

# Effects of diurnally oscillating $p\text{CO}_2$ on the calcification and survival of coral recruits

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Manipulative studies have demonstrated that ocean acidification (OA) is a threat to coral reefs, yet no experiments have employed diurnal variations in  $p\text{CO}_2$  that are ecologically relevant to many shallow reefs. Two experiments were conducted to test the response of coral recruits (less than 6 days old) to diurnally oscillating  $p\text{CO}_2$ ; one exposing recruits for 3 days to ambient (440  $\mu\text{atm}$ ), high (663  $\mu\text{atm}$ ) and diurnally oscillating  $p\text{CO}_2$  on a natural phase (420–596  $\mu\text{atm}$ ), and another exposing recruits for 6 days to ambient (456  $\mu\text{atm}$ ), high (837  $\mu\text{atm}$ ) and diurnally oscillating  $p\text{CO}_2$  on either a natural or a reverse phase (448–845  $\mu\text{atm}$ ). In experiment I, recruits exposed to natural-phased diurnally oscillating  $p\text{CO}_2$  grew 6–19% larger than those in ambient or high  $p\text{CO}_2$ . In experiment II, recruits in both high and natural-phased diurnally oscillating  $p\text{CO}_2$  grew 16 per cent larger than those at ambient  $p\text{CO}_2$ , and this was accompanied by 13–18% higher survivorship; the stimulatory effect on growth of oscillatory  $p\text{CO}_2$  was diminished by administering high  $p\text{CO}_2$  during the day (i.e. reverse-phased). These results demonstrate that coral recruits can benefit from ecologically relevant fluctuations in  $p\text{CO}_2$  and we hypothesize that the mechanism underlying this response is highly  $p\text{CO}_2$ -mediated, night-time storage of dissolved inorganic carbon that fuels daytime calcification.

**Keywords:** ocean acidification; calcification; diurnal; survivorship

## 1. INTRODUCTION

Ocean acidification (OA) arising from the dissolution of atmospheric  $\text{CO}_2$  in seawater is thought to be one of the most serious threats facing marine ecosystems [1–3], because most calcifying organisms deposit less  $\text{CaCO}_3$  at high  $p\text{CO}_2$  [4–6]. Some of the most striking examples of these effects are exhibited by scleractinian corals [7], yet despite the negative implications of these trends for coral reef ecosystems [8], progress has been slow in elucidating the mechanisms underlying the response of corals to high  $p\text{CO}_2$  [9–11].

Most evidence describing the effect of OA on corals has come from the exposure of adult colonies to seawater manipulated to create dissolved inorganic carbon (DIC) chemistries simulating the consequences of high  $p\text{CO}_2$  [8]. Most recently, attention has turned to early life stages of corals [12], because these may be more susceptible than adults to environmental challenges [13], and they play important roles in recruitment and population growth [14]. Early results from this effort show that larvae and newly recruiting corals can be affected negatively by OA [15–19]. An important advantage of studying early life stages is that they support an evaluation of the responses of environmentally naive organisms to novel conditions, thereby allowing the impacts of immediate conditions to be tested without the complexity of time-integrated effects that apply to studies of adults.

The experimental benefits of studying early life stages of corals are strong for calcification, for which a contrast of pelagic larvae and benthic recruits supports a contrast of uncalcified and calcified tissue.

To date, all experimental studies of the response of corals to OA have been accomplished through manipulations of pH or  $p\text{CO}_2$  in seawater, thereby creating steady levels of perturbed conditions [4]. While such studies have advanced understanding of the responses of corals to OA, they have overlooked natural variation in seawater  $p\text{CO}_2$ . The best-known example of this effect involves diurnal oscillations in  $p\text{CO}_2$  and pH in seawater flowing over shallow reefs [20–23], which can create changes between day and night of greater than 600  $\mu\text{atm}$   $p\text{CO}_2$  and pH 0.5 [22]. The magnitude of these oscillations is influenced first, by the benthic community structure, which drives DIC chemistry through metabolism and, second, by reef bathymetry, which determines the spatial extent of shallow habitats and the flux of seawater across them [22].

Oscillatory levels of physical and chemical conditions result in biological outcomes that can differ from those arising under stable conditions [24,25] and are more than the sum of exposure times to each extreme. For example, exposure of the corals *Pocillopora damicornis* and *Porites rus* to diurnally varying temperatures reduces their *Symbiodinium* content and dark-adapted quantum yield of PSII more than exposure to steady temperature perturbations similar in magnitude to those at the extremes of the diurnal treatment [26]. Similarly, exposure in tidal pools of *Acropora hyacinthus* to diurnally varying temperature increases their resistance to elevated

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thermal stress compared with corals in more homogeneous conditions [25]. Together, these results suggest that corals might respond to ecologically relevant natural oscillations in  $p\text{CO}_2$  in ways differing from those recorded under steady-state conditions.

The objective of this study was to test the response of newly settled coral recruits to diurnal oscillations in  $p\text{CO}_2$ . To achieve this objective, we settled brooded larvae of *Seriatopora caliendrum* onto artificial substrata and exposed the recruits to factorial combinations of high and ambient  $p\text{CO}_2$  in two series of experiments. Our treatments included diurnal oscillations of  $p\text{CO}_2$ , in which oscillatory exposure to high  $p\text{CO}_2$  was administered both at night (the natural phase) and day (a reverse phase) to gain insights into the interactive consequences of elevated  $p\text{CO}_2$  and daylight. The reversed-phase experiment was designed as the study progressed in order to explore processes potentially underlying the patterns that were emerging from the initial experiment.

## 2. METHODS

Larvae were obtained from the brooding coral *S. caliendrum*, which is a common branching coral on reefs along the southern coast of Taiwan and throughout the Indo-Pacific [27,28]. Colonies of *S. caliendrum* (approx. 20 cm in diameter) were collected from 5 to 7 m deep on Hobihu Reef, Nanwan Bay, in March and June of 2010, and transported to the National Museum of Marine Biology and Aquarium, where they were placed into individual flow-through seawater tanks and exposed to partially shaded sunlight at an intensity of  $131 \pm 13 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  (experiment I) or  $78 \pm 3 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  (experiment II; mean  $\pm$  s.e.m.). Overflow water from each tank passed through mesh-lined (110  $\mu\text{m}$ ) cups that captured larvae, which were released during the night [29]. Larvae were collected at 08.00, pooled across colonies and retained in a 1 l beaker until processed for the experiment.

Following collection, approximately 500 larvae were placed into plastic containers ( $12 \times 24 \times 12$  cm) at approximately 11.00, each of which was fitted with mesh windows (110  $\mu\text{m}$ ) to allow the passage of seawater, and floated in the same tanks containing the adult colonies. *Seriatopora caliendrum* larvae were settled onto clean pre-weighed glass microscope coverslips ( $22 \times 22 \text{ mm}^2$ , approximately 150  $\mu\text{m}$  thick). The coverslips allowed the size of the settled coral recruits to be assessed gravimetrically ( $\pm 0.0001 \text{ g}$ , Mettler-Toledo AX205). Six coverslips were placed in each container and the larvae left to settle for 24 h under a natural light–dark cycle. The following day greater than 90 per cent of larvae had settled on the coverslips, with up to 40 on each coverslip. The swimming larvae remaining were discarded, and coverslips with coral recruits ( $n = 18$ : experiment I;  $n = 36$ : experiment II) were assigned randomly to the  $p\text{CO}_2$  treatments.

In experiment I, treatments consisted of steady ambient  $p\text{CO}_2$ , steady high  $p\text{CO}_2$  and diurnally oscillating  $p\text{CO}_2$  on a natural phase (table 1); this design was augmented in experiment II by including a diurnally oscillating  $p\text{CO}_2$  on a reverse phase. The oscillatory treatments were created by moving the plastic container containing the coverslips (and attached recruits) between high and ambient  $p\text{CO}_2$  with the transfers accomplished at dawn and dusk (07.00 and 19.00 under the laboratory conditions) without exposing

Table 1. Seawater DIC chemistry in experiment I (March, 3 days duration) and experiment II (June, 6 days duration) based on samples collected daily in experiment I, and every other day in experiment II. Reverse-phase diurnally oscillating  $p\text{CO}_2$  in experiment II was identical to the natural phase. Values are means  $\pm$  s.e.m.,  $n = 3$  in experiment I and  $n = 4$  in experiment II.

experiment	$p\text{CO}_2$ treatment	TA ( $\mu\text{mol kgSW}^{-1}$ )	pH <sub>T</sub>	HCO <sub>3</sub> <sup>-</sup> ( $\mu\text{mol kgSW}^{-1}$ )	CO <sub>3</sub> <sup>2-</sup> ( $\mu\text{mol kgSW}^{-1}$ )	$\Omega_A$	$p\text{CO}_2$ ( $\mu\text{atm}$ )
experiment I	ambient	2160 $\pm$ 16	8.00 $\pm$ 0.05	1712.7 $\pm$ 55	180.4 $\pm$ 17.4	2.9 $\pm$ 0.3	456.0 $\pm$ 71.3
	natural-phase ambient	2193 $\pm$ 64	8.02 $\pm$ 0.03	1710.7 $\pm$ 35	195.1 $\pm$ 16.4	3.1 $\pm$ 0.3	420.4 $\pm$ 38.0
	high	2178 $\pm$ 26	7.88 $\pm$ 0.02	1813.8 $\pm$ 31	147.5 $\pm$ 7.8	2.4 $\pm$ 0.1	623.6 $\pm$ 47.2
experiment II	natural-phase high	2234 $\pm$ 15	7.90 $\pm$ 0.04	1843.5 $\pm$ 33	158.8 $\pm$ 11.5	2.5 $\pm$ 0.2	596.8 $\pm$ 57.3
	ambient	2187 $\pm$ 17	7.98 $\pm$ 0.01	1771 $\pm$ 17	169.8 $\pm$ 3.4	2.7 $\pm$ 0.1	464.0 $\pm$ 14.1
	natural-phase ambient	2211 $\pm$ 16	8.00 $\pm$ 0.01	1774 $\pm$ 11	178.6 $\pm$ 3.9	2.9 $\pm$ 0.1	448.2 $\pm$ 10.6
	high	2213 $\pm$ 19	7.77 $\pm$ 0.00	1932 $\pm$ 16	115.0 $\pm$ 1.6	1.9 $\pm$ 0.0	830.3 $\pm$ 11.2
	natural-phase high	2187 $\pm$ 15	7.76 $\pm$ 0.00	1915 $\pm$ 13	111.4 $\pm$ 1.4	1.8 $\pm$ 0.0	845.0 $\pm$ 8.0

the recruits to air. Containers in ambient and high  $p\text{CO}_2$  were also removed from treatment tanks in a similar fashion to control for movement in and out of tanks. In the diurnally oscillating  $p\text{CO}_2$  on a natural phase, high  $p\text{CO}_2$  was administered at night, and ambient  $p\text{CO}_2$  during the day, but in the diurnally oscillating  $p\text{CO}_2$  on a reverse phase, this sequence was reversed. The diurnally oscillating  $p\text{CO}_2$  on a natural phase mimicked the cycle of  $p\text{CO}_2$  in seawater passing over shallow reefs [23], and the diurnally oscillating  $p\text{CO}_2$  on a reverse phase was used to test coral calcification under novel conditions.

The  $p\text{CO}_2$  treatments were created in eight 30 l aquaria, with two maintained at ambient  $p\text{CO}_2$ , two at high  $p\text{CO}_2$  and two pairs of tanks retained at ambient and high  $p\text{CO}_2$  and used to create the oscillatory  $p\text{CO}_2$  treatment. All tanks were independently heated (Taikong Corporation), chilled (Aquatech Ac11) and mixed (Rio 1110 pumps) to generate temperatures of  $25.4 \pm 0.4^\circ\text{C}$  in March (experiment I,  $n = 128$ ) and  $25.1 \pm 0.2^\circ\text{C}$  in June (mean  $\pm$  s.e.m., experiment II  $n = 136$ ). Ambient seawater temperature was  $25.5 \pm 0.2^\circ\text{C}$  in March, and although it was warmer in June ( $27.8 \pm 0.2^\circ\text{C}$ ), the same incubation temperature was selected to strengthen the direct contrast between the two experiments. The tanks were filled with filtered seawater (1  $\mu\text{m}$ ), and the seawater was changed partially (10–15% volume) every night. All tanks were illuminated from 07.00 to 19.00 with lamps fitted with a metal halide bulb (Phillips 150 W 10 000 k) and two 39 W fluorescent bulbs (Phillips T5 460 nm) that provided light at an intensity of  $179 \pm 2 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  (mean  $\pm$  s.e.m.,  $n = 64$ ) in experiment I, and  $305 \pm 20 \mu\text{mol quanta}$  in experiment II ( $n = 120$ ; measured with an LI-192 sensor, LI-COR Biosciences, Lincoln, Nebraska).

#### (a) Experimental $p\text{CO}_2$ manipulation

The DIC content of seawater was manipulated by bubbling premixed gas of a known  $p\text{CO}_2$  or by bubbling unmodified air for the ambient treatment. To mix the gas for the high  $p\text{CO}_2$  treatments, a system employing a variable-timed solenoid valve was used, which controlled the flow of air and  $\text{CO}_2$  into a mixing chamber to reach the target  $p\text{CO}_2$  of 650  $\mu\text{atm}$  in experiment I. This target value was selected to provide a conservative estimate for the atmospheric  $p\text{CO}_2$  by 2100 following the business as usual emission scenario A1 [30]. In experiment II,  $p\text{CO}_2$  levels were increased to 800  $\mu\text{atm}$  to test the effects of diurnally oscillating  $p\text{CO}_2$  levels that exceeded what is found at Hobihu reef. The solenoid valve was connected to an infrared gas analyser (S151, Qubit Systems, Ontario Canada), which monitored the output gas and provided dynamic control of the duty cycle of the solenoid, thereby providing a consistent concentration of mixed gas to the treatment tanks. Refer to the electronic supplementary material for seawater chemistry and field sampling methods.

#### (b) Growth

Upon completion of the experiments, coverslips with coral recruits were placed in bleach (6% NaOCl) for 8 h to dissolve the tissue on the small corals and leave behind the  $\text{CaCO}_3$  skeleton. Coverslips were then rinsed with deionized water to remove the bleach and air-dried for 24 h at approximately  $27^\circ\text{C}$ . Calcification was measured using the summed weight of the  $\text{CaCO}_3$  deposited by recruits on each coverslip and also as the planar area of the basal plate of each recruit.

Coverslips without recruits but subjected to identical treatments served as procedural controls, and these did not change in weight in either experiment ( $t$ -test, experiment I,  $t = 2.80$ , d.f. = 11,  $p = 0.99$ ; experiment II,  $t = 0.48$ , d.f. = 3,  $p = 0.64$ ). In experiment I, the change in weight of each coverslip was divided by the number of corallites to provide a mean weight that was used as a statistical replicate. As some (approx. 5%) recruits died during the experiment, this technique slightly underestimated calcification. To remove this bias in experiment II, only recruits alive at the end of the experiment were used for growth measurements.

To measure the area of the recruits, bleached and dried corallites were photographed (Nikon Coolpix 4500, 4.0 megapixel resolution) through a compound microscope (Zeiss Axiolab E, 40 $\times$  magnification). Images were analysed using the IMAGE J software (Wayne Rasband, v. 1.42q), with approximately 65 corallites in each treatment measured for experiment I, and approximately 33 in each treatment for experiment II. To obtain an unambiguous indication of area, only corallites that were not touching neighbouring corallites were selected for analysis, and each corallite was treated as a statistical replicate.

#### (c) Survivorship

To evaluate survivorship during experiment II, recruits were photographed (Canon 40D, 10 megapixel resolution) every 2 days throughout the 6-day experiment. Images were used to score the recruits as alive or dead based on the presence of tissue, which is easily discernable from photographs. Survivorship was not measured in experiment I owing to logistical constraints.

#### (d) Statistical analysis

The weight and area of corallites were analysed with a nested ANOVA in which  $p\text{CO}_2$  treatment was the independent variable and replicate tanks were nested within treatments. To satisfy the normality assumptions of ANOVA, calcification on the weight scale in experiment I was fourth power transformed [31], to correct for positive skewing that was created by a small number of unusually heavy corallites. All other data met the homogeneity of variances (Levene's test) and normality assumptions of ANOVA (Shapiro–Wilks test). When tank effects were not significant ( $p > 0.05$ , which was true for all analyses), they were removed from the statistical model when the probability for this effect was greater than 0.25 [32] and the analysis completed using corals as independent replicates. While this does not avoid pseudoreplication, the demonstration of statistically identical results in independent tanks makes it unlikely that results are an artefact of dependence among corals. *Post hoc* analyses of calcification data were completed using a Tukey's test. To test for differences between natural- and reversed-phase diurnal  $p\text{CO}_2$ , a Bonferroni-adjusted one-tailed  $t$ -test ( $\alpha = 0.05$ ) was performed to test the hypotheses that any stimulatory effect of natural-phase diurnal  $p\text{CO}_2$  on calcification for both weight and area was diminished in the reverse phase.

To analyse survivorship in experiment II, a Kaplan–Meier (KM) product-limit analysis was used [33]. For this analysis, the probability of individual recruits surviving is assumed to be independent of all other recruits, and because KM analyses cannot accommodate nested experimental designs, replicate corallites were pooled within each treatment. Survival was analysed using the statistical program JMP

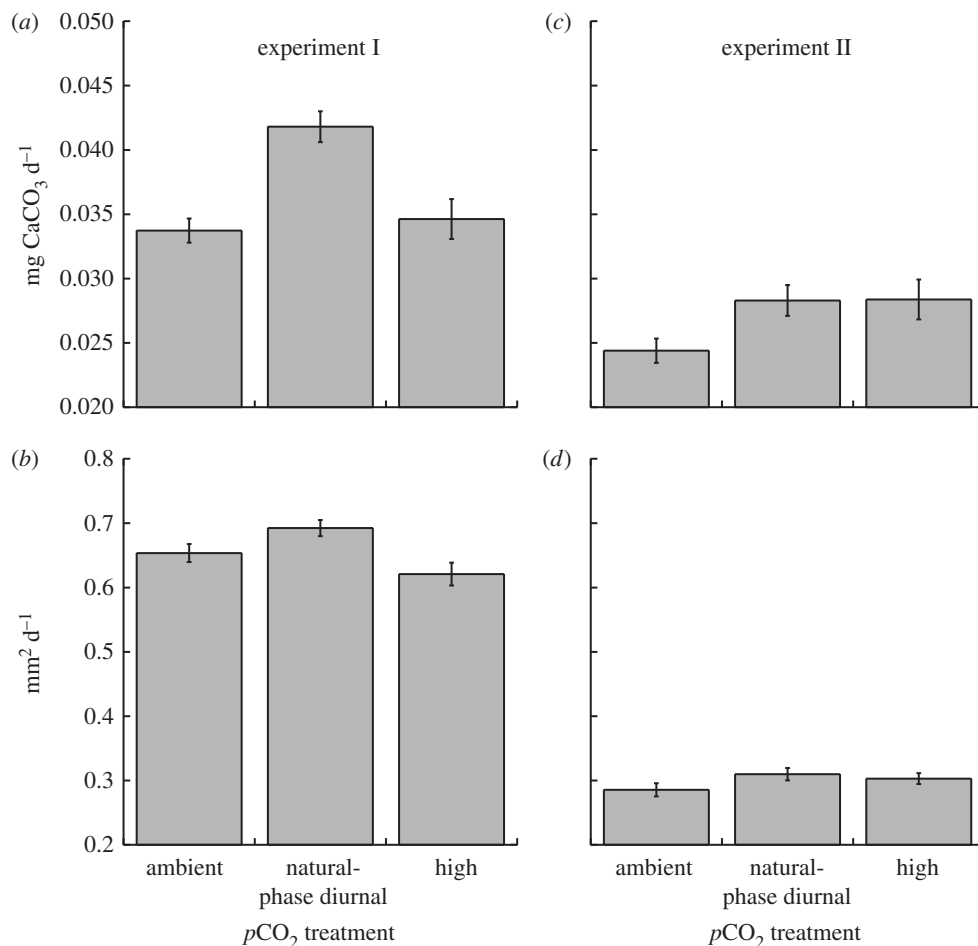


Figure 1. Calcification of *Seriatopora caliendrum* recruits in ambient, high and diurnally oscillating  $p\text{CO}_2$  treatments during experiments I and II. Calcification was measured by (a,c) weight and (b,d) area. Mean  $\pm$  s.e.m. displayed (weight  $n = 6\text{--}12$ , area  $n = 30\text{--}79$ ).

(v. 9.0.2, 2010, SAS Institute Inc.) and a log-rank test was used to test for differential survival among treatments.

### 3. RESULTS

#### (a) Treatment conditions

Experimental  $p\text{CO}_2$  conditions in experiment I (420–597  $\mu\text{atm } p\text{CO}_2$ , table 1) mimicked the conditions found at 6 m depth on Hobihu reef (365–515  $\mu\text{atm}$ , electronic supplementary material, figure S1), where parent colonies were collected. In experiment II, the  $p\text{CO}_2$  range was expanded (448–845  $\mu\text{atm } p\text{CO}_2$ ) to more closely match the experimental conditions to those occurring on shallow reefs [22].

#### (b) Growth

*Seriatopora caliendrum* larvae settled rapidly onto microscope coverslips at mean densities of  $3.4 \pm 1.5$  recruits  $\text{cm}^{-2}$  ( $\pm$  s.e.m.,  $n = 18$ ) in experiment I, and at  $6.3 \pm 2.2$  recruits  $\text{cm}^{-2}$  ( $\pm$  s.e.m.,  $n = 36$ ) in experiment II. Recruits began calcifying shortly after settlement, with septa and a basal plate visible within 24 h. In experiment I, single corallites weighed between 60 and 130  $\mu\text{g}$  with areas between 1.1 and 2.8  $\text{mm}^2$  after 3 days; in experiment II, corallites weighed between 110 and 210  $\mu\text{g}$  with areas ranging 1.0–3.0  $\text{mm}^2$  after 6 days. When standardized by time, the mean growth of recruits across all treatments

was 26 per cent slower in experiment II compared with experiment I (figure 1,  $t = 5.86$ , d.f. = 25,  $p < 0.001$ ).

In experiment I, the weight of *S. caliendrum* recruits was unaffected by the nested tank effect ( $p = 0.823$ ), which was therefore dropped from the analysis; weight was affected significantly by  $p\text{CO}_2$  treatments ( $F_{2,17} = 5.155$ ,  $p = 0.020$ ). While the area of the recruits was unaffected by tank ( $p = 0.080$ ), this effect could not be excluded from the analysis with the criterion applied ( $p > 0.25$ ). Area was not affected by  $p\text{CO}_2$  treatments when tanks were retained in the analysis ( $F_{2,3} = 2.832$ ,  $p = 0.204$ ), but when the tank effect was dropped based on a more liberal criterion (i.e.  $p > 0.05$ ),  $p\text{CO}_2$  treatments affected corallite area ( $F_{2,199} = 6.080$ ,  $p = 0.003$ ). For both weight and area, the highest growth occurred under diurnally oscillating  $p\text{CO}_2$  on a natural phase (figure 1). The increased growth under diurnally oscillating  $p\text{CO}_2$  on a natural phase amounted to a 17–19% increase by weight, and 6–10% larger area relative to recruits in the steady 456 and 624  $\mu\text{atm } p\text{CO}_2$  conditions; growth on weight and area scales were similar between steady 456 and 624  $\mu\text{atm } p\text{CO}_2$  (figure 1a,b).

In experiment II, the weight of corallites was again unaffected by the nested tank effect ( $p = 0.60$ ), which was dropped from the analysis; weight was affected significantly by  $p\text{CO}_2$  treatments ( $F_{2,30} = 3.899$ ,  $p = 0.032$ , figure 1c). Relative to steady ambient  $p\text{CO}_2$ , weight was elevated 16 per cent under both diurnally

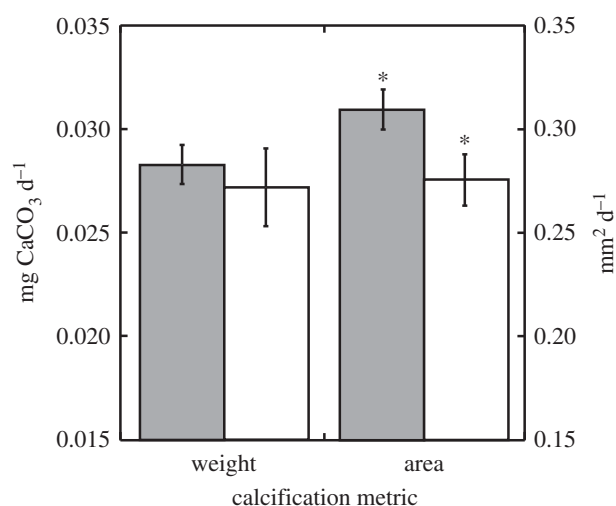


Figure 2. Calcification of *S. caliendrum* recruits in experiment II under natural-phase diurnal (grey bars) and reverse-phase diurnal  $p\text{CO}_2$  (white bars) treatments using weight (left ordinate) and area (right ordinate) scales. Significant paired  $t$ -tests between  $p\text{CO}_2$  treatments ( $p < 0.05$ ) denoted by an asterisk. Mean  $\pm$  s.e.m. displayed (weight:  $n = 32\text{--}36$ , area:  $n = 11\text{--}12$ ).

oscillating  $p\text{CO}_2$  on a natural phase and high  $p\text{CO}_2$ . The area of the corallites was unaffected by the tank effect ( $p = 0.494$ ), which was dropped from the analysis; area was unaffected by the  $p\text{CO}_2$  treatments ( $F_{2,99} = 1.786$ ,  $p = 0.173$ , figure 1d).

Coral recruits exposed to the diurnally oscillating  $p\text{CO}_2$  on a reverse phase in experiment II differed in area from those grown under diurnally oscillating  $p\text{CO}_2$  on the natural phase ( $t = 2.16$ , d.f. = 60,  $p = 0.018$ ). There was an 11 per cent reduction in mean recruit area in the diurnally oscillating  $p\text{CO}_2$  on a reserve phase relative to recruits in the natural phase. The weight of recruits did not differ between  $p\text{CO}_2$  regimes oscillating on either a reverse or natural phase ( $t = 0.49$ , d.f. = 17,  $p = 0.315$ , figure 2).

### (c) Survivorship

At the start of experiment II during which survivorship was measured, there were 331 recruits in the ambient  $p\text{CO}_2$  treatment, 138 in the high  $p\text{CO}_2$  treatment, 354 in the natural-phase diurnal  $p\text{CO}_2$  treatment and 402 in the reversed-phase diurnal  $p\text{CO}_2$  treatment. These recruits were scattered among a varying number of coverslips in each treatment, with a mean of  $30.1 \pm 1.7$  ( $\pm$  s.e.m.,  $n = 42$ ) recruits per coverslip. At the end of the 6-day experiment, there were 239 (72%) survivors in ambient  $p\text{CO}_2$ , 93 (67%) in the high  $p\text{CO}_2$ , 301 (85%) in the natural-phase diurnal  $p\text{CO}_2$  and 221 (55%) in the reverse-phase diurnal  $p\text{CO}_2$  (figure 3). Survivorship differed significantly among treatments ( $\chi^2 = 107$ , d.f. = 3,  $p < 0.001$ ) with the highest survival in the natural-phase diurnal  $p\text{CO}_2$  treatment.

## 4. DISCUSSION

Our experiments show for the first time that diurnally oscillating  $p\text{CO}_2$  can increase calcification and survival in coral recruits that are less than or equal to 6 days old. Despite the growing literature addressing the

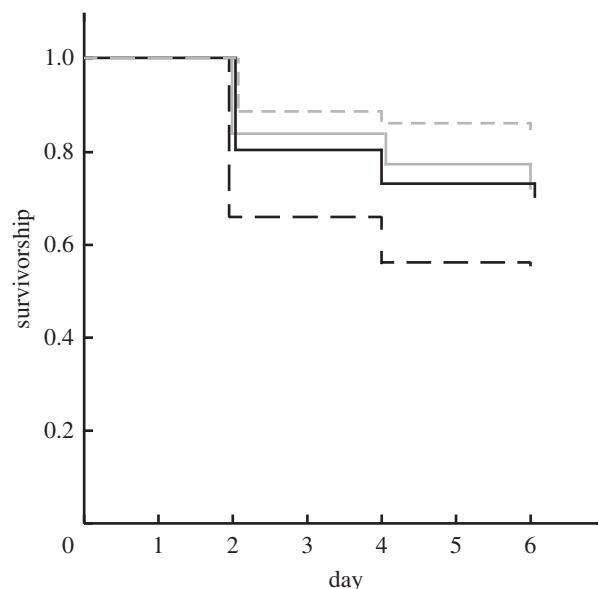


Figure 3. Survivorship of *S. caliendrum* recruits during experiment II as calculated by Kaplan–Meier product-limit analysis for ambient  $p\text{CO}_2$ , high  $p\text{CO}_2$ , diurnal  $p\text{CO}_2$  and reverse-diurnal  $p\text{CO}_2$  treatments. The numbers of recruits at the start of the experiment in each treatment are shown in the legend. Grey solid line, ambient  $p\text{CO}_2$ ,  $n = 331$ ; black solid line, high  $p\text{CO}_2$ ,  $n = 138$ ; grey dashed line, natural-phase diurnal  $p\text{CO}_2$ ,  $n = 354$ ; black dashed line, reverse-phase diurnal  $p\text{CO}_2$ ,  $n = 402$ .

response of corals to OA [7,8,12], to our knowledge, only one previous study has employed a measure of fitness as a response variable for corals exposed to high  $p\text{CO}_2$  [34]; this earlier work demonstrated the survivorship of *Porites panamensis* larvae was unaffected by 861–950  $\mu\text{atm}$   $p\text{CO}_2$ . Additionally, the impacts on corals of diurnally oscillating  $p\text{CO}_2$  with a natural-phase relationship—as occurs routinely on shallow reefs [20–23]—has not previously been considered. Instead, studies of the effects of OA on corals have employed steady DIC regimes mimicking those expected in open oceans under increased atmospheric  $p\text{CO}_2$  [8], and only recently have the limitations of these scenarios for the conditions in near-shore habitats been recognized [20]. Null or stimulatory effects of high  $p\text{CO}_2$  on coral calcification, similar to those reported here, have been reported for at least three species of corals studied as adult colonies. Anthony *et al.* [35] reported calcification of adult *Porites lobata* increased approximately 23 per cent under 520–700 ppm  $p\text{CO}_2$  at 28–29°C, Jury *et al.* [10] reported calcification in *Madracis auretenra* remained unaffected or increased up to 21 per cent when exposed to 876, 1406, 1480 ppm  $p\text{CO}_2$  at 27–28°C and Edmunds [36] reported area-normalized calcification in massive *Porites* spp. was unaffected by 815  $\mu\text{atm}$   $p\text{CO}_2$ . In the present study, we show that newly settled coral recruits grown for 3–6 days in diurnally oscillating  $p\text{CO}_2$  with a natural-phase relationship are fitter than their counterparts in static treatments of either ambient or elevated  $p\text{CO}_2$ .

Most scleractinian corals that have been studied respond to elevated  $p\text{CO}_2$  with decreased calcification [8], although a few exceptions to this trend have been found [10,35,36]. The present results contribute to these exceptions, with calcification of newly settled

*S. caliendrum* recruits elevated by weight (relative to ambient  $p\text{CO}_2$ ) in both diurnally oscillating  $p\text{CO}_2$  on a natural phase, and high  $p\text{CO}_2$  treatments. The stimulatory effects on calcification of diurnally oscillating  $p\text{CO}_2$  on the natural phase were more pronounced in experiment I compared with experiment II, where calcification decreased in all  $p\text{CO}_2$  treatments. The decreased calcification in experiment II was probably caused by the approximately  $2.7^\circ\text{C}$  decrease in temperature between ambient conditions ( $27.8^\circ\text{C}$ , when the research was conducted) and those chosen (i.e.  $25.1^\circ\text{C}$ ) to facilitate a contrast with experiment I. Furthermore, stimulatory effects on calcification of  $p\text{CO}_2$  as high as  $845\ \mu\text{atm}$  have not been observed in coral recruits, and instead the calcification of coral recruits (less than 50 days old and based on seven species) has been reported to decrease up to 84 per cent when exposed to  $p\text{CO}_2$  ranging from 560 to  $3585\ \mu\text{atm}$  [12]. Recruits of *Porites astreoides*, *Favia fragum* and *P. panamensis* departed somewhat from this trend, with calcification in *P. astreoides* and *F. fragum* remaining constant until  $p\text{CO}_2$  was greater than approximately  $800\text{--}850\ \mu\text{atm}$  (calculated from de Putron *et al.* [18]), and calcification in *P. panamensis* declining only 3 per cent at a  $p\text{CO}_2$  of  $926\ \mu\text{atm}$  [34]. It is important to note that corallite size determined from planar area or skeletal mass (as used in the present study and several others [16,18,34]) do not reflect the presence of biomass, which exerts a strong biological control on the process of mineralization [37] that can be altered to beneficial effect under OA conditions [36]. Thus, most contemporary studies of the effects of OA on coral recruits are limited in their capacity to ascribe process to the experimental outcomes and, likewise, here we are unable to determine the extent to which biomass contributed to the increased calcification in diurnally oscillating  $p\text{CO}_2$  on a natural phase, and high  $p\text{CO}_2$ .

While studies of the effects of OA on coral calcification are beginning to be published in large numbers, little has been done to determine the effects on fitness traits such as survivorship, fecundity and fertilization. There is evidence, however, that fertilization success in broadcast spawning corals declines with increased  $p\text{CO}_2$ , especially at low-sperm concentrations [38], although survival of coral recruits has been found unresponsive to high  $p\text{CO}_2$  [34]. In the present study, there were striking differences in the survival of *S. caliendrum* recruits, with the highest 6-day survivorship (85%) occurring in conditions which most closely mimic those on shallow reefs (diurnally oscillating  $p\text{CO}_2$  on a natural phase). Survivorship decreased to 67–72% in static 464 and  $830\ \mu\text{atm } p\text{CO}_2$ , and was only 55 per cent in the diurnally oscillating  $p\text{CO}_2$  on a reverse phase. For very young corals (i.e. colonies less than 2 cm), *in situ* survival is size-dependent [39], but this effect is unlikely to account for the present survivorship results, which were associated with only minor differences in the size of recruits in experiment II. Since our treatments did not result in a strong effect on the size of recruits, we infer that the treatment conditions acted directly on physiological processes closely related to organismic success. These processes may include the use of phenotypic plasticity, which presumably served to translate differential mass accretion into corallites of similar size. Increased light-enhanced dark respiration caused by high  $p\text{CO}_2$ , similar

to that recorded in adult *Acropora formosa* [40], could represent one physiological process affecting the survival of recruits by favouring the creation of an energetic deficiency.

While it was beyond the scope of this study to identify the mechanism underlying the stimulatory effects on calcification and survival of diurnally oscillating  $p\text{CO}_2$  on a natural phase, our results are consistent with at least two testable hypotheses. These focus on the extent to which calcification over 24 h is stimulated by low  $p\text{CO}_2$  during daylight, or high  $p\text{CO}_2$  at night. In the first hypothesis, low daytime  $p\text{CO}_2$  (with high night-time  $p\text{CO}_2$ ) could stimulate calcification directly by providing access to high concentrations of  $\text{HCO}_3^-$  (assuming this form of DIC is used in coral calcification [41]), or indirectly by promoting photosynthesis of *Symbiodinium* through the release of protons, which occurs during calcification [42]. We do not believe, however, that these mechanisms are in strong agreement with our results—even though there is a rich research history coupling high rates of photosynthesis and calcification in reef corals [11]—because both predict that calcification under natural-phase  $p\text{CO}_2$  oscillations should equal that under ambient (i.e. low)  $p\text{CO}_2$ . Our findings do not support this outcome, and instead we suggest they support a second hypothesis, that high night-time  $p\text{CO}_2$  leads to enhanced daytime calcification at a time when calcification (and perhaps photosynthesis) might otherwise become DIC limited. According to this hypothesis, nocturnal increases in seawater  $p\text{CO}_2$  favours the accumulation of DIC ( $\text{HCO}_3^-$ ,  $\text{CO}_2$ ) in the coral tissue at night through increased flux of DIC [43]. In turn, this leads to the creation of an intracellular DIC pool that could support calcification during the early part of the following day. In the present study, coral recruits in diurnally oscillating  $p\text{CO}_2$  on a natural phase were exposed to elevated DIC mostly in the form of  $\text{HCO}_3^-$  at night (table 1), which is thought to be used as the primary DIC source for calcification and photosynthesis in corals during the day [10,41,44–45]. During night,  $\text{HCO}_3^-$  might then be sequestered in the coral tissue.

In the coral *Stylophora pistillata*, the DIC pool equilibrates with surrounding seawater after approximately 3 h of calcification [11] (adapted from Furla *et al.* [43]), indicating active DIC storage is possible. Short-term storage of DIC would benefit corals if calcification becomes DIC-limited during periods of light-enhanced calcification [10,41,45], and potentially could sustain calcification until it became depleted. One means by which corals could store DIC is through storage in the cytosol, with the effect mediated by carbonic anhydrase (CA) in the oral ectoderm, which transports DIC from seawater into coral tissues [46]. Transport into coral tissue could be achieved by converting  $\text{HCO}_3^-$  to  $\text{CO}_2$  for passive transport across cell membranes, and then converting it back to  $\text{HCO}_3^-$  in the ectoderm to be later transferred throughout the coral for photosynthesis or calcification [46]. The hypothesized role of night-time DIC storage to explain stimulation of calcification in diurnally oscillating  $p\text{CO}_2$  on a natural phase also has the potential to account for the erosion of this effect in diurnally oscillating  $p\text{CO}_2$  on a reverse phase. Corals in this reverse-phase treatment calcified less than those in the natural-phase treatment, and this supports the ‘DIC

storage hypothesis' as these corals were not subjected to increased DIC at night, instead being subjected to ambient DIC.

Several other lines of evidence that allude to different mechanisms of daytime versus night-time calcification [43,47,48] may provide an alternative explanation for the physiological benefits described in the present study of diurnally oscillating  $p\text{CO}_2$  on coral calcification. To test the validity of the 'DIC storage hypothesis' versus alternative hypotheses (i.e. multiple calcification mechanisms and/or changes in photosynthesis), it might be productive to expose corals to combinations of labelled DIC in the form of either carbonate or bicarbonate over the course of several diurnal cycles and determine the incorporation of labelled DIC into host tissues and skeleton under ambient, high and natural-phased diurnally oscillating  $p\text{CO}_2$ . Such an experiment would reveal if there was a diurnal preference for DIC species, as well as DIC storage, which could support enhanced calcification in night or early morning as proposed above. Alternatively, measuring the net productivity of coral recruit's *Symbiodinium* sp. could help determine what role changes in photosynthesis may contribute to the observed calcification trends. Identifying the extent to which newly recruited juvenile and adult corals are able to benefit from diurnally oscillating  $p\text{CO}_2$  will play an important role in understanding the complexities of coral responses to ocean acidification and climate change.

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