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Mass Seed Production of *Macrobrachium idella idella* (Hilgendorf, 1898)

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Abstract

Mass larval culture experiments were carried out from naturally collected berried females and also unilateral eyestalk ablated females. Fecundity was more in the naturally collected berried females. It was ranged between 7,271 ± 17.34 and 28,520.60 ± 31.11. Comparatively the fecundity was little bit low in the eyestalk ablated females. It was ranged between 7,112.66 ± 19.19 and 28,225.33 ± 36.37. The hatching rate was maximum in the naturally collected berried females (97.65 ± 0.59). It was less (92.81 ± 0.69) in the eyestalk ablated females. The incubation period for naturally collected females was 14.00 ± 0.07 days and it was 14.79 ± 0.11 days in eyestalk ablated females. The larval cycle was completed within 40.86 ± 0.58 days in naturally collected berried female. Whereas it was 41.97 ± 0.38 days in eyestalk ablated females. The survival rate of hatchlings was higher in naturally collected brooder (74.04 ± 0.09%) and it was less (70.35 ± 0.21%) in the eyestalk ablated females.

Keywords: *Macrobrachium idella idella*; Larvae; Eyestalk ablated females; Farming

Introduction

Mass culture experiments were carried out mostly on commercially important bigger sized prawns like *M. rosenbergii* and *M. malcolmsonii.* Several other species belongs to the genus *Macrobrachium* are highly suitable for culture in India even though they are smaller in size [1-3]. The mass seed production technology is already well established and more than 300 commercial hatcheries are available for both east and west coast of India for the giant prawn, *M. rosenbergii* and some extend in monsoon river prawn, *M. malcolmsonii* [4]. As, these two species are very bigger in size and potential for aquaculture [2,5]. So the farmers paid more attention. Even though, *M. idella idella* plays major support in commercial fishery of considerable magnitude especially in Parangipettai area of Tamil Nadu. Farmers and scientist paid less attention to *M. idella idella*, *M. idae* and *M. scabriculum* because of their smaller size. In general there is a substitute for everything and prawns are no exception so *M. idella idella* are considered to be an alternative species for *M. rosenbergii* and *M. malcolmsonii.* Since there is no considerable information about the mass seed production of small prawns in general and *M. idella idella* in particular. So in the present study an attempt has been made to bring out the seed production technology for edible prawn, *M. idella idella* to popularize this species for aquaculture.

Materials and Methods

In this experiment the berried females were collected from Vellar estuary Tamilnadu, India. The collected berried females were brought to the laboratory and immediately immersed in a prophylactic dip of 20 ppm formalin for 30 minutes [6]. The berried females were kept singly in fiberglass tank (45 × 30 × 37 cm) containing brackish water with the salinity of 4-5 ppt. The water quality parameters were maintained as shown in Table 1. The atmospheric and water temperature were measured using a digital centigrade thermometer. Salinity was estimated with the help of a hand refractrometer (ERMA, Japan) and pH was measured using an ELICO Grip pH meter. Dissolved oxygen was estimated by the standard method. They were fed with oyster meat and also commercially available pelleted feed twice a day. Left out feed was removed half an hour after it was offered so as to avoid spoilage of rearing water. Every day half of the water was replaced. Once the egg mass became dark grey the larvae hatched out immediately at early morning. The hatched larvae were dispersed by the mother by the fanning movements of its pleopods. As soon as the hatching was completed the female was removed from the fiberglass tank and placed in another aquarium to avoid any predation by the mother itself.

Larval management

The larvae immediately after hatching were stocked at a density of 100 larvae/L in 50 L fiberglass tanks. They were siphoned very carefully so as to avoid the physical damage to the larvae. The optimum environmental parameters were maintained throughout the experiment. The water quality parameters followed are given in the Table 2.

Brackish water preparation and treatment

Filtered seawater and freshwater were mixed together and 12-14 ppt salinity was prepared. Sodium hypochloride solution (NaOCl) was added to the prepared 12-14 ppt water, which was then aerated for 24 hrs. Excess chlorine was removed by treating the water with sodium thiosulphate [7].

Water exchange

At every morning 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. Aeration was provided continuously through an air diffuser.

Live feed culture

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

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concentration of 0.1%. The nauplii were enriched for 12 hours and after washing in seawater the nauplii were fed to the zoeal stages (Plate- 1).

Artificial feed

Artificial feed was prepared in the combination of hen's egg, shrimp meal, cooked and smashed rice, milk powder, aminovit and minerals. The ingredients were dried and powdered separately. The pellets were prepared by mixing together; the required quantities of the finally powered materials and the mixture were kneaded well by adding minimum quantity of water to form dough. The 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 [8]. The proximate composition of the artificial feed was analyzed. The protein, carbohydrate and lipid contents were estimated by adopting the standard methods of [9-11] respectively. Ash and moisture was estimated by following the standard methods. The artificial feed composition and nutritive value are given in the Table 3.

Feeding

Even though newly hatched larvae could survive upto 5 days without food, they were provided with appropriate food from the second day onwards. *Artemia* nauplii were fed three times a day (6.00 am, 12 noon and 6.00 pm) from II zoeae to III zoeae at a rate of 5 nauplii/ml. Beginning from IV zoeae prepared feed was fed two times during the day time (6.00 am and 12 noon) and *Artemia* nauplii were fed only once in night (7.00 pm) (Table 4).

The amount of feed not consumed was carefully noted every morning and according to that the amount of next feeding were adjusted. The *Artemia* which were left out without consuming by the zoeae were carefully removed next day before providing the fresh feed since *Artemia* has the ability to grow faster than the zoeae and sometime it may consume the zoea.

Growth and developmental stages

Growth and developmental stages including hatching status of the larvae were observed under binocular microscope. Identification of larval stages and size range is presented in the Table 5.

Experiment II: Rearing of larvae by induced breeding technique

The induced breeding technique is well established in many marine shrimps. Whereas in freshwater prawns very few work was done mostly in larger prawns like *M. rosenbergii* and *M. malcolmsonii* [12]. There is no such work in *M. idella idella* till date. So in the present, study induced breeding technique (eyestalk ablation) was tried to know the effect of eyestalk ablation on maturation, spawning, hatching and survival percentage of zoeal stages in *M. idella idella.*

Selection of healthy brooder

For induced breeding technique matured and healthy brooder of *M. idella idella* was collected from Vellar estuary. They were acclimatized to the laboratory conditions in the fiber glass tank (45 × 30 × 37 cm). After acclimatization (temperature 28 ± 2°C, salinity 5 ppt., photophase 12 D:12 L) the matured females were selected for eyestalk ablation.

Method of eyestalk ablation

In the present study unilateral eyestalk ablation was performed by severing the left eyestalk from the body at the narrow proximal end, in the region of the articulating membrane, using a pair of fine sterilized scissors [13]. The wound was cauterized by placing a hot blunt needle over it in order to prevent the loss of heamolymph and mortality.

Maturation tanks

After eyestalk ablation the female was placed in a maturation tank. Optimum conditions were maintained in the maturation tank. The female was observed very carefully for its pre-mating- moult (Plate 19). The female was once ready for pre-mating moult the matured male was introduced into the tank. Immediately after introduction of the male the female undergone pre mating moult, which is attracted the male for mating. Since the female was very soft the male protect the female and was waiting for few hours until the female exoskeleton became slightly hard.

Mating, spawning and hatching

Immediately after mating, the females were laid their eggs within 24 hrs. The spawned females were transferred to the hatching tank and were maintained until the fertilized eggs were hatched into 1st zoeae. The total number of eggs, percentage of hatching and survival percentage were calculated for each berried female. Larval management and feeding were just like previous experiment. Triplicate was maintained for each experiment.

Fecundity

Immediately after extrusion of eggs to the brood chamber the eggs were manually removed from the abdomen of each female and egg clutch. The female weight was also determined separately. Egg clutch was weighed to the nearest 0.01 mg after excess water had been removed by repeated blotting. The independent sample from the clutch was counted under a microscope to estimate the total number

Water quality parameters	Optimum range
Temperature	28-30°C
Salinity	4-5 ppt
Dissolved oxygen	5.0 to 6.0 ppm
pH	7.0 to 8.5
Photoperiod	12/12 hL/D

Table 1: Water quality parameters of the brooders.

Water quality parameters	Optimum range
Temperature	28 ± 2°C
Salinity	12 to 14 ppt
Dissolved oxygen	5.0 to 6.0 ppm
pH	7.0 to 8.5
Photoperiod	12/12 hL/D

Table 2: Water quality parameters of the rearing larvae.

Component	Quality (%)	Nutrient content	%
Hens egg	5.0	Protein	35.0
Oyster meat	15.0	Carbohydrate	19.0
Shrimp meal	15.0	Fat	9.4
Cooked and mashed rice	8.0	Ash	7.3
Milk powder	4.5	Moisture	10.6
Aminovit	1.0		
Minerals	1.5		
Water	50.0		

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

of eggs. Fecundity was estimated as the number of eggs per female weight (eggs/g of female), as well as the percentage of effort devoted to reproduction per spawning event (egg clutch weight/female weight × 100).

Statistical Analysis

To Know the statistical significance, 't' test was applied for the survival of zoea to post-larval stages of naturally collected berried and eyestalk ablated female. It showed significant different ($P<0.05$).

Results

Fecundity rate

The fecundity of both females is displayed in the Table 6. Fecundity was more in the naturally collected berried females. It was ranged between 7,271 ± 17.34 and 28,520.60 ± 31.11. Comparatively the fecundity was little bit low in the eyestalk ablated females. It was ranged between 7,112.66 ± 19.19 and 28,225.33 ± 36.37 (Table 6).

Hatching rate

The hatching rate was maximum in the naturally collected berried females (97.65 ± 0.59%). It was less (92.81 ± 0.69%) in the eyestalk ablated females. Hatching of the eggs were observed in batches for both the experiments (Table 6).

Incubation period

The incubation period of the naturally collected females was about 14.00 ± 0.07 days. Whereas it was 14.79 ± 0.11 days in eyestalk ablated females (Table 6).

Rearing periods

The larval cycle was completed within 40.86 ± 0.58 days in naturally collected berried female. Whereas it was 41.97 ± 0.38 days in eyestalk ablated female to complete their larval cycle (Table 6).

Survival rate

The survival rate of hatchlings was higher in naturally collected brooder (74.04 ± 0.09%). And it was less (70.35 ± 0.21%) in the eyestalk ablated females (Table 7). The survival showed significant variation between naturally collected and eyestalk ablated females (Table 6 and 8).

Discussion

In decapod crustaceans, regulation of all organ systems depends on the integration of nervous and endocrine systems. The neuroendocrine system controls physiological processes related to moulting, growth and sexual maturation [14]. The X-organ–sinus gland (XO–SG) complex located in the eyestalk is the major neuroendocrine control center of crustaceans [15]. The synthesis and release of neurohormones are regulated by biogenic amines [16]. 5-Hydroxytryptamine (5-HT) is a neurotransmitter present in the crustacean nervous system [17,18]. It is a member of biogenic amines and plays a role in indirect regulation of various physiological processes, metabolism, reproduction, and moulting in crustaceans. It stimulates the release of a number of other hormones, such as crustacean hyperglycemic hormone [19,20], moult-inhibiting hormone [21], gonad-stimulating hormone [22,23] and red pigment-dispersing hormone [24,25]. Moulting frequency was more in unilaterally eyestalk ablated ones and the intermoult period in the subsequent moults become shorter when compared to intact control animales in *M. malcolmsonii* [12]. Mortality was high in bilaterally ablated prawns (*M. rosenbergii*; *M. malcolmsonii*) and survived upto first moult. They were feeding well and active after operation, but after that they became pale, weak and died at various time intervals [12]. Studies on the possible effects of

Zoeal stages	Quantity of *Artemia nauplii* feed per ml	Size of *Artemia nauplii* (mm)	Quantity of artificial feed (g)	Size of feed particles (µg)
II to III	5	0.3	-	-
IV to V	5	0.3	0.65 to 1.0	150 to 300
VI to VII	8	0.3	2.00 to 3.0	300 to 400
VIII to X	10	0.3	4.00 to 5.00	400 to 500
Post larvae	10	0.3 to 1.0	6.00 to 8.00	600 to 800

Table 4: Feeding schedule of both *Artemia nauplii* and artificial feed to the zoeal stage to post larvae.

Zoeal stages	Prominent characters	Size range (mm)
I	Sessile eyes	1.90-2.10
II	Stalked eyes	2.15-2.38
III	Rostrum with epigastric tooth, uropod developed	2.32-2.60
IV	Rostrum with 2 dorsal teeth	2.36-2.71
V	Buds of 4th pereiopod developed	2.74-2.94
VI	All pereiopods developed	3.12-4.10
VII	Uniramous buds of pleopods developed	3.82-4.46
VIII	Pleopods biramous	3.90-5.12
IX	1st and 2nd pereiopods chelate	4.67-5.88
X	Appendix interna developed	5.67-6.0
Post-larvae	Spines on ventral side of the rostrum developed	6.02-6.50

Table 5: Identification of larval stages.

Particulars	Naturally collected berried females	Eye stalked females
Fecundity rate (70-90 mm)	7,271 ± 17.34-28,520.60 ± 31.11	7,112.66 ± 19.19-28, 225.33 ± 36.37
Hatching rate (%)	97.65 ± 0.59	92.81 ± 0.69
Incubation period (%)	14.00 ± 0.07	14.79 ± 0.11
Rearing periods (%)	40.86 ± 0.58	41.97 ± 0.38

Table 6: Fecundity, hatching rate, incubation period, rearing periods and survival rate of *M. idella idella* larvae reared in both naturally collected and eyestalk ablated females.

	Natural collected berried		Eyestalk ablated	
Stage	Number of days after hatching	Survival (%)	Number of days after hatching	Survival (%)
Zoea I	0	99.15 ± 0.24	0	98.99 ± 0.15
Zoea II	2.00 ± 0.01	98.20 ± 0.23	2.00 ± 0.04	97.93 ± 0.17
Zoea III	5.71 ± 0.05	95.32 ± 0.19	5.75 ± 0.37	94.76 ± 0.26
Zoea IV	7.96 ± 0.03	92.25 ± 0.16	8.00 ± 0.06	91.03 ± 0.22
Zoea V	10.98 ± 0.15	89.11 ± 0.14	11.19 ± 0.58	88.04 ± 0.21
Zoea VI	14.48 ± 0.12	86.72 ± 0.24	14.69 ± 0.80	83.79 ± 0.20
Zoea VII	19.25 ± 0.19	83.31 ± 0.17	19.62 ± 0.86	81.39 ± 0.26
Zoea VIII	24.21 ± 0.11	80.83 ± 0.21	24.90 ± 1.13	78.09 ± 0.09
Zoea IX	30.94 ± 0.20	79.00 ± 0.27	31.23 ± 1.16	75.54 ± 0.31
Zoea X	35.22 ± 0.14	76.99 ± 0.13	36.16 ± 1.09	73.28 ± 0.21
Post- larval	40.86 ± 0.58	74.04 ± 0.09	41.97 ± 0.38	70.35 ± 0.21

Table 7: Larval development, moulting period and survival (%) of naturally collected and eyestalk ablated berried females of *M. idella idella* (values are mean of three values ± SE).

Survival rate	Degrees of freedom	't' value
I	2	1.71
II	2	0.66
III	2	4.78
IV	2	3.44
V	2	3.21
VI	2	7.10
VII	2	6.18
VIII	2	15.66
IX	2	49.94
X	2	16.21
Post-larva	2	15.01

Table 8: Result of 't' test for the survival of zoea to post- larval stages of naturally collected berried and eyestalk ablated female.

eyestalk removal on gonad development in crustaceans are suggested that eyestalk contains gonad inhibiting hormone which delays gonad development [13]. The gonad development is accelerated after eyestalk ablation by influencing Gonadotropin Releasing Hormone (GnRH). In *M. idella idella* also immediately after eyestalk ablation pre-mating moult and gonad development was initiated and therefore vitellogenesis and spawning takes place in very short period.

Hatching of eggs in batches was considered to be a common feature in *Macrobrachium* [26-29] observed that the occurrence of batch hatching is an index of unfavorable condition. According to [27], the zoea released from single batch was found to be uniformly healthy [28] remarked that the batch hatching of larvae shows slow growth rate and uneven stages [30] referred that last few batches were too weak to escape predation and to catch prey efficiently [27] also observed cannibalism in batch hatching. Batch hatching was common for both eyestalk ablated and naturally collected females of the present study. The hatching rate in naturally collected berried female was 97.65 ± 0.59% and in eyestalk ablated females it was about 92.81 ± 0.69%.

Most of the adults of *Macrobrachium* spp are known to migrate to the brackish water for breeding purpose [8]. The presence of less saline water (4-6 ppt) provides a better medium for hatching of *M. rosenbergii* eggs [8,12] and *M. malcolmsonii* [6,7,31] confirmed that the eggs of *M. idae* are able to "pick up" salts from brackish water more readily than from freshwater. In the present study for the experiment I the berried female collected from the natural environment, these berried females were mostly captured from the estuary where the salinity was above 5 ppt. It clearly shows that berried females migrate to suitable salinity area. In the second experiment also after mating the berried females were transferred to the tank had 5 ppt so that the embryos were developed healthy.

Successful culture of crustaceans depends on the surrounding environment, quality and types of feeds [32]. Variation of the nutritional values in live feeds is recognized and thus formulated diets have been introduced [33,34]. Recent improvement of the formulated diets has been shown to be as efficient as live *Artemia* in supporting growth and survival of the larvae of giant freshwater prawn, *M. rosenbergii* [35]. Crustacean larvae are aggressive feeders and feed primarily on live feeds, i.e., small zooplankton and larval stages of other aquatic invertebrates [36,37]. In the present study the rearing of the larvae are very successful because both live *Artemia* nauplii and formulated feeds are provided as earlier studies [12]. After metamorphosed into post larvae, they resemble miniature adults and gradually change from suspending in the water to dwelling at the bottom.

Artemia nauplii constitute the main live feed and are most efficient to date. However as use of *Artemia* increases, so does cost of production [6,38]. Therefore, from the second day of rearing, *Artemia* nauplii were fed three times a day and prepared feed was used as a substitute from eight day at 6.00 am and 12 noon and *Artemia* nauplii only once in night (7.00 pm). In the case of prepared feed, it is advisable to consider the following points, over feeding will pollute the culture water, and under feeding causes malnutrition and cannibalism. Cannibalism was observed when the larvae metamorphosed into post larvae. [7,12,39] also reported cannibalism in their experiments when post larvae appeared.

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 [40] 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 colour 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.

The newly hatched larva of *M. idella* is a typical zoea having close resemblance to those of other species of the genus *Macrobrachium*, which have a long larval history. Although, the specific identifications of the larvae of this species as well as those of the same genus is a difficult task. The relatively smaller size and characteristic colouration can help in separating larva of *M. idella idella* from those of others. The general pattern of larval development of *M. idella idella* has close similarities with that of *M. rosenbergii* [41-43], *M. carcinus* [44] and *M. malcolmsoni* [45]. In the present study it has been observed that the zoea of *M. idella idella* undergoes 10 morphological stages have been recognized and described [41] found 12 zoeal stages in *M. rosenbergii* but in a subsequent study grouped these larvae into 8 morphological stages [43] described 11 zoeal stages in the developmental of the same species from Japan [45] described 16 stages in *M. malcolmsoni* [46] found 10 morphological stages in the zoeal development of *M. acanthurus* and 12 stages in *M. carcinus* [44]. Ten morphological stages in the larval development of most of the species of *Macrobrachium* appear to be a common feature [47]. Reduction in the total length of larvae during its metamorphosis from last zoea to post-larva is noticed in several species of *Macrobrachium* [16]. In *M. idella idella* reduction of length of post-larva was not noticed for both the experiments in the present investigation and 10 zoeal stages were observed. This is very similar to the study of [48].

Among the three basic pattern of larval development noticed in Palaemonidae [49], perhaps *M. idella idella* is fits into the first category by virtue of its larger number of larval stages which are characteristic of the brackish water prawns producing relatively large number of eggs. This is in contrast with the abbreviated larval history of several purely freshwater species belonging to the same genera; in some of which the eggs hatched out even directly into post-larvae.

The newly hatched larvae of *M. idella idella* became mature and spawn within 120 days in the laboratory [48]. In nature the species may spawn at still shorter intervals. Once maturity is attained the species continues to spawn every 20 days, each time producing as many as 5000 larvae. These characteristic of the species together with its readiness to spawn in confined waters make it an ideal species for culture

operations in brackish waters. Besides, it would appear that *M. idella idella* is a species which can be cultivated entirely in brackish water environments [50] stated that approximately 8000 Km of brackish water areas presently unutilized along the coastal regions of India can be brought under effective prawn cultivation by adopting modern culture techniques. Commercial culture of this species in this area will, no doubt, be very profitable and it will certainly add new dimensions to the development of prawn fishery in the country.

In rearing *M. rosenbergii*, survival rates are dependent on several factors such as stocking densities [51], water volume/surface area [32], pH level of water [52], and food supplement [37]. The survival rate of PL is 74.04 ± 0.09 for the larvae reared from naturally collected berried females. It was 70.35 ± 0.21 for the larva from eyestalk ablated females. In both the experiments common environments parameters were maintained. But the survival rate was higher for naturally collected brooder rather than eyestalk ablated animal. It indicates that the survival rate of the larvae mainly depends on brooder collected either from natural source or performed eyestalk ablation.

In the present study it is clear that the larva of both the experiment doesn't have much variation. Even though the larva hatched from the naturally collected berried females had some advantage. But we cannot get the berried females throughout the year since it is restricted to some seasons. So in that case if we are going for hatchery production is better to adopt induced breeding technique which able to supply larvae irrespective of the season and this technique is already well established for other commercially important shrimps and prawns. The hatchery technology followed in this experiment is quite simple and can be applied to small-scale operations.

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