Journal of Experimental Marine Biology and Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Ocean acidification impacts the embryonic development and hatching success of the Florida stone crab, *Menippe mercenaria*

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ARTICLE INFO

Keywords:
Low pH
Climate change
Embryonic development
Hatching success
Crustacean
Stone crab

ABSTRACT

The deterioration of coastal habitats due to anthropogenic activities is being caused by nutrient rich runoff which will likely result in more frequent and severe extremes in seawater pH. The embryonic and larval stages of many coastal species may not have the physiological ability to tolerate these extreme shifts in pH forecasted for future oceans. The stone crab, *Menippe mercenaria*, was used to determine the impact of low seawater pH on embryonic development and hatching success. Ovigerous females were maintained in environments characteristic of both present-day and reduced seawater pH. Lower pH significantly reduced the rate of embryonic development (i.e., time to hatching) by ~24%, but had no effect on the size of developing embryos (i.e., embryonic volume). Larvae that successfully hatched were not morphologically different between treatments, although hatching success was reduced by 28% in lower pH seawater. Hatching success was also more variable in the reduced pH treatment indicating that some broods may be more tolerant to changes in seawater acidity. Variable hatching success under acidified conditions suggests that stone crab embryos may have the capacity to acclimatize to future seawater pH conditions.

1. Introduction

In coastal areas, anthropogenic influences including eutrophication, sewage inputs, and wetland degradation contribute to decreases in seawater pH (Millero et al., 2001; Bauer et al., 2013; Zhang and Fischer, 2014; Ekstrom et al., 2015), and influence the frequency, magnitude, and duration of extreme pH events (Harris et al., 2013; Hauri et al., 2013). The input of land-based runoff into many nearshore ecosystems can reduce seawater pH on daily, monthly, and seasonal temporal scales (Sammut et al., 1995; Zhang and Fischer, 2014). Although nearshore systems, especially estuaries, experience considerable diurnal and tidal fluctuations in pH (Hofmann et al., 2011), future extremes in pH may exceed the critical ecological and physiological thresholds of organisms that have adapted to less extreme environments (Attrill et al., 1999; Ringwood and Keppler, 2002; Hauton et al., 2009). Furthermore, the unprecedented increases in atmospheric carbon dioxide (CO_2) have already decreased seawater pH by 0.1 units (Calderia and Wickett, 2003). Current models forecast additional decreases in seawater pH of 0.14–0.35 units by 2100 and a decrease of 0.77 units within the next 300 years (Meehl et al., 2007; Ekstrom et al., 2015).

Decreases in seawater pH (elevated seawater pCO_2) have been shown to cause a myriad of adverse effects on the behavior, physiology, morphology, and survivorship of calcifying marine organisms (Kurihara et al., 2008; Kroeker et al., 2010; De la Haye et al., 2011). The positive, negative, mixed, and sometimes neutral responses of organisms to lower ocean pH differs among taxonomic groups (Ries et al., 2009; Kroeker et al., 2010) and among populations (Walther et al., 2009). These varied responses make extrapolating the results from elevated seawater pCO_2 experiments challenging when predicting the potential ecological and economic ramifications for a particular species or populations (Miller et al., 2016). Therefore, broad generalizations (i.e., 'winners' or 'losers') about the impacts of elevated pCO_2 on marine biota are problematic as future oceans will likely have some species or populations being more tolerant to lower seawater pH than other species (Carter et al., 2013; Hilmi et al., 2013).

A species' tolerance to low seawater pH may differ through different life-history stages (Whiteley, 2011) and be related to the individual's natural environment (Pane et al., 2008). For instance, low pH conditions impacted the physiology of embryos (e.g., slowed metabolic rate in *Petrolisthes cinctipes*, Carter et al., 2013), and reduced survival in juveniles (*P. cinctipes*, Ceballos-Osuna et al., 2013), but had no noticeable effect in larval survival (e.g., *P. cinctipes*, Ceballos-Osuna et al., 2013). Despite regular exposure to natural changes in pH within the intertidal zone, stage-1 larvae and juveniles of *P. cinctipes* showed enhanced acid-base regulation indicating a greater hypercapnia (e.g., blood pH becomes lower due to elevated CO_2 partial pressures)

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http://dx.doi.org/10.1016/j.jembe.2017.09.001
Received 7 April 2017; Received in revised form 4 August 2017; Accepted 5 September 2017

tolerance (Carter et al., 2013; Ceballos-Osuna et al., 2013). These results suggest that sensitivity to low pH may vary with developmental stage (Carter et al., 2013; Ceballos-Osuna et al., 2013). Similar mixed effects throughout a species' life-history are also reported in other non-decapod crustaceans (for reviews see Kroeker et al., 2013; Wittmann and Pörtner, 2013). Sensitivity to lower ocean pH conditions may be related to the ability of different life stages to regulate their blood hemolymph pH (i.e., acid-base balance), which can have negative effects on calcified structures leading to morphological abnormalities including reduced body size and spine deformities (Kurihara et al., 2008; Findlay et al., 2009; Long et al., 2013b).

Morphological abnormalities associated with decreases in seawater pH may negatively impact larval survival by reducing body size and defensive spines, which may in turn alter swimming behaviors including the ability to regulate buoyancy, maintain vertical position, and avoid predators. The varied responses to lower ocean pH reported for decapod crustaceans suggests that species-specific experimentation across different life history stages is still necessary to predict how species will respond to more frequent and extreme changes in seawater pH, especially in regions where anthropogenic activities continue to degrade coastal habitats (Bauer et al., 2013; Ekstrom et al., 2015; Miller et al., 2016). Identifying an organism's tolerance to extremes in seawater pH throughout different stages of its life history allows for determining if the species will be able to tolerate and eventually adapt to future decreases in seawater pH (Kurihara et al., 2008).

The Florida stone crab, *Menippe mercenaria*, is an important fishery in the southeastern United States. In Florida alone, stone crab landings support on average a US ~$25-million-a-year commercial and an active recreational fishery (FWC, 2000–2016). The stone crabs embryonic development, larval release, and post-larval recruitment occur within nearshore regions (Lindberg and Marshall, 1984; Krimsky and Epifanio, 2008; Krimsky et al., 2009; Gandy et al., 2010) some of which are being threatened by anthropogenic activities. For example, since 2005 the western fringes of Florida Bay (Florida, USA), have experienced a rate of decline in pH that is estimated to be three-times faster than the rate observed in the open ocean (Zhang and Fischer, 2014). Given the potential mixed effects reduced seawater pH may have on the development of decapod crustaceans, lower ocean pH could have damaging ramifications for economically important species like the Florida stone crab. This study examined the effects of low seawater pH on the embryonic development and hatching success of *M. mercenaria*. The hypothesis that exposure to acidified seawater conditions during embryonic development results in morphological abnormalities in newly-hatched larvae was also tested.

2. Materials and methods

2.1. Study site, collection and maintenance of experimental animals

All experiments were conducted from June–August 2012 at the Mote Tropical Research Laboratory in Summerland Key, Florida, USA (24° 39.69′ N, 81° 27.25′ W). Ovigerous *M. mercenaria* (carapace width $\bar{\mu}$ = 9.56 cm ± 1.06 SD) were collected within 17 km of the coast using commercial stone crab traps at different times throughout the summer. Following transport to the laboratory, crabs were randomly assigned to either the control (present-day pH) or low pH treatment. A total of 16 ovigerous stone crabs were caught in state approved commercial traps; eight were randomly assigned to the control treatment and eight were randomly assigned to the experimental treatment. Each brood was considered one replicate, thus N = 8 in each treatment level. The same eight females in the control and experimental treatment were used to evaluate responses to low seawater pH for the experiments listed below, and were run in parallel (i.e., balanced throughout experimentation) to ensure that differences in brood quality across the season did not bias the results (Supplemental Table 1). Only females with egg masses containing early-stage embryos (i.e., orange egg masses; Supplemental Table 2) were used in experiments. Ovigerous crabs were maintained in individual (40.6 × 20.3 × 25.4 cm) flow-through aquaria that hold 19 L of seawater (salinity 35) under a 14 h to 10 h light:dark cycle that approximated the photoperiod at the time of collection. Water temperatures were maintained within a narrow range (median ± median absolute deviation, MAD) by partially submerging the aquaria in a thermostatically controlled water bath, and approximated the median temperature at the collection site. Crabs were fed frozen shrimp every other day ad libitum, and aquaria were cleaned daily to remove wastes and uneaten food.

2.2. Experimental setup and seawater chemistry

Experiments were performed using a flow-through ocean-acidification system at the Mote Tropical Research Laboratory in Summerland Key, Florida. The system consisted of experimental aquaria (40.6 × 20.3 × 25.4 cm) that were sealed with acrylic lids. Treatment conditions within each aquarium were maintained by pumping seawater through 0.6 cm diameter inlet and outlet holes that were drilled into the acrylic lids. Saltwater for the control (pH = 8.0, ~540 µatm) and low pH treatment (pH = 7.5, ~2100 µatm) were obtained from a saltwater aquifer. The saltwater contained naturally high levels of CO_2, which resulted in a source of saltwater with a pH of 7.5 (± 0.07 SD), and a salinity of ~36 (± 0.6 SD) (Hall et al., 2012). Before being pumped into the system, saltwater passed through sand filters (100 lb) and biological filtration (3.8 cm Bio Balls) to remove particulates and nutrients. Next, saltwater was pumped into a second set of holding reservoirs (1000 L) where CO_2 was degassed with ambient air until near-present day (control) or future pH levels were achieved. A pH/CO_2 controller (Milwaukee, Aquatic Ecosystems, ± 0.2 accuracy, Apopka, FL) was used to regulate the CO_2 levels in each treatment.

Saltwater pH within each experimental chamber was monitored daily using a pH probe (SevenGo pro, Mettler-Toledo, ± 0.002 pH, Germany). Prior to use, a daily three-point calibration (at 26 °C) was performed on the pH probe using NBS buffers. Daily flow rates (ml min^{-1}) were measured by sampling the volume of seawater flowing into each experimental aquarium at ten-second intervals. Salinity of each aquaria for both treatments was monitored daily using a refractometer (resolution 0.2%, LW Scientific, Lawrenceville, GA), and temperature of each aquaria for both treatments was monitored using the same pH probe previously described. Water samples for total alkalinity (A_T) and phosphate measurements were collected from each experimental aquaria at the start (day 0), middle (~day 7), and at the end (after hatching, days 10–12) of each experiment. These water samples were stored at 4.4 °C for A_T and < − 40 °C for phosphate. All water samples were analyzed for total alkalinity within 10–14 days of sampling according to Environmental Protection Agency (EPA) approved method SM 2320 B-1997. Alkalinity samples were stored at 4.4 °C to reduce chances that A_T levels change due to contamination or microbial activity as HgCl was not available.

All seawater samples were processed by the Mote Marine Laboratory Analytical Chemical Ecology facility in Sarasota, FL. Phosphate levels were analyzed using a nutrient auto-analyzer (Bran Luebbe) according to Environmental Protection Agency (EPA) methods 353.2. A gran-titration method (EPA approved method SM 2320 B-1997) was used to estimate A_T values. Titrations were calibrated using a primary grade 0.05 N Na_2CO_3 solution. Three separate aliquots of the Na_2CO_3 solution were titrated to compute the normality of the acid. The average acid normality and ml of titrant were then used to compute A_T. Total alkalinity and pH values (NBS scale) were used to estimate pCO_2 levels for the control and low pH treatment using the CO2Sys program (v2.1; Pierrot et al., 2006). Stoichiometric dissociation constants defined by Mehrbach et al. (1973) and refit by Dickson and Millero (1987) were used during the CO2Sys calculation. Phosphate levels were incorporated into the CO2Sys calculations to better characterize the carbonate system during these experiments.