

## Embryonic Development of Commercially Important Portunid Crab *Portuns sanguinolentus* (Herbst)

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**Abstract:** In the present study an attempt was made to study the embryonic development of commercially important crab *Portunus sanguinolentus*. The newly spawned eggs were round and golden yellow in colour. The undeveloped and mass of undifferentiated cells were also found in the berry. The yolk granules were denser. The cleavage and gastrulation were not clear. The diameter of the freshly laid egg was 0.34mm. The multicell eggs were round, deep yellow or yellowish orange in colour. The space between the egg wall and the inner developing embryo was visible. The diameter of the egg was 0.41mm. The eye stage eggs were round and orange in colour. The yolk granules were not denser. Segmentation and organogenesis were clearly seen. The eye spots appeared as scarlet crescent. The egg diameter was 0.48mm. The pigment stage eggs were brown in colour with slightly elliptical shape. Appendages of the embryonic larvae were pigmented. The egg diameter was 0.57 mm. The Heart beat stage eggs were dark brown or black in colour. The eyes were round in shape. The heart started to beat vigorously. The diameter of the egg was 0.64 mm. In prehatching stage the rate of heartbeat was increased and the chromatophores were also found increased throughout the body. The diameter of the egg was 0.73mm.

**Key words:** *Portunus sanguinolentus*, cleavage, gastrulation, yolk granules and heart beat stage

### INTRODUCTION

Among crustaceans, crabs occupy third position, first and second being shrimps and lobsters based on their external and internal market demand (John Samuel, 2008). Even though, its present status is not comparable to that of shrimps, crabs support a sustenance fishery of appreciable importance. Portunid crabs are one of the good fishery resources of south-east Asian seas, out of which, mud crabs (*Scylla serrata* and *S. tranquebarica*) and blue swimming crabs (*P. pelagicus* – flower crab; *P. sanguinolentus* – three spot crab and *Charybdis feriata* – cross crab) are of commercial value. Crustacean embryonic and larval systems offer a unique and valuable tool for furthering our understanding of both developmental processes and physiological regulatory mechanisms (John Samuel *et al.*, 2004). The diverse array of developmental patterns exhibited by crustaceans allows species choice to be based on the specific questions being investigated, where defined larval forms are chosen based on their developmental pattern, degree of maturation or regulatory capabilities. However, this great diversity in developmental patterns, as well as crustacean diversity, can also confound one's ability to define or identify species for investigation. Embryonic studies are scanty for portunoid crabs in general and *P. sanguinolentus* in particular. So in the present study an attempt has been made to study the embryonic development in the portunid crab, *P. sanguinolentus*.

### MATERIALS AND METHODS

Gravid females of *P. sanguinolentus* with early broods (yellowish orange coloured eggs) were collected from the Parangipettai coastal waters and retained in separate tanks containing sea water (salinity - 35±1 ‰; temperature - 28 to 31°C; dissolved oxygen was up to the saturation level and photoperiod – 12 L:12 D). The crabs were fed with mussel and clam meat once in a day. Everyday the excess food, excreta and shed out eggs were siphoned out. Continuous aeration was given throughout the incubation period and the development of the egg was closely observed. Daily colour changes of the eggs during incubation period could be noted. Small clumps of eggs were snipped from random locations in each clutch using sharp scissors. All the developing embryos were examined with a MEIJI binocular dissecting microscope (100X) to ensure that only viable embryos were sampled and the colour change corresponding to development and length of incubation period was noted (Srinivasgam *et al.*, 2000; John Samuel, 2003). The time course of embryonic development, as indicated by the appearance of specific morphological features including the development of the compound eye, initiation of the heart beat, development of the limb pigmentation and initiation of limb twitches were monitored. The gradual change in the embryonic development and increase in the size of egg was recorded to understand the different developmental stages (Quinitio and Parado-Esteva, 2003). All these developmental stages

were photographed using the digital camera (Nikon, COOLPIX 990, Japan) attached with the microscope.

## RESULTS

The eggs of three-spot crab went through different colours with its gradual development. The newly spawned eggs were bright yellow and the number of eggs attached to each seta of the pleopod was not definite in number. The eggs were spherical and surrounded by two membranes, an inner and outer membrane. Both membranes were transparent and the yolk was visible as yellow granules with polygonal areas. Owing to the large size of the egg mass the abdomen was almost straight, continues with the cephalothorax and the telson was slightly tilted upwards. Fecundity ranged between 1, 68,730 and 3, 52,460 (1.68 to 3.52 lakhs).

The newly oviposited eggs contain all the necessary material for synthetic processes associated with embryogenesis and morphogenesis and all of the compounds required for oxidative metabolism and energy production. The egg contains nutritive reserves in the form of proteinaceous yolk and lipid vesicles scattered throughout the cytoplasm. The newly spawned eggs were bright yellow as the yolk contains carotenoid pigments. As the development progresses the bright yellow colour changes to dull yellow and finally to dark grey just one day before hatching. At this stage, the developing larvae with its occasional twitching movements were observed under the microscope. During this period there was considerable increase in the egg size also. The total days of incubation varied between 8-11 days. The process of embryonic development was divided into six stages, viz., newly spawned egg, multicell stage, eye stage, pigment stage, heart beat stage and prehatching stage.

**Stage 1: Newly spawned egg:** The eggs were round and golden yellow in colour. The undeveloped and mass of undifferentiated cells were also found in the berry. The yolk granules were denser. The cleavage and gastrulation were not clear. The diameter of the freshly laid egg was 0.34mm.

**Stage 2: Multicell stage:** The eggs were round, deep yellow or yellowish orange in colour. The space between the egg wall and the inner developing embryo was visible. The diameter of the egg was 0.41mm.

**Stage 3: Eye stage:** The eggs were round and orange in colour. The yolk granules were not denser. Segmentation and organogenesis were clearly seen. The eye spots appeared as scarlet crescent. The egg diameter was 0.48mm.

**Stage 4: Pigment stage:** Eggs were brown in colour with slightly elliptical shape. Appendages of the embryonic larvae were pigmented. The egg diameter was 0.57 mm.

**Stage 5: Heart beat stage:** The eggs were dark brown or black in colour. The eyes were round in shape. The heart started to beat vigorously. The diameter of the egg was 0.64mm.

**Stage 6: Prehatching stage:** In this stage the rate of heartbeat was increased and the chromatophores were also found increased throughout the body. The diameter of the egg was 0.73mm.

## DISCUSSION

In Decapod crustaceans, the embryos develop in broods that are carried by the females and experience the parental environmental conditions. In crabs, during mating, the sperms are transferred to the seminal receptacle, which act as a storage organ. Viable sperms are utilized during subsequent spawnings of that particular intermoult period. As the eggs are laid, they adhere to one another and to the setae of the endopodites of the abdominal segment, and the maturing egg mass/sponge is held between the reflexed abdomen and venter of the cephalothorax. The abdominal chamber acts as an incubation chamber for the developing eggs. Hamasaki *et al.* (1991) reported embryogenesis in *P. trituberculatus*, in which, females extrude their eggs from gonopores onto a bottom substratum such as sand where they bury themselves; then they attach the extruded eggs to the ovigerous setae of the endopods of the pleopods, which are moving forward and backward.

Needham (1950) classified the animal eggs into two categories based on the major substrate (fat or protein) utilized during embryogenesis. They are terrestrial or cleidic eggs utilizing fat during embryogenesis and aquatic or non-cleidic eggs utilizing proteins for oxidation. However, Pandian (1970) after working with several marine crustaceans discussed the merits and demerits of Needham's (1950) classification of eggs. Pandian (1970) classified the eggs according to the habitat of the species and the main substrate metabolized. They are: terrestrial eggs - protein metabolism is greatly suppressed and oxidation of fat is geared up; freshwater eggs - protein metabolism is predominant and limited fat metabolism; marine eggs - fat depletes heavily and considerable suppression of protein metabolism. Based on the egg size, Shakuntala and Ravichandra Reddy (1982) classified the crustacean embryogenesis into two groups as the larger eggs utilize more lipid than protein and the smaller ones utilize more protein than lipid. In most marine invertebrates, the newly laid eggs contain all the energy and reserves for embryonic development (Holland, 1978; Jaekle, 1995). Female nutritional and reproductive condition (Harrison, 1990; Palacios *et al.*, 1998, 1999), temperature (McLaren *et al.*, 1969; Wear, 1974; Steele and Steele, 1975) and salinity (Crisp and Costlow, 1963; Bas and Spivak, 2000) may affect oogenesis, embryogenesis and larval quality.

The number of eggs produced by crustaceans varies widely (Sastry, 1983). According to Parsons and Tucker (1986) fecundity can vary seasonally, annually and between areas. Among Brachyuran crabs, there is considerable variation in fecundity. In general, Portunids lay around 1 to 6 million eggs per spawning. In the present investigation the fecundity of *P. sanguinolentus* was found to be ranging from 1, 68,730 to 3, 52,460 eggs (1.68 to 3.52 lakhs). Whereas, previous reports on the fecundity of the same species were reported to be more or less similar. Pillai and Nair (1971) reported 1, 51,780 to 3, 07,500 eggs (1.5 to 3.07 lakhs) and later Radhakrishnan (1979) reported a total of 15,314 to 1,48,800 eggs (0.15 to 1.48 lakhs). Contrastingly higher fecundity levels were observed by Sukumaran and Neelakantan (1997) – 2, 88,162 to 9, 20,510 eggs (2.88 to 9.20 lakhs). But the allied species *P. pelagicus* was reported to be having 52,025 to 20,22,500 eggs (Prasad and Neelakantan, 1989), 3,18,720 to 5,21,450 eggs in southwest coast (Pillai and Nair, 1971) and 0.1 to 2.3 million eggs were reported by Srinivasagam *et al.* (2000). But at the same time the other portunid crabs such as *S. tranquebarica*, *S. serrata* have reported with comparatively higher fecundity (Pillai and Nair, 1971; Prasad and Neelakantan, 1989; Ingles and Braum, 1989; Srinivasagam *et al.*, 2000; John Samuel, 2003; Thirunavukkarasu, 2005). Several factors such as salinity, temperature, photoperiod, abundance of food in the environment and intrinsic state of the animal have been attributed to both interspecific and intraspecific variability of fecundity (Geise and Pearse, 1974). There has been considerable variation in the results obtained by various workers who studied fecundity of portunids from different regions (Dhawan *et al.*, 1976; Potter *et al.*, 1983). In several crustaceans there is a linear relationship between the number of eggs per brood and the size of the female. This has also been observed for the freshwater prawn *Macrobrachium lamarrei* (Shakuntala, 1977), the freshwater crayfish *Astacus leptodactylus* (Koksal, 1988), the crayfish *Procambarus (Austrocambarus) ilamasi* (Rodriguez-Serna *et al.* 2000) and the velvet swimming crab *Necora puber* (Norman and Jones, 1992). Prasad and Neelakantan (1989) showed a similar direct relationship between size and fecundity in *S. serrata* up to a size of 140mm carapace width. Churchill (2003) reported a significant correlation between crab size and fecundity, with larger crabs having higher fecundities. In *P. trituberculatus*, Hamasaki *et al.* (2006) emphasized that oocyte number increased with increasing female's body size and predicted estimates ranged between 0.8 and 4.5 million for carapace width of 130–240 mm.

As the embryo develops the size of the eggs increase gradually. The eggs swell as they develop so that by the time they are ready to hatch, they are roughly double their new-laid volume. The same trend was observed in *S. serrata* by Giles Churchill (2003) where egg diameter was not related in any way to female size and also the egg diameters increased at a relatively steady pace throughout ontogeny. There was an accelerated increase in egg

diameter when the embryonic heartbeat was first observed. Similar observations were made by Wear (1974) for green crab (*C. maenas*), the nut crab (*Ebalia turberosa*) and some lobsters (*Galathea dispersa* and *G. squamifera*). Under constant environmental conditions, the variability in egg size and biomass has been attributed to variation in female size or age (Qian and Chia, 1992; Stella *et al.*, 1996; Ito, 1997) and genetic factors (Eyster, 1979; Glazier, 1992; Mashiko, 1992).

Hamasaki *et al.* (2006) studied batch fecundity in *P. trituberculatus*, in which he emphasized that the size of eggs decreased with increasing temperatures, the number of first zoeae showed no fluctuation in the same-size females throughout the breeding season. He suggested that three patterns of reproductive characters may cause this phenomenon: (1) females invest the same amount of energy in the reproductive output throughout the breeding season, so that they increase egg number with decreasing egg size dependent on the trade-off between egg size and number (Hines), but lose more eggs between oviposition and hatching as the breeding season advances; (2) females produce similar egg numbers, and decrease egg size and energy investment for reproductive output as the breeding season advances; and (3) females decrease both egg size and number, and egg loss rate decreases as the breeding season advances. Seasonal and regional variability in egg size and number is known for a few brachyuran species. In the Japanese mitten crab, *Eriocheir japonica*, egg size varies within the breeding season; large eggs are spawned and developed at low temperature and small eggs at high temperature (Kobayashi and Matsuura, 1995). Brante *et al.* (2003) reported the egg number and size for the cancrid crab *C. setosus* distributed along the Chilean coast. Egg numbers produced by females showed no significant difference along a latitudinal (temperature) gradient, but egg size and reproductive output decreased in Northern Chile (high temperature). In this study, the number of eggs was examined using ovaries that developed during the overwintering period.

During the development, the colour of the egg changes through brown to grey as the yolk is used up and the outline of the embryo becomes visible. The eyes and pigment spots appear first followed by the outlines of the abdomen and cephalothorax. The eggs when deposited are yolky with bright yellow or yellowish orange, but they become brown and then dark brown or black before hatching. The colour change was caused by absorption of the yellow yolk and development of dark pigment in the eyes (Krishnan, 1988; Vijayakumar, 1992; Veera Ravi, 1994; Parimalam, 2001). Veera Ravi (1994) reported that as the progression of development occurs, the embryo decreases in dry weight stage by stage as it utilizes the yolk material. Subramoniam (1991) studied the yolk utilization during embryogenesis in *E. asiatica* and reported that the water content steadily increased; conversely, protein content showed a steady decline; protein-bound carbohydrates declined whereas the concentration of free carbohydrates and glycogen

exhibited an increasing trend; lipid level remained unaltered almost up to stage V, thereupon, the value fell precipitously, reaching the minimum in stage IX.

The embryonic development includes the stages of newly spawned egg, multicell stage, eye stage, pigment stage, heart beat stage and prehatching stage. The course of embryonic development includes cleavage, blastula, gastrula, segmentation, organogenesis, formation of appendages, formation and functioning of heart and formation of chromatophores all around the body. Similar pattern of embryonic development was reported for many portunid crab species (Krishnan, 1988; Vijayakumar, 1992; Veera Ravi, 1994; Parimalam, 2001; Quinitio and Parado-Esteva; 2003; John Samuel, 2003; Thirunavukkarasu, 2005).

Many workers have divided the crustacean egg stages based on the appearance of distinctive morphological features such as the eye, heart beat and appendage formation. However, such morphological characters only begin to appear mid-way during embryonic development. Cellular differentiation starts soon after gastrulation and requires enormous energy expenditure. Therefore Subramoniam (1991) emphasized the importance of a detailed classification of early development of decapod crustaceans to understand the changes in the metabolic pathways involving interconversion of already stored substrates within the closed system of egg development. In the embryology of *E. asiatica*, Subramoniam (1991) divided the egg development into nine stages based on colour change and other concomitant morphological features of the embryo. His study, reports on the biochemical alterations in the major organic substrates, as well as the activity of non-specific esterases during egg development leading to the release of the zoea.

Wu (1991) while studying the embryonic development in *S. serrata* observed different stages, which includes the stages of cleavage, blastula, gastrula, egg-nauplius, embryo with five pairs of appendages, embryo with seven pairs of appendages, embryo with compound eye pigments formation, and embryo almost ready to hatch. The cleavage evolves those from the spiral type to the superficial type. The dividing furrow can be seen from 2 cells. The gastrula is formed through invagination. Xue *et al.* (2001) studied the histology of embryonic development in *P. trituberculatus* and reported 5 stages, i.e., two egg-nauplius stages and three egg-zoea stages. The egg-nauplius stage could be divided into egg-nauplius I and egg-nauplius II. The egg-nauplius I includes the formation of optic lobe, antennule, antenna and mandible with the cleaving cells. These appendages are unsegmented; the thoracoabdominal process formed abdomen with proliferating cells; the labrum rudiment and labium rudiment formed in the tip of the stomodeum. The maxillule, maxilla, cavity abdomen, optic ganglia and antennule ganglia formed during the egg-nauplius II. The segmentation emerged between thorax and abdomen. The egg-zoea stages were divided into egg-zoea I, egg-zoea II and egg-zoea III. The carapace, stomach, hind gut,

compound eye and thoracic ganglia formed in the egg-zoea I. The antenna ganglia keep in touch with each other; each of the optic ganglia was independent; the abdomen was divided into 6 segments. The appendages segmented during the egg-zoea II. The ends of the appendages were divergent with setae; the parts of the compound eye have formed; the shape of the yolk sac was like butterfly. The pigment cells, maxilliped I, maxilliped II, heart, gonad and brain have formed. The mesoderm formed muscle and arranged themselves as beads in the next stage. The digestive system has formed except hepatopancreas. Compared with the egg-zoea I and egg-zoea II, the brain and carapace further developed in the egg-zoea III. The muscle and the hepatopancreas formed at the same time.

In the present study, the duration of embryonic development in *P. sanguinolentus* lasts for 8-11 days. Whereas in *P. pelagicus* the period reported was 8-10 days (Joseleen Jose, 2002). In general, the duration of embryonic development in the portunid crabs varies between 8 to 14 days depending on the environmental conditions. Wu (1991) have reported that the duration of embryonic development could be shortened by raising temperature in the range of 18-25°C. In most crustaceans the incubation period is highly dependent on temperature (Heasman and Fielder, 1983). Incubation periods of Moreton Bay (Australia) population of *S. serrata* are usually in early spring, at water temperatures of 18-20°C (Heasman and Fielder, 1983). Water temperatures in estuaries and coastal waters along the north coast of South Africa range between 17-22°C in winter and 23-30°C in summer (Robertson and Kruger, 1994). The egg incubation period of *S. serrata* exponentially decreased with increasing temperature (Hamasaki *et al.*, 2003).

Salinity also holds an important role in the embryonic development and egg hatching. During the course of current study, while maintaining the brooders of *P. sanguinolentus* at different salinities, it was observed that the hatching of eggs and release of larvae occurs in the waters of salinity ranging between 30-35‰. Campbell and Fielder (1987) opined that, in *P. sanguinolentus*, the occurrence of prezoae increased when eggs were hatched at salinities below the oceanic salinities that this species normally encounters in nature. Similarly, Sandoz and Hopkins (1944) found that prezoae were more prevalent in *C. sapidus*, when experimental salinities were below 20 ppt.

Among crustaceans; Brachyuran crabs carry the embryos for extended periods of time (Wear, 1974). During brooding, female crabs exhibit complex behaviors (Hoagland, 1979; Baeza and Fernandez, 2002), which seem to be mostly directed towards providing embryos with oxygen (Baeza and Fernandez, 2002). Since oxygen consumption of the embryos increases with progressing development, brooding females increase ventilation frequency providing oxygen to the embryos according to their demand (Baeza and Fernandez, 2002). This change in brooding behavior is related to a 2-fold increase in oxygen consumption of brooding females (after

discounting oxygen consumption of the embryo mass), when compared to nonbrooding females of similar size (Baeza and Fernandez 2002). This suggests that oxygen provision to the brood may account for a substantial fraction of total reproductive costs.

Hatching usually occurs in the early hours of the day in *P. sanguinolentus* as all the portunid crabs generally hatch. During the hatching process, the fully developed first zoea hatch out of the egg cases and swims freely in the water column. Davis (1965) reported the process of hatching, a period of swelling of the eggs followed by osmotic swelling of the inner egg membrane at the start of hatching. The swelling inner egg membrane then ruptures the chorion by pressure from within the larvae plays no part. The inner membrane is subsequently ruptured by mechanical action of the larval abdomen.

Water quality and feed are the important criterion in rearing the berried crabs. If they are not maintained properly diseases will attack which leads to the hatching of unhealthy larvae and their mortality in the early stages itself. Iin *et al.* (2003) observed that the egg quality, egg hatching rate and the quality of the newly hatched zoea are very good when berried crabs fed with formulated feeds when compared with normal diets. The mortality of the eggs has been attributed to fungus, predation, and suffocation in fouled water and changes in temperatures. On the average, only one out of every million eggs survives to become a mature adult (Van Engel, 1958).

The quality of the eggs produced by individual females has also been highly variable. In order to select the best eggs for incubation and subsequent rearing, an estimate of egg quality had to be established and this could be achieved immediately after extrusion. Churchill (2001) reported that, the newly hatched larvae could be subjected to a variety of stress tests including ammonium, salinity, formalin and starvation stress tests and the results of these tests would ultimately be the deciding factors in classifying eggs as good or poor quality.

The study on embryonic development in crabs is more important since the health of the newly hatched zoeae rely on the quality and health of the brooded eggs. The size and health of the ovigerous crabs along with good water quality parameters, like temperature and oxygen will play a major role in the production of healthy eggs and larvae which in turn is a prerequisite for successful production of crab juveniles in any hatchery. The water quality and feed are the important criteria in rearing the berried crabs, and if they are not maintained properly diseases will attack which leads to the hatching of unhealthy larvae and their mortality in the early stages itself.

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