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Rearing of larvae of giant freshwater prawn, *Macrobrachium rosenbergii* up to postlarvae using different feeds

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Abstract

Ability to produce seed (postlarvae) of giant freshwater prawn, *Macrobrachium rosenbergii*, for stocking in various culture facilities was a breakthrough in aquaculture in Sri Lanka. However, high cost of *Artemia* cysts and their occasional scarcity are the major concerns as live *Artemia* nauplii are considered to be the only suitable larval food for *M. rosenbergii.* During the present study, a feed for postlarvae of *M. rosenbergii* was prepared and its performance was evaluated in relation to some other feeds with a view to reduce the use of live *Artemia* nauplii in the rearing of larvae of this species. No larvae survived beyond the 6^{th} larval stage when they were fed 6 meal's day^{-1} with egg custard, egg and mussel meat custard, spray dried algal cells of *Schizochytrium* sp., a commercially available liquid shrimp feed and the prepared feed separately, each as single feed. The ingredients were 20% powdered milk, 6.8% corn flour, 40% eggs, 30% wet shrimp, 1.0% cod liver oil, 1.0% vitamin mineral mixture, 1.0% agar powder and 0.2% tetracycline. The best feeding regime was 6 meals of *Artemia* nauplii per day followed by the feeding regime of 3 meals of *Artemia* nauplii and 3 meals of prepared feed per day when percentage survival (10.35% and 9.28% respectively), mean total length achieved at the end of metamorphosis (9.078 $\pm$ 2.828 mm and 7.896 $\pm$ 2.812 mm respectively) and time taken to complete metamorphosis by 50% of larvae (40.42 $\pm$ 1.38 days and 48.42 $\pm$ 2.23 days respectively) were considered, when compared to the two other feeding regimes tested, viz, *Artemia* nauplii plus spray dried algal cells and *Artemia* nauplii plus liquid shrimp feed. Therefore, the feeding regime of 3 meals of *Artemia nauplii* and 3 meals of feed prepared during this study could be recommended in order to reduce the heavy use of costly *Artemia nauplii* in rearing the larvae of giant freshwater prawn, *M. rosenbergii.*

Introduction

The nutrition is highly significant, being second only to water quality, in the production of seed of any culturable species in sufficient numbers. Inadequate feeding will result in high morality of larvae, especially in species that have many larval stages as the larvae need more energy when they pass through each larval stage (New 1990).

The giant freshwater prawn, *Macrobrachium rosenbergii* is considered as a suitable candidate for both tropical fresh and brackishwater culture as it could fetch high prices due to its rich taste. The ability to produce its seed (postlarvae) in hatcheries was a break through. According to New (1990), due to long larval period, with twelve development stages it is difficult to provide nutrition to suit the requirements of each larval stage of giant freshwater prawn and even in most successful hatcheries the recorded survival is about 15% with live *Artemia* nauplii as the only feed. Sick and Beaty (1975) and Sandifer et al. (1987) pointed out that rearing of *M. rosenbergii* larvae relies almost completely on the use of live *Artemia* nauplii. High cost of *Artemia* cysts and their occasional scarcity are the major concerns in the expansion of *M. rosenbergii* hatcheries, particularly in the Asian region (Hagood and Willis 1976; Hanson and Goodwin 1977; Ong et al. 1977; Aniello and Singh 1982).

During the present study, a feed suggested by Chowdhury et al. (1993) was prepared and its performance was evaluated compared to some other feeds with a view to reduce the use of live *Artemia* nauplii in rearing the larvae of giant freshwater prawn, *M. rosenbergii* up to postlarvae.

Materials and Methods

Thirty cleaned glass aquaria measuring 75 cm x 30 cm x 30 cm, which were covered with black polythene to reduce penetration of light, were used in the experiment. Each aquarium was filled with 40 l of aged aerated water and stocked with 2000 one day old larvae of *M. rosenbergii*. The water used in the experiment had a salinity of 12 g l^{-1} and hardness of 100 g l^{-1}. The tanks were continuously aerated. The experiments were carried out at the Freshwater Prawn Breeding Centre of the National Aquaculture Development Authority of Sri Lanka at Pambala from March to September 2000.

In the first set of experiment, larvae were fed with six different feeds, which are given in Table 1. The feed types A, B, C, D, E and F were given at a ration of 30 ml day^{-1} which was divided in to six meals and offered at 06.00 h, 08.00 h, 10.00 h, 12.00 h, 14.00 h and 16.00 h. For the larvae fed with feed D, 200 ml of *Artemia* nauplii (with a density of 80 nauplii ml^{-1}) were given for each meal. Five replicates were arranged for each feed.

Table 1. Different types of feeds offered as single feed to larvae of *M. rosenbergii*.

Feed Type	Feed
A	Egg custard (Prepared by blending, steaming, reblending and sieving to obtain liquid food with particles of 100 μm – 200 μm)
B	Egg and mussel meat custard 1:1 by weight (Prepared in the same manner as egg custard)
C	Commercially available liquid shrimp diet (Salt Creek Revolution, USA)
D	*Artemia* nauplii (Commercially available *Artemia* cysts were hatched to get nauplii)
E	Prepared feed during this study (Prepared by steaming the liquid mixture of 20% powdered milk, 6.8% corn flour, 40% eggs, 30% wet shrimp, 1.0% cod liver oil, 1.0% vitamin mineral mixture, 1.0% agar powder and 0.2% tetracycline and sieving to have particles of 100 μm – 200 μm)
F	Commercially available spray dried algal cells of *Schizochytrium* sp. (prepared as a liquid)

Remaining feed and sediments were siphoned out daily with 1/3 of water from each aquarium and daily mortality (as the number of carcasses remaining) was recorded. Aquaria were filled again up to the initial level. The experiment was carried out over two production cycles.

Another set of experiment was carried out to find out the most suitable combination of feed for the larvae of *M. rosenbergii* in order to reduce the use of *Artemia*. In addition to *Artemia* nauplii as single feed (D_2) 3 other combinations of feed were tested. These are given in Table 2. These experiments were also carried out with 5 replicates. Here too the aquaria were arranged as described earlier and each was stocked with 2000 one day old larvae. Larvae in the first set of aquaria were fed with only *Artemia* nauplii (200 ml with 80 nauplii ml^{-1} per meal; D_2). Prepared feed was offered for the larvae in second set of aquaria at 6.00 h, 8.00 h and 10.00 h with 5 ml at a time and 200 ml of *Artemia* nauplii (80 nauplii ml^{-1}) per meal were given for the remaining three meals (E_2). Spray dried algal cells were offered for the third set of larvae (F_2) for the first three meals and *Artemia* nauplii were given for the last three meals. The fourth set of larvae were fed with the liquid shrimp diet (C_2) for the first three meals and *Artemia* nauplii were offered for the last three meals (Table 2).

The number of carcasses, survival of larvae, time taken to pass from one larval stage to another, time taken by larvae to complete metamorphosis and become post larvae and total body length of larvae were recorded for each aquarium separately. Water temperature,. dissolved oxygen content, pH, salinity and total ammonia were recorded between 09.00 h and 10.00 h daily using a Hatch kit (Model FF3).

Results were statistiscally analysed using one-way ANOVA followed by Tukey's pairwise comparison test.

Results

All the larvae fed with the feed types A and B could not survive beyond fourth larval stage and the larvae fed with feed types C, E and F were in sixth larval stage when 100% morality was recorded. In contrast, some larvae fed with *Artemia* nauplii (D) passed through all the larval stages (development stages) and became postlarvae.

Table 2 shows the mean number of carcasses of larvae recorded for the feeds D_2, E_2, F_2 and C_2. The mean number of carcasses recorded for the larvae fed only with *Artemia* nauplii was significantly higher ($P < 0.05$) than that of larvae fed with all the other combinations of feeds. Percentage survival of larvae at the end of experiment also was significantly higher ($P < 0.05$) when fed with only *Artemia* nauplii (Table 2). Significantly higher percentage survival was recorded for the larvae fed with *Artemia* nauplii combined with the prepared feed (E_2; $P < 0.05$) compared to the other two combinations of feeds (Table 2).

Prawn larvae fed with only *Artemia* nauplii (D_2) and the prepared feed in combination with *Artemia* nauplii (E_2) had comparatively shorter intermolt intervals (Table 3). Time taken by 50% of larvae to complete metamorphosis and become postlarvae also was significantly shorter ($P < 0.05$) when fed with feeds D_2, and E_2, compared to other two feeding regimes (Table 4).

Mean total lengths achieved by the larvae fed with feed D_2 and E_2, were significantly higher ($P < 0.05$) than those of the larvae fed with other two feeding regimes (Table 5).

During the present study, water temperature in aquaria varied between 26.5°C and 28.6°C while dissolved oxygen content ranged from 6 mg l^{-1} to 6.25 mg l^{-1}. Salinity of water was 12 g l^{-1}, pH varied between 7.5 and 8.5 and concentration of dissolved total ammonia content ranged from 0 to 0.02 mg l^{-1} (Table 6).

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Table 2. Mean number of carcasses of larvae recorded per day and survival of postlarvae of *M. rosenbergii* when fed with different feeds/feeding regimes (pooled data for the two production cycles). Mean values with different superscripts in a column are significantly different from each other at 5% level (Tukey's pairwise test)

Feed Type	Feeds/ Feeding regimes	Mean number of carcases recorded per day ± SE	Mean survival of post larvae (Total number and percentage) ± SE
D_2	*Artemia* nauplii (for all 6 meals)	$4.692^d \pm 4.817$	$206.96^a \pm 8.09$ (10.35%)
E_2	Three meals with *Artemia* nauplii and three meals with prepared feed during the present work	$2.088^e \pm 6.122$	$185.67^b \pm 9.73$ (9.28%)
F_2	Three meals with *Artemia* nauplii and three meals with spray dried algal cells	$2.059^c \pm 4.48$	$161.41^c \pm 8.02$ (8.07%)
C_2	Three meals with *Artemia* nauplii and three meals with liquid shrimp diet	$2.026^e \pm 7.169$	$163.33^c \pm 7.11$ (8.17%)

Discussion

During the present investigation, water quality parameters recorded in each experimental tank (water temperature, pH, dissolved oxygen content, salinity and total ammonia content) were within the acceptable ranges for the larvae of giant freshwater prawn as recommended by New and Singholka (1985), and Ang and Cheah (1986).

Formulation of the feed prepared and tested during this study was suggested by Chowdhury et al. (1993). However, there is no published literature to indicate that it has been employed in larval rearing of *M. rosenbergii.* Egg custard (Feed A), and egg and mussel meat custard (Feed B) are reported to be used by farmers in rearing larvae of giant freshwater prawn without much success. During the present study 100% cumulative mortality was observed at 4^th^ larval stage for these two feeds confirming their unsuitability to be used. Larvae fed with the prepared feed (Feed E), commercially available liquid shrimp diet (Feed C) and spray dried algal cells of *Schizochytrium* sp. (Feed F) also experienced 100% cumulative morality during the 6^th^ larval stage. Number of carcasses recorded for all these five feeds (A, B, C, E and F) were far lower than the number of larvae stocked indicating that under insufficient nutrition the larvae of giant

Table 3. The time taken by the larvae of *M. rosenbergii* to pass from one developmental stage to the next when fed with different feeds/feeding regimes (pooled data for the two production cycles).

Feed Type	Feeds/ Feeding regimes	Time taken to pass from one developmental stage to the next from the date of hatching (days)										
		i-ii	ii-iii	iii-iv	iv-v	v-vi	vi-vii	vii-viii	viii-ix	ix-x	x-xi	xi-xii
D_2	*Artemia* nauplii	1 – 4	3 – 7	6 – 10	8 – 13	11 - 21	17 - 22	20 - 26	23 – 34	24 - 40	36 - 40	36 – 56
E_2	*Artemia* nauplii and prepared feed during the present work	1 – 5	4 – 8	8 - 11	9 – 18	12 - 25	19 - 31	24 - 32	29 – 32	29 - 42	39 - 58	39 – 59
F_2	*Artemia* nauplii and spray dried algal cells	1 - 5	4 - 8	8 – 12	9 - 19	13 - 26	21 - 33	27 - 33	29 - 33	36 - 40	40 - 61	50 - 63
C_2	*Artemia* nauplii and liquid shrimp diet.	1 – 5	4 – 9	8 – 13	10 - 20	15 - 33	29 - 33	27 - 33	32 - 37	37 - 53	43 - 62	51 - 64

i to xii – Development stages of larvae (xii - postlarvae)

Table 4. Mean time taken by 50% of *M. rosenbergii* larvae to complete metamorphosis when fed with different feeds/ feeding regimes (pooled data for the two production cycles). Mean values with different superscripts are significantly different from each other at 5% level (Tukey's pairwise test).

Feed Type	Feeds/ Feeding regimes	Mean time taken by 50% larva to complete metamorphosis (days) ± SE
D_2	*Artemia* nauplii	$40.417^a \pm 1.381$
E_2	*Artemia* nauplii and prepared feed during the present work	$48.417^b \pm 2.234$
F_2	*Artemia* nauplii and spray dried algal cells	$57.583^c \pm 1.311$
C_2	*Artemia* nauplii and liquid shrimp diet.	$58.667^c \pm 1.303$

Table 5. The mean total length achieved by the larvae of *M. rosenbergii* when fed with different feeds/ feeding regimes (pooled data for the two production cycles). Mean values with different superscripts are significantly different from each other at 5% level (Tukey's pairwise test).

Feed Type	Feeds/ Feeding regimes	Mean total length of postlarvae (mm) ± SE
D_2	*Artemia* nauplii	$9.078^a \pm 2.828$
E_2	*Artemia* nauplii and prepared feed during the present work	$7.896^b \pm 2.812$
F_2	*Artemia* nauplii and spray dried algal cells	$5.671^c \pm 2.845$
C_2	*Artemia* nauplii and liquid shrimp diet.	$6.032^c \pm 2.792$

Table 6. Ranges of water quality parameters in experimental aquaria.

Parameter	Range
Temperature	26.5^0C - 28^0C
Dissolved oxygen content	$6 - 6.25$ mg l^{-1}
Salinity	12 g l^{-1}
pH	$7.5 - 8.5$
Dissolved ammonia content	$0 - 0.02$ mg l^{-1}

freshwater prawn become highly cannibalistic contributing greatly for the total mortality. Cannibalism has been identified as one of the major factors that reduces the production of postlarvae of *M. rosenbergii* which hinders the expansion of freshwater prawn farming (New 1990). In contrast, prawn larvae fed with only *Artemia* nauplii (Feed D) passed through all larval stages and became postlarvae. This is in agreement with the observation of Sick and Beaty (1975) and Sandifer et al. (1987) who pointed out that live *Artemia* nauplii are essential in rearing larvae of *M. rosenbergii.*

According to Hagood and Willis (1976), cost of feeding accounts for as much as 60% of the total production cost in the hatcheries of *M. rosenbergii.* High cost is the primary disadvantage of using *Artemia* nauplii as the sole food for larvae, particularly in developing countries (Achmad 1975; Ong et al. 1977; Solangi and Ogle 1977). Lovett and Felder (1987) stated that exuvia produced by *Artemia* nauplii could accumulate in culture vessels and bacterial degradation of these materials could foul the water when it is used in excess as larval food. In addition, these accumulated debris entangle crawling larvae contributing for their mortality. Therefore, feeding regimes developed with less number of meals with *Artemia* nauplii would offer positive results with regards to reduction of cost of production as well as to reduction of mortality in larvae of *M. rosenbergii* (Lovett and Felder 1987).

Hanson and Goodwin (1977), Murai and Andrews (1978), Aniello and Singh (1982) and many others have investigated the possibilities of using different feeds in rearing larvae of *M. rosenbergii.* However, none of these feeds readily replaced *Artemia* nauplii (Lovett and Felder 1987). Present study was carried out in glass aquaria where the limited space would have encouraged cannibalism while in commercial hatcheries larval rearing of *M. rosenbergii* is usually carried out in larger fiberglass tanks. In spite of this space limitation in glass aquaria, mean percentage survival recorded for the larvae fed only with *Artemia* nauplii (10.35%) and *Artemia* nauplii and prepared feed (9.28%) were quite satisfactory compared to the other two feeding regimes tested (Table 2) and the percentage survival recorded in commercial hatcheries, which is about 15% (New 1990).

Present study also shows that when mean percentage survival, length of intermolt intervals, time taken to complete metamorphosis by 50% of larvae and mean total length achieved are considered, three meals of *Artemia* naupli and three meals of prepared feed per day is the best feeding regime next to six meals of *Artemia* nauplii per day for the larval rearing of giant freshwater prawn (Tables 2, 3, 4 and 5). Harpaz and Schmalbuch (1986) pointed out that the shortening of the intermolt intervals could be regarded as an indicator of improved nutritional status since malnutrition or starvation usually results in extension of these intervals in larvae of giant freshwater prawn. Significantly higher mean number of carcasses recorded per day for the larvae fed only with *Artemia* nauplii (Table 2) shows that under sufficient nutrition larvae do not feed on dead individuals.

Results of the present investigation suggest that it would be useful to carry out further research on this formulated feed as a larval diet for *M. rosenbergii* after incorporating essential fatty acids, recommended growth promoters and feed attractants. Availability of essential fatty acids and feed attractants might reduce the cannibalism while contributing for growth. Deveresse and Coutteau (1989, unpublished) reported that enrichment of *Artemia* nauplii with Omega 3 unsaturated fatty acids resulted in better survival and offered vitality to the larvae of giant freshwater prawn.

The feed prepared during the present study consisted of locally available feed ingredients that could be purchased at a low cost, Since this prepared feed was tested as a wet feed, there is a tendency that it sinks to the bottom after sometime while early developmental stages of the prawn feed near the surface and later stages at the bottom. If this feed could be prepared as a dry feed which would float near the water surface, utilization of it by the early larval stages would be increased, in turn increasing the survival rate. It may be even possible to reduce the number of meals that should be supplied with *Artemia* nauplii per day further without a significant reduction in the performance of larvae by improving the nutritional and physical qualities of the feed tested in this investigation. Increased and consistent production of postlarvae that would be achieved at low cost may contribute for the expansion of farming of giant freshwater prawn in Sri Lanka, which has a considerable demand in the international market.

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