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Biogenic acidification reduces sea urchin gonad growth and increases susceptibility of aquaculture to ocean acidification

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ABSTRACT

Decreasing oceanic pH (ocean acidification) has emphasised the influence of carbonate chemistry on growth of calcifying marine organisms. However, calcifiers can also change carbonate chemistry of surrounding seawater through respiration and calcification, a potential limitation for aquaculture. This study examined how seawater exchange rate and stocking density of the sea urchin *Tripneustes gratilla* that were reproductively mature affected carbonate system parameters of their culture water, which in turn influenced growth, gonad production and gonad condition. Growth, relative spine length, gonad production and consumption rates were reduced by up to 67% by increased density (9–43 individuals.m^{-2}) and reduced exchange rates (3.0–0.3 exchanges.hr^{-1}), but survival and food conversion efficiency were unaffected. Analysis of the influence of seawater parameters indicated that reduced pH and calcite saturation state (ΩCa) were the primary factors limiting gonad production and growth. Uptake of bicarbonate and release of respiratory CO_2 by *T. gratilla* changed the carbonate chemistry of surrounding water. Importantly total alkalinity (A_T) was reduced, likely due to calcification by the urchins. Low A_T limits the capacity of culture water to buffer against acidification. Direct management to counter biogenic acidification will be required to maintain productivity and reproductive output of marine calcifiers, especially as the ocean carbonate system is altered by climate driven ocean acidification.

1. Introduction

The biological effects of anthropogenically-driven decreases in oceanic pH (ocean acidification) highlights how changes in seawater carbonate chemistry (pH, partial pressure of dissolved carbon dioxide [pCO_2] and calcite saturation state [ΩCa]) can reduce growth and fitness of calcifying organisms (Kroeker et al., 2013, 2010). Marine calcifiers can also alter carbonate chemistry through respiration, photosynthesis, calcification and dissolution of calcium carbonate (Anthony et al., 2011; Hurd et al., 2011; Schneider et al., 2011). In natural systems with low water exchange these processes can create greater changes in carbonate chemistry than that predicted for near future ocean acidification, as shown for boundary layers in algal beds and intertidal rock pool habitats (Hurd et al., 2011; Truchot and Duhamel-Jouve, 1980).

In closed aquaculture systems, acidification and reduced buffering capacity (low total alkalinity [A_T]) of culture water is also a serious limitation for aquaculture of calcifying organisms (Barton et al., 2012) as economics demand the highest possible densities of animals with the lowest practical water exchange rates (Mos et al., 2015). However, the extent of the effect of intensive production of calcifying organisms on the carbonate chemistry of their culture water and the potential for this to limit production of these organisms is poorly understood (Mos et al., 2015). It is critical to address this knowledge gap to predict how elevated atmospheric CO_2 levels and associated impacts on carbonate chemistry of the oceans may impact aquaculture production and to increase the efficiency and sustainability of the culture of marine calcifiers.

The extent to which calcifying organisms alter carbonate chemistry in aquaculture systems depends on the intensity of production and the rate of water turnover (Mos et al., 2015). Density and growth rates of organisms determine respiratory CO_2 production and the depletion of carbonate by calcification, whilst removal of CO_2 and replenishment of carbonate is governed by water turnover. Capacity to perturb carbonate systems is also likely to vary across life-history stages. For example, rapid calcification by fast-growing juveniles reduces A_T, limiting the capacity of their

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culture water to buffer changes in carbonate chemistry due to excretion of respiratory CO_2 (Mos et al., 2015), which is also high in juveniles (Mos et al., 2015). In contrast, adult calcifiers may have comparatively smaller effects on the A_T of culture water because their growth rates (and therefore calcification rates) are inherently lower (Schnute, 1981) or because they may allocate more effort to reproductive rather than somatic growth (Otero-Villanueva et al., 2004; Russell, 1998).

The impact of variation in seawater carbonate chemistry on organisms also changes across life history stages. Fertilisation and early embryos of marine invertebrates are generally robust to seawater acidification, but later calcifying larval stages are generally highly sensitive (Byrne and Przeslawski, 2013). The growth of newly metamorphosed (Wolfe et al., 2013) and juvenile (Albright et al., 2012; Asnaghi et al., 2014; Courtney et al., 2013; Holtmann et al., 2013; Mos et al., 2015) sea urchins is reduced under low pH/high pCO_2 conditions. However, little is known about how seawater acidification may affect the development of reproductive structures (Dupont et al., 2013; Kurihara et al., 2013; Siikavuopio et al., 2007b; Stumpp et al., 2012b; Suckling et al., 2015; Uthicke et al., 2014, 2013). This is a particularly important gap in our knowledge of the effects of acidification on the aquaculture of sea urchins because they are grown for their gonads.

This study investigated the impact of changes in seawater carbonate chemistry in culture systems during the period of growth to reproductive maturity of *Tripneustes gratilla*, a fast-growing, high value tropical sea urchin targeted for commercial aquaculture (Dworjanyn and Pirozzi, 2008; Dworjanyn et al., 2007; Mos et al., 2015, 2011; Scholtz et al., 2013; Seymour et al., 2013) and biocontrol (Westbrook et al., 2015). Specifically, we examined the effects of stocking density and seawater exchange rate on seawater physico–chemical parameters and, in turn, growth, survival, relative spine length, gonad production and gonad condition of the sea urchins. Data from three density and three exchange rate treatments were assessed with respect to temperature, pH, salinity, A_T, nitrate, DO, pCO_2 and calcite saturation state (ΩCa) in the culture system. We predicted that biogenic alteration of carbonate system parameters would have a major influence on growth and gonad production in *T. gratilla*, with implications for the culture of this and other calcifying organisms in the face of an acidifying ocean.

2. Materials and methods

2.1. Study organism

T. gratilla were cultured at the National Marine Science Centre, Southern Cross University, Coffs Harbour, Australia (30°12.5′S, 153°16.1′E) using established protocols (Mos et al., 2015, 2011) and gametes from five males and five females. Larvae were induced to settle using naturally derived biofilms and *Sargassum* sp. conditioned seawater in a flow-through seawater system at 24–25 °C and 35 ppt salinity. Settled juveniles consumed the biofilm until a test diameter (TD) of 5–10 mm when they were fed a seaweed diet (*Sargassum* sp.). *T. gratilla* were used in the experiment at the size when they began to produce gonads (~70 mm TD, approx. 7 months of age) (Mos et al., 2015). They were kept at 26–27 °C and not fed for two weeks prior to the experiment to standardise their nutritional condition.

2.2. Culture system

A fully factorial design was used to test the effects of three densities, low, medium and high (1, 3 and 5 individuals.replicate^{-1}, respectively) and three seawater exchange rates, low, medium and high (0.3, 1.0 and 3.0 exchanges.hr^{-1}, respectively) on the growth, gonad production, gonad condition, consumption rates, FCE and survival of *T. gratilla* over six weeks. Initial densities were equivalent to 9, 26 and 43 individuals.m^{-2} (also see Fig. S1).

Each treatment had five replicates consisting of 5 L rearing containers (180 mm Ø base, 200 mm H) with over-flow holes that maintained the water volume at 4 L. Replicates were randomly assigned positions in a water bath (100 mm deep) to maintain stable water temperatures. Filtered (20 μm) seawater was supplied from an 800 L header tank, heated (26–27 °C, optimal for growth and reproductive maturation Mos et al., 2015) using a looped flow-through 3000 Watt heater (Elecro Engineering TO3 Ti). Seawater exchange rates for each replicate were regulated using irrigation drip taps. Replicates were individually aerated (0.71 L.min^{-1}, regulated using flow control valves) and maintained under a 12:12 photoperiod ('cool white' fluorescent). Sea urchins were fed daily *ad libitum* with the seaweed *Sargassum* sp. which is a high preference food (Dworjanyn et al., 2007; Seymour et al., 2013).

2.3. Effects of density and seawater exchange rate on seawater parameters

Daily temperature, pH_{NIST} and salinity measurements were recorded for each replicate and the ambient seawater supplied to the system using Hach® HQ40d multi-controller, Hach® PHC101 pH probe and Hach® CDC101conductivity probe. Dissolved oxygen (DO) was measured three times a week (Hach® LDO101). Nitrite, nitrate and TAN (Total Ammonia Nitrogen) were measured from water samples collected daily, pooled from equal volumes taken from replicates in each treatment and from the ambient seawater supplied to the system (Palintest® photometer 7100, Ammonia AP152, Nitricol AP109 and Nitratest AP163). Total alkalinity (A_T) was measured in water samples (100 mL) collected weekly, pooled from equal volumes taken from replicates in each treatment and the ambient seawater supplied to the experiment, respectively. Samples were filtered (0.45 μm) and fixed with 5 μL of saturated $HgCl_2$. A_T was determined by potentiometric titration using a Metrohm 888 Titrando and certified reference standards (Dickson et al., 2007). Values for pCO_2 and ΩCa were calculated from weekly mean temperature, pH, salinity and A_T data using CO2SYS (Pierrot et al., 2006) using the dissociation constants of Mehrbach (1973) as refitted by Dickson and Millero (1987).

Estimates for the amount of CO_2 produced by *T. gratilla* during week six were compared to pCO_2 levels in the culture water (Table S4). The amount of CO_2 produced was estimated using regression coefficient (b) and constant (K) values for *T. gratilla* (Dy et al., 2002) under similar temperature and salinity conditions to this study, for the equation $M = KW^b$ relating body mass and metabolic rate (Webb et al., 1977).

2.4. Effects of density and seawater exchange rate on growth and survival

None of the urchins in the experiment died. To assess growth, test diameter (TD) and wet weight (WW) measurements were taken weekly using standard techniques (Dworjanyn et al., 2007; Mos et al., 2015; Watts et al., 2010). Urchins were weighed to the nearest 0.01 g after removal of excess seawater by placing them on dry paper towel for 30 s. Image analysis software, ImageJ (NIH, USA) was used to measure TD from digital photographs of the aboral side of the urchins. Two perpendicular measurements at the longest axis were taken for each urchin and the average used for statistical analysis. There was no significant difference in the initial TD ($F_{8, 44} = 1.54$, $P = 0.1769$; Mean = 71.1 mm ± 0.4 SE) or WW ($F_{8, 44} = 0.87$, $P = 0.5547$; Mean = 107.7 g ± 1.6 SE) of *T. gratilla* among treatments. After six weeks, Specific Growth Rate (SGR) and Linear