A Brief Study on the Development of the Purple Sea Urchin *Strongylocentrotus purpuratus*

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Introduction

The sea urchin *Strongylocentrotus purpuratus* is a member of the phylum Echinodermata and the class Echinoidea (Czihak 1971). Like other echinoderms it has five-fold (pentamerous) symmetry and moves by means of hundreds of tiny, transparent, adhesive tube feet. Within the echinoderms, sea urchins are classified as Echinoids, which are further defined by the presence of a modified regular spherical shape, interlocking calcareous plates, and movable appendages. *S. purpuratus* individuals are primarily found from the rocky intertidal to depths up to 160 meters. All individuals are free-spawning and have pelagic larvae called echinoplutei (Geise 1991).

The sea urchin embryo has played a key role in embryological studies due to its thorough documentation over the past century. This is mostly because these organisms are easy to obtain on almost any sea coast spawning readily and yielding large quantities of eggs at a time (Geise 1991). *S. purpuratus* has external fertilization (Hyman 1955). Eggs or sperm are shed through five gonopores on the aboral side of the sea urchin. Females release eggs which are surrounded by a jelly layer that hydrates and expands upon contact with seawater (Gonor 1973a). Males release sperm which exhibit a sudden increase in metabolism and motility as a result of a Na+ influx and H+ efflux. When a sperm cell encounters an egg, components of the jelly coat bind to specific "egg receptors" in the plasma membrane. This subsequently triggers a signal transduction cascade resulting in a series of events that facilitate fertilization (Okazaki 1975b).

In *S. purpuratus,* development proceeds via radial, holoblastic cleavage (Chatlynne 1969). Similar to embryonic cleavages of other invertebrates, *S. purpuratus* cleavage is reductive, producing more cells without an increase in the total cellular

cellular volume of the embryo. The first two cell cleavages are meridional. The plane passes through the animal-vegetal axis of the egg, with the second cleavage lying at right angles to the egg. Third cleavage is equatorial and divides the animal hemisphere from the vegetal one resulting in eight equal blastomeres (Geise 1991). The fourth cleavage, however, is very different from the first three. The four cells of the animal tier divide meridionally into eight blastomeres, each with the same volume. These cells are called mesomeres. The vegetal tier, however, undergoes an unequal equatorial cleavage to produce four large cells, the macromeres, and four smaller micromeres at the vegetal pole (Czihak 1971). As the 16-cell embryo cleaves, the eight mesomeres divide to produce two "animal" tiers, an_1 and an_2, one staggered above the other. The macromeres divide meridionally, forming a tier of eight cells below an_2. The micromeres also divide, albeit somewhat later, producing a small cluster beneath the larger tier. All the cleavage furrows of the sixth division are equatorial, and the seventh division is meridional, producing a 128-cell blastula (Geise 1991).

The blastula stage of sea urchin development begins at the 128-cell stage. Here the cells form a hollow sphere surrounding a central cavity, or blastocoel. By this time, all the cells are the same size, the micromeres having slowed down their cell division (Stearns 1974). The blastula eventually becomes ciliated and begins to rotate within the fertilization envelope and the embryo is now considered a free-swimming, hatched blastula (Okazaki 1975b).

Gastrulation is initiated by the inpocketing of the vegetal plate and proceeds via invagination where the vegetal plate bends inward and invaginates about one-fourth to one-half the way into the blastocoel. Then invagination suddenly ceases. The invaginated

region is called the archenteron (i.e. primitive gut), and the opening of the archenteron at the vegetal region is called the blastopore (Stearns 1974). Eventually, the archenteron makes contact with the blastocoel wall and a mouth is ultimately formed. The mouth fuses with the archenteron to create a continuous digestive tube. Thus, the blastopore marks the position of the anus, as is characteristic of deuterostomes (Geise 1991). Subsequently, the skeleton is laid down and *S. purpuratus* embryos change to form a prism stage and later develop a simple 4-armed echinopluteus larva (Okazaki 1975b).

The echinopluteus larva does not feed until it has developed into a relatively simple 4-armed pluteus. Feeding is accomplished by means of ciliary currents produced by ciliated bands on the larval arms that draw the water down. Particles crossing the ciliated bands are captured via ciliary reversal and are subsequently carried to the mouth (Ebert 1968). The echinopluteus larva continues to swim and feed until it ultimately develops into an 8-armed echinopluteus composed of pairs of posterodorsal and anterolateral arms and a pairs of post-oral and pre-oral arms with a ciliated band extending along the edges of the larval arms (Stearns 1974). The echinopluteus is competent to settle and metamorphose when a radical change in external shape and the formation of the echinus rudiment has developed which can be induced by the presence of microbial films. The echinus rudiment originates inside the vestibule, an outpocketing of the gut on the left side where the juvenile develops. Additionally, other metamorphosis stimulating conditions can be chemical cues in the saltwater, and in most cases the presence of a suitable substrate (Geise 1991).

Methods

Strongylocentrotus purpuratus specimens were collected on the Southside of Sunset Bay in Charleston, Oregon. Individuals were transported back to the lab and were injected with approximately two ml of 0.55M Potassium Chloride (KCl) around Aristotle's lantern using a 22 gauge needle. Individuals were then inverted over a beaker containing 9 degree Celsius filtered seawater to collect eggs and sperm. Sperm was diluted 1:10,000 in 9 degree Celsius filtered seawater and a sample was subsequently placed under a compound microscope to verify that they were motile. A glass Pasteur pipette was then used to transfer the eggs and dilute sperm to a small custard bowl where they were combined in approximately 100 ml of 0.45 micron filtered seawater. Three separate cultures were made accordingly, and kept in flowing sea water tables to keep the temperature at a constant 9 degrees Celsius.

Once embryos had finished gastrulation, *S. purpuratus* cultures were fed a combination of two types of algae, *Rhodomonas lens* and *Dunaliella tertiolecta*. Both algal species were raised in nutrient growth medium in one-liter Erlenmeyer flasks and bubbled to promote growth. Algae were prepared for the cultures by centrifuging and subsequently removing the supernatant. The supernatant was then mixed with a small quantity of filtered sea water. The cultures were fed every other day.

The cultures were changed every other day using reverse filtration to remove 90% of the filtered sea water. The larvae were then transferred to a new custard bowl, new filtered sea water was added and they were fed a combination of *R. lens* and *D. tertiolecta*. The cultures were also fed during each water change. Larval development was

recorded daily via pictures over the course of four weeks with the aid of dissecting and compound microscopes.

Results

The sperm of *Strongylocentrotus purpuratus* was determined active after brief observation under a compound microscope. Eggs and sperm of *S. purpuratus* were subsequently combined in culture dishes. Within seconds after the gametes were mixed, sperm underwent exocytosis of the acrosomal granule and extended acrosomal filaments. In addition, components of the egg jelly coat bound to specific "egg receptors" in the sperm plasma membrane. This subsequently triggered a signal transduction cascade resulting in a series of events that facilitated fertilization resulting in formation of the fertilization membrane (Figure 1).

Approximately three hours later, the first meridional cleavage occurred forming two cells of equal size (Figure 2). Two hours later, another meridional cleavage occurred at right angles to the egg resulting in second cleavage and the formation of four cells of equal size (Figure 3). Third cleavage was observed six hours post fertilization resulting in the division of the animal hemisphere from the vegetal hemisphere producing eight equal cells (Figure 4). Finally, a 16-cell embryo was observed approximately eight and a half hours post fertilization after four vegetal cells divided unequally to form four micromeres and four macromeres, while the animal half divided longitudinally into eight mesomeres, each with the same volume. The macromeres produced after fourth cleavage will contribute to the ectoderm and secondary mesenchyme cells which will be assimilated

into the echinopluteus. The micromeres are destined for contribution to the coelomic pouches and primary mesenchyme cells that will form the larval skeleton.

After 26 hours, the blastula of *S. purpuratus* had formed (Figure 5). The cells in the blastula form a hollow sphere surrounding a central cavity, or blastocoel. The blastula was also ciliated and rotating within the fertilization envelope and the embryo was now considered a free-swimming hatched blastula.

Approximately fifty-one hours post fertilization, gastrulation was initiated by the inpocketing of the vegetal plate and proceeded via invagination to form the tubular archenteron and the opening to the archenteron at the vegetal region called the blastopore (Figure 6). At ninety-six hours, the first skeletal spicules were observed and the gastrula had developed into a prism shaped larvae with a fully formed stomach, mouth and anus (Figure 7). The larval skeleton was laid down 8 days post fertilization. At this point, early development of postoral, anteriolateral rods and the dorsal arch in *S. purpuratus* larvae were observed.

After 17 days the prism developed into a simple 4-armed echinopluteus (Figure 8). The larvae were observed actively feeding on the algae at the bottom of their culture dish via the use of ciliary currents produced by ciliated bands on the larval arms that draw the water down and catch particles by means of ciliary reversal. An early 6-armed echinopluteus was observed at 19 days post fertilization (Figure 9) and a fully formed 6-armed echinopluteus was not observed until 21 days post fertilization. Finally, 27 days after fertilization an 8-armed echinopluteus was observed actively swimming within the culture dish with a juvenile rudiment visible within the vestibule at the anterior end of the echinopluteus larva (Figure 10).

Finally, 29 days after fertilization, the juvenile rudiment had appeared to develop, substantially filling a large percentage of the anterior end of the larva (Figure 11). Additionally, elongate echinopluteus arms were observed signifying a positive response to the substratum and competency to settle and metamorphose. Juvenile *S. purpuratus* individuals were observed 30 days post fertilization crawling independently along the bottom of the culture dish via the use of tube feet (Figure 12). A synopsis of the developmental timetable of *S. purpuratus* individuals observed in the laboratory is found in Table 1.

Discussion

The developmental timeline of *Strongylocentrotus purpuratus* is the hallmark of echinoid development and in turn deuterostome development. This is the product of its thorough documentation over the past century and its ubiquitous population that readily spawns, yielding large quantities of eggs (Geise 1991). Additionally, the developmental timeline post metamorphosis is an important determining factor for the success of natural recruitment rates within populations of *S. purpuratus* (Miller and Emlet 1999). However, due to the time constraints of a small lab culture, development post metamorphosis was not observed.

Nevertheless, the significance of post-metamorphic developmental events of juvenile *S. purpuratus* has been documented. The rapid formation of features such as coronal pedicellariae, sphaeridia, and a functional gut in addition to increased production of spines, test plates and podia are inherent in the success of juvenile survival during the first few weeks after metamorphosis (Miller and Emlet 1999). The development of these

features is also particularly important to the success of a young juvenile since they must form prior to feeding (Miller and Emlet 1999). The rates at which these momentous features develop are highly sensitive to temperature, salinity and food rations. Increased temperature and fluctuations in salinity levels cause juvenile development to retard (Miller and Emlet 1999). Lower food rations also slow the development of important post-metamorphic features such as coronal pedicellariae and sphaeridia (Miller and Emlet 1999).

Settlement and recruitment patterns of *S. purpuratus* in the field are not as easily determined. They range from sporadic to annually recurrent. For example, *S. purpuratus* recruits into Papalote Bay, Baja California, Mexico several times in the year with year-to-year variation in density (Ebert 1988). Additionally, *S. purpuratus* has a latitudinal variation in both settlement and recruitment patterns (Ebert 1988). However, in areas like the California Kelp forest large recruitments happen only once every 30 years with high mortality rates occurring for the remaining 29 years (Geise 1991 and Ebert 1988). Variations in recruitment rates may be a function of substrate type and oceanographic events since *S. purpuratus* juveniles are found in significantly greater numbers in boulder fields and downwelling areas (Ebert 1988).

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Table 1. Developmental timetable of *Strongylocentrotus purpuratus* fertilized at 10:30am on 4/2/08.

TIME POST FERTILIZATION	STAGE
0	Gametes mixed
<1 min	Formation of fertilization membrane
3 hours	First cleavage, 2-cell stage
5 hours	Second cleavage, 4-cell stage
6 hours	Third cleavage, 8-cell stage
8.5 hours	Fourth cleavage, 16-cell stage
26 hours	Formation of rotating blastula with cilia
51 hours	Appearance of archenteron and formation of anus
96 hours	Gastrula and appearance of skeletal spicules, Early Prism
8 days	Early echinopluteus
12 days	Echinopluteus
17 days	4-armed Echinopluteus
19 days	Early 6-armed Echinopluteus
21 days	6-armed Echinopluteus
27 days	8-armed Echinopluteus with rudiment visible
29 days	Late Metamorphosis
30 days	Juvenile