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Seed production of commercially valuable portunid crab *Portunus Sanguinolentus* (Herbst)

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ABSTRACT

Mass seed production technology was tried in the present study in three eyed crab, P. sanguinolentus. To enhance the survival, the larvae were offered with sufficient amount of enriched Artemia nauplii and B. plicatilis. During the experimental period the larvae were provided with cordial environmental parameters. In order to avoid cannibalism shelters were provided in the culture tanks. The total duration for zoea I and II required 3-4 days, while zoea III and IV took 2-3 days and megalopa required 6-7 days to metamorphose into crab instar stage. The complete larval development took a span of 15-18 days. The intermoult duration was 3.62±0.32, 3.68±0.37, 2.75±0.35, 2.56±0.51 and 6.50 ±0.40 days for zoea I, II, III, IV and megalopa respectively. The final survival rate from megalopa to first crab was 5.25%. The survival rate for each zoeal and megalopal stages are 71.75±2.36, 44.0±3.36, 34.5±4.79, 12.0±2.94 and 5.25% for zoea I, II, III, IV and megalopa respectively. A maximum of 28% of mortality was observed during the first zoeal stage itself and thereafter the mortality was gradually increased.

Key words: *Portunus sanguinolentus*, *Artemia nauplii*, Zoea, Megalopa, Crab instar.

INTRODUCTION

In the past, crabs were considered a secondary species to shrimps and finishes. However, crab culture gained its importance from the beginning of last decade due to great demand of live crabs and crab products in the export market. The crab culture is presently dependent on wild caught seeds that are not sufficient [1, 2]. The natural seed availability is declining due to

indiscriminate collection of juveniles for farming. The collected seeds are also not uniform in size and availability throughout the year is a big question mark. Many countries like Japan, Philippines, India, Indonesia, Thailand, Bangladesh, Vietnam, Australia and USA are actively involved in crab culture and research. However, in most of the countries to date, hatchery seed production of crab has been experimental, through the technology has developed for the production of crab seed. For the last three decades many hatcheries in Japan produce seeds of *P. trituberculatus* for the restocking progarmme. Philippines also actively involved in crab culture and contributed significantly in hatchery and farming technology for the mud crabs. However, there is no seed production technology is available for commercially important crab, *P. sanguinolentus*. To stop the depletion of the natural resources and to get uniform sized seeds throughout the year for farming. Seed production technology is badly needed. Hence, the present study is designed to develop a simple technology for the mass seed production of *P. sanguinolentus*. Since, *P. sanguinolentus* brooders are available throughout the year along the Parangipettai coast [3].

MATERIALS AND METHODS

Selection of brooders

Healthy gravid females of *P. sanguinolentus* with all the appendages intact and the characteristic yellow colured eggs were collected from the Parangipettai coastal waters. Crabs were kept in a holding tank at a salinity of 35±1‰, pH 8.2±0.1, temperature 27±2ºC and photophase 14 hours light and 10 hours darkness with continuous aeration. Filtered seawater was used for the entire operation and 50% of water is exchanged every day. Mussel and clam meat were given as food. Females about to hatch their eggs were identified by their egg colouration, abscence of yolk and vigorous limb movements in the embryo. Broodstock with gray 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. 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 [4]. 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.

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 / liter, 10,000 / 200 liters) and introduced into rearing tanks. The entire larval cycle (zoeae I to IV, megalopa and first crab stage) was carried out at 35‰ filtered seawater.

The Artemia nauplii were harvested from the Artemia hatching tank and placed in a plastic tub with required quantity of water. The enrichment solution (Culture Selco-INVE, Belgium) was added at a concentration of 0.1%. The nauplii were enriched for 12 hours and after washing in seawater the nauplii were fed to the crab larvae.

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 4 zoeal stages and megalops were fed with rotifers (*B. plicatilis*) and *Artemia* nauplii (OSI Brine shrimp eggs, USA) 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 (zoea III), 25-40 per ml (zoea IV) and 70-80 per ml (megalopa). In the evening thawed *Artemia* nauplii, 2-20 per larvae for zoea I, II, III and 20-50 per larvae for zoea IV and megalopa were provided.

The zoea IV on reaching the megalopa stage, were provided with pebbles as substrate, oyster shells were suspended by nylon ropes as hide in the rearing tanks. Soon after the first crab stage metamorphosed they were transferred to new rearing tanks having water of similar quality, parameters and conditions.

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.

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 O_2 ml/lit, temperature of 30±1ºC and pH of 7.3 to 8.2 were recorded during the study period.

The complete larval development of *P. sanguinolentus* consisted of four zoeal and one megalopal stages before moulting into crab instar stage. Total duration for zoea I and II required 3-4 days, while zoea III and IV took 2-3 days and megalopa required 6-7 days to metamorphose into crab instar stage. The complete larval development took a span of 15-18 days. The details of the intermoult duration of the different larval stages are given in Table. 1.

The details of the survival rate of different larval stages are presented in Table 2. The final survival rate from megalopa to first crab was 5.25%. A maximum of 28% of mortality was observed during the first zoeal stage itself and thereafter the mortality was gradually increased. The survival percentage shows that mortality was high in all zoeal and megalopa stages.

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 [5]. 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 defense against harmful bacterial contamination from other sources [6]. Water quality (temperature, salinity, nutrient and hygiene) is a significant factor in larval survival [7]. 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 [8]. 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 [9]. 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 [10] 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 [9] made similar study in *S. tranquebarica* 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. The results of the present study indicate that the most suitable range of temperature for crab larvae was found to be 30 to 32.5ºC. 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. 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[11]. The better survival evidenced in the present study with the larvae of *P. sanguinolentus* might also be due to the higher temperature (30±1ºC). The previous studies on the effect of temperature on larval rearing revealed that the larvae could not survive in low temperatures. The low survival rate was evident when the larvae of *S. serrata* reared at low mean temperatures, *i.e*., at 27.5ºC by Ong [12, 13], at 24ºC by Du Plessis [14], at 22ºC by Brick [10] and at 27ºC by Haesman and Fielder [15]. Temperature had a strong influence on the survival of *P. pelagicus* larvae, with marked negative effects at 22.5ºC.

The quantity and quality of food supply are the chief factors regulating the duration of larval development [16, 17]. 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 [17]. The seed production of aquatic species is almost entirely depending on the successful production of live food organisms, principally rotifers, followed by *Artemia.* 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 [18]. 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 the 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 favourable nutritional content [19]. The caloric content of rotifers per gram ash dry weight is not significantly different from that of *Artemia* nauplii[20]. The larvae of *P. sanguinolentus* were provided with *Artemia* only from 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. The smaller size and slower swimming speed of *B. plicatilis* apparently allow their capture and manipulation by small crab zoea and also newly hatched larvae of *C. sapidus* couldn't pass to the next stage when fed with *Artemia* nauplii [21]. Early larval stages of *Macrobrachium malcolmsonii* apparently graze on the appendages of *Artemia* nauplii but could consume entire rotifers [22]. The swimming crab *P. trituberculatus* was fed with *Artemia* nauplii from third zoea stage to avoid cannibalism [23].

Combination of *Artemia* nauplii and rotifer obtained mixed results when fed to the crab larvae by different authors. Brick [10] 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 [17] reported that *Rithropanopeus harrisii* larvae fed on rotifer could not metamorphose due to low lipid content and low feeding efficiency. Baylon and Failaman [24] demonstrated that the rotifers are more important than *Artemia* nauplii for maintaining the survival rate of the first and second zoeal stages, whereas 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 [25]. Successful seed production was reported in *P. trituberculatus* offered with rotifer and *Artemia* nauplii [23, 26, and 27]. Minagawa and Murano [28] recommended mixed diets (*Artemia* nauplii + rotifer) for mass seed production of frog crab, *Ranina ranina*. In the present study, both rotifer and *Artemia* nauplii have been offered to the larvae of *P. sanguinolentus* 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 [29]. 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 foods are 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[9,11,17,22,23,25,27,30-33,35,36]. Earlier study showed that feeding mud crab larvae with live food containing a low nutrition value, especially n-3 HUFA resulted in low survival and longer intermoult period. Hamasaki *et al.* [31]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 [23]. All the *Artemia* do not possess all the essential fatty acids in required concentrations, particularly 22:6 n-3 [37-39]. Similarly Watanabae *et al.* [40] 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 [36]. 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* [34] *S. paramamosain* [23, 27] and the swimming crab, *P. trituberculatus* [31, 41].

The final survival rate of *P. sanguinolentus* larvae in the present study was 5.3% from Megalopa to first crab instar. The survival rate is comparatively higher than that reported for *S. serrata* by Haesman and Fielder [15]. The reasons could be that the feeding schedules, incorporated combinations of *B. plicatilis* and *Artemia* nauplii. Similarly, Zainoddin [42] 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 Haesman and Fielder [15] on 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* [26]. Minagawaand Murano [28] recommended a combination of diets to mass rear the larvae of *R. ranina*.

In the present study mortality in the first two zoeal stages of *P. sanguinolentus* regardless of the feeding regimes is quite comparable with the results obtained by Joseleen Jose [43] and Soundarapandian *et al.* [25] in *P. pelagicus*. They point 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 IV and Megalopa stages was either before moulting, during moulting (includes incomplete moulting) in *P. sanguinolentus* larvae. The possible reason cited by Anger *et al.* [44] is that mortality due to depletion of reserves resulting in larval inability to catch the prey. Similarly, Rosenberg and Costlow [45]

suggested that the majority of the larval population is preparing for the premetamorphic moult to Megalopa. Likewise Costlow and Bookhout [46] and Christiansen and Costlow [47] 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 unfavourable 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 unfavourable conditions. Cannibalistic tendency was observed from megalopa onwards and it was the main reason for the higher mortality from megalopa to first crab stage. 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 to crablets. 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 [6, 10].

In the present study larval mortality was spread throughout the four 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; fourth to megalopa and megalopa to crab stage. This may be due to the inability of the larvae to break completely away from their casts. Many workers have reported that the mortality was high during the first zoea moult to second zoea in *S. serrata* [12, 15, 48].

Table 1. Intermoult duration of different larval stages

Larval stage	Intermoult duration (Days)
I Zoea to II Zoea	3.62±0.32
II Zoea to III Zoea	3.68±0.37
III Zoea to IV Zoea	2.75±0.35
IV Zoea to Megalopa	2.56±0.51
Megalopa to Crab instar	6.50±0.40
Total days	**15 - 18**

Table 2. Survival (%) of different larval stages

Larval stage	Survival (%)
I Zoea to II Zoea	71.75±2.36
II Zoea to III Zoea	44.0±3.36
III Zoea to IV Zoea	34.5±4.79
IV Zoea to Megalopa	12.0±2.94
Megalopa to Crab instar	5.25±0.50

Costlow *et al*. [49] also observed high larval mortality in the first zoeal stage in *Panopeus herbstii.* Anil [48] reported 40% mortality in the first zoeal stage in *S. oceanica.* Costlow and Bookhout [50] in *C. sapidus* and Raman *et al.* [51] in *P. pelagicus* have reported that high larval mortalities in the first two zoeal stages. In *Cyclograpsus cinereus,* found more mortality in later zoeal stages[52].

Hence, using the present study as database, it is clear that the larvae of *P. sanguinolentus* 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 *B. plicatilis* and enriched *Artemia* nauplii were given as feed for the zoeal stages and megalopa respectively. 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.

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