THE DEVELOPMENT OF THE PURPLE SEA URCHIN *STRONGYLOCENTROTUS PURPURATUS*

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INTRODUCTION

The sea urchin embryo has played a key role in the field of developmental biology both due to how easily it can be reared in a laboratory setting and for its transparency. At the turn of the century this transparent egg allowed for scientists to observe the stages of development, including the discovery of key events in fertilization, such as the fusion of the egg and sperm nuclei (Monroy 1986).

Strongylocentrotus purpuratus, the purple sea urchin is typically found intertidally to subtidally, no deeper than 525' (Harbo 1998). Like other echinoids, it is gonochoristic and releases its gametes externally through five aborally located gonophores. Hermaphrodites are rare, in fact Albert Tyler found only 20 specimens out of 100,000 with this characteristic (Geise et al. 1991). Echinoids are deuterostomes, so the eggs of *S. purpuratus* undergo radial cleavage and soon become larval echinopluteus', which settle and hatch out as mobile juvenile sea urchins. This paper discusses the development of *S. purpuratus*, which was observed in a comparative embryology/larval biology course at the Oregon Institute of Marine Biology.

MATERIALS & METHODS

Adult *Strongylocentrotus purpuratus* were collected from the rocky intertidal of South Cove, Cape Arago in Charleston, Oregon. On April 3rd, 2007 2cc of KCL were injected orally into the 5 radial portions of the parastomial membrane. This was done on 4 urchins to ensure a male and female were observed spawning. A monolayer of eggs were pipetted into a fingerbowl of filtered seawater. Two full pipettes of diluted sperm were added to the fingerbowl. Fertilization and cleavage were almost immediate. To avoid polyspermy water was decantered and replaced with fresh filtered sea water. The embryos were maintained in filtered seawater in a glass fingerbowl at 12°C. The culture was cleaned once a day until April 7th, after this it was cleaned every 2-3 days. Cleaning included suctioning most of the water through a 50μm mesh using a pastry baster and adding fresh filtered seawater to the remaining culture in a clean fingerbowl. Once *S. purpuratus* embryos hatched as larvae, they were fed 1 pipette of each *Rhodomonas lens* and *Dunaliella tertiolect* at time of cleaning. The embryos and larvae were observed several times before and after settlement and metamorphosis occurred.

RESULTS

The sperm of *Strongylocentrotus purpuratus* consist of an elongated head, a midpiece, and a flagellum. The head contains a large, conspicuous nucleus and an acrosomal vesicle. The midpiece contains mitochondria. At the base of the midpiece, a ring of lipid-like bodies adds extra energy for sperm longevity (Geise et al. 1991).

The eggs of *Strongylocentrotus purpuratus* are perfectly round, compact cells with a relatively large nucleus. They are yolky and lechithotrophic. Oogonium occurs in

small nests along the basal lamina of the ovarian germinal epithelium. Once ready, they are housed in the lumen of each gonad and spawned.

S. purpuratus undergoes external fertilization. A jelly layer that hydrates in seawater, which encompasses the vitelline layer and cortical layer of cytoplasmic granules, surrounds the eggs. When sperm and egg come in contact, the sperm undergoes exocytosis of the acrosomal granule in the apex of the sperm head and the extension of the acrosomal filament. This granule facilitates the movement of the sperm through the jelly coat to the vitelline layer, where it will fertilize the egg. This process took only seconds after egg and sperm were put together (Fig 1).

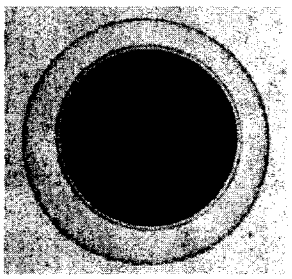

Fig 1: Fertilized egg

Early cleavage in *S. purpuratus* is typical of most deuterostomes, albeit rapid. Minutes after fertilization, the first meridional cleavage occurs forming two cells of equal size (Fig 2). The plane of the first cell division passes through the animal-vegetal axis of the egg. Several hours later, after another meridional cleavage and an equatorial cleavage, there are eight cells of equal size (Fig 3). This produces a 16-cell embryo in which the four vegetal cells divide unequally to form 4 micromeres and 4 macromeres, while the animal half longitudinally divides to consist of 8 mesomeres (Fig 4). The macromeres contribute to the ectoderm and vegetal plate of the blastula as well as

secondary mesenchyme incorporated in the echinopluteus. The micromeres will contribute to the coelomic sacs, and primary mesenchyme cells that will become the larval skeleton. By this point in development it is already difficult to distinguish blastomeres from each other through the microscope, especially at the center of the embryo, because of their three-dimensional nature.

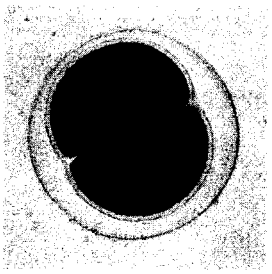
Fig 2: 2-cell embryo

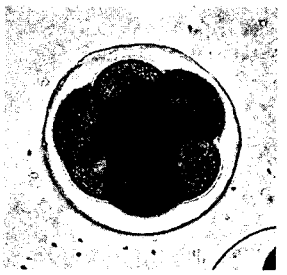
Fig 3: 8-cell embryo

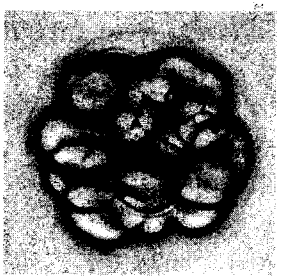
Fig 4: 16-cell

After 24 hours a blastula has formed with an apical tuft at the animal pole and a thickened plate of cells at the vegetal pole. Primary mesenchyme cells are clustered at the vegetal pole and appear to be migrating to the animal pole within the blastocoel (Fig 5).

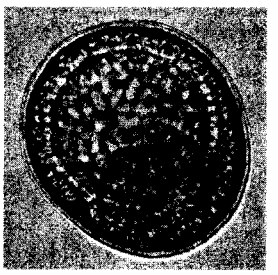
Fig 5: Late blastula

Two days after fertilization the gastrula has been formed through invagination at the vegetal pole, and elongation of the vegetal plate into a tubular archenteron that extends laterally across the blastocoel. Soon after, the gastrula was observed to develop

into a prism shaped larvae with a fully-formed gut. Its skeletal elements such as the dorsal arch, paired anteriolateral rods, and paired postoral rods are in early development, but will eventually support extended arms (Fig 6). The larvae at this point are observed to graze algae off the bottom of the culture dish. Red-pigment cells are also prevalent both dorsally and ventrally.

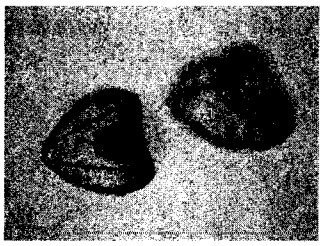

Fig 6: Prism

After 17 days the prism has developed into a 4-armed echinopluteus that moves quickly (Fig 7). It is not until 24 days after fertilization that a 6-armed echinopluteus is observed (Fig 8) and still 27 days after fertilization until an 8-armed echinopluteus is swimming around the culture.

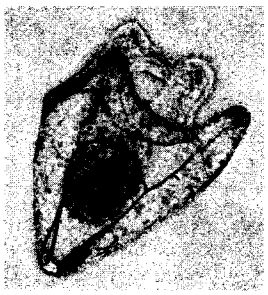

Fig 7: 4-armed echinopluteus

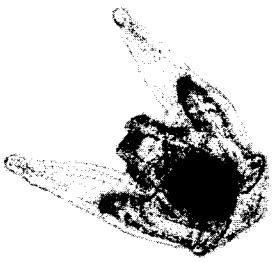

Fig 8: 6-armed echinopluteus

30 days after fertilization juvenile rudiment growth is apparent within an invaginated vestibule at the anterior end. The rudiment eventually develops to encompass a large portion of the anterior end, filling it almost in entirety (Fig 9). I observed flared arms in response to substratum indicating settlement, but it wasn't until 30 days after fertilization that metamorphosis was observed to be complete. Juvenile *S. purpuratus* were observed to be crawling independently with tube feet and spines, grazing the bottom of the culture dish (Fig 10). A developmental timetable for the individuals I observed in the laboratory is displayed in Table 1.

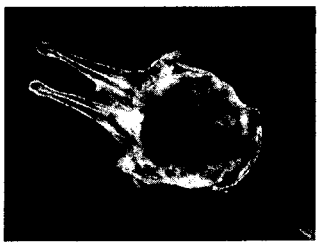

Fig 9: Large juvenile rudiment

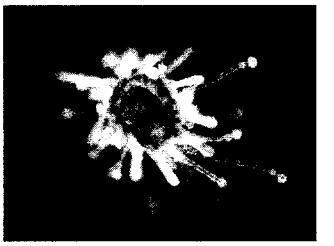

Fig 10: Settled juvenile

Table 1: Developmental timetable of *Strongylocentrotus purpuratus*. Fertilized at 10am on 4/3/2007.

TIME AFTER FERTILIZATION	STAGE
3 hours	2-cell
6 hours	4-cell and 8-cell
10 hours	16-cell and 32-cell
1 day	Late blastula.
2 days	Prism
4 days	Early Echinopluteus
17 days	4-armed Echinopluteus
24 days	6-armed Echinopluteus
28 days	8-armed Echinopluteus w/ visible rudiment
30 days	Late Metamorphosis
31 days	Juvenile

Discussion

Development after metamorphosis is an important determinant in the success of natural recruitment rates within a population. Unfortunately a small lab culture was not enough time to observe these patterns, but Miller & Emlet describe the significance of such events developmentally. They suggest that the formation of coronal pedicellariae, sphaeridia, a functional gut, test plates, spines, and podia are all new features that develop during the few weeks post-metamorphosis (1999). The development of such characteristics can determine a sea urchin's success as a tiny juvenile because it occurs prior to feeding. The rate at which these features develop has been traced to food rations and temperature during larval development. Those fed more during larval development generally have a shorter larval period and are larger at metamorphosis (Geise et al. 1991).

In the field however these settlement patterns do not determine survival. High mortality rates have been observed and large recruitments generally only happen once every 30 years (Ebert & Russel 1988). Ebert and Russel suggest that the differences between size frequency of settlement and recruitment in the field correlate with major topographic features such as capes and headlands (1988). These areas have upwelling and cold water plumes that show size frequencies that indicate low recruitment rates. Upwelling and jet formation coincides with the time in which larval *S. purpuratus* has reached competency.

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*Photos courtesy of Embryology class.