

Impacts of Ocean Acidification on the sand dollar, *Dendraster excentricus* Fertilization  
Success and Early Development

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## Abstract:

Ocean acidification (OA), a result of increased atmospheric CO<sub>2</sub>, is predicted to decrease the surface ocean pH by a value of 0.4 units by the year 2100. Decreases in pH have been shown to cause deleterious effects on many calcifying marine organisms, which may negatively affect ecosystem functions. Few studies, however, have investigated OA impacts on fertilization success of free-spawning marine organisms. This study focuses on the effects of OA on fertilization success in the sand dollar *Dendraster excentricus*. The goal of this study is to experimentally determine whether decreases in pH values lower fertilization success in *D. excentricus*. The two-pH treatments chosen for this experiment reflect the present day pH value (8.1) and the predicted pH value by the IPCC for the year 2100 (7.7). Gametes from adult *D. excentricus* collected from Crescent Bay, East Sound, Orcas Island, WA, were fertilized in filtered seawater with pH 8.1 and 7.7. Fertilization success was determined by the percentage of 2 and 4-cell cleaving embryos. A numerical model of reproductive success as a function of adult density and current conditions will be used to assess potential population-level effects of observed changes in fertilization success. Results of this investigation will aid future studies on the effects of climate change on marine organisms and ecological stability of marine communities.

## Introduction:

Global climate change, the result of rising atmospheric CO<sub>2</sub> from the burning of fossil fuels, has been predicted to decrease the oceanic pH by a value of 0.3- 0.4 units within the year 2100 (Orr et al. 2005). As the oceanic pH value declines, an increase in

hydrogen [H<sup>+</sup>] ions occurs and causes a decrease in carbonate [CO<sub>3</sub><sup>2-</sup>] ions; an occurrence known as ocean acidification (OA). The effects of OA may have deleterious consequences on calcifying marine organisms. Current research on the sand dollar larvae *Dendraster excentricus*, when exposed to lower pH values, revealed harmful morphological changes in the development of the larval stomach (Chan and Grunbaum, 2011).

Few studies, however, have investigated the impacts of OA on fertilization success of marine organisms (Gooding et al., 2009; Havenhand et al., 2008; Havenhand et al., 2009) with fertilization success being described by a percentage of embryos reaching the four-cell stage (Havenhand et al., 2008). The sensitivity of a lower pH on male and female gametes may be dependent on the species of study. Havenhand et al. (2008) investigated fertilization success of the sea urchin *Heliocidaris erythrogramma* and noted a decrease in fertilization by 25% when exposed to a pH of 7.7, the predicted pH value by the IPCC for the year 2100. Another study, however, did not find considerable effects of OA on fertilization for the oyster *Crassostrea gigas* (Havenhand et al., 2009).

Understanding fertilization success, and the implications it may have on the successful development through early life stages of marine species, is crucial for predicting the stability of ecological communities. In this study, the fertilization success of the sand dollar *Dendraster excentricus* will be measured when exposed to several pH treatments. The pH values (8.0 and 7.7) for the treatments were chosen to represent current day and predicted ocean values by the IPCC. Fertilization success in *D. excentricus* has been shown to increase when the egg size and the jelly-coat surrounding

the egg have a larger target surface area (Levitan and Irvine 2001). Sperm motility also contributes to successful fertilization. Sperm longevity has not been documented in *D. excentricus*; however, one study investigating sperm endurance and speed noted a correlation between a decrease in fertilization and sperm life history (Levitan 2000). If exposure to a decrease in pH interferes with egg quality or sperm motility than the number of fertilized embryos will decrease.

#### Methods:

This experiment was conducted at Friday Harbor Laboratories (FHL), Friday Harbor, WA. Adult sand dollars were collected on May 2012 from Crescent Bay, East Sound, Orcas Island, WA. Sand dollars were kept in flow through sea tables, with seawater temperature ranging from 10 to 12° C, until needed for spawning. Spawning occurred on 16 July 2012 to 24 July 2012 by injecting 1-2mL 0.53M KCl, depending on size, in the coelomic cavity. Eggs from two females were spawned directly into beakers containing 400mL of the pH treated water, 7.7 and 8.0. The pH water was taken from CO<sub>2</sub> generating tables from laboratory 7 at FHL. Dry sperm from one male was collected in 0.5mL centrifuge tubes and placed on ice. Eggs were collected for approximately three minutes into each beaker and covered tightly with parafilm.

Gametes were then quickly transported to a cold room kept at 10 to 11°C. Fertilization occurred within 25 minutes after spawning. Each 400mL beaker was gently stirred and half of the solution was poured into a separate beaker. Sperm suspensions were created by diluting 120µL of dry sperm into 12mL-filtered seawater (FSW) and

60 $\mu$ L of diluted sperm were used in each fertilization. Beakers were allowed to sit for 15 minutes to ensure fertilization.

Four six-well plates were used in each trial, 4 plates per female, and pH treatments were randomly assigned in each well. Approximately 10mL of the appropriate pH water was transferred into the 4 six-well plates. Exactly 1mL of solution from each beaker post-fertilization was placed into the designated well and well plates were sealed using electrical tape. Well plates were checked using a dissecting scope after a three-hour time period for 2-cell cleavage and an additional hour for 4-cell cleavage. Total counts of cleaving embryos were recorded from a subsample of each well. Through trial and error two of five trials were used in the experiment

#### Results:

Both females demonstrated a decrease in embryonic development from 2 to 4-cell stages across each pH treatment (figure 1). The percent values were plotted from dividing the total number of fertilized embryos by the total number of cleaving embryos in each respective pH and well plate treatment. Error bars were inserted using the standard deviations from each pH treatment within 2 and 4-cell cleavage. According to the Mann-Whitney U test, a significant difference across both pH treatments from the proportion surviving per mL between 2-cell to 4-cell stages was not found ( $p=0.061$ ) (figure 2). Sage models portray scenarios of the relationship between sperm concentrations and fertilization success of embryos (Figures 3 and 4).

## Discussion:

The effects of OA on calcifying marine organisms have been studied on many occasions (Kurihara and Shirayama 2004; Kurihara et al., 2007; Kurihara et al., 2009; Gooding et al., 2009; Havenhand et al., 2008; Havenhand et al., 2009). Fertilization success, however, on marine organisms when exposed to a more acidified environment is an upcoming field of study. In this study, the fertilization success between each pH did not show conclusive results. Each trial demonstrated a decrease in the number of cleaving embryos regardless of the pH value they were fertilized in. This may be due to variation in natural developmental stages or environmental factors such as the pH value the adults were acclimated to when taken from their habitat. Future studies are needed with larger sample sizes and maternal lineages to predict whether fertilization is affected in an acidified environment.

This study investigated the effects of ocean acidification on *D. excentricus* fertilization success and incorporated sage modeling as a tool to detect potential population dynamic scenarios. Statistical analysis showed no significant difference on a decrease in fertilization success across both pH treatments from 2-cell to 4-cell stages; this does not support my hypothesis. This finding may be related to the sample size (n=48) used in this experiment. Another limitation in the study was the inability to monitor pH once the fertilized embryos were sealed in the well plates and it is likely the pH treated water equilibrated with the surrounding environment. Since statistical findings did not detect a significant decrease, sage models were created on assumptions if the my hypothesis was supported (Figures 9 and 10). Previous literature noted an increase in

sperm rates but a decrease in sperm vitality when exposed to OA conditions (Chan personal communication). If sperm from adult male *D. excentricus* behave in the same manner then the number of eggs fertilized into embryos will decrease in a given population.

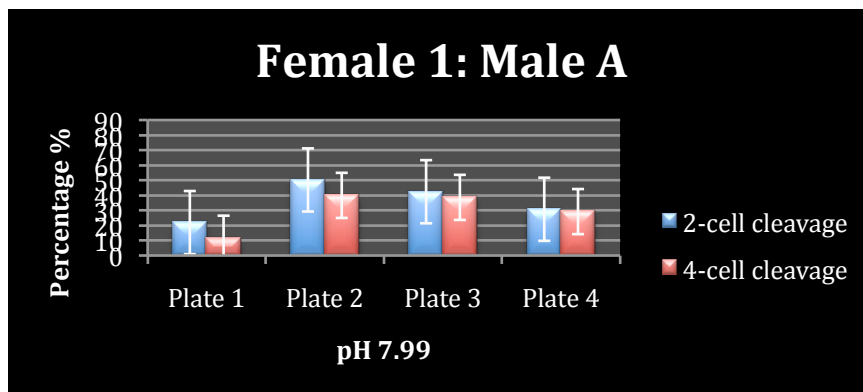
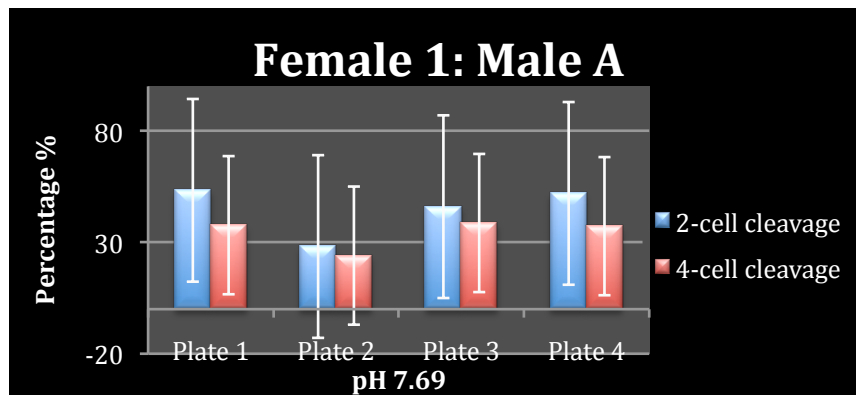
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#### References:

- Bos, A.R., Gumanao, G.S., Alipoyo, J.C.E. and Cardona., L.T. (2008). Population dynamic, reproduction and growth of the Indo-Pacific horned sea star, *Protoreaster nodosus*. *Mar. Biol.* **156**, 55-63.
- Chan, K.Y.K., Grunbaum, D. and O'Donnell, J. (2011). Effects of ocean-acidification-induced morphological changes on larval swimming and feeding. *J. Exp. Biol.* **214**, 3857-3867.
- Gooding, R.A., Harley, C.D.G. and Tang, E. (2009). Elevated water temperature and carbon dioxide concentration increase the growth of a keystone echinoderm. *PNAS* 106: 9316.
- Havenhand, J.N., Buttler, F.R., Thronyke, M.C. and Williamson, J.E. (2008). near-future levels of ocean acidification reduce fertilization success in a sea urchin. *Curr. Biol.* **18**, R651-R652.
- Havenhand, J.N. and Schlegel, P. (2009). Near-future levels of ocean acidification do not affect sperm motility and fertilization kinetics in the oyster *Crassostrea gigas*. *Biogeosci.* **6**, 3009-3015.
- Kurihara, H., Asai, T., Kato, S. and Ishimatsu, A. (2009). Effects of elevated pCO<sub>2</sub> on early development in the mussel *Mytilus galloprovincialis*. *Aquat. Biol.* **4**, 225-233.

- Kurihara, H., Kato, S. and Ishimatsu, A. (2007). Effects of increased sea- water pCO<sub>2</sub> on early development of the oyster *Crassostrea gi- gas*. *Aquat. Biol*, **1**, 91–98.
- Kurihara, H. and Shirayama, Y. (2004). Effects of increased atmospheric CO<sub>2</sub> on sea urchin early development. *Mar. Ecol.-Prog. Ser*, **274**, 161–169.
- Lauzon-Guay, J.S. and Scheibling, R.E. (2007). Importance of spatial population characteristics on the fertilization rates of sea urchins.
- Levitan, D.R. (2000). Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin *Lytechinus variegates*. *Proc. R. Soc. Lond. B*, **267**, 531-534
- Levitan, D.R. and Irvine, S.D. (2001). Fertilization selection on egg and jelly-coat size in the sand dollar *Dendraster excentricus*. *Evolution*. **55**, 2479-2483.
- Orr, J.C., Fabry, V.J., Aumont, O., Bopp, L., Doney, S.C., Feely, R.A., Gnanadesikan, A., Gruber, N., Ishida, A. and Joos, F. (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **473**, 681-686.



2-cell:P<0.0647 4-cell:P<0.0652

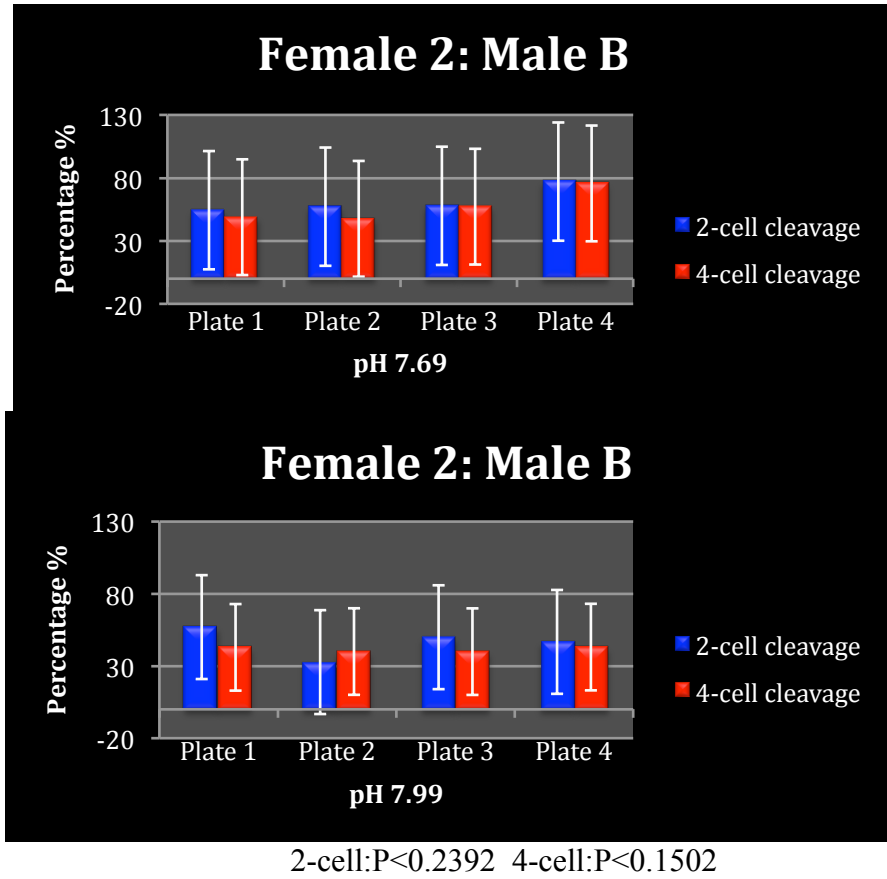


Figure 1. Percentages and standard deviation of total cleaving embryos per trial for each female. One way t-test values shown below after the second trial of each female.

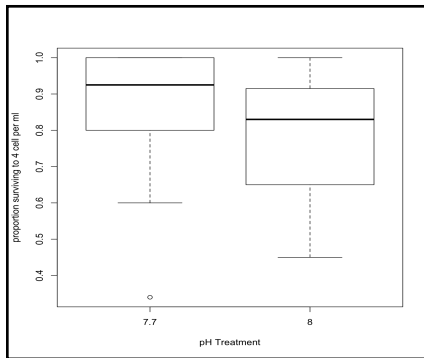


Figure 2: Boxplot of both pH treatments and proportion surviving per mL to 4-cell stage.

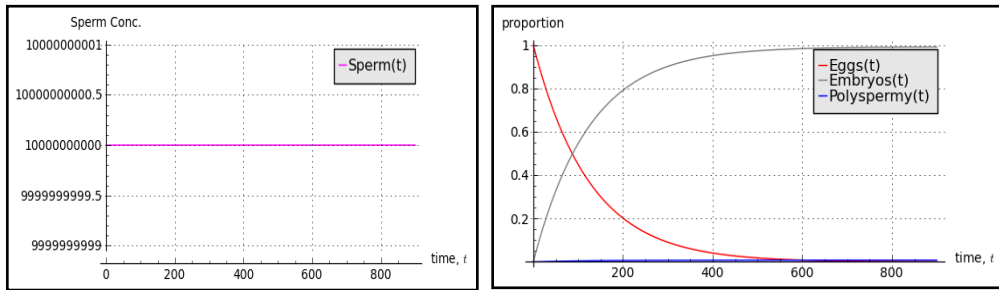


Figure 3: Sage model depicting relationship between sperm concentration (left) and eggs fertilized into embryos (right).

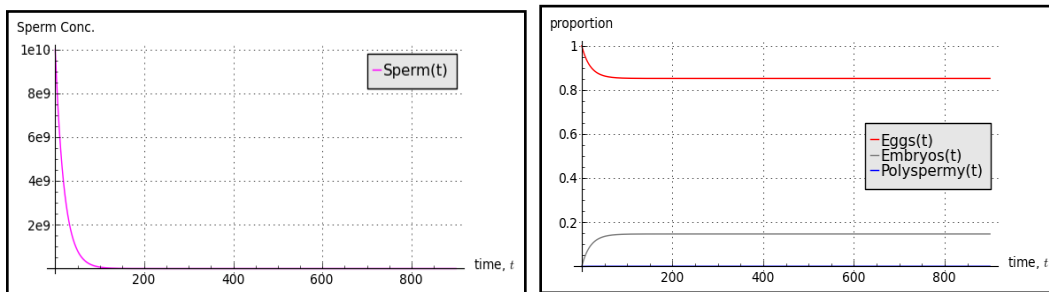


Figure 4: Sage model depicting relationship between a decrease in sperm concentration and eggs fertilized into embryos (right).