Heat-stressed coral microbiomes are stable and potentially beneficial at the level of taxa and functional genes

Laís Farias Oliveira Lima[1], Amanda Alker[1], Megan Morris[2], Robert Edwards[3], Samantha De Putron[4], and Elizabeth Dinsdale[3]

[1]San Diego State University
[2]Lawrence Livermore National Laboratory
[3]Flinders University
[4]Bermuda Institute of Ocean Sciences

February 3, 2023

Abstract

Coral reef health is tightly connected to the coral microbiome. Coral bleaching and disease outbreaks have caused an unprecedented loss in coral cover worldwide, particularly correlated to a warming ocean. Coping mechanisms of the coral holobiont under high temperatures are not completely described, but the associated microbial community is a potential source of acquired heat-tolerance. The relationship between stress and stability in the microbiome is key to understanding the role that the coral microbiome plays in thermal tolerance. According to the Anna Karenina Principle (AKP), stress or disease will increase instability and stochasticity among animal microbiomes. Here we investigate whether heat stress results in microbiomes that follow the AKP. We used shotgun metagenomics in an experimental setting to understand the dynamics of microbial taxa and genes in the surface mucous layer (SML) microbiome of the coral Pseudodiploria strigosa under heat treatment. The metagenomes of corals exposed to heat stress showed high similarity, indicating a deterministic and stable response of the coral microbiome to disturbance, in opposition to the AKP. We hypothesize that this stability is the result of a selective pressure towards a coral microbiome that is assisting the holobiont to withstand heat stress. The coral SML microbiome responded to heat stress with an increase in the relative abundance of taxa with probiotic potential, and functional genes for nitrogen and sulfur acquisition. These consistent and specific microbial taxa and gene functions that significantly increased in proportional abundance in corals exposed to heat are potentially beneficial to coral health and thermal resistance.

Introduction

Microbial symbioses are the engine of coral reef ecosystems. Corals associate with endosymbiotic dinoflagellates of the family *Symbiodiniaceae* and a diverse microbiome (e.g. bacteria, archaea, viruses) which function as a unit and form a holobiont (Rohwer et al. 2002). The coral holobiont depends on key services such as nitrogen and sulfur cycling mediated by the associated microbiome (Wegley et al. 2007; Siboni et al. 2008; Raina et al. 2013; Rädecker et al. 2015). The coral surface mucous layer (SML) sustains a diverse and abundant community of these microbial partners (Koren and Rosenberg 2006; Sharon and Rosenberg 2008; Garren and Azam 2012; Ainsworth et al. 2015; Lima et al. 2020, 2022). The coral microbiome benefits from the high nitrogen content and organic matter in the SML (Wild et al. 2005; Rädecker et al. 2015) and provides protection against coral pathogens via production of antimicrobials (Ritchie 2006; Krediet et al. 2013). However, coral-associated microbial communities are sensitive to environmental changes, especially to increased temperature, which disrupt the beneficial services provided to the holobiont (Thurber et al. 2009; Vega Thurber et al. 2014; Raina et al. 2016; Zaneveld et al. 2016).

Coral reefs are at great risk of collapse as coral bleaching (i.e., loss of algal symbionts) and disease outbreaks

have become more frequent in the last two decades, particularly correlated to rising seawater temperature, leading to major losses in coral cover worldwide (Willis et al. 2004; Maynard et al. 2015; Heron et al. 2016; Precht et al. 2016; Muller et al. 2018). These losses are pronounced on shallow water reefs of the Caribbean, where an overall decline in coral cover of up to 59 % has occurred since 1984 (Jackson et al. 2014). Corals live at their upper thermal limits, and therefore thermal thresholds may not be able to adjust to projected rises in seawater temperature in times of rapid environmental change (Berkelmans and Willis 1999; Fitt et al. 2001; Palumbi et al. 2014; Lough et al. 2018).

The mechanisms of resistance to environmental change in the coral holobiont are not completely understood, but the associated microbial community is a potential source of acquired heat-tolerance (Peixoto et al. 2017; Ziegler et al. 2017; Rosado et al. 2019; Doering et al. 2021; Santoro et al. 2021). There is also a potential evolutionary role of the microbiome as a source of genes (e.g. stress response genes) that can be used in thermal resilience and disease protection via mechanisms such as horizontal gene transfer (Webster and Reusch 2017). The use of metagenomics associated with physiological data in experimental settings is a recommended approach to further explore the role of the coral microbiome in heat-tolerance and stress response (Bourne et al. 2016). Another outstanding topic to be investigated is the relationship between stress and stability in the coral microbiome (Zaneveld et al. 2017).

According to the Anna Karenina Principle (AKP) for animal microbiomes, stress or disease will increase instability and result in low similarity among microbiomes exposed to the same disturbance (Zaneveld et al. 2017). In an analogy to a quote from Tostoy's novel *Anna Karenina* , the AKP states that 'all healthy microbiomes are similar; each dysbiotic microbiome is dysbiotic in its own way'. This increase in dissimilarity (i.e., microbial dispersion) among stressed microbiomes can be visualized in ordination methods (e.g., principal coordinate analysis) using a matrix of β-diversity distances between samples. When the AKP is shaping the microbial communities, healthy hosts present stable microbiomes that form tight clusters in ordination space, while stress leads to instability and higher β-diversity among microbiomes. The AKP seems to be shaping taxonomic composition in coral microbiomes under heat stress (Zaneveld et al. 2016; Ahmed et al. 2019), however, it needs to be further explored in the context of shotgun metagenomics for deeper coverage of microbial taxa and functional genes.

Here we investigate whether heat stress results in unstable coral SML microbiomes, as proposed by the AKP. We addressed these aims by exposing corals to a heat treatment and analyzing coral-algal physiological parameters and microbial taxa (genus level) and gene functions (stress response, nitrogen metabolism, and sulfur metabolism) associated with the coral SML using shotgun metagenomics.

Methods

Field sampling

We selected *Pseudodiploria strigosa* (Dana, 1846) as the coral host species because it is widely distributed across the Bermuda platform. In Bermuda, a high-latitude subtropical coral reef system, corals are exposed to a wide annual range in temperature fluctuations and reef-zone specific thermal regimes ranging from more fluctuating profiles in the inner lagoon patch reefs and milder conditions in the outer reefs (de Putron and Smith 2011a; Courtney et al. 2017, 2020; Lima et al. 2020; Wong et al. 2021). The reef zones sampled were approximately 8 km apart (Lima et al. 2020) and *P. strigosa* is a broadcast spawner; therefore, there is a high likelihood that gene flow between the coral hosts colonizing inner and outer reefs is maintained and that the host genetics is not structured into different populations. Indeed, studies on other species have indicated high genetic exchange among reef sites in Bermuda (Serrano et al. 2014, 2016). The sampling period occurred between May 18th and May 22nd, 2017, late spring in the northern hemisphere, when environmental conditions between the two reef zones, especially temperature, are similar. The environmental gradient assessed here are based on the knowledge that these two reef zones are exposed to different regimes on a seasonal basis, with the most striking fluctuations occurring in the winter and summer months (de Putron and Smith 2011b; Smith et al. 2013; Courtney et al. 2017). Therefore, we selected this period to capture a potential long-term acclimatization of the coral holobiont to their reef zones, and not their

immediate response to acute temperature fluctuations. The mucus from *P. strigosa* was collected from six colonies from the inner and outer reef zones (n = 12 colonies total) to describe the natural spatial variability in the coral microbiome (Lima et al. 2020, 2022). After the mucus sampling, each coral colony was collected using a hammer and chisel to be used in the experiments to assess heat resistance. The colonies (n = 12 total, 6 from each reef zone) were carefully placed in a cooler with seawater and transported to the Bermuda Institute of Ocean Sciences. The collections were performed via SCUBA diving at 4-6m depth.

Colony preparation and acclimation

After collection, the coral colonies were placed in an outdoor tank with opaque roof supplied with unfiltered flowing seawater to acclimate for a week. Each colony was fragmented in four pieces using a tile saw, tagged and all non-coral tissue area was cleaned and covered in reef-safe epoxy. The colony fragments, here referred as "coral nubbins", were left acclimating in the indoor system for two weeks (ambient temperature 25°C ± 1°C). Using coral nubbins in laboratorial experiments is a way to expose the same colony and microbiome to different treatments, allowing for assessment of genetic variability among treatments, as well as reducing the number of coral colonies collected. The nubbins were monitored and only healthy ones, assessed by recovery of the coring process (outward growth as opposed to tissue regression) and visual endosymbiont function as measured by color and fluorescence readings (Fv/Fm > 0.6) with a pulse amplitude modulated fluorometer (DIVING-PAM Walz Inc.) were used in the experiments.

Temperature treatments

After the acclimation period, twelve coral nubbins (6 from each reef zone) were exposed to a high temperature treatment (29°C ± 1°C), while the remaining were maintained at ambient conditions (control, 25°C ± 1°C). Temperature was gradually increased from ambient to heat conditions over two days then maintained within the target range. The indoor temperature-controlled tank system consisted of two fiberglass trays fed from a common header tank of filtered seawater. Each tray table was temperature controlled by aquarium chillers and heaters. Light intensity was controlled by aquarium lights (Storm and Storm X LED Controllers, CoraLux LLC) on a 12:12h photoperiod. Temperature was measured every 5 minutes throughout the experiment by pairs of HOBO data loggers (Onset Corp.) placed in each tray table and light intensity was measured by a LiCor PAR sensor.

Physiological measurements

Productivity and respiration rates were measured using dissolved oxygen probes in customized transparent glass chambers (approx. 4L) to detect oxygen evolution (production), and oxygen uptake (respiration rate). Each chamber had an oxygen sensor spot (PyroScience) glued to the inner chamber wall and a small in-line aquarium pump for water mixing during incubation. Oxygen concentration was monitored using a fiber optic sensor (PyroScience). Colonies were set individually inside the chamber that was bathed in and filled with the filtered, temperature-controlled seawater from the experimental tray table. Dissolved oxygen concentration was measured in the chamber during a 1h-dark period to calculate dark respiration rates (R) and subsequently during a light exposure period of 1h to calculate net productivity (NP). The difference of dissolved oxygen concentration at incubation the start and end of the experiment was normalized over time and colony surface area as described by Schneider & Erez (2006). Surface area was determined by the aluminum foil technique (Marsh, 1970) and image analysis (Image J, US National Institutes of Health) of planar digital images. The dark respiration and photosynthesis rates were analyzed according to their linearity over time for quality control. Gross productivity (GP) was calculated (gross productivity = net productivity + dark respiration). Photosynthesis to respiration (GP:R ratios) were calculated as a ratio of gross productivity (GP) to respiration (R) to estimate if production by the*Symbiodiniaceae* cells is exceeding maintenance requirements of both the symbiont and the coral host (Coles and Jokiel 1977). Maximum photochemical efficiency of photosystem II (i.e., effective quantum yield) was quantified via pulse-amplitude modulate (PAM) fluorometry (Warner et al. 1996; Jones et al. 1998). After an overnight dark acclimation period, fluorescence (Fm and F0) was measured by saturation pulses at three random spots on each colony to calculate the colony's average Fv/Fm. Maximum photochemical efficiency of the PSII (Fv/Fm, Fv = Fm

– F0) was calculated based on fluorescence measurements using a pulse amplitude modulated fluorometer (DIVING-PAM Walz Inc.).

Experiment design

Twelve acclimated coral nubbins (6 from each reef zone) were exposed to the high temperature treatment for one week while the remaining nubbins were maintained at ambient temperatures. Productivity and respiration rates and photochemical efficiency were measured the day before the heat treatment started (post-acclimation or pre-treatment) and on day 6, which is the final day of the experiment (post-treatment). The coral mucus was collected at the end of each incubation for respirometry using a two-way 50 ml syringe that is filled with 0.02 µm filtered seawater (Lima et al. 2020, 2022). The filtered seawater is flowed across the coral surface, dislodging the mucus and associated microbes, which are then sucked up via the recirculating tube, and resulting sample pushed through a 0.22 µm sterivex for DNA extraction.

Metagenomic analysis

Microbial DNA from the coral mucus collected on the 0.22-µm Sterivex was extracted using a modified Macherey-Nagel protocol using NucleoSpin column for purification. DNA was stored at - 20°C until quantification with Qubit (Thermo Fisher Scientific) (Dinsdale et al. 2008b). The Swift kit 2S plus (Swift Biosciences) was used for library preparation since it provides good results from small amounts of input DNA, characteristic of microbial samples collected from the surface of the host (Doane et al. 2017; Cavalcanti et al. 2018). All samples were sequenced by the Dinsdale lab on Illumina MiSeq at San Diego State University. The sequenced DNA was analyzed for quality control using PrinSeq (Schmieder and Edwards 2011) before annotation. The metagenomes were annotated through MG-RAST (Meyer et al. 2019), using the RefSeq database for taxonomic annotations and the SEED database for functional annotations.

For the taxonomic composition, the metagenomes were filtered in MG-RAST to include only Bacteria at the level of genera. For the functional composition, the metagenomes were filtered in MG-RAST for Stress Response, Nitrogen Metabolism, and Sulfur Metabolism. We selected these three broad functional gene groups (SEED subsystem level 1) because they have the greatest relevance for corals under heat stress (Wegley et al. 2007; Thurber et al. 2009; Littman et al. 2011; Raina et al. 2013; Nguyen-Kim et al. 2015; Cardini et al. 2016; Pogoreutz et al. 2017; Wang et al. 2018; Sun et al. 2022). The number of sequence hits for each microbial taxon or function is represented as the relative abundance by calculating the proportion of sequence hits for that parameter over the total number of sequences annotated for that metagenome. Metagenomes were compared using proportional abundance, which is preferred to rarefaction (McMurdie and Holmes 2014; Quince et al. 2017; Luz Calle 2019). Bacteria accounted for approximately 99 % of the annotation; therefore, we are only analyzing bacterial taxa and gene functions in this study. Metagenomes were sequenced from a subset of the coral nubbins used in the experiment (n = 8), representing four different coral colonies (n = 2 colonies per reef zone) that had their mucus sampled before and after the experimental treatments (n = 16 metagenomes total). These metagenomes were compared with the ones from the same colonies collected *in situ* (Lima et al. 2022).

Statistical Analysis

Statistical analyses were conducted using PRIMER v7 plus PERMANOVA, Statistical Analyses of Metagenomic profiles (STAMP) software (Parks et al. 2014), and R (R Project for Statistical Computing). Significant differences in the relative abundances of microbial genera and functions in the coral microbial communities sampled from inner and outer reefs were identified by permutational multivariate analysis of variance (PERMANOVA) using Bray-Curtis distances of normalized relative abundance obtained using a square-root transformation. A cluster analysis was created to visualize the similarity of the coral microbiome between temperature treatments. We also used PRIMER to calculate Pielou's evenness index (J') and Shannon's diversity index (H') of microbial genera. Principal Component Analyses (PCA) and the multiple comparisons of either taxa or functions across the three groups of metagenomes (i.e., pre-treatment, ambient, and heat) were conducted in STAMP using ANOVA/Tukey-Kramer, Welch's pairwise comparisons, and Benjamini-Hochberg FDR corrections. We used R to test parametric assumptions of normality (Shapiro-Wilk's test)

and homoscedasticity (Bartlett's test), and differences in the percent changes of maximum photochemical efficiency of photosystem II (Fv/Fm) and productivity to respiration ratios (GP: R) between temperature treatments (Student's T-Test).

Results

Coral-algal physiology

Corals showed a significantly lower photochemical efficiency (Fv/Fm) after exposure to the heat treatment (Supplemental Fig. 1.A, t = 4.37, df = 5.94, p-value < 0.005). GP:R ratios also decreased, although the mean difference was not statistically significant (Supplemental Fig. 1.B, t = 2.31, df = 3.39, p = 0.09). There were no visual signs of paling or bleaching of coral nubbins during the experiment.

Coral SML microbiome

The metagenomes associated with the coral SML of *P. strigosa* were sequenced at high coverage, ranging from 356,426 to 1,296,198 sequence counts (Supplemental Tab. 1). Principal Component Analyses of bacterial genera and genes related to stress response, nitrogen metabolism, and sulfur metabolism (Fig.1), showed that dispersion (β-diversity) was lower among metagenomes from heat-stressed corals than from pre-treatment and control groups. Pairwise PERMANOVAs concluded that relative abundances of microbial genera (t = 2.62, P(perm) < 0.03), nitrogen metabolism genes (t = 2.52, P(perm) = 0.03), and sulfur metabolism genes (t = 3.14, P(perm) = 0.03) were significantly different between pre-treatment and heat-stressed microbiomes. Metagenomes from corals at ambient conditions did not significantly change when compared to pre-experiment or heat treatments. Relative abundances of microbial stress response genes showed no significant change across treatments.

Heat exposure led to a significant increase in the relative abundances of *Ruegeria* (t = - 2.38, corrected p-value < 0.02),*Roseobacter* (t = - 2.15, corrected p-value < 0.001),*Oceanibulbus* (t = - 1.58 corrected p-value < 0.03),*Chromohalobacter* (t = - 0.84, corrected p-value < 0.02), and *Halomonas* (t = - 0.87, corrected p-value < 0.02), according to Welch's pairwise comparisons among the top 20 most abundant taxa in the coral microbiome (Fig. 2). In contrast, there was a significant decrease in the relative abundances of *Shewanella* (t = 0.82 corrected p-value < 0.001), *Synechococcus* (t = 0.88, corrected p-value < 0.04), and *Vibrio* (t = 0.284, p-value < 0.02) in the microbiome of corals exposed to heat treatment (Fig. 2). Heat-stressed coral SML metagenomes analyzed at the level of bacterial genera formed a cluster with the greatest similarity (Bray-Curtis index > 95%) across all samples (Supplemental Fig. 2). Microbial richness (S, number of genera) ranged from 578 to 587 genera and did not significantly change between microbiome samples collected *in situ* and across the experimental treatments (Supplemental Tab. 2). Diversity (H') was highest *in situ* due a decrease in evenness (J') under experimental conditions.

Within nitrogen metabolism, the relative abundances of microbial gene pathways related to amidase with urea and nitrile hydratase (t = - 0.363, corrected p-value < 0.0001), allantoin utilization (t = - 1.079, corrected p-value = 0.042), and nitrogen fixation (t = - 0.130, corrected p-value = 0.049) increased in the coral microbiome under heat stress, while nitrosative stress (t = 1.628, corrected p-value = 0.033), and ammonia assimilation (t = 4.51 corrected p-value = 0.037) decreased (Fig. 4.B).

Sulfur metabolism microbial genes also changed in relative abundance after heat exposure of corals. There was an increase in glutathione utilization (t = - 1.70, corrected p-value < 0.0001), sulfur oxidation (t = - 8.08, corrected p-value < 0.0001), and taurine utilization (t = - 1.44, corrected p-value < 0.0001), and a decrease in inorganic sulfur assimilation (t = 8.04, corrected p-value < 0.0001) (Fig. 4.C).

Discussion

Stability of the coral SML microbiome under heat stress

Thermal stress decreased coral-algal physiological performance and caused significant compositional and functional changes in the microbiome of *P. strigosa* , indicating holobiont dysbiosis (Levy et al. 2017). According to the AKP for animal microbiomes, stressful conditions disrupt the ability of the holobiont to

regulate the microbial community composition, resulting in an increase in dissimilarity (i.e., dispersion, β-diversity) among microbiomes experiencing dysbiosis (Zaneveld et al. 2017). Therefore, the AKP predicts an unstable and stochastic microbiome composition on hosts that were exposed to acute stress or disease, including corals under heat stress (Zaneveld et al. 2016, 2017; Ahmed et al. 2019). However, our results showed that β-diversity decreased in heat-stressed microbiomes at the level of bacterial genera, stress response, nitrogen metabolism, and sulfur metabolism genes (Fig.1). Heat-stressed coral SML microbiomes formed a cluster with the highest similarity among all metagenomes analyzed. The relative abundance of specific bacterial genera (Fig. 2) and gene functions (Fig. 3) changed consistently across coral replicates that were exposed to the heat treatment, while in the ambient treatment these changes were more variable and not significantly different from pre-treatment coral microbiomes. Diversity (H') remained high both in ambient and heat conditions (Supplemental Tab. 2). Here we present a case for heat stress having a more stable and deterministic effect on coral microbiomes at taxonomic and functional level, in opposition to the AKP.

Bacterial taxa

Ruegeria and *Roseobacter* significantly increased in relative abundance in response heat stress (Fig. 2). High abundances of Rhodobacterales such as *Ruegeria* and *Roseobacter* in juvenile and adult corals suggest they play a key role in coral fitness (Ceh et al. 2013a; Zhou et al. 2017), nitrogen acquisition and remineralization (Ceh et al. 2013b; McNally et al. 2017), and sulfur cycling (Raina et al. 2013). *Ruegeria* are among the three genera that are most frequently associated with coral species (Hugget and Appril 2019). Some strains of *Ruegeria* have probiotic potential as they can inhibit growth of pathogen *Vibrio coralliilyticus*(Miura et al. 2019) and support corals to withstand heat stress (Rosado et al. 2019, Kitamura et al. 2021, Santoro et al. 2021). In fact,*Vibrio* decreased in relative abundance in the coral microbiome under heat treatment (Fig. 2), indicating that *P. strigosa* was able to keep these potential pathogens in check. Lower abundances of*Synechococcus* after heat stress (Fig. 2) could be an indication that corals are using these cyanobacteria as an energy and nutrient source via heterotrophic feeding to compensate for lower productivity of the algal symbiont (Fig. 1). Corals can preferentially feed on*Synechococcus* , especially to recover from heat stress and bleaching (McNally et al. 2017; Meunier et al. 2019; Hoadley et al. 2021).

Stress response

Bacterial stress response genes in the coral SML metagenomes were not affected by heat stress (Fig. 3A). Metagenomics describes the relative abundance of genes in the microbiome which identifies functional genes that are potentially being selected by the microbiome under those conditions (Dinsdale et al. 2008a; Coelho et al. 2022) and it does not measure which functional genes are being expressed at the point the sample was taken. Therefore, bacterial stress response gene expression could be increased under heat stress, although the metagenomes show that they are potentially not being selected by the coral SML microbiome over the experiment to respond to stress. Future studies coupling metagenomics to metatranscriptomics could elucidate the changes in stress response genes in the coral microbiome under heat stress. However, our metagenomes showed that bacterial genes related to nitrogen and sulfur metabolism strongly responded to heat stress and were potentially selected in the coral SML microbiome of *P. strigosa* .

Nitrogen metabolism

Ammonia assimilation genes decreased in relative abundance after heat stress exposure, indicating that less ammonia is being used in the biosynthesis of bacterial compounds by the microbiome (Fig. 3B). Coral-associated bacteria compete with *Symbiodiniceae* for host-generated ammonia, which is the preferred form of nitrogen of the symbiotic algae (Rädecker et al. 2015; Bourne et al. 2016). Lower ammonia assimilation by the coral SML microbiome under heat stress is potentially beneficial to the holobiont because more ammonia may be available to the algal symbiont to support photosynthesis and prevent coral bleaching.

Nitrosative stress genes allow bacteria to detoxify nitric oxide (NO) and reactive nitrogen species (RNS) involved in the denitrification process (Poole 2005). Nitrosative stress genes decreased in relative abundance under heat stress and denitrification and NO synthase genes did not change in proportion (Fig. 3B). The-

refore, lower relative abundance of nitrosative stress genes could be a result of stability in the levels of NO and RNS in the coral SML microbiome.

Nitrogen incorporation genes, via allantoin utilization and nitrogen fixation, significantly increased in the coral SML post-heat stress (Fig. 3B). Allantoin is a urea-related compound produced by plants that can be a nitrogen source to bacteria and a form to transport fixed nitrogen to plants when nitrogen is limiting (Cobo-Díaz et al. 2015; Minami et al. 2016). Bacteria can provide about 11% of the nitrogen required by *Symbiodiniaceae* via nitrogen fixation in the coral holobiont (Bourne et al. 2016; Cardini et al. 2016). The significant increase in the proportions of allantoin utilization and nitrogen fixation genes could be a key beneficial service offered by the coral SML microbiome to supply nitrogen to the holobiont to withstand heat stress.

Sulfur metabolism

The relative abundances of bacterial genes related to glutathione utilization, sulfur oxidation and taurine oxidation, increased after heat stress in the coral SML microbiome (Fig. 3C). Glutathione is a key sulfur-based compound used by bacteria for protection against oxidative stress and by the coral holobiont as a source of organic sulfur (Masip et al. 2006; Wegley et al. 2007; Bourne et al. 2016). Taurine oxidation could be coupled to sulfur oxidation to increase sulfur availability in the coral holobiont. The degradation of the amino acid taurine can produce thiosulfate, which is converted to sulfate via sulfur oxidation (Robbins et al. 2019, Lima et al. 2022). Sulfur oxidation genes increase in relative abundance under stress and bleaching (Wegley et al. 2007; Littman et al. 2011). Taurine oxidation by *Ruegeria* was coupled to high primary productivity by planktonic dinoflagellates and plays a key role in the organic sulfur turnover in pelagic environments (Landa et al. 2019). Here, *Ruegeria* could be playing a similar role by increasing sulfur availability via taurine oxidation to the dinoflagellates *Symbiodiniaceae* in the coral holobiont under stress.

Conclusion

The future of coral reefs depends on a healthy coral microbiome, as coral bleaching and disease outbreaks are some of the leading causes of an unprecedented loss in coral cover worldwide, particularly in response to rising seawater temperature (Miller et al. 2009; Maynard et al. 2015; Heron et al. 2016; Precht et al. 2016). We used shotgun metagenomics in an experimental setting to understand the dynamics of microbial taxa and genes in heat-stressed corals, in the context of the AKP. The metagenomes of corals exposed to heat stress showed greater stability, therefore, not conforming to the AKP. Anti-AKP effects are expected when blooms of specific microbes or strong environmental filters are shaping animal microbiomes (Zaneveld et al. 2017). We hypothesize that this stability is the result of a selective pressure towards a beneficial microbiome that supports the holobiont to withstand stress (Fig 4.). Despite the significant decline in physiological performance, the coral colonies of *P. strigosa* from Bermuda showed an overall tolerance as none of the coral replicates bleached after exposed to high temperatures for one week. The microbiome of heat-stressed corals could have played a role in preventing bleaching by controlling opportunistic pathogens and providing nitrogen and sulfur to the algal symbiont and energy to the coral host (Fig. 4.).

Other factors could help to explain the anti-AKP effects seen here. AKP in coral microbiomes under heat stress were reported after long-term exposure (2 – 3 years) (Zaneveld et al. 2016; Ahmed et al. 2019), while this study tested for a shorter heat exposure (1 week). There could be host species-specific differences in the microbiome response of *P. strigosa* (present study) compared to *Siderastreasiderea* , *Porites* spp., and *Agaricia* spp. (Zaneveld et al. 2016) and to anemone *Aiptasia* used as a coral model (Ahmed et al. 2019). Also, these studies used amplicon metagenomics (16S rRNA) at the taxonomic level of family and order, contrasting with the shotgun metagenomics pipeline at the level of microbial genus and gene functions used here. Therefore, the AKP for coral microbiomes under heat stress need to be further investigated across different methodologies and systems.

Promising conservation efforts have been focusing on promoting and maintaining coral microbiome health (Damjanovic et al. 2017; Epstein et al. 2019; van Oppen and Blackall 2019), including the development of coral probiotics (Peixoto et al. 2017; Rosado et al. 2019; Santoro et al. 2021). Here we support these efforts

by showing that heat stress can lead to stable and potentially beneficial coral microbiomes.

Data Availability

The metagenomic data from this study is publicly available in the SRA database as BioProject PRJNA595374 (https://www.ncbi.nlm.nih.gov/bioproject/595374) and in MG-RAST as public study SDSU_BIOS_2017 (mgp81589; https://www.mg-rast.org/linkin.cgi?project=mgp81589).

Acknowledgements

We thank San Diego State University (SDSU)/University of California, Davis (UCD), Joint Doctoral Program in Ecology at SDSU/UCD, SDSU Graduate Travel Awards, and the Bermuda Institute of Science (BIOS) Grant-in-Aid Sydney L. Wright and Wolfgang Sterrer Fellowships for supporting Ph.D. candidates L.F.O.L. and A.T.A. with travel costs and research expenses at BIOS. We thank Yingqi Zhang for support in the coral physiology experiment as an undergraduate intern at BIOS. L.F.O.L. was also supported with a graduate student scholarship awarded by S. Lo & B. Billings Global Shark Research and Conservation fund. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Figures and Tables

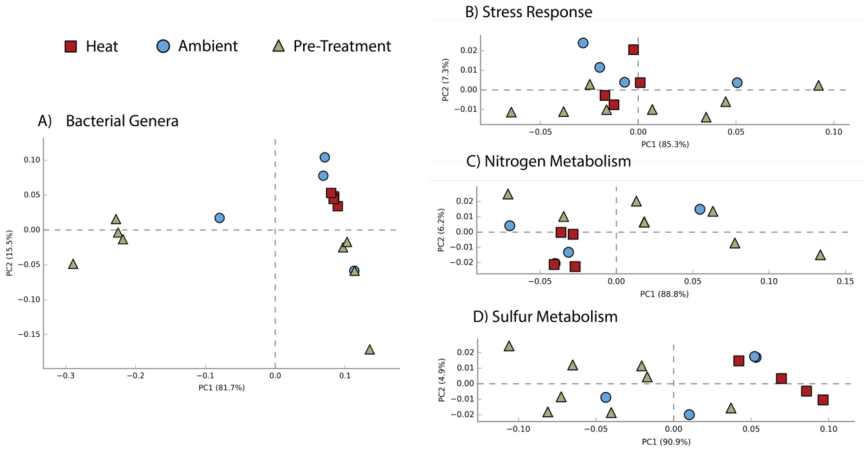


Figure 1. Principal Component Analysis of bacterial genera (A), stress response (B), nitrogen metabolism (C), and sulfur metabolism (D) genes in the coral SML of *P. strigosa* exposed to different temperature treatments for one week.

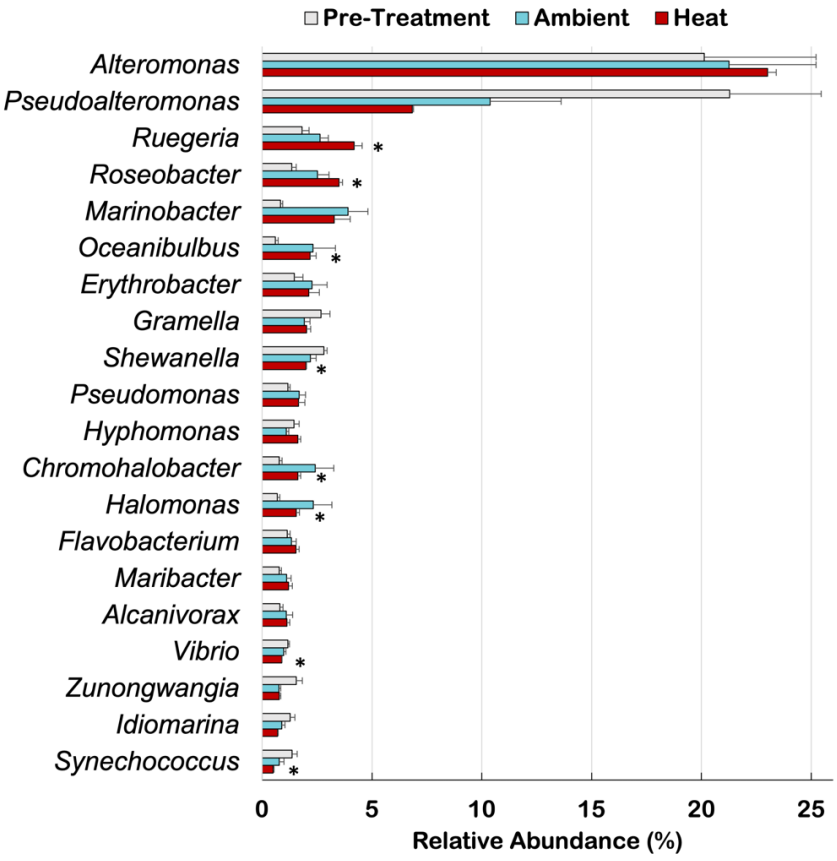


Figure 2 . Top 20 most abundant bacterial genera (mean ± SE) across different temperature treatments. Asterisks indicate a significant difference between pre-treatment and heat-treatment metagenomes according to Welch's pairwise comparisons, and Benjamini-Hochberg FDR corrections.

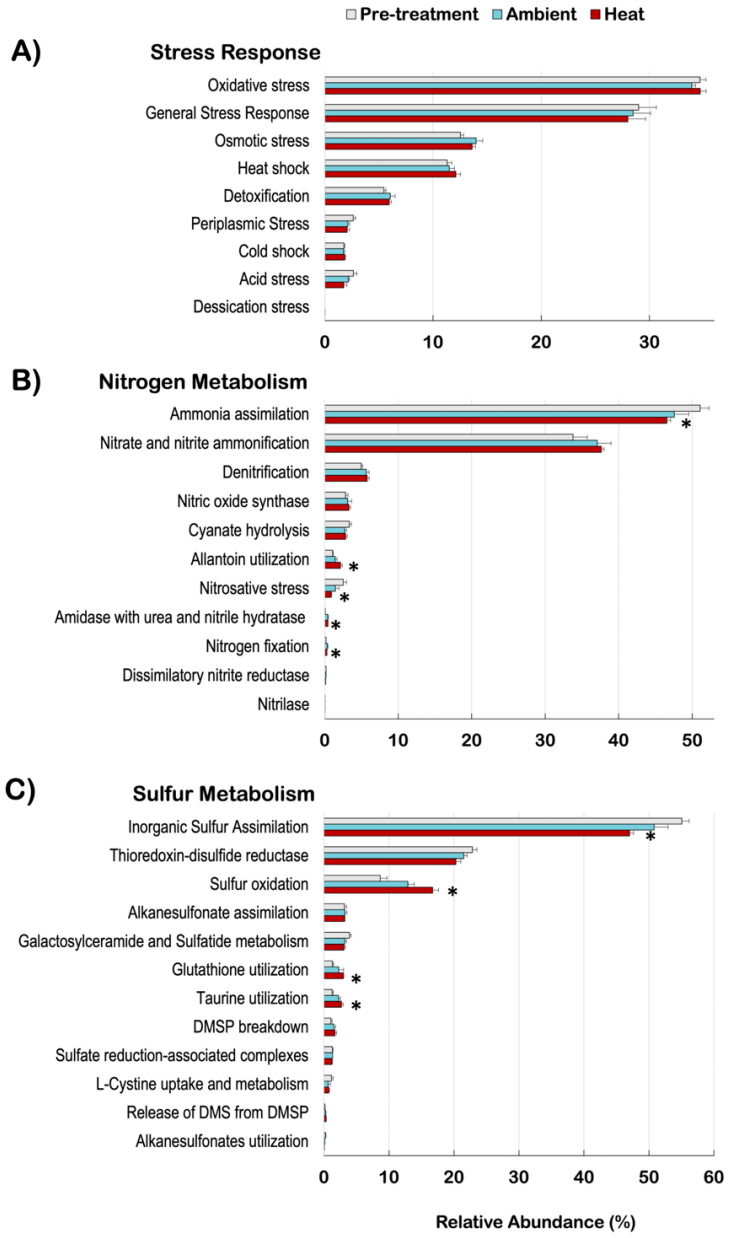


Figure 3. Relative abundances of bacterial genes related to stress response (A), nitrogen metabolism (B), and sulfur metabolism (C). Asterisks indicate a significant difference between pre-treatment and heat-treatment metagenomes according to Welch's pairwise comparisons, and Benjamini-Hochberg FDR corrections.

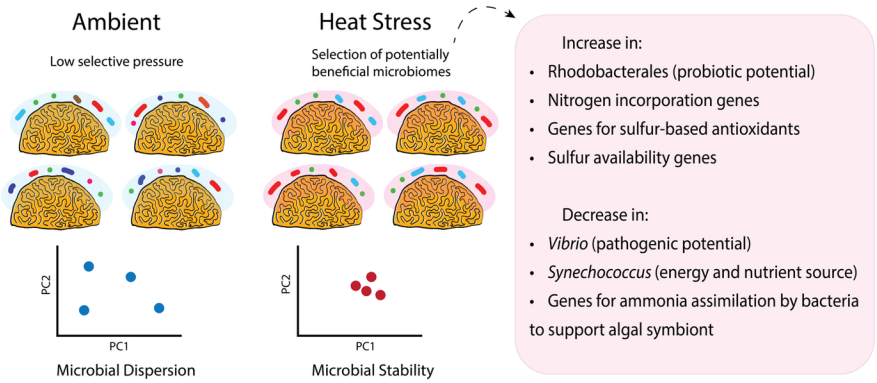
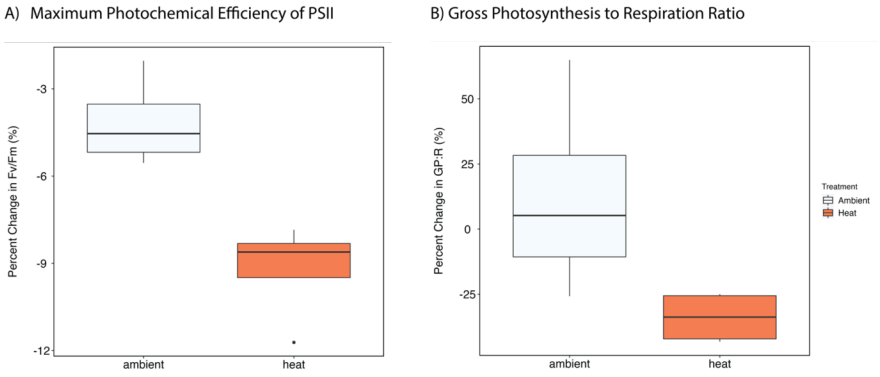
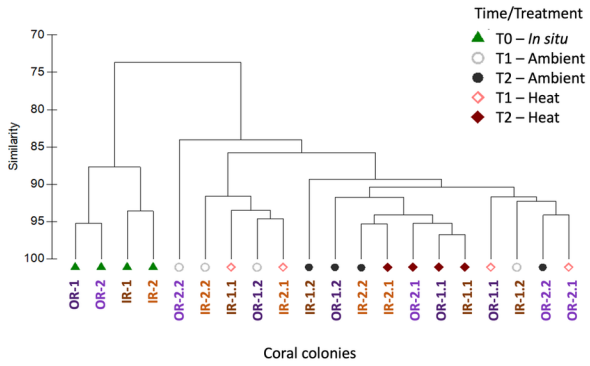


Figure 4. The coral SML microbiome of *P. strigosa*responded to heat stress with increased stability and a taxonomic and functional composition that was potentially beneficial to the holobiont. This response is in opposition to the AKP, that predicts greater microbial dispersion among stressed holobionts.

Supplemental Material



Supplemental Figure 1 . Percent change in A) maximum photochemical efficiency of photosystem II (Fv/Fm) and B) gross productivity to dark respiration ratios (GP: R) and of coral colonies (total n = 4 per treatment) after exposure to heat and ambient temperatures for one week.



Supplemental Figure 2 . A hierarchical cluster analysis of the metagenomes associated with the coral SML microbiome of *P. strigosa* exposed to different temperature treatments. The microbiomes of four coral colonies (OR-1, OR-2, IR-1, IR-2) were sampled in their natural environment (T0) before the coral colonies were removed and replicated into two fragments (coral nubbins) each (OR-1.1, OR-1.2, OR-2.1, OR-2.2, IR-1.1, IR-1.2, IR-2.1, IR-2.2). The microbiomes of the coral nubbins were sampled before (T1) and after (T2) being exposed to ambient and heat treatments for one week. The analysis was based on a Bray-Curtis similarity matrix using group average of the relative abundances of the bacterial genera.

Supplemental Table 1 . Metagenomic sequences coverage in MG-RAST server

Metagenome name	Coral colony ID	Coral nubbin ID	Treatment	Total number of sequence
IR1AA-1-Inner-Heat-R1-21Jun2017	IR-1	IR-1.1	Heat	683,893
IR1AA-1-Inner-Pre-R3-15Jun2017	IR-1	IR-1.1	Pre-treatment	368,182
IR1AA-4-Inner-Ambient-R3-21Jun2017	IR-1	IR-1.2	Ambient	356,426
IR1AA-4-Inner-Pre-R2-15Jun2017	IR-1	IR-1.2	Pre-treatment	453,083
IR3LL1-3-Inner-Heat-R3-21Jun2017	IR-2	IR-2.1	Heat	498,213
IR3LL1-3-Inner-Pre-R4-15Jun2017	IR-2	IR-2.1	Pre-treatment	460,170
IR3LL1-4-Inner-Ambient-R2-21Jun2017	IR-2	IR-2.2	Ambient	1,009,994
IR3LL1-4-Inner-Pre-R3-15Jun2017	IR-2	IR-2.2	Pre-treatment	936,937
OR2LL-1-Outer-Ambient-R2-21Jun2017	OR-1	OR-1.2	Ambient	715,686
OR2LL-1-Outer-Pre-R4-15Jun2017	OR-1	OR-1.2	Pre-treatment	674,319
OR2LL-2-Outer-Heat-R3-21Jun2017	OR-1	OR-1.1	Heat	1,164,365
OR2LL-2-Outer-Pre-R3-15Jun2017	OR-1	OR-1.1	Pre-treatment	535,735
OR3AA-3-Outer-Ambient-R1-21Jun2017	OR-2	OR-2.2	Ambient	1,296,198
OR3AA-3-Outer-Pre-R1-15Jun2017	OR-2	OR-2.2	Pre-treatment	845,883
OR3AA-4-Outer-Heat-R1-21Jun2017	OR-2	OR-2.1	Heat	840,108
OR3AA-4-Outer-Pre-R3-15Jun2017	OR-2	OR-2.1	Pre-treatment	580,876

Supplemental Table 2. Richness (S), Pielou's evenness index (J'), and Shannon's diversity index (H') of microbial genera from the coral SML metagenomes across different treatments.

Treatment	S	J'	H'
In situ (T0)	582 - 586	0.74 ± 0.02	4.72 ± 0.14
Heat (T2)	579 - 587	0.63 ± 0.004	4.04 ± 0.03
Ambient (T2)	580 - 585	0.62 ± 0.04	3.93 ± 0.24
Pre-treatment (T1)	578 - 585	0.55 ± 0.05	3.47 ± 0.32

References

Ahmed HI, Herrera M, Liew YJ, Aranda M (2019) Long-term temperature stress in the coral model Aiptasia supports the "Anna Karenina principle" for bacterial microbiomes. Front Microbiol 10:975

Ainsworth T, Krause L, Bridge T, Torda G, Raina J-B, Zakrzewski M, Gates RD, Padilla-Gamiño JL, Spalding HL, Smith C, Woolsey ES, Bourne DG, Bongaerts P, Hoegh-Guldberg O, Leggat W (2015) The coral core microbiome identifies rare bacterial taxa as ubiquitous endosymbionts. Isme J 9:2261

Berkelmans R, Willis BL (1999) Seasonal and local spatial patterns in the upper thermal limits of corals on the inshore Central Great Barrier Reef. Coral Reefs 18:219–228

Bourne DG, Morrow KM, Webster NS (2016) Insights into the Coral Microbiome: Underpinning the Health and Resilience of Reef Ecosystems. Annu Rev Microbiol 70:317–340

Cardini U, van Hoytema N, Bednarz VN, Rix L, Foster RA, Al-Rshaidat MMD, Wild C (2016) Microbial dinitrogen fixation in coral holobionts exposed to thermal stress and bleaching. Environ Microbiol 18:2620–2633

Cavalcanti GS, Shukla P, Morris M, Ribeiro B, Foley M, Doane MP, Thompson CC, Edwards MS, Dinsdale EA, Thompson FL (2018) Rhodoliths holobionts in a changing ocean: Host-microbes interactions mediate coralline algae resilience under ocean acidification. BMC Genomics 19:1–13

Ceh J, van Keulen M, Bourne DG (2013a) Intergenerational transfer of specific bacteria in corals and possible implications for offspring fitness. Microb Ecol 65:227–231

Ceh J, Kilburn MR, Cliff JB, Raina J, van Keulen M, Bourne DG (2013b) Nutrient cycling in early coral life stages: Pocillopora damicornis larvae provide their algal symbiont (Symbiodinium) with nitrogen acquired from bacterial associates. Ecol Evol 3:2393–2400

Cobo-Díaz JF, Fernández-González AJ, Villadas PJ, Robles AB, Toro N, Fernández-López M (2015) Metagenomic assessment of the potential microbial nitrogen pathways in the rhizosphere of a Mediterranean forest after a wildfire. Microb Ecol 69:895–904

Coelho LP, Alves R, del Río ÁR, Myers PN, Cantalapiedra CP, Giner-Lamia J, Schmidt TS, Mende DR, Orakov A, Letunic I, Hildebrand F, Van Rossum T, Forslund SK, Khedkar S, Maistrenko OM, Pan S, Jia L, Ferretti P, Sunagawa S, Zhao X-M, Nielsen HB, Huerta-Cepas J, Bork P (2022) Towards the biogeography of prokaryotic genes. Nature 601:252–256

Courtney TA, Kindeberg T, Andersson AJ (2020) Coral calcification responses to the North Atlantic Oscillation and coral bleaching in Bermuda. PLoS One 15:e0241854

Courtney TA, Lebrato M, Bates NR, Collins A, de Putron SJ, Garley R, Johnson R, Molinero JC, Noyes TJ, Sabine CL, Andersson AJ (2017) Environmental controls on modern scleractinian coral and reef-scale calcification. Sci Adv 3:

Damjanovic K, Blackall LL, Webster NS, van Oppen MJH (2017) The contribution of microbial biotechnology to mitigating coral reef degradation. Microb Biotechnol

Dinsdale EA, Edwards RA, Hall D, Angly F, Breitbart M, Brulc JM, Furlan M, Desnues C, Haynes M, Li L (2008a) Functional metagenomic profiling of nine biomes. Nature 452:629

Dinsdale EA, Pantos O, Smriga S, Edwards RA, Angly F, Wegley L, Hatay M, Hall D, Brown E, Haynes M (2008b) Microbial ecology of four coral atolls in the Northern Line Islands. PLoS One 3:e1584

Doane MP, Haggerty JM, Kacev D, Papudeshi B, Dinsdale EA (2017) The skin microbiome of the common thresher shark (Alopias vulpinus) has low taxonomic and gene function β-diversity. Environ Microbiol Rep 9:357–373

Doering T, Wall M, Putchim L, Rattanawongwan T, Schroeder R, Hentschel U, Roik A (2021) Towards enhancing coral heat tolerance: a "microbiome transplantation" treatment using inoculations of homogenized coral tissues. Microbiome 9:1–16

Epstein HE, Smith HA, Torda G, van Oppen MJH (2019) Microbiome engineering: enhancing climate resilience in corals. Front Ecol Environ 17:100–108

Fitt WK, Brown BE, Warner ME, Dunne RP (2001) Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. Coral reefs 20:51–65

Garren M, Azam F (2012) Corals shed bacteria as a potential mechanism of resilience to organic matter enrichment. ISME J 6:1159–1165

Heron SF, Maynard JA, Van Hooidonk R, Eakin CM (2016) Warming trends and bleaching stress of the world's coral reefs 1985–2012. Sci Rep 6:38402

Hoadley KD, Hamilton M, Poirier CL, Choi CJ, Yung C-M, Worden AZ (2021) Selective uptake of pelagic microbial community members by Caribbean reef corals. Appl Environ Microbiol 87:e03175-20

Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U (1998) Temperature-induced bleaching of corals begins with impairment of the CO2fixation mechanism in zooxanthellae. Plant, Cell Environ 21:1219–1230

Koren O, Rosenberg E (2006) Bacteria associated with mucus and tissues of the coral Oculina patagonica in summer and winter. Appl Environ Microbiol 72:5254–5259

Krediet CJ, Ritchie KB, Paul VJ, Teplitski M (2013) Coral-associated micro-organisms and their roles in promoting coral health and thwarting diseases. Proc R Soc London B Biol Sci 280:20122328

Landa M, Burns AS, Durham BP, Esson K, Nowinski B, Sharma S, Vorobev A, Nielsen T, Kiene RP, Moran MA (2019) Sulfur metabolites that facilitate oceanic phytoplankton–bacteria carbon flux. ISME J 13:2536–2550

Levy M, Kolodziejczyk AA, Thaiss CA, Elinav E (2017) Dysbiosis and the immune system. Nat Rev Immunol 17:219–232

Lima LFO, Alker AT, Papudeshi B, Morris MM, Edwards RA, de Putron SJ, Dinsdale EA (2022) Coral and Seawater Metagenomes Reveal Key Microbial Functions to Coral Health and Ecosystem Functioning Shaped at Reef Scale. Microb Ecol 1–16

Lima LFO, Weissman M, Reed M, Papudeshi B, Alker AT, Morris MM, Edwards RA, de Putron SJ, Vaidya NK, Dinsdale EA (2020) Modeling of the coral microbiome: the influence of temperature and microbial network. MBio 11:

Littman R, Willis BL, Bourne DG (2011) Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. Environ Microbiol Rep 3:651–660

Lough JM, Anderson KD, Hughes TP (2018) Increasing thermal stress for tropical coral reefs: 1871–2017. Sci Rep 8:6079

Luz Calle M (2019) Statistical analysis of metagenomics data. Genomics and Informatics 17:

Masip L, Veeravalli K, Georgiou G (2006) The many faces of glutathione in bacteria. Antioxid Redox Signal 8:753–762

Maynard J, Van Hooidonk R, Eakin CM, Puotinen M, Garren M, Williams G, Heron SF, Lamb J, Weil E, Willis B (2015) Projections of climate conditions that increase coral disease susceptibility and pathogen abundance and virulence. Nat Clim Chang 5:688

McMurdie PJ, Holmes S (2014) Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. PLoS Comput Biol 10:

McNally SP, Parsons RJ, Santoro AE, Apprill A (2017) Multifaceted impacts of the stony coral Porites astreoides on picoplankton abundance and community composition. Limnol Oceanogr 62:217–234

Meunier V, Bonnet S, Pernice M, Benavides M, Lorrain A, Grosso O, Lambert C, Houlbrèque F (2019) Bleaching forces coral's heterotrophy on diazotrophs and Synechococcus. ISME J 13:2882–2886

Meyer F, Bagchi S, Chaterji S, Gerlach W, Grama A, Harrison T, Paczian T, Trimble WL, Wilke A (2019) MG-RAST version 4—lessons learned from a decade of low-budget ultra-high-throughput metagenome analysis. Brief Bioinform 20:1151–1159

Miller J, Muller E, Rogers C, Waara R, Atkinson A, Whelan KRT, Patterson M, Witcher B (2009) Coral disease following massive bleaching in 2005 causes 60% decline in coral cover on reefs in the US Virgin Islands. Coral Reefs 28:925

Minami T, Anda M, Mitsui H, Sugawara M, Kaneko T, Sato S, Ikeda S, Okubo T, Tsurumaru H, Minamisawa K (2016) Metagenomic analysis revealed methylamine and ureide utilization of soybean-associated Methylobacterium. Microbes Environ ME16035

Muller EM, Bartels E, Baums IB (2018) Bleaching causes loss of disease resistance within the threatened coral species Acropora cervicornis. Elife 7:e35066

Nguyen-Kim H, Bouvier T, Bouvier C, Bui V, Le-Lan H, Bettarel Y (2015) Viral and Bacterial Epibionts in Thermally-Stressed Corals. J Mar Sci Eng 3:1272–1286

van Oppen MJH, Blackall LL (2019) Coral microbiome dynamics, functions and design in a changing world. Nat Rev Microbiol 17:557–567

Palumbi SR, Barshis DJ, Traylor-Knowles N, Bay RA (2014) Mechanisms of reef coral resistance to future climate change. Science (80-) 344:895–898

Parks DH, Tyson GW, Hugenholtz P, Beiko RG (2014) STAMP: statistical analysis of taxonomic and functional profiles. Bioinformatics 30:3123–3124

Peixoto RS, Rosado PM, Leite DC de A, Rosado AS, Bourne DG (2017) Beneficial microorganisms for corals (BMC): Proposed mechanisms for coral health and resilience. Front Microbiol 8:

Pogoreutz C, Rädecker N, Cárdenas A, Gärdes A, Voolstra CR, Wild C (2017) Sugar enrichment provides evidence for a role of nitrogen fixation in coral bleaching. Glob Chang Biol 23:3838–3848

Poole RK (2005) Nitric oxide and nitrosative stress tolerance in bacteria. Biochem Soc Trans 33:176–180

Precht WF, Gintert BE, Robbart ML, Fura R, Van Woesik R (2016) Unprecedented disease-related coral mortality in Southeastern Florida. Sci Rep 6:31374

de Putron SJ, Smith SR (2011a) Planula release and reproductive seasonality of the scleractinian coral porites astreoides in bermuda, a high-latitude reef. Bull Mar Sci 87:75–90

de Putron SJ, Smith SR (2011b) Planula release and reproductive seasonality of the scleractinian coral Porites astreoides in Bermuda, a high-latitude reef. Bull Mar Sci 87:75–90

Quince C, Walker AW, Simpson JT, Loman NJ, Segata N (2017) Corrigendum: Shotgun metagenomics, from sampling to analysis. Nat Biotechnol 35:1211

Rädecker N, Pogoreutz C, Voolstra CR, Wiedenmann J, Wild C (2015) Nitrogen cycling in corals: the key to understanding holobiont functioning? Trends Microbiol 23:490–497

Raina J-B, Tapiolas D, Motti CA, Foret S, Seemann T, Tebben J, Willis BL, Bourne DG (2016) Isolation of an antimicrobial compound produced by bacteria associated with reef-building corals. PeerJ 4:e2275

Raina JB, Tapiolas DM, Forêt S, Lutz A, Abrego D, Ceh J, Seneca FO, Clode PL, Bourne DG, Willis BL, Motti CA (2013) DMSP biosynthesis by an animal and its role in coral thermal stress response. Nature 502:677–680

Ritchie KB (2006) Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. Mar Ecol Prog Ser 322:1–14

Robbins SJ, Singleton CM, Chan CX, Messer LF, Geers AU, Ying H, Baker A, Bell SC, Morrow KM, Ragan MA, Miller DJ, Forêt S, Ball E, Beeden R, Berumen M, Aranda M, Ravasi T, Bongaerts P, Hoegh-Guldberg O, Cooke I, Leggat B, Sprungala S, Fitzgerald A, Shang C, Lundgren P, Fyffe T, Rubino F, van Oppen M, Weynberg K, Robbins SJ, Singleton CM, Xin Chan C, Messer LF, Geers AU, Ying H, Baker A, Bell SC, Morrow KM, Ragan MA, Miller DJ, Foret S, Voolstra CR, Tyson GW, Bourne DG, Voolstra CR, Tyson GW, Bourne DG, Consortium R (2019) A genomic view of the reef-building coral Porites lutea and its microbial symbionts. Nat Microbiol 4:2090–2100

Rohwer F, Seguritan V, Azam F, Knowlton N (2002) Diversity and distribution of coral-associated bacteria. Mar Ecol Prog Ser 243:1–10

Rosado PM, Leite DCA, Duarte GAS, Chaloub RM, Jospin G, Nunes da Rocha U, P Saraiva J, Dini-Andreote F, Eisen JA, Bourne DG (2019) Marine probiotics: increasing coral resistance to bleaching through microbiome manipulation. ISME J 13:921–936

Santoro EP, Borges RM, Espinoza JL, Freire M, Messias CSMA, Villela HDM, Pereira LM, Vilela CLS, Rosado JG, Cardoso PM (2021) Coral microbiome manipulation elicits metabolic and genetic restructuring to mitigate heat stress and evade mortality. Sci Adv 7:eabg3088

Schmieder R, Edwards R (2011) Quality control and preprocessing of metagenomic datasets. Bioinformatics 27:863–864

Serrano X, Baums IB, O'reilly K, Smith TB, Jones RJ, Shearer TL, Nunes FLD, Baker AC (2014) Geographic differences in vertical connectivity in the C aribbean coral M ontastraea cavernosa despite high levels of horizontal connectivity at shallow depths. Mol Ecol 23:4226–4240

Serrano XM, Baums IB, Smith TB, Jones RJ, Shearer TL, Baker AC (2016) Long distance dispersal and vertical gene flow in the Caribbean brooding coral Porites astreoides. Sci Rep 6:1–12

Sharon G, Rosenberg E (2008) Bacterial growth on coral mucus. Curr Microbiol 56:481–488

Siboni N, Ben-Dov E, Sivan A, Kushmaro A (2008) Global distribution and diversity of coral-associated Archaea and their possible role in the coral holobiont nitrogen cycle. Environ Microbiol 10:2979–2990

Smith SR, Sarkis S, Murdoch TJT, Croquer A, Bates NR, Johnson RJ, Putron S De, Andersson AJ (2013) Coral Reefs of the United Kingdom Overseas Territories. 4:

Sun H, Zheng H, Jiang Y, Liang J, Liao B, Wang R, Li A, Xiao B (2022) Elevated temperature alters bacterial community composition and metabolism in seawaters of coral reef ecosystem: An evidence of laboratory experiment with Acropora digitifera bleaching. Ecol Indic 139:108886

Thurber RV, Willner-Hall D, Rodriguez-Mueller B, Desnues C, Edwards RA, Angly F, Dinsdale E, Kelly L, Rohwer F (2009) Metagenomic analysis of stressed coral holobionts. Environ Microbiol 11:2148–2163

Vega Thurber RL, Burkepile DE, Fuchs C, Shantz AA, Mcminds R, Zaneveld JR (2014) Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. Glob Chang Biol 20:544–554

Wang L, Shantz AA, Payet JP, Sharpton TJ, Foster A, Burkepile DE, Vega Thurber R (2018) Corals and Their Microbiomes Are Differentially Affected by Exposure to Elevated Nutrients and a Natural Thermal Anomaly. Front Mar Sci 5:1–16

Warner ME, Fitt WK, Schmidt GW (1996) The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae in hospite from four different species of reef coral: A novel approach. Plant, Cell Environ 19:291–299

Webster NS, Reusch TBH (2017) Microbial contributions to the persistence of coral reefs. ISME J 11:2167–2174

Wegley L, Edwards R, Rodriguez-Brito B, Liu H, Rohwer F (2007) Metagenomic analysis of the microbial community associated with the coral Porites astreoides. Environ Microbiol 9:2707–2719

Wild C, Woyt H, Huettel M (2005) Influence of coral mucus on nutrient fluxes in carbonate sands. Mar Ecol Prog Ser 287:87–98

Willis BL, Page CA, Dinsdale EA (2004) Coral Disease on the Great Barrier Reef BT - Coral Health and Disease. In: Rosenberg E., Loya Y. (eds) Springer Berlin Heidelberg, Berlin, Heidelberg, pp 69–104

Wong KH, Goodbody-Gringley G, de Putron SJ, Becker DM, Chequer A, Putnam HM (2021) Brooded coral offspring physiology depends on the combined effects of parental press and pulse thermal history. Glob Chang Biol

Zaneveld JR, Burkepile DE, Shantz AA, Pritchard CE, McMinds R, Payet JP, Welsh R, Correa AMS, Lemoine NP, Rosales S, Fuchs C, Maynard JA, Thurber RV (2016) Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. Nat Commun 7:1–12

Zaneveld JR, McMinds R, Thurber RV (2017) Stress and stability: Applying the Anna Karenina principle to animal microbiomes. Nat Microbiol 2:

Zhou G, Cai L, Yuan T, Tian R, Tong H, Zhang W, Jiang L, Guo M, Liu S, Qian P (2017) Microbiome dynamics in early life stages of the scleractinian coral Acropora gemmifera in response to elevated pCO2. Environ Microbiol 19:3342–3352

Ziegler M, Seneca FO, Yum LK, Palumbi SR, Voolstra CR (2017) Bacterial community dynamics are linked to patterns of coral heat tolerance. Nat Commun 8:1–8