Diurnally Fluctuating pCO2 Modifies the Physiological Responses of Coral Recruits Under Ocean Acidification

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INTRODUCTION

Since the Industrial Revolution, about one third of human-emitted CO2 has been absorbed by the ocean, resulting in ocean acidification (OA), a phenomenon characterized by declines in seawater pH, carbonate concentration and saturation state of calcium carbonate (CaCO3) (Sabine et al., 2004). Relative to pre-industrial levels, average surface ocean pH has decreased by 0.1 units, and...
a further reduction of 0.3–0.4 units is projected by the end of this century based on “Business as usual” scenario (Orr et al., 2005; Gattuso et al., 2015).

Ocean acidification constitutes one of the most serious threats to various marine calcifying taxa because it reduces the availability of carbonate ions that are needed to accrete CaCO$_3$ (Orr et al., 2005); among them reef corals, which construct and maintain complex reef framework structures, are extensively studied (Chan and Connolly, 2013; Kroeker et al., 2013). Numerous laboratory experiments have demonstrated negative yet variable effects of OA on coral skeletal growth, with a mean decline in calcification of 15% per unit decrease in aragonite saturation state ($\Omega_{AR}$) (Chan and Connolly, 2013). In these empirical studies, reef corals have been exposed to static pH levels consistent with open ocean projections of seawater pH declines of 0.3–0.4 units by the year 2100. However, compared with pelagic ocean environments, the carbonate chemistry on coral reef ecosystems is highly dynamic. Large daily swings in seawater pH and $p$CO$_2$, mainly driven by biological activities (photosynthesis and respiration), have been recorded in many reef locations around the world. During daytime, uptake of CO$_2$ and HCO$_3^-$ by photosynthesis decreases seawater $p$CO$_2$ and elevates pH, while nighttime respiration decreases pH and increases $p$CO$_2$. Natural variability in carbonate chemistry is particularly characteristic of the shallow coastal reefs (Rivest et al., 2017), and pH and $p$CO$_2$ could vary by up to 0.7 units and 900 μatm, respectively, over a diel cycle (Santos et al., 2011; Shaw et al., 2012; Chen et al., 2015; Silverman et al., 2015). This environmental variability may greatly confound our current understanding and predictions of OA consequences on marine organisms, especially for those inhabiting high-variance ecosystems (Rivest et al., 2017). Therefore, focus is now shifting to how natural $p$CO$_2$ fluctuations will interact with increasing $p$CO$_2$ levels to affect the future performance of shallow marine organisms.

Recent evidence suggests that reef calcifiers respond very differently to constant elevated $p$CO$_2$ than to oscillating $p$CO$_2$ that simulates the daily variations in carbonate chemistry on shallow reefs. For instance, recruits of the reef coral Seriatopora caliendrum exposed to ecologically relevant $p$CO$_2$ fluctuations exhibited higher rates of calcification and survival compared to those under ambient or high $p$CO$_2$ (Dufault et al., 2012). Similarly, diel $p$CO$_2$ oscillations totally negate the inhibition of calcification by OA in corals Acropora formosa (Chan and Eggnis, 2017) and A. hyacinthus (Comeau et al., 2014b), and variable $p$CO$_2$ partially offset the OA-induced depression in calcification by crustose coralline algae (CCA) Porolithon onkodes (Johnson et al., 2014). In contrast, periods of high pH in the daytime and low pH at night act additively with OA to reduce the skeletal growth of adult and juvenile coralline algae Arthrocardia corymbosa (Cornwall et al., 2013; Roleda et al., 2015). Another recent study demonstrated that calcification rates of the coral Goniodora sp. and the CCA Hydroolithon reinholdii exhibited limited response to both OA and extreme pH fluctuations, possibly due to strong control over carbonate chemistry within the calcifying fluid (Cornwall et al., 2018). Differential calcification responses to diurnal $p$CO$_2$ fluctuations that are typical on shallow tropical reefs, together with OA, give rise to a clear need for more thorough studies of the influence of dynamic $p$CO$_2$ on calcification, especially under acidified seawater.

Furthermore, little is known about the biochemical mechanisms of these observed calcification responses of reef corals to future OA conditions. Symbiotic scleractinian corals are known to calcify faster in the light than in the dark, a phenomenon called “light enhanced calcification” (LEC) (Allemand et al., 2011). Photosynthesis by endosymbionts (i.e., zooxanthellae), which contributes greatly to the energy needs of coral holobiont (Muscatine et al., 1981), has been considered the primary cause of LEC. During daytime, photosynthesis consumes CO$_2$ and helps to maintain intracellular pH and elevated CO$_2^-$ and aragonite saturation state ($\Omega_{AR}$) (Gibbin et al., 2014). Together, this creates conditions favorable for calcification (McCulloch et al., 2012; DeCarlo et al., 2018). Meanwhile, release of hydroxide ions (OH$^-$) from photosynthesis makes the coelenteron an alkaline environment, supporting the titration of the protons (H$^+$) produced by calcification (Moya et al., 2008; Comeau et al., 2013a).

Carbonic anhydrase (CA), which catalyzes the interconversion between bicarbonate (HCO$_3^-$) and CO$_2$, is central to carbon supply for calcification, conversion of metabolic CO$_2$ to prevent night acidosis, and carbon concentrating for photosynthesis by zooxanthellae (Leggat et al., 1999, 2002; Moya et al., 2008; Bertucci et al., 2013). Moreover, Ca-ATPase and Mg-ATPase are hypothesized to transport Ca$^{2+}$ and Mg$^{2+}$ into the extracellular calcifying fluid (ECF) and simultaneously remove H$^+$ from ECF in reef corals (Ip and Lim, 1991; Ip et al., 1991; Meibom et al., 2004; Zoccola et al., 2004; Allison et al., 2011), thus driving the calcification reaction toward CaCO$_3$ precipitation (Al-Horani et al., 2003; Allemand et al., 2011). Although studies have shown that OA could dramatically change the gene expression of CA, Ca-ATPase, and Mg-ATPase (Kaniewska et al., 2012; Vidal-Dupiol et al., 2013; Kurihara et al., 2018), the effects of elevated and fluctuating $p$CO$_2$ on the functions of these crucial molecules, and more importantly, the way in which they may coordinate to regulate photosynthesis and calcification under future OA remain largely unexplored.

Further, OA has been demonstrated to increase reactive oxygen species (ROS) generation and induce oxidative stress in calcifying organisms (Matozzo et al., 2013; Mangan et al., 2017; Luz et al., 2018). As the energetic costs of calcification and acid-base regulation are expected to increase as $p$CO$_2$ rises (Cohen and Holcomb, 2009), this may reduce the energy availability for ROS scavenging by the antioxidant system, also a high energy-demanding process. Again, current literature provides little information on the effects of seawater acidification on the oxidative stress and antioxidant functioning for scleractinian corals.

To address these critical knowledge gaps, the present study investigated the physiological and biochemical responses of the reef coral Pocillopora damicornis to diel $p$CO$_2$ fluctuations that are characteristic of their natural settings. P. damicornis is a widely distributed and major reef-building coral on reef flats in the Indo-Pacific region and broods symbiotic planula larvae.
with maternally derived zooxanthellae (Veron, 1993). Several prior studies demonstrated that *P. damicornis* is resistant to OA, with unaffected calcification under high $p$CO$_2$ (Comeau et al., 2013b, 2014a, 2015). In our study site Luhuitou fringing reef, calcification by adult *P. damicornis* even responded positively to elevated $p$CO$_2$, suggesting the local acclimatization/adaptation to OA (Huang et al., 2014), while the post-settlement calcification and growth are highly susceptible to OA conditions (Jiang et al., 2015, 2018). Here, we extended our use of new recruits of *P. damicornis* and examined the effects of stable and fluctuating OA on a suite of physiological traits, including photochemical performance, survivorship and early development. In addition, oxidative stress and activities of CA, Ca-ATPase and Mg-ATPase putatively involved in photosynthesis and calcification were measured to illuminate the physiological changes of juvenile corals.

**MATERIALS AND METHODS**

**Study Site and in situ $p$CO$_2$ Profiling**

To quantify the pattern of diel $p$CO$_2$ oscillations currently experienced by corals, seawater was continuously pumped from 2 m depth on Luhuitou fringing reef into a flow-through tank on the beach. Seawater $p$CO$_2$ was measured *in situ* in the tank using a Picarro CRDS (Cavity Ring-Down Spectroscopy) analyzer. The accuracy of CRDS analyzer was verified by measuring certified reference gas standards, as per the manufacturer’s instructions. Seawater $p$CO$_2$ was monitored for 7 days from 20 to 27 August 2017.

**Coral Sampling and Larval Settlement**

Ten mature colonies of *P. damicornis* were collected at 2 m depth on Luhuitou reef (N18°12.7’, E109°28.5’) by snorkeling on August 26, 2017. Colonies were transported to the Tropical Marine Biological Research Station, placed into individual 20 L tanks with flow-through seawater at ambient temperature (28.6 ± 0.2°C) and exposed to partially shaded sunlight (noon irradiance, ca. 300 μmol photons m$^{-2}$ s$^{-1}$). The outflow of each tank was passed through a cup fitted with a 180 μm mesh on the bottom to trap larvae. Larvae were collected at 08:00 on August 30, 2017 and then pooled across colonies. Groups of approximately 60 larvae were introduced into plastic petri-dishes, and settlement was induced by small chips of crustose coralline algae *Porolithon onkodes*. Twelve hours later, unsettled larvae and algal chips were discarded. Five dishes with a total of approximately 200 primary polyps were allocated to each experimental tank. The newly settled corals were reared for 7 days under three $p$CO$_2$ treatments as described below.

**Experimental Setup**

Juvenile corals were exposed to three $p$CO$_2$ treatments: (1) steady and ambient $p$CO$_2$ (Control); (2) steady and elevated $p$CO$_2$ (Stable OA); and (3) diurnally fluctuating and elevated $p$CO$_2$ (Fluctuating OA). It is important to point out that the ambient $p$CO$_2$ of seawater measured here was much higher than the open ocean level. However, it just reflected the current conditions in this coastal reef ecosystem and was comparable to the values previously reported at this location (Zhang et al., 2013; Chen et al., 2015; Yan et al., 2016). The $p$CO$_2$ of the stable OA treatment was chosen based on the projections by the end of this century under RCP8.5 (Gattuso et al., 2015). The fluctuating OA treatment was created by superimposing the current diurnal variance onto the $p$CO$_2$ level predicted for 2100 (Cornwall et al., 2013; Camp et al., 2016). Limited facility precluded an additional, fluctuating treatment at ambient $p$CO$_2$, but this limitation did not affect the main objective of this study, i.e., comparing the physiological responses of juvenile corals to stable and oscillatory OA conditions. The experimental $p$CO$_2$ treatments were constructed in nine 25-L tanks, with three replicate tanks for each treatment. Each tank was filled with 0.5 μm-filtered and UV-sterilized seawater. Seawater within each tank was well mixed using submerged pumps (350 L h$^{-1}$) and partially (30%) changed at 20:00 every day. Each tank was covered with a transparent lid to minimize gas exchange and maintain experimental $p$CO$_2$ levels. The two steady regimes were established by bubbling with ambient air or elevated $p$CO$_2$ (1000 ppm), which was achieved by mixing air with CO$_2$ using a CO$_2$ enricher (CE100B, Ruihua, China). The fluctuating treatment was established by changing $p$CO$_2$ settings of the CO$_2$ enricher every 6 h. Preliminary high-resolution pH monitoring (every hour for 1 day) showed that this method successfully achieved step-wise and gradual decrease and increase in seawater pH, following a natural daily cycle. Mean pH of the fluctuating OA treatment was comparable to that in the stable OA treatment (7.82 vs. 7.81).

The seawater temperature in each tank was controlled independently with digital temperature controllers and titanium heaters at a targeted value of 29 ± 0.4°C (mean ± SD), which corresponded to the ambient and long-term mean summer temperature at the study site. Light was provided on a 12:12 h light-dark cycle between 07:00 and 19:00 h using T5 fluorescent lamps. The photosynthetically active radiation was about 200 μmol photons m$^{-2}$ s$^{-1}$, approximating that recorded in crevices preferred by juvenile corals at 2–3 m depths on Luhuitou reef (Lei Jiang, unpublished data). Seawater samples (100 mL) were collected from each tank at 06:00 and 18:00 every other day for the measurement of pH, salinity and total alkalinity. pH and salinity were measured with a Thermo Orion 5-star meter and the pH electrode was two-point calibrated with NBS buffers every other day. Total alkalinity (TA) was measured with an automatic titrator (AS-ALK2, Apollo, United States). The carbonate chemistry parameters were calculated using the CO2SYS program and seawater conditions for each treatment are presented in Table 1.

**Chlorophyll Fluorescence Measurement**

The photo-physiology of the symbionts was assessed using four distinct parameters associated with chlorophyll fluorescence. Five recruits from each replicate tank were randomly selected on the last day of the experiment, and a Diving-PAM (Pulse Amplitude Modulated) fluorometer (Walz GmbH, Germany) was used to assess the chlorophyll fluorescence parameters of *in Hopkins* symbionts. The fiber-optic probe was equipped with a plastic tube to ensure consistent probe orientation and
distance of 2 mm between probe and corals. Maximum and effective quantum yields (Fv/Fm and ΔF/Fm′) were measured for the same batch of recruits from each tank using the equations of Genty et al. (1989): 

\[ \frac{F_v}{F_m} = \frac{(F_m - F_o)/F_m}{(F_m' - F_i')/F_m'} \]

where \( F_m = \) maximum fluorescence yield, \( F_o = \) fluorescence yield in darkness, \( F_i' = \) fluorescence yield in actinic light and \( F_m' = \) maximum fluorescence yield in actinic light. \( F_v/F_m \) was measured at 06:00 to ensure enough time for dark adaptation and relaxation of photochemical quenching. 

\( F_v/F_m \) provides a measure of the maximum photochemical efficiency of photosystem II (PSII), with substantial declines indicating damage to the photosynthetic apparatus (Jones et al., 1998). \( \Delta F/F_m' \) was measured at 17:00 in a light-adapted state. This ratio assesses the actual light use efficiency to drive photochemical processes (Maxwell and Johnson, 2000). The depression of \( \Delta F/F_m' \) relative to \( F_v/F_m \) reflects the extent of non-photochemical quenching (NPQ), which is determined as 

\[ (F_m - F_m')/F_m' \]

Finally, maximum citation pressure over PSII (Qm) is determined from 

\[ 1 - \frac{[(\Delta F/F_m')/17:00]/(F_v/F_m)]}{[Q_m]} \]

with values close to 0 indicating that most of the reaction centers are open, while values close to 1 denoting mostly closed reaction centers and photo-inhibition (Iglesias-Prieto et al., 2004).

**Survival and Growth**

Recruits were checked daily and the number of dead corals was recorded based on loss of polyp tissue and presence of bare skeleton. On the last day of the experiment, 15−20 recruits were randomly selected from each tank, photographed under a dissecting microscope for the growth measurements, and the number of new buds counted for each recruit. Images with a scale bar were analyzed for lateral growth using ImageJ software (National Institutes of Health). Growth was estimated as the rates of change in planar area and number of new polyps over time (Dufault et al., 2012; Jiang et al., 2018). Upon completion of the experiment, 10 recruits were sampled from each tank and analyzed for skeletal weight and ash-free tissue biomass using a Mettler-Toledo ultramicrobalance at an accuracy of ±1 µg according to Aulaf et al. (2011).

**Enzymatic and Oxidative Stress Assays**

On the last day of the experiment, 4 groups of 15 recruits from each tank were randomly sampled using sterilized razor blades at 06:00 and 18:00. Two samples from each time were assayed for activities of Ca-ATPase and Mg-ATPase following modified protocols of Chan et al. (1986) and Prazeres et al. (2015). The remaining two samples were used to measure CA activities using the pH drift method (Weis et al., 1989). All samples were immediately snap-frozen in liquid nitrogen, transported on dry-ice to the lab in Guangzhou and preserved at −80°C. Detailed procedures of these assays are provided in the electronic Supplementary Material.

In addition, two batches of 10 recruits were sampled from each tank to analyze the signs of oxidative stress, including catalase (CAT) activity and malondialdehyde (MDA) content. Up-regulation of CAT reflects an organism’s capacity to detoxify ROS, and MDA is an indicator of cellular oxidative damage and lipid peroxidation. CAT was assayed using the Catalase assay kit (Beyotime, China) as per the manufacturer’s instructions. MDA content was determined as the thiobarbituric acid reactive metabolites (Cameo et al., 1998) using a Lipid Peroxidation Assay Kit (Sigma-Aldrich, United States).

**Data Analyses**

All response data were initially tested using a nested ANOVA with tank as a random factor nested within pCO₂ treatment. However, as the tank factor was non-significant (electronic Supplementary Material), it was dropped from the statistical model to enhance the power of the analysis (Quinn and Keough, 2002), and the analyses were repeated using corals as independent replicates. To assess the effects of pCO₂ treatments on photo-physiology, growth, CAT activity and lipid peroxidation, one-way ANOVAs were applied with Fisher’s Least Significant Difference (LSD) as planned post hoc multiple comparisons. The enzymatic activities were analyzed with two-way ANOVAs followed by Fisher’s LSD, with pCO₂ and time as fixed effects. Further post hoc tests for pairwise comparison of the effect of time on the enzymatic activities at each pCO₂ treatment were performed using Student’s t-test.

**RESULTS**

**Environmental and Experimental Seawater pCO₂**

Seawater pCO₂ at 2 m depth on Luhuitou fringing reef ranged from 215 to 1077 µatm over 7 days in summer (Figure 1A), averaging 528 ± 163 µatm (mean ± SD). A prominent diel cycle was present, with the lowest value before dusk and the highest value near dawn. Mean diurnal range of seawater pCO₂ was 518 ± 220 µatm (mean ± SD, range: 247–862 µatm).

Diurnal variations in seawater pH for each treatment are shown in Figure 1B. In the fluctuating OA treatment, pH decreased after 18:00 until reaching a minimum at 06:00 the next morning (Figure 1B). The pCO₂ values for the control, stable OA and fluctuating OA treatments were 508 ± 32, 1115 ± 77, and 1217 ± 621 µatm, respectively (mean ± SD, Table 1).

**TABLE 1** | Mean (±SD) physical and chemical parameters for each treatment.

| Treatment | pHNBS | Salinity (psu) | TA (µmol kg⁻¹) | DIC (µmol kg⁻¹) | pCO₂ (µatm) | Ω₂H₋₋cdc |
|-----------|-------|---------------|----------------|-----------------|-------------|-----------|
| Control   | 8.11 ± 0.02 | 33.4 ± 0.6    | 2245 ± 87      | 1981 ± 78       | 508 ± 32    | 3.13 ± 0.2 |
| Stable OA | 7.81 ± 0.02 | 33.2 ± 0.4    | 2195 ± 81      | 2074 ± 78       | 1115 ± 77   | 1.70 ± 0.12 |
| Fluct OA  | 7.82 ± 0.12 | 33.3 ± 0.6    | 2158 ± 126     | 2032 ± 146      | 1217 ± 621  | 1.77 ± 0.73 |
Biomass per individual ranged from 106 to 111 µg.

Figure 2C compared to the other treatments ($F_{2,15} = 0.051$), and reduced by 10% in the fluctuating OA treatment. Mean tissue biomass per individual varied from 106 to 111 µg, and was similar among treatments ($F_{2,87} = 0.20, p = 0.818$; Figure 2C). Lateral growth was affected by $pCO_2$ treatments ($F_{2,155} = 3.12, p = 0.047$), and reduced by 8% under stable OA relative to control and fluctuating OA (Figure 2D). $pCO_2$ treatments significantly influenced asexual budding ($F_{2,155} = 3.33, p = 0.038$), and the budding rate in the fluctuating OA treatment was only about half that in the other two treatments (Figure 2D).

**CAT and Lipid Peroxidation**

Host CAT activity was significantly elevated by OA treatments ($F_{2,15} = 4.44, P = 0.03$), but was similar between the stable and fluctuating OA treatments (Figure 3A). There was also a significant effect of $pCO_2$ treatments on MDA content in host tissue ($F_{2,15} = 6.39, P = 0.01$). MDA concentrations were 55 and 51% higher for corals under stable and fluctuating OA than that in the control, respectively (Figure 3B).

**Carbonic Anhydrase**

Both host and symbiont CA varied across $pCO_2$ treatments and between night and day, and treatment and time had no significant interactive effects (Table 2). Compared to the control, host CA was significantly elevated in the stable OA treatment and reduced in the fluctuating OA treatment (Figure 4A). Post hoc analyses showed that host CA in the fluctuating OA treatment differed significantly between light and dark conditions (Student $t$-test, $df = 10, t = 3.905, p = 0.003$). Furthermore, corals in both OA treatments exhibited significantly higher symbiont CA activities (Figure 4B). Post hoc analyses revealed that symbiont CA in the control and fluctuating OA treatments was significantly lower in the dark than that in the light (Student $t$-test, $df = 10, t = -4.885, p = 0.001$).

**Ca-ATPase and Mg-ATPase**

In general, there was a significant effect of $pCO_2$ treatments on the activities of Ca-ATPase and Mg-ATPase (Table 2), both of which were greatly elevated in the fluctuating OA treatment compared to the other 2 treatments. Relative to the control and stable OA treatments, Ca-ATPase activity in the fluctuating OA treatment increased by 35–43% and 78–91% during light and dark periods, respectively (Figure 4C). However, such pattern was less clear for Mg-ATPase activity, which was elevated in the fluctuating OA treatment by 21–41% and 25–33% in dark and light conditions, respectively. Furthermore, Ca-ATPase activity was unaffected by time (Table 2); by contrast, Mg-ATPase activities differed significantly between light and dark conditions (Table 2), largely driven by the higher nighttime Mg-ATPase activities under stable OA (Student $t$-test, $df = 10, t = -5.19, p < 0.001$; Figure 4D).

**DISCUSSION**

The present study revealed significant differences in the physiological responses of juvenile *P. damicornis* exposed to constant and fluctuating OA. Results showed that the fluctuating $pCO_2$ regime depressed the stimulatory effect of OA on photosynthetic activity. Further, corals significantly up-regulated the proton pumps and antioxidant CAT in the fluctuating OA treatment, in which calcification was only slightly reduced. However, asexual budding declined by 50% and oxidative damage still occurred under fluctuating OA conditions.
OA. Together, although the photochemical performance was enhanced by the fluctuating OA treatment, it was unable to fully compensate for the increased energy expense for coral recruits. More importantly, in the fluctuating OA treatment, corals appeared to compromise asexual reproduction and ROS detoxification to sustain skeletal growth, indicating potential trade-offs between calcification and other key physiological processes. These findings suggest that diurnal variability in pH/carbonate chemistry is likely to be an overriding factor influencing and determining the early success and recruitment of corals under future OA. Our study also highlights the importance of considering a broader spectrum of physiological traits in order to accurately and fully characterize the overall change in fitness and the possible trade-offs between different physiological functions when addressing corals’ responses to environmental stress.

Boosted Photo-Physiology and Potential Involvement of Symbiont CA Under OA

Consistent with our previous findings (Jiang et al., 2015, 2018), this study showed that OA did not influence $F_v/F_m$, indicating that there was no photo-inhibition or damage to the photosynthetic apparatus. Further, both OA treatments greatly improved the photosynthetic efficiency of in hospite symbionts, as evidenced by the elevated $\Delta F/F_m'$ and decreased $NPQ$ and $Q_m$. These findings suggest that, under elevated $pCO_2$, more electrons are being transported for carbon fixation and the photochemical process is more competitive for reaction centers than non-photochemical quenching. A similar photo-physiological response was observed in our previous study on P. damicornis recruits exposed to increased $pCO_2$ (Jiang et al., 2015).

Zooxanthellae possess the type II Rubisco with low CO$_2$ affinity, and are therefore carbon-limited under current $pCO_2$ levels (Leggat et al., 2002). However, CO$_2$ enrichment effects on photosynthesis are occasionally observed in reef corals. For example, Noonan and Fabricius (2016) showed that increased $pCO_2$ significantly promoted photosynthetic productivity in reef coral S. hystrix from central Great Barrier Reef, but not in A. millepora. In contrast, Strahl et al. (2015) reported that net photosynthesis of A. millepora and massive Porites spp. from volcanic CO$_2$ seeps in Papua New Guinea increased considerably with $pCO_2$, whereas this phenomenon was not observed in S. hystrix and P. damicornis. Additionally, parabolic responses of photochemical process to $pCO_2$ have also been documented (Crawley et al., 2010; Castillo et al., 2014). Hence, photo-physiological responses to OA in reef corals may be species-specific and context-dependent. Moreover, different $pCO_2$ levels and the diverse DIC utilization modes of symbionts...
Oxidative Stress in Response to OA

Despite the positive photochemical response to OA, we found that both OA regimes elicited a significant and similar increase in activity of the antioxidant CAT. Nevertheless, the elevated CAT activity was not effective against the damaging ROS, and lipid peroxidation still increased under OA, as evidenced by the significant differences in mean values among treatments.

> 50% increases in MDA contents, a specific end-product of the oxidative degradation of lipids. This supports previous reports on a range of calcifying marine invertebrates exposed to OA, including the reef coral P. capitata (Soriano-Santiago et al., 2013), the hydrocoral Millepora alcicornis (Luz et al., 2018) and the mussel Mytilus edulis (Mangan et al., 2017).

Reasons for this oxidative stress under OA conditions still remain unclear, particularly for corals under stable OA with considerable energetic benefits from higher photochemical activity. One parsimonious cause is that the increased photosynthetic electron flux under OA could induce the photo-reduction of oxygen, i.e., operation of the Mehler reaction at higher rates, ultimately resulting in the production of damaging hydrogen peroxide and superoxide which would subsequently diffuse into coral cytoplasm (Miyake and Yokota, 2000). ROS accumulates once the scavenging capacity of antioxidant system is exceeded, causing oxidative damage to a range of cell components, such as lipids, nucleic acids and proteins (Asada, 1999). If this is the case, the oxidative stress as measured by lipid peroxidation would have serious repercussions for the holobiont health and its capacity to cope with chronic OA exposure. Further research is clearly needed to pinpoint the exact mechanism behind the oxidative stress caused by OA in reef corals.

\( p\text{CO}_2 \) Regime Dictates Coral Calcification and Host CA Function Under OA

Paradoxically, while stable OA reduced lateral growth, it did not affect calcification, suggesting that linear extension and calcification may be decoupled in newly settled corals under OA. The lack of response in early development to static OA contrasts with prior work reporting that steady declines in pH...
FIGURE 4 | Enzymatic activities of *P. damicornis* recruits exposed to ambient pCO$_2$ (Control), steady-high pCO$_2$ (Stable OA), and fluctuating-high pCO$_2$ (Fluct OA). (A) Host CA; (B) symbiont CA; (C) Ca-ATPase; and (D) Mg-ATPase. Data shown as mean ± SE. Different letters indicate statistically significant differences in mean values among treatments, while asterisks denote significantly different means between night and day within each treatment.

Considerably reduced linear growth and calcification of coral recruits (Albright et al., 2010; Jiang et al., 2015, 2018). The most plausible explanation for this discrepancy is the short exposure duration in this study, during which energy reserves might be adequate to sustain calcification. Unexpectedly, calcification was more adversely affected by fluctuating OA than stable OA, which is in strong contrast to prior studies demonstrating either no, partial or complete mitigation of negative OA effects on calcification in adult corals by diel pCO$_2$ oscillations (Comeau et al., 2014b; Chan and Eggoins, 2017; Cornwall et al., 2018). Furthermore, Dufault et al. (2012) reported that calcification by new recruits of *S. caliendrum* from Hobihu, Taiwan responded positively to diurnally fluctuating pCO$_2$. It should be noted that the seawater pCO$_2$ range (365–515 µatm) at the study site of Dufault et al. (2012) was narrower than that at our location (215–1077 µatm). Moreover, OA treatments were more extreme in this study and the nighttime pCO$_2$ of the fluctuating OA treatment in this study was much higher than that of Dufault et al. (2012). Collectively, these large differences between pCO$_2$ histories, treatment conditions and also life stages likely account for this magnitude of pCO$_2$ fluctuations influence on coral skeletal growth.

The greater sensitivity of calcification by coral recruits to fluctuating OA than to stable OA can be attributed to at least three non-exclusive reasons. Firstly, the higher photosynthetic activity under stable OA could better promote and fuel calcification, either through direct energy supply for calcification or through the generation of OH$^-$ which help neutralize the H$^+$ released from ECF, and thus facilitate a higher pH gradient for CaCO$_3$ precipitation (Jokiel, 2011; Comeau et al., 2013a; Gibbin et al., 2014). Secondly, in light of the comparable tissue biomass and the decreased lateral growth in the stable OA treatment, area-normalized biomass was higher in stable OA than fluctuating OA. Hence, the thicker tissue layer of recruits under stable OA could create a better separation between ECF and low-Ω$_{ARG}$ ambient seawater and improve corals’ capacity to modulate the internal chemical microenvironment and buffer external acidification (Krief et al., 2010). Finally, it has been proposed that the nighttime storage of DIC mediated by host CA could stimulate daytime calcification of corals under diurnally fluctuating pCO$_2$ (Dufault et al., 2012). However, our data did not support this hypothesis. Instead, host CA under fluctuating OA was unaffected at night but reduced in light conditions, while host CA under stable OA was significantly up-regulated during the night. Although our study did not distinguish between the proportions of host CA utilized in calcification and photosynthesis, the up-regulation of Ca-ATPase under fluctuating OA (discussed below) most likely points to an increase in host CA functioning in calcification, particularly given that DIC assimilation and Ca$^{2+}$ pumping are tightly coupled in calcification (Tanbutte et al., 1996; Furla et al., 2000; Marshall and Clode, 2003). In other words, the declined host CA activity under fluctuating OA could simply reflect the reduced CA function in DIC uptake for photosynthesis.

This presumption is further evidenced by the increased H$^+$ pumping activities under fluctuating OA (discussed below) which would produce more CO$_2$ that can be recycled for photosynthesis (Furla et al., 2000; Moya et al., 2008). In this case, DIC uptake by host CA from the external seawater for photosynthesis may be
reduced to save energy. On the other hand, the increased host CA in the stable OA treatment at night suggests that more DIC could be transported into holobiont (Moya et al., 2008; Bertucci et al., 2013), and this situation would alleviate the daytime DIC competition between calcification and photosynthesis (Furla et al., 2000), further corroborating the unaltered calcification and higher photochemical efficiency under stable OA. It can be therefore concluded that CA activities within coral holobiont are potentially mediated by both environmental pCO₂ and associated changes in other cellular functions.

**Proton Pumping and the Trade-Off Between Calcification and Polyp Budding**

Interestingly, Ca- and Mg-ATPase activities (i.e., H⁺, Ca²⁺, and Mg²⁺ pumping) were unaffected in the stable OA treatment, in which calcification was maintained. Also, by using geochemical proxies to assess the chemical profiles within ECF in response to OA, Cornwall et al. (2018) found no change in Ca²⁺ pumping and calcification rates for the coral *Goniopora* sp. and coralline algae *H. reinboldii* under seawater acidification. To a large extent, the unresponsiveness of Ca- and Mg-ATPases to stable OA here could be ascribed to the enhanced photosynthetic performance, which will produce more OH⁻ for the titration of H⁺ from ECF and thus make H⁺ export easier (Jokiel, 2011; Comeau et al., 2013a; Yuan et al., 2018).

Studies of the role of Ca- and Mg-ATPases in corals’ response to OA have yielded inconsistent results. For instance, Vidal-Dupiol et al. (2013) found that the gene coding for CA-ATPase was upregulated at pH 7.8 and 7.4 but was down-regulated at pH 7.2 in the coral *P. damicornis*, while Kurihara et al. (2018) reported that gene expression of CA-ATPase in *A. digitifera* did not change in response to high pCO₂. Direct comparisons between these findings and our results are challenging because gene expression patterns may not reflect the exact content and function of proteins, due to post-translational modifications. On the other hand, de Barros Marangoni et al. (2017) observed a 1.6-fold increase in CA-ATPase activity in the hydrocoral *M. alcicornis* exposed to acidified seawater (pH < 7.5) for 30 days but not for 16 days; likewise, Prazeres et al. (2015) found that Ca- and Mg-ATPase activities of benthic foraminifera *Amphistegina lessonii* significantly increased (+50%) after 30 days at pH 7.6, while the 15-days exposure exerted no effect. However, another resistant species *Marginopora vertebralis* exhibited unaltered CA-ATPase activity and a 30% inhibition of Mg-ATPase activity following 30-days exposure to lowered pH (Prazeres et al., 2015). Taken together, treatment duration, together with species-specificity, appears to largely influence the response of these crucial enzymes to OA.

Notably, fluctuating OA elicited significant increases in activities of Ca- and Mg-ATPases, and this means that H⁺ pumping was highly activated to maintain pH within the ECF (pHₐ). As suggested by Comeau et al. (2018), seawater pH and DIC independently control the pHₐ of multiple calcifying species, including *P. damicornis*, and pHₐ declined with both decreasing seawater pH and increasing seawater DIC. Therefore, when seawater pH was lowest and [DIC] was highest at night in the fluctuating OA treatment, Ca- and Mg-ATPase activities were up-regulated to elevate pHₐ and Ωₐ, that is, to create a favorable physiochemical microenvironment for calcification (McCulloch et al., 2012; Cai et al., 2016). Nevertheless, given the up-regulation of H⁺ pumps under fluctuating OA, more metabolic CO₂ will be generated by mitochondria, further exacerbating the nighttime acidosis of calicoblastic cells and thus making H⁺ removal more difficult and skeleton more prone to dissolution (Furla et al., 2000; Moya et al., 2008; Jokiel, 2011). Surprisingly, activities of Ca- and Mg-ATPases in the fluctuating OA treatment also greatly increased during the daytime when seawater was [H⁺] lowest. The daytime up-regulation of H⁺ pumping under fluctuating OA may simply act as a strategy to promote light calcification and offset the possible night dissolution, thus maintaining overall calcification performance.

Additionally, the enhanced Ca- and Mg-ATPase activities indicate that more Ca²⁺ and Mg²⁺ would be delivered into ECF. It has been recently demonstrated that the ability to increase ECF [Ca²⁺] is a key mechanism enabling OA resistance in the reef coral *P. damicornis* (DeCarlo et al., 2018). Furthermore, Mg²⁺ is an important and basic component of center of calcification which controls the nucleation and serves as the basis for the growth of fibrous aragonite crystals (Meibom et al., 2004; Shapiro et al., 2018). The active [Mg²⁺] elevation would be, therefore, advantageous for calcification under fluctuating OA. However, the stronger biological control of pH, [Ca²⁺] and [Mg²⁺] within ECF was still paralleled by a 10% decline in calcification, suggesting that the negative effects of fluctuating OA on skeletal growth could not be fully counteracted by active H⁺ pumping. Moreover, this appeared to come at a cost on asexual reproduction, with a 50% decline in polyp budding, which is extremely energetically expensive for a newly settled coral (Graham et al., 2013). Thus, the increased cost associated with maintaining calcification under fluctuating OA may severely compromise energy investment in asexual budding. These results, together with our previous findings (Jiang et al., 2015, 2018), reaffirm the notion that energy is preferentially allocated to skeletal growth over asexual reproduction in juvenile corals when subjected to OA. Therefore, the measurement of calcification alone may be inadequate to detect the physiological maladaptation and overall changes in fitness of coral recruits under stressful conditions.

**CONCLUSION**

Overall, this study demonstrated significant impacts of pCO₂ fluctuations on the physiological and biochemical properties of juvenile *P. damicornis* in response to OA, and forged the links between coral physiology and the functions of CA, CA-ATPase, and Mg-ATPase under different pCO₂ regimes. Crucially, the boosted photosynthetic activity under fluctuating OA was insufficient to satisfy the increased energy demand for calcification, potentially causing disproportional energy allocation and arresting other key physiological processes, such as asexual budding and antioxidant system. Evidently,
fluctuating OA is more energetically costly than stable OA for the maintenance of newly settled corals. Since diel pCO₂ oscillations are expected to become more pronounced (Shaw et al., 2013), future OA conditions are likely to be more detrimental to the post-settlement development and early success of corals than predicted, especially in highly dynamic coastal reefs.

DATA AVAILABILITY STATEMENT
All datasets associated with this study are included in the manuscript and the Supplementary Files.

AUTHOR CONTRIBUTIONS
LJ and HH conceived and designed the study. LJ conducted the experiments and performed the laboratory analyses. LJ analyzed the data and drafted the manuscript. Y-JG, FZ, Y-YZ, LM, X-CY, X-ML, G-WZ, M-LG, LC, J-SL, P-YQ, and HH contributed to lab analysis and interpretation of the results. All authors commented on the draft and gave final consent for publication.

REFERENCES
Albright, R., Mason, B., Miller, M., Langdon, C., and Falkowski, P. G. (2010). Ocean acidification compromises recruitment success of the threatened caribbean coral Acropora palmata. Proc. Natl. Acad. Sci. U.S.A. 107, 20400–20404. doi: 10.1073/pnas.100273107
Al-Horani, F. A., Al-Moghrabi, S. M., and de Beer, D. (2003). The mechanism of calcification and its relation to photosynthesis and respiration in the scleractinian coral Galaxea fascicularis. Mar. Biol. 142, 419–426. doi: 10.1007/s00227-002-0981-8
Allemand, D., Tambutté, É, and Zoccola, D. (2011). “Coral calcification, cells to reefs,” in Coral Reefs: An Ecosystem in Transition, eds Z. Dubinsky and N. Stambler (Netherlands: Springer), 119–150. doi: 10.1007/978-94-007-0114-4_9
Allison, N., Cohen, I., Finch, A. A., Erezc, J., and Emif. (2011). Controls on Sr/Ca and Mg/Ca in scleractinian corals: the effects of Ca-ATPase and transcellular Ca channels on skeletal chemistry. Geochim. Cosmochim. Acta 75, 6350–6360. doi: 10.1016/j.gca.2011.08.012
Anlauf, H., D’Croiz, L., and O’Dea, A. (2011). A corrosive concoction: the combined effects of ocean warming and acidification on the early growth of a stony coral are multiplicative. J. Exp. Marine Biol. Ecol. 397, 13–20. doi: 10.1016/j.jembe.2010.11.009
Asada, K. (1999). The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50, 601–639. doi: 10.1146/annurev.arplant.50.1.601
Bertucci, A., Moya, A., Tambutté, S., Allemand, D., Supuran, C. T., Zoccola, D., et al. (2013). Carbonic anhydrases in anthozoan corals-a review. Bioorg. Med. Chem. 21, 1437–1450. doi: 10.1016/j.bmc.2012.10.024
Brading, P., Warner, M. E., Davey, P., Smith, D. J., Achterberg, E. P., Suggett, D. J., et al. (2011). Differential effects of ocean acidification on growth and photosynthesis among phylotypes of Symbiodinium. Limnol. Oceanogr. 56, 927–938. doi: 10.4319/lo.2011.56.3.0927
Brading, P., Warner, M. E., Smith, D. J., and Suggett, D. J. (2013). Contrasting modes of inorganic carbon accumulation among Symbiodinium (Dinophyceae) phylotypes. New Phytol. 200, 432–442. doi: 10.1111/nph.12379
Cai, W. J., Ma, Y., Hopkinson, B. M., Grottioli, A. G., Warner, M. E., Ding, Q., et al. (2016). Microelectrode characterization of coral daytime interior pH and carbonate chemistry. Nat. Commun. 7:11144. doi: 10.1038/ncomms11144
Camejo, G., Wallin, B., and Enojärvi, M. (1999). “Analysis of oxidation and antioxidants using microtiter plates,” in Free Radical and Antioxidant Protocols, ed. D. Armstrong (Totowa, NJ: Humana Press), 377–387. doi: 10.1385/0-89603-472-0:377
Camp, E. F., Smith, D. J., Evenhuis, C., Enochs, I., Manzello, D., Woodcock, S., et al. (2016). Acclimatization to high-variance habitats does not enhance physiological tolerance of two key Caribbean corals to future temperature and pH. Proc. R. Soc. B Biol. Sci. 283:20160442. doi: 10.1098/rspb.2016.0442
Castillo, K. D., Ries, J. B., Bruno, J. F., and Westfield, I. T. (2014). The reef-building coral Siderastrea siderea exhibits parabolic responses to ocean acidification and warming. Proc. R. Soc. B Biol. Sci. 281:20141856. doi: 10.1098/rspb.2014.1856
Chan, K. M., Delfert, D., and Junger, K. D. (1986). A direct colorimetric assay for Ca₂⁺–stimulated ATPase activity. Anal. Biochem. 157, 375–380. doi: 10.1016/0003-2697(86)90640-8
Chan, N. C. S., and Connolly, S. R. (2013). Sensitivity of coral calcification to ocean acidification: a meta-analysis. Glob. Chang. Biol. 19, 282–290. doi: 10.1111/gcb.12011
Chan, W. Y., and Eggins, S. M. (2017). Calcification responses to diurnal variation in seawater carbonate chemistry by the coral Acropora formosa. Coral Reefs 36, 1–10. doi: 10.1007/s00338-017-1567-8
Chen, X., Wei, G., Xie, L., Deng, W., Sun, Y., Wang, Z., et al. (2015). Biological controls on diurnal variations in seawater trace element concentrations and carbonate chemistry on a coral reef. Mar. Chem. 176, 1–8. doi: 10.1016/j.marchem.2015.06.030
Cohen, A. L., and Holcomb, M. (2009). Why corals care about ocean acidification: uncovering the mechanism. Oceanography 22, 118–127. doi: 10.5670/oceanog.2009.102
Comeau, S., Carpenter, R., and Edmunds, P. (2013a). Coral reef calcifiers buffer their response to ocean acidification using both bicarbonate and carbonate. Proc. R. Soc. B Biol. Sci. 280:20122374. doi: 10.1098/rspb.2012.2374
Comeau, S., Edmunds, P. J., Spindel, N. B., and Carpenter, R. C. (2013b). The responses of eight coral reef calcifers to increasing partial pressure of CO2 do not exhibit a tipping point. Limnol. Oceanogr. 58, 388–398. doi: 10.4319/lo.2013.58.1.0388
Comeau, S., Cornell, C. E., DeCarlo, T. M., Krieger, E., and McCulloch, M. T. (2018). Similar controls on calcification under ocean acidification across unrelated coral reef taxa. Glob. Chang. Biol. 24, 4857–4868. doi: 10.1111/gcb.14379

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Comeau, S., Carpenter, R. C., Nojiri, Y., Putnam, H. M., Sakai, K., Edmunds, P. J., et al. (2014a). Pacific-wide contrast highlights resistance of reef calcifiers to ocean acidification. Proc. R. Soc. B Biol. Sci. 281:20141339. doi: 10.1098/rspb.2014.1339

Comeau, S., Edmunds, P. J., Spindel, N. B., and Carpenter, R. C. (2014b). Diel pCO2 oscillations modulate the response of the coral Acropora hyacinthus to ocean acidification. Mar. Ecol. Prog. Ser. 501, 99–111. doi:10.3354/meps10690

Comeau, S., Edmunds, P. J., Spindel, N. B., and Carpenter, R. C. (2015). Fast coral reef calcifiers are more sensitive to ocean acidification in short-term laboratory incubations. Limnol. Oceanogr. 59, 1081–1091. doi: 10.4319/lo.2014.59.3.1081

Cornwall, C. E., Comeau, S., DeCarlo, T. M., Moore, B., D'Alessi, Q., McCulloch, M. T., et al. (2018). Resistance of corals and coralline algae to ocean acidification: physiological control of calcification under natural pH variability. Proc. R. Soc. B Biol. Sci. 285:20181168. doi: 10.1098/rspb.2018.1168

Comeau, S., Hepburn, C. D., Mccgraw, C. M., Currie, K. L., Piliditch, C. A., Hunter, K. A., et al. (2013). Diurnal fluctuations in seawater pH influence the rate of a calcifying macroalga to ocean acidification. Proc. R. Soc. B Biol. Sci. 280:20132201. doi: 10.1098/rspb.2013.2201

Crawley, A., Kline, D. I., Dunn, S., Anthony, K., and Dove, S. (2010). The effect of ocean acidification on symbiont photosorption and productivity in Acropora formosa. Glob. Chang. Biol. 16, 851–863. doi:10.1111/j.1365-2486.2009.01943.x

de Barros Marangoni, L. F., Calderon, E. N., Marques, J. A., Duarte, G. A. S., Pereira, C. M., Castro, C. B. E., et al. (2017). Effects of CO2-driven acidification of seawater on the calcification process in the calcareous hydrozoan Millepora alcicornis (Linnaeus, 1758). Coral Reefs 36, 1133–1141. doi:10.1007/s00338-017-1605-6

DeCarlo, T. M., Comeau, S., Cornwall, C. E., and McCulloch, M. T. (2018). Coral recruits under fluctuating OA resistance to ocean acidification linked to increased calcium at the site of calcification. Proc. R. Soc. B Biol. Sci. 285:20180564. doi: 10.1098/rspb.2018.0564

Dufault, A. M., Cumbo, V. R., Fan, T. Y., and Edmunds, P. J. (2012). Effects of diurnally oscillating pCO2 on the calcification and survival of coral recruits. Proc. R. Soc. B Biol. Sci. 279, 2951–2958. doi: 10.1098/rspb.2011.2545

Furla, P., Galgani, I., Durand, I., and Allemand, D. (2000). Sources and mechanisms of inorganic carbon transport for coral calcification and photosynthesis. J. Exp. Biol. 203, 3445–3457.

Gattuso, J.-P., Magnan, A., Billé, R., Cheung, W. W. L., Howes, E. L., Joos, F., et al. (2015). Contrasting futures for ocean and society from different anthropogenic CO2 emissions scenarios. Science 349:aac4722. doi: 10.1126/science.aac4722

Genty, B., Briantais, J.-M., and Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta Gen. Subj. 990, 87–92. doi:10.1016/S0005-2728(98)80016-9

Gibbin, E. M., Putnam, H. M., Day, S. K., and Gates, R. D. (2014). Intracellular pH and its response to CO2-driven seawater acidification in symbiotic versus non-symbiotic coral cells. J. Exp. Biol. 217, 1963–1969. doi:10.1242/jeb.099549

Graham, E. M., Baird, B. L., Willis, S. R., and Connolly, S. R. (2013). Effects of delayed settlement on post-settlement growth and survival of scleractinian coral larvae. Oecologia 173, 431–438. doi:10.1007/s00442-013-2635-6

Huang, H., Yuan, X. C., Cai, W. J., Zhang, C. L., Li, X., Liu, S., et al. (2014). Positive and negative responses of coral calcification to elevated pCO2: case studies of two coral species and the implications of their responses. Mar. Ecol. Prog. Ser. 502, 145–156. doi:10.3334/meps10720

Iglesias-Prieto, R., Beltran, V. H., Lajunesse, T. C., Reyes-Bonilla, H., and Thomé, P. E. (2004). Different algal symbionts explain the vertical distribution of dominant reef corals in the eastern Pacific. Proc. R. Soc. Lond. Ser. B Biol. Sci. 274, 1757–1763. doi:10.1098/rspb.2004.2757

Ip, Y. K., and Lim, A. L. L. (1991). Are calcium and strontium transported by the same mechanism in the hermatypic coral Galaxea fascicularis? J. Exp. Biol. 159, 507–513.

Ip, Y. K., Lim, A. L. L., and Lim, R. W. L. (1991). Some properties of calcium-activated adenosine triphosphatase from the hermatypic coral Galaxea fascicularis. Mar. Biol. 111, 191–197. doi:10.1007/BF01319700

Jiang, L., Huang, H., Yuan, X. C., Yuan, T., Zhang, Y. Y., Li, X. B., et al. (2015). Effects of elevated pCO2 on the post-settlement development of Pocillopora damicornis. J. Exp. Mar. Biol. Ecol. 473, 169–175. doi:10.1016/j.jembe.2015.09.004
by limitation of photosynthesis. Plant Cell Physiol. 41, 335–343. doi: 10.1093/pcp/41.3.335
Moya, A., Tambuté, S., Bertucci, A., Tambuté, E., Lotto, S., Vullo, D., et al. (2008). Carbonic anhydrase in the scleractinian Coral Stylophora pistillata: characterization, localization, and role in biomineralization. J. Biol. Chem. 283, 25475–25484. doi: 10.1074/jbc.M804726200
Muscatine, L., Mcloskey, L. R., and Marian, R. E. (1981). Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. Limnol. Oceanogr. 305, 369–401.
Noonan, S. H. C., and Fabricius, K. E. (2016). Ocean acidification affects productivity but not the severity of thermal bleaching in some tropical corals. ICES J. Mar. Sci. 73, 715–726. doi: 10.1093/icesjms/fsv127
Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., et al. (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437, 681–686. doi: 10.1038/nature04095
Prazeres, M., Uthicke, S., and Pandolfi, J. M. (2015). Ocean acidification induces biochemical and morphological changes in the calcification process of large benthic foraminifera. Proc. R. Soc. B Biol. Sci. 282:20142782. doi: 10.1098/rspb.2014.2782
Quinn, G., and Keough, M. (2002). Experimental Design and Data Analysis for Biologists. Melbourne: Cambridge University Press. doi: 10.1017/CBO9780511806384
Rivest, E. B., Comeau, S., and Cornwall, C. E. (2017). The role of natural variability in shaping the response of coral reef organisms to climate change. Curr. Clim. Chang. Rep. 3, 271–281. doi: 10.1007/s40641-017-0082-x
Rolda, M. Y., Cornwall, C. E., Feng, Y., McGraw, C. M., Smith, A. M., Hurd, C. L., et al. (2015). Effect of ocean acidification and pH fluctuations on the growth and development of coralline algal recruits, and an associated benthic algal assemblage. PLoS One 10:e0140394. doi: 10.1371/journal.pone.0140394
Sabine, C. L., Feely, R. A., Gruber, N., Key, R. M., Lee, K., Bullister, J. L., et al. (2004). The oceanic sink for anthropogenic CO2. Science 305, 367–371. doi: 10.1126/science.1097403
Santos, I. R., Glud, R. N., Maher, D., Erlert, D., and Eyre, B. D. (2011). Diel coral reef acidification driven by porewater advection in permeable carbonate sands, Heron Island, great barrier reef. Geophys. Res. Lett. 38:L03604. doi: 10.1029/2010GL046053
Shapiro, O. H., Kartvelishvily, E., Kramarsky-Winter, E., and Vardi, A. (2018). Magnesium-rich nanometric layer in the skeleton of Pocillopora damicornis with possible involvement in fibrous aragonite deposition. Front. Mar. Sci. 5:246. doi: 10.3389/fmars.2018.00246
Shaw, E. C., McNeil, B. I., and Tilbrook, B. (2012). Impacts of ocean acidification in naturally variable coral reef flat ecosystems. J. Geophys. Res. Oceans 117:C03038. doi: 10.1029/2011JC007655
Shaw, E. C., McNeil, B. I., Tilbrook, B., Matear, R., and Bates, M. L. (2013). Anthropogenic changes to seawater buffer capacity combined with natural reef metabolism induce extreme future coral reef CO2 conditions. Glob. Chang. Biol. 19, 1632–1641. doi: 10.1111/gcb.12154
Silverman, J., Kline, D. I., Johnson, L., Rivlin, T., Schneider, K., Erez, J., et al. (2015). Carbon turnover rates in the one tree island reef: a 40-year perspective. J. Geophys. Res. Biogeosci. 117:3023.