An investigation into the effects of rearing salinity on blastocoel development in pre-pluteus *Dendraster excentricus* larvae

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National Science Foundation – Research Experience for Undergraduates

Summer 2011

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INTRODUCTION

Many benthic marine invertebrates hatch as a swimming, or pelagic, larva. While this larval form differs between phyla, development of early swimming is common to many of these taxa (Strathmann 1987). Despite marine invertebrates differing by taxa on whether their larvae are feeding or non-feeding, this trend holds for the majority of larvae. In many species of marine invertebrates with feeding larvae, the ability to swim develops before the ability to feed, suggesting this early development is motivated by something other than the need for nutrients.

The pelagic larva typically serves as the dispersal form: it is the means by which benthic marine organisms disperse to established habitats and colonize new areas (Pennington & Emlet 1986, Metaxas & Young 1998a, b, Forward & Tankersley 2001, Welch & Forward 2001, Forward et al., 2003, Shanks et al., 2003, Clay et al., 2004). Larvae swim away from the parent population, locating new places to settle and metamorphose.

Temperature, density, risk of predation, food availability, and UV exposure all vary strongly with vertical position within the water column. Thus, it is likely that larval survival depends on their ability to swim vertically; away from predators, towards food and proper exposure.

Vertical swimming requires orienting upwards and maintaining that orientation. Three primary mechanisms are available for larval orientation: hydrodynamic, gravitational, or by active steering. Larvae vertically orient hydrodynamically by water interacting with body shape, much the way air exerting torque on a shuttlecock causes it to orient with the vanes pointing upward. However, at very early stages, the larvae are

blob-shaped and soft, yet still exhibit strong, stable vertical swimming. Actively steering requires some feedback from a gravity-sensing organ, such as a statolith. However, many larvae lack such organs.

Vertical orientation through gravity requires the center of buoyancy to be anterior to the center of gravity (Alexander 1968). That is, the larva must be bottom-heavy. The blastocoel, which develops prior to swimming in many marine invertebrate larvae, serves as a morphological compartment: if the blastocoel differs in density from the surrounding tissue, its placement and size may shift the centers of buoyancy and gravity, potentially resulting in more stable gravitational orientation.

Further speculation has been made about the larval blastocoel in *Dendraster excentricus*: The larvae of this species may be actively altering the density of the fluid in their blastocoel. As these larvae develop, the blastocoel shifts from an anterior position to a posterior position. To maintain vertical orientation through gravitational stability, the fluid in the blastocoel should be less dense in the anterior position and denser in the posterior position. The current theory proposes *D. excentricus* larvae can actively transport ions into and out of their blastocoel to alter the internal density of the fluid therein. With the blastocoel serving such an important function in a relatively undifferentiated blob, larval orienting ability may rely heavily on its location and volume. If larval survival depends on larval swimming, and larval swimming relies on larval orientation, and larval orientation is enhanced or retarded by blastocoel characteristics, the blastocoel may determine whether a species thrives or dies out.

In this study, I aimed to test whether raising *Dendraster excentricus* larvae developing in different salinities differ in blastocoel volume. I used two salinity

treatments: salinity approximately 3‰ higher than that of sea water from Friday Harbor, WA, and salinity approximately 3‰ lower than that of sea water from Friday Harbor, WA. Prior to direct observations, I modeled a suite of larval shapes, blastocoel volumes, and densities, predicting the effects of these features on larval swimming. Results of the models indicated that water density affected swimming, and that altering blastocoel density compensated for these effects. Using this information, I was able to narrow down physically feasible blastocoel volumes and fluid densities, which helped shape my hypotheses. My hypotheses were further shaped by the natural history of *D. excentricus* larvae: In the earliest stages of the larva, the blastocoel is anteriorly located. This means that the fluid within the blastocoel should be less dense than the larval body in order to ensure correct orientation. Based on the models, I hypothesized that there would exist a direct correlation between the rearing salinity and the larvae's internal density. Based on the developmental process, I further postulated that larval blastocoel volume would increase as rearing salinity decreased.

MATERIALS & METHODS

Effect of Rearing Salinity on Blastocoel Development

In this experiment, *Dendraster excentricus* were force-spawned and larvae were raised at two different salinity treatments, with two replicates for each treatment. Once larvae reached the "target stage" defined under the *Experimental*, samples were taken and the larvae within each sample were killed. One half of each sample was used to determine blastocoel diameter. The other half was injected into a water column containing a strong salinity gradient (<0.5‰ – 117.4‰). After 5-10 minutes, the larvae

were extracted from the column by water level. This procedure was performed thrice: approximately 20 (TS1), 23 (TS2), and 26 (TS3) hours after force-spawning.

We performed the entire experimental procedure in an Environmental Chamber set to 15^{o}C, approximately the temperature of the bay.

***Modeling multiple stages of* D. excentricus *prior to experimentation*.** My models were based on three major assumptions: low Reynolds numbers, shear as a description of turbulence, and a cilial envelope as an adequate representation of the compound effect of individual cilia. I assumed low Reynolds numbers because of the negligible mass of *D. excentricus* larvae. For this same reason I assumed that shear, which tilts larvae by unbalancing the forces acting on the larval body (Grünbaum & Strathmann 2003, Strathmann & Grünbaum 2006, Clay & Grünbaum 2010), was the best approximation of how larvae experience turbulence (Kiøerboe & Saiz 1995, Tennekes & Lumley 1972, Kundu & Cohen 2002, Emlet 1991), on the order of .01-10s^{-} (Yamazaki et al. 2002, Metaxas et al. 2009). Because of the importance of the vertical dimension, we can also assume that the shear that these larvae are most affected in the vertical direction and that all other shear is relatively insignificant.

Initial models were based upon micrographs from the internet. These images were traced and scanned. I digitized shapes using Image J (National Institute of Health). After digitizing the scanned image, I used pixel values to generate a list of X-R points. I built a mesh from this list using the open-source package Gmsh (Geuzaine & Remacle 2011). Using a MATLAB (MATLAB R2011a) script, the meshes were virtually "swum" through various water densities. Within the script, a blastocoel fluid density could be

specified and I observed the hydrodynamic effects of altering the density and volume of the blastocoel within various morphologies of the target stage.

Investigation of the effects of rearing salinity on blastocoel development. All larvae used in the procedure were brooded from the same spawning event. *D. excentricus* adults were collected from Friday Harbor in May 2011 and force-spawned in late July 2011. Force-spawning was achieved by injecting a male and female with 1mL of KCl, placing the adults upside down with gonopores submerged in filtered seawater with a salinity of 29.3‰. The respective gametes were collected in embryo-safe (E-ware) culture dishes. Approximately half of the collected eggs were placed in a large glass E-ware beaker and combined with a drop of sperm. I confirmed fertilization by checking that the eggs' fertilization envelopes had lifted using microscopy immediately upon mixing gametes. We transferred 1mL of the fertilized egg solution into each treatment replicate.

Each treatment was a formed with a base mixture of 100mL of filtered sea water, 1mL of high salinity water, or "heavy water" (approximately132.5‰), and 1mL of Reverse Osmosis (RO) water (<0.05‰). I altered this base by titrating RO or heavy water to attain salinities of 26.3‰ (Treatment 1) and 32.5‰ (Treatment 2) at 15^{o}C.

The "target stage" of larvae that would be used in the experiment was defined to be the radially symmetrical, swimming, non-feeding, non-pluteus form, which developed approximately 20 hours after fertilization. Approximately 6 hours later, larvae began entering the pluteus stage and I declared them past the target stage.

Once larvae reached the target stage, I withdrew a 1mL sample from each replicate of each treatment. The larvae were killed and initially fixed with one drop (approximately 0.1mL) of Lugol's iodine solution per sample. I measured the diameters

of the larval body and blastocoel for n=12-18 individuals per sample using microscopy at 100x magnification. I calculated the volumes of the larva and blastocoel assuming both were spheres, as exact values were not required to test my hypotheses.

RESULTS

Modeling multiple stages of* D. excentricus *prior to experimentation. Initial models were based upon photographs gathered from the internet. Three models of larval bodies were generated and named for their general shape: Oval (Figure 1), Sphere (Figure 2), and Helmet (Figure 3). Each model included a blastocoel, added as an inclusion with distinct density. Using data from McDonald & Grünbaum (2010), I used average densities of 1070-1080g/mL as a basis for testing feasible densities of larval bodies and blastocoel fluids. I also observed an effect of water density on larval swimming, which could be altered by changing the density of the blastocoel inclusion.

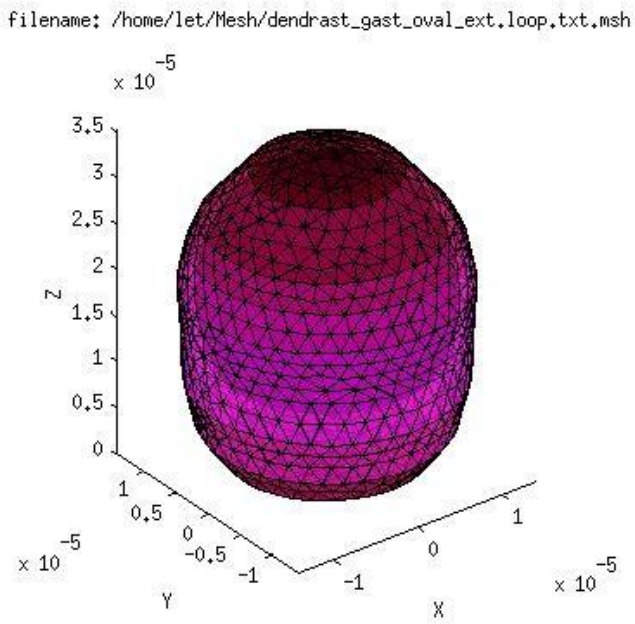

Figure 1. The preliminary model based upon an oval-shaped Dendraster excentricus *larva. The blastocoel is incorporated as a blue inclusion.*

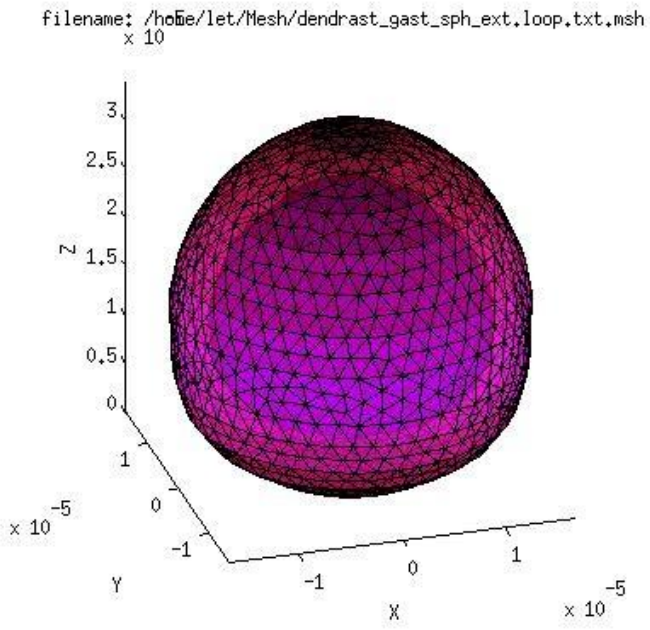

Figure 2. The preliminary model based upon a sphere-shaped Dendraster excentricus larva. The blastocoel is incorporated as a blue inclusion.

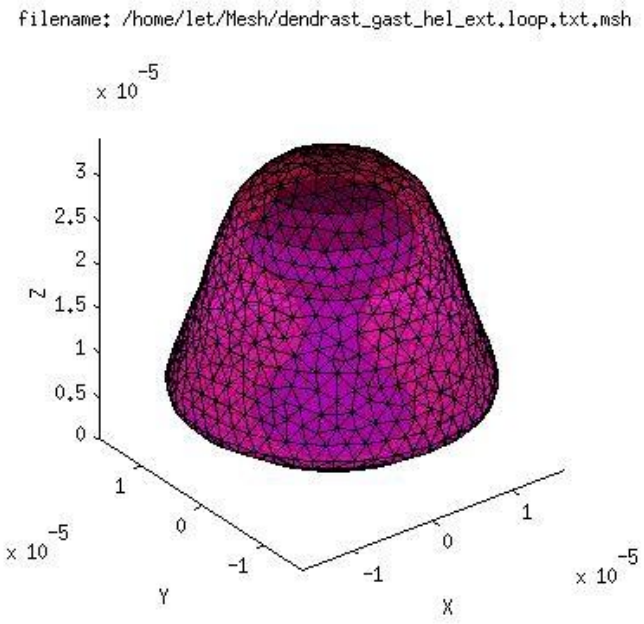

Figure 3. The preliminary model based upon a sphere-shaped Dendraster excentricus larva. The blastocoel is incorporated as a blue inclusion.

Investigation of the effects of rearing salinity on blastocoel development. Larval body diameters and blastocoel diameters were obtained for each treatment at TS1, TS2, and TS3. These values were used to estimate larval body volumes and blastocoel volumes. I used the estimated volumes of body and blastocoel to calculate the percent of the larval volume that is composed of blastocoel (PB). Averaged values for each treatment at TS1, TS2, and TS3 are shown in Table 1 and Table 2. Combining treatment replicates, I ran Mann-Whitney Rank Sum tests comparing the blastocoel volumes between salinity treatments at TS1, TS2, and TS3 (Figure 4). I also ran Mann-Whitney Rank Sum tests to compare the PB between salinity treatments at TS1, TS2, and TS3 (Figure 5). My results indicate a significant difference in both blastocoel volume and PB

between treatments at TS3, with larvae reared in the higher salinity treatment having significantly lower blastocoel volumes and significantly lower PB values.

Table 1. Average blastocoel volume (BV) for each treatment at TS1, TS2, and TS3, and whether the values exhibit a significant difference between treatments.

TS1	**BV (in mm^3)**	**Difference**
Treatment 1	0.166	Not significant
Treatment 2	0.164	
TS2		
Treatment 1	0.196	Not significant
Treatment 2	0.208	
TS3		
Treatment 1	0.252	Significant; $p < 0.001$
Treatment 2	0.152	

Table 2. Average percent of larval body (PB) constituted by the blastocoel for each treatment at TS1, TS2, and TS3, and whether the values exhibit a significant difference between treatments.

TS1	**PB**	**Difference**
Treatment 1	57.74%	Not significant
Treatment 2	50.95%	

TS2		
Treatment 1	62.47%	Not significant
Treatment 2	62.55%	
TS3		
Treatment 1	72.35%	Significant; $p < 0.001$
Treatment 2	45.78%	

DISCUSSION

My results support my hypotheses: I did see a significant difference in blastocoel development, with larvae reared in the higher salinity developing a blastocoel of significantly smaller volume compared to larvae reared in lower salinity. This difference in blastocoel volume was not paired with a significant difference in larval body diameter, assuring me that a higher rearing salinity does not result in significantly smaller larvae.

The results of my experiment add support to the theoretical relationship between blastocoel and ionic alteration of blastocoel density. By showing a difference in blastocoel development corresponding to salinity, which is a measure of ions within the water, and blastocoel volume, I have tentatively illustrated a relationship between blastocoel volume and ionic content.

If this relationship holds, the morphological change seen in my results may have biomechanical implications. In lower salinity water, where ions are less readily available, my results indicate a larger blastocoel will develop. This larger blastocoel may allow a larva to exert tighter control on ionic content as a larger volume could provide

more fluid and space to alter more gradually. This ability could prove advantageous when a larva is faced with changing environmental conditions, such as changes in salinity or turbulence as it struggles to orient within a medium of changing density and hydrodynamics.

As a larva swims away from its parent population, it must be able to reach resources, despite such environmental changes. From McDonald & Grunbaüm (2010), this need to reach resources inflicts a "swimming standard" on larvae: If a larva cannot successfully orient and swim in the face of environmental challenges, then it does not meet the swimming standard. Due to the changeability of the marine environment, this standard is not fixed. Rather, it represents a plasticity that allows larvae to swim efficiently in a multitude of physiological states and waters of varied energetics.

This plasticity is expressed as a suite of morphologies that vary to promote new forms and mitigate the negative impacts of these new forms. Plasticity in *D. excentricus* larvae is tightly regulated (Kaern et al. 2005). For example, *D. excentricus* larvae develop a variety of cilia patterns (Masuda & Sato 1984, Damen & Dictus 1994) but may be altering their physiology or behavior to maintain swimming ability (Sameoto et al. 2010). In my study, I saw significant differences in blastocoel volume. As blastocoel volume changes, the ability to maintain an optimal internal density may also be changing, possibly affecting gravitational stability. However, I observed this difference in blastocoel volume at TS3, when the larval shape was changing to a form that is better able to hydrodynamically stabilize. Thus, blastocoel volume changed when the larvae was developing a shape that could possibly mitigate the effects of a different blastocoel. In this way, adherence to the theoretical swimming standard is retained.

FUTURE WORK

With new micrographs from observations made during the experiment, I will construct a "consensus form." By digitizing multiple images of each TS, I will build a model that lacks the eccentricities of any individual larva. These models will prove invaluable as I begin to investigate the effects of rearing salinity on larval swimming. I hope to test these models in turbulence by modeling them in shear to examine the relationship between orienting in shear and blastocoel volume. After modeling larvae swimming in shear, I would like to implement an experimental component.

I have already begun an investigation of rearing salinity on internal density. Data gathered from these experiments will also be used to improve the realism of my larval models.

Currently, I am eager to begin working on swimming experiments. I plan to observe and quantify speed of larvae swimming through a series of haloclines, and analyze blastocoel characteristics of those larvae that swim through these haloclines fastest.

ACKNOWLEDGMENTS

This research was conducted at University of Washington Friday Harbor Laboratories under the National Science Foundation Research Experience for Undergraduates (REU) program. Funding was provided by the National Science Foundation. Additional funding was provided by Danny Grünbaum. I thank Sophie George and Adam Summers for heading the REU program at Friday Harbor Labs; Scott Schwinge and Kathy Cowell

for arranging my travel and stay at Friday Harbor; my fellow REU students, who offered constructive criticism and support throughout the project; Richard Strathmann for training in force-spawning *Dendraster excentricus* and subsequent advice on larval culturing. I owe special thanks to my program mentor, Danny Grünbaum for training in building, programming, and using models, as well as support and advice in the experimental methods.

REFERENCES

Alexander, R. M. 1968. *Animal Mechanics*. University of Washington Press, Seattle, WA, p 346.

Clay, T. W., Bollens, S. M., Bochdansky, A. B. & T. R. Ignoffo. 2004. The effects of thin layers on the vertical distribution of larval Pacific herring, *Clupea pallasi*. *Journal of Experimental Marine Biology and Ecology*. **305**: 171-189.

Clay, T. W. & D. Grünbaum. 2010. Morphology-flow interactions lead to stage-selective vertical transport of larval sand dollars in shear flow. *The Journal of Experimental Biology*. **213:** 1281-1292.

Damen, P. & W. J. A. G. Dictus. 1994. Cell lineage of the prototroch of *Patella vulgate* (Mollusca, Gastropoda). *Developmental Biology*. **162**: 364-383.

Emlet, R. E. 1991. Functional constraints on the evolution of larval forms of marine invertebrates: experimental and comparative evidence. *American Zoologist*. **31**: 707-725.

Forward, R. B. & R. A. Tankersley. 2001. Selective tidal-stream transport of marine animals. *Oceanography and Marine Biology: An Annual Review*. **39**: 305-353

Forward, R. B., Tankersley, R. A. & J. M. Welch. 2003. Selective tidal-stream transport of the blue crab *Callinectes sapidus*: an overview. *Bulletin of Marine Science*. **72**: 347-365.

Geuzaine, C. & J.-F. Remacle. 2009. Gmsh: a three-dimensional finite element mesh generator with built-in pre- and post-processing facilities. *International Journal for Numerical Methods in Engineering*. **79**: 1309-1331.

Grünbaum, D. & R. R. Strathmann. 2003. Form, performance, and trade-offs in swimming and stability of armed larvae. *Journal of Marine Research*. **61**: 659-691.

Kaern, M., Elston, T., Blake, W. & J. Collins. 2005. Stochasticity in gene expression: from theories to phenotypes. *Nature Reviews Genetics*. **6**: 451-464.

Kiørboe, T. & E. Saiz. 1995. Planktivorous feeding in calm and turbulent environments, with an emphasis on copepods. *Marine Ecology Progress Series*. **122**: 135-145.

Kundu, P. K. & I. M. Cohen. 2002. *Fluid Mechanics*. Academic Press, San Diego, CA.

Masuda, M. & H., Sato. 1984. Asynchronization of cell division is concurrently related with ciliogenesis in sea urchin blastulae. *Development, Growth & Differentiation*. **26**: 281-294.

McDonald, K. A. & D. Grünbaum. 2010. Swimming performance in early development and the "other" consequences of egg size for ciliated planktonic larvae. *Integrative and Comparative Biology*. **50**: 589-605.

Metaxas, A., Mullineaux, L. S. & J. Sisson. 2009. Distribution of echinoderm larvae relative to the halocline of a salt wedge. *Marine Ecology Progress Series*. **377**: 157-168.

Metaxas, A. & C. M. Young. 1998a. Behavior of echinoid larvae around sharp haloclines: effects of salinity gradient and dietary conditioning. *Marine Biology*. **131**: 443-459.

Metaxas, A. & C. M. Young. 1998b. Responses of echinoid larvae to food patches of different algal densities. *Marine Biology*. **130**: 433-445.

Pennington, J. T. & R. B. Emlet. 1986. Ontogenetic and diel vertical migration of a planktonic echinoid larva, *Dendraster excentricus* (Eschscholtz): occurrence, problems, and probable consequences. *Journal of Experimental Marine Biology and Ecology*. **104**: 69-95.

Sameoto, J. A., Ross, T. & A. Metaxas. 2010. The effect of flow on larval vertical distribution of the sea urchin, *Strongylocentrotus droebachiensis*. *Journal of Experimental Marine Biology and Ecology*. **383**: 156-163.

Shanks, A. L., Largier, J. & J. Brubaker. 2003. Observations on the distribution of meroplankton during an upwelling event. *Journal of Plankton Research*. **25**: 645-667.

Strathmann, M. 1987. *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast*. Seattle: University of Washington Press.

Strathmann, R. R. & D. Grünbaum. 2006. Good eaters, poor swimmers: compromises in larval form. *Integrative and Comparative Biology*. **46**: 312-322.

Tennekes, H. & J. L. Lumley. 1972. *A First Course in Turbulence*. The MIT Press, Cambridge, MA.

Welch, J. M. & R. B. Forward. 2001. Flood tide transport of blue crab, *Callinectes sapidus*, postlarvae: behavioral responses to salinity and turbulence. *Marine Biology*. **139**: 911-918.

Yamazaki, H., Mackas, D. L. & K. L. Denman. 2002. Coupling small-scale physical processes with biology. In: Robinson, A. R., McCarthy, J. J., Rothschild, B. J. (eds) *The Sea, Volume 12*. John Wiley & Sons, New York, NY, p 248-286.