6. SEED PRODUCTION OF *C.FERIATA*

6.1. Introduction

The crab *C. feriata is* a portunid crab formerly classified as *C.cruciata* and commonly known as the crucifix crab. It is a commercially important species but is not being cultured commercially. *C.feriatus* is a good potential aquaculture species because of its meat quality, taste and size. However in recent years it is noticed that the catch of the crucifix crab is decreasing and for future development and conservation of the species, aquaculture is a right choice to increase the production. In India very few studies have been attempted concern with reproductive biology and population characteristic of crucifix crab (Padayatti, 1990; Josileen, 2011).No studies have been reported on the life cycle and seed production of the species. Few attempts have been made in the west Asian countries on the larval stages and seed production (Motoh and Villaluz, 1976;, Hu *et al.,* 1983; Parado-Estepa *et al.*, 2002, 2007). Knowledge on the life history of the species is very important for captive seed production.Considering the potential of the species seed production experiment was initiated in the present study on the crucifix crab, *C.feriata*.

6.2. Materials and Methods

Broodstock Maintenance

Healthy gravid females of *C.feriata* with all the appendages intact and the characteristic yellow colured eggs were collected from the Parangipettai coastal waters (Plate 5). They were disinfected with 200 ppm formalin for 30 minutes. Crabs were kept in holding tanks at a salinity of 35±1‰, pH 8.2±0.1, temperature 27±2°C and photophase 14 hours light and 10 hours darkness with continuous aeration. Filtered seawater was used for the entire operation and 50% of water was exchanged every day. Mussel and clam meat were given as food. Females about to hatch their eggs were identified by their egg colouration, absence of yolk and vigorous limb movements in the embryo. Broodstock with grey eggs were transferred to the hatching tanks. Eggs hatched during the early hours of the day by jerking movements of the abdomen which is probably to disperse the newly hatched larvae.

Structure of Larval Rearing Tanks

Circular fiberglass tanks (flat bottom) with the capacity of 250 litres were used (size 3 feet height X 2 feet in diameter).

Water Quality Management

Seawater was brought to the laboratory and filtered by a 0.5mm mesh size cloth sock. The seawater was allowed to settle in a sedimentation tank for 24 hours and passed through sand filters. The water was disinfected by adding calcium hypochlorite (10.8 grams for 500 litres) and allowed to stand for another 24 hours (Lagoc, 1990). The water was vigorously aerated for the next 24 hours and passed through cotton filters at the rate of five litres per hour. Antibiotics such as oxytetracyclin and ciproflaxin were added to the rearing water. During the experimental period, salinity, dissolved oxygen, pH and temperature were recorded by using a Centuary Water Analyzer Kit Model CK 711.

Larval Culture

Immediately after hatching the aeration was stopped for few minutes for settling the hatching waste and the weak larvae.The newly hatched active photopositive zoeae I congregate along water interfaces.They were siphoned into glass beakers and counted. The number of larvae was estimated (at the rate of 50 / litre,) and introduced into rearing tanks.The entire larval cycle (zoeae I to megalopa) was carried out at 35‰ filtered seawater.

Live Feed Culture

Cultures of algae, *C. marina* and rotifer, *B. plicatilis* were raised simultaneously in the wet laboratory.The culture details of *C. marina*, rotifer and also *Artemia* hatching were already mentioned earlier.

Feeding

Newly hatched zoeae I was fed three hours after stocking. Larvae were fed twice daily in the morning (8:00 AM) and evening (5:00 PM). All the zoeal stages and megalops were fed with rotifers (*B. plicatilis*) and *Artemia* nauplii daily. In the morning larvae were fed with *B. plicatilis* at the rate of 5-15 per ml (zoea I& II), 15-25 per ml (zoeae III), 25-40 per ml (zoeae IV-VI) and 70-80 per ml (megalopa). In the evening thawed *Artemia* nauplii, 2-20 per larvae for zoeae I- III and 20-50 per larvae for zoeae IV to megalopa were provided.The zoeae VI on reaching the megalopa stage, were provided with pebbles as substrate, oyster shells were suspended by nylon ropes as hide in the rearing tanks.

Daily the rearing water was exchanged up to three fourths of the tank capacity. Dead larvae and exuviae were siphoned out during this time to prevent contamination. A cloth sock was used to prevent the loss of live zoeae. Mild and continuous aeration was provided (10 to 15

bubbles per minute) to the rearing tanks by an air compressor. Care was taken to prevent irregular aeration using a generator.

Larval numbers were estimated daily by counting 8 replicates taken in 500 ml beakers from the rearing tanks. Assessment of each zoeal stage was done at completion of different levels of metamorphosis to determine feeding and survival rates. Thus mass rearing from different broods was carried out simultaneously in 5 rearing tanks in order to replicate the experiment.

Statistical Analysis

The data were subjected to One- way analysis of variance (ANOVA) and difference between means were determined by Duncan's multiple range tests ($P<0.05$) using SPSS version 17.0.

6.3. Results

The regular monitoring of water quality parameters in the culture medium did not show much variation. Parameters like salinity - 35±1ppt, dissolved oxygen - 5±1 ppm, temperature - 30±1°C and pH - 7.3 to 8.2 were recorded during the study period. The larval development of *C.feriata* includes six zoeal stages and a megalopa stage

(Plate 6). Each zoea has a long rostrum, a dorsal spine and a pair of short lateral spines on the carapace. Zoeae resemble the typical portunid larva in morphology and are very active and photopositive. The total duration of the zoeal phase varied between 23-26 days during different trails. The stages were idenitified based on the setation of the telson, number of abdominal segments and pleopods of the abdominal somites.

Identification of Larval Stages and Intermoult Duration

Zoea-I

Eyes are sessile. Abdomen is five segmentated plus the telson. Telson forked and each fork bearing one inner and dorsal spine. Inner margin of each fork bears three, long serrated setae (3+3). It took 4-5 days to reach next stage.

Zoea-II

Development of stalked eyes. Abdomen is five segmented as in the previous stage.Abdominal somites 3-5 bears more distinct lateral spines. Telson bears a pair of short, plumose setae on median margin of clept part (4+4). It took 3-4 days to reach next stage.

Zoea-III

Abdomen develops 6 segments and lateral spines on 3-5 somites are longer.Telson is similar to that of previous stage (4+4). It took 3-4 days to reach next stage.

Zoea-IV

Abdomen is as in the previous stage.Pleopod buds just started appearing at the ventral posterior end of somites 2-5. Telson adds one additional short setae on inner margin (4+1+4). It took 3-4 days to reach next stage.

Zoea-V

Pleopod buds on the abdominal somite second to sixth developed. Telson has developed additional short setation inner margin (5+5). It took 3-4 days to reach next stage.

Zoea-VI

Abdominal somites and telson are similar to previous stage.Pleopod buds well developed, biramous on somites 2-5 and

uniramous on somite 6. Telson setation same as in previous stage (5+5). It took 3-4 days to reach next stage.

The complete larval development took a span of 22-26 days.According to Duncans multiple range test the larval duration is more or less same for I & III zoeal stages.Similarly II, IV,V &VI zoeal stages are also similar.However, I & III and II, IV,V &VI zoeal stages are significantly varied between each other. The details of the intermoult duration of different larval stages are given in table 5 & fig.3.

Table 5. Intermoult Duration of Different Larval Stages

Larval Stage	Intermoult Duration (Days)
I Zoea	4.52^{b}±0.41
II Zoea	3.72^{c}±0.36
III Zoea	4.01^{b}±028
IV Zoea	3.97^{c}±0.58
V Zoea	3.48^{c}±0.83
VI Zoea	3.49^{c}±0.48
Megalopa	5.20^{a}±0.12
Total days	**23-26**

Means with different superscript are significantly different ($P<0.05$; Duncan's multiple range test).

Survival

The details of the survival rate of different larval stages are presented in Table 6. A maximum of 95% of survival was observed during the first zoeal stage and thereafter the survival was gradually decreased.The survival percentage shows that mortality was high in all zoeal and megalopa stages.The survival rate of zoeal and megalopal stages are significantly varied each other.

Table 6. Survival (%) of Different Larval Stages

Larval stage	Survival (%)
I Zoea	$95.00^{a}\pm1.46$
II Zoea	$71.75^{b}\pm2.36$
III Zoea	$44.0^{c}\pm3.36$
IV Zoea	$34.5^{d}\pm4.79$
V Zoea	$25.5^{e}\pm0.50$
VI Zoea	$12.0^{f}\pm0.50$
Megalopa	$5.25^{g}\pm0.50$

Means with different superscript are significantly different ($P<0.05$; Duncan's multiple range test).

6.4. Discussion

In order to reduce the gap between supply and increasing demand through the commercialization of captive raised organisms, one special constraint must be overcome – larval mass rearing (Calado *et al.*, 2003). To date broodstock development and hatchery seed production of crabs (in terms of percentage of survival) have been experimental, though the technology has developed for the production of crab seeds in many countries. Several studies related to the survival of the commercial portunid crab larvae have used brine shrimp, rotifers and algae as food, since the nutrition turns to be vital to the larval survival. Apart from the live feed, the water quality parameters such as salinity and temperature will also play an important role in the larval growth and survival during mass culture experiments.

Seawater appears to be an excellent medium for bacterial survival and the microbiological safety of all sea- and freshwater used must be assured to make it the first line of defence against harmful bacterial contamination from other sources (Blackshaw, 2001). Water quality (temperature, salinity, nutrient and hygiene) is a significant factor in larval survival (Motoh *et al.*, 1977). Since the nutritional aspects of the hatcheries have been standardized much, it is hypothesized that the microbiological environment in the cultures is

now the most significant constraint on the achievement of consistently high levels of survival through the larval cycle at production scale (Mann, 2001). So, now a days the role of antibiotics is much felt in the mass scale culture and they are found to be enhancing the premetamorphic survival of zoeae while rearing rate of zoeal development and success of metamorphosis to megalopa unaltered (Thirunavukkarasu, 2005; Nunnam John Samuel *et al.*, 2011;Josileen, 2011). In the present study, the antibiotics such as oxytetracyclin and ciproflaxin were used to control the microbial load in the rearing tanks and also to provide a healthy environment to the larvae to grow and metamorphose successfully with less intermoult duration. The survival and the health of the larvae were improved after applying these two antibiotics to the rearing medium. Brick (1974) made a preliminary study on antibiotics such as penicillin-G, streptomycin and polymycin-B, individually and in combination, on survival and development of the crab larvae of *S. serrata*. Thirunavukkarasu (2005) and Nunnam John Samuel *et al.* (2011) made similar study in *S. tranquebarica* and *P.sanguinolentus* by treating water with ciproflaxin and oxytetracyclin to control the microbial load in the larval rearing medium.

Survival and longevity of marine invertebrate larvae are influenced by abiotic factors such as water temperature and salinity,

and by biotic factors such as food availability, food quality and predation. In the present study the water salinity was maintained at 35 ppt since the spawning, embryogenesis and hatching of eggs generally takes place in coastal regions as in *P.pelagicus* (Soundarapandian *et al.*, ,2007) and *P.sanguinolentus* (Nunnam John Samuel *et al.*, 2011). The results of the present study indicate that the most suitable range of temperature for crab larvae was found to be 30 to 32.5°C. The higher mortality rate of the zoea I, especially during the first three days of culture might be due to fluctuations in water temperature in brooder's tank and the mass culture tanks. Mann *et al.* (2000) have also noticed that the temperature shock caused larval stress and mortality has been surmised when there is an unintentional temperature fluctuation of 5^0C's due to equipment failures that lead to abnormally high mortality rates. The better survival evidenced in the present study with the larvae of *C.feriata* might also be due to the higher temperature (30±1°C). The previous studies on the effect of temperature on larval rearing revealed that the larvae could not survive in lower temperatures. The lower survival rate was evident when the larvae of *S. serrata* reared at low mean temperatures, *i.e.*, at 27.5°C by Ong (1964, 1966), at 24°C by Du Plessis (1971), at 22°C by Brick (1974) and at 27°C by Heasman and Fielder (1983).

The quantity and quality of food supply are the chief factors regulating the duration of larval development (Roberts, 1974; McConaugha, 1985). Insufficient food supply will prolong larval development, thus, increasing the risks of larval mortality due to predation and starvation. At first feeding, larvae usually restrict the size of the food particles that can be ingested. Providing prey of a suitable size is one of the more important feeding strategy aspects for crustacean larvae, which hatch with little or no yolk reserve. Suitable prey for larvae should meet three general criteria: namely, they should be an appropriate size for easy capture and consumption, they should be present at an adequate concentration, and they should contain essential dietary nutrients (McConaugha, 1985). The seed production of aquatic species is almost entirely depending on the successful production of live food organisms, principally rotifers, followed by *Artemia* and that too enriched live food organisms of improved quality. The superiority of the live food organisms in larval nutrition over existing compounded diet is partly due to the availability of exogenous enzymes through the live food, which in combination with endogenous enzymes of the animal lead to efficient digestibility (Chen and Lin, 1992). Young animals with less developed digestive system benefit more from exogenous enzymes than do adults. The exact quantity of food required at each stage cannot be prescribed as it depends on the

utilization of feed by the larvae and must be judged visually by the operator.

In the present study the zoea were initially fed with *B. plicatilis*, since the small size of first zoea refused to feed on *Artemia* nauplii. *B. plicatilis* is small in size and can be ingested completely by small decapod crustacean larvae. Rotifer gut is usually filled with bacteria and algae, which could provide additional nutrition for the larval forms of decapods. It has a short life cycle with simple dietary requirements can be cultured in high densities and has a favorable nutritional content (Lovett and Fielder, 1988). Emmerson (1984) reported that caloric content of rotifers per gram ash dry weight is not significantly different from that of *Artemia* nauplii. The larvae of *C.feriata* were provided with *Artemia* nauplii only later stages especially from III zoea onwards. The studies on the crab larvae showed that the absence of small prey during the early zoeal stage of *C. sapidus* resulted in high mortalities. Sulkin (1975) reported that the smaller size and slower swimming speed of *B. plicatilis* apparently allow their capture and manipulation by small crab zoea. He also found out that newly hatched larvae of *C. sapidus* couldn't pass to the next stage when fed with *Artemia* nauplii. Soundarapandian *et al*. (1998) observed that the *M. malcolmsonii* early larval stages apparently graze on the appendages of

Artemia nauplii but could consume entire rotifers. The swimming crab *P. trituberculatus* was fed with *Artemia* nauplii from third zoea stage to avoid cannibalism (Takeuchi *et al.*, 1999).

Combination of *Artemia* nauplii and rotifer obtained mixed results when fed to the crab larvae by different authors. Brick (1974) showed that mud crab larvae fed on *Artemia* nauplii alone had a higher survival rate than those fed on rotifers. He suggested that the addition of rotifers might have contributed to the deterioration of the culture medium, through oxygen consumption or release of metabolites, without providing any nutritional benefit for the larvae. McConaugha (1985) reported that *Rithropanopeus harrisii* larvae fed on rotifer could not metamorphose due to low lipid content and low feeding efficiency. Baylon and Failaman (1999) demonstrated that the rotifers are more important than *Artemia* nauplii for maintaining the survival rate of the first and second zoeal stages, where as supplying *Artemia* or rotifers as the sole prey failed to maintain the survival rate of mud crab. In most of the previous studies, successful seed productions obtained when rotifer and *Artemia* nauplii were used as feed (Soundarapandian *et al.*, 2007). Successful seed production was reported in *P. trituberculatus* offered with rotifer and *Artemia* nauplii (Hue *et al.*, 1972; Takeuchi, 2000; Kobayashi *et al.*, 2000). Minagawa and Murano (1993)

recommended mixed diets (*Artemia* nauplii + rotifer) for mass seed production of frog crab, *R. ranina*. In the present study, both rotifer and *Artemia* nauplii have been offered to the larvae of *C.feriata* as experimented in the previous study. However, the survival rate is not encouraging. Various reasons are attributed for the lower survival even though standard live foods were used.

Information on larval nutritional requirements is important for the establishment of successful seed production technology. Improving the food value of *Artemia* through enrichment prior to feeding of fish or crustacean larvae is now a common practice among marine hatcheries for improved survival, growth and stress resistance (Sorgeloos and Leger, 1992). The advantage of using *Artemia* nauplii for feeding the larval crab is that it could have contribute to the lipid and energy resulting in a high feeding efficiency. In general live food is lack of n-3 HUFA, without which the growth of the developing larvae will not be optimized. Although data on the nutritional requirements of brachyuran crabs in the larval stage is limited, the essential fatty acid requirements has been revealed for several species (Levine and Sulkin, 1984; McConaugha, 1985; Hamasaki *et al.*, 1998, 2002; Soundarapandian *et al.*, 1998; Takeuchi *et al.*, 1999 a, b, 2000; Kobayashi *et al.*, 2000, 2001; Mann *et al.*, 2000; Suprayudi *et al.*,

2002, 2004; Kannupandi *et al.*, 2003; Thirunavukkarasu, 2005; Soundarapandian *et al.*, 2007; Nunnam John Samuel *et al.*, 2011). Earlier study showed that feeding mud crab larvae with live food containing a low nutrition value, especially n-3 HUFA resulted in lower survival and longer intermoult period. Hamasaki *et al.* (1998) emphasized that as the amount of n-3 highly unsaturated fatty acids (n-3HUFA) increases in the feed, the larval survival, growth and velocity of development were also been improved in the larvae of swimming crab,*P. trituberculatus*. The swimming crab larvae fed with *Artemia* containing n-3 HUFA from the 3rd stage to obtain high survival rate (Takeuchi, 2000). All the *Artemia* do not possess all the essential fatty acids in required concentrations, particularly 22:6 n-3 (Yone, 1978; Leger *et al.*, 1985; Bell *et al.*, 1986). Similarly Watanabe *et al.* (1983) reported that rotifer cultured with the yeast were quiet low in n-3 unsaturated fatty acids. The larvae fed with cuttle fish liver oil enriched *Artemia* nauplii and rotifer showed accelerated growth, and survival (Kannupandi *et al.*, 2003). Larval growth and morphogenesis might be controlled by the nutritional conditions of the prey and the larva itself. Although it has not been shown for brachyuran larvae that the morphogenesis is affected by nutritional factors, there are a few reports that dietary n-3HUFA improves the growth which was represented by the carapace width of the first crab stage in the larval rearing of *S.*

serrata (Suprayudi *et al.*, 2002), *S. paramamosain* (Takeuchi *et al.*, 2000; Kobayashi *et al.*, 2000) and the swimming crab, *P. trituberculatus* (Hamasaki *et al.*, 1998; Takeuchi *et al.*, 1999, 2000).

The larval development of *C.feriata* resembles other portunid crabs with variation in number of zoeal stages (Greenwood and Fielder, 1983; Fielder *et al.*, 1984; Anil, 1997;Josileen and Menon, 2004).*C. feriata* crab had six zoeal stages, the maximum number recorded for a portunid crab in the Indo-Pacific region. The larval stages recorded in the present study are found to be similar to the larval stages of *C.feriata*, reported from other regions (Motoh and Villaluz, 1976; Pardo-Estepa *et al.*, 2002, 2007; Josileen, 2011). The final survival rate of *C.feriata* larvae in the present study was 5.25% from VI zoea to megalopa. The survival rate is comparatively higher than that reported for *S. serrata* by Heasman and Fielder (1983). Almost similar report obtained in *P.sanguinolentus* (Nunnam John Samuel *et al.*, 2011) and *C.feriata* (Joslieen, 2011).The reasons could be that the feeding schedules, incorporated combinations of B. *plicatilis* and *Artemia* nauplii. Similarly, Zainoddin (1992) also pointed out that the combination of *B. plicatilis* and *Artemia* nauplii served as feed gave better survival. The survival rate in the zoeal stages (I to IV zoeae, 65 to 35 per cent) achieved by Heasman and Fielder (1983) in the larvae of *S.*

serrata was due to high concentration of *Artemia* nauplii (5 to 30 nos. per ml) used once in a day. Larvae of the related family *P. trituberculatus* have been reared with success (over 60%) on the same species of rotifer in combination with *Artemia* by Hue *et al*. (1972). Minagawa and Murano (1993) recommend a combination of diets to mass rear the larvae of *R. ranina*.

In the present study mortality in all zoeal stages of *C.feriata* regardless of the feeding regimes is quite comparable with the results obtained by Josileen (2002, 2011) in *C.feriata* and Soundarapandian *et al*. (2007) in *P. pelagicus* and Nunnam John Samuel *et al*. (2011) in *P.sanguinolentus*. They pointed out from their experiments that initial mortality during the first two days of the experiment occurred often and relatively high regardless of feeding regimes and are rather unpredictable. They related the mortality to the low viability of the individual larva. Similarly mortality during the zoea VI and megalopa stages was either before moulting, during moulting (includes incomplete moulting) in *C.feriata* larvae. The possible reason cited by Anger *et al*. (1981) is that mortality due to depletion of reserves resulting in larval inability to catch the prey. Similarly, Rosenberg and Costlow (1979) suggested that the majority of the larval population is preparing for the premetamorphic moult to megalopa. Likewise Costlow

and Bookhout (1971) and Christiansen and Costlow (1975) have observed high mortalities in the larvae of *R. harrisii* at the premetamorphic stage. They attribute two reasons for such mortality – 1. The larvae at this premetamorphic stage are extremely susceptible to unfavorable environmental conditions at this time of life cycle and 2. The metabolic cost of metamorphosis is very high and appears to decrease the capacity of larvae to counteract these unfavorable conditions. Cannibalistic tendency was observed in all zoeal and megalopal stages and it was the main reason for the higher mortality from Ist zoea to megalopa. The shelter provided to this larval stage was found effective and reduced the cannibalism to some extent.

The maintenance of good water quality and hygiene during the larval culture results in higher survival percentages. The hygiene begins with the preparation of the broodstock and continues up to the metamorphosis of megalopa. The aim is to restrict the growth of potential pathogens, including bacteria, fungi, viruses and protozoa in the culture system. It can be safely assumed that all inputs into a culture tank are potential sources of infection that may reduce rates of larval survival and metamorphosis. All tanks and equipment used in the culture must first be effectively sterilized following standard

methods before use as a simple precautionary measure (Brick, 1974; Blackshaw, 2001).

In the present study larval mortality was spread throughout the six zoeal and megalopa stages and was not confined to any one particular stage of development. However, highest mortality occurred during the transition from first zoea to second and sixth zoea to megalopa. This may be due to the inability of the larvae to break completely away from their casts. Similar observations were already made in *P.sanguinolentus* (Nunnam John Samuel *et al.*, 2011) and *C.feriata* (Josileen, 2011) Many workers have reported that the mortality was high during the first zoeal moult to second zoea in *S. serrata* (Ong, 1964; Heasman and Fielder, 1983; Anil, 1997).

Costlow *et al.* (1962) also observed high larval mortality in the first zoeal stage in *Panopeus herbstii.* Anil (1997) reported 40% mortality in the first zoeal stage in *S. oceanica.* Costlow and Bookhout (1959) in *C. sapidus* and Raman *et al.* (1987) in *P. pelagicus* have reported high larval mortalities in the first two zoeal stages.Costlow and Fagetti (1967) found more mortality in later zoeal stages of *Cyclograpsus cinereus.*

The crab *C.feriata* has been listed one of the six suitable species for stock enhancement and culture in the international forum on the culture of portunid crabs (Williams and Primavera, 2001). The present study also found that *C.feriata* suitable for seed production. Hence, using the present study as database, it is clear that the larvae of *C.feriata* can be cultured on a large scale by adding more importance to the water quality to prevent mortality by bacterial infection and with the combination of *B. plicatilis* and *Artemia* nauplii as feed.The rotifer and the enriched *Artemia* nauplii were found to be supporting the larval development very much and hence better survival could be observed. No ciliate and bacterial infection was noted during the study period.