4. EMBRYONIC DEVELOPMENT OF *C.FERIATA*

4.1. Introduction

After the disease outbreak in shrimp farming, crabs are became a candidate species for aquaculture because of their export potential. In order to develop a hatchery technology, the crab larval biology should be thoroughly studied to produce good quality eggs and healthy zoeae from the mother crab. So far there is no reliable hatchery for crabs because of the mass mortality of the zoeal stages. Hence, embryological study forms a base line to get healthy first zoeae from developing eggs. So in the present study an attempt has been made to study the embryonic development of *C.feriata*

4.2. Materials and Methods

Gravid females of C.*feriata* 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 and Soundarapandian, 2009).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 eggs were recorded to understand the different developmental stages (Quinitio and Parado-Estepa, 2003). All these developmental stages were photographed using the digital camera (Nikon, COOLFIX 990, and Japan) attached with the microscope.

4.3. Results

The eggs of C.*feriata* 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.

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 eggs at the time of oviposition were quite distinct and large. They could be divided into six stages, viz., blastula, gastrula, eye placode, pigment, heart beat and freshly hatched first zoea (Amsler and George, 1984).

Stage – I – Blastula (Plate 4)

Eggs were round, golden yellow in colour and were undeveloped and mass of undifferentiated cells are found. Yolk granules were denser. Cleavage and gastrulation were not clear. The diameter of the freshly la id *C.feriata* egg was 0.36mm – 0.37mm.

Stage – II – Gastrula (Plate 4)

Eggs were round and 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 0.38mm – 0.39mm.

Stage – III – Eye placode (Plate4)

Eggs were round orange in colour. Yolk granules were not denser. Segmentation and organogenesis were distinct. The eyespots were appeared as scarlet crescent. The diameter of egg was 40mm – 41mm.

Stage – IV – Pigment (Plate 4)

Eggs were brown in colour with slightly elliptical in shape. Appendages of embryonic larvae were pigmented. The diameter of the egg was 0.42mm – 0.43mm.

Stage – V – Heart Beat (Plate 4)

The eggs were dark brown or black in colour. Eyes were round in shape.Heart starts to beat vigorously. The diameters of *C.feriata* eggs were 0.44mm – 0.45mm.

Stage – VI – Newly Hatched First Zoea (Plate 4)

The freshly hatched I zoea moved freely in the water and its carapace length ranges from 1.05mm - 1.25mm. The hatching success of freshly hatched I zoea is 95%.

4.4. 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 cledoic eggs utilizing fat during embryogenesis and aquatic or non-cledoic 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 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; Jaeckle, 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.

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 egg size of nine species of brachyuran crabs did not vary significantly within species and mean egg size was not correlated with female body size (Hines, 1991). 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 they 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. They 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, 1982; Nakaoka, 2003), 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 (Warner, 1977).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 (Sundaramoorthy, 1987; Krishnan, 1988; Vijayakumar, 1992; Lin *et al.*, 1994; 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 *Emerita asiatica* and reported that the water content steadily increased; conversely, protein content showed a steady decline; protein-bound carbohydrates declined where as 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 (Sundaramoorthy, 1987; Krishnan, 1988; Vijayakumar, 1992; Lin *et al.*, 1994; Veera Ravi, 1994; Parimalam, 2001; Quinitio and Parado-Estepa; 2003; Thirunavukkarasu, 2005; John Samuel and Soundarapandian, 2010).

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.

Sun *et al.* (1989) 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 *C.feriata* lasts for 8-11 days.Almost similar duration was also reported in *P.sanguinolentus* (John Samuel and Soundarapandian, 2010.) Whereas in *P. pelagicus* the period reported was 8-10 days (Josileen, 2002). In general, the duration of embryonic development in the portunid crabs varies between 8 to 14 days depending on the environmental conditions. Sun *et al.* (1989) have reported that the duration of embryonic development could be shortened by raising temperature in the range of 18-25ºC. In most crustaceans the incubation period is highly dependent on temperature (Heasman and Fielder, 1983; Hines, 1986). 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 *C,feriata* 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 prezoeae increased when eggs were hatched at salinities below the oceanic salinities that this species normally encounters in nature. Similarly, Sandoz and Rogers (1944) found that prezoeae were more prevalent in *C. sapidus*, when experimental salinities were below 20‰.

Among crustaceans; brachyuran crabs carry the embryos for extended periods of time (Wear, 1974; Hines, 1986). During brooding, female crabs exhibit complex behaviours (Hoagland, 1979; Hartnoll and Paul, 1982; Naylor *et al.*, 1999; Fernandez-Vergaz *et al.*, 2000; 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 behaviour 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 *C.feriata* 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 compared than 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. Giles Churchill (2003) 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.