J. Agrofor. Environ. 6 (1): 143-147, 2012 ISSN 1995-6983

Growth, survival and diseases stress of *Macrobrachium rosenbergii* larvae at different stocking densities in cemented tank under hatchery condition

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Abstract: Experimental stocking density of *Macrobrachium rosenbergii* in larval rearing was conducted in Shrimp Research Station Prawn Hatchery, Bagerhat, Bangladesh. The research work was conducted using six cemented tanks having capacity of 2.6mX1.3mX1m ($3m^3$) each. Stocking density was maintained in the three experimental setup as 200, 150 and 100 ind/L of the T_1, T_2 and T_3 respectively with one replicate each. The larvae were fed with *Artemia* nauplii, Custard. Water quality was maintained by exchanging 20-30% (12ppt saline water) daily. During the study period, temperature, pH, DO, salinity, nitrite-nitrogen, ammonia and alkalinity were maintained from 28.5-31.5ºC, 7.5-7.8, 5.8-5.9mg/L, 12-13ppt, 0.14-0.2 mg/L, 0.22-0.3mg/L, and 140-160mg/L respectively. The growth rates of larvae at 11^{th} stage were recorded in terms of body length 0.115, 0.136, and 0.169 mm/day whereas body weight were observed 0.000115, 0.000180, and 0.000240g/day. The survival rate of larvae was found 21.8%, 30.4% and 51.3% in treatments T_1, T_2 and T_3 respectively. PL was obtained as 43, 45, and 51PL/L and days required of 41, 38 and 34 days in stocking density of 200, 150, and 100ind/L respectively. It was found that the minimum of 34 days was required to attain the PL (12^{th} stage) using the stocking density of 100 individuals/L. Cannibalism, *Zoothamnium and* Mid Cycle Disease (MCD) were found to be the threat to the hatchery operation that might responsible for potential larval damages which can be reduced by lowering the stocking densities in larval rearing tank that also increased the survival and growth rate.
Key words: Growth, survival, *Macrobrachium rosenbergii*, cemented tank, hatchery condition

Introduction

Prawn farming (in *gher*) in the south-west region of Bangladesh is taken as a traditional custom and alternative income source. Aquaculture of freshwater giant prawn, *Macrobrachium rosenbergii*, has gained a significant momentum with an expansion of farm lands from 3,500 ha in mid 80s to about 50,000 ha at present. There has been a trend of increasing this area at the rate of 10-20% annually (Alam, 2009). Production of shrimp and freshwater prawn for the year 2007 - 2008 was 223,095 mt of which 23% was cultured prawns. In the year 2007 - 2008 total quantity of export frozen shrimp and prawn was 49,907 mt, which earned Tk. 2863.92 crore sharing 2.68% to total export earning (DoF, 2009). With the rapid expansion of prawn farming in last two decades, demand of PL in recent times throughout the country has reached around 1,500 million, which may increase with further expansion of farming area and its intensification. At Present prawn hatcheries contributing only about 15% of total demand though it is facing numerous constraints and the rest bulk portion are collected from natural sources of rivers (Khondaker, 2009). Aquaculture production of prawn depends on quality of PL stocking from the nature and hatcheries. For achieving big marketable size in the shortest possible time *Macrobrachium rosenbergii* requires excellent operation and management practices in its culture system especially in hatchery for artificial propagation. The success of this system depends on its proper operation and management. The maintenance of proper stocking density is important for the optimum production in hatchery condition. Stocking density plays an important role in larvae rearing of freshwater prawn at hatchery condition. It affects survival, growth, disease and larval quality as well. Under or low stocking density or over stocking density brings low production or hampers the total system. Under or over stocking density causes economic losses. Proper stocking density is key factor in larval rearing of *Macrobrachium rosenbergii*.It helps to create maximum economic efficiency, healthy and disease resistant fry and finally excellent aquaculture products. So, the maintenance of the right level of stocking density of cultured *Macrobrachium rosenbergii* in the larval rearing tanks should be considered more carefully for the supreme success of the hatchery. Now in captivity if the environment is carefully controlled and the larvae are reared with optimum food and temperature, there is some variation of larvae on growth and survivability. Considering the prospect and importance of the study and its field of application in our country encouraged to under take such research work. The objective of the present study was to find out the growth, survival rate and disease stress of *Macrobrachium* larvae at different stocking densities by maintaining water quality and balance feed in hatchery condition.

Materials and Methods

The study area: The study was conducted in the experimental tanks of Shrimp Research Station (SRS) prawn Hatchery in Bagerhat district. Communication system of the hatchery was very suitable for the transportation of the PL and the required hatchery materials. It was conducted for the period of 07 weeks (40-45 days) from 21, April to 05^{th} June, 2012

Experimental design: The experimental set-up consisted of six cemented tanks of size 2.6mX1.3mX1m ($3m^3$) situated in the indoor hatchery complex. Three treatments (T_1, T_2 and T_3) were arranged with one replicate for each treatment. The concrete tanks as well as all components of the hatchery were cleaned and treated with the bleaching powder before stocking the Brood. Then all the tanks were washed by the freshwater.

Brine storage and Mixing: Ninety five ppt (95‰) were collected from the Munshigonj, Shathkhira and stored. To get 12‰ saline water, required quantities of brine was mixed with freshwater. After cleaning, drying and formalin wash, 12ppt saline water was used for larval rearing of *M. rosenbergii*.

Experimental larvae: Required number of larvae were treated with 25ppm formalin for 30minutes with vigorous aeration and then transferred into the LRT at 200, 150 and 100ind/l of the T_1, T_2 and T_3 respectively. Dead larvae were picked daily and recorded.

Feeding of fish: *Artemia* and custard were used as live and formulated feed that was made from locally available ingredients (Table 1). The basic guideline used in estimating the amount of feed to be given to the larvae was 10-15% of the total body weight. Depending on the daily actual food consumption, feeding was adjusted accordingly. The larval feeding programme used was as described in Table 2.

Monitoring of water quality: Siphoning has been done daily morning and afternoon for removal of waste particles. To maintain an optimum environment for larval growth, 20-30% volume water exchange has been done every day. The water removed has been replaced by ready mix, aerated, 12 ppt salinity of water at the same temperature as that of the larval tank. Water quality was monitored by observing the physico-chemical parameters *viz.* water temperature, water pH, Dissolved Oxygen (DO), Salinity, and Alkalinity, Nitrite- nitrogen (NO_2- N) and Ammonia (NH_3). OTC (4ppm to 4.75ppm) and Formalin (25ppm) were used in two days intervals for preventive measure (periodic medication) to avoid undesirable situation.

Table **1.** Ingredients of Formulated diet (Custard) for Larvae

Ingredients	Quantity of ingredients
Powered milk	200.0 g
Corn flour	60.0 g
Egg (6 nos)	210.0 g
Prawn/ Shrimp	180.0 g
Cod liver oil	10.5 ml
MultiVitamin	1.5 g
Agar powder	6.0 g

Table 2. Feeding schedule used for the treatments 1, 2, and 3 in larval rearing

Feed Items	Feeding times	Larval stages											
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	PL1
Artemia nauplii	Two times (8.00, 18.00)	4-5 nauplii/ml				2-3 npi/ml							
Custard	Two times (8.00, 18.00)					5-10 g/ton		15-20 g/ton					

Growth and survival rate monitoring: By the volumetric estimation of stocked larvae were monitored daily to observe the survival rate. The growth was measured with centimeter scale for length and weighing by electrical balance. The larval behavior was regularly visually checked twice a day, especially after feeding, and early morning to determine the larval conditions (active swim, pigmentation, accumulates at the tank bottom) after stopped aeration. One hundred larvae were sampled daily and observed under an electrical (binocular stereo) microscope to cleanliness of the gills, stomach content, present or absence of necrosis, and filamentous infection on the appendages.

Results and Discussion

Water quality: During the study period, water temperature in different larval rearing tanks was ranged from 28.5°C -31°C in the morning and 28.5°C -31.5°C in the afternoon. The mean water temperature was recorded as 30.1°C, 30.2°C, and 30.1 °C in T_1, T_2 and T_3 respectively. During this study, the water temperatures were ranged from 28.5°C to 31.5°C (Chowdhury *et. al.* 1993). New and Singholka (1985) reported that the optimal temperature in prawn larval rearing water which could range from 26-31°C. Water pH values were ranged (6.5-8.4) in the morning and (6.8-8.2) in the evening. In the present study, the pH was ranged from 6.5 to 8.4 where the various authors suggested 7.0 to 8.5 for prawn larval rearing (Ling, 1969), Aquacop (1984) and New (1990). The mean DO values were recorded 5.8 mg/L, 5.8 mg/L, and 5.9 mg/L in different treatments T_1, T_2 and T_3. The mean values of NO_2-N concentration were found 0.19 mg/L, 0.18 mg/L, and 0.15 mg/L in treatments T_1, T_2 and T_3 respectively. The mean values of NH_3 concentration were found 0.29 mg/L, 0.26 mg/L, and 0.23 mg/ in treatments T_1, T_2 and T_3 respectively. The mean values of alkalinity were recorded 145 mg/L, 150 mg/L, and 155 mg/L in T_1, T_2 and T_3 respectively. The research was designed to maintain the standard salinity 12‰ for larval rearing in all treatments until complete metamorphosis which was strongly recommended (New, M. B. and Singholka S. 1985). The optimum salinity range (13-15‰) for *M. rosenbergii* larvae (Fujimura and Okamoto, 1972; Ling, 1969). NH_3 is the principal excretory metabolic of prawns and is generally considered a major cause of death in hatchery condition. In a conditioned system, a bacterial nitrification process converts NH_3 to relative non-toxic NO_3 where as the intermediate product; NO_2 is highly toxic to aquatic vertebrates. Observed NO_2-N concentration was ranged from 0.1-0.3mg/l where the *Macrobrachium* larvae can tolerate 1.8mg/l concentration (Armstrong *et al.* 1976).

Growth rate: In the total study period the growth rate of the larvae observed. Growth rate in terms of body length was 0.126 mm/day, 0.136 mm/day, and 0.169 mm/day in T_1, T_2 and T_3 respectively whereas growth rate in terms of body weight was observed 0.000115g/day, 0.000180g/day, and 0.000240g/day in T_1, T_2 and T_3 respectively. From the beginning of rearing (stage I) until the stage VI there was no significant difference in the growth rate of larvae (0.3mm/day) in each treatment. In stage VII and VIII faster (1.017mm/day) growth was observed whereas in stage IX to XI moderate (0.367mm/day) growth was recorded. The overall growth rate of larvae in treatment T_3 was faster than T_1, and T_2. By the present investigation it was reveal that 22-45 days were required to complete metamorphosis (11 larval phases) of the *Marcobrachium* larvae. The result of the experiment was similar with

studies of previous study (Uno and Soo, 1969). It has been needed 15-60 days for prawn larvae to pass 11 molting phases (Thang, 1995). Mean growth rate in terms of weight and length was higher in T_3 than T_1 and T_2. After metamorphosed to postlarvae, the average size (length) was 6.7, 6.9, and 7.4 mm in T_1, T_2 and T_3 respectively (Fig. 1) whereas the average weight was 0.00045, 0.00066, 0.00078 gm (Fig. 2). The weight of early postlarve is 0.006-0.009 gm and 7-10 mm in length 4 (D'Abramo *et. al.* 2003). After metamorphosis the larvae appears about 7 mm length.

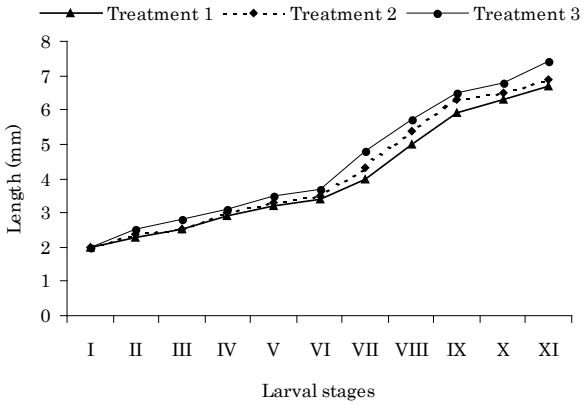

Fig. 1. Larval growth (length) in 3 different treatments

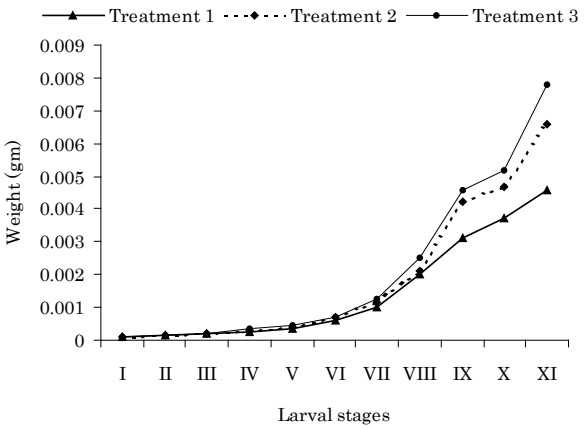

Fig. 2. Larval growth (weight) in 3 different treatments

Survival rate: The first postlarvae were observed after 26, 24, and 22 days of rearing in T_1, T_2 and T_3 respectively (Fig. 3). When the postlarval stage appeared, it was easier to identify by the change of swimming to crawling on the bottom or the wall of the tank. However, 100% larvae (survived) were metamorphosed at the 41st, 38th and 34th day for treatment T_1, T_2 and T_3 respectively. Though the initial stocking density of the larvae was 200 larvae/L, 150 larvae/L, and 100 larvae/L, in the treatments T_1, T_2 and T_3 respectively, the average post larvae were 43PL/L, 45PL/L, and 51PL/L during harvest. At the end of rearing cycle the survival rate of larvae were found 21.8%, 30.4% and 51.3% in treatments T_1, T_2 and T_3 respectively (Table 3). So, the survival rate was comparatively higher in T_3 than T_2 and T_1.

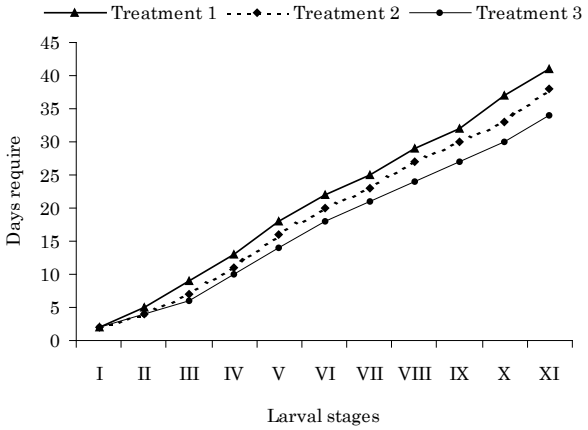

Fig. 3. Larval development (days) in 3 different treatments

Survival rate was nearly 50% in hatcheries, but less than 1% in the wild (Malecha *et al.*, 1980). In the present trial, 21.8%, 30.4% and 51.3% survival rate was achieved in treatments T_1, T_2 and T_3 respectively where the initial stocking density of larvae was 200 larvae/l, 150 larvae/l, and 100 larvae/l for respective treatment. During late larval stages, mortality can occur due to cannibalism (Suharto and Djajadiredja, 1980). Similar to that report, in the present trail cannibalism was observed when the larvae metamorphosed into post larvae immediately after larval molting. The mortality caused by cannibalism was high when the larval population was well dispersion or was insufficient nutrition or both due to the high stocking densities. Mortality of larvae was also observed as larvae jumped and got stranded on tank wall above the water surface. Larvae were seen to start jumping after stage VIII, especially after feeding with prepared feed. To reduce this mortality, aeration was adjusted and the stranded larvae on the tank wall were more frequently rinsed.

From the start of *Macrobrachium* larval rearing, the stocking density was of much concern in this trail. However, rearing density depended on the technique applied. In clear water open system, the postlarval production varied widely and was reported 19PL/L (Ling, 1969), 11PL/L (Fujimura and Okamoto, 1972), 30PL/L (Malecha, 1982), 10-20PL/L (New and Singholka, 1985). In this study, the average postlarvae were 43PL/L, 45PL/L, and 51PL/L during harvest in treatments T_1, T_2 and T_3 respectively (Table 3). Survival rate of *Macrobrachium* larvae expressed in percentage was no longer a real indicator of success in prawn larval culture (Menasveta, 1980). The yield of post larvae per unit volume of water was more realistic.

Disease: Different diseases *viz.*, Zoothanium (Protozoan disease), Mid Cycle Disease (MCD) were diagnosed in this trial. Severe Zoothanium infestation was observed in all treatments which colonized in the culture media and exoskeleton of larvae causing mortality in these trials. For the prevention, 25pp Formalin as a 24 hours static bath was applied in controlling larval Zoothanium infestation. Then to improve the water quality 30% water has been changed. MCD was observed in late stage (IV-XI) larvae

and early post larvae in the T_1 where the highest number larvae were initially stocked. The cause of MCD is Unknown, but *Enterobacter aerogenes* has been associated with the disease. For these reasons, nutrients enriched foods and multivitamins (0.25-0.50 ppm) were incorporated in their diet. Besides by the microscopic observation, BN was found in the few larvae of T_1 during IV and V stages. Then preventive measure was taken by using (4.0-4.75) ppm OTC.

Table 3. Summery of the result that was found in three different treatments

Tank No	Stocking density/L	PL occurred (days after hatching)	Total PL production/Tank	PL /L	Survival (%)
T1	200	38	131000	43	21.8
T2	150	37	137000	45	30.4
T3	100	34	154000	51	51.3

In addition, the mortality caused by disease was the most serious case. Though, different disease viz., *Zoothanium*, MCD, BN were diagnosed in this trail, but the *Zoothanium* had the severe effects on larvae than other occurred diseases and their presence was observed in the water and exoskeleton of larvae causing mortality in the all treatments, *Zoothanium* was found all stage of the larval development. T_1 resulted in mass mortality due to the high socking density and occurrence of *Zoothanium*, MCD and BN diseases. By this investigation, it was observed that MCD affects late stage larvae and early postlarvae where the affected larvae were unable to free appendages, eyes or rostrum from the exuvia in which they became entrapped. Other larvae, which shed the exuvia, have malformed appendages and die shortly after molting. This observation was completely coincided with Brock (1983, 1988). Clinical sign of the BN disease were a bluish colour or discoluoration, empty stomach, weak larvae falling to the bottom of the tank, and brown spots at the antennae and newly formed appendages. This disease was more severe in early stages of larvae and identified as major threat to cause massive mortality that was observed in T_1. BN disease affecting *Macrobrachium* larvae (stage IV-V) and causing up to 100% mortality in 48 hours. Another important finding was that MCD and BN only occurred in T_1 and there was no presence of MCD and BN in T_2 and T_3 where the initial stocking was relatively low than T_1 which reveals that by lowering stocking densities with optimum water quality management in LRT, MCD and BN could prevent (Aquacop, 1977).

Acknowledgement: Heartiest thanks to our dedicated hatchery technicians for their heard labor and support during the experiment. Thanks to the researcher M. Nazim Uddin and their associate, they were prepared a research article published in *AQUANET* which helped in completion of the experiment.

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