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Early Development of the Sea Cucumber *Holothuria leucospilota*

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Abstract: This study presents preliminary results on the early development and growth of the sea cucumber *Holothuria leucospilota* in the Bostane equatic ecosystem on the coast of Persian Gulf. For this purpose, spawning was induced by thermal stimulation after fertilization, embryos were incubated under optimal conditions (salinity between 38-40 and temperature 33 degrees) until reached the late uricholaria stage. In order to study the developmental stages and the timing of each stage including cleavage, morula, blastula, gastrula, early auricularia, auricularia, late auricularia the embryos were studied by light microscopic studies. Observations showed that after 120 h from fertilization time, the embryos developed to late auricularia stage.

Key words: *Holothuria leucospilota*, developmental stages, timing schedule, Persian Gulf, embryos, growth

INTRODUCTION

The sea cucumber or otherwise known as the *Holothurian* is a very important part of aquatic life; this invertebrate is classified under Echinoderms phylum. Echinoderm comprises 5 classes, Asteroidea (sea stars), Ophiuroidea (Brittle stars), Crinoidea (sea feather), Echinoidea (sea urchins) and Holothuroidea (sea cucumbers) (Abdel-Razek *et al*., 2005). Sea cucumbers are long, soft-bodied, marine invertebrates in the class Holothuroidea. Their skeleton has been reduced to small calcarious pieces (ossicles) in the body wall which have distinct species-specifc shapes. Male and female sexes are usually separate (Despalotovic *et al*., 2004) but there have been a few reports of hermaphrodites in some species. Sea cucumber can reproduce sexually and asexually (Uthicke, 1997; Abdel-Razek *et al*., 2005). Environmental conditions play a main role in invertebrate larval development (Asha and Muthiah, 2005). There is much variety among the habitats in which sea cucumbers reside. Some species inhabit quiet, still waters while some others are found in areas that are constantly swept by tidal currents. Some holothuroids are tropical reef dwellers and others live in temperate or polar waters (Hamel and Mercier, 1995). Even the depth at which these animals can be found varies greatly. Many species live within the intertidal zone but a few sea cucumbers live deep in the ocean, some among hydrothermal vents (Smirnov *et al*., 2000). Regardless of the environment in which they are found, sea cucumbers have become highly adapted to life in that environment. At present, nearly 1400 species of sea cucumbers are known from the seas in the world oceans of which nearly 30 species are edible (James, 2001). The holothurian, *Holothuria leucospilota* is a common component of fauna of the water of southern of Iran and it is a deposit-feeding sea cucumber that is mainly found on reefs and sandy bottoms along the northern coast of the Persian Gulf. This study with aim to assess the early developmental stages and determination of the timing schedule of the sea cucumber *Holothuria leucospilota* a new step will be to perform other biological research in this field.

MATERIALS AND METHODS

Adult *Holothuria leucospilota* were collected from the rocky intertidal at the research station (31°26 latitude North, 50°54 Eastern longitude) in the coastal Western Persian Gulf, bostaneh area on April-September 2009. Then, samples were shipped to the Persian Gulf. After adult transfer to lab to eliminate stress, the samples were stored inside tanks containing sea water (salinity between 38-40 and temperature 33 degrees) with appropriate Aeration for 24 h. Spawning induced through thermal stimulation so that the first samples were placed in water temperature 5°C for 1 h and then were transferred to the water temperature over 30°C and therefore fertilization occurs inside the spawning tank. After spawning, sea cucumbers are removed from the spawning tanks and the fertilized eggs are collected and then the fertilized eggs diluted to a density of 200 eggs mL^{-1}. After past the enough time for first cleavage, the embryos were added to solution to reach the final concentration of 20 embryo mL^{-1}. After dilution embryos were incubated

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under optimal conditions until reached the uricholaria stage. In order to the timing determination of each developmental stages (first cleavage, morula, blastula, gastrula, early uricholaria, uricholaria and late uricholaria larva), we were tested the samples and after observe the obvious characteristics of each stage, the timing point was recorded. To reduce the error rate for the results, experiments were repeated with 3 replicates.

RESULTS AND DISCUSSION

Investigation of developmental stages in *Holothuria leucospilota*: When sperm and mature eggs are placed near each other, sperm penetrated to the oocytes and fertilization occurred. Obvious characteristic of fertilization is the formation of membrane around the fertilized oocytes. The 1st cleavage furrow passed through the animal-vegetal axis and forming two cells of equal size while that the second cleavage followed the first one with the cleavage plane perpendicular to that of the first division (Fig. 1 and 2).

The 3rd and the 4th cleavages, planes of which were equatorial and meridional, respectively gave rise ultimately to 16 equal blastomeres (Fig. 3). Up to the 7th cleavage each blastomere divides, rather synchronously. After the 7th cleavage the divisions became asynchronous and the intervals were prolonged.

The surface of the blastula was not wrinkled (Fig. 4). About 15 h after fertilization, invagination at the vegetal pole was initiated as gastrulation proceeded,

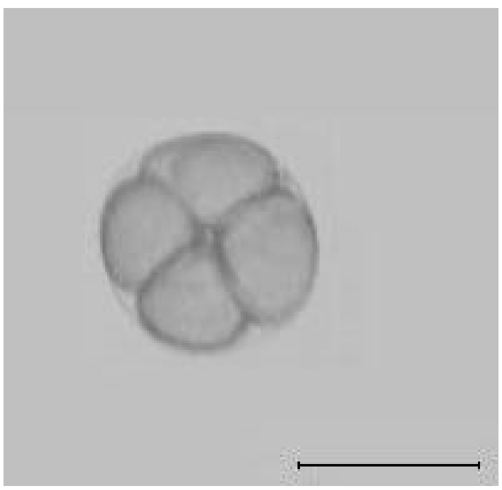

Fig. 1: Four-cell embryo (100 μm)

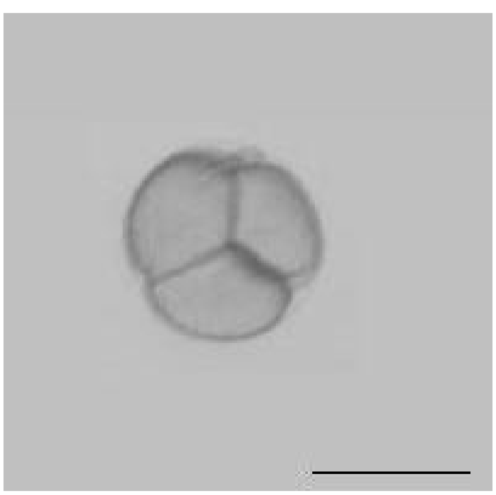

Fig. 2: Four-cell embryo

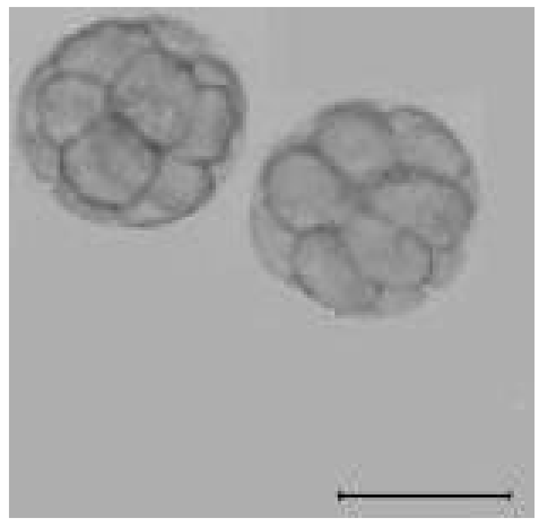

Fig. 3: Sixteen-cell embryo (100 μm)

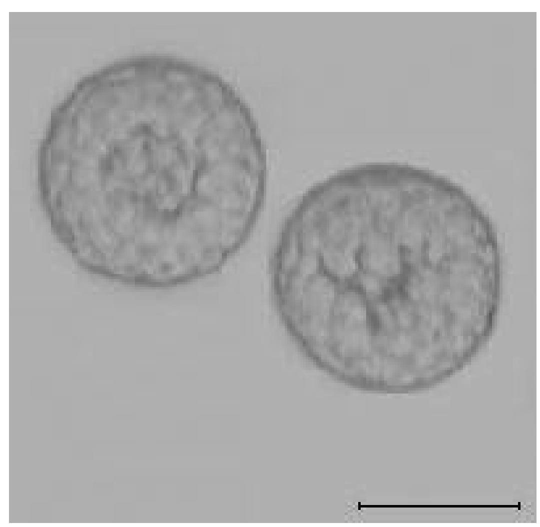

Fig. 4: Blastula stage (100 μm)

Fig. 5: Gastrula stage (100 μm)

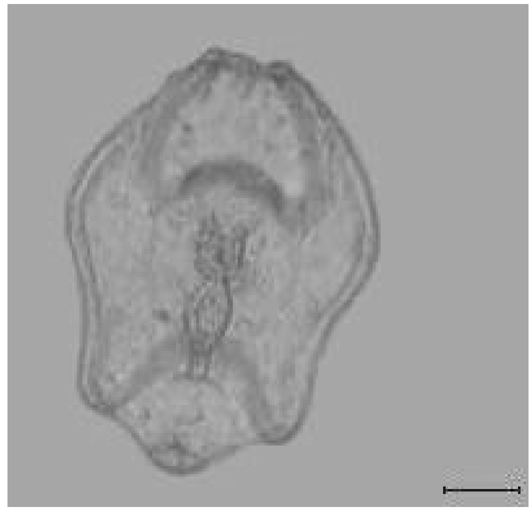

Fig. 7: Auricularia larvae

Fig. 6: Early auricularia larvae (100 μm)

Fig. 8: Late auricularia larvae

mesenchymal cells began migrating from the archenteron tip into the blastocoel and at finally after 24 h the embryos developed to the gastrula (Fig. 5).The stomodeum at the ventral surface was attached by the bent tip of the archenteron and cells at the tip attached to the median dorsal gastrula wall and formed the dorsal pore. At 48 h, the larvae were at the early auricularia stage (Fig. 6). About 96 h after fertilization, early auricularia has developed into a auricularia and at this time, it can observe the position of the anus shifted to the ventral side and appeared a small birefringent granule that is a calcareous structure. At about 52 h, a tubular pore canal connected the dorsal pore with the coelom at the position of the oesophagus and at 96 h, the larvae developed to the typical auricularia (Fig. 7). The gut was divided into three parts that is including esophagus, stomach and intestine and then began rhythmic contraction of the stomach. These larvae swam and finally at 120 h, the larvae developed to the late auricularia (Fig. 8). Bars indicate 100 μm. Reproduction characteristic is one of the most important, aspects in dealing with the biology of sea cucumber.

This process has been observed in the environment (natural spawning) and also in the laboratory (Ocana and Sanchez-Tocino, 2005). Environmental factors such as Temperature, food availability, photoperiod and lunar cycle play a main role in controlling spawning (Morgan, 2000). Water temperature is often cited as a primary factor

Table 1: Developmental timing of *Holothuria leucospilota*

Developmental stages	Time after fertilization (h)
First cleavage	60
Blastula	6
Gastrula	24
Early uricholaria	48
Uricholaria	96
Late uricholaria	120

that influences spawning in holothuroids (Tanaka, 1958; Morgan, 2000; Battaglene *et al*., 2002; Giraspy and Ivy, 2005). In some investigation similar to this study, spawning was induced by thermal stress. Some others of stress can spawning induce in the sea cucumber including the stress comes from the collection and transport them (Battaglene *et al*., 2002, *Schizochytrium* sp.). In most cases similar to this study, males released sperm first and for >30 min and followed by the males, the females release their eggs for less than a minute. This shows that males are readily to induce to spawn and females are stimulated through the presence of sperm (Battaglene *et al*., 2002; Mceuen, 1988).

Based on the timing chart obtained from developmental stages of *Isostichopus fuscus* (Table 1), 120 h after fertilization, the embryos developed to the late uricholaria larvae stage. The other researchers have been reported various developmental stages with different timing. For example, Giraspy and Ivy showed that in *H. scabra versicolor* after 9 h passed from fertilization, the embryos become to the blastula and developed to the gastrula then after 2 days. After 3 days, 12 h embryos developed to the early uricholaria and 8 days developed to uricholaria and finally, late uricholaria larvae developed after 14 days.

In the present study, *Isostichopus fuscus* larvae were fed with *Chaetoceros calcitrans* and *Isochrysis galbana* at different developmental stages. In an investigation fed *scabra versicolor* larvae with *Rhodomonas salina*, *Chaetoceros calcitrans*, *C. mulleri*, *Tetraselmis chui*, *Isochrysis galbana* and *Pavlova lutheri* at different developmental stages. In other studies that conducted by Battaglene *et al*. (2002) *H. scabra larvae* fed with microalgal species such as *Rhodomonas salina*, *Chaetoceros muelleri*, *C. calcitrans*, *P. salinai* and *Tetraselmis chuii* while James (2001) used mixed cultures of *Chaetoceros* sp. and *Isochrysis galbana* for on *H. scabra*. Density of eggs in study is 20 egg mL^{-1} for *Isostichopus fuscus* While this density in a study conducted by James has been suggested of 0.5-1 egg mL^{-1}. Also in another study indicated the suitable larval density as 1 egg mL^{-1}.

CONCLUSION

The importance of detailed studies on Holothuroids is necessary due to their important role in the conservation of the marine environment. They are economically of important value and used as food also they are important members of the benthic communities and responsible for causing significant changes in the composition of the sea floor. They have also medical importance include treating weakness, impotence, debility of the aged, constipation due to intestinal dryness and frequent urination (Abdel-Razek *et al*., 2005).

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