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±6.62 µm. The embryo developed into the gastrula stage on the 2nd day with a mean egg diameter of 337.10±8.37 µm. 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±6.57 µm. On the 4th day, the eye-spot became crescent and there was a clearer tissue formation with a mean egg diameter of 358.45±14.80 µm. 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±11.50 µm.

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 ranks (3 tonne capacity of 138 cm width×321 cm length×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 ×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 ± 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 329.91µm ±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±8.37 µm. 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±8.67 µm.

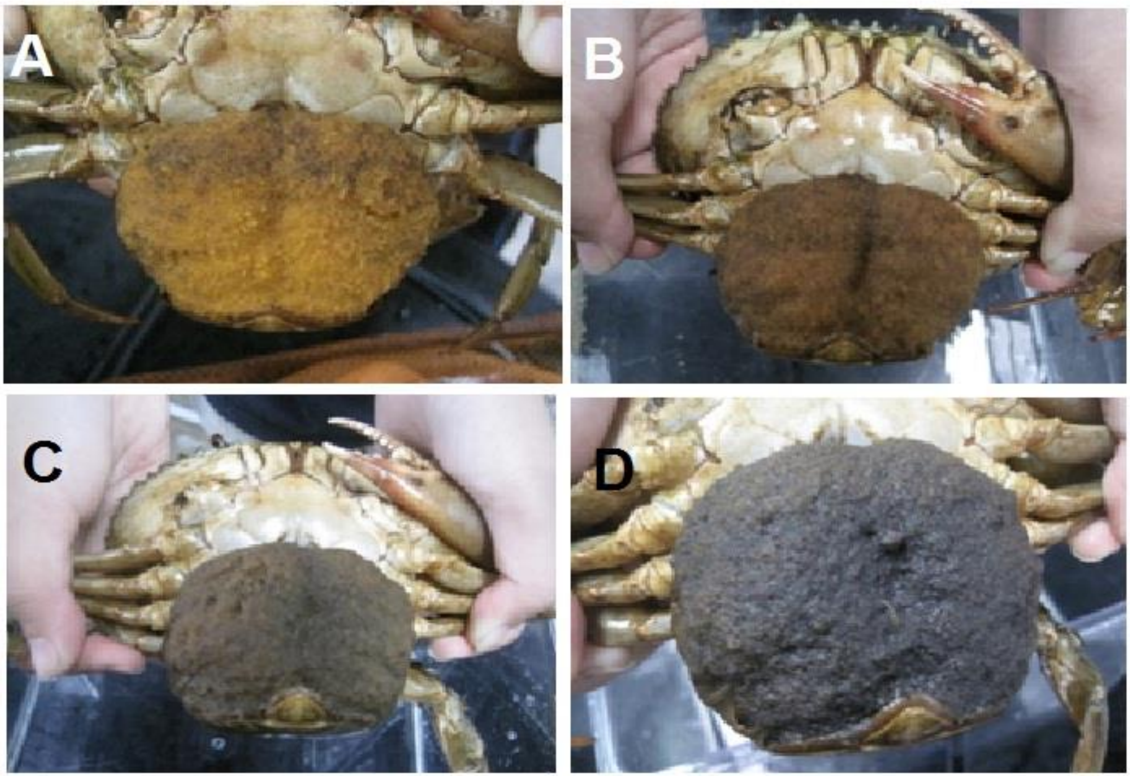

Figure 1: Eggs colour changes of *Scylla olivacea*. (A) First day berried female (orange); (B) Fourth day (brown); (C) Fifth day (grey); and (D) The day before hatching (dark grey).

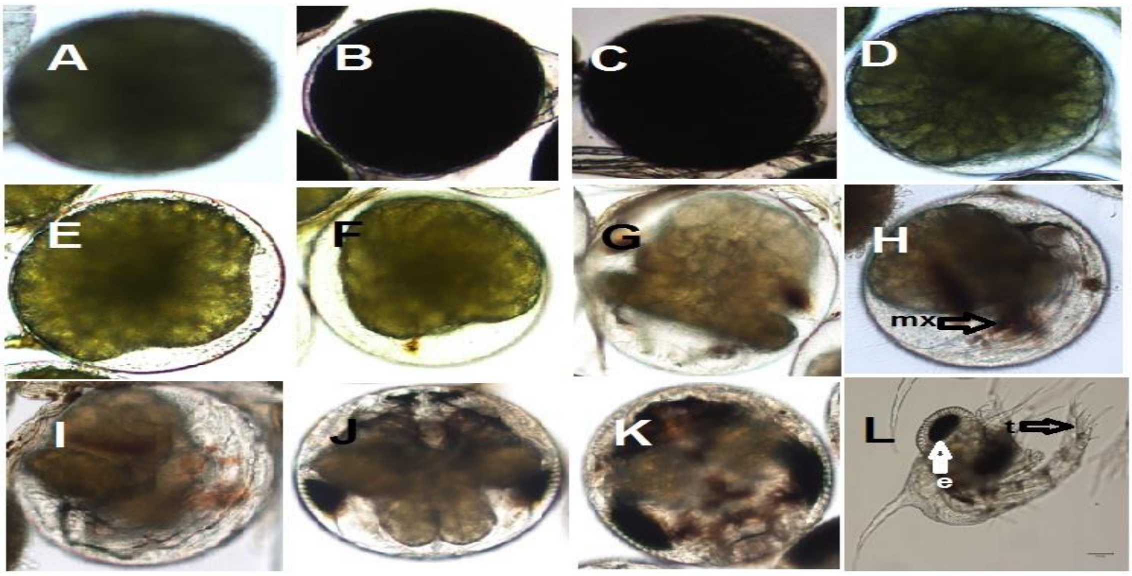

Figure 2: Stages of development embryos of *Scylla olivacea* (A) unfertilized egg, (B) fertilized egg before cleavage, (C) blastula, (D, E) gastrula, (F) 3 days embryo, (G, H) 4 days embryo; e, eye; mx, maxillipeds (I) 5 days embryo, (J) 6 days embryo, (K) 7 days embryo, (L) zoeae hatching; e, eye; T, 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 (1st day)	329.91 ± 6.62	-Blastula stage
48 (2nd day)	337.1 ± 8.37	-Gastrula stage -Gastrulation took placed by epiboly
60 (2nd day)	326.81 ± 8.67	-Yolk-free portion increases
72(3rd day)	338.16 ± 6.57	-Evidence of eyes observed -A further increased in the yolk-free portion
96 (4th day)	358.45 ± 14.80	-Clearer tissue formation -Utilization of the yolk -Exposing the cephalothorax
108 (4th day)	355.93 ± 6.84	-Heart beat first observed with slow jerking movements
120 (5th day)	370.48 ± 13.36	-Chromatophores had appeared on the abdomen.
132 (5th day)	381.67 ± 12.94	-Chromatophores continue to increase.
144 (6th day)	368.60 ± 11.11	-Heart beating rapidly
156 (6th day)	389.44 ± 7.96	-Eyes grew bigger and complex
168 (7th day)	377.26 ± 11.50	-Chromatophores further increased -Heart beating more vigorously
192 (8th day)	1027.27 ± 90.01	-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±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±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±6.84 µm (Fig. 2).

Later, the abdomen was divided into segments (metameres) with chromatophores on it with mean egg diameter of 370.48±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±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±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±11.50 µm (Fig. 2). Hatching occurred on day 8 (Fig. 2). Before hatching, the larvae moved vigorously especially inside the egg shell specificially the abdominal part. The mean hatching rate of *S. olivacea* was 92.56±3.72% and the mean length of the newly hatched zoea was 1027.27±90.01 µm.

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 protozoea or zoea larvae. During hatching, there is period of swelling of inner egg membrane at the beginning of the process (Davis, 1965). The protozoea has an elongated segmented abdomen, but swims by means of its antennae, while the zoea swims by means of its thoracic lims. 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±6.62 µm and increased to 377.26±11.50 µm 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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References

Arshad, M.S., Efrizal, A., Kamarudin, M.S. and Saad, C. R., 2006. Study on fecundity, embryology and larval development of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) under laboratory conditions. *Research Journal of Fisheries and Hydrobiology*, 1, 35-44.

Babu, D.E., 1987. Observation on the embryonic development and energy source in the crab *Xantho bidentatus. Marine Biology*, 95, 123-127.

Bennett, D.B., 1973. The effect of limb loss and regeneration on the growth of edible crab, *Cancer pagurus*. *Journal of Experimental Marine Biology and Ecology*, 13, 45-53.

Davis, C.C., 1965. A study of the hatching process in aquatic invertebrates: XX. The blue crab, *Callinectes sapidus*, Rathbun, XXI. The nemertean, *Carcinonemertes carcinophila* (Kölliker). *Chesapeake Science*, 6, 201-208.

Gilbert, S.F., 2006. Developmental biology. 8th ed. Sunderland, USA: Sinaeur Associates.

Guerrero, M.G. and Hendrickx, M.E., 2006. Embryology of decapods crustaceans III: embryonic development *Eurypanopeus canalensis* (Abele and Kim, 1989) and *Panopeus chilensis* (H. Milne Edwards and Lucas, 1844) (Decapoda, Brachyura, Panopeidae). *Belgian Journal of Zoology*, 136, 249-253.

Ikhwanuddin, M., Bachok, Z., Mohd Faizal, W.W.Y., Azmie, G. and Abol-Munafi, A.B., 2010. Size at maturity of mud crab *Scylla olivacea* (Herbst, 1796) from mangrove areas of Terengganu coastal waters. *Journal of Sustainability Science and Management*, 5, 134-147.

Ikhwanuddin, M., Azmie, G., Juariah, H.M., Zakaria, M.Z. and Ambak, M.A., 2011. Biological information and population features of mud crab, genus *Scylla* from mangrove areas of Sarawak, Malaysia. *Fisheries Research*, 108, 299-306.

Ikhwanuddin, M., Noor Baiduri, S., Wan Norfaizza, W.I. and Abol-Munafi, A.B., 2014. Effect of water salinity on mating success of orange mud crab, *Scylla olivacea* (Herbst, 1796) in captivity. *Journal of Fisheries and Aquatic Science*, 9, 134-140.

Islam, S. and Kurokura, H., 2012. Male reproductive biology of mud crab *Scylla olivacea* in a tropical mangrove swamps. *Journal of Fisheries and Aquatic Sciences*, 7, 194-204.

Kosuge, T., 2001. Brief assessment of stock mud crabs *Scylla* spp. in Matang Mangrove Forest, Malaysia and proposal for resource management. *Japan Agricultural Research Quarterly*, 35, 145-148.

Noor Baiduri, S., Nurul Akmal, S. and Ikhwanuddin, M., 2014. Mating success of hybrid trials between two mud crab species, *Scylla tranquebarica* and *Scylla olivacea*. *Journal of Fisheries and Aquatic Science*, 9, 85-91.

Pandian, T.J., 1970. Ecophysiological studies on the developing eggs and embryos of the European lobster *Homarus gammarus. Marine Biology*, 5, 154-167.

Quinitio, E.T., Pedro, J. and Estepa, D.P., 2007. Ovarian maturation stages of the mud crab *Scylla serrata. Aquaculture Research*, 38(**14**), 1434-1441.

Reveberi, G., 1971. Experimental embryology of marine and freshwater invertebrates. Amsterdam, Holland: North-Holland Publishing Company. 587P.

Samuel, N. J., and Soundarapandian, P., 2009. Development of commercially important portunid crab *Portunus sanguinolentus* (Herbst). *International Journal of Animal and Veterinary Advances*, 1(**2**), 32-38.

Samuel, N.J. and Soundarapandian, P., 2010. Embryology of commercially important portunid crab *Scylla serrata* (Forskal). *Current Research Journal of Biological Sciences*, 2(**1**), 35-37.

Sarker, M. M., Islam, M.S. and Uehara, T., 2009. Artificial insemination and early embryonic development of the mangrove crab *Perisesarma bidens* (De Haaan) (Crustacea: Brachyura). *Zoological Studies*, 48(**5**), 607-618.

Satheeshkumar, P., 2012. Mangrove vegetation and community structure of brachyuran crabs as ecological indicators of Pondicherry coast, South east coast of India. *Iranian Journal of Fisheries Sciences*, 11(**1**), 184-203.

Shelley, C., 2008. Capture-based aquaculture of mud crabs (*Scylla* spp.). In Lovatelli, A. and Holthus, P. F. editor. Capture-based aquaculture. Global overview. FAO Fisheries Technical Paper. No. 508. Rome, FAO. pp. 255-269.

Soundarapandian, P. and Singh, R.K., 2008. Biochemical composition of the eggs of commercially important crab *Portunus pelagicus* (Linnaeus). *International Journal of Zoological Research*, 4(**1**), 53-58.

Soundarapandian, P. and Tamizhazhagan, T., 2009. Embryonic development of commercially important swimming crab *Portunus pelagicus* (Linnaeus). 1(**3**), 106-108.

Warner, G.F., 1977. The biology of crabs. London. Paul Elek, Ltd., 202P.

Wear, R.G., 1974. Incubation in British decapods crustacean and the effects of temperature on the rate of success of embryonic development. *Journal of the Marine Biological Association of the United Kingdom*, 54, 745-762.

Zeng, C., 2007. Induced out-of-season spawning of the mud crab, *Scylla paramamosain* (Estampador) and effects of temperature on embryonic development. *Aquaculture Research*, 38, 1478-1480.