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Seed Production of Commercially Important Blue Swimming Crab *Portunus pelagicus* (Linnaeus)

P. Soundarapandian, E. Thamizhazhagan and N. John Samuel
CAS in Marine Biology, Annamalai University, Parangipettai-608 502, Tamil Nadu, India

Abstract: To establish a commercial hatchery in crabs a mass seed production experiment was conduced in the blue swimming crab, *Portunus pelagicus*. It undergone 5 zoeal and I megalopa stage. The larvae took 24.3 days to complete their cycle. The rotifer (*Brachionus plicatilis*) and *Artemia* nauplii were provided as feed for the larval forms. The survival rate decreases with increasing larval stages. While, larval duration increases with increasing larval stages. Maximum survival (71.6%) was reported in I zoea and minimum (4.3%) was in Megalopa when metamorphosed into Ist crab instar. Initially the larvae took 3 days to reach the next stage (I-IV stage). It was 4-5 days for the later stages (IV-Ist crab instar). Mortality and cannibalism is a common problem encountered during the study period. The reason for the mortality and cannibalism is discussed in detail.

Key words: *Portunus pelagicus*, *Artemia* nauplii, *Brachionus plicatilis*, megalopa, gastrula

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 (Keenan, 1999; Fortes, 1999). 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, though 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. pelagicus*. 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. pelagicus*. Since, *P. pelagicus* brooders are available throughout the year along Parangipettai coast (John Samuel *et al*., 2004).

MATERIALS AND METHODS

Broodstock Management

Healthy, sponge-bearing females of *P. pelagicus* were collected from the Parangipettai coast (Lat. 11° 29'N and Long. 79° 46') and brought to the laboratory. They were immediately immersed

Corresponding Author: P. Soundarapandian, CAS in Marine Biology, Annamalai University, Parangipettai-608 502, Tamil Nadu, India

Table 1: Water quality parameters of the brooders

Water quality parameters	Optimum range
Salinity	33-35 ppt
Temperature	28-31°C
Dissolved oxygen	5.0 to 6.0 mg L^{-1}
pH	7.5 to 8.0
Photoperiod	12/12 h L/D

Table 2: Feeding schedule for the larval stages of *P. pelagicus*

	Larval stages					
Food	I zoea	II zoea	III zoea	IV zoea	V zoea	Megalopa
Rotifer (inds mL^{-1})	5-10	5-10	10-15	10-15	15-20	-
Artemia nauplii (inds mL^{-1})	-	-	5-10	15-20	15-25	20-25
Bivalve meat	-	-	-	-	-	5% of BW

in a prophylactic dip of 200 ppm formalin for 30 min (Parado Estepa Emilia *et al*., 2002). The crabs with yellow colour eggs were kept in 50 L fiberglass tank. The physico chemical parameter maintained during the experimental period is given in the Table 1. During incubation period, the brooders were fed with oyster meat (*Crassostrea madrasensis*). At every morning left over feed and faeces from the tank was removed and half of the water was replaced with fresh seawater. Larvae hatched during the early hours of the day.

Larval Rearing

Stocking

The newly hatched healthy zoeae were stocked at a density of 50 zoeae L^{-1} in a 50 L plastic tuff (2 feet height and 3 feet diameter). During the experimental period, the salinity of the water was 33-35 ppt, temperature 28-31°C, pH 7.5-8.0 and dissolved oxygen close to saturation (5-6 mg L^{-1}).

Feeding

All the 5 zoeal and megalopa stages were fed with rotifers (*B. plicatilis*) and *Artemia* nauplii (San Francisco Bay strain). The I zoeae were fed three hours after stocking. The larvae were fed with *B. plicatilis* at the rate of 5-10 per mL (zoea I, II). The III and IV zoeae were fed with rotifers at the rate of 10-15 per mL. In addition to rotifers, *Artemia* nauplii also provided from III zoea onwards. The addition of rotifers was stopped once the zoea reached V stage. The megalopa was provided with *Artemia* nauplii and bivalve meat. The amount of feed not consumed was carefully noted every morning and the amount of feed was adjusted accordingly. Aeration was given through out the experiment (Table 2).

Artemia cyst (San Francisco Bay strain) was hatched in a glass jars containing filtered seawater of 35 ppt. One gram of cyst was added in 1 L of seawater and was provided with a light source and vigorous aeration. After 18 h *Artemia* cyst hatched out into nauplii. The freshly hatched nauplii was washed in fresh seawater and offered to the zoeal stages of crab. Rotifers were raised in the laboratory following the method of Soundarapandian and Kannupandi (1998).

Water Treatment

Seawater of required amount was brought to the laboratory and was allowed to settle in a tank for 24 h and disinfected by adding 10-20 ppm Calcium hypochlorite. The water was vigorously aerated for 24 h and excess chlorine was treated with sodium thiosulphate. This treated sea water was used for brooders as well as larvae.

Water Exchange

Fifty percent of water was changed daily. To remove left over feed/detritus and dead larvae each day, the aeration was stopped temporarily and settled particles were removed from the tank bottom by siphoning. Soon after the appearance of first crab stage they were transferred to new rearing tanks having similar water quality parameters and conditions. Survival rate and larval duration was calculated once each zoea metamorphosed into next stage. The same experiment was repeated three times.

Identification of Larval Stages

The developmental stages (Table 3) of the larvae were observed under binocular microscope and classified according to Shinkarenko (1979).

Statistical Analysis

The data was analysed for statistical significance by Two-way analysis of variance (ANOVA) using SPSS/PC$^+$ package.

RESULTS

Survival Rate

The survival rate was higher in I zoea stages (71.6%). The survival rate was decreased regularly when the development proceeds (Table 3). The survival rate was very low when the V zoea metamorphosed into megalopa (12.6%). Similarly low survival was observed when megalopa moulted into first crab instar (4.3%) (Fig. 2). The two-way AVONA showed significant variations between trails as well as between zoeal stages (Table 4).

Larval Duration

The larvae took 24.3 days to complete their development. The larvae spent approximately 3 days to reach the next stage from I zoeae stage to IV zoeae stage. For reaching of IV zoeae stage to megalopa took 4 days. However, from megalopa to first crab it was 5 days. For earlier zoeal stages growth was fast and later stages the growth was slow (Table 3). The two-way ANOVA showed significant variations between trails and non-significant variations between different zoeal stages (Table 5).

Table 3: Survival rate and larvae duration of *P. pelagicus*

Zoeal stages	Survival rate (Mean±SD)	Time duration (Mean±SD)
I zoea	71.6±1.25	3.0±0.38
II zoea	65.3±2.05	3.7±0.47
III zoea	57.7±2.05	3.0±0.00
IV zoea	51.7±2.05	3.3±0.47
V zoea	27.7±2.05	4.0±0.00
Megalopa	12.6±2.10	4.7±0.47
Megalopa-Ist crab instar	4.3±1.25	5.6±0.47

Table 4: Two-way analysis of variances (ANOVA) for the larval survival of *Portunus pelagicus*

Source of variations	SS	df	MS	F	p-value	F crit
Trials	12862	6	2143.667	9003.4	3.36	2.996177
Zoeal stages	77.80952	2	38.90476	163.4	1.97	3.88529
Error	2.857143	12	0.238095			
Total	1294143	20				

Table 5: Two-way analysis of variances (ANOVA) for the larval duration of *Portunus pelagicus*

Source of variations	SS	df	MS	F	p-value	F crit
Trials	17.90476	6	2.984127	7.673469	0.001482	2.996177
Zoeal stages	0	2	0	0	1	3.88529
Error	4.666667	12	0.388889			
Total	22.57143	20				

DISCUSSION

The swimming crab, *P. pelagicus* has 5 zoeal and 1 megalopa stages. It took 17-20 days for the completion of first 5 zoeal stages and 5-6 days for the megalopa to first crab instar. Previous observations in various crabs are comparable in the present investigation with reference to larval duration (Raman *et al*., 1987; CMFRI, 1984; CIBA News, 1998, 2000; Bryars, 1997).

In the present study larval mortality was started from I zoea onwards. The mortality in the larval rearing of crabs has not been well documented. Several reasons were assigned for the low survival of the larvae. Many authors have reported mortality in the larval rearing of mud crab (Marichamy and Rajapackiam, 1984, 1992). In the present study high mortality was observed in IV, V and megalopa stages of *P. pelagicus* is comparable with earlier reports (Anger *et al*., 1981; Ingles and Braum, 1989; Bryars, 1997; Hamasaki *et al*., 2002; Suprayudi *et al*., 2002). The possible reason for the mortality is due to depletion of reserves resulting in larval inability to catch the prey (Anger *et al*., 1981). But this reason would not fit for the mortality during the later stages of the present study as the larvae were fed in excess to avoid causality due to depletion of reserves.

Rosenberg and Costlow (1979) and Hamsa (1982) suggested that majority of larval population preparing for the premetamorphic moult to megalopa. Christiansen and Costlow (1975) have observed high mortalities in the larva of *Rhithropanopeus harrisii* at the premetamorphic stage. They attribute reasons for such mortality is the larvae at this stage are extremely susceptible to unfavourable environmental conditions. The metabolic cost of metamorphosis is very high and appears to decreases the capacity of the larvae to counteract these unfavorable conditions. Larvae suspended in the water column are very sensitive to stress, which cause them to fall to the bottom of the tank, forming dense clumps. When aggregated on the tank bottom, the larvae are liable to damage one another as a result of their abdominal flipping action. The aggregation also brings them into contact with any biofilm, which may be present in the tank. Close contact with the biofilm increases the chances of the larvae becoming fouled or attacked to the biofilm mucus.

According to Jamari (1992), sudden death of the larvae occurred due to the inability to moult. The possible reasons for the mortality may be due to chitin destroying bacteria and fungal attack near the carapace (Ting *et al*., 1981; Shields and Wood, 1993; Hamasaki *et al*., 2002). High mortality rate was observed during the later zoeal stages of the present study. A similar result was reported by Frank *et al*. (1975) during the larval development of *R. harrisii*. The reason for high mortality in the later stage is the increased metabolic activity, which resulted in the increase in energy requirements and normal diets failing to provide the required nutrients. Similarly McConaugha (1982) described that later stage is a critical period for which proper diet was required for the normal development of megalopa (Leger *et al*., 1987; Sorgeloos and Leger, 1992; Kannupandi *et al*., 2003). Mann and Paterson (2004) and Davis *et al*. (2001) explained that the appearance of moult-death syndrome during IVth zoea transformed into megalopa stage is one of the reasons for mortality.

Feed and feeding schedule is very important for the seed production of any aquatic organisms. In the present investigation, two types of live feeds are offered to the larvae of *P. pelagicus*. Live feeds are still the food of choice in most hatcheries. The superiority of 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 prescribed as it depends on the utilization of the feed by the larvae and must be judged visually by the operator.

The studies on the crab larvae showed that the absence of small prey during the early zoeal stage of *Callinectes sapidus* results in high mortalities. In the present study the zoea were initially fed with

B. plicatilis. The small size of first zoea refused to feed on *Artemia* nauplii, since it is bigger in size. To overcome this problem the *P. pelagicus* were provided with only on later stage especially from III zoea onwards (Table 2). The smaller size and slower swimming speed of *B. plicatilis* apparently allow their capture and manipulation by small zoea (Sulkin, 1975). Since, *B. plicatilis* is small in size and can be ingested completely by small decapod crustacean larvae. Soundarapandian *et al*. (1998) observed that the *Macrobrachium malcomsonii* early larval stages apparently graze on the appendages of *Artemia* nauplii but could consume entire rotifers. Rotifer gut is usually filled with bacteria and algae, which could provide additional nutrition for the larval forms of decapods. Sulkin (1975) reported that newly hatched larvae of *C. sapidus* couldn't pass to the next stage when fed with *Artemia* nauplii. The swimming crab, *P. trituberculatus* was fed with *Artemia* nauplii from third zoea stage to avoid cannibalism (Takeuchi *et al*., 1999).

Mixed results are obtained when *Artemia* nauplii and rotifers were used as feed for different authors. Brick (1974) showed that mud crab larvae fed on *Artemia* nauplii alone had on 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. Baylon and Failaman (1999) demonstrated that 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 production obtained when rotifer and *Artemia* nauplii was used as feed. Successful seed production was reported in *P. trituberculatus* offered with rotifer and *Artemia* nauplii (Takeuchi, 2000; Kobayashi *et al*., 2000). Minagawa and Murano (1993) recommended mixed diets (*Artemia* nauplii + rotifer) for the mass seed production of *R. ratina*.

As in the previous study, rotifer and *Artemia* nauplii have been offered to *P. pelagicus*. However, the survival rate is not encouraging. Various reasons are attributed for the lower survival eventhough standard live foods are used. McConaugha (1985) reported that *R. harrisii* fed on rotifer could not metamorphosis due to low lipid content and low feeding efficiency. The advantage of using *Artemia* nauplii for last feeding of larval mud crab is that it could have contribute to the lipid and energy resulting in a high feeding efficiency. In general the live food lack of n-3 HUFA will not optimize the growth of the developing larvae. 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. The swimming crab larva 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 (Leger *et al*., 1985; Bell *et al*., 1986). The larvae fed with cuttle fish liver oil enriched *Artemia* nauplii and rotifer showed accelerate growth and survival (Kannupandi *et al*., 2003). So poor survival in the present study may be lack of n-3 HUFA in the live feeds used. So, to improve the survival live feeds should be enriched with fatty acids.

Cannibalism is a serious problem in decapod larval rearing in general and crabs larval rearing in particular. The occurrence of cannibalism is usually associated with heterogeneous size variation, limited food availability, high population density, limited space and light conditions (Hecht and Pienae, 1993). The photoperiod and light intensity affected the survival rate and cannibalism in Australian giant crab *Pseudocarcinus giga* (Gardner and Maguire, 1998). Increasing prey density produced a high survival rate with an accelerated intermoult period and metamorphosis. Minagawa and Murano (1993) reported that the survival and metamorphosed rate of *Ranina rantha* increased with increasing prey density and low prey density generally produced a low survival rate. The survival rate from the fifth zoeal to megalopa stage in the present study is lower than from first to fifth stage. This phenomenon may be explained by high frequency of cannibalism that occurred when megalopa that moulted one day earlier grasped and fed on remaining fifth zoeae (Hamasaki *et al*., 1998).

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