Hydrodynamic capabilities of *Pisaster ochraceus* larvae raised low salinity

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Abstract

Environmental variations act as stressors on organisms, and can strongly influence the geographic ranges of species. A source of environmental variation in the marine environments of the San Juan Islands is salinity concentrations. Echinoderms, namely *Pisaster ochraceus*, are heavily impacted by these fluctuations, and past experiments have shown that they can directly influence larval morphology and distribution within a vertical water column. We investigated whether the differences in vertical distributions were due to salinity's impacts on their hydrodynamic capabilities by tracking their instantaneous swimming patterns in a vertical water column and in a halocline. The analysis is still in progress, but the results could lend insight into the effects a less saline environment might have on an echinoderm's capabilities of distribution.

Introduction

Environmental variations are stressors on marine organisms, and often have direct effects on species' geographic ranges. For many marine invertebrates, a large proportion of species distribution occurs in the larval stages of their life cycles. For example, echinoderms are rather sedentary as juveniles and adults, but their larval stages distribute through the water column. An environmental variant that has been shown to effect echinoderms is salinity. Exposure to low salinity can have a large impact on echinoderms because they are stenohaline, ie. organisms that only tolerate small fluctuations in salinity (Stickle and Diehl, 1987). During the summer months, the salinity around the San Juan Islands varies between 20 and 30 PSU (Carrington, FHL weather station). Studies have

shown that similar variations in salinity affect the timing of metamorphosis and larval shape of sand dollars, as well as survival and developmental rates of larval sea urchins (Metaxes, 1998; George and Walker, 2007).

Held and Harley (2009) have shown that populations of the sea star, *Pisaster ochraceus*, can acclimatize to different salinities. In particular, *P. ochraceus* larvae develop unique morphologies when exposed to low salinity, and the extent of these changes depends on both duration and larval age during exposure. Altering the length of exposure to low salinity during the bipinnaria stage can result in a wider body and increased ciliated band length, or significantly smaller stomachs and shorter postero-lateral arms. These morphological differences can extend into the juvenile stage, although the period of the most drastic difference occurs during the larval stages. Therefore, the stages most heavily influenced by exposure to low salinity during development occur when *P. ochraceus* is most capable of widespread distribution (Pia et al. 2012).

Variation in larval shape has been shown to directly affect swimming capabilities of various larvae, and *P. ochraceus'* morphology directly affects its swimming capabilities (Crawford and Jackson, 2002; Grünbaum and Strathmann, 2003). Bipinnaria *P. ochraceus* larvae swim in a corkscrew fashion, rotating while swimming in a specific direction. This basic swimming pattern remains relatively consistent throughout development, but differences in the shape of the larvae correlate with differences in swimming patterns. Morphologically deformed *Pisaster ochraceus* larvae rotate more frequently than healthy larvae, and smaller larvae rotate more quickly than healthy larvae. The effects of these morphological changes can also be seen in long-term larval *Pisaster ochraceus* distribution in a halocline (Lee and George 2011). Larvae that are not exposed

to low salinity are able to breach a halocline more quickly than larvae that are exposed to low salinity, and they also repeatedly congregate near the surface. In comparison, a 14 day exposure to low salinity during the bipinnaria stage of development results in significantly fewer larvae reaching the surface of a halocline, even after 24 hours.

The goal of this experiment is to understand whether these differences in distributions among different salinity treatments are morphological in nature. We are testing two hypotheses: (1) hydrodynamic capabilities differ between salinity treatments and (2) The presence of a halocline will affect these differences. The metrics we will use to test these hypotheses are the overall swimming speeds of the larvae and the ratios of vertical to horizontal movement over time. Understanding these variations in swimming patterns can lend insight the relationships between changes larval morphology and changes in vertical distributions.

Methods

Spawning and embryo concentration

Eight adult *Pisaster ochraceus* were collected from Friday Harbor Laboratories (San Juan Island, WA, USA) and kept in a sea table. Two days following their collection, 7 animals were injected with 2mL of 10^{-4}M 1-methyl adenine to induce spawning. Two females and one male spawned between 1.5 and 4.5 hours post injection, and gametes were identified using a [type of microscope] microscope. Fertilization occurred in the same tank as the adults with 99% success, and the zygotes were divided into [] jars, each containing 2000ml of 0.45µm filtered seawater. Two days post-fertilization, embryos were examined using the [type of microscope] microscope to determine that they were in

the gastrula stage. Embryo concentration was calculated using three samples, and resulted in an approximate count of 24,285 healthy embryos.

Feeding and maintenance

The larvae were fed a diet of *Rhodomonas*, *Dunaliella*, and *Isochrysis*, a diet known to maintain a higher survival rate, and a system of swinging paddles was used to keep the larvae and algae in a constant state of suspension**.** Algae were grown in 300mL Erlenmeyer flasks in filtered 30‰ sea water, and kept next to a fluorescent light. *Rhodomonas* was occasionally removed from the light for 6 hours a day and was aerated to increase its health. All larvae were fed every Monday, Wednesday, and Friday, and each stock jar was fed an equivalent amount of food based on algal cell concentrations and the volume of water in the stock jar.

Larval stock jars were kept in a sea table to maintain a constant and healthy temperature, and sea water in the larval stock jars was replaced once a week by reverse filtration. Filter tubes covered with 150μm nitex mesh were used to remove two thirds of the sea water in the jar. The remaining third of the original sea water contained all of the larvae, and was poured into a new jar containing fresh sea water of the appropriate salinity. A few days later, more filtered sea water was added to the jars. As the larvae entered the braciolaria stage and became larger, a gentler method was used. The stock jar would sit unstirred until most of the larvae had gathered at the surface. Then 1000mL of the stock jar would be poured into a new jar containing 1000mL of fresh sea water of the appropriate salinity. Another 500mL of the stock jar would be poured into the new jar after the larvae had once again gathered at the surface. The process would be repeated to

a final 400mL, and then any remaining larvae would be pipetted into the new jar. Fresh sea water of the appropriate salinity would be added until the total volume of the stock jar was 3000mL. After the water changes, jars were moved to randomized positions on the water table to reduce the effects of possible variations within the sea tables.

Experimental design

Two days post-fertilization, larvae were separated into two treatments: a low 20‰ salinity treatment, and a control 30‰ salinity treatment. 31 days post fertilization, one jar from each treatment was divided to create two replicates per treatment. Beginning 53 days post fertilization, braciolaria larvae from each replicate were put into two types of water columns: a control water column containing either filtered 30‰ sea water throughout or filtered 20‰ sea water throughout, and a 20‰/30‰ halocline. The columns that contained a consistent salinity acted as controls for each treatment. Their salinity was the same as the culture salinity of the larvae being inserted. For each run, two control columns and two haloclines were used, allowing a sample of each treatment in each environment to run simultaneously. The four columns used were rectangular prisms 34cm in height. They were kept within a jacket of flowing sea water from the sea tables in order to maintain a relatively constant and healthy temperature throughout the experiment. In order to keep the surface of the water below the surface of the jacket, the water within the columns was only filled to around 24cm each time.

Haloclines were created by filling columns to 8cm with 20‰ filtered sea water, and then slowly siphoning 30‰ filtered sea water into the bottom of the columns until the columns were filled to approximately 21cm. Control columns were filled to 21cm

with their respective salinities by pouring the water in the top. Larvae were added to all columns by siphoning them along with 40mL of their original sea water. This may have led to a natural increase in initial upward movement for the low salinity treatment being inserted into the 20‰/30‰ halocline. The larvae were added one column at a time from column one to four with a gap of approximately 3 to 4mins between siphoning the first group of larvae and recording.

To reduce stress on the larvae, they were not collected via pipetting. Therefore, the number of larvae inserted into each column was not precise. Instead, the stock jars were allowed to sit until a large proportion of the larvae had swam to the surface, and a sterile 50ml beaker was used to scoop up approximately 20mL of sea water and larvae. Two beakers were acquired per jar, and were then mixed with each other to even the number of larvae between them. Each beaker was then filled to 40mL with the larvae's treatment salinities, and each beaker was siphoned into a designated column.

Larvae were imaged by two leopardboard cameras that were mounted on a computer controlled platform. A C program, fosica, was used to capture and analyze video, and a perl script, StepItUSB.pl, was used to manipulate the computer controlled platform. Those two functions were controlled in tandem with a shell script, profile_stepper6. The height of the columns was divided vertically into two areas of video capture: bottom and the surface**.** Two columns could be recorded at once, creating four areas of video capture: bottom left, bottom right, top left, top right. Over a period of approximately 1.5 to 2hrs, larvae were recorded almost continually, and then once an hour for an additional 2 to 3hrs. Each area of video capture was recorded for 300frames. Infrared lighting was used to eliminate any light-induced swimming patterns.

Analyzing the videos

Each 300 frame video was processed in fosica by going through a series of filters. An infrared De-Bayer was first used to even the infrared lighting throughout the image. Then, all except a singular column in the area of video capture was cropped out, and a series of contrasts and a convolution was used as a background subtraction step. Next, a threshold filter and a find-particles filter were used to create a binary image which the computer could interpret as particle movement over 300 frames.

Preliminary Results

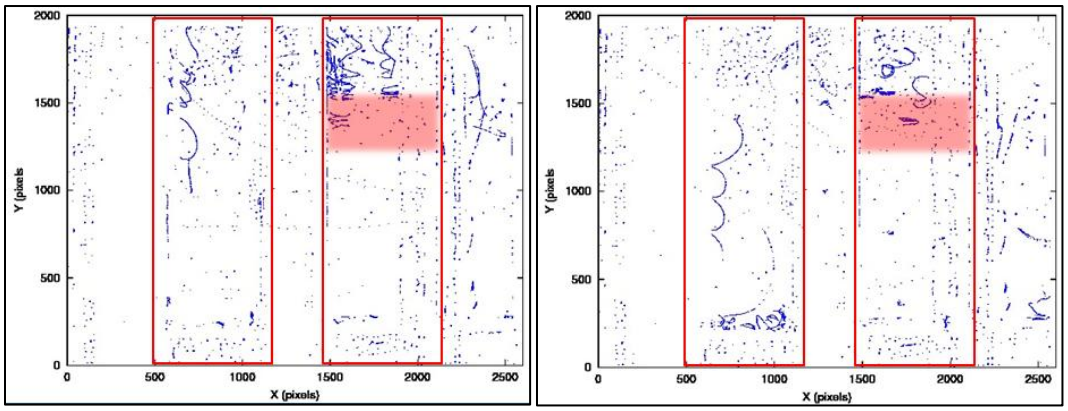

Figure 1: The particle trajectories of larvae raised in constant 30‰ on the surface of the first two columns. Columns 1 and 2 (left to right on each graph) are outlined by red boxes, and the halocline is marked by the translucent pink rectangle.

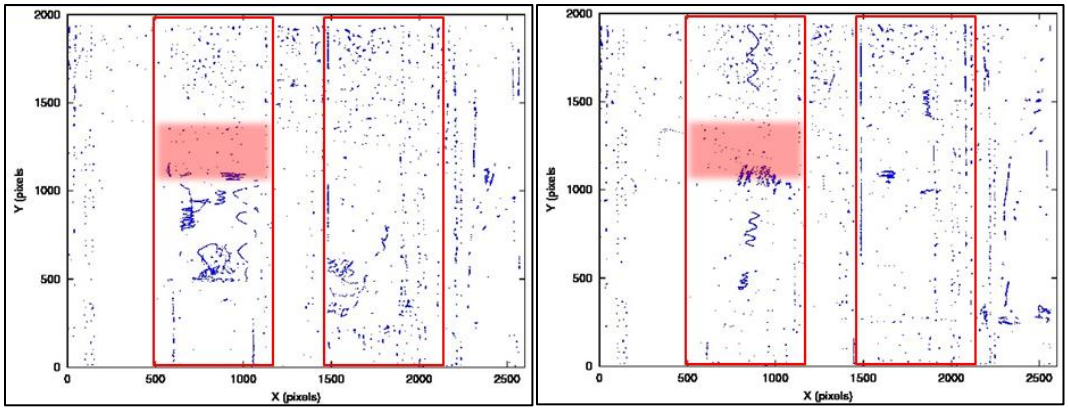

Figure 1: The particle trajectories of larvae raised in constant 20‰ on the surface of the first two columns. Columns 1 and 2 (left to right on each graph) are outlined by red boxes, and the halocline is marked by the translucent pink rectangle.

Particle trajectories for each 300 frame capture showed helical swimming patterns of pisaster larvae. The ratio of vertical to horizontal movement decreased within and around the halocline in both treatments, as seen in figures 1 and 2.

Preliminary results suggest that there might be a smaller overall ratio of vertical to horizontal movement in the constant 20‰ treatment when compared to the trajectories of the constant 30‰ treatment.

Discussion

Analysis of the videos is still in progress, and we have yet to complete any statistical analysis.

It would be beneficial to repeat this experiment with a greater sample size over a greater number of runs. It would also be beneficial to examine the effects that briefer periods of exposure to low salinity would have on the larvae. Using the same ranges of

exposure found in Pia et al., 2012 would more accurately reflect current salinity fluctuations found around the San Juan Islands.

References

Bearon RN, Grünbaum D, Cattolico RA. 2004. Relating cell-level swimming behaviors to vertical population distributions in Heterosigma akashiwo (Raphidophyceae), a harmful alga. American Society of Limnology and Oceanography 49(2): 607–613.

Crawford BJ and Jackson D. 2002. Effect of microgravity on the swimming behaviour of larvae of the starfish Pisaster ochraceus. Canadian Journal of Zoology 80: 2218- 2225.

George SB and Walker D. 2007. Short-term fluctuation in salinity promotes rapid development and metamorphosis of *Dendraster excentricus* larvae. Journal of Experimental Marine Biology and Ecology 349(2007): 113–130.

Grünbaum D and Strathmann RR. 2003. Form, performance and trade-offs in swimming and stability of armed larvae. Journal of Marine Research 61(5): 659–691.

Held MBE and Harley CDG. 2009. Responses to low salinity by the sea star Pisaster ochraceus from high- and low-salinity populations. Invertebrate Biology 128(4): 381–390.

Lee D and George SB. 2011. Vertical distribution of Pisaster ochraceus larvae in haloclines. Blinks Research Fellowship 2011.

Metaxas A. 1998. The effect of salinity on larval survival and development in the sea urchin Echinometra lucunter. Invertebrate Reproduction & Development 34(1998): 2-3, 323-330.

Pia TS, Johnson T, George SB. 2012. Salinity-induced morphological changes in Pisaster ochraceus (Echinodermata: Asteroidea) larvae. Journal of Plankton Research 34(7): 590–601.

Stickle WB and Diehl WJ. 1987. Effects of salinity on echinoderms. Echinoderm studies 2(1987): 235-285.