Ocean acidification compromises recruitment success of the threatened Caribbean coral Acropora palmata

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Ocean acidification (OA) refers to the ongoing decline in oceanic pH resulting from the uptake of atmospheric CO2. Mounting experimental evidence suggests that OA will have negative consequences for a variety of marine organisms. Whereas the effect of OA on the calcification of adult reef corals is increasingly well documented, effects on early life history stages are largely unknown. Coral recruitment, which necessitates successful fertilization, larval settlement, and postsettlement growth and survivorship, is critical to the persistence and resilience of coral reefs. To determine whether OA threatens successful sexual recruitment of reef-building corals, we tested fertilization, settlement, and postsettlement growth of Acropora palmata at pCO2 levels that represent average ambient conditions during coral spawning (~400 μatm) and the range of pCO2 increases that are expected to occur in this century (~560 μatm [mid-CO2] and ~800 μatm [high-CO2]). Fertilization, settlement, and growth were all negatively impacted by increasing pCO2, and impairment of fertilization was exacerbated at lower sperm concentrations. The cumulative impact of OA on fertilization and settlement success is an estimated 52% and 73% reduction in the number of larval settlers on the reef under pCO2 conditions projected for the middle and the end of this century, respectively. Additional declines of 39% (mid-CO2) and 50% (high-CO2) were observed in postsettlement linear extension rates relative to controls. These results suggest that OA has the potential to impact multiple, sequential early life history stages, thereby severely compromising sexual recruitment and the ability of coral reefs to recover from disturbance.

carbon dioxide | climate change | pH | juvenile | elkhorn coral

The susceptibility of reef-building corals to increasing CO2 levels has been a central issue in the context of global climate change. Present-day atmospheric CO2 (pCO2) levels are estimated at 387 ppm, 30% higher than the natural range over the last 650,000 y (1). pCO2 is increasing at an annual rate of 0.5% (2), 200 times faster than any changes that occurred during the last eight glacial cycles (1). Approximately one-third of all CO2 emissions from the past 200 y has been absorbed by the oceans (3). On dissolution in seawater, CO2 reacts with H2O, triggering a series of chemical reactions that alter the seawater carbonate chemistry: [CO2][aq] and [HCO3−] increase, and [CO32−], pH, and the carbonate saturation state (Ω) decrease, causing ocean surface waters to become more acidic. Increasing atmospheric CO2 concentrations have already depleted seawater carbonate concentrations by ~30 μmol kg−1, simultaneously reducing the pH of the ocean’s surface waters by 0.1 units relative to the pre-industrial era (a 30% increase in [H+]2) (2). Further reductions of 0.3–0.5 pH units are projected by the end of this century as the oceans continue to absorb anthropogenic CO2 (2, 3).

Mounting experimental evidence suggests that ocean acidification (OA) will have negative consequences for numerous marine organisms, primarily oceanic calcifiers that rely on the delicate balance of dissolved inorganic carbon species for the formation of their shells and skeletons (4–7). Although recent research efforts have aimed to constrain the mechanisms and effects (both physiological and ecological) of elevated pCO2 on adult corals, comparatively little attention has been given to the effect of OA on earlier life history stages. The majority of reef-building corals rely on external fertilization and the development, survival, and settlement of lecithotrophic (i.e., energy-limited) planula larvae (8). Coral larvae spend hours to days developing in the water column before they are capable of settling on the reef. Larval settlement requires the recognition of water-soluble and substrate-bound chemical cues, physical attachment to the substrate, and subsequent metamorphosis. Recruitment (identification and inclusion in a population) necessitates survival and growth of the newly settled individual. Successful coral recruitment is determined by three sequential life history stages: (i) larval availability (including gamete production and successful fertilization), (ii) settlement ecology (related to larval and substrate condition), and (iii) postsettlement ecology (growth and survival) (9). Environmental factors that disrupt these various processes can result in compromised recruitment or recruitment failure and profoundly affect marine population dynamics (10–12).

The objective of the present study was to investigate the effect of pCO2 on the three sequential life history stages that are critical to successful sexual recruitment of broadcast-spawning reef corals. The experimental coral chosen for this study was the ecologically significant Caribbean elkhorn coral Acropora palmata (Lamark). This fast-growing, branching species has historically functioned as a primary framework builder on many Caribbean shallow reefs. However, Caribbean acroporid populations have experienced widespread decline over the last several decades due to hurricanes, disease, bleaching, and predation (13, 14). A drastic reduction in population size resulted in the designation of A. palmata as a threatened species under the US Endangered Species Act in 2006 (15). Recovery of A. palmata populations likely will require high rates of recruitment (13, 16, 17). Although this coral’s primary reproductive mode is asexual fragmentation, sexual recruitment is critical for maintaining the genetic diversity of future populations. To determine the effects of OA on the sexual recruitment of this species, we tested fertilization, settlement, and postsettlement growth at three pCO2 levels, representing average conditions during the spawning season [400 μatm (control)] and the range of pCO2 increases expected during this century [560 μatm (mid-CO2) and 800 μatm (high-CO2)], as determined by the Intergovernmental Panel on Climate Change (2).

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Results

Fertilization Assays. Fertilization success decreased with increasing CO₂ concentration, with average reductions (averaged across all sperm concentrations) of 12% at the high-CO₂ level and 13% at the mid-CO₂ level. At the highest sperm concentration (3.21 × 10⁶ sperm mL⁻¹), fertilization was reduced by 7% at the mid-CO₂ level and by 12% at the high-CO₂ level. As sperm concentration declined, the reduction in fertilization success was exacerbated at elevated CO₂ levels: fertilization was reduced by 29% at the mid-CO₂ level and by 15% at the high-CO₂ level at 1.61 × 10⁵ sperm mL⁻¹ and by 64% at the mid-CO₂ level and by 63% at the high-CO₂ level at 6.41 × 10⁴ sperm mL⁻¹. At the most dilute sperm concentration (3.20 × 10⁵ sperm mL⁻¹), fertilization was reduced by 59% at the high-CO₂ level. Data from this sperm concentration were unreliable for the mid-CO₂ level due to sperm contamination and thus were omitted from the regression analysis.

Nonlinear regressions were fit to percent fertilization at sperm concentration data (Fig. 1B). The model parameters, listed in Table 1, are valid for _A. palmata_ fertilization under the laboratory conditions as described in Materials and Methods, using sperm concentrations ranging from 10⁵ to 10⁶ sperm mL⁻¹. A comparison-of-fit test rejected the null hypothesis (H0) that one curve best fits all data sets ($F_{4,103} = 13.69, P < 0.0001$).

Settlement Assays. Compared with control, percent settlement was reduced by 45% at the mid-CO₂ level and by 69% at the high-CO₂ level. A significant linear relationship was seen between CO₂ concentration and percent larval settlement ($F_{1,20} = 41.79, P < 0.0001$, linear regression analysis of arcsine-transformed data using least squares residuals) (Fig. 1C).

Postsettlement Growth. Compared with control, there was a significant reduction in linear extension, of 39% at the mid-CO₂ level and 50% at the high-CO₂ level ($F_{1,23} = 4.86, P < 0.05$, linear regression analysis using least squares residuals) (Fig. 1D). The reduced sample sizes at the mid- and high-CO₂ levels resulted from greater partial and full mortality at these levels.

Discussion

This study demonstrates that OA will affect three sequential life history phases necessary for successful coral recruitment of _A. palmata_: (i) larval availability (by compromising fertilization), (ii) settlement ecology (by reducing settlement success), and (iii) postsettlement ecology (by impeding postsettlement growth). Our results indicate that OA has the potential to reduce fertilization by 12–13% (averaged across all sperm concentrations) and to decrease settlement success by 45–69% at pCO₂ concentrations expected for the middle and the end of this century. The compounding effect of OA on these early life history stages translates into a 52–73% reduction in the number of larval settlers on the reef. The net impact on recruitment likely will be even greater, given that depressed postsettlement growth is likely to result in elevated rates of postsettlement mortality.

Sexual reproduction of many sessile marine invertebrates occurs through the release (spawning) of gametes into the water, followed by external fertilization. Consequently, water chemistry can greatly affect fertilization success. Elevated pCO₂ has been shown to impair fertilization success of other marine invertebrates, including oysters (18) and sea urchins (19, 20). Reduced sperm flagellar motility, which has been shown to occur in both
on the fertilization of broadcast-spawning marine invertebrates (22, 23). Thus, it is likely one of the key factors limiting the reproductive success of sessile, broadcast-spawning marine invertebrates. At our highest sperm concentration (approaching 2200 hours. Gamete bundles disintegrated ≥30 min after release, whereupon eggs and sperm from each colony were separated via pipet. Eggs were washed between five and seven times with filtered seawater to remove residual sperm. Equal volumes of eggs from each genotype were combined to create a “stock” egg batch for use in fertilization experiments. The same was done with the sperm.

**Materials and Methods**

The fertilization experiments described in this report were conducted in field laboratories in Key Largo, FL. Settlement and growth experiments were conducted at the Climate Change Laboratory at the Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, FL.

**Spawn Collection.** Two stands of previously genotyped Acropora palmata in the Upper Florida Keys were monitored for spawning during August 2009. Gamete bundles were collected from two distinct genets at Elbow Reef and one at Sand Island. Corals spawned at ≈2200 hours. Gamete bundles disintegrated ≥30 min after release, whereupon eggs and sperm from each colony were separated via pipet. Eggs were washed between five and seven times with filtered seawater to remove residual sperm. Equal volumes of eggs from each genotype were combined to create a “stock” egg batch for use in fertilization experiments. The same was done with the sperm.

**Seawater Chemistry.** Seawater chemistry was manipulated via direct bubbling with CO₂-enriched air to create three target conditions: 400 μatm (control), 560 μatm (mid-CO₂), and 800 μatm (high-CO₂). Actual chemical and physical
conditions that persisted during each experiment are outlined in Table 2. The control was bubbled only with air and approximates the average ambient seawater chemistry that was observed at 0-4 h postspawning (2000-0200 hours, hours of maximum sperm activity and highest potential for fertilization) on Elbow Reef, Key Largo, FL (the site of gamete collection) as determined by hourly water sampling at 11 d postspawning (n = 4 hourly samples) (Table 2). Treatment water was filtered (0.2 μm) before use in experiments, to limit respiratory alterations of desired CO2 levels. Water samples were obtained and analyzed at the start of fertilization and settlement experiments to verify distinct treatments; samples were obtained weekly for growth experiments. Water samples were analyzed for TA and pH. TA was determined in duplicate using automated, open-cell Gran titration (SOP3b; ref. 39), and accuracy was checked against certified seawater reference material (from A. Dickson, Scripps Institute of Oceanography). pH on the total scale was determined using an Orion ROSS combination pH electrode (Thermo Scientific) calibrated at 25 °C against a seawater buffer (SOP6b; ref. 39). Concentrations of CO2−, Ca2+, and ΩCaCO3 were computed from TA, pH, temperature, and salinity using the CO2SYS program (from E. Lewis, Brookhaven National Laboratory), dissociation constants for carbonate determined by Mehrbach et al. (40) as reft by Dickson and Millero (41), and the dissociation constant for boric acid determined by Dickson (42). pH is reported on the total scale, the scale on which K1 and K2 were determined in the Gran functions.

**Fertilization Assays.** Concentrated sperm (3.21 × 10^6 sperm mL−1), representing equal contributions from three parental genotypes, were diluted to 3.21 × 10^6, 1.61 × 10^6, 6.41 × 10^5, and 3.20 × 10^5 sperm mL−1 in filtered treatment water. Optimal sperm concentrations for other species of broadcast-spawning corals have been reported to range between 10^6 and 10^8 sperm mL−1 (43). After 1-ml subsamples of each dilution were fixed in 10% formalin for verification of sperm concentrations (least replicate counts by hemocytometer), 10 mL of each sperm dilution–treatment combination was transferred via pipette into each of 10 replicate 15-ml glass vials. A 10-ml volume of sperm-free seawater was transferred into an extra vial as a negative (no-sperm) control. Then 100 μL of eggs (~200–250 eggs) was immediately added to each vial, and the mixture was swirled. Fertilization experiments were initiated within 3 h of spawning and conducted at 28.1 ± 0.1 °C. Embryos were subsampled at 4 h and fixed in 10% formaldehyde. Subsequently, 100–200 undamaged eggs from each subsample were examined using a dissecting microscope and classified as either fertilized (showing normal cleavage patterns of cell division) or unfertilized (showing no signs of cleavage).

Nonlinear regressions (exponential rise to maximum, two parameter) were fit to percent fertilization at sperm concentration data. Regressions were fit separately for each CO2 concentration using least squares residuals. The data were fit to the following model:

\[
\% \text{Fert} = \max \left(0, \% \text{Fert}_0 - e^{-C_0} \right)
\]

where \%Fert is the percent fertilization at sperm concentration (SC), \%Fert_0 is the asymptotic average maximum percent fertilization, and C is a rate coefficient that determines how quickly (or slowly) the maximum is attained (i.e., the slope). Justification for the use of this model is provided in SI Text. A comparison-of-fit test was conducted on arcsine-transformed data to test the null hypothesis (H0) that one curve best fits all data sets.

Data from the 0.32 × 10^5 sperm mL−1, 673 μatm treatment were omitted from the regression (**) in Fig. 52b, resulting in 30 df at 673 μatm. Reliable data were not available for this cell due to sperm contamination. This notion is supported by the model results, which indicate an expected value much lower than what was found for that cell. In addition, one outlier was removed from the 998-μatm treatment, resulting in 39 df (** in Fig. 52c); this outlier was >2.5 SD away from the mean. It is important to note that omitting these points did not change the statistical outcome of the regression analyses, but did provide a better fit for the model.

**Settlement Assays.** Gametes not used for fertilization experiments were fertilized, reared to competency in ambient seawater, and used in assays to test the effects of pCO2 on settlement success. Five-day-old larvae (5 days after spawning [dAS]) were introduced to settlement assays, where they were offered limestone settlement tiles that had been preconditioned for 40 d in recirculating aquaria corresponding to the three CO2 treatments. A single source of live rock was divided equally among the three treatment aquaria to provide a consistent source of crustose coralline algae and microfauna during the conditioning phase of the experiment. Settlement assays were conducted in six-well nontreated polystyrene tissue culture dishes for 7 d. The number of larvae per settlement assay was standardized for each CO2 treatment, and results were reported as the mean number of larvae per dish ± SD. When the percentage settlement of larvae is expressed as a percentage of the maximum number of larvae necessary to achieve 100% settlement, \%settlement is calculated using the following formula:

\[
\% \text{settlement} = \left( \frac{N}{N_{\text{max}}} \right) \times 100
\]

where N represents the number of larvae attached and N_{\text{max}} represents the maximum number of larvae necessary to achieve 100% settlement.
plates (BD Biosciences) using the same treatment water as used for substrate conditioning. One settlement tile, 10 mL of treatment water, and 10 larvae were randomly added to each well. Four 24-well plates were used per treatment. The plates were securely covered and submerged in treatment tanks to ensure temperature control (28 °C) and to prevent gas exchange (i.e., equilibration of mid- and high-CO$_2$ levels with atmosphere). Water was exchanged by syringe every 48 h, with care taken to not disturb larvae.

Tiles were examined at 6, 8, and 11 dA. Previous studies have shown that the first permanent larval settlement (i.e., metamorphosed and permanently settled juvenile polyps) of two acroporid species occurred at 5–6 dAS, and most larval settlement (85–97% of total) occurred within 9–10 dAS (44). The number of settled larvae on the top and bottom of each tile was counted using a dissecting microscope. Larvae were classified as “settled” when they had fully metamorphosed (i.e., a flat-disk-shaped appearance rather than pear-shaped), with little or no possibility of active detachment and further migration (11). Per-cent-age data were arcine-transformed and analyzed using regression analysis.

**Linear Extension.** After settlement, juveniles from the settlement experiments were reared in recirculating treatment aquaria over the course of 50 d. Linear extension was routinely measured using an optical micrometer, capable of measuring linear extension rates to a precision of 0.5 μm. During each measurement period, juveniles were briefly removed from the treatment aquaria, fitted onto the stage of the micrometer, and advanced through the path of the optical micrometer. Each measurement was conducted in less than 3 min, thereby minimizing the time that spat were out of the water. Linear extension was calculated as the change in elevation over time. Individuals exhibiting partial or full mortality were excluded from the analysis. Data were analyzed by regression analysis.

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