

The Effects of Ocean Acidification on the Development of *Lytechinus variegatus* (Green Sea Urchins)

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ABSTRACT

The purpose of this experiment was to study the effects of ocean acidification on early spicule formation and development in Lytechinus variegatus (green sea urchins). After induced spawning of adult green sea urchins occurred the larvae were placed in a control (8.1pH) and experimental (7.6 pH) tank of equivalent salinity, dissolved oxygen (DO), temperature, volume, and sea water. Soon after fertilization was confirmed ten randomly selected larvae from each tank were observed and recorded using a compound microscope. Our results showed similar rates of growth in the early stages of development (Fertilization - hour 6) with significant differences in later development (hours 12 - 22). Overall, this experiment was successful in observing the effects of ocean acidification in developing Lyechinus variegatus sea urchins; however, more experiments should be conducted to verify the results and reduce the extinction rate of the acidic environment.

Marine environments can possess a variety of stressors to both adult and particularly, larval organisms. A major stressor marine organisms often encounter is acidic waters formed by a process known as ocean acidification (1). Ocean acidification is a consequence of elevated levels of dissolved carbon dioxide in the water which then forms carbonic acid and further dissociates into bicarbonate and carbonate ions; releasing H⁺ ions and ultimately lowering the pH (2). The present level of atmospheric carbon dioxide (approximately 300-380 ppm) is predicted to increase dramatically to levels between 450 - 1,000 ppm by 2100 (3). This increase can be attributed to human fossil fuel combustion and deforestation rates which have increased by an order of magnitude faster than in the last few thousand years (1).

Calcifying marine invertebrates require carbonate ions for the production of

their skeletons, but with an increase in acidification the carbonate ions become less available, reducing not only the size but also strength of the organism's skeleton (4). If the ocean remains at a constant salinity, the calcium concentration will also remain nearly constant due to the direct relationship between calcium and salinity; and as such the calcification process is primarily dependent on the level and availability of carbonate ions (4).

The survival of most marine species depends on the ability of the species larvae to locate a compatible settlement site with suitable water chemistry (5). In echinoderms, another important factor in species survival is production of appropriate egg size; however, ocean acidification can cause egg size to decrease to an un-survivable state (6). Specifically, direct developing sea urchins, which do not feed and begin transformation into the adult form after a single day, are greatly

affected (7). After insemination, sea urchin zygotes typically lower their pH. However, molecular carbon dioxide caused by acidification easily diffuses into the gametes additionally decreasing the intracellular pH and preventing subsequent development (8). During the sea urchins planktonic stage and early development they spend an extended period of their growth in the water column where the water chemistry and temperature of the water have major impacts on development (9). Echinoderms are highly susceptible to ocean acidification because they lack both an impermeable barrier between the outside seawater and their internal cavity and they also use active transport mechanisms meaning they have little mechanisms for buffering changes in the acid-base balance of their fluids (10).

PROCEDURE

This experiment was broken into a pretrial stage and experimental stage.

Pre-trial

One twenty gallon tank was used to house the adult *Lytechinus varigeatus* sea urchins; it was equipped with an Aqueon QuietFlow 20 filter, 19 ± 1 gallon of 1 micron filtered sea water, and a heater. Two ten gallon tanks equipped with an Aqua Tech 10-20 Power filter and filled 9 ± 1 gallon of 1 micron filtered sea water were also set up as the control and experimental tanks. All tanks were monitored for a one week acclimation period. Water quality parameters included measuring: dissolved oxygen (DO), salinity, temperature, and pH with an YSI model 556 MPS portable meter. The YSI meter was calibrated prior to the start of the trial and calibrated for DO before every use. DO was maintained at $85\% \pm 3\%$, salinity $32 \text{ ppt} \pm 1.75 \text{ ppt}$, and temperature were also maintained at $22 \pm 2 \text{ }^\circ\text{C}$. A carbon dioxide tank and pH meter were attached to the experimental tank which was calibrated to maintain a

pH of $7.6 \pm .02$. Both the adult sea urchin tank (20 gallon tank) and the control tank were maintained at a pH of 8.1 ± 0.2 . During the course of the experiment adult sea urchins were each individually fed once daily.

Experimental trial

Six *Lytechinus varigeatus* sea urchins were randomly selected and, using a syringe, each sea urchin was injected with 1.5 mL of a 0.05M solution of potassium chloride into the central cavity to induce spawning. After injection the sea urchins were swirled and inverted over a cup for a maximum of 15 minutes or until gametes were released. Female gametes, which appeared slightly yellow in color, were added to 25 mL of 1 micron filtered sea water and washed by allowing the eggs to sink to the bottom of the cup. After the eggs sank the water was decanted and the washing process was repeated several times to reduce the eggs' membrane barrier. Male gametes, which appeared white in color, were not washed. To confirm gamete sex each cup's contents were observed under a microscope. After washing the eggs, the male and female gametes were combined to induce fertilization. Once fertilization was confirmed under a compound microscope the contents of the cup were evenly divided into the control and experimental tank. Sea urchin development was observed and documented at specific intervals for three days: 1 hour after fertilization, 3 hours after fertilization, 6 hours after fertilization, 12 hours after fertilization, 22 hours after fertilization, 24 hours after fertilization and every 24 hours thereafter until the completion of the experiment. Before each observation water quality was tested and recorded. To observe larvae, a pipette was used to extract each larva from the bottom of each tank. The first 10 randomly selected larvae from each pH treatment were observed and each individual's stage

of development was documented. After observation, larvae were disposed of to avoid the possibility of counting the same larva twice in the experiment. Results of the acid treatment were then compared to a normal pH treatment which should have had a normal triangular shaped body with two arms and minimal asymmetry.

RESULTS AND DISCUSSION

Echinoderms are highly susceptible to ocean acidification because they lack an impermeable barrier between the outside seawater and their internal cavity. Further, they use active transport mechanisms, meaning they have little ability to buffer changes in the acid-base balance of their fluids (10). Because of this, I expected development to be slowed in the experimental tank when compared to the control tank. The developmental stages one hour after fertilization showed there was no difference between the acid and control pH levels of development (Figure 1) and as such these data were not significant (chi square for 1 and 3 hours, $p > .05$). As stated in previous studies, early larvae development (development from fertilization to roughly ten hours after fertilization) is rarely significantly affected by increasing pH levels (6). Results of the experiment showed a slower pace of development in the acidic treatment with fewer noticeable differences at the early stages of development (Figure 2). A two-way chi square test used to analyze these data was not significant ($p > .05$). After six hours in the pH treatment the larvae in the acid treatment started to show signs of a slower pace of development (Figure 3); however, results of the results were not significant ($p > .05$). Fisher's exact test was used for the rest of the calculations because the data did not meet the assumptions of the two-way chi square test (zero value or lack of observation for a specific developmental

stage at that time).

These non-significant results, although not predicted, can be understood by considering echinoderms' ability to buffer the fluids in and around their internal cavity which delayed the effects of ocean acidification (10). The increase in the amount of time spent in the acid treatment started to have more significant negative effects on the rate of development as time increased ($p < .05$; see Figures 4 and 5 and Table 2). This positive correlation between decreased pH level and pace of development is thought to be a result of the length of time spent in the treatment along with the complexity of development. As the zygotes spent more time in the acidic treatment, the availability of calcium ions necessary to complete their calcium bicarbonate skeleton diminished, decreasing the rate of development.

At 22 hours after fertilization mass mortality occurred in the acidic pH treatment [see Figure 5; results confirmed by Fisher's exact test $p < .05$]. This was hypothesized to occur due to the low levels of calcium in the water which prevented subsequent development and further use of calcium in the water. Because the experimental pH value was at an extreme level, once the availability of calcium decreased it was possible for the acidic water to dissolve the calcium already formed around the zygote, reducing and eventually removing the protective barrier which resulted in mortality for these sea urchins.

Overall this experiment was successful in determining how sea urchins may be affected by ocean acidification; however, more experiments are necessary to determine more possible causes for mass mortality at a pH level of 7.6. This study also shows, as others have previously, that further research is necessary when considering the overall effects of ocean acidification on echinoderms. This can be

achieved in many ways, including looking at the specific calcium concentration available in the water as sea urchins develop to determine the potential for those organisms to survive and by examining sea urchins variability and adaptability potential. Other studies which look at different classes of echinoderms, species that display varying habitat, or alternative life cycles will help further understand the

effects of ocean acidification on a deeper level. However, based on this study, it is now clear that ocean acidification will have a negative effect on the development of *Lytechinus variegatus*.

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Figures

The pH effects on the development of Green Sea Urchin embryos. Figures below are separated by the amount of hours after fertilization development was observed for each pH treatment. Each figure displays the calculated standard deviation for each pH treatment at each hour of development.

Figure 1.

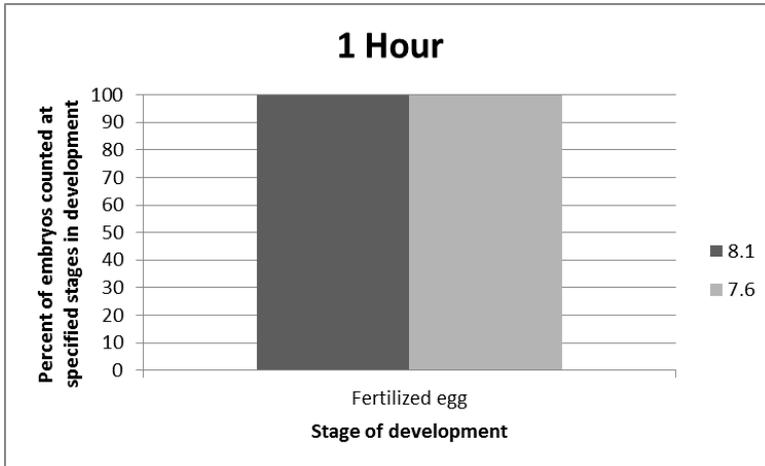


Figure 2.

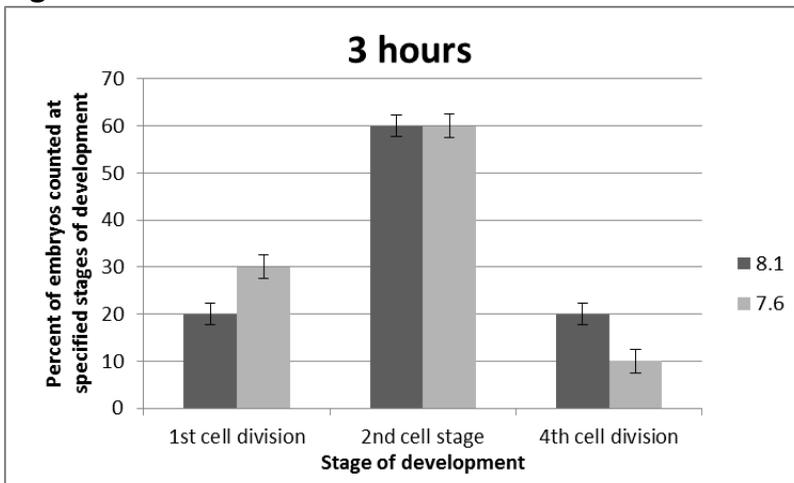


Figure 3.

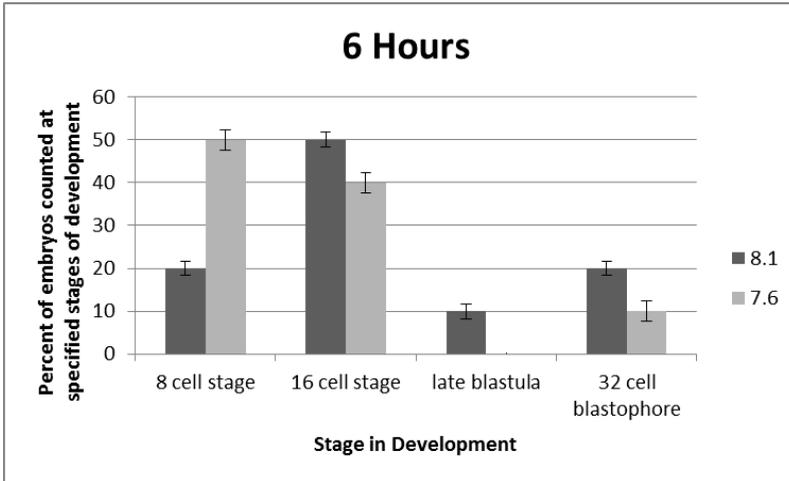


Figure 4.

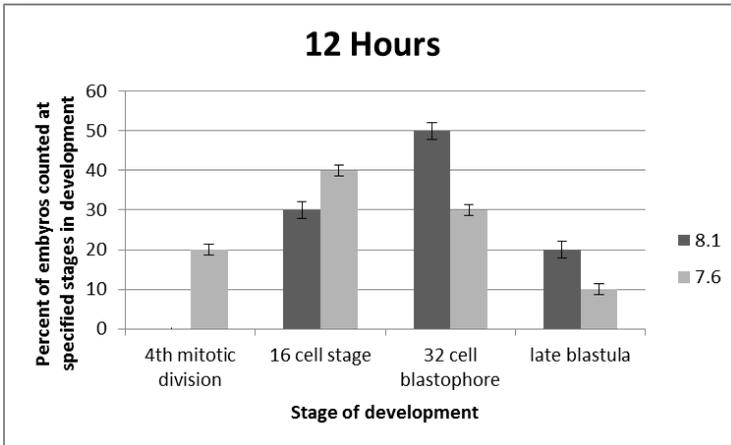
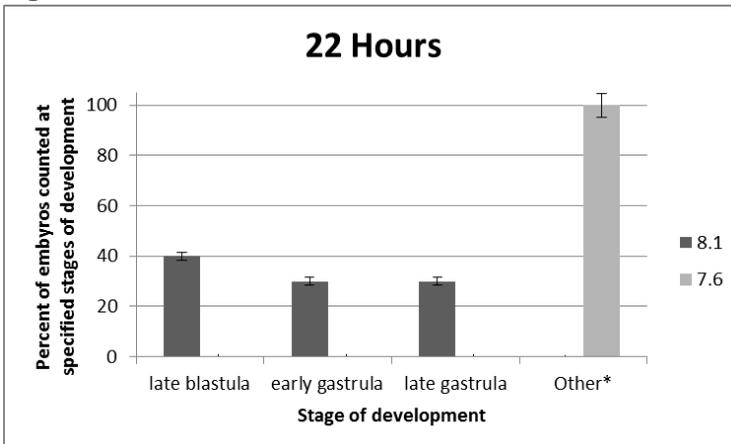


Figure 5.



*p < .05; *Other represents the amount of embryos which disintegrated at this hour of development.

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