Global Change Biology
celebrating 20 years
Global Change Biology (2015) **21**, 236–249, doi: 10.1111/gcb.12706

# Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals

RACHEL N. SILVERSTEIN[1], ROSS CUNNING[1] and ANDREW C. BAKER[1,2]
[1]*Department of Marine Biology and Ecology, Rosenstiel School of Marine and Atmospheric Science, 4600 Rickenbacker Cswy, Miami, FL 33149, USA,* [2]*Wildlife Conservation Society, International Conservation - Marine Program, 2300 Southern Boulevard, Bronx, NY 10460, USA*

## Abstract

Mutualistic organisms can be particularly susceptible to climate change stress, as their survivorship is often limited by the most vulnerable partner. However, symbiotic plasticity can also help organisms in changing environments by expanding their realized niche space. Coral–algal (*Symbiodinium* spp.) symbiosis exemplifies this dichotomy: the partnership is highly susceptible to 'bleaching' (stress-induced symbiosis breakdown), but stress-tolerant symbionts can also sometimes mitigate bleaching. Here, we investigate the role of diverse and mutable symbiotic partnerships in increasing corals' ability to thrive in high temperature conditions. We conducted repeat bleaching and recovery experiments on the coral *Montastraea cavernosa*, and used quantitative PCR and chlorophyll fluorometry to assess the structure and function of *Symbiodinium* communities within coral hosts. During an initial heat exposure (32 °C for 10 days), corals hosting only stress-sensitive symbionts (*Symbiodinium* C3) bleached, but recovered (at either 24 °C or 29 °C) with predominantly (>90%) stress-tolerant symbionts (*Symbiodinium* D1a), which were not detected before bleaching (either due to absence or extreme low abundance). When a second heat stress (also 32 °C for 10 days) was applied 3 months later, corals that previously bleached and were now dominated by D1a *Symbiodinium* experienced less photodamage and symbiont loss compared to control corals that had not been previously bleached, and were therefore still dominated by *Symbiodinium* C3. Additional corals that were initially bleached without heat by a herbicide (DCMU, at 24 °C) also recovered predominantly with D1a symbionts, and similarly lost fewer symbionts during subsequent thermal stress. Increased thermotolerance was also not observed in C3-dominated corals that were acclimated for 3 months to warmer temperatures (29 °C) before heat stress. These findings indicate that increased thermotolerance post-bleaching resulted from symbiont community composition changes, not prior heat exposure. Moreover, initially undetectable D1a symbionts became dominant only after bleaching, and were critical to corals' resilience after stress and resistance to future stress.

*Keywords:* bleaching, climate change, community disturbance, coral–algal symbiosis, functional redundancy, heat tolerance, resilience, *Symbiodinium*

*Received 7 May 2014 and accepted 13 June 2014*

## Introduction

Corals form ecologically critical, but environmentally sensitive, symbioses with diverse, dinoflagellate algal partners (*Symbiodinium* spp.). In recent decades, as a result of climate change, episodes of coral 'bleaching,' or the stress-induced breakdown of symbiosis typically resulting from high temperature conditions, have contributed to dramatic declines in coral cover worldwide (~50–80% declines since the 1970s; Gardner, 2003; Bruno & Selig, 2007). To reverse this decline and to persist in warmer oceans, corals will need to rapidly increase their tolerance, in particular, to more frequent episodes of anomalously high temperatures that underlie most coral bleaching events.

Accurate assessments of the adaptive capacity of reef corals in future warmer oceans are critical to evaluating coral survival trajectories and managing reefs for climate change over the next century (Donner *et al.*, 2005; Baskett *et al.*, 2009; Logan *et al.*, 2013). The relative contributions of various acclimatization mechanisms in corals have been debated in the literature (e.g. Hoegh-Guldberg, 1999; Goulet, 2006; Ortiz *et al.*, 2012; Logan *et al.*, 2013). Here, we investigate the role of (i) low-abundance (or newly acquired), thermally tolerant algal symbionts; (ii) prior bleaching (community disturbance); and (iii) warm acclimation, in corals' adaptive responses to heat stress. The term 'adaptive' refers to any trait or process that provides a benefit or

Correspondence: Rachel N. Silverstein,
e-mail: rsilverstein@rsmas.miami.edu

advantage, and is therefore distinct from 'adaptation' in the Darwinian evolutionary sense (see Baker, 2004; Buddemeier *et al*., 2004; Fautin & Buddemeier, 2004 for discussion of this definition).

The genus *Symbiodinium* is divided into nine subgeneric clades (named A-I, Pochon & Gates, 2010). Reef-building corals typically host *Symbiodinium* in clades A-D (Baker, 2003), although additional partnerships with clades F and G have also been documented. Within these clades, over 400 Internal Transcribed Spacer-2 (ITS2) rDNA 'types' have been reported (see Correa & Baker, 2009; Thornhill *et al*., 2013; Tonk *et al*., 2013; LaJeunesse *et al*., 2014). Several ITS2 types have been shown to have distinct physiological tolerances, such as *Symbiodinium* D1a (also D1-4 or *Symbiodinium trenchi*, LaJeunesse *et al*., 2010b; 2014), which can have bleaching resistant and/or thermotolerant traits (LaJeunesse *et al*., 2009; Wang *et al*., 2012; Kemp *et al*., 2014).

Symbiotic plasticity (the capacity to host different symbionts) is known to occur in coral–algal symbiosis (e.g. Baker, 2001; Chen *et al*., 2005; Berkelmans & van Oppen, 2006; Thornhill *et al*., 2006a; Baker & Romanski, 2007 for review; Jones *et al*., 2008; and see Abrego *et al*., 2008), but its capacity to influence reef futures on a large scale in an era of climate change has been questioned (Goulet, 2006; Hoegh-Guldberg *et al*., 2007; LaJeunesse *et al*., 2010a; Hill & Hill, 2012; McGinley *et al*., 2012). Recent surveys using high-sensitivity molecular techniques, such as quantitative PCR (qPCR), have revealed that diverse, low-abundance, background symbionts are more common in reef corals than previously thought, indicating that the potential for symbiotic plasticity could be robust (Mieog *et al*., 2007; Correa *et al*., 2009; Silverstein *et al*., 2012). These studies hypothesized that low-abundance symbionts could provide functional redundancy during stress and increase holobiont (host and associated symbionts considered together) resistance to, and resilience after, environmental stress (Mieog *et al*., 2007; Correa *et al*., 2009; Silverstein *et al*., 2012). We explore this hypothesis by experimentally manipulating replicate coral clones of the same host genotype to contain different *Symbiodinium* communities (dominated by either heat-sensitive or heat-tolerant symbionts), which we then use to test the role of symbiont change and prior thermal history in coral bleaching resistance.

In this study, we do not differentiate between low-abundance symbionts that were undetectable prior to bleaching and symbionts newly acquired from the environment post-bleaching. While there are different ecological implications depending on the source of these initially undetected symbionts, the findings reported in this study do not depend on distinguishing between them.

To control for the potential contribution of prior heat exposure on thermotolerance, corals were initially bleached with either heat at 32 °C or the herbicide DCMU (*N*′-(3,4-dichlorophenyl-*N*,*N*-dimethylurea, or Diuron™) at 24 °C. DCMU inhibits photosynthetic function by blocking electron transport at the reaction center of photosystem II (Bowyer *et al*., 1991), which leads to the formation of reactive oxygen species (ROS; Jones, 2004), triggering bleaching (Weis, 2008). Here, DCMU-bleached corals serve as controls for corals that bleached, but were still naïve to heat exposure.

While symbiotic plasticity is a critical element of mutualists' response to changing environments (Six & Bentz, 2007; Wernegreen & Wheeler, 2009), mutualistic partnerships also link hosts and symbionts to a shared fate, potentially increasing the number of species that could experience detrimental effects from climate change (Six, 1998; Kiers *et al*., 2010). Our study tests the role of mutualisms in rapid, adaptive responses to climate change stressors. We test how disturbance promotes symbiont community change, resulting in emergent thermotolerant traits. We demonstrate that symbiotic diversity, even when initially undetectable in prestressed corals, can have profound implications for the community ecology of microbial mutualisms during and after stress. The role of these diverse symbiotic partnerships should therefore continue to be studied and accurately incorporated into predictive models of corals' survivorship in climate change conditions.

## Materials and methods

### *Experimental setup*

Nine colonies of *Montastaea cavernosa* were collected (Florida Fish and Wildlife Conservation Commission Special Activity License #SAL-11-1182-SRP) from a 20 m depth off of West Palm Beach, FL, cored into replicate 2.5 cm diameter cores, individually labeled and affixed to ceramic plugs (See Supplementary Information for more details). Corals were housed in an indoor facility in flow-through 284 l fiberglass tanks, supplied with sand- and UV-filtered seawater pumped from Biscayne Bay, although incoming seawater was not completely devoid of free-living *Symbiodinium* (Jones, 2014). Corals were maintained at 24 °C ± 1 °C using heater/chillers (TR20 SeaChill, TECO, São Paulo, Brazil) in each of four identical tanks. Seawater flowed into the tanks at a rate of 10 ml $s^{-1}$ and two pumps circulated water within each tank.

Light (190–280 micromoles quanta $m^{-2}$ $s^{-1}$, as measured by an Apogee Quantum Meter MQ-100) was provided using 400 W metal halide pendant lights (IceCap Inc., USA) on a 12 hour light–dark cycle. Light and temperature levels were

recorded every 15 min using HOBO temperature and light pendant data loggers throughout the study (Onset Corp UA-002–64). Corals were grouped together in the center of the tanks to control for any potential within-tank variation in light levels and water flow, and cores were rotated regularly within individual tanks (2–3x per week). Corals were fed Reef Chili (a dried coral food mixture comprised of zooplankton, phytoplankton, *Artemia nauplii*, daphnia, rotifers, and copepods, available: Bulk Reef Supply), which was added directly to tanks 2–3 times per week. Experimental cores were randomly allocated among treatment groups (6 groups, $N$ = 24–25 cores per group), such that at least two cores from each coral colony (genotype) were present in each treatment group (see Table S2).

### *Initial bleaching (heat or DCMU) treatment*

Corals were initially exposed to either: (i) heat stress (32 °C for 10 days, $N$ = 48), using Jaeger and ViaAqua 300 W aquarium heaters, or (ii) herbicide (at 24 °C, 450 μg $L^{-1}$ DCMU for 10 days, $N$ = 48). Additional control cores ($N$ = 24-25) were also maintained, without acute stress exposure, at either (iii) 24 °C ('cool control'), or (iv) placed at 29 °C ('warm control'). Conditions were the same for all recovery and treatment tanks throughout the experiment (see description in 'Experimental Setup' section and Supplementary Information), except for the DCMU treatment, which used a 20 l closed system tank with a circulating pump and an airstone immersed in the larger 24 °C tank, which acted as a water bath. Water was changed

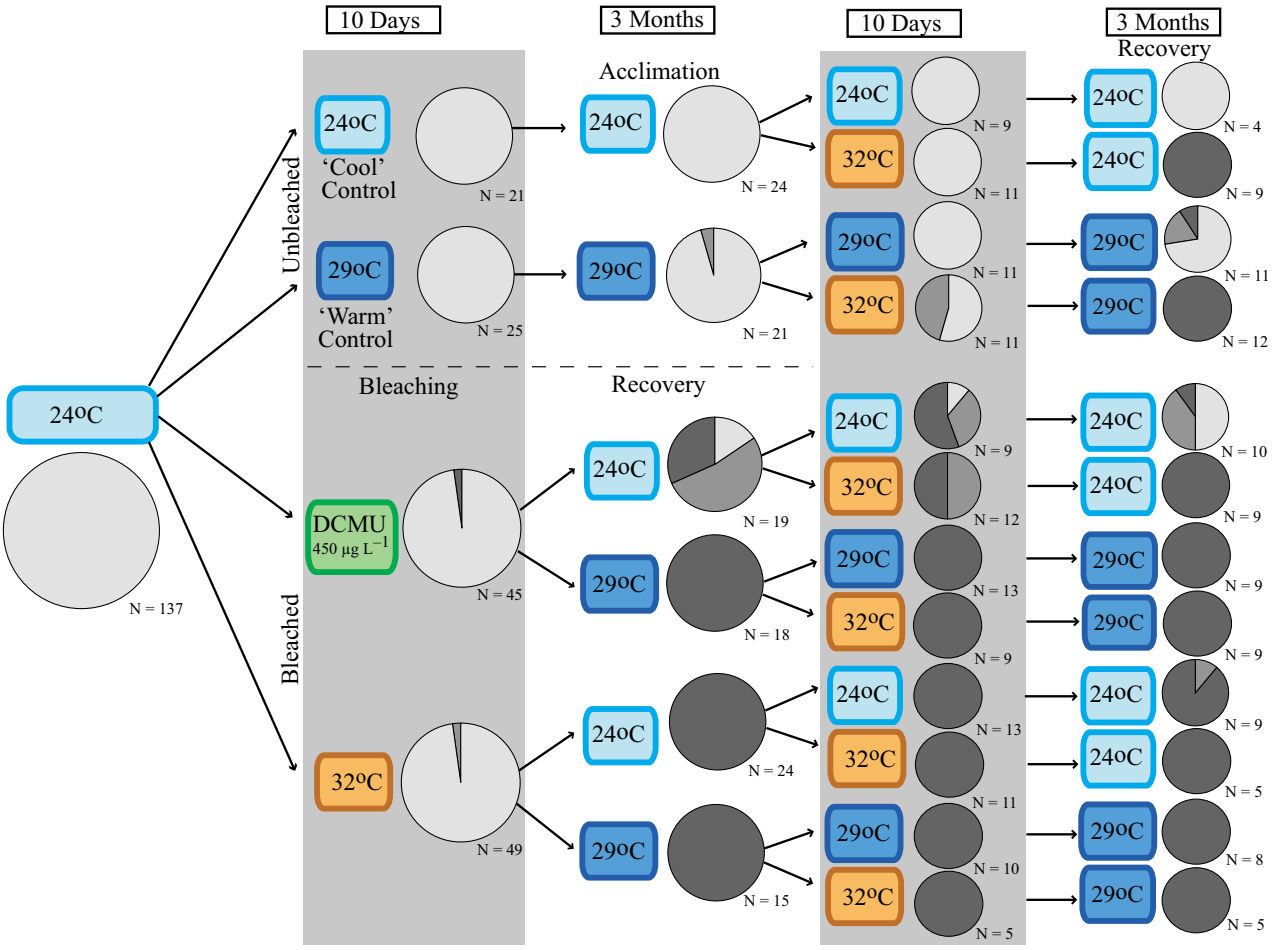


**Fig. 1** Experimental design. All corals were acclimated to 24 °C for 2 months prior to the start of the experiment. Corals were then either (i) not bleached and maintained at 24 °C for 10 days (light blue) or (ii) not bleached and maintained at 29 °C for 10 days (dark blue), (iii) bleached with DCMU at 24 °C (green) for 10 days, or (iv) bleached with heat at 32 °C (orange) for 10 days. Corals were then allowed to recover in one of two replicate tanks at either 24 °C or 29 °C for 3 months. After recovery, half of the corals from each treatment group were exposed to a second heat stress event (32 °C for 10 days), identical to the first heat stress (orange). Corals were again allowed to recover in the same replicate tanks at either 24 °C or 29 °C for another 3 months. Stress exposure periods are shown in gray boxes. Pie charts display the proportion of colonies at a given time point and treatment that are dominated by >90% clade C (light gray), a mixture of clade C and D (gray), or >90% clade D (dark gray). N represents the number of coral cores represented, i.e. those for which qPCR amplification was successful.

in the 20 l tank every 48 h, with fresh aliquots of DCMU added at each water change (Figure S1).

### *Initial recovery conditions*

After the first 10 day bleaching treatment (by heat or herbicide), bleached corals were placed into either 24 °C or 29 °C recovery tanks (one of two replicate tanks at each temperature), along with the 'cool' and 'warm' control corals, respectively, and were continuously maintained at these temperatures for 89 days of recovery. DCMU-exposed corals were flushed with clean, running seawater for 2 min before being added to the recovery tanks.

### *Second bleaching (heat only) treatment*

After a 3 month recovery period, experimental *M. cavernosa* cores belonged to one of six treatment groups: unbleached and acclimated to (i) 24 °C or (ii) 29 °C, heat bleached and recovered at (iii) 24 °C or (iv) 29 °C, or DCMU bleached and recovered at (v) 24 °C or (vi) 29 °C (Fig. 1, $N$ = 23–27 cores per group). For each of these groups, half of the cores ($N$ = 11–14) were maintained at the recovery temperature (24 °C or 29 °C) for an additional 3 months, while the other half of the cores were exposed to a second, 10 day thermal stress event (32 °C), before being returned back to their respective recovery temperatures (24 °C or 29 °C, see Fig. 1) for a further 3 month recovery period. Recovery conditions during this second recovery period were, in all respects, identical to those used during the initial recovery period.

### *Symbiont community function*

*Chlorophyll fluorometry*. Measurements of maximum quantum yield of photosystem II (Fv/Fm) were taken using an Imaging Pulse Amplitude Modulated Fluorometer (I-PAM, Walz, Effeltrich, Germany) at 08:00 hours, before lights were turned on. A saturating pulse was administered at 2800 μmol photons $m^{-2}$ $s^{-1}$ with an LED array at 460 nm for 800 milliseconds. During the first 10 day bleaching period, measurements were taken immediately prior to stress (day 0), and then on days 3 and 10. During the second bleaching period, measurements were taken immediately prior to stress (Day 92), and then on days 95, 99, and 102. I-PAM measurements during recovery after both bleaching events were then taken every 1–2 weeks.

### *Symbiont community structure*

*Tissue sampling*. Small (~1–3 $mm^2$) tissue samples were taken from each core using a new razor blade immediately before and after each of the two 10 day stress exposure periods, and also at the end of both 3 month recovery periods. Tissue samples were immediately transferred to DNAB containing 1% SDS and heated to 65 °C for 60–90 min to produce a cell lysate for archival purposes. DNA was extracted using established organic protocols (Baker & Rowan, 1997).

### *Symbiont identification and quantification*

For a subset of colonies from each time point and treatment, the Internal Transcribed Spacer-2 (ITS2) region of ribosomal DNA (rDNA) was amplified and *Symbiodinium* ITS2 types were identified using denaturing gradient gel electrophoresis (DGGE) and sequencing, per the revised methods described in Silverstein *et al.* (2012), based on LaJeunesse (2001). See Supplementary Information for more details.

To calculate the relative abundance of each symbiont clade in each sample, quantitative PCR (qPCR) assays were used to quantify the number of copies of actin loci of (i) *Symbiodinium* clade C, (ii) *Symbiodinium* clade D, and (iii) the coral host. Clade-specific assays for clade C and clade D symbionts were performed following the methods of (Cunning & Baker, 2013), modified for 10 μl reaction volumes. The *M. cavernosa*-specific assay was designed from cloned actin gene sequences from five colonies using Allele ID 7.7 (Premier Biosoft, Palo Alto, CA, USA) and Primer Express (Applied Biosystems, *Genbank* Accession Number for representative *M. cavernosa* sequence: KF447732). Unlabeled primers were synthesized by Integrated DNA Technologies, and fluorescently labeled MGB Taqman probes by Applied Biosystems (F: 5′-CGT TGA CAT CCG TAA GGA TCT CTAT-3′, R: 5′-CAA TGA TCT TAA TCT TCA TGG TTG GT- 3′, Probe: 5′-NED- CCA ACA CTG TCC TCT CT-MGB-3′). *M. cavernosa* assay primers were used at a final concentration of 150 nm forward primer, 200 nm reverse primer, and 150 nm probe. (For qPCR assay conditions and run method, see Supplementary Information.)

Positive amplifications were scored as those that exceeded a fluorescence threshold value of 0.01 in <40 cycles in both replicate wells. Samples for which Ct values of technical replicates were more than 2–3 cycles apart were rerun. If replicates were still dissimilar, the sample was excluded from the analysis. Between 7 and 16% of samples were excluded at each time point because they either did not amplify (even after re-extraction), replicates did not agree (even after re-extraction and/or repeated assays), or the calculated symbiont to host cell ratio was a statistical outlier, defined as occurring at least two standard deviations away from the mean. Typically, this was due to late amplification of the host DNA, indicating likely PCR inhibition in the sample. Each plate included two 'no template control' (NTC) reactions for each primer set (with $ddH_2O$ substituted for DNA). Each plate also included two positive control reactions for each assay that amplified plasmid DNA containing the exact primer and probe sequence matches.

### *Data analysis*

The number of symbiont actin copies was translated into symbiont cell numbers by determining the average number of copies of actin gene per symbiont cell for each clade (See Supplementary Information). Relative abundance of each symbiont clade was then calculated as the number of symbiont cells per host actin copy. (Because no single-copy gene for *M. cavernosa* is available, host actin copy number could not be determined and therefore absolute cell numbers also could not be determined. However, calculating absolute numbers of symbiont cells per host cell, e.g. Mieog *et al.*,

2009; Cunning & Baker, 2013, is not necessary for the analyses presented here, as we compare only relative changes in symbiont abundance over time between clades.) 'Total symbiont abundance' is calculated as the abundance (symbiont cells/host copy) of clade C *Symbiodinium* plus the abundance of clade D *Symbiodinium*.

Raw qPCR cycle threshold (Ct) values were adjusted for differences in fluorescence intensity between assays before analysis (see Supplementary Information for more details). Symbiont abundance ratios are log-distributed and therefore symbiont abundance data were log-transformed prior to analysis. Maximum quantum yield data (Fv/Fm) were arcsine-transformed prior to analysis. Because >90% of colonies were >90% dominated by either clade C or clade D symbionts, corals were categorized as either 'C-dominated' (>90% clade C) or 'D-dominated' (>90% clade D) for analysis. Cores not dominated >90% by either clade were categorized as 'C+D' mixtures.

Arcsine square root transformed Fv/Fm values were compared among treatments by ANOVA with *post hoc* Tukey's HSD tests, either at single time points or as relative change between time points. When multiple experimental factors were tested in the same model (i.e. bleaching mode, recovery temperature, and/or dominant clade), two-way ANOVAs or multiple regression models were used. The effects of dominant symbiont clade, acclimation temperature, and/or initial bleaching stressor on relative symbiont loss during bleaching (log final/initial symbiont abundance) were each tested by ANCOVA models with prestress symbiont abundance as a continuous covariate. During the initial stress exposure, all corals were clade C dominated. However, during the second stress exposure when

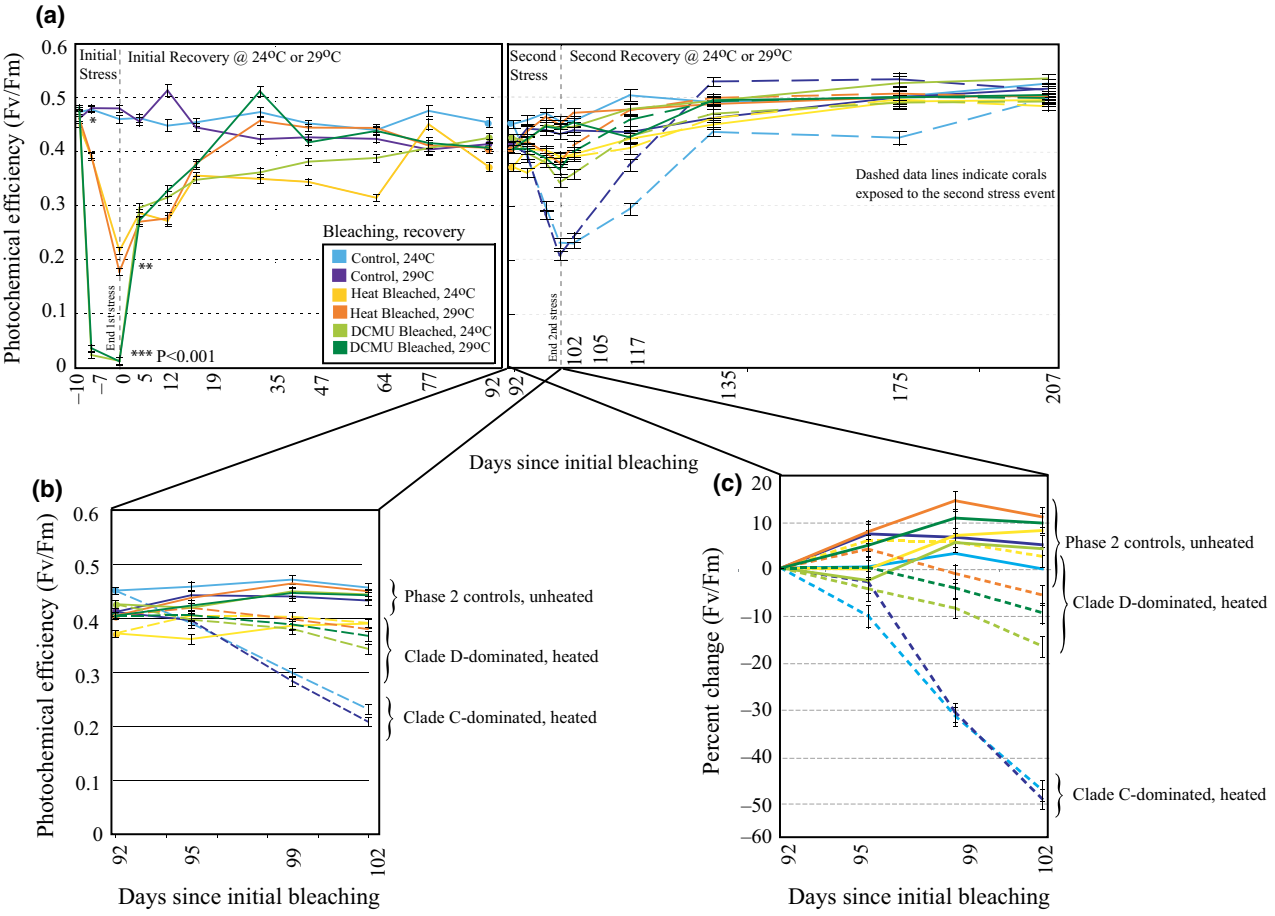


**Fig. 2** Maximum quantum yield of photosystem II (Fv/Fm) of dark-adapted *Symbiodinium* in experimental corals. Vertical dashed lines indicate the end of the 10 day periods of stress exposure, followed by the 3 month recovery periods. Unbleached ('warm' and 'cool' control) corals are shown in blue (dark blue: 29 °C, light blue: 24 °C); heat-bleached corals are shown in orange (29 °C recovery) and yellow (24 °C recovery); DCMU-bleached corals are shown in green (dark green: 29 °C recovery, light green: 24 °C recovery). (a) Mean Fv/Fm values within each treatment during both the first and second bleaching and recovery periods (b) Expansion of days 92–102 (second stress exposure period) to show detail (c) Change in Fv/Fm values during days 92–102, expressed as percent change over values at day 92. Error bars represent standard error. Dashed, colored, data lines indicate the portion of the treatment group exposed to the second heat stress event. $N$ = 23–27 for each data point at each time point during the first bleaching and recovery phases. $N$ = 11–14 for each data point at each time point during the second stress and recovery phases.

multiple clades were present, effects of acclimation temperature and bleaching stressor on change in symbiont abundance and photochemical efficiency were analyzed either within a single clade (i.e. within C-dominated or D-dominated corals only), between clades (with dominant clade as a factor), or with proportion clade D (arcsine square root transformed) as a response variable. Coral host genotype was initially included as a factor in statistical models, but was found not to be significant (partial *F*-test; $P > 0.05$) and was removed. An alpha value of 0.05 was used for all tests, unless otherwise stated. All statistical tests were performed in JMP (version 10.0.0, SAS).

## Results

### *Initial bleaching*

Prior to initial bleaching, all corals ($N = 139$) hosted clade C *Symbiodinium* exclusively (ITS2 type: C3), except for two cores that contained trace amounts (<1%) of clade D *Symbiodinium* (Figs 1 and 3).

Symbionts exposed to DCMU showed greater percent decline from starting Fv/Fm values (−92.8 ± 1.6%), than heat-exposed corals (−37.7 ± 1.6%) after

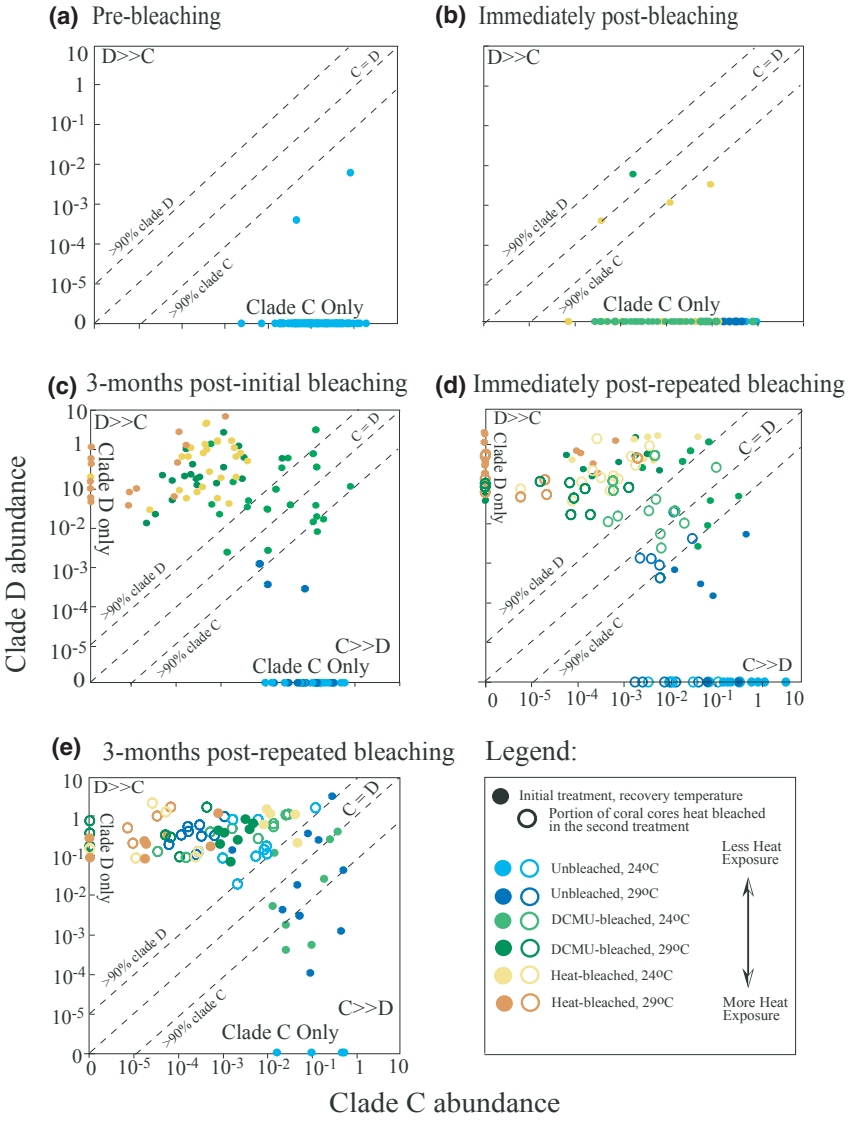


**Fig. 3** Cell ratio abundance of clades C and D symbionts. Each data point represents the symbiont community composition of a single colony at a single time point. Colonies on the upper left of the graph host more clade D than clade C, and colonies on the lower right corner host more clade C than clade D *Symbiodinium*. Colonies along the central diagonal, dashed line host approximately equivalent clade C and clade D *Symbiodinium*. Logarithmic plots of symbiont community structure in experimental corals: (a) prior to bleaching, (b) immediately postinitial bleaching, (c) 3 months after first bleaching, (d) immediately after second bleaching (open symbols) or controls at recovery temperatures (closed symbols), (e) 3 months after the second bleaching (i.e. 6 months after the first bleaching). Each data point represents a single colony.

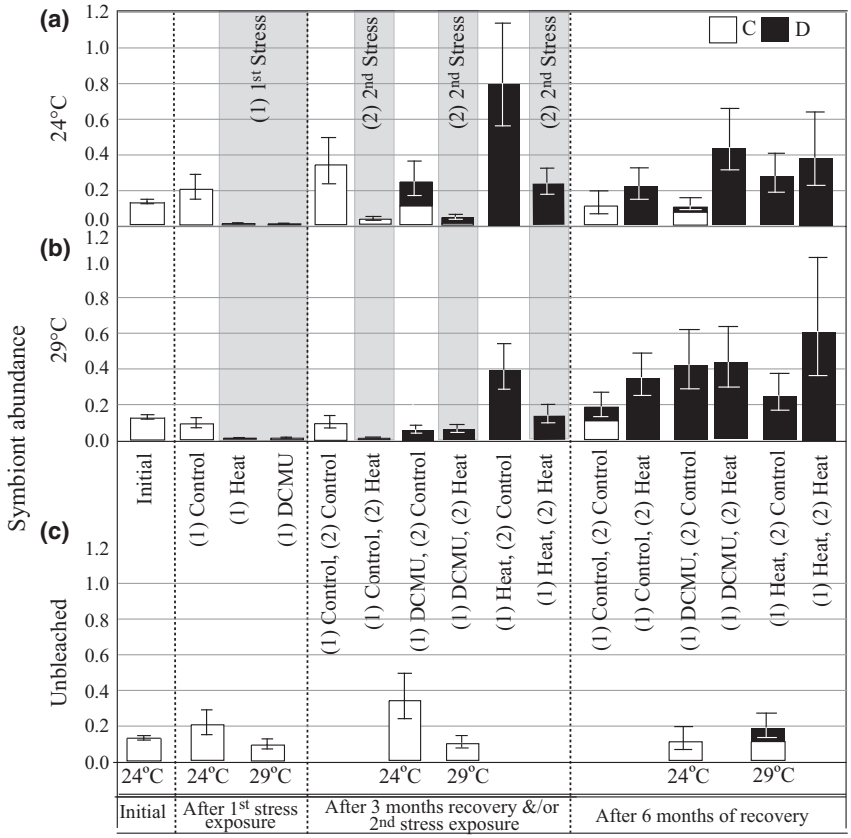


**Fig. 4** Total symbiont abundance (height of bar) and proportion of clade C (white) and D (black) symbionts in unbleached, heat-bleached, and DCMU-bleached corals at (a) 24 °C and (b) 29 °C recovery temperatures, and (c) unbleached corals acclimated to either 24 °C or 29 °C. Gray shaded areas indicate stress exposure periods. Labels show: (1) treatment during the first stress event followed by (2) treatment during the second stress event [e.g. a coral bleached with DCMU in the first stress event and heat in the second would read: (1) DCMU, (2) Heat]. Error bars represent standard error for the total symbiont community (clades C + D).

10 days ($P < 0.0001$, ANOVA, see Fig. 2), although corals in both treatments lost the same proportion of symbionts [DCMU: 96.2% (± 1 SE 97.5–95.3%), Heat: 95.6% (± 1 SE 96.9–94.5%); $P = 0.67$, ANCOVA, see Figs 3 and 4]. In both heat and DCMU treatments, corals with higher initial symbiont abundances lost a greater proportion of their symbiont communities during stress (Cunning & Baker, 2013).

Corals that were transferred from 24 °C to 29 °C ('warm controls'), without acute heat stress, lost 47.1% (± 1 SE 33.1–67.0%) of their symbionts after 10 days, despite no visual bleaching and no decline in symbiont photochemical efficiency. Rather, a slight Fv/Fm increase was observed (after 10 days: 2.6 ± 0.006% warm controls vs. −1.6 ± 0.005% in cool controls; $P < 0.0001$, Fig. 2).

### *Recovery from initial bleaching*

Despite falling to lower levels during DCMU exposure, the photochemical efficiency of symbionts within DCMU-bleached corals recovered to the same levels as symbionts within heat-bleached corals after just 5 days of recovery (ANOVA, $P = 0.15$, Fig. 2). Recovery temperature did not affect Fv/Fm during this 5 day period (ANOVA, $P > 0.06$, Fig. 2). However, after 19 days of recovery, symbionts within bleached corals recovering at 29 °C had higher Fv/Fm values than those at 24 °C (0.37 ± 0.005 vs. 0.35 ± 0.005; ANOVA, $P = 0.002$), regardless of bleaching stressor (ANOVA, $P = 0.47$, Fig. 2). After 35 days, the Fv/Fm of symbionts within bleached corals recovering at 29 °C exceeded control levels (0.48 ± 0.007 vs. 0.42 ± 0.01), but this recovery took 2–3 times longer (from 74 days to 3 months) for symbionts within corals recovering at 24 °C (See Fig. 2).

After 3 months of recovery, all bleached corals hosted *Symbiodinium* communities dominated by clade D (ITS2 type D1a), but most still contained some C3 *Symbiodinium* as well (Fig. 1,3,4). Clade D-dominated corals hosted higher densities of symbionts than clade C-dominated corals (ANOVA, $P < 0.0001$, Fig. 4). Exposure to elevated temperatures, either during bleaching (32 °C) or recovery (29 °C), resulted in corals with

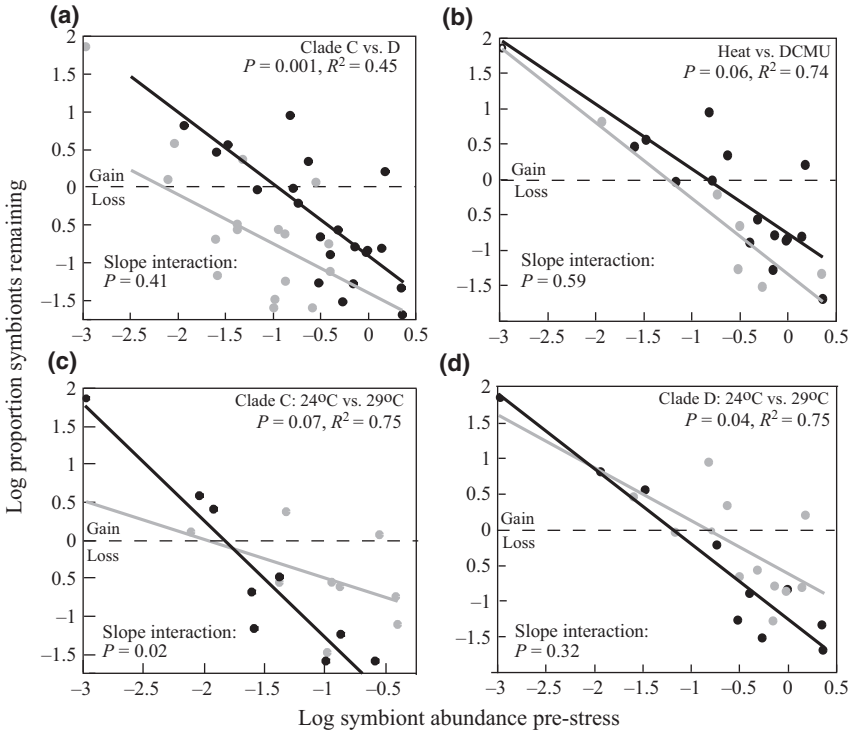


**Fig. 5** Proportional symbiont abundance change during the second heat stress event (ANCOVAS) comparing: (a) clade D (black)- and clade C (gray)-dominated corals. Corals hosting mainly clade D symbionts lost fewer symbionts than corals hosting clade C symbionts; (b) clade D-dominated corals initially bleached with heat (black) or DCMU (gray); There was no difference in symbiont abundance changes between initially heat-bleached and DCMU-bleached corals; (c) clade C-dominated corals acclimated to 24 °C (gray) or 29 °C (black). No significant effect of acclimation temperature on symbiont loss; (d) clade D-dominated corals acclimated to either 24 °C (gray) or 29 °C (black). Corals acclimated to 29 °C lost more symbionts than those at 24 °C. For all tests, corals with higher initial symbiont abundances lost more symbionts during stress ($P < 0.0003$).

equal proportions of D1a (>99%) at the end of the recovery period (ANCOVA, $P > 0.07$, Table S1, Figs 1,3,4). *Symbiodinium* D1a comprised >99% of the symbiont community in corals that were heat bleached and recovered at either warm (29 °C) or cool (24 °C) temperatures, and in corals that were bleached with DCMU and recovered at 29 °C (Figs 1,3,4). Bleached corals that were heat-naïve, those that were DCMU-exposed and recovered at 24 °C, were also dominated by D1a, but contained significantly lower proportions (63.5 ± 3.3%, ANCOVA, $P < 0.0001$, Figs 1,3,4) than those heat bleached and/or recovered at 29 °C. Similarly, higher proportions of clade C symbionts were observed in heat-bleached corals that recovered at 24 °C, compared to heat-bleached corals and recovered at 29 °C (ANOVA, $P < 0.02$, Fig. 4, see Table S1).

Unbleached corals at 24 °C ('cool controls') had no detectable clade D symbionts after 3 months, while 'warm controls' hosted low (but detectable) levels of clade D symbionts in 3 of 20 cores after 3 months (mean of three cores containing clade D: 6.1 ± 4.2%, mean clade D among all 20 'warm control' cores at 29 °C: 0.9 ± 0.7%).

### *Repeat bleaching*

During the second heat stress event, corals that were not previously bleached (and remained dominated by C3 symbionts) lost more symbionts (C3: −88.3% ± 1 SE −88.5% to −88.1%; D1a: −36.1% ± 1 SE −36.4 to −35.6%; ANCOVA, $P < 0.001$, Fig. 5a) and showed greater reductions in photochemical efficiency (C3: −31.6 ± 0.01%; D1a: −2.0 ± 0.1%, ANOVA, $P < 0.0001$, Fig. 2) than corals that had previously bleached and recovered with D1a-dominated communities. Symbiont loss was equivalent between D1a-dominated corals that were previously DCMU bleached and heat bleached (ANCOVA, $P = 0.06$, Figs 4 and 5b). However, those that previously bleached by heat maintained higher Fv/Fm values (0.38 ± 0.004) than those initially DCMU bleached (0.36 ± 0.004, ANOVA, $P < 0.0001$, Fig. 2).

Less photodamage (change in Fv/Fm, 24 °C: 0.001 ± 0.006, 29 °C: −0.041 ± 0.004; ANOVA, $P < 0.0001$, Fig. 2) and less symbiont loss (24 °C: 26.7% ± 1 SE 0 – 46.9%; 29 °C: 75.1% ± 1 S.E. 64.5 – 82.5% symbionts lost; ANCOVA, $P = 0.04$, Fig. 5d) was also observed among D1a-dominated corals that had

recovered from the first event at 24 °C, compared to 29 °C. Conversely, there was no effect of prior acclimation temperature on Fv/Fm (ANOVA: $P = 0.92$, Fig. 2) or symbiont loss (ANCOVA, $P = 0.07$, Fig. 5c) among C3-dominated corals. Corals (both dominated by clade C or D) with higher initial symbiont abundances lost a greater proportion of their symbiont communities overall (ANCOVA, $P < 0.0003$).

### *Recovery from repeat bleaching phase*

'Cool controls' still hosted no detectable clade D symbionts after 6 months at 24 °C (Figs 1,3,4). However, 'warm control' corals, which were maintained at 29 °C, had all acquired some clade D *Symbiodinium* after 6 months (41.9 ± 7.7% of the total symbiont community, see Figs 1,3,4).

Among corals that were bleached for the first time during the second heat exposure (i.e. still C3 dominated), those that recovered at 24 °C had slightly lower proportions of D1a symbionts after 3 months than those at 29 °C (96.3 ± 0.9% at 24 °C vs. 99.9 ± 0.3%, $P < 0.0001$, ANOVA), similar to the first recovery phase (Fig. 1). Fv/Fm exceeded control values after 33 days of recovery at 29 °C, but took 3 months to recover to control levels at 24 °C (Fig. 2). In contrast, D1a-dominated corals during the second heat stress had Fv/Fm values recover to control levels after 14-33 days at either 24 or 29 °C (Fig. 2).

Most corals that were not exposed to the second stress event showed no change in total symbiont abundance during the second 3 month recovery period (ANCOVA, $P > 0.1$), regardless of bleaching history. However, corals that were initially DCMU bleached and recovered at 24 °C for 6 months (i.e. were naïve to heat during bleaching and recovery) lost clade D symbionts during this period, dropping from 63.5 ± 3.3% clade D after 3 months, to 34.3 ± 6.7% after 6 months.

Similar to the first recovery phase, clade D-dominated corals hosted higher densities of symbionts than the clade C-dominated corals after the second recovery phase (ANOVA, $P < 0.0001$, Fig. 4). No coral mortality was observed in any phase of the experiment.

### *Coral genotype analysis*

Analysis of the nine *M. cavernosa* colonies with eight microsatellite markers (Table S4, see Serrano *et al*., 2014 for genotyping methods) showed the colonies to be distinct genotypes, not clones.

### *Actin copy numbers in Symbiodinium*

*Symbiodinium* C3 was estimated to have 50 (50.5 ± 4.2) copies of the targeted actin locus per symbiont cell, while *Symbiodinium* D1a was estimated to have a copy number of 3 (3.3 ± 0.2). Relative symbiont abundance calculations were adjusted by these values prior to analysis.

### *ITS2 genotyping*

All initial samples were dominated by *Symbiodinium* C3. Bleached corals recovered with dominant communities of *Symbiodinium* D1a. No other ITS2 types were detected by DGGE.

## Discussion

### *Disturbance promotes rapid community change and thermotolerance*

Initially populated only with *Symbiodinium* C3, *M. cavernosa* corals recovered from bleaching with dominant communities of *Symbiodinium* D1a, regardless of the type of stress experienced (heat or DCMU) or the recovery temperature (24 or 29 °C). When exposed to a subsequent episode of heat stress 3 months later, these D1a-dominated corals were more resistant to bleaching – experiencing less photodamage (Fig. 2) and losing fewer symbionts (Fig. 5a) – compared to control corals that had not previously bleached, and therefore still hosted *Symbiodinium* C3.

*Symbiodinium* D1a was less abundant in unbleached corals (Figs 1,3,4), even in those exposed to warm conditions for 3 months. Unbleached corals did not host enough D1a symbionts to avoid severe bleaching during subsequent heat exposure, and did not acquire thermotolerance from long-term acclimation to 29 °C (Figs 1,3). Bleaching, therefore, provided an adaptive benefit for corals by allowing the proliferation of D1a symbionts and the emergence of associated heat-tolerant traits (Figs 1,3). The role of bleaching in enhancing rapid symbiont community turnover supports findings from previous theoretical and experimental studies (e.g. Buddemeier & Fautin, 1993; Baker, 2001; Toller *et al*., 2001; Buddemeier *et al*., 2004; Fautin & Buddemeier, 2004; Berkelmans & van Oppen, 2006; Jones *et al*., 2008), including a previous study of Indo-Pacific *Acropora*, which found that corals increased their thermotolerance by 1–1.5 °C after bleaching and changing from clade C to clade D-dominated *Symbiodinium* communities (Berkelmans & van Oppen, 2006).

Disturbance (bleaching) also increased diversity within *Symbiodinium* communities, changing them from monocultures of C3 to mixed communities of C3 + D1a postrecovery. The increase in community diversity after disturbance also implies that these taxa differ in their

relative competitive and/or colonization abilities (Connell, 1978; Tilman, 1994). Some members of clade D (e.g. D1a) may have opportunistic physiological traits (as reviewed in Stat & Gates, 2010), which allow them to colonize tissues first after bleaching, but which also result in inferior competitive ability under benign conditions. This may lead to a reversion away from opportunistic symbiont types during long periods of 'undisturbed' conditions (Chen *et al.*, 2005; Thornhill *et al.*, 2006a; LaJeunesse *et al.*, 2009). After 6 months, for example, we observed community reversion back toward a C3-dominated community in bleached corals that were never exposed to heat (DCMU bleached and recovery at 24 °C, Figs 1,3,4), unlike the consistently high (>99%) or increasing clade D abundance after initial heat exposure , 29 °C recovery conditions, and/or repeated bleaching (Fig. 3). These results suggest that, with frequent disturbance or sustained warming, opportunists and colonizers may be favored, resulting in the proliferation of stress-tolerant, but potentially less efficient, partnerships involving *Symbiodinium* in clade D. Such transitions may already be occurring on stressed reefs (Baker *et al.*, 2004).

Our finding that corals recovered with overall higher symbiont population levels post-bleaching (Fig. 4) is consistent with other studies (Kemp *et al.*, 2014). Kemp *et al.*, 2014 hypothesizes that this could be due to dysfunction in host–symbiont regulation due to high levels of intracellular, inorganic nutrients that accumulated after a period of low symbiont abundance during bleaching. Clade D population levels (or possibly any post-bleaching symbiont communities) could be harder for the host to control; alternatively, the host may require more of the inefficient clade D symbionts to meet its energetic needs (See below 'Tradeoffs to hosting stress-tolerant symbionts'). Both explanations could potentially result in higher symbiont abundance post-bleaching.

Few 'warm control' corals hosted any D1a symbionts after 3 months at 29 °C, but most of these corals did host some clade D symbionts after 6 months (albeit not dominated by clade D). However, it is not known whether thermotolerance would have been observed in these corals because they were not heat stressed at the 6 month time point. Future studies should examine thresholds at which accumulated D1a symbionts result in emergent thermotolerant traits.

### *Clade D abundance increases with heat exposure*

Even among bleached corals, clade D *Symbiodinium* were present at greater abundances within colonies that were exposed to elevated temperatures, either during bleaching or recovery (i.e. heat bleaching and/or recovered at 29 °C; Fig. 1,3,4). This indicates that all bleaching events may not result in the same symbiont community reassembly outcomes. Rather than being only a response to vacant niche space created during bleaching, D1a proliferation instead appears to be influenced by environmental conditions, both during and after stress.

Wooldridge, 2012 proposed a model of symbiont shuffling based on symbiont growth rates (mitotic index, MI), which are positively correlated with temperature (Strychar *et al.*, 2004). Corals may favor slow growing symbionts (low MI, such as clade D) in higher temperatures, possibly as a way to regulate symbiont population levels (Baghadasarian & Muscatine, 2000), and resulting in the observed high clade D abundance in heat-bleached and 29 °C-acclimated corals. Higher abundances of clade D present after heat exposure could be due to: (i) the enhanced ability of clade D symbionts to invade heat-bleached tissues or proliferate in warm environments; (ii) the decreased ability of clade C symbionts to thrive in heat-bleached tissues or warm environments, altering the competitive landscape between clade C and D symbionts (e.g. Rowan, 2004); (iii) a decrease in the host's ability to regulate symbiont populations (e.g. Wooldridge, 2012; Kemp *et al.*, 2014), or (iv) a decreased ability for corals to recognize symbiont partners on a molecular level after heat stress, allowing opportunistic (clade D) symbionts to proliferate (sensu Wood-Charlson *et al.*, 2006).

### *Previously undetectable symbionts can become dominant*

This experiment does not distinguish, in absolute terms, whether the D1a *Symbiodinium* that became dominant after bleaching were acquired from the environment, or were already present in corals at extreme low abundance. Regardless of the source, these data do show that symbionts that may be difficult or impossible to detect prior to stress, even using high-sensitivity techniques, can be critical components of coral recovery. LaJeunesse *et al.*, (2009) also found that clade D symbionts in *M. cavernosa* were undetectable by qPCR prior to a bleaching event, but became dominant community members 4–10 months post-bleaching. These studies suggest that *M. cavernosa* either routinely hosts clade D symbionts at low abundance, perhaps in spatially isolated pockets of tissue that were not sampled by either study, or are readily capable of acquiring clade D from the environment. Future studies should attempt to tease apart questions of newly acquired symbiont sources in the field (see Lewis & Coffroth, 2004 for a study on octocorals; see Coffroth *et al.*, 2010 for a lab study). Either way, this underappreciated newly-acquired symbiotic potential (Hoegh-Guldberg *et al.*, 2007) should continue to be studied and included

in estimates of corals' adaptive potential to changing environments, including warming oceans (Baskett *et al.*, 2009; Logan *et al.*).

### *Increased thermotolerance is due to symbiont identity, not thermal history*

By comparing high and low temperature bleaching, as well as warm and cool recovery/acclimation temperatures, we were able to demonstrate that the observed increase in thermotolerance was due to changes in symbiont identity, rather than exposure to prior heat stress or warm acclimation. Because warm acclimated corals (29 °C) experienced a less severe thermal elevation to reach heat stress temperatures (32 °C) than cool acclimated corals (24 °C), we had expected that they might be less affected by the acute heat exposure. This was not the case, however. Instead, it seems that the 3 month exposure to constant, warm temperatures may have acted as a mild stressor, which exhausted corals' and/or symbionts' capacity to mitigate future thermal stress, ultimately resulting in more severe bleaching (Figs 2, 5d). In addition, higher recovery temperatures and potentially associated, faster symbiont division rates may have hindered symbiont population control by the host, resulting in a more severe breakdown of symbiosis during stress (Wooldridge, 2012). The rapid recovery of Fv/Fm values in symbionts at 29 °C seems to support this idea, indicating faster proliferation or metabolic rates at elevated temperatures (Fig. 2).

Previous studies report mixed effects of chronic and/or punctuated preacclimation on holobiont thermal tolerance. Corals preconditioned for a short time (days to weeks) have been shown to avoid bleaching via upregulation of physiological mechanisms to deal with stress (Brown, 2002; Middlebrook *et al.*, 2008; Bellantuono *et al.*, 2011). Others report that, as in this study, preconditioning to stress (without symbiont change) did not improve, or even lowered, stress tolerance (Saxby *et al.*, 2001; Berkelmans & van Oppen, 2006; Middlebrook *et al.*, 2012). Thermal variability, rather than chronic temperature elevation, may more effectively promote acclimatization responses in corals and symbionts (McClanahan *et al.*, 2007; Oliver & Palumbi, 2011). Future studies should seek to measure the abundance, longevity, and upregulation of cellular acclimatization mechanisms (e.g. heat shock proteins) in both the host and symbiont during longer term, warm acclimations (e.g. Abrego *et al.*, 2008).

### *Tradeoffs to hosting stress-tolerant symbionts*

Cost–benefit ratios within symbiotic partnerships may be altered by climate change (Kiers *et al.*, 2010). In coral–algal symbiosis, symbionts within clade D may allow corals to avoid bleaching or aposymbiosis during stress, but have also been linked to less-efficient translocation of sugars (Cantin *et al.*, 2009), slower coral growth (Little *et al.*, 2004; Jones & Berkelmans, 2010; Gillette, 2012), and lower fecundity (Jones & Berkelmans, 2011). Therefore, while some members of clade D provide a clear benefit to coral hosts during stress, they are also characterized as 'selfish opportunists' and a potentially 'ominous sign' for corals (Stat & Gates, 2010 for review). This has provoked recent cost–benefit analyses of corals hosting clade D symbionts (Jones & Berkelmans, 2010, 2011; Grottoli *et al.*, 2012; Ortiz *et al.*, 2012). In some studies, even in warm conditions, corals hosting clade D symbionts exhibit slower growth and decreased lipid stores (Little *et al.*, 2004; Jones & Berkelmans, 2011). In other studies, however, differences in growth rates between clade C- and clade D-dominated corals decrease or disappear at the higher temperatures where thermotolerant symbionts may have an advantage. For example, in laboratory studies of *Pocillopora damicornis*, growth benefits at 26 °C for corals hosting C1b-c over D1 symbionts disappear at 30 °C (Gillette, 2012; Cunning, 2014). Tradeoffs among different symbiont types are therefore likely to be environmentally dependent, and resulting cost–benefit analyses may continue to shift under climate change conditions. We suggest that symbiotic plasticity generally, and community shifts in coral–algal symbioses specifically, might be considered all of the following, depending on context: (i) an adaptive response, (ii) a proliferation of a selfish partner, and (iii) a neutral consequence following environmental disturbance (Wernegreen & Wheeler, 2009).

### *Symbiotic flexibility and thermotolerance in other systems*

In addition to reef corals, there are several other examples of flexible mutualisms that promote host survival during stress. For example, pea aphids (*Acyrthosiphon pisum*) typically associate with a primary bacterial symbiont, but 35–80% of hosts also contain a secondary bacterial symbiont which, under normal conditions, can be detrimental to host fitness (Koga *et al.*, 2003). If the primary symbiont is removed, however, the secondary, low-abundance symbiont can proliferate within the aphid and assume the general function of the primary symbiont (Koga *et al.*, 2003). Secondary symbionts also confer increased thermotolerance to aphids exposed to heat stress (Montllor *et al.*, 2002; Russell & Moran, 2006). In some cases, relationships are environmentally mediated, for example, in the mutualism between bark beetles (*Dendroctonus ponderosae*), and two species of

fungi, *Grosmannia clavigera* and *Ophiostoma montium* (Six & Bentz, 2007; Kiers *et al*., 2010). These fungi supplement their hosts' diet, and there is some evidence that the fungi have differential effects on beetle fitness (Six, 1998). The relative dominance of the fungal species within *D. ponderosae* also appears to be mediated by temperature. As most fungi have relatively limited temperature ranges, hosting 'cool' and 'warm' species of fungi help beetles to expand their environmental ranges (Six & Bentz, 2007).

As with corals, these hosts routinely associate with multiple symbiotic partners, sometimes undergoing shifts in dominance, with varying effects on host fitness. For corals, hosting multiple *Symbiodinium* types may provide similar functional redundancy (Yachi & Loreau, 1999) and realized thermal niche expansion, increasing the likelihood that at least one symbiosis will be preserved during stress. Even if the remaining symbiont confers a lesser or suboptimal partnership, the host may still benefit overall by avoiding potential mortality resulting from aposymbiosis (Six & Bentz, 2007).

### *Coral–algal symbiosis in a changing world*

We used coral–algal symbiosis as a model to investigate how symbionts can expand the realized thermal niches of their hosts and rapidly increase resilience and resistance during and after stress. Our study also confirms previous reports, using photochemical data from symbionts in hospite, that the *Symbiodinium* ITS2 type D1a, a common and widespread symbiont, is thermotolerant, performs well at high temperature, and is resistant to bleaching, compared to other *Symbiodinium* types, such as C3 (Wang *et al*., 2012; Kemp *et al*., 2014). Furthermore, by using different recovery temperatures and bleaching stressors, we accounted for the effect of prior heat exposure as a confounding factor in the acquisition of thermotolerant traits. This allowed us to identify changes in the symbiotic community post-bleaching as the primary mechanism underlying the increased heat tolerance that we observed.

Using high-sensitivity qPCR techniques, which also quantified intra- and interclade symbiont dynamics over time, we showed symbionts that are extremely rare, or absent, in corals prior to bleaching can become dominant after disturbance and influence overall host physiology. Therefore, studies that sample only healthy corals at a single time point, even using qPCR, may be underestimating the full range of possible symbiotic associations – and the adaptive potential that this represents. This study also supports prior hypotheses regarding the critical role of disturbance in promoting beneficial symbiont community change (Buddemeier & Fautin, 1993).

Additional stressors that combine with high temperatures to exacerbate coral decline, such as ocean acidification, nutrient pollution, and disease, may still limit the extent to which corals can persist, even when undergoing adaptive symbiont community shifts (e.g. Howells *et al*., 2013). The ability of reef corals to exploit latent functional redundancy in their symbiont communities is also highly likely to be site-, species- and region-dependent (Pandolfi *et al*., 2011; Baker *et al*., 2013; Howells *et al*., 2013). Symbiont community changes do not necessarily occur after every bleaching event, and some host species appear more readily able to partner with diverse symbionts (Toller *et al*., 2001; Thornhill *et al*., 2006b; Stat *et al*., 2009; McGinley *et al*., 2012). However, where it does occur, symbiont community changes may grant corals some extended survivorship, hopefully in time for global threats to be addressed and for the effects of climate change to be mitigated. Symbiotic flexibility as a potential source of adaptive potential, therefore, should continue to be examined (Baker, 2001; Baker *et al*., 2004; Berkelmans & van Oppen, 2006; Jones *et al*., 2008; Howells *et al*., 2013) and to be incorporated into future assessments of corals' ability to persist in climate change conditions (Baskett *et al*., 2009; Logan *et al*., 2013).

## Acknowledgements

We thank X. Serrano for genotyping the *M. cavernosa*, N. Formel, K. Montenero, K. Ondrasik, Z. Schwartz, P. Jones, R. Winter, R. Okazaki, L. Gordon, K. Dziedzic, and N. Guy for help with aquaria maintenance and assistance with PAM fluorometry, and D. Thornhill for comments on the manuscript. R.N.S and R.C. were funded by Graduate Research Fellowships from the National Science Foundation, and by University of Miami Graduate Fellowships. R.N.S. is also funded by the RSMAS Alumni Award, the Captain Harry D. Vernon Memorial Scholarship, The Garden Club of America Ecological Restoration Fellowship, the Reitmeister Award, and the Rowlands Research Fellowship. A.C.B was funded by NSF (OCE-0547169), the Wildlife Conservation Society, the Lenfest Ocean Program and a Pew Fellowship in Marine Conservation.

## References

Abrego D, Ulstrup K, willis B, van Oppen MJH (2008) Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 2273–2282.

Baghadasarian G, Muscatine L (2000) Preferential expulsion of dividing algal cells as a mechanism for regulating algal-cnidarian symbiosis. *Biological Bulletin*, **199**, 278–286.

Baker AC (2001) Reef corals bleach to survive change. *Nature*, **411**, 765–766.

Baker AC (2003) Flexibility and specificity in coral-algal symbiosis, diversity, ecology and biogeography of *Symbiodinium*. *Annual Review of Ecology, Evolution, and Systematics*, **4**, 661–689.

Baker AC (2004) Symbiont diversity on coral reefs and its relationship to bleaching resistance and resilience. In: *Coral Health and Disease* (eds Rosenberg E, Loya Y), pp. 177–191. Springer-Verlag, Berlin.

Baker AC, Romanski AM (2007) Multiple symbiotic partnerships are common in scleractinian corals, but not in octocorals: comment on Goulet (2006). *Marine Ecology Progress Series*, **335**, 237–242.

Baker AC, Rowan R (1997) Diversity of symbiotic dinoflagellates (zooxanthellae) in scleractinian corals of the Caribbean and eastern Pacific. *Proceedings of the 8th International Coral Reef Symposium*, **2**, 1301–1306.

Baker AC, Starger CJ, McClanahan TR, Glynn PW (2004) Coral reefs: corals' adaptive response to climate change. *Nature*, **430**, 741–741.

Baker AC, McClanahan TR, Starger CJ, Boonstra RK (2013) Long-term monitoring of algal symbiont communities in corals reveals stability is taxon dependent and driven by site-specific thermal regime. *Marine Ecology Progress Series*, **479**, 85–97.

Baskett M, Gaines S, Nisbet R (2009) Symbiont diversity may help coral reefs survive moderate climate change. *Ecological Applications*, **19**, 3–17.

Bellantuono AJ, Hoegh-Guldberg O, Rodriguez-Lanetty M (2011) Resistance to thermal stress in corals without changes in symbiont composition. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 1100–1107.

Berkelmans R, van Oppen MJH (2006) The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 2305–2312.

Bowyer JR, Camilleri P, Vermaas W (1991) *Photosystem II and its interaction with herbicides*. Elsevier Science Publishers BV, Amsterdam, the Netherland, 59. pp.

Brown B (2002) Exploring the basis of thermotolerance in the reef coral *Goniastrea aspera*. *Marine Ecology Progress Series*, **242**, 119–129.

Bruno JF, Selig ER (2007) Regional decline of coral cover in the Indo-Pacific: timing, extent, and subregional comparisons. *PLoS ONE*, **2**, e711.

Buddemeier RW, Fautin DG (1993) Coral bleaching as an adaptive mechanism. *BioScience*, **43**, 320–326.

Buddemeier RW, Baker AC, Fautin DG, Jacobs JR (2004) The adaptive hypothesis of bleaching. In: *Coral Health and Disease* (ed. Rosenberg E), pp. 427–444. Springer-Verlag, Berlin.

Cantin NE, van Oppen MJH, Willis BL, Mieog JC, Negri AP (2009) Juvenile corals can acquire more carbon from high-performance algal symbionts. *Coral Reefs*, **28**, 405–414.

Chen A, Wang JT, Fang LS, Yang YW (2005) Fluctuating algal symbiont communities in *Acropora palifera* (Scleractinia: Acroporidae) from Taiwan. *Marine Ecology Progress Series*, **295**, 113–121.

Coffroth MA, Poland DM, Petrou EL, Brazeau DA, Holmberg JC, Thompson R (2010) Environmental symbiont acquisition may not be the solution to warming seas for reef-building corals. *PLoS ONE*, **5**, e13258.

Connell JH (1978) Diversity in tropical rain forests and coral reefs. *Science*, **199**, 1302–1310.

Correa AMS, Baker AC (2009) Understanding diversity in coral-algal symbiosis: a cluster-based approach to interpreting fine-scale genetic variation in the genus *Symbiodinium*. *Coral Reefs*, **28**, 81–93.

Correa AMS, McDonald MD, Baker AC (2009) Development of clade-specific *Symbiodinium* primers for quantitative PCR (qPCR) and their application to detecting clade D symbionts in Caribbean corals. *Marine Biology*, **156**, 2403–2411.

Cunning R, Baker AC (2013) Excess algal symbionts increase the susceptibility of reef corals to bleaching. *Nature Climate Change*, **2**, 1–4.

Cunning R (2014) Not Just Who, But How Many: The Importance of Partner Abundance in Reef Coral Symbioses. Doctoral Dissertation. University of Miami, Miami, FL.

Donner SD, Skirving WJ, Little CM, Oppenheimer M, Hoegh-Guldberg O (2005) Global assessment of coral bleaching and required rates of adaptation under climate change. *Global Change Biology*, **11**, 2251–2265.

Fautin DG, Buddemeier RW (2004) Adaptive bleaching: a general phenomenon. *Hydrobiologia*, **530**, 459–467.

Gardner TA (2003) Long-term region-wide declines in Caribbean corals. *Science*, **301**, 958–960.

Gillette P (2012) Genetic variation in thermal tolerance in the coral *Pocillopora damicornis* and its effects on growth, photosynthesis and survival. University of Miami Master's Thesis.

Goulet T (2006) Most corals may not change their symbionts. *Marine Ecology Progress Series*, **321**, 1–7.

Grottoli A, Warner ME, Levas S, Scheopf V, Aschaffenburg M, McGinley M, Matsui Y (2012) What combinations of coral species and *Symbiodinium* are more resilient to repetitive bleaching? 12th International Coral Reef Symposium.

Hill M, Hill A (2012) The magnesium inhibition and arrested phagosome hypotheses: new perspectives on the evolution and ecology of *Symbiodinium* symbioses. *Biological Reviews*, **87**, 804–821.

Hoegh-Guldberg O (1999) Coral bleaching, climate change and the future of the world's coral reefs. *Marine and Freshwater Research*, **50**, 839–866.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ *et al.* (2007) Coral reefs under rapid climate change and ocean acidification. *Science*, **318**, 1737–1742.

Howells EJ, Berkelmans R, van Oppen MJH, Willis B, Bay LK (2013) Historical thermal regimes define limits to coral acclimatization. *Ecology*, **94**, 1078–1088.

Jones R (2004) Testing the 'photoinhibition' model of coral bleaching using chemical inhibitors. *Marine Ecology Progress Series*, **284**, 133–145.

Jones P (2014) Biotic and Abiotic Influences on Algal Symbiont (Symbiodinium spp.) Community Dynamics in Reef Corals Recovering from Disturbance. Open Access Dissertations. Paper 1172.

Jones A, Berkelmans R (2010) Potential costs of acclimatization to a warmer climate: growth of a reef coral with heat tolerant vs. sensitive symbiont types (ed. DeSalle R). *PLoS ONE*, **5**, e10437.

Jones AM, Berkelmans R (2011) Tradeoffs to Thermal Acclimation: energetics and Reproduction of a Reef Coral with Heat Tolerant *Symbiodinium* Type-D. *Journal of Marine Biology*, **2011**, 1–12.

Jones AM, Berkelmans R, van Oppen MJH, Mieog JC, Sinclair W (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proceedings of the Royal Society B: Biological Sciences*, **275**, 1359–1365.

Kemp DW, Hernandez-Pech X, Iglesias-Prieto R, Fitt WK, Schmidt GW (2014) Community dynamics and physiology of *Symbiodinium* spp. before, during, and after a coral beaching event. *Limnology and Oceanography*, **59**, 788–797.

Kiers ET, Palmer TM, Ives AR, Bruno JF, Bronstein JL (2010) Mutualisms in a changing world: an evolutionary perspective. *Ecology Letters*, **13**, 1459–1474.

Koga R, Tsuchida T, Fukatsu T (2003) Changing partners in an obligate symbiosis: a facultative endosymbiont can compensate for loss of the essential endosymbiont *Buchnera* in an aphid. *Proceedings of the Royal society of London. Series B. Biological Sciences*, **270**, 2543–2550.

LaJeunesse TC (2001) Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: in search of a "species" level marker. *Journal of Phycology*, **37**, 866–880.

LaJeunesse TC, Smith RT, Finney J, Oxenford H (2009) Outbreak and persistence of opportunistic symbiotic dinoflagellates during the 2005 Caribbean mass coral 'bleaching' event. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 4139–4148.

LaJeunesse TC, Smith R, Walther M *et al.* (2010a) Host-symbiont recombination versus natural selection in the response of coral-dinoflagellate symbioses to environmental disturbance. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 2925–2934.

LaJeunesse TC, Pettay DT, Sampayo EM *et al.* (2010b) Long-standing environmental conditions, geographic isolation and host-symbiont specificity influence the relative ecological dominance and genetic diversification of coral endosymbionts in the genus *Symbiodinium*. *Journal of Biogeography*, **37**, 785–800.

LaJeunesse TC, Wham DC, Pettay DT, Parkinson JE, Keshavmurthy S, Chen CA (2014) Ecologically differentiated stress-tolerant endosymbionts in the dinoflagellate genus *Symbiodinium* (Dinophyceae) Clade D are different species. *Phycologia*, **53**, 305–319.

Lewis C, Coffroth MA (2004) The acquisition of exogenous algal symbionts by an octocoral after bleaching. *Science*, **304**, 1490–1492.

Little AF, van Oppen MJH, Willis BL (2004) Flexibility in algal endosymbioses shapes growth in reef corals. *Science*, **304**, 1492–1494.

Logan CA, Dunne JP, Eakin CM, Donner SD (2013) Incorporating adaptive responses into future projections of coral bleaching. *Global Change Biology*, **20**, 125–139.

McClanahan TR, Ateweberhan M, Muhando CA, Maina J, Mohammed MS (2007) Effects of climate and seawater temperature variation on coral bleaching and mortality. *Ecological Monographs*, **77**, 503–525.

McGinley MP, Aschaffenburg MD, Pettay DT, Smith RT, LaJeunesse TC, Warner ME (2012) *Symbiodinium* spp. in colonies of eastern Pacific *Pocillopora* spp. are highly stable despite the prevalence of low-abundance background populations. *Marine Ecology Progress Series*, **462**, 1–7.

Middlebrook R, Hoegh-Guldberg O, Leggat W (2008) The effect of thermal history on the susceptibility of reef-building corals to thermal stress. *Journal of Experimental Biology*, **211**, 1050–1056.

Middlebrook R, Anthony KRN, Hoegh-Guldberg O, Dove S (2012) Thermal priming affects symbiont photosynthesis but does not alter bleaching susceptibility in *Acropora millepora*. *Journal of Experimental Marine Biology and Ecology*, **432–433**, 64–72.

Mieog J, van Oppen MJH, Cantin N (2007) Real-time PCR reveals a high incidence of *Symbiodinium* clade D at low levels in four scleractinian corals across the Great Barrier Reef: implications for symbiont shuffling. *Coral Reefs*, **26**, 449–457.

Mieog JC, van Oppen MJH, Berkelmans R, Stam WT (2009) Quantification of algal endosymbionts (*Symbiodinium*) in coral tissue using real-time PCR. *Molecular Ecology Resources*, **9**, 74–82.

Montllor CB, Maxmen A, Purcell AH (2002) Facultative bacterial endosymbionts benefit pea aphids *Acyrthosiphon pisum* under heat stress. *Ecological Entomology*, **27**, 189–195.

Oliver TA, Palumbi SR (2011) Do fluctuating temperature environments elevate coral thermal tolerance? *Coral Reefs*, **30**, 429–440.

Ortiz JC, González-Rivero M, Mumby PJ (2012) Can a thermally tolerant symbiont improve the future of Caribbean coral reefs? *Global Change Biology*, **19**, 273–281.

Pandolfi JM, Connolly SR, Marshall DJ, Cohen AL (2011) Projecting coral reef futures under global warming and ocean acidification. *Science*, **333**, 418–422.

Pochon X, Gates RD (2010) A new *Symbiodinium* clade (Dinophyceae) from soritid foraminifera in Hawai'i. *Molecular Phylogenetics and Evolution*, **56**, 492–497.

Rowan R (2004) Thermal adaptation in reef coral symbionts. *Nature*, **430**, 742–742.

Russell JA, Moran NA (2006) Costs and benefits of symbiont infection in aphids: variation among symbionts and across temperatures. *Proceedings of the Royal Society B: Biological Sciences*, **273**, 603–610.

Saxby T, Dennison W, Hoegh-Guldberg O (2001) Photosynthetic responses of the coral Montipora digitata to cold temperature stress. Thesis, University of Queensland.

Serrano X, Baums IB, O'Reilly K *et al.* (2014) Geographic differences in vertical connectivity in the Caribbean coral *Montastraea cavernosa* despite high levels of horizontal connectivity at shallow depths. (accepted). *Molecular Ecology*, **23**, 4226–4240.

Silverstein RN, Correa AMS, LaJeunesse TC, Baker AC (2011) Novel algal symbiont (*Symbiodinium* spp.) diversity in reef corals of Western Australia. *Marine Ecology Progress Series*, **422**, 63–75.

Silverstein RN, Correa AMS, Baker AC (2012) Specificity is rarely absolute in coral–algal symbiosis: implications for coral response to climate change. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 2609–2618.

Six DL (1998) Effects of mycangial fungi and host tree species on progeny survival and emergence of *Dendroctonus ponderosae* (Coleoptera: Scolytidae). *Environmental Entomology*, **27**, 1391–1401.

Six DL, Bentz BJ (2007) Temperature determines symbiont abundance in a multipartite bark beetle-fungus ectosymbiosis. *Microbial Ecology*, **54**, 112–118.

Stat M, Gates RD (2010) Clade D *Symbiodinium* in Scleractinian Corals: a 'Nugget' of Hope, a selfish opportunist, an ominous sign, or all of the above? *Journal of Marine Biology*, **2011**, Article ID 730715.

Stat M, Loh WKW, LaJeunesse TC, Hoegh-Guldberg O, Carter DA (2009) Stability of coral–endosymbiont associations during and after a thermal stress event in the southern Great Barrier Reef. *Coral Reefs*, **28**, 709–713.

Strychar KB, Coates M, Sammarco PW (2004) Loss of *Symbiodinium* from bleached Australian scleractinian corals (*Acropora hyacinthus, Favites complanata* and *Porites solida*). *Marine and Freshwater Research*, **55**, 135–144.

Thornhill D, LaJeunesse T, Kemp D, Fitt W (2006a) Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. *Marine Biology*, **148**, 711–722.

Thornhill DJ, Fitt WK, Schmidt GW (2006b) Highly stable symbioses among western Atlantic brooding corals. *Coral Reefs*, **25**, 515–519.

Thornhill DJ, Lewis AM, Wham DC, LaJeunesse TC (2013) Host-Specialist lineages dominate the adaptive radiation of reef coral endosymbionts. *Evolution*, **68**, 352–367.

Tilman D (1994) Competition and biodiversity in spatially structured habitats. *Ecology*, **75**, 2–16.

Toller WW, Rowan R, Knowlton N (2001) Repopulation of zooxanthellae in the Caribbean corals *Montastraea annularis* and *M. faveolata* following experimental and disease-associated bleaching. *Biological Bulletin*, **201**, 360–373.

Tonk L, Bongaerts P, Sampayo EM, Hoegh-Guldberg O (2013) SymbioGBR: a web-based database of *Symbiodinium* associated with cnidarian hosts on the Great Barrier Reef. *BMC Ecology*, **13**, 7.

Wang J-T, Chen Y-Y, Chen CA, Meng P-J (2012) Determination of the thermal tolerance of *Symbiodinium* using the activation energy for inhibiting photosystem II activity. *Zoological Studies*, **51**, 137–142.

Weis VM (2008) Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. *Journal of Experimental Biology*, **211**, 3059–3066.

Wernegreen JJ, Wheeler DE (2009) Remaining flexible in old alliances: functional plasticity in constrained mutualisms. *DNA and Cell Biology*, **28**, 371–381.

Wood-Charlson EM, Hollingsworth LL, Krupp DA, Weis VM (2006) Lectin/glycan interactions play a role in recognition in a coral/dinoflagellate symbiosis. *Cellular Microbiology*, **8**, 1985–1993.

Wooldridge SA (2012) Breakdown of the coral-algae symbiosis: towards formalising a linkage between warm-water bleaching thresholds and the growth rate of the intracellular zooxanthellae. *Biogeosciences Discussions*, **9**, 8111–8139.

Yachi S, Loreau M (1999) Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis. *Proceedings of the National Academy of Sciences*, **96**, 1463–1468.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Diagram of the DCMU-exposure tank setup.
**Table S1.** Statistically significant differences in symbiont communities after 6 months of recovery.
**Table S2.** Distribution of host genotypes between treatment groups.
**Table S3.** Mean HOBO-recorded temperatures in tanks.
**Table S4.** Microsatellite markers used to identify Montrastraea cavernosa genotypes.