Effects of Bicarbonate Addition on *Montipora capricornis* Growth Rate and Photosynthesis

Team Falcon

Saint Stephen's Episcopal School

USA

Meredith Moore, Hannah Arendt

ashields@saintstephens.org

Abstract

Within the past century, exponential increases in anthropogenic carbon dioxide release to the atmosphere has lowered the ocean's pH, ultimately preventing coral reefs from absorbing sufficient calcium carbonate needed to maintain healthy skeletal structures. While it is generally accepted that corals utilize carbonate (CO_3^{2-}) from their surrounding water to calcify, evidence suggests that bicarbonate (HCO_3^-) may also support the DIC needs of calcification, either directly or indirectly by converting HCO_3^- to CO_3^{2-} at the calcification site (Comeau, S., et al., 2012). Here we examined what concentration of $NaHCO_3$ was able to maximize the growth rate, calcification, and photosynthesis of *Montipora capricornis*. We found that increased bicarbonate concentrations of 5mM, on average, tripled *M. capricornis* growth rate. Growth rate began to decline as concentrations exceeded 5mM. Photosynthesis also became saturated at 5mM concentrations.

Purpose

Coral reefs, often referred to as "rainforests of the sea" are known to harbor greater biodiversity than nearly any other ecosystem on the planet. They play a main role in the carbon cycle as they lead to a calcium carbonate production of about 10^{12} kg per year, therefore performing over half of the world's biological $CaCO_3$ precipitation (Milliman and Droxler, 1996, p. 496-504). Apart from their environmental benefits, coral reefs provide food and resources for over 500 million people in countries all across the globe and therefore are essential to the economies of tropical maritime nations. In the past, coral reefs have been able to rebound from natural disaster and climate change however within the past century, anthropogenic activities have expedited climate change to a point where reefs may not be able to keep up with the pace of the changing ocean chemistry and temperatures. Atmospheric CO_2 is estimated to reach twice its preindustrial level by 2065 (Herfort, Lydie, et al., 2008, p. 91-98). Ten percent of coral reefs have already been damaged beyond repair and it is projected that over 90% will be in danger by 2030.

When CO_2 is absorbed by the ocean, it reacts with seawater to form carbonic acid which ultimately dissociates into HCO_3^- and H^+ ions. As pH is a measurement of hydrogen ions in a solution, this reaction has the effect of lowering seawater pH. Additionally, the excess H^+ ions consume carbonate (CO_3^{2-}), thus lowering the aragonite saturation state (Ω_{ar}) of the seawater that is essential to the deposition of calcium carbonate skeletons. The saturation state of aragonite – the mineral form of $CaCO_3$, — is a concept often used by physical chemists to describe the condition of seawater in relation to the mineral form of $CaCO_3$ precipitated by coral reefs. The term Ω_{ar} is defined by:

$$\Omega_{ar} = \frac{[Ca^{2+}][CO_3^{2-}]}{K_{sp}} \qquad (1)$$

Where K_{sp} is the solubility product of aragonite (Jokiel, 2011, p. 639-657). Since Ca^{2+} is highly concentrated in seawater and affected little by external influences, Ω_{ar} is generally limited solely by CO_3^{2-} availability.

An organic matrix (OM), biosynthesized using both photosynthetic products of zooxanthellae and heterotrophic sources as shown by Allemand *et. al* (2001) is a prerequisite step for the growth and formation of skeletal biomolecules. Specifically, a number of acidic proteins within the OM catalyze the precipitation of $CaCO_3$ and induce the nucleation of $CaCO_3$ crystals (Allemand *et. al*, 2001). The skeletal growth of scleractinian corals involves two different processes. The first process occurs at night, in which a calcium carbonate crystal framework is laid down. This initial mineralization is characterized by randomly-oriented microgranular components. The next day, crystallographic alignment and nucleation of the new crystals determines the arrangement of the aragonite fibers and results in increased skeletal density (Marubini, et al., 1998).

While it is generally accepted that corals utilize carbonate (CO_3^{2-}) from their surrounding water to calcify, evidence suggests that bicarbonate (HCO_3^-) may also support the DIC needs of calcification, either directly or indirectly by converting HCO_3^- to CO_3^{2-} at the calcification site (Comeau, S., et al., 2013). In either case, an increase in the concentration of DIC surrounding scleractinian corals may be able to accelerate the calcification process, showing that calcification rate is limited by DIC availability. Previous studies have shown that the addition of 2 mM bicarbonate to tanks of the branching coral, *Porites porites*, has doubled the calcification rate of the coral skeleton (Marubini et al., 1999).

Materials and Methods

Biological material

Biological materials used for the present experiment were cloned colonies of the coral *Montipora capricornis* cut into ~2.5cm × 2.5cm fragments from parent colonies propagated in the laboratory. The coral were originally acquired from the Pacific Coral Reef Display at Mote Marine Aquarium. Two separate genotypes, red and green, were tested with a 2:1 ratio of red to green. Prior to the experiment, the coral were stored in an aquarium with ambient seawater pulled from Sarasota Bay. All seawater was filter-sterilized, ozonated, and aerated before use.

Abiotic conditions

Thirty-six *M. capricornis* were divided evenly into 12 separate 9.46-liter tanks, each with 2 red genotypes and 1 green genotype. Ambient seawater (26.5°C, salinity 35ppt) was kept in constant circulation within tanks and temperature was controlled by keeping the tanks partially submerged in one main tank. Daily water quality measurements were taken with a YSI ProDSS to ensure consistent temperature, salinity, pH, and dissolved oxygen levels. Fluorescent lighting with a L:D cycle of 9:15 and photosynthetic active radiation (PAR) of 150 $\mu molm^{-2}s^{-1}$ was directly above the tanks.

Bicarbonate additions

The bicarbonate exposure period lasted 31 days. A stock solution was prepared every two days from lab-grade $NaHCO_3$ powder and alkaline bromine (BrO^-) water and distributed proportionally to each tank, creating tank $NaHCO_3$ concentrations of 0mM, 2mM, 5mM, and 8mM. Tank water changes and cleaning also occurred every two days.

Growth measurements

Coral growth was measured using the buoyant weight technique in which the living coral are weighed as they are suspended in a buoyant medium of seawater. The sensitivity of this method can detect changes in mass over short time intervals (Jokiel et. al., 1978, p. 529-541). Buoyant weight measurements were taken at 0 days, 14 days, and 31 days for the present experiment.

Fluorescence measurements

Photosynthetic efficiency was measured through different fluorescence-based parameters including PAR, electron transport (ETR), and Y(II) — the quantum yield of photochemical energy conversion in photosystem II which gives insight into the photosystem's photon-use ability. The Imaging-PAM fluorometer used provides a quick assessment of overall photosynthetic state by administering a series of saturating light pulses and subsequently measuring the coral's fluorescent output (Warner, et. al., 2010).

Statistical analysis and curve-fitting

For buoyant weight measurements, the results are expressed as percent change over time found using:

$$((M_2 - M_1) \div M_1) \times 100 \quad (2)$$

in which M_1 represents initial mass and M_2 represents final mass. The data was transformed ($grams^{1/2}$) to normalize before testing for homogeneity. Photosynthetic measurements of quantum yield and ETR slope were transformed using a boxcox transformation from the R GenABEL package. Maximum ETR data was not transformed. Differences with $P<0.05$ were considered statistically significant.

Results

Growth

The growth responses to HCO_3^- addition showed that *M. capricornis* growth rate maximized at DIC concentrations of +5 mM with an average increase of 52.2% over the 4-week exposure. The *M. capricornis* not dosed with any HCO_3^-, by contrast, only grew an average of 16.3% over the 4 weeks. Figure 1 illustrates the average percent change of buoyant weight for each treatment. A bit unexpectedly, growth rate began to decline slightly as concentrations exceeded +5mM. Post hoc statistical testing showed that there were significant differences between the 5mM treatment and the Control group (F = 13.53, DOF = 3, P = 0.0000299) as well as between the 5mM treatment and the 2mM treatment (F = 13.53, DOF = 3, P = 0.0000370). Significant differences were also noted between the 8mM treatment and the Control group (F = 13.53, DOF = 3, P = 0.0405998) and the 8mM and 2mM treatments (F = 13.53, DOF = 3, P = 0.0480789).

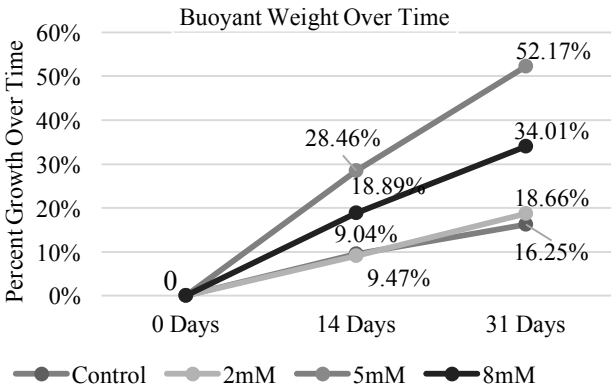

Figure 1: Percent change of initial to final buoyant weight. Values were calculated by averaging percent changes over time of all coral in each treatment.

Electron transport rate

ETR of *M. capricornis* also maximized in the 5mM treatment with a maximum ETR of 25.34814815 µmol electrons $m^{-2}s^{-1}$ and an ETR slope of 0.10780382. The control group showed to have the lowest average ETR, maximizing at 22.25185185 µmol electrons $m^{-2}s^{-1}$ with a slope of 0.070864448. There was a significant difference among treatments for ETR max. The post hoc test showed that the 5mM treatment was different than the 2mM (F = 5.798, DOF = 3, P = 0.0011281) and the Control (F =5.798, DOF = 3, P = 0.0255222). Post hoc tests also showed significant differences in ETR slope among treatments. The 5mM treatment was significantly different from the Control (F = 10.246, DOF = 3, P = 0.0016232) and the 2mM (F = 10.246, DOF = 3, P = 0.0000504). The 8mM treatment also showed to be significantly different from the Control (F = 10.246, DOF = 3, P = 0.0204818) and the 2mM (F = 10.246, DOF = 3, P = 0.0010508) for ETR slope.

Quantum yield of PS II photochemical energy conversion

Although the 5mM treatment showed to have the greatest ETR and reached maximum ETR most rapidly, these coral on average showed to have the lowest fraction of energy photo-chemically converted in PS II at 0.45074074. The coral in the 2mM treatment showed to have the highest average PS II quantum yield at 0.47462963. Differences of significance were noted between the 2mM and 5mM concentrations (F = 4.492, DOF = 3, P = 0.0023267).

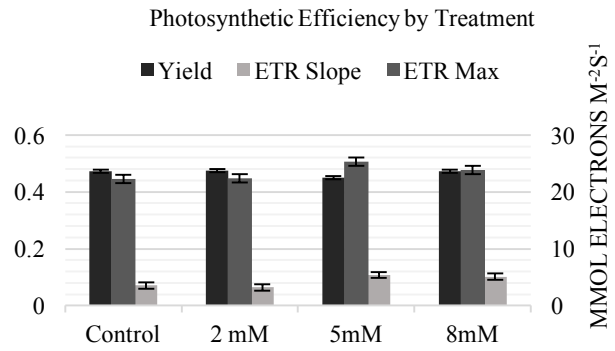

Figure 2: The photochemical yield, ETR maximum, and slope of increasing electron transport for each treatment.

Conclusion

Coral growth and electron transport efficiency both increased linearly with $NaHCO_3$ concentrations under conditions of constant pH. These findings are in agreement with the results of previous studies investigating the effects of bicarbonate concentration on coral growth (Marubini et al., 1999; Schneider and Erez, 2006) and suggest that coral growth in ambient seawater is carbon-limited and can, in fact, be accelerated when natural seawater DIC levels are increased. Such increases allow for a greater seawater Ω_{ar} and therefore, provide a greater supply of the resource utilized by the coral in their process of calcification. As $NaHCO_3$ concentrations exceeded 5mM, however, growth and electron transport began to decrease, perhaps due to slight pH decreases caused by the dissociation of HCO_3^- into CO_3^{2-} and H^+ ions. Based on the "proton flux hypotheses" (Jokiel, 2011), higher H^+ concentrations in the water result in a decreasing efflux of H^+ through boundary membranes, thus reducing efficiency of basic physiological processes, such as calcification and photosynthesis. It therefore may hold true that coral growth can be expedited proportionally to increased DIC levels beyond 5mM. Further experimentation with higher DIC levels and conditions of constant pH would be needed to identify the DIC saturation of calcification and photosynthesis.

While HCO_3^- addition stimulated both growth and electron transport, it resulted in a slightly lower energy yield in Photosystem II. This suggests that the chloroplasts within the zooxanthellae of treated coral ran more efficiently yet had a lower use of photons during energy capture. Several studies based on CO_2 and O_2 measurements of marine plants, however, have reported increased photosynthesis in DIC concentrations higher than ambient seawater, suggesting that DIC may play a larger role in the process of photosynthesis. (Gao et al. 1993, Weis 1993, Herfort et al. 2002) Therefore although photochemical energy conversion was decreased slightly under higher HCO_3^- concentrations, the extra availability of CO_3^{2-} as a form of DIC still shows to be beneficial to the photosynthetic process of coral.

With the unprecedented bleaching and death rates of coral reefs across the globe today, research into the factors surrounding coral health as well as new methods for fast-paced coral regeneration are needed more than ever. The results of this experiment highlight the positive effects that increased DIC levels, in the form of $NaHCO_3$, have on coral calcification and zooxanthellae photosynthesis and ultimately provide evidence that dosing laboratory-propagated corals with $NaHCO_3$ may expedite growth rate and allow for quicker and more abundant transplantation back to natural reef habitats. It would, however, be important to investigate the acclimation of corals grown in a DIC-enriched environment back into natural seawater before utilizing this DIC-addition method for coral regeneration. This experiment also poses the question of what other forms of DIC may be useful in expediting coral calcification and enhancing other general coral physiological processes, such as photosynthesis.

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