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Effects of Ocean Acidification on Bleaching, Survival, and Calcification of *Porites porites* and *P. astreoides* in Cartagena, Colombia [†]

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Abstract: Estimations of the ocean acidification-OA effects on marine environments indicate that coral reefs' structure will collapse. This study aimed to determine the effects of OA, and its associated carbon chemistry in the sea water, on corals near the Colombian Caribbean city of Cartagena, taking as model organisms of the species *Porites astreoides* and *P. porites*. For each species, the effect of OA on bleaching, survival, and calcification was determined using artificial systems with pH of 7.879 ± 0.004 and 7.789 ± 0.007. The results showed that under the first pH, the bleaching of *P. astreoides* increased by 24.92% and its survival decreased by 80.56%, while at lowest pH, bleaching increased in 32.78% and survival decreased by 87.5%. In the case of *P. porites*, at first pH bleaching increased by 29.42% and survival decreased by 30.56% and at the lowest, bleaching increased in 37.32% and survival decreased by 13.39%. In both species, calcification was reduced in more than 90% at 7.879 ± 0.004 and their skeleton began to dissolve at 7.789 ± 0.007. This study represents the first effort to determine OA effects on Colombian Caribbean's marine biota.

Keywords: coral reefs; climate change; Colombian Caribbean; *Porites* spp

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1. Introduction

In the last 50 years, the capture of CO_2 by the ocean has risen from 0.9 to 2.93 PgC yr^{-1}, generating a new component of climate change known as ocean acidification (OA) (Bolin and Eriksson 1958; Keeling et al. 1965; McNeil et al. 2003; Manning and Keeling 2006; Mikaloff et al. 2009; Ballantyne et al. 2012). It is estimated that in the year 2100, the acidification of the ocean will increase by 407% (reduction of pH to 7.5), and the solubility of CO_3^{-2} will decrease by 39%, affecting the corals that depend of this balance to produce their skeleton, gain space, and develop structures that help their survival (Feely and Chen 1982; Kleyplas et al. 1999; Feely et al. 2004; Sabine et al. 2004; Caldeira and Wickett 2005; Langdon et al. 2000; Hubbard et al. 2016; Allemand and Osborn 2019). Although ocean acidification affects mainly corals' calcification and growth, the energy imbalance also affects their reproduction, survival, and resistance to bacterial infections (Anthony et al. 2008; Jokiel et al. 2008; Cigliano et al. 2010; Kavousi et al. 2016). There is no information about the phenomenon of OA for the Colombian Caribbean, its effects, and which coral areas are most susceptible to it (Kleyplas et al. 1999; Orr et al. 2001). Considering the above, this study aimed to determine the effect of acidic stress on bleaching, calcification, and survival of two amply distributed coral reef builders, near the Colombian Caribbean city of Cartagena.

2. Materials and Methods

2.1. Study Area

The samples were collected from three Colombian Caribbean sites located between Bocachica (natural entrance to Cartagena's Harbour; 10° 18′ 35,3′′ N–75° 35′ 21,5′′ W) and the Barú Peninsula (North-East border of Rosario and San Bernando National Park; 10° 16′28′′ N–75° 37′ 56′′ W) (Vivas-Aguas et al. 2015). The coral species *Porites astreoides* (Lamarck 1816) and *P. porites* (Pallas 1766) were used as model organisms and four samples of both species were collected from each site. The samples were cut in pieces of ≈ 50 cm^2, separating each sample of each site in 3 marine systems where got acclimatize for a month. The marine systems got optimal parameters for coral growth (Moe 1989) and temperature, salinity, pH, alkalinity and nutrients were monitored.

2.2. CO_2 Treatments and Emulation of Acidic Conditions in Aquaria

Ocean acidification was emulated with CO_2 injections into aquaria, lowering the pH until it reflected A1B and A1F1 scenarios of projection model ISAM (Integrated Science Assessment Model for Climate Change) for 2100 (Jain et al. 1994; IPCC 2013). For scenario A1B the pH calculated was 7.896 ± 0.002, and for scenario A1F1 was 7.787 ± 0.001 (Robbins et al. 2010). A paired *t*-test was used to compare the physical-chemical conditions in each treatment with the optimal conditions for coral development.

2.3. Differences on Bleaching and Survival with Respect to OA Scenarios

The bleaching and percentage of survival were monitored by photographing the specimens with a high-resolution camera at 15 cm from the water. The bleaching level was determined using a coral health card on a decreasing scale from 6 to 1 (Siebeck et al. 2006). The survival (percentage) was defined as the ratio of living colonies between the beginning and the end of the experiment. The bleaching and survival data normality was checked with the Kolmogorov-Smirnov test and the homocestacity with the Levene statistic ($p > 0.05$). To determine the effect of pH on coral health, an ANOVA was made for each species (*Porites astreoides* and *P.porites*) and each response variable (bleaching and survival), taking as experimental factor the CO_2 concentrations (control, A1B, A1F1). To determine any potential effect from the collection sites on the response of each species to the treatments, a second ANOVA was performed for *P. astreoides* and a t-test for *P. porites* with each response variable (bleaching and survival), using as experimental factor the collection sites.

2.4. Differences on Calcification with Respect to the OA Scenarios

The calcification rate was estimated three times per month as the change in the water's alkalinity, produced by the fixation of the carbonate, following Langdon and Atkinson (2005). To determine the fixation of the carbonate, each of the coral samples was placed in an incubator for 1 h in 0.35 ± 0.05 Kg of water at 27 °C, with 35 g Kg^{-1} of salinity, 3.1 meq L^{-1} of alkalinity, 700 μmol photons m^{-2} s^{-1} of light, and the experimental pH to which the samples were being exposed (scenarios A1B, pH 7.896 ± 0.002; A1F1, pH 7.787 ± 0.001). Alkalinity was measured before and after incubation by adapting the titration by open-cell and approximation by least squares of Dickson and Sabine (2007). The area for *Porites porites* samples was estimated by using the formula for the area of a cylinder ($2\pi Hr$) and those of *P. astreoides* by flat photographic projection (Naumann et al. 2009). A Kruskall-Wallis test was performed to determine the potential differences in calcification with respect to the OA treatments.

3. Results

Data regarding the physical-chemical parameters, expected to be controlled at optimal ranges, did not show significant differences with respect to the optimal ranges for coral growth. During the experiments, and once the pH was stabilized, no significant differences (T-tests T_{A1B}: −0.13; T_{A1F1}: −0.55; $p > 0.05$; df: 2) were found between the theoretical and experimental pH corresponding to the scenarios.

3.1. Differences on Bleaching and Survival with Respect to the OA Scenarios

Less than 1% of the variation in *P. astreoides* health measures were explained by the collection sites and besides controls of *P. porites,* no significant difference was found. In the case of *P. astreoides*, pH had a significant effect on bleaching ($r^2 = 44.52\%$; F: 14.85; $p < 0.05$; df: 2) and survival ($r^2 = 63.46$; F: 19.97; $p < 0.05$; df: 2). There was also a significant effect of pH on bleaching for *P. porites* ($r^2 = 41.60$; F = 9.26; $p < 0.05$, df:2). However, differences in survival of *P. porites* were not significantly related to changes in pH ($r^2 = 2.91\%$; F: 0.36; $p > 0.7$; df: 2). The control samples of *P. astreoides* showed an improvement of up to 44.22% in their health during the experiments, with 100% survival. In the case of the respective A1B (700 ± 50 ppm = 7.894 ± 0.024 pH) and A1F1 (950 ± 50 ppm = 7.781 ± 0.019) scenarios, *P. astreoides* showed a 47.88% and 55.74% increase in bleaching compared to the controls and a decrease in survival of 80.56% and 87.50%, respectively. The control samples of *P. porites* were 11.48% healthier than the *P. astreoides* controls, but showed 11.11% less survival. At A1B, *P. porites* bleaching increased by 63.86%, and its survival decreased by 30.56% compared to the controls. At A1F1, its bleaching increased by 71.76%, and its survival decreased by 63.00%. Comparisons between the species showed that *P. astreoides* had 4.5% less bleaching than *P. porites* colonies in both acidification scenarios, but presented higher mortality at A1B (38.89%) and A1F1 (63%).

3.2. Differences on Calcification with Respect to the OA Scenarios

The Kruskal-Wallis test were used to establish differences on calcification rates of the samples between treatments, between sites, and between species (*Porites astreoides* and *P.porites*). In the case of *P. astreoides,* no site effect was found (H: 0.14; $p > 0.9$; df: 2) and the average calcification rate decreased significantly with pH (H = 6.77; $p < 0.05$; df: 2). The calcification under scenario A1B decreased by 80.19% ($4.28 \pm 3.28 \times 10^{-3}$ μE Hour^{-1} mm^{-2}), and under scenario A1F1 there was negative calcification ($-2.42 \pm 3.46 \times 10^{-3}$ μE Hour^{-1} mm^{-2}) with 11.17% of skeletal tissue dissolving. The average calcification rate in *Porites porites* also decreased significantly with pH (H: 6.55; $p < 0.05$; df: 2). There were not significant differences between collection sites under scenario A1B (H: 0.87; $p > 0.5$; df: 2), with all the coral samples showing a 99.43% decrease in calcification ($0.46 \pm 1.34 \times 10^{-3}$ μE Hour^{-1} mm^{-2}). However, under scenario A1F1 the calcification rate decreased by 99.34% in Baru ($0.53 \pm 1.24 \times 10^{-3}$ μE Hour^{-1} mm^{-2}) and there was negative calcification in Bocachica ($-5.33 \pm 0.913 \times 10^{-4}$ μE Hour^{-1} mm^{-2}) with 0.65% of skeletal dissolution.

4. Discussions

During the experiments, both corals increased their proportion of bleaching under acidic conditions. This increase in bleaching observed under OA conditions agrees with the results obtained by Anthony et al. (2008), who recorded a 20–50% increase in bleaching under concentrations of 520-1360 ppm pCO$_2$ (7.6–7.95 pH). Regarding the validity of the data with respect to the natural environment, bleached corals can continue to obtain energy by feeding on suspended particles in the short to medium term (Grottoli et al. 2006; Colombo-Pallota and Rodríguez-Román, 2010). Doo et al. (2019), suggest that neighbour crustose coralline algae and other reef calcifyers may be more sensible to pH changes than average corals, and under natural environments they can reduce the direct effect that lower pH waters have on calcification by adding their own diluted $CaCO_3$ to the surrounding water (Doo et al. 2019). Therefore, under OA scenarios, the effect on the calcification and energy demand of a coral could be less. However, due to the evolutionary dependency of corals on zooxanthellae for their nutrition and survival, corals will tend to die in the long term. The data obtained suggest that *P. astreoides* kept their zooxanthellae under acidic environments (lower bleaching) and calcifyed longer than *P. porites*. However, their survival was potentially affected by the oxidative stress from the reoxygenation produced by the symbionts. Previous studies confirm that resilience and survival of *P. astreoides* decrease with pH (Hall et al. 2015; Bove et al. 2019), while its calcification rate only drops up to 66% (6.7–7.3 pH) (Crook et al. 2012; Martinez et al. 2019). It was observed that the calcification rate in control samples of *P. porites* was in some cases null or negative, an indication of a strategy to minimize stress due to the change between the aquaria and the incubators, because by decreasing their calcification rate, there was a lower energy demand in RC (rapid calcification zones), which, together with zooxanthellae's expulsion, reduces oxidative stress in tissue reoxygenation and increase survival (Chuanyu and Jackson 2002; Downs et al. 2002; Richier et al. 2003; Jokiel 2011).

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