***In Situ* Effects of Low pH and Elevated HCO_3^- on Juvenile Massive *Porites* spp. in Moorea, French Polynesia**

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Abstract. Juvenile colonies of massive *Porites* spp. were exposed to manipulated pH and bicarbonate ($[\text{HCO}_3^-]$) *in situ* to test the hypothesis that ocean acidification (OA) does not affect respiration and calcification. Incubations lasted 28 h and exposed corals to ambient temperature and light with ecologically relevant water motion. Three treatments were applied: (1) ambient conditions of pH 8.04 and $1751 \mu\text{mol HCO}_3^- \text{ kg}^{-1}$ (Treatment 1), (2) pCO_2 -induced ocean acidification of pH 7.73 and $2011 \mu\text{mol HCO}_3^- \text{ kg}^{-1}$ (Treatment 2), and (3) pCO_2 and HCO_3^- -enriched seawater of pH 7.69 and $2730 \mu\text{mol HCO}_3^- \text{ kg}^{-1}$ (Treatment 3). The third treatment providing elevated $[\text{HCO}_3^-]$ was used to test for stimulatory effects of dissolved inorganic carbon on calcification under low pH and low saturation of aragonite (Ω_{arag}), but it does not reflect conditions expected to occur under CO_2 -driven OA. Calcification of juvenile massive *Porites* spp. was affected by treatments, with an 81% elevation in Treatment 3 *versus* Treatment 1, but no difference between Treatments 1 and 2; respiration and the metabolic expenditure concurrent with calcification remained unaffected. These findings indicate that juvenile massive *Porites* spp. are resistant to short exposures to OA *in situ*, and separately, that they can increase calcification at low pH and low Ω_{arag} if $[\text{HCO}_3^-]$ is elevated. Juvenile *Porites* spp. may therefore be limited by dissolved inorganic carbon under ambient pCO_2 conditions.

Introduction

Coral reefs are threatened by natural and anthropogenic factors, including global climate change and ocean acidification (OA) (Hoegh-Guldberg *et al.*, 2007; Wilkinson, 2008). OA poses a particularly severe threat, because if corals do not first succumb to rising temperature (Hoegh-Guldberg *et al.*, 2007), OA challenges their capacity to produce calcareous skeletons (Hofmann *et al.*, 2010). OA originates in the dissolution of atmospheric CO_2 in seawater, resulting in decreases in seawater pH, the concentration of carbonate ($[\text{CO}_3^{2-}]$), and the saturation state of aragonite (Ω_{arag}), and an increase in the concentration of bicarbonate ($[\text{HCO}_3^-]$) (Kleypas *et al.*, 1999). Together, these effects impair the ability of corals to deposit CaCO_3 (Jury *et al.*, 2010; Hofmann *et al.*, 2010; Edmunds *et al.*, 2012; Comeau *et al.*, 2013b), and diminish their capacity to function as ecosystem engineers (Wild *et al.*, 2011).

Laboratory studies have contributed much to the understanding of the response of corals to OA (Andersson and Gledhill, 2013), particularly with regard to the underlying physiological mechanisms (Erez *et al.*, 2011). However, laboratory experiments typically are performed under conditions that do not replicate the natural environment perfectly, notably by providing light at a constant low intensity throughout the day, and with a spectral composition differing from ambient sunlight (Kinzie *et al.*, 1984; Schlacher *et al.*, 2007). Light stimulates coral calcification (Gattuso *et al.*, 1999), fuels *Symbiodinium* photosynthesis, and facilitates autotrophic nutrition by the holobiont (Pearse and Muscatine, 1971). Also of great importance to corals is the hydrodynamic regime of reef environments (Monismith, 2007), which is difficult to recreate in tanks and yet is

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Abbreviations: Ω_{arag} , saturation state of aragonite; OA, ocean acidification; PAR, photosynthetically active radiation; TA, total alkalinity.

critical for multiple aspects of coral physiology (Dennison and Barnes, 1988; Patterson *et al.*, 1991). Given the limitations of laboratory experiments, there is a need to evaluate the effects of OA on corals in the natural environment (Langdon and Atkinson, 2005; Holcomb *et al.*, 2010). *In situ* experiments have been used to study the physiology of corals and coral reefs for decades (Porter, 1980; Pearson *et al.*, 1984; Carpenter, 1985; Patterson *et al.*, 1991; Ralph *et al.*, 1999; Levy *et al.*, 2006), but to our knowledge, there have been only two studies in which *in situ* approaches have been used to study the effects of OA on corals (Chauvin *et al.*, 2011; Klein *et al.*, 2012). Natural CO₂ seeps afford the potential to study aspects of the effects of OA in the natural environment (Hall-Spencer *et al.*, 2008; Fabricius *et al.*, 2011), but the rarity of such locations and their suitability mostly for mensurative analyses (*sensu* Hurlbert, 1984) limits the general application of this approach.

The objective of this study was to utilize chambers placed *in situ* to examine the effects of low pH and high [HCO₃⁻] on juvenile massive *Porites* spp. Three hypotheses were tested: (1) low pH and high [HCO₃⁻] have no effect on respiration or calcification, (2) the energetic expenditure concurrent with calcification is unaffected by OA, and (3) low pH impairs calcification at high [HCO₃⁻] (2730 μmol kg⁻¹ seawater). As rationales for these hypotheses, we reasoned that OA typically reduces coral calcification (Comeau *et al.*, 2013b) and can increase the rate of respiration (Crawley *et al.*, 2010; but see Edmunds, 2012), with the energetic cost of depositing CaCO₃ referred to as a cause of stimulatory effects of OA on respiration (Fabry *et al.*, 2008; Erez *et al.*, 2011). As elevated [HCO₃⁻] can mitigate the negative effects of OA *in vitro* (Marubini *et al.*, 2008; Comeau *et al.*, 2012), it was compelling to test for similar effects *in situ*.

Materials and Methods

Experimental design

We used three seawater treatments of contrasting pH and carbonate chemistry to examine the effects of OA and elevated [HCO₃⁻] on juvenile massive *Porites* spp. Juvenile massive *Porites* spp. in Moorea is a functional group consisting of about 85% *P. lutea* (Edwards and Haime, 1860) and about 15% *P. lobata* (Dana, 1846) (Edmunds, 2009), which are difficult to distinguish from each other while alive. Experiments were performed in three trials (beginning on 4, 6, and 8 February 2012), with six corals in each trial exposed to treatments ($n = 2$ corals treatment⁻¹) for 28 h in acrylic chambers at 1.5-m depth on a fringing reef (Fig. 1). Seawater treatments contrasted (1) ambient pH and ambient [HCO₃⁻] (Treatment 1, the control against which the other treatments were gauged); (2) low pH and high [HCO₃⁻] (Treatment 2); and (3) low pH and very high [HCO₃⁻] (Treatment 3). Treatment 1 represented present

seawater conditions on the shallow reefs of Moorea (~pH 8.00 and 1750 μmol HCO₃⁻ kg⁻¹); Treatment 2 reflected the seawater conditions projected to occur in 100 y (~pH 7.70 and 2000 μmol HCO₃⁻ kg⁻¹) under representative concentration pathway (RCP) 6.0 (~95 Pa pCO₂) (van Vuuren *et al.*, 2011); and Treatment 3 (~pH 7.70 and 2700 μmol HCO₃⁻ kg⁻¹) tested the hypothesis that elevated [HCO₃⁻] stimulates calcification at reduced Ω_{arag} and low pH. Although Treatment 3 is not a feasible combination of future seawater conditions attributable to OA, it allowed further exploration of the role of HCO₃⁻ in coral calcification (*sensu* Comeau *et al.*, 2012), as well as consideration of the possibility that elevated [HCO₃⁻] alleviates the effects of low pH (Comeau *et al.*, 2012). We reasoned that the results of Treatment 3 would therefore be valuable in designing future experiments focused on the mechanism by which corals can respond to OA.

Nine chambers (2.47 l) were made from custom-cast, UV-transparent acrylic (Industrial Plastics, California), which transmits UV A and B radiation (280–400 nm) and photosynthetically active radiation (PAR; 400–700 nm) (Gleason *et al.*, 2006). A test of the light transmission qualities (for UV A and PAR) of the acrylic polymer was performed using a cosine-corrected sensor attached to a fiber optic cable and a spectrophotometer (USB-2000, Ocean Optics, Dunedin, Florida) and operated using a PC running the manufacturer's software (Spectra Suite 2.0, Ocean Optics). Light was measured with spectral resolution between 350 and 1000 nm and corrected against a known color temperature (°Kelvin). There was minimal attenuation of UV A and PAR, with greater than 96% transmission through the acrylic of light between 350 nm and 1000 nm wavelength (Fig. 2); transmission of light at wavelengths below 350 nm was not measured.

Three chambers were allocated to each treatment, with two exposing corals to treatments ($n = 1$ coral chamber⁻¹), and one filled with seawater alone as a control. All chambers were filled with fresh seawater at the start of each trial ($n = 3$), and stirred with a motor (Playmobil, Zirndorf, Germany) that provided a mean flow speed of 15.8 ± 1.3 cm s⁻¹ ($\pm$ SE, $n = 12$) in the chamber. Flow speeds were determined in a preliminary study in which hydrated *Artemia* spp. eggs within the chambers were photographed (after Sebens and Johnson, 1991). At the start of each trial, chambers were filled with treatment seawater, and bubbles were removed before the chambers were sealed and placed on the reef. Chambers remained on the reef for 28 h, but were retrieved at 4-h intervals to replenish treatment seawater and batteries in the stirring motors. This procedure generally took less than 10 min, and corals were kept submerged at all times. Light intensity (PAR) and seawater temperature adjacent to the chambers were recorded at 10-min intervals using a 4π spherical quantum sensor (MkV-L, JFE Advantech Co., Kobe, Japan) and Hobo Wa-

Figure 1. Photo of (A) juvenile massive *Porites* spp., (B) fringing reef off the Richard B. Gump South Pacific Research Station in Cook's Bay, Moorea, and (C) the 2.47-l experimental chambers (23 x 23 x 8 cm) each housing a single coral colony *in situ*. i = Location of chambers on the fringing reef at 1.5-m depth, ii = juvenile colony attached to mesh with epoxy, and iii = submersible stirring motor. (D) Schematic illustrating the location of coral and stirring motor within chambers. Photo credits (A) D. Liittschwager, used with permission; (B–D) C. Wall.

ter Temp Pro v2 loggers (± 0.2 °C, Onset Computer, Massachusetts), respectively.

Coral collection and carbonate chemistry

Juvenile *Porites* spp. (≤ 4.0 -cm diameter, $n = 18$) were collected from 2–3-m depth in the back reef of Moorea, between 28 January 2012 and 1 February 2012. Corals were transported to the Richard B. Gump South Pacific Research Station, attached to plastic bases with epoxy (Z-Spar A788), and placed in a flow-through tank (1000 l) to recover. This tank was filled with filtered seawater (50 μm) at about 27.5

°C and illuminated with two 75-W LED lamps (Sol White LED Module, Aquillumination, Iowa) that supplied light at $515 \pm 30 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (mean $\pm$ SE, $n = 75$), as measured underwater using a spherical quantum sensor (Li-Cor LI-193). Colonies remained in the recovery tank until used in the trials.

To create pH and $[\text{HCO}_3^-]$ treatments, the pCO_2 of seawater was manipulated by bubbling ambient air or CO_2 -enriched air into three 150-l reservoirs supplied with filtered seawater (50 μm). Gas mixtures were created using a solenoid-controlled system (Model A352, Qubit Systems, On-

Figure 2. The spectral comparison of the relative irradiance of sunlight measured from 350 to 1000 nm and corrected against a known color temperature ($^{\circ}\text{Kelvin}$) for natural sunlight (black) and sunlight transmitted through UV-transmitting acrylic polymer (gray).

tario, Canada), as detailed in Edmunds (2012). Seawater for Treatment 1 was un-manipulated; seawater for Treatment 2 was equilibrated with pCO_2 at 98.9 Pa; seawater for Treatment 3 was obtained by augmenting Treatment 2 seawater with baking soda ($0.9 \text{ mmol l}^{-1} \text{ NaHCO}_3$, Church and Dwight Co., California) to increase $[\text{HCO}_3^-]$ to $2730 \text{ } \mu\text{mol kg}^{-1}$ at pH_T of 7.69. Seawater conditions within the three reservoirs were monitored for salinity, pH on the total scale (pH_T), total alkalinity (TA, $\mu\text{mol kg}^{-1}$), and carbonate chemistry for one week prior to the start of the experiment, and daily during the experiment (4–9 February). Analyses of seawater were conducted according to standard operating procedure (SOP) 3b of Dickson *et al.* (2007), as detailed in Comeau *et al.* (2012), with final values for carbon chemistry calculated using the seacarb script in R (R Foundations for Statistical Computing; Lavigne and Gattuso, 2011). Titrations of certified reference materials (Batch 105) provided by A. G. Dickson (Scripps Institution of Oceanography) revealed determination of TA to be within $0.3\text{--}4.5 \text{ } \mu\text{mol kg}^{-1}$ of certified TA values (*i.e.*, $\leq 0.2\%$).

Calcification and respiration

After 24-h incubations, the response of the corals was assessed as rates of calcification and respiration. Calcification was measured *in situ* as the change in TA of seawater over 4 h (after Smith and Key, 1975) from about 1000 to 1400 h, with the volume of the stirring motor and coral subtracted from each seawater sample. Calcification was

adjusted for the change in TA in the control chambers, and standardized by time and the surface area of the coral tissue ($\mu\text{g CaCO}_3 \text{ cm}^{-2} \text{ h}^{-1}$). Surface area of the corals was determined using aluminum foil (Marsh, 1970) and was completed after the respiration measurements.

After measurements of calcification (at $\sim 1500 \text{ h}$), corals were transferred to respirometers (0.24 l) filled with filtered seawater ($50 \text{ } \mu\text{m}$) representing the same conditions as the *in situ* treatments, and kept in darkness for about 30 min to reduce the effects of light on respiration (Edmunds and Davies, 1988). After dark acclimation, respiration of corals was measured at 28°C with a stir bar used to create a flow speed of $3.0 \pm 0.2 \text{ cm s}^{-1}$ (mean $\pm$ SE, $n = 16$) determined as described above. Changes in pO_2 in seawater were measured with optrodes (Foxy-R, 1.58-mm diameter, Ocean Optics) calibrated against water-saturated air (100%) and a zero solution (saturated sodium sulfite in 0.01 mol l^{-1} sodium tetraborate), and connected to spectrophotometers (USB2000 and NeoFox, Ocean Optics) that logged O_2 saturation onto a PC running Ocean Optics software (OOISensors, ver. 1.00.08 and NeoFox Viewer ver. 2.3, Ocean Optics). O_2 saturation was converted to $\mu\text{mol O}_2 \text{ ml}^{-1}$ using tabulated values for O_2 solubility [N. Ramsing and J. Gundersen at Unisense (Aarhus, Denmark), based on Garcia and Gordon (1992)], yielding a saturated oxygen content of $0.1999 \text{ } \mu\text{mol O}_2 \text{ ml}^{-1}$ at 28°C , a salinity of 36, and 101.3 kPa atmospheric pressure. Respiration rates were determined by regressing O_2 concentration against time, correcting for O_2 fluxes in control chambers, and standardizing oxygen consumption to the surface area of the coral tissue (cm^2).

Respiration rates were used to estimate the metabolic energy expenditure concurrent with calcification by converting hourly O_2 consumption to energy units (joules) using a conversion of $440 \text{ J mmol O}_2^{-1}$ (Elliot and Davison, 1975), assuming lipids are the primary respiratory substrate (Patton *et al.*, 1977). Respiratory energy expenditure ($\text{J cm}^{-2} \text{ h}^{-1}$) was normalized to the calcification rate ($\mu\text{g CaCO}_3 \text{ cm}^{-2} \text{ d}^{-1}$) and expressed as $\text{J g}^{-1} \text{ CaCO}_3$ to evaluate the energy expenditure concurrent with calcification.

Statistical analyses

Calcification, respiration, energy expenditure concurrent with calcification, and incubation conditions (pH_T and $[\text{HCO}_3^-]$) were compared among treatments using one-way ANOVA. As consecutive trials were performed over 3 d with potential differences in physical conditions among days, a one-way ANOVA was used to test for differences in PAR and temperature among trials. *Post hoc* analyses were performed using Tukey's Honestly Significant Difference (HSD) test, and statistical assumptions of normality and homoscedasticity required for ANOVA were tested by graphical analysis of residuals. Analyses were completed

Table 1

Summary of physical and chemical conditions for three treatments of *Porites* spp. colonies maintained in 2.47-l acrylic chambers in situ at 28.8 °C between 4 and 9 February 2012

Treatment	pH _{total}	TA ($\mu\text{mol kg}^{-1}$)	pCO ₂ (Pa)	HCO ₃ ⁻ ($\mu\text{mol kg}^{-1}$)	CO ₃ ²⁻ ($\mu\text{mol kg}^{-1}$)	DIC ($\mu\text{mol kg}^{-1}$)	Ω_{arag}
1	8.04 ± 0.01	2359 ± 3	41.1 ± 0.8	1751 ± 6	246 ± 2	2008 ± 4	3.95 ± 0.03
2	7.73 ± 0.04	2358 ± 2	98.9 ± 9.5	2011 ± 25	141 ± 10	2177 ± 17	2.27 ± 0.15
3	7.69 ± 0.03	3131 ± 8	146.4 ± 10.5	2730 ± 21	171 ± 8	2938 ± 16	2.75 ± 0.12

Values are mean ± SE ($n = 6$ for carbonate chemistry; TA = total alkalinity; DIC = dissolved inorganic carbon; Ω_{arag} = aragonite saturation state; Treatment 1 = ambient pH, ambient HCO₃⁻; Treatment 2 (e.g., end of century pCO₂) = low pH, high HCO₃⁻; Treatment 3 (e.g., pCO₂ and HCO₃⁻ enriched) = low pH, very high HCO₃⁻. Carbonate chemistry was calculated using the seacarb package in R.

with Systat 11 software (Systat, Inc., Illinois) using a Windows operating system. Power analysis was performed on select results according to Zar (2010).

Results

The chemical conditions of seawater used to fill the chambers were maintained with a high degree of precision (Table 1) and differed significantly among treatments in terms of pH_T ($F_{2,15} = 44.84$, $P < 0.001$) and [HCO₃⁻] ($F_{2,15} = 720.79$, $P < 0.001$). Although the chemical conditions were affected by the incubations, notably for oxygen concentration (through the effects of respiration and photosynthesis) and TA (through the effects of calcification), these effects were minimized by changing the seawater every 4 h. Mean salinity throughout the treatments was 35.9 ± 0.1 (± SE, $n = 16$), and the mean temperature was 28.8 ± 0.2 °C ($n = 514$, pooled among days). However, temperature differed among trials ($F_{2,86} = 35.75$, $P < 0.001$) and was slightly warmer during the last trial (8 February, 29.4 ± 0.2 °C) than the first trial (4 February, 28.2 ± 0.2 °C) (both mean ± SE, $n = 170$ – 171). The mean light intensity during the daylight portion of the incubations was 1072 ± 51 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (SE, $n = 227$ measurements over 3 trials), but sustained maximum intensities reaching about 2300 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Integrated each day, the light regime provided 52.3 ± 8.5 mol photons $\text{m}^{-2} \text{d}^{-1}$ (± SD, $n = 3$ days). Over the 4-h period when calcification was measured (i.e., 1000–1400 h), the mean irradiance at 1.5-m depth was 1769 ± 60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (± SE, $n = 86$) and light did not differ among trials ($F_{2,83} = 2.17$, $P = 0.121$).

All corals appeared healthy and showed no visible signs of stress or loss of pigmentation during the experiments; however, the loss of two seawater samples in Treatment 2 reduced calcification measurements to four corals. The *in situ* incubations proved successful in exposing corals to seawater of contrasting carbonate chemistry under physical conditions similar to those in the natural environment. Rates of calcification in the light (mean ± SE) for control corals

(e.g., Treatment 1) were 67.92 ± 14.48 $\mu\text{g CaCO}_3 \text{cm}^{-2} \text{h}^{-1}$ and 52.19 ± 8.76 $\mu\text{g CaCO}_3 \text{cm}^{-2} \text{h}^{-1}$ and 123.04 ± 18.12 $\mu\text{g CaCO}_3 \text{cm}^{-2} \text{h}^{-1}$ in Treatment 2 and Treatment 3, respectively. Hourly calcification rates differed significantly among treatments ($F_{2,13} = 6.56$, $P = 0.011$) (Fig. 3). *Post hoc* analyses revealed that hourly calcification was increased significantly in Treatment 3 (HCO₃⁻-enriched) compared to Treatment 1 (ambient conditions) ($P > 0.05$), but no other contrasts were significant ($P > 0.50$). Calcification in Treatment 3 (HCO₃⁻-enriched) was significantly higher than in other treatments and was elevated 82% and 113% relative to Treatment 1 and Treatment 2, respectively (Fig. 3). Mean respiration (± SE) ranged from 0.39 ± 0.05 $\mu\text{mol O}_2 \text{cm}^{-2} \text{h}^{-1}$ (Treatment 2) to 0.46 ± 0.06 $\mu\text{mol O}_2 \text{cm}^{-2} \text{h}^{-1}$ (Treatment 3), but it did not vary significantly among treatments ($F_{2,15} = 0.37$, $P = 0.697$) (Fig. 3), although the power ($1-\beta$) of this test was low (<0.30).

Converting respiratory O₂ consumption to joules revealed a mean energy expenditure of 0.17 to 0.22 J $\text{cm}^{-2} \text{h}^{-1}$ for corals across treatments, which corresponded to mean energy expenditures per colony of 3.30 to 4.38 J h^{-1} , and calcification rates of 52.19 μg and 123.04 $\mu\text{g CaCO}_3 \text{cm}^{-2} \text{h}^{-1}$. Together these values revealed that the energy expenditure coincident with calcification ranged from (± SE) 1804 ± 260 to 3461 ± 459 J $\text{g}^{-1} \text{CaCO}_3$ in Treatment 3 and Treatment 2, respectively (Fig. 3), although these values did not differ among treatments ($F_{2,13} = 2.35$, $P = 0.135$).

Discussion

Laboratory-based mesocosms have dominated studies of the effects of ocean acidification (OA) on corals, in part due to the logistical challenges of manipulating seawater chemistry, but such approaches have limited utility in evaluating how corals respond to similar conditions *in situ*. While laboratory experiments are designed to create conditions resembling those found in the natural environment at the site where study corals are collected, many fall short of attaining this objective. For instance, the common use of low-intensity artificial lighting does not reflect conditions

Figure 3. Calcification, respiration, and the metabolic expenditure concurrent with calcification in juvenile massive *Porites* spp. exposed for 28 h to three treatments (ambient seawater [SW], ocean acidification conditions for the year 2100, and HCO₃⁻-enriched/low pH treatment) in chambers maintained *in situ*. (A) Calcification (left ordinate) and respiration (right ordinate) normalized to surface area (cm²). (B) Metabolic expenditure concurrent with calcification. Treatments significantly affected calcification ($P = 0.011$), with letters indicating treatments that differed as determined from *post hoc* analysis; there was no effect of treatment on respiration ($P = 0.697$) or metabolic expenditure concurrent with calcification ($P = 0.135$). Values displayed are mean $\pm$ SE.

on shallow reefs or simulate the intensity and spectral composition of sunlight reaching the study depth (Kinzie *et al.*, 1984). As the quality and quantity of light has important implications for photoautotrophic nutrition and calcification in corals (Kinzie *et al.*, 1984; Barnes and Chalker, 1990; Grottoli and Wellington, 1999) and affects the response of coral fragments (Suggett *et al.*, 2013) and coral recruits (Dufault *et al.*, 2013) to pCO₂, it is clear that light is one physical parameter that would benefit from closer attention. To address this limitation (and others) of laboratory-based experiments, here we emphasize the utility of simple *in situ* approaches (Chauvin *et al.*, 2011). Our *in situ* approach has

the potential to support tests for short-term biological effects of OA under physical conditions closely resembling those found on coral reefs, and may provide a means to evaluate effects of various light treatments (both UV and PAR) on coral calcification under ambient and elevated pCO₂.

The mean intensity of PAR adjacent to the incubation chambers at 1.5-m depth ($1072 \pm 51 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) was 2–10 times higher than irradiances reported in OA studies in which incubations have been conducted in laboratory mesocosms. For instance, Edmunds (2012) reported an intensity of $537 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, Schneider and Erez (2006) reported a light intensity of about $350 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, Crawley *et al.* (2010) employed a maximum irradiance of about $110 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, and Albright *et al.* (2008) used a mean light intensity of less than $10 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. However, a few OA experiments have been performed under natural irradiances in outdoor mesocosms on land (noon irradiances $700 \mu\text{mol}–1200 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$; Anthony *et al.*, 2008) and in flumes ($17 \text{ mol}–40 \text{ mol photons m}^{-2} \text{ d}^{-1}$; Langdon and Atkinson, 2005). In our study, not only was the light intensity greater than in most previous studies using corals from shallow reefs, but our incubations also included UV radiation, which is important for the rhythmicity of *Symbiodinium* photosynthesis *in hospite* (Sorek and Levy, 2012), coral bleaching (Lesser, 1997), and photooxidative stress in *Symbiodinium* (Shick *et al.*, 1995). Moreover, exposure to ecologically relevant intensities of UV radiation may favor slight reductions in calcification rates (Jokiel and York, 1982; Roth *et al.*, 1982) that are relevant to corals under natural conditions. While explicitly evaluating the role of UV radiation on coral calcification was beyond the scope of this study, we note that *in situ* chambers create the potential for such analyses in the future using UV-transparent and UV-absorbing polymers (Shick *et al.*, 1995; Gleason *et al.*, 2006). This would not be possible in the laboratory using metal-halide and LED lamps that do not emit significant radiation at less than 400 nm (Schubert and Kim, 2005), and instead would require specialized UV lights (Shick *et al.*, 1999). We suggest that our *in situ* approach could be used to examine OA effects on corals using light conditions (PAR and UV) of similar intensity and flux (*i.e.*, diel oscillations) to those experienced by corals on the reef. Striving for ecologically relevant light treatments should be a priority of OA research in the laboratory.

In addition to the advantages of being able to manipulate UV light to test for interactive effects with OA on corals, *in situ* chambers provide access to high intensities of PAR that are important to symbiotic corals for photosynthesis (Davies, 1991) and calcification (Barnes and Chalker, 1990; Anthony *et al.*, 2002; Allemand *et al.*, 2004). Light intensity also modulates the response of corals to OA (Marubini *et al.*, 2001; Suggett *et al.*, 2013), with recent work character-

izing a pCO_2 -dependent shift in the nonlinear relationship between light and calcification in recruits of *Pocillopora damicornis* (Dufault *et al.*, 2013). For these recruits, light intensity affected calcification rates with an effect at 50 versus 89 Pa pCO_2 at intermediate light intensities ($\sim 70 \mu\text{mol photons m}^{-2} \text{s}^{-1}$), but reduced effects at lower and higher light intensities ($31 \mu\text{mol}$ vs. $226 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) (Dufault *et al.*, 2013). It has been hypothesized that the effect of light intensity on the response of corals to OA is driven by a light-dependent utilization of HCO_3^- that stimulates calcification and reduces the negative implications of decreasing $[\text{CO}_3^{2-}]$ at low pH (Gattuso *et al.*, 1999; Comeau *et al.*, 2012). The high rates of calcification in Treatment 3 (HCO_3^- -enriched) of the present study lend support to this possibility, particularly since ecologically relevant high light intensities were attained with our *in situ* design.

Few studies have reported hourly calcification rates for massive *Porites* spp., and no study has attempted to measure *in situ* calcification under pCO_2 enrichment and ambient light intensities. As a result, there are no comparable data with which our results can be contrasted for the purpose of testing for generality. However, hourly calcification rates recorded in the light during the present study are within the range reported for *Porites* (Comeau *et al.*, 2013a), and when extrapolated to a 24-h day assuming calcification in the light is 3-fold greater than in the dark (Gattuso *et al.*, 1999) and each day consists of 12-h light and 12-h dark, daily calcification for juvenile *Porites* spp. is similar to that previously reported for this taxon in Moorea (Edmunds, 2011, 2012). Therefore, it is reasonable to interpret our results as ecologically relevant, with treatment effects that are insightful for understanding how corals respond to similar conditions on the reef.

The calcification rate of juvenile massive *Porites* spp. in the light under ambient conditions (e.g., Treatment 1) did not differ significantly from calcification under conditions mimicking those expected by the end of the present century (e.g., Treatment 2). The Treatment 2 result is inconsistent with studies showing that OA reduces coral calcification (Langdon and Atkinson, 2005; Schneider and Erez, 2006; Comeau *et al.*, 2013b), but is consistent with reports that *Porites* is resistant to OA (Fabricius *et al.*, 2011; Edmunds, 2012; Edmunds *et al.*, 2012; Comeau *et al.*, 2013a). However, interpreting Treatment 2 results relative to previous studies is complicated by the measurement here of calcification at high mid-day irradiances that probably would have maximized both calcification and photosynthesis. It remains possible that the present results might have differed during longer incubations lasting weeks or months that would also have captured the effects of reduced dark calcification and skeletal dissolution at low saturation of aragonite (Ω_{arag}).

In Treatment 3, HCO_3^- -enrichment ($2730 \mu\text{mol kg}^{-1}$) increased calcification 81% relative to ambient conditions,

despite coinciding with reduced Ω_{arag} and low pH. Stimulation of calcification in corals through elevated $[\text{HCO}_3^-]$ has been reported previously (Marubini and Thake, 1999; Herfort *et al.*, 2008; Comeau *et al.*, 2012); furthermore, under OA conditions, elevated $[\text{HCO}_3^-]$ increases coral calcification (Marubini *et al.*, 2008; Jury *et al.*, 2010; de Putron *et al.*, 2011; Comeau *et al.*, 2012). At constant pH_T , for example, calcification in *Madracis auretenra* declined with reduced $[\text{HCO}_3^-]$ but was stimulated with increased $[\text{HCO}_3^-]$, yet it was unresponsive to pH_T (7.6–8.1) and Ω_{arag} (1.7–4.3) (Jury *et al.*, 2010). HCO_3^- enrichment (2.0 mmol l^{-1} above ambient seawater) stimulated calcification in *Stylophora pistillata* at pH_{sw} 7.6, 8.0, and 8.2, suggesting that calcification may be carbon-limited in this species (Marubini *et al.*, 2008). While the exposure period for the treatments was short in the current study (28 h), short incubations ($<3 \text{ h}$) have been useful in past experiments examining the effects of nutrients and pCO_2 enrichment on corals (Furla *et al.*, 2000; Langdon and Atkinson, 2005; Chauvin *et al.*, 2011; Cumbo *et al.*, 2013). While the implications of the short incubations in the present study deserve attention, our finding nevertheless suggests that calcification in juvenile *Porites* spp. may not be DIC saturated *in situ* at ambient $[\text{HCO}_3^-]$.

In tropical corals, calcification requires substantial amounts of metabolic energy and may account for 13%–30% of the entire metabolic costs (Allemand *et al.*, 2011). These costs are met by the supply of ATP from aerobic respiration (with smaller amounts of ATP presumably from glycolysis), and therefore aerobic respiration is likely to reflect changes in the cost of calcification. The extent to which shifts in costs of calcification can be detected through measurements of aerobic respiration depends, however, on the magnitude of the changes involved and the degree to which the demands of other energy-requiring processes covary. In the present study, treatments had no effect on aerobic respiration, and we were unable to detect a change in the total metabolic costs concurrent with calcification, even though calcification increased 81% with elevated $[\text{HCO}_3^-]$. The biological meaning of the null effect of OA (Treatment 2) and HCO_3^- enrichment (Treatment 3) on coral respiration is uncertain, in part due to low statistical power to detect an effect of the treatment on this dependent variable.

Given the effect of $[\text{HCO}_3^-]$ on calcification in Treatment 3 and a mean metabolic cost of calcification of $1804 \text{ J g}^{-1} \text{ CaCO}_3$, aerobic respiration should have increased from 0.22 J to $0.41 \text{ J cm}^{-2} \text{ h}^{-1}$ to equal the metabolic costs scaled proportionately from the control costs (i.e., Treatment 1) associated with calcification (e.g., $\text{J g}^{-1} \text{ CaCO}_3$). In contrast to this prediction, the mean aerobic respiratory demand in Treatment 3 was indistinguishable from that of corals in other treatments, indicating that elevated $[\text{HCO}_3^-]$ stimulated calcification without affecting metabolic expen-

diture. Resolving the causes of this thermodynamic paradox (*i.e.*, the increase in a metabolically costly process without an increased demand for metabolic energy) was beyond the scope of this study, but we suspect it might reflect hyperoxic stimulation of respiration (Shick, 1990) after maximum photosynthesis in the control corals, which could conceal the additional costs associated with enhanced calcification. Alternatively, it is possible that respiration rates were mass-transfer-limited in the small respiration chambers employed, where we were only able to create a flow speed of 3.0 cm s^{-1} , which is lower than the 15.8 cm s^{-1} in the *in situ* chambers where calcification was measured. Notwithstanding these limitations, our results for the respiration of corals exposed to OA are at least inconsistent with the notion that the metabolic costs of calcification are elevated by high pCO_2 (Erez *et al.*, 2011).

With the important caveats that our short incubations were conducted in the natural environment where we cannot separate the effects of incubation duration from the effects of ecologically relevant physical conditions relative to previous studies, our results suggest that the respiration of massive *Porites* spp. is resistant to short-term exposure to OA conditions. Previously, using laboratory-based mesocosms, we have shown that respiration of juvenile *Porites* spp. is depressed 36% after longer exposures (11 d) to 87 Pa pCO_2 (but not to 77 Pa pCO_2), although, as reported here, the metabolic costs concurrent with calcification were unaffected by pCO_2 (Edmunds, 2012). Analyses of the effects of pCO_2 on the dark respiration of corals generally have provided equivocal results, with most studies reporting no effects at pCO_2 levels as high as 207 Pa (Reynaud *et al.*, 2003; Anthony *et al.*, 2008; Godinot *et al.*, 2011; S. Comeau, California State University Northridge, pers. comm.), one study reporting a trend for increased light-enhanced respiration with high pCO_2 (Crawley *et al.*, 2010), and a few studies reporting metabolic depression at 77–87 Pa pCO_2 (Edmunds, 2012; Edmunds *et al.*, 2013). Clearly there is scope for further research on the impacts of OA on coral respiration, specifically to resolve the effects of exposure duration, pCO_2 regime, and the assemblage of physical and chemical conditions that collectively determine the ecological relevancy of manipulative experiments. The completion of the necessary experiments *in situ* rather than *in vitro* will need to be an important part of this agenda and will likely be critical to advance a mechanistic appreciation of the effects of OA on marine organisms (Kelly and Hofmann, 2013). *In situ* chambers, such as the ones we use here, can play an important part in this effort.

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