

# Preliminary Evaluation of the Potential of *B. plicatilis* for Use as a Live Food for Freshwater Prawn Larvae

Shivananda Murthy H<sup>1</sup>, Yogeeshababu MC<sup>1</sup> and Tejpal CS<sup>2\*</sup>

<sup>1</sup>Department of Aquaculture, Karnataka Veterinary, Animal and Fisheries Sciences University, College of Fisheries, Mangalore 575002, India

<sup>2</sup>National Fisheries Development Board, Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, GOI, India

## Abstract

*Artemia* is predominantly used as a live feed in freshwater prawn hatcheries. In view of high cost of cysts and their occasional scarcity, the dependence on *Artemia* is a major concern in the expansion of *Macrobrachium rosenbergii* hatcheries. Efforts are made to replace *Artemia* by other live food organisms and inert feeds. In the present study, an attempt was made to evaluate rotifer, *Brachionus plicatilis* to replace *Artemia* either partially or fully in prawn larval rearing unit. The experiment was carried out in triplicate groups with four feed treatments. Prawn larvae were fed with *Artemia* alone (100%) T<sub>1</sub> (A100), T<sub>2</sub> *Artemia* (70%) and *Brachionus plicatilis* (30%) (A70 and B30), T<sub>3</sub> *Artemia* and *B. plicatilis*, (50% each) (A50:B50) and T<sub>4</sub> *B. plicatilis* alone (100%) (B100). Group T<sub>2</sub> (A70 and B30) resulted in good survival, growth and mean larval stage (MLS) of prawn larvae, which was not significantly different from treatment T<sub>1</sub> group fed with *Artemia* alone. The survival obtained in A50:B50 and *B. plicatilis* (B100) alone was not satisfactory, and differ significantly from the other treatments.

**Keywords:** Larval nutrition; Survival; Growth; Rotifer; *Artemia nauplii*; *Brachionus plicatilis*; *Macrobrachium rosenbergii*

## Introduction

*Artemia nauplii* are the predominant live food used in shrimp and prawn hatcheries. Though supplementation of *Artemia* with prepared feed has been reported [1,2], no substitutes have yet become standard in freshwater prawn hatcheries. In view of high cost of cysts coupled with their occasional scarcity, the dependence on *Artemia* is a major concern in the sustainable seed production of *M. rosenbergii* in hatcheries. Further, the exuvia and capsules (outer calcareous layer of cysts) accumulate in the larval rearing tanks. Bacterial degradation of these materials fouls the water; accumulated debris entangles larvae and leads to increased larval mortalities. The cysts or shells which are ingested by the larvae cannot be digested and they may cause blockage of the gut or have other deleterious effects [3]. Although partial success has been achieved in the development of formulated feed to supplement and replace *Artemia* in prawn larval culture [1], the use of these diets has limited success in promoting sustained larval production [4]. In contrast, rotifer *B. plicatilis* has a short life cycle, can be cultured in high densities and has favourable nutritional contents [5]. Since it is small in size, it can be consumed completely by small decapods crustacean larvae. The individuals of *M. rosenbergii* in early larval stages (I to III) apparently graze on the appendages of *Artemia* but are not able to consume entire nauplii [6]. In contrast to this *Brachionus plicatilis* found to be good live diet for *M. rosenbergii* because early larval stages could consume entire rotifer simply due to its smaller size. In this direction, an attempt was made in this study to evaluate rotifer, *B. plicatilis* to replace *Artemia* either partially or fully in prawn hatcheries.

## Materials and Methods

### Experimental animal

One day old larvae of *Macrobrachium rosenbergii* were procured from the College of Fisheries hatchery, Mangalore, India and were used for the study.

### Experimental design

18,000 one day old prawn larvae were randomly distributed into

four groups. Four treatment groups namely, T<sub>1</sub> (100% *Artemia* (A 100)); T<sub>2</sub> (70% *Artemia* and 30% *B. plicatilis* (A70: B30)); T<sub>3</sub> (50% *Artemia* and 50% *B. plicatilis* (A50: B 50%)) and T<sub>4</sub> (100% *B. plicatilis* alone (B100)) were arranged in triplicates following a Complete Randomized Design (CRD) design and fed respective diets. The total volume of water in each tank was maintained at 50 l throughout the experimental period. In all the treatments, larvae were fed with live food organisms twice a day at 8.30 hrs and 17.30 hrs at the rate of 3 organisms per ml of tank water and the number of *Artemia* and *B. plicatilis* varied according to the treatment. Round the clock aeration and water recirculation was provided.

### Experimental diet

The study was conducted to evaluate the nutritive value of *B. plicatilis* (rotifer) and its effect in the feeding of *M. rosenbergii* by replacing *Artemia*. The experiment consisted of four dietary treatments in triplicate groups. The larvae fed with 100% *Artemia* (A 100) T<sub>1</sub>, 70% *Artemia* and 30% *B. plicatilis* (A 70: B 30) T<sub>2</sub>, 50% *Artemia* and 50% *B. plicatilis* (A 50: B 50%) T<sub>3</sub> and 100% *B. plicatilis* alone (B 100) T<sub>4</sub>. *B. plicatilis* samples collected from nearby Nethravathy estuary were segregated and multiplied in the laboratory by providing chlorella and yeast as food. After 5-7 days of inoculation in nutrient rich media, *B. plicatilis* attained a peak density of 100-150 individuals/ml and were harvested with a scoop net (100-150  $\mu$ ) early in the morning or late in the evening when they were at the surface. The harvested biomass of *B. plicatilis* was washed thoroughly and fed to the prawn larvae. *Artemia* cysts were decapsulated and hatched in the laboratory and fed to prawn larvae.

**\*Corresponding author:** Tejpal CS, National Fisheries Development Board, Department of Animal Husbandry, Dairying and Fisheries, Ministry of Agriculture, GOI, India, E-mail: [tejpal.arun@rediffmail.com](mailto:tejpal.arun@rediffmail.com)

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## Proximate analysis

Experimental diets were analyzed using standard methods [7] for crude protein, lipid and ash. Crude protein by Kjeldtec semi-automatic system (Tecator); lipid by Soxhlet system (Model SD2, 1045, Tecator) and ash by muffle furnace incineration at 550°C for 6 h were analysed. The proximate composition of *Artemia* and rotifer were analyzed in triplicates.

## Water analysis

Physico-chemical parameters of water was analysed at weekly intervals. Temperature of water was measured using mercury in glass thermometer having an accuracy of 0.1°C. The pH was measured using a laboratory lovibond comparator. Salinity of the water was estimated by refractometer with 1 ppt accuracy. Dissolved oxygen, free carbon dioxide and total ammonia were determined following standard methods [8].

## Mean larval stage (MLS) and relative percentage survival

Mean larval stage (MLS) and relative percentage survival were estimated every second, third and fourth day respectively for the first, second and third week onwards. Thirty randomly sampled larvae from each treatment were identified following the descriptions given by Uno and Kwon [9]; Murai and Andrews [10]. Development of the larvae was determined by calculating the mean larval stage (MLS) by the formula given by Lovett and Felder [6].

$$MLS = \sum(S \times PS)$$

Where 'S' is the larval stage number and PS is the proportion of the larvae at stage 'S'.

The relative survival of larvae in each tank was estimated by taking random samples. One liter of water from each tank was taken ten times and average number is multiplied to whole volume. The experiment was terminated when more than 95% of the larvae metamorphosed to post-larvae. All the post larvae were harvested from each tank and counted to calculate the percentage survival. After termination of the experiment, 50 post larvae were randomly taken from each tank to measure individual total length (from tip of the rostrum to the end of the telson) and total weight.

## Statistical analysis

The data were statistically analyzed by one way ANOVA and Duncan's multiple range test by statistical package SPSS version 11 to determine the significant difference between the treatments comparisons were made at 5 % probability level [11,12].

## Results

Water quality parameters analyzed during the course of study are given in the (Table 1). Water temperature, pH, dissolved oxygen and ammonia in different treatments varied from 24.3 to 26.7°C, 7-7.8, 5.3-5.85 mg/l and 0.03 to 0.07 mg/l respectively. Free carbon dioxide was not detectable in any of the experimental tanks.

The survival rate of *Macrobrachium rosenbergii* larvae fed with T<sub>1</sub> group (*Artemia* alone (A100%)) showed the highest survival (43.33%) followed by (42.22%) in the T<sub>2</sub> group (A70:B30 (70% *Artemia* and 30% *B. plicatilis*)). Lowest survival was recorded in T<sub>4</sub> group when larvae fed with (*B. plicatilis* (B100%)) alone (Table 2). However, there was no significant difference in the larval survival recorded between T<sub>1</sub> and T<sub>2</sub> treatment groups. Whereas, the survival rates obtained in T<sub>1</sub> (A100)

Parameters	Treatments			
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
Temperature (°C)	25.70 ± 0.44	25.61 ± 0.46	25.65 ± 0.62	25.71 ± 0.53
Dissolved oxygen (mg/l)	5.66 ± 0.11	5.54 ± 0.08	5.60 ± 0.07	5.56 ± 0.08
pH	7.41 ± 0.10	7.31 ± 0.06	7.43 ± 0.09	7.36 ± 0.09
Ammonia (mg/l)	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01

**Table 1:** Water quality recorded in different experimental tanks (values are means three replicate groups in each treatment)

Treatment	Post-larval production				
	Larvae stocked		Total post-larvae Obtained	Post-larvae re-recorded per liter	% survival
	Total Nos.	No./ liter			
T1	1500	30	650 ± 50.0a	13 ± 1.0a	43.33 ± 3.3a
T2	1500	30	633 ± 28.0a	12.67 ± 0.57a	42.22 ± 1.9a
T3	1500	30	400 ± 36.0b	8.00 ± 1.73b	26.66 ± 5.7b
T4	1500	30	133 ± 18c	2.66 ± 0.57 c	8.89 ± 1.9c

Different superscripts (abc) in the same column indicate significant difference (P<0.05) treatment groups (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>) (Duncan's multiple range test  $\alpha$  = 0.05). The value expressed as a mean ± S.E. (n = 6)

**Table 2:** Post-larval production of *M. rosenbergii* in different feed treatments (values are means SD)

and T<sub>2</sub> (A70:B30) were significantly different from the treatments T<sub>3</sub> (A50:B50) and T<sub>4</sub> (B100) groups.

The mean larval stage (MLS) of *Macrobrachium rosenbergii* in different treatment groups are given in the (Table 3). The MLS showed significantly (p<0.05) higher among two treatment groups (T<sub>1</sub> & T<sub>2</sub>). The development of the larvae is expressed as the mean larval stage (MLS). In the treatment T<sub>1</sub> and T<sub>2</sub> groups larvae took relatively less time to reach the next stage, where as in the T<sub>3</sub> and T<sub>4</sub> treatment groups larvae took more time to reach the next stage. The highest MLS value was recorded in the T<sub>1</sub> followed by T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. There was no significant difference in the MLS values of larvae fed with T<sub>1</sub> (A100) and T<sub>2</sub> (A70:B30). But, the other two treatments T<sub>3</sub> (A50:B50) and T<sub>4</sub> (B100) differed significantly from T<sub>1</sub> and T<sub>2</sub>.

The recorded length and weight of post-larvae fed with different diets was highest in T<sub>1</sub> fed *Artemia* alone, than T<sub>2</sub> followed by T<sub>3</sub> and T<sub>4</sub> (Table 4). Data pertaining to the proximate composition of *Artemia* and Rotifer (*B. plicatilis*) is presented in table 5. Crude protein estimated in *Artemia* and rotifer were 48.43 ± 1.36 and 30.90 ± 1.0, respectively the fat content was 19.00 ± 0.26 and 5.99 ± 0.20 and ash was 7.43 ± 0.21 and 19.14 ± 0.67 respectively.

## Discussion

In the present study, all the physico-chemical parameters of water namely temperature, pH, DO, CO<sub>2</sub>, total alkalinity and ammonia-nitrogen were found well within the optimum range of requirement for the growth of *Macrobrachium rosenbergii* larvae. The survival rate of *Macrobrachium rosenbergii* was significantly better in the T<sub>1</sub> and T<sub>2</sub> groups when compared with T<sub>3</sub> and T<sub>4</sub> groups. Lovett and Felder [6] observed no significant difference in the survival of the larvae fed *Artemia* alone and combination of *Artemia* and *B. plicatilis*. The mean larval stages (MLS) of larvae fed with different diets vary significantly among the treatment group. MLS showed better values in the T<sub>1</sub> and T<sub>2</sub> groups which were fed with *Artemia* and *Artemia* and *B. plicatilis* (70+30%) when compared with T<sub>3</sub> and T<sub>4</sub> groups fed with *Artemia* and *B. plicatilis* (50+50%) and *B. plicatilis*. Our finding of this study are comparable to results of Alam et al., [13] who reported that higher MLS values for larvae fed *Artemia* alone and combination of *Artemia* and

Treatment	Mean larval stages on different days										
	3	5	7	10	13	16	20	24	28	32	36
T <sub>1</sub>	2.03 ± 0.02 <sup>a</sup>	2.23 ± 0.02 <sup>a</sup>	3.20 ± 0.0 <sup>a</sup>	4.17 ± 0.02 <sup>a</sup>	4.83 ± 0.0 <sup>a</sup>	5.70 ± 0.04 <sup>a</sup>	6.830.01 <sup>a</sup>	8.13 ± 0.02a	8.90 ± 0.0 <sup>a</sup>	9.76 ± 0.02 <sup>a</sup>	10.63 ± 0.01 <sup>a</sup>
T <sub>2</sub>	2.03 ± 0.02 <sup>a</sup>	2.23 ± 0.03 <sup>a</sup>	3.17 ± 0.0 <sup>a</sup>	4.13 ± 0.01 <sup>a</sup>	4.80 ± 0.0 <sup>a</sup>	5.67 ± 0.0 <sup>a</sup>	6.80 ± 0.0 <sup>a</sup>	8.08 ± 0.0a	8.83 ± 0.0 <sup>a</sup>	9.67 ± 0.02 <sup>b</sup>	10.60 ± 0.04 <sup>a</sup>
T <sub>3</sub>	2.03 ± 0.02 <sup>a</sup>	2.20 ± 0.02 <sup>a</sup>	3.13 ± 0.0 <sup>b</sup>	4.05 ± 0.03 <sup>b</sup>	4.66 ± 0.0 <sup>b</sup>	5.46 ± 0.0 <sup>b</sup>	6.60 ± 0.0 <sup>b</sup>	7.86 ± 0.0b	8.63 ± 0.0 <sup>b</sup>	9.30 ± 0.0 <sup>c</sup>	10.40 ± 0.01 <sup>b</sup>
T <sub>4</sub>	2.03 ± 0.02 <sup>a</sup>	2.20 ± 0.01 <sup>a</sup>	3.10 ± 0.0 <sup>b</sup>	4.00 ± 0.04 <sup>b</sup>	4.61 ± 0.0 <sup>b</sup>	5.33 ± 0.02 <sup>c</sup>	6.47 ± 0.0 <sup>c</sup>	7.76 ± 0.01c	8.43 ± 0.0 <sup>c</sup>	9.17 ± 0.02 <sup>d</sup>	10.23 ± 0.02 <sup>c</sup>

Different superscripts (abc) in the same column indicate significant difference (P<0.05) treatment groups with respective Mean larval stages on different days (T1, T2, T3 and T4) (Duncan's multiple range test a = 0.05). The value expressed as a mean ± S.E. (n = 6)

**Table 3:** Comparison of mean larval stages (MLS) of *M. rosenbergii* in different feed treatments (values are means and SD of 3 replicate groups)

Treatment	Length (mm)	Weight (mg)
T1	9.66 ± 0.42	9.75 ± 0.64
T2	9.58 ± 0.38	9.67 ± 0.66
T3	9.28 ± 0.29	9.55 ± 0.58
T4	9.26 ± 0.42	9.53 ± 0.73

**Table 4:** Length and weight of post- larvae recorded under different feeding regimes (values are means of three replicate groups) (n = 6)

Live feed organisms	Protein	Fat	Ash
<i>Artemia nauplii</i>	48.43 ± 1.36	19.00 ± 0.26	7.43 ± 0.21
<i>B. plicatilis</i>	30.90 ± 1.0	5.99 ± 0.20	19.14 ± 0.67

**Table 5:** Proximate composition of *Artemia* and *B. plicatilis* (% dry weight)

*Moina* as compared to larvae fed *Moina* alone.

Prawn larvae fed *Artemia* alone showed better survival, MLS and growth and took shorter time to reach the next stage in the present study. This is attributed to high lipid content of *Artemia* than rotifers (Sulkin, 1975) [14] (Table 5) and presence of higher levels of n-3 HUFA's [15]. Further, the caloric content of *Artemia* is better than rotifers (Emmerson, 1984). In the present study, as the percentage of *Artemia* in the feed decreased the survival of the larvae also reduced. However, survival and growth recorded by feeding T<sub>2</sub> (A70:B30) was not significantly different from T<sub>1</sub> (A100) treatment. The survival of larvae obtained by feeding T<sub>3</sub> and T<sub>4</sub> was not satisfactory because, 50% of rotifer or rotifer alone (100) was not sufficient to fulfill the nutritional requirement of prawn larvae. The rotifer, *B. plicatilis* found to contain less amount of n-3 HUFA's and energy compared to *Artemia* [16].

The findings of the present study demonstrate that combination of *Artemia* and *B. plicatilis* in the ratio of A70:B30 respectively was found the best combination for larval rearing of *M. rosenbergii* in view of its cost effectiveness. Based on the results, it was possible to reduce 30% of cost on use of *Artemia* cysts. Rotifer, *B. plicatilis* could be raised in the laboratory without much involvement of cost and labour. Further research is needed in this direction to study the replacement of *Artemia* by rotifer and other zooplankton in the larval rearing of freshwater prawn.

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