

## Simple Technology for the Hatchery Seed Production of Giant Palaemonid Prawn *Macrobrachium rosenbergii* (De Man)

P. Soundarapandian, K.S. Prakash and G.K. Dinakaran  
Centre of Advanced Study in Marine Biology, Annamalai University,  
Parangipettai- 608 502, Tamil Nadu, India

**Abstract:** The optimum salinity requirement of *Macrobrachium rosenbergii* brooders for better hatching and survival of hatchlings is studied. The berried females of *M. rosenbergii* were kept in different salinities (0, 5, 10, 15 and 20 ppt) during incubation period. Maximum hatching rate (98 % and 97 %) was recorded when the berried females were kept in 0 ppt and 5 ppt salinities respectively. The post larvae appeared at very short periods of 22 and 24 days where brooders kept in 0 and 5 ppt salinities. The larval cycle completed within 35 and 37 days when the brooders acclimatized in 0 and 5 ppt respectively. Survival percentages of 82 and 79 were also high from the brooders kept in 0 and 5 ppt. Inferior hatching rate, extended larval batch and lower survival rate is observed from brooders kept in 10, 15 and 20 ppt salinities. The hatchery technology proposed is quite simple and can be applied to small-scale operations.

**Key words:** *Macrobrachium rosenbergii*, salinities, hatching rate, brooders, *Artemia* nauplii and formalin

### INTRODUCTION

The Giant freshwater prawn, *M. rosenbergii*, has great demand for both in national and international markets. The recent spate of disease outbreak in penaeid shrimp culture has forced farmers to invest in freshwater prawn farming. The natural seed resources are not sufficient to meet the demand and are also not uniform in size. Their identification is a major problem in the post-larval stage. So economically viable technology is the need of the hour. Salinity is the most important factor that influences mating, fertilization, incubation and hatching rhythm of freshwater prawns (Jeyalakshmy and Natarajan, 1996). However, no serious attempt has been made on the optimum salinity requirement of the berried females of freshwater prawns. So the present study is aimed to know the optimum salinity requirement of the berried females for better hatching and survival of the hatchlings.

### MATERIALS AND METHODS

**Brood stock management:** Brooders of *M. rosenbergii* were collected from private hatchery at Karuvampadi near Parangipettai (Lat. 11° 29' N and Long. 79° 46' E). They were carefully transported to the laboratory by using oxygenated plastic bags (Soundarapandian *et al.*, 1995) immediately immersed in a prophylactic dip of 20-ppm formalin for 30 minutes. They were kept in 5 different test salinities, ranging from 0 to 20 ppt (increasing the salinity by 5 ppt at each step), until hatching. For all experiments 50 L fiberglass tank were used. Water quality parameters maintained are shown in Table 1.

During incubation period, the brooders were fed with oyster meat (*Crassostrea madrasensis*). At every morning

left over feed and faeces were removed from the tank and half of the water was replaced. Aeration was provided continuously through an air diffuser. Once the egg mass turned dark grey, the larvae hatched out immediately at early morning.

**Larval management Stocking:** The larvae hatched in different experimental salinities were separately stocked at a density of 100 larvae/L in 50 L fiberglass tanks. The optimum salinity (12±1 ppt) was maintained in all tanks during the experimental period (Soundarapandian *et al.*, 2002). The water quality parameters followed is given in Table 2.

**Brackish water preparation and treatment:** Filtered seawater and freshwater were mixed to prepare 12 ppt salinity water. Sodium hypochloride solution was added to the prepared 12 ppt water, which was then aerated for 24 h. Excess chlorine was removed by treating the water with sodium thiosulphate (Soundarapandian *et al.*, 1997).

**Water exchange:** On every morning, left over feed, detritus and dead larvae were removed by turning off the aeration and siphoning the settled particles from the tank bottoms. Fifty percent of the water was exchanged each day.

**Feeding:** Both live *Artemia* nauplii and formulated feed were fed to the larvae. The composition and nutrient value is given in Table 3. The ingredients were dried and powdered separately. The pellets were prepared by mixing together; the required quantities of the finely powdered materials and the mixture were kneaded well by adding minimum quantity of water to form dough. The

Table 1: Water quality parameters for the brooders.

Water quality parameters	Optimum range
Temperature	29 to 31° C
Dissolved oxygen	5.0 to 6.0 ml/L
pH	7.0 to 8.5
Photoperiod	12 / 12 h L / D
Nitrate nitrogen	< 20 ppm.
Nitrite nitrogen	< 0.1 ppm.
Ammonia	< 0.1 ppm.

Table 2: Water quality parameters of the larval rearing.

Water quality parameters	Optimum range
Temperature	28 ± 2° C
Salinity	12 ± 1 ppt.
Dissolved oxygen	5.0 to 6.0 ppm.
PH	7.0 to 8.5
Nitrate nitrogen	< 20 ppm.
Nitrite nitrogen	< 0.1 ppm.
Ammonia	< 0.1 ppm.
Hardness	100 ppm.
Photoperiod	12 / 12 h L / D

Table 3: Composition and nutritive value of the formulated feed.

Component	Quality	Nutrient content	%
Hen's egg	4.0%	Protein	29.0
Oyster meat	15.0%	Carbohydrate	17.0
Shrimp meal	10.0%	Fat	8.1
Water	50.0%	Moisture	11.9
Beef meat	15.0%	Ash	6.2
Milk powder	3.0%		
Aminovit	1.0 %		
Minerals	1.5%		
Antibiotic	0.5%		

Table 4: Nutritive value of *Artemia* nauplii.

S. No	Nutrient content	%
1.	Protein	62.00
2.	Carbohydrate	22.00
3.	Fat	15.28
4.	Ash	15.29
5.	Moisture	73.65

dough was cooked in a pressure cooker for 30 minutes and the cooked material was extruded through a hand pelletizer with required perforation in the form of noodles on a filter paper and oven dried at 60 °C. The dried pellets were broken into pieces of required length and stored in polythene bags for future use (John Samuel *et al.*, 1997). The protein, carbohydrate, fat, moisture and ash content of the formulated feed and *Artemia* nauplii was determined by using standard methods of Raymont *et al.*(1964), Dubois *et al.*(1956), Folch *et al.*(1956), Soundarapandian (1996) and Paine (1964) respectively.

*Artemia* nauplii (Sunshine Brand, Gujarat, India) eggs were hatched following the instructions given on pack itself, by putting 1g of cyst in a 1L beaker and providing vigorous aeration for 18 to 22 h (Salinity 28-32 ppt, pH 8.2 – 8.5, Temperature 28-30°C). The hatched *Artemia* nauplii were separated from the cyst and stored in the refrigerator for further use. The nutritive value of *Artemia* nauplii is given in Table 4.

*Artemia* nauplii were fed three times a day (07.00, 10.00, 13.00 hrs.) for the first four days at a rate of 5 nauplii/ml (Table 5). Beginning from the fifth day, prepared feed was fed three times during the day time (07.00, 10.00 and 13.00 hrs.) and *Artemia* nauplii were fed only once in night (20.00 h).

The amount of feed not consumed was carefully noted every morning and the amount of feed was adjusted accordingly. Aeration was given throughout the experiment.

**Harvesting:** Post larvae were harvested from the larval tanks by reducing the water level and covering most of the tank. The post larvae concentrated in the illuminated area and could be easily removed with dip nets. Three replicates were maintained for each experiment.

**Statistical analysis:** To know the statistical significance, the data were analyzed by one-way analysis of variance. Differences in treatment means were determined by Duncan's multiple range test (P<0.05) using SPSS/PC<sup>+</sup> package.

## RESULT

**Hatching rhythm:** The hatching rhythm was studied under different salinities (0-20 ppt). Complete hatching of eggs in a single batch was noted from the brooders kept in 0 ppt and 5 ppt. Where as at 10, 15 and 20 ppt, complete hatching required two to four batches in successive nights.

**Hatching rate:** The results of optimum salinity requirement of *M. rosenbergii* brooders for hatching are presented in Table 6. The hatching rate was significantly higher in the brooders kept in 0 ppt (98.66 %) and 5 ppt (97.33 %), than that of 10 ppt (95.00 %), 15 ppt (93.66 %) and 20 ppt (91.00 %). But according to Duncan's multiple range test the hatching rate did not show any significant difference between the brooders kept in 0 ppt (98.66 %) and 5 ppt (97.33 %) and also 10 ppt (95.00 %) and 15 ppt (93.66 %).

**Post-larval appearance:** Post larvae appeared from the brooders kept in different salinities are shown in Table 6. The first post larvae appeared significantly in shorter period where brooders, maintained in 0 ppt (22.66 days) and 5 ppt (24.66 days) than brooders kept in other salinities viz., 10 ppt (27.33 days), 15 ppt (29.66 days) and 20 ppt (31.33 days). However, the brooders kept in 0 ppt (22.66 days) and 5 ppt (24.66 days) and also in 15 ppt (29.66 days) and 20 ppt (31.33 days) salinities did not show any significant difference between their first post larval appearances.

**Rearing periods:** The larval cycle was completed significantly quicker in brooders kept in 0 ppt salinity (35.33 days) rather than the brooders kept in other salinities viz., 5 ppt (37.66 days), 10 ppt (41.33 days), 15 ppt (44.66 days) and 20 ppt (47.66 days). The brooders kept in 20 ppt salinity took 47.66 days to complete their larval cycle (Table 6).

**Survival rate:** The survival rate of hatchlings was significantly higher from the brooders placed in 0 ppt (82.66 %). It shows significant discrepancy with other

Table 5: Feeding (per 5000 larvae) and larval stages of *M. rosenbergii* during rearing.

Zoeal stages	Quantity of <i>Artemia</i> naupliifed per ml	Size of <i>Artemia</i> nauplii(mm)	Quantity of formulated feed(g)	Size of feed particles(mg)
I to II	5	0.3	-	-
III to V	5	0.3	0.70 to 1.0	200 to 400
VI to VII	5	0.3	3.00 to 5.0	400 to 600
IX to X	10	0.3	6.00 to 8.0	600 to 800
XI to PL	10	0.3 to 1.0	12.00 to 14.0	800 to 1000

Table 6: Hatching rate, first post larval appearance, rearing periods and survival rate of *M. rosenbergii* brooders kept in different salinities.

Salinity(ppt)	Hatching(%)	Time of first post larvae (days)	Rearing period(days)	Survival(%)
0	98.66±0.33 <sup>c</sup>	22.66±0.33 <sup>a</sup>	35.33±0.88 <sup>a</sup>	82.66±0.66 <sup>c</sup>
5	97.33±0.88 <sup>c</sup>	24.66±0.33 <sup>a</sup>	37.66±0.33 <sup>b</sup>	79.33±0.33 <sup>d</sup>
10	95.00±0.57 <sup>b</sup>	27.33±0.88 <sup>b</sup>	41.33±0.88 <sup>c</sup>	70.66±0.88 <sup>e</sup>
15	93.66±0.33 <sup>b</sup>	29.66±0.66 <sup>c</sup>	44.66±0.33 <sup>d</sup>	68.33±0.33 <sup>b</sup>
20	91.00±0.57 <sup>a</sup>	31.33±0.88 <sup>c</sup>	47.66±0.66 <sup>e</sup>	59.66±0.33 <sup>a</sup>

Means with different superscripts in any column are statistically different (P < 0.05).

brooders kept in different salinities viz., 5 ppt (79.33 %), 10 ppt (70.66 %), 15 ppt (68.33 %) and 20 ppt (59.66 %). The lowest survival rate of hatchlings was seen in the brooders kept in 20 ppt (59.66 %) (Table 6).

## DISCUSSION

Among various environmental parameters, salinity plays a vital role for hatching and survival of hatchlings in *M. rosenbergii*. According to Jayalakshmy and Natarajan (1996), an ideal salinity was found to be very important for hatching of *Macrobrachium* species. In the present study, hatching and hatching rhythm was observed under different salinities (0, 5, 10, 15 and 20 ppt). The brooders kept in 0 ppt shows, higher hatching rate (98.66%) and hatching was completed in a single batch (Table 6). The brooders of *Macrobrachium* sp. are usually freshwater in habitat. Hence in the present study, the brooders show high hatching rate in 0 ppt. The brooders acclimatized in freshwater did not show any stress, since they are already collected from freshwater. John Samuel *et al.* (1997) observed that larvae hatched very well from *M. rosenbergii* brooders kept in 0.5 ppt rather than brooders kept in 2-10 ppt. Similar result was already reported in *Macrobrachium* sp. by Ibrahim (1962) Kewalramani (1972) and Rao (1986). Whereas hatching took two to four batches from the brooders kept in 10, 15 and 20 ppt salinities.

Hatching of eggs in batches was considered to be a common feature in *Macrobrachium* (Katre and Pandian, 1972; Katre *et al.*, 1980; Balasundaram and Pandian, 1981; Mathavan and Murugadass, 1988; Jeyalakshmy and Natarajan, 1996; Ranjeet and Kurup, 2000). Ignatius and Thampy (1990) observed that the occurrence of batch hatching is an index of unfavorable condition. According to Jayalakshmy and Natarajan (1996), the zoea released from single batch was found to be uniformly healthy. Ranjeet and Kurup (2000) remarked that the batch hatching of larvae shows slow growth rate and uneven stages. Pandian and Katre (1972) referred that last few batches were too weak to escape predation and to catch prey efficiently. Jayalakshmy and Natarajan (1996) also observed cannibalism in batch hatching, as observed in the larvae that hatched out from the brooders kept in 10, 15 and 20 ppt salinities of the present work.

Most of the adults of *Macrobrachium* sp. are known to migrate to the brackish water for breeding purpose (John Samuel *et al.*, 1997). In the present study, the brooders kept in 5 ppt salinity shows better hatching as brooders kept in 0 ppt (Table 6). The presence of little saline water (4-6 ppt) provides a better medium for hatching of *M. rosenbergii* eggs (Soundarapandian *et al.*, 1995b; John Samuel *et al.*, 1997) and *M. malcolmsonii* (Soundarapandian and Kannupandi, 2000, 2002). Katre and Pandian (1972) conformed that the eggs of *M. idae* are able to "pick up" salts from brackish water more readily than from freshwater.

The larvae hatched from the brooder kept in different salinities (0-20 ppt) were transferred to optimum salinity (12 ppt). In normal hatchery practice, the hatched I zoea is first acclimatized in lower salinity (5-10 ppt) as the growth proceeds the optimum salinity (12 ppt) was maintained. When the larvae metamorphosed into post larvae the salinity was decreased from saline to freshwater. In the present study, the larvae hatched from lower salinities (0-10 ppt) being transferred to larval rearing tank having lower salinity would not have (5-10 ppt) stress and no initial mortality. However, larvae hatched from higher salinity (15-20 ppt) faced stress problem, ultimately some initial mortality. To prevent this, instead of acclimatizing the I zoea in lower salinity (5-10 ppt), it will be directly stocked at higher salinity (15-20 ppt) then the salinity is decreased to optimum salinity (12 ppt).

The first post larvae appeared in 22<sup>nd</sup> and 24<sup>th</sup> day when the brooders kept in 0 ppt and 5 ppt salinities respectively (Table 6). Soundarapandian *et al.* (1995b) obtained post larvae on 19<sup>th</sup> to 24<sup>th</sup> day in *M. rosenbergii*. They acclimatized brooders in 4-6 ppt during incubation period. In the present study rearing periods ranged from 35 to 47 days (0-20 ppt). The rearing period was also limited in the larvae taken from the brooders kept in 0 ppt and 5 ppt. It is coincide with the study of Soundarapandian *et al.* (1995b), kept *M. rosenbergii* brooders in 5-6 ppt salinity before hatching. However, survival rate in the present study is higher than that of the previous study by Soundarapandian *et al.* (1995b) in *M. rosenbergii* larvae and Soundarapandian *et al.* (1997) in *M. malcolmsonii* larvae. In general the survival rate (59-82 %) of the present study is higher than commercial

hatcheries. This may be due to limited stocking, exposing the larvae to favourable environmental conditions (Table 2) better feed and feed management (Table 5).

The time for a larval batch to metamorphose varied according to feeding and environmental condition. Feed quality and feeding technique would be an important factor for successful larval rearing. The exact quantity of the feed required at each meal cannot be prescribed (New and Singholka, 1985) since it depends on the utilization of the feed by the larvae and must be judged visually by the operator. Larvae in "poor" conditions were sluggish, did not respond well to feed, were not strong enough to swim against the air bubbles, accumulated at the bottom of the tank, were often bluish in color and sometimes jumped out of water. Healthy larvae swam at the water surface, fed actively, had reddish brown pigmentation and were not observed to cannibalize each other. They swim tail first, head down and ventral side up.

*Artemia* nauplii constitute the main live feed and are most efficient to date. However as use of *Artemia* increases, so does the cost of production (Sorgeloos, 1976; Soundarapandian and Kannupandi, 2000). Therefore, from the fifth day of rearing, *Artemia* nauplii were fed only once a day (during night) and prepared feed was used as a substitute. In the case of prepared feed, it is advisable to consider the following points: over feeding will pollute the culture water, while under feeding causes malnutrition and cannibalism. Cannibalism was observed when the larvae metamorphosed into post larvae. Aquacop (1977) and Soundarapandian *et al.* (1995b, 1997, 2002) also reported cannibalism in their experiment when post larvae appeared. The hatchery technology followed is quite simple and can be applied to small-scale operations.

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