

The embryonic development of orange mud crab, *Scylla olivacea* (Herbst, 1796) held in captivity

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Abstract

This study attempts to observe and record early embryonic developments of the orange mud crab, *Scylla olivacea*. The period taken by the eggs to hatch was 8 days and the colour of the eggs gradually changed from yellow to brown, gray and dark gray. During the embryonic development, the developing embryos reached the blastula stage within 24 hours with a mean egg diameter of $329.91 \pm 6.62 \mu\text{m}$. The embryo developed into the gastrula stage on the 2nd day with a mean egg diameter of $337.10 \pm 8.37 \mu\text{m}$. Eyes were consequently observed on the 3rd day and there was a further increase in the yolk-free portion with a mean egg diameter of $338.16 \pm 6.57 \mu\text{m}$. On the 4th day, the eye-spot became crescent and there was a clearer tissue formation with a mean egg diameter of $358.45 \pm 14.80 \mu\text{m}$. Meanwhile, on the 7th day prior hatching, there were many chromatophores present, mostly dark in colour and the yolk granules had further reduced in size. The heart beats faster than previous days before and the embryo occupied most of the available egg volume with a mean egg diameter of $377.26 \pm 11.50 \mu\text{m}$.

Keywords: Aquaculture, Crustacean, Embryonic development, Mud crab, *Scylla olivacea*.

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Introduction

Decapoda crustaceans are very common invertebrates inhabiting the marine environment (Satheeshkumar, 2012). Mud crab as a decapoda crustacean, has been practice cultured in China since the past 100 years and spread throughout Asia for the last 30 years. Mud crabs of the genus *Scylla* are commercially important and conspicuous crustaceans that provide basic source of income for coastal fishing communities throughout the Indo-Pacific region (Islam and Kurokura, 2012). FAO statistics shows that the mud crab aquaculture is well developed especially in China and Philippines (Shelley, 2008). Due to the virtue of its meat quality and the large size, mud crabs are sought after as a quality food item wherever they occur, with continued growth expected in the crab fishery in the future (Ikhwanuddin *et al.*, 2011). As the development of mud crab hatchery for the commercial scale has only occurred in few countries, farms in most countries are depending on wild caught stocks (Shelley, 2008). Increasing human population and market demand led to the limitation supply of seed stocks caused by over exploitation.

Mud crab are increasingly known to be part of the nowadays aquaculture cultivated species (Ikhwanuddin *et al.*, 2014); fattening and sea farming are now being conducted in Asian countries to supplement the reduction of mud crab stock (Kosuge, 2001).

Unfortunately, stocking of juveniles in mangrove ecosystem is difficult to rebuild in its natural habitat. The collected wild seeds are not uniform in size and consistently unavailable throughout the year thus made this questionable for the enough supply for the market. Hence, to solve this problem, hatchery technology is very much needed; the crab larval biology needs to be studied thoroughly to produce good quality eggs and healthy zoea from the mother crab (Soundarapandian and Tamizhazhagan, 2009). Despite the increasing interest in mud crab farming, very little information exists on mud mud crab breeding in detailed (Noor Baiduri *et al.*, 2014). Study regarding early embryonic development have been done on several species such as blue swimming crab, *Portunus pelagicus*, coastal lagoon crab, *Eurypanopeus canalensis*, *Panopeus chilensis*, *Paralithodes platypus*, *P.sanguinolentus*, red-clawed crab, *Perisesarma bidens* (Arshad *et al.*, 2006; Guererro and Hendrickx, 2006; Samuel and Soundarapandian, 2009; Sarker *et al.*, 2009) and recently *S. serrata* (Samuel and Soundarapandian, 2010). However, there has been no study specifically on orange mud crab, *S. olivacea* in captivity.

In an effort to develop mud crab hatchery technology with special reference to *S. olivacea*, comprehensive knowledge on embryogenesis may help in obtaining healthy zoea from

developing eggs. Hence, the aim of this study is to characterize the early embryonic development of *S. olivacea*.

Materials and methods

5 mature crabs of *S. olivacea* were obtained from Setiu Wetlands, Terengganu, Malaysia (5.65° N; 102.77° E) and induced to mate to produce berried female crabs. The mean crab size used were 8.97 cm carapace width (CW) ± 0.41 (n=5) for mature males and 8.72 cm CW ± 0.48 (n=5) for mature female. Sexually matured crabs were identified based on the size at maturity of *S. olivacea* sampled from Setiu Wetland by Ikhwanuddin *et al.* (2010) characterizing the size of maturity of male at 8.97 cm CW and 9.06 cm CW for female. *S. olivacea* broodstocks were cultured in a fibreglass culture tanks (3 tonne capacity of 138 cm width \times 321 cm length \times 60 cm height) supplied with adequate aeration and daily fed with fresh blood cockles, *Anadara granosa* at 10% biomass. Meanwhile, culture conditions were prepared based on Samuel and Soundarapandian (2010) where water salinity was maintained at 30-35 psu, temperature of 28-30°C, pH of 7.8-8.2 and dissolved oxygen level of more than 5 ppm.

Crabs were induced to moult by autotomizing the appropriate walking legs by crushing the carpus or the merus; the chelae were removed by crushing the propodus (Bennett, 1973). Once the female crabs reached pre-

moult stage, with the sign of growing limb buds and turning black, the crabs were quickly transferred into another individual tank. With an inter-moult matured, a male crab was introduced after then. Activities from pre-copulation until post copulation stage such as the male approached the female, turning the female upside down and until the male had released the female all were closely monitored. Then, the male was removed from the tank once the female was released. The newly mated females were cultured until it spawns to produce berried female crabs. Berried females were cultured individually in 300 liters capacity High-Density Polyethylene (HDPE) black tank supplemented with a tray of sand (29 cm width \times 42 cm length and filled with 3 cm thickness of the sand). Cultured conditions were prepared as what has been designed by Samuel and Soundarapandian (2010). Samples of minimum at 10 eggs were withdrawn from the female in the morning and night at every 12 hours interval. Daily colour changes (if any) in eggs during incubation period were noted. Diameters of the eggs were measured daily and pictures were taken. Each stage of embryonic development such as blastula, gastrula, generation of eye placode, presence of pigment and heartbeat was identified according to Samuel and Soundarapandian (2010). All data collected were expressed in mean and standard deviation. Maximum

and minimum data were calculated for the period of mating.

Results

Fig. 1 shows the colour of the eggs after spawning appeared to be orange on day 1 and became brown on day 4, grey on day 5 and finally dark grey on the day before hatching (day 7) (Fig. 1). Meanwhile, the unfertilized eggs (Fig. 2) had one layer of membrane closely attached to the egg body. The thin distinct periplasm was homogeneous throughout the egg. On the other hand, the fertilized egg was macrolecithal, centrolecithal, spherical and had a uniform dark olive green (Fig. 2). The fertilization rate was $88.22 \pm 7.17\%$.

Table 1 shows the development chronology of *S. olivacea* under laboratory condition. The developing embryos reached the blastula stage (Fig. 2) within 24 hours with mean egg

diameter of $329.91\mu\text{m} \pm 6.62$. The cleavage of eggs soon formed after fertilization. The egg cell was divided into 2 daughter cells known as cleavage cells or blastomeres and the cell continued to divide: from 2 to 4, thus producing 8, 16, 32 blastomeres onwards. When approximately more than 128 blastomeres were produced the embryo developed into gastrula stage (Fig. 2) on day 2 with mean egg diameter of $337.10 \pm 8.37 \mu\text{m}$. The phase between blastula and gastrula was not clearly marked until the gastrulation took place by epiboly with increase of the yolk-free portion. The yolk-free portion continued to increase (Fig. 2) and there was a cluster of presumptive primordial cells began to form as patch located in the ventral position with mean egg diameter of $326.81 \pm 8.67 \mu\text{m}$.

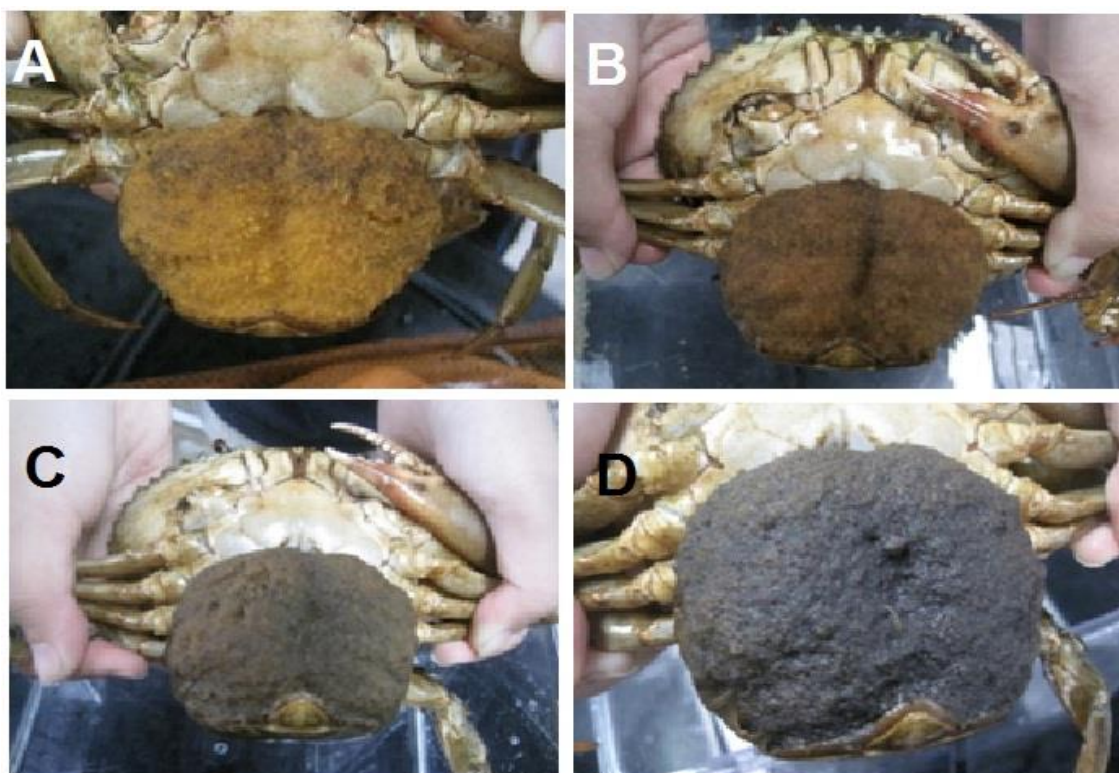


Figure 1: Eggs colour changes of *Scylla olivacea*. (A) First day berried female (orange); (B) Fourth day (brown); (C) Fifth day (grey); and (D) The day before hatching (dark grey).

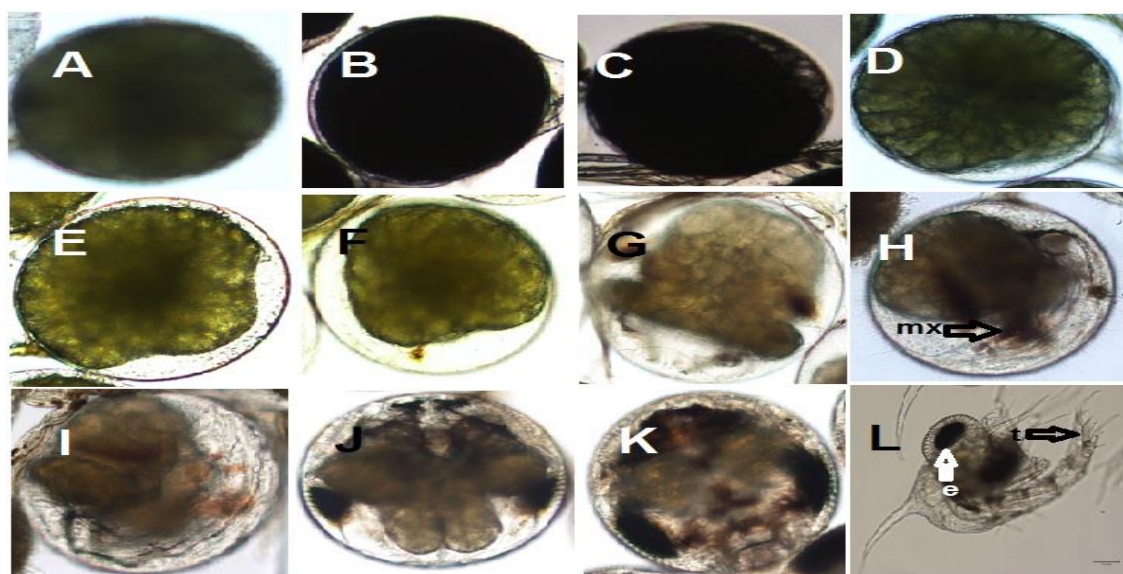


Figure 2: Stages of development embryos of *Scylla olivacea* (A) unfertilized egg, (B) fertilized egg before cleavage, (C) blastula, (D, E) gastrula, (F) 3 days embryo, (G, H) 4 days embryo; e, eye; mx, maxillipeds (I) 5 days embryo, (J) 6 days embryo, (K) 7 days embryo, (L) zoeae hatching; e, eye; T, telson.

Table 1: Embryonic development chronology of *Scylla olivacea* under laboratory conditions at 30 - 35 psu water salinity and 28-30°C water temperature.

Estimated hours after spawning (day)	Mean egg diameter (μm) ($n=10$)	Remarks and stages of development
24 (1 st day)	329.91 \pm 6.62	-Blastula stage
48 (2 nd day)	337.1 \pm 8.37	-Gastrula stage
60 (2 nd day)	326.81 \pm 8.67	-Gastrulation took place by epiboly
72 (3 rd day)	338.16 \pm 6.57	-Yolk-free portion increases
96 (4 th day)	358.45 \pm 14.80	-Evidence of eyes observed -A further increase in the yolk-free portion
108 (4 th day)	355.93 \pm 6.84	-Clearer tissue formation -Utilization of the yolk -Exposing the cephalothorax
120 (5 th day)	370.48 \pm 13.36	-Heart beat first observed with slow jerking movements
132 (5 th day)	381.67 \pm 12.94	-Chromatophores had appeared on the abdomen.
144 (6 th day)	368.60 \pm 11.11	-Chromatophores continue to increase.
156 (6 th day)	389.44 \pm 7.96	-Heart beating rapidly
168 (7 th day)	377.26 \pm 11.50	-Eyes grew bigger and complex -Chromatophores further increased
192 (8 th day)	1027.27 \pm 90.01	-Heart beating more vigorously -Hatching of zoeae

Eyes were then observed to be developed on day 3 and there was further increment in the yolk-free portion with mean egg diameter of 338.16 \pm 6.57 μm . The cluster began to differentiate into many transparent embryo structures which are ocular, antennules-antenna, maxillule-maxilla, maxilliped and thoracic-abdominal which separated by slits. However, they were not clearly distinguished (Fig. 2). On the 4th day, the eye-spots became crescent and there was clearer tissue formation with mean egg diameter of 358.45 \pm 14.80 μm (Fig. 2). Utilization of yolk by the developing embryos was notable when abdominal and cephalothoracic primordial had increased in size and started to separate. Antennules-antenna and maxillule-maxilla were not clearly seen, however, maxillipeds were clearly observed as tiny buds rising below and back of the

optical primordial structures with mean egg diameter of 355.93 \pm 6.84 μm (Fig. 2).

Later, the abdomen was divided into segments (metameres) with chromatophores on it with mean egg diameter of 370.48 \pm 13.36 μm (Fig. 2). The yolk components were arranged in 4 lobes. Further development of the heart and heartbeat were observed with mean of 146.20 \pm 7.76 bpm. Eye pigmentation was more intense and differentiated in cornea and retina during night time. The next day, heartbeat became more vigorous with mean of 187.90 \pm 2.13 bpm and it was observed that the eyes grew bigger and had a distinct triangular shape while the chromatophores continued to expand. On day 7, many chromatophores appeared, mostly dark in colour and the yolk granules had further reduced in size. The heart beat faster than the day

before and the embryo occupied most of the available egg volume with mean egg diameter of $377.26 \pm 11.50 \mu\text{m}$ (Fig. 2). Hatching occurred on day 8 (Fig. 2). Before hatching, the larvae moved vigorously especially inside the egg shell specifically the abdominal part. The mean hatching rate of *S. olivacea* was $92.56 \pm 3.72\%$ and the mean length of the newly hatched zoea was $1027.27 \pm 90.01 \mu\text{m}$.

Discussion

During mating, male crab transferred the sperm to female's body via gonopods and stored on spermatheca. Spermatozoa (sperm) are stored in spermatheca where it could be retained through a moult and remain viable for a long period (Samuel and Soundarapandian, 2009). In brachyuran crabs, females incubate the eggs in the body cavity from oviposition until hatching. The female released the eggs into the abdominal cavity followed by sperm and fertilization will be occurred. Therefore, the fertilization pattern of crabs can be regarded as incomplete internal fertilization (Samuel and Soundarapandian, 2010). The family of Penaeidae do not carry the eggs, but shed freely into water body. Meanwhile, in some decapods crustaceans, the eggs are fertilized when they passed through the spermatheca (Reveberi, 1971; Warner, 1977).

Number of eggs per batch is generally very large and varies

according to size and species of mangrove tree crab, *Aratus pisonii* of 1.6 cm CW lays about 5,000 eggs and a 3.6 cm CW striped shore crab, *Pachygrapsus crassipes* lays about 48,000 eggs. Large crabs like brown crab, *B. pagurus* lay up to 3 million eggs when fully grown (Warner, 1977). In general, Portunids lay around 1 to 6 million eggs per spawning period (Arshad *et al.*, 2006; Ikhwanuddin *et al.*, 2010; Samuel and Soundarapandian, 2010; Ikhwanuddin *et al.*, 2011). The colour of the eggs immediately after spawning appeared orange at first and became brown, grey and finally to dark grey before hatching. This was due to the absorption of the yellow yolk and the development of dark pigment in the eyes and appearance of chromatophores on the abdomen (Samuel and Soundarapandian, 2010). The utilization of yolk by developing embryos has two purposes that are as an energy source and for tissues and organs differentiation (Babu, 1987).

There is no definite standard in staging the embryos of brachyurans. Some findings showed 5 embryonic stages (Samuel and Soundarapandian, 2010), 10 embryonic stages and even as many as fifteen embryonic stages before hatching (Babu, 1987). In present study, the continuous daily progress of developing embryos was defined. The transition between blastulation and gastrulation took place in epiboly. In this case, epiboly

occurred through the cells changing shape and the increasing yolk-free portion. Moreover, epiboly also occurred through cell division or by any intercalating of several layers into fewer layers (Gilbert, 2006).

In general, the incubation period of *Scylla* spp. eggs was between 7 to 13 days depending on the conditions maintained (Samuel and Soundarapandian, 2010). The reduction of incubation period might be due to the adequate food supply and good water quality parameters. The temperature during the incubation should range between 24.5–28.0°C which was usually colder in the morning compared to the evening period with the same temperature and water. The result of incubation period in present study was almost the same as study done by Samuel and Soundarapandian (2010) on *S. serrata* which took 7-9 days at 28-30°C. On the other hand, incubation period of *P. bidens* was 17 days at 25°C (Sarker *et al.*, 2009). Temperature is known to regulate the rate of egg development in various brachyuran species (Wear, 1974). A study by Zeng (2007) on *S. paramamosain* showed that abnormal cell division was observed at both low (10°C) and high (35°C) temperatures and the embryogenic development were retarded around the gastrula stage at 15°C.

Oxygen consumption is one of the factors affecting the incubation period. The amount of oxygen consumed by an

embryo provides a measure of its metabolic rate and can be used to study the effect of environmental conditions on embryonic metabolism. The dry weight of an embryo should diminish as respiratory substrates are oxidized. However, the embryos of crustacean in saline habitats could be able to maintain the dry weight by taking up salts (Reveberi, 1971). Furthermore, the highest biochemical composition in the eggs of blue swimming crab, *P. pelagicus* and crab, *Xantho hidentatus* was protein (Soundarapandian and Singh, 2008). Protein content of yolk is crucial for tissue differentiation and organization particularly for the cuticle layers, muscles, digestive and nervous systems.

It can be observed that lipid content decreased significantly at the late stage in crab, *X. bidentatus*; lipid was used as reorganized in embryo rather than as energy source (Babu, 1987). However, lipids are highly efficient source of energy in a way that it contains more than twice the energy of carbohydrates and proteins (Soundarapandian and Singh, 2008). Crustacean eggs can be divided into terrestrial, marine and freshwater according to their habit (Pandian, 1970). The protein metabolism is prominent in marine and freshwater eggs as compared to terrestrial eggs where the oxidation of the eggs is high. Lipid was also found to be the main energy source during development of demersal marine crustaceans' eggs.

Many decapods hatched as protozoa or zoea larvae. During hatching, there is period of swelling of inner egg membrane at the beginning of the process (Davis, 1965). The protozoa has an elongated segmented abdomen, but swims by means of its antennae, while the zoea swims by means of its thoracic limbs. Well-developed lateral compound eyes and 6 pairs of appendages are present in the zoea (Reveberi, 1971). In the present study, the mean eggs diameter of *S. olivacea* on the first day was $329.90 \pm 6.62 \mu\text{m}$ and increased to $377.26 \pm 11.50 \mu\text{m}$ on the day before hatching, increasing about 15.15%. On the other hand, previous study had pointed out that the largest mean egg diameter of *E. canalensis* and *P. chilensis* (Guerrero and Hendrickx, 2001) and *P. bidens* (Sarker *et al.*, 2009) were 320, 380, 340 and 410 μm , respectively.

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