Early Development of the Deep-Sea Echinoid *Cidaris blakei* (A. Aggasiz, 1878)

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Introduction

The order of echinoids known as Cidaroida has been relatively well-studied, as an example of both shallow and deep-water fauna extending from about 10°N to the far south of the Antarctic Ocean (Emlet 1987). The extant population of Cidaroids is comprised of at least 27 genera and 148 species and subspecies (Mortensen 1938). In the time since Mortensen's tabulations, a number of new Cidaroid species have been described, increasing the known number of genera and species (e.g. Holland 1967, Tyler & Gage 1984). Examples of Cidaroids are found in the fossil record as far back as the Cretaceous, and they are widely considered to be living representatives of "primitive" urchins and evolutionary precursors to modern sea urchins, or "euechinoids" (Schroeder 1981).

Although the development of Cidaroids in general is well documented, the embryology of the deepwater urchin *Cidaris blakei* (A.Agassiz, 1878) has not been described. In his study of the development of the shallow-water Cidaroid *Eucidaris tribuloides*, Schroeder (1981) noted that there were major differences between traditional Euechinoid development and that of *E. tribuloides*. Most notably, he cites a lack of primary mesenchyme (which gives rise to the larval skeleton in other echinoids), a virtual absence of a hyaline layer or apical tuft, and small, irregular numbers of micromeres. In a detailed description of larval form and metamorphosis of *Eucidaris thouarsi*, Emlet (1988) described differences between rudiment development in Cidaroids and Euechinoids. There is great variation in embryonic development among genera of Cidaroids. Additional descriptions of Cidaroid species will allow us to construct a more complete phylogeny, and therefore better infer relationships between this ancient lineage and the Euechinoids.

In addition to their value as a study organism for the echinoid ancestral condition, deep water Cidaroids also provide mechanism for a foray into the reproductive biology of deep-sea echinoids. Describing a timeline for development will help us to understand larval dispersal and position of larvae within the water column (Young & Cameron 1989).

Materials and Methods

Adult specimens of the deepwater urchin, *Cidaris blakei*, were collected using the manned submersible Johnson Sea-Link II (Harbor Branch Oceanographic Research Institute, Fort Pierce, FL, U.S.A.). The organisms were collected at a depths ranging of 800-1400 feet. The specimens were immediately transferred to an on-ship cold room. Spawning was induced using intracoelomic injections of 0.55M KCl. After fertilization, cultures were kept in the cold room with an ambient temperature of 13°C (at the time of spawning, the cold room temperature was 8°C. A day later, we increased the temperature to 13°C, and maintained it for the duration). Cultures were cleaned every other day using

Nytex mesh and 0.45 um filtered seawater. Samples from the cultures were photographed at regular intervals, and notes were taken describing development.

Twelve days after fertilization, cultures were shipped home to Oregon where they were divided into two cultures. One culture is kept at the ambient (Oregon) seawater temperature of 11°C and the other is kept in an incubator at 16°C.

Results

The unfertilized ova of *C. blakei* have a diameter of 150μm (Fig. 1a). First cleavage of the embryos was nine hours after fertilization (Fig. 1b). When development seemed arrested at the two-cell stage, we raised the cold-room temperature to 13°C and the embryos were at morula stage 24 hours post-fertilization (Fig. 1c).

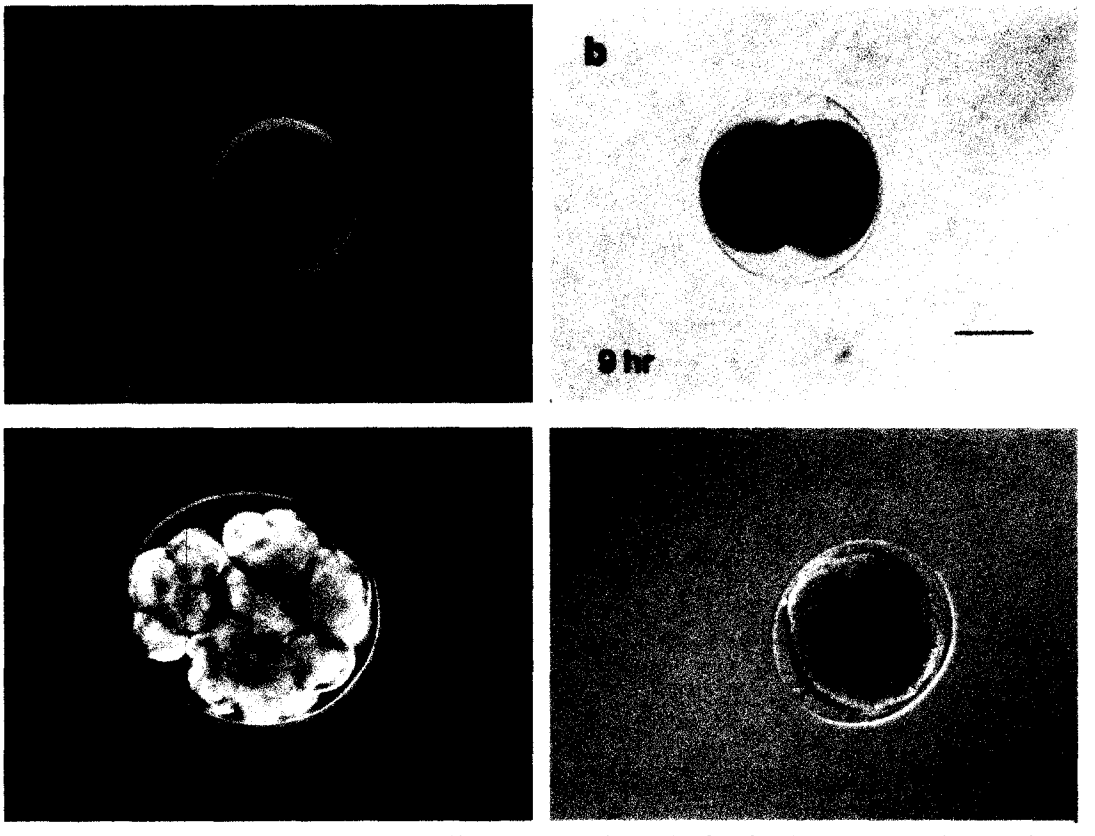

Fig. 1 *An unfertilized egg (a), two-cell stage is reached 9 h after fertilization (b). The morula is evident after 28 h, and the macromeres still have a characteristic "disorganized" appearance (c), unhatched blastula (d)*

Morula stage, and the multi-cellular stages just prior to it, showed extremely disorganized macromeres. It was thought at the time that the cultures were abnormal. Because the cultures contained swimming embryos, they were not thrown away. Two days later hatched and un-hatched blastulae were observed. The blastulae appeared normal, with a distinctive and concentric blastocoel (Fig. 1d, 2a).

Fig. 2 *Three days after fertilization, there are hatched blastulae in culture (a). A gastrula with visible archenteron 7 days after fertilizaion.*

The gastrula stage, with a well-developed and visible archenteron, was present 7 days after fertilization (Fig. 2b). At 8 days, there is clearly formation of the larval skeleton, as well as a defined apical tuft (Fig. 3a,4). The four-armed pluteus showed clearly fenestrated post-oral arms (Fig. 3d). Currently, 28 days after fertilization, the larvae are at the six-arm plutei stage. Of our two current cultures of *C. blakei* that we have in Oregon (11°C and 16°C, respectively), the 16°C initially showed a faster rate of development, but they now appear to be the same.

Discussion

In general, a small egg diameter is indicative of a lack of parental investment, (Emlet et al. 1987). The 150 μm egg size of *C. blakei* implies planktotrophic development.

Delayed development at the time of earliest cleavage can probably be attributed to the low cold room temperature. When ambient temperature of the cold-room was increased, further cleavage in the embryos occurred relatively rapidly. The current culture being kept in a 16°C incubator also showed an initial increase of developmental rate. The deceleration of this rate at the six-arm pluteus stage could be indicative of stage-specific thermal tolerances, as described by Young et al. (1998). In their study, they tested the thermal tolerances of embryos and larvae of two deep-sea echinoids, *Archaeopneustes hystrix* and *Stylocidarais lineata*, and used their data to predict depths at which development is unlikely to occur. It may be that all temperatures at which these particular cultures of *C. blakei* were kept is too cold for normal development. We do not know where in the water column the planktotrophic larvae of *C. blakei* develop, but further experimentation with temperature tolerances may give us more clues.

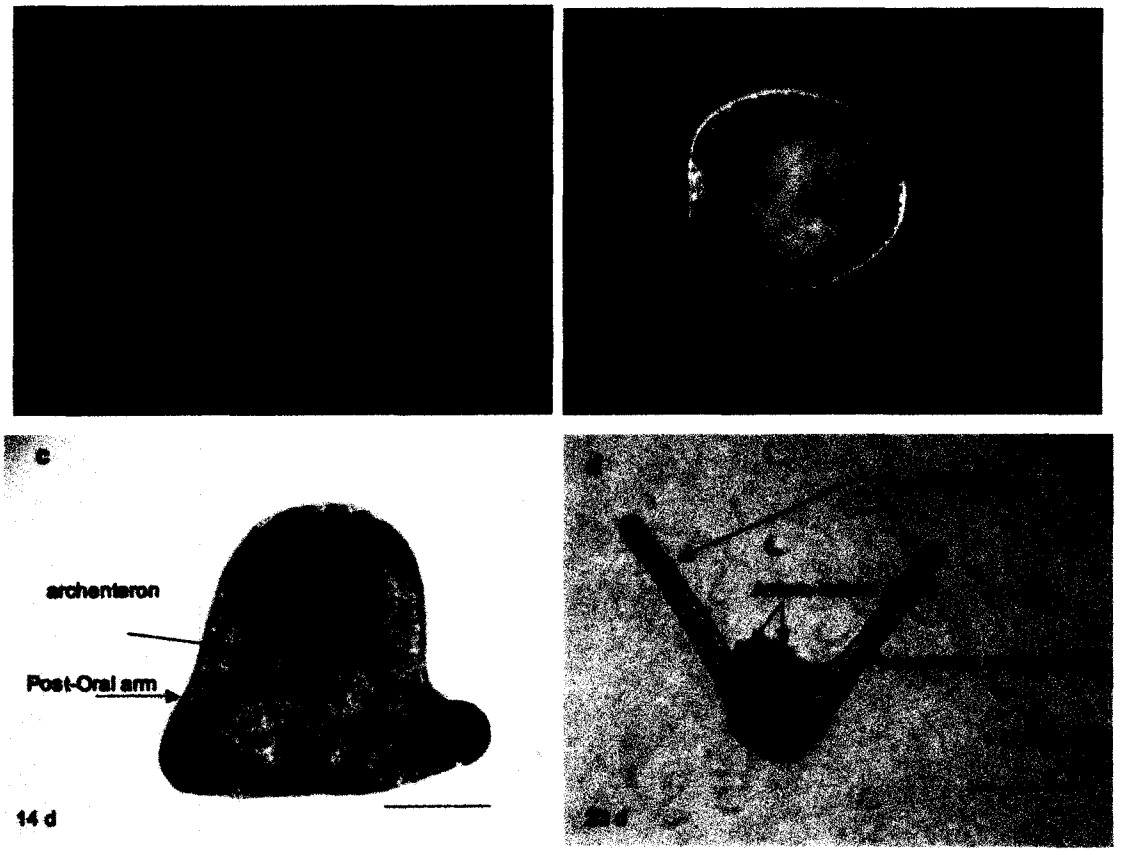

Fig. 3 *Clear development of the larval skeleton after 8 days (a), there is an apical tuft during the gastrula stage of C. blakei (b), prism stage (c), early four-arm pluteus (d)*

The opacity of the *C. blakei* egg and embryos made it difficult to compare some aspects of development with the observations of *Eucidaris tribuloides* made by Schroeder (1981). We were unable to determine the presence of primary mesenchyme, or to compare the number and organization of micromeres. It is important to note that the disorganized macromeres that almost caused us to dispose of our cultures were well described by Schroeder, who attributes it to "the virtual absence of a hyaline layer." An examination of *C. blakei* embryos with a scanning electron microscope (SEM) may allow us to better make these comparisons. One key difference between the embryos of *E. tribuloides* and *C. blakei* is the presence of an apical tuft during the gastrula stage of *C. blakei*, which is absent in *E. tribuloides*.

A more thorough understanding of the development of *C. blakei* and other Cidaroids offers a great potential benefit both to our understanding of evolutionary processes and of deep-sea larval ecology.

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