Effects of Diet on the Larval Development of *Dendraster excentricus*

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INTRODUCTION

The sand dollar *Dendraster excentricus* is a close relative of the purple sea urchin, and is likewise a good candidate for studies on early development. This is due to its relatively rapid radial cleavage and development of these gonochoristic organisms. In addition, KCl has proven to be a reliable method for inducing spawning on *D. excentricus*. Researchers have conducted various experiments with this species to determine how diet effects larval development and survival. One such study done by Rietzel et al. (2003) set out to determine whether larval development of *D. excentricus* is limited by food quality and/or quantity. The experiment involved two separate treatments. Larvae in treatment #1 were cultured in natural seawater at depths of one meter and 20 meters. For the second treatment larvae were cultured in filtered seawater and fed a monoculture diet of *Dunaliella tertiolecta*. Results indicated that larvae were least food limited in treatment #2, followed by treatment #1 at the one-meter depth, and treatment #1 at the 20-meter depth. The presence of adequate food quantity in both treatments suggests that food quality may be the limiting factor in larval development for this experiment.

Another study by Schiopu et al. (2005) investigated the effect of single versus mixed algal diets on the development of *D. excentricus*. Single algal diets included *Dunaliella tertiolecta, Isochrysis galbana* and *Rhodomonus sp.* In addition, larvae were fed four different mixed algal diets including *D. tertiolecta* and *Rhodomonas sp., I. galbana* and *D. tertiolecta, I. galbana* and *Rhodomonas sp., D. tertiolecta, I. galbana* and *Rhodomonas sp.* The study found that a mixed diet of *I. galbana* and *D. tertiolecta* produced the best results in terms of larval size, survival and development to metamorphosis. The authors of the study explain that though *D. tertiolecta* is considered a good diet for echinoderm larvae, it lacks EPA and DHA, two essential long chain PUFAs found in both *I. galbana* and *Rhodomonas sp.*

A study by Miner (2006) investigated the effects of added algal cells, algal exudates, and polystyrene beads (comparable in size to algal cells) on the development of feeding structures of pre-feeding echinoid larvae. Results showed shorter arm growth in pre-feeding *D. excentricus* larvae when algal cells or algal exudates were present, while the polystyrene beads had no observable effect on arm length. These results suggest that larvae may gain information about environmental food concentration before they have the ability to feed.

Such studies provide evidence that not only the timing of spawning in relation to food availability, but also the quantity and quality of food present at the time of spawning has a significant effect on the developmental timetable of *D. excentricus* larvae.

METHODS

Adult Dendraster excentricus were collected on the morning of April 3rd 2007 from South Cove, Cape Arago and brought back to the lab where they were kept in a flow-through sea table at 13 degrees C. In the afternoon spawning was induced by injecting KCl. Eggs were pipetted into a small dish and a dilute sperm solution was added. Percent fertilization was checked and more sperm added until fertilization reached roughly 95-100%. The culture was stored in a sea table at 13-15 degrees C. Every few days, bacteria and other impurities were removed through a sieve and new filtered seawater was added.

Once larvae were observed moving, they were fed a mixed diet of *Dunaliella tertiolecta* and *Rhodomonus lens*. Algae cultures were aerated and kept under natural light. During feeding, one 15 ml sample of each alga was collected and centrifuged for 10 minutes. The medium was then discarded and filtered seawater reincorporated. One pipette-full of each algae solution was then added to the culture directly after bacteria and impurities were removed. On day 10 of observations, the quantity was increased to two pipettes-full.

RESULTS

Developmental stages of *D. excentricus* larva observed during this experiment include early cleavage, blastula, gastrula, early 4-armed, early 8-armed, late 8-armed and juvenile (see Table 1, fig. 1). Many individuals died and individuals remaining on the final day of observation were at various stages of development. I observed a total of 6 juveniles on the final day of observation, while many were still at the 6 or 8 –armed stage.

Developmental Time Table of Dendraster excentricus

Day of observation	Observed stage of development
1	Cleavage through 8 cell stage
2	Blastula
3	Gastrula
4	Early 4-armed echinopluteus
13	Early 6-armed echinopluteus
16	Early 8-armed echinopluteus
19	8-armed and rudiment
23	Juvenile

Table 1: Timetable of larval development of *D. excentricus* fed a mixed diet of *D. tertiolecta* and R. lens.

Table 2: Comparison Developmental Time Table for D. Excentricus

raven.zoology.washington.edu/celldynamics/downloads/urchinlab.html - 35k

Dendraster excentricus (sand dollar)

Stage	10-11 C	11-12 C	13-14 C	17 C	22 C
Two cells	3 hr	2 hr			
Four cells	4 hr	3 hr	2 hr		
Eight cells	5.5 hr				
Sixteen cells	7 hr		3 hr		
Hatching	30-32 hr	19-23 hr	17 hr	15 hr	10.5 hr
Mesenchyme blastula	36 hr	27-29 hr			
Gastrula	38-42 hr	32-35 hr	20 hr		
Prism	55-60 hr	40-46 hr	32 hr	24 hr	17 hr
Early pluteus (2 arms)	65 hr	57 hr		35.5 hr	24 hr
4-arm pluteus		4 day		2.5 day	2 day
6-arm pluteus		8 day	6 day	4 day	2.5 day
Pluteus with juvenile rudiment			11 day		

Photographic Journal of Development

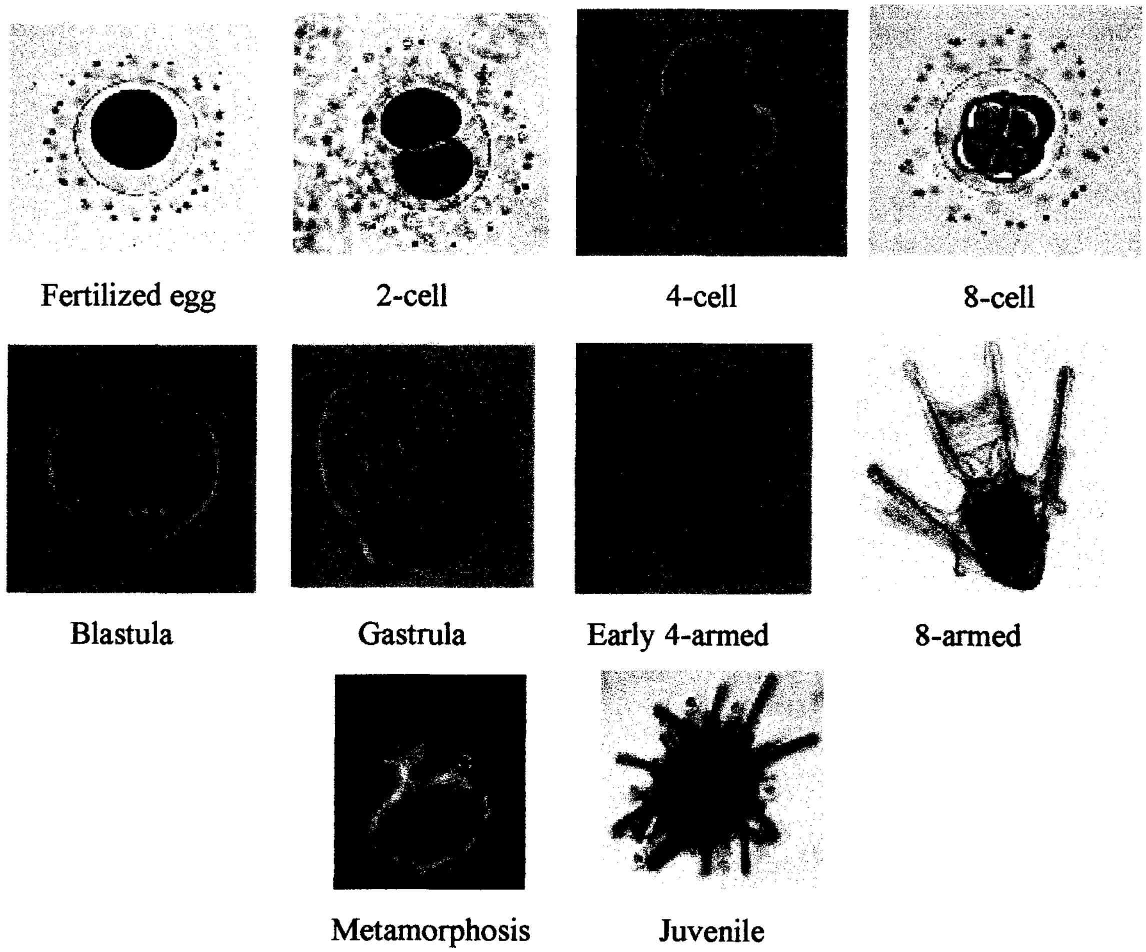

Figure 1: Photographic journal showing observed developmental stages of *D. Excentricus.*

DISCUSSION

The developmental timetable recorded in the current study is comparable to a developmental timetable recorded by the Zoology department at the University of Washington. The timing of cleavage and early echinopluteus stages are particularly consistent with one another. The timetables begin to differ a bit in later stages, with the consistent with one another. The timetables begin to differ a bit in later stages, with the current study showing a slightly slower development. Differences could be the result of several factors such as varying food type and quantity, or a difference in rearing methods.

The significant effect that food type and availability have on echinoid larvae illustrates the delicacy of the ecosystem. A change in timing of food availability relative to spawning, or a sudden difference in food quantity or quality could alter the development of echinoid larvae. In contrast, a study by Hoegh-Guldberg et al. (1995) concluded that water temperature, even more so than food availability or other variables, is the most likely explanation for latitudinal differences in developmental rates of marine larvae. Whether this is the case or not it brings to the surface some important questions about the impact various environmental factors may have on marine larval development. Climate change, pollution and the introduction of invasive species may all have serious implications for the suitability of larval habitat and the development of *D. excentricus* larvae. It would be interesting to conduct further studies on the effect of ocean temperature on larval development, as well as the potential impact that more frequent and intense storms due to global warming might have on fertilization success and settlement of marine larvae.

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