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Developmental stages of *Echinometra mathaei* (Blainville, 1825) reared under laboratory conditions from Karachi coast (northern Arabian Sea)

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Citation: Ahmed Q., Ali Q.M., Shaikh I., Ghory F. S., Bat L., Mubarak S., Qazi H., Baloch H. (2023) Developmental stages of Echinometramathaei (Blainville, 1825) (Echinodermata-Echinoidea) Reared under laboratory conditions from Karachi coast (northern Arabian Sea), J. Mater. Environ. Sci., 14(5), 626-634. Abstract: The sea urchins (Echinoidea) are keystone species in an ecosystem with the simplest form of development. The mature specimens of *Echinometra mathaei* (Blainville, 1825) were collected from Cape Monze on 17th March and kept in the laboratory. Spawning occurred after same day, at room temperature 28°C in filtered seawater with a salinity of 37 ppt and a pH of 8. After fertilization the developmental stages of embryos and the timing of each stage including cleavage, morula, blastula, gastrula, prism and pluteus larvae were studied under the microscope. This study represents the first successful analysis on embryonic, larval, and pre- juvenile development of *E. mathaei*. Understanding of ontogeny and life-history strategies will make it easier to develop techniques for breeding, seed production, and culture of sea urchins in captivity conditions also the findings would greatly be helpful for future biologist.

Keywords: Echinometra mathaei; developmental stages; Karachi coast (northern Arabian sea);

1. Introduction

Sea urchins belongs to class Echinoidea, they are small, spiny, and globular animals also known as sea hedgehogs due to their superficial resemblance to the spiny mammalian species. sea-urchin grazing exerts a major control on the structure and functioning of the coastal benthic ecosystems (Hill *et al.*, 2003; Tuya *et al.*, 2004a; Hereu Fina, 2007; Eklof *et al.*, 2008; Gianni *et al.*, 2017; Yorke *et al.*, 2019). They are also modifying agents for erect algae community structure is well known (Watanabe and Harrold, 1991; Filbee-Dexter and Scheibling, 2014; Miller *et al.*, 2018). Although there are other herbivores recognized as relevant grazing agents, their functional activity is lower or insignificant compared to sea urchins (Cordeiro *et al.*, 2020).

The biodiversity of sea urchins is ecologically important since they retain levels of heavy metals in their bodies (Freeman, 2004). The sea urchin aquaculture is mainly based on the production of

marketable gonads, which are valuable seafood product in Asian and European markets (Klumppetal., 1993; Buitrago *et al.*, 2005; Tsuchiya *et al.*, 2009).

Echinometramathaei (Blainville, 1825) is one of the world's most abundant sea urchin distributed both in tropical and sub-tropical zones (Mortensen, 1943; Clark, 1973). The feeding behavior, ecology, and growth lines of sea urchins, especially *E mathaei*, were studied by Kelso, 1971, Ebert 1975, 1982, 1988; Muthiga, 1996; Lawrence and Bazhin, 1998; Drummond, 2020. Most of echinoderms have a planktonic larvae stage and an adult benthic stage. The planktonic period can last from hours to months and its duration differs between species. Most echinoids are free spawners and produce large numbers of small, yolk-poor eggs that develop into planktonic, obligate planktotrophic larvae known as **echinoplutei** (McEdward and Miner, 2001). After the planktonic stages, larvae seek a suitable substrate to settle on and undergo metamorphosis. The development from post-larvae to juvenile and adult and consequent sexual maturation will give start to a new cycle. The life cycle of the sea urchin can be divided into six more-or-less distinct phases. (1) The fertilized egg, (2) development through blastula and gastrula to pluteus, at which time egg nutrients are usually consumed, (3) growth and development of the feeding pluteus to a mature larva, (4) development of the embryonic urchin inside the growing larva, (5) metamorphosis, and (6) growth of the young urchin to a reproductive adult. Most other Echinoderm groups develop in a similar way.

No data available on ecology, reproductive biology, and developmental stages on *E.mathaei* from Pakistan except (Siddique and Ayub, 2019, 2021) who have studied reproduction and population dynamics of the *E.mathaei*, thus this is first attempt to report the developmental stages (embryonic, larval, and pre-juvenile) of *E.mathaei* under controlled laboratory conditions.

2. Methodology

2.1 Sample collection

Six adult specimens of *E. mathaei* were collected from the rocky beach of Cape Monze (Lat. $24^{\circ}50'02"$ N Long. $66^{\circ}39'24"E$) (Figure 1) on 17^{th} March, 2022 from intertidal zone during low tide (0.2m) with a salinity of 37 (ppt), pH of 7.9 and water temperature (27 °C). The urchins were taken in plastic containers and filled with seawater. The specimens were immediately transported to laboratory and shifted with aerated aquarium.

2.2 Spawning and fertilization in laboratory

Same day after settlement in the aquarium *E. mathaei* started to shed their gametes (Time; 6:00pm), room temperature (28°C), water temperature (26°C), salinity of 37 and a pH of (8). The male urchin starts releasing creamy white mass from the dorsal side and then female spilled the eggs through the aboral side as yellowish color solution. The sperms and eggs were fertilized at room temperature. Fertilized eggs were transferred in to beaker mild aeration was provided and the beaker was partially covered with black cloth to reduce the light penetration (technique followed by Jose *et al.*, 2007). Free-swimming blastula stages obtained after 12 hours of fertilization were transferred to glass aquarium filled with disinfected seawater the aquarium containing the fertilized eggs were maintained at temperature 27- 28°C and the salinity was 37 - 38ppt for the growth and development of eggs. One day after fertilization, 4 triplicate sets with 100 numbers of 'prism stage' larvae were monitored. Water exchange was carried out at the rate of 50% in two days using suitable filter and the aquarium were covered using black cloth.



Figure 1. Map showing the collection site (Map developed by Abrar Ali, Marine Reference Collection and Resource Centre, University of Karachi).

3. Results and Discussion

3.1 Larval and pre-juvenile development

The morphological changes that occurred during the embryonic development of E. mathaei were observed in captivity (Figure 2A, B. and Table 1.). Photographed were made by using a Nikon (LABOPHOT-2) upright microscope at (10 x 20 magnifications). The mature eggs are transparent, spherical in shape, loose, and yellowish, while the sperm are whitish. Sperm and eggs have come closer, the sperm penetrated the egg and fertilization occurred in 5 minutes after contact of sperm and egg. The formation of the fertilization membrane around the egg is the most visible feature of the fertilization process. The first cleavage was observed after one hour of fertilization, and the second cleavage was known to be four to eight cells of equal size 80µmin diameter after 2:50 hours, followed by a 16-cell embryo as the fourth cleavage after 3:30hrs. Cell division took 5 hours, after which cell masses were transformed into a morula. Notice further development in the cells as a blastula at 6:35 hours after observing the morula. The embryo, observed after 15 hours of fertilization, formed a gastrula through invaginating at the vegetal pole. Embryos entered the prism size 13µm, after fertilization for 24 hours and formed the skeletal elements. In such conditions, the stage of the embryo was prismatic, and the primitive gut was observed. The development of the 2-armed pluteus larvae with the size 175 µm began in the prism stage, and after a calculation of 34 hours, the 4-armed pluteus larvae having size 275 µm formed on the third day. In this stage, the pluteus front, short arms, and a pair of dorsal long arms of larvae were observed, and pigmented cells were visible under the upright microscope. The temperature was maintained at 26- 30°C and the salinity was 37ppt for the growth and development of an embryo. The following larval stages 6-arm with size 265µm and 8-arm was 300 um stages were observed on the 15th and 20th day of post-fertilization.



Figure 2. A. Mature Female B. Mature Male of E. Mathaei (Blainville, 1825).

Developmental stages	Time after insemination
Fertilization occur	5 minutes
First cleavage	01:00 hrs.
Second cleavage	02:50 hrs.
16 Cells	03:30 hrs.
Morula	5 hrs.
Blastula	6:35 hrs.
Gastrula	15:00 hrs.
Prism	24 hrs.
Pluteus 2-armed	34 hrs.
Pluteus 4-armed	3 rd day
Pluteus 6-armed	15 th day
Pluteus 8-armed	20 th day
Pre-juvenile	33 rd day

 Table 1. Developmental stages of Echinometra mathaei under laboratory condition.

On day 22, when the larvae reached the 8-arm stage, they demonstrated "competence" by observing the bottom of the beaker or aquarium (**Figure 3**). Microalgae were offered as food. The larval survival rate did not differ significantly during the first days of feeding during the competency stage, the rudiment developed tube feet and spines that became active inside the larval body, but no pedicellariae were formed on the larval surface body, as is common in competent larvae of regular echinoids. After the third day of observation of competency, newly settled juvenile sea urchins are nearly 630 μ m in width, have developed mouth parts, and have begun feeding on benthic diatoms. The present study investigated the larval developmental stages of *E. mathaei* under laboratory conditions from Karachi coast (northern Arabian Sea). The developmental timing of the hatching blastulae period (6:35 hrs. at 27–28°C), Similar to other echinoids with planktotrophic larvae *E. mathaei* embryo, and larval development undergo cleavages (6 h at 29°C) by Ghorani*et al.* (2013), *Lytechinus variegatus* (6 h at 23°C) (Strathmann, 1987) and *Clypeastersub depressus* (7 h at 26°C) (Vellutini and Migotto, 2010) the developmental timing of *Salmacis sphaeroides* hatching blastulae took longer to mature (08.45 h at 24°C) (Rahman *et al.*, 2012).



Figure 3. Embryonic developmental stages of *E. mathaei*(A) Ovum after fertilization (B) Fertilized egg with complete fertilization membrane (C) 4-cell stage (D) 8-cell stage (E) Morula stage enclosed with fertilization membrane (F) Blastula stage (G) Gastrula stage (H, I) Prism stage (J) 2-arm pluteus (K, L) 4-arm pluteus (M) 6-arm pluteus (N) 8-arm pluteus (O) Juvenile stage.

Later stages of development exhibited the same patterns but somewhat varied from those of *Lytechinus variegatus* species from the Caribbean at 23°C (Strathmann,1987) and other Echinoidea species *Colobocentrotus mertensii* species from the Pacific at 27°C (Thet *et al.*, 2004). Gastrulation is strong with red-pigmented cells starting at the vegetal pole and moving through the ectoderm to the apical plate as the archenteron continues to elongate. Comparable events were seen in the tropical sea urchin *E. mathaei* (Takata and Kominami, 2004) and the sea biscuit *Clypeastersub depressus* (Vellutini and Migotto,2010). These cells may be involved in the morphological alterations that take place during pluteus larvae's prism development and early axis determination (Thet *et al.*, 2004;Takata and Kominami, 2004).

The ideal salinity and temperature for the growth of *E. mathaei* embryos, in the port city Bandar Abbas, a warm region of Iran, respectively, were 29°C and 39 ppt. whereas we observed the growth and development of the embryo at 27–28°C and 37ppt salinity. *E. Mathaei* (Ghorani*et al.*, 2013) and similar to the *Arbacia punctulata* (sea urchin), the first cleavage happened after 60 minutes of fertilization (Shimek, 2003). Moreover, King and Riddle (2001) demonstrated that the blastula develops into the embryo in *Sterechinus neumayeri* 2-3 days after fertilization, but in *Paracentrotus lividus* in 12 hrs. (Russo et al., 2003) and while *E. mathaei*, in 6 hours (Ghorani*et al.*, 2013) respectively. In the present study, the embryos developed into the gastrula 15 hours after fertilization, whereas the *E. mathaei* developed into gastrula in 14hrs. *E. mathaei* (Ghorani, 2011) and the *Arbacia punctulata* embryos grew into the gastrula after 24 hrs. (Shimek, 2003).

Prism in *E. mathaei* happened 24 hrs. after conception, but Shimek (2003) stated that embryos mature into prism after 20 hours. According to the studies, *Paracentrotus lividus* and *Sterechinus neumayeri* need 48 hours and 20 days after fertilization to reach the pluteus larvae (Russo *et al.*, 2003; King and Riddle, 2001), but *E. mathaei* takes 34 hours, in comparison to other sea urchin species, *E. mathaei* has a shorter developmental period and the larvae develop in a shorter period, according to Ghorani*et al.* (2013) findings.

Competent larvae of *Salmacissphaeroides*contain pedicellariae during the late larval phase and after metamorphosis, as in *Paracentrotus lividus* (Gosselin and Jangoux, 1998), and *Strongylocentrotus franciscanus* (Miller and Emlet,1999), but pedicellariae of *Strongylocentrotus* sometime after metamorphosis. On the other hand, competent larvae of *Echinocardium cordatum* lack spines and pedicellariae (De Amaral P. Nunes and Jangoux, 2007), whilst those of *Clypeastersub depressus* have spines but no pedicellariae (Vellutini and Migotto, 2010). Similar to *Colobocentrotus mertensii* (Thet *et al.*, 2004), *Paracentrotus lividus* (Gosselin and Jangoux, 1998), and *Lytechinus pictus* (Hinegardner, 1969), the digestive system and maybe other internal organs developed approximately 4-5 days after settling, and the urchin began to eat, while in the present study the newly settled young sea urchins, *E. mathaei* are nearly competent after the third day of observation. *E. mathaei*, newly settled juvenile sea urchins, are almost 630µm wide, have grown mouth parts, and have begun eating on benthic diatoms. Siddique and Ayub, (2019) studies reproduction of the sea urchin of *E. mathaei* from Pakistani coastal waters, results showed that the spawning season of *E. mathaei* and female were spawn in March.

In conclusion, this is the first productive examination of the embryonic, larval, and postmetamorphic juvenile development of *E. mathaei* in Karachi, Pakistan.

Conclusion

The present study represents the first successful analysis on embryonic, larval, and pre-juvenile development of *Echinometra mathaei* (Blainville, 1825) from Karachi coast of the northern Arabian Sea reared under laboratory conditions. Detailed descriptions of embryonic and larval development led to preliminary results that are very promising for domesticating this species and advancing future production diversification. The results will also be of tremendous use to future biologists.

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