



# Antibiotics Alter *Pocillopora* Coral-Symbiodiniaceae-Bacteria Interactions and Cause Microbial Dysbiosis During Heat Stress

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Symbioses between eukaryotes and their associated microbial communities are fundamental processes that affect organisms' ecology and evolution. A unique example of this is reef-building corals that maintain symbiotic associations with dinoflagellate algae (Symbiodiniaceae) and bacteria that affect coral health through various mechanisms. However, little is understood about how coral-associated bacteria communities affect holobiont heat tolerance. In this study, we investigated these interactions in four *Pocillopora* coral colonies belonging to three cryptic species by subjecting fragments to treatments with antibiotics intended to suppress the normal bacteria community, followed by acute heat stress. Separate treatments with only antibiotics or heat stress were conducted to compare the effects of individual stressors on holobiont transcriptome responses and microbiome shifts. Across all *Pocillopora* species examined, combined antibiotics and heat stress treatment significantly altered coral-associated bacteria communities and caused major changes in both coral and *Cladocopium* algal symbiont gene expression. Individually, heat stress impaired *Pocillopora* protein translation and activated DNA repair processes, while antibiotics treatments caused downregulation of *Pocillopora* amino acid and inorganic ion transport and metabolism genes and *Cladocopium* photosynthesis genes. Combined antibiotics-heat stress treatments caused synergistic effects on *Pocillopora* and *Cladocopium* gene expression including enhanced expression of oxidative stress response genes, programmed cell death pathways and proteolytic enzymes that indicate an exacerbated response to heat stress following bacteria community suppression. Collectively, these results provide further evidence that corals and their Symbiodiniaceae and bacteria communities engage in highly coordinated metabolic interactions that are crucial for coral holobiont health, homeostasis, and heat tolerance.

**Keywords:** coral, holobiont, transcriptome, microbiome, antibiotics, bacteria, dysbiosis

## INTRODUCTION

Microbial symbioses are fundamental to the structure and functioning of marine ecosystems, and influence marine organisms' ecology, evolution, and stress responses (O'Brien et al., 2019; Wilkins et al., 2019; Apprill, 2020). Healthy reef-building corals (Order Scleractinia) maintain associations with endosymbiotic algae (Family Symbiodiniaceae) and bacteria, and are popular models for the study of complex microbial symbioses that underpin marine ecosystem processes (Blackall et al., 2015; Thompson et al., 2015).

Coral-associated bacteria communities exhibit patterns of phyllosymbiosis and cophylogeny (Pollock et al., 2018) and demonstrate genomic signatures that are suggestive of highly interdependent symbiotic relationships between corals and bacteria (Robbins et al., 2019). Recent studies have also uncovered evidence for intimate associations between bacteria and Symbiodiniaceae, indicating that diverse multi-partner symbiotic interactions are present in coral holobionts (Motone et al., 2020; Maire et al., 2021). Certain coral-associated bacteria have been investigated for their roles in affecting corals' responses to heat stress, with different bacteria taxa exerting either negative or positive effects on coral holobiont resistance and resilience (Tout et al., 2015; Zaneveld et al., 2016; Ziegler et al., 2017, 2019; Epstein et al., 2019; Avila-Magaña et al., 2021). Other environmental stressors such as nutrient enrichment can also alter coral bacteria communities (Pogoreutz et al., 2018; Ziegler et al., 2019), but the interactive effects of different stressors applied in combination and their effects on coral health are still the focus of much research (Maher et al., 2019).

In contrast to other environmental stressors, broad-spectrum antibiotics treatments have previously been used to directly suppress the coral-associated bacteria community and generate hypotheses about how bacteria might affect coral holobiont health and homeostasis (Hodgson, 1990; Gilbert et al., 2012; Sweet et al., 2014; Sweet and Bythell, 2015; Glasl et al., 2016). Altogether, these studies have documented negative effects of antibiotics on coral health and hypothesized that antibiotic suppression of the native microbiota enables the proliferation of potential pathogens. These results have led to concerns about antibiotics as environmental pollutants on coral reefs (Zhang R. et al., 2018, 2019), which merit special consideration as antibiotics treatments are now being broadly deployed as field interventions to combat stony coral tissue loss disease (SCTLD) in the Caribbean (Neely et al., 2020). Despite these novel applications and concerns over the use of antibiotics on coral reefs, the specific genes and pathways involved in coral holobiont responses to antibiotic suppression of the bacteria community have not been explored. The experimental study of these processes can inform our knowledge of risks that antibiotics pose to coral health in the field and can also improve our understanding of coral holobiont interactions under normal conditions.

The coral genus *Pocillopora* is among the most widespread and important reef-building coral taxa in the world, with a distribution from east Africa to western Central America. For this reason, *Pocillopora* corals have become popular experimental

model species and *Pocillopora* holobiont responses to heat stress and other environmental stressors have been thoroughly studied (Traylor-Knowles et al., 2011; Mayfield et al., 2014; Vidal-Dupiol et al., 2014; Zhou et al., 2018; Poquita-Du et al., 2019; Li et al., 2020). These studies suggest that *Pocillopora* coral transcriptomes are generally more responsive to heat stress than their associated Symbiodiniaceae transcriptomes, and that expression of genes involved in metabolism, protein folding and immune pathways such as heat shock proteins (HSPs) and tumor necrosis factor receptors (TNFRs) are important components of the *Pocillopora* coral heat stress response (Zhou et al., 2017; Zhang Y. et al., 2018). Other studies have assessed *Pocillopora*-associated bacteria community shifts that occur during heat stress, nutrient enrichment, and treatment with putative beneficial microbes (Tout et al., 2015; Pogoreutz et al., 2018; Rosado et al., 2018; Maher et al., 2019; Ziegler et al., 2019; Li et al., 2020, 2021; Zhang Y. et al., 2021). However, despite the critical importance of coral-associated bacteria to holobiont health, no study has simultaneously assessed coral holobiont transcriptome and microbiome responses to acute heat stress following direct antibiotic suppression of the native bacteria community. These studies are needed to better understand the role of coral-associated bacteria in coral holobiont health and heat tolerance, and to inform ongoing efforts that seek to conserve and restore coral reef ecosystems by enhancing corals' natural resilience through the application of beneficial microbes (Peixoto et al., 2021; Voolstra et al., 2021).

To investigate how antibiotics treatments suppress the native coral bacteria community and affect coral holobiont interactions and acute heat stress responses, we performed an experiment with four *Pocillopora* coral colonies (genotypes) collected from Kenting National Park in southern Taiwan. These four distinct genotypes belonged to three morphologically cryptic *Pocillopora* species: *Pocillopora acuta* ( $n = 2$ ) and *Pocillopora damicornis* ( $n = 1$ ) (Schmidt-Roach et al., 2014), and the undescribed species *Pocillopora* type 8a ( $n = 1$ ) (Gélin et al., 2017; Johnston et al., 2017). In this experiment we examined the effects of antibiotics, acute heat stress, and combined antibiotics-heat stress treatments on coral host and *Cladocopium* symbiont gene expression through RNAseq and bacteria composition through 16S gene amplicon sequencing. Through this work, we identified genes involved in coral-bacteria interactions to gain a better understanding of how coral-associated bacteria communities affect coral holobiont stress responses. We found that there were differences between the four *Pocillopora* genotypes from distinct cryptic species and sites, as expected. However, we also found that there were shared responses across all genotypes and species examined. Most notably, for all genotypes we found that both antibiotics and combined antibiotics-heat stress treatments altered *Pocillopora* coral bacteria community composition and affected *Pocillopora* coral and *Cladocopium* symbiont gene expression. We hypothesize that the changes in bacteria community composition exacerbated the holobiont heat stress responses causing microbial dysbiosis and the activation of programmed cell death pathways. These results have implications for understanding how multi-partner interactions affect coral holobiont health and homeostasis and highlight the utility of

antibiotics treatments as an experimental tool to suppress and manipulate coral-associated bacteria communities.

## MATERIALS AND METHODS

### Coral Study Site, Collection, Fragmentation, and Acclimation

Colonies of *Pocillopora* corals were collected from two reefs within Kenting National Park in southern Taiwan (Kenting National Park Permit #10621218300) Houwan (22° 2'29" N, 120°41'43" E) and Wanglitung (21°59'43" N, 120°42'22" E) (**Supplementary Figure 1**). Two *Pocillopora* colonies with morphological similarity to *P. damicornis* were collected at each site, Houwan and Wanglitung, and colonies (hereafter referred to as HW1, HW2, WT1, and WT2) were collected in July 2017 (**Table 1**). Colonies were acclimated in a flow-through system receiving ambient seawater under natural sunlight at the National Museum of Marine Biology and Aquarium (NMMBA), and then split into a minimum of 12 replicate 2 cm<sup>2</sup> fragments per colony that were affixed to numbered plastic tags and allowed to recover for 3 weeks before the experiment.

### Heat Stress, Antibiotics, and Antibiotics-Heat Stress Treatment Experiment

Three experimental treatments (heat stress, antibiotics, and antibiotics-heat stress) and a control were designed to examine the separate and interactive effects of acute heat stress and antibiotic bacteria suppression on the *Pocillopora* holobiont transcriptome and bacteria community composition (**Figure 1**). Triplicate fragments from each coral genotype were placed into individual autoclaved 400 mL glass jars filled with filtered seawater (1 μm) and randomly assigned to experimental or seawater control treatments (**Figure 1**). Jars were placed into two recirculating systems, one for the control and antibiotics treatment and another for the heat stress and antibiotics-heat stress treatments. Blue and white LED lights were operated on a 12 h:12 h light:dark cycle with even distribution across fragment jars. At 9:00 am in the morning of each day of the 3-day experiment, partial water changes were completed

using 100 mL of filtered seawater (1 μm). The control system was maintained at 28°C for the full 72 h, to mimic the mean summer seawater temperature in Kenting National Park (Keshavmurthy et al., 2019).

### Acute Heat Stress Treatment

The acute heat stress treatment was maintained for 48 h at 32°C (~0.9 DHWs) after an initial 24 h at 28°C and a 90-min temperature ramp to 32°C. Temperatures in the control system and heat stress system were recorded at 1-min intervals with HOBO® Pendant data loggers (Onset, MA, United States) (**Figure 1**). This acute heat stress was done to contrast the short-term transcriptomic responses of healthy corals with normal bacteria communities against those with bacteria communities that had been previously suppressed by antibiotics treatment.

### Antibiotics Treatment

The antibiotics treatment consisted of a combined exposure to 100 μg/mL ampicillin (Sigma-Aldrich A9393) and 100 μg/mL streptomycin sulfate (Sigma-Aldrich 6501), which were administered together to each jar as 400 μL of 100 mg/mL stock solution. These antibiotics were selected for their broad-spectrum antibacterial activity and different modes of action against bacterial cell wall cross-linking and protein synthesis, respectively. Previous studies that have used ampicillin and streptomycin indicate that the chosen concentrations of these antibiotics are non-toxic to the coral host or associated Symbiodiniaceae, and preliminary experiments supported this observation (Soffer et al., 2008; Gilbert et al., 2012; Sweet et al., 2014). Coral fragments were exposed to the antibiotic solution for a total of 72 h before fragment sampling and preservation. For daily partial water changes, 100 mL of antibiotic-treated seawater was used to maintain consistent levels of antibiotic exposure.

### Antibiotics-Heat Stress Treatment

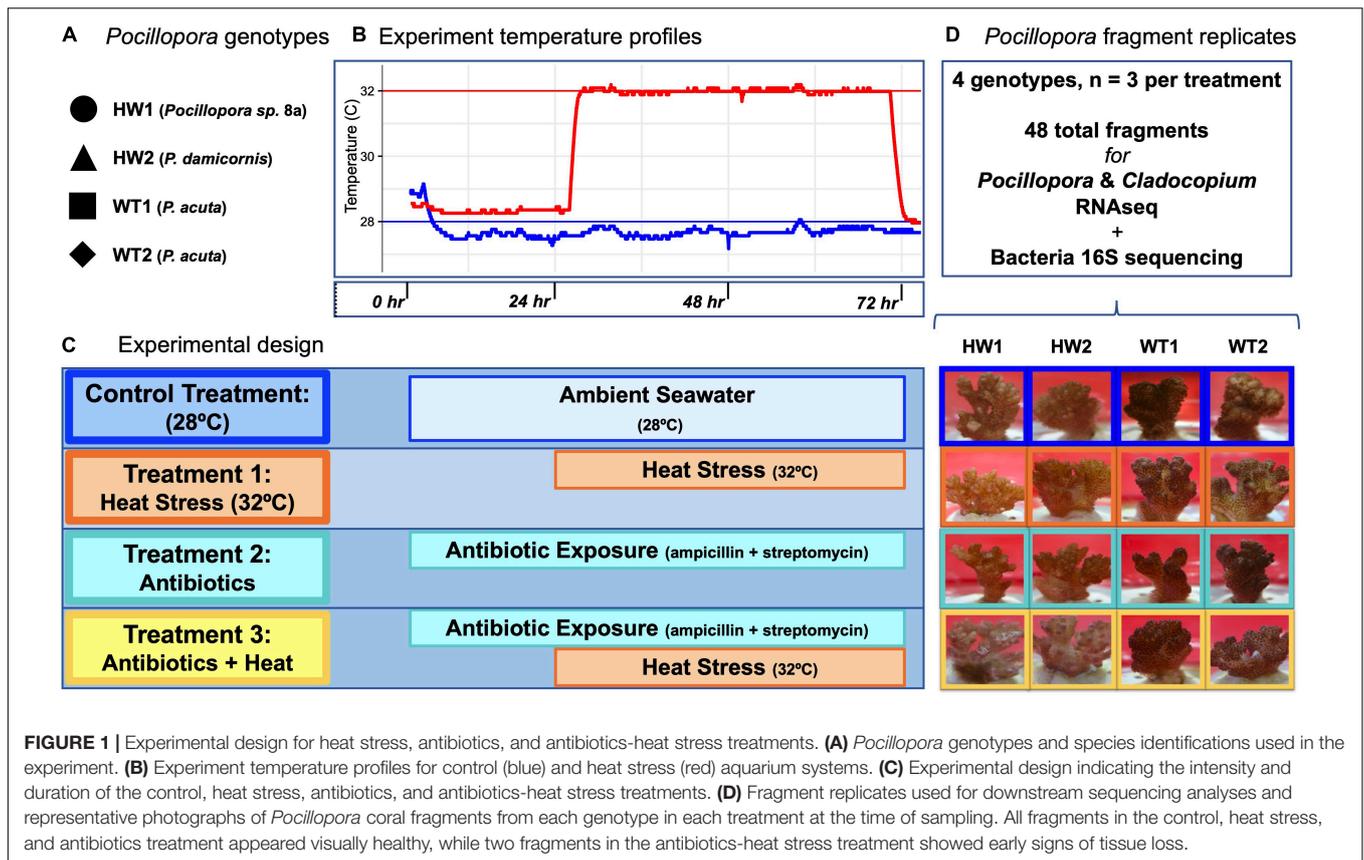
A combined antibiotics-heat stress treatment was conducted to parse apart the relative importance of stability within the coral-associated bacteria community toward the holobiont heat stress response. The antibiotics-heat stress treatment consisted of the same combined ampicillin and streptomycin treatment as in the individual antibiotics treatment. Corals were incubated in the antibiotic cocktail for 24 h at 28°C before exposure to acute heat stress at 32°C for 48 h (**Figure 1**), to allow 24 h for the antibiotic suppression of the coral bacteria community to occur before the start of the heat stress. For daily partial water changes, 100 mL of antibiotic-treated seawater was used to maintain consistent levels of antibiotic exposure for a total of 72 h through the entire experiment (**Figure 1**).

## Collection and Preservation of Coral Tissue Samples

After the experiment was completed, fragments were photographed, and 1 cm<sup>2</sup> coral fragments were cut using stainless steel bone cutters that were cleaned in 70% ethanol between samples (**Supplementary Figure 4**). These fragments were placed into tubes with 5 mL of RNA stabilization solution consisting of 70% w/v ammonium sulfate, 20 mM EDTA, and

**TABLE 1** | Origin sites and species designations of the *Pocillopora* coral colonies used in the antibiotics and heat stress experiment.

Colony Site	<i>Pocillopora</i> nominal species ( <i>sensu</i> Schmidt-Roach et al., 2014)	<i>Pocillopora</i> mtORF type ( <i>sensu</i> Pinzón et al., 2013)	<i>Pocillopora</i> ORF type ( <i>sensu</i> Géliin et al., 2017)
HW1	Houwan <i>Pocillopora</i> sp. type 8a	8a	ORF23
HW2	Houwan <i>Pocillopora damicornis</i>	4a	ORF09
WT1	Wanglitung <i>Pocillopora acuta</i>	5a	ORF18
WT2	Wanglitung <i>Pocillopora acuta</i>	5a	ORF18



**FIGURE 1 |** Experimental design for heat stress, antibiotics, and antibiotics-heat stress treatments. **(A)** *Pocillopora* genotypes and species identifications used in the experiment. **(B)** Experiment temperature profiles for control (blue) and heat stress (red) aquarium systems. **(C)** Experimental design indicating the intensity and duration of the control, heat stress, antibiotics, and antibiotics-heat stress treatments. **(D)** Fragment replicates used for downstream sequencing analyses and representative photographs of *Pocillopora* coral fragments from each genotype in each treatment at the time of sampling. All fragments in the control, heat stress, and antibiotics treatment appeared visually healthy, while two fragments in the antibiotics-heat stress treatment showed early signs of tissue loss.

25 mM sodium citrate adjusted to pH 5.2 with 1.0 M H<sub>2</sub>SO<sub>4</sub>. Fragments were incubated at room temperature for 30 min and frozen at -80°C. Frozen samples were then transported to the University of Miami Rosenstiel School of Marine and Atmospheric Science in Miami, FL, United States, in coolers on dry ice for further processing.

### RNA Extraction, cDNA Library Preparation and Sequencing

Total RNA was isolated from the coral tissue using the Qiagen RNeasy Kit and eluted in 30 µL of RNase-free water. RNA concentration and purity were quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher). cDNA libraries were then created using the Illumina TruSeq RNA Library Prep Kit v2 with Poly-A selection according to the manufacturer's protocol. cDNA concentrations were quantified using the Qubit fluorometer and Qubit dsDNA BR kit (Thermo Fisher Q32853). cDNA libraries were shipped overnight on dry ice to the University of Utah's Huntsman Cancer Center for 50 bp single-end sequencing on the Illumina HiSeq 2000.

### *Pocillopora* Mitochondrial Open Reading Frame Type Determination and Symbiodiniaceae Community Profiling

*Pocillopora* mitochondrial open reading frame (mtORF) consensus sequences were extracted for each genotype and

used to identify the closest mtORF haplotype in GenBank as described previously (Connelly et al., 2020), and these mtORF haplotypes were used to assign species designations following the most recent taxonomic revision to the genus *Pocillopora* (Schmidt-Roach et al., 2014). The genus-level composition of the Symbiodiniaceae community was assessed by aligning non-coral reads against reference sequences of Symbiodiniaceae clade-delineating loci (ITS1, ITS2, and cp23S) with BWA (Li and Durbin, 2009) as in Ladner et al. (2012) to show that all colonies were dominated by *Cladocopium goreauii* (Supplementary Table 2).

### *Pocillopora* and *Cladocopium* Dual RNAseq Data Analysis

Raw FASTQ files were checked for base pair quality using the FASTQC program<sup>1</sup>, then Illumina adapter sequences and reads with poor-quality bases were removed using Trimmomatic v0.36 (Bolger et al., 2014). Trimmed reads were aligned against the *P. damicornis* genome using the program STAR v2.5.3a in two-pass mode (Dobin et al., 2013; Cunning et al., 2018), and the remaining unaligned reads were then aligned against the *C. goreauii* genome (Liu et al., 2018). Unsorted BAM files containing the *Pocillopora* or *Cladocopium*-aligned transcripts were quantified at the gene level using the featureCounts command in the Subread program (Liao et al., 2014), with only

<sup>1</sup><https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>

transcripts that were assigned as accurately single-mapping being retained for gene expression analysis in R version 4.0.3 (R Core Team, 2020).

*Pocillopora* and *Cladocopium* gene counts were filtered to include only genes with counts  $\geq 10$  in at least 90% of samples, and counts were normalized using the variance-stabilizing transformation (vst) in the R package DESeq2 (Love et al., 2014). Principal coordinates analysis (PCoA) of Manhattan distances was used to visualize the overall transcriptome patterns of coral and symbiont normalized counts, and pairwise PERMANOVA tests in the R package vegan (Oksanen et al., 2019; Martínez Arbizu, 2020) were used to test for significant differences in transcriptome profiles between experimental treatments.

Statistical analysis of differential gene expression was conducted using generalized linear models implemented in DESeq2. Average log<sub>2</sub> fold change (LFC) for each gene was estimated by comparing normalized gene expression counts between treatments, and significantly differentially expressed genes (DEGs) were identified based on a Benjamini–Hochberg false discovery rate-adjusted *p*-value cutoff of  $<0.05$ . The set properties of the DEGs lists produced by each experimental treatment contrasted against the control treatment were visualized using the R package VennDiagram (Chen and Boutros, 2011).

To identify modules of co-expressed *Pocillopora* and *Cladocopium* genes across all treatments, weighted gene co-expression network analysis (WGCNA) was performed. The R package WGCNA (Langfelder and Horvath, 2008) was used to construct a signed co-expression network using vst-normalized gene counts and the bi-weight mid-correlation statistic. The resulting adjacency matrix was transformed into connection strengths using a soft-thresholding power of 20 for *Pocillopora* and 5 for *Cladocopium*, based on a scale-free topology fit index. Modules were identified using a cut height of 0.99 on the topological overlap matrix and a minimum module size of 50 genes, and modules with  $>70.0\%$  similar expression profiles were merged. The expression of each network module “eigengene” was correlated against a binary matrix containing categorical sample treatment information to identify modules with expression patterns linked to a given treatment.

Gene ontology (GO) and Eukaryotic Orthologous Group (KOG) class annotations were obtained for the *P. damicornis* and *C. goreauii* genome using the eggNOG-mapper online tool<sup>2</sup> (Huerta-Cepas et al., 2017). Fisher’s exact tests for enrichment of GO terms and KOG categories associated with transcriptome changes among sets of differentially expressed genes and co-expression module genes were conducted using GO\_MWU<sup>3</sup> and the R package KOGMWU (Wright et al., 2015; Barfield et al., 2018; Matz, 2019).

## Bacteria DNA Extraction and 16S rRNA Gene Amplicon Sequencing

Total DNA was extracted using the Qiagen Dneasy PowerSoil kit from all coral fragment samples in the experiment

(Weber et al., 2017). PCR amplification of the V4 region of the bacterial 16S gene was completed with the PCR primers 515F and 806R with single-indexed Illumina adapters according to the Earth Microbiome Project protocols (Apprill et al., 2015). PCR amplicons were checked for quality and size distribution on a 1.5% TE-agarose gel, and amplicon DNA concentration was determined using a Qubit fluorometer and Qubit dsDNA BR kit (Thermo Fisher Q32853). 16S amplicons from all samples and controls were purified using AMPure XP beads (Beckman Coulter A63881) and 80.0% ethanol washes to remove contaminants, then final DNA amplicon products were normalized to 4 nM concentration and pooled before submission to the University of Miami Center for Genome Technology (CGT) for 300 bp paired-end sequencing on the Illumina MiSeq.

## Bacteria Community Data Analysis

Raw 16S sequence reads were processed using the QIIME2 pipeline version 2020.2 (Bolyen et al., 2018). The QIIME2 plugin q2-demux was used to visualize read quality and the plugin DADA2 (Callahan et al., 2016) was used to remove primer sequences, trim poor-quality bases, dereplicate reads, identify chimeric sequences and merge paired-end reads. The plugin q2-feature-table (McDonald et al., 2012) was used to generate sequence summary statistics based upon sample metadata. Bacterial 16S rRNA sequences were taxonomically classified using the plugin q2-feature-classifier (Bokulich et al., 2018) and a Naïve Bayes Classifier previously trained on the SILVA 16S rRNA database (Quast et al., 2013) version 132 QIIME2 release based on the 515F/806RB primer pair<sup>4</sup> at the 99.0% similarity amplicon sequence variant (ASV) level. The plugins q2-alignment (Kato and Standley, 2013) and q2-phylogeny (Price et al., 2010) were used to create a phylogenetic tree for further downstream analyses, and the plugin q2-taxa was used to remove all mitochondria and chloroplast sequences from the dataset. The plugin q2-diversity was used to assess the core diversity metrics of the dataset and create sequence depth rarefaction plots in the QIIME2 environment. Finally, the output tables containing ASV counts, phylogenetic trees and sample metadata were exported from QIIME2 and imported into R for statistical analyses.

All bacteria community statistical analyses were conducted in R using the packages phyloseq (McMurdie and Holmes, 2013), CoDaSeq (Gloor and Reid, 2016), vegan (Oksanen et al., 2019), ggplot2 (Wickham, 2016), and the tidyverse (Wickham et al., 2019). ASVs with a mean count  $< 3$  were filtered from the dataset and ASV abundances were adjusted with the package zCompositions before the centered log-ratio (clr) transformation was performed for compositional data analysis (Palarea-Albaladejo and Martín-Fernández, 2015; Gloor et al., 2017). Alpha-diversity metrics were calculated for the unfiltered dataset using the Chao1 richness, Simpson evenness and Shannon diversity indices, and non-parametric Kruskal–Wallis tests were used to test for significant differences between treatments, reef sites, and coral genotypes (Xia and Sun, 2017; Ziegler et al., 2019). Beta-diversity was calculated using Aitchison dissimilarity matrices and visualized using

<sup>2</sup><http://eggnog-mapper.embl.de/>

<sup>3</sup>[https://github.com/z0on/GO\\_MWU](https://github.com/z0on/GO_MWU)

<sup>4</sup><https://data.qiime2.org/2020.2/common/silva-132-99-515-806-nb-classifier.qza>

principal coordinates analysis (PCA) (Gloor et al., 2017). Tests for homogeneity of multivariate dispersions (PERMDISP) and permutational analysis of molecular variance (PERMANOVA) (Anderson, 2017) were used to test for differences in bacteria community beta-diversity between treatments, reef sites and coral genotypes. Tests for differential abundance of bacteria ASVs between experimental treatments and the control treatment were completed with the R package ALDEx2 (Fernandes et al., 2013, 2014).

## RESULTS

### *Pocillopora* and *Cladocopium* Transcriptome Profiling Reveals Strong Species and Genotype Effects on Coral and Symbiont Gene Expression

RNAseq of 24 *Pocillopora* tissue samples yielded approximately 647.1 million raw reads ranging from 8.2 to 20.4 million reads per sample (Supplementary Table 1 and Supplementary Figure 5). Approximately 69.3% of total reads aligned to the *P. damicornis* genome while 16.1% of total reads aligned to the *C. goreauii* genome (Supplementary Figure 2). This was supported by preliminary alignments of non-coral reads to algal ITS2 reference sequences that suggested that the symbiont community was dominated by *C. goreauii* (Supplementary Table 2) as has been previously observed for *Pocillopora* corals at these sites (Keshavmurthy et al., 2014; Brener-Raffalli et al., 2018). The 14.6% of total reads that did not align to either reference were discarded from downstream analysis. Filtering only genes with counts  $\geq 10$  in at least 90% of samples left 12,867 *Pocillopora* genes and 9,818 *Cladocopium* genes remaining for downstream expression analyses.

Principal coordinates analysis visualization of vst-normalized gene counts indicated that *Pocillopora* genotype accounted for the most variance in overall *Pocillopora* and *Cladocopium* gene expression (Figures 2A,B). PCoA visualization showed the different genotypes clustering separately along PC1 with HW1 (*Pocillopora* sp. 8a) as the most dissimilar from the other three genotypes and HW2 (*P. damicornis*) in between HW1 and the WT1 and WT2 genotypes (*P. acuta*). PERMANOVA tests revealed significant effects of coral genotype (*Pocillopora*:  $F = 8.21$ ,  $R^2 = 0.36$ ,  $p < 0.001$ ; *Cladocopium*:  $F = 8.36$ ,  $R^2 = 0.37$ ,  $p < 0.001$ ) and experimental treatment (*Pocillopora*:  $F = 3.64$ ,  $R^2 = 0.20$ ,  $p < 0.001$ ; *Cladocopium*:  $F = 2.36$ ,  $R^2 = 0.14$ ,  $p < 0.001$ ) on overall transcriptome patterns.

### Differential Gene Expression Analysis Reveals Effects of Acute Heat Stress, Antibiotics, and Antibiotics-Heat Stress Treatments

In addition to *Pocillopora* host genotypic effects, PCoA and PERMANOVA revealed a combined effect of the antibiotics-heat stress treatment on *Pocillopora* and *Cladocopium* gene expression. Samples in the antibiotics-heat stress treatment grouped separately from samples in the control treatment and the

individual heat stress or antibiotics treatments in the PCoA plot (Figure 2). Furthermore, pairwise PERMANOVA tests between treatments only identified significant differences ( $p < 0.001$ ) in *Pocillopora* transcriptome profiles between the antibiotics-heat treatment and the individual control, antibiotics, and heat stress treatments, which were not significantly different from each other (Supplementary Table 3).

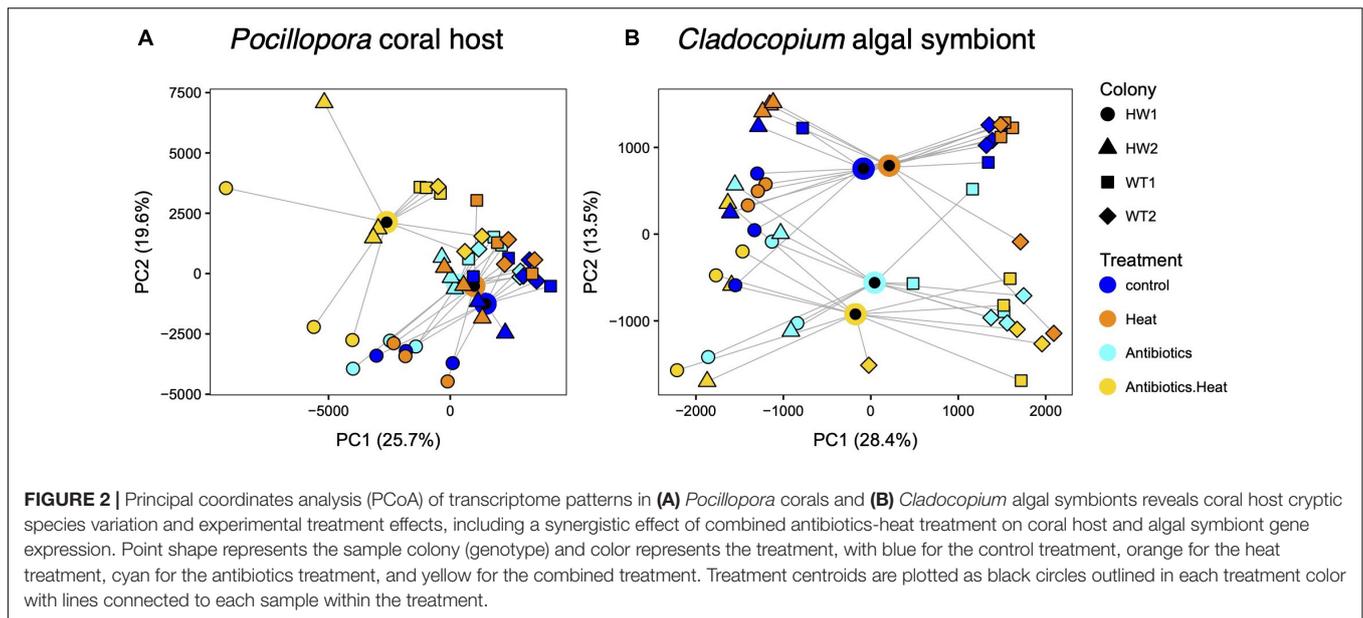
This pattern was confirmed via the DESeq2 differential gene expression analysis that identified *Pocillopora* and *Cladocopium* DEGs between each of the heat stress, antibiotics, and antibiotics-heat stress treatments versus the control treatment for each genotype and all genotypes analyzed together (Table 1). The all-genotypes analysis detected 702 coral DEGs (185 up and 517 down) and 35 symbiont DEGs (11 up and 24 down) between the heat stress treatment and the control treatment, 730 coral DEGs (369 up and 361 down) and 1,614 symbiont DEGs (804 up and 810 down) between the antibiotics treatment and control treatment, and 6,118 coral DEGs (3,117 up and 3,001 down) and 2,936 symbiont DEGs (1,558 up and 1,378 down) between the antibiotics-heat stress treatment and the control treatment (Table 1 and Supplementary Table 3). Venn diagram analysis of the DEG set intersects revealed that many *Pocillopora* DEGs were uniquely differentially expressed in the antibiotics-heat stress treatment (5,044, 82.4%, Figure 3A), indicating a synergistic effect of antibiotics and heat stress on the *Pocillopora* transcriptome (Gunderson et al., 2016). This same pattern was also observed in the *Cladocopium* DEGs in the antibiotics-heat stress treatment (1,971, 67.1%, Figure 3B).

### Eukaryotic Orthologous Group and Gene Ontology Enrichment Identifies *Pocillopora* and *Cladocopium* Biological Processes Affected by Heat Stress, Antibiotics, and Antibiotics-Heat Stress Treatments

Enrichment analysis of the *Pocillopora* and *Cladocopium* DEGs detected in treatment contrasts that had annotations to 23 non-overlapping KOG categories revealed distinct biological responses to the heat stress, antibiotics, and antibiotics-heat stress treatments (Figure 3C).

*Pocillopora* upregulated DEGs in the heat stress treatment were not significantly enriched ( $p_{adj} < 0.05$ ) for any KOG categories or GO terms (Figure 3C). However, *Pocillopora* downregulated DEGs in the heat stress treatment were enriched for the KOG categories 'translation, ribosomal structure and biogenesis' and 'inorganic ion transport and metabolism' (Figure 3C) and this was supported by enriched biological process (BP) GO terms ( $n = 36$ ,  $p_{adj} < 0.05$ ), including 'protein localization to endoplasmic reticulum,' 'cytoplasmic translation,' and 'ribosome biogenesis' (Supplementary Table 4).

*Pocillopora* upregulated DEGs in the antibiotics treatment were enriched for the KOG categories 'intracellular trafficking, secretion, and vesicular transport' and 'posttranslational modification, protein turnover, chaperones' (Figure 3C), and were enriched for BP GO terms ( $n = 20$ ,  $p_{adj} < 0.05$ )



including ‘endoplasmic reticulum to Golgi vesicle-mediated transport,’ ‘response to topologically incorrect protein,’ ‘canonical Wnt signaling pathway,’ and ‘regulation of hydrolase activity’ (Supplementary Table 4). *Pocillopora* downregulated DEGs in the antibiotics treatment were enriched for the KOG categories ‘inorganic ion transport and metabolism’ and ‘cytoskeleton’ (Figure 3C), however no BP GO terms were significantly enriched at the  $p_{adj} < 0.05$  level (Supplementary Table 4). Notable downregulated *Pocillopora* DEGs in the antibiotics treatments included five amino acid transporters (pdam\_00001802, pdam\_00001810, pdam\_00011663, pdam\_00012562, and pdam\_00017019), one ammonium transporter (pdam\_00014954), and several enzymes involved in amino acid biosynthetic pathways including glutamate carboxypeptidase 2 (pdam\_00006146), adenosylhomocysteinase (pdam\_00017020), S-adenosylmethionine synthase (pdam\_00004044), and alanine aminotransferase I (pdam\_00024643) (Supplementary Table 3).

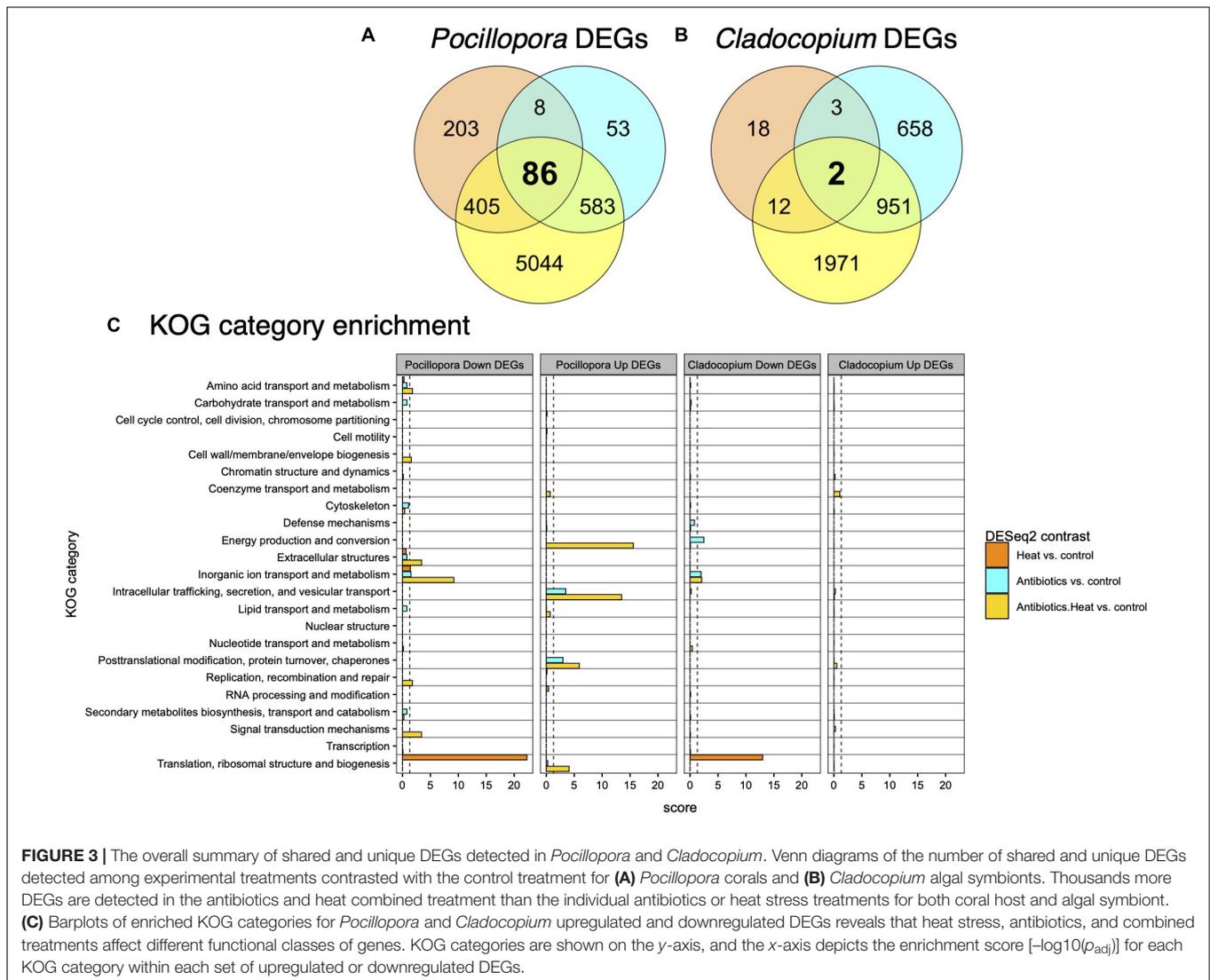
*Pocillopora* upregulated DEGs in the antibiotics-heat stress treatment were enriched for the KOG categories ‘energy production and conversion,’ ‘intracellular trafficking, secretion and vesicular transport,’ ‘posttranslational modification, protein turnover, chaperones,’ and ‘translation, ribosomal structure and biogenesis’ (Figure 3C), as well as many BP GO terms ( $n = 92$ ,  $p_{adj} < 0.05$ ), among which the most significantly enriched were ‘cellular respiration,’ ‘electron transport chain,’ and ‘establishment of protein localization to mitochondrion.’ Other highly enriched terms included ‘antigen processing and presentation,’ ‘generation of precursor metabolites and energy,’ ‘oxidation-reduction process,’ and ‘proteolysis’ (Supplementary Table 4).

*Pocillopora* downregulated DEGs in the antibiotics-heat stress treatment were enriched for the KOG categories ‘inorganic ion transport and metabolism,’ ‘extracellular structures,’ ‘signal transduction mechanisms,’ ‘amino acid transport and metabolism,’ ‘replication, recombination and repair,’

and ‘cell wall/membrane/envelope biogenesis’ (Figure 3C), and these processes were supported by the most highly enriched BP GO terms ( $n = 154$ ,  $p_{adj} < 0.05$ ) including ‘sodium ion transport,’ ‘metal ion transport,’ ‘microtubule organizing center organization,’ ‘response to wounding,’ and ‘cell–cell adhesion via plasma-membrane adhesion molecules’ (Supplementary Table 4).

The few *Cladocopium* upregulated DEGs in the heat stress treatment were not enriched for any KOG categories or BP GO terms, whereas *Cladocopium* downregulated DEGs in the heat stress treatment were enriched for the KOG category ‘translation, ribosomal structure and biogenesis’ and the BP GO terms ( $n = 4$ ,  $p_{adj} < 0.05$ ) ‘cytoplasmic translation,’ ‘amide biosynthetic process,’ ‘maturation of LSU-rRNA,’ and ‘ribosomal large subunit biogenesis’ (Supplementary Table 4).

*Cladocopium* upregulated DEGs in the antibiotics treatment were also not enriched for any KOG categories or BP GO terms. *Cladocopium* downregulated DEGs in the antibiotics treatment were enriched for the KOG categories ‘energy production and conversion’ and ‘inorganic ion transport and metabolism’ and BP GO terms ( $n = 4$ ,  $p_{adj} < 0.05$ ) ‘cytoplasmic translational elongation,’ ‘photosynthesis,’ ‘cellular response to hydrogen peroxide,’ and ‘cellular response to toxic substance.’ Among these *Cladocopium* downregulated DEGs was a chloroplastic aminomutase (SymbC1.scaffold4424.2), a chloroplastic delta-aminolevulinic acid dehydratase (SymbC1.scaffold548.4), six aminotransferases (SymbC1.scaffold31.2, SymbC1.scaffold2551.4, SymbC1.scaffold2895.2, SymbC1.scaffold2898.1, SymbC1.scaffold2207.2, and SymbC1.scaffold1758.2), an amino acid permease (SymbC1.scaffold7668.1), a chloroplastic glutamate synthase (SymbC1.scaffold1423.11), a type-3 glutamine synthase (SymbC1.scaffold371.1), a mitochondrial basic amino acid transporter (SymbC1.scaffold1251.1), and a fumarate reductase enzyme (SymbC1.scaffold10507.1).



**FIGURE 3 |** The overall summary of shared and unique DEGs detected in *Pocillopora* and *Cladocopium*. Venn diagrams of the number of shared and unique DEGs detected among experimental treatments contrasted with the control treatment for (A) *Pocillopora* corals and (B) *Cladocopium* algal symbionts. Thousands more DEGs are detected in the antibiotics and heat combined treatment than the individual antibiotics or heat stress treatments for both coral host and algal symbiont. (C) Barplots of enriched KOG categories for *Pocillopora* and *Cladocopium* upregulated and downregulated DEGs reveals that heat stress, antibiotics, and combined treatments affect different functional classes of genes. KOG categories are shown on the y-axis, and the x-axis depicts the enrichment score  $[-\log_{10}(p_{adj})]$  for each KOG category within each set of upregulated or downregulated DEGs.

*Cladocopium* upregulated DEGs in the antibiotics-heat stress treatment were not enriched for any KOG categories or BP GO terms, whereas *Cladocopium* downregulated DEGs in the antibiotics-heat stress treatment were enriched for the KOG category ‘inorganic ion transport and metabolism,’ and no BP GO terms (Supplementary Table 4).

### WGCNA Reveals Modules of Co-expressed Immunity, Symbiosis, and Stress Response Genes Correlated to Experimental Treatments

WGCNA based on vst-transformed counts identified 23 *Pocillopora* and 13 *Cladocopium* co-expressed gene modules that were labeled according to module size along a standard sequence of colors within the WGCNA R package (Figure 4). Of these, 12 *Pocillopora* modules and 8 *Cladocopium* modules displayed module eigengene expression that was significantly correlated to one or more treatments ( $p < 0.05$ ).

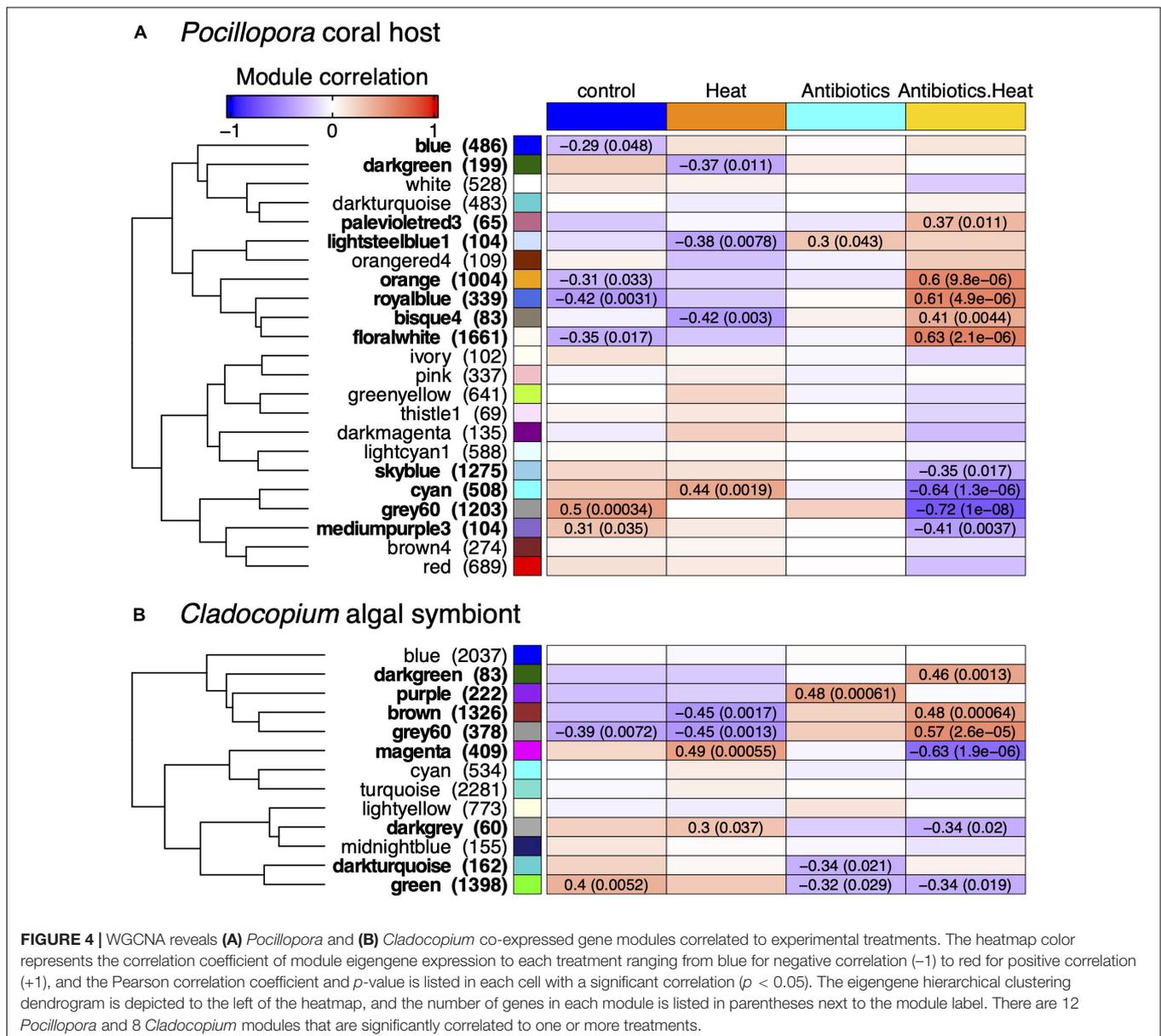
For *Pocillopora*, there were 2 positively (‘grey60’ and ‘mediumpurple3’) and 4 negatively (‘royalblue,’ ‘floralwhite,’ ‘orange,’ and ‘blue’) correlated modules to the control treatment, 1 positively (‘cyan’) and 3 negatively (‘bisque4,’ ‘lightsteelblue1,’ and ‘darkgreen’) correlated modules to the heat stress treatment, 1 module positively correlated to the antibiotics treatment (‘lightsteelblue1’), and 5 positively (‘floralwhite,’ ‘royalblue,’ ‘orange,’ ‘bisque4,’ and ‘palevioletred3’) and 4 negatively (‘grey60,’ ‘cyan,’ ‘mediumpurple3,’ and ‘skyblue’) correlated modules to the antibiotics-heat stress treatment (Figure 4A).

For *Cladocopium*, there was 1 positively (‘green’) and 1 negatively (‘grey60’) correlated modules to the control treatment, 2 positively (‘magenta’ and ‘darkgrey’) and 2 negatively (‘grey60’ and ‘brown’) correlated modules to the heat stress treatment, 1 positively (‘purple’) and 2 negatively (‘darkturquoise’ and ‘green’) correlated modules to the antibiotics treatment, and 3 positively (‘grey60,’ ‘brown,’ and ‘darkgreen’) and 3 negatively (‘magenta,’ ‘darkgrey,’ and ‘green’) correlated modules the antibiotics-heat stress treatment (Figure 4B).

Among all the significantly correlated *Pocillopora* modules, the 'lightsteelblue1' module ( $n = 104$  genes) was the only module positively correlated to the antibiotics treatment and was also negatively correlated to the heat stress treatment. This module was enriched for the KOG category 'signal transduction mechanisms' and BP GO terms ( $n = 38, p_{adj} < 0.05$ ) related to immune processes such as 'activation of immune response,' 'leukocyte activation,' and 'response to bacterium' (Supplementary Tables 5, 6). The module hub gene was an endoribonuclease (ZC3H12B, pdam\_00012426) (Table 2) that may function as an RNase involved in immunomodulation via mRNA decay, and other co-expressed genes included the immune-related transcription factors ETS-related transcription factor Elf-4 (ELF4, pdam\_00016137), ETS domain-containing protein Elk-1 (ELK1, pdam\_00003296), baculoviral IAP

repeat-containing protein 2 (BIRC2, pdam\_00007333), and neural proliferation differentiation and control protein 1 (NPDC1, pdam\_00019344).

The 'floralwhite' module was the largest module ( $n = 1,661$  genes) and was negatively correlated to the control treatment and positively correlated to the antibiotic-heat treatment (Figure 4A). This module was highly enriched for the KOG categories 'energy production and conversion,' 'intracellular trafficking, secretion, and vesicular transport,' 'posttranslational modification, protein turnover, chaperones' and 'translation, ribosomal structure and biogenesis,' as well as the BP GO terms ( $n = 131, p_{adj} < 0.05$ ) 'generation of precursor metabolites and energy,' 'protein polyubiquitination,' and 'proteasome assembly' (Supplementary Tables 5, 6). The 'floralwhite' module hub gene was a proteolytic subunit of the



**TABLE 2** | Number of *Pocillopora* and *Cladocopium* DEGs (# of genes DESeq2  $p_{adj} < 0.05$  [up, down]) detected in contrasts between each experimental treatment and the control treatment for each genotype and all genotypes together.

Holobiont partner	DESeq2 contrast	HW1	HW2	WT1	WT2
<i>Pocillopora</i>	Heat vs. control	0 [0, 0]	2 [0, 2]	58 [12, 46]	71 [8, 63]
<i>Pocillopora</i>	Antibiotics vs. control	210 [96, 114]	75 [59, 16]	6 [4, 2]	14 [11, 3]
<i>Pocillopora</i>	Antibiotics heat vs. control	1124 [572, 552]	2677 [1315, 1362]	2000 [848, 1152]	929 [487, 442]
<i>Cladocopium</i>	Heat vs. control	0 [0, 0]	0 [0, 0]	6 [1, 5]	4 [2, 2]
<i>Cladocopium</i>	Antibiotics vs. control	1 [1, 0]	0 [0, 0]	10 [4, 6]	699 [346, 353]
<i>Cladocopium</i>	Antibiotics heat vs. control	144 [91, 53]	149 [86, 63]	701 [336, 365]	1453 [730, 723]

The increased number of DEGs for both the coral host and algal symbiont in the combined antibiotics and heat stress treatment relative to the separate treatments indicates that there is a synergistic effect of antibiotics and heat stress on both coral and algal symbiont gene expression.

mitochondrial ATP-dependent Clp protease complex (CLPP, pdam\_00002241) (Table 2), and other co-expressed genes within the ‘floralwhite’ module were involved in immune-related processes including the master immune regulator NF- $\kappa$ B (pdam\_00003205), tumor necrosis factor receptor-associated factor 6 (TRAF6, pdam\_00017873), and mannan-binding lectin-associated serine protease 1 (MASP1, pdam\_00000447). Additional upregulated and co-expressed genes were involved in pro-apoptosis pathways and protein degradation such as apoptosis regulator BAX (pdam\_00019537), programmed cell death 6-interacting protein (pcdip6, pdam\_00015525), matrix metalloproteinase 25 (MMP25, pdam\_00023163), and multiple 26S proteasome subunits.

The ‘orange’ module ( $n = 1,004$  genes) was also negatively correlated to control treatment and positively correlated to antibiotics-heat stress treatment (Figure 4A). The ‘orange’ module was enriched for the KOG category ‘cytoskeleton,’ and the most highly enriched BP GO terms ( $n = 74$ ,  $p_{adj} < 0.05$ ) included ‘response to oxidative stress,’ ‘cellular amino acid catabolic process,’ and ‘response to temperature stimulus’ (Supplementary Tables 5, 6). Although the module hub gene (pdam\_00024070) lacked annotation, other highly connected and upregulated genes were involved in the oxidative stress response, such as catalase (pdam\_00001882), peroxidase (pdam\_00000351), peroxiredoxin-6 (pdam\_00009060) and apoptosis-inducing factor 2 (AIFM2, pdam\_00001271), which is involved in directly triggering programmed cell death pathways during oxidative stress.

Conversely, the ‘grey60’ ( $n = 1,203$  genes) and ‘mediumpurple3’ ( $n = 104$  genes) modules were both positively correlated to the control treatment and negatively correlated to the antibiotics-heat treatment. The ‘grey60’ module was enriched for the KOG categories ‘extracellular structures,’ ‘inorganic ion transport and metabolism,’ ‘signal transduction mechanisms,’ and ‘transcription’ as well as many BP GO terms ( $n = 305$ ,  $p_{adj} < 0.05$ ) related to cellular housekeeping functions and homeostasis maintenance (Supplementary Tables 5, 6). The ‘mediumpurple3’ module was enriched for the KOG category ‘signal transduction mechanisms’ and several BP GO terms ( $n = 25$ ,  $p_{adj} < 0.05$ ) involved in epithelial immune processes such as ‘regulation of antibacterial peptide biosynthetic process,’ ‘positive regulation of humoral immune response,’ ‘cell–cell adhesion,’ and ‘columnar/cuboidal epithelial cell

development.’ The module hub gene was a fibroblast growth factor receptor (FGFR, pdam\_00020781), and there were numerous other tyrosine kinase receptors within the module (Supplementary Tables 5, 6).

The ‘cyan’ module ( $n = 508$  genes) was positively correlated to the control treatment and heat stress treatment and negatively correlated the antibiotics-heat treatment, and was enriched for the KOG categories ‘cell cycle control, cell division, and chromosome partitioning,’ ‘chromatin structure and dynamics,’ ‘cytoskeleton,’ ‘nucleotide transport and metabolism,’ ‘replication, recombination, and repair,’ and ‘secondary metabolites biosynthesis, transport, and catabolism,’ and the top enriched BP GO terms ( $n = 209$ ,  $p_{adj} < 0.05$ ) included numerous DNA damage response and repair processes (Supplementary Tables 5, 6).

For *Cladocopium*, only the ‘purple’ module ( $n = 222$  genes) was positively correlated with antibiotics treatment, however, no KOG categories or BP GO terms were significantly enriched (Figure 4B). The module hub gene was a 26S proteasome regulatory subunit (SymbC1.scaffold7524.1) (Table 2), and other genes in the module that were also upregulated in the antibiotics treatment included a D-amino acid dehydrogenase small subunit protein (SymbC1.scaffold805.5), an isoleucine-tRNA ligase (SymbC1.scaffold2776.4), a vacuolar amino acid transporter associated with ATP-mediated isoleucine uptake (SymbC1.scaffold2075.3), and two sodium-dependent amino acid transporters (SymbC1.scaffold8224.5 and SymbC1.scaffold8948.3).

The largest significant *Cladocopium* module was the ‘green’ module ( $n = 1,398$  genes), which was positively correlated to the control treatment and negatively correlated to both the antibiotics and antibiotics-heat stress treatment, and was enriched for the KOG category ‘energy production and conversion’ and BP GO terms ‘photosynthesis’ and ‘nucleoside monophosphate biosynthetic process.’ The ‘darkturquoise’ module ( $n = 162$  genes) was also negatively correlated to the antibiotics treatment and had a chloroplastic hub gene of fucoxanthin-chlorophyll binding protein E (SymbC1.scaffold3718.1), an element of the light-harvesting complex associated with photosystem II.

Both the *Cladocopium* ‘magenta’ ( $n = 409$  genes) and ‘darkgrey’ ( $n = 60$  genes) modules were positively correlated to the heat stress treatment and negatively correlated to the antibiotics-heat stress treatment (Figure 4B). The ‘magenta’

module was enriched for the KOG category ‘signal transduction mechanisms,’ however, no BP GO terms were enriched. Conversely, the ‘brown’ ( $n = 1,326$  genes) and ‘grey60’ ( $n = 378$  genes) modules were negatively correlated to the heat stress treatment and positively correlated to the antibiotics-heat stress treatment. The ‘brown’ module was enriched for the KOG category ‘translation, ribosomal structure and biogenesis’ and BP GO terms ‘cytoplasmic translation,’ ‘membrane docking,’ ‘ncRNA metabolic process,’ and ‘RNA/ncRNA processing,’ and its hub gene was autophagy-related protein 9 (ATG9, SymbC1.scaffold517.1), a protein involved in autophagic vesicle formation. Genes related to ncRNA metabolism and processing included piRNA biogenesis protein Exd1 (SymbC1.scaffold5866.5), an Elav-like RNA-binding protein associated with miRNA processing (SymbC1.scaffold526.10), and an insulin-like mRNA-binding protein capable of shielding target transcripts from microRNA-mediated degradation (SymbC1.scaffold2518.1).

## Antibiotics Alters *Pocillopora*-Associated Bacteria Communities

Sequencing of bacteria 16S rRNA gene V4 region on the Illumina MiSeq at the University of Miami CGT was completed in April 2019. A total of 3,716,629 demultiplexed reads were obtained from all 48 samples, with an average sequence length of 300 bp (Supplementary Figure 3). After read denoising, classification against the SILVA database and filtering of mitochondria and chloroplast-derived sequences, 470 coral-associated ASVs were detected representing 17 phyla, 31 classes, 84 orders, and 135 bacterial and archaeal families. The most abundant bacterial phyla were Proteobacteria, Bacteroidetes, and Cyanobacteria, and the most abundant families within the phylum Proteobacteria were Endozoicomonadaceae, Rhodobacteraceae, and Alteromonadaceae (Figure 5B). Filtering out low-abundance taxa with mean counts  $< 3$  left 36 high-abundance ASVs remaining for compositional data analysis of bacteria community alpha and beta-diversity. Antibiotics treatments caused significant changes in coral-associated bacteria community beta-diversity according to PCA visualization of center-log ratio transformed abundances, as PC1 separated samples in both antibiotics treatments from the control and heat stress treatment samples and explained 24.8% of the variance (Figure 5A). PC2 explained 20.8% of the variance and partially separated the *P. acuta* genotypes (WT1 and WT2) from the *P. damicornis* genotypes (HW1 and HW2), in part due to a higher relative abundance of *Endozoicomonas* bacteria (Figures 5A,B).

Non-parametric Kruskal–Wallis tests detected significant differences in bacteria community alpha-diversity metrics between experimental treatments (Chao1, Kruskal–Wallis chi-squared = 8.62,  $p = 0.035$ ), with lower alpha-diversity observed in antibiotics-treated samples. PERMDISP tests revealed inhomogeneity of dispersions between treatments ( $F = 3.44$ ,  $p = 0.027$ ), and PERMANOVA tests also revealed significant differences between treatment centroids ( $F = 5.28$ ,  $R^2 = 0.26$ ,

$p < 0.001$ ), indicating differences in bacteria community composition in antibiotics-treated samples.

Specifically, antibiotics treatments reduced the relative abundance of bacteria in the family Rhodobacteraceae while bacteria in the family Alteromonadaceae persisted and increased in relative abundance (Figure 5B and Supplementary Figure 4). Bacteria in the genus *Endozoicomonas* were also observed to persist through antibiotics treatment but did not increase in relative abundance (Figure 5B and Supplementary Figure 4).

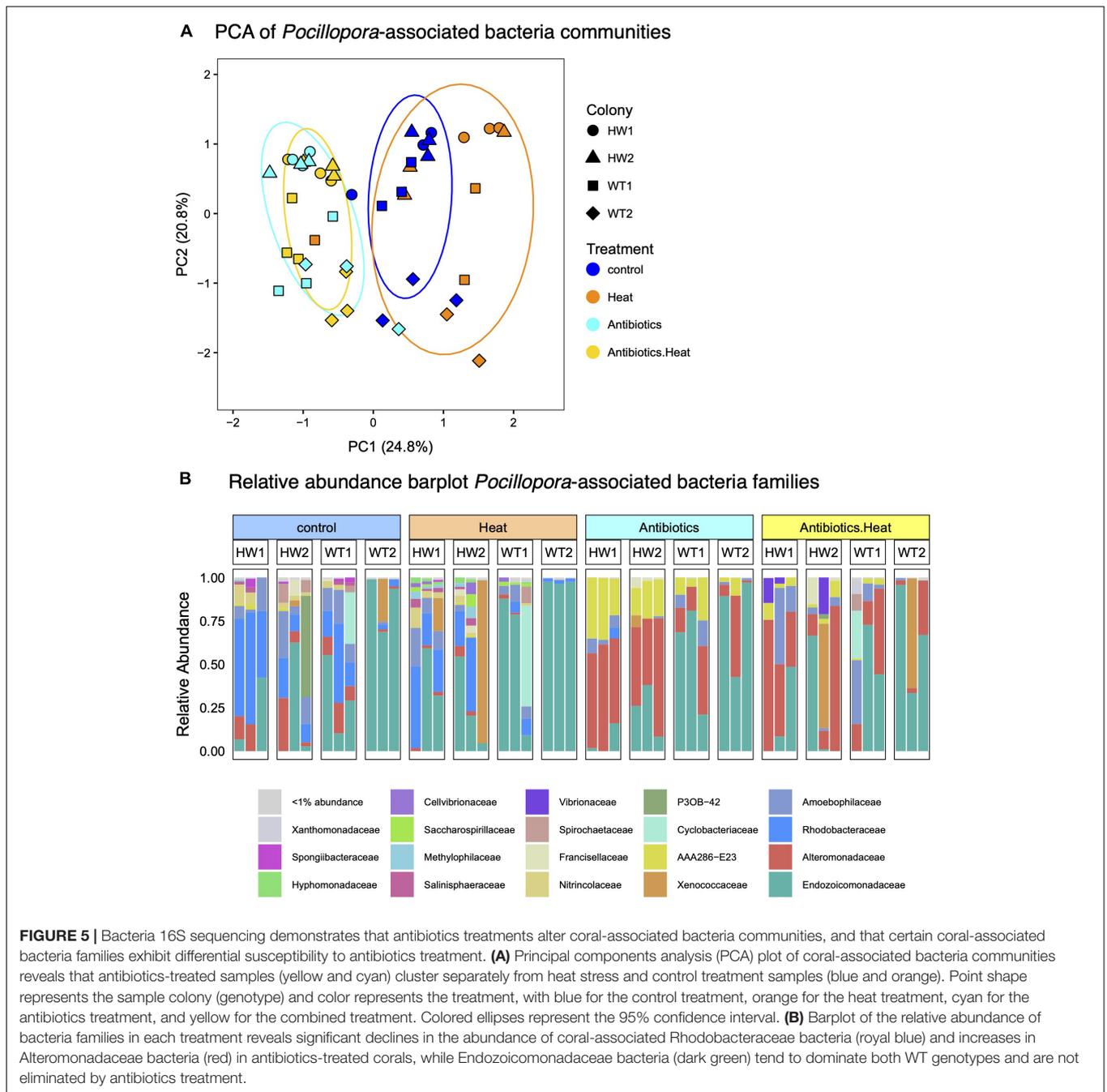
Furthermore, paired ALDEx2 tests that contrasted each experimental treatment with the control identified specific differentially abundant ASVs. Three ASVs in the family Rhodobacteraceae and one ASV in the genus *Neptuniibacter* (family Nitriticolaceae) were significantly reduced in both antibiotics and antibiotics-heat stress treatment samples (Supplementary Figure 5). Two ASVs in the family Alteromonadaceae had increased relative abundance in the antibiotics treatment and antibiotics-heat stress treatment (Supplementary Figure 5). BLASTn searches of these ASVs against the NCBI 16S rRNA database uncovered 100% sequence similarity ( $e$ -values  $< 3e^{-123}$ ) to *Alteromonas aestuariivivens*, an ampicillin-resistant bacterium isolated from a South Korea tidal flat (Park et al., 2017) and *Aestuuriibacter aggregatus*, another ampicillin-resistant bacterium isolated from Yellow Sea (Wang et al., 2010).

## DISCUSSION

On coral reefs, antibiotics are increasingly being considered for use in coral disease intervention strategies and as environmental pollutants that are potentially damaging to coral health (Zhang R. et al., 2018; Zhang R. et al., 2019; Neely et al., 2020; Walker et al., 2021). Our experiment reveals that antibiotic suppression of coral-associated bacteria communities produces dramatic changes in *Pocillopora* coral and *Cladocopium* symbiont gene expression and bacteria community composition, resulting in microbial dysbiosis and diminished holobiont heat tolerance. These findings corroborate results from previous studies that documented negative effects of antibiotics on coral health (Gilbert et al., 2012; Glasl et al., 2016), and provide new insights into the complex multi-partner interactions that occur within *Pocillopora* coral holobionts (Brener-Raffalli et al., 2018; Pogoreutz et al., 2018; Geissler et al., 2021; Haydon et al., 2021; Li et al., 2021).

## Antibiotics Treatment Disrupts Expression of Genes Involved in Coral Holobiont Metabolic Interactions

*Pocillopora* and *Cladocopium* transcriptome responses to antibiotics treatment revealed changes in intracellular trafficking and vesicular transport and inorganic ion transport and metabolism genes and upregulation of immune-related genes in the *Pocillopora* ‘lightsteelblue1’ module. These changes suggest that disruptions of the coral-associated bacteria community negatively affect coral holobiont metabolic exchanges and inter-partner signaling, resulting in immune



inflammation and microbiome dysregulation (Figures 4, 5 and Supplementary Table 3). This transcriptomic evidence also supports the notion that these holobiont metabolic interactions entail the exchanges of amino acids, inorganic ions, and other secondary metabolites among the *Pocillopora* coral host, its *Cladocopium* algal symbionts and specific bacteria within the coral holobiont (Matthews et al., 2020; Peixoto et al., 2021). Previous studies have identified diazotrophic bacteria as a source of fixed nitrogen to coral holobionts (Olson et al., 2009; Benavides et al., 2017; Lesser et al., 2018; Rådecker et al., 2021), and have implicated coral-associated

bacteria in dimethylsulfoniopropionate (DMS) metabolism (Raina et al., 2010; Tandon et al., 2020) and the production of siderophores capable of alleviating iron and trace metal limitation (Hopkinson and Morel, 2009; Schalk et al., 2011; Amin et al., 2017; Reich et al., 2020; Peixoto et al., 2021). Importantly, while transcriptome read alignments suggest these *Pocillopora* genotypes were *Cladocopium*-dominated, direct measurement of Symbiodiniaceae community composition via ITS2 amplicon sequencing (Boulotte et al., 2016; Hume et al., 2019) in future studies would be preferred for assessing interactions of different Symbiodiniaceae genera with associated bacteria.

While the magnitude of *Pocillopora* gene expression changes were similar for the acute heat stress treatment and antibiotics treatment relative to the control treatment, *Cladocopium* gene expression had more DEGs in response to antibiotics treatment than to the heat stress treatment (Figures 2B, 3B). This difference was driven in part by the downregulation of genes involved in energy production and photosynthesis in the *Cladocopium* 'green' and 'darkturquoise' modules that were negatively correlated to antibiotics treatment, such as the light-harvesting fucoxanthin-chlorophyll A-C binding proteins E and F, a carotenochlorophyll A-C-binding protein and several photosystem I proteins (Figures 3C, 4B and Supplementary Table 4).

We hypothesize that the downregulation of *Cladocopium* photosynthesis and metabolism genes in the antibiotics treatment may be related to disruptions in nitrogen cycling and amino acid exchanges between the *Pocillopora* coral, *Cladocopium* symbionts and members of the associated bacteria community. Five *Pocillopora* amino acid transporters were downregulated in the antibiotics treatment (Supplementary Table 3), however, for *Cladocopium* three amino acid transporters and an isoleucine tRNA ligase were upregulated in the 'purple' module, and other genes involved in amino acid metabolism were downregulated in the 'green' and 'darkturquoise' modules. The role of amino acids in generating energy via the TCA cycle (Akram, 2014) or alternatively as metabolites in chlorophyll biosynthesis (Vavilin and Vermaas, 2002) could potentially play a role in *Cladocopium* photosynthetic impairment. This second hypothesis is consistent with observations of decreased chlorophyll a in nitrogen-starved *C. goreauii* (Zhou et al., 2021) and stable photochemical efficiency and dramatically increased growth rates in *Brevolium minutum* cultures supplemented with casein amino acids (Kirk et al., 2020). Although the *Pocillopora* and *Cladocopium* genomes possess pathways for synthesizing most amino acids and heterotrophic feeding may account for substantial input of essential amino acids to the coral holobiont (Fox et al., 2019; Ferrier-Pagès et al., 2021), these observations suggest that certain coral-associated and/or Symbiodiniaceae-associated bacteria may provide *in hospite* Symbiodiniaceae with additional nitrogenous compounds that benefit holobiont metabolism (Benavides et al., 2017; Geissler et al., 2021; Glaze et al., 2021; Rådecker et al., 2021). While more experimental evidence is required to validate this conclusion, *Cladocopium* symbionts were recently shown to associate with diverse bacteria located within algae cells and on external cell surfaces, which supports the idea that Symbiodiniaceae-bacteria metabolic exchanges may be more common than previously recognized (Camp et al., 2020; Matthews et al., 2020; Maire et al., 2021).

## Antibiotics Treatment Alters *Pocillopora*-Associated Bacteria Communities

Treatments of *Pocillopora* corals with the broad-spectrum antibiotics ampicillin and streptomycin caused significant changes in coral-associated bacteria community composition (Figure 5A), as antibiotics treatment substantially reduced the relative abundance of bacteria in the family Rhodobacteraceae

while bacteria in the family Alteromonadaceae persisted and increased in relative abundance (Figure 5B).

Bacteria community analysis of these *Pocillopora* corals from southern Taiwan revealed similar results to previous studies demonstrating that *P. acuta* corals in Taiwan and elsewhere in the Indo-Pacific have high relative abundances of *Endozoicomonas* bacteria (Brener-Raffalli et al., 2018; Epstein et al., 2019; Connelly et al., 2020), however, further studies on how variation in coral host species, site effects and environmental factors affect *Pocillopora* corals' associations with *Endozoicomonas* bacteria are needed to corroborate this trend. Furthermore, these results indicate that *Endozoicomonas*-dominated microbiomes may be more resistant to experimental antibiotic perturbation (Figure 5B and Supplementary Figure 4). The persistence of *Endozoicomonas* bacteria throughout multiple stressors suggests that these bacteria are either resistant to environmental perturbations or perhaps are effectively shielded from external stressors because of their location as cellular aggregates within *Pocillopora* coral tissues (Work and Aeby, 2014; Neave et al., 2017; Pogoreutz et al., 2018).

Furthermore, it appears that other bacteria that persist through antibiotics treatment, such as the bacteria ASVs similar to the ampicillin-resistant *Alteromonas* spp. and *A. aggregatus*, might act as opportunistic pathogens that activate coral immune responses and cause microbial dysbiosis during antibiotics-heat stress treatment (Wang et al., 2010; Park et al., 2017; Zaneveld et al., 2017). More in-depth research on the taxonomic diversity, localization, and metabolic functions of *Pocillopora* coral-associated bacteria communities is necessary to fully understand how interactions with specific bacteria influence coral holobiont health, and these results highlight the potential of antibiotics treatments as an experimental tool to manipulate these associations.

## Antibiotics-Heat Stress Treatment Causes Microbial Dysbiosis and Exacerbates *Pocillopora* and *Cladocopium* Transcriptome Stress Responses

Despite the strong influence of coral genotype on both *Pocillopora* and *Cladocopium* gene expression, differential gene expression and gene co-expression network analyses revealed distinct effects of the separate acute heat stress and antibiotics treatments as well as a strong combined effect of the antibiotics-heat treatment (Figure 2 and Table 1). Samples in the antibiotics-heat stress treatment clustered separately from the heat stress, antibiotics, and control treatment samples in PCoA, and thousands more DEGs were detected in the antibiotics-heat treatment than either individual heat stress or antibiotics treatment (Table 1 and Figures 2, 3). This dramatic transcriptional activation of *Pocillopora* and *Cladocopium* energy conversion and stress response genes suggest that in the antibiotics-heat stress treatment, bacteria community disruption negatively impacts *Pocillopora* coral holobiont metabolism and immune homeostasis such that opportunistic microbes flourish and the corals' ability to

collectively mount an efficient heat stress response and defend against infection is diminished (Figures 3C, 5B). This is supported by functional enrichment analyses of *Pocillopora* DEGs and the co-expressed ‘floralwhite,’ ‘orange,’ ‘grey60,’ ‘mediumpurple3,’ and ‘cyan’ modules that indicate *Pocillopora* corals’ responses to the antibiotics-heat stress treatment involved increases in energy production and conversion, immune inflammation, proteolysis and apoptosis at the expense of diminished holobiont metabolism, epithelial immune integrity, ion transport, inter-partner signal transduction, and DNA maintenance (Figure 3C). This suggests that in the antibiotics-heat treatment, oxidative damage signals together with a dysregulated bacteria community triggers programmed cell death pathways through the activation of tumor necrosis factor and caspase pathways, and proteolytic effectors such as MMPs and the 26S proteasome (Wecker et al., 2018; Chuang, 2020; Jiang et al., 2020). Indeed, many of these energy conversion, oxidative stress response and proteolysis genes were co-expressed within the *Pocillopora* ‘floralwhite’ and ‘orange’ modules and the *Cladocypium* ‘brown’ module, making the hub genes of each of these modules appealing targets for future functional characterization, pharmacological inhibition and gene knockdown/knockout studies (Table 3) (Cleves et al., 2020a,b; Jiang et al., 2020).

### Implications for Coral Conservation and Disease Intervention Strategies

The results on the effect of antibiotic suppression of coral bacteria community and host-microbe interactions lead us to warn coral reef conservation practitioners to be cautious in considering field-based applications of antibiotics treatments as

potential disease therapies, as there may be numerous unintended consequences for coral health. Current mitigation strategies being used throughout the Caribbean for treating stony coral tissue loss disease (SCTLD) involve treatments with antibiotics-infused epoxies (Voss et al., 2019; Walker and Pitts, 2019; Neely et al., 2020; Shilling, 2020). Our results suggest that ampicillin and streptomycin based antibiotic treatments do not non-selectively eliminate pathogenic bacteria, and more investigation into the efficacy and safety of these approaches should be conducted.

Future laboratory work should attempt to carefully manipulate associations between different coral genotypes and microbial symbiotic partners (including Symbiodiniaceae and bacteria) to move toward the generation of gnotobiotic (i.e., known-microbiome) corals for use in functional experiments. This has been shown to be feasible in cnidarian model systems such as *Hydra*, *Nematostella*, and *Aiptasia* (Murillo-Rincon et al., 2017; Domin et al., 2018; Medrano et al., 2019; Costa et al., 2021; Dungan et al., 2021), but is only beginning to be explored in reef-building corals and other cnidarians (Lin et al., 2019; Cuning and Baker, 2020; Weiland-Bräuer et al., 2020). Experiments that apply a combination of antibiotics treatments followed by targeted probiotic delivery or “microbiome recovery” treatments will yield insights into whether disturbed coral-associated bacteria communities and host stress response phenotypes can be “rescued” by the re-introduction of one or several beneficial microbes (Peixoto et al., 2017, 2021; Rosado et al., 2018; Damjanovic et al., 2019; Assis et al., 2020; Santoro et al., 2021). Future work would also benefit from the inclusion of baseline samples and additional time points to track the temporal changes in bacteria community composition and transcriptome responses across coral colonies.

**TABLE 3 |** Hub genes of *Pocillopora* and *Cladocypium* WGCNA modules with significant correlations to experimental treatments.

Holobiont partner	Module	Hub gene	UniProt best match
<i>Pocillopora</i>	Blue	pdam_00016931	Cct6a: T-complex protein 1 subunit zeta ( <i>Mus musculus</i> )
<i>Pocillopora</i>	Darkgreen	pdam_00015892	RPS11: 40S ribosomal protein S11 ( <i>Canis lupus familiaris</i> )
<i>Pocillopora</i>	Palevioletred3	pdam_00006619	SAMD15: sterile alpha motif domain-containing protein 15 ( <i>Macaca fascicularis</i> )
<i>Pocillopora</i>	Lightsteelblue1	pdam_00012426	ZC3H12B: probable ribonuclease ZC3H12B ( <i>Homo sapiens</i> )
<i>Pocillopora</i>	Orange	pdam_00024070	Unknown function
<i>Pocillopora</i>	Royalblue	pdam_00014622	PPP2CA: serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform ( <i>Gallus gallus</i> )
<i>Pocillopora</i>	Bisque4	pdam_00010339	wnt8a: protein Wnt-8a ( <i>Danio rerio</i> )
<i>Pocillopora</i>	Floralwhite	pdam_00002241	Clpp: ATP-dependent Clp protease proteolytic subunit%2C mitochondrial ( <i>Mus musculus</i> )
<i>Pocillopora</i>	Skyblue	pdam_00015203	SYT11: synaptotagmin-11 ( <i>Homo sapiens</i> )
<i>Pocillopora</i>	Cyan	pdam_00002625	ZP domain-containing protein (Acropora millepora)
<i>Pocillopora</i>	Grey60	pdam_00009922	Unknown function
<i>Pocillopora</i>	Mediumpurple3	pdam_00020781	FGFR: fibroblast growth factor receptor ( <i>Strongylocentrotus purpuratus</i> )
<i>Cladocypium</i>	Darkgreen	SymbC1.scaffold9599.2	50S ribosomal protein L15 OS = <i>Prochlorococcus marinus</i> (strain MIT 9215) GN = rplO PE = 3 SV = 1
<i>Cladocypium</i>	Purple	SymbC1.scaffold7524.1	26S protease regulatory subunit 4 homolog OS = <i>Oryza sativa</i> subsp. <i>japonica</i> GN = TBP2 PE = 2 SV = 2
<i>Cladocypium</i>	Brown	SymbC1.scaffold517.1	Autophagy-related protein 9 OS = <i>Dictyostelium discoideum</i> GN = atg9 PE = 2 SV = 1
<i>Cladocypium</i>	Grey60	SymbC1.scaffold1162.13	Unknown function
<i>Cladocypium</i>	Magenta	SymbC1.scaffold4557.4	Glycine-tRNA ligase OS = <i>Symbiodinium microadriaticum</i> GN = glyQS PE = 4 SV = 1
<i>Cladocypium</i>	Darkgrey	SymbC1.scaffold2117.1	Unknown function
<i>Cladocypium</i>	Darkturquoise	SymbC1.scaffold3718.1	Fucoxanthin-chlorophyll a-c binding protein E, chloroplastic OS = <i>Macrocyctis pyrifera</i> GN = FCPE PE = 2 SV = 1
<i>Cladocypium</i>	Green	SymbC1.scaffold36579.1	Twitchin OS = <i>Symbiodinium microadriaticum</i> GN = unc-22 PE = 4 SV = 1

Collectively, the results of this experiment highlight the vital importance of coral-associated bacteria communities to coral holobiont metabolism, immunity, and acute heat stress responses, and provide a basis for future mechanistic studies of how symbiotic interactions between multiple partners shape coral holobiont health, evolution, and ecological resilience (Engelberts et al., 2021; Voolstra et al., 2021).

## DATA AVAILABILITY STATEMENT

The mRNA and 16S sequencing datasets generated for this study can be found in the NCBI Sequence Read Archive under accession PRJNA587509. All scripts used in the RNAseq and 16S data analysis are available on GitHub: [https://github.com/michaelconnelly/EAPSI\\_Pocillopora\\_AxH](https://github.com/michaelconnelly/EAPSI_Pocillopora_AxH).

## AUTHOR CONTRIBUTIONS

MC and NT-K designed the research. MC, CJM, and P-JL conducted the experiments. MC and CEM analyzed the data. MC, CEM, and NT-K wrote the manuscript. All authors participated in the editing of the manuscript.

## REFERENCES

- Akram, M. (2014). Citric acid cycle and role of its intermediates in metabolism. *Cell Biochem. Biophys.* 68, 475–478. doi: 10.1007/s12013-013-9750-1
- Amin, S. A., Green, D. H., Hart, M. C., Kupper, F. C., Sunda, W. G., and Carrano, C. J. (2017). Photolysis of iron – siderophore chelates promotes bacteria – algal mutualism. *Photolysis of iron – siderophore chelates promotes bacterial – algal mutualism. Environ. Sci.* 106, 2–7.
- Anderson, M. J. (2017). “Permutational multivariate analysis of variance (PERMANOVA),” in *Wiley StatsRef Stat. Ref. Online*, eds N. Balakrishnan, T. Colton, B. Everitt, W. Piegorsch, F. Ruggeri, and J. L. Teugels (Hoboken, NJ: John Wiley & Sons), 1–15. doi: 10.1002/9781118445112.stat07841
- Apprill, A. (2020). The role of symbioses in the adaptation and stress responses of marine organisms. *Ann. Rev. Mar. Sci.* 12, 291–314. doi: 10.1146/annurev-marine-010419-010641
- Apprill, A., McNally, S., Parsons, R., and Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquat. Microb. Ecol.* 75, 129–137. doi: 10.3354/ame01753
- Assis, J. M., Villela, H., Abreu, F., Duarte, G., Roj, L., and Peixoto, R. (2020). Delivering beneficial microorganisms for corals (BMCs): rotifers as carriers of coral probiotics. *Front. Microbiol.* 11:608506. doi: 10.3389/fmicb.2020.608506
- Avila-Magaña, V., Kamel, B., DeSalvo, M., Gómez-Campo, K., Enríquez, S., Kitano, H., et al. (2021). Elucidating gene expression adaptation of phylogenetically divergent coral holobionts under heat stress. *Nat. Commun.* 12:5731. doi: 10.1038/s41467-021-25950-4
- Barfield, S. J., Aglyamova, G. V., Bay, L. K., and Matz, M. V. (2018). Contrasting effects of *Symbiodinium* identity on coral host transcriptional profiles across latitudes. *Mol. Ecol.* 27, 3103–3115. doi: 10.1111/mec.14774
- Benavides, M., Bednarz, V. N., and Ferrier-Pagès, C. (2017). Diazotrophs: overlooked key players within the coral symbiosis and tropical reef ecosystems? *Front. Mar. Sci.* 4:10. doi: 10.3389/fmars.2017.00010
- Blackall, L. L., Wilson, B., and Van Oppen, M. J. H. (2015). Coral—the world’s most diverse symbiotic ecosystem. *Mol. Ecol.* 24, 5330–5347. doi: 10.1111/mec.13400
- Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., et al. (2018). Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2’s q2-feature-classifier plugin. *Microbiome* 6, 1–17. doi: 10.1186/s40168-018-0470-z

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2021.814124/full#supplementary-material>

- Bolger, A. M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114–2120. doi: 10.1093/bioinformatics/btu170a
- Bolyen, E., Dillon, M., Bokulich, N. A., Abnet, C., Al-Ghalith, G., Alexander, H., et al. (2018). QIIME 2: reproducible, interactive, scalable, and extensible microbiome data science. *PeerJ Preprints* 6:e27295v2. doi: 10.7287/peerj.preprints.27295
- Boulotte, N. M., Dalton, S. J., Carroll, A. G., Harrison, P. L., Putnam, H. M., Peplow, L. M., et al. (2016). Exploring the *Symbiodinium* rare biosphere provides evidence for symbiont switching in reef-building corals. *ISME J.* 10, 2693–2701. doi: 10.1038/ismej.2016.54
- Brener-Raffalli, K., Clerissi, C., Vidal-Dupiol, J., Adjeroud, M., Bonhomme, F., Pratlong, M., et al. (2018). Thermal regime and host clade, rather than geography, drive *Symbiodinium* and bacterial assemblages in the Scleractinian coral *Pocillopora damicornis* sensu lato. *Microbiome* 6:39. doi: 10.1186/s40168-018-0423-6
- Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: high resolution sample inference from Illumina amplicon data. *Nat. Methods* 13, 581–583. doi: 10.1038/nmeth.3869
- Camp, E. F., Kahlke, T., Nitschke, M. R., Varkey, D., Fisher, N. L., Fujise, L., et al. (2020). Revealing changes in the microbiome of Symbiodiniaceae under thermal stress. *Environ. Microbiol.* 22, 1294–1309. doi: 10.1111/1462-2920.14935
- Chen, H., and Boutros, P. C. (2011). VennDiagram: a package for the generation of highly-customizable Venn and Euler diagrams in R. *BMC Bioinformatics* 12:35. doi: 10.2307/2689606
- Chuang, P. (2020). Signaling pathways in the coral polyp bail-out response. *Coral Reefs* 39, 1535–1548. doi: 10.1007/s00338-020-01983-x
- Cleves, P. A., Shumaker, A., Lee, J. M., Putnam, H. M., and Bhattacharya, D. (2020a). Unknown to known: advancing knowledge of coral gene function. *Trends Genet.* 36, 93–104. doi: 10.1016/j.tig.2019.11.001
- Cleves, P. A., Tinoco, A., Bradford, J., Perrin, D., Bay, L. K., and Pringle, J. (2020b). Reduced heat tolerance in a coral carrying CRISPR-induced mutations in the gene for a heat-shock transcription factor. *Proc. Natl. Acad. Sci. U.S.A.* 117, 28899–28905. doi: 10.1073/pnas.1920779117
- Connelly, M. T., McRae, C. J., Liu, P.-J., and Traylor-Knowles, N. (2020). Lipopolysaccharide treatment stimulates *Pocillopora* coral genotype-specific

- immune responses but does not alter coral-associated bacteria communities. *Dev. Comp. Immunol.* 109:103717. doi: 10.1016/j.dci.2020.103717
- Costa, R. M., Cárdenas, A., Loussert-fonta, C., Toullec, G., Meibom, A., Voolstra, C. R., et al. (2021). Surface topography, bacterial carrying capacity, and the prospect of microbiome manipulation in the sea anemone coral model *Aiptasia*. *Front. Microbiol.* 12:637834. doi: 10.3389/fmicb.2021.637834
- Cunning, R., and Baker, A. C. (2020). Thermotolerant coral symbionts modulate heat stress-responsive genes in their hosts. *Mol. Ecol.* 29, 2940–2950. doi: 10.1111/mec.15526
- Cunning, R., Bay, R. A., Gillette, P., Baker, A. C., and Traylor-Knowles, N. (2018). Comparative analysis of the *Pocillopora damicornis* genome highlights role of immune system in coral evolution. *Sci. Rep.* 8:16134. doi: 10.1038/s41598-018-34459-8
- Damjanovic, K., van Oppen, M. J. H., Menéndez, P., and Blackall, L. L. (2019). Experimental inoculation of coral recruits with marine bacteria indicates scope for microbiome manipulation in *Acropora tenuis* and *Platygyra daedalea*. *Front. Microbiol.* 10:1702. doi: 10.3389/fmicb.2019.01702
- Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., et al. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* 29, 15–21. doi: 10.1093/bioinformatics/bts635
- Domin, H., Zurita-Gutiérrez, Y. H., Scotti, M., Buttlar, J., Humeida, U. H., and Fraune, S. (2018). Predicted bacterial interactions affect in vivo microbial colonization dynamics in *Nematostella*. *Front. Microbiol.* 9:728. doi: 10.3389/fmicb.2018.00728
- Dungan, A. M., van Oppen, M. J., and Blackall, L. L. (2021). Short-term exposure to sterile seawater reduces bacterial community diversity in the sea anemone, *Exaiptasia diaphana*. *Front. Mar. Sci.* 7:599314. doi: 10.3389/fmars.2020.599314
- Engelberts, J. P., Robbins, S. J., Damjanovic, K., and Webster, N. S. (2021). Integrating novel tools to elucidate the metabolic basis of microbial symbiosis in reef holobionts. *Mar. Biol.* 168, 1–19. doi: 10.1007/s00227-021-03952-6
- Epstein, H. E., Torda, G., Munday, P. L., and van Oppen, M. J. H. (2019). Parental and early life stage environments drive establishment of bacterial and dinoflagellate communities in a common coral. *ISME J.* 13, 1635–1638. doi: 10.1038/s41396-019-0358-3
- Fernandes, A. D., Macklaim, J. M., Linn, T. G., Reid, G., and Gloor, G. B. (2013). ANOVA-Like differential expression (ALDEx) analysis for mixed population RNA-Seq. *PLoS One* 8:e67019. doi: 10.1371/journal.pone.0067019
- Fernandes, A. D., Reid, J. N. S., Macklaim, J. M., McMurrrough, T. A., Edgell, D. R., and Gloor, G. B. (2014). Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. *Microbiome* 2, 1–13. doi: 10.1186/2049-2618-2-15
- Ferrier-Pagès, C., Martínez, S., Grover, R., Cybulski, J., Shemesh, E., and Tchernov, D. (2021). Tracing the trophic plasticity of the coral–dinoflagellate symbiosis using amino acid compound-specific stable isotope analysis. *Microorganisms* 9:182. doi: 10.3390/microorganisms9010182
- Fox, M. D., Elliott Smith, E. A., Smith, J. E., and Newsome, S. D. (2019). Trophic plasticity in a common reef-building coral: insights from  $\delta^{13}\text{C}$  analysis of essential amino acids. *Funct. Ecol.* 33, 2203–2214. doi: 10.1111/1365-2435.13441
- Geissler, L., Meunier, V., Rädcker, N., Perna, G., Rodolfo-Metalpa, R., Houlbrèque, F., et al. (2021). Highly variable and non-complex diazotroph communities in corals from ambient and high CO<sub>2</sub> environments. *Front. Mar. Sci.* 8:754682. doi: 10.3389/fmars.2021.754682
- Gélin, P., Postaire, B., Fauvelot, C., and Magalon, H. (2017). Reevaluating species number, distribution and endemism of the coral genus *Pocillopora* Lamarck, 1816 using species delimitation methods and microsatellites. *Mol. Phylogenet. Evol.* 109, 430–446. doi: 10.1016/j.ympev.2017.01.018
- Gilbert, J. A., Hill, R., Doblin, M. A., and Ralph, P. J. (2012). Microbial consortia increase thermal tolerance of corals. *Mar. Biol.* 159, 1763–1771. doi: 10.1007/s00227-012-1967-9
- Glasl, B., Herndl, G. J., and Frade, P. R. (2016). The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. *ISME J.* 10, 2280–2292. doi: 10.1038/ismej.2016.9
- Glaze, T. D., Erler, D. V., and Siljanen, H. M. P. (2021). Microbially facilitated nitrogen cycling in tropical corals. *ISME J.* 16, 68–77. doi: 10.1038/s41396-021-01038-1
- Gloor, G. B., and Reid, G. (2016). Compositional analysis: a valid approach to analyze microbiome high-throughput sequencing data. *Can. J. Microbiol.* 703, 692–703.
- Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., and Egozcue, J. J. (2017). Microbiome datasets are compositional: and this is not optional. *Front. Microbiol.* 8:2224. doi: 10.3389/fmicb.2017.02224
- Gunderson, A. R., Armstrong, E. J., and Stillman, J. H. (2016). Multiple stressors in a changing world: the need for an improved perspective on physiological responses to the dynamic marine environment. *Ann. Rev. Mar. Sci.* 8, 357–378. doi: 10.1146/annurev-marine-122414-033953
- Haydon, T. D., Seymour, J. R., Raina, J. B., Edmondson, J., Siboni, N., Matthews, J. L., et al. (2021). Rapid shifts in bacterial communities and homogeneity of symbiodiniaceae in colonies of *Pocillopora acuta* transplanted between reef and mangrove environments. *Front. Microbiol.* 12:756091. doi: 10.3389/fmicb.2021.756091
- Hodgson, G. (1990). Tetracycline reduces sedimentation damage to corals. *Mar. Biol.* 104, 493–496.
- Hopkinson, B. M., and Morel, F. M. M. (2009). The role of siderophores in iron acquisition by photosynthetic marine microorganisms. *BioMetals* 22, 659–669. doi: 10.1007/s10534-009-9235-2
- Huerta-Cepas, J., Forslund, K., Coelho, L. P., Szklarczyk, D., Jensen, L. J., Von Mering, C., et al. (2017). Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. *Mol. Biol. Evol.* 34, 2115–2122. doi: 10.1093/molbev/msx148
- Hume, B. C. C., Smith, E. G., Ziegler, M., Hugh, J., Burt, M. W. J. A., and Voolstra, C. R. (2019). SymPortal: a novel analytical framework and platform for coral algal symbiont next – generation sequencing ITS2 profiling. *Mol. Ecol. Resour.* 19, 1063–1080. doi: 10.1111/1755-0998.13004
- Jiang, S., Zhou, Z., Sun, Y., Zhang, T., and Sun, L. (2020). Coral gasdermin triggers pyroptosis. *Sci. Immunol.* 5:eabd2591. doi: 10.1126/SCIIMMUNOL.ABD2591
- Johnston, E. C., Forsman, Z. H., Flot, J.-F., Schmidt-Roach, S., Pinzón, J. H., Knapp, I. S. S., et al. (2017). A genomic glance through the fog of plasticity and diversification in *Pocillopora*. *Sci. Rep.* 7:5991. doi: 10.1038/s41598-017-06085-3
- Katoh, K., and Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30, 772–780. doi: 10.1093/molbev/mst010
- Keshavmurthy, S., Kuo, C., Huang, Y., and Carballo-Bolaños, R. (2019). Coral reef resilience in taiwan: lessons from long-term ecological research on the coral reefs of Kenting national park (Taiwan). *J. Mar. Sci. Eng.* 7:388. doi: 10.3390/jmse7110388
- Keshavmurthy, S., Meng, P. J., Wang, J. T., Kuo, C. Y., Yang, S. Y., Hsu, C. M., et al. (2014). Can resistant coral-*Symbiodinium* associations enable coral communities to survive climate change? A study of a site exposed to long-term hot water input. *PeerJ* 2:e327. doi: 10.7717/peerj.327
- Kirk, A. L., Clowez, S., Lin, F., Grossman, A. R., and Xiang, T. (2020). Transcriptome reprogramming of symbiodiniaceae *Breviolum minutum* in response to casein amino acids supplementation. *Front. Physiol.* 11:574654. doi: 10.3389/fphys.2020.574654
- Ladner, J. T., Barshis, D. J., and Palumbi, S. R. (2012). Protein evolution in two co-occurring types of *Symbiodinium*: an exploration into the genetic basis of thermal tolerance in *Symbiodinium* clade D. *BMC Evol. Biol.* 12:217. doi: 10.1186/1471-2148-12-217
- Langfelder, P., and Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics* 9:559. doi: 10.1186/1471-2105-9-559
- Lesser, M. P., Morrow, K. M., Pankey, S. M., and Noonan, S. H. C. (2018). Diazotroph diversity and nitrogen fixation in the coral *Stylophora pistillata* from the Great Barrier Reef. *ISME J.* 12, 813–824. doi: 10.1038/s41396-017-0008-6
- Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25, 1754–1760. doi: 10.1093/bioinformatics/btp324
- Li, J., Long, L., Zou, Y., and Zhang, S. (2020). Microbial community and transcriptional responses to increased temperatures in coral *Pocillopora damicornis* holobiont. *Soc. Appl. Microbiol.* 23, 826–843. doi: 10.1111/1462-2920.15168
- Li, J., Long, L., Zou, Y., and Zhang, S. (2021). Microbial community and transcriptional responses to increased temperatures in coral *Pocillopora damicornis* holobiont. *Environ. Microbiol.* 23, 826–843.
- Liao, Y., Smyth, G. K., and Shi, W. (2014). FeatureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* 30, 923–930. doi: 10.1093/bioinformatics/btt656

- Lin, M. F., Takahashi, S., Forêt, S., Davy, S. K., and Miller, D. J. (2019). Transcriptomic analyses highlight the likely metabolic consequences of colonization of a cnidarian host by native or non-native *Symbiodinium* species. *Biol. Open* 8, 1–11. doi: 10.1242/bio.038281
- Liu, H., Stephens, T. G., González-Pech, R. A., Beltran, V. H., Lapeyre, B., Bongaerts, P., et al. (2018). *Symbiodinium* genomes reveal adaptive evolution of functions related to coral-dinoflagellate symbiosis. *Commun. Biol.* 1:95. doi: 10.1038/s42003-018-0098-3
- Love, M. I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 15:550. doi: 10.1186/s13059-014-0550-8
- Maher, R. L., Rice, M. M., McMinds, R., and Burkepile, D. E. (2019). Multiple stressors interact primarily through antagonism to drive changes in the coral microbiome. *Sci. Rep.* 9:6834. doi: 10.1038/s41598-019-43274-8
- Maire, J., Girvan, S. K., Barkla, S. E., David, A. P., Blackall, L. L., and Van Oppen, M. J. H. (2021). Intracellular bacteria are common and taxonomically diverse in cultured and in hospite algal endosymbionts of coral reefs. *ISME J.* 15, 2028–2042. doi: 10.1038/s41396-021-00902-4
- Martínez Arbizu, P. (2020). *pairwiseAdonis: Pairwise Multilevel Comparison Using Adonis. R Package Version 0.4*.
- Matthews, J. L., Raina, J. B., Kahlke, T., Seymour, J. R., van Oppen, M. J. H., and Suggett, D. J. (2020). Symbiodiniaceae-bacteria interactions: rethinking metabolite exchange in reef-building corals as multi-partner metabolic networks. *Environ. Microbiol.* 22, 1675–1687. doi: 10.1111/1462-2920.14918
- Matz, M. V. (2019). *KOGRAMWU: Functional Summary and Meta-Analysis of Gene Expression Data. R Package Version 1.2*. Available online at: <https://cran.r-project.org/web/packages/KOGRAMWU/index.html> (accessed February 19, 2019).
- Mayfield, A. B., Wang, Y. B., Chen, C.-S., Lin, C. Y., and Chen, S. H. (2014). Compartment-specific transcriptomics in a reef-building coral exposed to elevated temperatures. *Mol. Ecol.* 23, 5816–5830. doi: 10.1111/mec.12982
- McDonald, D., Clemente, J. C., Kuczynski, J., Rideout, J. R., Stombaugh, J., Wendel, D., et al. (2012). The Biological Observation Matrix (BIOM) format or: how I learned to stop worrying and love the ome-ome. *Gigascience* 464, 1–6. doi: 10.1186/2047-217X-1-7
- McMurdie, P. J., and Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One* 8:e61217. doi: 10.1371/journal.pone.0061217
- Medrano, E., Merselis, D. G., Bellantuono, A. J., and Rodriguez-Lanetty, M. (2019). Proteomic Basis of symbiosis: a heterologous partner fails to duplicate homologous success in a novel cnidarian- Symbiodiniaceae mutualism. *Front. Microbiol.* 10:1153. doi: 10.3389/FMICB.2019.01153
- Motone, K., Takagi, T., Aburaya, S., Miura, N., Aoki, W., and Ueda, M. (2020). A zeaxanthin-producing bacterium isolated from the algal phycosphere protects coral endosymbionts from environmental stress. *MBio* 11:e01019-19.
- Murillo-Rincon, A. P., Klimovich, A., Pemöller, E., Taubenheim, J., Mortzfeld, B., Augustin, R., et al. (2017). Spontaneous body contractions are modulated by the microbiome of Hydra. *Sci. Rep.* 7:15937. doi: 10.1038/s41598-017-16191-x
- Neave, M. J., Rachmawati, R., Xun, L., Michell, C. T., Bourne, D. G., Apprill, A., et al. (2017). Differential specificity between closely related corals and abundant *Endozoicomonas* endosymbionts across global scales. *ISME J.* 11, 186–200. doi: 10.1038/ismej.2016.95
- Neely, K. L., Macaulay, K. A., Hower, E. K., and Dobler, M. A. (2020). Effectiveness of topical antibiotics in treating corals affected by Stony Coral Tissue Loss Disease. *PeerJ* 8:e9289. doi: 10.7717/peerj.9289
- O'Brien, P. A., Webster, N. S., Miller, D. J., and Bourne, D. G. (2019). Host-Microbe coevolution: applying evidence from model systems to complex marine invertebrate holobionts. *MBio* 10:e02241-18. doi: 10.1128/mBio.02241-18
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., et al. (2019). *vegan: Community Ecology Package*.
- Olson, N. D., Ainsworth, T. D., Gates, R. D., and Takabayashi, M. (2009). Diazotrophic bacteria associated with Hawaiian *Montipora* corals: diversity and abundance in correlation with symbiotic dinoflagellates. *J. Exp. Mar. Biol. Ecol.* 371, 140–146. doi: 10.1016/j.jembe.2009.01.012
- Palarea-Albaladejo, J., and Martín-Fernández, J. A. (2015). ZCompositions – R package for multivariate imputation of left-censored data under a compositional approach. *Chemom. Intell. Lab. Syst.* 143, 85–96. doi: 10.1016/j.chemolab.2015.02.019
- Park, S., Choi, S. J., Park, J. M., and Yoon, J. H. (2017). *Alteromonas aestuariivivens* sp. nov., isolated from a tidal flat. *Int. J. Syst. Evol. Microbiol.* 67, 2791–2797. doi: 10.1099/ijsem.0.002023
- Peixoto, R. S., Rosado, P. M., Leite, D. C., de, A., Rosado, A. S., and Bourne, D. G. (2017). Beneficial microorganisms for corals (BMC): proposed mechanisms for coral health and resilience. *Front. Microbiol.* 8:341. doi: 10.3389/fmicb.2017.00341
- Peixoto, R. S., Sweet, M., Villela, H. D. M., Cardoso, P. M., Thomas, T., Voolstra, C. R., et al. (2021). Coral probiotics: premise, promise, prospects. *Annu. Rev. Anim. Biosci.* 9, 265–288. doi: 10.1146/annurev-animal-090120-115444
- Pinzón, J. H., Sampayo, E., Cox, E., Chauka, L. J., Chen, C. A., Voolstra, C. R., et al. (2013). Blind to morphology: genetics identifies several widespread ecologically common species and few endemics among Indo-Pacific cauliflower corals (*Pocillopora*, Scleractinia). *J. Biogeogr.* 40, 1595–1608. doi: 10.1111/jbi.12110
- Pogoreutz, C., Rådecker, N., Cárdenas, A., Gärdes, A., Wild, C., and Voolstra, C. R. (2018). Dominance of *Endozoicomonas* bacteria throughout coral bleaching and mortality suggests structural inflexibility of the *Pocillopora verrucosa* microbiome. *Ecol. Evol.* 8, 2240–2252. doi: 10.1002/ece3.3830
- Pollock, F. J., McMinds, R., Smith, S., Bourne, D. G., Willis, B. L., Medina, M., et al. (2018). Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. *Nat. Commun.* 9:4921. doi: 10.1038/s41467-018-07275-x
- Poquita-Du, R. C., Huang, D., Chou, L. M., Mrinalini, and Todd, P. A. (2019). Short term exposure to heat and sediment triggers changes in coral gene expression and photo-physiological performance. *Front. Mar. Sci.* 6:121. doi: 10.3389/fmars.2019.00121
- Price, M. N., Dehal, P. S., and Arkin, A. P. (2010). FastTree 2 – approximately maximum-likelihood trees for large alignments. *PLoS One* 5:e9490. doi: 10.1371/journal.pone.0009490
- Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., et al. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* 41, 590–596. doi: 10.1093/nar/gks1219
- R Core Team (2020). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing.
- Rådecker, N., Pogoreutz, C., Gegner, H. M., Cárdenas, A., Perna, G., Geißler, L., et al. (2021). Heat stress reduces the contribution of diazotrophs to coral holobiont nitrogen cycling. *ISME J.* doi: 10.1038/s41396-021-01158-8
- Raina, J. B., Dinsdale, E. A., Willis, B. L., and Bourne, D. G. (2010). Do the organic sulfur compounds DMSP and DMS drive coral microbial associations? *Trends Microbiol.* 18, 101–108. doi: 10.1016/j.tim.2009.12.002
- Reich, H. G., Rodriguez, I. B., LaJeunesse, T. C., and Ho, T. Y. (2020). Endosymbiotic dinoflagellates pump iron: differences in iron and other trace metal needs among the Symbiodiniaceae. *Coral Reefs* 39, 915–927. doi: 10.1007/s00338-020-01911-z
- Robbins, S. J., Singleton, C. M., Chan, C. X., Messer, L. F., Geers, A. U., Ying, H., et al. (2019). A genomic view of the reef-building coral *Porites lutea* and its microbial symbionts. *Nat. Microbiol.* 4, 2090–2100. doi: 10.1038/s41564-019-0532-4
- Rosado, P. M., Leite, D. C. A., Duarte, G. A. S., Chaloub, R. M., Jospin, G., Nunes da Rocha, U., et al. (2018). Marine probiotics: increasing coral resistance to bleaching through microbiome manipulation. *ISME J.* 13, 921–936. doi: 10.1038/s41396-018-0323-6
- Santoro, E. P., Borges, R. M., Espinoza, J. L., Freire, M., Messias, C. S. M. A., Villela, H. D. M., et al. (2021). Coral microbiome manipulation elicits metabolic and genetic restructuring to mitigate heat stress and evade mortality. *Sci. Adv.* 7, 19–21. doi: 10.1126/sciadv.abg3088
- Schalk, I. J., Hannauer, M., and Braud, A. (2011). New roles for bacterial siderophores in metal transport and tolerance. *Environ. Microbiol.* 13, 2844–2854. doi: 10.1111/j.1462-2920.2011.02556.x
- Schmidt-Roach, S., Miller, K. J., Lundgren, P., and Andreakis, N. (2014). With eyes wide open: a revision of species within and closely related to the *Pocillopora damicornis* species complex (Scleractinia; Pocilloporidae) using morphology and genetics. *Zool. J. Linn. Soc.* 170, 1–33. doi: 10.1111/zooj.12092
- Shilling, E. N. (2020). *Stony Coral Tissue Loss Disease Intervention Strategies for Montastrea cavernosa*. Master's thesis. Ann Arbor, MI: ProQuest.
- Soffer, N., Gibbs, P. D. L., and Baker, A. C. (2008). "Practical applications of contaminant-free *Symbiodinium* cultures grown on solid media," in *Proceedings of the 11th International Coral Reef Symposium*, Ft. Lauderdale, FL, 159–163.

- Sweet, M. J., Croquer, A., and Bythell, J. C. (2014). Experimental antibiotic treatment identifies potential pathogens of white band disease in the endangered Caribbean coral *Acropora cervicornis*. *Proc. R. Soc. B Biol. Sci.* 281, 20140094–20140094. doi: 10.1098/rspb.2014.0094
- Sweet, M., and Bythell, J. C. (2015). White syndrome in *Acropora muricata*: nonspecific bacterial infection and ciliate histophagy. *Mol. Ecol.* 24, 1150–1159. doi: 10.1111/mec.13097
- Tandon, K., Lu, C.-Y., Chiang, P.-W., Wada, N., Yang, S.-H., Chan, Y.-F., et al. (2020). Comparative genomics: dominant coral-bacterium *Endozoicomonas acroporae* metabolizes dimethylsulfoniopropionate (DMS). *ISME J.* 14, 1290–1303. doi: 10.1038/s41396-020-0610-x
- Thompson, J. R., Rivera, H. E., Closek, C. J., and Medina, M. (2015). Microbes in the coral holobiont: partners through evolution, development, and ecological interactions. *Front. Cell. Infect. Microbiol.* 4:176. doi: 10.3389/fcimb.2014.00176
- Tout, J., Siboni, N., Messer, L. F., Garren, M., Stocker, R., Webster, N. S., et al. (2015). Increased seawater temperature increases the abundance and alters the structure of natural *Vibrio* populations associated with the coral *Pocillopora damicornis*. *Front. Microbiol.* 6:432. doi: 10.3389/fmicb.2015.00432
- Traylor-Knowles, N., Granger, B. R., Lubinski, T. J., Parikh, J. R., Garamszegi, S., Xia, Y., et al. (2011). Production of a reference transcriptome and transcriptomic database (PocilloporaBase) for the cauliflower coral, *Pocillopora damicornis*. *BMC Genomics* 12:585. doi: 10.1186/1471-2164-12-585
- Vavilin, D. V., and Vermaas, W. F. J. (2002). Regulation of the tetrapyrrole biosynthetic pathway leading to heme and chlorophyll in plants and cyanobacteria. *Physiol. Plant.* 115, 9–24. doi: 10.1034/j.1399-3054.2002.1150102.x
- Vidal-Dupiol, J., Dheilly, N. M., Rondon, R., Grunau, C., Cosseau, C., Smith, K. M., et al. (2014). Thermal stress triggers broad *Pocillopora damicornis* transcriptomic remodeling, while *Vibrio coralliilyticus* infection induces a more targeted immuno-suppression response. *PLoS One* 9:e107672. doi: 10.1371/journal.pone.0107672
- Voolstra, C. R., Suggett, D. J., Peixoto, R. S., Parkinson, J. E., Quigley, K. M., Silveira, C. B., et al. (2021). Extending the natural adaptive capacity of coral holobionts. *Nat. Rev. Earth Environ.* 2, 747–762. doi: 10.1038/s43017-021-00214-3
- Voss, J. D., Shilling, E. N., and Combs, I. R. (2019). *Intervention and Fate Tracking for Corals Affected by Stony Coral Tissue Loss Disease in the Northern Florida Reef Tract*. Miami, FL: Florida DEP, 1–23.
- Walker, B. K., Turner, N. R., Noren, H. K. G., Buckley, S. F., and Pitts, K. A. (2021). Optimizing Stony Coral Tissue Loss Disease (SCTLD) intervention treatments on *Montastraea cavernosa* in an endemic zone. *Front. Mar. Sci.* 8:666224. doi: 10.3389/fmars.2021.666224
- Walker, B., and Pitts, K. (2019). *SE FL Reef-Building-Coral Response to Amoxicillin Intervention and Broader-Scale Coral Disease Intervention*. Miami, FL: Florida DEP, 12.
- Wang, Y., Wang, H., Liu, J., Lai, Q., Shao, Z., Austin, B., et al. (2010). *Aestuuriibacter aggregatus* sp. nov., a moderately halophilic bacterium isolated from seawater of the Yellow Sea. *FEMS Microbiol. Lett.* 309, 48–54. doi: 10.1111/j.1574-6968.2010.02011.x
- Weber, L., DeForce, E., and Apprill, A. (2017). Optimization of DNA extraction for advancing coral microbiota investigations. *Microbiome* 5:18. doi: 10.1186/s40168-017-0229-y
- Wecker, P., Lecellier, G., Guibert, I., Zhou, Y., Bonnard, I., and Berteaux-Lecellier, V. (2018). Exposure to the environmentally-persistent insecticide chlordecone induces detoxification genes and causes polyp bail-out in the coral *P. damicornis*. *Chemosphere* 195, 190–200. doi: 10.1016/j.chemosphere.2017.12.048
- Weiland-Bräuer, N., Pinnow, N., Langfeldt, D., Roik, A., Güllert, S., Chibani, C. M., et al. (2020). The native microbiome is crucial for offspring generation and fitness of *Aurelia aurita*. *MBio* 11:e02336-20. doi: 10.1128/mBio.02336-20
- Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. New York, NY: Springer-Verlag New York.
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., et al. (2019). Welcome to the {tidyverse}. *J. Open Source Softw.* 4:1686. doi: 10.21105/joss.01686
- Wilkins, L. G. E., Leray, M., Dea, A. O., Yuen, B., Peixoto, R., Pereira, T. J., et al. (2019). Host-associated microbiomes drive structure and function of marine ecosystems. *PLoS Biol.* 17:e3000533. doi: 10.1371/journal.pbio.3000533
- Work, T. M., and Aeby, G. S. (2014). Microbial aggregates within tissues infect a diversity of corals throughout the Indo-Pacific. *Mar. Ecol. Prog. Ser.* 500, 1–9. doi: 10.3354/meps10698
- Wright, R. M., Aglyamova, G. V., Meyer, E., and Matz, M. V. (2015). Gene expression associated with white syndromes in a reef building coral, *Acropora hyacinthus*. *BMC Genomics* 16:371. doi: 10.1186/s12864-015-1540-2
- Xia, Y., and Sun, J. (2017). Hypothesis testing and statistical analysis of microbiome. *Genes Dis.* 4, 138–148. doi: 10.1016/j.gendis.2017.06.001
- Zaneveld, J. R., Burkepile, D. E., Shantz, A. A., Pritchard, C. E., McMinds, R., Payet, J. P., et al. (2016). Overfishing and nutrient pollution interact with temperature to disrupt coral reefs down to microbial scales. *Nat. Commun.* 7:11833. doi: 10.1038/ncomms11833
- Zaneveld, J. R., McMinds, R., and Thurber, R. V. (2017). Stress and stability: applying the Anna Karenina principle to animal microbiomes. *Nat. Microbiol.* 2:17121. doi: 10.1038/nmicrobiol.2017.121
- Zhang, R., Yu, K., Li, A., Wang, Y., and Huang, X. (2019). Antibiotics in corals of the South China Sea: occurrence, distribution, bioaccumulation, and considerable role of coral mucus. *Environ. Pollut.* 250, 503–510. doi: 10.1016/j.envpol.2019.04.036
- Zhang, R., Zhang, R., Yu, K., Wang, Y., Huang, X., Pei, J., et al. (2018). Occurrence, sources and transport of antibiotics in the surface water of coral reef regions in the South China Sea: potential risk to coral growth. *Environ. Pollut.* 232, 450–457. doi: 10.1016/j.envpol.2017.09.064
- Zhang, Y., Yang, Q., Ling, J., Long, L., Huang, H., Yin, J., et al. (2021). Shifting the microbiome of a coral holobiont and improving host physiology by inoculation with a potentially beneficial bacterial consortium. *BMC Microbiol.* 21:130. doi: 10.1186/s12866-021-02167-5
- Zhang, Y., Zhou, Z., Wang, L., and Huang, B. (2018). Transcriptome, expression, and activity analyses reveal a vital heat shock protein 70 in the stress response of stony coral *Pocillopora damicornis*. *Cell Stress Chaperones* 23, 711–721. doi: 10.1007/s12192-018-0883-4
- Zhou, Z., Yu, X., Tang, J., Wu, Y., Wang, L., and Huang, B. (2018). Systemic response of the stony coral *Pocillopora damicornis* against acute cadmium stress. *Aquat. Toxicol.* 194, 132–139. doi: 10.1016/j.aquatox.2017.11.013
- Zhou, Z., Zhang, G., Chen, G., Ni, X., Guo, L., Yu, X., et al. (2017). Elevated ammonium reduces the negative effect of heat stress on the stony coral *Pocillopora damicornis*. *Mar. Pollut. Bull.* 118, 319–327. doi: 10.1016/j.marpolbul.2017.03.018
- Zhou, Z., Zhang, K., Wang, L., Su, Y., Wang, J., Song, T., et al. (2021). Nitrogen availability improves the physiological resilience of coral endosymbiont *Cladocopium goreauii* to high temperature. *J. Phycol.* 57, 1187–1198. doi: 10.1111/jpy.13156
- Ziegler, M., Grupstra, C. G. B., Barreto, M. M., Eaton, M., BaOmar, J., Zubier, K., et al. (2019). Coral bacterial community structure responds to environmental change in a host-specific manner. *Nat. Commun.* 10:3092. doi: 10.1038/s41467-019-10969-5
- Ziegler, M., Seneca, F. O., Yum, L. K., Palumbi, S. R., and Voolstra, C. R. (2017). Bacterial community dynamics are linked to patterns of coral heat tolerance. *Nat. Commun.* 8:14213. doi: 10.1038/ncomms14213

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