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Effects of Temperature and pCO_2 on Population Regulation of *Symbiodinium* spp. in a Tropical Reef Coral

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Abstract. This study tested the bleaching response of the Pacific coral *Seriatopora caliendrum* to short-term exposure to high temperature and elevated partial pressure of carbon dioxide (pCO_2). Juvenile colonies collected from Nanwan Bay, Taiwan, were used in a factorial experimental design in which 2 temperatures (~27.6 °C and ~30.4 °C) and 2 pCO_2 values (~47.2 Pa and ~90.7 Pa) were crossed to evaluate, over 12 days, the effects on the densities and physiology of the symbiotic dinoflagellates (*Symbiodinium*) in the corals. Thermal bleaching, as defined by a reduction of *Symbiodinium* densities at high temperature, was unaffected by high pCO_2. The division, or mitotic index (MI), of *Symbiodinium* remaining in thermally bleached corals was about 35% lower than in control colonies, but they contained about 53% more chlorophyll. Bleaching was highly variable among colonies, but the differences were unrelated to MI or pigment content of *Symbiodinium* remaining in the coral host. At the end of the study, all of the corals contained clade C *Symbiodinium* (either C1d or C15), and the genetic variation of symbionts did not account for among-colony bleaching differences. These results showed that high temperature causes coral bleaching independent of pCO_2, and underscores the potential role of the coral host in driving intraspecific variation in coral bleaching.

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Introduction

The dynamic symbiosis between cnidarians and dinoflagellates (*Symbiodinium* spp.) is fundamental to the survival of coral reefs in oligotrophic tropical seas (Muscatine and Porter, 1977). In reef corals, population density and physiology of *Symbiodinium* are regulated by host- and symbiont-driven mechanisms that vary in response to environmental conditions that include temperature, nutrients, and light intensity (Lesser, 2004; Davy *et al.*, 2012). Extreme changes in these conditions can disrupt the symbiosis and cause bleaching (Lesser, 2011), as defined by transparency of coral tissue due to a reduction in *Symbiodinium* density (Jokiel, 2004) and/or loss of photosynthetic pigments (Dove *et al.*, 2006). Persistent bleaching can result in coral mortality (Anthony *et al.*, 2007), and large-scale bleaching episodes have serious consequences for the coral reef ecosystem (Hoegh-Guldberg, 1999; Wild *et al.*, 2011). Of the global threats facing coral reefs, increased seawater temperature and lowered seawater pH associated with anthropogenic increases in atmospheric pCO_2 (Intergovernmental Panel on Climate Change (IPCC), 2014) are expected to have the most severe effects (Hoegh-Guldberg *et al.*, 2007).

Coral species, however, are not equally affected by high temperature (Guest *et al.*, 2012) or low pH (Comeau *et al.*, 2014). Variations in corallum morphology, host physiology, and the genotypes of *Symbiodinium* represent proximal mechanisms used by corals, with the ultimate effect of stabilizing the symbiosis in the face of environmental challenges (Loya *et al.*, 2001; Jones *et al.*, 2008; Baird *et al.*, 2009; Logan *et al.*, 2014). Such mechanisms also can influence acclimatization to changing conditions (Gates and Edmunds, 1999).

Ultimate outcomes are facilitated by a variety of processes including respiratory physiology, cellular biochemistry, animal and algal pigment concentrations, antioxidative compounds, heat shock proteins, and regulation of algal and host cell cycles (Strychar *et al.*, 2004; Dove *et al.*, 2006; Lesser, 2006; Venn *et al.*, 2006; Edmunds, 2008; Crawley *et al.*, 2010; Meyer and Weis, 2012; Palumbi *et al.*, 2014). These mechanisms of acclimatization are modulated by the genetic variability of the host and can expedite the adaptive responses of corals to environmental change (Edmunds and Gates, 2008; Pandolfi *et al.*, 2011; Kenkel *et al.*, 2013; Parkinson *et al.*, 2015). Intraspecific variation in gene expression as a function of environmental conditions is well established (Bay *et al.*, 2013; Kenkel *et al.*, 2013; Palumbi *et al.*, 2014), and may provide insight into mechanisms associated with adaptation of corals to climate change.

Adaptation in symbiotic corals can involve the host and/or its diverse symbiotic partners (Baker, 2003; Stat *et al.*, 2008; Baird *et al.*, 2009; Kenkel *et al.*, 2013; Parkinson *et al.*, 2015). Adaptation also may occur through shifts in the type of symbionts harbored within the coral tissue (Buddemeier *et al.*, 2004), followed by natural selection acting on the functional unit of the holobiont (Margulis and Fester, 1991). In the case of *Symbiodinium* symbionts, there are stable populations in some hosts (*e.g.*, Thornhill *et al.*, 2006, 2009; McGinley *et al.*, 2012), but evidence shows that changes are already occurring in some tropical corals through shifts in the types of dinoflagellates they contain, and that these changes are helping to resist recurrent thermal stress (Stat and Gates, 2011; Lesser *et al.*, 2013; Stat *et al.*, 2013). These observations suggest that changes in the *Symbiodinium* complement can have adaptive value (Buddemeier and Fautin, 1993; Buddemeier *et al.*, 2004). Further, these changes in the types of dinoflagellates may be viewed in the context of adaptation or acclimatizaton of symbionts (and/or holobionts) to environmental changes through modified algal division rates (Baghdasarian and Muscatine, 2000; Strychar *et al.*, 2004), photosynthetic pigment concentrations (Strychar and Sammarco, 2012; Hoadley *et al.*, 2016), and other cellular parameters to manage reactive oxygen species (Weis, 2008).

Our study tested the effects of high temperature and elevated pCO_2 on the *Symbiodinium* population in the common Pacific coral *Seriatopora caliendrum* Ehrenberg, 1834. Our hypothesis was based on predictions of increasing large-scale coral bleaching episodes (Lesser, 2011; Ainsworth *et al.*, 2016), particularly resulting from upward thermal anomalies, and the possibility that this stress response might be exacerbated by high pCO_2 (Hoegh-Guldberg *et al.*, 2007). Evidence that high pCO_2 levels and high temperature have interactive effects on bleaching in corals is equivocal; some studies suggest that high pCO_2 promotes bleaching (Anthony *et al.*, 2008; Kaniewska *et al.*, 2012) while other work shows no additive effect (Wall *et al.*, 2014). Given this uncertainty, we reasoned that further study of the effects of high temperature combined with high pCO_2 on *Symbiodinium* densities in reef corals was warranted. We sought to answer three questions: 1) To which extent is thermal bleaching modulated by high pCO_2? 2) How does cell division in *Symbiodinium* contribute to the bleaching process? and 3) What is the role of the physiology and genotypes of *Symbiodinium* in mediating bleaching?

Materials and Methods

Collection and maintenance of corals

Forty-eight juvenile colonies of *Seriatopora caliendrum* (< 40 mm in diameter) were collected at a 3–5-m depth on Hobihu Reef (21°56′48″ N, 120°44′58″ E), Nanwan Bay, Taiwan, on 22 June 2012. After transport to the National Museum of Marine Biology and Aquarium in Pingtung, Taiwan, corals were suspended from nylon monofilament in a 1000-liter tank filled with seawater, then allowed to recover for 4 d before the experiment began. Recovery was assessed by visual inspection of the tissue and detection of tissue regeneration at the site of fracture. The tank received a constant flow of filtered seawater (6 l min^{-1}, 50 μm-filter-pore size) pumped from the Taiwan Strait (adjacent to the aquarium) and maintained at a constant mean temperature of 27.7 ± 0.1 °C (± SE, $n = 26$), which was the ambient temperature on the reef when the experiment was conducted. Ambient seawater had a pH of 8.013 ± 0.004, pCO_2 of 43.89 ± 0.55 Pa, total alkalinity (TA) of 2202.1 ± 9.9 μmol kg^{-1}, and aragonite saturation state (Ω_{arag}) of 2.92 ± 0.04 (all means ± SE, $n = 8$; see next section for measurement details). Light was supplied to the tank using lamps fitted with metal halide bulbs (150W; Royal Philips, Amsterdam, the Netherlands) and fluorescent bulbs (18W, TL-D Blue; Royal Phillips), which created a mean light intensity of 165 ± 5 μmol photons m^{-2} s^{-1} (as measured by LI-COR LI-193 spherical quantum sensor, ± SE, $n = 4$ measurements) (LI-COR Environmental, Lincoln, NE) where the corals were suspended in the tank.

Experimental overview and treatments

A factorial design was used in which two temperatures, ambient (AT) and high (HT), and two pCO_2 values, ambient (ACO_2) and high (HCO_2), were crossed. Treatments assessed the effects of temperature and pCO_2 in four combinations: ambient temperature–ambient pCO_2 (AT-ACO_2), ambient temperature–high pCO_2 (AT-HCO_2), high temperature–ambient pCO_2 (HT-ACO_2), and high temperature–high pCO_2 (HT-HCO_2) levels. In this design, duplicate tanks were treated as a nested factor in each treatment and were used to evaluate the impacts of pseudoreplication on the experimental outcomes (Hurlbert, 1984). The tanks (77 × 77 × 30 cm) contained 130 l of 1.0-μm filtered seawater and were independently heated, chilled, recirculated, and supplied with ambient

air or a gas blend with elevated pCO_2. Twenty percent of the seawater in each tank was replaced daily (at 12:00 h) with freshly filtered seawater from the Taiwan Strait. Light intensities were maintained on a 12 h:12 h light:dark photoperiod, using the fluorescent (18W, TL-D Blue; Royal Phillips) and metal halide bulbs (150W; Royal Phillips). Light intensity was measured at 12:00 h using the LI-COR LI-193 spherical quantum sensor adjusted to a target intensity of ~250 μmol photons m^{-2} s^{-1}. This intensity was similar to the maximum light intensity recorded in June on the reef (at 5-m depth), where the corals were collected (Dufault *et al.*, 2012). Six coral colonies were suspended by nylon monofilament in each of 8 experimental tanks for 12 d, the duration of the experiment. In this paired design, each colony was sampled at the start and end of the experiment. Colony was treated as a factor in the statistical analysis to increase the ability to test for treatment effects when replicate colonies differed from one another.

Seawater temperature in each tank was maintained using AquaController microprocessor-based regulators (Neptune Systems, San Jose, CA) connected to an Aquatek Aquasystems chiller (Aquatek International Corp., Canonsburg, PA) and a 300-W heater (Taikong Corp., Taipei, Taiwan). Temperatures were monitored daily at 09:00, 12:00, and 17:00 h by a traceable digital thermometer (± 0.05 °C) (model no. 15-077-8; Thermo Fisher Scientific, Waltham, MA). The system was adjusted to contrast a target ambient temperature of ~28.0 °C (about the ambient temperature of Nanwan Bay at the time of coral collection) with a target high temperature of ~30.5 °C. The 2.5 °C temperature increase was effective in inducing bleaching in *Seriatopora caliendrum* collected from the same area (Wall *et al.*, 2014), and was within the IPCC and Representative Concentration Pathways (RCP) Scenario 6.0 projection for increased sea surface temperatures (Intergovernmental Panel on Climate Change (IPCC), 2014). Salinity was monitored daily at 09:00, 12:00, and 17:00 h with a YSI 3100 conductivity meter (YSI, Yellow Springs, OH).

Partial pressure of carbon dioxide was maintained by bubbling ambient air or CO_2-enriched air into the tanks. For high pCO_2, 99% CO_2 was mixed with ambient air using a solenoid-controlled gas regulation system (model A352; Qubit Systems, Islamabad, Pakistan). In the mixture, pCO_2 was measured with an infrared gas analyzer (model S151; Qubit Systems) calibrated with certified reference gas (1793 ppm CO_2; San Ying Gas Co., New Taipei, Taiwan), and supplied at a flow rate adjusted by solenoid valve to achieve the desired target value. The pCO_2 level in the mixture was recorded using LabPro software (Vernier Software & Technology, Beaverton, OR). Ambient and CO_2-enriched air were delivered to the tanks at about 15 l min^{-1}, and the system was set to achieve target pCO_2 (ambient) values of ~45 Pa and elevated pCO_2 values of ~90 Pa (consistent with values reported in the Intergovernmental Panel on Climate Change (IPCC), 2014 and RCP Scenario 6.0 for 2100).

Symbiodinium *density and tissue protein content*

For sample processing, tissue was stripped from the coral colonies using an Iwata-Medea airbrush (Anest Iwata-Medea, Portland, OR) filled with about 20–40 ml of 1.0-μm filtered seawater. The tissue slurry was homogenized (Polytron PT2100 homogenizer; Thermo Fisher Scientific) and the *Symbiodinium* was concentrated by centrifugation (1500 × *g* for 60 s) using an Eppendorf 5810 R centrifuge (Thermo Fisher Scientific) before being resuspended in filtered seawater and counted using a hemocytometer (n = 8 counts $sample^{-1}$). *Symbiodinium* density was standardized to the area of coral tissue, as determined by wax dipping (Stimson and Kinzie, 1991), and is expressed as cells × 10^6 cm^{-2}. Density was also standardized to total protein, expressed as cells × 10^6 mg $protein^{-1}$.

The supernatant obtained from the centrifuged tissue slurry was used to measure soluble protein in the host coral tissue. Soluble protein concentration was determined spectrophotometrically using absorbances at 235 nm and 280 nm (Whitaker and Granum, 1980) and standardized to the area of the measured coral (final units of mg protein cm^{-2}).

Mitotic index of Symbiodinium

To evaluate the population growth of *Symbiodinium in hospite* (within the host), the number of cells undergoing mitosis were measured by scoring the number of dividing cells (*i.e.*, doublets), as shown by the presence of a division plate (*i.e.*, mitotic index (MI) (Wilkerson *et al.*, 1988). The MI was calculated by scoring the number of doublets from a minimum of 1000 cells in a total of 8 to 10 subsamples of the suspension. Screening for doublets was accomplished at 400× magnification using an Olympus CH-2 microscope (Olympus, Tokyo, Japan), and MI was expressed as a percentage of the total cell count. A preliminary sampling of the *Symbiodinium* in *Seriatopora caliendrum* (n = 8 corals, sampled every 3 h, at 1000 cells per sample) showed that MI did not vary over a 24-h period, although subsequent sampling was standardized to 09:00 h each day.

Chlorophyll determination

To measure chlorophyll content in the *Symbiodinium* extracted from *Seriatopora caliendrum*, duplicate 4-ml aliquots of the resuspended *Symbiodinium* were filtered under a light vacuum onto a cellulose acetate membrane filter (25-mm, 3-μm pore size; Critical Process Filtration, Nashua, NH). The chlorophyll was extracted in 3 ml of 90% acetone at 4 °C over 24 h (Jeffrey and Humphrey, 1975). Following extraction, samples were centrifuged (1500 × *g* for 3 min). Absorbance of the supernatant at 630 nm and 663 nm was used to calculate chlorophyll *a* and chlorophyll c_2 concentrations using equations for dinoflagellates (Jeffrey and Humphrey, 1975).

Symbiodinium *genotyping*

Symbiodinium samples collected for measurement of physiological parameters included a subsampling for genotyping. DNA was extracted from the samples in guanidinium buffer (Pochon *et al.*, 2001). DNAs were amplified for Roche 454 pyrosequencing (Roche Sequencing, Pleasanton, CA) of the nrDNA Internal Transcribed Spacer 2 (ITS2) region at Research and Testing Laboratory, LLC (RTL Genomics, Lubbock, TX) (for more details, see Edmunds *et al.*, 2014). Sequences were split by sample with a maximum barcode error of 0, maximum primer mismatch of 5, minimum sequence length of 150 base pairs (bp), a quality score window of 50, and removal of reverse and forward primers prior to clustering. Data were analyzed using a 97% similarity clustering (UCLUST; Edgar, 2010) in QIIME (Caporaso *et al.*, 2011). The 97% operational taxonomic units (OTUs) were identified *via* BLAST (e-value = $1e^{-5}$) to an internal reference database of ITS2 sequences from GenBank (Edmunds *et al.*, 2014; Appendix Fig. A1).

Statistical analyses

Symbiodinium density (normalized to surface area and protein), MI, total chlorophyll content, and the ratio of chlorophyll *a* to chlorophyll c_2 were analyzed using a four-way split-plot ANOVA. Here, temperature and pCO_2 were fixed main effects, tank was a random effect nested in both main effects, and colony was a within-subjects factor processed as a repeated-measures effect. The statistical assumptions of equal variance and normality were tested by graphical analyses of residuals. Following analysis of the experiment for all main effects and their interactions, *post hoc t*-tests were used in an exploratory manner to evaluate differences in each treatment between the start and end of the experiment. These tests were applied in a design paired by colony to address differences among coral colonies (see Results). All statistical tests of physiology were completed using Systat ver. 11.0 (Systat Software, San Jose, CA) for Windows. *Symbiodinium* OTUs were assessed with multidimensional scaling (MDS) to visualize differences in communities between treatments and over time. Because time was not significant, permutational multivariate ANOVA (PERMANOVA) was used to test the hypothesis that there was no difference in symbiont communities between treatments at each time point. Statistical testing of *Symbiodinium* communities was carried out using the vegan package in the statistical program R ver. 2.15.1 (R Core Team, 2012; Supplementary File, view online).

Results

Experimental treatments

Treatments were precisely regulated (Table 1) to compare a mean temperature of 27.61 ± 0.05 °C *versus* 30.46 ± 0.02 °C, and a mean pCO_2 of 47.2 ± 0.4 Pa *versus* 89.3 ± 0.7 Pa (all ± SE; $n = 32$). Temperatures differed between treatments ($P < 0.001$) but not among tanks within each temperature ($P = 0.901$). There were differences in pCO_2 between treatments ($P < 0.001$) but not between temperatures within a pCO_2 level ($P = 0.307$) or between tanks within a treatment combination ($P = 0.397$). There was no interactive effect of temperature and pCO_2 ($P = 0.143$). Salinity was constant among all levels of the statistical model ($P \geq 0.064$). pH differed between pCO_2 treatments ($P < 0.001$) but not between temperatures ($P = 0.113$), and total alkalinity (TA) was similar in pCO_2 treatments ($P = 0.885$) and temperatures ($P = 0.060$). However, both pH and TA differed between tanks within each treatment combination ($P \leq 0.027$). In both cases, the tank effects for pH and TA were associated with numerically small differences in mean values for each variable (≤ 0.024 and ≤ 34 μmol kg^{-1}, respectively).

Juvenile colonies of *Seriatopora caliendrum* suspended in these tanks had a mean diameter of 18.4 ± 0.8 mm (± SE; $n = 48$) and consisted of several small branches that were dark brown at the start of the experiment. A coral from one of the ambient temperature–ambient pCO_2 (AT-ACO_2) tanks died on the third day of the study, and the experiment was continued with the remaining 47 corals.

Symbiodinium *density and tissue protein content*

Colonies at the start of the experiment contained *Symbiodinium* at 0.93 ± 0.05 × 10^6 cells cm^{-2} and soluble protein at 1.16 ± 0.05 mg cm^{-2}. When these dependent variables were combined, protein-normalized *Symbiodinium* densities were 0.81 ± 0.03 × 10^6 cells mg $protein^{-1}$ (all means ± SE; $n = 47$).

Following the incubations, corals in the high-temperature treatments were pale whereas corals in the remaining treatments were brown. Area-normalized *Symbiodinium* densities ranged from 0.53 ± 0.04 × 10^6 cells cm^{-2} in the high temperature–ambient pCO_2 (HT-ACO_2) treatment to 0.57 ± 0.06 × 10^6 cells cm^{-2} in the high temperature–high pCO_2 (HT-HCO_2) treatment (Fig. 1A). The densities differed among coral colonies and between temperature treatments, but no other main effects or their interactions were significant (Table 2); differences in tank effects also were not significant ($P \geq 0.250$). The temperature effect reflected declines in density at high temperature; densities declined 40.5% ± 2.1% (mean ± SD; $n = 24$). *Post hoc*, paired sample *t*-tests (Table 3) revealed a significant decline in *Symbiodinium* densities in the two high-temperature (HT-ACO_2 and HT-HCO_2) groups but not in the ambient-temperature (AT-ACO_2 and AT-HCO_2) groups (Fig. 1A).

Soluble protein in the coral tissue remained at about 1.2 mg cm^{-2} following the incubations (Fig. 1C) and was unaffected by pCO_2 and temperature or their interaction (Table 2). There were no differences between paired initial and final protein

Table 1

Incubation conditions during the 12-day experiment

Treatment	Temp °C	Irradiance (μmol photons m^{-2} s^{-1})	pH	pCO_2 (Pa)	TA (μmol kg^{-1})	Ω_{Arag}
AT–ACO_2	27.64±0.07	229±4	8.022±0.005	47.10±0.66	2235±7	3.05±0.04
HT–ACO_2	30.51±0.03	246±3	8.064±0.003	47.29±0.43	2262±10	3.42±0.03
AT–HCO_2	27.59±0.08	224±5	7.774±0.007	90.00±1.36	2217±12	1.88±0.04
HT–HCO_2	30.41±0.03	243±6	7.826±0.002	88.64±0.55	2254±9	2.19±0.02

Values are means ± SE. AT–ACO_2 treatment, $n = 11$. For the other three treatments, $n = 12$ each.

AT-ACO_2, ambient temperature–ambient pCO_2; AT-HCO_2, ambient temperature–high pCO_2; HT-ACO_2, high temperature–ambient pCO_2; HT-HCO_2, high temperature–high pCO_2.

concentrations (Table 3). However, protein content differed among tanks and among colonies, that is, there was a colony × tank interaction (Table 2). The differences among the tanks were reflected in the variation in protein content between individual corals that were allocated to pairs of tanks in each treatment; the magnitude of this effect ranged from 10.8% to 25.2%. This variation was within the same range of differences between tanks that was observed in *Symbiodinium* density (13.8%–23.4%) and colony surface area (20.3%–32.5%).

When *Symbiodinium* densities were normalized to soluble protein (Fig. 1B), initial densities were $0.81 \pm 0.03 \times 10^6$ cells mg $protein^{-1}$. Following the incubations, densities ranged from $0.55 \pm 0.04 \times 10^6$ cells mg $protein^{-1}$ at HT-HCO_2 to $0.67 \pm 0.04 \times 10^6$ cells mg $protein^{-1}$ at AT-ACO_2 (mean ± SE; $n = 12$ and 11, respectively). Protein-normalized *Symbiodinium* densities differed among colonies and temperatures (Table 2), and mean values were significantly depressed compared to initial values in AT-ACO_2 (23.5% reduction), HT-ACO_2 (31.3% reduction), and HT-HCO_2 (27.8% reduction), but not in AT-HCO_2 (Table 3, Fig. 1B). None of the differences in the interactive effects or the tank effects was significant (Table 2).

Mitotic index of Symbiodinium

At the start of the incubations, mean MI of *Symbiodinium* was 2.96% ± 0.16% (± SE; $n = 47$), but varied by colony from 1.41% to 6.17%. Following the incubations, MI varied from 1.60% ± 0.13% at HT-ACO_2 to 2.73% ± 0.28% at AT-ACO_2 (mean ± SE; $n = 12$ and 11, respectively). Overall, MI differed among coral colonies by temperature, but this effect was varied under different pCO_2 conditions (*i.e.*, there was a three-way interaction between coral, temperature, and pCO_2 (Table 4)). The MI also was influenced by the main effect of colony and temperature (Table 4). *Post hoc*, paired sample *t*-tests revealed a significant reduction in MI under HT-ACO_2 (by 44%; $P = 0.003$) and AT-HCO_2 (by 35%; $P = 0.043$) as compared to initial values (Table 3; Fig. 2).

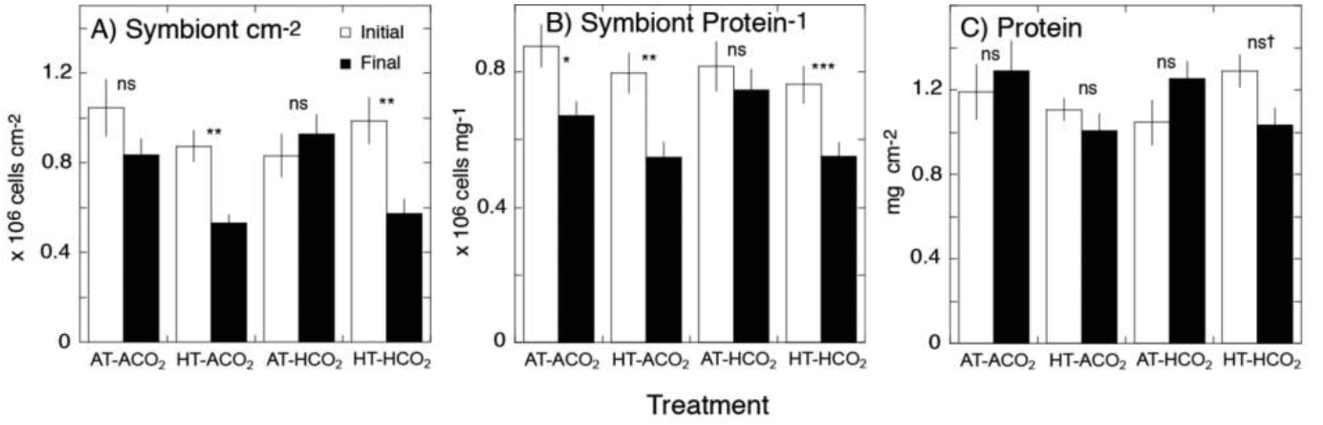

Figure 1. Effects of 12 d of incubation under combinations of temperature and pCO_2 for *Seriatopora caliendrum*. (A) *Symbiodinium* population density normalized to area (Symbiont cm^{-2}); (B) *Symbiodinium* population density normalized to protein content (Symbiont $protein^{-1}$); and (C) protein content of coral host. Data are means ± SE; $n = 12$ for all bars. Results of *post hoc*, paired sample *t*-tests (for final *vs.* initial values) shown as ns (not significant; $P > 0.05$), $^*P < 0.05$, $^{**}P < 0.01$, and †(ns, $P < 0.10$). AT-ACO_2, ambient temperature–ambient pCO_2; AT-HCO_2, ambient temperature–high pCO_2; HT-ACO_2, high temperature–ambient pCO_2; HT-HCO_2, high temperature–high pCO_2.

Table 2

Statistical analysis of symbiont density (normalized to area and protein) and protein in Seriatopora caliendrum *incubated at two temperatures and two* pCO_2 *values, with each treatment created in duplicate tanks*

Variable	Type	Source	SS	df	MS	*F*	*P*-value
Symbiodinium density (area normalized)	Between subjects	Temperature	0.618	1	0.618	7.725	**0.049**
		pCO_2	0.005	1	0.005	0.063	0.814
		Temperature × pCO_2	0.093	1	0.093	1.163	0.342
		Tank (temperature × pCO_2)	0.321	4	0.080	0.770	0.552
		Error	4.064	39	0.104		
	Within subjects	Coral	1.093	1	1.093	116.768	< **0.001**
		Coral × temperature	0.612	1	0.612	6.442	0.064
		Coral × pCO_2	0.077	1	0.077	0.811	0.419
		Coral × temperature × pCO_2	0.209	1	0.209	2.200	0.212
		Coral × tank (temperature × pCO_2)	0.378	4	0.095	1.338	0.273
		Error	2.757	39	0.071		
Symbiodinium density (protein normalized)	Between subjects	Temperature	0.308	1	0.308	10.621	**0.031**
		pCO_2	0.001	1	0.001	0.034	0.863
		Temperature × pCO_2	0.003	1	0.003	0.103	0.764
		Tank (temperature × pCO_2)	0.116	4	0.029	0.573	0.684
		Error	1.964	39	0.050		
	Within subjects	Coral	0.773	1	0.773	33.609	**0.004**
		Coral × temperature	0.057	1	0.057	2.478	0.191
		Coral × pCO_2	0.040	1	0.040	1.739	0.258
		Coral × temperature × pCO_2	0.012	1	0.012	0.522	0.510
		Coral × tank (temperature × pCO_2)	0.093	4	0.023	0.913	0.466
		Error	0.989	39	0.025		
Protein (area normalized)	Between subjects	Temperature	0.130	1	0.130	0.389	0.566
		pCO_2	0.007	1	0.007	0.021	0.891
		Temperature × pCO_2	0.174	1	0.174	0.521	0.521
		Tank (temperature × pCO_2)	1.335	4	0.334	2.687	**0.045**
		Error	4.844	39	0.124		
	Within subjects	Coral	0.005	1	0.005	0.022	0.889
		Coral × temperature	0.612	1	0.611	2.728	0.174
		Coral × pCO_2	0.002	1	0.002	0.009	0.929
		Coral × temperature × pCO_2	0.113	1	0.113	0.505	0.517
		Coral × tank (temperature × pCO_2)	0.897	4	0.224	3.823	**0.010**
		Error	2.286	39	0.059		

Corals (*Seriatopora caliendrum*) ($n = 6$ tank^{-1}) were analyzed at the start and end of the incubation period and are treated as a repeated within subject effect. Main effects and their interactions were tested over the tank (temperature × pCO_2) effect (between subjects) or the coral × tank (temp. × pCO_2) effect (within subjects). Significant differences are in **bold**. df, degrees of freedom; *F*, *F*-statistic; MS, mean sum of squares; SS, sum of squares.

Chlorophyll concentration determination

The concentration of chlorophyll in the *Symbiodinium* showed a mixed response to the treatments. Sometimes the concentration was unaffected; at other times, it changed significantly. The overall chlorophyll content at the beginning of the experiment was 94.3 ± 6.1 μg 10^6 cells^{-1} (mean ± SE; $n = 47$), and following the incubations it varied from 154.1 ± 34.3 μg 10^6 cells^{-1} at HT-HCO_2 to 92.4 ± 6.8 μg 10^6 cells^{-1} at AT-ACO_2 (mean ± SE; $n = 12$ and 11, respectively) (Fig. 3). Following the incubations, chlorophyll content was affected significantly by temperature but not by other main effects or their interactions (Table 5). *Post hoc*, paired sample *t*-tests showed that the observed increase in chlorophyll content (by 53%) at HT-ACO_2 was significant ($P = 0.035$), but none of the other experimental treatments significantly affected total chlorophyll levels (Table 3).

At the beginning of the experiment, the ratio of chlorophyll *a* to chlorophyll c_2 was 1.25 ± 0.07 (mean ± SE; $n = 47$); following the incubations, this ratio varied from 0.91 ±

Table 3

Summary of paired sample t*-tests comparing initial and final values in each treatment combination after pooling between tanks*

Treatment	*Symbiodinium*/ area (cm^{-2})			*Symbiodinium*/ protein ($mg^{-1}cm^{-2}$)			Protein ($mg \times cm^{-2}$)			Mitotic index (%)			Total *chlorophyll* $g \times 10^6$ $cells^{-1}$			Chlorophyll *a*: Chlorophyll c_2		
	t	df	*P*	*t*	df	*P*	*t*	df	*P*	*t*	df	*P*	*t*	df	*P*	*t*	df	*P*
AT–ACO_2	1.691	10	0.122	2.864	10	0.017	0.805	10	0.440	0.040	10	0.969	1.029	10	0.328	1.179	10	0.266
HT–ACO_2	4.250	11	**0.001**	4.232	11	**0.001**	1.173	11	0.266	3.854	11	**0.003**	2.400	11	**0.035**	2.937	10	**0.015**
AT–HCO_2	0.723	11	0.485	0.801	11	0.440	1.702	11	0.117	2.294	11	**0.043**	0.938	11	0.369	2.246	11	**0.046**
HT–HCO_2	4.147	11	**0.002**	5.836	11	<**0.001**	2.131	11	0.057	1.946	11	0.078	1.264	10	0.235	2.165	11	0.053

AT–ACO_2, ambient temperature–ambient pCO_2; AT–HCO_2, ambient temperature–high pCO_2; HT–ACO_2, high temperature–ambient pCO_2; HT–HCO_2, high temperature–high pCO_2; df, degrees of freedom; *t*, Student's *t* statistic. Significant differences are in **bold**.

0.07 at HT-ACO_2 to 1.11 ± 0.06 at AT-ACO_2 (mean ± SE) (Fig. 3B). The ratio also varied among colonies both as a main effect and in two- and three-way interactions between temperature, pCO_2, and temperature × pCO_2 (Table 4); no other main or interactive effects were significant. *Post hoc*, paired sample *t*-tests showed that, compared to pretreatment values, the depressed ratio of chlorophyll *a* to chlorophyll c_2 in HT-ACO_2 (by 30.1%) and AT-HCO_2 (by 26.1%) was significant ($P = 0.015$ and 0.046 respectively), but there was no shift in this ratio in the other two treatments (Table 3).

Symbiodinium *genotypes*

Symbiodinium ITS2 sequencing generated 344,746 sequences, most of which represented clade C (99.99%), with < 0.1% representing clades A and D combined. Using BLAST, 144 OTUs (97% similarity) aligned to 55 types (2012) (Supplementary File, view online). Further analysis of *Symbiodinium* from the corals identified the dominant types as subclade C1d (93.4%) and C15c (2.3%). There was no spatial separation of the initial and final samples, indicating no concerted shift in community during the experiment (Appendix Fig. A2). The communities of *Symbiodinium* did not vary between temperatures or pCO_2 levels at either the first or last time points (Table 6).

Discussion

This study evaluated bleaching in *Seriatopora caliendrum* as a result of short-term exposure to high temperature and elevated pCO_2. Although much is known about the mechanisms of coral bleaching caused by high temperature (Jokiel, 2004; Lesser, 2007; Brown and Dunne, 2015), little is understood about the ways in which the bleaching response will be affected by ocean acidification (Hoegh-Guldberg *et al.*, 2007). While some researchers expect to see a positive synergy be-

Table 4

Statistical analysis of mitotic index (arcsine transformed) of Symbiodinium *in* Seriatopora caliendrum *exposed to two temperatures and two* p*CO2 values, with each treatment performed in duplicate tanks*

Type	Source	SS	df	MS	*F*	*P*-value
Between subjects	Temperature	0.0035	1	0.0035	8.750	**0.042**
	pCO_2	<0.0001	1	<0.0001	<0.250	0.643
	Temperature × pCO_2	0.0004	1	0.0004	1.000	0.374
	Tank (temperature × pCO_2)	0.0017	4	0.0004	0.607	0.659
	Error	0.0028	39	0.0007		
Within subjects	Coral	0.0129	1	0.0129	21.500	**0.010**
	Coral × temperature	0.0010	1	0.0010	1.667	0.266
	Coral × pCO_2	<0.0001	1	<0.0001	<0.167	0.704
	Coral × temperature × pCO_2	0.0051	1	0.0051	8.500	**0.043**
	Coral × tank (temperature × pCO_2)	0.0022	4	0.0006	0.684	0.607
	Error	0.0318	39	0.0008		

Corals (*Seriatopora caliendrum*; $n = 6$ $tank^{-1}$) were analyzed at the start and end of the incubation period and are treated as a repeated measures, within subject effect. Main effects and their interactions were tested over the Tank (temperature) × pCO_2) effect (between subjects) or the Coral × Tank (temperature × pCO_2) effect (within subjects). df, degrees of freedom; *F*, *F*-statistic; MS, mean sum of squares; SS, sum of squares. Significant differences are in **bold**.

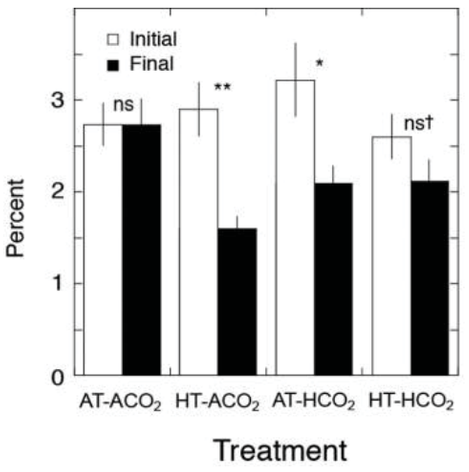

Figure 2. Effect of 12 d of incubation under combinations of temperature and pCO_2 on the mitotic index of *Symbiodinium* in *Seriatopora caliendrum*. Data are means ± SE; $n = 12$ for all bars. Results of *post hoc*, paired sample *t*-tests (for final *vs.* initial values) shown as ns (not significant; $P > 0.05$), $*P < 0.05$, $**P < 0.01$, and †(ns, $P < 0.10$). AT-ACO_2, ambient temperature–ambient pCO_2; AT-HCO_2, ambient temperature–high pCO_2; HT-ACO_2, high temperature–ambient pCO_2; HT-HCO_2, high temperature–high pCO_2.

tween these stressors (Anthony *et al.*, 2008; Kaniewska *et al.*, 2012), support for this outcome is equivocal (Wall *et al.*, 2014; Noonan and Fabricius, 2016). In the present study, corals were warmed to 30.4 °C and exposed to a high pCO_2 value expected to occur by the end of this century, assuming the moderate RCP 6.0 scenario predicted by the Intergovernmental Panel on Climate Change (IPCC) (2014). We used their responses to these conditions to answer our three questions posed in the Introduction.

First, we found that thermal bleaching was unaffected by high pCO_2. *Seriatopora caliendrum* bleached at 30.4 °C, that is, *Symbiodinium* density standardized to surface area and protein declined, but this response was unaffected by pCO_2. Second, we discovered that the division of *Symbiodinium* (*i.e.*, MI) was affected by increased temperature (although not during the high temperature–high pCO_2 combination [HT-HCO_2]). Generally, *Symbiodinium* that remained in thermally bleached corals divided more slowly than those in the controls. Third, we found evidence that *Symbiodinium* physiology, as evaluated indirectly by chlorophyll content, was affected by temperature, and pigment levels were elevated in the cells remaining in bleached corals. Moreover, because there was little genetic variation in the *Symbiodinium* types found in *S. caliendrum*, the extent of bleaching was unrelated to differences in *Symbiodinium* genotypes. Finally, a strong component of the answers to all three of our questions was the extent to which colonies differed in their responses to the treatments. This finding emerged as a significant main effect for virtually every dependent variable. Together, these results showed that high temperature, independent of pCO_2 level, was a primary cause of coral bleaching, and suggest that variations in bleaching among coral colonies (Edmunds, 1994; Baird *et al.*, 2009) are attributed to intraspecific genetic variation in cnidarian hosts.

Loss of Symbiodinium *from the coral host*

High temperature caused bleaching in juvenile *Seriatopora caliendrum.* However, increased pCO_2 by itself or with increased temperature had no effect on *Symbiodinium* density, and thus had no role in causing bleaching (*sensu* whitening of corals due to loss of *Symbiodinium*). The consequences of elevated temperature on *Symbiodinium* density in the present study are well known (Fitt *et al.*, 2001; Jokiel, 2004; Lesser, 2007), but the results of quantitative analyses of the effects of

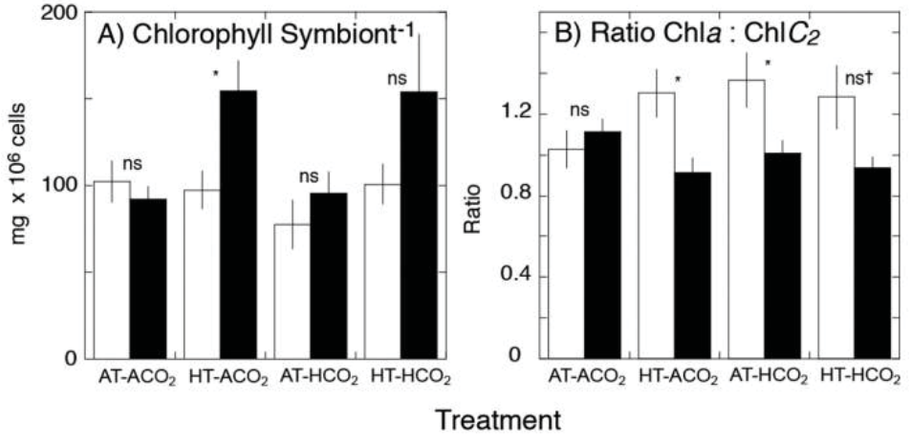

Figure 3. Effects of 12 d of incubation under combinations of temperature and pCO_2 treatments for chlorophyll content of *Symbiodinium* from *Seriatopora caliendrum*. (A) Total chlorophyll (chlorophyll symbiont^{-1}), and (B) ratio of chlorophyll *a* to chlorophyll c_2 (Chl*a* : Chlc_2). Results of *post hoc*, paired sample *t*-tests (for final *vs.* initial values) shown as ns (not significant; $P > 0.05$), $*P < 0.05$, $**P < 0.01$, and ns†($P < 0.10$). AT-ACO_2, ambient temperature–ambient pCO_2; AT-HCO_2, ambient temperature–high pCO_2; HT-ACO_2, high temperature–ambient pCO_2; HT-HCO_2, high temperature–high pCO_2.

Table 5

Statistical analysis of chlorophyll content (normalized to Symbiodinium *density) and the ratio of chlorophyll* a *to chlorophyll* c_2 *in* Seriatopora caliendrum *exposed to two temperatures and two* pCO_2 *values, with each treatment combination created in duplicate tanks*

Variable	Type	Source	SS	df	MS	*F*	*P*
Total chlorophyll	Between subjects	Temperature	15833.4	1	15833.4	20.304	**0.010**
		pCO_2	0.394	1	0.394	0.005	0.947
		Temperature × pCO_2	72.467	1	72.467	0.094	0.774
		Tank (Temperature × pCO_2)	3119.561	4	779.891	0.418	0.795
		Error	70978.4	38	1867.853		
	Within subjects	Coral	11282.0	1	11282.0	4.512	0.101
		Coral × temperature	7622.913	1	7622.913	3.049	0.156
		Coral × pCO_2	42.660	1	42.660	0.017	0.903
		Coral × temperature × pCO_2	5334.577	1	5334.577	2.134	0.218
		Coral × tank (temperature × pCO_2)	10000.7	4	2500.176	1.325	0.278
		Error	71725.8	38	1887.521		
Chlorophyll a:c_2	Between subjects	Temperature	0.047	1	0.047	0.385	0.569
		pCO_2	0.195	1	0.195	1.598	0.275
		Temperature × pCO_2	0.023	1	0.023	0.189	0.686
		Tank (temperature × pCO_2)	0.486	4	0.122	1.059	0.390
		Error	4.364	38	0.115		
	Within subjects	Coral	1.541	1	1.541	102.733	<**0.001**
		Coral × temperature	0.348	1	0.348	23.200	**0.009**
		Coral × pCO_2	0.192	1	0.192	12.800	**0.023**
		Coral × temperature × pCO_2	0.377	1	0.377	25.133	**0.007**
		Coral × tank (temperature × pCO_2)	0.061	4	0.015	0.123	0.974
		Error	4.696	38	0.124		

Corals (*Seriatopora caliendrum*; $n = 6$ tank^{-1}) were analyzed at the start and end of the incubation period and are treated as a repeated measures, within subject effect. Main effects and their interactions were tested over the Tank (Temperature × pCO_2) effect (between subjects) or the Coral × tank (Temperature × pCO_2) effect (within subjects). Chlorophyll a:c_2, ratio of chlorophyll a to chlorophyll c_2; df, degrees of freedom; *F*, *F*-statistic; MS, mean sum of square; SS, sum of squares. Significant differences are in **bold**.

high pCO_2 on bleaching are equivocal. For example, Anthony *et al.* (2008) incubated *Acropora intermedia* and *Porites lutea* under combinations of two temperatures (25–26 °C *vs.* 28–29 °C) and three pCO_2 regimens (38.5, 52.7–70.0, and 101.3–131.7 Pa) at ecologically relevant irradiances (1000 μmol photons m^{-2} s^{-1}), and found that bleaching was enhanced during high pCO_2 at values similar to those used in the present study (~89.2–92.2 Pa). However, because Anthony *et al.* (2008) evaluated bleaching using a luminance colorimetric scale rather than using *Symbiodinium* densities or measurements of their pigment content, their results are difficult to compare with our own (Table 1). Kaniewska *et al.* (2012) reported a bleaching response to elevated pCO_2 in *Acropora millepora*, but observed a decline in *Symbiodinium* densities only at the highest pCO_2 treatments (102.3–136.8 Pa), which were higher than our own in this study. In contrast, Wall *et al.* (2014), who also worked with *Seriatopora caliendrum* in Taiwan, found that bleaching (*i.e.*, decline in *Symbiodinium* density and their chlorophyll content) occurred when corals were incubated at 27.7 °C and 30.5 °C, but saw no direct or synergistic effect of high pCO_2 (~84.8 Pa). Similarly, Noonan and Fabricius (2016) showed no effect of elevated pCO_2 (~79.0 Pa) on bleaching in *Acropora millepora* or *Seriatopora hystrix* when corals were incubated at 28 °C and 30 °C. Hoadley *et al.* (2016) established various physiological impacts of the interaction of increased temperature and higher pCO_2 levels on *Symbiodinium trenchii*, but no such interactive effect was observed in the case of symbiont densities. Although variation among coral species and dissimilar light intensities can vary the extent of bleaching among corals (Weis, 2008), mounting evidence suggests that bleaching will not be accentuated by the expected increase in pCO_2 over the next century.

Changes in the mitotic index of Symbiodinium

The division rates of *Symbiodinium* in the corals following treatments were reduced 45% at high temperature and ambient pCO_2 (HT-ACO$_2$ treatment) and reduced 35% in high pCO_2 and ambient temperature (AT-HCO$_2$ treatment), all compared to initial values. Mitotic indices (MIs), however, remained unchanged in controls and combined high temperature and high pCO_2 (HT-HCO$_2$ treatment) (Fig. 2). The mean

Table 6

Statistical analysis of Symbiodinium *community composition in* Seriatopora caliendrum *exposed to two temperatures and two* pCO_2 *values*

A) Initial time point

Variable	Source	SS	df	MS	*F*	*P*-value
Symbiodinium community composition	Temperature	0.02396	1	0.02396	0.98126	0.4115
	pCO_2	0.05047	1	0.05047	2.06647	0.0735
	Temperature × pCO_2	0.02898	1	0.02898	1.18665	0.2847
	Error	0.51286	21	0.024422		

B) Final time point

Variable	Source	SS	df	MS	*F*	*P*-value
Symbiodinium community composition	Temperature	0.01210	1	0.01210	0.59798	0.8018
	pCO_2	0.02282	1	0.02282	1.12747	0.3479
	Temperature × pCO_2	0.01063	1	0.01063	0.52506	0.8609
	Error	0.42501	21	0.020239		

Corals (*Seriatopora caliendrum*; $n = 6–7$ treatment^{-1}) were analyzed at (A) the start and (B) end of the incubation period, using a permutation test with pseudo *F*-ratio (number of permutations:9999). df, degrees of freedom; *F*, pseudo-*F* statistics; MS, mean sum of squares; SS, sum of squares.

MI of *Symbiodinium* in corals prior to the incubations (2.87% ± 1.03% [± SD]) was within the range of values recorded for other reef corals (*i.e.*, 1.1%–14.1% [Wilkerson *et al.*, 1988]). Mitotic indices are variable and may be host and/or *Symbiodinium* species specific (Wilkerson *et al.*, 1988; Starzak *et al.*, 2014); variation under experimental conditions may be exacerbated by differences in incubation temperature (Strychar *et al.*, 2005). In the present study, the decline in MI of the *Symbiodinium* in corals exposed to thermal stress only (AT-ACO_2 *vs.* AT-HCO_2 treatment; Fig. 2) differed from the results of previous studies. For instance, Strychar *et al.* (2004) examined *Symbiodinium* in the corals *Acropora hyacinthus*, *Favites complanata*, and *Porites solida* following 48 h of exposure to increased temperature (30 °C, 32 °C, and 34 °C) but with no pCO_2 treatments, and reported a variable and species-specific increase of MI as a linear function of temperature from 28–34 °C. However, they found no difference in MI between 28 °C and 30 °C or 30 °C and 32 °C, but suggested an overall increase in MI based on the larger temperature range (*i.e.*, 6 °C). The present study, which lasted 12 d, and employed a seawater temperature of ~2.9 °C above ambient, was conducted under a moderate range of ecological conditions as predicted by the IPCC 2014 RCP scenario 6.0 (Intergovernmental Panel on Climate Change (IPCC). 2014) . This difference, along with differences in the coral species considered, likely explains the discrepancy between our findings and those of Strychar *et al.* (2004).

Strychar *et al.* (2004) observed a preferential loss of dividing *Symbiodinium* following increased temperature in *Acropora hyacinthus* and *Favites complanata*, but not *Porites solida*. This outcome was similar to our previous work (Baghdasarian and Muscatine, 2000), in which *Pocillopora damicornis* and the sea anemone *Aiptasia pulchella* were incubated for 15.5 h at 29.5 °C in Hawaii (when ambient seawater was at 27 °C). Baghdasarian and Muscatine (2000) showed that dividing *Symbiodinium* were preferentially released during bleaching, and suggested that this form of release represented a mechanism for regulating algal-cnidarian symbiosis during fluctuating temperatures. Although neither Baghdasarian and Muscatine (2000) nor Strychar *et al.* (2004) incubated corals for > 48 h, Strychar *et al.* (2004) suggested that the release of dividing symbionts occurred primarily in the first 36 h of exposure to high-temperature stress, declining thereafter to undetectable levels. We hypothesize that the initial rapid releases of dividing *Symbiodinium* represent a functional response favoring convergence to a new equilibrium population density. In this context, the MI of *Symbiodinium* remaining *in hospite* following bleaching (as in the present case) could reflect an aspect of acclimation to the new temperature regimen.

Although the main effect of pCO_2 did not affect MI in the present study, there was a temperature × pCO_2 interaction that varied among corals (*i.e.*, the three-way interaction was significant). This effect was revealed in the *post hoc* contrasts of the initial and final values, which showed that the MI of *Symbiodinium* remaining in the corals differed (and was reduced after the incubations) under high pCO_2 at ambient temperature (AT-HCO_2) but not at high pCO_2 and temperature (HT-HCO_2), and that this effect varied among corals. In the case of the AT-HCO_2 treatment, depressed MI did not reflect the expulsion of dividing *Symbiodinium* (as noted above and in Baghdasarian and Muscatine [2000]), because bleaching did not occur in this treatment (Figs. 1, 2). Therefore, the reduction in MI may have reflected a change in the division rate of *Symbiodinium* when the holobiont was exposed to high pCO_2. Alternatively, this difference in MI among treatments could be influenced by *Symbiodinium* type. While no significant differences in *Symbiodinium* communities were found among the treatments, the AT-HCO_2 treatment showed the

highest frequency of *Symbiodinium* C15. This type has demonstrated a low capacity for being cultured *in vitro* and depressed population growth in comparison to other C types (Krueger and Gates, 2012), which could partly explain the low MI seen in the present study. However, the presence of C15 did not appear to be affected by the external conditions. The lack of sensitivity of the symbiont community within corals to increasing pCO_2 is also supported by comparison of symbionts in five coral species across a natural CO_2 gradient, where no difference in symbiont community was observed within each of the species due to CO_2 exposure (Noonan *et al.*, 2013). Coral hosts can also modulate symbiont physiology (*e.g.*, photosynthesis), through acidification of the symbiosome surrounding the algal cells, to a pH as low as ~4 (Barott *et al.*, 2015), suggesting that smaller fluctuations in pH of seawater may not significantly affect algal growth rates. Finally, free-living phytoplankton, including dinoflagellates, cryptophytes, diatoms, and prymnesiophytes, show no effect of lowered pH (within a range of ~7.0–8.5) on growth rates (Berge *et al.*, 2010), emphasizing the lack of algal growth sensitivity to high pCO_2 exposure. The results of the aforementioned studies, along with our observations of reduced MI in response to increases in temperature and pCO_2 levels, underscore the potential role of the cnidarian host in mediating symbiont division. Further investigation is needed.

Our results for MI as well as those cited above suggest that the division of *Symbiodinium* in corals is depressed following perturbed environmental conditions as a result of at least three mechanisms that cannot be distinguished. One mechanism involves the expulsion of dividing cells, as was seen in the high temperature–ambient pCO_2 treatment. A second mechanism is a direct result of high pCO_2 depressing *Symbiodinium* division under conditions that do not favor bleaching (at AT-HCO_2). Finally, it is possible that *Symbiodinium* remaining in the coral following bleaching—when large numbers of *Symbiodinium* cells are lost—undergo division at rates no different from the controls. If this is true, it suggests that bleaching under these conditions (*i.e.*, HT-HCO_2) may be mechanistically different from bleaching under HT-ACO_2. Whereas bleaching at HT-ACO_2 probably resulted from the expulsion of large numbers of dividing cells, bleaching at HT-HCO_2 may have involved the expulsion of a wide selection of *Symbiodinium* cells relative to the stage of their progression through the classic stages of the cell cycle (Murray and Hunt, 1993). Further experiments are necessary to distinguish among these three mechanisms, and it is likely that the research will be insightful to fundamental mechanisms driving variation in the coral bleaching response.

Changes in chlorophyll

High temperature was associated with increased total chlorophyll concentrations in the *Symbiodinium* remaining in corals at the end of the experiment, but not in those corals that were exposed to elevated pCO_2, either individually or combined with elevated temperature (Fig. 3). High temperature and high pCO_2 resulted in a decline in the chlorophyll *a* to chlorophyll c_2 ratio (Fig. 3B), caused primarily by increased concentrations of chlorophyll c_2 *versus* chlorophyll *a* (G. Baghdasarian, unpubl. data).

The temperature-induced increases in chlorophyll content of *Symbiodinium* remaining *in hospite* in the present study were inconsistent with some previous findings. Takahashi *et al.* (2008) maintained cultured *Symbiodinium* at 31–34 °C, and found that type OTcH-1 was unaffected by increased temperature, but type CS-73 lost chlorophyll over 24 h. However, chlorophyll loss in CS-73 was attributed to photobleaching rather than thermal bleaching (Hoegh-Guldberg, 1999), because Takahashi *et al.*'s light intensities were 5-fold higher than what we used in the present study (*i.e.*, 200 *vs.* 40 μmol quanta m^{-2} s^{-1}). Strychar and Sammarco (2012) also reported reduced chlorophyll concentrations in *Symbiodinium* as a result of high temperatures, but this effect was only observed at temperatures of at least 32 °C (to 34 °C); they found no effect at a temperature of ~30 °C, which was close to the high temperature used in our current study. Another experiment reporting high temperature-induced declines in the chlorophyll content of *Symbiodinium* in reef corals also used a high temperature of 32 °C (Dove *et al.*, 2006). A number of factors, including interspecific effects and thermal history of corals, may influence the temperature at which chlorophyll concentrations change. Yet, in the case of the aforementioned studies, a temperature of about 32 °C might have served as a threshold for impairment of photophysiological function in *Symbiodinium*. It may explain the higher chlorophyll levels observed in the above-cited studies than in our own. However, our finding that pCO_2 did not affect chlorophyll concentration in *Symbiodinium* was consistent with studies of *Seriatopora* spp. and *Stylophora pistillata* (Burris *et al.*, 1983; Tremblay *et al.*, 2013; Wall *et al.*, 2014). The finding may either reflect the insensitivity of photosynthesis to the small changes in pH involved or the capacity of the coral host to maintain the pH of the microenvironment around the *Symbiodinium* at levels favoring photosynthesis (Gates *et al.*, 1995, 1999; Barott *et al.*, 2015). Our differences in the response of *Symbiodinium* to increased temperature under ambient *versus* high pCO_2 treatments were similar to those described for *Symbiodinium trenchii* associated with the coral *Turbinaria renifomis* (Hoadley *et al.*, 2016). In this case, and similar to our study, chlorophyll concentrations under HT-HCO_2 (*i.e.*, ~31.5 °C; pCO_2 ~77 Pa) remained unchanged over the experimental period, although other cellular and physiological parameters of the symbionts did change in response to high pCO_2. Our study and the aforementioned findings emphasize the differences in holobiont response to thermal stress under elevated *versus* ambient pCO_2. All suggest that the interactive effects of temperature, pCO_2, and available light are complex, and that a complete understanding of the mechanics of the physiological response of the corals to environmental change remains equivocal.

The changes in chlorophyll concentration that were seen in our study likely reflected treatment effects on photophysiology of the *Symbiodinium* remaining in the corals, with the potential capacity to shift maximum rate of photosynthesis in the symbionts and affect the overall metabolic state of the holobiont under changed temperature, pCO_2 and light levels. For example, increased concentrations of chlorophyll c_2 in *Symbiodinium* may be associated with reorganization of the photosynthetic units (PSUs) to favor saturation at lower irradiance levels (*i.e.*, lower saturation irradiance (I_k) (Shick, 1991). Increased concentrations of chlorophyll *a* in *Symbiodinium* are typically associated with higher numbers of PSUs, which can increase the maximum rate of photosynthesis (P_{max}) (Shick, 1991). However, the means by which these chlorophyll concentration changes were made remain uncertain, because we could not distinguish the pigment changes in the *in hospite Symbiodinium* from those attributed to the preferential release of *Symbiodinium* with different pigment concentrations. Future study of the adaptive and/or acclimation potential of these physiological responses will also need to consider *Symbiodinium* released from hosts during the experimental period. We acknowledge that a more detailed study to differentiate the physiological effects of temperature and high pCO_2 as they relate to photosynthesis in *Symbiodinium* is needed. That study should include measurement of chlorophyll content (as we did here) and of photosynthesis irradiance (PI) curves, and also measurement of quantum efficiency (F_v/F_m) under the different environmental conditions.

Variation among coral colonies

Increased temperature both with and without high pCO_2 caused a variable bleaching response among the 48 colonies of *Seriatopora caliendrum*. This variation was illustrated by

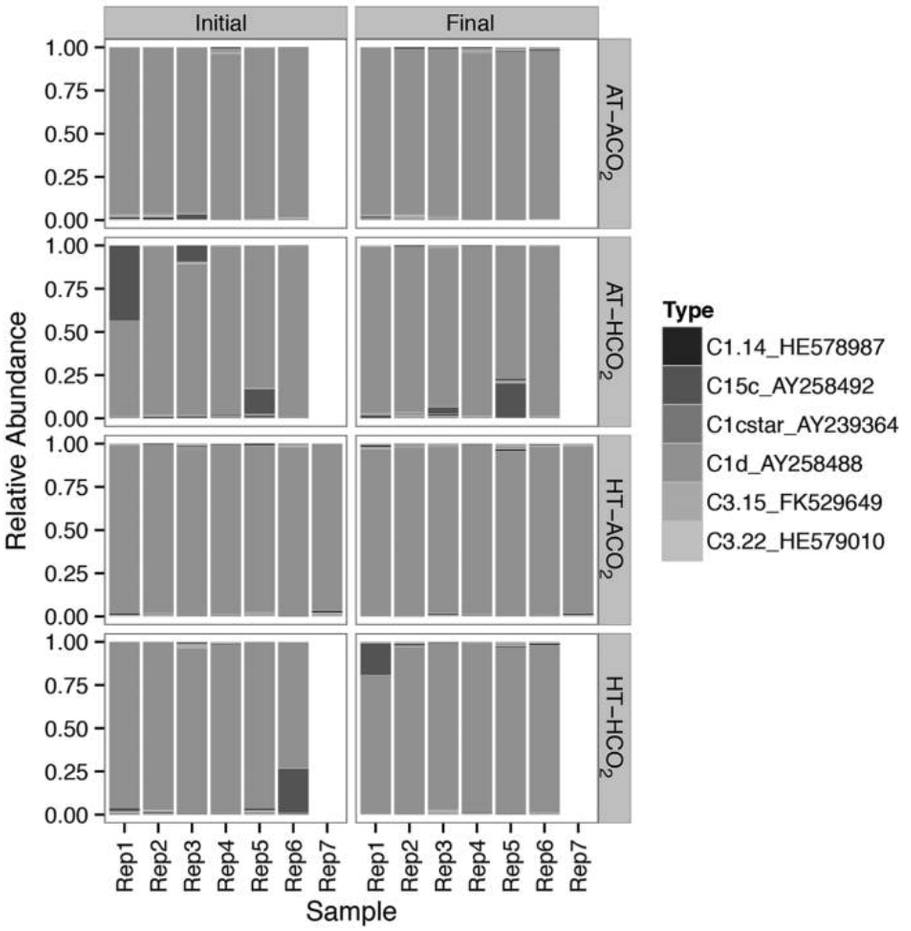

Figure 4. *Symbiodinium* types from the start (Initial) and end (Final) of the experiment. BLAST identification of the top 10 operational taxonomic units (OTUs) resulted in identification of 6 types, which comprised > 98.8% of all sequences. Visualization of the top 10 OTUs before taxonomic assignment shows similar results, with no clear pattern among treatments or over time (Appendix Fig. A1). AT-ACO_2, ambient temperature–ambient pCO_2; AT-HCO_2, ambient temperature–high pCO_2; HT-ACO_2, high temperature–ambient pCO_2; HT-HCO_2, high temperature–high pCO_2. Rep1–Rep7, replicate coral samples; C1.14 –C3.22, *Symbiodinium* internal transcribed spacer (ITS) type identified for each OTU, with corresponding accession number after each underscore.

Symbiodinium densities that decreased 77% at HT-ACO_2 in one colony while increasing 9% in another coral. Although genetic identification of the cnidarian hosts was beyond the scope of our study, our choice of study species and the collection regimen we used increased the likelihood that the colonies were genetically distinct. *Seriatopora caliendrum* is fecund at the location of this study, Nanwan Bay, Taiwan (Cumbo *et al.*, 2012). Because there is no evidence of substantial asexual larval production by this species in Taiwan, we assume that colonies arising from larval settlement are likely to be genetically distinct. Further, by collecting small colonies that were separated from one another on a scale of a few meters (2–15 m), we reduced the likelihood of sampling colonies within single clones that arose from fragmentation (Highsmith, 1982). If our colonies were genetically distinct, then the among-colony phenotypic variation described herein (*i.e.*, variation in the response to treatments) may be genetically determined by the coral host and thus be subject to natural selection. This possibility is important, because it suggests that the population-level response of *Seriatopora caliendrum* to climate change and ocean acidification can occur more quickly through evolutionary processes than was previously supposed (Buddemeier *et al.*, 2004; Guest *et al.*, 2012).

Because all of the corals in this study contained Clade C *Symbiodinium*—primarily a single type, C1d (> 90%)—variation in *Symbiodinium* genetics does not seem to explain why the colonies differed in their response to treatments. Although C15 *Symbiodinium* has been associated with tolerance to high temperature in multiple coral genera (but not previously in *Seriatopora*) at our study site in Taiwan (Keshavmurthy *et al.*, 2014), these *Symbiodinium* were present in low numbers (2.3%) in our study corals, and variation in their distribution was not consistent with differences in bleaching severity (Fig. 4; Supplementary File, view online). Given the constancy of *Symbiodinium* types among the treatments, it is more likely that the changes in their MI and photosynthetic pigment content represented either acclimation to favor protection against bleaching or possibly a benign byproduct of environmental change in their cellular physiology (Dove *et al.*, 2006; Venn *et al.*, 2006). The alternate explanation, supported by our data, is that variation in bleaching response of *Seriatopora caliendrum* to treatment conditions is a function of differences in the host cnidarian. Morphologically hidden host genetic variations amongst corals is well established (Ladner and Palumbi, 2012; Warner *et al.*, 2015), and may explain at least some cases of variation in bleaching among conspecific coral colonies. It could also represent genetically determined variation in capacity for phenotypic plasticity (Barshis *et al.*, 2013; Granados-Cifuentes *et al.*, 2013). Previous studies have implicated the role of the host in coral bleaching (Edmunds, 1994; Edmunds and Gates, 2003; Fitt *et al.*, 2009). Further, interspecific variation in the stress response to cold shock has been established in *Acropora palmata*, with isoclonal *Symbiodinium* showing variation in photochemical efficiency and protein production (Parkinson *et al.*, 2015). It has also been established in *Porites astreoides* through diverse molecular response when it was transplanted among different environments (Kenkel and Matz, 2016). Based on the current study and the literature cited above, we reemphasize the importance of future work to consider the role of the coral host genotype, and both interspecific and intraspecific coral physiological differences in determining bleaching sensitivity.

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Literature Cited

Ainsworth, T. D., S. F. Heron, J. C. Ortiz, P. J. Mumby, A. Grech, D. Ogawa, C. M. Eakin, and W. Leggat. 2016. Climate change disables coral bleaching protection on the Great Barrier Reef. *Science* **352:** 338–342.

Anthony, K. R. N., S. R. Connolly, and O. Hoegh-Guldberg. 2007. Bleaching, energetics, and coral mortality risk: effects of temperature, light, and sediment regime. *Limnol. Oceanogr.* **52:** 716–726.

Anthony, K. R. N., D. I. Kline, G. Diaz-Pulido, S. Dove, and O. Hoegh-Guldberg. 2008. Ocean acidification causes bleaching and productivity loss in coral reef builders. *Proc. Natl. Acad. Sci.USA* **105:** 17442–17446.

Baghdasarian, G., and L. Muscatine. 2000. Preferential expulsion of dividing algal cells as a mechanism for regulating algal-cnidarian symbiosis. *Biol. Bull.* **199:** 278–286.

Baird, A. H., R. Bhagooli, P. J. Ralph, and S. Takahashi. 2009. Coral bleaching: the role of the host. *Trends Ecol. Evol.* **24:** 16–20.

Baker, A. C. 2003. Flexibility and specificity in coral-algal symbiosis: diversity, ecology, and biogeography of *Symbiodinium*. *Annu. Rev. Ecol. Evol. Syst.* **34:** 661–689.

Barott, K. L., A. A. Venn, S. O. Perez, S. Tambutté, and M. Tresguerres. 2015. Coral host cells acidify symbiotic algal microenvironment to promote photosynthesis. *Proc. Natl. Acad. Sci. USA* **112:** 607–612.

Barshis, D. J., J. T. Ladner, T. A. Oliver, F. O. Seneca, N. Traylor-Knowles, and S. R. Palumbi. 2013. Genomic basis for coral resilience to climate change. *Proc. Natl. Acad. Sci. USA* **110:** 1387–1392.

Bay, L. K., A. Guérécheau, N. Andreakis, K. E. Ulsrup, and M. V. Matz. 2013. Gene expression signatures of energetic acclimatization in the reef building coral *Acropora millepora*. *PLoS One* **8:** e61736.

Berge, T., N. Daugbjerg, B. B. Andersen, and P. J. Hansen. 2010. Effect of lowered pH on marine phytoplankton growth rates. *Mar. Ecol. Prog. Ser.* **416:** 79–91.

Brown, B. E., and R. P. Dunne. 2015. Coral bleaching. Pp. 266–283 in *Diseases of Coral*, C. M. Woodley, C. A. Downs, A. W. Bruckner, J. W. Porter, and S. B. Galloway, eds. John Wiley, Hoboken, NJ.

Buddemeir, R. W., and D. G. Fautin. 1993. Coral bleaching as an adaptive mechanism. *BioScience* **43:** 320–326.

Buddemeier, R. W., A. C. Baker, D. G. Fautin, and J. R. Jacobs. 2004. The adaptive hypothesis of bleaching. Pp. 427–444. in *Coral Health and Disease*, E. Rosenberg and Y. Loya, eds. Springer, New York.

Burris, J. E., J. W. Porter, and W. A. Laing. 1983. Effects of carbon dioxide concentration on coral photosynthesis. *Mar. Biol.* **75:** 113–116.

Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer, and R. Knight. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. USA* **108:** 4516–4522.

Comeau, S., P. J. Edmunds, N. B. Spindel, and R. C. Carpenter. 2014. Fast coral reef calcifiers are more sensitive to ocean acidification in short-term laboratory incubations. *Limnol. Oceanogr.* **59:** 1081–1091.

Crawley, A., D. I. Kline, S. Dunn, K. Anthony, and S. Dove. 2010. The effect of ocean acidification on symbiont photorespiration and productivity in *Acropora formosa*. *Global Change Biol.* **16:** 851–863.

Cumbo, V. R., T.-Y. Fan, and P. J. Edmunds. 2012. Physiological development of brooded larvae from two pocilloporid corals in Taiwan. *Mar. Biol.* **159:** 2853–2866.

Davy, S. K., D. Allemand, and V. M. Weis. 2012. Cell biology of cnidarian-dinoflagellate symbiosis. *Microbiol. Mol. Biol. Rev.* **76:** 229–261.

Dove, S., J. C. Ortiz, S. Enríquez, M. Fine, P. Fisher, R. Iglesias-Prieto, D. Thornhill, and O. Hoegh-Guldberg. 2006. Response of holosymbiont pigments from the scleractinian coral *Montipora monasteriata* to short-term heat stress. *Limnol. Oceanogr.* **51:** 1149–1158.

Dufault, A. M., V. R. Cumbo, T. Y. Fan, and P. J. Edmunds. 2012. Effects of diurnally oscillating pCO_2 on the calcification and survival of coral recruits. *Proc. Biol. Sci.* **279:** 2951–2958.

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26:** 2460–2461.

Edmunds, P. J. 1994. Evidence that reef-wide patterns of coral bleaching may be the result of the distribution of bleaching-susceptible clones *Mar. Biol.* **121:** 137–142.

Edmunds, P. J. 2008. The effects of temperature on the growth of juvenile scleractinian corals. *Mar. Biol.* **154:**153–162.

Edmunds, P. J., and R. D. Gates. 2003. Has coral bleaching delayed our understanding of fundamental aspects of coral-dinoflagellate symbioses? *BioScience* **53:** 976–980.

Edmunds, P. J., and R. D. Gates. 2008. Acclimatization in tropical reef corals. *Mar. Ecol. Prog. Ser.* **361:** 307–310.

Edmunds, P. J., X. Pochon, D. R. Levitan, D. M. Yost, M. Belcaid, H. M. Putnam, and R. D. Gates. 2014. Long-term changes in *Symbiodinium* communities in *Orbicella annularis* in St. John, US Virgin Islands. *Mar. Ecol. Prog. Ser.* **506:** 129–144.

Fitt, W. K., B. E. Brown, M. E. Warner, and R. P. Dunne. 2001. Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. *Coral Reefs* **20:** 51–65.

Fitt, W. K., R. D. Gates, O. Hoegh-Guldberg, J. C. Bythell, A. Jatkar, A. G. Grottoli, M. Gomez, P. Fisher, T. C. Lajuenesse, O. Pantos *et al.* 2009. Response of two species of Indo-Pacific corals, *Porites cylindrica* and *Stylophora pistillata*, to short-term thermal stress: the host does matter in determining the tolerance of corals to bleaching. *J. Exp. Mar. Biol. Ecol.* **373:** 102–110.

Gates, R. D., and P. J. Edmunds. 1999. The physiological mechanisms of acclimatization in tropical reef corals. *Am. Zool.* **39:** 30–43.

Gates, R. D., O. Hoegh-Guldberg, M. J. McFall-Ngai, K. Y. Bil, and L. Muscatine. 1995. Free amino acids exhibit anthozoan "host factor" activity: they induce the release of photosynthate from symbiotic dinoflagellates *in vitro*. *Proc. Natl. Acad. Sci. USA* **92:** 7430–7434.

Gates, R. D., K. Y. Bil, and L. Muscatine. 1999. The influence of an anthozoan "host factor" on the physiology of a symbiotic dinoflagellate. *J. Exp. Mar. Biol. Ecol.* **232:** 241–259.

Granados-Cifuentes, C., A. J. Bellantuono, T. Ridgway, O. Hoegh-Guldberg, and M. Rodriguez-Lanetty. 2013. High natural gene expression variation in the reef-building coral *Acropora millepora*: potential for acclimative and adaptive plasticity. *BMC Genomics* **14:** 228–240.

Guest, J. R., A. H. Baird, J. A. Maynard, E. Muttaqin, A. J. Edwards, S. J. Campell, K. Yewdall, Y. A. Affendi, and L. M. Chou. 2012. Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. *PLoS One* **7:** e33353.

Highsmith, R. C. 1982. Reproduction by fragmentation in corals. *Mar. Ecol. Prog. Ser.* **7:** 207–226.

Hoadley, K. D., D. T. Pettay, A. G. Grottoli, W.-J. Cai, T. F. Melman, S. Levas, V. Schoepf, Q. Ding, X. Yuan, Y. Wang *et al.* 2016. High-temperature acclimation strategies within the thermally tolerant endosymbiont *Symbiodinium trenchii* and its coral host, *Turbinaria reniformis*, differ with changing pCO_2 and nutrients. *Mar. Biol.* **163:** 1–13.

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. *Mar. Freshw. Res.* **50:** 839–866.

Hoegh-Guldberg, O., P. J. Mumby, A. J. Hooten, R. S. Steneck, P. Greenfield, E. Gomez, C. D. Harvell, P. F. Sale, A. J. Edwards, K. Caldeira *et al.* 2007. Coral reefs under rapid climate change and ocean acidification. *Science* **318:** 1737–1742.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr.* **54:** 187–211.

Intergovernmental Panel on Climate Change (IPCC). 2014. *Climate Change 2013: the Physical Science Basis*. Contribution of working group I to the fifth Assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge.

Jeffrey, S. W., and G. F. Humphrey. 1975. New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanz.* **167:** 191–194.

Jokiel, P. L. 2004. Temperature stress and coral bleaching. Pp. 401–425 in *Coral Health and Disease*, E. Rosenberg and Y. Loya, eds. Springer, New York.

Jones, A. M., R. Berkelmans, M. J. H. van Oppen, J. C. Mieog, and W. Sinclair. 2008. A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proc. R. Soc. B* **275:** 1359–1365.

Kaniewska, P., P. R. Campbell, D. I. Kline, M. Rodriguez-Lanetty, D. J. Miller, S. Dove, and O. Hoegh-Guldberg. 2012. Major cellular and physiological impacts of ocean acidification on a reef building coral. *PLoS One* **7:** 334659.

Kenkel, C. D., and M. V. Matz. 2016. Gene expression plasticity as a mechanism of coral adaptation to a variable environment. *Nat. Ecol. Evol.* **1:** 0014.

Kenkel, C. D., G. Goodbody-Gringley, D. Caillaud, S. W. Davies, E. Bartels, and M. V. Matz. 2013. Evidence for a host role in thermotolerence divergence between populations of the mustard hill coral (*Porites astreoides*) from different reef environments. *Mol. Ecol.* **22:** 4335–4348.

Keshavmurthy, S., P.-J. Meng, J-T. Wang, C.-Y. Kuo, S.-Y. Yang, C.-M. Hsu, C.-H. Gan, C.-F. Dai, and C. A. Chen. 2014. Can resistant coral-*Symbiodinium* assocations enable coral communities to survive climate change? A study of a site exposed to long-term hot water input. *PeerJ*. **2:** e327.

Krueger, T., and R. D. Gates. 2012. Cultivating endosymbionts—host environmental mimics support the survival of *Symbiodinium* C15 *ex hospite*. *J. Exp. Mar. Biol. Ecol.* **413:** 169–176.

Ladner, J. T., and S. R. Palumbi. 2012. Extensive sympatry, cryptic diversity and introgression throughout the geographic distribution of two coral species complexes. *Mol. Ecol.* **21:** 2224–2238.

Lesser, M. P. 2004. Experimental biology of coral reef ecosystems. *J. Exp. Mar. Biol. Ecol.* **300:** 217–252.

Lesser, M. P. 2006. Oxidative stress in marine environments: biochemistry and physiological ecology. *Annu. Rev. Physiol.* **68:** 253–278.

Lesser, M. P. 2007. Coral reef bleaching and global climate change: can corals survive the next century? *Proc. Natl. Acad. Sci. USA* **104:** 5259–5260.

Lesser, M. P. 2011. Coral bleaching: causes and mechanisms. Pp. 405–419 in *Coral Reefs: an Ecosystem in Transition*, Z. Dubinsky and N. Stambler, eds. Springer, New York.

Lesser, M. P., M. Stat, and R. D. Gates. 2013. The endosymbiotic dinoflagellates (*Symbiodinium* sp.) of corals are parasites and mutualists. *Coral Reefs* **32:** 603–611.

Logan, C. A., J. P. Dunne, C. M. Eakin, and S. D. Donner. 2014. Incorporating adaptive responses into future projections of coral bleaching. *Global Change Biol.* **20:** 125–139.

Loya, Y., K. Sakai, K. Yamazato, Y. Nakano, H. Sambali, and R. van Woesik. 2001. Coral bleaching: the winners and the losers. *Ecol. Lett.* **4:** 122–131.

Margulis, L., and R. Fester, eds. 1991. *Symbiosis as a Source of Evolutionary Innovation: Speciation and Morphogenesis*. MIT Press, Cambridge, MA.

McGinley, M. P., M. D. Aschaffenburg, D. T. Pettay, R. T. Smith, T. C. LaJeunesse, and M. E. Warner. 2012. *Symbiodinium* spp. in colonies of eastern Pacific *Pocillopora* spp. are highly stable despite the prevalence of low-abundance background populations. *Mar. Ecol. Prog. Ser.* **462:** 1–7.

Meyer, E., and V. M. Weis. 2012. Study of cnidarian-algal symbiosis in the "omics" age. *Biol. Bull.* **223:** 44–65.

Murray, A., and T. Hunt. 1993. *The Cell Cycle*. Oxford University Press, New York.

Muscatine, L., and J. W. Porter. 1977. Reef corals: mutualistic symbioses adapted to nutrient-poor environments. *BioScience* **27:** 454–460.

Noonan, S. H. C., and K. E. Fabricius. 2016. Ocean acidification affects productivity but not the severity of thermal bleaching in some tropical corals. *ICES J. Mar. Sci.* **73:** 715–726.

Noonan, S. H. C., K. E. Fabricius, and C. Humphrey. 2013. *Symbiodinium* community composition in scleractinian corals is not affected by life-long exposure to elevated carbon dioxide. *PLoS One* **8:** 63985.

Palumbi, S. R., D. J. Barshis, N. Traylor-Knowles, and R. A. Bay. 2014. Mechanisms of reef coral resistance to future climate change. *Science* **344:** 895–898.

Pandolfi, J. M., S. R. Connolly, D. J. Marshall, and A. L. Cohen. 2011. Projecting coral reef futures under global warming and ocean acidification. *Science* **333:** 418–422.

Parkinson, J. E., A. T. Banaszak, N. S. Altman, T. C. LaJeunesse, and I. B. Baums. 2015. Intraspecific diversity among partners drives functional variation in coral symbioses. *Sci. Rep*. **5:** 15667.

Pochon, X., J. Pawlowski, L. Zaninetti, and R. Rowen. 2001. High genetic diversity and relative specificity among *Symbiodinium*-like endosymbiotic dinoflagellates in soritid foraminiferans. *Mar. Biol.* **139:** 1069–1078.

R Core Team. 2012. *R: A Language and Environment for Statistical Computing* [Online]. R Foundation for Statistical Computing, Vienna, Austria. Available: http://www.R-project.org/ [2014, January 19].

Shick, J. M. 1991. *A Functional Biology of Sea Anemones*. Springer, New York.

Starzak, D. E., R. G. Quinnell, M. R. Nitschke, and S. K. Davy. 2014. The influence of symbiont type on photosynthetic carbon flux in a model cnidarian–dinoflagellate symbiosis. *Mar. Biol.* **161:** 711–724.

Stat, M., and R. D. Gates. 2011. Clade D *Symbiodinium* in scleractinian corals: a "nugget" of hope, a selfish opportunist, an ominous sign, or all of the above? *J. Mar. Biol.* **2011:** 730715.

Stat, M., E. Morris, and R. D. Gates. 2008. Functional diversity in coral-dinoflagellate symbiosis. *Proc. Natl. Acad. Sci. USA* **105:** 9256–9261.

Stat, M., X. Pochon, E. C. Franklin, J. F. Bruno, K. S. Casey, E. R. Selig, and R. D. Gates. 2013. The distribution of the thermally tolerant symbiont lineage (*Symbiodinium* clade D) in corals from Hawaii: correlations with host and the history of ocean thermal stress. *Ecol. Evol.* **3:** 1317–1329.

Stimson, J., and R. A. Kinzie III. 1991. The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. *J. Exp. Mar. Biol. Ecol.* **153:** 63–74.

Strychar, K. B., and P. W. Sammarco. 2012. Effects of heat stress on phytopigments of zooxanthellae (*Symbiodinium* spp.) symbiotic with the corals *Acropora hyacinthus*, *Porites solida*, and *Favites complanta*. *Int. J. Biol.* **4:** 3–19.

Strychar, K. B., M. Coates, and P. W. Sammarco. 2004. Loss of *Symbiodinium* from bleached Australian scleractinian corals (*Acropora hyacinthus, Favites complanata* and *Porites solida*). *Mar. Freshw. Res.* **55:** 135–144.

Strychar, K. B., M. Coates, P. W. Sammarco, T. J. Piva, and P. T. Scott. 2005. Loss of *Symbiodinium* from bleached soft corals *Sarcophyton ehrenbergi, Sinularia* sp. and *Xenia* sp. *J. Exp. Mar. Biol. Ecol.* **320:** 159–177.

Takahashi, S., S. Whitney, S. Itoh, T. Maruyama, and M. Badger. 2008. Heat stress causes inhibition of the *de novo* synthesis of antenna proteins and photobleaching in cultured *Symbiodinium*. *Proc. Natl. Acad. Sci. USA* **105:** 4203–4208.

Thornhill, D. J., T. C. LaJeunesse, D. W. Kemp, W. K. Fitt, and G. W. Schmidt. 2006. Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. *Mar. Biol.* **148:** 711–722.

Thornhill, D. J., Y. Xiang, W. K. Fitt, and S. R. Santos. 2009. Reef endemism, host specificity and temporal stability in populations of symbiotic dinoflagellates from two ecologically dominant Caribbean corals. *PLoS One* **4:** e6262.

Tremblay, P., M. Fine, J. F. Maguer, R. Grover, and C. Ferrier-Pagès. 2013. Photosynthate translocation increases in response to low seawater pH in a coral-dinoflagellate symbiosis. *Biogeosciences* **10:** 3997–4007.

Venn, A. A., M. A. Wilson, H. G. Trapido-Rosenthal, B. J. Keely, and A. E. Douglas. 2006. The impact of coral bleaching on the pigment profile of the symbiotic alga, *Symbiodinium*. *Plant Cell Environ.* **29:** 2133–2142.

Wall, C. B., T.-Y. Fan, and P. J. Edmunds. 2014. Ocean acidification has no effect on thermal bleaching in the coral *Seriatopora caliendrum*. *Coral Reefs* **33:** 119–130.

Warner, P. A., M. J. H. van Oppen, and B. L. Willis. 2015. Unexpected cryptic species diversity in the widespread coral *Seriatopora hystrix* masks spatial-genetic patterns of connectivity. *Mol. Ecol.* **24:** 2993–3008.

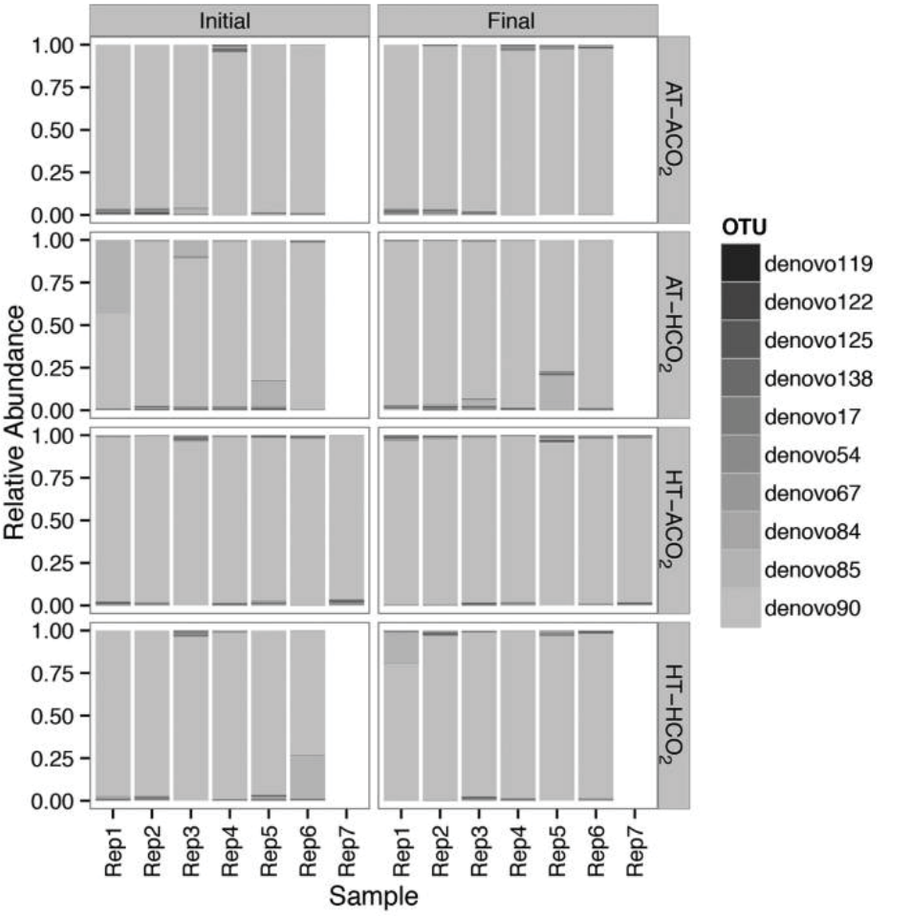
Weis, V. M. 2008. Cellular mechanisms of cnidarian bleaching: stress causes the collapse of symbiosis. *J. Exp. Biol.* **211:** 3059–3066.

Whitaker, J. R., and P. E. Granum. 1980. An absolute method for protein determination based on difference in absorbance at 235 and 280 nm. *Anal. Biochem.* **109:** 156–159.

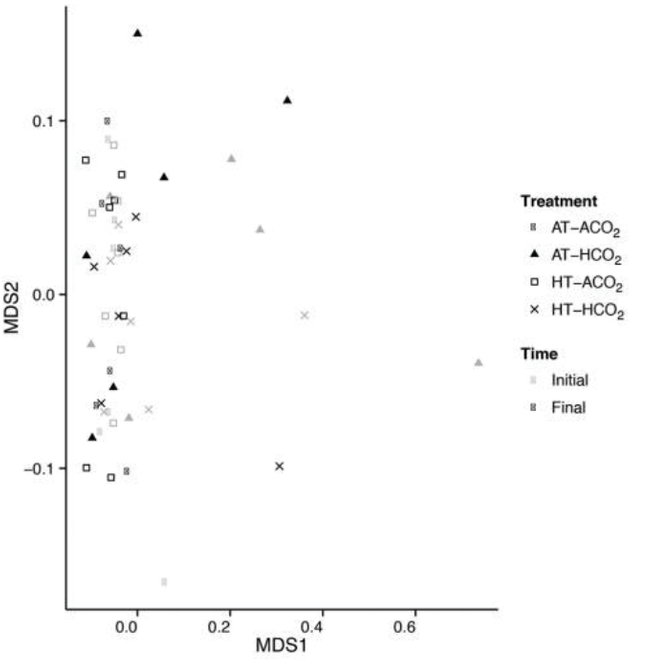
Wild, C., O. Hoegh-Guldberg, M. S. Naumann, M. F. Colombo-Pallotta, M. Ateweberhan, W. K. Fitt, R. Iglesias-Prieto, C. Palmer, J. C. Bythell, J.-C. Ortiz *et al.* 2011. Climate change impedes scleractinian corals as primary reef ecosystem engineers. *Mar. Freshw. Res.* **62:** 205–215.

Wilkerson, F. P., D. Kobayashi, and L. Muscatine. 1988. Mitotic index and size of symbiotic algae in Caribbean reef corals. *Coral Reefs* **7:** 29–36.

Appendix



Appendix Figure A1. The relative abundance of *Symbiodinium* types from the start (Initial) to the end (Final) of the experiment for 6 samples per treatment (except for HT-ACO_2, where $n=7$). Visualization of the top 10 operational taxonomic units (OTUs) before taxonomic assignment shows no clear pattern among treatments or over time. AT-ACO_2, ambient temperature–ambient pCO_2; AT-HCO_2, ambient temperature–high pCO_2; HT-ACO_2, high temperature–ambient pCO_2; HT-HCO_2, high temperature–high pCO_2; denovo 17–138, identification number of *Symbiodinium* OTUs; Rep1–Rep7, replicate coral samples.



Appendix Figure A2. Non-metric multidimensional scaling (NMDS) ordination plot of the samples from four experimental treatments based on Bray Curtis dissimilarity of the square root-transformed relative abundance data of *Symbiodinium* operational taxonomic units (OTUs). Gray symbols indicate initial samples and black symbols are final samples. AT-ACO_2, ambient temperature–ambient pCO_2; AT-HCO_2, ambient temperature–high pCO_2; HT-ACO_2, high temperature–ambient pCO_2; HT-HCO_2, high temperature–high pCO_2; MDS1, MDS2, multidimensional scaling1, 2.