Zooplanktivory ameliorates the effects of ocean acidification on the reef coral *Porites* spp.

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Abstract

    I tested the hypothesis that the effects of high pCO_2 and temperature on massive *Porites* spp. (Scleractinia) are modified by heterotrophic feeding (zooplanktivory). Small colonies of massive *Porites* spp. from the back reef of Moorea, French Polynesia, were incubated for 1 month under combinations of temperature (29.3°C vs. 25.6°C), pCO_2 (41.6 vs. 81.5 Pa), and feeding regimes (none vs. ad libitum access to live *Artemia* spp.), with the response assessed using calcification and biomass. Area-normalized calcification was unaffected by pCO_2, temperature, and the interaction between the two, although it increased 40% with feeding. Biomass increased 35% with feeding and tended to be higher at 25.6°C compared to 29.3°C, and as a result, biomass-normalized calcification statistically was unaffected by feeding, but was depressed 12–17% by high pCO_2, with the effect accentuated at 25.6°C. These results show that massive *Porites* spp. has the capacity to resist the effects on calcification of 1 month exposure to 81.5 Pa pCO_2 through heterotrophy and changes in biomass. Area-normalized calcification is sustained at high pCO_2 by a greater biomass with a reduced biomass-normalized rate of calcification. This mechanism may play a role in determining the extent to which corals can resist the long-term effects of ocean acidification.

Current atmospheric pCO_2 is unprecedented over the last 800,000 yr (Lüthi et al. 2008), and, depending on future emission scenarios, will rise to 53.0–92.0 Pa by 2100 (Meehl et al. 2007). In addition to functioning as a greenhouse gas driving global warming (Meehl et al. 2007), atmospheric CO_2 equilibrates with seawater to form carbonic acid, which depresses pH and causes ocean acidification (OA) (Kleypas et al. 1999). OA describes a suite of processes beginning with increased pCO_2 and depressed seawater pH, but ultimately it also causes increased $[HCO_3^-]$, reduced $[CO_3^{2-}]$, and a lowering of the $CaCO_3$ saturation state (Ω). Ω determines the ease with which mineralization occurs, and is the product of $[Ca^{2+}]$ and $[CO_3^{2-}]$ divided by K'_{sp}, the stoichiometric solubility product for the mineral form of $CaCO_3$ being considered; when $\Omega < 1$, $CaCO_3$ is undersaturated in seawater and dissolution is favored (Kleypas et al. 1999).

There are multiple mechanisms through which OA affects marine taxa, depending on the extent to which they are sensitive to pCO_2, pH, $[HCO_3^-]$, or $[CO_3^{2-}]$ (Kleypas et al. 2006; Przeslaswki et al. 2008). Arguably, however, OA poses the greatest threat to calcifying organisms, whose rates of calcification decline with Ω (Hofmann et al. 2010), classically because it is thermodynamically less favorable to mineralize at low Ω (Atkinson and Cuet 2008). This relationship is well known for coral reefs (Erez et al. 2011), where the data available have been used to suggest that reefs might cease to exist as net calcifying systems towards the end of this century (Hoegh-Guldberg et al. 2007; Silverman et al. 2009). Although such dire projections may be overly pessimistic (Pandolfi et al. 2011), nevertheless, most empirical research reveals a strongly negative effect on scleractinian calcification of depressed Ω_{arag} (which is relevant to aragonite-depositing corals), with

calcification declining 5–40% for each unit decline in Ω_{arag} (Kleypas and Langdon 2006), or more broadly, with a 40% reduction predicted when preindustrial pCO_2 doubles to 56.0 Pa (Hoegh-Guldberg et al. 2007). However, there are still few published studies relevant to evaluating the effects of OA on corals, with recent reviews based on ≤ 21 taxa (Kleypas and Langdon 2006; Erez et al. 2011) drawn from studies that are challenging to synthesize because they have been completed under dissimilar conditions.

New studies of the effects of OA on coral calcification are beginning to appear in greater number, but some of the results appear incongruent under geochemical vs. biological views of mineralization. For example, from a geochemical perspective, coral calcification declines with pH because of the concomitant reductions in $[CO_3^{2-}]$ and Ω_{arag} (Kleypas et al. 2006). In contrast, aspects of the response of corals to OA best fit a model influenced by biological control of mineralization (Erez et al. 2011). For instance, calcification is enhanced by $[HCO_3^-]$ in *Porites porites*, *Acropora* sp. (Herfort et al. 2008) and *Madracis mirabilis* (Jury et al. 2010), low temperature ameliorates the negative effects of elevated pCO_2 on calcification in *Stylophora pistillata* (Reynaud et al. 2003) and *Acropora intermedia* (Anthony et al. 2008), and in recruits of *Favia fragum* and *Porites astreoides*, calcification is unresponsive to declining Ω_{arag} until a threshold value is reached (de Putron et al. 2011). Further, the observations for scleractinians that they can endure low pH and high pCO_2 when nutrient concentrations are elevated (Atkinson et al. 1995), that increased concentrations of nitrogen and phosphorus lessen the steepness of their calcification–Ω_{arag} relationship (Atkinson and Cuet 2008), and that heterotrophy can protect calcification against high pCO_2 (Holcomb et al. 2010), indicate that the response of corals to OA can be modulated by their nutritional status. Although the mechanism underlying this response is

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unclear, it is likely to involve the interplay between heterotrophy and the allocation of resources to biomass vs. calcification (Ferrier-Pagès et al. 2011), and the stimulatory effect of nutrients on *Symbiodinium* density (Dubinsky et al. 1990). The enhancement of calcification and *Symbiodinium* populations by heterotrophy underscores the long-standing perplexing nature of the positive association between calcification and light intensity, *Symbiodinium* content, and photosynthesis (Allemand et al. 2011), which has recently been revisited as a means to explain why corals display a range of responses to OA (Holcomb et al. 2010).

Motivated by the prominence of biological control over mineralization in scleractinians, the objective of this study was to test the hypothesis that the effects of OA on massive *Porites* spp. were unaffected by temperature and zooplankton food. Massive *Porites* spp. was studied because of the importance of this functional group of corals on reefs in the Pacific and Caribbean (Done and Potts 1992; Green et al. 2008), and because this group is relatively resistant to environmental assaults (Loya et al. 2001; Edmunds 2010), including calcification responses under moderate increases in pCO_2 (52.0–70.5 Pa) (Anthony et al. 2008; Fabricius et al. 2011). Calcification was selected as the primary response variable because of its prominence in the discussion of OA effects on coral (Erez et al. 2011), and it was normalized to area and biomass to improve the capacity to understand the phenomenon of calcification (i.e., mass of $CaCO_3$ per area of coral) based on the biology of the processes through which it occurs (i.e., mass of $CaCO_3$ per quantity of coral tissue).

Methods

Study organism and experimental overview—This study was completed during April and May 2010 in Moorea, French Polynesia, using 80 juvenile colonies ($<$ 4-cm diameter) of massive *Porites* spp. collected on 08 April 2010 from 3–4-m depth in the back reef. Juvenile colonies cannot reliably be identified to species in situ when alive and, therefore, were analyzed as a functional group consisting of 85% *Porites lutea* and 15% *Porites lobata* (Edmunds 2009). In the lab, the corals were epoxied (Z-Spar A788) onto 20-mm lengths of 13-mm-diameter polyvinyl chloride pipe with a 6-mm-diameter nylon screw projecting from the bottom, thereby creating nubbins. The nubbins were fitted into racks that each held five nubbins separated by $\sim$ 2 cm, and used in a partly nested analysis of variance (ANOVA) design with two between-plots effects (temperature and pCO_2), one within-plot factor (feeding) based on tanks as plots, and duplicate tanks serving as a nested factor in each combination of temperature and pCO_2. A feeding treatment was created using two racks in each tank, with one rack allocated at random to feeding on *Artemia* spp. and the other to a procedural control. The effects of the treatments were assessed after 1 month using calcification and biomass.

Treatments—Treatments were created in eight custom-made (Aqua Logic) 150-liter tanks that were independently heated, chilled, and mixed with a pump (Rio 8HF, 2082 L h^{-1}). Tanks were illuminated with 400-W metal halide lamps (True 10,000K, Hamilton Technology) and filled with seawater filtered through a 50-μm filter. This seawater was partially (13%) replaced daily, and fully exchanged approximately weekly to facilitate cleaning of the tanks and plumbing. Four treatments crossed two temperatures and two pCO_2 levels, and each combination was created in duplicate tanks. The tanks were adjusted to achieve target light intensities of $\sim$ 600 μmol quanta m^{-2} s^{-1} (on a 12 : 12 light : dark cycle), target temperatures of 29.2°C and 25.5°C, and target pCO_2 treatments of 38.4 and 80.8 Pa. The light intensity provided a daily integrated intensity of photosynthetically active radiation (PAR; 26 mol quanta m^{-2} d^{-1}) similar to that found where the corals were collected (22 mol quanta m^{-2} d^{-1} on 05 May 2009, recorded with a logging cosine light sensor [Compact LW, JFE Advantech] at 2-m depth), the temperatures spanned the seasonal range in the back reef of Moorea, and the pCO_2 treatments represented current conditions and those expected within 100 yr (Meehl et al. 2007). These conditions were used to create treatments defined as high temperature–high pCO_2 (HT–HCO$_2$), high temperature–low pCO_2 (HT–LCO$_2$), low temperature–high pCO_2 (LT–HCO$_2$), and low temperature–low pCO_2 (LT–LCO$_2$). The HT–LCO$_2$ treatment corresponded to ambient conditions on the reef when the experiment was conducted, and therefore it served as the control.

The pCO_2 treatments were established by bubbling ambient air or CO_2-enriched air into the tanks. The CO_2-enriched air was created with a solenoid-controlled gas regulation system (Model A352, Qubit Systems) that received 99% pure CO_2 from a gas cylinder (at 17 kPa) and ambient air from a compressor (103 kPa). Automated mass-flow controllers and a solenoid valve with a variable duty cycle were used to blend air and CO_2 in a mixing chamber from which gas was drawn to measure pCO_2 with an infrared (IR) gas analyzer. The IR gas analyzer was calibrated with certified reference gases, and served to dynamically adjust the duty cycle of the solenoid valve to maintain the desired pCO_2. The output pCO_2 was logged (in ppm) on a PC running LabPro software (Vernier Software and Technology). The final gas mixture was pumped (using a Model DOA-P704-AA Gast pump) through tubing that was split among four tanks that received elevated pCO_2 at $\sim$ 10–15 L min^{-1}; air was supplied to the other four tanks at a similar rate.

To evaluate the efficacy of the treatments, tanks were monitored for light intensity (PAR), temperature, salinity, and dissolved inorganic carbon (DIC) chemistry. Light intensity was recorded daily below the surface of the seawater using a 4-π quantum sensor (LI-193, Li-Cor Biosciences) and meter (Li-1400, Li-Cor Biosciences), and temperature was recorded 2–6 times d^{-1} with a certified digital thermometer (Fisher Scientific, 15-077-8, $\pm$ 0.05°C), with continuous temperature records (0.001 Hz) obtained using loggers (Hobo Water Temp Pro v2, precision $\pm$ 0.2°, Onset Computer). The continuous records were used retrospectively to evaluate thermal stability and the results are not reported here.

DIC chemistry of the seawater was analyzed every third day for total alkalinity (TA) using potentiometric titration according to standard operating procedure (SOP) 3b (Dickson et al. 2007). Titrations were completed with an automatic titrator (Model T50, Mettler-Toledo) fitted with a DG115-SC pH probe (Mettler-Toledo) that was three-point calibrated with pH 4.00, 7.00, and 10.00 NBS buffers (Fisher), and filled with certified acid titrant (~ 0.1 mol L^{-1} HCl and 0.6 mol L^{-1} NaCl, from A. Dickson Laboratory, Scripps Institution of Oceanography). Seawater was analyzed in single samples (~ 400 mL) drawn from each tank at $\sim 09{:}00$ h. Samples were equilibrated to the laboratory temperature, and were processed for salinity (YSI 3100 Conductivity Meter), pH (using a spectrophotometric method with m-cresol purple dye [SOP 6; Dickson et al. 2007]), and TA, usually within 2–3 h of collection. The results were processed in a Microsoft Excel spreadsheet (Fangue et al. 2010) to determine TA (μmol kg^{-1}), and the accuracy and precision of the determinations evaluated by analyzing certified reference material (CRM) (Batch 99 from A. Dickson Laboratory, Scripps Institution of Oceanography) with each series of samples. Over the course of the study, CRMs were analyzed with mean accuracy of 0.7%. TA, pH, salinity, and temperature were used to calculate DIC parameters using CO2SYS (Lewis and Wallace 1998) with the constants of Mehrbach et al. (1973) and pH expressed on the total scale.

Feeding treatment—The feeding treatment was created by exposing half the corals in each tank to *Artemia* spp. for $\sim$ 1 h every second day. This treatment began after dark ($\sim$ 21:00 h) when the polyps of the corals were expanded, and was conducted under intermittent red light. Treatments were created in paired 7.5-liter containers filled with filtered seawater (50 μm), one pair for each combination of temperature and pCO_2, with one container in each pair augmented with ~ 300 mL of a culture of live *Artemia* spp. The *Artemia* spp. were provided as 24-h-old nauplii that were grown from ~ 2 g of eggs in 1.25 liters of seawater to create a concentration of $\sim 1.6 \times 10^4$ nauplii L^{-1} following dilution (without washing) in the feeding containers. This concentration was not intended to be ecologically relevant (Jacobson and Edmunds 2010); rather, it was designed to provide ad libitum access to zooplankton in order to explore the potential of food to affect the response to OA. The seawater in the feeding containers was decanted from the respective treatment tanks so that temperature and pCO_2 were minimally perturbed, and the containers were aerated during the incubations. One rack of five corals from each tank was allocated to *Artemia* spp. exposure and the other to filtered seawater (as a procedural control), and both groups were rinsed in filtered seawater (50 μm) prior to being returned to their treatment tanks. Thus, all corals had access to food derived from photosynthesis of their *Symbiodinium* symbionts, dissolved organic material present naturally in reef seawater, and particulate food that passed through the 50-μm filter; "fed" corals received food over and above these sources through the capture of *Artemia* spp.

Experimental incubations and dependent variables—Following preparation as nubbins, corals were placed into two

tanks at ~ 600 μmol quanta m^{-2} s^{-1} and $\sim 29^\circ$C to acclimate for 4 d; these conditions were similar to ambient conditions where the corals were collected. Following acclimation, on 12 April 2010 corals were buoyant weighed (± 1 mg; Spencer Davies 1989) and on 13 April 2010 they were placed into the treatment tanks. Initially all tanks were $29.2 \pm 0.2^\circ$C (mean $\pm$ SE, $n = 8$), but four tanks were then reduced to the target temperature of $\sim 25.5^\circ$C over 12 h. Thereafter, treatments were applied for 1 month, with tanks cleaned every 5–7 d.

At the end of the experiment on 11 May 2010, the buoyant weight of the corals was again recorded (± 1 mg). The difference in buoyant weight between the start and the end of the experiment was converted to dry weight using an aragonite density of 2.93 g cm^{-3} (Spencer Davies 1989) and used as a measure of calcification, which was normalized to area and biomass. Tissue area was determined using aluminum foil (Marsh 1970). Biomass was measured on half of the corals in each treatment (with corals selected at random), with the mean biomass (mg cm^{-2}) in each treatment used to normalize calcification. To measure biomass, corals were fixed in 5% formalin and decalcified in $\sim 5\%$ HCl until a tunic of tissue remained. Endolithic taxa were removed, and the tunic homogenized in 5 mL of distilled water with ultrasonic vibration (model 15-338-550, Fisher; fitted with a 3.2-mm-diameter probe, Fisher 15-338-67). Two 1-mL aliquots of the slurry from each sample were dried at 60°C, and the mean weight used to calculate biomass per area (mg cm^{-2}). Two additional aliquots (800 μL) were fixed in 5% formalin and analyzed for *Symbiodinium* content. Counts were completed using a hemocytometer, with 5–8 replicate counts for each aliquot, with the average cell density in the two aliquots used to calculate *Symbiodinium* content; cell densities were standardized by area ($\times 10^6$ cells cm^{-1}) and biomass ($\times 10^5$ cells mg^{-1}).

Statistical analysis—Physical and DIC conditions in the tanks were compared with a two-way ANOVA with treatment as a fixed effect and tank a random factor nested in each treatment. Calcification was analyzed with four-way ANOVAs in which temperature and pCO_2 were fixed, between-plot effects; tank was a random factor nested in each treatment; and feeding was a fixed, split-plot effect in each tank. Tank effects were removed from all statistical models when they were not significant at $p \geq$ 0.250 (Quinn and Keough 2002). Biomass and *Symbiodinium* content were analyzed with a three-way ANOVA using temperature, pCO_2, and feeding as fixed effects. The statistical assumptions of normality and equal variance were evaluated through graphical analyses of residuals, and all analyses were completed with Systat 9 software on a Windows platform. Power calculations for select nonsignificant results were calculated according to Zar (2010).

Results

Conditions within the tanks were precisely regulated (Table 1), with a mean light intensity of 599 ± 7 μmol quanta m^{-2} s^{-1}, and mean salinity of 34.5 ± 0.1 (pooled

Table 1. Summary of conditions in 8 tanks between 13 Apr 2010 and 11 May 2010. Mean $\pm$ SE (n) shown except where SE < 0.1.

Treatment	Tank	Temperature (°C)*	pH*	pCO_2 (Pa)	TA (μmol kg^{-1})	Ω_{arag}
HT–HCO$_2$	1	29.3 (81)	7.80 (10)	78.1$\pm$5.2 (10)	2089$\pm$44 (11)	2.11$\pm$0.17 (10)
	2	29.3 (80)	7.80 (10)	80.9$\pm$2.1 (10)	2054$\pm$49 (11)	1.93$\pm$0.07 (10)
HT–LCO$_2$	3	29.2 (80)	8.02 (10)	41.6$\pm$0.7 (10)	1981$\pm$60 (10)	2.96$\pm$0.16 (10)
	4	29.4 (81)	8.02 (10)	42.0$\pm$1.2 (10)	1982$\pm$53 (10)	2.92$\pm$0.13 (10)
LT–HCO$_2$	5	25.7 (81)	7.74 (10)	83.2$\pm$2.2 (10)	2135$\pm$24 (11)	1.78$\pm$0.05 (10)
	6	25.5 (80)	7.73 (10)	83.8$\pm$3.3 (10)	2064$\pm$35 (10)	1.70$\pm$0.06 (10)
LT–LCO$_2$	7	25.4 (81)	7.99 (10)	41.3$\pm$0.9 (10)	2038$\pm$42 (11)	2.69$\pm$0.09 (10)
	8	25.7 (81)	7.99 (10)	41.6$\pm$0.9 (10)	2046$\pm$45 (11)	2.68$\pm$0.10 (10)

* SE < 0.1.

across times and tanks, $\pm$ SE, n = 224 and 212, respectively). Temperature differed between treatments ($F_{1,6}$ = 2438.303, $p < 0.001$), and between tanks within treatments ($F_{6,637}$ = 12.726, $p < 0.001$), although the tank effect was small (means < 0.3°C). Analysis of DIC parameters (Table 1) revealed that pH differed between treatments ($F_{1,6}$ = 127.338, $p < 0.001$), and although it also differed between tanks ($F_{6,72}$ = 3.868, p = 0.002), the variation between paired tanks was trivial (means differing ≤ 0.01 pH); pCO_2, TA, and Ω_{arag} all differed between treatments ($F_{1,83} \geq 5.838$, $p \leq 0.018$) but not between paired tanks ($F_{6,77} \leq 1.069$, $p \geq 0.388$). Overall, the treatments contrasted 29.3°C (seawater ambient) with 25.6°C (means with SEs < 0.1, n = 322–323), and 41.6 Pa pCO_2 (ambient for laboratory seawater) with 81.5 Pa pCO_2.

The corals appeared healthy throughout the experiment, and all survived; one developed a small dead patch that was colonized by algae. Mean area-normalized calcification varied from 0.85 $\pm$ 0.07 mg cm^{-2} d^{-1} (LT–HCO$_2$, unfed) to 1.50 $\pm$ 0.10 mg cm^{-2} d^{-1} (LT–LCO$_2$, fed) (both $\pm$ SE, n = 10), and was affected by feeding (Table 2), with consumption of *Artemia* spp. increasing calcification 40%

(Fig. 1A). Area-normalized calcification was unaffected by pCO_2, tanks nested within treatments, or any other main effect or their interactions (Table 2). In the test of pCO_2 effects, the power (1 $-$ β) of the test for detecting a difference of 0.1 mg cm^{-2} d^{-1} (9% of the grand mean) was $\sim$ 0.83, and the test provided a 90% confidence of detecting a difference of 0.23 mg cm^{-2} d^{-1}. Although there was a trend for calcification to decline at high pCO_2 compared to low pCO_2 in the fed corals, an exploratory post hoc analysis of the fed corals alone, did not detect a significant effect of pCO_2 ($F_{1,36}$ = 3.096, p = 0.087, power $\sim$ 0.45 for detecting a difference of 0.1 mg cm^{-2} d^{-1}).

Biomass was quantified in half of the corals in each treatment, with these corals selected at random, and the corals remaining allocated to alternative analyses. The use of half of the corals for biomass determination precluded the use of tank as a nested factor in the statistical analysis. Biomass of individual corals ranged from 1.9 to 10.1 mg cm^{-2}, with means varying from 3.9 $\pm$ 0.6 mg cm^{-2} (HT–LCO$_2$, unfed) to 7.5 $\pm$ 0.9 mg cm^{-2} (LT–HCO$_2$, fed) (both $\pm$ SE, n = 10; Fig. 1B). Biomass differed between feeding regimes ($F_{1,36}$ = 7.17, p = 0.011) and tended to vary between temperatures ($F_{1,36}$ = 3.59, p =

Table 2. Comparison of calcification normalized to area (mg cm^{-2} d^{-1}) and biomass (mg mg^{-1} d^{-1}) for massive *Porites* spp. among treatments. Analyses were completed with a partly nested ANOVA with two crossed between-plots effects (pCO_2 and temperature [temp]) and one within-plot factor (feeding). SS = sum of squares, df = degrees of freedom, and MS = mean sum of squares.

| Dependent variable	Source	Effect	SS	df	MS	F	p
Area-normalized calcification (mg cm^{-2} d^{-1})	Between plots	pCO_2	0.279	1	0.279	2.653	0.108
		Temp	0.022	1	0.022	0.208	0.650
		$pCO_2 \times$ temp	0.097	1	0.096	0.920	0.341
	Within plots	Feeding	3.050	1	3.050	29.050	<0.001
		Feeding$\times pCO_2$	0.188	1	0.188	1.792	0.185
		Feeding$\times$temp	0.098	1	0.098	0.935	0.337
		Feeding$\times pCO_2 \times$temp	0.051	1	0.051	0.486	0.488
		Error	7.559	72	0.105		
Biomass-normalized calcification (mg mg^{-1} d^{-1})	Between plots	pCO_2	0.022	1	0.022	6.397	0.014
		Temp	0.038	1	0.038	11.353	0.001
		$pCO_2 \times$ temp	0.0002	1	0.0002	0.070	0.792
	Within plots	Feeding	0.001	1	0.001	0.305	0.583
		Feeding$\times pCO_2$	0.00003	1	0.00003	0.009	0.925
		Feeding$\times$temp	0.0007	1	0.0007	0.196	0.660
		Feeding$\times pCO_2 \times$temp	0.003	1	0.003	0.785	0.378
		Error	0.243	72	0.003		
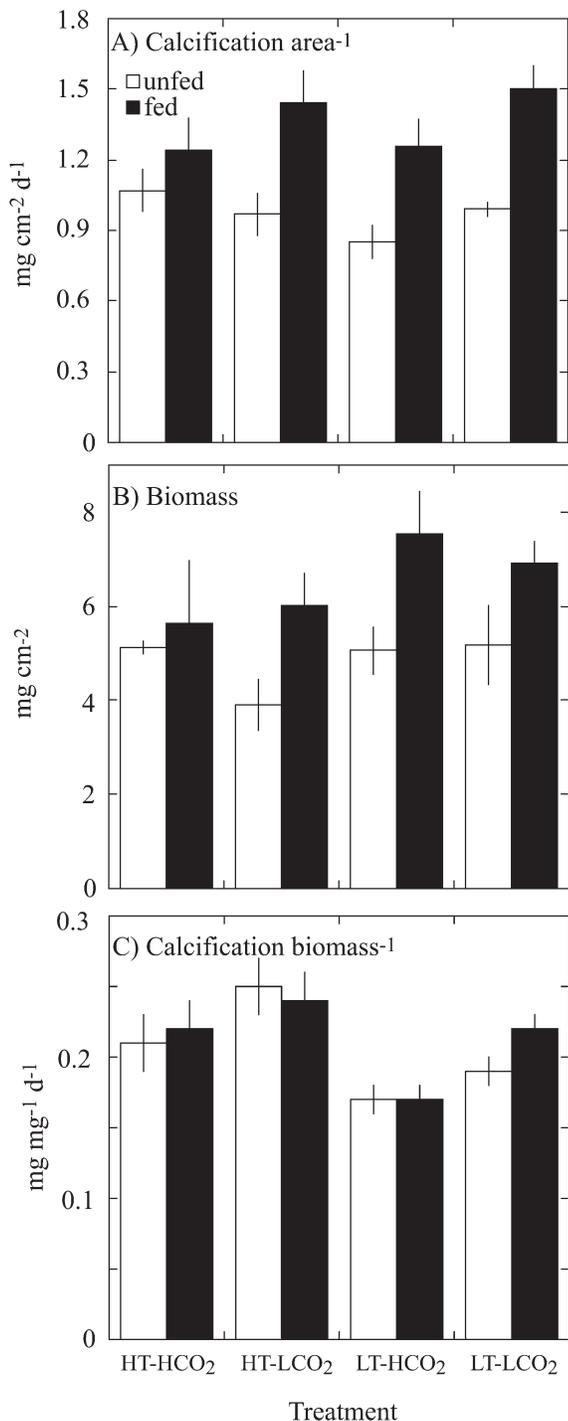
Fig. 1. Calcification and biomass of massive *Porites* spp. after 1 month exposure to combinations of temperature, pCO_2, and feeding (unfed or fed with *Artemia* spp.): HT–HCO$_2$, 29.3°C and 81.6 Pa; HT–LCO$_2$, 29.3°C and 41.6 Pa (ambient conditions); LT–HCO$_2$, 25.7°C and 81.6 Pa; and LT–LCO$_2$, 25.7°C and 41.6 Pa. (A) Calcification normalized to area, (B) biomass normalized to area, and (C) calcification normalized to biomass; mean ± SE ($n = 10$) displayed. Refer to Table 2 for statistical contrasts.

0.066), but not between pCO_2 treatments ($F_{1,36} = 0.27$, $p = 0.609$); it was unaffected by all interactions ($p > 0.285$). Feeding increased mean biomass 35%, from 4.9 ± 0.3 mg cm^{-2} to 6.5 ± 0.5 mg cm^{-2}, and low temperature was associated with a 19% increase in mean biomass, from 5.2 ± 0.4 mg cm^{-2} to 6.2 ± 0.4 mg cm^{-2} (± SE, pooled across treatments, $n = 40$).

Area-normalized calcification was expressed per biomass by division with the mean biomass of corals in the respective treatment groups (Fig. 1B). Mean biomass-normalized calcification (Fig. 1C) ranged from 0.17 ± 0.01 mg mg^{-1} d^{-1} (LT–HCO$_2$, fed and unfed) to 0.25 ± 0.02 mg mg^{-1} d^{-1} (HT–LCO$_2$, unfed) (both ± SE, $n = 10$), and was affected by pCO_2 and temperature. In the test of pCO_2 effects, the power $(1 - \beta)$ of the test for detecting a difference of 0.02 mg mg^{-1} d^{-1} (10% of the grand mean) was ~ 0.38, and the test provided a 90% confidence of detecting a difference of 0.04 mg mg^{-1} d^{-1}. Mean biomass-normalized calcification was depressed 17% by high vs. ambient pCO_2 at 25.6°C, 12% by high vs. ambient pCO_2 at 29.3°C, and 20% by 25.6°C vs. 29.3°C (pooled across pCO_2 treatments); it was unaffected by tank ($p \geq 0.415$) or any of the interactive effects (Table 2).

Mean *Symbiodinium* spp. content on the area scale ranged from 0.52 ± 0.05 × 10^6 cells cm^{-2} (HT–HCO$_2$, unfed) to 1.33 ± 0.23 × 10^6 cells cm^{-2} (LT–HCO$_2$, fed), and on the biomass scale ranged from 1.00 ± 0.09 × 10^5 cells mg^{-1} (HT–HCO$_2$, unfed) to 2.22 ± 0.03 × 10^5 cells mg^{-1} (HT–HCO$_2$, fed) (all ± SE, $n = 10$; Fig. 2). Area-normalized *Symbiodinium* spp. content was affected by temperature ($F_{1,32} = 5.47$, $p = 0.026$), declining 18% at the high temperature, and by feeding ($F_{1,32} = 32.52$, $p < 0.001$), increasing 81% as a result of feeding, and there was a trend for it to be affected by pCO_2 in a pattern dependent on feeding ($F_{1,32} = 3.11$, $p = 0.087$); no other effects were significant ($p \geq 0.278$). On the biomass scale, *Symbiodinium* spp. content was affected by feeding ($F_{1,32} = 7.26$, $p = 0.011$), increasing 33% as a result of feeding, and also by pCO_2 in a pattern dependent on feeding ($F_{1,32} = 4.51$, $p = 0.032$); no other effects were significant ($p \geq 0.107$).

Discussion

Against the backdrop of the consensus opinion that scleractinian corals will be severely affected by OA (Hoegh-Guldberg et al. 2007; Erez et al. 2011), this study was designed to explore the effects of OA on massive *Porites* spp. under ecologically relevant conditions of temperature, light, and seawater motion. The experiment was conducted at a light intensity greater than that used in most previous studies, in large volumes of vigorously agitated seawater, and for a duration that allowed the corals to equilibrate to the treatment conditions. The results describe a novel response of a scleractinian coral to OA, in which biomass plasticity is utilized to sustain area-normalized calcification at high pCO_2 (81.5 Pa).

The maintenance of area-normalized calcification during 1 month exposure to 81.5 Pa pCO_2 is ecologically relevant as this is the critical measure of coral growth that describes the deposition of CaCO$_3$ per area of benthic surface. With a
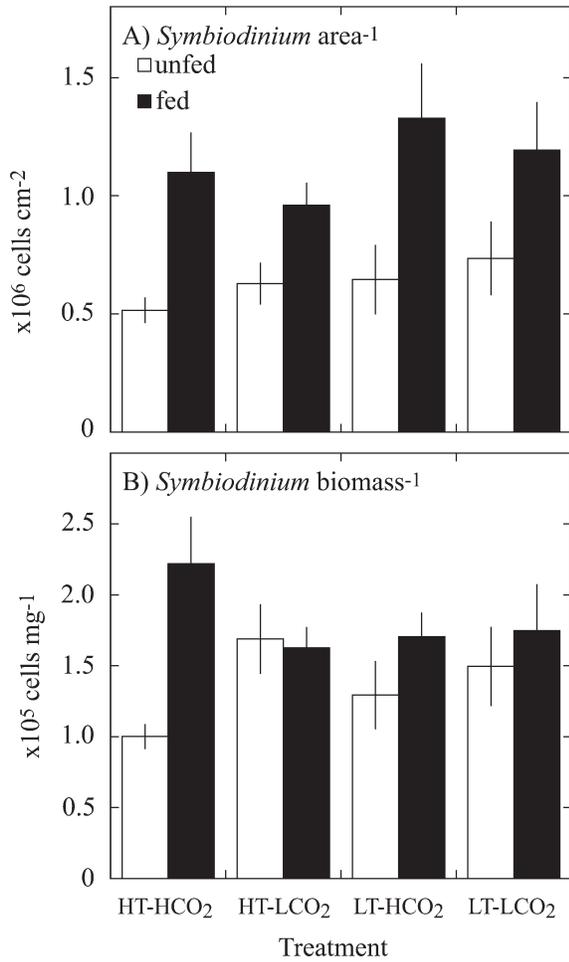
Fig. 2. *Symbiodinium* spp. content of massive *Porites* spp. after 1 month exposure to combinations of temperature, pCO_2, and feeding regimes (unfed or fed with *Artemia* spp.) as described in Fig. 1. (A) *Symbiodinium* spp. content normalized to the area and (B) *Symbiodinium* spp. content normalized to biomass (Fig. 1B); mean $\pm$ SE ($n = 10$) displayed.

skeletal density of 1.28 g cm^{-3} (Lough and Barnes 2000), the present calcification rates of $\sim$ 1 mg cm^{-2} d^{-1}, regardless of treatment conditions, correspond to a linear extension of $\sim$ 3 mm yr^{-1}. This is slower than the growth rates of this taxon in situ (ca. 13 mm yr^{-1}; Lough and Barnes 2000), where a spectrum of heterotrophic food is available to stimulate calcification (Ferrier-Pagès et al. 2011), and TA is higher than was sustained in the present treatments. Reef water in Moorea, for example, typically has a TA of $\sim$ 2350 μmol kg^{-1} (Gattuso et al. 1997; P. J. Edmunds unpubl.), and was probably depleted in the incubations by fauna in the seawater system from which the seawater was drawn and by calcification of the corals themselves. Regardless of these effects, the proportional depression of growth in the tanks does not affect the conclusions pertaining to the relative effects of treatments on calcification. Feeding on *Artemia* spp. augmented biomass and *Symbiodinium* content of massive *Porites* spp. in a way similar to that occurring in scleractinians

during cooler months (Fitt et al. 2000), and when some bleached corals feed on zooplankton (Grottoli et al. 2006). Further, *Artemia* spp. consumption enhanced area-normalized calcification more (40%) than biomass (35%), suggesting that ecologically relevant light intensities promoted the allocation of resources from heterotrophic feeding to skeletogenesis (Ferrier-Pagès et al. 2011).

Feeding on *Artemia* spp. was not, however, the sole cause of the changes in biomass of massive *Porites* spp. Independent of feeding, temperature also affected biomass, with a 19% increase at 25.6°C relative to 29.3°C. This increase may, in part, reflect metabolic savings accrued at low temperature in poikilothermic organisms like corals (Edmunds 2009). It is interesting, however, that pCO_2 had no effect on biomass, which implies that OA does not affect the allocation of resources to tissue, even though the consequences of OA on calcification are believed to be thermodynamically costly (Atkinson and Cuet 2008; Erez et al. 2011). Although such costs have yet to be demonstrated empirically, one reason they are thought to be biologically significant is the greater energetic costs of mineralizing at low Ω_{arag} (Erez et al. 2011), which under normal conditions accounts for 13–30% of the energy supplied by respiration (Allemand et al. 2011). Assuming, therefore, that elevated metabolic costs are incurred at low Ω_{arag}, they raise the possibility that OA prompts a reallocation of resources in massive *Porites* spp. to favor biomass, either to the detriment of other functions (e.g., reduced fecundity) or through enhanced energetic influxes (e.g., through the stimulation of *Symbiodinium* photosynthesis by HCO_3^-; Herfort et al. 2008).

Based on work with *Astrangia poculata*, it has been hypothesized (Holcomb et al. 2010) that nutrient-replete corals benefit photosynthetically from additional DIC associated with OA, allowing calcification to be sustained while $[CO_3^{2-}]$ and Ω_{arag} decline; with nutrients, *Symbiodinium* become DIC limited and, therefore, photosynthesis is stimulated by HCO_3^-. In this model, the positive association between photosynthesis and calcification operates through the conversion of HCO_3^- to CO_2 prior to fixation in the Calvin cycle of the *Symbiodinium*, with the OH^- liberated from HCO_3^- neutralizing protons generated by calcification (Allemand et al. 2011), or the use of energy from photosynthetically fixed carbon to assist with the metabolic costs of calcification. In contrast, under nutrient limitation, which may be common in corals (Dubinsky et al. 1990), DIC at low pCO_2 is sufficient to support photosynthesis and calcification, but the rate of calcification is dictated by Ω_{arag}, which declines (Holcomb et al. 2010).

In the present study, massive *Porites* spp. obtained nutrients through feeding on *Artemia* spp., and as predicted for nutrient-limited *Symbiodinium* (Dubinsky et al. 1990), algal populations increased. *Symbiodinium* density also responded to temperature, increasing on an area-normalized scale at 25.6°C compared to 29.3°C, and declining at high pCO_2 when food was withheld (but not when fed). Nutrient stimulation of *Symbiodinium* has been hypothesized to confer resistance to high pCO_2 (Holcomb et al. 2010), and although this was the case for area-normalized

calcification, the same outcome was observed in unfed corals, and further, biomass-normalized calcification was susceptible to high pCO_2 regardless of feeding. It is unlikely that the unfed corals were benefiting greatly from access to dissolved mineral nutrients and particulate materials, because dissolved nutrients occur at low concentration in the coastal waters of Moorea (e.g., 0.72 ± 0.20 μmol L^{-1} for NO_2^- and NO_3^-; Alldredge and Carlson 2009), and only particulates $< 50\text{-}\mu m$ diameter were able to enter the incubation water following filtration. The results for massive *Porites* spp. suggest that nutrients do not directly mitigate the effects on calcification of elevated pCO_2 as in *A. poculata* (Holcomb et al. 2010), and instead, nutrients appear to function in concert with other abiotic factors to alter biomass in ways that conserve area-normalized calcification.

It is unclear why the biomass of massive *Porites* spp. differs among treatments in its ability to calcify, but the low biomass-normalized calcification rates under high pCO_2 suggest that it may be more costly to mineralize under these conditions. This is consistent with the enhanced thermo-dynamic challenge of depositing $CaCO_3$ at lower Ω_{arag}, which was reduced 31% under the HT–HCO_2 treatment and 41% under the LT–HCO_2 treatment (both relative to the control, HT–LCO_2). To evaluate this possibility, it would be interesting to measure the metabolic rate of the holobiont under elevated pCO_2 in order test the hypothesis that that the increased costs of mineralization are reflecting in enhanced respiration. Although data on this topic are sparse, this does seem to be the case for *Acropora formosa*, in which light-enhanced dark respiration (LEDR; but not dark respiration) increases 81% at 111 Pa CO_2 compared to 38 Pa CO_2 (Crawley et al. 2010). Because LEDR is coincident with light-enhanced calcification (Allemand et al. 2011), the results from *A. formosa* are consistent with the hypothesis that greater LEDR at high pCO_2 reflects the costs of calcification. However, a similar outcome can result from the immediate catabolism of photosynthetically fixed carbon (Edmunds and Spencer Davies 1988), which might also be accentuated if carbon limitation of *Symbiodinium* is alleviated at high pCO_2 (Crawley et al. 2010). Thus, to interpret the effects of increased pCO_2 on LEDR, it will be necessary to develop techniques to resolve the contribution to LEDR of increased metabolic costs (i.e., through calcification), vs. enhanced supply of respiratory substrate (i.e., through fixed carbon). If indeed a large proportion of LEDR reflects the costs of calcification, it is likely that such effects will be well developed in massive *Porites* spp., because the long transport distances from the seawater to tissue deep within the skeleton might accentuate the costs of transporting the ions necessary for calcification at high pCO_2 (Allemand et al. 2011).

When normalized to area, the calcification results for massive *Porites* spp. are inconsistent with the notion that corals calcify more slowly because of OA (Hoegh-Guldberg et al. 2007; Erez et al. 2011). Although the present study addressed changes in seawater DIC chemistry that are predicted to occur as a result of OA occurring on a shorter time horizon ($\sim$ 100 y) than used in many other studies (Anthony et al. 2008; Jury et al. 2010), the null finding is not without precedent (Anthony et al. 2008; Holcomb et al. 2010; Jury et al. 2010). Based on a 1-month exposure to 81.5 Pa pCO_2, massive *Porites* spp. appears to differ from other corals that offer little reason to suspect that they will be resistant to OA, and indeed, in comparative analyses, calcification of *A. intermedia* was more sensitive to pCO_2 than that of *P. lobata* (Anthony et al. 2008). The results presented here suggest it might be productive to evaluate the distinctive features of massive *Porites* spp. and ask how they might facilitate OA resistance. Of these, one of the most striking is the highly perforate skeleton and its labile interactions with the tissue that allow it to penetrate $\sim$ 5 mm into the skeleton (Lough and Barnes 2000). It might be valuable, therefore, to explore the roles of skeleton porosity and variable biomass in the response of scler-actians to OA. The present study suggests that some perforate corals might have the capacity to tolerate the effects of OA, at least during short exposures to conditions expected to occur this century.

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