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Preconditioning to high CO_2 exacerbates the response of the Caribbean branching coral *Porites porites* to high temperature stress

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ABSTRACT: Climate change stress on coral reefs occurs as a result of increased temperature and ocean acidification. However, these stressors do not act uniformly: acidification is a 'press' disturbance characterized by chronic increases in CO_2, whereas thermal stress is a 'pulse' disturbance characterized by acute episodes of anomalously warm temperatures. Therefore future episodes of thermal stress will develop within the context of pre-existing acidification. Many studies have investigated the effect of combined temperature and CO_2 on corals, but no study has yet investigated whether pre-exposing corals to elevated CO_2 affects their response to high temperature. We investigated this for the first time using replicate fragments of the Caribbean coral *Porites porites* preconditioned to either 390 ppm or 900 ppm CO_2 at 26°C for 3 mo. After this period, half of the corals from each CO_2 level were exposed to 31°C (i.e. 31°C/390 ppm or 31°C/900 ppm) for 2 mo, while the other half were maintained in their original treatments (26°C/390 ppm or 26°C/900 ppm). Calcification, feeding rate, and photochemical efficiency were measured. Corals preconditioned to high CO_2 before thermal stress (i.e. 31°C/900 ppm) showed 44% lower calcification rates than the control group, but single stress treatment groups did not experience significant growth reductions. Feeding rates increased for corals exposed to either high CO_2 or high temperature singularly, but not when thermal stress was applied following CO_2 preconditioning. Photochemical efficiency decreased by 25% for all treatment groups compared to the control. Together, these data suggest that preconditioning to elevated CO_2 worsens holobiont response to thermal stress, potentially exacerbating the effects of climate change stressors on coral reefs.

KEY WORDS: Climate change · Ocean acidification · Coral bleaching · Calcification · Heterotrophy · Photochemical efficiency

INTRODUCTION

Rising levels of atmospheric greenhouse gases are driving the world's oceans towards conditions that have not occurred for the last 300 million years, increasing the risk of long-term ecological transformation (Hoegh-Guldberg & Bruno 2010, Hönisch et al. 2012). Greenhouse gases have increased the global mean temperature by 0.2°C decade^{-1} over the last 3 decades (Hansen et al. 2006). This increased warming is predicted to cause severe coral bleaching (resulting from the loss of algal endosymbionts and/or a reduction in their per-cell pigment concentration) on an annual basis by the middle of the century, leading to widespread coral mortality (Frieler et al. 2013). In addition to absorbing heat, the ocean has absorbed one-third of the CO_2 produced by anthropogenic activities to date, resulting in a decrease of 0.02 pH units decade^{-1} over the last 3 decades (Sabine et al. 2004, Hoegh-Guldberg & Bruno 2010). Ocean acidification (OA) is expected to reduce coral calcification and possibly push coral reefs into a negative carbon-

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ate balance (i.e. net dissolution as opposed to net accretion) (Hoegh-Guldberg et al. 2007). A decline in coral calcification occurs because increased CO_2 dissolved in seawater results in an increased abundance of hydrogen ions, which react with carbonate ions to form bicarbonate, thereby decreasing the abundance of the carbonate ion in bulk seawater and depressing the aragonite saturation state (Ω_{arag}) (Langdon & Atkinson 2005, Kleypas et al. 2006). Present-day tropical surface seawater pH is expected to decline from 8.08 to 7.8 by the year 2100, leading to a reduction in Ω_{arag} from 4.0 to 2.8 (Kleypas et al. 1999, Gattuso et al. 2015). This reduction threatens reef ecosystems because rates of coral calcification are positively related to Ω_{arag} (Marubini et al. 2001, Langdon & Atkinson 2005, Chan and Connolly 2013). Reduced pH and Ω_{arag} make it energetically more difficult for corals to calcify because more energy must be expended to elevate pH at the site of calcification in order to precipitate calcium carbonate (Cohen & Holcomb 2009, Venn et al. 2013).

The effects of high temperature and CO_2 will not occur uniformly in space and time across the world's reef regions (Pandolfi et al. 2011, Albright et al. 2013, van Hooidonk et al. 2014). Acute bleaching stress caused by episodic temperature anomalies will be superimposed on chronic acidification stress due to gradual increases in the partial pressure of carbon dioxide (pCO_2) (Buddemeier et al. 2004). Consequently, future bleaching events will occur at elevated CO_2 levels relative to the present-day level of ~400 ppm. In recognition of these dual stressors, many studies have investigated the effect of combined temperature and pCO_2 on reef corals (Anthony et al. 2008, Edmunds 2011, Edmunds et al. 2012, Schoepf et al. 2013). To date, however, no study has investigated whether pre-exposure to elevated CO_2 affects reef corals' response to high temperatures by first acclimating the corals to high CO_2. Edmunds & Gates (2008) argued that the key to understanding how corals will fare in this era of global climate change is understanding the extent to which acclimatization to these stressors is important. Acclimatization refers to phenotypic changes occurring under naturally varying conditions, but when these changes are investigated experimentally by manipulating a single environmental factor, the responses are termed acclimation (Prosser 1991).

In this study, the response of the holobiont to CO_2 acclimation was measured as calcification, and the response of the *Symbiodinium* populations was measured as maximum photochemical efficiency of open reaction centers in Photosystem II (F_v/F_m), as in Edmunds (2014) who studied a Pacific *Porites* spp. We also measured coral host feeding rates since coral heterotrophy has been shown to be a good indicator of resilience to both thermal (Grottoli et al. 2006, Rodrigues & Grottoli 2007, Palardy et al. 2008, Anthony et al. 2009), and OA stress (Cohen & Holcomb 2009, Holcomb et al. 2010, Edmunds 2011, Towle et al. 2015). However, we do not yet know how effectively corals can increase feeding rates under thermal and OA stress, or if a threshold of stress exposure exists where feeding can no longer compensate. Palardy et al. (2005) found that degree of coral heterotrophy is plastic and varies with changes in the physical environment. Such plasticity may allow for shifts in energy input from autotrophy to heterotrophy under thermal and/or OA stress. Coral species that exhibit this plasticity may be able to offset decreased energy inputs from their algal symbionts during stress events and thereby increase their resilience (Palardy et al. 2005).

Here, we assessed whether host calcification and feeding rate and symbiont photochemical efficiency were affected by preconditioning to high CO_2 prior to thermal stress in the common Caribbean finger coral *Porites porites*. This work fills a gap in our knowledge of the relationship between symbiont photochemical efficiency, CO_2 levels, and coral feeding rate, all of which may contribute to resilience to climate change stress. This study therefore improves our understanding of how reefs of the future will respond to climate change stress in a more ecologically relevant way than has been tested to date.

MATERIALS AND METHODS

Coral collection

A total of 8 colonies of the Caribbean branching coral *Porites porites* were collected at a depth of ~4 to 5 m by SCUBA divers in April 2012 from Evan's Reef near Broad Key in the northern Florida Keys (25.332° N, 80.200° W) under permit BISC-2011-SCI0022 issued by Biscayne National Park. *P. porites* was chosen because it is a common coral in the Florida reef tract and has been shown to be thermally sensitive (Wagner et al. 2010), making it relevant to studies projecting the fate of coral reefs in the future. *P. porites* is also not a listed species in the US Endangered Species Act, so it is relatively easy to obtain permits to collect, and easy to fragment into multiple experimental units. Corals were transported to the Corals and Climate Change Facility at the University

of Miami's Rosenstiel School of Marine and Atmospheric Science on Virginia Key, Florida, where they were fragmented into 80 nubbins (10 nubbins fragmented per colony, each ~5 cm in branch height) and attached to polyvinyl chloride (PVC) sleds using a 2-part epoxy (All-Fix, Cir-Cut). Nubbins were allowed to recover from fragmentation under control conditions (26°C and 390 ppm CO_2) for 1 mo prior to the start of the experiment.

Experimental design

The 5 mo study took place from May to October 2012, and consisted of 2 phases: a preconditioning phase and a warming phase. On 15 May 2012, the preconditioning phase of the study began with 2 treatments replicated 4 times for a total of 8 experimental tanks. Each 60 l tank contained 10 coral nubbins allocated haphazardly from each of the 8 original colonies. During preconditioning, tanks were maintained at constant temperature (26°C) and 2 different CO_2 levels: 4 tanks at 390 ppm CO_2 (ambient) and 4 tanks at 900 ppm CO_2 (elevated). After 3 mo (15 August 2012), the warming phase began: 2 tanks from each of the 2 treatments in the preconditioning phase were ramped to 31°C, creating 2 new treatment conditions: 31°C/390 ppm and 31°C/900 ppm, each replicated twice. Temperatures in these tanks were ramped at a rate of 0.5°C d^{-1} over 10 d. The warming phase of the experiment therefore consisted of 4 treatments: 26°C/390 ppm, 26°C/900 ppm, 31°C/390 ppm, and 31°C/900 ppm, each replicated twice across a total of 8 tanks (Fig. 1). The warming phase lasted 2 mo, until 15 October 2012. Severe annual bleaching in the Florida reef tract is usually observed beginning in August, and if severe, can persist through early October before temperatures cool down (authors' pers. obs.). Therefore, the reason for the 2 mo warming phase was to represent a severe summer thermal stress event. The arbitrary 3 mo preconditioning phase was considered a proxy for 'long-term' pre-exposure to high CO_2, and its length was determined by the availability of experimental facilities. In summary, the corals in the 26°C/390 ppm and the 26°C/900 ppm group were maintained under those initial conditions for the entire 5 mo study. The corals in the 31°C/390 ppm and the 31°C/900 ppm groups were held at 26°C/390 and 26°C/900 ppm, respectively, for the first 3 mo of the study, and then exposed to thermal stress for the last 2 mo of the study (Fig. 1). The target of 31°C for the warming phase was chosen as the closest whole number that exceeded 1°C above the maximum monthly mean of the local climatology (29.64°C), the threshold used for NOAA's Coral Reef Watch, and is consistent with a local bleaching threshold in the Upper Florida Keys of approximately 30.4 °C (Manzello et al. 2007). The treatment carbon dioxide level (900 ppm) was chosen to represent the approximate value projected for the year 2075 under a 'business as usual' trajectory (IPCC 2013).

Water chemistry and temperature in each experimental tank was controlled independently following best-practices recommended in Riebesell et al. (2010) and Cornwall & Hurd (2015). Each experimental tank was supplied by a separate 250 l sump tank bubbled with CO_2-enriched air to achieve the desired CO_2 level. Temperature in each sump tank was controlled with an OMEGA CN7533 controller with an accuracy of 0.1°C. Superimposed over the treatment temperature was a natural diel swing in temperature related to the amount of insolation received each day. Typically the temperature would increase by 0.1 to 0.3°C during the day and cool by the same amount during the night. CO_2 levels were achieved by bubbling the water in a sump tank with CO_2-enriched air produced using mass flow controllers (Model 810C, Sierra Instruments). A pump circulated the CO_2-treated water into the experimental tank. The metabolism of the corals resulted in a natural diel swing in

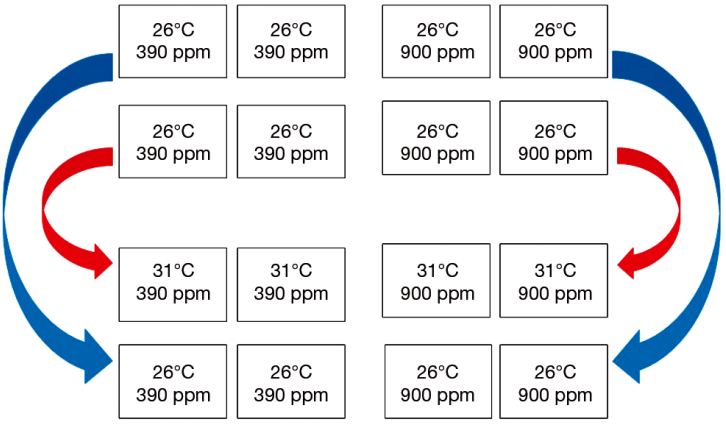

Fig. 1. Schematic of the 5 mo experimental design. The upper 8 tanks represent the preconditioning phase of the experiment (first 3 mo) where all corals were held at 26°C and half were exposed to ambient CO_2 (390 ppm) and the other half exposed to high CO_2 (900 ppm). The bottom 8 tanks represent the warming phase of the experiment (last 2 mo) where 2 of the 4 tanks at 26°C/390ppm were ramped to 31°C (represented by red arrows), while the other 2 remained the same (represented by blue arrows), and 2 of the 4 tanks at 26°C/900ppm were ramped to 31°C while the other 2 remained the same

pCO_2 that decreased during the day and increased during the night. All tanks were connected to a HOBO U30 data logger, which recorded temperature and light measurements every 5 min for the entire study. Average daily light level over the course of the 5 mo study was 200 µmol photons m^{-2} s^{-1}, achieved using a shade cloth and targeted to represent light levels at approximately 5 m depth on the turbid, inshore South Florida patch reef where experimental corals were collected. Coral nubbins were fed a commercial coral diet (Ziegler's Larval APO) twice weekly (Towle et al. 2015), and were rotated within tanks to minimize positional effects within tanks during the twice weekly tank cleanings.

Seawater chemistry

In order to monitor seawater chemistry parameters, water samples (250 ml) were taken from each tank once a week and poisoned with 100 µl mercuric chloride following collection. Total alkalinity (TA) was measured in duplicate on an automated Gran titrator and standardized using certified reference materials obtained from Dr. A. Dickson (Scripps Institute of Oceanography). Dissolved inorganic carbon (DIC) was measured in duplicate using a DIC analyzer (Apollo SciTech) standardized to the same certified reference seawater. A Licor infrared gas analyzer-based pCO_2 equilibrator system was used to directly measure the pCO_2 of the water in the experimental tanks (Bates et al. 1998). This system was used to document the diel variability of pCO_2 in the tanks and was rotated from tank to tank during the weekly water sampling to obtain a measurement of pCO_2 at the same time that discrete water samples were collected for TA and DIC analysis. Salinity was measured using a YSI meter calibrated against a 50 000 µSiemen standard solution. Mean temperature, salinity, TA, and DIC were then used to calculate pCO_2, pH, and Ω_{arag} for each treatment using the program CO_2SYS using K_1 and K_2 from Mehrbach et al. (1973) refit by Dickson & Millero (1987) per Lewis & Wallace (1998).

Calcification

Coral buoyant weights (Davies 1989) were measured every 2 wk during the 8 wk warming phase of the study. A skeletal density of 1.46 g cm^{-3} based on 4 *P. porites* fragments sacrificed at the beginning of the experiment was used to calculate colony weight in air. Calcification rate (mg $CaCO_3$ d^{-1}) was calculated by regressing buoyant weight against date and determining the slope of the best-fit straight line through the data. The area-normalized calcification rates (mg d^{-1} cm^{-2}) were calculated by dividing the slopes by colony surface area.

Feeding rates

To quantify capture rates of prey ingested by *P. porites*, initial and final concentrations of live rotifers were measured following the 8 wk warming phase, and feeding rates were calculated based on Coughlan (1969), similar to methods described in Towle et al. (2015). This method has an advantage over those published by Palardy et al. (2005) and Grottoli et al. (2006) in that it is non-destructive. Briefly, twelve 1 l beakers were used, 10 containing a coral and 2 controls (prey only, without coral) to account for any changes in rotifer density independent of coral feeding. Each beaker had the same flow rate (controlled by magnetic stir plates), light conditions, and initial rotifer density from the same stock solution. Corals were allowed to feed for 1 h after sunset as in Grottoli et al. (2006) and were observed to have extended tentacles in the presence of rotifers, indicating feeding was occurring. While the majority of previous feeding rate studies have used a lower initial concentration of prey (ca. 1000 to 2000 zooplankters l^{-1} (Houlbrèque et al. 2003, 2015, Ferrier-Pagès et al. 2010, Gori et al. 2015), in this study initial concentrations of rotifers were approximately 10 000 cells l^{-1}. This concentration was not meant to be ecologically relevant, but rather it was chosen to mimic a level closer to that used in Edmunds (2011) for a Pacific *Porites* species (~1.6×10^4 nauplii l^{-1}), in order to provide corals with ad libitum access to zooplankton to test for potential differences in feeding due to thermal stress, with and without preconditioning to high CO_2. After 1 h, 4 replicate 15 ml water samples were taken from each beaker, fixed with Lugol's solution, and final rotifer concentrations quantified via microscopy using a Petri dish with gridlines and a cell counter. Feeding rates were normalized to the surface area of each coral in the 10 experimental beakers.

Chlorophyll fluorometry

In order to assess photochemical efficiency, the maximum quantum yield of Photosystem II (F_v/F_m) of the algal symbionts was measured. This non-invasive

metric was quantified using an Imaging Pulse Amplitude Modulated (I-PAM) fluorometer (Walz) weekly during the bleaching phase of the experiment, following the methods of Maxwell & Johnson (2000). Corals were dark-adapted for 45 min after sundown prior to fluorescence measurements, and analyzed using ImagingWin software (Walz). Generally, corals were dark-adapted after sundown from 20:00 to 20:45 h, and florescence measurements began at 20:45 h for each weekly measurement.

Surface area

To minimize damage to corals, non-invasive procedures were used to measure coral surface area. We analyzed photos using the software program ImageJ (National Institute of Health) as opposed to more traditional methods such as wax dipping, which destroy or damage coral. Photographs of each experimental nubbin were taken at the beginning and end of the study and analyzed for surface area using stereophotography (3D) with 2 cameras mounted on a fixed frame. All photographs included a 15 cm ruler used as a reference to convert the areal measurements into cm^2. Stoltenberg (2012) compared results from this method with those obtained using the wax dipping method (Veal et al. 2010) and found good agreement between the 2 methods in *P. porites* (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m546p075_supp.pdf; Pearson's r = 0.88, p < 0.0001, n = 79 coral nubbins).

Statistical analyses

A 2-way fully-factorial nested ANOVA was used to test for effects of temperature, CO_2, their interaction, and the nested effect of tank within temperature and CO_2 level on calcification rate, feeding rate and the final time point for photochemical efficiency. Post hoc Tukey's HSD tests were used to determine differences between individual groups. A repeated measures MANOVA was used to test for the effects of time, temperature, CO_2, their interaction, and tank on photochemical efficiency. All prerequisite normality tests, homoscedasticity tests, ANOVA, MANOVA, and post hoc statistical analyses were carried out using the statistical software package JMP v.12.0.0 (SAS Institute). We used a significance level of $\alpha = 0.05$ for all tests.

RESULTS

Calcification

Mean seawater chemistry parameters are summarized in Table 1 for the preconditioning phase, and Table 2 for the warming phase. There was no significant difference in the calcification rates of *Porites porites* at 31°C/390 ppm (4.88 mg cm^{-2} d^{-1}) and 26°C/900 ppm (5.18 mg cm^{-2} d^{-1}) compared to the control (26°C/390 ppm; 5.10 mg cm^{-2} d^{-1}), (Fig. 2, Table 3). However, calcification rates were significantly lower compared to all other treatment groups in corals pre-

Table 1. Seawater chemistry parameters (mean ± SD) for the preconditioning phase of the experiment (May to August 2012). Water samples were taken weekly over the 3 mo period. Ω_{arag}: aragonite saturation state; TA: total alkalinity; DIC: dissolved inorganic carbon; SW: seawater

Treatment	Temperature (°C)	Salinity (psu)	CO_2 (ppm)	pH	Ω_{arag}	TA (µmol kg^{-1} SW)	DIC (µmol kg^{-1} SW)
26°C, 390 ppm	26.2 ± 0.3	31.0 ± 1.0	360 ± 36	8.06 ± 0.04	3.2 ± 0.4	2070 ± 148	1797 ± 127
26°C, 900 ppm	26.2 ± 0.2	31.0 ± 1.0	907 ± 188	7.73 ± 0.09	1.8 ± 0.4	2116 ±100	1987 ± 127

Table 2. Seawater chemistry parameters for the warming phase of the experiment (August to October 2012). Water samples were taken weekly over the 2 mo period

Treatment	Temperature (°C)	Salinity (psu)	CO_2 (ppm)	pH	Ω_{arag}	TA (µmol kg^{-1} SW)	DIC (µmol kg^{-1} SW)
26°C, 390 ppm	26.1 ± 0.4	32.0 ± 1.0	364 ± 39	8.04 ± 0.05	3.0 ± 0.5	2000 ± 191	1741 ± 161
26°C, 900 ppm	26.2 ± 0.9	32.0 ± 1.0	914 ± 187	7.73 ± 0.09	1.8 ± 0.4	2135 ± 151	2004 ± 129
31°C, 390 ppm	30.8 ± 0.8	32.0 ± 1.0	380 ± 34	8.06 ± 0.03	3.6 ± 0.5	2225 ± 157	1919 ± 126
31°C, 900 ppm	30.9 ± 0.2	32.0 ± 1.0	937 ± 203	7.73 ± 0.09	2.0 ± 0.5	2205 ± 146	2059 ± 119

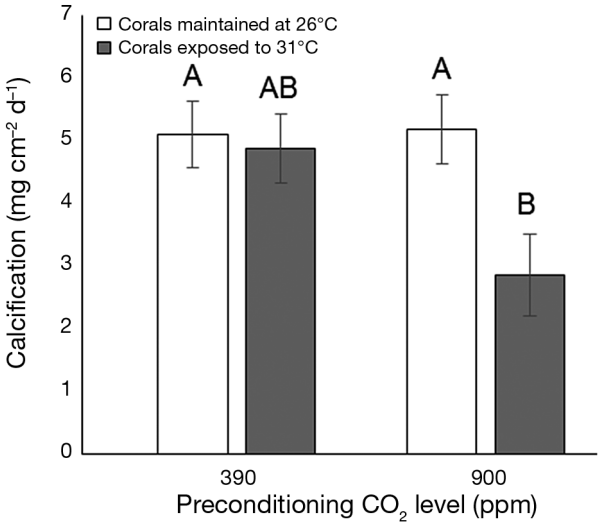

Fig. 2. Mean calcification rates of *Porites porites* at the 4 treatment levels during the 2 mo warming phase of the study. Letters represent statistical differences based on post hoc Tukey's HSD test ($p < 0.05$); n = 20 coral nubbins treatment^{-1}

Table 3. ANOVA and effect tests (2-way ANOVA) of temperature (T), carbon dioxide (CO_2), their interaction (T × CO_2), and tank nested within T and CO_2 on calcification rate, feeding rate, and the final time point for photochemical efficiency. Significant p-values ($p \leq 0.05$) are given in **bold**

Source	df	SS	*F*	p
Calcification				
Model	7	55.6472	1.4765	0.1945
Error	56	301.5143		
Total	63	357.1615		
T	1	23.978263	4.4535	**0.0393**
CO_2	1	15.498953	2.8786	0.0953
T × CO_2	1	18.238217	3.3874	0.0710
Tank[T,CO_2]	4	7.534373	0.3498	0.8431
Feeding				
Model	8	6.4534	4.2970	**0.0008**
Error	41	7.6969		
Total	49	14.1504		
T	1	0.0312409	0.1664	0.6854
CO_2	1	0.0592640	0.3157	0.5773
T × CO_2	1	2.9718816	15.8306	**0.0003**
Tank[T,CO_2]	5	1.2632664	1.3458	0.2646
Photochemical efficiency (final time point)				
Model	7	0.27293135	4.0186	**0.0010**
Error	65	0.63065441		
Total	72	0.90358575		
T	1	0.10740713	11.0702	**0.0014**
CO_2	1	0.03246268	3.3458	0.0720
T × CO_2	1	0.08138506	8.3882	**0.0051**
Tank[T,CO_2]	4	0.03632396	0.9360	0.4488

conditioned to high CO_2 and then exposed to thermal stress (31°C/900 ppm treatment) (2.85 mg cm^{-2} d^{-1}; Tukey's HSD, $p < 0.05$; Fig. 2, Table 3).

Feeding

The interaction between temperature and CO_2 on feeding rate was significant (ANOVA, $p < 0.05$; Table 3). Corals preconditioned to ambient CO_2 levels prior to thermal stress (31°C/390 ppm treatment) fed at rates 3 times greater than the 26°C/390 ppm corals (0.87 vs. 0.24 rotifers h^{-1} cm^{-2} ml^{-1}; Fig. 3). *P. porites* exhibited the highest feeding rates in the 26°C/900 ppm treatment, feeding at approximately 4 times the rate of the 26°C/390 ppm corals (1.05 vs. 0.24 rotifers h^{-1} cm^{-2} ml^{-1}, Tukey's HSD, $p < 0.05$; Fig. 3, Table 3). However, corals preconditioned to high CO_2 prior to thermal stress (31°C/900 ppm treatment) did not have significantly higher feeding rates compared to the 26°C/390 ppm corals (Fig. 3, Table 3).

Photochemical efficiency

There was a significant effect of time, temperature, CO_2, and the interaction between all 3, on photochemical efficiency (MANOVA, $p < 0.05$; Fig. 4A, Table 4). At the final time point, photochemical efficiency was significantly lower in all 3 treatment groups compared to the control (treatment means of 0.36 to 0.40 compared to control mean of 0.51; Tukey's HSD, $p < 0.05$; Fig. 4B, Table 4).

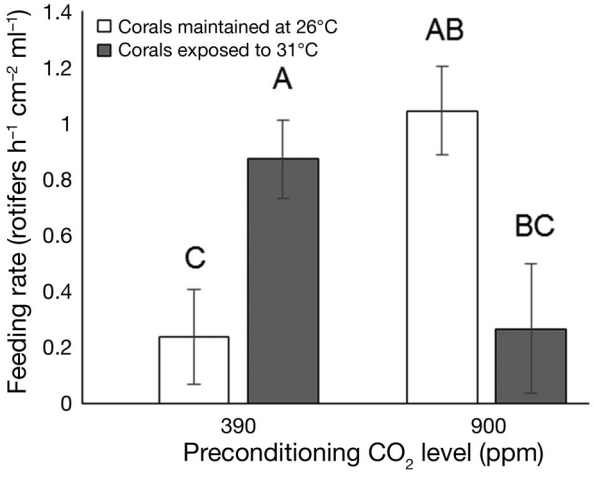

Fig. 3. Mean feeding rates of *Porites porites* at the 4 treatment levels over the 2 mo warming phase of the study. Letters represent statistical differences based on post hoc Tukey's HSD test ($p < 0.05$); n = 20 coral nubbins treatment^{-1}

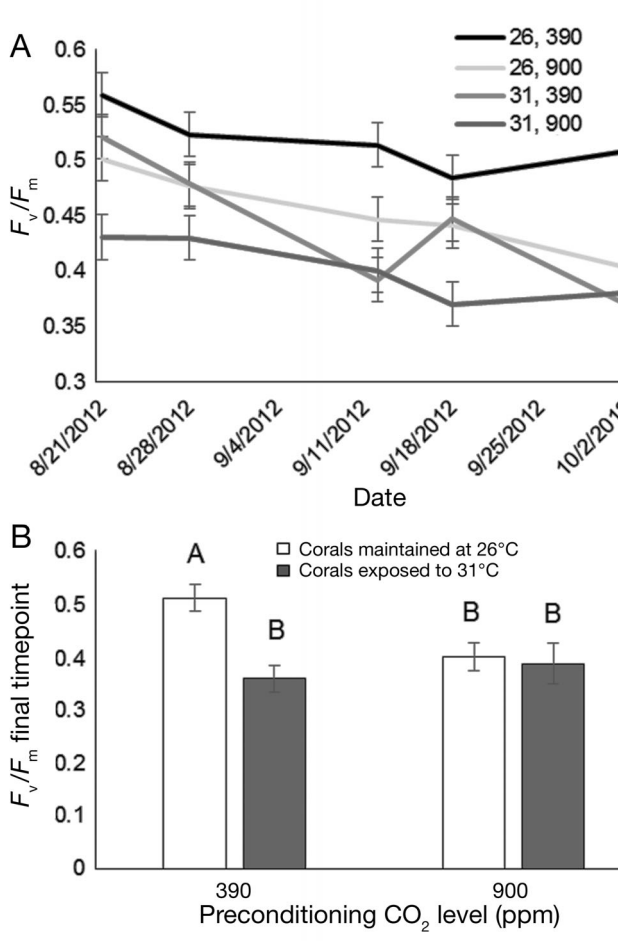

Fig. 4. Mean photochemical efficiency (F_v/F_m) of *Porites porites* symbionts (A) across 5 time points following the start of the warming phase of the study, and (B) at the final time point for the 4 treatment levels. Letters in (B) represent statistical differences based on post hoc Tukey's HSD test ($p < 0.05$); n = 20 coral nubbins treatment^{-1}

Table 4. Repeated measures MANOVA for photochemical efficiency testing the effects of temperature (T), carbon dioxide (CO_2), their interaction (T × CO_2), and tank nested within T and CO_2 against time. Significant p-values ($p \leq 0.05$) are given in **bold**

Source	*F*	df_{Num}	df_{Den}	p-value
Between subjects				
All between	3.3710	7	65	**0.0040**
T	12.9114	1	65	**0.0006**
CO_2	8.4434	1	65	**0.0050**
T × CO_2	0.7510	1	65	0.3893
Tank[T,CO_2]	0.2754	4	65	0.8928
Within subjects				
Time	18.2789	4	62	**<0.0001**
Time × T	2.5629	4	62	**0.0470**
Time × CO_2	2.8317	4	62	**0.0319**
Time × T × CO_2	8.5376	4	62	**<0.0001**

DISCUSSION

The goal of this study was to determine how preconditioning *Porites porites* to high CO_2 levels before thermal stress exposure would affect its calcification, feeding rate, and photochemical efficiency. Preconditioning to high CO_2 is a more realistic approximation of real-world scenarios facing corals in the coming decades, i.e. exposure to a chronic CO_2 stress prior to exposure to an acute thermal stress. As such, we wanted to compare these preconditioned corals to corals that experienced only chronic CO_2 stress, only acute thermal stress, or no perceived stress. Our results showed that preconditioning to high CO_2 prior to high temperature stress significantly decreased calcification rates in *P. porites* by about 44% compared to the controls. Interestingly, we did not see significant decreases in calcification rates in corals that experienced only high CO_2 without thermal stress for a total of 5 mo, nor did we see significant decreases in calcification rates in corals that experienced only thermal stress for 2 mo without prior exposure to high CO_2. This finding suggests that corals may be able to compensate for reductions in growth during long-term (2 to 5 mo) singular stress events, but will suffer decreases in calcification when experiencing long-term thermal stress following long-term high CO_2 exposure. We hypothesize that this compensation may come from extra energy input from heterotrophy, as feeding rates increased approximately 3- and 4-fold under thermal stress alone and OA stress alone, respectively. This finding agrees with Edmunds (2011), who showed that a massive Pacific *Porites* species was able to buffer the effects of 1 mo exposure to high CO_2 through heterotrophy. Castillo et al. (2014) found that another Caribbean coral species, *Siderastrea siderea*, was also able to maintain calcification rates under high CO_2 when fed on a regular basis, but did not assess the combined effects of both thermal and OA stress.

The decrease in calcification rate and lack of increased feeding rate presented here for *P. porites* in the 31°C/900 ppm treatment contrasts with data from Towle et al. (2015), who found that calcification of fed *Acropora cervicornis* at 30°C/900 ppm was not significantly different than fed controls at 26°C/390 ppm. However, the corals in Towle et al. (2015) were not preconditioned to high CO_2 before exposure to thermal stress, suggesting that the CO_2 preconditioning in advance of thermal stress may be the cause of this dramatic reduction in calcification. These differences in the responses of *P. porites* and *A. cervicornis* may also have to do with the ability of these species to use

heterotrophy. It is possible that *P. porites* in the high CO_2 alone treatment was feeding close to its maximal rates (a 4-fold increase over controls). Energetic costs of feeding such as polyp extension (Levy et al. 2006) may be considerable, and when corals are exposed to both high temperature and CO_2 (i.e. 31°C/900 ppm treatment group), feeding rates may not be able to increase from basal (control) levels due to the combined stress. In contrast, *A. cervicornis* may not yet have been feeding at its maximal rates in the 2015 study. More research is needed to understand what the physiological basis for these differences may be, recognizing that different coral species will vary both in their feeding plasticity (Palardy et al. 2005) and in their algal symbionts' responses to stress, particularly if different clades of *Symbiodinium* are involved (Baker et al. 2004).

We found that the photochemical efficiency of the algal symbionts of *P. porites* was reduced by ~25% compared to the control in all treatments by the end of the warming phase. Corals that were preconditioned to high CO_2 before experiencing thermal stress initially had lower F_v/F_m values than those in single stress treatments, but after 6 wk of stress exposure, F_v/F_m showed similar reductions across all treatments relative to controls. These results suggest that there may be a common mechanism in the symbionts' response to thermal and OA stress. Weis (2008) provided a detailed description of increases in intracellular reactive oxygen and nitrogen species (ROS and RNS) in host cells due to thermal bleaching, and Kaniewska et al. (2012) described metabolic suppression characterized by similar increases in oxidative stress, ROS, and RNS under OA stress. Nevertheless, additional research is needed to understand how *Symbiodinium* will respond to thermal stress with and without OA stress.

It remains unclear why corals that were preconditioned to high CO_2 prior to thermal stress suffered reductions in calcification while corals in single stress treatments did not, even though all treatments experienced the same reductions in F_v/F_m. We hypothesize that host heterotrophy may help explain this difference. Photoinactivation is initially detected as a significant drop in F_v/F_m (Warner et al. 1996), and may lead to lower rates of photosynthate translocation from symbiont to coral host and/or reduced photosynthate quality. Coral heterotrophy has been shown to stimulate an increase in symbiont density per unit surface area, chlorophyll *a* and photosynthetic rates (Ferrier-Pagès et al. 2003, Houlbrèque et al. 2003, 2004a), indicating that heterotrophy may help offset reductions in the symbionts' physiological responses to stress. Hughes et al. (2010) provided further evidence that endosymbiotic algae depend on heterotrophically-derived carbon in response to temperature stress and that this dependence can last for at least 11 mo post-bleaching (Hughes & Grottoli 2013). Schoepf et al. (2013) also attributed heterotrophy as the reason for lack of reduced calcification in some coral species under high temperature and CO_2, despite reductions in symbiont density and/or chlorophyll *a* content.

These data suggest that an increased feeding rate provides energy to maintain ambient calcification rates when photochemical efficiency is reduced, but this response has limits that we cannot fully explain yet. Exposure to 2 mo of high temperature stress following 3 mo of high CO_2 stress with no increase in coral feeding rate resulted in a dramatic (44%) decline in calcification. However, exposure to long-term (5 mo) elevated CO_2 without thermal stress, as well as 2 mo exposure to thermal stress without CO_2 stress, resulted in increased host feeding rates and no reduction in calcification. More research using invasive metrics (e.g. chl *a*, symbiont density, lipid content, etc.) is needed to identify why corals in the 31°C/900 ppm treatment were unable to increase their feeding rates. Additionally, more research in general is needed to assess the ecologically relevant pre-exposure of corals to long-term CO_2 stress before exposure to acute thermal stress. Finally, these results highlight the pressing need to reduce CO_2 emissions on a global scale to maximize coral survivorship over the coming century.

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