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Growth Performance and Survival Rate of *Macrobrachium rosenbergii* (De Man, 1979) Larvae Using Different Doses of Probiotics

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Abstract: The efficiency of probiotics (Ecomarine) in rearing of *Macrobrachium rosenbergii* larvae was evaluated in a commercial prawn hatchery for five weeks. Stage-1 (zero age) larvae (of length: 2 mm; weight: 0.12 mg) were stocked at the rate of 100 L^{-1}. The experiment determined the growth rate, survival rate of the larvae for the both treatment and control groups. Final average weight were found 8.39±3.28E-04 and 8.18±2.86E-04 mg and length were found 9.08±0.649 and 9.02±0.081 mm for treatment and control group respectively. Comparatively higher growth performance was observed in treatment than control. Post Larvae (PL) was first observed 20th days of culture in treatment tanks whereas PL in control tanks was found 24th days of culture. Survival rate was found 58 and 46% in treatment and control group respectively. There was significant ($p<0.05$) survival rate between two experiment groups. This study revealed that probiotics could be better in quality seed production of *M. rosenbergii* while significant changes were not noticed in the physic-chemical parameters i.e., water temperature, salinity, DO, pH, nitrate-NO_2, hardness and alkalinity observed in both the treatments.

Key words: Probiotics, growth performance, survival rate, *Macrobrachium rosenbergii*, commercial hatchery

INTRODUCTION

Aquaculture is an important economic activity in many countries especially in Asian part of the globe (Ahmed *et al*., 2012, 2013; Hossain *et al*., 2013). It is an increasingly important source of animal protein (Wang, 2007). In last few years, the fresh water prawn *M. rosenbergii* was familiar as a species with great aquaculture value (Keysami *et al*., 2012). In large-scale production, where aquatic animals are exposed to stressful conditions, problems related to diseases and environmental deterioration often occur which finally result in serious economic losses. In recent decades, the use of veterinary medicine and chemical additives are significantly increased for disease prevention and control (Wang *et al*., 2008). Moreover, Wang (2007) mentioned that the abuse of antimicrobial drugs, pesticides and disinfectants in aquacultural disease prevention and growth performance was led to the evolution of resistant strains of bacteria and it's a question of safety. In addition, there are environmental problems associated with the chemical additives as well (Verschuere *et al*., 2000). Therefore, the use of probiotics for aquatic animals is increasing with the demand for environment-friendly sustainable aquaculture (Gatesoupe, 1994). The use of probiotics in aqua feeds received considerable attention in recent years. The rationale of their use in aquaculture is to improve feed intake, survival and to minimize feed wastage and water pollution (Verschuere *et al*., 2000).

The benefit of probiotics supplements include improved food value, enzymatic contribution to the digestive system and inhibition of pathogenic microorganisms, growth promoting factors and increased immune response (Verschuere *et al*., 2000). In intensive culture systems, usually increase load of material in the culture bottom due to the uneaten feed, feces and organisms die-offs. Thus, water quality in intensive systems is a key issue which controlled by the microbial biodegradation of organic residues (Avnimelech *et al*., 1995). Microbial process affects water quality mainly due to utilization of oxygen, regeneration of inorganic nutrients and produce toxic substanceslike ammonia,

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nitrite and sulphide (Devaraja *et al*., 2002). Therefore, microbes play a critical role in aquaculture systems, in both the hatchery and the grow out stage, because water quality and disease control are directly related and closely affected by microbial activity. Probiotics increases survival rate and decreases mortalityas well as occur early molting (Venkat *et al*., 2004). Probiotics bacteria in feed or additive form were proved to be beneficial for growth and survival of shrimp and prawn (Rengpipat *et al*., 1998). This study was aimed to evaluate the effect of probiotics on larval growth and survival rate of *Macrobrachium rosenbergii*.

MATERIALS AND METHODS

Experimental design: The experiment was conducted in a commercial hatchery. Same size plastic tanks (250 L) were used for larval rearing in which one treatment and a control group with three replicates each. The tanks were identified TA and CA. There was no alternative way to maintain optimum environment for larval growth and survival without daily water exchange. No water exchanged first two days of stocking. Water exchange rate was 70-80% (first week to fourth week) and 80% was last week that is until harvesting. This volume of water was exchanged at every afternoon. Probiotics were applied after water exchange. Whole duration of experiment was 35 days from larval rearing tank preparation to PL harvesting.

Probiotics and application: Ecomarine probiotics was used for experiment. Its main component of five beneficial bacteria are *B. subtilis*, *B. pumilus*, *B. amyloliquefaciens*, *B. megaterium*, *B. licheniformis*. This probiotic was applied every day after exchange of water until PL harvesting. Doses were increased with increasing age of larvae. Probiotics was directly applied in the rearing water.Application doses of Ecomarine probiotic during larval rearing have been furnished in Table 1.

Feeds and feeding: Furthermore, live feed and formulated feed were given for larvae. Live feed (*Artemia nauplii*) was given at the rate of 5 n mL^{-1} (first week) and then 4 n mL^{-1} daily two times (morning and evening) until harvesting in all experiment groups. In addition, custard was given daily three times (early morning, morning and afternoon) from 9th day to harvesting at the following rate 1.4 g $tank^{-1}$ (second week), 2.14 g $tank^{-1}$ (third week) and finally 2.85 g $tank^{-1}$ until harvesting into all experiment groups.

Table 1: Application doses of ecomarine probiotic during larval rearing

Day of culture	Dosage $tank^{-1}$ (ppm)	Usage and frequency
First week	0.30	Everyday after exchange of water
Second week	0.50	Everyday after exchange of water
Third week	0.85	Everyday after exchange of water
Fourth week	1.35	Everyday after exchange of water
Fifth week	1.80	Everyday after exchange of water

Water quality maintenance: Physico-chemical parameters were monitored every day. Siphoning and water exchange were performed daily. Water exchange was done 80% volume of every day. The aeration system in the backyard hatchery was performed with a compressor. Water quality was maintained by controlling the physio-chemical parameters of the rearing tanks. The following parameters such as water temperature, salinity, DO, pH, nitrate-NO_2 and hardness were recorded regular interval and maintained optimum condition. Among these nitrate-NO_2 and hardness were recorded every three days interval and remaining test were recorded daily according to the APHA (1992) by test kits and manually.

Growth and weight analysis: Increment of weight was started from 8th day and continued for fifth week. So the increment of weight was recorded after the first week and continued for fifth week with a weekly interval. For measuring weight, 30 larvae (15 for each LRT) were sampled for Treatment and Control group, respectively. Larvae were weighted by electronic balance.

The increment of length was recorded after the 1st week and continued for fifth weeks with a week interval. For measuring length, 30 larvae (15 for each LRT) were sampled for Treatment and control group, respectively. Larvae were measured by millimeter scale.

Specific growth rate (SGR): The specific growth rate was determined from the following equation as advocated Sinha (1981):

$$SGR\ (\%/day) = \frac{\log w_t - \log w_0}{t} \times 100$$

Where:

wt = Mean body weight (g) at time t
w_0 = Mean body weight (g) at time 0
t = Times in day

Mean daily growth rate (g day^{-1}): Four separate mean daily growth rate values were recorded from 1st week to 5th week for every corresponding week of each Treatment and control and finally the overall mean daily growth rate (g day^{-1}) value was determined for the both Treatment and Control. Mean daily growth rate in terms of length and weight were determined from a simple mathematical equation as advocated by Sinha (1981):

$$\text{Mean daily growth rate (g/day)} = \frac{W_t - W_0}{t}$$

$$\text{Mean daily growth rate (mm day}^{-1}) = \frac{L_t - L_0}{t}$$

where, W_t = Mean weight (g) time t, W_0 = Mean weight (g) at time 0, L_t = Mean length (mm) at time t, L_0 = Mean length (mm) at time 0, t = times in day.

Survival rate (%): Survival rate was recorded after the 1st week and continued for next five weeks with a weekly interval. Survival rate was calculated by using the following equation (Narasimham, 1970):

$$\text{Survival rate (\%)} = \frac{\text{Total population}}{\text{Total No. of larvae stocked}} \times 100$$

Statistical analysis: Recorded data were analyzed using Microsoft Excel 2007 software. Survival rate between initial number of fries and alive number of fries after a week wasanalysed using regression analysis and level of significance was $p<0.05$ (95% confidence level).

RESULTS AND DISCUSSION

Physico-chemical parameters: Waters parameters are important factors to provide an ideal rearing environment for any kind of shellfish larvae. The water temperature was recorded from 28-31°C in both experiments in the present study (Table 2). Chowdhury *et al.* (1993) recorded the optimum temperature was 28-31°C for better survival rate. The concentration of dissolve oxygen was also fairly well as no stocked organisms showed any sign of oxygen deficiency. In this study dissolved oxygen content was found from 5.6-7.5 and 5.3-7.4 mg L^{-1} in treatment and control, respectively (Table 2). The mean values of DO were 6.39 and 6.29 in treatment and control, respectively. Suitable range of DO was 3.0-6.1 mg L^{-1} for *M. rosenbergii* (Hossain and Paul, 2007).

Water pH indicates acidic or alkaline condition of water. It is an important factor for rearing prawn larvae. The water pH was recorded from 6.9-8.2 and 6.8-8.2 in treatment and control, respectively. The mean values of water pH were 7.63 and 7.62 in treatment and control, respectively. Chowdhury *et al.* (1993) reported that pH ranged from 7.0-8.5 was suitable for *Macrobrachium rosenbergii* larvae. Chowdhury *et al.* (1993) reported 0.1 ppm NO_2-N is required for better growth. The range of NO_2 –N was measured from 0.01-0.15 mg L^{-1} and 0.07 mg t^{-1} to 0.15 mg L^{-1} in both treatment and control.

Table 2: Mean value of physico-chemical parameters of treatment and control

Parameters	Treatment	Control
Temperature (°C)	29.700	29.200
DO (mg L^{-1})	6.390	6.290
Salinity (ppt)	12.000	12.000
Water P^H	7.630	7.600
Nitrate-NO_2	0.094	0.085
Hardness ppm $CaCO_3$	107.460	110.190
Alkalinity (ppm)	104.270	110.000

Increase of weight and length: At the end of the five weeks, higher mean weight (8.39±3.28E-04 mg) was recorded in treatment and lower mean weight (8.18±2.86E-04 mg)was recorded in control. At the end of the experiment, higher mean length (9.08±0.649 mm) was recorded in Treatment and lower mean length (9.02±0.081 mm) was recorded in control group.

Specific growth rate (SGR): Specific growth rate was determined after first week. Specific growth rate was recorded from 3.34±1.237-26.13±0.986% day^{-1} and 2.76±0.0547% day^{-1} to 25.76±0.838 in treatment and control respectively during 2nd to 5th week. Overall specific growth rate was higher in treatment (13.28% day^{-1}) than in control (12.76% day^{-1}). Daily mean growth in terms of weight and daily mean growth in terms of length were measured as well. Overall daily mean growth in terms of length was higher in treatment (0.289 mm day^{-1}) than in control (0.251 mm day^{-1}).

Growth performance, survival rate and probiotic: In the present study, growth performance of prawn larvae using probiotics in treatment was showed better performance than the larvae using in control (negative control) where no chemical was used. Mean growth in terms of weight of prawn larvae was higher in treatment than control in every sampling. The final mean weight of the larvae in treatment and control was 8.39 mg and 8.18 mg respectively and the final mean length of the larvae in treatment and control was 9.069 and 9.021 mm respectively. These data is coincide with the finding of (D'Abramo *et al.*, 2003) who reported that after metamorphosis to post larvae, the prawns resemble miniature adults, having a total body length of 7-10 mm and weighing 6 to 9 mg.

In the present study first 10% PL observed in treatment after 20 days of stocking and in control after 24 days of stocking. After 33 days of stocking, 100% PL in treatment and 95% PL in control were observed. Chowdhury *et al.* (1993) reported that before metamorphosis, the larvae passes through 11 distinct stages and takes 35-50 days. FAO (2002) found that most of the prawn larvae should have metamorphosed into PL by 25-35 days at the recommended temperature of 28-31°C and it is not usually economically viable to maintain any batch longer than 32-35 days.

Table 3: Survival rate of prawn larvae of treatments and control group

Sampling time (week)	Treatment name	Initial No. of alive fry	Alive No. of fry (avg)	Survival rate % (avg)
1st	TT	25000	21087	84
	CT	25000	17300	69
2nd	TT	21087	16420	77
	CT	17300	11062	63
3rd	TT	16420	10440	63
	CT	11062	5797	52
4th	TT	10440	6487	62
	CT	5797	3035	52
5th	TT	6487	3782	58*
	CT	3035	1407	46*

*Final survival rate obtained by probiotics. TT = Treatment tanks, CT: Control tanks

Table 4: Regression analysis of survival rate on the corresponding initial and final larvae stocks

Regression statistics					
Multiple R					0.982
R square					0.965
Adjusted R square					0.964
Standard error					1234.293
Observations					40
ANOVA	df	SS	MS	F	p-value
Regression	1	1577405263	1577405263	1035.396	0.003
Residual	38	57892252.57	1523480.33		
Total	39	1635297516			

The survival rate of fresh water prawn larvae was 58 and 46% in treatment and control group respectively in the present study (Table 3). In this research, treatment group showed better survival rate (Table 4). This might be due to good water quality as well as using of probiotics than control. Chowdhury *et al.* (1993) noted that larvae stocking 100 L^{-1}, keeping all these parameters favorable and by controlling management accordingly, the survival rate was 30-40 PL L^{-1} fed on brine shrimp nauplii and custard. Survival rate of 15 to 20 PL L^{-1} seems to be about an average level. Although, FAO (2002) reported that 40-60% survival rate was more normal in practice by using Brine Shrimp Nauplii (BSN) and Egg Custard (EC) as a larval feed. Phuong *et al.* (2006) found that, 27.4% survival rate in the re-circulating water system at a stocking density of 120/L fed on egg custard.

CONCLUSION

It was observed that by using of probiotics in hatchery, production volume was increased by about 12% rather than as usual (without any additives) production practice in hatchery. Furthermore, probiotics is a welcome addition to protection of diseases as well as reduce various gases in rearing tanks. Finally, the success of aquaculture in future might be expanded with the success of probiotics applications.

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