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-tank 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. In-terestingly, 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 more sensitive to acidifica-tion, 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.

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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 supprotting 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
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 20% in the high-CO2/high-temperature treatment. In this species, there was a strong synergy between CO2 and temperature as the high-CO2/low-temperature corals showed <10% bleaching (Fig. 1G). High temperature thus amplified the bleaching responses by 10–20% in CCA and Acropora, and up to 50% in Porites.

**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, 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. 1F and I). 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), photospirosis (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 photosynthesis, and increasing its supply may increase rates of photosynthesis in organisms that are CO₂ limited. Many aquatic organisms however, take up HCO₃⁻ relatively efficiently using 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 acquisition 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. anckodes*, 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 hoesaw. 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 regimes were regulated by a custom-built CO₂ dosing (bubbling) and temperature control system (Campbell Scientific, Australia) set to pH target values of 7.85–7.95 and 7.60–7.70, corresponding to CO₂ concentrations of 520–705 ppmV and 1000–1300 ppmV for the intermediate- and high-CO₂ dosing regimes, 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 environments matched high categories IV and above VI for IPCC CO₂ stabilization scenarios, with peaking CO₂ in years 2020–2060 and 2060–2090, respectively. Five replicate tanks were nested within tanks. However, because the tank factor was nonsignificant (data not shown), tanks were pooled in subsequent analyses and species 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 post hoc test with the Shaffer procedure (46). Standard errors of the daily net rates of photosynthesis (*Pₕₑₜ*) were determined using standard Monte Carlo procedure (programmed in Matlab v. 6, Mathworks) in which sets of values for *Pₜₐₜₐₜₐ₉₉* and *rₚₕ₉ₕₕₖ* 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 *Pₚₙₑₜ* (Eq. 1). Rate of calcification was determined as differences in buoyant (underwater) weight between the first and last days 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.

**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 experiments 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 (*Pₜₐₜₐₜₐ₉₉* 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 (*Rₙₑₛₑₜ*) (8 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, Pesens, 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 Centre). 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)

\[
P_{\text{Net}} = \int_{t=0}^{t=12} P_{\text{Net Max}} \tanh \left( \frac{E(t)}{E_k} \right) dt = \int_{t=12}^{t=24} r_{\text{Dark}} dt
\]

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 substraturation irradiances, *Ek*, for the three study species based on results of studies of photosynthesis in similar light habitats. *P. anckodes*, 205 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 net rates of photosynthesis (*Pₚₙₑₜ*) were determined using standard Monte Carlo procedure (programmed in Matlab v. 6, Mathworks) in which sets of values for *Pₜₐₜₐₐ₉₉* and *rₚₕₕₕₕₖ* 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 *Pₚₙₑₜ* (Eq. 1). Rate of calcification was determined as differences in buoyant (underwater) weight between the first and last days 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. Species were nested within tanks. However, because the tank factor was nonsignificant (data not shown), tanks were pooled in subsequent analyses and species 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 heterogeneity using Levene’s test and normality using the Kolmogorov–Smirnov one-sample test.

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