Ocean acidification causes bleaching and productivity loss in coral reef builders

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Ocean acidification represents a key threat to coral reefs by reducing the calcification rate of framework builders. In addition, acidification is likely to affect the relationship between corals and their symbiotic dinoflagellates and the productivity of this association. However, little is known about how acidification impacts on the physiology of reef builders and how acidification interacts with warming. Here, we report on an 8-week study that compared bleaching, productivity, and calcification responses of crustose coralline algae (CCA) and branching (Acropora) and massive (Porites) coral species in response to acidification and warming. Using a 30-30 experimental system, we manipulated CO2 levels to simulate doubling and three- to fourfold increases [Intergovernmental Panel on Climate Change (IPCC) projection categories IV and VI] relative to present-day levels under cool and warm scenarios. Results indicated that high CO2 is a bleaching agent for corals and CCA under high irradiance, acting synergistically with warming to lower thermal bleaching thresholds. We propose that CO2 induces bleaching via its impact on photoprotective mechanisms of the photosystems. Overall, acidification impacted more strongly on bleaching and productivity than on calcification. Interestingly, the intermediate, warm CO2 scenario led to a 30% increase in productivity in Acropora, whereas high CO2 lead to zero productivity in both corals. CCA were most sensitive to acidification, with high CO2 leading to negative productivity and high rates of net dissolution. Our findings suggest that sensitive reef-building species such as CCA may be pushed beyond their thresholds for growth and survival within the next few decades whereas corals will show delayed and mixed responses.

climate change | global warming | carbon dioxide | Great Barrier Reef

The concentrations of atmospheric CO2 predicted for this century present two major challenges for coral-reef building organisms (1). Firstly, rising sea surface temperatures associated with CO2 increase will lead to an increased frequency and severity of coral bleaching events (large-scale disintegration of the critically important coral–dinoflagellate symbiosis) with negative consequences for coral survival, growth, and reproduction (2). Secondly, >30% of the CO2 emitted to the atmosphere by human activities is taken up by the ocean (3, 4), lowering the pH of surface waters to levels that will potentially compromise or prevent calcium carbonate accretion by organisms including reef corals (1, 5), calcifying algae (6, 7) and a diverse range of other organisms (8). Ocean acidification research has focused mainly on the consequences of shifting ocean chemistry toward suboptimal saturation states of aragonite and calcite (9) and how this will affect the calcification processes of organisms in the pelagic (10) and benthic (11, 12) environments. Previous studies have shown dissolution of coral skeletons (13) and reduced rates of reef calcification (14) with increasing CO2 concentrations. Ocean acidification, however, is likely to also impact on other physiological processes in key reef-building species, but little is known about these responses and their biotic consequences. Here, we investigate and compare the effects of ocean acidification on three key physiological processes in reef-building organisms. Firstly, we examine CO2 impacts on bleaching, which is a phenomenon mainly associated with thermal stress (2, 15), although early unpublished work suggested a possible link between CO2 and coral bleaching (16). Secondly, we investigate effects on organic productivity, which is expected to be influenced by bleaching state, and thirdly, we compare the patterns of these organic responses with effects on rates of calcification. Three groups of reef builders were used, representing some of the most common and functionally important benthic organisms on coral reefs: staghorn corals (Acropora intermedia), massive corals (Porites lobata), and crustose coralline algae (Porolithon onkodes). Crustose coralline algae (CCA), and in particular P. onkodes, play an important role in reef building and the consolidation of dead reef matrix (17, 18) and have recently demonstrated reduced growth and recruitment under elevated CO2 (7).

The present study is based on an 8-week experiment on Heron Island (Southern Great Barrier Reef, Australia) during the austral summer of 2007 (February–March) using a system of 30 flow-through aquaria with controlled CO2 dosing and temperature regimes. To cover the broad range of CO2 environments projected for the century, we used experimental CO2-dosing scenarios that represent present-day (control, 380 ppm atmospheric CO2), intermediate (high category IV, 520–700 ppm), and high-end (above category VI, 1000–1300 ppm) CO2 stabilization scenarios by the IPCC (19). To examine how CO2 interacts with warming, the experimental design also incorporated two temperature treatments (25–26°C and 28–29°C) representing low- and high-average summer temperatures for the region. In summary, the experimental design consisted of two CO2 dosing regimes and a control crossed with two temperature treatments, each replicated by 5 aquaria holding 3–5 specimens of each study species. The aquaria were organized randomly to control for spatial heterogeneity in light regimes, which averaged 1000 μmol photon m⁻² s⁻¹ over the day (see Methods for further details).

Results

Bleaching Responses. High-CO2 dosing led to 40–50% bleaching for the CCA and Acropora after 8 weeks of experimentation (Fig. 1A and D). The response was highly significant as the bleaching metric (luminance scale representing variation in chlorophyll content, see Methods) showed low variation among the 15–25 specimens within each treatment combination [see also ANOVA results in supporting information (SI) Table S1]. Intermediate-CO2 dosing led to marginally more bleaching (~30% for CCA and 20% for Acropora) relative to 20 and 10% for controls, respectively. Interestingly, for the CCA and Acropora, the effect of CO2 dosing on bleaching was stronger than the effect of

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temperature. Specifically, high-CO₂ dosing led to a two- to threefold increase in bleaching relative to the control, whereas high temperature led to only 20% increase in bleaching for these species. *Porites* was less sensitive and bleached to a maximum of 40% in the high-CO₂/high-temperature treatment. In this species there was a strong synergy between CO₂ and temperature as the CO₂ dosing led to dramatic reductions in Productivity Responses.

**Productivity Responses.** CO₂ dosing led to dramatic reductions in daily productivity (as hourly rates of photosynthesis minus respiration integrated over the day) of the CCA. At low temperature, intermediate-CO₂ dosing (pH 7.85–7.95) resulted in a 50% reduction in productivity relative to the control. High-CO₂ dosing (pH 7.60–7.70) led to a further reduction in productivity to near zero (Fig. 1B). Interestingly, acidification affected net rate of photosynthesis (daytime measurements) only, whereas rates of dark respiration varied <10% across treatments (data not shown). The acidification effect on the CCA was exacerbated by warming. At the control CO₂ (highest pH), warming led to a 45% drop in productivity, and at the warm, intermediate-CO₂ dosing scenario, productivity fell to below zero. At the highest CO₂ dosing under warm conditions, productivity of CCA was 160% reduced relative to the warm control conditions—i.e., daily rates of respiration far exceeded daily rates of photosynthesis.

Interestingly, productivity of *Acropora* was enhanced under the intermediate, warm CO₂ dosing regime but was suppressed in *Porites* (Fig. 1E and H). Again, productivity patterns were driven by variation in net rates of photosynthesis only, and dark rate of respiration varied by <10%. In *Acropora*, intermediate-CO₂ dosing had no impact on productivity at low temperature, but was 40% increased in the warm treatment. At the highest CO₂ dosing, however, productivity dropped to near zero for both temperature groups (Fig. 1E). The significant interaction between CO₂ and temperature in the productivity response for *Acropora* (see ANOVA results in Table S1) was driven mainly by the high productivity maximum in the warm, intermediate-CO₂ regime. In *Porites*, productivity was marginally enhanced by warming at the control CO₂, but fell by 80% in the warm, intermediate-CO₂ group—opposite to the pattern for *Acropora* (Fig. 1H). High CO₂ led to a 30% drop in productivity in *Porites* (relative to the control) in cool conditions and dropped to near zero at the highest CO₂ dosing, analogous to the pattern for *Acropora*.

**Calcification Responses.** CCA calcification was highly sensitive to the highest CO₂ dosing and the effect was exacerbated by warming (Fig. 1B). Intermediate-CO₂ dosing and warming led to a 50% drop in the CCA calcification rate, but the temperature effect was not significant. High-CO₂ dosing led to 130 and 190% reductions in calcification rate relative to control conditions at low and high temperatures. Rates of calcium carbonate dissolution by CCA in the warm, high-CO₂ scenario were thus as high as their rates of accretion at present-day conditions.

Compared to their bleaching and productivity responses, the calcification responses of *Acropora* and *Porites* to CO₂ dosing were relatively weak (Fig. 1E and F). In *Acropora*, for example, rate of calcification in the high-CO₂ dosing regime was ~40% lower than at control conditions. Warming suppressed the calcification rate of *Acropora* significantly, but only for the high-CO₂ treatment (~25%). The calcification response of *Porites* to CO₂ dosing was almost identical to that of *Acropora*, but calcification in *Porites* did not show a clear response to warming.

**Discussion**

Our results indicated that prolonged CO₂ dosing (representative of CO₂ stabilization categories IV and VI by the IPCC) (19) causes bleaching (loss of pigmentation) in two key groups of reef-building organisms. The bleaching results indicate that future predictions of bleaching in response to global warming must also take account of the additional effect of acidification and suggests that any potential adaptation and acclimatization by coral reef organisms to thermal stress (20, 21) may be offset or overridden by CO₂ effects. Previous studies of CO₂ enrichment and warming in corals and algae have not observed a bleaching response (22, 23). One explanation is that this study used a higher natural irradiance (average of ~1000 μmol photons m⁻² s⁻¹), which is a key bleaching agent in corals (24), thereby bringing organisms closer to their bleaching thresholds. Also, the experimental period of CO₂ dosing used in this experiment was longer than that of for example the study by Reynaud et al. (2003) (22), thereby allowing time for the buildup of physiological stress. The process by which high CO₂ induces bleaching is unknown, but
could involve a number of possible mechanisms such as changes to the carbon-concentrating mechanism (25), photorespiration (26), and/or direct impacts of acidosis (27). Results of a recent study using the same experimental conditions (A. Crawley, S.D., and K.R.N.A., unpublished data) indicate that high CO2 and/or lowered pH disrupt the photoprotective mechanisms of coral symbionts or algal chloroplasts by lowering rates of photorespiration and the capacity for thermal dissipation. The implications are that CO2 concentration and irradiance interact to trigger bleaching under the naturally high light level used in our experiment (noon irradiances of >1200 µmol photons m⁻² s⁻¹). Importantly, the study by Reynaud et al. (2003) (22), which showed an increase in chlorophyll content under elevated CO2, used an irradiance level of only 350 µmol photons m⁻² s⁻¹—a third of that used in this study and potentially below the threshold for combined CO2/irradiance-induced bleaching. Also, the study by Schneider and Erez (2006) (23) found no effect of CO2 dosing on rates of photosynthesis and respiration, but similar to Reynaud et al. (2003) (22) used an experimental irradiance of only 350 µmol photons m⁻² s⁻¹.

The productivity responses of the CCA and corals to CO2 dosing are likely to be a result of a series of opposing mechanisms. Initial loss of pigmentation in the corals can result in increased productivity per remnant symbiont or per chlorophyll because of subtle increases in temperature or an increased internal light field (29, 30). As severe bleaching takes over, the decline in the symbiont population (or the chlorophyll pool) overrides the increased photosynthetic efficiencies, leading to a drop in areal productivity. Alternatively, CO2 is the substrate for carbonic anhydrase to interconvert to CO2 and bridge membranes in a carbon-concentrating mechanism that ultimately delivers CO2 to rubisco for carbon fixation (25, 31, 32). The potential effects of increasing CO2 and/or impacts of acidosis on inorganic carbon accumulation are likely to be highly variable in different organisms, as are the thermal thresholds that dictate whether an increase in temperature leads to a negative or positive response. In CCAs, increasing CO2 led to a dramatic monotonic decline in productivity, and this decline was exacerbated by warming. This productivity pattern suggests that the CO2-stabilization scenario predicted for the IPCC category IV (CO2 peaking years 2020–2060), here represented by the warm, intermediate-CO2 regime, will be unsustainable for CCA, and thresholds for survival of this important functional group will be far exceeded under the category VI scenario (CO2 peaking years 2020–2090). Our data are consistent with the recent findings that elevated CO2 leads to lowered growth and recruitment of CCA (7). A decline in CCA abundance can potentially have dramatic ecological consequences because of the roles they play in coral reefs. CCA are an important settlement cue for invertebrate larvae including corals and contribute significantly to reef accretion and cementation (33).

Interestingly, the productivity of Acropora was maximized at the intermediate-CO2 regime (Fig. 1 E and H), suggesting that rate of photosynthesis is stimulated either directly by increased CO2 supply, and/or by an increase in excitation pressure driven by bleaching-induced increases in internal light fields (34). The large drop in productivity at the highest CO2 dosing suggests that the positive effect of high-CO2 supply is here overridden by the disruption of photophysiological processes and as a consequence of bleaching and thereby loss of photosynthetic capacity. Low pH may interfere with the preferred pathway for CO2 accumulation at the site of rubisco within intracellular symbionts or directly with electron transport through the destabilization of thylakoid proton gradients thereby directly affecting the ability of the individual symbionts to fix carbon. Productivity in the massive coral (Porites) displayed an almost opposite pattern to the branching Acropora with respect to temperature. Under warm conditions, productivity in Porites also dropped dramatically at the highest CO2 level, but only 20% (and nonsignificantly) lower than at control conditions, suggesting a generally weaker response to acidification than Acropora.

Calcification responses of the CCA were analogous to their response in terms of productivity, further supporting the prediction that the niche boundaries of CCA will be exceeded under the intermediate-CO2 scenario. One explanation for the high sensitivity of CCA is that their skeletons consist of magnesian calcite, which has higher solubility and requires a higher saturation state for deposition (and hence potentially more metabolic energy) than does aragonite and calcite (35, 36). The high rate of dissolution of CCA in the high-CO2 dosing treatments, in combination with the low estimated saturation states for aragonite (<2, Table 1), suggest that the CCA were approaching undersaturation in this scenario. Also, being fully autotrophic, any loss of photosynthetic capacity because of bleaching is likely to translate more directly to reduced physiological performance and mortality than in corals that have dual trophic modes (37). Our results are consistent with the productivity pattern of mixed epilithic and endolithic algal communities under elevated CO2 (38), but also indicate that temperature is a critical covariate determining survivorship.

Calcification of Acropora and Porites, however, was less responsive to CO2 than was bleaching and organic productivity. This is an important result as coral calcification and biogeochemistry has been used as the key response variable for predicting risks of ocean acidification to coral reefs (1, 39). The results of this study suggest that impacts of high CO2 on the photophysiology and energy balance of reef organisms are as important in defining acidification threats to reefs as are impacts on calcification and reef geochemistry. The observation that CO2 triggers bleaching in sympathy with warming under high light, and thereby partly drives patterns of net productivity, indicates that predictions of survival thresholds for reef builders under
climate change must take account of acidification–warming interactions in the integrated biological and biogeochemical response.

Methods

Study Species. To represent three of the most important framework builders on Indo-Pacific coral reefs, we used a species of CCA commonly found on forereefs and reef-crest habitats, P. onkodes, and two common species of branching, A. intermedia, and massive, P. lobata, scleractinian coral. Between 125 and 220 specimens of each species were collected from the reef slope (2–3 m below lowest astronomical tide) from 3–5 different reef sites on Heron Reef. Collecting was conducted from as large an area at each site as possible to maximize the number of genotypes represented. For the CCA, we used 3 cm by 3 cm large chips chiselled off the substrate, and for A. intermedia, we used 6–7 cm long terminal branches. Specimens of P. lobata consisted of 3.5 cm diameter plugs collected by holesaw. All specimens were transferred to aquaria with running seawater and left for 6 weeks to recover from handling at light and temperature conditions similar to those in the field. Acropora branches were suspended from their tips by thin monofilament line, which allowed the healed tissue to completely cover their skeleton. Mortality during the acclimation phase was <5% for all species.

Experimental Setup. The experimental facility consisted of 30 flow-through aquaria (20 litres) under a natural light regime (noon irradiance ranging from 700 to 1200 μmol photons m−2 s−1) receiving unfiltered reef seawater from six temperature-controlled CO2 mixing tanks. Levels of acidification and temperature were regulated by a custom-built CO2 dosing (bubbling) and temperature control system (Campbell Scientific, Australia) set to pH target stabilization scenarios, with peaking CO2 in years 2020–2060 and 2060–2090, respectively. pH was measured on seawater scale using 12 polarographic sensors (±0.01 pH unit), each connected to the logger/controller unit via a MicroChem interface (TPS Australia). The experimental CO2 dosing regimes were crossed with two temperature regimes, and above VI for IPCC CO2 stabilization scenarios, with peaking CO2 in years 2020–2060 and 2060–2090, respectively (16). The control pH (zero CO2 dosing, corresponding to an atmospheric CO2 of 380 ppmV) ranged from 8.0 to 8.4, resulting in an estimated pCO2 range of 130–465 ppm reflecting the diurnal variability of the intake water from the reef. To determine interactions with temperature in a factorial design, CO2 treatments were crossed with two temperature regimes representing low and high average summer temperatures for the region (25–26 °C and 28–29 °C) (15). On the basis of measurements of pH, temperature, and total alkalinity (measured using the Gran alkalinity method (40) on a Mettler Toledo T50 automated titrator using 0.1M HCL for 130 g seawater samples), and salinity (measured using a Bellingham Stanley refractometer), the distribution of carbon species and aragonite saturation state were estimated for all treatments (Table 1) using the program CO2SYS (41). Five replicates were tested per CO2–temperature combination, each with three temperature-controlled CO2 mixing tanks. Levels of acidification and temperature as fixed factors and tanks as replicates. Specimens were not fed during the acclimation and experimental phases as the running seawater directly from the reef was unfiltered.

Response Variables. Bleaching was quantified colorimetrically from digital photographs (42) at the end of the 8-week experimental period and quantified as the reduction in luminescence relative to maximum (representing maximum symbiont or chlorophyll density). Ideally, chlorophyll samples should be used directly as a bleaching metric, but all biological samples from the experiment were lost in a fire. Because bleaching is a progressive response (because of gradual chlorophyll depletion over time) (43) effects on productivity were also analyzed at the end of the experiment. Net productivity was estimated from daytime assays of maximum net rates of photosynthesis (PNetMax 10 a.m.–3 p.m.) under controlled artificial lighting (200 W metal-halide lamp, AquaMedic, Germany) simulating daytime environmental irradiances of 1000 μmol photons m−2 s−1 in situ and nighttime assays of dark respiration (PDark), 3 p.m.–2 a.m. Photosynthesis and respiration measurements were conducted using four sealed, recirculating respirometry chambers with flow regimes simulating natural conditions (44), each chamber connected to a high-precision optical oxygen sensor (optode) and logging system (Oxy-4, Presens, Germany). Oxygen fluxes of all specimens were normalized to tissue surface area determined from geometric analyses of digital photographs (Image Tools, The University of Texas Health Science Center). To construct daily budgets for oxygen fluxes, hourly net rates of photosynthesis were integrated over the 24-hour light-dark cycle using the hyperbolic tangent function (45)

\[
P_{\text{Net}} = \int_{0}^{t} P_{\text{NetMax}} \tanh \left( \frac{E(t)}{E_K} \right) \text{dt} - \int_{t}^{24} \text{P}_{\text{Dark}} \text{dt}
\]

with irradiance at time t, E(t) based on average daily light profiles from four loggers (PAR sensor, Odyssey, Dataflow Systems, New Zealand) deployed in each of four aquaria across the setup. We used subsaturation irradiances, EK, for the three study species based on results of studies of photosynthesis in vivo under high irradiances: P. onokodes 350 μmol m−2 s−1 (18), A. intermedia, 350 μmol m−2 s−1 (28), and P. lobata, 177 μmol m−2 s−1 (46). Standard errors of the daily rates of photosynthesis (PNet) were determined using standard Monte Carlo procedure (programmed in Matlab v. 6, Mathworks) in which sets of values for PNetMax and PDark were sampled from the normal distributions specified by their parameter estimates and associated variances. The sampling procedure was repeated 1000 times for each species to produce error ranges for PNet (Eq. 1). Rate of calcification was determined as differences in buoyant (underwater) weight between the first and last day of the 8-week experiment (47). Because standardized fragment sizes were used, and because buoyant weight scales directly with skeletal weight, calcification rate was expressed as the relative monthly change in buoyant weight.

Data Analysis. All response data to CO2 and temperature treatments were tested using a two-factor nested analysis of variance (ANOVA) with CO2 dosing and temperature as fixed factors and tanks as replicates. Specimens were nested within tanks. However, because the tank factor was non-significant (data not shown), tanks were pooled in subsequent analyses and specimens were used as replicates (28), hence increasing the power of the analysis. Data from the latter analyses are presented here. When significant interactions between CO2 and temperature occurred, t-tests or independent one-way ANOVAs were used to examine effects. All ANOVAs were followed by a multiple comparisons’ test to identify significant groups. Data were tested for variance homogeneity using Levene’s test and normality using the Kolmogorov–Smirnov one-sample test.

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