Survival and Growth of *Macrobrachium rosenbergii* (de Man) Juvenile in Relation to Calcium Hardness and Bicarbonate Alkalinity

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Abstract

Survival and growth of *Macrobrachium rosenbergii* juvenile (0.045±0.007 g, 25 days old) were investigated under different calcium hardness levels from 92 to 384 mg/L $CaCO_3$ for 42 days. The maximum survival rate (100%) was observed at a calcium hardness level of 92 mg/L $CaCO_3$, while the lowest survival rate (60%) was recorded at the highest calcium hardness level of 384 mg/L $CaCO_3$. Maximum growth of the prawn (11.6±2.7 mg/day) was observed at 132 mg/L $CaCO_3$. However, there were not significant ($P>0.05$) changes in growth at calcium hardness levels of 92 and 192 mg/L $CaCO_3$. The growth of the prawn declined significantly ($P<0.05$) at calcium hardness level of 228 mg/L $CaCO_3$ and above. Survival and growth study of *M. rosenbergii* juvenile (0.12±0.03 g, 60 days old) was also done under different bicarbonate levels from 100 to 370 mg/L $CaCO_3$ for 60 days. Survival rate of the prawn was highly impaired at the highest bicarbonate levels tested while maximum survival rate (90%) was recorded at bicarbonate level of 100 mg/L $CaCO_3$. The highest growth (4.92±0.13 mg/day) of the prawn was recorded at lowest bicarbonate level of 100 mg/L $CaCO_3$ tested and the growth significantly ($P<0.05$) declined at 205 mg/L $CaCO_3$ bicarbonate alkalinity and above.

Key words: survival rate, growth, *Macrobrachium rosenbergii*, hardness, alkalinity.

Introduction

Alkalinity is an important criterion for determining the effect and concentration of water quality constituents and criteria; and therefore, the general suitability of a water source for the culture of fish and prawns. Water of low alkalinity (<20 mg/L $CaCO_3$) is considered to be less suitable for fish and prawn culture due to the associated unstable water chemistry. The upper limit of alkalinity is largely defined by individual species requirements and the magnitude of the concomitant increase in pH value. In general, freshwater fish in hard water (100-150 mg/L $CaCO_3$) tend to spend less energy on osmoregulation, resulting in a better growth. The upper limit of alkalinity may also be related to its effect on osmoregulation at high ion concentrations (Department of Water Affairs and Forestry, 1996).

Hardness is a measure of water's calcium and magnesium. Calcium and magnesium are very important to freshwater prawns in keeping exoskeletons strong. When hardness is too low prawns will take longer time after a molt for the exoskeleton to harden. While soft prawns are vulnerable to mechenical damage and predator. Prawns exposed to soft water are more susceptible to other water quality conditions also. Very hard water can also be a problem. Ideal ranges of water hardness could be 50 to 200 mg/L $CaCO_3$ for optimum growth of prawns (Wetzel, 2001).

Wickins (1972) suggested a hardness range of 65 to 200 mg/L $CaCO_3$ for satisfactory culture of *M. rosenbergii* while New and Singholka (1985) recommended on hardness levels of 40 to 100 mg/L $CaCO_3$. A greater inhibition of growth at hardness levels above 65 mg/L $CaCO_3$ was reported by Cripps and Nakamura (1979). Brown *et al.* (1991) also reported that the growth of juvenile prawn was maximum at <53 mg/L $CaCO_3$, and did not change significantly at lower hardness levels but decreased at higher levels. Hardness levels between 940 and 1060 mg/L $CaCO_3$ did not adversely affect the growth, though the water had relatively low alkalinity between 58 and 86 mg/L $CaCO_3$ (Bartlett and Enkerlin, 1983). However, Howlader and Turjoman (1984) also reported an adversely affected growth at high hardness levels of 688 to 987 mg/L $CaCO_3$. Thus it is evident that hardness and alkalinity play an important role on the growth of freshwater prawn *M. rosenbergii*. The present paper reports about survivability and growth of *M. rosenbergii* in relation to calcium hardness and bicarbonate alkalinity.

Materials and Methods

The present investigation has been carried out at the Central Institute of Freshwater Aquaculture (CIFA).

Effect of calcium hardness on the growth of *M. rosenbergii*

Two hundred juveniles of giant freshwater prawn were collected from the Giant freshwater prawn hatchery of the Institute in September 2002. Twenty -five days old juveniles having the average weight of 0.045 g of the same stock were used. They were brought to the laboratory in a bucket having well oxygenated water, where they were stocked in three tanks (30×30×38 cm) with 34.2 liters maximum capacity each, which had 25 liters of well-oxygenated tap water. The juveniles were acclimatized in the experimental condition for 1 week.

One thousand ppm stock solution of calcium was made from $CaCl_2.2H_2O$ (Merck, Mumbai, India) for the calcium treatment. Six concentrations of calcium hardness (92, 132, 192, 228, 288 and 384 mg/L) were used for the present experiment. Three replicates were kept for each concentration of calcium hardness.

Eighteen cylindrical (radius 11 cm, height 30 cm) tanks were used for this study. Eight litre well oxygenated tap water was filled in each tank. Eight prawns were stocked in each tank and each tank was connected with aerator for 18 hours per day. After stocking, the calcium treatment was given. The mussel shells and small plastic pipes were used at the bottom in each tank to allow prawn to distribute themselves better throughout the water in order to reduce cannibalism associated with crowding. Prawns were fed with pelleted feed at the rate of 10% body weight twice daily at 08:00 and 18:00 h. The feed energy composition was protein 40%, fat 6%, nitrogen free extract 35%, moisture 6% and ash 13%. The diameter of feed pellets was 1-2 mm. The feed was prepared using Pearson-Hand square method. The unconsumed feed and faecal matter was siphoned off on every alternate day. The half of the experimental media also renewed on alternate days. The physico-chemical parameters of water were measured at every five-day intervals following the standard methods (APHA 1989) and the data are presented in Table 1. The experiment was continued for 42 days. Prawns were collected on 43rd day by siphoning off the tank's water and weight was taken by an electronic balance (Sartorius, Bangalore, India).

Effect of alkalinity on *M. rosenbergii*

Two hundred juveniles of giant freshwater prawn were collected from the Institute's prawn hatchery in September 2004. The juveniles were of 60 days old having the average weight of 0.120 g of same stock. They were brought to the laboratory in a bucket having oxygenated water where they were stocked in three tanks (30 x 30 x 38 cm) with 34.2 litres maximum capacity, each which had 25 litres of oxygenated tap water. The juveniles were acclimatized in the experimental condition for 1 week.

One thousand ppm stock solution of sodium bicarbonate was made for the alkalinity experiment. Four concentrations of bicarbonate alkalinity (100, 205, 285 and 370 mg/L as $CaCO_3$) were selected for the present study. Three replications were maintained for each alkalinity level.

In this experiment twelve cylindrical (radius-11 cm, height-30 cm) tanks were used. Eight litre well oxygenated tap water was filled in each tank. Eight numbers of prawns were stocked in each tank and each tank was connected with aerator for 18 h day^{-1}. After stocking in each tank, sodium-bicarbonate treatment was provided to get the different alkalinity levels. The mussel shells and small plastic pipes were also used at the bottom in each tank in the present study like the previous experiment. The prawns were fed with the pelleted feed at the rate of 4% body weight of the prawn. The feed was provided at 08:00 h and 18:00 h daily. The feed energy composition was same as calcium hardness experiment except feed pellet diameter, which was 3 mm in this study. The unconsumed feed and faecal matter was siphoned off on every alternate day. The half of the test solutions was renewed on alternate days. The experiment was continued for 60 days. Prawns were collected 61st day by siphoning off the tank's water and weight was taken. The physico-chemical parameters of water were measured at every five days intervals following the standard methods (APHA, 1989) and the data are presented in Table 4.

Statistical analysis

Multiple regression analysis was performed using SPSS software to find out the significant differences among treatment means for growth studies at different calcium hardness levels. One way analysis of variance (ANOVA) with Duncan multiple range test (DMRT) was applied to find out the significant differences among treatment means using the SAS computer software for growth studies of prawns at different bicarbonate alkalinity levels.

Results

Some important water quality parameters from *M. rosenbergii* juveniles exposed to waters of different calcium levels are presented in Table 1. The water quality parameters were optimum for the growth of the prawn. In the present study, highest calcium hardness level (384 mg/L) of water recorded low survivability (60%) of the prawn while 100% survival was observed at lowest calcium hardness level (92 mg/L). At intermediate calcium hardness levels, between 132 and 192 mg/L $CaCO_3$, survival was also very good, ranging from 80 to 90%. Though the prawn survived at high calcium hardness levels (288 to 384 mg/L), growth of these prawns was reduced to less than 5.7 mg/day (Table 2). The growth rates at the calcium hardness levels between 132 and 192 mg/L $CaCO_3$ were significantly ($P<0.05$) greater than the growth at higher hardness levels ($r=-0.8185$; slope=-0.0228; Intercept=10.7555; $r^2=0.669$). No significant differences were observed in growth rate

(4.4 to 8.3 mg/day) between the lowest (92 mg/L) and higher (between 288 to 384 mg/L) calcium hardness levels. The maximum growth rate (11.6 mg/day) occurred at 132 mg/L calcium hardness level. The mean final live weight of prawns at 132 mg/L calcium hardness level was maximum while the minimum mean final live weight was occurred at 384 mg/L calcium hardness level. The mean live weight of prawns at various calcium hardness levels was statistically significant ($P<0.05$) ($r=-0.8159$; slope=-0.0009; intercept=0.477; $r^2=0.666$).

It is evident from Table 3 that the survivability of the prawn was maximum (90%) at an alkalinity level of 100 mg/L $CaCO_3$ and the least survivability (10%) was recorded at an alkalinity level of 370 mg/L $CaCO_3$. The growth of the prawn was also maximum (4.92 mg/day) at the alkalinity of 100 mg/L $CaCO_3$ while the least growth rate (2.33 mg/day) was recorded at the highest alkalinity level of 370 mg/L $CaCO_3$. The growth of the prawns at 100 mg/L alkalinity was significantly higher than the growth of the prawns at other alkalinity levels ($F=5.973$; $P<0.05$). Important water quality parameters from *M. rosenbergii* juveniles exposed to different bicarbonate alkalinity levels are presented in Table 4. The water quality parameters were optimum for the growth of the prawn.

Table 1. Some important water quality parameters from *M. rosenbergii* juveniles exposed to waters of different calcium levels

Calcium hardness (mg/L as $CaCO_3$)	92	132	192	228	288	384
pH	8.0± 0.2	7.8± 0.1	7.7± 0.1	7.9± 0.2	7.7± 0.2	7.7± 0.1
Electrical conductivity (ds/m)	0.33±0.07	0.44± 0.06	0.58± 0.04	0.68± 0.06	0.77± 0.04	0.94± 0.06
Temperature (°C)	24± 1.2	24± 1.2	25± 1.6	24± 1.4	25± 1.4	25± 1.6
Ammonium nitrogen (mg/L)	0.13± 0.02	0.11± 0.03	0.16± 0.02	0.09± 0.03	0.11± 0.02	0.06± 0.02
Nitrate nitrogen (mg/L)	0.46± 0.04	0.43± 0.07	0.30± 0.05	0.35± 0.05	0.23± 0.06	0.23± 0.07
Soluble ortho-phosphate (mg/L)	0.08± 0.02	0.08± 0.02	0.09± 0.01	0.10± 0.01	0.09± 0.02	0.08± 0.02
Potassium (mg/L)	10.2± 0.6	10.2± 0.8	9.6± 0.6	11.2± 0.6	9.6± 0.6	10.0± 0.4

Table 2. Survival and growth of *M. rosenbergii* juveniles in waters with different calcium hardness levels

Calcium hardness (mg/L as $CaCO_3$)	92	132	192	228	288	384
Survival (%)	100	80	90	80	70	60
Mean growth rate (mg/ day)	8.3±3.0	11.6±2.7	10.1±2.1	5.7±1.7	5.6±1.4	4.4±1.3
Mean final weight (g)	0.38±0.15	0.51±0.09	0.45±0.07	0.27±0.10	0.28±0.08	0.22±0.14

Table 3. Survival and growth of *M. rosenbergii* juveniles in waters of different bicarbonate alkalinity levels

Bicarbonate alkalinity (mg/L as $CaCO_3$)	100	205	285	370
Survival (%)	90	40	20	10
Mean growth rate (mg/ day)	4.92±0.13	2.8±0.79	2.4±1.02	2.3±0.18
Mean final weight (g)	0.53±0.01	0.30±0.06	0.29±0.03	0.26±0.04

Table 4. Some important water quality parameters from *M. rosenbergii* juveniles exposed to waters of different bicarbonate alkalinity levels

Bicarbonate alkalinity (mg/L as $CaCO_3$)	100	205	285	370
pH	7.45± 0.15	7.75± 0.12	7.85± 0.10	7.86± 0.14
Temperature (°C)	22± 1.5	21± 1.2	21± 1.3	22± 1.2
Electrical conductivity (dSm^{-1})	0.31± 0.09	0.39± 0.06	0.51± 0.09	0.70± 0.08
Ammonium nitrogen (mg/L)	0.06± 0.01	0.08± 0.01	0.07± 0.02	0.10± 0.01
Nitrate nitrogen (mg/L)	0.17± 0.03	0.19± 0.02	0.16± 0.02	0.20± 0.03
Soluble orthophosphate (mg/L)	0.07± 0.02	0.08± 0.01	0.09± 0.02	0.08± 0.02
Potassium (mg/L)	10.2± 0.8	9.8± 0.6	10.0± 0.6	10.2± 0.8

Discussion

In the current study, high calcium hardness level (>228 mg/L as $CacO_3$) and high bi-carbonate alkalinity level (>205 mg/L as $CaCO_3$) affected the survival of *M. rosenbergii*. Earlier studies have shown that high calcium level could affect the growth of the prawn, but these studies have not reported the effect of calcium on survival rate of the prawns. This could be related to the relative sizes of prawns and the method used in different studies. In the tank-based study of Cripps and Nakamura (1979), the prawns used were very much larger than those used in the present study. Other trials were based on pond studies in which survival was not so easily determined.

In the present study the growth of *M. rosenbergii* was maximum at 132 mg/L calcium hardness level. The growth of the prawn declined significantly at and above 192 mg/L $CaCO_3$ level. Brown *et al*. (1991) reported that growth of *M. rosenbergii* was maximum at < 53 mg/L $CaCO_3$, and did not change significantly at lower hardness levels though it decreased at higher levels. Cripps and Nakamura (1979) also observed reduction in growth of the prawn at hardness above 65 mg/L $CaCO_3$. The variations in growth rate at different calcium levels may be due to the changes in metabolic activities of the prawn. Calcium is very important to freshwater prawns in keeping exoskeletons strong. When hardness is too low, prawns will take longer time for the exoskeleton to harden after a molt. While water is soft, prawns are vulnerable to mechanical damage and predators. Too much hard water can also be a problem. Therefore, Wetzel (2001) recommended a range of hardness from 50 to 200 mg/L, which is ideal for freshwater prawn culture. Also the present study demonstrated that the growth of the prawn was reduced above 205 mg/L bicarbonate alkalinity level. Bartlett and Enkerlin (1983) reported that hardness levels between 940 and 1060 mg/L $CaCO_3$ did not adversely affect the prawn growth; but their water had relatively low alkalinity of 58-86 mg/L $CaCO_3$.

Thus the present study indicated that the freshwater prawn, *M. rosenbergii* is sensitive to higher levels of water calcium and higher levels of bicarbonate alkalinity as they affected both survivability and growth. The present findings are also supported by New and Singholka (1985) whose recommended levels were between 40 and 100 mg/L $CaCO_3$ for successful farming operation. Low calcium content has often been considered as a constraint to freshwater crustaceans distribution, but in case of *M. rosenbergii*, the effect of high hardness water appears to be more of a handicap, particularly when carbonate alkalinity is also elevated (Brown *et al*., 1991). The present study demonstrated that successful culture of *M. rosenbergii* should be between 92 and 192 mg/L level of calcium hardness and also should be around 100 mg/L $CaCO_3$ of bicarbonate alkalinity. Therefore, for successful prawn culture development, the water hardness and alkalinity should be properly monitored and maintained for optimum and sustainable production.

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