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CO_2-induced ocean acidification impairs calcification in the tropical urchin *Echinometra viridis*

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ABSTRACT

Atmospheric carbon dioxide (pCO_2) has risen from approximately 280 to 400 ppm since the Industrial Revolution, due mainly to the combustion of fossil fuels, deforestation, and cement production. It is predicted to reach as high as 900 ppm by the end of this century. Ocean acidification resulting from the release of anthropogenic CO_2 has been shown to impair the ability of some marine calcifiers to build their shells and skeletons. Here, we present the results of ocean acidification experiments designed to assess the effects of an increase in atmospheric pCO_2 from ca. 448 to 827 ppm on calcification rates of the tropical urchin *Echinometra viridis*. Experiments were conducted under the urchin's winter (20 °C) and summer (30 °C) water temperatures in order to identify seasonal differences in the urchin's response to ocean acidification. The experiments reveal that calcification rates decreased for urchins reared under elevated pCO_2, with the decline being more pronounced under wintertime temperatures than under summertime temperatures. These results indicate that the urchin *E. viridis* will be negatively impacted by CO_2-induced ocean acidification that is predicted to occur by the end of this century. These results also suggest that impact of CO_2-induced ocean acidification on urchin calcification will be more severe in the winter and in cooler waters.

1. Introduction

Atmospheric pCO_2 has increased from approximately 280 to 400 ppm since the Industrial Revolution, primarily due to the anthropogenic combustion of fossil fuels, deforestation, and cement production (IPCC Assessment 4, 2007; Keeling, 1960; Keeling et al., 2009; Neftel et al., 1985; Rahmstorf et al., 2007; Worrell et al., 2001). This post-Industrial increase in atmospheric pCO_2 has caused the pH of surface seawater to decrease by approximately 0.1 units (Raven et al., 2005). Current estimates predict that atmospheric pCO_2 could reach as high as 900 ppm by the end of this century, resulting in a decrease in seawater pH of up to 0.3–0.4 units (Brewer, 1997; Caldeira and Wickett, 2005; IPCC Assessment 4, 2007; Raven et al., 2005). Recent studies suggest that these reductions in seawater pH will impair shell and skeletal production within many calcifying marine organisms (e.g., Doney et al., 2009; Fabry et al., 2008; Iglesias-Rodriguez et al., 2008; Kleypas et al., 2006; Ries et al., 2009; Rodolfo-Metalpa et al., 2010; Wood et al., 2008).

Sea urchin tests are composed of large, optically aligned crystals of high magnesium (~5–8%) calcite and occluded glycoproteins (<0.1% by mass) that form complex, rounded vesicular structures filled with living tissue (Politi et al., 2004; Wilt, 1999, 2002). Recent investigations focused on larval urchin development and adult urchin spine regeneration report that urchin biomineralization is mediated by organic matrices (DuBois and Chen, 1989; Politi et al., 2004; Wilt, 1999, 2002), where vesicles transport protein-stabilized amorphous calcium carbonate (ACC) into a syncytium composed of primary mesenchyme cells (Politi et al., 2004; Wilt, 2002). This syncytium controls ion balance, liquid volume, and shape of the mineralization site, thereby regulating the crystallization of high-magnesium calcite into the complex framework that composes the urchin endoskeleton (DuBois and Chen, 1989; Politi et al., 2004; Wilt, 2002).

Yet despite urchins' apparently strong biomineralogical control, previous work has shown that their calcification can be strongly influenced by the physical properties of seawater (Borremans et al., 2009; Dickson, 2002; McClintock et al., 2011; Ries, 2004; Weber, 1969, 1973). The effects of seawater pH, in particular, on the growth and calcification of echinoderms have been investigated in numerous studies (e.g., Brennand et al., 2010; Gooding et al., 2009; Ries et al., 2009; Shirayama and Thornton, 2005; Wood et al., 2008). One study (Gooding et al., 2009), conducted on the sea star *Pisaster ochraceus*, found that calcified mass decreased relative to the control (380 ppm pCO_2) when the sea star was reared under acidified conditions (780 ppm pCO_2). However, total growth rates remained constant due to increased soft tissue production under acidified conditions. This same study also examined the effects of temperature on total growth rates and found that total growth

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decreased linearly with temperature from 21° to 5 °C at 380 ppm pCO_2. The study revealed no other deleterious impacts on health during the experimental period, despite the decrease in calcified mass.

Conversely, a study conducted on the brittlestar *Amphiura filiformis* revealed that calcification rates increased while rates of muscle mass production decreased when seawater was acidified from pH 8.0 to 6.8 (Wood et al., 2008), suggesting that the maintenance of elevated rates of calcification under the high-pCO_2 conditions came at the metabolic expense of muscle generation. A study on the effects of ocean acidification on the growth of the urchin *Echinometra mathaei* revealed a decrease in net calcification under high-pCO_2 conditions (modern atmospheric $pCO_2 + 200$ ppm) compared with net calcification rates under modern atmospheric pCO_2 (Shirayama and Thornton, 2005). Ries et al. (2009) reared the tropical urchin *Eucidaris tribuloides* and the temperate urchin *Arbacia punctulata* under pCO_2 levels of ca. 400, 600, 900, and 2850 ppm. They found that the tropical urchin *E. tribuloides* exhibited a 'threshold-negative' response to elevated pCO_2 (with the decline evident at 2850 ppm) while the temperate urchin *A. punctulata* exhibited a 'parabolic' response (i.e., calcification increased from 400 to 900 ppm pCO_2 and declined at 2850 ppm). These studies collectively indicate that echinoderms exhibit variable responses to moderately acidified conditions ($pCO_2 < 900$ ppm), with a more unanimous decline in health (calcification and tissue generation) when exposed to highly acidified conditions ($pCO_2 > 900$ ppm).

Here, we report on experiments investigating the effects of CO_2-induced ocean acidification on the reef- and rocky-substrate-dwelling tropical urchin *Echinometra viridis*, which is widely distributed throughout the Caribbean Sea, from Southern Florida to the West Indies to Venezuela (McPherson, 1969). We reared this tropical urchin under control pCO_2 conditions (ca. 480 ppm) and under predicted end-of-century pCO_2 conditions (ca. 800 ppm), under both winter (ca. 20 °C) and summer temperatures (ca. 30 °C), in order to determine how *E. viridis* may respond to end-of-century acidification during both the warm and cold season.

2. Materials and methods

2.1. Collection and acclimation

Approximately 44 specimens of *Echinometra viridis* were collected in late January 2011 off Key Largo in Southern Florida and transported to the University of North Carolina at Chapel Hill by airplane. Temperatures near the collection site ranged from ca. 15 to 33 °C in 2010 (NOAA Station KYWFl-8724580). Specimens were acclimated to laboratory conditions for ca. 45 days in a 55 gallon holding tank that contained seawater formulated at a salinity of ca. 32 and maintained at a temperature of ca. 25 °C. The acclimating urchins were fed every other day with approximately 270 mg dehydrated marine algae.

2.2. Seawater formulation

Each experimental aquarium contained 34 liters of seawater formulated at a salinity of 32.06 ± 0.02 with *Instant Ocean Sea Salt* mixed with deionized water. Deionized water was also periodically added to the experimental aquaria in order to replenish water lost due to evaporation. 250 mL seawater samples were taken weekly for analysis of dissolved inorganic carbon and total alkalinity and were replaced with ca. 32 salinity seawater.

2.3. Aquarium conditions

Four sets of three-way replicated treatments were illuminated for 10 hr/day with T8 6500°K fluorescent lighting, which generated an average irradiance (± standard error) of 884 (±38) Lux at the base of each aquarium. The seawater in each aquarium was filtered with a hanging power filter rated at 757 L/h that utilized activated carbon contained within a floss-filter cartridge. Each aquarium was covered with a thin, transparent plexiglass sheet. Cellophane wrap was used to seal the top of each aquarium and filtration system, which minimized evaporation and gas exchange with external air. The low-temperature treatments were circulated and cooled to 20.42 ± 0.07 °C by pumping the aquarium seawater through coiled tubes that passed through a water bath maintained at a sufficiently low temperature by an *Oceanic 1 HP Aquarium Chiller (Model 01505)*. The high-temperature seawaters were maintained at 29.93 ± 0.04 °C with 50-W heaters and were circulated within each aquarium with powerheads at 400 L/h.

2.4. Feeding

The urchins were fed to the point of satiation every other day with approximately 60 mg of dehydrated marine green algae per tank. Glass beads were used to weigh down the algal sheets on the base of the tank and the urchins were placed on top of the sheets at each feeding. Excess food was removed from the aquaria prior to the next feeding.

2.5. Experimental conditions

Urchins were reared for 60 days under three-way replicated low (ca. 480 ppm) and high (ca. 800 ppm) pCO_2 treatments (Table 1), which were each maintained at low (ca. 20 °C) and high temperature levels (ca. 30 °C). The actual pCO_2 and temperature values for the four treatments (± standard error) were: (1) 20.3 ± 0.1 °C and 524 ± 33 ppm; (2) 20.56 ± 0.09 °C and 827 ± 37 ppm; (3) 30.00 ± 0.06 °C and 448 ± 27 ppm; and (4) 29.86 ± 0.06 °C and 783 ± 45 ppm (Table 1). The high-pCO_2 gas was formulated by mixing compressed CO_2 gas with compressed air using *Aalborg* mass flow controllers. The low CO_2-gas was compressed air supplied to the laboratory through an in-house airline and was sourced from air outside of the laboratory building. The gases were bubbled into the aquaria via 6-in. porous ceramic airstones that were fastened to the bottom of each tank. For a given pCO_2 treatment, calculated pCO_2 levels for the lower temperature seawaters were greater than those for the higher temperature seawaters because the solubility of CO_2 gas in seawater increases with decreasing temperature (Weiss, 1974).

2.6. Carbonate system parameters

2.6.1. Measured parameters

Temperature within the experimental aquaria was measured every other day (Table 1) with a NIST-calibrated partial-immersion organic-filled glass thermometer. Salinity was measured every other day (Table 1) with a *YSI 3200* conductivity meter with a *YSI 3440* cell ($K = 10$) that was calibrated with seawater standards of known salinity provided by the laboratory of Prof. A. Dickson of Scripps Institute of Oceanography. Seawater pH was measured every other day (Table 1) with a *Thermo Scientific Orion 2 Star* benchtop pH meter and *Orion 9156BNWP* probe, calibrated with 7.00 and 10.01 Orion NBS buffers traceable to NIST standard reference material (for slope of the calibration curve) and with seawater standards of known pH provided by the laboratory of Prof. A. Dickson of Scripps Institute of Oceanography (for *y*-intercept of the calibration curve). Seawater dissolved inorganic carbon (DIC) and total alkalinity (TA) were measured weekly (Table 1) with a VINDTA 3C (*MARIANDA* corporation). Seawater DIC was measured via coulometry (*UIC 5400*) and TA was measured via closed-cell potentiometric titration.

2.6.2. Calculated parameters

Seawater pCO_2, pH, carbonate ion concentration ($[CO_3^{2-}]$), bicarbonate ion concentration ($[HCO_3^-]$), aqueous CO_2, and aragonite saturation state (Ω_A) were calculated (Table 1) with the program *CO_2SYS* (Lewis and Wallace, 1998), using Roy et al. (1993) values for the K_1 and K_2