

*Observations of Growth of Dendraster excentricus  
in a Laboratory Setting*

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## Introduction

Culturing embryos and caring for them is a necessary skill of any student of developmental biology. The University of Oregon's course at the Oregon Institute of Marine Biology entitled Comparative Embryology and Larval Development gives undergraduate and graduate students the opportunity to grow and study their own cultures of larvae. This gives the student hands on understanding of how marine larvae develop. The student learns to care for the larvae, as well as, witnesses a timetable of development for many different marine species. It is the intent of this paper to detail the development of one of the cultured embryos, *Dendraster excentricus*, as well as research factors that effect the survival, growth, and development of the larvae, both in the laboratory and in their natural environment. Egg and jelly coat size and diet of the larvae are factors that deem investigation when studying the growth of larvae in laboratory cultures.

Jelly coat and egg size vary among females within a species. Any increase in size is beneficial, be it from egg size or jelly coat size. These two factors are under direct selection for fertilization success but the selection for egg size is stronger than on jelly coat. In fact the selection pressure was measured as a selection gradient and was found to be about 922% stronger for egg size versus jelly coat size. The study found that thicker jelly coats were more likely to be fertilized in low sperm concentrations suggesting that jelly coat size is under selection pressures to increase fertilization in limited sperm situations. Moreover, decreases in the amount of spawned sperm available can result in selection for larger size. However, there are potential drawbacks, a larger jelly coat makes penetration of the egg harder for the sperm and increased egg size is a trade off

because the animals tend to spawn less eggs when the eggs are larger (Levitan and Irvine, 2001).

Once a *Dendraster excentricus* embryo hatches into a larva it possesses a ciliated band that can reverse to engulf their food. Each species of embryo has an optimal mixture of phytoplankton to develop optimally. It is possible to supplement a laboratory larva with a mixture of algae so that the two types fed to the cultures were complimentary and provide the necessities for growth. Daniela Schioppa and her colleagues (2006) have studied how different alga combinations provide needed fatty acids in the development of *D.excentricus*. They measured physical characteristics of larvae fed different diets and found that body length was not affected by changes in diet but arm length and rudiment size are sensitive to fatty acid changes (Schioppa et al, 2006).

These scientists fed their lab reared larvae a mix of *Rhodomonas sp.* and *Dunaliella tertiolecta* and found that that particular mix resulted in the smallest rudiments of all the mixes or monoculture diet. Their data support a 31-48% variation in rudiment size is due to diet. A dietary mix of *Isochrysis galbana* and *D. tertiolecta* was found to be the best mix of alga. The second best diet was a monoculture of *Rhodomonas sp.* and the third a *D. tertiolecta* monoculture (Schioppa et al, 2006).

To determine why these treatments were so successful Schioppa (2006) and her team analyzed the algae for their amino acid content. *D. tertiolecta* lacks the essential long chain polyunsaturated fatty acids (PUFA), EPA and DHA. *Rhodomonas sp.* was found to be sufficient in EPA and DHA. *Rhodomonas sp.* was found to have the lowest saturated fatty acids, a component necessary for a nutrient sufficient diet. Not only are SUFA found in great abundance in the body of the larva, short chain PUFA are also

present. Linolenic acids were found in highest abundance in *D. tertiolecta* (Schiopu et al, 2006). Therefore, the mixed diet of *R. lens* and *D. tertiolecta* fed in the OIMB lab to the *D. excentricus* cultures are sufficient to provide necessary fatty acids.

Fatty acids are not the only component in the laboratory algae treatments. The phytoplankton contains chlorophyll A, B and C. When chlorophyll concentrations were measured at the surface of the water and at 20 meters below the water it was found to have higher concentrations of both Chl A and Chl C. There was not significant difference in Chl B concentrations. Chl C is found in diatoms and dinoflagellates and because these organisms are poor in nutrients they cause slower growth. McDonald (2004) state that growth and development are influenced by sub-lethal toxicity due to unstable food sources like diatoms. She concludes that the laboratory reared larvae of her experiments were less food limited than the larvae observed at the water surface or 20 meters below it. The laboratory raised larvae grew the fastest and their rudiment size was larger than the rudiments of the surface water larva. McDonald believes that the vertical placement of a larva in the water column affects the food types the animal encounters and therefore affects larva size and time of development (McDonald, 2004).

## Methods

In our laboratory setting we cultured *D. excentricus* by injecting both males and females with 0.5mol KCl to induce spawning of their gametes. The cultures were kept in Petrie dishes in the laboratory sea table between 12-15°C. Cultures were changed in the first 4 weeks every other day and the last 6 weeks 2 times per week. The water was removed using reverse filtration with Nitex mesh of no greater than 75µm. After filtering

the embryos and larvae were examined under the compound scope when they were small and then under the dissecting scope when they grew larger. The date, time, and a drawing or picture was recorded at each observation. After examination the larvae were transferred to a clean Petrie dish and filtered seawater was added. The *D. excentricus* larvae were fed a mixed diet of *Dunaliella tertiolecta* and *Rhodomonas lens*. Once in the clean Petrie dish the larvae were given 2 generous Pasteur pipettes full of each alga. The algae were stored in large aerated Erlenmeyer flask in a nutrient rich solution. Prior to feeding the alga cells suspended in the solution were centrifuged so the alga went to the bottom and the nutrient rich solution formed a supernatant layer on the top. The top layer was decanted off leaving the alga cells concentrated in the bottom of the centrifuge vile. Filtered seawater was then added to the cell and the vials were shaken. After feeding, the cultures were then placed back in the sea table for further development.

## **Results**

Day one we fertilized the eggs and witnessed first cleavage. The second day the embryos were blastulas and some were rotating early blastulas. By day 8, four arms had formed making the larvae a four armed echinopleuteus. The first appearance of the juvenile rudiment was on day 15. The larva grew two more arms by day 17 and was a 6 armed echinopleuteus. The 8 armed echinopleuteus was first seen on day 22 with juvenile rudiments almost completely formed inside the larvae. On day 22 the first juveniles were seen. Observations of day 32 showed the juveniles had grown and their fenestrated spikes and podia were considerably longer than on day 22. The final observation was taken on Day 50 and the juvenile was more advanced with long podia

and fenestrated spikes and the animal had the petal like design characteristic of the sand dollar.

## **Discussion**

It appears that the suggested diet in the OIMB lab of *D. tertiolecta* and *R. lens* is an excellent choice to feed these developing animals. Studies done by Schiopu et al. (2006) and Mc Donald (2004) give us needed information about nutrient content of the algae and dietary requirements of echinoid larva. By feeding the laboratory cultures of *D. excentricus* the mix of *D. tertiolecta* and *R. lens* the class was able to supply the needed DHA and EPA required for growth. By feeding these essential nutrients to the larva the class was able to have several different species reach juvenile stage. If we were feeding the larva a monoculture of algae then it is likely less species of juveniles would have been seen.

Taking care of embryos is a time consuming undertaking that requires students to look closely to see very small structures. My biggest shortfall was that I was inexperienced in caring for larva and also lacked good microscope skills. This was challenging to me at first but as I continued to care for them I became better skilled at both changing the cultures and looking at the larva under a microscope. These are skills I can take with me to future endeavors in the field of Biology.



Fig 1 : Juvenile rudiment inside echinopluteus

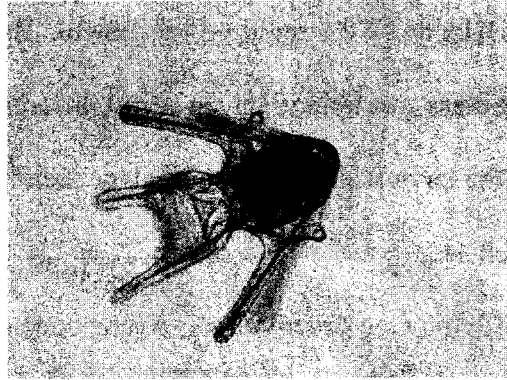
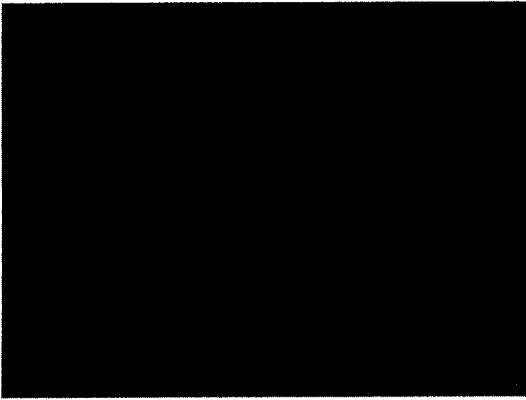


Fig 2 : 8-armed echinopleuteus

## References

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