# Embriogenesis and Larval Stages of *Dendraster excentricus*

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## INTRODUCTION

The sand dollar *Dendraster excentricus* (Class Echinoidea, Order Clypeasteroida, Family Dendrasteridae) is abundant in localized aggregations on sandy or sandy –mud bottoms, low intertidal to subtidal zones in sheltered bays: subtidal to 40 m (rarely to 90 m) in open coastal areas. Its distribution is from southeastern Alaska to central west coast of Baja California, with unconfirmed records from the Gulf of California (Morris et al.1992). Individuals reach test sizes of 7.5 to 8 cm (Morris et al. 1992, Kozloff 1993) with a pale gray-lavender, medium brown, red-brown, or dark purplish black color. The aboral surface (upper side) is distinguished by a flowerlike pattern, with the madreporite at its center. The genital pores are located on this side or surface, on the interambulacral zone (Morris et al. 1992).

Sexes are separate, but occasional hermaphrodites are found (Morris et al. 1992). No external morphological differences can be observed to separate the two sexes (Strathmann 1987). Individuals reproduce seasonally for several years, but major spawning occurs from May through July (Pearse & Cameron 1991, Morris et al. 1992). The time and period of spawning may depend on the location and size of each individual. Previous studies have shown that most individuals reach sexual maturity by four years of age. Individuals can be aged by counting growth rings on the plates of the test. Average mature females produced 356,000-379,000 eggs per year (Morris et al. 1992). Eggs and sperm are released into the water column, where fertilization occurs (Kozloff 1993). Sperm are usually milky white in color, and are considered to be the primitive flagellated

type with a large acrosome at the tip of a conical head (Summers et al. 1975). Eggs are pale orange, 110-125μm in diameter and covered by a 60-80μm thick jelly coat. The jelly has small peripheral red granules, which are PIGMENT cells (Chia & Atwood 1982). According to previous studies, the jelly deters sand dollar from feeding on their own eggs (though later they eat their own larvae without hesitation).

Embriogenesis and larval development has been carried out entirely in the laboratory. According to Strathman (1987), at temperatures fluctuating from 7 to 17°C, the period from fertilization through metamorphosis and settlement lasted 68-162 days. Once fertilization has occurred, the fertilization envelope increases in size to avoid polyspermy (Emlet et al. 2002). Two, four, eight and sixteen cell embryos are produced usually within 24 hours after fertilization has occurred. A few hours later, the embryo hatches, emerging as a blastula, which is characterized by the presence of the blastocoel in the middle of the body. One to two days later, this blastula will have metamorphosed into a gastrula, with the formation of the archaenteron or primitive gut and the blastopore. The blastopore will become the anus of the future larvae. This primitive gut is produced by an apical constriction of the vegetal or lower cells, which produces an invagination. The top of the invagination is then pulled closer to the animal pole by elongations from the secondary mesenchyme cells, while the primary mesenchyme cells produce the triradiate spicules that will provide the larval "skeleton". Seventeen to 70 hours later, this gut will reach the outer layer producing a second pore, which will become the mouth of the larvae. This larval stage is called a prism. After this stage the embryo will develop two arms transforming itself into an echinopluteus larva. This is followed by the development of arms, until it reaches 8 arms all together. The arms of the echino pluteus

are supported by calcium carbonate spicules that may be simple or fenestrated rods (Wray 1992). The echinopluteus stage takes approximately 24 to 64 hours to reach and lasts for two to 9 days. After this the larva develops an echinus or juvenile rudiment, which as can be inferred will become the juvenile. Detailed time lines of embryogenesis and larval development at different temperature are presented in Table 1 (Strathmann 1987).

Previous studies were mostly done in laboratories at a stable temperature. This study attempted to culture *D. excentricus* larvae in water temperatures that approximate actual environmental conditions found in the rocky intertidal.

## METHODS

Adult individuals were collected by hand from the North Spit, which is a sandy protected beach located in the northern area of the Coos river mouth. Adults were transported to the lab and placed in a flow-through sea table for several days. Afterwards, since the sex of the specimens cannot be determined externally with any certainty, several adult individuals were injected with 1-2 ml of 0.5 M Potassium Chloride (KCL) to induce spawning (Strathman 1987). This injection was delivered with a 23-gauge hypodermic needle and was inserted through the perioral membrane and injected in the coelom. Individuals were than placed upside down on top of small glass dishes with enough cold filtered seawater to cover the gonopores to collect the gametes. Once the gametes were collected from both sexes, enough eggs were pipetted into a separate glass dish with cold filtered seawater to cover the dish bottom with a monolayer. To increase the percentages of fertilization an excess of sperm was pipetted into the dish with eggs. Once gametes

from both sexes were pipetted in the glass dish, cultures were placed in the sea water table. To prevent polyspermy, excess sperm and seawater were removed several minutes after fertilization had taken place. The glass dish and the filtered seawater of the cultures were changed every day. The filtered seawater was removed using a piece of 53 μm-nylon net glued to the bottom of a small plastic dish. The dish was dipped into the seawater and the water was extracted from within the plastic dish with a "turkey baster" or large pipette, leaving only a small fraction of the filtered seawater and embryos in the glass dish. This small fraction of seawater was then placed in a clean glass dish with clean filtered seawater. Once the larvae where able to feed, approximately 1 ml of concentrated *Rhodemonas lens* and *Dunaliella tertiolecta*, which are two micro algae species, were daily pipetted into the culture. The culture was observed daily with the use of a dissecting and compound microscope. Finally, photographs of the culture were taken, and temperature (°C) and salinity of the seawater were measured periodically.

## RESULTS

Temperature of the seawater varied from 12 to 15 °C, and the salinity was approximately 33 units throughout the culturing of the larvae. The eggs spawned were pale orange and the sperm milky white. The sperm fertilized the egg in less than one hour. Percentage of fertilization was higher than 50%. Fertilization was confirmed when the fertilization envelope was observed (Fig. 1a). The egg presented small peripheral red granule-like cells, which were present up to the Blastula stage. First division began two hours after fertilization had occurred (Fig. 1b). Four, eight and 16-cell stages were reached four, 12 and 18 hours after fertilization had occurred, respectively (Fig. 1c, d, e).

Blastula stage was reached 24 hours after fertilization. Early and late gastrula stages were reached 36 and 48 hours (respectively) after fertilization had occurred, with prism stage occurring approximately two hours later. Early Echinopluteus, two to four arms, was reached approximately 72 hours after fertilization had occurred (Fig. 1f). Late Echinopluteus, eight arms, was reached eight days after fertilization had occurred (Fig. 1g). The juvenile rudiment was first observed 14 days after fertilization had occurred (Fig. 1h). Finally metamorphosis and settlement occurred 26 days after fertilization had occurred. A detailed list of times in which division and metamorphosis occurred is presented in Table 1.

**DISCUSSION**

Similar embryological and larval stages observed in previous studies were observed in this study. However, the time during which each stage occurred varied greatly. This was obvious when comparing to the previous studies that worked at temperatures between 12 and 15°C (R. Langelan pers. comm., L. McEdward pers. comm., M. Strathmann unpubl, Whiteley & Whiteley 1972). In this study, it took the larvae approximately 50 hours to reach the prism stage, and 26 days to settle. While in previous study it took the larvae between 20 and 46 hours to reach the prism stage and approximately 11 days to settle. The difference in the timing of events became significant once the larvae reached the 2-armed echinopluteus stage. The reasons for the difference in the time of development could have been several. These reasons could have been that the above-mentioned previous studies raised the larvae at a stable temperature or raised echinoid hybrids could have had an effect on the timing of events. These stable temperatures or echinoid hybrids,

however, might not be common in the rocky intertidal, and thus the time tables reached in the present study should be considered more realistic.

## REFERENCES


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**Table 1.** Times of division and metamorphosis of *Dendraster excentricus* found in previous and present studies. Past study data taken from Strathmann 1987. Data obtained from: Ozaki 1965; R. Langelean pers. comm. (10-11°C) R. Langelan pers. Comm; L. McEdward pers. Comm (11-12°C), M. Strathmann unpubl (13-14°C), Whiteley & Whiteley 1972 (15°C), and L. McEdward pers. Comm (17 and 22°C)

| | Past Studies | | | | | | Present Study |
|---|---|---|---|---|---|---|---|
| Stage Reached | 10-11°C | 11-12°C | 13-14°C | 15°C | 17°C | 22°C | 12-15°C |
| Fertilization | 0 hrs | 0 hrs | 0 hrs | 0 hrs | 0 hrs | 0 hrs | 0 hrs |
| Two cells | 2 hrs | 2 hrs | | | | | 2-3 hrs |
| Four cells | 4 hrs | 3 hrs | 2 hrs | | | | 4-6 hrs |
| Eight cells | 5.5 hrs | | | | | | 12-14 hrs |
| Sixteen cells | 7 hrs | | 3 hrs | | | | 18-21 hrs |
| Blastula | 36 hrs | 27-29 hrs | | 19 hrs | | | 24-30 hrs |
| Early Gastrula | 38-42 hrs | 32-35 hrs | 20 hrs | | | | 36-41 hrs |
| Late Gastrula | | | | | | | 42-48 hrs |
| Prism stage | 55-60 hrs | 40-46 hrs | 32 hrs | 31-33 hrs | 24.5 hrs | 17 hrs | 48-50 hrs |
| Echinopluteus, 2-armed | 65 hrs | 57 hrs | | 40 hrs | 35.5 hrs | 23.5 hrs | 72 hrs |
| Echinopluteus, 4-armed | 72 hrs | 97 hrs | | 47 hrs | 63 hrs | 47.5 hrs | 96 hrs |
| Echinopluteus, 6-armed | | 199hrs | 144 hrs | | 107 hrs | 63 hrs | 7 days |
| Echinopluteus, 8-armed | | | | | | | 8 days |
| Echinopluteus with juvenile rudiment | | | 11 days | | | | 14 days |
| Settlement | | | | | | | 26 days |

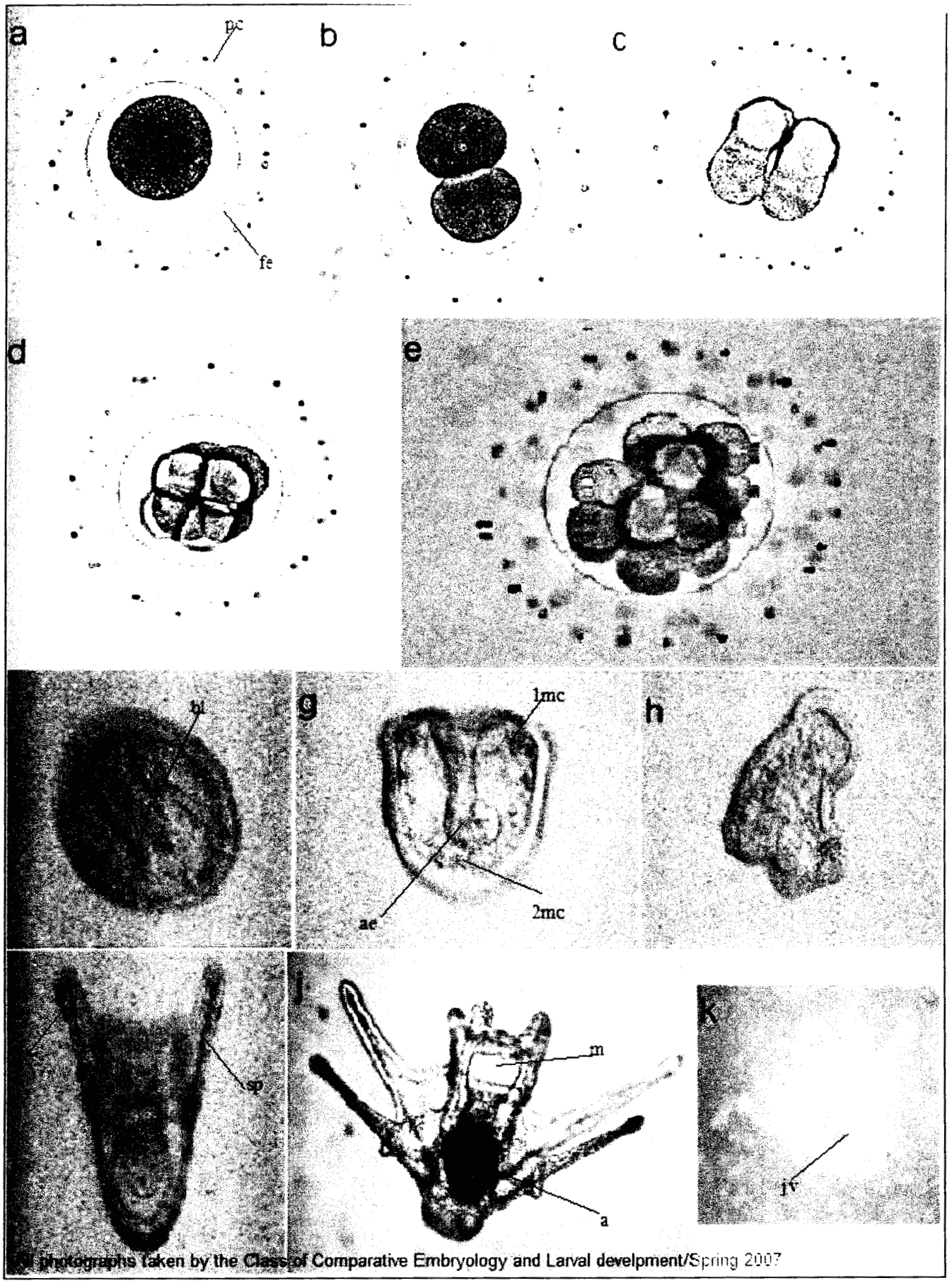


**Fig. 1.** *Dendraster excentricus* stages: (a) fertilized egg. (b) 2-cell stage. (c) 4-cell stage. (d) 8-cell stage. (e) 16-cell stage. (e) blastula stage. (f) early gastrula stage. (h) late blastula stage. (I) 4-armed echinopluteus. (j) 8-armed echinopluteus. (k) echinopluteus with juvenile rudiment. Abbreviations: anus (a). Archaenteron (ae). arm (ar). fertilization envelope (fe). juvenile rudiment (jr). mouth (m). peripheral cells (pc). spicule (sp). primary mesenchyme cells (1mc). secondary mesenchyme cells (2mc).