

## SUPPLEMENTARY METHODS

### Temperature Trends

Prior to sampling there were multiple thermal anomalies starting in December 2018 in which temperatures exceeded the thermal stress accumulation threshold level of 29 °C (Leinbach et al., 2021; Pratchett et al., 2013; Speare et al., 2021) by >1°C. Temperature trends were analyzed using the Mo'orea Coral Reef Long Term Ecological Research (MCR LTER) daily average water temperature data. Time-series data were collected from 3 sites on the MCR LTER fore-reef: FOR1, FOR4 and FOR5 (GPS location: 17°28'30.0"S 149°50'13.2"W; 17°32'49.2"S 149°46'08.4"W; 17°34'55.2"S 149°52'30.0"W; respectively). From each location, measurements from five sensors ("upper water column", "middle water column", "bottom water column", "temperature shallow", and "temperature deeper") was used to calculate the average temperature +/- one standard deviation. Bleaching was first observed in the corals adjacent to Gump Station, Mo'orea, in April 2019 (Leinbach et al., 2021) (Figure 1B). Accumulated degree heating days reached a maximum of 17°C-Days in mid-April before rapidly decreasing (Burgess et al., 2021). By the start of field collection on May 8th, 2019, the temperatures dropped below the 29 °C threshold. Corals had experienced a total of 110 days of temperatures exceeding the threshold in a period of five months (151 days).

### Field Collections

*Pocillopora verrucosa* and *Acropora pulchra* nubbins were collected from a common garden LTER1 site on the back-reef of Paopao Bay, Mo'orea, French Polynesia (17°28'45.0"S 149°50'10.44"W). *Porites rus* nubbins were collected from the fringing reef north of Gump Station research facility (17°29'11.6"S 149°49'31.1"W). Nubbins were collected under permits issued by the French Polynesian Government (Délégation à la Recherche) and the Haut-commissariat de la République en Polynésie Française (DTRT) (Protocole d'Accueil 2005-

2021). After collection, corals were transported to the Gump Station research facility and acclimated to ambient conditions in a 1300 L flow-through water table for three days.

#### Pre-Treatment in Flow-Through Aquaria

At the start of the pre-treatments, coral nubbins were loosely situated in silicon holders and placed into 1.5 L polycarbonate aquaria containing 1.4 L of unfiltered water. To mimic reef-wide bleaching/thermal stress signals, two nubbins from each of the three coral species at a given bleaching phenotype were combined in individual aquaria for a total of six coral fragments in each of the 12 aquaria. Influent water from a flow-through seawater system (sourced from a depth of 6 m directly adjacent to the Gump Station fringing reef) was pumped into the aquaria at a constant rate using a peristaltic pump with platinum cured silicone tubing yielding a final residence time of five hours in the aquaria. Water in each aquaria was recirculated with 4.8 W pumps moving 240 L hr<sup>-1</sup>. Aquaria (n=3 per treatment) were exposed to six days of either ambient (28.6 °C) or elevated water temperatures (32 °C +/- 0.2 °C) and ambient light levels. The water tables holding aquaria were heated using four 300 W and two 800 W Finnex heaters. The combination of bleaching level and temperature yielded four treatments representing a factorial cross of prior bleaching phenotype and temperature: “Control”, “Heated”, “Bleached”, and “Bleached + Heated” (Fig A.I and A.II). Additionally, two water-only control aquaria, one for each temperature treatment, were included (“Negative Control” and “Negative Control + Heated”). Because we hypothesized that recent bleaching and subsequent experimental heat stress are additive rather than interactive effects inducing four distinct coral physiologies, we considered these treatments as four distinct categories in all downstream statistics.

#### Symbiodiniaceae Quantification

Samples were frozen at -40 °C for 14 days prior to transportation to University of Hawai‘i at Mānoa campus where they were frozen at -80 °C for 23 months prior to flow cytometry

processing. In brief, samples were thawed, briefly homogenized using a vortex machine, and 200  $\mu$ L of each sample being aliquoted into 96-well round-bottom flow cytometry plates. Symbiodiniaceae slurries were run on a Beckman Coulter CytoFLEX S Flow Cytometer (Beckman Coulter, Product No: B78560).

The Chlorophyll-a emissions PMT and the two scatter detectors for the violet laser were used in conjunction to count Symbiodiniaceae cells, delineated as distinct populations of large cells with high specific *chlorophyll a* content. Voltages and gating were manually determined to enable easy identification of this population using a FSC gain of 100, a SSC gain of 100, and a Chla gain of 50. Chla values had a lower threshold of 20,000. Density plots of Chla vs. FSC were gated on the easily distinguishable population of SYBR green stained bacteria (Figure S1b).

#### Metabolomics Sample Collection

Prior to sampling, 200 mg mass Bond Elut-PPL (Agilent) cartridges were soaked overnight in LC-MS grade methanol followed by a series of washes (2x washes of methanol, 2x washes of LC-MS grade water, 1x wash of methanol) before air-drying the cartridges. In the field, PPL cartridges were activated with 1x methanol wash followed by 2x pH 2 LC-MS grade water washes. Samples were loaded on the cartridges by pumping the acidified filtrate over the PPL cartridges at 8mL/min. Cartridges were desalinated using a 3x pH2 LC-MS grade water wash and dried using N<sub>2</sub> gas.

#### Bacterioplankton Abundance and Flowcytometry Settings

Samples for bacterioplankton abundance were flash-frozen at -40 °C for 14 days prior to transportation to University of Hawai'i at Mānoa campus where they were frozen at -80 °C for six months prior to flow cytometry processing. Fixed microbial abundance samples were

thawed, and 200  $\mu$ L of each sample aliquoted and stained with 2  $\mu$ L 100X SYBR Green to be run on an Attune Acoustic Focusing Cytometer (Applied Biosystems, Part No. 4445280ASR) at University of Hawai'i at Mānoa to enumerate bacterial cell counts.

The BL1 detector for the blue laser (488 nm) and the SSC detector for the violet laser (405 nm) were used in conjunction to elucidate bacterial abundances. Voltages and gating were manually determined to enable easy identification of SYBR green stained bacterioplankton populations using a BL1 voltage of 2,625 mV and a SSC voltage of 2,500 mV. Density plots of BL1 vs. SSC were gated on the easily distinguishable population of SYBR green stained bacteria (Figure S1a).

#### Water Collection for Bacterial Community Composition, Dissolved Organic Carbon and Metabolite Solid Phase Extraction

Sterivex filters were frozen at -40 °C for 14 days prior to transportation to University of Hawai'i at Mānoa campus where they were transferred to -80 °C for six months prior to DNA extraction. DOC samples were kept at room temperature for 14 days prior to transportation to the University of California, Santa Barbara (UCSB). At UCSB the DOC samples were stored at 15 °C in the dark for six months prior to sample processing. PPL Cartridges were stored at -80 °C until samples were processed at UCSD in spring 2021.

#### Microbial Community DNA Extraction, Library Prep, and Sequencing

To extract bacterial DNA from the Sterivex filters, the filter was removed from the plastic casing using sterile pliers, scalpels, and tweezers, and added to MP Biomedicals Lysing Matrix A (No. 116910100) tubes with 0.5 mL MC 1 lysis buffer and homogenized using a MP Biomedicals FastPrep-96 bead beater. A portion of the homogenate (0.4 mL) was recovered and DNA extractions were completed using the Macherey-Nagel NucleoMag Plant Extraction Kit

(No. 744400.4) with KingFisher Accessory Kit (No. 744951). DNA samples were eluted to a final volume of 110  $\mu$ L.

515F (Parada et al., 2016) and 806R (Apprill et al., 2015) Earth Microbiome Project primers were used according to Walters et al., 2016 with barcodes on the 515F primer. Amplicons were generated from a single round of PCR using primers that include Illumina spacers, Illumina adapters, index sequences (on the forward primers), and 16S rRNA gene template region. PCR reagents included 7.2  $\mu$ L DNase free water, 10  $\mu$ L PLAT II MM (2X) (Invitrogen, Platinum II Hot-Start PCR Master Mix, Catalog No: 14000012), 0.4  $\mu$ L forward primer (10  $\mu$ M), 0.4  $\mu$ L reverse primer (10  $\mu$ M), and 2  $\mu$ L DNA template. PCR was performed on an Applied Biosystems SimpliAmp (Catalog No: A24811) system using an initial denature of 94  $^{\circ}$ C for 2:00 min followed by 35 cycles of 94  $^{\circ}$ C for 15 s, 54  $^{\circ}$ C for 15 s, 68  $^{\circ}$ C for 7 s, followed by a final extension at 68  $^{\circ}$ C for 3 min. DNA extraction blanks and no-template control blanks were included as negative controls. Mock communities (ZymoBIOMICS Microbial Community DNA Standard, Cat No: D6305) were included as positive controls to detect contaminants from kits or library preparation. Total amplicons per sample were normalized to between 12.5 and 15 ng using Charm Biotech Just-a-Plate PCR purification and normalization kit.

#### 16 Amplicon Bioinformatics

Raw paired fastq reads were preprocessed using the DADA2 R package (Callahan et al., 2016a). We truncated forward reads at position 220 and reverse reads at position 190 and discarded them if they contained a number of expected errors above three using the filterAndTrim() function. Denoising was performed with the learnError() and dada() functions with default parameters. Using the mergePairs() function, we merged reads if they overlapped by at least 20 bases, and allowed for 1 mismatch at most. Duplicate technical replicates were then merged bioinformatically. We used mothur (Schloss et al., 2009) along with the Silva (release 132) database (Quast et al., 2013) to align and annotate the sequences, respectively.

Sequences with a start or stop position outside the 5th-95th percentile range (over all sequences) were discarded. We removed potential chimeras with chimera.vsearch(). Taxonomies were assigned using classify.seqs() and classify.otus(). We removed all mitochondrial or chloroplast OTUs, as well as sequences with no annotations at the domain level. Using sub.sample(), we normalized the abundance in each sample by subsampling to 12,000 sequences. OTUs were defined as unique “amplicon sequence variants” (100% clustering OTUs) by DADA2 (Callahan et al., 2016). We used the lulu R package to remove artefactual OTUs (Frøslev et al., 2017): we merged two OTUs if all of the 3 following conditions were satisfied: 1) They co-occur in every sample, 2) One of the two OTUs has a lower abundance than the other in every sample and 3) they share a sequence similarity of at least 97%. Finally, we discarded OTUs represented by two or less reads across the 243 samples included in this library. UniFrac distance matrices were constructed from the OTU data and used to assess multivariate differences between microbial communities (Lozupone & Knight, 2005). At the final time point, two outlier samples were identified and removed from downstream 16S analysis (outliers were defined as samples whose log10 distance from the centroid of a treatment  $\geq 1.5$  SD above the mean log10 distance from the centroid for a given treatment).

#### Metabolomics Chemoinformatic Methods

Untargeted LC-MS/MS data pre-processing was performed with MzMine3 v3.2.8 (Pluskal et al., 2010). Mass detection was performed using the “centroid” algorithm. Intensity thresholds of 1E5 and 1E3 were for used for MS1 and MS2, respectively. Chromatograms were built using the ADAP chromatogram builder with a min group size of four, group intensity threshold of 2E5, minimum peak intensity of 1E5, and m/z tolerance of 0.0015 Da or 10 ppm. Extracted Ion Chromatograms (XICs) were deconvoluted using the local minimum search algorithm with a chromatographic threshold of 85% , a search minimum in RT range of 0.08 min, and a median m/z center calculation with m/z range for MS2 pairing of 0.1 and RT range for

MS2 scan pairing of 0.15. Isotope peaks were grouped and features from different samples were aligned with 0.001 Da or 5 ppm mass tolerance and 0.1 min retention time tolerance. MS1 peak lists were joined using an m/z tolerance of 0.0015 Da or 10 ppm and retention time tolerance of 0.15 min. Alignment was then performed by placing a weight of one on RT and a mobility weight of one. The feature table of peak areas were exported as a .csv file and the corresponding consensus MS/MS spectra were exported as a .mgf file.

### Bacterial Differential Abundance Analysis

In order to directly elucidate which specific bacterial taxa were driving these differences, we performed DESeq2, a method for analysis of differential expression of count data derived from high throughput sequencing, on a subset of the data that only included the four coral DOM treatments (Love et al., 2014). DESeq2 requires raw read inputs prior to reads per sample normalization and was thus run on raw read counts prior to the subsampling and Lulu steps of our bioinformatic pipeline. In order to eliminate low abundance and prevalence of OTUs prior to DESeq2, OTUs were removed so that only those with raw abundance  $\geq 50$  in three or more samples or a raw abundance  $\geq 1000$  in one or more samples were included, which comprised a subset of 187 OTUs. Given that OTU abundances in the stressed coral treatments were going to be compared to the coral controls, we further removed 28 highly variable OTUs within the Ccontrol coral treatment. Specifically, all OTUs with a coefficient of variation (CV) greater than 1 standard deviation of the mean CV of all OTUs were culled, yielding a final count of 159 OTUs to be run through DESeq2.

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