

The following supplement accompanies the article

## **Patterns of bleaching and recovery of *Montipora capitata* in Kāneʻohe Bay, Hawaiʻi, USA**

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### **Supplement.**

All data and scripts to reproduce analyses and figures presented in this study are available at: <http://github.com/jrcunning/kbayrecov2014>

### **qPCR assay development for *Montipora capitata* PaxC intron**

Primers and a Taqman MGB probe targeting a 121 bp conserved region of the *M. capitata* PaxC intron were developed based on five sequences from GenBank (AY313475-9; van Oppen et al. 2003) (Table S1). Replicate standard curves were performed using a tenfold dilution series of two cloned PaxC intron sequences as template, and the slopes of these standard curves were used to calculate the assay's amplification efficiency using the formula  $E=(10^{(-1/\text{slope})}-1)*100$ . Results indicated 105.7% amplification efficiency.

Table S1. Primers and Taqman MGB probe used for qPCR of the *M. capitata* PaxC intron.

Oligo	Sequence
Mcap-PaxC-For	5'-GTGCAGGTGAGATTGAGTCTTATAACA
Mcap-PaxC-Rev	5'-CGGTTGAGCTTCGCTAAACAG
Mcap-PaxC-Probe	5'-FAM-CAGTTCCTTCCAACAATG-MGB

## Standard curves and fluorescence normalization

Different Taqman probes have variable fluorescence intensity that must be accounted for in order to use a fixed  $\Delta R_n$  threshold across multiple assays for relative quantification. To quantify these differences, we performed standard curves with known quantities of target DNA over a 5- $\log_{10}$  dilution series, using two replicate template preparations of each target, each run in triplicate reactions. A linear model of the  $C_T$  values as a function of template concentration and target indicated that for the same template concentration, the *M. capitata* PaxC assay and clade C *Symbiodinium* assay amplified 0.848 and 2.268 cycles later than the clade D *Symbiodinium* assay. These values were subtracted from  $C_T$  data for these assays to normalize differences due to variable fluorescence.

## Actin gene copy number estimation

To estimate the number of copies of the targeted locus in clade C and D *Symbiodinium* (copies cell<sup>-1</sup>), we quantified DNA extracted from known numbers of cells using standard curves of known numbers of copies of target DNA. Tissue from six *M. capitata* colonies with varying proportions of clade C and D *Symbiodinium* was extracted with an airbrush, homogenized, and loaded onto a haemocytometer to count *Symbiodinium* cells. After four replicate counts of 0.5 mm<sup>2</sup> to determine cell concentration in each sample, DNA was extracted from three replicate aliquots of  $0.7-1 \times 10^5$  cells in constant volumes (n=18 extractions), and eluted in 50  $\mu$ L TE buffer. Each extraction was then assayed for clade C and D *Symbiodinium* with three technical replicates on each of two plates with standard curves of known concentrations of each target. The number of copies of clade C and D target present in each sample (averaged among technical replicates) on each plate was calculated from the standard curve, and an equation was constructed for each sample in the form of:

$$(\text{No. C copies}) / (\text{C copies cell}^{-1}) + (\text{No. D copies}) / (\text{D copies cell}^{-1}) = \text{No. cells}$$

where only C copies cell<sup>-1</sup> and D copies cell<sup>-1</sup> are unknown. The overdetermined system of 36 equations was then solved by singular value decomposition to estimate the number of copies per cell for each target. This yielded values of 32.85 and 2.75 for clade C and D, respectively; therefore, integer values of 33 and 3 were used and assumed constant for all samples in the study. These values are consistent with other studies that have quantified copy number of these actin loci in other clade C and D *Symbiodinium* (Cunning & Baker 2013, Silverstein et al. 2014).

### qPCR data quality filtering

qPCR data were screened to identify low quality sample runs. All samples with a standard deviation greater than one among technical replicates of any target were rerun, and the run yielding the lowest average standard deviation of technical replicates was retained in the dataset. For samples with a mean  $C_T$  value for the host assay greater than one standard deviation above the mean for all samples, DNA was re-extracted from the tissue sample archive and subsequently rerun. This was done because high host  $C_T$  values may indicate low DNA yield or carryover of PCR inhibitors during extraction. The extraction yielding the lowest host  $C_T$  values was kept in the dataset; other runs were discarded. After these steps, samples that still had a high outlying host  $C_T$  value ( $1.5 \times \text{IQR} > 75^{\text{th}}$  percentile) were used in qualitative but not quantitative analyses.

### ITS2 sequences from *Symbiodinium* in *M. capitata*

From a subset of 3 bleached clade C-dominated colonies, 3 non-bleached clade C-dominated colonies, and 3 non-bleached clade D-dominated colonies, we analyzed *Symbiodinium* ITS2 sequence assemblages (Fig. S1) obtained from paired-end Illumina libraries sequenced at Research and Testing Laboratory (Lubbock, TX) using the primers *its-dino* and *its2rev2* (Stat et al. 2009). Paired reads were merged using *illumina-utils* Eren et al. (2013) with a minimum overlap of 150 bp, Q30-check, and maximum mismatch of 3 bp. QIIME (Caporaso et al. 2010) was used to remove chimeric sequences, cluster OTUs at both 97% and 100% similarity, and assign taxonomy to the most abundant sequence from each OTU using BLAST with a custom *Symbiodinium* ITS2 reference database and e-value cutoff of 0.001. With *Symbiodinium* sequences, 97% and 100% similarity OTU tables were generated and imported into R using the *phyloseq* package (McMurdie and Holmes 2013). OTUs not observed at least 3 times in at least 1 sample were removed, and counts were transformed to relative abundance. In clade C colonies, >95.8% of sequences in each sample belonged to the same 97%-OTU, which was identified as ITS2 type C31 (AY258496). In clade D colonies, >98.4% of sequences in each sample belonged to the same 97%-OTU identified as D1a (AF499802) (Fig. S1). These results indicate that colonies are dominated by a single symbiont type, as 97% similarity in the ITS2 region is associated with single clonal lineages of *Symbiodinium* (Arif et al. 2014). However, to be conservative we also analyzed 100%-OTUs: the dominant 97%-OTUs broke down into several closely related OTUs, though all clade C- or D-dominated colonies still had the same numerically dominant sequence (C31 and D1a, respectively), and similar rank-abundance order of additional sequence types (Fig. S1). PERMANOVA detected no difference in community structure between bleached and non-bleached C-dominated colonies using either 97% or 100% OTUs, but differences between non-bleached C and non-bleached D-dominated colonies were

significant ( $p < 0.05$ ) at both levels of resolution. While identifying C31 and D1a as the particular clade C and D types in these corals, these results also provide evidence that clade C-dominated colonies have the same symbionts regardless of whether they bleached or not.

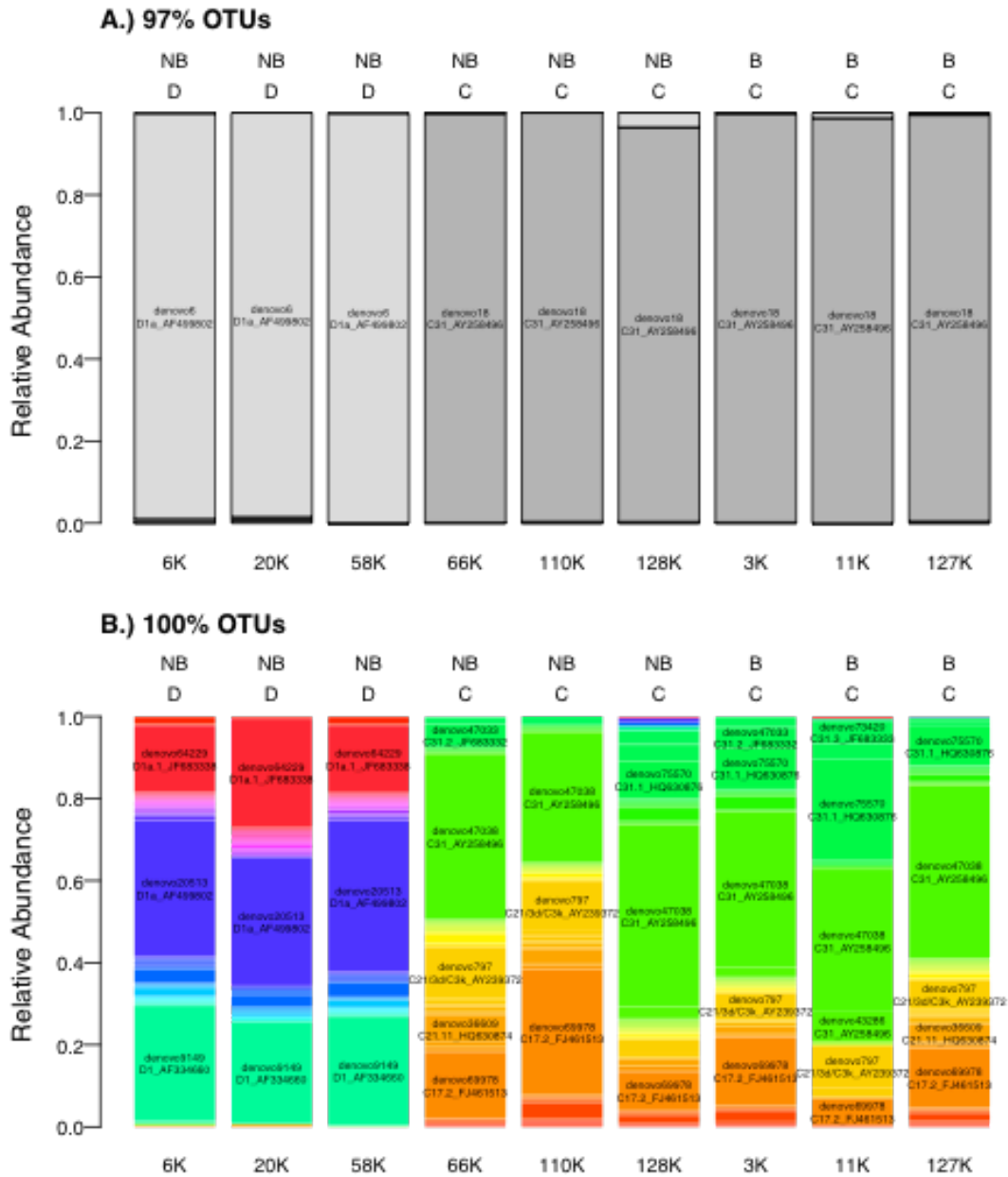


Figure S1. Relative abundance of ITS2 sequence OTUs clustered at A) 97% and B) 100% identity for each of the bleached (“B” label above column) and non-bleached (“NB”) colonies dominated by clade C (“C”) or clade D (“D”) *Symbiodinium*. Each OTU is represented by a unique grayscale or color, and those with a relative abundance of 4% or more within a sample are labeled with their unique OTU identifier (assigned by QIIME) and closest BLAST hit from the reference database

### **Actin sequences of clade C *Symbiodinium* in *M. capitata***

Due to the high biological and sequence diversity within *Symbiodinium* clade C, we confirmed that primer and probe sequences previously developed to target an actin gene locus in other members of clade C (Cunning and Baker 2013) would also successfully amplify the clade C symbionts in *M. capitata* (ITS2 type C31, see above). (Assays have already been validated and performed for *Symbiodinium* D1a, see Silverstein et al. 2015). Universal actin forward primer 2 (5'-CGGCTACTCCTTYACCACMA-3') and universal actin reverse primer (5'-TCRCCCTTGGAGATCCACAT-3') (Mieog et al. 2009) were used to amplify actin sequences from 20 colonies of *M. capitata* containing clade C *Symbiodinium*. Amplicons were cloned using the pGEM®-T Easy Vector System (Promega, Madison, WI, USA), and inserts were amplified using M13 primers. Inserts of the expected length for *Symbiodinium* clade C actin (~1 kb) were sequenced in the forward direction at the Pacific Biosciences Research Center at the University of Hawaii. After removing failed or low quality sequences (5 total), a total of 60 sequences were analyzed (2-4 sequences from each of 19 colonies, and 8 sequences from one colony), 55 of which were exact matches to the clade C primers and probe. Four sequences, each from a different colony, displayed 1 unique mismatch, while a single sequence displayed 7 mismatches. Mismatching sequences may represent 1) PCR or sequencing errors, 2) other actin loci or pseudogenes from genomes that also contain the matching locus, or 3) clade C *Symbiodinium* types that do not match the qPCR assay. The fact that each of the 4 single mismatches were different suggests these may be errors, and the sequence with 7 mismatches returned an actin pseudogene as its closest BLAST hit on NCBI. While the existence of low abundance uncaptured diversity cannot be ruled out completely, these results, combined with the uniform dominance of ITS2 type C31 (see above), suggest the clade C actin qPCR assay utilized here is capturing most if not all of the clade C *Symbiodinium* present in *M. capitata*.

### **Environmental data**

Temperature and light data were collected at 2 m depth at each reef site (Ritson-Williams and Gates 2016a,b). Temperature data were collected using calibrated HOBO Pendant UA-002-08 loggers (Onset Corp., Bourne, MA, USA). Light data were collected using Odyssey Integrating PAR sensors (Dataflow Systems PTY Limited, Christchurch, New Zealand) calibrated with a cosine-corrected LICOR PAR sensor (LI-192, LICOR Biosciences, Lincoln, NE, USA). Due to logistical reasons, reef 25 light data were not obtained during the study period, and temperature data are missing for a portion of the study.

Daily mean values for temperature ( $^{\circ}\text{C}$ ) and light ( $\text{mol PAR m}^{-2} \text{d}^{-1}$ ) were calculated for each reef and plotted along with 7-day moving averages in Fig. S2. Linear mixed models found no significant difference ( $p > 0.01$ ) in temperature among reefs, however, irradiance was  $\sim 21\%$  higher at HIMB than reef 44 ( $p < 0.001$ ) between December 2014 and May 2015.

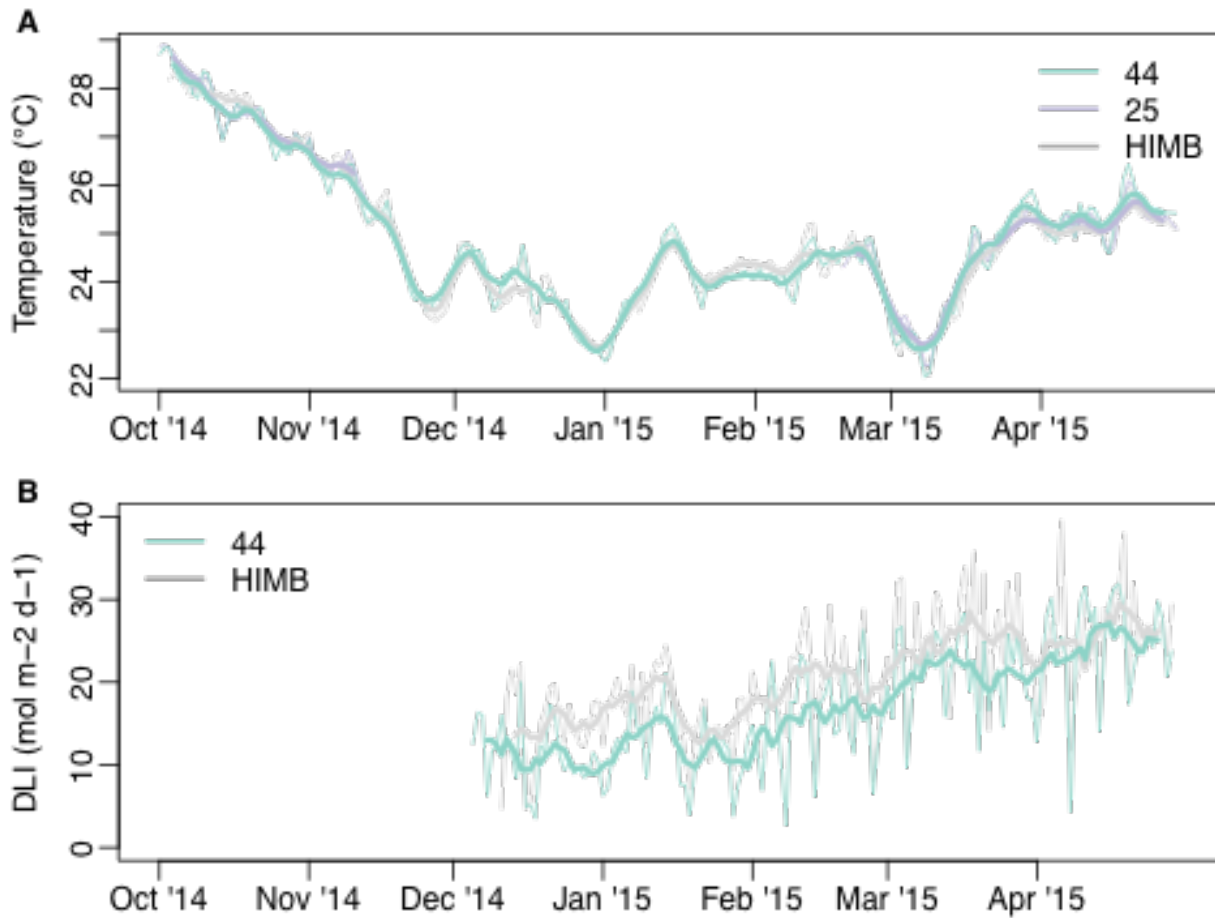


Figure S2. (A) Daily mean temperature and (B) daily light integrals (DLI) from the three reef sites during the study period (data gaps are due to logistical reasons). Thin lines are daily mean values and thick lines are 7-day moving averages.

## Literature Cited

- Arif C, Daniels C, Bayer T, Banguera-Hinestroza E, Barbrook A, Howe CJ, LaJeunesse TC, Voolstra CR (2014) Assessing *Symbiodinium* diversity in scleractinian corals via next-generation sequencing-based genotyping of the ITS2 rDNA region. *Mol Ecol* 23:4418–4433
- Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 7:335–336
- Cunning R, Baker AC (2013) Excess algal symbionts increase the susceptibility of reef corals to bleaching. *Nat Clim Chang* 3:259–262
- McMurdie PJ, Holmes S (2013) phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* 8:e61217
- Mieog JC, Oppen MJH van, Berkelmans R, Stam WT, Olsen JL (2009) Quantification of algal endosymbionts (*Symbiodinium*) in coral tissue using real-time PCR. *Mol Ecol Res* 9:74–82
- Ritson-Williams R, Gates RD (2016a) Kaneohe Bay Seawater Temperature Data 2014 and 2015. Zenodo. Available from <http://dx.doi.org/10.5281/zenodo.46366>.
- Ritson-Williams R, Gates RD (2016b) Kaneohe Bay Light Data 2014 and 2015. Zenodo. Available from <http://dx.doi.org/10.5281/zenodo.46367>
- Silverstein RN, Cunning R, Baker AC (2015) Change in algal symbiont communities after bleaching not prior heat exposure, increases heat tolerance of reef corals. *Glob Chang Biol* 21:236–249
- Stat M, Pochon X, Cowie ROM, Gates RD (2009) Specificity in communities of *Symbiodinium* in corals from Johnston Atoll. *Mar Ecol Prog Ser* 386:83–96
- van Oppen MJH, Koolmees EM, Veron JEN (2003) Patterns of evolution in the scleractinian coral genus *Montipora* (Acroporidae). *Mar Biol* 144:9–18