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Prior exposure to elevated pCO_2 does not affect calcification of a tropical scleractinian when returned to ambient pCO_2

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ABSTRACT

Coral reefs experience biologically-driven pCO_2 oscillations that are predicted to become more extreme in magnitude and duration under ocean acidification (OA) regimes. Understanding the plasticity of responses in common reef-building corals to oscillations in pCO_2 will allow for better predictions of their function in future seawater conditions. This study explored the effects of variation in seawater pCO_2 on coral calcification using experiments conducted over one month between 9 April 2018 and 18 May 2018. Branches (~4-cm long) of *Acropora retusa* were sampled from colonies at 10-m depth on the fore reef of Mo'orea, French Polynesia (17° 28′ 53.9004" S, 149° 49′ 50.5992" W). We tested the hypothesis that depressed calcification caused by elevated pCO_2 (~1000 µatm) is relaxed (i.e., calcification increases) upon return to ambient pCO_2 (~400 µatm). Corals first were incubated in ambient or elevated pCO_2 for 19 days, with the result that calcification integrated over this period was reduced by 31% under elevated pCO_2. The same corals were then incubated at ambient pCO_2 for 11 days, during which calcification was independent of the experimental pCO_2 exposure history. Our results suggest that a quick relaxation of pCO_2-depressed calcification in *A. retusa* following cessation of high pCO_2 indicates that corals are capable of a reversible plastic response of calcification when confronted by pCO_2 oscillations.

1. Introduction

Ocean acidification (OA) is projected to approximately double pCO_2 in seawater relative to current levels by 2100 under the scenario described by Representative Concentration Pathway (RCP) 8.5 (IPCC, 2014). This translates to a depression of the pH of surface seawater by a further ~0.3 units, and a reduction of the concentration of carbonate ions [CO_3^{2-}] and the saturation state (Ω) of calcium carbonate [as aragonite (Ω_{arag}) and calcite (Ω_{ca})]. These trends ecologically are problematic in reducing rates of biogenic calcification worldwide including, for example, by crustaceans (Arnold et al., 2009; Walther et al., 2010; Courtney et al., 2013), molluscs (Orr et al., 2005; Ries et al., 2009b), echinoderms (Doo et al., 2012; Byrne et al., 2013), and calcifying algae (Martin and Gattuso, 2009).

The tendency for OA to reduce biogenic calcification is of great concern for tropical scleractinian corals, for which rapid calcification is of central importance to their role as ecosystem engineers (Wild et al., 2011). Reef-building corals provide the framework that numerous other species use as habitat to alleviate the risks of predation and competition (Huber and Coles, 1986), and enhance resource acquisition (Beukers and Jones, 1997). The stringency of OA conditions projected for the end of the 21st century under RCP 8.5 (IPCC, 2014) likely will impair the ecosystem engineering function of scleractinians, because calcification of most corals may decline as seawater pCO_2 rises above ~450 µatm (Erez et al., 2011). Despite great attention to the effects of OA on coral reefs, much of the current understanding of OA is based on laboratory experiments that implement treatment levels that are predicted to be characteristic of oligotrophic open oceans. In oligotrophic open oceans, seawater pCO_2 can vary by ~25 µatm over a month (calculated from Hofmann et al., 2011, assuming pH_t range of 0.024, seawater temperature of 27 °C, salinity of 35, and a total alkalinity of 2380 µmol kg^{-1}). Although OA experiments completed under stable pCO_2 conditions have played important roles in understanding the implications of OA for tropical reefs, accurate projections of the biogenic effects of OA require close attention to ecological relevance and diel (and longer) variation in ambient pH (McElhany and Busch, 2013).

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In contrast to the oligotrophic open ocean, near-shore coral reefs may experience seawater pCO_2 that exhibits diel variability of ~236 μatm (calculated from Page et al., 2017, assuming pH_t daily maximum and minimum of 7.99 and 7.84 units, seawater temperature of 27 °C, salinity of 35, and total alkalinity values of 2100 and 2168 μmol kg^{-1} that approximately correspond to pH_t values previously described). Community metabolism (i.e., calcification, photosynthesis, and respiration) of coral reefs largely is responsible for these diel oscillations, and also drives seasonal variation in seawater pCO_2 (Kayanne et al., 2005). Such seasonal effects can arise from changes in the abundance of macroalgae (Anthony et al., 2011), or through variation in the metabolism of macroalgae and corals driven by temperature and light (Bates, 2002; Falter et al., 2012). Because the calcification, photosynthesis, and respiration of coral reefs, and the interactions among these processes, are influenced by light, which varies in quantity on multiple spatio-temporal scales, the feedback between community metabolism and seawater chemistry creates oscillations in seawater pCO_2. The amplitude and periodicity of these oscillations can also be affected by changes in cloud cover, transitions from day to night, hydrodynamic variability (e.g. offshore wave height and reef flat length are inversely related to variation in carbonate chemistry), and input of solar radiation into the ocean that varies among seasons (Falter et al., 2012, 2013).

Under the pessimistic atmospheric pCO_2 projection of RCP 8.5 (~1000 μatm by 2100) (IPCC, 2014), exposure of coral reefs to natural oscillations in pCO_2 predicted for the future (e.g., as large as ~200–1400 μatm diurnally) (Shaw et al., 2012) could attenuate the magnitude of the depression of coral calcification that is observed under elevated pCO_2 regimes at a stable value of ~1000 μatm (Comeau et al., 2013; Dove et al., 2013; Silbiger and Donahue, 2015). Evidence that corals might respond differentially to treatments contrasting oscillatory versus stable pCO_2 has motivated inclusions of such variation into experimental studies (Dufault et al., 2012; Price et al., 2012). The results have been incongruent between treatments (Dufault et al., 2012; Comeau et al., 2014), for example, with net calcification of the coral, *Acropora hyacinthus*, 27% higher for individuals exposed to diel oscillatory pCO_2 (400 and 2000 μatm) compared to those maintained under a stable elevated pCO_2 of 1000 μatm after 6 weeks (Comeau et al., 2014). The implications of this result are accentuated by the expectation that the rates of calcification should have been similar between stable and oscillatory pCO_2 treatments, at least based on the cumulative exposure time to elevated pCO_2 (Comeau et al., 2014). Evidence that the pCO_2 of seawater over shallow coral reefs oscillates over months and seasons (Kayanne et al., 2005; Manzello, 2010) has yet to lead to experimental tests of these effects on corals.

While seawater pCO_2 higher than the current ambient (i.e., > 450 μatm) generally depresses coral calcification over weeks-to-months (Kroeker et al., 2010, 2013), it is unknown whether these effects might abate should pCO_2 be reduced during the declining phase of natural pCO_2 oscillations superimposed on the long-term trend for globally rising pCO_2. In the present study, we assessed how a monthly-scale fluctuation in seawater pCO_2 (as might occur within seasons [Kayanne et al., 2005; Manzello, 2010]) affects calcification of a common Indo-Pacific coral, *Acropora retusa*. We tested the hypothesis that depressed calcification caused by a 19-day exposure to elevated pCO_2 is subsequently relaxed (i.e., calcification increases) when the same corals are returned for 11 days to seawater maintained at ambient pCO_2. In addition to measuring calcification as a response variable, net photosynthesis was measured to gain insight into the ways by which autotrophic nutrition of the coral host (*sensu* Muscatine, 1990) might be associated with the response of calcification to the sequence of exposures to pCO_2 regimes.

2. Methods

2.1. Coral collection

This study was conducted in the austral autumn between 9 April and 18 May 2018. On 9 April, 20 branches (~4-cm tall) of *A. retusa* were collected from separate colonies (1 branch $colony^{-1}$) at 10-m depth on the fore reef of the north shore of Mo'orea, French Polynesia (17°28′30.06" S, 149°50′13.44" W). Colonies that were ≥ 2 m apart were haphazardly selected for sampling to increase the likelihood that each was genetically unique, assuming that each colony was a product of the settlement of a sexual larva. The branches of *A. retusa* were transported to the lab in a container shielded from direct sunlight, and attached upright to plastic stands using cyanoacrylate adhesive (EcoTech Marine Coral Glue). Prepared branches were allowed to recover and adjust to laboratory conditions in a large aquarium (1000L) for 4 days prior to the first calcification measurement.

2.2. Aquaria conditions

This experiment was conducted in aquaria at Richard B. Gump South Pacific Research Station. A single 1000-L aquarium was used during the laboratory adjustment and relaxation phases, and two 150-L aquaria were used during the high-pCO_2 exposure phase (experimental phases described below). The physical and chemical conditions in the incubation seawater were recorded during high-pCO_2 exposure and relaxation phases. The relaxation phase allowed corals to recover from handling and manipulations prior to commencing treatments. All aquaria were supplied at ~150 mL min^{-1} with filtered seawater (pore size ~100 μm) freshly pumped from Cook's Bay, and were maintained at ~29.0 °C using chillers and heaters. This temperature was close to the ambient seawater temperature at the collection site (~28.7 °C) when the experiment was conducted in April 2018. The temperature of the seawater in aquaria was recorded several times daily using a digital thermometer (± 0.05 °C certified, model 15–077, Fisher Scientific, Pittsburgh, PA, USA).

Four 75 W light-emitting diode (LED) lamps (Sol White LED Module; Aqua-illumination) provided overhead light to each aquarium on a 12:12 h light:dark photoperiod. Photosynthetically active radiation (PAR) was increased gradually from 06:00 to 10:00 h, maintained close to the maximum PAR typically recorded at 10-m depth on the fore reefs of Mo'orea (~645 μmol quanta m^{-2} s^{-1}, recorded with a 4π quantum sensor) from 10:00 to 14:00 h, and then decreased gradually from 14:00 to 18:00 h to mimic natural light conditions on the reef. Maximum irradiances at the bottom of the aquaria were ~ 679 μmol quanta m^{-2} s^{-1} (measured with a 4π quantum sensor (Li Cor, LI-193) attached to a Li-Cor LI-1400 hand-held meter). During the laboratory adjustment phase, coral branches were kept in a large aquarium (1000 L), within which they were supported on a circular table that rotated once every 12 h to expose them to similar levels of PAR, and prevent position effects.

The total alkalinity (A_T) of the seawater in ambient and elevated pCO_2 aquarium was measured every 2 days during the pCO_2 exposure and relaxation phases using potentiometric titration (Dickson et al., 2007). The accuracy (deviation from the certified value of reference material) and precision (the SD between duplicated measurements) of the titrations were evaluated by processing certified reference material (batch 158 from A. Dickson Laboratory, Scripps Institute of Oceanography). The accuracy and precision of our measurements were 4.9 and 1.9 μmol kg^{-1} (n = 6), respectively. Salinity of the seawater was measured using a conductivity meter (Thermo Scientific Orion A212). The carbonate chemistry parameters of the seawater in the treatment

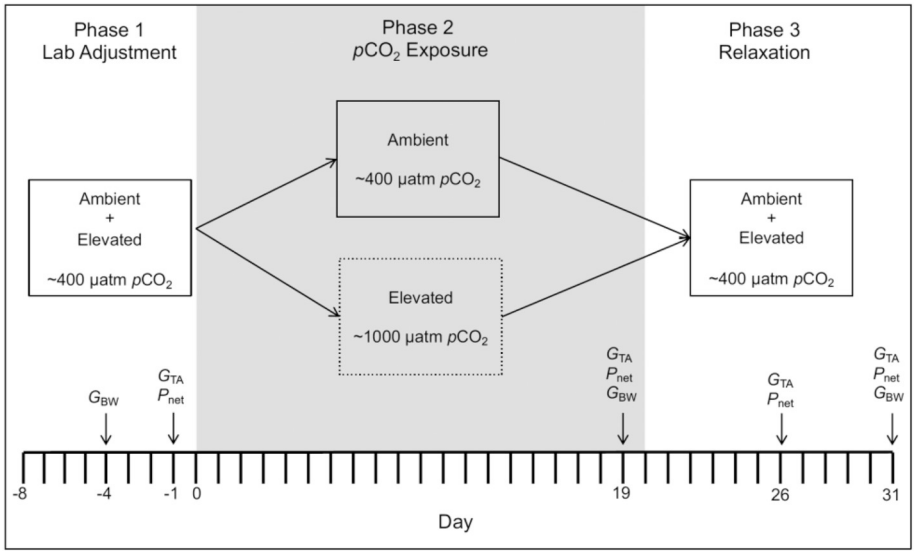

Fig. 1. Schematic representation of the experimental design with shading distinguishing the three phases of the experiment extending along a chronology of: (1) -8 to 0 days, the laboratory adjustment phase, 2) 0–20 days, the pCO_2 exposure phase, and 3) 21–31 days, the relaxation phase. Ambient and elevated pCO_2 aquaria are shown by solid and dashed rectangular outlines, respectively. The timing of long-term integrated calcification (G_{BW}), short-term calcification (G_{TA}), and net photosynthesis (P_{net}) measurements are indicated along the abscissa.

aquarium were calculated from temperature, pH_T, A_T, and salinity using the Seacarb package (Lavigne and Gattuso, 2013) in R software. Carbonic acid dissociation constants of Lueker et al. (2000), the K_2SO_4 concentration for the bisulfate ion from Dickson (1990), and the K_f constant of Perez and Fraga (1987) were used to calculate carbonate chemistry parameters.

2.3. Experimental approach

2.3.1. Overview

To test the hypothesis that depressed calcification due to elevated pCO_2 is relaxed when corals are placed into ambient pCO_2 seawater, an experiment was designed in three phases: Phase 1 allowed adjustment of freshly-collected corals to laboratory conditions ("laboratory adjustment"), Phase 2 exposed lab-adjusted corals to pCO_2 treatments ("pCO_2 exposure"), and Phase 3 exposed pCO_2-treated corals to ambient pCO_2 ("relaxation") (Fig. 1). *Acropora retusa* was used for all experiments, as acroporids were relatively abundant (i.e., ~5% cover of stony corals in 2018) on the fore reef (< 10-m depth) on the north shore of Mo'orea, French Polynesia (17° 28′ 53.9004" S, 149° 49′ 50.5992" W), where this study was conducted in 2018 (Edmunds, 2018). Moreover, calcification for acroporids in Mo'orea is more sensitive to elevated pCO_2 than for other coral genera (Comeau et al., 2013, 2014), which increased the likelihood of detecting a negative response during the high-pCO_2 treatment phase. Such sensitivity was required to test for relaxation of the elevated pCO_2 treatment effect following a return to ambient pCO_2, testing for which was the objective of this study.

2.3.2. Laboratory adjustment phase

During the laboratory adjustment phase, corals freshly collected from the field were placed in a single aquarium exposed to laboratory conditions of seawater flow, light, and temperature over 8 days. Net calcification and photosynthesis of all coral branches was measured under ambient pCO_2, which corresponded to the present day atmospheric concentration (~400 µatm). Details of net calcification and photosynthesis measurements are described in Section 2.4.

2.3.3. pCO_2 exposure phase

In the pCO_2 exposure phase, lab-adjusted corals were exposed to ambient and elevated pCO_2 to test for an effect of elevated pCO_2 on calcification. During this phase, net calcification and photosynthesis was measured under ambient and elevated pCO_2 following a 19-day exposure to the treatments. Elevated pCO_2 (~1000 µatm) corresponded to the conditions expected by the end of the 21st Century under RCP 8.5 (IPCC, 2014). Net calcification under ambient and elevated pCO_2 was measured using two techniques that provided different temporal resolution (described below in Sections 2.4.1 and 2.4.2) in resolving calcification dynamics following cessation of pCO_2 treatments. Net photosynthesis also was measured concurrently with net calcification to gain insight into the possibility that variation in net calcification was functionally associated with net photosynthesis. For example, photosynthetically fixed carbon from dinoflagellate symbionts may be used to enhance calcification by their host coral (Allemand et al., 2011). Net photosynthesis measurements are described below in Section 2.4.3.

The pCO_2 exposure phase was conducted in two aquaria (each 150 L), with half of the coral branches allocated to each of the ambient pCO_2 (~400 µatm) and the elevated pCO_2 (~1000 µatm) aquaria (n = 10 corals treatment^{-1}). Elevated pCO_2 was created by bubbling CO_2 gas into one of the aquarium, with the flow of CO_2 adjusted with a digital controller (Apex, Neptune Systems, USA) that regulated seawater pH using a solenoid supplying CO_2 gas to the aquarium. The regulation system was adjusted to ensure that the pH stat technique was associated with an oscillation in pH_T of only ~0.2 (~60 µatm pCO_2) over 4–5 min. An ecologically relevant diurnal cycle in seawater pH was not created as our study was designed to understand the effects of longer-term exposure of pCO_2 on coral calcification. Seawater pH (total scale; pH_T) in the aquaria was measured several times each day using a portable pH meter (Orion 3-star, Thermo Scientific, USA) fitted with a pH probe (Mettler, Mettler-Toledo, USA) that was calibrated using Tris/HCl buffers (Dickson et al., 2007). Accuracy was evaluated by measuring seawater pH spectrophotometrically on three occasions using m-cresol dye (Standard operating procedure 6b, Dickson et al., 2007). pH measured spectrophotometrically differed ≤0.03 units from the pH measured with the pH probe, which indicated that routine measurements with the pH probe provided an accuracy sufficient for the physiological experiment we wished to conduct. The ambient pCO_2 treatment contained seawater (with a natural pCO_2 of ~435 µatm) freshly pumped from Cook's Bay, as described above. Ambient air was bubbled into both aquaria.

2.3.4. Relaxation phase

After 19 days of corals being exposed to ambient or elevated pCO_2, the relaxation phase was initiated when corals from both treatments were transferred to a single aquarium under ambient pCO_2 seawater conditions for 11 days. During the relaxation phase, net calcification of coral branches previously exposed to ambient and elevated pCO_2 was measured using two techniques that provided different temporal resolution (described below in 2.4.1 and 2.4.2). Net photosynthesis was

also measured concurrently with net calcification.

2.4. Response parameters

2.4.1. Integrated net calcification

Net calcification over a time scale of weeks was measured using buoyant weighing (hereafter G_{BW}) (Spencer Davies, 1989), which integrates calcification that is completed in the day and night (Schoepf et al., 2017). G_{BW} of coral branches (n = 10 corals treatment^{-1}) during the pCO_2 exposure and relaxation phases of the experiment were compared to evaluate whether depressed calcification in corals exposed to elevated pCO_2 subsequently increased when corals were returned to ambient pCO_2 (Fig. 1). The differences in buoyant weight were converted to dry weight of coral skeleton using the density of aragonite (2.93 g cm^{-3}), and dry weight was standardized to the surface area of live tissue by wax dipping (Stimson and Kinzie, 1991) after completing the relaxation phase of the study. This approach assumes that growth over the short experiment did not appreciably contribute to coral area. Finally, dry weights were standardized by time (i.e., 1 day) to estimate net calcification (mg $CaCO_3$ cm^{-2} day^{-1}).

2.4.2. Short-term net calcification

Net calcification (G) was measured using two techniques that differed in temporal resolution to better describe patterns of calcification emerging following the elevated pCO_2 exposure phase. The total alkalinity (A_T) anomaly method (after Smith, 1973) was used to measure short-term net calcification over ~1 h in the light (hereafter G_{TA}) for a subset of the coral branches exposed to ambient and elevated pCO_2 (n = 7 treatment^{-1}) (Fig. 1). The A_T anomaly method has the resolution to measure calcification occurring over minutes-to-days, at least when the incubation volumes are relatively small, and the coral biomass is relatively large (Gattuso et al., 1996; Shaw et al., 2015). Insights into calcification (or dissolution) in the dark were obtained by combining measurements of short-term calcification in the light with temporally integrated (i.e., light and dark) calcification. The A_T anomaly method was first employed during the laboratory adjustment phase to test for similarity of calcification among the corals prior to exposure to ambient and elevated pCO_2. During the pCO_2 exposure phase, G_{TA} was measured after 19 days to test for the effects of pCO_2 on calcification. Finally, during the relaxation phase, G_{TA} was measured after 6 and 11 days after corals from both pCO_2 treatments had been returned to ambient pCO_2 (Fig. 1).

G_{TA} was measured by incubating coral branches in a sealed 0.24-L acrylic chamber that was surrounded by a water jacket to maintain temperatures close to experimental conditions (~29.0 °C). The chamber was fitted with a stir bar to create flow (~7.5 cm s^{-1}), which was measured in a separate trial from the mean time required for neutral density particles (hydrated brine shrimp eggs) to complete one revolution around the perimeter of the chamber. The filtered seawater (45 μm) in each chamber was obtained from the respective treatments for each sampled coral, and the incubations lasted ~1 h. G_{TA} was measured using Eq. (1) (after Smith, 1973):

$$G_{TA} = \frac{-0.5\Delta A_T V_\rho}{\Delta t SA} \quad (1)$$

where ΔA_T is the change in total alkalinity (μmol kg^{-1}), V is the volume of seawater in the acrylic chamber (L), ρ is the density of the seawater (kg L^{-1}), Δt is the time interval of the incubations (h), and SA is the surface area of live coral tissue (cm^2). A_T of incubated seawater was calculated using the Seacarb package (Lavigne and Gattuso, 2013) in R software after performing potentiometric titrations. The potentiometric titrations provided for calculation of A_T (Dickson et al., 2007). If titrations for TA analysis were not performed immediately after collecting the seawater samples, they were sealed with parafilm in glass bottles and placed into darkness at 4 °C to minimize evaporation and biological activity. Samples were returned to room temperature prior to completing titrations, and all analyses were completed within one day of collecting the samples. The volume of seawater in each acrylic chamber used for coral incubations was calculated by subtracting the estimated volume of each coral, stir bar, and coral stand from the total chamber volume. G_{TA} was standardized to the surface area of live tissue as described above, and to 12 h of daylight (i.e., daylight hours, "DH") when the majority of daily calcification occurs (reviewed by Gattuso et al., 1999) (mg $CaCO_3$ cm^{-2} DH^{-1}). Logistical constraints necessitated measuring G_{TA} under daytime PAR levels during night time hours (i.e., ~18:00–06:00 h). During evenings when incubations were performed, aquaria lights were left at full intensity so that corals would not undergo a dark-to-light adjustment as they were moved from aquaria to incubation chambers. The mean (± SE) irradiance of the bottom of the incubation chambers used was 643 (± 6.3) μmol quanta m^{-2} s^{-1}. Control incubations were completed in identical ways as coral incubations, except that the chambers contained only seawater.

2.4.3. Net photosynthesis

Net photosynthesis (P_{net}) was measured concurrently with G_{TA} (Fig. 1) by recording changes in O_2 concentration of seawater in the sealed incubation chambers every minute. O_2 concentration was measured using oxygen probes (Foxy-R, Ocean Optics) that were connected to spectrophotometers (USB2000, Ocean Optics) and operated using the manufacturers software (OOISensors ver. 1.00.08, Ocean Optics). Probes were calibrated to 100% air saturated water (O_2 saturated) and 0% O_2 obtained with a chemical zero (0.01 M sodium tetraborate and sodium sulphite). P_{net} was calculated as:

$$P_{net} = \left[\frac{(m_A)}{100} V_A S_{O_{2A}}\right] - \left[\frac{(m_C)}{100} V_C S_{O_{2C}}\right] \quad (2)$$

where m_A is the rate of change of O_2 saturation values for incubation chambers that contained corals (%O_2 hr^{-1}), m_C is the rate of change of O_2 saturation values for incubation chambers that did not contain corals (%O_2 hr^{-1}), V_A is the volume of seawater in the experimental acrylic chamber (L) (i.e., contained corals) (seawater volume was calculated by subtracting the estimated volume of each coral, stir bar, and coral stand from the total chamber volume), V_C is the volume of seawater in the control acrylic chamber (L) (i.e., without corals) (seawater volume was calculated by subtracting the volume of stir bar and coral stand from the total chamber volume) and $S_{O_{2A}}$ and $S_{O_{2C}}$ are the tabulated values of O_2 solubility (N. Ramsing and J. Gundersen at Unisense, Aarhus, Denmark, based on Garcia and Gordon, 1992) that correspond to measured seawater temperature and salinity within experimental and control chambers, respectively (μmol O_2 L^{-1}). P_{net} was normalized to the area of each coral branch, as determined by wax dipping (Stimson and Kinzie, 1991), and to 12 DH (μmol O_2 cm^{-2} DH^{-1}).

2.5. Analyses

A one-way analysis of variance (ANOVA) was used to test for variation in mean seawater pCO_2 among treatments. A t-test was used to test for differences in short-term calcification (G_{TA}) between treatments following the laboratory adjustment phase, and before corals were placed into pCO_2 conditions. This analysis tested for intrinsic differences in calcification among corals prior to initiation of the treatments.

To evaluate whether corals exposed to elevated pCO_2 exhibited depressed calcification rates compared to corals that were not exposed to elevated pCO_2, integrated calcification (G_{BW}) was first compared between pCO_2 treatments at the end of pCO_2 exposure phase using a t-test. G_{BW} of corals was analyzed using a 2-way, repeated measure (RM) ANOVA in which pCO_2 treatment (ambient vs. elevated) was a between-subject fixed effect, and time (throughout pCO_2 exposure and relaxation phases) was the RM effect.

G_{TA} was also analyzed using a 2-way RM ANOVA using the same factors listed above, modified through the addition of levels to the RM

factor (4 time periods throughout Phases 1–3). To test for the effect of pCO_2 on P_{net}, a 2-way RM ANOVA was used in which pCO_2 treatment (ambient vs. elevated) was a between-subject effect, and time (4 time periods during phases 1–3) was the RM effect. To resolve the effects of pCO_2 exposure history on calcification and P_{net}, planned comparisons using a paired *t*-test (Quinn and Keough, 2011) were conducted. Treatment levels were contrasted across the four incubations that occurred during the lab adjustment, pCO_2 exposure, and relaxation phases of the experiment using G_{TA} and P_{net} as response variables. If the main effect of time significantly affected mean G_{TA} or P_{net}, paired *t*-tests were planned to orthogonally test for differences in the response variables between all combinations of time periods when incubations were performed (6 combinations) (Quinn and Keough, 2011).

Although coral branches within a single aquarium during the three phases of the experiment were pseudoreplicated (*sensu* Hurlbert, 1984), the potential biases associated with this effect were reduced by the large volume of the aquaria (~150 and 1000 L), the constant flow (~150 mL min^{-1}) of fresh seawater into the aquaria, and the relatively small biomass of the corals (~5 mg cm^{-2} for *Acropora pulchra* [from Comeau et al., 2013]). Based on the rationale that the branches effectively were independent of one another in each aquarium, the coral branches were treated as statistical replicates.

The statistical assumptions of normality and equality of variances required for ANOVA were tested through graphical analyses of residuals, and an alpha value of < 0.05 was considered statistically significant. In the case of the six planned comparisons, a Bonferroni correction was made to downwardly adjust alpha to 0.008 (Quinn and Keough, 2011). All analyses were performed using the nlme package (Pinheiro et al., 2018) running in R software (R Foundation for Statistical Computing).

3. Results

Two corals exposed to ambient pCO_2 were dropped from the analyses of G_{BW} because they bleached, and G_{TA} was measured using 6 coral branches $treatment^{-1}$ during the last day of incubations (day 31 of the experiment). All other coral branches retained pigmentation similar to that observed in the field, and exhibited polyp expansion throughout the incubation periods.

3.1. Physical and chemical parameters

The physical and chemical conditions of seawater in the 150-L aquaria in which pCO_2 treatments were maintained are reported in Table 1. During the pCO_2 exposure phase, the treatments contrasted mean (± SE) pCO_2 values of 476 ± 7 µatm ($n = 19$) and 1086 ± 15 µatm ($n = 19$), and in the 1000-L aquaria used during the relaxation phase, the mean pCO_2 was 435 ± 12 µatm ($n = 5$). pCO_2 differed among aquaria ($F_{2,40} = 867.13$, $p < .01$), and was higher in the elevated versus the ambient pCO_2 aquaria in the pCO_2 exposure and relaxation phases (Tukey HSD, $p < .01$). The two ambient pCO_2 aquaria used in the pCO_2 exposure and relaxation phases were not significantly different from one another (Tukey HSD, $p = .22$).

3.2. Calcification

Short-term calcification (G_{TA}) of *A. retusa* in the ambient and elevated pCO_2 groups was statistically similar during the laboratory adjustment phase prior to allocating them to pCO_2 treatments (2-sample *t*-test, $t_{16} = 0.001$, $p = .99$).

3.2.1. Integrated net calcification

Elevated pCO_2 negatively affected G_{BW} during the pCO_2 exposure phase ($t_{16} = 2.36$, $p = .03$). After 19 days, G_{BW} of corals exposed to elevated pCO_2 was depressed by 31% (elevated pCO_2 treated corals: 0.40 ± 0.04 mg $CaCO_3$ cm^{-2} day^{-1}; ambient pCO_2 treated corals: 0.57 ± 0.06 mg $CaCO_3$ cm^{-2} day^{-1}) (mean ± SE) (Fig. 2a). After 31 days, G_{BW} was significantly affected by time (pCO_2 exposure versus relaxation phases: $F_{1,16} = 35.90$, $p < .01$), but not pCO_2 (ambient versus elevated: $F_{1,16} = 2.01$, $p = .18$), or the interaction between time and pCO_2 ($F_{1,16} = 1.65$, $p = .22$). Overall, mean G_{BW} was higher during the relaxation phase compared to the pCO_2 exposure phase (0.70 ± 0.06 mg $CaCO_3$ cm^{-2} day^{-1} vs. 0.47 ± 0.04 mg $CaCO_3$ cm^{-2} day^{-1}, respectively) (mean ± SE) (Fig. 2a). G_{BW} of corals in the elevated pCO_2 treatment increased by 0.27 ± 0.03 mg $CaCO_3$ cm^{-2} day^{-1} compared to 0.17 ± 0.08 mg $CaCO_3$ cm^{-2} day^{-1} for corals in the ambient pCO_2 treatment, but this effect was not significant (time × pCO_2: $F_{1,16} = 1.65$, $p = .22$) (Fig. 2a).

3.2.2. Short-term net calcification

Over the course of the experiment, G_{TA} was significantly affected by time ($F_{1,38} = 7.89$, $p = .01$), but not pCO_2 ($F_{1,12} = 0.17$, $p = .68$), or the interaction between the two ($F_{1,38} = 0.96$, $p = .33$) (Fig. 2b). For a complete list of pairwise comparisons of G_{TA} among the pCO_2 exposure and relaxation phases see Table 2.

3.3. Net photosynthesis

Across all four sampling periods, RM ANOVA indicated that P_{net} was not affected by time ($F_{1,38} = 1.92$, $p = .17$), pCO_2 ($F_{1,12} = 1.58$, $p = .23$), or the interaction between the two ($F_{1,38} = 2.26$, $p = .14$). However, graphical exploration of P_{net} over the course of the experiment (Fig. 2b) indicated that after exposing corals to elevated pCO_2 for 19 days, mean P_{net} was 34% greater than in corals exposed to ambient pCO_2 for the same duration. A paired *t*-test revealed this difference was significant ($t_{12} = 4.26$, $p < .01$), but planned comparisons between pCO_2 treatments during other phases of the experiment were not significant (laboratory adjustment phase day −1: $t_{12} = 0.90$, $p = .39$; relaxation phase day 26: $t_{12} = 0.54$, $p = .60$; relaxation phase day 31: $t_{10} = 1.37$, $p = .20$) (Fig. 2b).

4. Discussion

A month-long study was conducted to test for interactive effects between pCO_2 treatment (i.e., present vs. predicted future: ~400 vs. ~1000 µatm), and pCO_2-exposure history on net calcification of a tropical scleractinian. As expected from the generalized inhibition of coral calcification by high pCO_2 (Erez et al., 2011; Chan and Connolly, 2013;

Table 1
Seawater carbonate chemistry in the aquaria employed in two of the pCO_2 exposure and the relaxation phases (Fig. 1) of the experiment. Carbonate chemistry was not measured during the laboratory adjustment phase. All values were discrete measurements, or were calculated from discrete measurements. Set points of the aquarium regulation system were adjusted several times daily to minimize temporal variation. The values reported are mean ± standard error (SE).

pCO_2 Treatment	Phase	n	pCO_2 (µatm)*	A_T (µmol kg^{-1} SW)**	pH_T**	Ω_{arag}*	Temperature (°C)**	Light (µmol quanta $m^{-2}s^{-1}$)**
Ambient	pCO_2 exposure	19	476 ± 7	2296 ± 6	7.98 ± 0.01	3.44 ± 0.03	29.1 ± 0.1	621 ± 6
Elevated	pCO_2 exposure	19	1086 ± 15	2286 ± 4	7.67 ± 0.01	1.90 ± 0.02	29.0 ± 0.1	612 ± 8
Ambient	relaxation	5	435 ± 12	2355 ± 3	8.02 ± 0.01	3.79 ± 0.07	29.1 ± 0.1	656 ± 6

* Calculated using the Seacarb package (Lavigne and Gattuso, 2013) in R software.
** Directly measured.

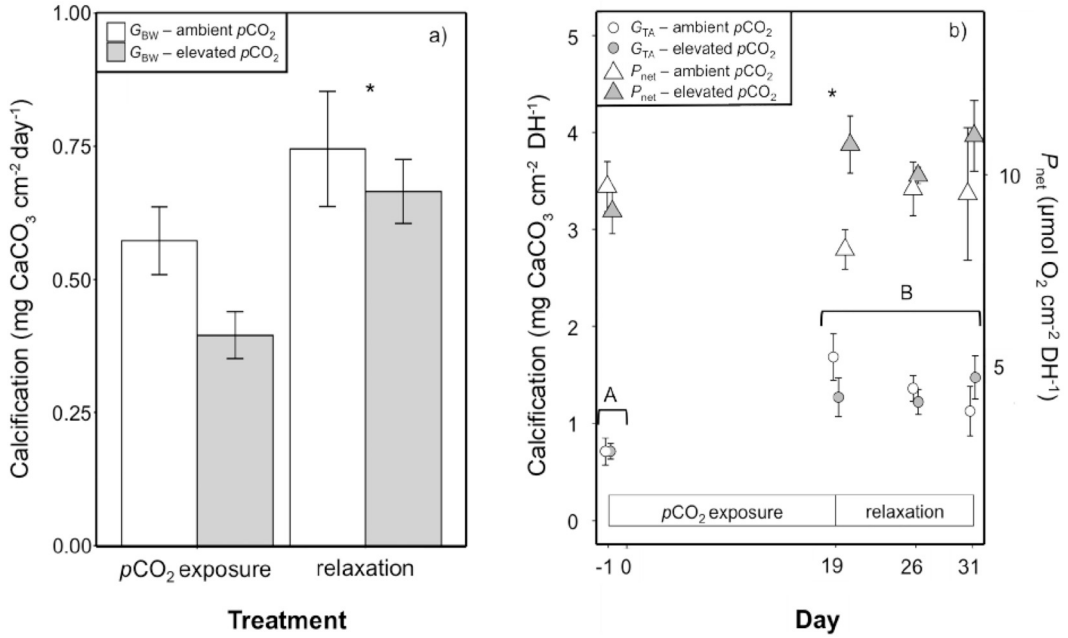

Fig. 2. Calcification and photosynthesis of *Acropora retusa* exposed to the experimental design shown in Fig. 1. (A) Integrated net calcification (G_{BW}) (mean ± SE mg $CaCO_3$ cm^{-2} day^{-1}) over time ($n = 8$ and $n = 10$ coral branches exposed to ambient and elevated pCO_2, respectively). As revealed by the repeated measures ANOVA, a significant difference in G_{BW} between the pCO_2 exposure and relaxation phases is indicated with an asterisk ($P < .01$). (B) Short-term (~1 h) calcification (G_{TA}) standardized to 12 daylight hours (i.e., DH) and net photosynthesis (P_{net}) (mean ± SE) over the full experiment lasting 32 days ($n = 7$ corals treatment^{-1} for day −1, 19, and 26, and $n = 6$ corals treatment^{-1} for day 31). Differing letters denote significant differences among experimental phases, and asterisks denote significant differences between calcification or photosynthesis within a phase ($p < .01$) revealed on day 19 of the pCO_2 exposure phase.

Table 2
Pairwise comparison of mean G_{TA} between phases 1 (lab adjustment: day −1), 2 (pCO_2 exposure: day 19), and 3 (relaxation: days 26 and 31), as outlined in Fig. 1. Significant *p*-values (Bonferroni adjusted alpha < .008) are indicated by an asterisk.

Days compared	Δ *mean*	*df*	*t*	*p*
Days −1 and 19	−0.74	11	4.44	.001*
Days −1 and 26	−0.63	11	4.43	.001*
Days −1 and 31	−0.64	11	3.41	.006*
Days 19 and 26	0.10	11	0.71	.483
Days 19 and 31	0.10	11	0.47	.643
Days 26 and 31	−0.01	11	0.05	.955

Kornder et al., 2018), *Acropora retusa* exposed to elevated pCO_2 exhibited integrated net calcification (G_{BW}) that was reduced by ~31% compared to corals retained in ambient pCO_2. This effect was not evident in hour long records of calcification (i.e., G_{TA}) measured in daylight, which suggests that the depression of G_{BW} was an emergent property associated with the duration of exposure to high pCO_2. During the relaxation phase of the present experiment, the depression of G_{BW} by elevated pCO_2 that was recorded during the pCO_2 exposure phase, was reduced. As a result, within 11 days the G_{BW} of corals previously retained under elevated pCO_2 was statistically indistinguishable from the G_{BW} of corals previously retained under ambient pCO_2. Evidence here of quick relaxation of pCO_2-depressed G_{BW} in *A. retusa* indicates that at least some corals are capable of a fully reversible plastic response (*sensu*Liew et al., 2018) of net calcification to elevated pCO_2 over short periods (i.e., ≤ 19 days).

In the present study, two techniques for measuring coral calcification were employed to resolve treatment effects on two temporal scales. Buoyant weighing (BW) resolved changes in aragonite mass over weeks, and integrated the effects of light and dark calcification (Spencer Davies, 1989; Schoepf et al., 2017), as well as skeletal dissolution that is expected to be greatest in the dark (Yates and Halley, 2006; Silverman et al., 2007). The total alkalinity (A_T) method (Smith and Key, 1975) resolved changes in net calcification over hours and, therefore, captured fine-scale effects attributed to the light regime. By focusing on calcification in daylight, the A_T method provided insight into the processes potentially occurring in the dark (i.e., dissolution) that were captured through the temporal integration inherent in the BW approach. After scaling G_{TA} from the ~1 h over which measurements were recorded, to 12 daylight hours of a 24-hour day, the difference between 24 h-G_{BW} and 12 h-G_{TA} yield estimates of nighttime net calcification (Schoepf et al., 2017). When this approach was applied to the 19-day pCO_2 exposure phase of the present study, 24 h-G_{BW} was ~69% lower than 12 h-G_{TA}. It is reasonable to suspect that the observed difference between the two calcification measurements may have been caused by the incorporation of nighttime dissolution into values of G_{BW}, and because G_{TA} was measured under maximum daily irradiance which would promote calcification (Allemand et al., 2011). Similarly, calcification of a coral community constructed in aquaria was reduced by ~100% at night when a pCO_2 treatment equivalent to future levels predicted by RCP scenario 8.5 (i.e., ~1000 μatm) was implemented compared to current levels of pCO_2 (i.e., ~400 μatm) (Dove et al., 2013). Additionally, corals that were exposed to elevated pCO_2 may have adjusted calcification rates throughout the pCO_2 exposure phase to ameliorate the negative effects of OA. This effect could have resulted in a mismatch between instantaneous calcification (G_{TA}) observed at the end of the experiment and integrated calcification (G_{BW}) observed or the entire experiment.

This study was performed to better understand how calcification of corals living under naturally oscillating pCO_2 regimes will be affected by future OA. Previous studies of the effects of high pCO_2 on corals (Marubini and Atkinson, 1999; Leclercq et al., 2002; Anthony et al., 2008; Ries et al., 2009a), including those conducted with *Acropora* in Mo'orea (Comeau et al., 2013; Comeau et al., 2016), show that calcification of corals exposed to ≥1000 μatm pCO_2 typically are depressed compared to corals exposed to ambient pCO_2 (i.e., ~400 μatm). The rapid relaxation of depressed calcification following cessation of elevated pCO_2 in the present study suggests that calcification of tropical

corals may positively track oscillations of pCO_2 that are super-imposed on long-term trends of increasing atmospheric pCO_2 caused by anthropogenic activities. The sum of the integrated effect of varying pCO_2 on coral calcification could potentially reduce the effects of a consistently high pCO_2 regime, which are frequently observed to be negative for calcifying taxa (Ries et al., 2009b; Kroeker et al., 2013).

Net photosynthesis (P_{net}) was measured to explore potential mechanisms underlying variation in G_{TA} and G_{BW} between treatments, notably with respect to how they might be mediated by the supply of photosynthetically-fixed carbon. Calcification in symbiotic corals is stimulated by light (Allemand et al., 2011), and this effect is thought to arise from multiple pathways, a number of which are likely associated with production of oxygen through photosynthesis by the dinoflagellate symbionts. Such photosynthesis could promote calcification by supporting the translocation of carbon to the animal host, which then can be included in the organic matrix that facilitates calcification (Allemand et al., 2011; Tambutté et al., 2011), or might be used as a respiratory substrate fueling the production of ATP to meet the metabolic costs of calcification (Allemand et al., 2011; Frieder et al., 2016). Through the uptake of intracellular CO_2 by photosynthesis, symbiotic dinoflagellates might also be able to modify the pH in the sub-calicoblastic space, thus accelerating calcification (Allemand et al., 2011).

In this present study, mean P_{net} measured over ~1 h was 34% higher in corals exposed to elevated versus ambient pCO_2 at the end of the pCO_2 exposure phase, and it was similar between the treatment groups during the other phases of the experiment. As indicated by higher rates of P_{net} observed in corals exposed to elevated pCO_2 during the pCO_2 exposure phase, this effect raises the possibility that surplus carbon available to corals accelerated short-term, daylight calcification (i.e., G_{TA}) under elevated pCO_2. Since the mechanisms causing P_{net} to vary over time in corals exposed to ambient pCO_2 are unknown, it is premature in this study to speculate about functional connections between P_{net} and calcification.

4.1. Ecological significance

There is great interest in the possibility that scleractinian corals might be able to reduce their sensitivity to elevated pCO_2 through phenotypic adjustments within a generation (i.e., acclimatization) (Crook et al., 2013; Putnam et al., 2016; Eirin-Lopez and Putnam, 2019), or among generations through adaptation (Liew et al., 2018). Corals have the mechanisms necessary to acclimate (*sensu*Edmunds and Gates, 2008) and adapt to OA (Liew et al., 2018), as well as other environmental challenges (Edmunds and Gates, 2008; Torda et al., 2017). However, it is unclear whether those mechanisms will support ecologically meaningful reductions in the negative implications of climate change and OA for reef corals. Ultimately, the persistence of corals, and other organisms, through those challenges may rely not only on their ability to respond, but also the rate at which they are able to respond, to their rapidly changing environment (Bell, 2013).

The present study tested for biological implications of a single oscillation in seawater pCO_2 that are similar to those occurring in several coastal ecosystems (Hofmann et al., 2011; Duarte et al., 2013; Guadayol et al., 2014; Page et al., 2018; Silbiger and Sorte, 2018). Our month-long experiment specifically evaluated whether prior exposure to stable elevated pCO_2 has carry-over effects that modulate the subsequent response of coral calcification to ambient pCO_2. While marine organisms are confronted by long-term (i.e., years to decades) trends of rising seawater pCO_2 due to OA (Orr et al., 2005; Hoegh-Guldberg et al., 2007), their response to these conditions may, in part, depend on how they respond to periods of declining pCO_2 that reflect natural oscillations in this variable that occur on scales of days to months (Kayanne et al., 2005; Hoffman et al., 2011). Furthermore, the long-term rise in seawater pCO_2 will not occur independent of variation in other physical, chemical, and biological conditions in seawater. Understanding, for example, how natural pCO_2 oscillations interact with ocean warming, as well as OA, will be important in characterizing the long-term response of corals to a diversity of local and global stressors. The interaction between zooplankton abundance and OA may also be important in understanding the trajectory of coral populations into the future, because heterotrophy through zooplanktivory by corals can ameliorate some of the negative effects of OA (Edmunds, 2011; Towle et al., 2015). Consideration of the effects of the magnitude and duration of prior pCO_2 exposures on calcification of tropical corals are needed to more accurately project the summed effects of coral calcification (i.e., net reef accretion) into the future.

Author statement

All authors designed the experiment. SG collected and analyzed the data. SG and PE wrote the first draft the manuscript, and all authors contributed to manuscript revisions.

Data accessibility

The data for this project are hosted at the MCR-LTER site: http://mcr.lternet.edu(https://doi.org/10.6073/pasta/c7f2f8abd2eaa83b6f882e01b286382b).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Allemand, D., Tambutté, E., Zoccola, D., Tambutté, S., 2011. Coral calcification: Cells to reef. In: Dubinsky, Z., Stambler, N. (Eds.), Coral Reefs: An Ecosystem in Transition. Springer, Netherlands, pp. 119–150. https://doi.org/10.1007/978-94-007-0114-4_9.

Anthony, K.R.N., Kline, D.I., Diaz-Pulido, G., Dove, S., Hoegh-Guldberg, O., 2008. Ocean acidification causes bleaching and productivity loss in coral reef builders. Proc. Natl. Acad. Sci. U. S. A. 105, 17442–17446. https://doi.org/10.1073/pnas.0804478105.

Anthony, K.R.N., Maynard, J.A., Diaz-Pulido, G., Mumby, P.J., Cao, L., Marshall, P.A., Hoegh-Guldberg, O., 2011. Ocean acidification and warming will lower coral reef resilience. Glob. Change Biol. 17, 1798–1808. https://doi.org/10.1111/j.1365-2486.2010.02364.x.

Arnold, K.E., Findlay, H.S., Spicer, J.I., Daniels, C.L., Boothroyd, D., 2009. Effect of CO_2-related acidification on aspects of the larval development of the European lobster, *Homarus gammarus* (L.). Biogeosciences 6, 1747–1754. https://doi.org/10.5194/bg-6-1747-2009.

Bates, N.R., 2002. Seasonal variability of the effect of coral reefs on seawater CO_2 and air-sea CO_2 exchange. Limnol. Oceanogr. 47, 43–52. https://doi.org/10.4319/lo.2002.47.1.0043.

Bell, G., 2013. Evolutionary rescue and the limits of adaptation. Philos. Trans. R. Soc. B 368, 1–6. https://doi.org/10.1098/rstb.2012.0080.

Beukers, J.S., Jones, G.P., 1997. Habitat complexity modifies the impact of piscivores on a coral reef fish population. Oecologia 114, 50–59. https://doi.org/10.1007/s004420050419.

Byrne, M., Gonzalez-Bernat, M., Doo, S., Foo, S., Soars, N., Lamare, M., 2013. Effects of ocean warming and acidification on embryos and non-calcifying larvae of the invasive sea star Patiriella regularis. Mar. Ecol. Prog. Ser. 473, 235–246. https://doi.org/10.3354/meps10058.

Chan, N.C., Connolly, S.R., 2013. Sensitivity of coral calcification to ocean acidification: a meta-analysis. Glob. Change Biol. 19, 282–290. https://doi.org/10.1111/gcb.12011.

Comeau, S., Edmunds, P.J., Spindel, N.B., Carpenter, R.C., 2013. The responses of eight

coral reef calcifiers to increasing partial pressure of CO_2 do not exhibit a tipping point. Limnol. Oceanogr. 58, 388–398. https://doi.org/10.4319/lo.2013.58.1.0388.
Comeau, S., Edmunds, P.J., Spindel, N.B., Carpenter, R.C., 2014. Diel pCO2 oscillations modulate the response of the coral *Acropora hyacinthus* to ocean acidification. Mar. Ecol. Prog. Ser. 501https://doi.org/10.3354/meps10690. 99—11.
Comeau, S., Carpenter, R.C., Lantz, C.A., Edmunds, P.J., 2016. Parameterization of the response of calcification to temperature and pCO_2 in the coral *Acropora pulchra* and the alga *Lithophyllum kotschyanum*. Coral Reefs 35, 929–939. https://doi.org/10.1007/s00338-016-1425-0.
Courtney, T., Westfield, I., Ries, J.B., 2013. CO_2-induced ocean acidification impairs calcification in the tropical urchin *Echinometra viridis*. J. Exp. Mar. Biol. Ecol. 440, 169–175. https://doi.org/10.1016/j.jembe.2012.11.013.
Crook, E.D., Cohen, A.L., Rebolledo-Vieyra, M., Hernandez, L., Paytan, A., 2013. Reduced calcification and lack of acclimatization by coral colonies growing in areas of persistent natural acidification. Proc. Natl. Acad. Sci. U. S. A. 110, 11044–11049. https://doi.org/10.1073/pnas.1301589110.
Dickson, A.G., 1990. Standard potential of the reaction: AgCl(s) + $1.2H_2$(g) = Ag(s) + HCl (aq), and the standard acidity constant of the ion HSO_4^- in synthetic sea water from 273.15 to 318.15. J. Chem. Thermodyn. 22, 113–127. https://doi.org/10.1016/0021-9614(90)90074-Z.
Dickson, A.G., Sabine, C.L., Christian, J.R. (Eds.), 2007. Guide to Best Practices for Ocean CO_2 Measurements. North Pacific Marine Science Organization, Sidney, Canada.
Doo, S.S., Dworjanyn, S.A., Foo, S.A., Soars, N.A., Byrne, M., 2012. Impacts of ocean acidification on development of the meroplanktonic larval stage of the sea urchin *Centrostephanus rodgersii*. ICES J. Mar. Sci. 69, 460–464. https://doi.org/10.1093/icesjms/fsr123.
Dove, S., Kline, D.I., Pantos, S., Angly, F.E., Tyson, G.W., Hoegh-Guldberg, O., 2013. Future reef decalcification under a business-as-usual CO_2 emission scenario. Proc. Natl. Acad. Sci. U. S. A. 110, 15342–15347. https://doi.org/10.1073/pnas.1302701110.
Duarte, C.M., Hendriks, I.E., Moore, T.S., Olsen, Y.S., Steckbauer, A., Ramajo, L., Carstensen, J., Trotter, J.A., McCulloch, M., 2013. Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. Estuar. Coasts 36, 221–236. https://doi.org/10.1007/s12237-013-9594-3.
Dufault, A.M., Cumbo, V.R., Fan, T., Edmunds, P.J., 2012. Effects of diurnally oscillating pCO_2 on the calcification and survival of coral recruits. Proc. R. Soc. B 279, 2951–2958. https://doi.org/10.1098/rspb.2011.2545.
Edmunds, P.J., 2011. Zooplanktivory ameliorates the effects of ocean acidification on the reef coral *Porites* spp. Limnol. Oceanogr. 56, 2402–2410. https://doi.org/10.4319/lo.2011.56.6.2402.
Edmunds, P. of Moorea Coral Reef LTER, 2018. MCR LTER: Coral Reef: Long-term Population and Community Dynamics: Corals, ongoing since 2005. knb-lter-mcr.4.35. https://doi.org/10.6073/pasta/263faa48b520b7b2c964f158c184ef96.
Edmunds, P.J., Gates, R.D., 2008. Acclimatization in tropical reef corals. Mar. Ecol. Prog. Ser. 361, 307–310. https://doi.org/10.3354/meps07556.
Eirin-Lopez, J.M., Putnam, H.M., 2019. Marine environmental epigenetics. Annu. Rev. Mar. Sci. 11, 335–368. https://doi.org/10.1146/annurev-marine-010318-095114.
Erez, J., Reynaud, S., Silberman, J., Schneider, K., Allemand, D., 2011. Coral calcification under ocean acidification and global change. In: Dubinsky, Z., Stambler, N. (Eds.), Coral Reefs: An Ecosystem in Transition. Springer, Netherlands, pp. 151–176. https://doi.org/10.1007/978-94-007-0114-4_10.
Falter, J.L., Lowe, R.J., Atkinson, M.J., Cuet, P., 2012. Seasonal coupling and de-coupling of net calcification rates from coral reef metabolism and carbonate chemistry at Ningaloo Reef, Western Australia. J. Geophys. Res. 117, 1–14. https://doi.org/10.1029/2011JC007268.
Falter, J.L., Lowe, R.J., Zhang, Z., McCulloch, M., 2013. Physical and biological control on the carbonate chemistry of coral reef waters: effects of metabolism, wave forcing, sea level, and geomorphology. PLoS One 8, 1–19. https://doi.org/10.1371/journal.pone.0053303.
Frieder, C.A., Applebaum, S.L., Pan, T.C.F., Hedgecock, D., Manahan, D.T., 2016. Metabolic cost of calcification in bivalve larvae under experimental ocean acidification. ICES J. Mar. Sci. 74, 941–954. https://doi.org/10.1093/icesjms/fsw213.
Garcia, H.E., Gordon, L.I., 1992. Oxygen solubility in seawater: better fitting equations. Limnol. Oceanogr. 37, 1307–1312. https://doi.org/10.4319/lo.1992.37.6.1307.
Gattuso, J.P., Pichon, M., Delesalle, B., Canon, C., Frankignoulle, M., 1996. Carbon fluxes in coral reefs. I. Lagrangian measurement of community metabolism and resulting air-sea CO_2 disequilibrium. Mar. Ecol. Prog. Ser. 145, 109–121. https://doi.org/10.3354/meps145109.
Gattuso, J.P., Allemand, D., Frankignoulle, M., 1999. Photosynthesis and calcification at cellular, organismal and community levels in coral reefs: a review on interactions and control by carbonate chemistry. Am. Zool. 39, 160–183. https://doi.org/10.1093/icb/39.1.160.
Guadayol, O., Silbiger, N.J., Donahue, M.J., Thomas, F.I.M., 2014. Patterns in temporal variability of temperature, oxygen, and pH along an environmental gradient in a coral reef. PLoS One 9, 1–12. https://doi.org/10.1371/journal.pone.0085213.
Hoegh-Guldberg, O., Mumby, P.J., Hooten, A.J., Steneck, R.S., Greenfield, P., Gomez, E., Harvell, C.D., Sale, P.F., Edwards, A.J., Caldeira, K., Knowlton, N., Eakin, C.M., Iglesias-Prieto, R., Muthiga, N., Bradbury, R.H., Dubi, A., Hatziolos, M.E., 2007. Coral reefs under rapid climate change and ocean acidification. Science 318, 1737–1742. https://doi.org/10.1126/science.1152509.
Hofmann, G.E., Smith, J.E., Johnson, K.S., Send, U., Levin, L.A., Micheli, F., Paytan, A., Price, N.N., Peterson, B., Takeshita, Y., Matson, P.G., Crook, E.D., Kroeker, K.J., Gambi, M.C., Rivest, E.B., Frieder, C.A., Yu, P.C., Matz, T.R., 2011. High-frequency dynamics of ocean pH: a multi-ecosystem comparison. PLoS One 6, 1–11. https://doi.org/10.1371/journal.pone.0028983.
Huber, M.E., Coles, S.L., 1986. Resource utilization and competition among the 5 Hawaiian species of *Trapezia* (Crustacea, Brachyura). Mar. Ecol. Prog. Ser. 20, 21–31. https://doi.org/10.3354/meps030021.
Hurlbert, S.H., 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54, 187–211. https://doi.org/10.2307/1942661.
IPCC, 2014. Climate change 2014: synthesis report. In: Team, Core Writing, Pachauri, R.K., Meyer, L.A. (Eds.), Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, IPCC, 630 Geneva, pp. 151.
Kayanne, H., Hata, H., Kudo, S., Yamano, H., Watanabe, A., Ikeda, Y., Nozaki, K., Kato, K., Negishi, A., Saito, H., 2005. Seasonal and bleaching-induced changes in coral reef metabolism and CO_2 flux. Glob. Biogeochem. Cycles 19, 1–11. https://doi.org/10.1029/2004GB002400.
Kornder, N.A., Riegl, B.M., Figueiredo, J., 2018. Thresholds and drivers of coral calcification responses to climate change. Glob. Change Biol. 24, 5084–5095. https://doi.org/10.1111/gcb.14431.
Kroeker, K.J., Kordas, R.L., Crim, R.N., Singh, G.G., 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecol. Lett. 13, 1419–1434. https://doi.org/10.1111/j.1461-0248.2010.01518.x.
Kroeker, K.J., Kordas, R.L., Crim, R.N., Hendriks, I.E., Ramajo, L., Singh, G.S., Duarte, C.M., Gattuso, J., 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Glob. Change Biol. 19, 1884–1896. https://doi.org/10.1111/gcb.12179.
Lavigne, H., Gattuso, J.P., 2013. Seacarb, seawater carbonate chemistry with R. R package version 2.4.10. Available at: https://CRAN.Rproject.org/package=seacarb (accessed 1 April 2018).
Leclercq, N., Gattuso, J.P., Jaubert, J., 2002. Primary production, respiration, and calcification of a coral reef mesocosm under increased CO_2 partial pressure. Limnol. Oceanogr. 47, 558–564. https://doi.org/10.4319/lo.2002.47.2.0558.
Liew, Y.J., Zoccola, D., Li, Y., Tambutté, E., Venn, A.A., Michell, C.T., Cui, G., Deutekom, E.S., Kaandorp, J.A., Voolstra, C.R., Forêt, S., Allemand, D., Tambutté, S., Aranda, M., 2018. Epigenome-associated phenotypic acclimatization to ocean acidification in a reef-building coral. Sci. Adv. 4, 1–10. https://doi.org/10.1126/sciadv.aar8028.
Lueker, T.J., Dickson, A.G., Keeling, C.D., 2000. Ocean pCO_2 calculated from dissolved inorganic carbon, alkalinity, and equations for K_1 and K_2; validation based on laboratory measurements of CO_2 in gas and seawater at equilibrium. Mar. Chem. 70, 105–119. https://doi.org/10.1016/S0304-4203(00)00022-0.
Manzello, D.P., 2010. Ocean acidification hot spots: spatiotemporal dynamics of the seawater CO_2 system of eastern Pacific coral reefs. Limnol. Oceanogr. 55, 239–248. https://doi.org/10.4319/lo.2010.55.1.0239.
Martin, S., Gattuso, J.P., 2009. Response of Mediterranean coralline algae to ocean acidification and elevated temperature. Glob. Change Biol. 15, 2089–2100. https://doi.org/10.1111/j.1365-2486.2009.01874.x.
Marubini, F., Atkinson, M.J., 1999. Effects of lowered pH and elevated nitrate on coral calcification. Mar. Ecol. Prog. Ser. 188, 117–121. https://doi.org/10.3354/meps188117.
McElhany, P., Busch, D.S., 2013. Appropriate pCO_2 treatments in ocean acidification experiments. Mar. Biol. 160, 1807–1812. https://doi.org/10.1007/s00227-012-2052-0.
Muscatine, L., 1990. The role of symbiotic algae in carbon and energy flux in coral reefs. In: Dubinsky, Z., Stambler, N. (Eds.), Ecosystems of the World. Elsevier Science Publishing Company, Inc., Netherlands, pp. 75–87.
Orr, J.C., Fabry, V.J., Aumont, O., Bopp, L., Doney, S.C., Feely, R.A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R.M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R.G., Plattner, G., Rodgers, K.B., Sabine, C.L., Sarmiento, J.L., Schlitzer, R., Slater, R.D., Totterdell, I.J., Weirig, M., Yamanaka, Y., Yool, A., 2005. Anthropogenic Ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437, 681–686. https://doi.org/10.1038/nature04095.
Page, H.N., Courtney, T.A., Collins, A., De Carlo, E.H., Andersson, A.J., 2017. Net community metabolism and seawater carbonate chemistry scale non-intuitively with coral cover. Front. Mar. Sci. 4. https://doi.org/10.3389/fmars.2017.00161.
Page, H.N., Courtney, T.A., De Carlo, E.H., Howins, N.M., Koester, I., Andersson, A.J., 2018. Spatiotemporal variability in seawater carbon chemistry for a coral reef flat in Kāne'ohe Bay. Hawai'i. Limnol. Oceanogr. 64, 913–934. https://doi.org/10.1002/lno.11084.
Perez, F.F., Fraga, F., 1987. Association constant of fluoride and hydrogen ions in seawater. Mar. Chem. 21, 161–168. https://doi.org/10.1016/0304-4203(87)90036-3.
Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., Core Team, R., 2018. Nlme: linear and nonlinear mixed effects models. R package version 3, 1–137. Available at: https://CRAN.R-project.org/package=nlme, Accessed date: 1 April 2018.
Price, N.N., Martz, T.R., Brainard, R.E., Smith, J.E., 2012. Diel variability in seawater pH relates to calcification and benthic community structure on coral reefs. PLoS One 7, 1–9. https://doi.org/10.1371/journal.pone.0043843.
Putnam, H.M., Davidson, J.M., Gates, R.D., 2016. Ocean acidification influences host DNA methylation and phenotypic plasticity in environmentally susceptible corals. Evol. Appl. 9, 1165–1178. https://doi.org/10.1111/eva.12408.
Quinn, G.P., Keough, M.J., 2011. Experimental Design and Data Analysis for Biologists. Cambridge University Press.
Ries, J.B., Cohen, A.L., McCorkle, D.C., 2009a. A nonlinear calcification response to CO_2-induced ocean acidification by the coral *Oculina arbuscula*. Coral Reefs 29, 661–674. https://doi.org/10.1007/s00338-010-0632-3.
Ries, J.B., Cohen, A.L., McCorkle, D.C., 2009b. Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. Geol. 37, 1131–1134. https://doi.org/10.1130/G30210A.1.
Schoepf, V., Hu, X., Holcomb, M., Cai, W., Li, Q., Wang, Y., Xu, H., Warner, M.E., Melman, T.F., Hoadley, K.D., Pettay, D.T., Matsui, Y., Baumann, J.H., Grottoli, A.G., 2017.

Coral calcification under environmental change: a direct comparison of the alkalinity anomaly and buoyant weight techniques. Coral Reefs 36, 13–25. https://doi.org/10.1007/s00338-016-1507-z.

Shaw, E.C., McNeil, B.I., Tilbrook, B., 2012. Impacts of ocean acidification in naturally variable coral reef flat ecosystems. J. of Geophys. Res. 117, 1–14. https://doi.org/10.1029/2011JC007655.

Shaw, E.C., Phinn, S.R., Tilbrook, B., Steven, A., 2015. Natural in situ relationships suggest coral reef calcium carbonate production will decline with ocean acidification. Limnol. Oceanogr. 60, 777–788. https://doi.org/10.1002/lno.10048.

Silbiger, N.J., Donahue, M.J., 2015. Secondary calcification and dissolution respond differentially to future ocean conditions. Biogeosciences 12, 567–578. https://doi.org/10.5194/bg-12-567-2015.

Silbiger, N.J., Sorte, C.J.B., 2018. Biophysical feedbacks mediate carbonate chemistry in coastal ecosystems across spatiotemporal gradients. Sci. Rep. 8, 796. https://doi.org/10.1038/s41598-017-18736-6.

Silverman, J., Lazar, B., Erez, J., 2007. Community metabolism of a coral reef exposed to naturally varying dissolved inorganic nutrient loads. Biogeochemistry 84, 67–82. https://doi.org/10.1007/s10533-007-9075-5.

Smith, S.V., 1973. Carbon dioxide dynamics: a record of organic carbon production, respiration, and calcification in the Eniwetok reef flat community. Limnol. Oceanogr. 18, 106–120.

Smith, S.V., Key, G.S., 1975. Carbon dioxide and metabolism in marine environments. Limnol. Oceanogr. 20, 493–495.

Spencer Davies, P., 1989. Short-term growth measurements of corals using an accurate buoyant weighing technique. Mar. Biol. 101, 389–395. https://doi.org/10.1007/BF00428135.

Stimson, J., Kinzie, R.A., 1991. The temporal pattern and rate of release of zooxanthellae from the reef coral *Pocillopora damicornis* (Linnaeus) under nitrogen-enrichment and control conditions. J. Exp. Mar. Biol. Ecol. 153, 63–74. https://doi.org/10.1016/S0022-0981(05)80006-1.

Tambutté, S., Holcomb, M., Ferrier-Pages, C., Reynaud, S., Tambutté, E., Zoccola, D., Allemand, D., 2011. Coral biomineralization: from the gene to the environment. J. Exp. Mar. Bio. Ecol. 408, 58–78. https://doi.org/10.1016/j.jembe.2011.07.026.

Torda, G., Donelson, J.M., Aranda, M., Barshis, D.J., Bay, L., Berumen, M.L., Bourne, D.G., Cantin, N., Foret, S., Matz, M., Miller, D.J., Moya, A., Putnam, H.M., Ravasi, T., van Oppen, M.J.H., Vega Thurber, R., Vidal-Dupiol, J., Voolstra, C.R., Watson, S., Whitelaw, E., Willis, B.L., Munday, P.L., 2017. Rapid adaptive responses to climate change in corals. Nat. Clim. Chang. 7, 627–636.

Towle, E.K., Enochs, I.C., Langdon, C., 2015. Threatened Caribbean coral is able to mitigate the adverse effects of ocean acidification on calcification by increasing feeding rate. PLoS One 10, 1–17. https://doi.org/10.1371/journal.pone.0139398.

Walther, K., Anger, K., Portner, H.O., 2010. Effects of ocean acidification and warming on the larval development of the spider crab *Hyas araneus* from different latitudes (54° vs. 79° N). Mar. Ecol. Prog. Ser. 417, 159–170. https://doi.org/10.3354/meps08807.

Wild, C., Hoegh-Guldberg, O., Naumann, M.S., Colombo-Pallotta, M.F., Ateweberhan, M., Fitt, W.K., Iglesias-Prieto, R., Palmer, C., Bythell, J.C., Ortiz, J.C., Loya, Y., van Woesik, R., 2011. Climate change impedes scleractinian corals as primary reef ecosystem engineers. Mar. Freshw. Res. 62, 205–215. https://doi.org/10.1071/MF10254.

Yates, K.K., Halley, R.B., 2006. CO_3^{2-} concentration and pCO_2 thresholds for calcification and dissolution on the Molokai reef flat, Hawaii. Biogeosciences 3, 357–369. https://doi.org/10.5194/bg-3-357-2006.