Global Change Biology

Global Change Biology (2014), doi: 10.1111/gcb.12658

The cumulative impact of annual coral bleaching can turn some coral species winners into losers

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Abstract

Mass coral bleaching events caused by elevated seawater temperatures result in extensive coral loss throughout the tropics, and are projected to increase in frequency and severity. If bleaching becomes an annual event later in this century, more than 90% of coral reefs worldwide may be at risk of long-term degradation. While corals can recover from single isolated bleaching and can acclimate to recurring bleaching events that are separated by multiple years, it is currently unknown if and how they will survive and possibly acclimatize to annual coral bleaching. Here, we demonstrate for the first time that annual coral bleaching can dramatically alter thermal tolerance in Caribbean corals. We found that high coral energy reserves and changes in the dominant algal endosymbiont type (*Symbiodinium* spp.) facilitated rapid acclimation in *Porites divaricata*, whereas low energy reserves and a lack of algal phenotypic plasticity significantly increased susceptibility in *Porites astreoides* to bleaching the following year. Phenotypic plasticity in the dominant endosymbiont type of *Orbicella faveolata* did not prevent repeat bleaching, but may have facilitated rapid recovery. Thus, coral holobiont response to an isolated single bleaching event is not an accurate predictor of its response to bleaching the following year. Rather, the cumulative impact of annual coral bleaching can turn some coral species 'winners' into 'losers', and can also facilitate acclimation and turn some coral species 'losers' into 'winners'. Overall, these findings indicate that cumulative impact of annual coral bleaching could result in some species becoming increasingly susceptible to bleaching and face a long-term decline, while phenotypically plastic coral species will acclimatize and persist. Thus, annual coral bleaching and recovery could contribute to the selective loss of coral diversity as well as the overall decline of coral reefs in the Caribbean.

Keywords: annual coral bleaching, energy reserves, *Symbiodinium* type, winners and losers

Received 21 February 2014; revised version received 15 May 2014 and accepted 19 May 2014

Introduction

Tropical coral reefs are presently in serious decline due to global warming (Hoegh-Guldberg, 1999; Eakin *et al.*, 2009; Veron *et al.*, 2009) and other stressors (Veron *et al.*, 2009; Fabricius, 2011; Hughes *et al.*, 2011). At elevated seawater temperatures, many scleractinian corals lose substantial numbers of their photosynthetic endosymbiotic dinoflagellates (*Symbiodinium* spp.) giving the colony a pale (hence bleached) appearance and often resulting in mortality. Bleaching susceptibility varies among species, depths, and locations (e.g. Fisk & Done, 1985; Marshall & Baird, 2000; Stimson *et al.*, 2002; Grottoli *et al.*, 2004), and is influenced by coral morphology (Wilkinson & Hodgson, 1999; Loya *et al.*, 2001), the physiological response of both the animal and the endosymbionts (Grottoli *et al.*, 2006; Rodrigues & Grottoli, 2007; Fitt *et al.*, 2009; Dunn *et al.*, 2012), and the type(s) of endosymbionts harbored by the coral (e.g. Warner *et al.*, 1999; Glynn *et al.*, 2001; Howells *et al.*, 2011). Factors associated with the ability to recover from bleaching include coral energy reserves (Rodrigues & Grottoli, 2007; Anthony *et al.*, 2009) and heterotrophic feeding capacity (Grottoli *et al.*, 2006; Houlbreque & Ferrier-Pagès, 2009; Levas *et al.*, 2013).

Symbiodinium can provide up to 100% of a healthy coral's daily fixed carbon requirements (Muscatine *et al.*, 1981; Grottoli *et al.*, 2006). However following bleaching, recovering corals may rely heavily on

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alternate sources of fixed carbon acquired via catabolism of energy reserves (Fitt *et al.*, 2000; Rodrigues & Grottoli, 2007) and/or increased heterotrophy (Grottoli *et al.*, 2006; Palardy *et al.*, 2008; Levas *et al.*, 2013). In addition, the distribution of certain types of *Symbiodinium* can change substantially post bleaching via internal shuffling or possible acquisition of new algae (Rowan *et al.*, 1997; Jones & Berkelmans, 2010). In particular, studies have shown that many *Symbiodinium* within the D-lineage are thermally tolerant and persist during bleaching (Baker *et al.*, 2004; Berkelmans & Van Oppen, 2006; LaJeunesse *et al.*, 2009).

At the current rate of CO_2 emissions, models predict that reefs globally will experience annual bleaching events by 2040, and that parts of the Caribbean and tropical western Pacific will experience annual bleaching as soon as 2025 (Van Hooidonk *et al.*, 2013). Yet, it is unknown if corals can acclimatize to such rapid increases in bleaching frequency, or if the traits that confer resilience to single isolated bleaching also impart resilience to annually recurring bleaching. While thermal preconditioning may reduce bleaching susceptibility (Middlebrook *et al.*, 2008; Bellantuono *et al.*, 2012; Guest *et al.*, 2012; Maynard *et al.*, 2012; McClanahan & Muthiga, 2014), these studies to date have focused only on the outcome of single isolated bleaching events or on natural bleaching events that are separated by several years. However, if recovery from single bleaching takes longer than 1 year, annual bleaching may overwhelm the capacity of corals to recover between bleaching events unless they are able to rapidly acclimate to yearly thermal stress. For the first time, we experimentally simulated annually recurring coral bleaching on ecologically relevant time scales. We hypothesized that the coral holobiont (i.e. the animal and endosymbiont) could survive and acclimate to consecutive annual bleaching. We define acclimation as the improved performance of the physiological variables measured here from being significantly lower than the control corals after bleaching to being no different from controls after bleaching the following year.

The hypothesis was tested using the Caribbean corals *Porites divaricata*, *Porites astreoides*, and *Orbicella faveolata*, with a period of short-term elevated temperature exposure in two consecutive summers followed by recovery on the reef. Endosymbiont density, endosymbiont type, total energy reserves, and calcification rates were assessed immediately after the elevated temperature exposure and again after 6 weeks on the reef at ambient temperature each year. In addition, the carbon acquired via photosynthesis and heterotrophy relative to respiratory demand was calculated immediately after bleaching each year as well.

Materials and methods

Experimental design

In July 2009, 10 coral fragments from nine healthy colonies of *Porites divaricata* (branching morphology), *Porites astreoides* (mounding/encrusting morphology), and *Orbicella faveolata* (formerly *Montastraea faveolata* (Budd *et al.*, 2012)) (large, mounding morphology) were collected near Puerto Morelos, Mexico (20°50′N, 86°52′W region, see details in Table S1). Fragments were mounted on labeled PVC tiles, and randomly placed in 10 shaded (600 μmol photons m^{-2} s^{-1}) outdoor flow-through seawater tanks, allowed to acclimate for 5 days, and then buoyantly weighed (Fig. 1a). The temperature in five tanks was gradually raised to 31.5 °C ± 0.20 (single bleaching treatment) over 7 days then maintained at the elevated temperature, while the other five tanks received ambient reef water (controls; 30.6 °C ± 0.24) (Fig. 2a, b). After 15 days, one treatment and one control fragment from each parent colony of each species were buoyantly weighed and then frozen at −80 °C (0 weeks on the reef) (Fig. 1a). The remaining fragments were transplanted back to the reef at 4.9 m depth (20°52.815′N, 86°50.989′W) (Fig. 1a). After 6 weeks on the reef, one additional treatment and control fragment from each colony of each species were collected, buoyantly weighed, and frozen at −80 °C, while the remaining fragments stayed on the reef for a full year at ambient reef temperatures (Figs 1a and 2a).

The following summer (2010), the experiment was repeated with the treatment corals from the previous year exposed to elevated temperatures again (31.6 °C ± 0.24) (repeat bleaching treatment) while the control fragments from 2009 maintained at ambient temperature (30.4 °C ± 0.23) (Figs 1a and 2a, c). After 17 days (0 weeks on the reef), one treatment and one control fragment from each colony of each species were buoyantly weighted, then frozen at −80 °C (Fig. 1a). A second treatment and control fragment from each colony of each species were used to quantify photosynthesis, respiration, and feeding rates (see Photosynthesis, respiration, and feeding section) (Fig. 1a). The remaining fragments were returned to the reef for 6 weeks then collected, buoyantly weighed, and frozen at −80 °C (Fig. 1a). All frozen samples were analyzed for endosymbiontic algal concentration, *Symbiodinium* type identification, and total energy reserve concentration.

Calcification, endosymbiotic algae concentration, total energy reserves, and Symbiodinium identification

Calcification rates were calculated from the buoyant weight data (Jokiel *et al.*, 1978) and standardized to surface area. Endosymbiont cell concentration (Warner *et al.*, 2006), and total soluble lipid, soluble animal protein, and soluble animal carbohydrates (Rodrigues & Grottoli, 2007; Levas *et al.*, 2013) were measured on all frozen fragments. Total energy reserves were calculated as the sum of total lipids, protein, and carbohydrates and reported in Joules (Gnaiger & Bitterlich, 1984) per gram ash free dry weight of coral tissue. As polyp structure and the coral tissue thickness of each species are

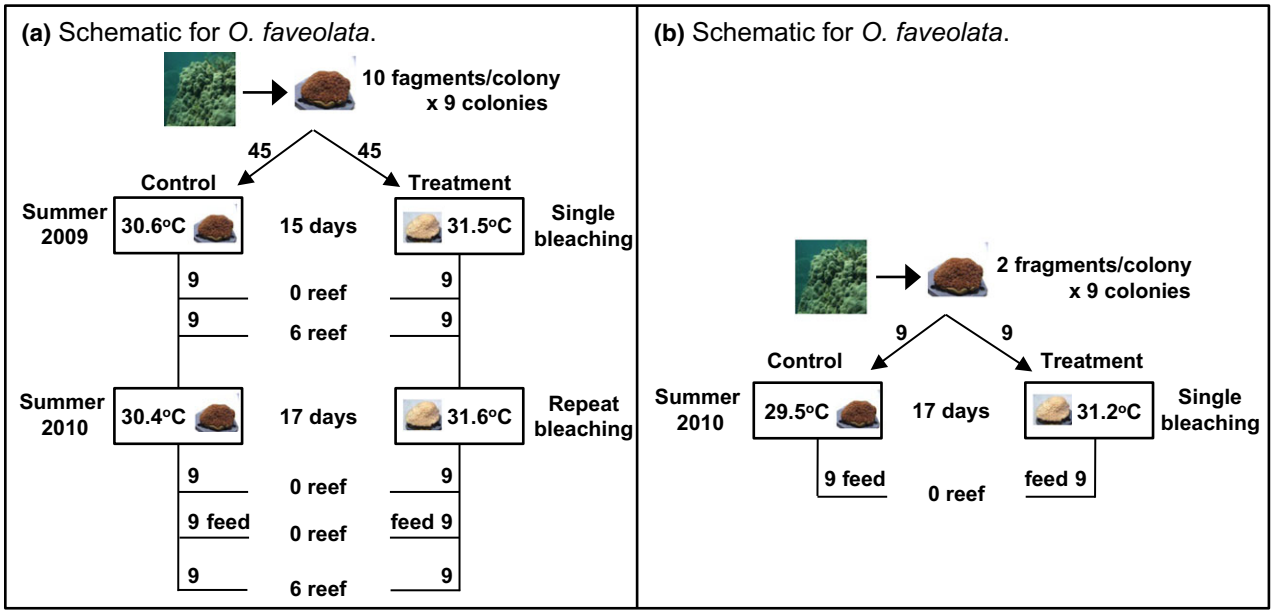

Fig. 1 Flow diagram of experimental design and coral fragments collected in (a) the single and repeat bleaching experiment of 2009–2010 and (b) the single bleaching experiment of 2010. *Orbicella faveolata* pictured. This schematic also applies to *Porites divaricata* and *Porites astreoides*. days = days in the tanks, reef = weeks on the reef, feed = fragments used for photosynthesis, respiration, and feeding measurements.

different, this normalization facilitates interspecies comparisons (Edmunds & Gates, 2002). Genetic characterizations of *Symbiodinium* were determined by amplification of the internal transcribed spacer two region (ITS2), followed by denaturing gradient gel electrophoresis and cycle sequencing (Warner *et al*., 2006). This method reliably identifies the dominant symbiont type in healthy and bleached corals (LaJeunesse *et al*., 2004, 2009; Warner *et al*., 2006) and provides qualitative identification of other background *Symbiodinium*, either within different clades (e.g. endosymbionts A3 vs. B1) or at the intracladal scale (e.g. endosymbionts A3 and A13) within the same coral fragment. The dominant ITS2 types (intracladal designations) are listed for each coral species in the text, while for statistical analyses (described below) the dominant symbiont for each coral fragment treatment^{-1} was grouped by clade. Specific quantitative PCR for all clades confirmed the accuracy of scoring dominant bands by DGGE analysis (data not shown, McGinley, 2012).

Photosynthesis, respiration, and feeding

The tissue slurries needed to standardize the photosynthesis, respiration, and feeding rates in the singly bleached coral fragments in 2009 were inadvertently discarded. Therefore, to produce singly bleached corals in 2010, a second experiment was conducted in early July with two new coral fragments collected from nine healthy colonies from the same populations of *P. divaricata*, *P. astreoides*, and *O. faveolata* and subjected to the same experimental treatments as above (Figs 1b and 2a, c, Table S1). Maximal photosynthesis and respiration rates were measured via changes in dissolved oxygen on each individual coral fragment immediately following their respective thermal stress then standardized to ash free dry weight (Rodrigues & Grottoli, 2007). All fragments were placed back on the reef and then feeding rates of each coral fragment were determined using methods in Palardy *et al*. (2008). In late July, the entire procedure was conducted again with repeat bleached treatment fragments and their respective controls described in the previous section (Fig. 1a 'feed' fragments).

Photosynthesis and respiration were used to calculate the percent Contribution of Zooxanthellae (*Symbiodinium* spp.) to Animal Respiration (CZAR) (Muscatine *et al*., 1981), while respiration and feeding rates were used to calculate the percent Contribution of Heterotrophy to Animal Respiration (CHAR) (Grottoli *et al*., 2006; Palardy *et al*., 2008). The Contribution of the Total acquired fixed carbon relative to Animal Respiration (CTAR) was calculated as the sum of CZAR and CHAR.

Statistical analyses

Coral health was determined by comparing all measured variables in treatment and controls of each species at each sampling time point. If a variable was significantly different between the average treatment compared to control values immediately following bleaching (i.e. 0 weeks on the reef), then no longer differed after 6 weeks, that variable was deemed to have fully recovered from that bleaching episode.

For endosymbiont cell density, total energy reserve concentration, and calcification rates, residual values for each variable and species were calculated and tested for normality using a Shapiro–Wilk's test and homogeneity of variance was assessed with plots of expected vs. residual values. Data

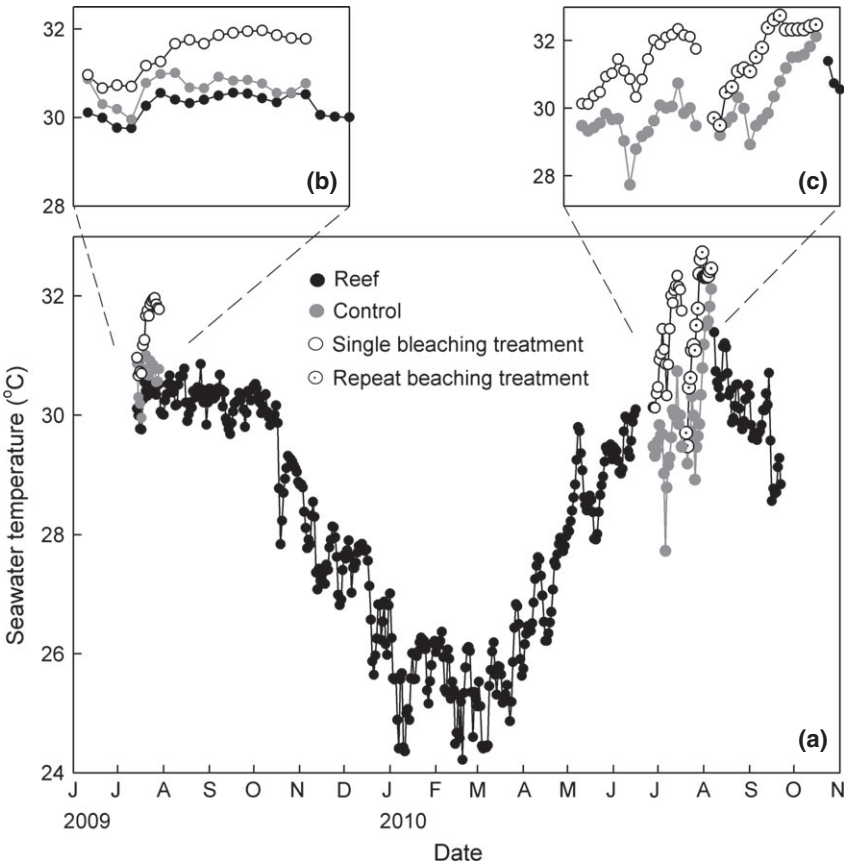

Fig. 2 Average daily seawater temperature records (a) throughout the study. Inset boxes show details of average daily temperature profiles of the treatment and control tanks during (b) the 2009 bleaching and (c) the 2010 bleaching portions of the study. Months are indicated by their first letter and the year is indicated at the first listed month for that year.

failing to meet assumption of normality were transformed. In two cases, transformation did not result in normality and 1 and 2 outliers were removed, respectively, to achieve normality and homogeneity of variance prior to statistical analysis. Three-way analysis of variance (ANOVA) was used to test for significant status, time, and genotype effects for each species. Status (two levels: treatment, control) and time (four levels: 0 and 6 weeks on the reef in 2009, 0 and 6 weeks on the reef in 2010) were fixed effects and fully crossed, while genotype was a random effect (nine levels: one for each genotype). The purpose of including the genotype in the ANOVA model was to determine if any single genotype was systematically different from all others for a given variable. In cases where significant genotype effects were detected, Tukey's tests revealed that the distribution of the genotype average values completely overlapped such that no one genotype was completely different from all of the others. As such, we concluded that the selected colonies represented the natural variation in the population well as no single or group of genotypes were consistently different from the others. This is reassuring as full exploration of any genotype interaction terms was not possible because genotype was not replicated within cells. Thus, interaction terms involving genotype were not included in the ANOVAS. *Post hoc* slice tests [i.e. test of simple effects (Winer, 1971)] were used to determine significant differences between treatment and control averages within a species at each time point. Bonferroni corrections were not used due to increased likelihood of false negatives (Moran, 2003). Statistical analyses were generated using SAS software version 9.2, where $P < 0.05$ was considered significant.

CZAR, CHAR, and CTAR data were computed from measurements collected from two separate experiments (see Fig. 1 'feed' fragments). In addition, inherit variability in the CHAR measurements (i.e. not all corals feed every night) make it impossible to achieve normality or homogeneity of variance in CHAR, and by extension CTAR values. Therefore, possible significant differences in CZAR, CHAR, and CTAR between treatment and control corals of each species each year were analyzed using the nonparametric Kruskal–Wallis test. Statistical analyses were generated using SAS software version 9.2, where $P < 0.05$ was considered significant.

Furthermore, each *Symbiodinium* clade was scored for each colony and converted to dichotomous data (dominant/non-dominant). To determine if the proportion of fragments dominated by a given *Symbiodinium* clade changed over the duration of the study within treatment and control fragments, data were analyzed using the Cochran's Q test using SPSS Statistics (v.21, IBM) with significance determined at $P < 0.05$.

Finally, nine replicate colonies were used across all treatments and time points reducing the overall variation between treatments. As all coral fragments were reared under the same conditions except for the few weeks in the tanks each year, differences between treatment and control coral fragments for any variable at any given sampling time were due to the temperature treatments alone and independent of natural seasonal variation.

Results

Treatment fragments of all three species of corals were visibly paler compared to their controls immediately following the first bleaching in 2009, with *O. faveolata* being the palest (Fig. 3a–c). After 6 weeks on the reef, treatment fragments were not visibly different from their controls (Fig. 3d–f). However, a mild natural bleaching event in the late summer of 2009 caused some paling in the control fragments of *O. faveolata* (Fig. 3f), but had no visible effect on the control fragments of the other two species (Fig. 3d–e). Following repeat bleaching the next year, treatment *P. divaricata* fragments did not appear to differ in color from their controls, treatment *O. faveolata* fragments were slightly paler than their controls, and treatment *P. astreoides* fragments were much paler than their controls (Fig. 3g–i). After 6 weeks on the reef, the treatment *P. divaricata* and *O. faveolata* fragments did not visibly differ in color from their controls (Fig. 3j, l). However, treatment *P. astreoides* were much paler than they had been immediately after the end of the thermal event (i.e. 0 weeks on the reef) and were dramatically paler than their controls (Fig. 3k).

In 2009, treatment *P. divaricata* showed a 50% decline in endosymbiont cell density and a 24% decline in calcification compared to the controls (Fig. 4a, c; Table S2). After 6 weeks on the reef, total energy reserves declined by 25%, but endosymbiont cell density and calcification had fully recovered (Fig. 4a–c; Table S2). When bleached again 1 year later, treatment corals were not significantly different from controls during the 6 weeks on the reef (Fig. 4a–c; Table S2).

Treatment *P. astreoides* suffered an initial 46% loss in endosymbionts and a 36% decline in calcification compared to controls – both of which fully recovered to

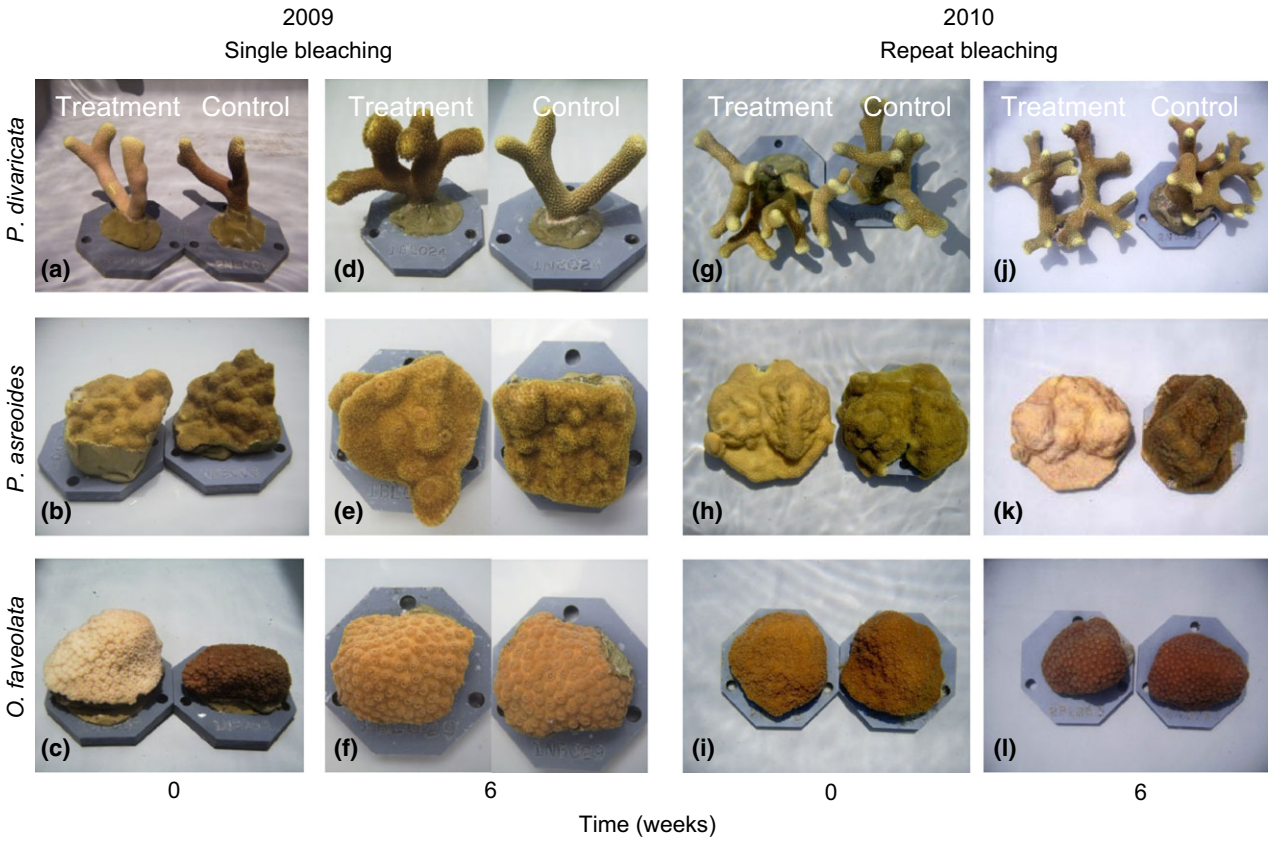

Fig. 3 Photographs of representative coral fragments. *Porites divaricata*, *Porites astreoides*, and *Orbicella faveolata* following (a–c) 0 and (d–f) 6 weeks on the reef in 2009 (single bleaching event) and after (g–i) 0 and (j–l) 6 weeks on the reef in 2010 (repeat bleaching event). Tiles are 6.3 cm in diameter for scale. Photographs by Grottoli, Levas, and Schoepf.

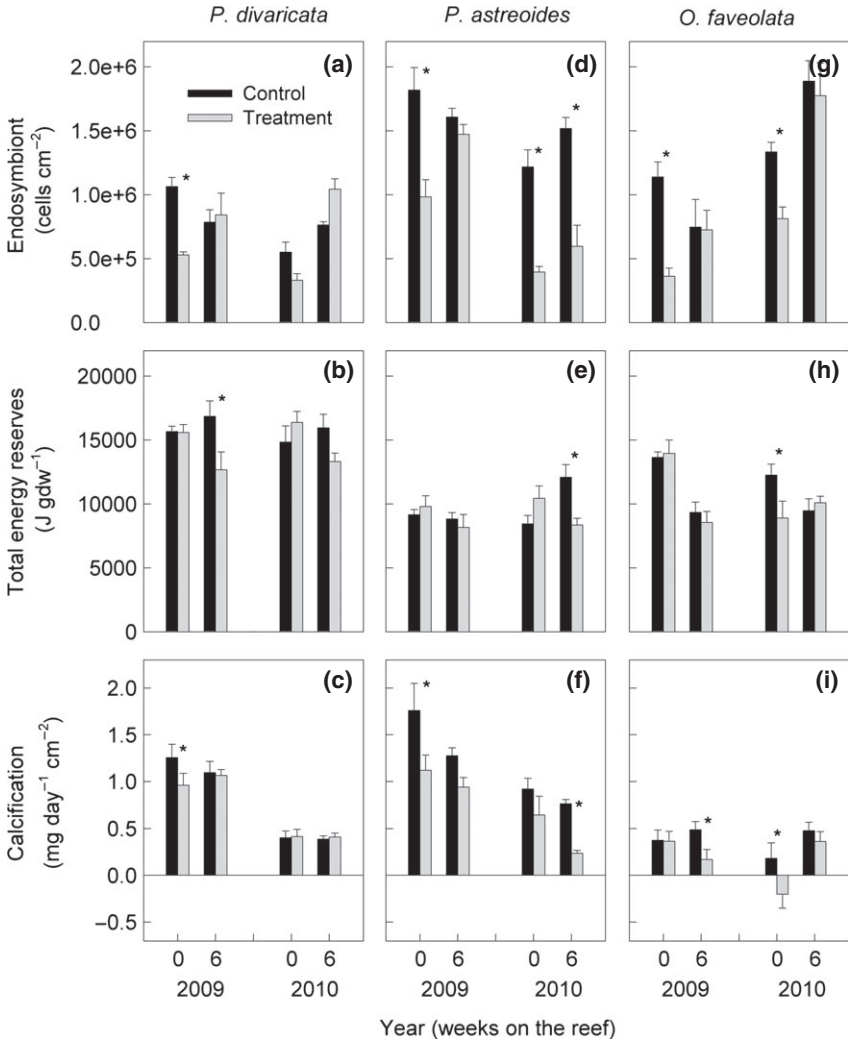

Fig. 4 Endosymbiont algal density, total energy reserves, and calcification. Average values (± SEM) for control (black bars) and treatment (gray bars) fragments of (a–c), *Porites divaricata*, (d–f), *Porites astreoides*, and (g–i), *Orbicella faveolata* after 0 and 6 weeks on the reef in 2009 (single bleaching event) and 2010 (repeat bleaching event) (see Experimental design in Fig. 1a). gdw = grams dry weight, * = significant difference between control and treatment averages within a sampling time point and species. Statistical results of the corresponding ANOVA analyses in Table S2.

control levels after 6 weeks on the reef in 2009 (Fig. 4d and f; Table S2). When bleached once more the next year, this species lost 68% of its endosymbionts compared to controls (Fig. 4d). After 6 weeks on the reef, these low concentrations of endosymbionts persisted in the treatment corals and were coupled with a 31% and 69% decline in total energy reserves and calcification, respectively, relative to the controls (Fig. 4d–f; Table S2).

Finally, treatment *O. faveolata* displayed an initial 68% decline in endosymbionts relative to controls, which fully recovered after 6 weeks in 2009 (Fig. 4g; Table S2). At the same time, total energy reserves did not differ between treatment and control fragments, while calcification rates declined by 65% (Fig. 4h, i; Table S2). When bleached again the following year, endosymbiont concentration, energy reserves, and calcification all significantly declined initially in treatment fragments by 39%, 27%, and 212% relative to controls, respectively, but all variables fully recovered after 6 weeks (Fig. 4g–i; Table S2).

Fixed carbon acquisition

In the single bleaching-treated *P. divaricata*, CZAR declined by 47% relative to controls and CTAR values were below 100% because the dramatic decreases in CZAR were not compensated for by increased CHAR (Fig. 5a–c). In repeat bleached-treated corals, CZAR declined by 32% relative to controls. Neither CHAR nor

CTAR were impacted by repeat bleaching stress (Fig. 5a–c).

Relative to average control values, single bleaching treatment had no effect on CZAR in *P. astreoides* (Fig. 5d). Despite a trend of increased feeding rates (CHAR increased by 147%) and CTAR (increased by 104%), this pattern was not statistically significant (Fig. 5e–f). The following year, repeat bleaching-treated *P. astreoides* maintained 100% of their metabolic demand (i.e. CTAR) (Fig. 5f) due to sustained photosynthesis rates (and therefore CZAR) in the remaining endosymbionts.

Finally, single bleaching-treated *O. faveolata* displayed a 67% decline in CZAR relative to controls (Fig. 5g). As CHAR did not change, treatment *O. faveolata* corals did not meet 100% of their metabolic demand as indicated by the 64% reduction in CTAR relative to controls (Fig. 5h, i). When bleached again the following year, CTAR significantly declined again by 47% relative to controls largely due to a trend in lower CZAR ($P = 0.07$) (Fig. 5g–i).

Symbiodinium type

In *P. divaricata*, 90% of the coral fragments were dominated by *Symbiodinium* C47 at the beginning of the study (Fig. 6a). Over the course of the 2 years, the number of treatment corals dominated by *Symbiodinium* C47 declined, while those dominated by *Symbiodinium* A4 increased significantly (Table S3). By the end of the

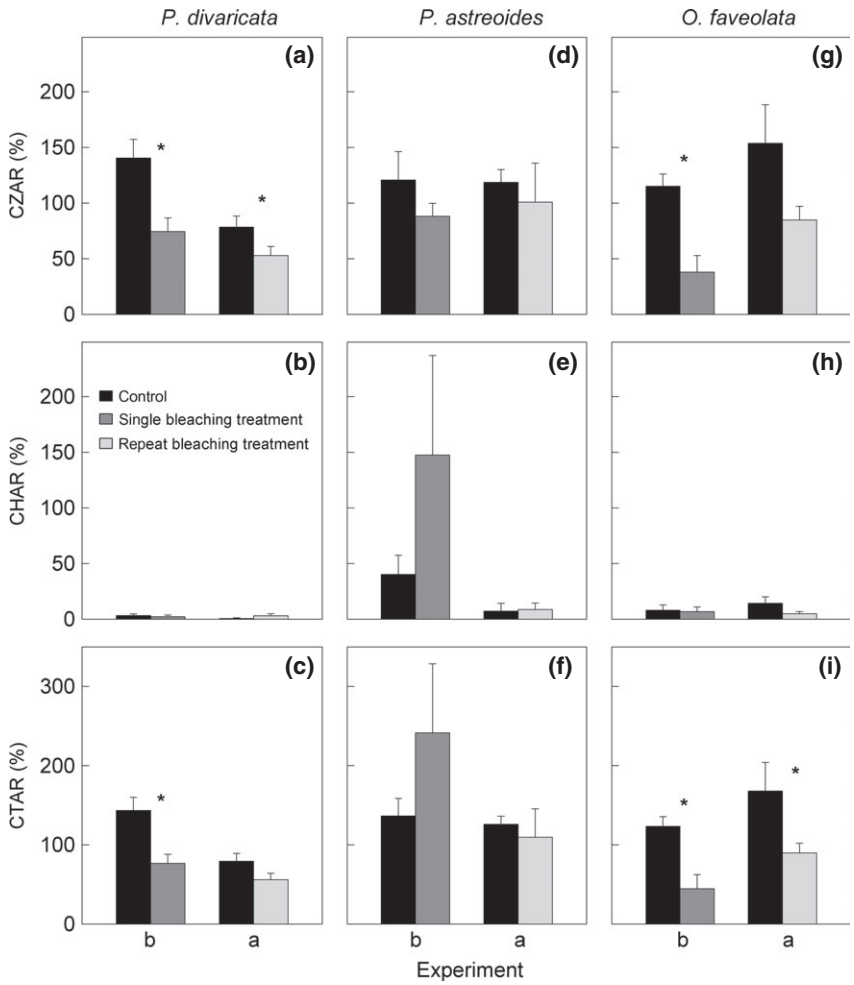

Fig. 5 CZAR, CHAR, and CTAR. Average values (± SEM) for control (black bars), single bleaching treatment (dark gray bars), and repeat bleaching treatment (light gray bars) fragments of (a–c), *Porites divaricata*, (d–f), *Porites astreoides*, and (g–i), *Orbicella faveolata* from experiment *b* where treatment corals were singly bleached (see Fig. 1b for experiment details) and experiment *a* where treatment corals were repeat bleached (see Fig. 1a for experiment details). CZAR = per cent Contribution of Zooxanthellae Acquired carbon to daily animal Respiration, CHAR = per cent Contribution of Heterotrophically Acquired carbon to daily animal Respiration, CTAR = per cent Contribution of Total Acquired carbon to daily animal Respiration. * = significant difference between control and treatment averages within each experiment and species as determined by individual Kruskal–Wallis tests.

study, A4 had fully replaced C47 in the repeat bleached treatment corals (Fig. 6a). Interestingly, there was no change in *Symbiodinium* composition in the single bleaching-treated *P. astreoides* in 2009 and only minimal change from *Symbiodinium* A4 to *Symbiodinium* C3 in repeat bleaching-treated fragments in 2010 (Fig. 6b; Table S3).

In 2009, treatment *O. faveolata* fragments displayed significant increases in *Symbiodinium* D1a and dramatic decreases in B17 relative to the controls (Fig. 6c; Table S3). Both the treatment and control fragments lost their C7 algae. The following year, *Symbiodinium* D1a, A3, and A13 largely dominated the repeat bleaching-treated corals (Fig. 6c; Table S3). Interestingly after 6 weeks on the reef, type B *Symbiodinium* was no longer detectable in treatment corals (Fig. 6c).

Discussion

This is the first study to examine the effect of annually recurring coral bleaching. We find that yearly bleaching can dramatically alter the thermal tolerance of corals. In the case of *P. divaricata*, even though endosymbiont cell density, energy reserves, calcification, CZAR, and CTAR all decreased in singly bleached treatment fragments compared to controls, only CZAR was moderately affected by the recurrence of bleaching stress the following year (Figs 4a–c and 5a–c). However, the total carbon budget (i.e. CTAR) was unaffected by repeat bleaching stress indicating that the combination of CZAR and CHAR together maintained CTAR at levels comparable to controls. Interestingly, *P. divaricata* had energy reserves that were 20–45% higher than either of the other two species throughout the study (Fig. 4b, e, h) – a feature known to reduce coral susceptibility to bleaching and increase recovery rates (Anthony *et al.*, 2009). This high energy reserve content was especially obvious during the repeat bleaching year of 2010. At the same time, there was a substantial shift in *Symbiodinium* dominance from C47 to A4 in the repeat bleaching-treated corals (Fig. 6a) such that the A4 symbiont was always dominant according to the DGGE analysis, while *Symbiodinium* C47 was only faintly visible or completely missing from denaturing gradient gels. Thus, a shift in *Symbiodinium* dominance coupled with high energy reserve concentrations appears to underlie the minimal impact of repeat bleaching stress and rapid acclimation of this species. Recent modeling evidence suggests that branching *Porites* in the Caribbean have the capacity for adaptation when bleaching events were separated by 5 years (Smith *et al.*, 2013). In the Pacific, observations following natural bleaching events separated by many years indicate that branching species that were historically more susceptible to single bleaching appear to have acclimatized in some cases (Guest *et al.*, 2012; Maynard *et al.*, 2012; Pratchett *et al.*, 2013). Our work here is congruent with these findings and extends this idea by showing that at least one branching Caribbean *Porites* species can acclimate on much shorter timescales.

Porites astreoides was only modestly impacted by single bleaching in 2009 as only two of the six measured variables declined initially and had recovered after 6 weeks on the reef (Figs 4d–f and 5d–f). In addition to the known initial thermal tolerance of the *Symbiodinium* in this species when singly bleached (Warner *et al.*, 2006), the increased trend in feeding rates, which produced a trend of increased CHAR and CTAR

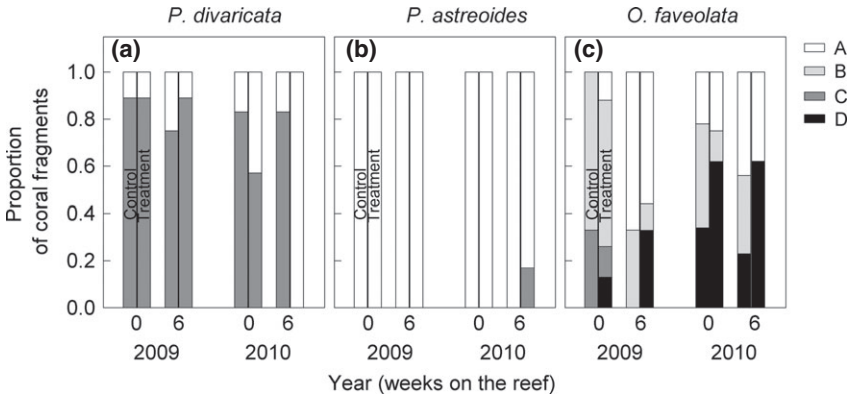

Fig. 6 *Symbiodinium* clades. Proportion of coral fragments dominated by a particular clade of *Symbiodinium* in (a), *Porites divaricata*, (b), *Porites astreoides*, and (c), *Orbicella faveolata* corals after 0 and 6 weeks on the reef in 2009 (i.e. single bleaching event) and 2010 (repeat bleaching event). Within each sampling time point, control and treatment results are plotted in the left and right bars, respectively. Clade A = white, clade B = light gray, clade C = dark gray, clade D = black. In *P. divaricata*, the A and C types are A4 and C47. In *P. astreoides* the A and C types are A4 and C3. In *O. faveolata*, the A, B, C and D types are A3, A13, B1, B17, C7, and D1a, respectively. Statistical results of the corresponding Cochran's *Q* tests in Table S3.

(Fig. 5d–f), may have helped to maintain energy reserves throughout the 6 weeks on the reef and to promote the rapid recovery of the endosymbionts and calcification in the single bleaching treatment fragments (Fig. 4d–f). However, this species was severely affected by repeat bleaching the following year, and its condition worsened for 6 additional weeks. Interestingly, this species exhibited a virtually static *Symbiodinium* composition throughout the study (Fig. 6b) and on average had energy reserve concentrations that were 14% and 61% lower than those of *O. faveolata* and *P. divaricata*, respectively (Fig. 4b, e, h). While *P. astreoides* has a history of high temperature tolerance to single bleaching experiments (Warner *et al.*, 2006) and is increasing in dominance in some Caribbean locations (Green *et al.*, 2008), our findings indicate that due to the combination of low energy reserves and low potential for endosymbiont change, this species is unlikely to acclimatize to annual bleaching, thus casting doubt on its long-term survival. This is consistent with field observations of increased bleaching susceptibility and mortality among Pacific mounding *Porites* corals to repeat bleaching separated by several years (Guest *et al.*, 2012).

Finally, while the proportion of *O. faveolata* fragments dominated by *Symbiodinium* D1a steadily increased over the course of the study (Fig. 6c), the corals were actually more susceptible to repeat bleaching stress. Despite its brown color following the second bleaching stress in 2010, *O. faveolata* experienced declines in total *Symbiodinium* density, energy reserves, calcification, and a strong trend of decreased CZAR immediately after repeat bleaching (Figs 4g–i and 5g). Additional visual *post hoc* exploration of the data revealed that these declines after repeat bleaching appear to be due to fragments that did not contain *Symbiodinium* D1a. While the sample size was too low to make any statistically supported conclusions ($n = 3$ fragments not dominated by D1a), a shift in *Symbiodinium* dominance to D1a may be associated with resilience and acclimation to repeat bleaching stress in *O. faveolata*. In addition, when bleaching stress occurred in two consecutive summers, *Symbiodinium* D1a dominated this coral for much longer than had previously been observed (Thornhill *et al.*, 2006; LaJeunesse *et al.*, 2009), suggesting that D1a persistence is linked to bleaching frequency and confers long-term bleaching resistance to the holobiont. Irrespective of endosymbiont type and severity of initial effects of repeat bleaching, *O. faveolata* still fully recovered within 6 weeks (Figs 4g–i). Thus, *O. faveolata* have the capacity to recover from repeat bleaching stress and may be able to persist in a future with recurring annual bleaching. While the results of *P. divaricata* and *O. faveolata* show the benefits of hosting multiple endosymbiont types, larger studies investigating the long-term physiological impacts of repeated thermal stress in the same coral colonies for time periods greater than used here will be needed to fully understand how these endosymbioses change through ecological time.

Collectively, these results show that the capacity of corals to resist and recover from single isolated bleaching is not a reliable predictor for resistance and recovery potential from annually recurring bleaching. The ability to acclimate to annually recurring bleaching was species-specific: one acclimated (*P. divaricata*), one did not acclimate (*P. astreoides*), and for another acclimation was complex (*O. faveolata*). Interestingly, acclimation and resistance to annual bleaching in branching *P. divaricata* is in stark contrast to susceptibility patterns established for single isolated bleaching events (Loya *et al.*, 2001), but in keeping with repeated natural bleaching events separated by 3–5 years (Guest *et al.*, 2012; Maynard *et al.*, 2012; Pratchett *et al.*, 2013).

While heterotrophy did not have any impact in maintaining CTAR, nor did it facilitate acclimation to annual bleaching, higher energy reserves were associated with acclimation. In addition, change in the dominant endosymbiont type, as opposed to *in situ* endosymbiont acclimation (Berkelmans & Van Oppen, 2006; Howells *et al.*, 2011), was a key feature underlying the potential for acclimation to annual bleaching stress. Although changes in endosymbiont type are often considered a positive force for acclimatization (Buddemeier & Fautin, 1993), physiological trade-offs for harboring some *Symbiodinium* species, such as those within the D-lineage, do exist (Jones & Berkelmans, 2010). This study is the first to show that the severe negative short-term repercussions of repeat bleaching in *O. faveolata* appear to have minimal long-term negative impacts toward coral health. Meanwhile, the complete loss of *Symbiodinium* C47 and shift in dominance to A4 in *P. divaricata* did not occur until it was exposed to repeated bleaching stress, yet the acclimation of this coral to repeat thermal stress was dramatic. The only species that did not express any significant change in the dominant endosymbiont type (i.e. *P. astreoides*) was also the only one unable to acclimate to repetitive bleaching.

Interestingly, the shift to A4 in *P. divaricata* was associated with acclimation, whereas the maintenance of A4 in *P. astreoides* was not associated with acclimation. These findings suggest that (i) the host modifies the performance of endosymbionts, (ii) that there is a host by endosymbiont type interaction that impacts holobiont performance that is species specific, (iii) that these may be different populations of A4 *Symbiodinium* (individual genotypes within a single ITS2 category) with considerable physiological variability that do not represent one clonal symbiont as recent laboratory work has shown among other *Symbiodinium* genotypes

(MW personal observation), or (iv) some combination of all three. Regardless of which mechanisms underlie the performance of endosymbionts in corals, these findings indicate that the *Symbiodinium* type–coral species combination plays a critical role in the acclimation potential of corals to single and repeat bleaching stress. Thus, predictive modeling studies that incorporate *Symbiodinium* population genetics should also account for the coral species interaction with a particular endosymbiont.

Conclusions

Both shifts in the dominant *Symbiodinium* type and high concentrations of energy reserves appear to minimize the impact of annually recurring bleaching stress on the coral holobiont. This emphasizes the role of both the endosymbiotic algae and the animal host in determining how the coral holobiont responds to bleaching stress. In addition, neither reproductive strategy (Baker *et al.*, 2008), morphology (Loya *et al.*, 2001), or starting endosymbiont type composition were predictors of the acclimation potential of *P. divaricata*, *P. astreoides*, and *O. faveolata* to annually recurring bleaching stress. The logical next step would be to conduct studies with annual bleaching over many years to determine if there are cumulative impacts on corals that appear to have acclimated to two consecutive bleaching stress events, and to survey a wider range of variables and species to form a more comprehensive assessment of annual bleaching on coral physiology and resilience.

In the Caribbean, a + 1–1.5 °C acclimatization and/or adaptation over the coming decades is required for corals to keep up with ocean warming (Teneva *et al.*, 2012). As such, our findings showing a + 1 °C experimental acclimation via holobiont phenotypic plasticity within 1 year, is very encouraging. Coral capacity for acclimation would significantly delay the onset of frequent bleaching for some species (Logan *et al.*, 2014). However, determining the percentage of reefs at risk to long-term degradation worldwide will require further evidence for acclimatization over several years of annual bleaching in other species of corals and in other ocean basins that support high topographic complexity and structural stability of reefs in general. Critically, no studies to date have considered the consequences of decreasing bleaching thresholds such as seen here in *P. astreoides* on predicted future coral mortality, reef degradation, and frequency of mass bleaching events. Thus, current model predictions of the rate of reef degradation may be optimistic. As additional threats such as ocean acidification, overfishing, and pollution may compromise coral reefs even further (Hoegh-Guldberg *et al.*, 2007) and possibly increase bleaching susceptibility (Anthony *et al.*, 2008; Frieler *et al.*, 2012), the persistence of coral reefs beyond the 21st century remains uncertain.

Acknowledgements

We thank Universidad Nacional Autonoma de Mexico's Instituto de Ciencias del Mar y Limnologia (UNAM-ICML) in Puerto Morelos, Mexico and A. Banaszak and R. Iglesias-Prieto for local assistance; Teresa Huey, Dana Borg, Leslie Irwin, and Elizabeth Zebrowski for field and laboratory assistance. We thank James Bauer and Christine Ferrier-Pages for comments on the manuscript. We thank Steven Naber and James Palardy for statistical advice. Funding was provided by the US National Science Foundation to A.G.G. (OCE#0825490) and M.E.W (OCE#0825413). Additional support to students was provided from the Ford Foundation Fellowship to S. E. L. and from the OSU Presidential Fellowship to V. S. All collections and experiments were conducted following the rules and regulations of Mexico and imported to the USA under CITES permits held by UNAM-ICML and the Ohio State University. Data deposited with www.bco-dmo.org at www.bco-dmo.org/project/516103.

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Supporting Information

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

Table S1. Parent colony collection information from Puerto Morelos Reef National Park, Mexico.
Table S2. Endosymbiont density, energy reserves, and calcification ANOVAs for *Porites divaricata*, *Porites astreoides*, and *Orbicella faveolata*. Status (two levels: treatment, control) and time (four levels: 0 and 6 weeks on the reef in 2009, 0 and 6 weeks on the reef in 2010) were fixed effects and fully crossed, while genotype was a random effect (nine levels: one for each genotype). df = degrees of freedom, SS = sum of squares, energy res = energy reserves. Endosymbiont values of *O. faveolata* were log transformed prior to analysis to achieve normality.
Table S3. *P*-values from Cochran's *Q* tests for *Porites divaricata*, *Porites astreoides*, and *Orbicella faveolata*. na = not applicable. A $P \leq 0.05$ indicates a significant change in the dominant *Symbiodinium* clade at some point over the course of the 2-year study.

Table S1 Parent colony collection information from Puerto Morelos Reef National Park, Mexico.

Species	Colony	Date	Depth (m)	Location	Coordinates
a) ***Collections in 2009***					
P. divaricata	1-9	7/5/09	2.7	El Islote	20°55.607'N, 86°49.882'W
P. astreoides	1-9	7/4/09	3	El Islote	20°55.607'N, 86°49.882'W
O. faveolata	1-4, 6, 7	7/9/09	2.4	Radio Pirata	20°51.260'N. 86°51.909'W
O. faveolata	5,9	7/6/09	7.9	The Wall	20°49.432'N, 86°52.664'W
O. faveolata	8	7/9/09	4	Jardines	20°50.045'N, 86°52.694'W
b) ***Collections in 2010***					
P. divaricata	1-9	6/18/10	3	El Islote	20°55.607'N, 86°49.882'W
P. astreoides	1-9	6/18/10	3	El Islote	20°55.607'N, 86°49.882'W
O. faveolata	1-4, 6, 7	6/18/10	4.6	Radio Pirata	20°51.260'N. 86°51.909'W
O. faveolata	5,9	6/18/10	4.9	The Wall	20°49.432'N, 86°52.664'W
O. faveolata	8	6/18/10	4.9	Jardines	20°50.045'N, 86°52.694'W

Table S2 Endosymbiont density, energy reserves, and calcification ANOVA's for *Porites divaricata*, *Porites astreoides*, and *Orbicella faveolata* . Status (two levels: treatment, control) and time (four levels: 0 and 6 weeks on the reef in 2009, 0 and 6 weeks on the reef in 2010) were fixed effects and fully crossed, while genotype was a random effect (nine levels: one for each genotype). df = degress of freedom, SS = sum of squares, energy res = energy reserves. Endosymbiont values of *O. faveolata* were log transformed prior to analysis in order to achieve normality.

Variable	Effect	df	SS	F-statistic	p-value
P. divaricata					
Endosymbiont	Model	15, 47	4.7×10^{12}	5.22	<0.0001
	Status	1	18×10^{10}	2.98	0.0910
	Time	3	1.7716	9.93	<0.0001
	Genotype	8	10×10^{11}	2.10	0.0551
	Status x Rec	3	1.3923	7.81	0.0002
Energy res.	Model	15, 47	249×10^{6}	2.70	0.0048
	Status	1	24×10^{6}	3.88	0.0548
	Time	3	18×10^{6}	1.00	0.4019
	Genotype	8	122×10^{6}	2.48	0.0248
	Status x Rec	3	63×10^{6}	3.43	0.0245
Calcification	Model	15, 45	9.1494	10.05	<0.0001
	Status	1	0.0630	1.04	0.3140
	Time	3	6.7725	37.21	<0.0001
	Genotype	8	1.4721	3.03	0.0080
	Status x Rec	3	0.2992	1.64	0.1930
P. astreoides					
Endosymbiont	Model	15, 54	1.7×10^{13}	10.87	<0.0001
	Status	1	7.9×10^{12}	75.34	<0.0001
	Time	3	5.6×10^{12}	17.95	<0.0001
	Genotype	8	2.0×10^{12}	2.41	0.0266
	Status x Rec	3	1.7×10^{12}	5.55	0.0022
Energy res.	Model	15, 55	173×10^{6}	2.35	0.0111
	Status	1	3×10^{6}	0.62	0.4356
	Time	3	27×10^{6}	1.82	0.1539
	Genotype	8	63×10^{6}	1.60	0.1454
	Status x Rec	3	82×10^{6}	5.55	0.0021

Calcification	Model	15, 52	14.8823	5.89	<0.0001
	Status	1	3.3785	20.06	<0.0001
	Time	3	8.4858	16.80	<0.0001
	Genotype	8	2.7678	2.05	0.0577
	Status x Rec	3	0.4664	0.92	0.4362
O. faveolata					
Endosymbiont	Model	15, 54	5.1751	6.32	<0.0001
	Status	1	0.7940	14.55	0.0004
	Time	3	3.0008	18.32	<0.0001
	Genotype	8	0.0478	1.09	0.3820
	Status x Rec	3	0.7588	4.63	0.0059
Energy res.	Model	15, 52	394×10^6	4.63	<0.0001
	Status	1	8×10^6	1.41	0.2407
	Time	3	242×10^6	14.17	<0.0001
	Genotype	8	103×10^6	2.27	0.0367
	Status x Rec	3	40×10^6	2.36	0.0817
Calcification	Model	15, 52	4.5021	3.00	0.0017
	Status	1	0.8056	8.06	0.0065
	Time	3	1.9716	6.57	0.0008
	Genotype	8	1.7971	2.25	0.0384
	Status x Rec	3	0.4431	1.48	0.2316

Table S3 P-values from Cochran's Q tests for *Porites divaricata*, *Porites astreoides*, and *Orbicella faveolata*. na = not applicable. A $p \leq 0.05$ indicates a significant change in the dominant *Symbiodinium* clade at some point over the course of the two-year study.

Clade	Control	Bleached
P. divaricata		
A	1.0	0.002
C	1.0	0.002
P. astreoides		
A	0.392	0.029
C	*na*	0.029
O. faveolata		
A	0.012	0.172
B	0.261	0.029
C	0.112	0.039
D	0.145	0.017