Effects of predicted future acid conditions on calcification and polyp health of *Pocillopora damicornis*

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INTRODUCTION

Present-day atmospheric carbon dioxide levels are estimated to be 30% higher than the natural range over the last 650,000 years, during which most marine organisms evolved; these levels are projected to double by the end of the century (Meehl *et al.* 2007). Increased carbon dioxide (CO_2) emissions have led to growing concern for the world's coral reefs due to a process known as ocean acidification (OA), which refers to the increase in acidity of the ocean's waters resulting from oceanic uptake of atmospheric CO_2. About 25% of anthropogenic CO_2 emitted into the atmosphere is absorbed by the oceans (Canadell *et al.* 2007), where it reacts with water to form carbonic acid. Carbonic acid then dissociates to form bicarbonate ions and hydrogen ions, which in turn react with carbonate ions to produce more bicarbonate ions, thus reducing the availability of carbonate to organisms like coral that require it to form their calcium carbonate skeletons (Silverman 2009). In this way, OA reduces calcification rates of corals (Hoegh-Guldberg *et al.* 2007) by decreasing aragonite saturation essential to building the calcium carbonate skeletons that constitute reefs. Already, increased atmospheric CO_2 has depleted seawater carbonate concentrations by ~30 mmol kg^{-1} seawater and acidity by 0.1 pH unit (Meehl *et al.* 2007).

Coral reefs are among the most biologically diverse and economically important ecosystems on the planet (Connell 1978; Peterson & Lubchenco 1997). Reefs provide services such as coastal protection and the potential for new pharmaceuticals through incredible biodiversity, as well as providing a direct livelihood to thousands through abundant fisheries and building materials. Non-monetary benefits humans derive from coral reefs such as existence value are difficult to quantify (Spurgeon 1992), but the GBR provides an estimated AUD$5 billion per annum in to the Australian economy through tourism, recreational boating and fishing, and direct use (Access Economics 2008).

For this study we tested how increased CO_2 emissions might affect *Pocillopora damicornis* in particular in order to assess the effects OA might have on the reef as a whole. *P. damicornis* was chosen as a model organism because it has been studied extensively and is common on Great Barrier Reef (GBR) reef flats (Clausen & Roth 1975; Stoddart 1983; Yeoh & Dai 2010). It has relatively fast growth rates, small polyps, and thin tissue, ideal for this short-term

experiment. *P. damicornis* is a highly colonial shallow-water species, one of the brooding minority. *P. damicornis* is an especially successful recruiter; it settles and grows quickly, particularly in marginal areas, making it important species for recovering or struggling reefs. Every species plays its role in retaining nutrients on the reef, and numerous symbiotic species depend upon *P. damicornis* for survival (Stimson 1990; Stimson 1997). Given that recent and future rates of change dwarf even those of the ice age transitions (Hoegh-Guldberg *et al.* 2007), it is likely that these changes will exceed the capacity of *P. damicornis* to adapt. If it is unable to build its skeleton under future acid conditions, it will not be *P. damicornis* alone that is affected, and a ripple effect could be seen throughout the reef.

Here, we tested two reduced pH conditions to observe the effects on calcification and tissue recovery of *P. damicornis* and the extent to which the species may be able to withstand increased acidity according to mid- and late-century predictions. We hypothesized that elevated pCO_2 would slow calcification, increase dissolution, and decrease polyp health of *P. damicornis*.

METHODS

CO_2 treatments and general protocol

The experiment was conducted at Heron Island Research Station (HIRS, **Figure 1**), on the southern GBR 72 km northeast of Gladstone, Queensland, from 17 to 27 October 2012. Corals were settled in ambient seawater and grown in a flow-through aquarium system following standard protocols for OA research (Gattuso *et al.* 2010). Corals were subjected to three treatments, one control (pH 8.05) and two elevated (pH 7.82 and pH 7.63). These levels were chosen based on A1F1 projections of future acid conditions for mid-century and late-century by the IPCC (Meehl *et al.* 2007) and are standard for OA research at HIRS. These levels are worst-case projections but do not represent unlikely scenarios; current CO_2 emissions are tracking the highest carbon intensive levels (A1F1) predicted by the IPCC (Le Quere *et al.* 2009).

Figure 1. Heron Island, the site of HIRS (Credit**:** Image by Robert Simmon).

We used an OA system at the HIRS which was a model system for these predicted ppm of CO_2 and pH values. The sea water system pumped unfiltered seawater directly off the reef flat into the tubs, allowing for a constant flow of fresh seawater to the micronubbins with no water recirculation. Six tubs (Tubs 3 through 8) in the Ocean Acidification System were used. Two tubs (Tubs 4 and 6) received control seawater at ambient pH (averaging 8.05). This seawater was unfiltered seawater pumped directly off the reef flat into the tubs with no alteration. The pH was monitored using pH probes. Two tubs (Tubs 3 and 8) received experimental seawater with pH reduced by dissolving additional CO_2 to an average 7.82. Two tubs (Tubs 5 and 7) received experimental seawater with pH reduced to an average 7.63 by dissolving additional CO_2. For the experimental seawater, the unfiltered seawater pumped directly off the reef flat was first adjusted to 7.8 and 7.6 (from 0.3 to 0.5 units below the current ambient pH value according to the predicted decrease in pH by ca. 2100) using an automated CO_2 injection system to elevate the dissolved CO_2 concentrations before being pumped into the tubs. The experimental seawater was still unfiltered seawater pumped directly off the reef flat, however, the CO_2 levels were first increased by using adjusting the pH in separate, 200 mL sumps. A pH control unit (Aquatronica, AEB technologies, Italy) was used to monitor and maintain the pH at the desired level. The pH control unit was connected to a pH probe in each sump and to an electronic solenoid connected to a cylinder of CO_2. Whenever the pH of the experimental seawater rose above the desired pH, the solenoid valves were opened and injected bubbles of CO_2 into a diffuser rapidly dissolving CO_2 into the experimental sea water. The pH probes were cleaned

and calibrated using Mettler-Toledo calibration buffers to 0.01 pH units. Data were logged every 3 hours to provide average pH values for the duration of the experiment. Tanks were shared with a fellow researcher, conducting a similar experiment on the coral *Stylophora pistillata*. Walls of the aquaria were cleaned regularly to minimize algal growth.

Coral collection and preparation

An adult colony of *Pocillipora damicornis* was collected by HIRS researchers on the reef flat and housed in a flow-through outdoor aquarium facility of unfiltered seawater at HIRS. A total of ninety polyp micronubbins ranging approximately 2-6 mm were sliced from branches using straight razors; slides were prepared of three micronubbins each. These slides were acclimated to ambient seawater for three days before being randomly assigned among the replicate tanks in the experimental system, where they remained for the course of the experiment. Mortality occurred during transportation, randomly between groups (2 micronubbins in the ambient treatment, 5 in the 7.6 treatment, and 1 in the 7.8 treatment). These micronubbins were knocked off the slides while they were transported from the tanks to the lab for photographing.

Data analyses

Photographs of each specimen were taken at the beginning and end of the experiment, and growth estimates were made using ImageJ software (http://rsbweb.nih.gov/ij/). Data were analyzed using a one-way ANOVA.

Polyp condition was analyzed qualitatively, on a standardized scale from 1-5. A score of 1 indicated complete bleaching; a 2 was given to micronubbins with zooxanthellae present only on polyp tentacles; micronubbins with a score of 3 had zooxanthellae only on polyps and sparsely elsewhere; a 4 was given to relatively healthy micronubbins with zooxanthellae present throughout and only small areas bleached; and a score of 5 indicates a healthy, completely unbleached micronubbin. Change in condition from the start to the end of the experiment was calculated (e.g. from a score of 5 to 3 was a change of -2) and averaged to assess polyp health for each of the treatments.

RESULTS

Although a trend was observed indicating some skeletal growth in the control treatment and mild dissolution in the experimental treatments, results of ANOVA indicate that the change in skeletal area of the micronubbins was not significantly different between pH treatments, as is shown in **Figure 2** (ANOVA, $p=0.42$).

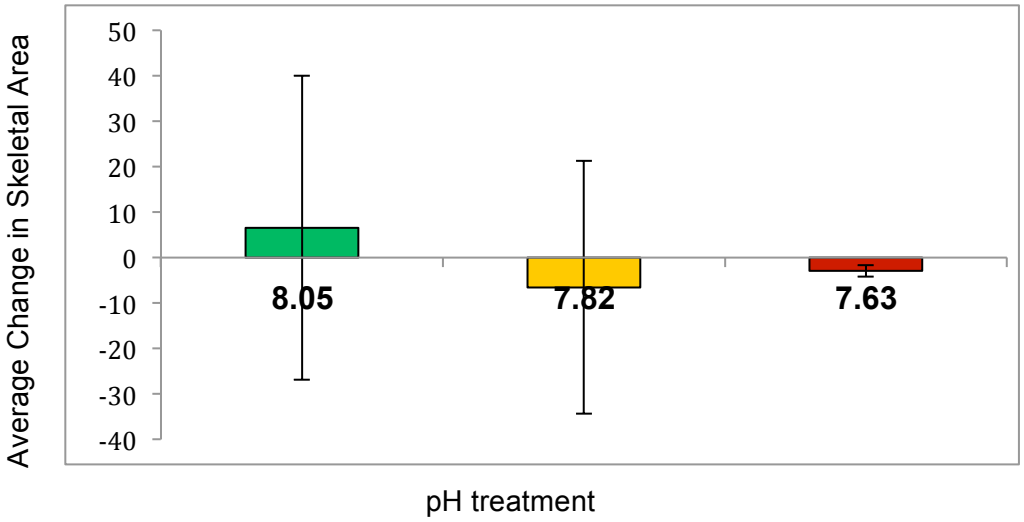

Figure 2. Average change in skeletal area of micronubbins expressed as percentage of initial surface area.

The difference in polyp condition between pH treatments shown in **Figure 3** indicates a trend toward increased bleaching and decreasing overall polyp health with decreasing pH; however, statistical analysis showed that this trend is not significant (ANOVA, p=0.089).

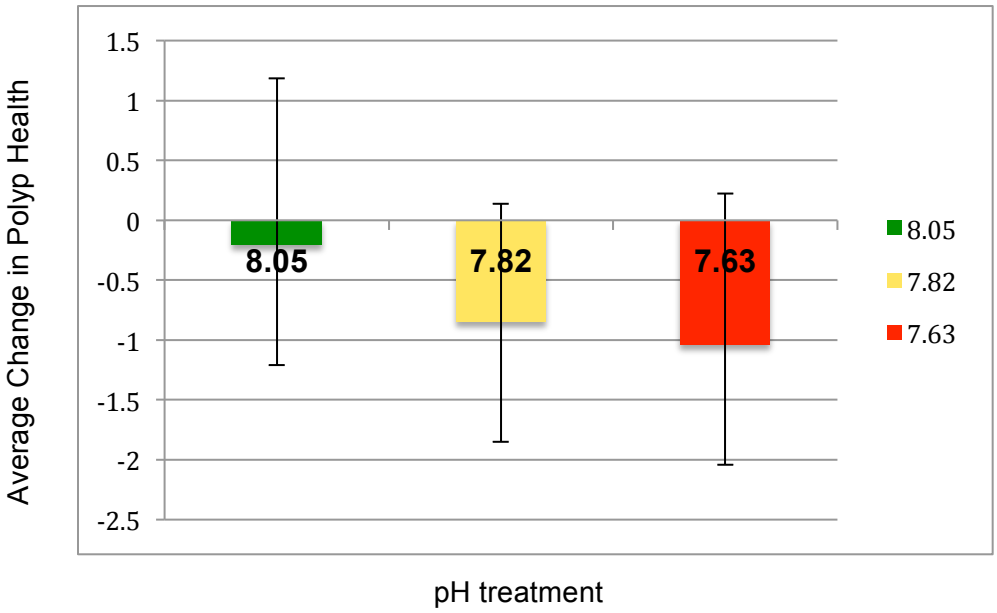

Figure 3. Average change in polyp condition for each treatment expressed as difference between initial and final condition on 1-5 scale.

DISCUSSION

Despite the statistically non-significant findings of this study, it is well documented that changing ocean chemistry as a result of increased oceanic uptake of atmospheric CO_2 has profound effects on the physiology of many marine organisms, and on corals in particular (Orr *et al.* 2005; Kleypas *et al.* 2006; Anthony *et al.* 2008; Pelejero *et al.* 2010; Suwa *et al.* 2010). The consistently-observed trend toward decreased calcification of coral skeletons under reduced pH conditions is visible in this study (**Figure 2**), though it was not statistically significant. The trend shows that micronubbins in the control treatment showed modest growth, while the micronubbins in the two reduced pH treatments showed negative growth, or dissolution of their calcium carbonate skeletons. It is possible that had the experiment continued for more than two weeks, this trend would have become significant.

Also consistent with previous studies, though not statistically significant here, bleaching was more prevalent and overall polyp health was poorer in the two reduced pH treatments than in the control treatment at ambient pH (Anthony *et al.* 2008) (**Figure 3**). Bleaching occurs when corals, under stress, release their zooxanthellae. These corals are less aesthetically pleasing, which could dramatically impact the tourism industry in Australia; bleaching can also affect populations of species that live on the reef and may have widespread, unpredictable effects on biodiversity. Individuals and communities that rely on the reef for enjoyment and survival could be deeply impacted by future bleaching events resulting from reduced pH.

Limitations of this study warrant further work. Importantly, a longer experiment likely would have yielded significant results for both change in skeletal area and polyp health. A larger sample size would also improve this study and generate more data; mortality during transportation further reduced sample size, and methods could be developed to avoid this problem in the future.

Further research might improve these conditions and also test additional variables. For example, in this study we wished to count the number of polyps in each micronubbin at the start and end of the experiment to assess differences between treatments in asexual reproduction, but this proved impossible due to the quality of photographs and the three-dimensionality of the micronubbins. Future researchers may also wish to consider pH changes combined with temperature changes, according to IPCC projections in association with global warming and sea surface temperature (SST) rise. It would be especially interesting to test these variables against an additional tank in which the pCO_2 and temperature are adjusted to pre-industrial levels; this method would illustrate the effects humans have already had on coral calcification and polyp health.

It is important to conduct further research in this field as humans continue destructive practices that change the natural environment and alter marine chemistry. It is clear from current research that ocean acidification exacerbates local pressures such as sedimentation and overexploitation, pushing reefs further toward functional collapse.

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