

Intergenerational effects of macroalgae on a reef coral: major declines in larval survival but subtle changes in microbiomes

Deanna S. Beatty, Cody S. Clements, Frank J. Stewart, Mark E. Hay*

School of Biological Sciences and Aquatic Chemical Ecology Center, Georgia Institute of Technology, Atlanta, GA 30332-0230, USA

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 a 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 macroalgal-dominated area exhibited higher pre-settlement mortality and reduced settlement compared with 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.

KEY WORDS: Coral microbiome · Coral larvae · Coral–algae interactions · Marine protected areas · *Pocillopora damicornis*

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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 1970s (Gardner et al. 2003, Jackson et al. 2014) and by >50% in the Pacific since the 1980s (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 & Hay 2010), potentially harming corals 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, the 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 of concern 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. 2018). 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 on 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 ~2000 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 with 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 3 ocean systems, while Zaneveld et al. (2016) found that herbivore exclusion plots had higher algal abundances and more variable coral microbiomes compared with 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 m. We conducted experiments in long-term (>10 yr) MPAs and adjacent fished areas (2 MPAs and 2 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 with larvae from the fished area, (4) post-settlement juvenile coral experience higher survivorship in an MPA compared with 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 MPAs 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 2 small (0.5–0.8 km²), 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 3 km 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 ($\leq 2\%$) 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 d 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 l of water from the respective collection site and monitored at dusk for larval release. Four colonies from the MPA and 4 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) seawater (FSW), preserved separately in RNAlater (ThermoFisher Scientific), and stored at -20°C . We simultaneously collected 4 clippings from each adult coral colony that released larvae and preserved these in the same manner.

Of the 8 colonies used for microbiome analysis, 4 colonies from the MPA and 3 from the fished area produced sufficient numbers of larvae (≥ 100 per colony) for use in subsequent larval survival and settlement experiments (see text below and Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m589p097_supp.pdf 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 d in the lab with water changes every 2–3 d (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 to 16 d due to larval release occurring on different days. Any inactive larvae that failed to exhibit swimming behavior after 3 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 μ l universal pipette tips: wide bore, ThermoFisher Scientific).

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). To account for intra-colony variation, DNA from 5 larvae and 4 clippings of adult coral branches were extracted individually per colony. For the larval survival experiment (see below), DNA from 2 larvae per dish was extracted individually (with one exception when only one larva was alive at the end of the experiment). In addition, for each sample, we centrifuged the residual RNA later solution ($9391 \times g$, 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 μ l of Platinum PCR SuperMix (Life Technologies, Thermo Scientific), 3 μ l of template DNA (of 100 μ l total DNA elution volume), and 1 μ l 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 s), primer annealing at 50°C (45 s), primer extension at 72°C (90 s), and a final extension at 72°C (10 min). Amplicons were cleaned and DNA concentrations were normalized using SequalPrep plates (ThermoFisher Scientific). Amplicons were then pooled at equimolar concentrations and sequenced on Illumina's MiSeq platform using a 500 cycle kit (250 \times 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! (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 (Mago & 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: 1650 for coral larvae, coral adults, and water and 1175 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 (PCOA) 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 version 7.

Two-factor ANOVA (factor 1: area of origin; factor 2: life stage), implemented via the `aov` function within the `lm` package in RStudio version 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 3.0, on proportion data.

We first included only those taxa (family level) contributing to 2% or greater relative abundance within at least 1 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 2-factor permutation ANOVA (factor 1: area of origin; factor 2: life stage). A Bonferroni 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 1-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 I 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 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 on 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 m 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 ~1–2 m above the benthos and changed daily for the first 5 d 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 avoided settling on the polystyrene surfaces of the dishes (as commonly observed with other coral species, K. B. Ritchie pers. comm.). We recorded metamorphosis (on the dish or in the water column, which was rare) daily for 6 d and assessed survivorship at the end of the 6 d experiment. Larvae were considered alive if they exhibited swimming behavior after 3 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 3 times in FSW, 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 2-factor ANOVA via the `aov` function within the `lm` package of RStudio 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 1-factor ANOVA (factor: water area of origin). Primer E (Clarke 1993) was used to perform 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 version 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 haphazardly chosen 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 3 types of substrate were fouled with comparable amounts of crustose coralline algae (CCA) and short (<0.5 cm) turf. 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 3 types of rubble collected (MPA rubble without macroalgae, fished-area rubble without macroalgae, and fished-area rubble with macroalgae) were tested with a 1-factor ANOVA in JMP Pro 13 software (SAS Institute). Rubble fouled with macroalgae had short algal fronds ~0.5–4.0 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 600 ml 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 m above the benthos. These 2 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 4 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 3 km 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 4 and 26 d on the reef (when experimental coral were 8 and 30 d post-settlement, respectively). Recruitment to control

rubble was low in each area (0–0.1 recruit per 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, 9 replicates were lost due to rubble becoming unattached from the benthos over 26 d 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 (1) the MPA (without macroalgae), (2) the fished area but without macroalgae, or (3) the fished area but with macroalgae ($n = 14$ – 15 for each treatment). Experimental procedures were the same as in the previous 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 m above the benthos. Settlement was assessed at 24 and 48 h. Survivorship of newly settled juvenile coral was assessed 4 d after the initial 48 h 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 per 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) was used to analyze larval metamorphosis, larval settlement, and larval and juvenile survivorship. Larval metamorphosis and

survivorship were analyzed by a 2-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 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 (Fig. 1A, PERMANOVA: sample type $p = 0.001$, area of origin $p = 0.426$, sample type \times area of origin $p = 0.803$). Water and larval microbiomes were more diverse than adult microbiomes (Fig. 1B, number of OTUs: sample type $p < 0.001$, area of origin $p = 0.764$, sample type \times area of origin $p = 0.909$; Fig. 1C, Shannon diversity index: sample type $p < 0.001$, area of origin $p = 0.539$, sample type \times area of origin $p = 0.282$), despite under-sampling water microbial communities at a rarefaction depth of 1650 (see rarefaction curve in Fig. S2 in the Supplement). Findings were similar when 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) (Fig. S3B, PERMANOVA: life stage $p = 0.001$, area of origin $p = 0.338$, life stage \times 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 (Fig. S3A,B). However, differences detected with PERMANOVA are partially due to dispersion differences among groups, with microbiome composition exhibiting lower dispersion among MPA adults compared with dispersion among fished-area adults, or to larvae from either area (Fig. S3B PERMDISPERSION: area of origin $p = 0.743$, life stage $p = 0.002$, area of origin \times life stage $p = 0.013$, see Table S1 in the Supplement 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 (Fig. S3C,D, Monte Carlo PERMANOVA: adults $p = 0.246$, larvae $p = 0.588$).

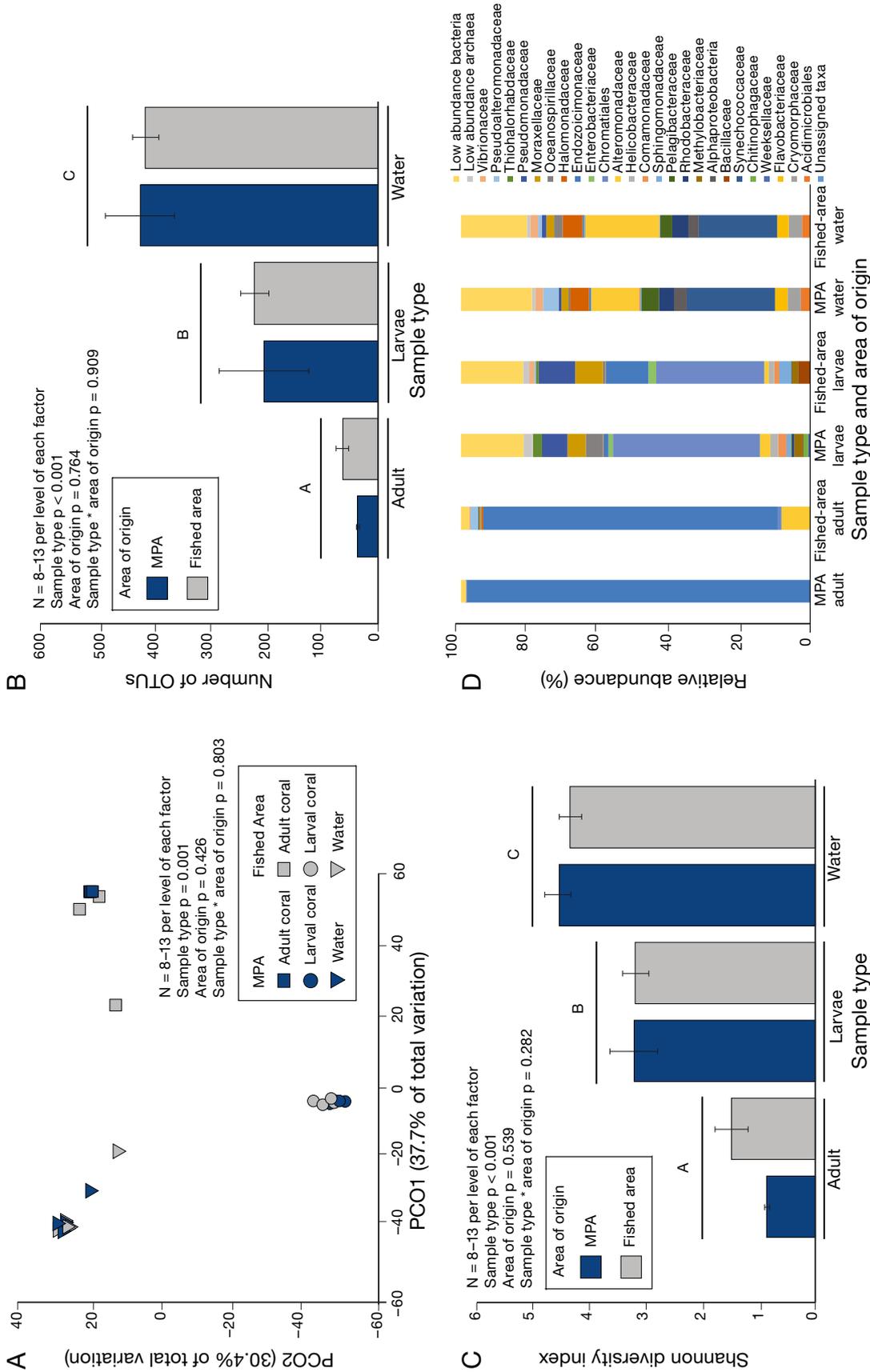


Fig. 1. (A) PCO and PERMANOVA analysis of Bray-Curtis dissimilarity matrix of coral *Pocillopora damicornis* larvae and adults and water microbiomes from the MPA and fished area. (B) OTU richness in coral and water samples (mean \pm SE). (C) Shannon diversity index for coral and water samples (mean \pm SE). OTUs and Shannon diversity were analyzed by a 2-factor ANOVA with Tukey's 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. In (A), each coral larva and coral adult data point represents the mean community composition for a single replicate

We also compared each common taxonomic group that comprised $\geq 2\%$ relative abundance and the pooled group of uncommon bacterial and archaeal taxa ($< 2\%$ relative abundance) between MPA and fished-area sites (Fig. 1D, Tables S2A,B & S3A,B). 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 datasets. In contrast, certain taxonomic groups differed notably in relative abundance between adults and larvae. Endozoicimonaceae were enriched 13-fold in adults compared with larvae ($\sim 90\%$ vs. $\sim 7\%$; 2-factor ANOVA: source area $p = 0.722$, life stage $p < 0.001$, source area \times 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 S2A,B). Larvae were also enriched 8-fold in low abundance bacteria ($p = 0.001$) and 90-fold in low abundance archaea ($p = 0.002$) compared with adult coral (Table S2A,B).

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, 2 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: Responses of coral microbial communities' regarding limitations of 16S-based classification of microbial species) were the only potential coral pathogens in more than 1 of the 16 coral replicates. We did not detect *V. shilonii* in any of our MPA coral at a sampling depth of 1650; however, it occurred at low ($< 1\%$), but significantly higher, relative abundances in the fished-area coral (Fig. 2A, Table S4, 2-factor ANOVA: coral area of origin $p = 0.009$, life stage $p = 0.116$, coral area of origin \times 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 (Fig. 2B, Table S4, 2-factor ANOVA: coral area of origin $p = 0.003$, life stage $p = 0.041$, coral area of origin \times life stage $p = 0.234$). Vibrionaceae

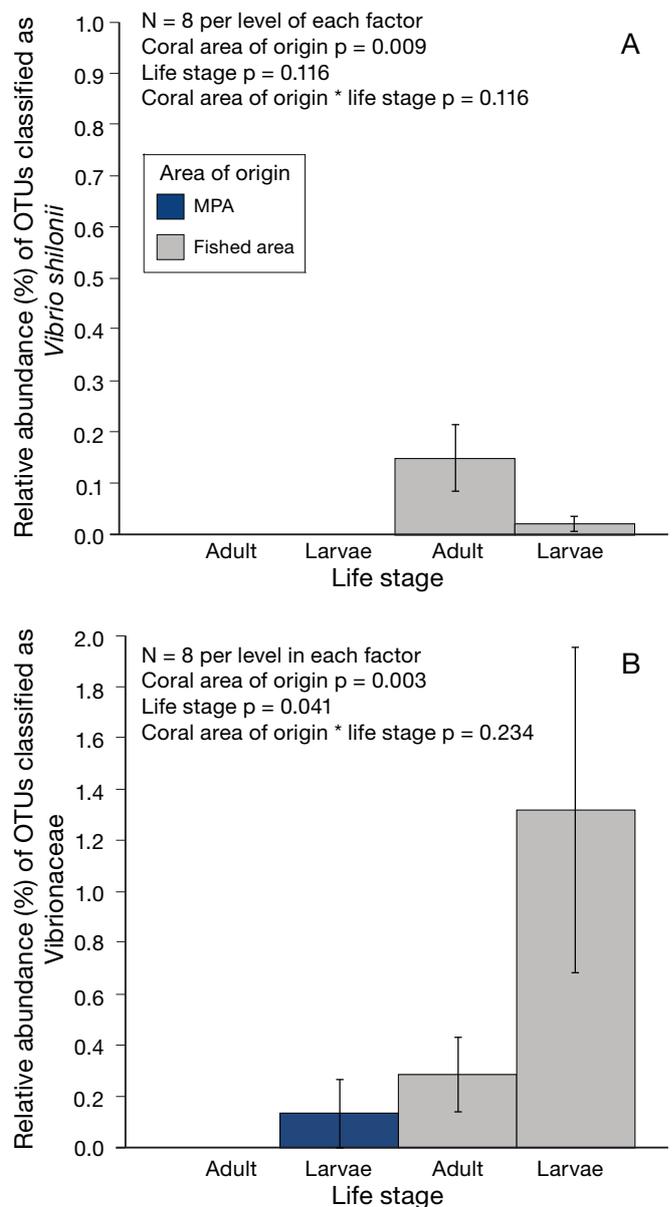
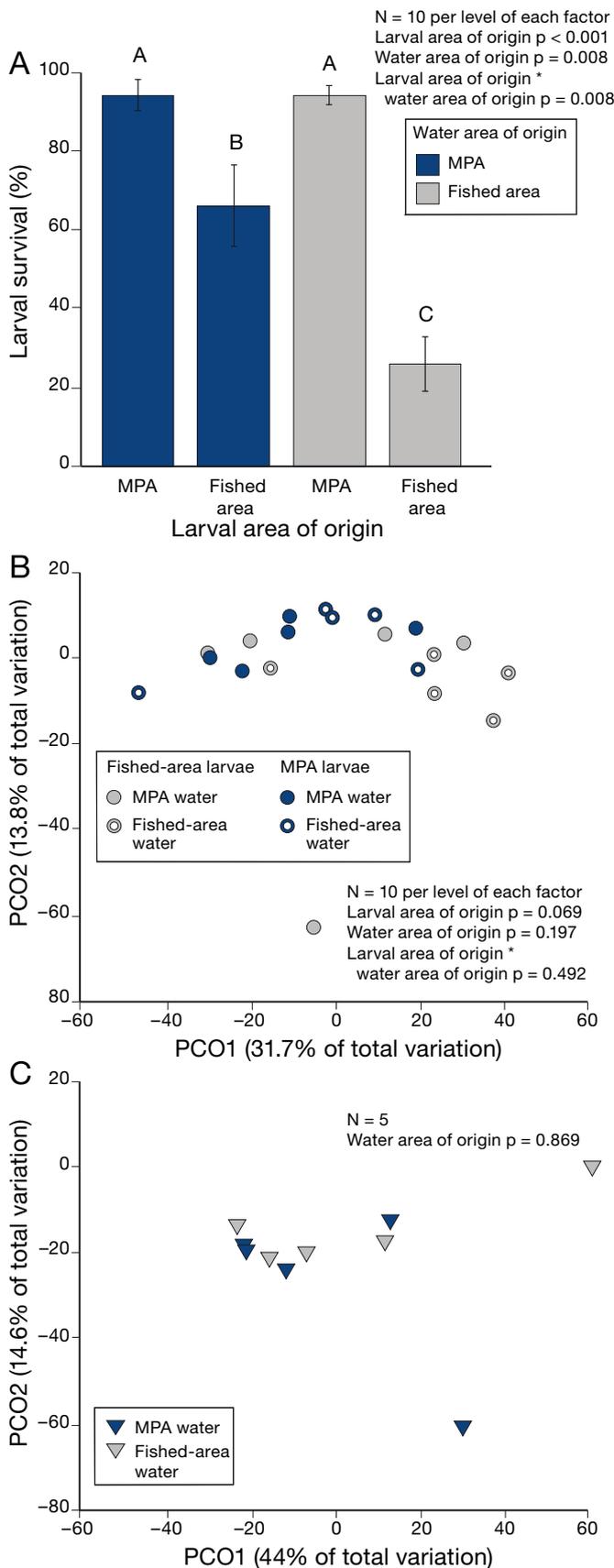


Fig. 2. Abundances (mean \pm SE) and 2-factor ANOVA analyses of (A) *Vibrio shilonii* and (B) taxa within the family Vibrionaceae in coral *Pocillopora damicornis* (adults and larvae) from the MPA and fished area

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 S4, $p = 0.269$, $n = 5$). The abundances of Vibrionaceae also did not differ significantly between MPA and fished-area water (Table S4, $2.08 \pm 0.66\%$ vs. $0.93 \pm 0.39\%$, respectively, $p = 0.898$, $n = 5$).



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 6 d 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 (Fig. 3A, 2-factor ANOVA, Tukey’s HSD post-hoc analysis; larval area of origin $p < 0.001$, water area of origin $p = 0.008$, larval area of origin \times 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 (2-factor ANOVA: larval area of origin $p = 0.332$, water area of origin $p = 0.332$, larval area of origin \times water area of origin $p = 0.332$).

When comparing the microbiomes of larvae that survived the 6 d 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 (Fig. 3B, PERMANOVA: larval area of origin $p = 0.069$, water area of origin $p = 0.197$, larval area of origin \times water area of origin $p = 0.492$), nor between the MPA and fished-area water in which the larvae were held (Fig. 3C, $p = 0.869$). The only suggested coral pathogens found on larvae in this experiment were OTUs classified as *V. 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 S4 in the Supplement). *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 S4). These abundances did not differ significantly among treatments (2-factor ANOVA: larval

Fig. 3. (A) Survival (mean \pm SE) and 2-factor ANOVA analysis of coral *Pocillopora damicornis* larvae from the MPA and fished area maintained in MPA or fished-area water for 6 d ($n = 10$ per level of each factor). Letters above bars indicate significant groupings by Tukey’s 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 6 d. (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 6 d

area of origin $p = 0.177$, water area of origin $p = 0.551$, larval area of origin \times 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 ($0.53 \pm 0.14\%$) water (Table S4), 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 S4, $2.08 \pm 0.66\%$ vs. $0.93 \pm 0.39\%$, respectively, $p = 0.898$, $n = 5$).

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 (Fig. 4A, Table 1A, larval area of origin \times 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 (Fig. 4A, Table 1A, substrate type \times 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 (Fig. 4B, Table 1B, substrate out-plant treatment \times time $p < 0.001$). Survival on fished-area substrate out-planted to the fished area was 12–29% by Day 4 and 5–8% by Day 26. In contrast, survival on MPA substrate out-planted to the MPA was 49–64% on Day 4 and 22–39% on Day 26. Surprisingly, given lower survivorship of fished-area larvae pre-settlement (Fig. 3A), larvae from fished-area adults survived better as newly settled juveniles when out-planted to the field than did those from MPA adults (Fig. 4B, Table 1B, larval area of origin $p = 0.007$, larval area of origin \times 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 $\leq 4\%$ and did not differ among treatments (larval area of origin $p = 0.336$, substrate type $p = 0.747$, larval area of origin \times substrate type $p = 0.747$).

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.

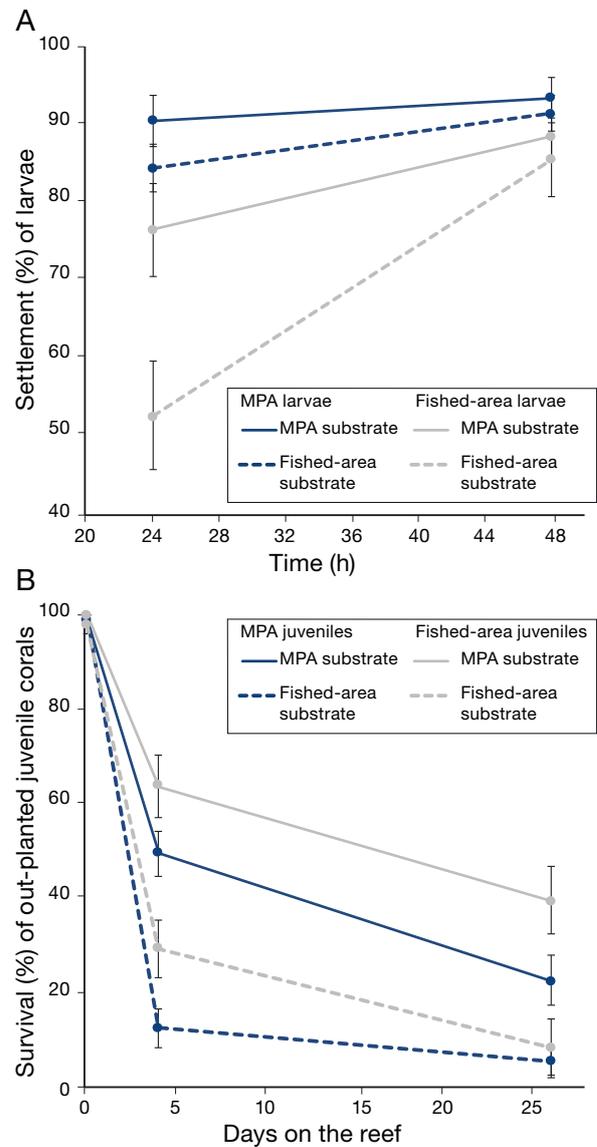


Fig. 4. (A) Settlement (mean \pm SE) of MPA and fished-area coral *Pocillopora damicornis* larvae on rubble from the MPA without macroalgae and from the fished area with macroalgae at 24 and 48 h ($n = 20$ per level of each factor; absolute percentages provided). See Table 1A for statistical analyses of repeated-measures ANOVA on square-root transformed proportion data. (B) Survival (mean \pm 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 4 d old ($n = 13$ –18 per level of each factor due to loss of zip-tied rubble on the reef over time). See Table 1B for statistical analyses of repeated-measures ANOVA on proportion data

differences in abundances of macroalgae or CCA 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, $79\% \pm 0.04$,

Table 1. Repeated-measures ANOVA on (A) square-root transformed settlement of coral *Pocillopora damicornis* 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 d 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 × Substrate type	1	2.10	0.156
Larval area of origin × Time	1	14.26	<0.001
Substrate type × Time	1	7.40	0.010
Larval area of origin × Substrate type × Time	1	3.72	0.062
B			
Larval area of origin	1	8.16	0.007
Substrate out-plant treatment	1	46.39	<0.001
Time	2	446.69	<0.001
Larval area of origin × Substrate out-plant treatment	1	0.77	0.387
Larval area of origin × Time	2	4.67	0.013
Substrate out-plant treatment × Time	2	22.53	<0.001
Larval area of origin × Substrate out-plant treatment × Time	2	1.15	0.322

84% ± 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 (Fig. 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 (Fig. 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 macro-

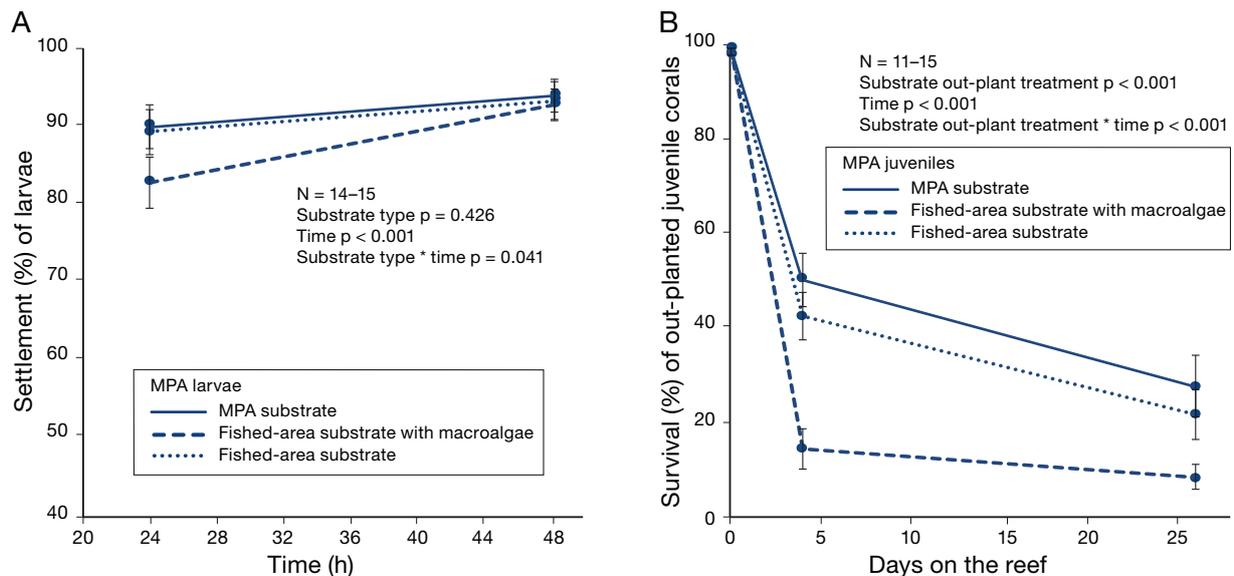


Fig. 5. (A) Percent settlement (mean ± SE) of MPA coral *Pocillopora damicornis* larvae on rubble from the MPA without macroalgae, the fished area without macroalgae, and the fished area with macroalgae at 24 and 48 h (n = 15 with the exception of 1 lost replicate due to sloughing of macroalgae during 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 4 d old. Repeated-measures ANOVA was performed on proportion data (n = 11–15 due to loss of replicates planted on the reef over time)

algae survived similarly well whether placed in the MPA or fished area (Fig. 5B, 43–51% survival on Day 4 and 22–28% on Day 26). In contrast, those on macroalgae-fouled rubble in the fished area experienced only 15% survival to Day 4 and 9% to Day 26 (Fig. 5B). On Days 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 with the fished area on Days 4 and 26, respectively.

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 yr) 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 m 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+ yr we have worked on these reefs (M. E. Hay pers. obs.).

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 *Hali-medea*], 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, 2013, 2017, Vega Thurber et al. 2012, Nelson et al. 2013). Specifically, algae are predicted to affect corals through dissolved organic matter 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, 2012, Pratte et al. 2018). 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 m 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). In addition, corals in the fished area grow as well as those in the MPA when macroalgae within ~15 cm of the coral colonies are removed (Clements et al. 2018). This suggests minimal effects of macroalgal dissolved organic matter on growth of corals at the scale of ≥ 15 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. S. Beatty unpubl. 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, Vega 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 area 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. 2017), including *P. damicornis* (Bayer et al. 2013). We also detected similar abundances of Endozoicimonaceae (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 FSW before larval release; therefore, we do not know whether Endozoicimonaceae were rapidly acquired from the environment or vertically transferred to brooded larvae. However, larvae were rinsed in FSW 3 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 (Tout et

al. 2015, Lee et al. 2017, Morrow et al. 2017), 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 with 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 *Ruminooccus gnnavus* as an indicator species of fished-area larvae. *R. gnnavus* is an anaerobic gut microbe that has been implicated in human disease and is capable of breaking down mucins (Croft 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 (Zaneveld et al. 2016, 2017), 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 with 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 (Vega 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 et

al. 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 previous evidence showing that larvae of *P. damicornis* are packed with photosynthate-providing *Symbiodinium* and that larvae can settle in under 2 h or remain viable in the plankton for 100 d (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 6 d 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 (Fig. 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 *V. shilonii* on coral larvae that experienced higher mortality. It is possible that *V. 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 6 d 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 (Fig. 3B,C), 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 & Hay 2008, Vega 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, Dixon 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 in the fished area (Fig. 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 Days 4 and 26 was ~190 and 150% higher, respectively, if on fished-area rubble without macroalgae than on fished-area rubble fouled with macroalgae (Fig. 5B, days 4 and 26). In contrast, the increase in survival due to site was modest (Fig. 5B, Days 4 and 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. In addition, larval and adult *P. damicornis* from the MPA had significantly lower abundances of Vibrionaceae and OTUs classified as the coral pathogen *V. 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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