Clements et al. 2018). All sites experience comparable flushing of reef water, with oceanic water flowing over the reef crest at high tide and washing out through deep channels at low tide. The MPAs have high coral cover (~57%) and low macroalgal cover (≤ 2%) on hard substrates; the fished areas have low coral cover (4-16%) and high macroalgal cover (50-90%) on hard substrates (Rasher et al. 2013). Consequently, coral contact with macroalgae is 5-15 times more frequent and 23-67 times more extensive in the fished areas than in the MPAs (Bonaldo & Hay 2014). MPAs also have 2-3 times higher diversity and 7-17 times higher biomass of herbivorous fishes than fished areas (Rasher et al. 2013).

Coral collection and maintenance of coral larvae

Between 29 October and 6 November 2014 (1-10 days before the full moon), portions from individual *P. damicornis* colonies were collected from the MPA and adjacent fished area at Votua village (12 colonies per area, collected with permissions from the Korolevu-i-Wai District Environment Committee). Collection locations for MPA versus fished area coral were separated by ~100 to 500 m. Each coral was placed in a separate bucket with approximately 19 liters of water from the respective collection site and monitored at dusk for larval release. Four colonies from the MPA and four from the fished area released larvae at dusk on the day they were collected. To characterize the microbiome of larvae from the MPA and fished area, we collected 10 larvae per colony upon release. Each larva was rinsed 3 times in 0.22 µm filter-sterilized (Corning disposable vacuum filter/storage systems 0.22 µm cellulose acetate 45 mm filter, ThermoFisher Scientific, Waltham, MA) seawater (FSW), preserved separately in RNAlater (ThermoFisher Scientific, Waltham, MA), and stored at -20º C. We simultaneously collected four clippings from each adult coral colony that released larvae and preserved these in the same manner.

Of the eight colonies used for microbiome analysis, four colonies from the MPA and three from the fished area produced sufficient numbers of larvae ($\geq$ 100 per colony) for use in subsequent larval survival and settlement experiments (see text below and Figure A.1 for a diagram of the experimental design). These larvae were pooled by area (MPA or fished area) and maintained in 600 mL polystyrene plastic containers filled with 400 mL of unfiltered water collected from a deep channel on the back reef that is open to the outer reef. Larvae of *P. damicornis* are packed with *Symbiodinium* and can remain viable for 100 days in the lab with water changes every 2-3 days (Richmond 1987, Isomura & Nishihira 2001). We changed water daily until the start of all experiments (which were all run simultaneously). Larval age at the start of experiments ranged from 7-16 days due to larval release occurring on different days. Any inactive larvae that failed to exhibit swimming behavior after three gentle pipette aspirations in the plastic dish were not used in any experiments. All larvae were transferred with sterile wide bore pipette tips (Axygen 1000 μL universal pipette tips: wide bore, ThermoFisher Scientific, Waltham, MA).

DNA extractions and amplicon sequencing of the 16S gene

Sequencing of the 16S rRNA gene was used to compare microbiome composition between MPA and fished area coral and seawater. DNA was extracted from coral larvae and adults using the PowerSoil DNA extraction kit and from water samples (polyethersulfone filters) using the PowerWater DNA extraction kit (both kits from MoBio Laboratories, QIAGEN, Carlsbad, CA). To account for intra-colony variation, DNA from five larvae and four clippings of adult coral branches were extracted individually per colony. For the larval survival experiment (see below), DNA from two larvae per dish was extracted individually (with one exception when only one larva was alive at the end of the experiment). Additionally, for each sample, we centrifuged the residual RNAlater solution (10,000 rpm, 10 min) to collect any dissociated cells, re-suspended the resulting

pellet in solution C1 (MoBio Laboratories, QIAGEN), and added these cells to the power bead tube. PCR reactions were performed in triplicate with dual-barcoded primers (F515 and R806) targeting the V4 region of the 16S rRNA gene, following standard protocols described in Kozich et al. (2013). PCR reactions included 45 µL of Platinum PCR SuperMix (Life Technologies, Thermo Scientific, Waltham, MA), 3 µL of template DNA (of 100 µL total DNA elution volume), and 1 µL each of forward and reverse primer. The thermal cycling protocol was as follows: initial denaturation at 94°C (3 min), followed by 35 cycles of denaturation at 94°C (45 sec), primer annealing at 50°C (45 sec) primer extension at 72°C (90 sec), and a final extension at 72°C (10 min). Amplicons were cleaned and DNA concentrations were normalized using SequalPrep plates (ThermoFisher Scientific, Waltham, MA). Amplicons were then pooled at equimolar concentrations and sequenced on Illumina's MiSeq platform using a 500 cycle kit (250 X 250 nt paired end reads) spiked with 10% PhiX to increase nucleotide diversity. Raw sequence reads can be found under NCBI bioproject number PRJNA382809.

Amplicon data analyses

We used Trim Galore! (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) to demultiplex, trim (100 bp cutoff length), and filter low-quality reads (Phred score cutoff 25), and FLASH (Magoč & Salzberg 2011) to merge paired-end reads (read length 250 bp, fragment length 300, fragment standard deviation 30). QIIME (Caporaso et al. 2010) was used to assess community composition based on merged reads. Briefly, chimeric sequences were identified and removed in QIIME using USEARCH (Edgar 2010). Amplicons were clustered into Operational Taxonomic Units (OTUs) at 97% similarity using the UCLUST algorithm (Edgar 2010) in open-reference OTU picking. The Greengenes database (McDonald et al. 2012, Werner et al. 2012) was used to assign taxonomy to OTUs. Chloroplast-affiliated OTUs were removed from downstream analyses. A total of

1,066,315 sequences (from 6,012,330 originally) remained after quality filtering and removal of chimeras and chloroplast sequences. The number of sequences per sample ranged from 235 to 20,812 for the initial collection of coral larvae and adults, 970 to 46,941 for coral larvae maintained in MPA or fished area water, and 31,816 to 59,734 for water samples. To avoid confounding sequencing depth with biological or environmental variables, as discussed recently in Weiss et al. (2017), diversity analyses were performed using a uniform sequence count identified as the highest count permitted without losing any replicates for a given experiment: 1,650 for coral larvae, coral adults, and water and 1,175 for larvae maintained in MPA or fished area water, and water samples. OTU abundances from each non-independent subsample were collapsed on the mean for a given independent replicate to avoid pseudoreplication (where coral colonies are spatially segregated and confounded within factor: area of origin) using the QIIME script collapse_samples.py. All of the following analyses were performed on the mean OTU abundance for each replicate.

Primer E (Clarke 1993) was used to perform principal coordinates analysis (PCO) on Bray-Curtis dissimilarity matrices from OTU tables based on 97% similarity clusters of 16S rRNA gene sequences. Statistical significance of a priori groupings were tested with PERMANOVA and PERMDISPERSION within Primer E v 7.

Two-factor ANOVA (factor 1: area of origin, factor 2: life stage), implemented via the aov function within the lm package in RStudio v 3.0, was used to test for differences in the relative abundances of microbial taxa among adult and larval coral using proportion data. When groups did not meet the parametric assumption of homoscedasticity, we applied a permutation ANOVA, via the aovp function within the lm package of RStudio v 3.0, on proportion data. We first included only those taxa (Family level) contributing to 2% or greater relative abundance within at least one sample group, using data from coral larvae and adults from the MPA and fished area, collected when

larvae were initially released. Taxa contributing < 2% were pooled to generate 'Low Abundance Bacteria' and 'Low Abundance Archaea' datasets that were also tested by two-factor permutation ANOVA (factor 1: area of origin, factor 2: life stage). A Bonferonni correction was implemented to account for multiple comparisons (critical *p*-value $p < 0.004$). Upon detecting large differences between adult and larval coral microbial taxa, we chose to also conduct an additional evaluation of the effects of area of origin on relative abundances of taxa contributing to 2% or greater, 'Low Abundance Bacteria' pooled, and 'Low Abundance Archaea' pooled with a one-factor (area of origin) ANOVA or permutation ANOVA (if data were not homoscedastic) on proportion data for adult coral and larval coral separately. This additional testing increases our risk of a type one statistical error (discussed in the context of our findings in the results section below) but allowed us to examine our samples for any microbial taxa that may differ by area of origin within each coral life stage, while reducing the number of factors and contrasts involved in the analyses.

We also tested for indicator OTUs of coral from the MPA and fished area, analyzing adult coral and larval coral separately with multi-level pattern analysis within the indicspecies package in RStudio v 3.0.

Two-factor ANOVA was also used as above to test for differences in the relative abundances of potential pathogens among sample groups. OTU tables (species level) were screened for bacterial groups that have been described as coral pathogens, both those verified with Koch's postulates and those that have not been verified (see the following reviews for described coral pathogens: Harvell et al. 2007, Rosenberg et al. 2007, Rosenberg & Kushmaro 2011). We detected bacterial OTUs (97% similarity clusters) most closely related to *Vibrio shilonii*, a bacterium previously shown to cause disease in *Oculina patagonica* (Kushmaro et al. 2001) and closely related to bacterial strains that cause bacterial bleaching (Ben-Haim et al. 2003b, Harvell et al. 2007) and

white syndromes (Sussman et al. 2008) in *P. damicornis* and other coral species. These were the only OTUs closely affiliated with a known coral pathogen, with the exception of an OTU most closely related to *Serratia marcescens* (posited coral pathogen of coral species in the Caribbean, Harvell et al. 2007), which was present in only 1 adult sample at 0.06% relative abundance. We therefore tested for differences in the abundances of OTUs identified as belonging to the Vibrionaceae family, and to *V. shilonii* specifically (based upon Greengenes classification) in adults and larvae (factor 1: area of origin, factor 2: life stage).

Larval survivorship in MPA or fished area water

To test for the effect of water from the MPA versus fished area on larval survivorship before settlement, MPA and fished area larvae were aliquoted in a full factorial design into 600 mL polystyrene dishes with 400 mL unfiltered water collected ~1-2 meters above the benthos daily from the MPA or fished area and used immediately in water changes. There were 10 replicate dishes per level of each factor (factor 1: larval area of origin; factor 2: water area of origin), dishes were randomly interspersed, and each replicate dish held 10 larvae. To maintain similar conditions between experiments and to reduce the influence of 'home reef' (i.e., enhanced larval preferences for or survival on or within substrates or water from the site where the parent coral was collected) effects on larval responses (survival or settlement), we collected water for both experiments from the MPA and fished area of Vatu-o-lalai village, approximately 3 km from where adults that released these larvae were collected at Votua village. Water was collected approximately 1-2 meters above the benthos and changed daily for the first five days of the experiment. A 250 mL aliquot of this freshly collected water was filtered through a 0.22 µm polyethersulfone filter each day, and the filter was preserved in RNALater for microbiome analysis (i.e., non-filtered water was used to hold the larvae, but the filter was used to assess the water's microbiome). No settlement substrate was

added during the experiment, and larvae avoid settling on the polystyrene surfaces of the dishes (KB Ritchie, personal communication). We recorded metamorphosis (on the dish or in the water column, which was rare) daily for six days and assessed survivorship at the end of the six-day experiment. Larvae were considered alive if they exhibited swimming behavior after three gentle pipette aspirations within the dish. Larvae alive at the end of the experiment were collected (n = 10 independent samples per level of each factor in our design, i.e., dishes considered independent, not individual larvae from within dishes), rinsed three times in filter-sterilized seawater, and preserved in RNAlater individually for microbiome analysis. Following DNA extraction and sequencing of the 16S gene, MPA and fished area larvae maintained in MPA or fished area water were screened for potential coral pathogens. OTUs identified as *V. shilonii* were the only hypothesized coral pathogens detected in these samples. We tested for differences in the abundance of taxa identified as *V. shilonii* and Vibrionaceae with a two-factor ANOVA via the aov function within the lm package of RStudio v 3.0 (factor 1: larval area of origin, factor 2: water area of origin). Lastly, we tested for differences in the abundance *V. shilonii* and Vibrionaceae in water samples by a one-factor ANOVA (factor: water area of origin). Primer E (Clarke 1993) was used to perform principal coordinates analysis (PCO) on Bray-Curtis dissimilarity matrices from OTU tables based on 97% similarity clusters of 16S rRNA gene sequences. Statistical significance of a priori groupings were tested with PERMANOVA within Primer E v 7.

Settlement behavior and post-settlement survivorship of MPA and fished area larvae offered MPA and fished area substrates

To test for the effects of MPA vs. fished area substrates on larval settlement and survivorship, we set up a full-factorial experiment with MPA and fished area larvae offered coral rubble from either the MPA or the fished area. To prevent confounding home reef with MPA vs. fished area effects, we collected rubble pieces from the MPA

and fished area at Vatu-o-lalai village (approximately 3 km from sites where adult colonies were collected) and used these in settlement assays with larvae from the MPA and fished area at Votua village. Rubble pieces were similar in size and collected from haphazard locations throughout the MPA and fished area. All rubble collected from the MPA was naturally free of macroalgal fouling, whereas rubble collected from the fished area was either fouled with some macroalgae (characteristic of the benthos in the fished area - Rasher et al. 2013, Bonaldo & Hay 2014) or free of fouling. All three types of substrate were fouled with comparable amounts of CCA and short (< 0.5 cm) turf. Crustose coralline algae (CCA) may stimulate settlement of coral larvae; therefore, we also quantified CCA cover on rubble from each location. Photos of rubble collected from the MPA and fished area were analyzed with Coral Point Count Software (Nova Southeastern University, Kohler & Gill 2006). CCA abundances between the three types of rubble collected (MPA rubble without macroalgae, fished area rubble without macroalgae, and fished area rubble with macroalgae) were tested with a one-factor ANOVA in JMP Pro 13 software (SAS Institute Inc.). Rubble fouled with macroalgae had short algal fronds ~0.5-4 cm in height. These pieces of rubble were used to test for the mean effect of naturally occurring multi-species assemblages of macroalgae on larval settlement and post-settlement survival. Water for these experiments was collected from the MPA and fished area at Vatu-o-lalai simultaneously with the rubble and then daily thereafter for use in the larval settlement experiments described below.

For the first settlement experiment, larvae from MPA and fished area adults were separately aliquoted to 600mL polystyrene plastic dishes (10 larvae per dish; n = 20 dishes per level of each factor, dishes randomly interspersed) and offered only MPA substrate (without macroalgae) with 400 mL of unfiltered water from the MPA or only fished area substrate (with macroalgae) with 400 mL of unfiltered water from the fished area, with daily water changes. All water used in experiments was collected from ~1-2

meters above the benthos. These two substrates were chosen for the first experiment because they are typical of the MPA vs. fished area site differences (Rasher et al. 2013, Bonaldo & Hay 2014). Within each replicate, larvae could either settle on the added substrate or remain in the water column. Settlement was recorded at 24 and 48 h. After 48 h, all non-settled larvae were removed and the settled coral were held on their substrate in the lab. The effect of settlement substrate on post-settlement survival was assessed on day four following the 48 h settling period; surviving juveniles were then out-planted to the reef.

Juveniles on MPA rubble were out-planted to the MPA and juveniles on fished area rubble were out-planted to the fished area. To reduce the possibility of home reef effects confounding MPA vs. fished area effects, juvenile coral were out-planted to MPA and fished area sites at Vatu-o-lalai village approximately 3km from Votua, where the fragments of adult coral colonies had initially been collected. Zip-ties were used to attach the rubble to u-nails driven into the reef bottom, with each rubble piece containing 4-9 juveniles at the time of out-planting. For each replicate, similarly sized pieces of control rubble (without any juvenile coral) were attached to the benthos in the same manner as above to test for natural coral recruitment to rubble (MPA rubble without macroalgae in the MPA and fished area rubble with macroalgae in the fished area) that might be confused with, and falsely increase survivorship rates of, our out-planted juveniles. Survivorship of out-planted juveniles and natural recruitment to control pieces of rubble were recorded after four and twenty-six days on the reef (when experimental coral were eight and thirty days post-settlement). Recruitment to control rubble was low in each area (0-0.1 recruit/replicate), and average recruitment to control rubble was deducted from the appropriate treatment before calculating the proportion of surviving juveniles at each time-point. Across all treatment combinations, nine replicates were lost due to rubble becoming unattached from the benthos over 26 days on the reef.

Differences between survivorship of juvenile coral on MPA substrate planted in the MPA vs. fished area substrate planted in the fished area could be due to differences in macroalgal abundance on the settlement substrate or due to other unrecognized physical or biotic differences between the MPA and fished area sites (hereafter referred to as 'site' effects). To test for a site effect vs. the effect of macroalgae on the settlement rubble, we performed a second experiment to test for settlement of MPA larvae (too few larvae remained from fished area adults to conduct this experiment with those larvae) on similarly sized rubble from either i) the MPA (without macroalgae), ii) the fished area but without macroalgae, or iii) the fished area but with macroalgae (n = 14 - 15 for each treatment). Experimental procedures were the same as in the settlement experiment. Briefly, larvae from MPA adults were aliquoted to 600 mL polystyrene plastic dishes with 400 mL of unfiltered water and substrate from either the MPA or fished area (10 larvae per dish, dishes randomly interspersed). Water changes were performed daily with freshly collected unfiltered water from the MPA or fished area collected ~1-2 meters above the benthos. Settlement was assessed at 24 and 48 hours. Survivorship of newly-settled-juvenile coral was assessed four days after the initial 48-hour settlement experiment. Juvenile coral that had settled on these substrates were then out-planted into the field (MPA rubble to the MPA site and fished area rubble to the fished area site) using the procedures described above. Again, natural recruitment to MPA or fished area control rubble at both sites was low (0.0-0.07 recruit/replicate) and was deducted before calculating the proportion of surviving juvenile coral. Four replicates planted in the MPA became detached and were lost by day 26. No replicates from the fished area were lost.

Statistical analyses of larval behavior and recruit survivorship

JMP Pro 12 (SAS Institute Inc.) was used to analyze larval metamorphosis, larval settlement, and larval and juvenile survivorship. Larval metamorphosis and survivorship were analyzed by a two-factor ANOVA on proportion data. Settlement was analyzed with

repeated measures ANOVA on square root transformed proportion data. Juvenile coral survivorship was analyzed with repeated measures ANOVA on proportion data. All data were homoscedastic; when needed, square root transformations were performed to improve normality.

Results

Coral and water microbiomes from coral-dominated Marine Protected Areas (MPAs) and macroalgae-dominated fished areas

Microbiome community composition of adult coral, larvae, and water did not differ as a result of collection site (MPA or fished area), but did differ between sample types (Figure 1.1A PERMANOVA sample type $p = 0.001$, area of origin $p = 0.426$, sample type * area of origin $p = 0.803$). Water and larval microbiomes were more diverse than adult microbiomes (Figure 1.1B, number of OTUs: sample type $p < 0.001$, area of origin $p = 0.764$, sample type * area of origin $p = 0.909$; Figure 1.1C, Shannon diversity index: sample type $p < 0.001$, area of origin $p = 0.539$, sample type * area of origin $p = 0.282$), despite under-sampling water microbial communities at a rarefaction depth of 1,650 (see rarefaction curve in supplementary Figure A.2). Findings were similar if water samples were excluded from the analyses; microbial community composition was dictated by life stage (adult or larvae), not by the area (MPA vs. fished area) (Figure A.3B, PERMANOVA life stage $p = 0.001$, area of origin $p = 0.338$, life stage * area of origin $p = 0.584$). Findings were also similar whether based on the mean for each independent replicate colony or all subsamples from each independent colony (Figure A.3A and A.3B). However, differences detected with PERMANOVA are partially due to dispersion differences among groups, with microbiome composition exhibiting lower dispersion among MPA adults compared to dispersion among fished area adults, or to larvae from either area (Figure A.3B PERMDISP area of origin $p = 0.743$, life stage $p = 0.002$, area of origin * life stage $p = 0.013$, see Table A.1 for pairwise comparisons). We also tested

for area of origin effects using adults alone and larvae alone and did not detect effects in either analysis (adults p = 0.246; larvae p = 0.588, Monte Carlo PERMANOVA, Figure A.3C-D).

We also compared each common taxonomic group that comprised ≥ 2% relative abundance and the pooled group of uncommon bacterial and archaeal taxa (< 2% relative abundance) between MPA and fished area sites (Figure 1.1D, Table A.2.1 – A.2.2, and A.3.1 – A.3.2). None of these taxonomic groups differed significantly between MPA and fished area sites and this was true whether adults and larvae were tested together (2-factor ANOVA) or separately (1-factor ANOVA), despite biasing our analyses toward a higher probability of a false positive through multiple statistical tests on these data sets. In contrast, certain taxonomic groups differed notably in relative abundance between adults and larvae. Endozoicimonaceae were enriched 13-fold in adults compared to larvae (~ 90% vs ~ 7%; two-factor ANOVA source area $p = 0.722$, life stage $p < 0.001$, source area * life stage $p = 0.113$), whereas larvae contained 58-243 fold more Chromatiales ($p < 0.001$), Methylobacteriaceae ($p = 0.001$), Sphingomonadaceae ($p < 0.001$), Pseudomonadaceae ($p = 0.003$), and Helicobacteraceae ($p = 0.002$) (Table A.2.1 and A.2.2). Larvae were also enriched 8-fold in low abundance bacteria ($p = 0.001$) and 90-fold in low abundance archaea ($p = 0.002$) compared to adult coral (Table A.2.1 and A.2.2).

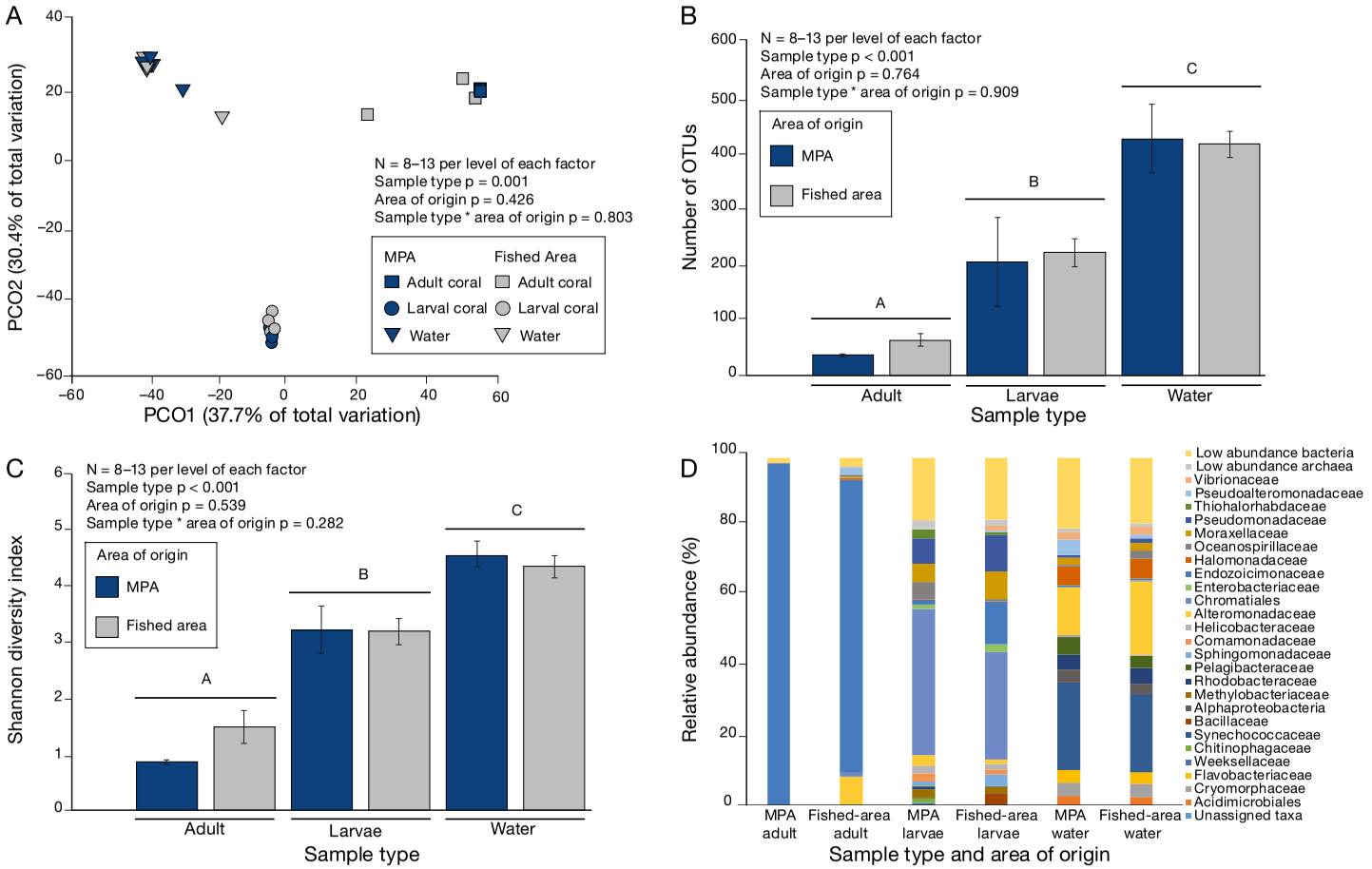

Figure 1.1: A) PCO and PERMANOVA analysis of Bray Curtis dissimilarity matrix of coral larvae, coral adults, and water microbiomes from the MPA and fished area. B) Diversity of OTUs in coral and water samples. C) Shannon Diversity Index for coral and water samples. OTUs and Shannon Diversity were analyzed by a two-factor ANOVA with Tukey post-hoc analysis. D) Taxonomic groups that contribute to 2% or greater of the microbial community composition of coral adults, coral larvae, and water are depicted at the level of family with the exception of Chromatiales (order). Low abundance taxa, contributing less than 2% of community composition were pooled to generate 'Low Abundance Bacteria' and 'Low Abundance Archaea' groups. For analyses in A-D above, each coral larva, and coral adult data point represents the mean community composition for a single replicate.

Indicator OTU analysis on coral from the MPA and fished area did not find any OTU that was enriched in MPA or fished area adults. However, two OTUs are indicative of fished area larvae. An OTU classified as *Ruminococcus gnavus* within the family Lachnospiraceae, and an unclassified OTU within the family Lachnospiraceae were found to have high specificity (100% and 91%, respectively, of the reads for each OTU were found in fished area larvae) and high fidelity (each of these OTUs were found in 100% of fished area larvae).

OTUs classified as *Vibrio shilonii* (at 97% clustering; see discussion below regarding limitations of 16S-based classification of microbial species) were the only potential coral pathogens in more than one of our 16 coral replicates. We did not detect *V. shilonii* in any our MPA coral at a sampling depth of 1,650; however, it occurred at low (< 1%), but significantly higher, relative abundances in our fished area coral (Figure 1.2A, Table A.4, two-factor ANOVA coral area of origin $p = 0.009$, life stage $p = 0.116$, coral area of origin * life stage $p = 0.116$). We also detected higher abundances of taxa within the Vibrionaceae family in fished area versus MPA coral, especially in larvae (Figure 1.2B, Table A.4, two-factor ANOVA coral area of origin $p = 0.003$, life stage $p = 0.041$, coral area of origin * life stage $p = 0.234$). Vibrionaceae were not detected on MPA adults but were detected at low (mean $\pm$ SE; 0.13 $\pm$ 0.13%) abundances on their larvae. We detected low abundances of *V. shilonii* in both MPA (0.32 $\pm$ 0.12%) and fished area (0.53 $\pm$ 0.14%) water, with these values not differing significantly (Table A.4, $p = 0.269$, $n = 5$). The abundances of Vibrionaceae also did not differ significantly between MPA and fished area water (Table A.4, 2.08 $\pm$ 0.66% vs. 0.93 $\pm$ 0.39%, respectively, $p = 0.898$, $n = 5$).

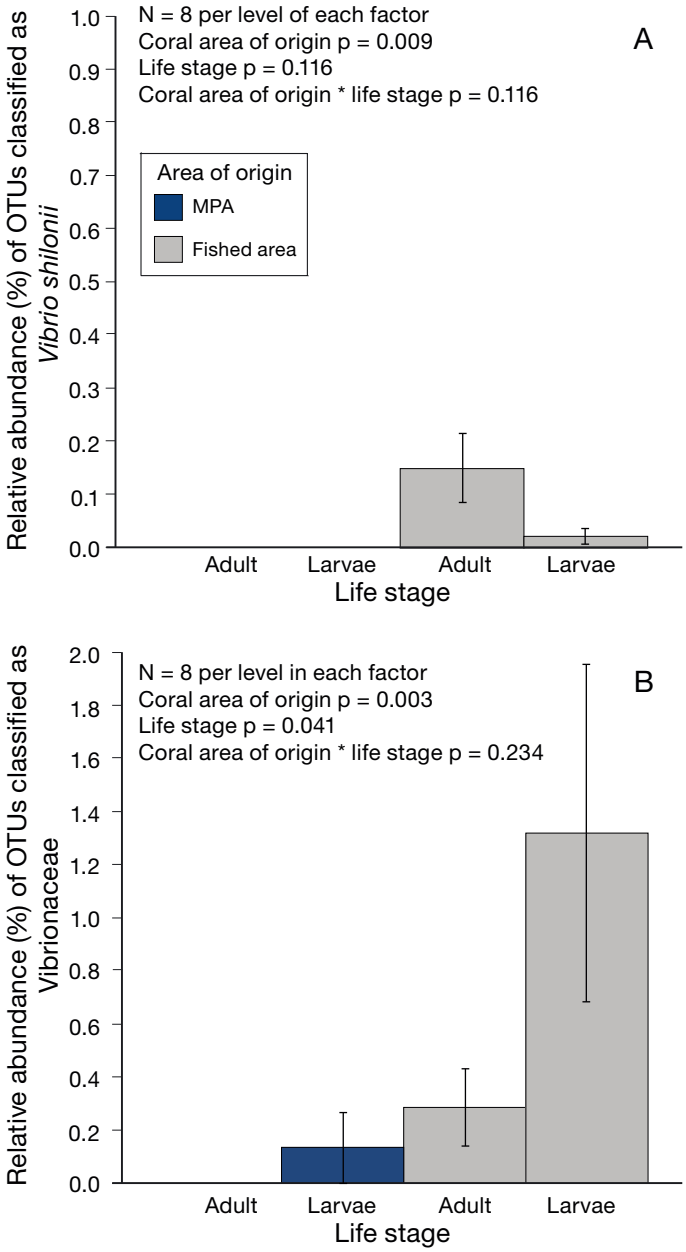

Figure 1.2: Abundances and two-factor ANOVA analyses of *Vibrio shilonii* (A) and taxa within the family Vibrionaceae (B) in corals (adults and larvae) from the MPA and fished area.

Larval microbiomes, survivorship, settlement, and post-settlement survival

Despite the similarity of coral microbiomes between the coral-dominated MPA and macroalgae-dominated fished sites, when held in the lab for six days in MPA or fished area water, survivorship of MPA larvae was 94% regardless of water source while survivorship of fished area larvae was significantly lower – only 26%, when in fished area water and 66% when in MPA water (Figure 1.3A; two-factor ANOVA, Tukey HSD post-hoc analysis; larval area of origin $p < 0.001$, water area of origin $p = 0.008$, larval area of origin * water area of origin $p = 0.008$). Larval metamorphosis (in the water column or on the plastic dish) did not bias these results, as < 1% of individuals underwent metamorphosis in any treatment, and this percentage did not differ significantly among treatments (two-factor ANOVA larval area of origin $p = 0.332$, water area of origin $p = 0.332$, larval area of origin * water area of origin $p = 0.332$).

When comparing the microbiomes of larvae that survived the 6-day experiment, we did not detect differences among larvae from the fished area or the MPA when maintained in water from the fished area or from the MPA (Figure 1.3B: PERMANOVA larval area of origin $p = 0.069$, water area of origin $p = 0.197$, larval area of origin * water area of origin $p = 0.492$), nor between the MPA and fished area water in which the larvae were held (Figure 1.3C; $p = 0.869$). The only suggested coral pathogens found on larvae in this experiment were OTUs classified as *Vibrio shilonii*. The mean relative abundance ($\pm$ SE) of *V. shilonii* on MPA larvae was 0.0 $\pm$ 0.0% for larvae held in MPA water and 0.01 $\pm$ 0.01% for larvae in fished area water (Table A.4). *V. shilonii* abundance on fished area larvae was 4.16 $\pm$ 4.14% for larvae in MPA water and 0.28 $\pm$ 0.17% for larvae in fished area water (Table A.4). These abundances did not differ significantly among treatments (two-factor ANOVA larval area of origin $p = 0.177$, water area of origin $p = 0.551$, larval area of origin * water area of origin $p = 0.495$). We detected low abundances of *V. shilonii* in both MPA (0.32 $\pm$ 0.12%) and fished area

(0.53 ± 0.14%) water (Table A.4), with these values not differing (p = 0.269, n = 5). Furthermore, the abundance of Vibrionaceae as a group did not differ significantly between MPA and fished area water (Table A.4, 2.08 ± 0.66% vs. 0.93 ± 0.39%, respectively, p = 0.898, n = 5).

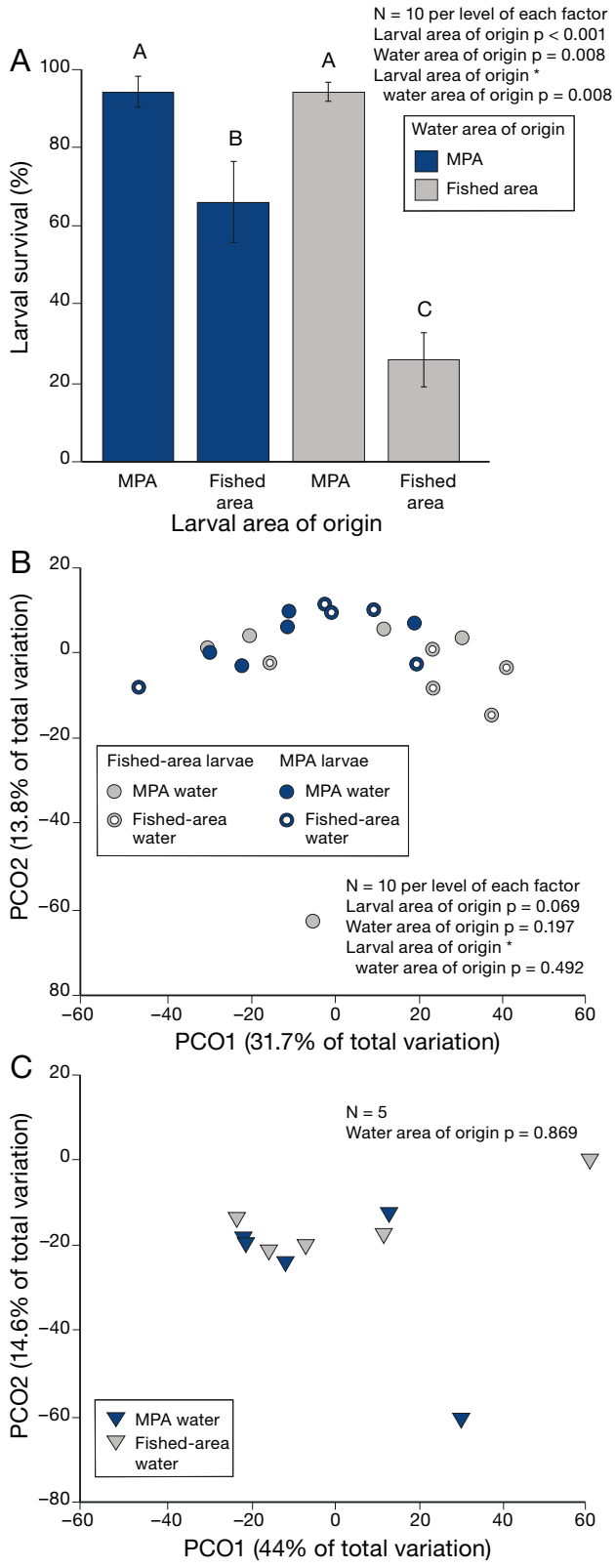

Figure 1.3: A) Survival and two-factor ANOVA analysis of larvae (mean ± SE) from the MPA and fished area maintained in MPA or fished area water for six days (n = 10 per level of each factor). Letters above bars indicate significant groupings by Tukey HSD

post-hoc analysis. B) PCO and PERMANOVA analysis of Bray Curtis dissimilarity matrix of microbiomes from surviving larvae from the MPA or fished area maintained in MPA or fished area water for six days. C) PCO and PERMANOVA analysis of Bray Curtis dissimilarity matrix of water microbiomes from the MPA and fished area, used to maintain larvae in the lab from the MPA or fished area for six days.

When larvae were offered rubble from either the MPA or fished area as settlement substratum in a no-choice experiment (i.e., larvae are given the option to settle on the type of rubble provided or remain in the water column), MPA larvae settled more rapidly than larvae from the fished area (Figure 1.4A, Table 1.1A larval area of origin * time interaction $p < 0.001$). For both MPA and fished area larvae, settlement was more rapid in response to MPA than to fished area substrate (Figure 1.4A, Table 1.1A, substrate type * time $p = 0.010$). For MPA larvae, 84-90% had settled by 24 h, whereas 52-76% of fished area larvae settled in this time period. After 48 h of isolation with a particular substrate type, 85-93% of all larvae had settled regardless of larval origin or substrate type.

When recently-settled juvenile coral were out-planted to the sites from which their settlement substrates had been collected (i.e., MPA substrate to the MPA, fished area substrate to the fished area), survival was higher in the MPA than in the fished area regardless of larval area of origin (Figure 1.4B, Table 1.1B, substrate out-plant treatment * time $p < 0.001$). Survival on fished area substrate out-planted to the fished area was 12-29% by day four and 5-8% by day 26. In contrast, survival on MPA substrate out-planted to the MPA was 49-64% on day four and 22-39% on day 26. Surprisingly, given lower survivorship of fished area larvae pre-settlement (Figure 1.3A), larvae from fished area adults survived better as newly-settled juveniles when out-planted to the field than did those from MPA adults (Figure 1.4B, Table 1.1B, larval area of origin $p = 0.007$; larval area of origin * time $p = 0.013$). Greater post-settlement survivorship of fished area larvae was not due to selective pre-settlement mortality of less hardy individuals among

the fished area larvae. Mortality of larvae during the initial settlement experiment (48 h) was ≤ 4% and did not differ among treatments (larval area of origin $p = 0.336$, substrate type $p = 0.747$, larval area of origin * substrate type $p = 0.747$).

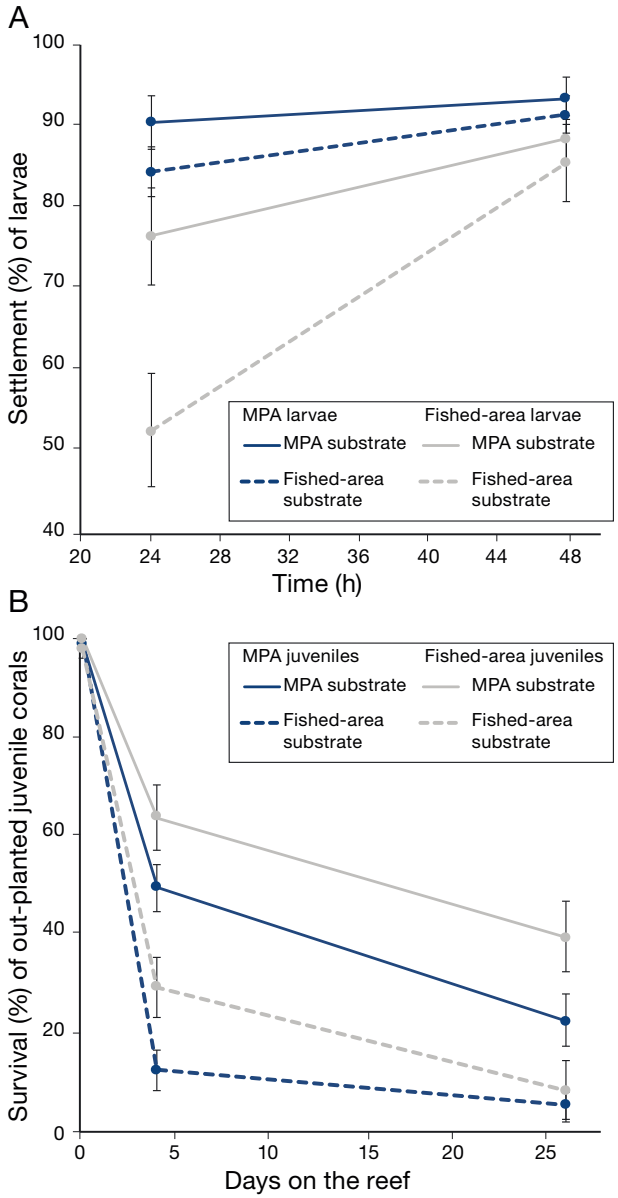

Figure 1.4: A) Settlement (mean ± SE) of MPA and fished area larvae on rubble from the MPA without macroalgae and from the fished area with macroalgae at 24 and 48 h (n = 20 per level of each factor; absolute percentages provided). See Table 1.1A for statistical analyses of repeated measures ANOVA on square root transformed proportion data. B) Survival (mean ± SE) of newly settled MPA and fished area juvenile corals on MPA versus fished area substrates that were out-planted to their corresponding reef (MPA rubble planted in the MPA and fished area rubble planted in the fished area) when corals were four and 26 days old (n = 13 – 18 per level of each factor due to loss of zip-

tied rubble on the reef over time). See Table 1.1B for statistical analyses of repeated measures ANOVA on proportion data.

Table 1.1 Repeated measures ANOVA on A) square root transformed settlement of larvae (originating from the MPA or fished area) on substrate from the MPA (no macroalgae) or fished area with macroalgae and B) survival of recently settled juvenile corals over 26 days on the reef. Juveniles that settled on MPA substrate were out-planted to the MPA and juveniles that settled on the fished area substrate were out-planted to the fished area.

Source	df	F	p
A			
Larval area of origin	1	15.75	<0.001
Substrate type	1	5.95	0.020
Time	1	31.62	<0.001
Larval area of origin x Substrate type	1	2.10	0.156
Larval area of origin x Time	1	14.26	<0.001
Substrate type x Time	1	7.40	0.010
Larval area of origin x Substrate type x Time	1	3.72	0.062
B			
Larval area of origin	1	8.16	0.007
Substrate type	1	46.39	<0.001
Time	2	446.69	<0.001
Larval area of origin x Substrate type	1	0.77	0.387
Larval area of origin x Time	2	4.67	0.013
Substrate type x Time	2	22.53	<0.001
Larval area of origin x Substrate type x Time	2	1.15	0.322

Lower survivorship of juvenile coral (regardless of larval area of origin) planted in the fished area versus the MPA could be due to larger-scale site differences, or smaller-scale differences of the substrate used (i.e., differences in abundances of macroalgae or crustose coralline algae on rubble). CCA cover did not contribute to differences between MPA and fished area rubble. CCA cover was high and did not differ among treatment groups (ANOVA $p = 0.308$, 66% $\pm$ 0.12 SE, 79% $\pm$ 0.04, 84% $\pm$ 0.03 on fished area rubble with macroalgae, fished area rubble without macroalgae, and MPA rubble, respectively). To assess the potentially confounding factors of site differences and macroalgal presence on rubble, we conducted a second, no-choice settlement experiment that ran simultaneously, but used only MPA larvae (due to insufficient larvae

produced by fished area adults). In this experiment, larvae settled more rapidly (by 24 h) on rubble without macroalgae than rubble with macroalgae, even if both types of rubble originated from the fished area; this difference disappeared by 48 h (Figure 1.5A; substrate type * time $p = 0.041$). Mortality of larvae during the 48 h settlement experiment was low (<3%) and did not differ among treatments (substrate type $p = 0.841$). When these juveniles were out-planted back to their respective field sites (MPA rubble to the MPA and fished area rubble to the fished area), survival of juveniles differed between treatment types (Figure 1.5B, substrate out-plant treatment $p < 0.001$), with the lowest survival occurring for juveniles on substrate fouled with macroalgae within the fished area. Coral on rubble not fouled by macroalgae survived similarly well whether placed in the MPA or fished area (Figure 1.5B, 43-51% survival on day four and 22-28% on day twenty-six). In contrast, those on macroalgae-fouled rubble in the fished area experienced only 15% survival to day four and 9% to day 26 (Figure 1.5B). On day 4 and 26 after out-planting, survival of juvenile coral in the fished area was ~190% and ~150% higher, respectively, if on fished area rubble without macroalgae than on fished area rubble fouled with macroalgae. In contrast, survivorship of juvenile coral on non-macroalgal fouled rubble was only ~20% to 30% higher when out-planted to the MPA compared to the fished area on day 4 and 26, respectively.

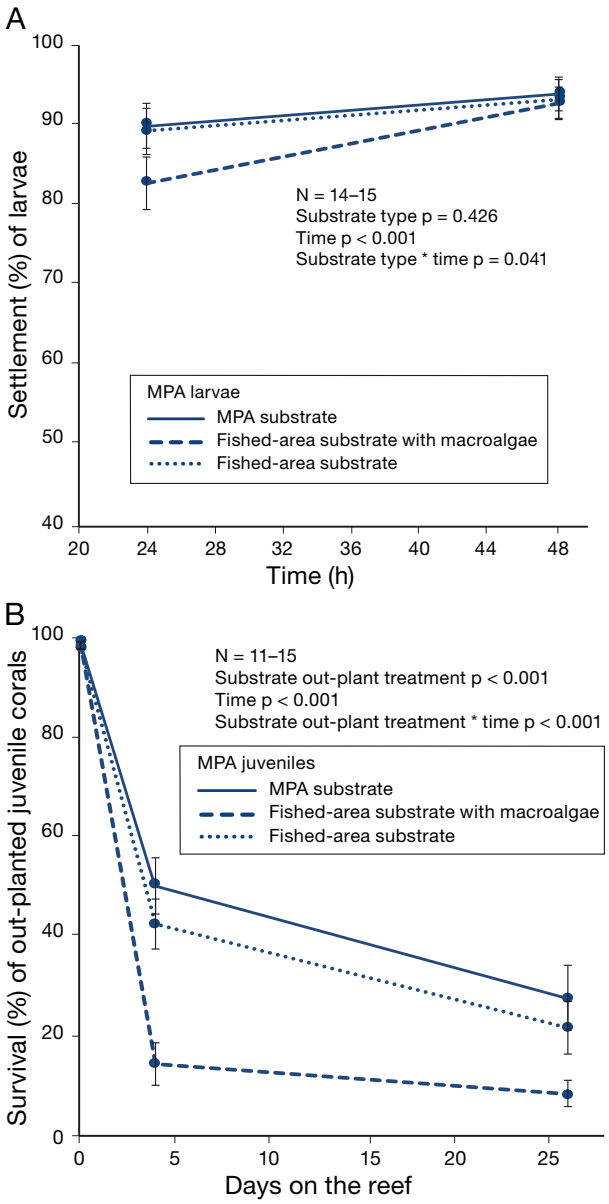

Figure 1.5: A) Percent settlement (mean ± SE) of MPA larvae on rubble from the MPA without macroalgae, the fished area without macroalgae, and the fished area with macroalgae at 24 and 48 hours (n = 15 with the exception of one lost replicate due to sloughing of macroalgae during the settlement experiment; absolute percentages provided). Repeated measures ANOVA was performed on square root transformed proportion data. B) Percent survival (mean ± SE) of newly settled juvenile corals on rubble from the MPA without macroalgae, the fished area without macroalgae, and the fished area with macroalgae that were out-planted to their corresponding reef (MPA rubble planted in the MPA and fished area rubble planted in the fished area) when the juvenile corals were four days old. Repeated measures ANOVA was performed on proportion data (n = 11 - 15 due to loss of replicates planted on the reef over time).

Discussion

Our experiments within an MPA dominated by corals and an adjacent fished area dominated by macroalgae allowed an assessment of how microbiomes of the coral *Pocillopora damicornis* are shaped by chronically (up to 12 years) higher macroalgal abundances and whether these habitat differences are correlated with changes in larval behavior or survivorship. By sampling coral from coral- versus macroalgae-dominated reefs that are only ~100-500 meters apart, we were able to examine microbiomes on degraded and healthy reefs that are not confounded in time or by large spatial scales. These study areas differ dramatically in the extent (23-67 fold greater) and frequency (5-15 fold greater) of coral-macroalgae contact (Bonaldo & Hay 2014), and these differences have persisted for the 7+ years we have worked on these reefs (M.E. Hay, personal observation).

Responses of coral microbial communities

Despite the large difference in algal cover (primarily brown seaweeds [*Sargassum, Turbinaria, Dictyota*], and a lesser abundance of red and green seaweeds [*Galaxaura, Amphiroa, Liagoria,* and *Halimeda*], Rasher et al. 2013) between the fished area and MPA, the microbiome composition of adult and larval *P. damicornis* did not differ between the coral-dominated MPA and the macroalgae-dominated fished area. This result contrasts with evidence suggesting that macroalgae alter the physiochemical environment, the microbial load, and community composition in surrounding seawater (Wild et al. 2010, Haas et al. 2011, Nelson et al. 2013), and the microbiome of associated corals (Wild et al. 2010, Haas et al. 2011, Morrow et al. 2012, Thurber et al. 2012, Morrow et al. 2013, Nelson et al. 2013, Morrow et al. 2017). Specifically, algae are predicted to affect corals through DOM release that promotes microbial growth in surrounding seawater, declines in local oxygen concentrations, and enrichment of copiotrophic and pathogenic microbes that may overwhelm the native coral microbiota

(Dinsdale & Rohwer 2011, Barott & Rohwer 2012). Algae may also release allelochemicals that alter coral microbial communities on contact (Morrow et al. 2011, 2012, 2017), or act as vectors for pathogenic microbes (Nugues et al. 2004, Sweet et al. 2013). All of these mechanisms may operate on relatively small spatial scales, exerting strongest effects in zones of direct algae-coral contact (Barott et al. 2009, Barott et al. 2011). Here, our sampling did not assess microbiome variation relative to algal contact sites. Nonetheless, the similarity of microbiomes (both coral and water) from sites with ~ 2% cover of macroalgae versus ~90% cover of high-biomass macroalgae (Rasher et al. 2013) suggests that enhanced algal coverage at the reef scale does not systemically alter the microbiome of *P. damicornis*. Lack of differences in water microbiomes from our coral- and algae-dominated reefs may result from sampling water ~1-2 meters above the benthos, where reef water is readily exchanged with oceanic water flowing over the reef crest. The positioning of small protected areas within the larger background of fished areas may also facilitate dispersal and mixture of microbes at scales of hundreds of meters, helping to homogenize both coral and seawater microbiomes across degraded algae-dominated and protected coral-dominated reefs. However, if this is the case, it is not suppressing corals in the MPAs, where coral cover on hard substrates is nearly 60% (Rasher et al. 2013). Additionally, corals in the fished area grow as well as those in the MPA when macroalgae within 50 cm of the coral colonies are removed (Clements et al. in press). This suggests minimal effects of macroalgal DOM on growth of corals at the scale of $\geq$ 50 cm.

It is also possible that our results are specific to *P. damicornis* (but preliminary data for other corals from these sites suggest that this is not the case; D. Beatty unpublished data). Previous work has indicated that coral-algae interactions and their outcomes are often species-specific, with effects of macroalgae on coral microbiomes varying from undetectable to strong (Morrow et al. 2012, 2013, Thurber et al. 2012).

Here, in contrast to numerous non-Pocilloporid coral taxa, which decline in abundance at macroalgae-dominated sites, the abundance of Pocilloporid coral does not differ significantly between the MPA and fished areas at Votua village (Bonaldo & Hay 2014). This persistence may be due in part to the ability of Pocilloporids to maintain a stable microbiome in spite of drastic differences in benthic cover. In support of this hypothesis, we found similarly high relative abundances (>80%) of Endozoicimonaceae bacteria in adult *P. damicornis* coral from both healthy and degraded reefs. Recent evidence suggests that these bacteria are functionally important members of the healthy coral holobiont in multiple coral species (Meyer et al. 2014, Lee et al. 2015, Ding et al. 2016, Neave et al. 2016), including *P. damicornis* (Bayer et al. 2013). We also detected similar abundances of Endozoicimonacaeae (1-12%) on larvae from both the MPA and fished area, adding further support for the hypothesized importance of these bacteria in *P. damicornis* persistence. Adult corals were not maintained in filter-sterilized seawater (FSW) before larval release; therefore, we do not know if Endozoicimonaceae were rapidly acquired from the environment or vertically transferred to brooded larvae. However, larvae were rinsed in FSW three times before preservation, so it is unlikely that the presence of Endozoicimonaceae represents contamination from seawater because this group's abundance was <0.5% in our seawater samples.

While we did not detect a significant community-level shift in coral microbiomes between our macroalgae-dominated and coral-dominated sites, we did detect differences in the abundances, although rare, of Vibrionaceae, with this bacterial family being significantly enriched in both adults and larvae from the macroalgae-dominated reef compared to those from the coral-dominated MPA. The enriched bacteria included OTUs classified, as *Vibrio shilonii*, a demonstrated coral pathogen (Kushmaro et al. 2001). Caution should be taken when interpreting ecological function and pathogenicity when using the 16S rRNA gene for classification because bacterial strains identified as

the same species by this method can vary in genome size and functional gene content, including genes involved in pathogenicity (discussed in Franzosa et al. 2015, Land et al. 2015). However, it is interesting to note that the 16S rRNA gene of *V. shilonii* shares 96.6% similarity with that of a *P. damicornis* pathogen (*Vibrio coralliilyticus*) that causes coral bleaching (Ben-Haim et al. 2003a) and with other *Vibrio* spp. that cause white syndromes in many Indo-Pacific coral species (Sussman et al. 2008). The overall abundance of taxa falling within the Vibrionaceae family was low ($\leq$ 2%) and comparable to abundances (0-3%) found in healthy corals (Lee et al. 2017, Morrow et al. 2017, Tout et al. 2015), even in the macroalgae-dominated area, suggesting that the sampled *P. damicornis* were not in a 'diseased' state. Nevertheless, the differences in Vibrionaceae abundance may indicate that coral in the protected area are more resistant to colonization by potentially harmful bacteria, consistent with a recent investigation by Lamb et al. (2016) that found lower abundances of coral disease in no-take reserves. While our findings provide evidence of proportionally lower abundances of Vibrionaceae on coral in a no-take protected area compared to an adjacent fished reef, more work is needed to confirm a pathogenic role for the detected bacteria and the reproducibility of findings in other coral species and protected areas. Indeed, *Vibrio* species are also found in healthy corals (Chimetto et al. 2008, Raina et al. 2009) and may function as coral mutualists by providing fixed nitrogen (Ceh et al. 2013). We also found *R. gnavus* as an indicator species of fished area larvae. *R. gnavus* is an anaerobic gut microbe that has been implicated in human disease and is capable of breaking down mucins (Crost et al. 2013). Its impact on adult coral or their larvae is unknown.

We also found that microbiomes of adult coral from the macroalgae-dominated fished area were more variable in community composition than those from the coral-dominated MPA. This is consistent with Zaneveld et al. (2016) who found that corals in experimental plots where macroalgal cover increased due to the absence of fish grazing

exhibited greater microbial beta diversity. Thus, microbiome variance may be an early indicator of coral stress, but further investigations are needed to test this hypothesis. We also found that while adult MPA coral were less variable in their microbiome composition compared to their larvae, levels of inter-individual microbiome variability did not differ between adult and larval coral from the macroalgae-dominated fished area. Taken together, these patterns suggest that *P. damicornis* adults from the MPA have more constrained microbial communities than their adult counterparts from the fished area and than juveniles from both areas, adding support to the notion that coral-algae interactions may increase the variance (Thurber et al. 2012, Zaneveld et al. 2016) of coral microbiomes. However, greater inter-individual variability in coral microbiomes could indicate either 1) the loss of regulatory mechanisms within the coral holobiont, thereby predisposing corals to microbial dysbiosis (Krediet et al. 2013, Thompson et al. 2015), or 2) the holobiont's adaptive response to counter local biotic or abiotic stressors (Rosenberg 2007). To better understand our microbial community data in the context of coral fitness and health, we concurrently investigated how more frequent and chronic algal interactions (in the macroalgae-dominated fished area) affected larval behavior, and larval and juvenile survivorship.

Effects of parentage, habitat, and substrate on larval survival

Based on prior evidence showing that larvae of *P. damicornis* are packed with photosynthate providing *Symbiodinium* and that larvae can settle in under two hours or remain viable in the plankton for 100 days (Richmond 1987, Isomura & Nishihira 2001), we expected high larval survivorship over the short duration of our larval survival experiment. In contrast, we found rapid mortality within some treatments. During six days of exposure to MPA or fished area water without a choice of appropriate settlement substrates, larvae from MPA adults experienced only 6% mortality regardless of water source, while larvae from fished area adults experienced significantly higher, 74% and

34%, mortality in both fished area and MPA water, respectively (Figure 1.3A). Thus, larvae produced by adults in the fished area appear less robust than those produced by adults in the MPA. We failed to detect differences in the relative abundance of potentially pathogenic bacterial OTUs classified as *Vibrio shilonii* on coral larvae that experienced higher mortality. It is possible that *Vibrio shilonii* OTUs could have been at greater abundance on, and differentially impacted survivorship of, fished area larvae but that we failed to detect differences in bacterial relative abundances because we analyzed only the less infected, or most resistant, larvae living at the end of the six-day experiment. Microbiomes of dead larvae were not analyzed due to rapid shifts in microbial communities following mortality.

Given that we were unable to document significant differences in potential pathogens or microbial community composition between MPA and fished area larvae or between MPA and fished area water (Figure 1.3B & 1.3C), it may be that differential mortality is due to differential larval provisioning by adults rather than microbial effects. Dense macroalgae, which is typical of the fished area, commonly suppress coral recruitment, growth, and survivorship (Hughes et al. 2007, Burkepile and Hay 2008, Thurber et al. 2012, Zaneveld et al. 2016). However, to our knowledge, this is the first documentation of negative intergenerational effects of algal dominance on coral.

Experimental studies indicate that many species of macroalgae deter coral larval settlement (Kuffner et al. 2006, Vermeij et al. 2009, Diaz-Pulido et al. 2010, Dixson et al. 2014). However, as reefs globally continue to degrade, larvae may not be able to avoid settlement near macroalgae. We found that both MPA and fished area larvae settled more rapidly on MPA substrate free of macroalgae than on fished area substrate fouled with macroalgae. However, by the end of the 48 h experimental period, almost all larvae had settled, regardless of substrate type. When newly-settled juvenile coral were out-planted to the sites from where their substrates originated, juvenile survivorship was ~5

times greater in the MPA than the fished area (Figure 1.4B day 30), confirming a strong positive effect of the no-take MPA on juvenile coral survival.

However, lower survivorship of juveniles in the fished area could have been due to macroalgae on the substrate onto which they settled, other differences between the MPA and fished area (site differences), or both. We therefore investigated the relative impact on juvenile survival of site and of macroalgal presence on the settlement substrate. Survivorship of juveniles in the fished area on day 4 and 26 was ~190% and 150% higher, respectively, if on fished area rubble without macroalgae than on fished area rubble fouled with macroalgae (Figure 1.5B day 4 & 26). In contrast, the increase in survival due to site was modest (Figure 1.5B day 4 & 26, 20-30% higher). Thus, nearby macroalgae on the same piece of rubble–not general traits of the macroalgae-dominated area (i.e., site effects)–were largely responsible for reductions in juvenile survivorship in the fished area.

Although pre-settlement larvae from MPA adults experienced greater survival than larvae from fished area adults, this relationship was reversed for post-settlement survivorship in the field. This pattern occurred regardless of settlement substrate type (MPA or fished area origin) or the site into which the coral were out-planted. This was not due to selective mortality of less hardy fished area larvae during the initial 48 h settlement experiment; in that period, mortality was low ($\leq$ 4%) and did not differ between treatments. It is possible that degraded reefs have selected for hardier post-settlement populations of *P. damicornis*, but this hypothesis is difficult to reconcile with the lower survival of fished area larvae during the pre-settlement period. If degraded reefs have selected for hardier coral, then these populations may become increasingly valuable as global change and other anthropogenic stressors continue to impact reefs.

Conclusion

The composition of *P. damicornis* microbial communities did not differ significantly between the MPA and fished area despite drastic differences in benthic cover between these sites and substantial differences in larval survivorship. However, adults within the coral-rich MPA exhibited lower variability in their microbial community composition than those from the macroalgae-dominated fished area. Additionally, larval and adult *P. damicornis* from the MPA had significantly lower abundances of Vibrionaceae and OTUs classified as the coral pathogen *Vibrio shilonii*. Taken together, our findings indicate that coral within a coral-dominated MPA with abundant and diverse herbivore populations and low abundances of macroalgae experience greater larval survivorship, reduced variability in their adult microbial community composition, and reduced abundances of rare but potentially harmful bacteria. However, overall microbial community composition remained relatively uniform despite reef protection status and a 45-fold difference in macroalgal cover (~2% vs 90%) between these sites. Reproductive adults were only collected from one MPA and one fished area (following permitting guidelines). Further studies will be needed to understand how frequency of coral-algae interactions in natural reef environments affects coral microbiomes and coral fitness in other species of reef-building corals and if findings are reproducible among other coral- and algae-dominated areas. At present, our study suggests that investigating macroalgal impacts on coral health via alterations of their microbiomes may require understanding the importance of subtle microbiome alterations such as changes in rare taxa of potential pathogens or changes in variability of coral microbial communities rather than drastic differences in microbial community composition.

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CHAPTER 2

LOCAL MANAGEMENT TO SUPPRESS MACROALGAE ENHANCES CORAL CHEMICAL DEFENSE AGAINST A THERMALLY-REGULATED BLEACHING PATHOGEN

Abstract

Bleaching and disease are causing unprecedented declines of coral reefs, especially when warming promotes virulence in coral bleaching pathogens, such as *Vibrio coralliilyticus.* We demonstrate that corals are chemically defended against *V. coralliilyticus*, but that defenses are compromised at elevated temperature. For an ecologically sensitive species within the critical *Acropora* genus, its resistance to *V. coralliilyticus* is enhanced by ~75-154% if it occurs on coral-dominated reefs protected from fishing versus adjacent fished reefs dominated by macroalgae. Increased anti-*Vibrio* potency in *Acropora* was associated with less variable microbiomes and with a strain of Endozoicimonaceae, a bacterial family proposed as facultatively symbiotic with coral. Defenses of an ecologically robust poritid coral and a weedy pocilloporid coral were not affected by macroalgal abundance. For some critical, but bleaching susceptible, corals like *Acropora*, local management to enhance fishes that suppress macroalgae may provide greater coral resistance to global stressors such as bacteria-induced coral bleaching during warming events.

Introduction

Coral reefs are biodiverse and productive ecosystems upon which coastal societies rely for ecosystem services such as protection from storm surge, tourism-generated revenue, and as a critical source of protein (Moberg and Folke 1999). However, reefs are declining worldwide, notably from a five-fold increase in the frequency of thermal stress events associated with atmospheric CO_2 increases (Hughes et al. 2018a). Coral decline is further exacerbated by other stressors, including ocean

acidification, disease, pollution, and increases of harmful macroalgae following removal of herbivorous fishes (Bellwood et al. 2004, Hughes et al. 2010). These stressors can harm or kill corals through diverse mechanisms, including alterations in how corals interact with microorganisms (Rosenberg and Ben-Haim 2002, Ritchie 2006, Harvell et al. 2007, Krediet et al. 2013). Determining how coral-microbe interactions change in response to stress, potentially either through loss of beneficial microbes or suppression of coral defenses against harmful microbes, is challenging, but may be vital for understanding and mitigating losses of coral in a changing ocean.

A wealth of evidence suggests that coral health is affected by interactions with bacterial associates. For example, bacterial mutualists can help corals obtain nutrients (Thompson et al. 2015) and may inhibit the growth of pathogens (Ritchie 2006, Krediet et al. 2013). Coral susceptibility to pathogens, such as bacteria of the genus *Vibrio*, increases when waters warm (Rosenberg and Ben-Haim 2002, Harvell et al. 2007), generating the hypothesis that temperature-related stress in corals may occur due to the shifts in the beneficial bacteriome at high temperature (Ritchie 2006, Harvell et al. 2007, Zaneveld et al. 2016). Stressors other than temperature, including algal-induced blooms of virulent bacteria and direct toxicity from allelochemical-producing seaweed that becomes abundant on degraded reefs, are also hypothesized to operate by destabilizing the protective coral bacteriome (Barott and Rohwer 2012, Morrow et al. 2012). Indeed, it has been suggested that corals regulate their microbiomes via production of antimicrobial peptides, sloughing of mucus, direct consumption of microbes, or by hosting predatory microbes that consume microbial pathogens (Krediet et al. 2013, Thompson et al. 2015, Welsh et al. 2015). As thermal stress events become more frequent, and algal cover increases (Hughes et al. 2010, Hughes et al. 2018a), corals may lose the ability to regulate their microbiomes (McDevitt-Irwin et al. 2017, Peixoto et

al. 2017, Webster and Reusch 2017), and may lose chemical defenses produced by commensal microbes (Ritchie 2006).

Microbial dysbiosis (the loss of beneficial, or increase of harmful, microbes) is common on reefs with abundant macroalgae (Dinsdale et al. 2008). Macroalgae are suggested to disrupt coral microbiomes via transfer of allelochemicals (Morrow et al. 2012) or microbes (Nugues et al. 2004, Sweet et al. 2013, Pratte et al. 2017), or release of dissolved organic carbon that affects microbial growth (Barott and Rohwer 2012). Indeed, bacterial density and virulence gene abundance are significantly enriched in the benthic water (within ~25 cm of the reef surface) of algal-dominated compared to coral-dominated reefs (Kelly et al. 2014, Haas et al. 2016). Furthermore, coral microbiomes of algal-dominated sites can be more variable than those of coral-dominated sites (Zaneveld et al. 2016, Beatty et al. 2018). These patterns suggest that algal overgrowth, like high temperature, may impair a coral's ability to regulate its microbiome (Zaneveld et al. 2016, Zaneveld et al. 2017) and possibly the ability of the coral, or it's microbiome, to control pathogen adhesion and persistence (Krediet et al. 2013).

The hypothesis that macroalgae may suppress coral chemical defense can be explored through comparisons among coral-dominated and macroalgal-dominated areas of closely adjacent reefs – thus avoiding contrasts across large spatial scales and differing physical regimes that could confound results. We utilized paired reefs that differed in benthic community composition as a result of local management for 10-12 years prior to our study (during that period, fishing was prohibited, herbivory increased by 3-6 fold, and macroalgae was reduced by 75-95% in protected versus fished areas) (Rasher et al. 2013, Bonaldo and Hay 2014, Bonaldo et al. 2017). We tested for a mechanism (chemical defense) by which coral may inhibit the thermally-regulated coral bleaching pathogen *Vibrio coralliilyticus* and assessed whether the potency of coral chemical defense differed between coral-dominated marine protected areas (MPAs;

fishing prohibited) and adjacent macroalgal-dominated areas (fishing allowed). We also evaluated whether changes in algal cover and coral chemical defense correlated with changes in coral microbiomes that would be suggestive of a microbial role in antibiotic production, as observed in a diversity of other invertebrates (including insects, isopods, shrimp, bryozoans, and tunicates; reviewed in (Lopanik 2014)).

V. coralliilyticus is an ecologically realistic assay pathogen because it targets diverse coral groups (Agaricids, Acroporids, and Pocilloporids), is distributed worldwide (Kimes et al. 2012), becomes virulent under elevated temperatures (Ben-Haim et al. 2003, Kimes et al. 2012), and causes coral bleaching and mortality throughout the Indo-Pacific (Ben-Haim et al. 2003, Sussman et al. 2008, Bourne et al. 2014). Moreover, the bacterium is congeneric with other species that cause coral bleaching (Bourne et al. 2014), and therefore may be useful for exploring coral response to warming in general.

On our study reefs, coral and algal cover differed dramatically between adjacent fished and MPA areas due to differences in the abundance of herbivorous fishes (Rasher et al. 2013, Bonaldo and Hay 2014), and these fished and MPA areas were replicated (n = 3) and interspersed. This framework facilitated tests in the absence of confounding physical variables between sites. We found that the inhibitory effects of coral water (obtained via 20 sec agitation of coral fragments in reef water and hereafter called “coral water”; see methods) against *V. coralliilyticus* differed significantly based on temperature, coral species, and inoculum density of the pathogen. We also found that reef state (algal- versus coral-dominated sites) altered chemical defense for a coral of the genus *Acropora*, one of the major reef-building coral groups worldwide. Differences in reef state and chemical defense coincided with differences in key indicator bacterial strains and with variation in microbiome stability. We discuss these findings as potentially linked to subtle changes in the beneficial microbiome or to microbiome-independent chemical changes in the coral holobiont.

Material and Methods

Sites and species

We investigated how reef state affects coral microbiomes and chemical defense against the coral pathogen *Vibrio coralliilyticus* (ATCC BAA-450) using the common corals *Porites cylindrica, Acropora millepora*, and *Pocillopora damicornis*. Corals were collected haphazardly throughout three small (0.5-0.8km^2), no-take MPAs and their adjacent fished reefs at Namada (18°11.30' S, 177°37.10' E), Vatu-o-lalai (18°12.26' S, 177°41.26' E) and Votua (18°13.08' S, 177°42.59' E) villages along the southwest coast of Viti Levu, Fiji between October and December of 2014 (8-12 December; 21-24 October; 25-29 October, respectively at each village). Protection of reef herbivores within the MPAs in the decade before our study resulted in benthic communities differing drastically between MPAs and fished areas at each village (Rasher et al. 2013, Bonaldo and Hay 2014, Bonaldo et al. 2017). MPAs have 38-56% coral cover and $\leq$3% macroalgal cover, while fished areas have only 4-16% coral cover but 50-90% macroalgal cover on hard substrates (Rasher et al. 2013).

We collected 2 to 3 individuals for each coral species from MPAs and adjacent fished areas each day to assure that samples across species and between MPA and fished areas were interspersed in time, and we repeated this over multiple days at each village until acquiring a total of 10 individuals of each species from each area at each village. This allowed testing for differences in coral traits between MPAs and fished areas. We could not collect from all villages at the same time; thus, village and time are confounded and should be interpreted cautiously.

Antipathogen activity of corals

As many marine invertebrates harbor microbes that may produce chemical defenses (Lopanik 2014), we investigated anti-pathogen activity in corals and evaluated how this related to changes in the coral's microbiome. In most previous studies of

marine chemical defenses, entire organisms have been exhaustively extracted in strong solvents over long periods of time [i.e., multiple rounds of extraction that can last upwards of 24 hours (Paul and Fenical 1986, Pawlik et al. 1995, Hay et al. 1998)]. These extracts are then concentrated away from the solvents and tested against enemies. In contrast to these exhaustive methods, we simply agitated coral samples in reef water for 20 seconds and used this water to assess its effects on the coral pathogen *Vibrio coralliilyticus*. We collected 50 ml displacement volume of coral per colony (n = 10 colonies per species per site) within each of the three MPA and three fished area sites). Each coral sample was volumetrically displaced in a 1:1 ratio with reef water collected adjacent to each colony and agitated in a glass jar for 20 sec. This "coral water" (reef water containing coral mucus, and anything else released during agitation, such as antibacterial compounds (Geffen and Rosenberg 2005)), was decanted into a 50 mL sterile polystyrene tube and frozen at -20°C. This procedure should generate a conservative sample of the coral's anti-pathogen activity because a pathogen landing on, or present within, coral tissues would likely experience the full concentration of the coral's mucus or any chemicals associated with the host or symbiotic dinoflagellates, while our samples were mostly washed from the surface and were diluted with 50 ml of seawater.

As the seawater we used from each site to generate the coral water sample might itself contain anti-pathogen traits, we also collected 50 mL of reef water adjacent to each colony (frozen as above) as a control for our bioassays with *V. coralliilyticus*. A random subset of three coral water samples and three reef water (control) samples from each of the three MPAs and each of the three fished areas (i.e., n = 9 samples per species per area type) were selected for bioassays against *V. coralliilyticus*.

For the bioassay, 100 μL of coral water, or adjacent reef water (the control), was aliquoted per well in sterile 96-well round bottom plates, lyophilized on a freeze dryer, and UV-radiated for 90 sec to kill any microbes that survived lyophilization. This procedure allows a test of the coral water chemical traits without any confounding biological interactions. However, this should produce a conservative estimate of the holobiont's chemical defense against *V. coralliilyticus* because it introduces a single, diluted dose (see above) that may degrade or be metabolized during the assay; in nature, the holobiont would presumably be continuously producing chemical defenses. To the samples of dried coral water in the bottom of each well, we then added 100 μL of *V. coralliilyticus* bacterial cell suspension. These inoculum cultures were grown in Marine Broth (Difco™ 2216) and added to the wells to span a gradient of cell densities, from 10^3-10^1 cells per mL (with additional concentrations of 10^6-10^4 cells per mL for *Acropora millepora* only).

Assays were conducted at temperatures of 24°C and 28°C. These temperatures are near the upper and lower limits of the seasonal means in Fiji and represent temperatures at which *V. coralliilyticus* is less (24°C) versus more (28°C) virulent (Kimes et al. 2012). Tetrazolium chloride (TTC) was added (0.05 μg/μl final concentration) to each well and the plates were incubated for 24 h. Reduction of TTC by cellular respiration produces a red compound, triphenylformazan, allowing a direct measurement of metabolic activity. We used this method rather than measuring turbidity associated with cell density or direct cell counts because density assesses both live and dead, healthy and unhealthy cells, whereas this method assesses only those cells that are metabolically active. Reduction of TTC by *V. coralliilyticus* was quantified by absorbance at 490 nm using a BioTek ELx800 absorbance reader. Background absorbance was measured in blanks containing lyophilized and UV-radiated coral water

or reef water reconstituted in Marine Broth with TTC but without bacteria. Blank-corrected measurements were used to determine relative *V. coralliilyticus* metabolism, expressed as a ratio of metabolism in the coral water compared to the control (reef water collected adjacent to the coral). Values >1 indicate that coral water is stimulatory, and values <1 indicate that coral water is inhibitory (confirmed by comparing coral water to reef water with functions aov or aovp in package lm v 2.1.0 implemented within RStudio3 with false discovery rate [FDR] corrections for multiple comparisons; Table B.1.1 & B.1.2). Linear mixed effects models within package nlme v 3.1-137 implemented within RStudio 3 were implemented with Akaike Information Criterion (AIC) for model selection for each species to determine how area of origin (MPA or fished area), temperature (24°C or 28°C), and bacterial inoculum concentration (cells per mL) influenced each species antipathogen activity (via relative metabolism of TTC by *V. coralliilyticus*).

DNA extractions and sequencing of the 16S gene to test for differences in coral or benthic reef microbiomes

Fragments of coral (~1 g) from the same colonies used to obtain coral water were preserved in RNAlater® (QIAGEN) in the field and stored at -20°C until DNA extraction. Our previous analyses at these sites demonstrated that water collected from ~1 m above the benthos did not differ in microbiome community composition (Beatty et al. 2018) but previous studies at other reefs found microbiome differences in water collected from within ~25 cm of the reef surface on macroalgal- versus coral-dominated reefs (Kelly et al. 2014). Thus, we collected benthic water samples from each habitat type to evaluate whether microbiome contrasts on our study reefs differed in some fundamental way from those observed in previous studies. Benthic water samples (n =10 per site) from within 1 cm of the benthos were collected haphazardly at each site using a 240 mL syringe. Water was filtered through a 0.22 µm polyethersulfone filter (total

volumes ranged 100-240 mL depending on filter clogging), which was then preserved in RNAlater® and frozen at -20°C.

We performed Illumina sequencing of the 16S rRNA gene to characterize the microbial community in our samples. DNA was extracted from approximately 250 mg of coral using the MoBio PowerSoil Kit and from water filters using the MoBio PowerWater kit (MoBio Laboratories, QIAGEN, Carlsbad, CA). For each sample, residual RNAlater® solution was centrifuged at 10,000 rpm for 10 minutes to pellet dissociated microbial cells. This pellet was re-suspended with C1 solution and added to the powerbead tube (MoBio Laboratories, QIAGEN). Dual-barcoded primers (F515 and R806) appended with Illumina sequencing adapters (see Kozich et al. 2013 (Kozich et al. 2013)) were used to amplify the V4 region of the microbial 16S rRNA gene. PCR reactions were carried out in triplicate. Total reaction volume was 50 µL, containing 45 µL of Platinum PCR SuperMix (Life Technologies, Thermo Scientific, Waltham, MA), 1 µL each of forward and reverse primer, and 3 µL of template DNA. Thermal cycling involved initial denaturation at 94°C (3 min), 35 cycles of denaturation at 94°C (45 sec), primer annealing at 50°C (45 sec), primer extension at 72°C (90 sec), and final extension at 72°C (10 min). SequalPrep plates (ThermoFisher Scientific, Waltham, MA) were used to remove impurities and normalize DNA concentrations across samples. Pooled amplicons were sequenced on an Illumina MiSeq using a 500-cycle kit (250 X 250 nt) spiked with 10% PhiX to introduce sequence diversity. Raw sequence reads were deposited at NCBI (bioproject number PRJNA476581).

Microbiome data analyses

TrimGalore! (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) was used to demultiplex and trim (100 bp cutoff length) sequence reads, and to remove low quality reads (Phred score cutoff 25). FLASH (Magoč and Salzberg 2011) was used to merge

paired-end reads using the criteria: read length >250 bp, fragment length >300 bp, fragment standard deviation <30 bp). Chimeras were identified and removed with USEARCH (Edgar 2010) in QIIME (Caporaso et al. 2010). Remaining sequences were then clustered into Operational Taxonomic Units (OTUs) with 97% similarity clusters using the UCLUST algorithm (Edgar 2010), followed by open-reference picking to assign taxonomy based on the Greengenes database (McDonald et al. 2012, Werner et al. 2012). After quality filtering 12,057,258 sequences remained from 21,520,596 sequences generated by the MiSeq run, with per-sample counts ranging from 44 - 131,802 for benthic water samples and 1,191 - 216,258 for coral samples. Microbiome analyses were performed on OTU tables after rarefaction to a uniform sequence count of 7700 for coral samples and 17700 for water samples (Figure B.6).

Alpha (number of OTUs, Shannon Diversity) and beta diversity (Bray Curtis dissimilarity) were calculated in QIIME (Caporaso et al. 2010). Aov or aovp functions in package lm v 2.1.0 implemented within RStudio 3 were used to test for differences in alpha diversity between coral or water samples from MPAs or fished areas by a two-factor ANOVA or permutation ANOVA if data were heteroscedastic (factor 1: area of origin, factor 2: village, where village is confounded in time). Upon detecting differences in *A. millepora* microbiome alpha diversity among villages, we subsequently tested for differences in *A. millepora* microbiome alpha diversity between MPA and fished areas independently at each village using a one-factor ANOVA. Principal coordinate analysis (PCO) and corresponding tests for differences in microbiome composition (PERMANOVA) and variability (PERMADISPERSION) were implemented in Primer E (Clarke 1993) for coral and water samples via two factor tests (factor one: area of origin, factor two: village). Upon detecting differences in microbiome composition in *A. millepora* among villages, we independently tested for area of origin effects via a one factor PERMANOVA test at each village. Coral OTU tables were also filtered to obtain

only Endozoicimonaceae OTUs (via QIIME script filter_otus.py) and tested for differences in Endozoicimonaceae OTU communities for each coral species via two-factor (area of origin and village) PERMANOVA and PERMADISPERSION in Primer E. Multi-level pattern analysis was implemented within the indicspecies package v 1.7.6 implemented within RStudio 3.0 to test for MPA or fished area indicator OTUs for coral species and water samples. OTUs were considered as indicators if their fidelity value was 0.50 or greater.

Results

We tested how sterilized coral water from the abundant reef building corals *Acropora millepora, Porites cylindrica,* and *Pocillopora damicornis* affected the metabolic activity of *Vibrio coralliilyticus* as a function of temperature (24°C and 28°C), inoculum concentration of the pathogen, and whether corals came from coral-dominated MPAs or algal-dominated fished areas. Coral water significantly altered *V. coralliilyticus* activity, with the effect varying considerably based on coral species, temperature, and the concentration of the *V. coralliilyticus* inoculum (Figure 2.1 and Table B.1.1 – B.1.2; linear mixed effect model for each species). *P. cylindrica* inhibited activity by 94-98% at 24°C and 35-94% at 28°C; *A. millepora* inhibited activity by 61-87% at 24°C and 22-68% at 28°C; and *P. damicornis* inhibited activity by 8-19% at 24°C, but stimulated activity by 6-12% at 28°C. Suppression of *V. coralliilyticus* declined with increasing inoculum concentration for all corals, but especially for *P. cylindrica* at 28°C.

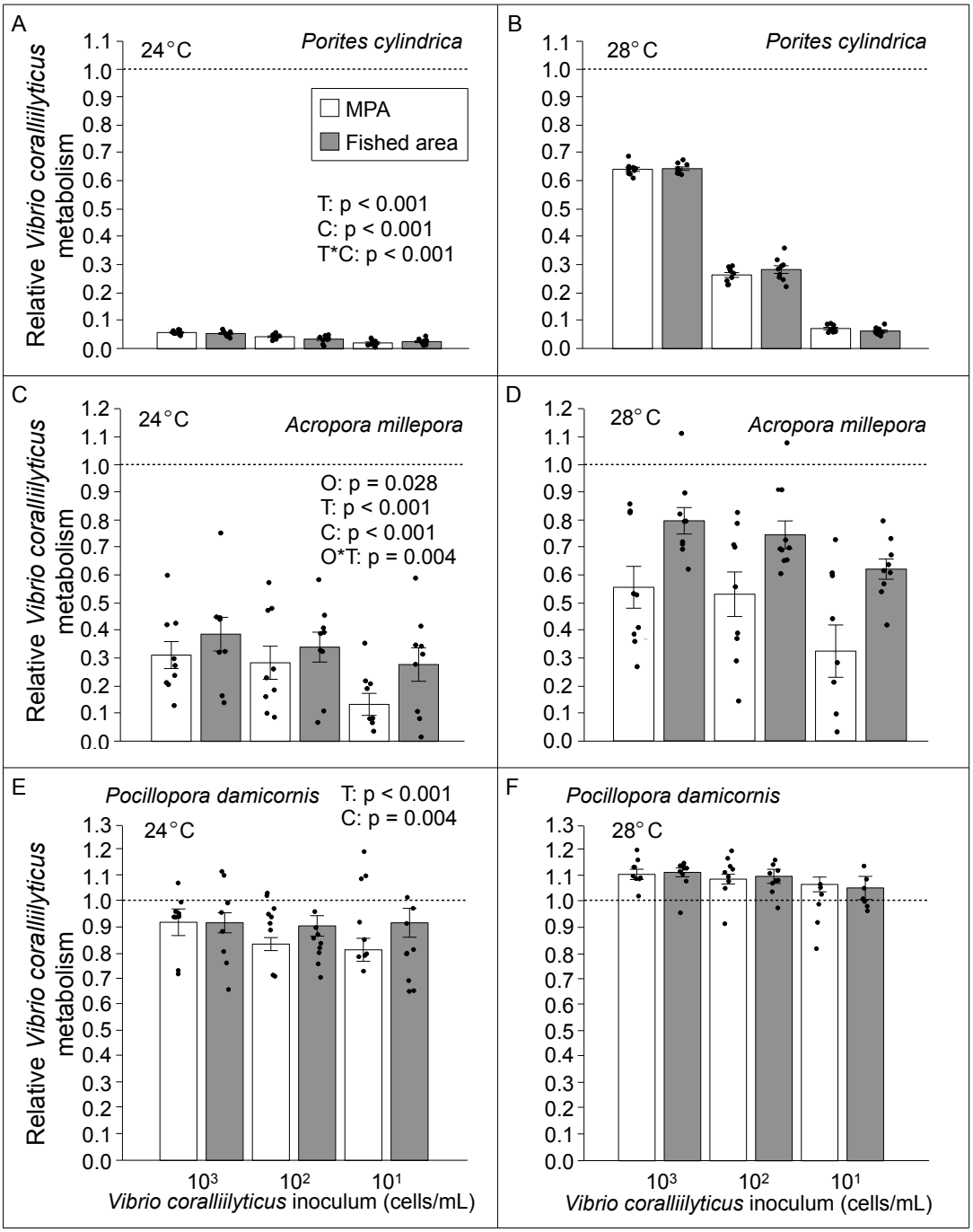

Figure 2.1: Mean (±SE) activity of coral water relative to reef water against *V. coralliilyticus* at 24°C and 28°C for *Porites cylindrica (A-B)*, *Acropora millepora (C-D)*, and *Pocillopora damicornis (E-F)*. The dashed line at 1.0 is the expected value if there is no effect. *P*-values are from linear mixed effects models implemented with Akaike Information Criterion (AIC) for model selection (n = 9). Factors remaining for each species' model after the model selection process are provided. O, T, and C represent origin (MPAs or fished areas), temperature, and concentration of pathogen inoculum, respectively. Dots indicate individual data points. One data point with negative values (after reduction of the optical density of sterilized coral water, see methods for

information on data reduction) is not depicted in each of C (data point value is -0.040) and in D (data point value is -0.078) for the 10 cells per mL concentration MPA samples.

For *A. millepora*, inhibition differed significantly between individuals from coral-dominated MPAs versus macroalgae- dominated fished areas (Figure 2.1C & D, linear mixed effect model), especially at the higher temperature. At 28°C, coral water of *A. millepora* from MPAs was 1.8-2.5 times more inhibitory than that from individuals in fished areas (Figure 2.1D & Figure B.1.B); this effect persisted across pathogen inoculum densities (Figure B.1.B). At 24°C, patterns were similar but less dramatic (Figure 2.1C & B.1.A).

Composition of coral microbiomes differed significantly among coral species (PERMANOVA $p = 0.001$), but not between conspecifics from MPAs and fished areas (Figures 2.2 & B.2). The large difference in anti-pathogen potency of *A. millepora* from MPAs versus fished areas was not paralleled by a significant difference in the coral's microbiome between areas. There were no between-habitat type differences in the composition of *A. millepora* microbiomes whether all *A. millepora* samples were tested together via two-factor analysis (Figure 2.2B PERMANOVA Origin $p = 0.101$) or independently at each village by one-factor analysis (PERMANOVA on factor Origin at Namada, $p = 0.679$; at Vatu-o-lalai, $p = 0.111$; at Votua, $p = 0.132$). Microbiome composition of *A. millepora* differed among villages (Figure 2.2B PERMANOVA Village $p = 0.001$), but collections across villages were taken at different times, making it impossible to distinguish location versus time effects. However, in *A. millepora* (where anti-*Vibrio* activity was weaker in fished areas), dispersion (variance) in microbiome composition was significantly higher in fished areas compared to MPAs (Figure 2.2B PERMADISPERSION Origin $p = 0.013$), and also elevated in comparison to the other

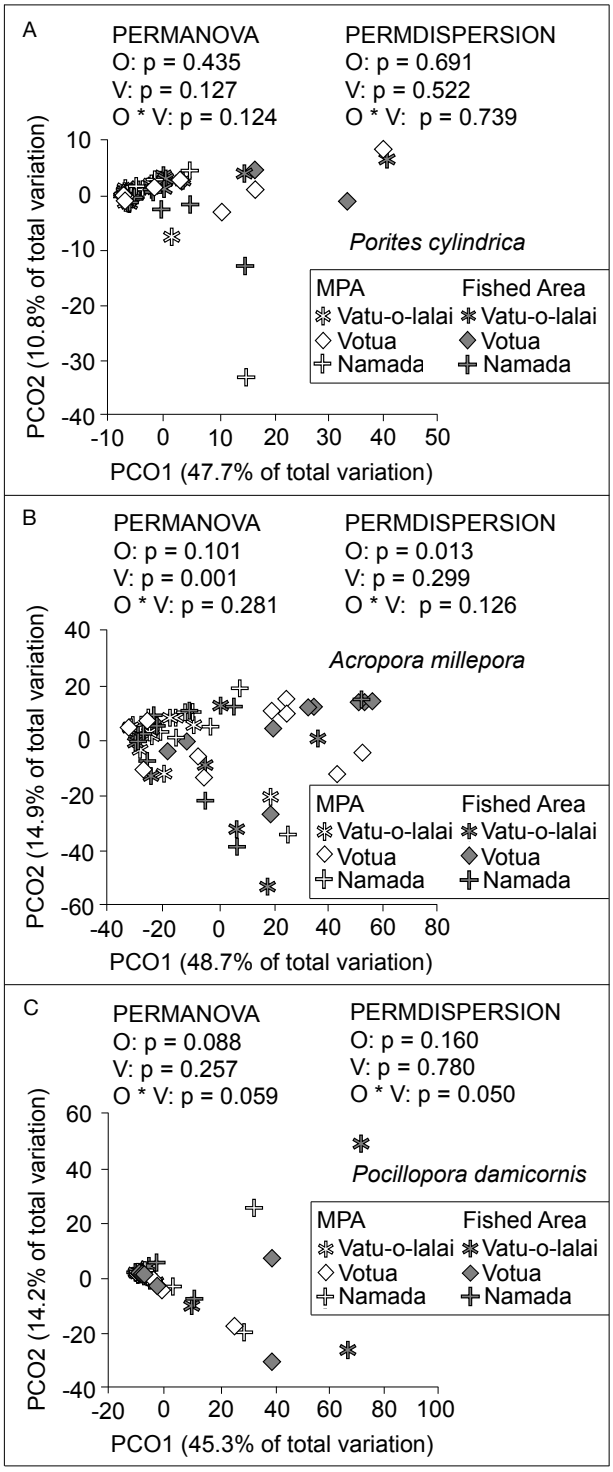

Figure 2.2: Principal coordinate analysis with PERMANOVA and PERMDISPERSION tests of microbial community compositio and dispersion on operational taxonomic unit (OTU) tables rarefied to a uniform sequencing depth of 7,700 sequences per sample. (A) *Porites cylindrica* (n = 28, 30 for MPA and fished area coral), (B) *A. millepora* (n = 29, 28 for MPA and fished area coral), (C) *Pocillopora damicornis* (n = 26, 23 for MPA and fished area coral). Abbreviations O and V represent factors origin (MPA or fished area) and village, respectively.

two coral species (Table B.2.B & C). For *P. cylindrica* and *P. damicornis*, dispersion did not differ between areas (Figure 2.2A and 2.2C).

Alpha diversity (operational taxonomic unit [OTU] richness and Shannon diversity) did not differ between coral microbiomes from MPAs and fished areas, except for *P. damicornis*, which exhibited higher OTU richness in fished areas (78-135 OTUs) compared to MPAs (68-90 OTUs; Figure B.3E). This difference was not associated with a difference in *P. damicornis* anti-*Vibrio* potency between corals from MPAs versus fished sites. The microbiomes of all three coral species were characterized by >80% relative abundance of OTUs in the Endozoicimonaceae (Gammaproteobacteria, 1,404 total OTUs across datasets; Figure B.2, Table B.3), with each species harboring a unique Endozoicimonaceae community (PERMANOVA coral species $p = 0.001$ overall, and for each pairwise test $p = 0.001$). Indicator analyses identified Endozoicimonacae OTU (987) as an indicator of *A. millepora* from MPAs (specificity 85.9%, fidelity 55.2%), making it a candidate for production of *Vibrio* suppressive compounds. OTU (922761) of the Enterobacteriaceae (Gammaproteobacteria) was an indicator of fished area *A. millepora* (specificity 89.7%, fidelity 50.0%), and OTU (823476) of the genus *Alteromonas* (Gammaproteobacteria) was an indicator of fished area *P. damicornis* (specificity 97.7%, fidelity 82.6). Significant indicator OTUs were not detected for *P. cylindrica*.

Despite the lack of intraspecific differences in community composition of coral microbiomes between coral- versus macroalgal-dominated sites, the taxonomic composition of microbiomes from benthic water (water from within 1 cm of hard bottom surfaces) did differ significantly between MPAs and fished areas, as well as between villages (Figure B.4, PERMANOVA Origin $p = 0.001$, Village $p = 0.001$, Origin*Village $p = 0.001$). We identified 269 OTUs as indicators of MPA benthic water, and 502 OTUs as indicators of fished area water. OTU richness was greater in fished areas (3215-3488

OTUs) than MPAs (2875-2986 OTUs) (Figure B.5A, two-factor permutation ANOVA Origin $p = 0.018$). The lack of differences in microbiome composition with reef state for all three corals, despite the large differences in microbiome composition of benthic water, suggests that corals are regulating their microbiomes in spite of considerable biotic differences in their surroundings. Shannon diversity and among-sample dispersion in microbiome composition of benthic water did not differ based on reef state or village site (Figure B.4 & B.5B, permutation two-factor ANOVA).

Discussion

Coral reefs are undergoing precipitous decline, with functionally and evolutionarily critical species, such as acroporids, often exhibiting the greatest losses (Marshall and Baird 2000, Hughes et al. 2018b). Previous studies have suggested that coral microbiomes are critical in protecting corals from pathogenic microbes and may be compromised by elevated temperatures (Ritchie 2006, Krediet et al. 2013, Zaneveld et al. 2016), and that macroalgae may chemically destabilize and fundamentally alter coral microbiomes (Barott and Rohwer 2012, Morrow et al. 2012). Here, we show that: i) all three corals we tested possessed a chemical defense against a coral-bleaching pathogen, ii) this defense was more effective at 24°C than at 28°C, iii) high abundances of seaweeds compromised the chemical defense of the ecologically important coral genus, *Acropora*, but not the resistant genus *Porites* (one of the most persistent genera on degraded reefs) or the weedy genus *Pocillopora*, and iv) the variance in potency of coral water from *Acropora* collected from macroalgal- versus coral-dominated areas coincides with different indicator strains (within Endozoicimonaceae and Enterobacteriaceae) and with declines in microbiome stability, rather than with large-scale changes in microbiome community composition.

All corals suppressed the pathogen *V. coralliilyticus* at 24°C, but suppression was compromised to varying degrees at 28°C. As we tested the effects of chemistry from sterilized coral water rather than effects of the living coral or its living microbiome, this suggests that chemical defenses are compromised at elevated temperatures. This might occur due to compound degradation at elevated temperatures, or to more rapid growth of *Vibrio* at elevated temperatures and the single dose of compound(s) being bound to, or degraded by, these more abundant cells.

Although the potency of *P. cylindrica* and *P. damicornis* defenses was unaffected by reef state (coral versus macroalgal domination), coral water of *A. millepora* from algal-dominated reefs exhibited a 43-61% decline in inhibition compared to conspecifics from coral-dominated reefs and this decline was greater at elevated temperature. The decline of an acroporid coral's chemical defense against a thermally regulated coral pathogen on algal-dominated reefs is especially worrisome given that thermal stress events have increased in frequency by five-fold over the last 40 years (Hughes et al. 2018a), and that acroporids are disproportionately important in generating structure on reefs (Kerry and Bellwood 2015), are associated with increased diversity of other critical reef species such as fishes (Bellwood et al. 2017), and have suffered high losses associated with ocean warming and disease (Marshall and Baird 2000, Harvell et al. 2007). It is equally interesting that the potency of *P. cylindrica* defenses were high and unaffected by reef state; this genus is commonly one of the most persistent on degraded reefs (Green et al. 2008, Adjeroud et al. 2009).

Despite not differing in community composition, *A. millepora* within algal-dominated areas did exhibit an increase in microbiome compositional variability (dispersion) relative to conspecifics in the MPAs. This is consistent with increased variability of host microbiomes in response to stressors in a variety of species, including

corals, mice, chimpanzees, and humans (Zaneveld et al. 2017). The increased variability of *A. millepora* microbiomes within fished areas could indicate a decline of regulatory mechanisms that constrain the coral microbiome (Krediet et al. 2013, Zaneveld et al. 2017). In support of this hypothesis, *A. millepora* from fished areas were relatively depleted in an indicator bacterium of the Endozoicimonaceae, a family hypothesized to play mutualistic roles in coral health (McDevitt-Irwin et al. 2017). In contrast, fished area *A. millepora* were enriched in an indicator bacterium of the family Enterobactericeae, a ubiquitous bacterial family with members previously associated with coral disease (Sunagawa et al. 2009, Daniels et al. 2015). The functional significance of these nuanced shifts in OTUs between MPA and fished area corals are unknown, but are consistent with recent investigations (Beatty et al. 2018, Ramirez et al. 2018) suggesting that minor alterations in microbiome composition may lead to large biotic consequences.

Unlike *A. millepora* microbiomes, those of *P. cylindrica* and *P. damicornis* did not differ in variability between macroalgal-dominated fished areas and coral-dominated MPAs. This lack of change for *P. cylindrica* may help explain its common persistence on degraded reefs (Green et al. 2008, Adjeroud et al. 2009). *P. damicornis* is a rapidly colonizing weedy species; microbiomes from fished areas had higher OTU richness compared to conspecifics from MPAs. Elevated microbiome diversity and richness in response to stressors is common (McDevitt-Irwin et al. 2017) and could reflect a decline of regulatory mechanisms or attempts to adjust to changing conditions via alterations in the protective microbiome.

Despite drastic differences in benthic algal abundances (Rasher et al. 2013) and benthic seawater microbiome composition (Figure B.4), we did not detect community-level differences in coral microbiome composition between MPAs and fished areas for any of the species investigated (though a few differences in dispersion or indicator OTUs

were detected). This result contrasts with previous work indicating that coral-seaweed interactions cause the microbiome to shift towards one enriched in copiotrophic and virulent bacteria (Barott and Rohwer 2012, McDevitt-Irwin et al. 2017) or become more similar to seaweed microbiomes upon direct seaweed-coral contact (Pratte et al. 2017). We did not test for responses to direct seaweed contact, but our results indicate that dramatic differences in macroalgal abundance and in the surrounding microbial community may have more nuanced effects (e.g., on indicator taxa and dispersion) within coral microbiomes, while dominant community members (Figure B.2) appear to persist, even under even strongly diverging field conditions.

Reef state impacts corals in species-specific ways. For an acroporid coral from macroalgal-dominated areas, we detected a decline in chemical defense against a coral bleaching pathogen, loss of a potentially beneficial symbiont in the Endozoicimonaceae, and increases in microbiome variability. In contrast, chemical defense and microbiome variability did not differ in *P. damicornis* and *P. cylindrica* between fished areas and MPAs, suggesting greater resistance to algal-induced stress. Regardless of collection site, the microbiomes of all three corals were dominated by Endozoicimonaceae, with each coral harboring a distinct Endozoicimonaceae community. Host-specific Endozoicimonaceae compositions may therefore play a role in coral resistance to pathogens. Only nine Endozoicimonaceae occurred at $\geq$1% average abundance, making them likely candidates for tests of their functional roles in coral microbiomes. OTU987 was an indicator of *A. millepora* from MPAs; it may be linked to the elevated antipathogen activity of *A. millepora* from MPAs.

It is presently uncertain whether the antibiotic effects documented for all three corals, or the variable effects for *A. millepora* from MPAs versus fished areas, are host, or symbiont, produced. The compound(s) involved are unidentified, but our assays are likely conservative in terms of documenting potency due to our collection method, which

involved dilution of the coral products with 50 mL of seawater. Additionally, live organisms often produce chemical defenses continuously; our tests were run using a single dose (the chemicals present at the time of collection) that likely declined as it was metabolized or degraded over the course of storage and deployment in the experiment. Thus, the antipathogen effects observed *in vitro* may be diminished compared to those in corals on the reef. Determining the compounds responsible for these effects, as well as their concentration and potency *in situ* and across other host taxa, would be useful.

Conclusion

Processes like ocean warming, overfishing, pollution, and other anthropogenic stresses not only suppress corals and advantage seaweeds, but also create positive feedbacks that suppress coral reef resilience (Mumby and Steneck 2008). Acroporid corals are disproportionately important for reef recovery. They provide the critical topographic complexity (Kerry and Bellwood 2015) that facilitates diversification of numerous groups of reef fishes (Bellwood et al. 2017), and their rapid growth and high abundance make them critically important for maintaining reef accretion through time. These corals are also among the most threatened, and rapidly declining, due to bleaching and disease (Marshall and Baird 2000, Harvell et al. 2007). Here, *Acropora millepora* exhibited a decline in defense against *Vibrio coralliilyticus* when collected from algal-dominated, fished reefs. At our study sites, recovery of herbivores and declines in macroalgae following protection from fishing (Rasher et al. 2013, Bonaldo et al. 2017) were associated with coral water from *A. millepora* being 75-154% more inhibitory to *V. coralliilyticus* at 28°C. This suggests that local management to suppress macroalgae may benefit reefs in the face of global pressures by promoting chemical defense against climate induced coral pathogens for critical acroporid species during warming events.

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CHAPTER 3

ACCLIMATIZATION OF CORAL ANTI-PATHOGEN CHEMICAL DEFENSE IN RESPONSE TO RAPID SHIFTS BETWEEN CORAL AND MACROALGAL DOMINATED REEFS

Abstract

Coral reefs are undergoing precipitous decline due to coral bleaching and disease following warming events, often causing shifts from coral to macroalgal reef dominance. We reciprocally transplanted three coral species between spatially paired coral dominated marine protected areas (MPAs) and algal dominated fished areas to test for the effects of reef origin (historical) and current reef (acclimatization) on coral chemical defense toward the common coral bleaching pathogen *Vibrio coralliilyticus.* For an ecologically sensitive acroporid species, both historical reef state and current reef state influenced the potency of chemical defense, but for an ecologically hardy poritid coral or a weedy pocilloporid coral, chemical potency was not altered. *Acropora millepora* that originated from, or were transplanted into, coral dominated MPAs exhibited 46% and 36% increase, respectively, in inhibition of *V. coralliilyticus* relative to those that originated from, or were transplanted into, macroalgal-dominated fished areas. *A. millepora* also exhibited historical reef effects on their microbial community composition, notably by persistently higher relative abundances of Vibrionaceae among individuals that originated from macroalgal dominated fished areas compared to individuals that originated from coral dominated MPAs. For some ecologically important but bleaching susceptible species like acroporids, macroalgal dominated reefs may suppress coral chemical defense against *Vibrio* bleaching pathogens and facilitate blooms of *Vibrio* bacteria that may harm corals following periods of stress.

Introduction

Coral reefs are among Earth's most biodiverse ecosystems. Despite covering only 0.2% of the ocean floor (Cesar et al. 2003), coral reefs contain ~ 2,800 species of reef fishes in the IndoPacific (Mora et al. 2003) and ~570 in the Caribbean (Weigt et al. 2012), with about three billion humans depending upon a subset of these fishes as a critical source of protein (Cesar et al. 2003). Coral reefs also provide a diversity of other ecosystem goods and services including protection from storm surge, tourism-generated revenues, and potential drugs from marine organisms (Cesar et al. 2003). Goods and services provided by coral reefs are valued at >$30 billion USD per year globally (Spalding et al. 2017), but are threatened by thermally-induced bleaching events (Chen et al. 2015) that have increased in frequency by 5-fold over the last four decades owing to increases in anthropogenic CO_2 (Hughes et al. 2018a). Consequently, reefs are undergoing rapid decline, largely due to the loss of critical reef building species within the family Acroporidae following bleaching events (Marshall and Baird 2000, Hughes et al. 2018b). The relative roles of coral loss from direct heat stress versus complex interactions among heat stress and disease, or other factors, is unclear, but coral-bacterial interactions are postulated to play an important role in coral decline or persistence in response to climate change (Harvell et al. 2007, Krediet et al. 2013, McDevitt-Irwin et al. 2017, Peixoto et al. 2017).

It is becoming increasingly clear that corals host beneficial microbes within their microbiomes that provide nutrients to their host, may protect the coral against harmful bacteria, and appear critical for host health and persistence (Ritchie 2006, Krediet et al. 2013, Thompson et al. 2015). Corals regulate their microbiome via mechanisms including: sloughing of mucus, consumption of microbes, production of antimicrobial peptides, and via hosting predatory bacteria that may selectively prey on pathogenic bacteria within the coral microbiome (Krediet et al. 2013, Thompson et al. 2015, Welsh et al. 2015). Thus, it is concerning that warming may alter these regulatory processes,

resulting in loss of beneficial bacterial associates and of their protective roles (Ritchie 2006, Zaneveld et al. 2016).

Bacterial dysbiosis (loss of beneficial bacteria or increases in harmful bacteria) is occurring with increasing frequency on degraded reefs (Dinsdale et al. 2008) and increasing abundances of macroalgae on these reefs are hypothesized to be a driver of coral dysbiosis (Barott and Rohwer 2012, Morrow et al. 2012, McDevitt-Irwin et al. 2017). Algae may harm corals via multiple mechanisms, including direct toxicity (Morrow et al. 2012), releasing dissolved organics that lead to blooms of virulent bacteria in surrounding waters (Barott and Rohwer 2012, Haas et al. 2016), and by vectoring algal bacteriomes to corals following contact (Pratte et al. 2017). In some cases algae may vector pathogenic microbes to corals, though additional stressors are likely to play a critical role in disease initiation and progression (Nugues et al. 2004, Sweet et al. 2013). Increases in the frequency of warming events and interactions with algae on degraded reefs may lead to irreversible changes to corals' beneficial bacteriome and to corals' ability to withstand various stressors.

Recent work indicates that coral-dominated reefs resulting from protection status can bolster an acroporid coral's chemical defense toward a thermally-regulated coral bleaching pathogen, *Vibrio coralliilyticus*, compared to fished reefs dominated by macroalgae (Beatty et al. chapter 2). Enhanced chemical defense coincided with decreased microbiome variability and increases in a putative beneficial bacterium in the family Endozoicomonaceae (Beatty et al. chapter 2). However, it is unclear how coral chemical defense may change in response to rapid shifts in reef state (e.g. changes from coral to macroalgal dominance) that can occur after disturbances such as heat generated bleaching events, damaging cyclones, or disease-related coral die-offs. Understanding how corals respond to rapid changes in reef state may be helpful for predicting reef trajectories under current and future climate scenarios.

By reciprocally transplanting three coral species between replicated pairs of adjacent coral versus macroalgal dominated reefs, we investigated how coral bacteriomes and coral chemical defense toward the thermally-regulated bleaching pathogen, *V. coralliilyticus,* responded to rapid changes in benthic community composition. We utilized two pairs of marine protected areas (MPAs) and adjacent fished areas that differed in benthic community composition as a result of local management. Prohibiting fishing in marine protected areas for 10-12 years prior to this study resulted in 2 to 5 fold increases in herbivory, 75-95% reductions in algal cover, a 3 fold increase in coral recruitment, and 2 to 10 fold more coral cover (Rasher et al. 2013, Bonaldo et al. 2017). These reefs are interspersed along the southwestern coast of Viti Levu, Fiji and allow for tests of benthic community composition on coral chemical defense without confounding physical or oceanographic features.

V. coralliilyticus is an ecologically realistic assay pathogen because it is distributed globally (Kimes et al. 2012), targets diverse groups of corals (pocilloporids, agaricids, and acroporids (Sussman et al. 2008, Bourne et al. 2015), and becomes more virulent under elevated water temperatures causing bleaching and mortality of corals across the IndoPacific (Ben-Haim et al. 2003, Sussman et al. 2008, Kimes et al. 2012). An acroporid coral transplanted from macroalgal to coral dominated areas exhibited enhanced potency of its chemical defenses against *V. coralliilyticus* after 28 days, compared to those transplanted from coral to macroalgal reefs. However, 28 days after transplanting, historical reef state (reef of origin) also continued to influence this coral's chemical defense. We discuss these findings in relation to changes in this coral's bacteriome and in comparison to a resistant poritid and a weedy pocilloporid coral whose anti-*Vibrio* chemical defenses did not change in response to experimentally-induced shifts in reef state following reciprocal transplantation between coral and algal dominated reefs.

Material and Methods

Coral species and sites

We investigated how historical (origin) and the current (transplant area) reef state impact coral microbiomes and coral chemical defense against the coral pathogen *Vibrio coralliilyticus* by reciprocally transplanting corals between coral-dominated marine protected areas (MPAs) and algal-dominated fished areas. We utilized three common corals, *Porites cylindrica, Acropora millepora*, and *Pocillopora damicornis*, that were reciprocally transplanted between adjacent pairs of MPAs and fished areas at two villages, Vatu-o-lalai (18°12.26' S, 177°41.26' E) and Votua (18°13.08' S, 177°42.59' E), along the southwest coast of Viti Levu, Fiji. MPAs were coral-dominated and macroalgae depauperate (38-56% live coral cover and $\leq$3% macroalgal cover), while fished areas were algal-dominated and coral poor (4-16% live coral cover and 50-90% macroalgal cover). Differences in coral versus macroalgal dominance resulted from local management that prohibited fishing for 10-12 years prior to our study, resulting in 6 – 16 fold greater biomass of herbivorous fishes, 2 – 5 fold greater herbivory, and 75 -95% reductions in cover of macroalgae (Rasher et al. 2013, Bonaldo and Hay 2014, Bonaldo et al. 2017).

Coral was collected haphazardly from each reef (n =10 colonies per species per MPA or fished area at each village), fragmented into ~30-50g clones (six per colony), and planted in plastic bottle tops with Emerkit® epoxy (following methods of (Clements and Hay 2015). Briefly, inverted lids of the bottle were attached to the reef substrate by driving a nail through the lid's center; corals epoxied into the sawed-off top of a plastic bottle could then be attached to the benthos by screwing the bottle top into the bottle lid. After fragmenting and epoxying corals into bottle tops, corals were attached to lids on the benthos of their reef of origin and allowed to recover for 28 days. After this recovery period, corals were reciprocally transplanted between MPAs and fished areas at each

village. This occurred on 21-24 October 2014 at Vatu-o-lalai village and 25-29 October 2014 at Votua village, with equal numbers of each species planted each day.

Ten spatially blocked sites for transplantation were located haphazardly within each reef area, with blocks separated by ~4-5m and corals within a block separated by 20-50cm. Treatments within each block consisted of origin (MPA or fished area) crossed with transplant area (MPA or fished area), such that half of the fragments for each species stayed on their reef of origin and half moved to their adjacent paired reef at each village. To maintain equal handling of corals among treatments, at the end of the recovery period all corals were unscrewed from the benthos, attached to boards used to transfer corals between sites, and treated to equivalent movement patterns before reattachment to the benthos in their various treatment sites. These procedures allowed investigation of how area of origin (historical reef) and transplant area (current reef) influence coral microbiomes and anti-pathogen chemical defense. Corals were sampled 28-29 days after transplanting, with equal numbers of each species and treatment sampled each day. Approximately one-gram samples from each coral were preserved in RNAlater® (QIAGEN) and frozen at -20°C until DNA extraction and sequencing to assess composition of the microbiome. Additionally, ~50 mL volumetric displacement of each coral was collected to assess the coral's anti-pathogen chemical defenses (see below).

Antipathogen activity of corals

To test for coral chemical inhibition of the thermally regulated pathogen *Vibrio coralliilyticus* (ATCC BAA-450), each coral was volumetrically displaced in a 1:1 ratio with seawater collected from the reef and agitated in a glass jar for 20 seconds (as performed in chapter 2). The resulting "coral water" containing coral mucus and any other material, such as antibacterial compounds released during agitation (Geffen & Rosenberg 2005), was decanted into a sterile polystyrene tube and frozen at -20°C. We

screened coral water against *V. coralliilyticus,* using artificial seawater (Instant Ocean®) as a control. Artificial seawater does not differ from seawater collected from the reef (n = 27 permutation ANOVA p = 1.0) when tested against *V. coralliilyticus* using methods listed below. 100 μL of coral water or artificial seawater was aliquoted into wells of sterile 96-well round bottom plates, frozen and dried under vacuum. We then sterilized the dried material by UV-radiation for 90 seconds to assure that we were testing chemical traits without biotic interactions among live microbes confounding results. Sterilized and dried coral water or artificial seawater was reconstituted in 100 μL Marine Broth (Difco™ 2216) containing 100 cells per mL of *V. coralliilyticus.* Tetrazolium chloride (TTC, final concentration 0.05 μg/μl) was supplemented to each well as an indicator of pathogen growth; microbial metabolism reduces tetrazolium chloride (colorless) to triphenylformazan (red pigment) which can be quantified via absorbance. We chose this method over cell counts or measures of turbidity because these latter methods assess both live and dead or active and inactive cells, while this method measures only metabolically active cells. Plates were incubated at 28°C for 16 hours. We selected 28°C for the incubation temperature because *V. coralliilyticus* upregulates virulence at 27°C and higher. 28°C is also commonly experienced during the summer months along the reefs in Fiji and this temperature is effective for detecting variance in coral chemical defense among differing environments (chapter 2). We measured absorbance of triphenylformazen at 490 nm a BioTek ELx800 absorbance reader. To determine absorbance due solely to bacterial metabolism, background absorbance of lyophilized and UV-radiated coral water or artificial seawater supplemented with TTC but without bacteria was deducted from each well containing bacteria. These blank-corrected values were compared statistically via ANOVA or permutation ANOVA in RStudio3 package lm perm v 2.1.0 to determine if coral water altered microbial metabolism compared to

artificial seawater. Relative *V. coralliilyticus* metabolism, expressed as a ratio of microbial metabolism in response to coral water relative to artificial seawater was utilized to test for differences due to the historical reef state (origin), current reef state (transplant area), and village via a three factor ANOVA or permutation ANOVA in RStudio3 within package lm perm v 2.1.0. Values greater than 1 in relative metabolism indicate the coral water is stimulatory and values less than 1 indicate the coral water is inhibitory.

DNA extraction and sequencing of the 16S rRNA gene

DNA extraction and sequencing of the 16S rRNA gene from the frozen coral chips was utilized to characterize the microbial communities associated with each coral sample. Corals preserved in RNAlater® (QIAGEN) were subjected to DNA extraction with MoBio PowerSoil kits (MoBio Laboratories, QIAGEN, Carlsbad, CA). We pelleted any dissociated microbial cells in the RNALater solution by centrifugation at 10,000 rpm for 10 minutes. We reconstituted the pellet with solution C1 and added this to the powerbead tube with approximately 250mg of coral (MoBio Laboratories, QIAGEN). Dual-barcoded Illumina Nextera fusion primers F515 and R926 were used to amplify the V4-V5 region of the 16S gene as described in Comeau et al. (2017). Briefly, 25 μL PCR reactions were carried out in duplicate using high-fidelity polymerase and 2 μL of template DNA. Thermal cycling involved initial denaturation at 98°C (30 s), 30 cycles of denaturation at 98°C (10 s), primer annealing at 55°C (30 sec), primer extension at 72°C (30 sec), and final extension at 72°C (4:30 min). PCR products verified on an Invitrogen 96-well E-gel, amplicons were then cleaned and normalized on Invitrogen SequalPrep 96-well plates. Amplicons were sequenced on an Illumina MiSeq at the Centre for Comparative Genomics and Evolutionary Bioinformatics at Dalhousie University.

Microbiome Data Analyses

TrimGalore! (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) was implemented to trim sequence reads, removing Nextera adapters (100 bp minimum read length) and to filter reads with low quality scores (Phred score cutoff 25). Trimmed read-pairs were merged using FLASH with the criterion of >250bp input read length and >300 bp merged fragment length, with standard deviation of <30 bp. Merged reads were imported in QIIME2 using a manifest file. Deblur (Amir et al. 2017) was implemented within QIIME2 to remove erroneous sequences, trim sequences to 120bp, and remove chimeras. We extracted reads from SILVA 99% similarity consensus taxonomy with our primer pair (F515 & R926), trimmed to 120bp, and trained sequences with a Naïve Bayes model within QIIME2. This classifier was implemented to classify our sequence variants with single nucleotide resolution. After quality filtering, 1,434,438 sequences remained from 6,694,241 sequences generated by the MiSeq run, with per-sample counts ranging from 30-65,514 sequences per sample. Sequence variant tables were rarefied to a uniform depth of 1,185 sequences per sample to avoid confounding sequencing depth with a biological signal (Weiss et al. 2017).

Alpha diversity (number of sequence variants and Shannon Diversity) and beta diversity (Bray Curtis) were calculated in PrimerE (Clarke 1993). We tested for differences in alpha diversity within RStudio3 package lm perm by a three factor ANOVA or permutation ANOVA if data were not homoscedastic (factors: origin, transplant area, village). We tested for differences in beta diversity by PERMANOVA and PERMDISPERSION on log transformed [Log(x+1)] in PrimerE by three factor analysis (factors: origin, transplant area, village). Indicator species analysis (multilevel pattern analysis) was performed within RStudio3 indicspecies package to determine indicator sequence variants (single nucleotide changes are classified as unique sequence variants) among groupings of origin crossed with transplant area. Sequence variants are considered indicators if their fidelity scores were 0.50 or greater (i.e. occurrence in ≥

50% of corals from a treatment group). We implemented random forest analysis, a supervised machine learning classifier, within QIIME2 (command line: sample-classifier classify-samples) to determine taxa that are predictors of sample groupings (origin x transplant area). Briefly, the sequence variant table was first collapsed at the family level, randomly split into training and test groups (0.80 & 0.20, respectively), trained with 100 decision trees using a random forest algorithm and validated with a stratified k-fold cross-validation scheme. Following identification of predictive taxa by random forest analysis, we tested these predictive taxa by a two factor ANOVA or permutation ANOVA in RStudio3 package lm perm for differences between origin and transplant area.

Results

Antipathogen activity

Coral water from *P. cylindrica, A. millepora*, and *P. damicornis* inhibited *V. coralliilyticus* by 46-67%, 18-46%, and 0-29%, respectively, relative to controls (Figure 3.1A-C, permutation ANOVA $p < 0.001$ for each species comparing coral water to artificial seawater). Inhibition did not differ between origin or transplant area for *P. cylindrica* (Figure 3.1A, ANOVA Origin $p = 0.742$, Transplant Area $p = 0.940$, Village $p = 0.486$) or *P. damicornis* (Figure 3.1C, permutation ANOVA Origin $p = 0.332$, Transplant Area $p = 0.745$, Village $p = 0.060$). In contrast, both origin and transplant area influenced *A. millepora* chemical defense against *V. coralliilyticus* (Figure 3.1B, ANOVA Origin $p = 0.002$, Transplant Area $p = 0.009$, Village $p = 0.568$, Origin*Transplant Area $p = 0.758$). *A. millepora* that originated from, or were planted into, coral-dominated MPAs exhibited 46% and 36% higher, inhibition of *V. coralliilyticus,* respectively, relative to those that originated from or were planted into macroalgal-dominated fished areas.

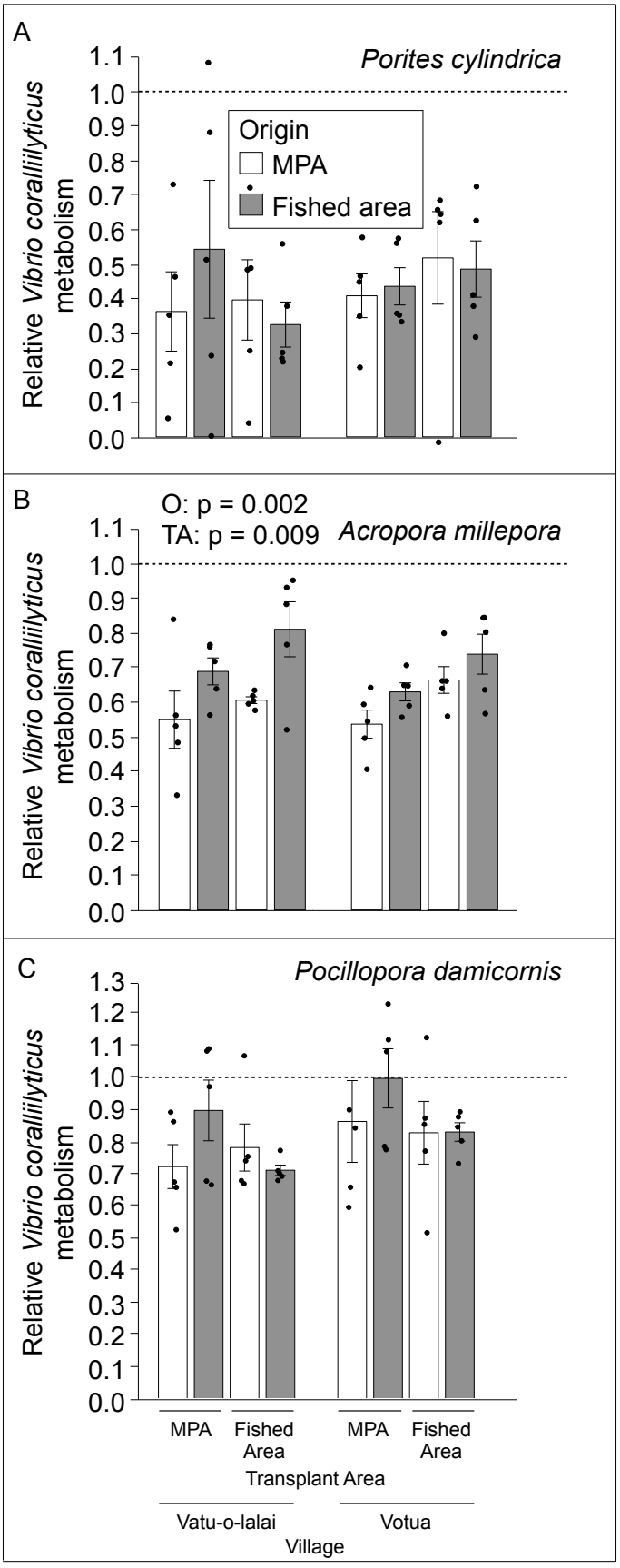
A
Porites cylindrica
Origin
MPA
Fished area
Relative Vibrio coralliilyticus metabolism
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
B
O: p = 0.002
TA: p = 0.009
Acropora millepora
Relative Vibrio coralliilyticus metabolism
C
Pocillopora damicornis
Relative Vibrio coralliilyticus metabolism
1.3
1.2
MPA
Fished Area
MPA
Fished Area
Transplant Area
Vatu-o-lalai
Votua
Village

Figure 3.1: Mean (±SE) activity of coral water relative to artificial seawater against *V. coralliilyticus* at 28°C for *Porites cylindrica (A)*, *Acropora millepora (B)*, and *Pocillopora damicornis (C)*. The dashed line at 1.0 is the expected value if there is no effect. P-values are from ANOVA or permutation ANOVA (n = 20 per level of each factor). Significant factors for each species' are provided. O, TA, and V represent origin (MPAs or fished areas), transplant area (MPAs or fished areas), and village, respectively. Dots indicate individual data points.

Microbiomes

When we investigated if coral microbiomes correlated to anti-pathogen activity, we found that *Porites cylindrica*, whose anti-pathogen activity did not differ across treatments, also did not differ across treatments in microbiome community composition. Neither origin, transplant area, nor village influenced *P. cylindrica* microbiome composition or dispersion (Figure 3.2A, PERMANOVA Origin p = 0.360, Transplant Area p = 0.755, Village p = 0.279; PERMDISP p = 0.418, Transplant Area p = 0.782, Village p = 0.353). The anti-pathogen activity of *A. millepora* varied as a function of both origin and transplant area, but its microbiome varied only as a function of origin and village, not as a function of transplant area despite that treatment's significant effect on anti-pathogen activity (Figure 3.2B, PERMANOVA Origin p = 0.042, Transplant Area p = 0.210, Village p = 0.001, Origin*Transplant Area p = 0.211, Origin* Village p = 0.172, Transplant Area*Village p = 0.913, Origin*Transplant Area*Village p = 0.321). Differences between *A. millepora* microbiomes at each village were due in part to greater variability among *A. millepora* microbiomes at Vatu-o-lalai village compared to Votua village (PERMDISP Origin p = 0.153, Transplant Area p = 0.733, Village p = 0.003). Although the anti-pathogen activity of *P. damicornis* did not differ across treatments, origin, transplant area, and the interaction between these factors significantly influenced the composition of its microbiome (Figure 3.2C, PERMANOVA Origin p = 0.033, Transplant Area p = 0.001, Village p = 0.064, Origin*Transplant Area p = 0.038, Origin* Village p = 0.205, Transplant Area*Village p = 0.113, Origin*Transplant

Area*Village p = 0.502). Differences in *P. damicornis* microbiomes between transplant areas, occurred in part due to greater variability among *P. damicornis* microbiomes planted in MPAs compared to fished areas (PERMDISP Origin p = 0.091, Transplant Area p = 0.018, Village p = 0.326).

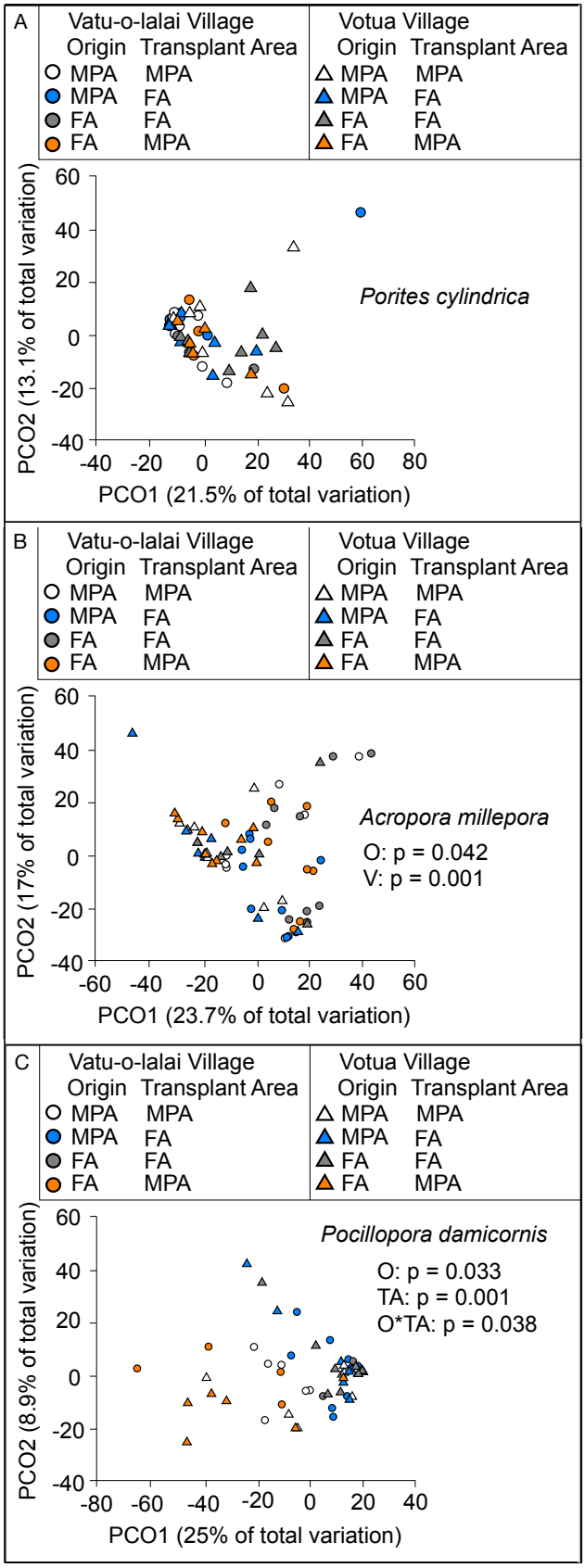
A
Vatu-o-lalai Village
Origin Transplant Area
MPA MPA
MPA FA
FA FA
FA MPA
Votua Village
Origin Transplant Area
MPA MPA
MPA FA
FA FA
FA MPA
PCO2 (13.1% of total variation)
PCO1 (21.5% of total variation)
Porites cylindrica
B
Vatu-o-lalai Village
Origin Transplant Area
MPA MPA
MPA FA
FA FA
FA MPA
Votua Village
Origin Transplant Area
MPA MPA
MPA FA
FA FA
FA MPA
PCO2 (17% of total variation)
PCO1 (23.7% of total variation)
Acropora millepora
O: p = 0.042
V: p = 0.001
C
Vatu-o-lalai Village
Origin Transplant Area
MPA MPA
MPA FA
FA FA
FA MPA
Votua Village
Origin Transplant Area
MPA MPA
MPA FA
FA FA
FA MPA
PCO2 (8.9% of total variation)
PCO1 (25% of total variation)
Pocillopora damicornis
O: p = 0.033
TA: p = 0.001
O*TA: p = 0.038

Figure 3.2: Principal coordinate analysis with PERMANOVA tests of microbial community composition on sequence variant tables rarefied to a uniform sequencing depth of 1,185 sequences per sample. (A) *Porites cylindrica* (n = 25-29 per village), (B) *A. millepora* (n = 32-34 per village), (C) *Pocillopora damicornis* (n = 21-33 per village). Abbreviations O, TA, and V represent origin (MPAs or fished areas), transplant area (MPAs or fished areas), and village, respectively.

Microbiomes of all three coral species were characterized by high abundances of Endozoicimonaceae (ranging 33-99% of community composition), with each species harboring a unique Endozoicimonaceae community (Figure 3.3A-C, Table C.1). Four, eight, and four unique sequence variants comprised the dominant ($\geq$2%) Endozoicimonaceae for *Porites cylindrica* (Endozoicimonaceae abundance 78-96%), *Acropora millepora* (Endozoicimonaceae abundance 66-99%), and *Pocillopora damicornis* (Endozoicimonaceae abundance 33-53%), respectively. *P. damicornis* microbiomes were also characterized by high abundances of Gammaproteobacteria (23-46%) and Vibrionaceae (0.17-37.75%; Figure 3.3C; Table C.2). Alpha diversity (number of sequence variants) or Shannon diversity did not differ with origin or transplant area for any species, with the exception of *P. damicornis* having higher Shannon diversity when planted into MPAs (Figure C.1F, permutation ANOVA Origin p = 0.961, Transplant Area p = 0.012, Village p = 1.000, Origin*Transplant Area p = 0.066, Origin*Village p = 0.941, Transplant Area*Village p = 0.540, Origin*Transplant Area*Village p = 1.000).

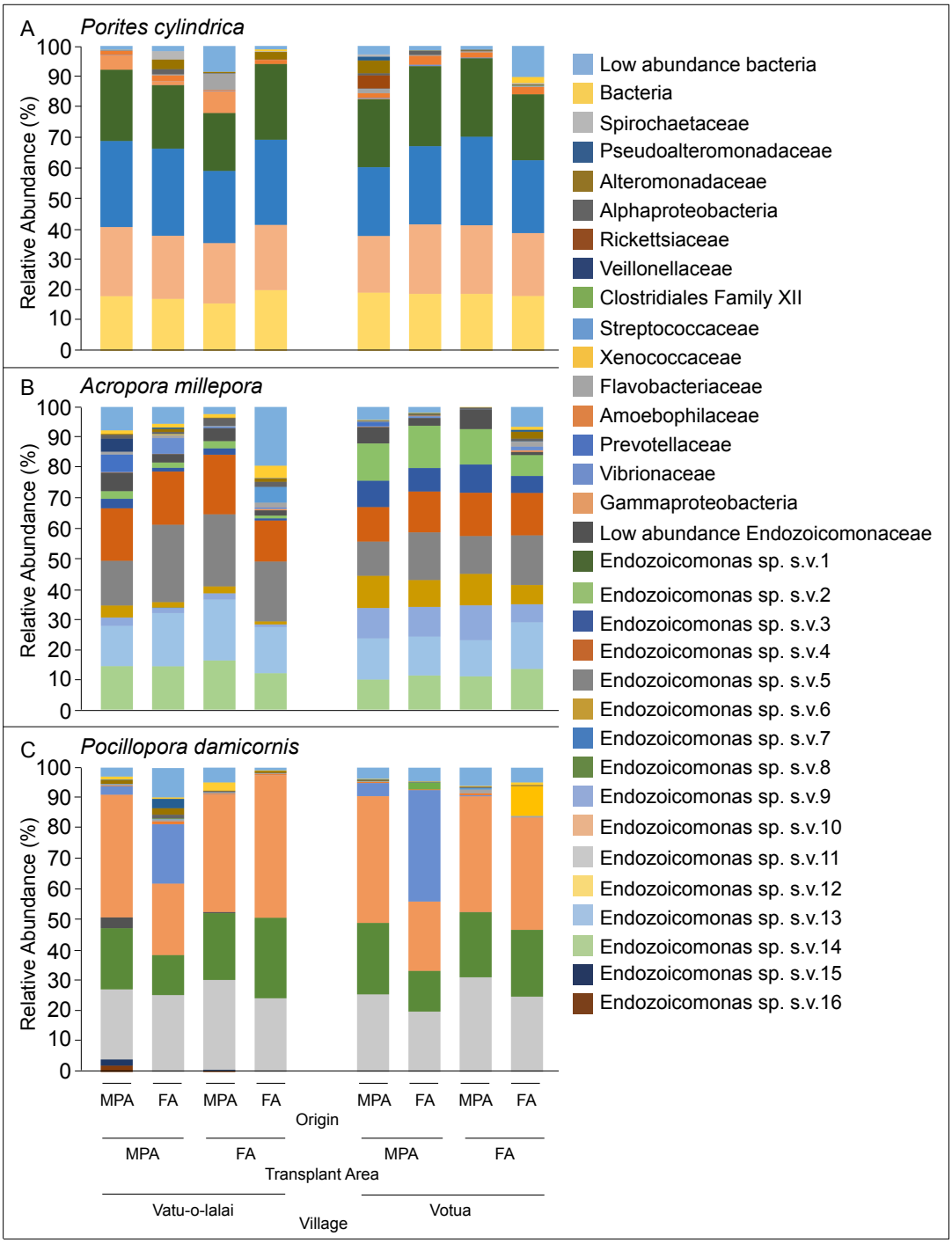

Figure 3.3: Microbial community composition for the three corals species. Taxa of <2% relative abundance were pooled by and depicted as 'low abundance bacteria'. Endozoicimonaceae are depicted at the sequence variant level for taxa contributing to 2% or greater composition, with all remaining Endozoicimonaceae sequence variants pooled to generate 'low abundance Endozoicimonaceae'.

Although microbial community composition did not change appreciably with changes in coral anti-pathogen activity, it is still possible that changes in particular microbes could explain the differences in potency. We conducted random forest analyses to assess this possibility. This analysis indicated that Endozoicimonaceae, Bacteroidia, Vibrionaceae, Alphaproteobacteria, Alteromonadaceae, and Flavobacteriaceae best predicted origin and transplant area for *A. millepora* (Figures C.2, C.3, importance scores: 0.098, 0.077, 0.073, 0.071, 0.050, 0.048, respectively, overall predictive accuracy 64%). Four predictive taxa occurred in higher relative abundances in coral from fished areas (Figure C.3, Bacteroidia, 13.73 times higher, O $p < 0.001$, TA $p = 0.064$, O*TA $p = 0.015$; Vibrionaceae 10.85 times higher, O $p = 0.004$, TA $p = 0.182$, O*TA $p = 0.062$; Alteromonadaceae 8.75 times higher, O $p < 0.001$, TA $p = 0.368$, O*TA $p = 0.256$; and Flavobacteraceae 4.13 times higher, O $p < 0.022$, TA $p = 0.510$, O*TA $p = 0.074$; Figure 3.3B, C.3). In contrast, Alphaproteobacteria were found in higher abundances in *A. millepora* planted into fished areas (2.74 times higher, O $p = 0.667$, TA $p = 0.009$, O*TA $p = 1.0$; Figure C.3). Endozoicimonaceae occurred in higher abundances for *A. millepora* from the MPA compared to those from fished area, but only when planted into fished areas (Figure C.3, 1.29 times higher, O $p = 0.139$, TA $p = 0.439$, O*TA $p = 0.016$).

Although we did not detect differences in *P. damicornis* chemical defense with reef state, its microbiome did change significantly as a function of reef state, which could best be explained by abundances of Vibrionaceae, Gammaproteobacteria, Arcobacteraceae, Endozoicimonaceae, Alteromonadaceae, Flavobacteraceae (Figure C.4, Random Forest Analysis importance scores: 0.105, 0.080, 0.073, 0.069, 0.044, 0.040, respectively, overall predictive accuracy 82%). Endozoicimonaceae occurred in higher abundances in corals from MPAs (Figure C.5, 1.24 times higher, O $p = 0.008$, TA $p = 0.079$, O*TA $p = 0.319$). Gammaproteobacteria occurred in higher abundances for *P.*

damicornis coral from MPAs, with the effect most pronounced for coral planted back into MPAs (Figure C.5, 1.79 times higher for coral from MPAs planted into MPAs compared to corals from fished areas planted into MPAs, O $p = 0.002$, TA $p = 0.014$, O*TA $p = 0.011$). Arcobacteraceae and Alteromonadaceae occurred in higher abundances in *P. damicornis* coral planted into MPAs regardless of their reef of origin (Figure C.5, Arcobacteraceae, 157.72 times higher, O $p = 0.980$, TA $p < 0.001$, O*TA $p = 0.667$; Alteromonadaceae, 3.82 times higher, O $p = 0.706$, TA $p < 0.001$, O*TA $p = 0.564$). Vibrionaceae occurred in higher abundances in corals from fished areas with the effect most pronounced for coral planted into MPAs (Figure C.5, Table C.2, 8.18 times higher in fished area coral planted into MPAs compared to MPA coral planted into MPAs, O $p < 0.001$, TA $p < 0.001$, O*TA $p < 0.001$).

P. cylindrica did not differ with reef state in either microbial community composition or potency of chemical defense, and microbial taxa were not good predictors of reef state by Random Forest Analysis (overall predictive accuracy 27%).

Indicator species analysis provided a second approach to addressing whether specific sequence variants were associated with the variable anti-pathogen activity of *A. millepora* with reef state. This analysis found a Bacteroidia sequence variant with high fidelity (53%) and specificity (86%) for *A. millepora* coral that originated from macroalgal dominated fished areas and were planted back into these fished areas. An Alphaproteobacteria sequence variant also occurred with high fidelity (50%) and specificity (86%) for *A. millepora* coral that were planted into fished areas (originating from both MPAs and fished areas). There were also differences in indicator sequences variants occurring with reef state in *P. damicornis*, a species that also exhibited microbiome shifts but not differences in anti-*Vibrio* activity with reef state. Nine *Vibrio* sequence variants were found with high fidelity (ranging 50-100%) and specificity (ranging 79-100%) in *P. damicornis* originating from fished areas transplanted into

MPAs. An *Algicola* sequence variant and an *Arcobacter* sequence variant were also found with high fidelity (50 & 70%, respectively) and specificity (89 & 81%, respectively) for *P. damicornis* from fished areas transplanted into MPAs. One *Vibrio* sequence variant was an indicator of *P. damicornis* originating from MPAs or fished areas transplanted into MPAs (fidelity 71%, specificity 99%). Relative abundances of indicator sequence variants are provided in table C.3. There were no indicator sequence variants for *P. cylindrica* from MPAs and fished areas.

Discussion

Thermally-induced bleaching of corals has increased in frequency by 5-fold over the last 40 years (Hughes et al. 2018a), resulting in unprecedented losses of acroporid corals, which can be catastrophic for reefs due to the central role that acroporids play in building reef structural complexity and providing habitat for other species (Kerry and Bellwood 2015, Bellwood et al. 2017, Hughes et al. 2018b). While the relative roles of coral loss from direct heat stress versus complex interactions among heat stress and disease, or other factors, is often unclear, a recent investigation by Beatty et al. (see chapter 2) indicates that an acroporid coral's chemical defense toward a *Vibrio* bleaching pathogen may be compromised by increased macroalgal abundance, which is a common trait of degraded reefs. However, it is uncertain if coral chemical defenses can acclimate, and likewise, if historical reef state will continue to influence coral chemical defense following rapid changes in reef state (e.g., from coral to macroalgal dominance following severe stress events such as bleaching or cyclone impact). Here we demonstrate that *i*) an acroporid coral demonstrated significant acclimatization to its current reef state within 28 days, via enhanced chemical defense toward a *Vibrio* bleaching pathogen for individuals planted into a coral dominated MPA compared to a macroalgal dominated fished area, *ii*) historical reef state also impacted this acroporid coral's chemical defense 28 days after transplanting, exhibited by reduced anti-*Vibrio*

chemical defenses for individuals that originated from algal dominated fished areas, iii) chemical defenses of a resistant poritid coral and a weedy pocillorid coral were not impacted by the historical or current reef state, and *iv*) declines in the acroporid coral's anti-*Vibrio* chemical defense coincided with persistently higher relative abundances of Vibrionaceae for individuals that originated from reefs with higher algal cover regardless of the current reef state.

Previously (Beatty et al. – see chapter 2) and again here, we found that all three corals we investigated possessed chemical defenses against *V. coralliilyticus,* but that potency varied among species. Chemical defenses for *P. cylindrica* and *P. damicornis* were unaffected by reef state in Beatty et al. (chapter 2) and this is also true for *P. cylindrica* and *P. damicornis* following fragmentation, acclimation for 28 days on their reef of origin, and reciprocal transplantation between coral- and algal-dominated reefs (Figure 3.1A & C). In contrast to the chemical defensive traits of *P. damicornis*, we did detect differences in microbiomes of reciprocally transplanted *P. damicornis* colonies with reef state (Figure 3.2C) that we did not detect previously (chapter 2). It is possible that fragmentation (despite 28 days of recovery on their reef of origin) generates a degree of susceptibility to microbiome alterations in response to reef state that does not occur for corals naturally occurring within our sites. This may also be true of *A. millepora*, where previously we detected only changes in microbiome variability with reef state (chapter 2), but here, document shifts in *A. millepora* microbiome composition with historical reef state (Figure 3.2B). Chapter 2 experiments and those here were also performed a month apart, thus, we cannot exclude the possibility that differences between findings may be due to differing field conditions during the time of experimentation.

By experimentally generating rapid changes in reef state via reciprocally transplanting corals between coral and macroalgal dominated reefs, we detected both

historical and current (within 28 days) reef effects on anti-pathogen chemical defenses of *A. millepora*. *A. millepora* from macroalgal dominated areas exhibited greater anti-*Vibrio* chemical defense if planted into coral dominated areas; the opposite occurred for corals planted into macroalgal dominated areas (Figure 3.1B). *A. millepora* also retained anti-*Vibrio* traits from their reef of origin, indicating that historical reef state can continue to impact coral chemical defense for >28 days after a rapid shift in reef state. These findings suggest that acroporid corals may be more susceptible than other groups to thermally-induced pathogens on degraded reefs; this increased susceptibility could further exacerbate the direct stress of heat during, or following, periods of warming.

Reef of origin (historical reef) effects on *A. millepora* chemical defense coincided with differences in coral microbiomes (e.g. beta diversity and relative abundances of bacterial taxa), notably by a 10-fold increase in relative abundances of Vibrionaceae among coral that originated from macroalgal dominated fished areas. Additionally, *A. millepora* originating from coral dominated MPAs maintained higher relative abundances of putative beneficial Endozoicimonaceae bacteria compared to *A. millepora* originating from fished areas when both were planted in macroalgal dominated fished areas. However, we did not detect significant shifts in *A. millepora* microbial community composition (i.e. changes in beta diversity) in response to transplant area.

The legacy effects of reef of origin on *A. millepora* microbiomes may, in part, reflect persistent bacterial infections or presence of nuisance bacteria that established while the coral resided within degraded, macroalgal dominated reefs. After colonization by harmful or nuisance bacteria, it may prove more difficult to clear these bacterial residents, even after coral are removed from degraded reefs and placed in healthier, coral dominated reefs. Further, bacterial associates may only become pathogenic under certain conditions (e.g. warming water temperatures that promote growth or virulence of bacteria, (Ben-Haim et al. 2003, Kimes et al. 2012) or when corals experience additional

stressors (Harvell et al. 2007)). Indeed, *V. coralliilyticus* upregulates virulence in response to temperature, increasing pathogenicity at 27°C and higher (Ben-Haim et al. 2003, Kimes et al. 2012). While *V. coralliilyticus* did not show up in our sequence variant tables, 16S gene analyses may be unreliable for taxonomic identification at the bacterial species level (strains identified as the same species by 16S can differ in both genome size and functional gene content as discussed in (Franzosa et al. 2015) and (Land et al. 2015)). Additionally, caution should be taken when interpreting these findings, as *Vibrio* bacteria can be found in healthy corals, with some strains playing beneficial roles by providing fixed nitrogen to the coral (Chimetto et al. 2008, Ceh et al. 2013).

In contrast to *A. millepora*, *P. cylindrica* and *P. damicornis* did not differ in their chemical defense against *V. coralliilyticus* between individuals from coral versus macroalgal dominated reefs. *P. cylindrica* exhibited the most potent chemical defense (46-67% inhibition) against *V. coralliilyticus.* Poritid corals are among the most persistent coral genera on degraded reefs and are often resistant to thermally-induced bleaching (Marshall and Baird 2000, Adjeroud et al. 2009, Adjeroud et al. 2018). Lack of decline in chemical defense or changes to microbiomes among poritid corals may, in part, facilitate their persistence on degraded reefs and their resistance to bleaching. *P. damicornis* exhibited the weakest chemical defense (0-29% inhibition) against *V. coralliilyticus*, and this did not vary as a function of reef state despite this coral exhibiting the greatest changes in microbiome composition (i.e. beta diversity) between coral and macroalgal dominated reefs (both origin and transplant area impacted microbiomes, Figure 3.2C). In addition to shifts in *P. damicornis* microbiomes with reef state, *P. damicornis* corals from macroalgal dominated fished areas exhibited a decline in relative abundances of putative beneficial Endozoicimonaceae and increases in Vibrionaceae. While pocilloporids are bleaching susceptible, they often rapidly recolonize reefs following disturbances and exhibit faster growth rates after warming events that may allow for their

recovery and persistence on degraded reefs (Berumen and Pratchett 2006, Glynn et al. 2014, Tortolero-Langarica et al. 2017, Adjeroud et al. 2018). This life strategy may lessen the need for anti-pathogen chemical defenses, as the coral may rely more on rapid growth following non-lethal bleaching episodes or larval dispersal to new habitats rather than defense within its current habitat, though further work is needed to test this hypothesis.

Although we found that only *A. millepora* varied in its chemical defense against a bleaching pathogen as a function reef state, macroalgal effects within the *Acropora* genus alone could still strongly impact the structure and function of Pacific reefs because acroporids are the dominant reef-building species upon which much of reef biodiversity depends (Kerry and Bellwood 2015, Bellwood et al. 2017). As examples, congruence in the fossil record suggests that *Acropora* played a critical role in the rapid diversification of several lineages of reef fishes (Bellwood et al. 2017) and present-day studies indicate that the structures produced by acroporid corals are critical for numerous fishes on modern reefs (Kerry and Bellwood 2015). Consequently, if other species of *Acropora* respond similarly to rapid changes in reef state, losses due to bleaching will impact not only these corals, but also a host of other reef species.

The limits to chemical defense under heating are not known, but under modest levels of warming, acroporids on coral dominated reefs may be more resistant to bleaching than those on macroalgal dominated reefs. Beyond certain thermal thresholds, e.g. the 3-4° heating-weeks observed on the Great Barrier Reef during the 2016 heat wave (Hughes et al. 2018b), benefits of herbivory and reductions in algal cover will likely prove insufficient for coral resistance to bleaching. At this time, it is unclear whether the observed chemical defense in this study is produced by the coral animal or its prokaryotic or microeukaryotic symbionts. However, previous investigators have suggested that antibiotic defenses may arise from the coral's microbiome (Ritchie

2006, Krediet et al. 2013) and this seems reasonable given that microbes associated with numerous marine and terrestrial invertebrates produce metabolites that defend the host against natural enemies (Lopanik 2014, Van Arnam et al. 2018). It is unclear how warming may impact the anti-*Vibrio* defenses if these are produced by the coral animal itself, its bacteriome, its dinoflagellate symbionts, or any combination of these components.

Our findings are consistent with a growing body of evidence that algal contact can induce shifts in coral microbiomes, often in a species-specific manner (Morrow et al. 2012, Thurber et al. 2012, Morrow et al. 2013). However, we only detected shifts in *A. millepora* microbiomes with historical reef state (differing for those originating from coral vs. macroalgal dominated reefs) but not with current reef state (after 28 days). It is possible that greater time must transpire to detect differences in *A. millepora* microbiomes following a rapid change in reef state. However, this was not the case for *P. damicornis*; this species exhibited microbiome differences within 28 days of a rapid change in reef state, but these changes did not coincide with changes in the potency of its anti-pathogen defenses. Several hypotheses have emerged to explain shifts in coral microbiomes following contact with algae. These include algal vectoring of microbes upon contact (Nugues et al. 2004, Pratte et al. 2017), direct toxicity of algal allelochemicals (Morrow et al. 2012), and algal release of dissolved organic carbon that stimulates bacterial growth and virulence genes in the surrounding seawater (Barott and Rohwer 2012, Haas et al. 2016). Indeed, benthic water microbiomes differ from macroalgal and coral dominated reefs (chapter 2 & (Haas et al. 2016)). However, despite drastically different benthic community composition in both macro ((Rasher et al. 2013, Bonaldo and Hay 2014) and microorganisms (chapter 2) at our sites, we found that certain species, e.g. *P. cylindrica*, are unimpacted by reef state in both their anti-*Vibrio* chemical defense and microbiomes. Even for corals that did exhibit differences in

their microbiomes in response to reef state, e.g. *A. millepora* and *P. damicornis*, dominant sequence variant community members (i.e. Endozoicimonaceae sequence variants) largely maintained similar relative abundances (Figure 3.3, Table C.1) regardless of reef state. Thus, differences may be resulting from changes in lower relative abundance taxa or rare community members rather than in dominant community members.

Conclusion

Coral reefs are losing functionally important reef building coral species within the Acroporidae family at unprecedented rates due to thermally-induced bleaching events (Marshall and Baird 2000, Hughes et al. 2018b) and disease (Aronson and Precht 2001). We found that an acroporid coral was able to acclimate to its current reef state via enhanced chemical defense toward a *Vibrio* bleaching pathogen within 28 days of planting into coral dominated MPAs compared to adjacent macroalgal dominated fished areas. However, *A. millepora'*s reef of origin continued to influence chemical defense 28 days after transplanting via reductions in chemical defense and persistently higher relative abundances of Vibrionaceae for individuals that originated from macroalgal dominated fished areas. In contrast, we did not detect differences in anti-*Vibrio* chemical defenses for a weedy pocilloporid coral or a resistant poritid coral in response to rapid shifts in reef state. These findings will help inform our understanding of reef resilience as corals continue to face stress from rising algal abundances and increasing frequency of warming events.

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APPENDIX A

Supplemental figures and tables for chapter 1

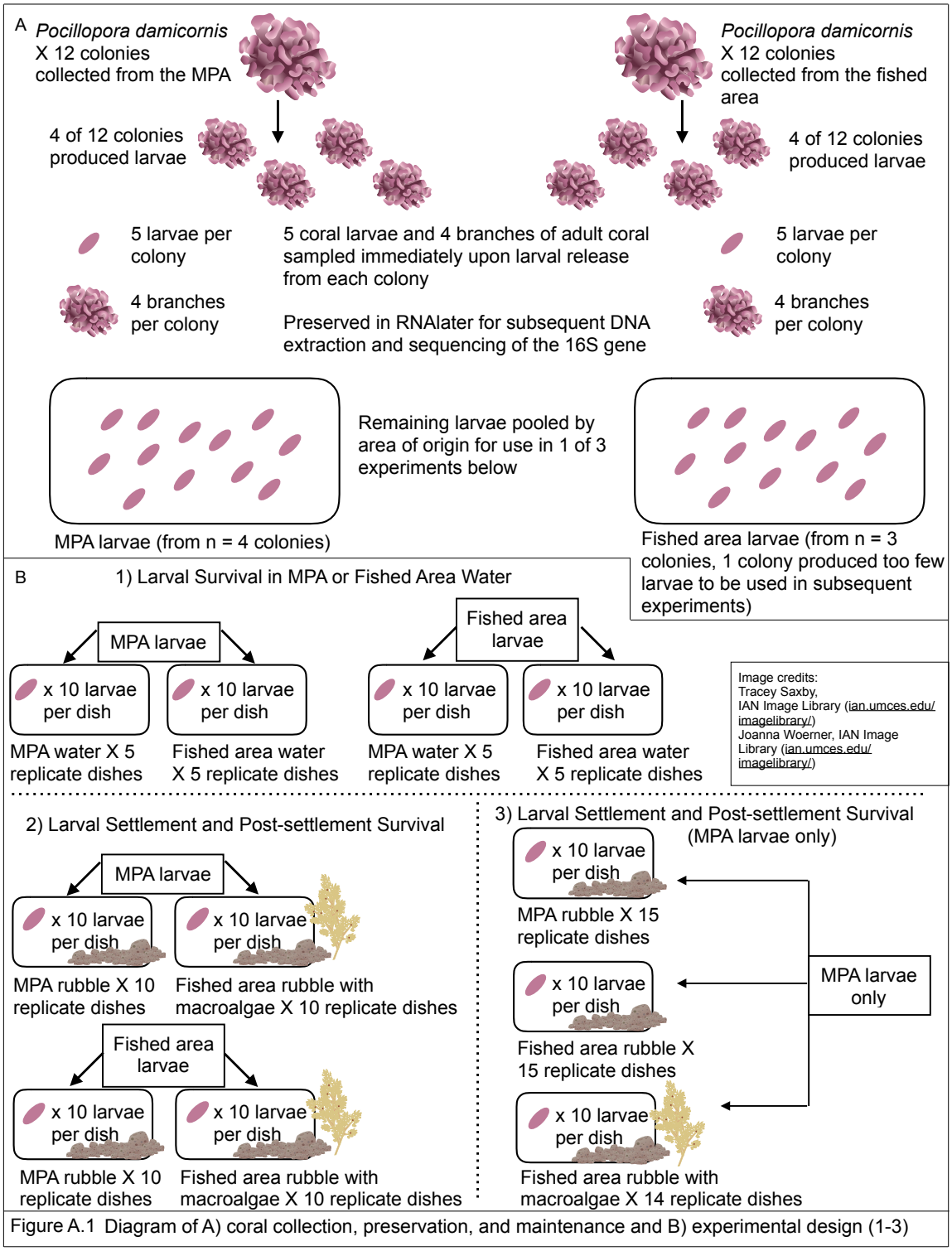


Figure A.1 Diagram of A) coral collection, preservation, and maintenance and B) experimental design (1-3)

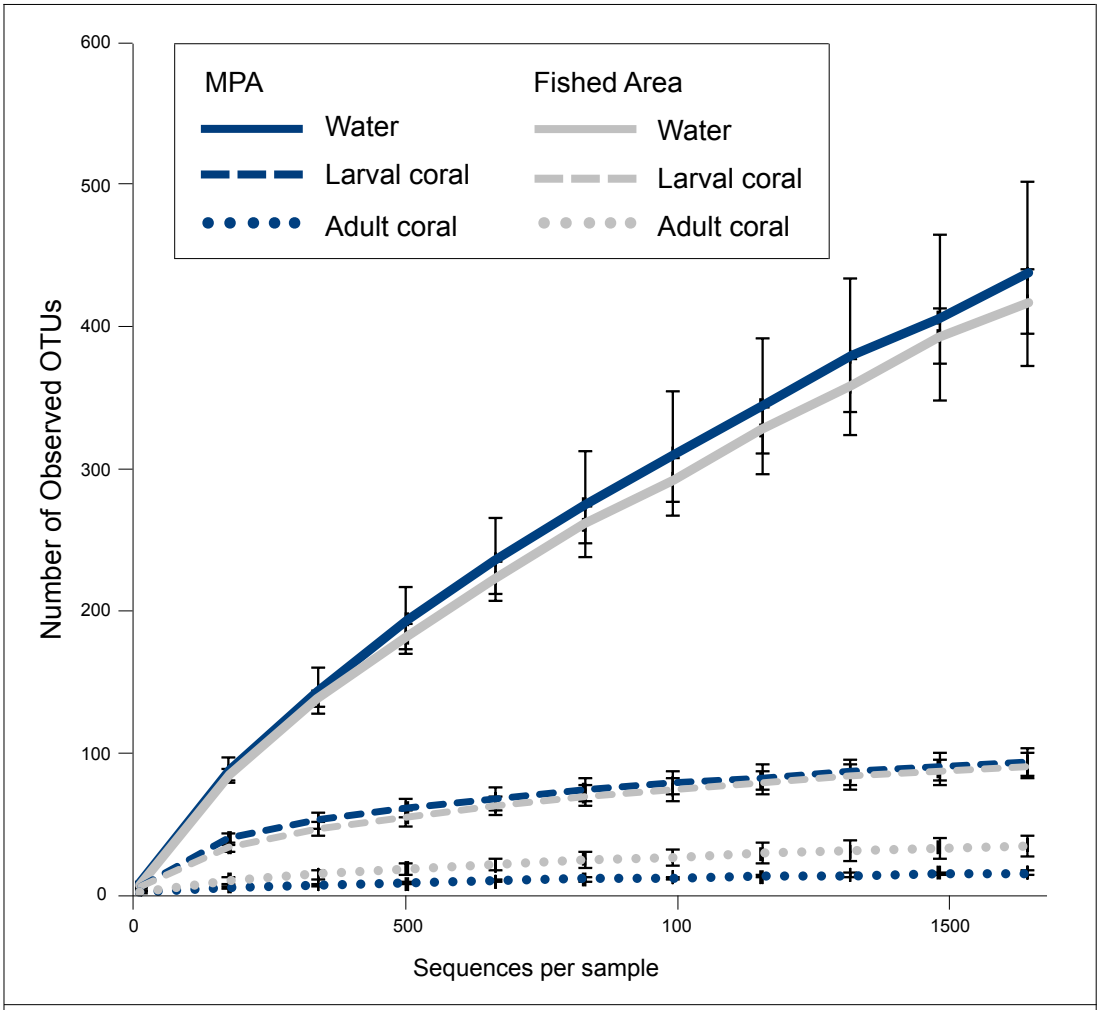


Figure A.2 Rarefaction curves for water, larval coral, and adult coral samples (mean +/- standard error).

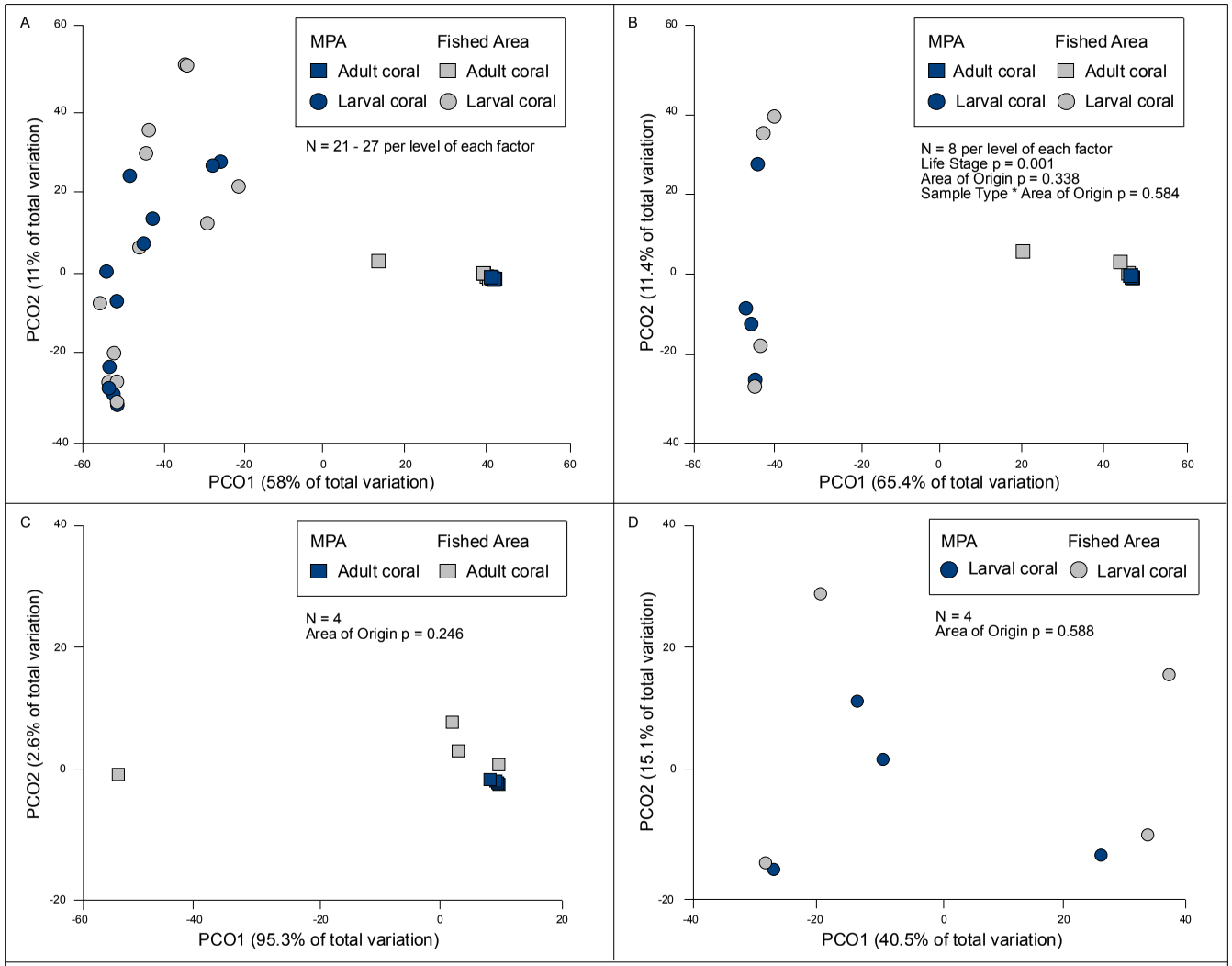


Figure A.3 PCO of Bray Curtis dissimilarity matrix of coral larvae and adults from the MPA and fished area
A) PCO includes all subsamples before collapsing subsamples to generate a mean community composition for each replicate (coral colony) for adults and larvae. PCO and PERMANOVA analysis after collapsing subsamples on the mean for each replicate (coral colony) for adults and larvae (B), adults only (C), and larvae only (D).

Table A.1 PERMDISPERSION distance from centroid means and pairwise comparison permutation p values

	Mean distance from centroid	Standard error
MPA adult	2.426	0.327
Fished area adult	22.453	7.065
MPA larvae	36.181	2.214
Fished area larvae	40.614	1.084

Pairwise comparisons

Group 1	Group 2	p(perm)
MPA adult	Fished area adult	0.037
MPA adult	MPA larvae	0.026
MPA adult	Fished area larvae	0.015
MPA larvae	Fished area adult	0.247
MPA larvae	Fished area larvae	0.083
Fished area adult	Fished area larvae	0.179

Table A.2.1 Fold changes in relative abundances

In bold type font are taxa that significantly differ between adults and larvae

* taxa contributes to less than 2% of the community

** classified up to order

*** only present in coral larvae

A. Taxa enriched in adult coral	Average relative proportion abundance in adult corals (MPA + fished area adults)	Average relative proportion abundance in coral larvae (MPA + fished area larvae)	Fold change in relative proportion abundance (adult/larvae)
Endozoicimonaceae	0.90202	0.06656	12.55
Pseudoalteromonadaceae	0.01092	0.00231	3.73
Alteromonadaceae	0.04658	0.02022	1.30

B. Taxa enriched in coral larvae	Average relative proportion abundance in coral larvae (MPA + fished area larvae)	Average relative proportion abundance in adult coral (MPA + fished area adults)	Fold change in relative proportion abundance (larvae/adults)
Helicobacteraceae	0.01953	0.00008	243.13
Methylobacteriaceae	0.02044	0.00014	145.00
Thiohalorhabdaceae	0.01708	0.00013	130.38
Comamonadaceae	0.01783	0.00015	117.87
Low abundance archaea*	0.02087	0.00023	89.74
Sphingomonadaceae	0.02270	0.00033	67.79
Chromatiales**	0.35638	0.00595	58.90
Pseudomonadaceae	0.08598	0.00147	57.49
Moraxellaceae	0.06503	0.00270	23.09
Oceanospirillaceae	0.02707	0.00127	20.31
Low abundance bacteria*	0.22581	0.02555	7.84
Unassigned taxa	0.01331	0.00249	4.35
Bacillaceae***	0.01887	0.00000	

C. Taxa enriched in MPA corals	Average relative proportion abundance in MPA corals (adults & larvae)	Average relative proportion abundance fished area coral (adults & larvae)	Fold change in relative proportion abundance (MPA/fished area)
Oceanospirillaceae	0.02386	0.00448	4.33
Unassigned taxa	0.01211	0.00369	2.28
Thiohalorhabdaceae	0.01263	0.00458	1.76
Comamonadaceae	0.01140	0.00658	0.73
Methylobacteriaceae	0.01211	0.00847	0.43
Helicobacteraceae	0.01124	0.00836	0.34
Low abundance archaea*	0.01201	0.00909	0.32
Chromatiales**	0.20548	0.15684	0.31
Endozoicimonaceae	0.49837	0.47021	0.06

D. Taxa enriched in fished area corals	Average relative proportion abundance in MPA corals (adults & larvae)	Average relative proportion abundance fished area coral (adults & larvae)	Fold change in relative proportion abundance (MPA/fished area)
Bacillaceae***	0.01870	0.00017	109.00
Pseudoalteromonadaceae	0.01284	0.00039	31.92
Alteromonadaceae	0.05239	0.01441	2.64
Sphingomonadaceae	0.01674	0.00629	1.66
Moraxellaceae	0.04155	0.02618	0.59
Pseudomonadaceae	0.05182	0.03563	0.45
Low abundance bacteria*	0.13365	0.11771	0.14

Table A.2.2 Two-factor ANOVA or permutation ANOVA p value

In bold type font are taxa that significantly differ between adults and larvae ($p < 0.004$ for statistical significance with Bonferroni correction for multiple comparisons).

Permutation ANOVA p values are provided for taxa that are not homoscedastic. Taxa that are not homoscedastic are in italics.

	Area of origin	Life stage	Area of origin * Life stage
Low Abundance Archaea	0.8431	**0.0018**	0.6863
Low Abundance Bacteria	0.6667	**0.0006**	0.9020
Unassigned	0.5102	0.1901	0.0727
Bacillaceae	0.3220	0.3140	0.3220
Methylobacteriaceae	0.6230	**0.0010**	0.6429
Sphingomonadaceae	0.2927	**0.0002**	0.2508
Comamonadaceae	0.5389	0.0386	0.5142
Helicobacteraceae	0.6154	**0.0024**	0.5811
Alteromonadaceae	0.3820	0.5400	0.2160
Chromatiales	0.3438	**0.0004**	0.3223
Endozoicimonaceae	0.7220	**<0.0001**	0.1130
Moraxellaceae	0.3300	0.0042	0.7255
Pseudomonadaceae	0.5102	**0.0026**	0.6545
Oceanospirillaceae	0.3380	0.209	0.3060
Thiohalorhabdaceae	0.1343	0.0040	0.0808
Pseudoalteromonadaceae	0.164	0.325	0.3

Table A.3.1 One-factor (area of origin) ANOVA or permutation ANOVA p values for adult coral

Permutation ANOVA p values are provided for taxa that are not homoscedastic. Taxa that are not homoscedastic are in italics.

	Area of origin
Low Abundance Archaea	0.537
Low Abundance Bacteria	0.243
Unassigned	0.153
Bacillaceae	Not present in adult coral
Methylobacteriaceae	0.246
Sphingomonadaceae	0.143
Comamonadaceae	0.356
Helicobacteraceae	0.356
Alteromonadaceae	0.293
Chromatiales	0.348
Endozoicimonaceae	0.265
Moraxellaceae	0.315
Pseudomonadaceae	0.344
Oceanospirillaceae	0.104
Thiohalorhabdaceae	0.237
Pseudoalteromonadaceae	0.239

Table A.3.2 One-factor (area of origin) ANOVA or permutation ANOVA p values for larval coral

Permutation ANOVA p values are provided for taxa that are not homoscedastic. Taxa that are not homoscedastic are in italics.

	Area of origin
Low Abundance Archaea	0.634
Low Abundance Bacteria	0.835
Unassigned	0.141
Bacillaceae	0.342
Methylobacteriaceae	0.57
Sphingomonadaceae	0.266
Comamonadaceae	0.538
Helicobacteraceae	0.576
Alteromonadaceae	0.501
Chromatiales	0.439
Endozoicimonaceae	0.255
Moraxellaceae	0.534
Pseudomonadaceae	0.614
Oceanospirillaceae	0.341
Thiohalorhabdaceae	0.159
Pseudoalteromonadaceae	0.339

Table A.4 Relative abundance of Vibrionaceae

Relative abundance (%) of *Vibrio shilonii* in adults and larvae at time point of larval release

	MPA adult	MPA larvae	Fished area adult	Fished area larvae
mean	0.00	0.00	0.15	0.02
standard error	0.00	0.00	0.07	0.01

Relative abundance (%) of *Vibrio shilonii* in water collected from the MPA or fished area

	MPA water	Fished area water
mean	0.32	0.53
standard error	0.12	0.14

Relative abundance (%) of *Vibrio shilonii* in larvae maintained in MPA or fished area water for six days

	MPA larvae maintained in MPA water	MPA Larvae maintained in fished area water	Fished area larvae maintained in MPA water	Fished area larvae maintained in fished area water
mean	0.00	0.01	4.16	0.28
standard error	0.00	0.01	4.14	0.17

Relative abundance (%) of *Vibrionaceae* in adults and larvae at time point of larval release

	MPA adult	MPA larvae	Fished area adult	Fished area larvae
mean	0.00	0.13	0.29	1.33
standard error	0.00	0.13	0.15	0.64

Relative abundance (%) of *Vibrionaceae* in water collected from the MPA or fished area

	MPA water	Fished area water
mean	2.08	0.93
standard error	0.66	0.39

Relative abundance (%) of *Vibrionaceae* in larvae maintained in MPA or fished area water for six days

	MPA larvae maintained in MPA water	MPA Larvae maintained in fished area water	Fished area larvae maintained in MPA water	Fished area larvae maintained in fished area water
mean	0.75	0.51	4.37	1.65
standard error	0.66	0.46	4.30	0.89

APPENDIX B

Supplemental figures and tables for chapter 2

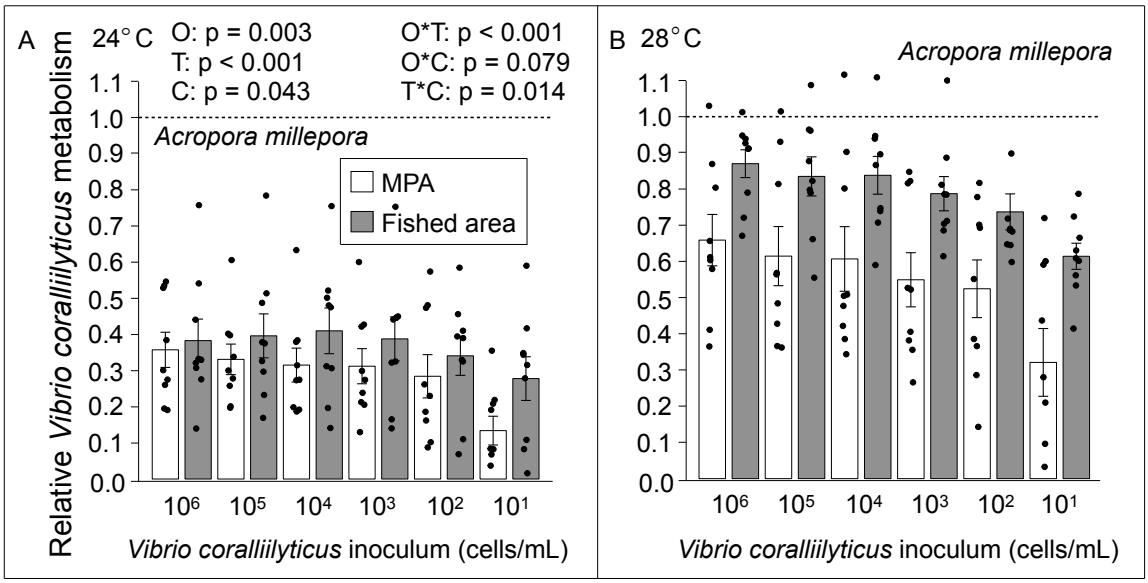


Figure B.1: Mean (±SE) activity of coral water relative to reef water against *V. coralliilyticus* at 24°C and 28°C for *Acropora millepora* as a function of pathogen inoculum concentration. The dashed line at 1.0 is the expected value if there is no effect. *P*-values are from a linear mixed effects model implemented with Akaike Information Criterion (AIC) for model selection (n = 9). Factors remaining after the model selection process are provided. O, T, and C represent origin (MPAs or fished areas), temperature, and concentration of pathogen inoculum, respectively. Dots indicate individual data points. One data point with negative values (after reduction of the optical density of sterilized coral water, see methods for information on data reduction) is not depicted in each of A (data point value is -0.040) and in B (data point value is -0.078) for the 10 cells per mL concentration MPA samples.

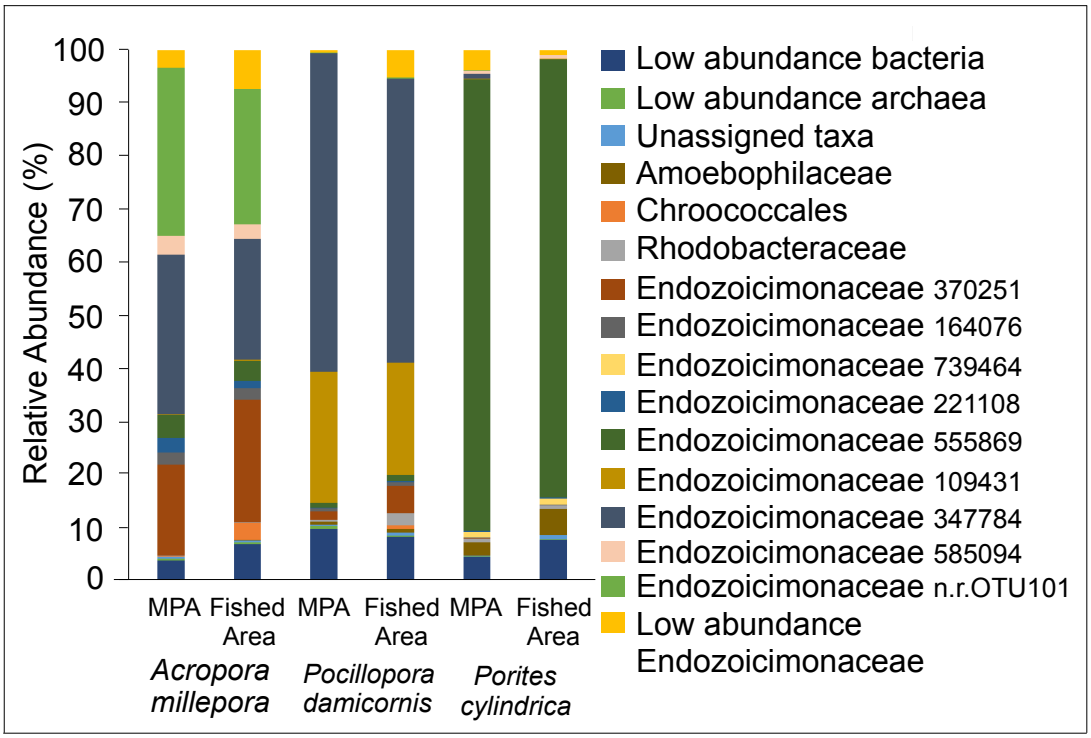


Figure B.2: Microbial community composition for the three corals samples. Taxa of <2% relative abundance were pooled by domain and depicted as 'low abundance bacteria' and 'low abundance archaea'. Endozoicimonaceae are depicted at the OTU level for taxa contributing to 1% or greater composition, with all remaining Endozoicimonaceae OTUs pooled to generate 'Low abundance Endozoicimonaceae'.

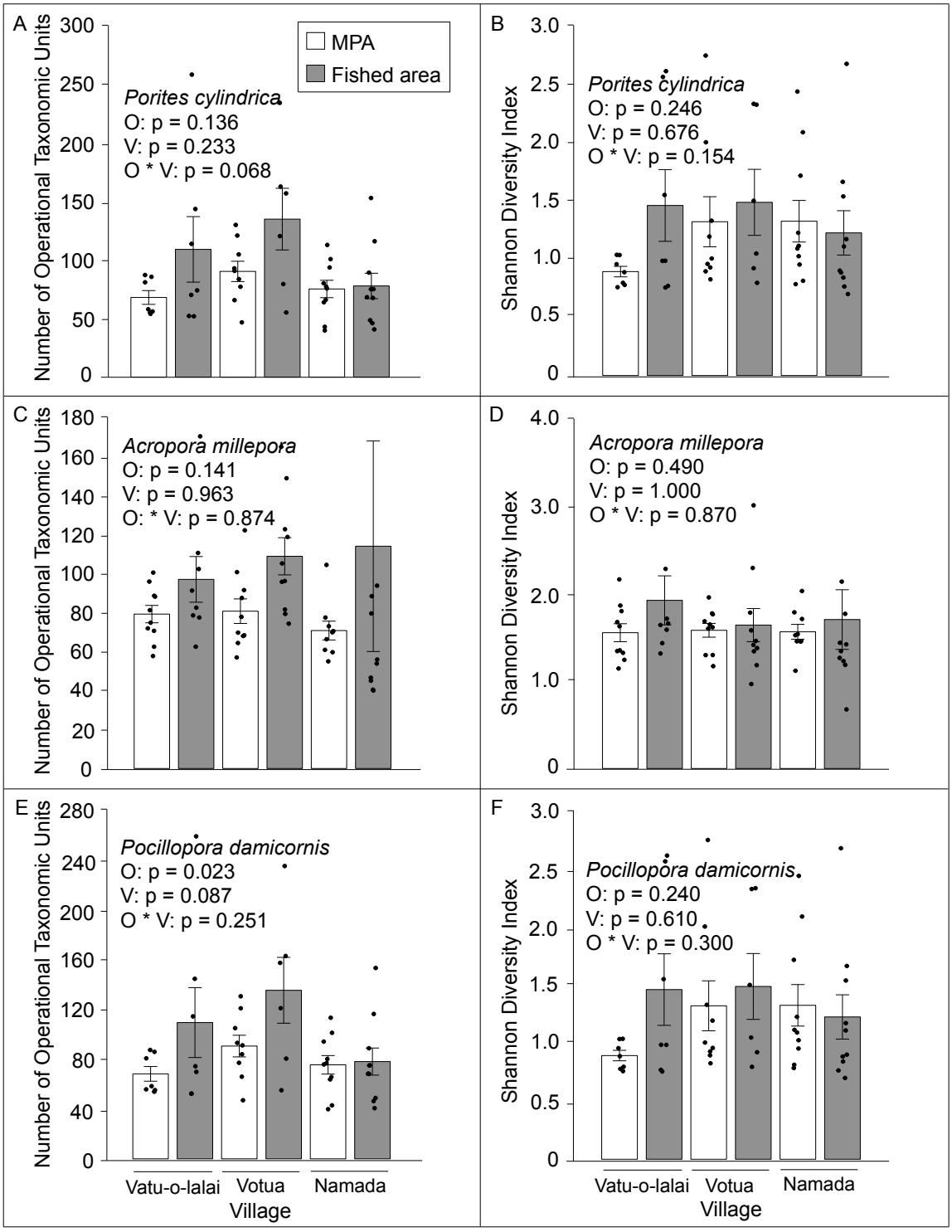


Figure B.3: Graphs on left show operational taxonomic unit (OTU) richness and on right show Shannon diversity (mean ± SE) for (A-B) *Porites cylindrica* (n = 28, 30 for MPA and fished area coral), (C-D) *A. millepora* (n = 29, 28 for MPA and fished area coral), (E-F) *Pocillopora damicornis* (n = 26, 23 for MPA and fished area coral). Analyses by two factor ANOVA or permutation ANOVA. O indicates origin (MPA or fished area) and V represents village. Dots show the individual data points. One (off the graph) data point

is not depicted in C and D above for Namada fished area (600 OTUs and 4.583 Shannon diversity).

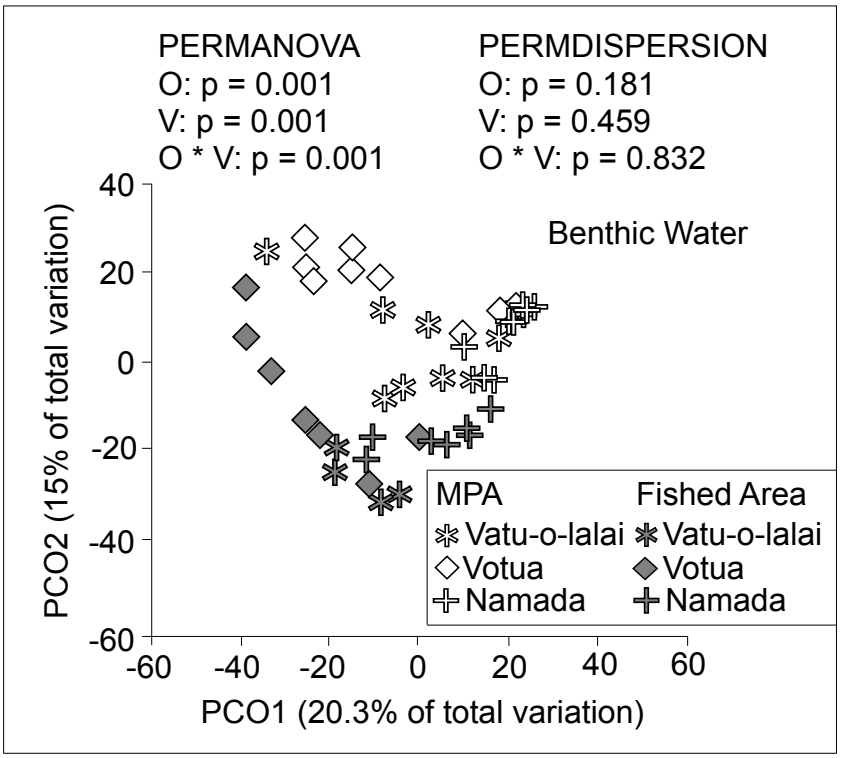


Figure B.4: Principal coordinate analysis with PERMANOVA and PERMDISPERSION tests of microbial community composition and dispersion for benthic water samples on operational taxonomic unit (OTU) tables rarefied to a uniform sequencing depth of 17,700 sequences per sample (n = 27, 18 for MPA and fished area samples). O and V represent origin and village, respectively, with village confounded by time (i.e., samples in different villages were taken on different dates).

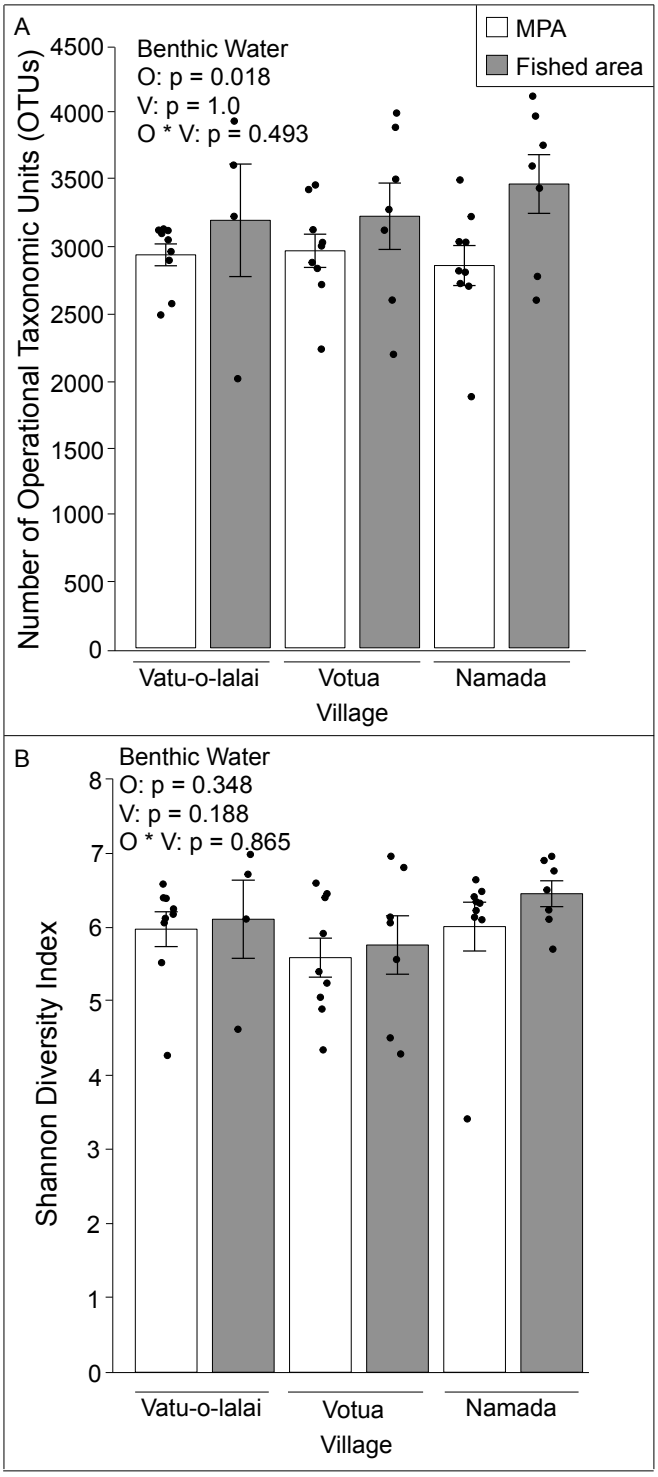


Figure B.5: OTU richness (A) and Shannon diversity (B) for benthic water samples (n = 27, 18 for MPA and fished area samples). Analyses by two factor permutation ANOVA. Abbreviations O and V represent factors origin and village, respectively, with village confounded by time.

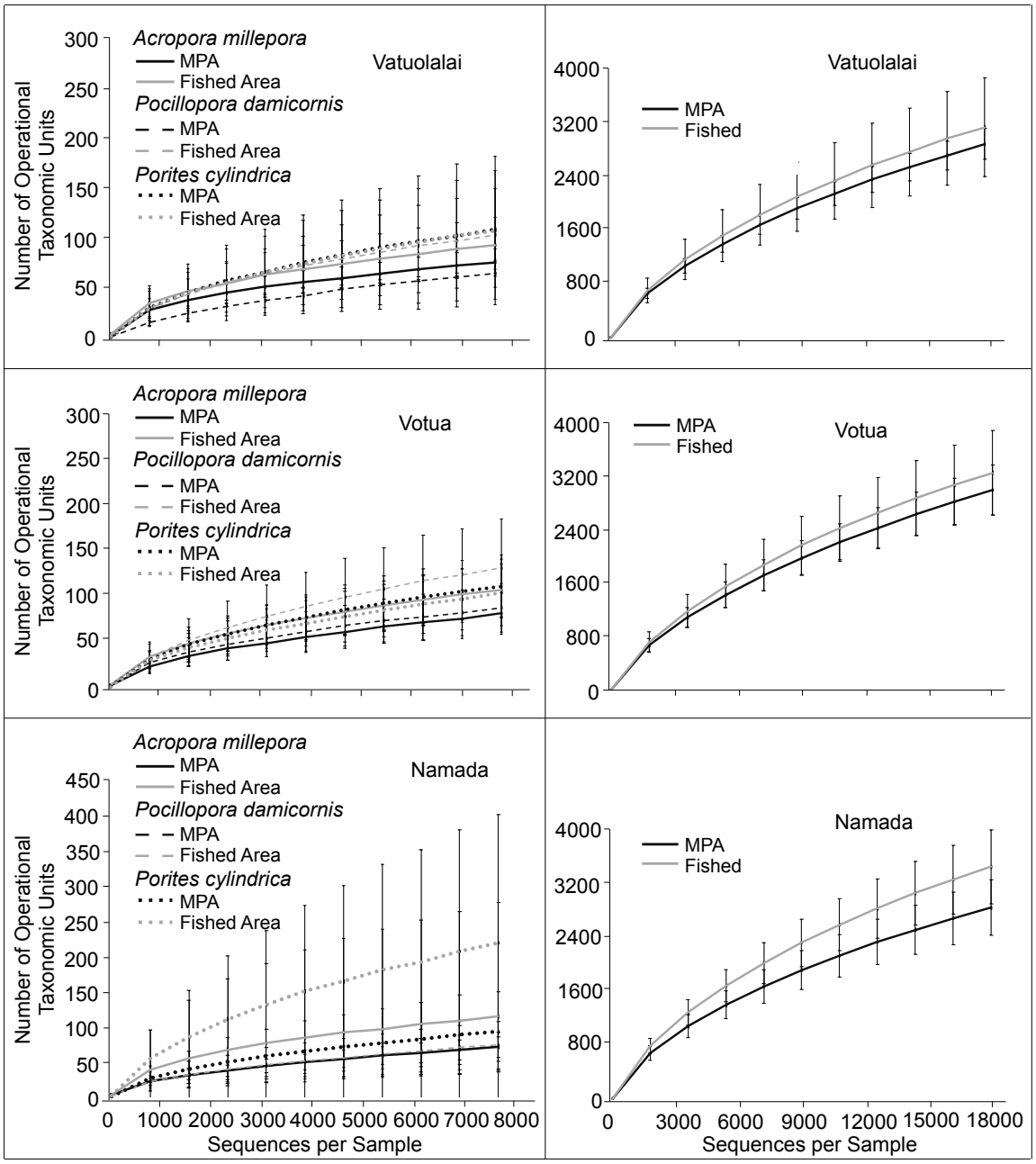


Figure B.6: Rarefaction curves (mean ± SEM) for *Porites cylindrica* (n = 28, 30 for MPA and fished area coral), *A. millepora* (n = 29, 28 MPA and fished area coral), *Pocillopora damicornis* (n = 26, 23 MPA and fished area coral) at each village (left graphs) and for benthic water (n = 27, 18 MPA and fished area samples) from each village (right graphs).

Table B.1.1 Comparisons of treatment (coral water) to control (reef water) at 24C

Coral Species:	*Acropora millepora*	Coral Species:	*Pocillopora damicornis*	Coral Species:	*Porites cylindrica*
Area:	MPA	Area:	MPA	Area:	MPA
Temperature:	24 Celsius	Temperature:	24 Celsius	Temperature:	24 Celsius
Concentration of *Vibrio coralliilyticus* cells/mL	ANOVA or permutation ANOVA FDR-corrected p value (coral water compared to seawater)	Concentration of *Vibrio coralliilyticus* cells/mL	ANOVA or permutation ANOVA FDR-corrected p value (coral water compared to seawater)	Concentration of *Vibrio coralliilyticus* cells/mL	ANOVA or permutation ANOVA FDR-corrected p value (coral water compared to seawater)
1000000	<0.001	1000	**0.192**	1000	<0.001
100000	<0.001	100	<0.001	100	<0.001
10000	<0.001	10	0.001	10	**<0.001**
1000	<0.001				
100	<0.001				
10	**<0.001**				

Coral Species:	*Acropora millepora*	Coral Species:	*Pocillopora damicornis*	Coral Species:	*Porites cylindrica*
Area:	Fished Area	Area:	Fished Area	Area:	Fished Area
Temperature:	24 Celsius	Temperature:	24 Celsius	Temperature:	24 Celsius
Concentration of *Vibrio coralliilyticus* cells/mL	ANOVA or permutation ANOVA FDR-corrected p value (coral water compared to seawater)	Concentration of *Vibrio coralliilyticus* cells/mL	ANOVA or permutation ANOVA FDR-corrected p value (coral water compared to seawater)	Concentration of *Vibrio coralliilyticus* cells/mL	ANOVA or permutation ANOVA FDR-corrected p value (coral water compared to seawater)
1000000	<0.001	1000	0.092	1000	<0.001
100000	<0.001	100	0.038	100	<0.001
10000	**<0.001**	10	**0.192**	10	**<0.001**
1000	<0.001				
100	<0.001				
10	<0.001				

permutation p values are in bold

Table B.1.2 Comparisons of treatment (coral water) to control (reef water) at 28C

Coral Species:	*Acropora millepora*	Coral Species:	*Pocillopora damicornis*	Coral Species:	*Porites cylindrica*
Area:	MPA	Area:	MPA	Area:	MPA
Temperature:	28 Celsius	Temperature:	28 Celsius	Temperature:	28 Celsius
Concentration of *Vibrio coralliilyticus* cells/mL	ANOVA or permutation ANOVA FDR-corrected p value (coral water compared to seawater)	Concentration of *Vibrio coralliilyticus* cells/mL	ANOVA or permutation ANOVA FDR-corrected p value (coral water compared to seawater)	Concentration of *Vibrio coralliilyticus* cells/mL	ANOVA or permutation ANOVA FDR-corrected p value (coral water compared to seawater)
1000000	<0.001	1000	<0.001	1000	<0.001
100000	<0.001	100	**<0.001**	100	<0.001
10000	<0.001	10	**0.063**	10	<0.001
1000	<0.001				
100	<0.001				
10	<0.001				

Coral Species:	*Acropora millepora*	Coral Species:	*Pocillopora damicornis*	Coral Species:	*Porites cylindrica*
Area:	Fished Area	Area:	Fished Area	Area:	Fished Area
Temperature:	28 Celsius	Temperature:	28 Celsius	Temperature:	28 Celsius
Concentration of *Vibrio coralliilyticus* cells/mL	ANOVA or permutation ANOVA FDR-corrected p value (coral water compared to seawater)	Concentration of *Vibrio coralliilyticus* cells/mL	ANOVA or permutation ANOVA FDR-corrected p value (coral water compared to seawater)	Concentration of *Vibrio coralliilyticus* cells/mL	ANOVA or permutation ANOVA FDR-corrected p value (coral water compared to seawater)
1000000	0.014	1000	**<0.001**	1000	<0.001
100000	0.008	100	0.002	100	<0.001
10000	**0.011**	10	**0.251**	10	<0.001
1000	0.011				
100	<0.001				
10	<0.001				

permutation p values are in bold

Table B.2 PERMANOVA and PERMADISPERSION results for coral microbial community composition and dispersion

A. PERMANOVA pairwise tests	t	p (permutation)
Porites cylindrica - Acropora millepora	14.69	0.001
Porites cylindrica - *Pocillopora damicornis*	20.12	0.001
Acropora millepora - Pocillopora damicornis	7.89	0.001

B. PERMADISPERSION Averages	Average distance from centroid	Standard Error
Acropora millepora	36.79	2.07
Pocillopora damicornis	21.57	2.88
Porites cylindrica	12.86	1.32

C. PERMADISPERSION pairwise tests	t	p (permutation)
Porites cylindrica - Acropora millepora	9.79	0.001
Porites cylindrica - Pocillopora damicornis	2.90	0.015
Acropora millepora - Pocillopora damicornis	4.38	0.002

Table B.3 Coral Microbial Community Composition

	Relative Abundances (%)					
	Acropora		Pocillopora		Porites	
	MPA	Fished Area	MPA	Fished Area	MPA	Fished Area
Amoebophilaceae	0.01	0.01	0.55	0.70	2.50	4.87
Chroococcales	0.10	3.24	0.01	0.71	0.00	0.00
Rhodobacteraceae	0.17	0.18	0.35	2.28	0.64	0.68
Low abundance Endozoicimonaceae	3.31	7.32	0.41	5.19	3.83	0.90
Endozoicimonaceae 370251	17.17	23.11	1.63	5.21	0.10	0.07
Endozoicimonaceae 164076	2.25	2.11	0.51	0.69	0.12	0.05
Endozoicimonaceae 739464	0.03	0.04	0.02	0.02	1.02	1.16
Endozoicimonaceae 221108	2.78	1.43	0.10	0.13	0.25	0.22
Endozoicimonaceae 555869	4.33	3.80	0.99	1.17	85.28	82.75
Endozoicimonaceae 109431	0.10	0.16	24.67	21.22	0.12	0.06
Endozoicimonaceae 347784	30.10	22.76	60.13	53.62	0.86	0.08
Endozoicimonaceae 585094	3.54	2.83	0.02	0.03	0.58	0.60
Endozoicimonaceae New Reference OTU101	31.81	25.53	0.20	0.18	0.08	0.07
Unassigned Taxa	0.32	0.45	0.26	0.52	0.16	0.80
Low Abundance Archaea	0.28	0.28	0.51	0.18	0.06	0.13
Low Abundance Bacteria	3.69	6.74	9.63	8.16	4.39	7.56

APPENDIX C

Supplemental figures and tables for chapter 3

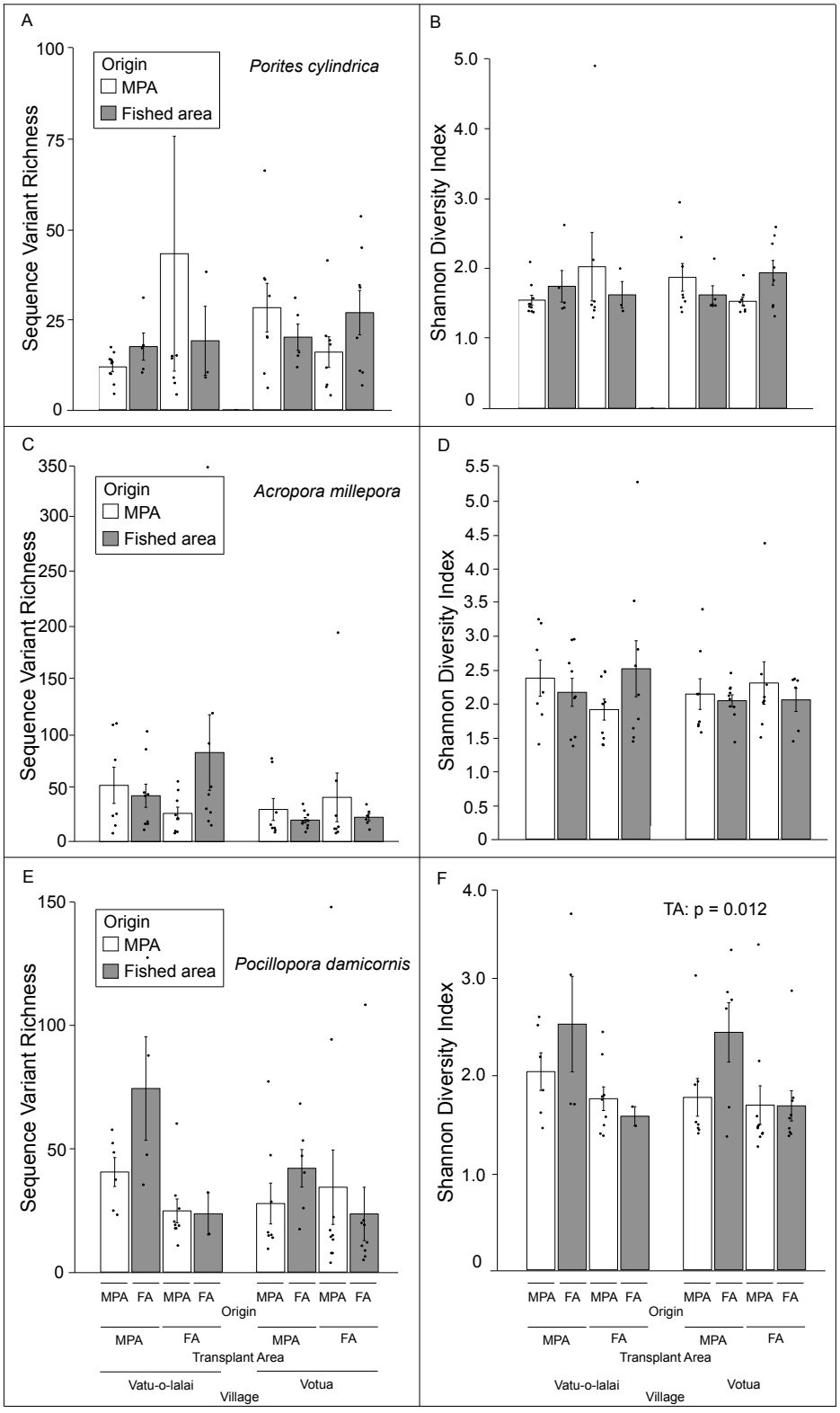
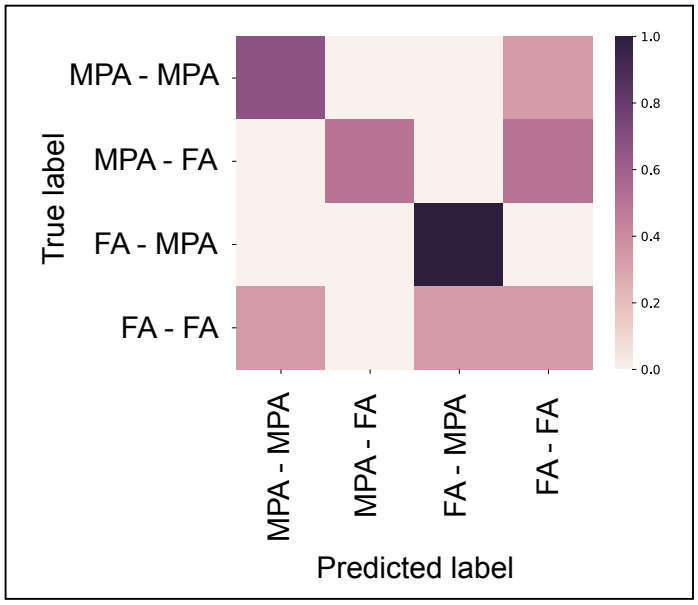


Figure C.1: Graphs on left show sequence variant richness and on right show Shannon diversity (mean ± SE) for (A-B) *Porites cylindrica* (n = 25-29 per village), (C-D) *A. millepora* (n = 32-34 per village), (E-F) *Pocillopora damicornis* (n = 21-33 per village). Analyses by three factor ANOVA or permutation ANOVA, with p-values provided for significant factors. Abbreviations O, TA, and V represent origin (MPAs or fished areas), transplant area (MPAs or fished areas), and village, respectively. Dots show the individual data points.

A.

Origin - Transplant Area	MPA-MPA	MPA-FA	FA-MPA	FA-FA	Overall Accuracy
MPA-MPA	0.67	0.00	0.00	0.33	
MPA-FA	0.00	0.50	0.00	0.50	
FA-MPA	0.00	0.00	1.00	0.00	
FA-FA	0.33	0.00	0.33	0.33	
Overall Accuracy					0.64
Baseline Accuracy					0.29
Accuracy Ratio					2.25

B.



C.

Taxonomic grouping	Importance scores
Endozoicomonadaceae	0.098
Bacteroidia	0.077
Vibrionaceae	0.073
Alphaproteobacteria	0.071
Alteromonadaceae	0.050
Flavobacteriaceae	0.048

Figure C.2: Random forest analysis on *Acropora millepora* on feature table collapsed to family level. A) & B) predictive accuracy of the consensus model in predicting treatment groups (origin*transplant area), as proportion (A) and heatmap (B) from bacterial family

or class groups. C) Taxa ranked according to their predictive performance in the consensus model (higher scores are better predictors).

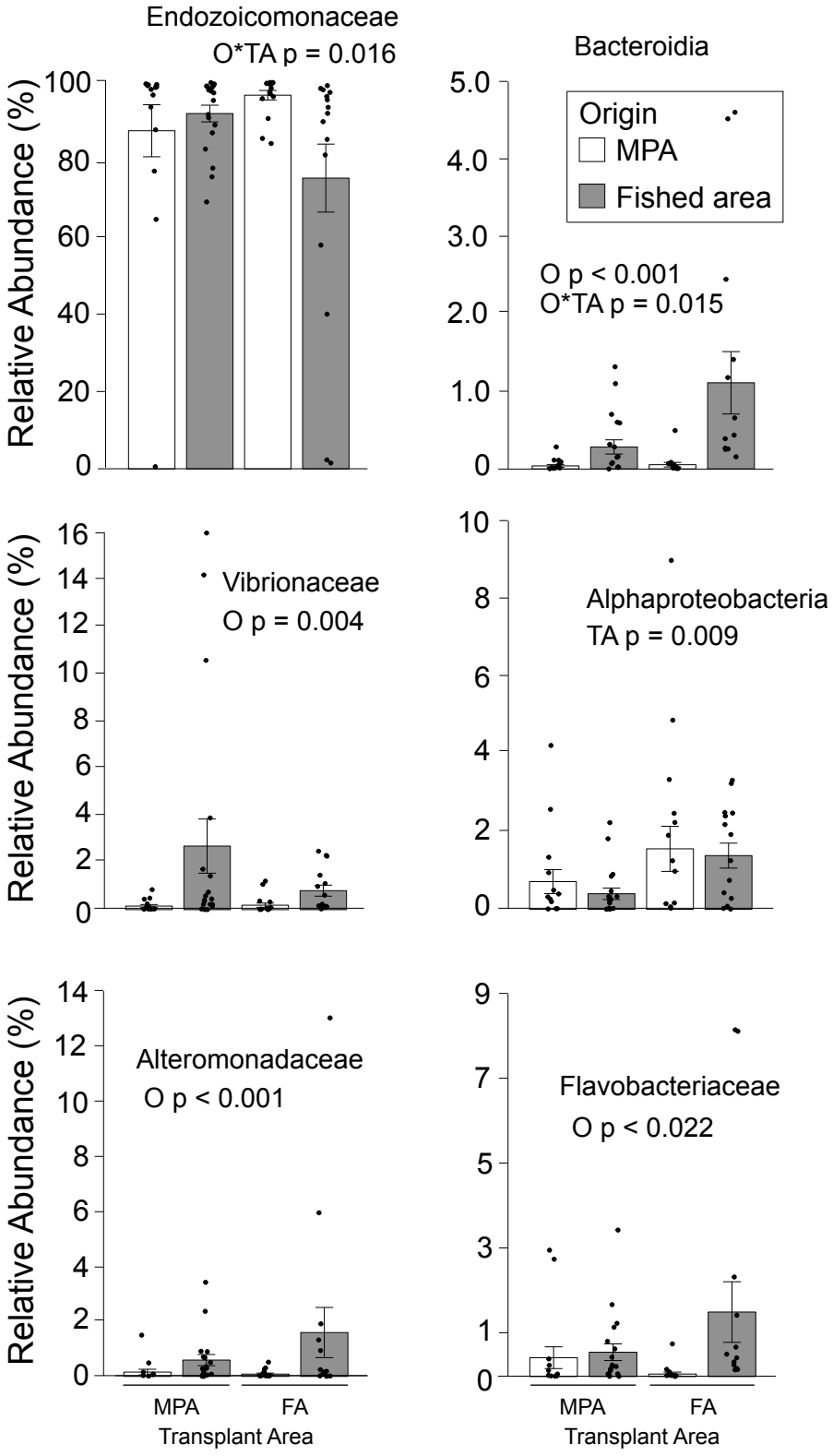
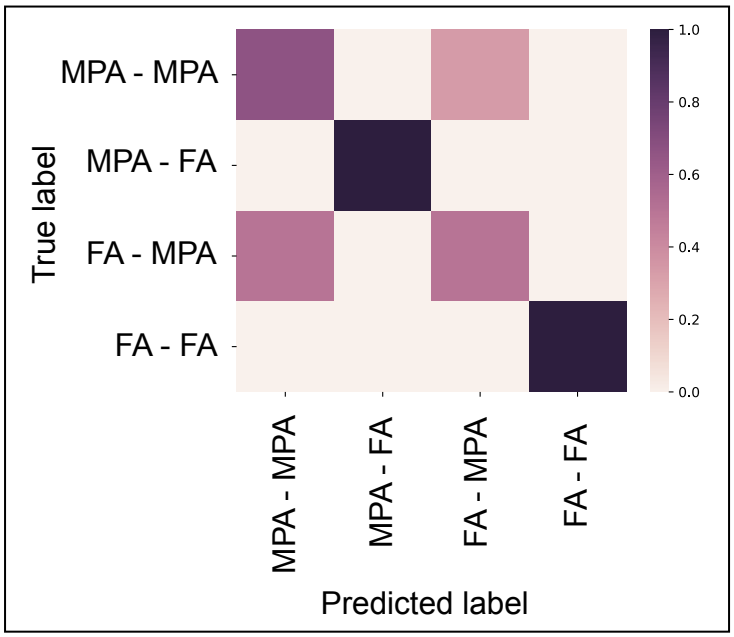


Figure C.3: Relative abundances of predictive taxa from random forest analysis on *Acropora millepora* on feature table collapsed to family level. Significant factors from a two factor ANOVA or permutation ANOVA on relative abundances are provided. Abbreviations O, and TA represent origin (MPAs or fished areas), and transplant area (MPAs or fished areas). Dots show the individual data points.

A.

Origin - Transplant Area	MPA-MPA	MPA-FA	FA-MPA	FA-FA	Overall Accuracy
MPA-MPA	0.67	0.00	0.33	0.00	
MPA-FA	0.00	1.00	0.00	0.00	
FA-MPA	0.50	0.00	0.50	0.00	
FA-FA	0.00	0.00	0.00	1.00	
Overall Accuracy					0.82
Baseline Accuracy					0.36
Accuracy Ratio					2.25

B.



C.

Taxonomic grouping	Importance scores
Vibrionaceae	0.105
Gammaproteobacteria	0.080
Arcobacteraceae	0.073
Endozoicomonadaceae	0.069
Alteromonadaceae	0.044
Flavobacteriaceae	0.040

Figure C.4: Random forest analysis on *Pocillopora damicornis* on feature table collapsed to family level. A) & B) predictive accuracy of the consensus model in predicting treatment groups (origin*transplant area), as proportion (A) and heatmap (B) from bacterial family or class groups. C) Taxa ranked according to their predictive performance in the consensus model (higher scores are better predictors).

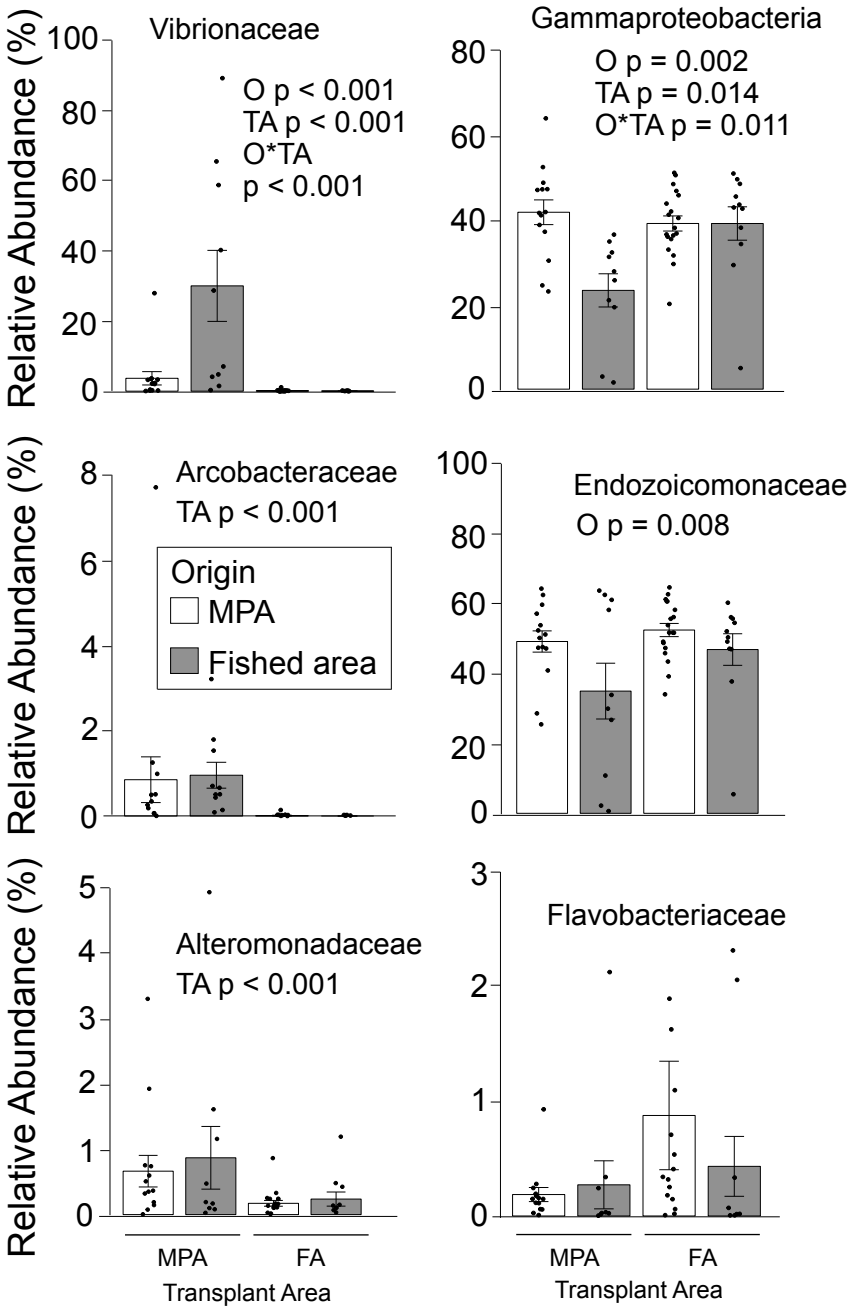


Figure C.5: Relative abundances of predictive taxa from random forest analysis on *Pocillopora damicornis* on feature table collapsed to family level. Significant factors from a two factor ANOVA or permutation ANOVA on relative abundances are provided. Abbreviations O, and TA represent origin (MPAs or fished areas), and transplant area (MPAs or fished areas). Dots show the individual data points.

Table C.1 Relative abundances of dominant (≥2% contribution) and rare (<2%) Endozoicomonaceae

	Porites cylindrica							
	Vatuolalai village				Votua village			
Origin:	MPA	FA	MPA	FA	MPA	FA	MPA	FA
Transplant Area:	MPA		FA		MPA		FA	
Endozoicomonas sp. s.v.1	23.37	20.78	18.99	24.83	22.28	26.23	25.69	21.63
Endozoicomonas sp. s.v.7	28.21	28.55	23.63	27.95	22.53	25.65	29.04	23.88
Endozoicomonas sp. s.v.10	22.70	20.70	19.82	21.38	18.62	22.81	22.51	20.63
Endozoicomonas sp. s.v.12	17.95	17.11	15.59	19.95	19.12	18.71	18.74	18.05
Rare Endozoicomonaceae summed	0.00	0.08	0.00	0.00	0.08	0.00	0.00	0.00
Total Endozoicomonaceae abundance	92.24	87.23	78.03	94.11	82.63	93.40	95.98	84.19

	Acropora millepora							
	Vatuolalai village				Votua village			
Origin:	MPA	FA	MPA	FA	MPA	FA	MPA	FA
Transplant Area:	MPA		FA		MPA		FA	
Endozoicomonas sp. s.v.2	2.56	1.73	2.40	0.89	12.29	14.00	11.65	6.87
Endozoicomonas sp. s.v.3	3.14	1.23	2.07	0.73	8.72	7.75	9.39	5.62
Endozoicomonas sp. s.v.4	17.36	17.60	19.72	13.56	11.38	13.50	14.33	14.06
Endozoicomonas sp. s.v.5	14.79	25.58	23.86	19.81	11.38	15.75	12.49	16.38
Endozoicomonas sp. s.v.6	3.97	1.81	2.24	0.97	10.63	8.92	10.39	6.37
Endozoicomonas sp. s.v.9	2.73	1.64	2.07	0.89	10.05	9.83	11.48	5.96
Endozoicomonas sp. s.v.13	13.31	17.60	20.13	15.26	13.62	12.83	11.99	15.47
Endozoicomonas sp. s.v.14	14.38	14.31	16.24	12.01	9.88	11.25	10.98	13.40
Rare Endozoicomonaceae summed	6.12	2.96	4.31	1.79	5.48	2.58	6.54	1.08
Total Endozoicomonaceae abundance	78.35	84.46	93.04	65.91	93.44	96.42	99.25	85.19

	Pocillopora damicornis							
	Vatuolalai village				Votua village			
Origin:	MPA	FA	MPA	FA	MPA	FA	MPA	FA
Transplant Area:	MPA		FA		MPA		FA	
Endozoicomonas sp. s.v.8	20.05	13.14	21.97	26.43	23.46	13.42	21.36	21.95
Endozoicomonas sp. s.v.11	23.03	25.29	29.60	24.24	25.54	19.83	31.14	24.77
Endozoicomonas sp. s.v.15	2.07	0.00	0.41	0.00	0.00	0.00	0.00	0.00
Endozoicomonas sp. s.v.16	2.07	0.00	0.25	0.00	0.00	0.00	0.00	0.00
Rare Endozoicomonaceae summed	3.56	0.00	0.33	0.00	0.00	0.00	0.08	0.00
Total Endozoicomonaceae abundance	50.79	38.44	52.57	50.67	49.00	33.25	52.59	46.72

Table C.2 Relative abundances of dominant (≥2% contribution) and rare (<2%) Vibrionaceae

	Porites cylindrica							
	Vatuolalai village				Votua village			
Origin:	MPA	FA	MPA	FA	MPA	FA	MPA	FA
Transplant Area:	MPA		FA		MPA		FA	
Rare Vibrionaceae summed	0.00	0.08	0.33	0.00	0.33	0.67	0.50	0.00

	Acropora millepora							
	Vatuolalai village				Votua village			
Origin:	MPA	FA	MPA	FA	MPA	FA	MPA	FA
Transplant Area:	MPA		FA		MPA		FA	
Rare Vibrionaceae summed	0.41	5.76	0.58	0.73	0.50	0.92	0.08	1.82

	Pocillopora damicornis							
	Vatuolalai village				Votua village			
Origin:	MPA	FA	MPA	FA	MPA	FA	MPA	FA
Transplant Area:	MPA		FA		MPA		FA	
***Vibrio* sp. s.v.1**	0.58	2.08	0.08	0.00	0.67	2.75	0.08	0.00
Vibrio sp. s.v.2	0.17	2.00	0.00	0.00	0.00	0.00	0.00	0.00
***Vibrio* sp. s.v.3**	0.08	0.25	0.00	0.00	0.92	5.25	0.00	0.00
Vibrio sp. s.v.4	0.00	0.08	0.00	0.00	0.08	2.83	0.00	0.00
***Vibrio* sp. s.v.5**	0.08	0.17	0.00	0.00	0.58	4.50	0.00	0.00
***Vibrio* sp. s.v.6**	0.33	0.75	0.08	0.00	0.08	2.50	0.00	0.00
***Vibrio* sp. s.v.7**	0.50	2.41	0.00	0.08	0.25	3.50	0.08	0.00
***Vibrio* sp. s.v.8**	0.00	0.08	0.00	0.00	0.00	3.83	0.00	0.00
***Vibrio* sp. s.v.9**	0.66	2.00	0.00	0.00	1.08	3.00	0.08	0.08
Rare Vibrionaceae summed	1.66	10.48	0.17	0.08	1.33	9.58	0.74	0.17
Total Vibrionaceae abundance	4.06	20.30	0.33	0.17	4.99	37.75	0.99	0.25

In bold are sequence variants that are indicators of origin and transplant area groupings

Table C.3 Relative abundances (%) of indicator sequence variants

	Pocillopora damicornis							
	Vatuolalai village				Votua village			
Origin:	MPA	FA	MPA	FA	MPA	FA	MPA	FA
Transplant Area:	MPA		FA		MPA		FA	
***Vibrio* sp. s.v.1**	0.58	2.08	0.08	0.00	0.67	2.75	0.08	0.00
***Vibrio* sp. s.v.3**	0.08	0.25	0.00	0.00	0.92	5.25	0.00	0.00
***Vibrio* sp. s.v.5**	0.08	0.17	0.00	0.00	0.58	4.50	0.00	0.00
***Vibrio* sp. s.v.6**	0.33	0.75	0.08	0.00	0.08	2.50	0.00	0.00
***Vibrio* sp. s.v.7**	0.50	2.41	0.00	0.08	0.25	3.50	0.08	0.00
***Vibrio* sp. s.v.8**	0.00	0.08	0.00	0.00	0.00	3.83	0.00	0.00
***Vibrio* sp. s.v.9**	0.66	2.00	0.00	0.00	1.08	3.00	0.08	0.08
Vibrio sp. s.v.10	0.00	0.33	0.00	0.00	0.00	0.67	0.00	0.00
Vibrio sp. s.v.11	0.00	0.58	0.08	0.00	0.00	0.67	0.00	0.00
Vibrio sp. s.v.12	0.08	0.75	0.00	0.00	0.25	0.42	0.08	0.08
Algicola sp. s.v.1	0.08	1.16	0.00	0.00	0.08	0.08	0.00	0.00
Arcobacter sp. s.v.1	0.08	0.42	0.00	0.00	0.17	0.25	0.00	0.00

	Acropora millepora							
	Vatuolalai village				Votua village			
Origin:	MPA	FA	MPA	FA	MPA	FA	MPA	FA
Transplant Area:	MPA		FA		MPA		FA	
Bacteroidia s.v.1	0.00	0.00	0.08	0.24	0.00	0.08	0.00	0.33
Alphaproteobacteria s.v.1	0.08	0.08	0.50	0.24	0.08	0.08	0.08	0.17

In bold are sequence variants that are found at ≥ 2% in at least one origin and transplant area grouping