Bangladesh J. Fish. (2019) **31**(2) : 279-286

Suitability of artificial seawater and brine solution as media for culture of giant freshwater prawn (*Macrobrachium rosenbergii*) larvae

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Abstract. An experiment was conducted for a period of 42 days to evaluate the suitability of artificial seawater and brine as media for culture of giant freshwater prawn larvae. Prawn larvae were reared under three treatments with three replications. The treatments were: T1- brine solution, T2-artificial seawater-1 (complex formula) and T3-artificial seawater-2 (simple formula). The cost of 12 ppt brine water was 12US$/m3, artificial seawater-1 was 95US$/m3 and artificial seawater-2 was 71US$/m3. The survival rate of prawn larvae was 56.45%, 55.63% and 55.78% for T1, T2 and T3respectively. There were no significant differences ($p < 0.05$) among the survival rates and metamorphosis time of different treatments. The production cost in T1 (US$8.97) was significantly lower ($p < 0.05$) (1000PL) followed by T2 (US$13.42) and T3 (US$11.53). The results of this study revealed that brine solution is the bestlarvae culture media for an inland prawn hatchery. Where brine solution is not available or costly, artificial seawater can be used for prawn PL production. In this case simple formula of artificial seawater is more suitable than complex formula due to low cost, well defined and easily replicated by farmers.
Keywords: Giant freshwater prawn, Artificial sea water, Brine solution, Larvae

Introduction

The giant freshwater prawn *Macrobrachium rosenbergii*, locally called *"golda"* is a very popular food item all over the world, which is indigenous to South and Southeast Asia, parts of Oceania and some Pacific islands. However the production of prawn from natural sources is decreasing day by day due to various man-made and natural causes. In former time, this prawn was highly abundant in rivers, lagoons, freshwater reservoirs and brackish water areas. A drastic reduction of the wild stocks has been observed because of water pollution and overfishing which could have been due to the increasing demand of freshwater prawns in the global market. In the past, it was possible to transport larvae from the source to anywhere because of low transportation cost. Unfortunately, because of the continuing increase in the transportation cost for saline water, cost effective PL production is becoming less feasible. Furthermore, lower survival rate of larvae can occur due to stress from long hours of transportation, hence the growing need for local hatcheries. However, inland prawn hatcheries still need to transport concentrated seawater from the source, which still commands high transportation cost and in turn makes it difficult for local hatcheries to continue larval production. Therefore, it is necessary to find an alternative source of saline water to lower the cost of operation. Thus, the use of artificial sea water and brine solution constitutes a cornerstone in prawn culture. Such systems can reduce the costs of using seawater, allowing the establishment of hatcheries in areas distant from the coast, where land is less expensive and large consumer markets are located (Nair and Hameed 1992, Valenti 1993, Alam and Alam 2014). To address this problem, the use of commercial artificial and brine solution has been investigated in this study.

In addition, use of artificial seawater has the advantage of eliminating the incidence of toxic substances from sea pollution, parasites, predators and competitors of the prawns in larvae rearing tanks (New 2002). New (1990) reported that hatcheries in inland Thailand and Philippines obtained brackish water by the dilution of rock salt and evaporated sea salt. Daniels *et al.* (1992) reported that brackish water produced from commercially manufactured salt mixtures can be used successfully.Some research has been conducted to produce *M. rosenbergii* post larvae using commercial artificial seawater (Reddy *et al.* 1991, Nair and Hameed 1992). Commercial production of *M. malcolmsonii* seeds using artificial seawater water and crude common salt was also tested by Soundarapandian *et al.* (1994). Artificial sea water was used by Soundarapandian *et al*. (2006) for freshwater prawn PL production and they found the survival rate from 39.7-63%.Kanaujia and Mohanty (1996) established seed production of *M. malcolmsonii* in artificial seawater and found the post-larval production in synthetic and natural seawater did not show any significant differences. Giant freshwater prawn post larvae were successfully produced in Nagaland, India in *in vitro* conditions but cost was quite high due to use of artificial sea water of complex formula (DoF 2011). So far no research has been done to evaluate and performed a comparative performance between brine solution, artificial sea water with different formula constituents on cost benefit ratio. The main objective of this study was to assess the performance of the artificial seawater versus brine solution relative to culture duration, metamorphosis rate, and productivity in the freshwater prawn hatchery carried out in closed system. The comparison between low cost and high cost formula of artificial seawater and economic analysis for PL production was also investigated.

Materials and Methods

Experimental design: This experiment was carried out at Jononi prawn hatchery of Bangladesh for a period of 42 days from 28 April to 8 June 2015 to evaluate the suitability of artificial sea water for the production of post larvae of freshwater prawn, *M. rosenbergii*. Nine 300 liter fiber glass tanks were used for this experiment. The experiment was designed with three treatments each with three replications. Three types of saline water were used in this experiments viz. brine solution- T_1, artificial seawater-1 (complex formula) - T_2 and artificial seawater-2 (simple formula) T_3. High cost artificial sea water was prepared by using complex formula according to Spotte (1979)and low cost artificial sea water was prepared by using simple formula according to New (2002).

Preparation of brine solution: Brine (150 ppt) was collected from salt pans of Cox's Bazar, Bangladesh and kept into a brine holding tank for storage. After settling down the brine solution was transferred into a water treatment tank (30 tons) and was mixed with freshwater to prepare 12 ppt saline water. The brackish water was bleached with 60% chlorinated bleaching powder at a dose of 12 ppm and aeration was performed for 24 hours and treated with 6 ppm sodium thio-sulphate and air was blown for 24 hours to remove the chlorine. The supernatant clear water was then collected for rearing of larvae. Cost of 1 ton (1 m^3)12 ppt brine water was 12 US$.

Preparation of artificial seawater: Two types of artificial seawater were prepared by mixing the different ingredients in two cemented tanks. The amounts of different ingredients of artificial seawater A (cost high) and B (low cost) is given in Table I and Table IIrespectively. Cost of 1 ton (1 m^3) 12 ppt artificial seawater-1 was 95 US$ and of artificial seawater-2 was 71 US$.

Stocking of prawn larvae: Female prawns bearing gray colored egg were collected from Pyra river, Bangladesh and kept in fibre glass tank containing 6 ppt saline water. The prawn were treated with 30 ppm formalin for 10-15 min for minimizing any contamination. Air was blown in the hatching tank from the aerator. To obtain a larval batch with synchronized development, larvae from a singleovernight spawning were stocked randomly into each experimental larvae rearing tank at a density of 80 larvae/l (Pramanik and Haldar 1996).

Table I. Ingredients of artificial sea water-1 of 35 ppt (Spotte 1979) used in Jononi hatchery during April - June 2015

Major salts	
Ingredients	Quantity (kg)
Sodium chloride (NaCl)	27.59
Magnesium sulphate ($MgS0_4.7H_2O$)	6.89
Magnesium chloride ($MgC1_2.6H_2O$)	5.39
Calcium chloride ($CaCI_2.2H_2O$)	1.37
Potassium chloride (KCl)	0.599
Sodium bicarbonate ($NaHC0_3$)	0.209
Minor salts	
Ingredients	Quantity (g)
Strontium chloride ($SrCl_2$.$6H_2O$)	19.84
Manganese sulphate ($MnS0_4$. H_2O)	3.96
Sodium phosphate ($NaHl0_4.7H_2O$)	3.96
Lithium chloride (Li CI)	0.992
Sodium molybdate($NaMo0_4$. H_2O	0.992
Sodium thiosulphate ($Na_2Sp_3.5$ H_2O)	0.992
Trace salts	
Ingredients	Quantity (g)
Potassium bromide (KBr)	26.875
Aluminiumsulphate [$Al_2(S0_4)$ 3.18 H_2O]	0.859
Rubidium chloride (RbCl)	0.148
Zinc sulphate ($ZnS0_4.7$ H_2O)	0.095
Cobalt sulphate ($CoS0_4.7$ H_2O)	0.089
Potassium iodide (KI)	0.089
Cupric sulphate ($CuS0_4$• $5H_2O$)	0.089

Table II. Ingredients of artificial sea water-2 of 12 ppt (New 2002) used in Jononi hatchery during April - June 2015

Salts	Quantity (g/ton)
NaCl	9200
$MgS0_4.7H_2O$	2300
$MgCl_2.6H_2O$	1800
$CaCl_2.H_2O$	467
Kcl	200
$NaHCO_3$	67
KBr	9

***Feed and feeding**:* *Artemia* nauplii were used as initial food for prawn larvae. The nauplii were produced from the commercially available canned *Artemia* cysts following standard protocol. Larvae were fed *Artemia* nauplii twice a day at the rates shown in Table III.

Table III. Amount of *Artemia* given at different ages of the larvae in Jononi hatchery during April - June 2015

Age of larvae (days)	Stage of larvae	Number of *Artemia* nauplii/ larvae/day
03	II - III	5
04	II - III	10
5 - 6	III - IV	15
7 - 8	IV - V	20
9	IV – VI	30
10 - 11	V – VII	35
12	VI – VII	40
13 - 14	VI – VIII	45
15 - 24	VII – PL	50
25 - 30	VIII – PL	40
31 - 35	IX - PL	30

Egg custard was fed after 10 days in all the treatments until the end of the experiment together with *Artemia* to minimize the feeding cost. The ingredients of egg custard are given in Table IV. The ingredients were mixed by a blender and baked for 30 min to make a cake. Then the egg custard was sieved through small meshed net and fed the larvae as shown in Table V.

Water quality management and sampling: Physico-chemical parameters, such as water temperature (°C), dissolved oxygen (mg/L), pH, alkalinity (mg/L), ammonia nitrogen (mg/L), nitrite (mg/L), salinity (ppt) were recorded regularly throughout the study period. Larvae rearing tanks were siphoned to remove uneaten food and dirt in every morning and afternoon and 30% water was changed after every 3 days.

Table IV. Ingredients of egg custard used in Jononi hatchery during April - June 2015

Ingredients	Amount
Powder milk	30%
Egg	35%
Cornflower	10%
Prawn	20%
Agar powder	2%
Cod liver oil	1.5 %
Vitamin premix	1%
Oxytetracycline	0.5%
Total	100%

Table V. Feeding chart of prawn larvae used in Jononi hatchery during April - June 2015

Age (Day)	Stage	Feeding time								
		7:30 am	9:00 am	11:00 am	12:00 pm	1:00 pm	2:00 pm	3:00-5.00 pm	6:00 pm	10:00 pm
02-08	II-V	AN		-	AN	-	-	Siphoning	AN	-
09-11	VI-VII	PF_1	AN	PF_1	AN	PF_1	PF_1	"	AN	-
12-19	VIII-X	PF_2	AN	PF_2	AN	PF_2	PF_2	"	AN	AN
20-35	XI-PL	PF_3	AN	PF_3	AN	PF_3	PF_3	"	AN	PF_3

Note: AN = Artemia *nauplii*, PF_1= Prepared feed retained on 230 micron sieve, PF_2= Prepared feed retained on 350 micron sieve and PF_3 = Prepared feed retained on 600 micron sieve

Monitoring of larval growth and health: Larval health condition and pathogenic attack were checked every day at hatchery laboratory using microscope. The larvae were reared up to post larval (PL) stage. At the end of the experiment, the PL were collected and counted for survival rate as:

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

Statistical analysis: One way analysis of variance (ANOVA) was performed to determine the treatment effects. Significant differences between treatments were determined by using Duncan's multiple range test (DMRT) at 5% level of significance.

Results

Water quality parameters: The average value of water quality parameters viz., water temperature, dissolved oxygen (DO), pH, alkalinity, NH_3 and NO_2in three different treatments are shown in Table VI. The mean values of water temperature in T_1, T_2 and T_3 were 31.18± 0.95°C, 31.10±0.42°C, 31.52±0.41°C, respectively. The mean values of dissolved oxygen concentration were 6.16±0.12 mg/L in T_1, 6.24±0.13 in T_2 and 6.18±0.21 in T_3. The observed mean values of pH were 8.16±0.37, 8.12±0.26, and 8.35±0.14 in T_1, T_2 and T_3, respectively. The mean values of alkalinity in T_1, T_2 and T_3 were 147.5±2.12, 155.3±2.61 and 143.7±2.15 ppm, respectively. Alkalinity of different larvae rearing tanks were more or less same and slightly increasing with time. The mean values of ammonia in T_1, T_2 and T_3 were 0.25 ±0.02, 0.21±0.03 and 0.24±0.03 ppm, respectively. Ammonia concentration of larvae rearing tanks gradually increased in most treatment but in some case decreased. Ammonia gasses were increased due to metabolic activity of larvae, decomposition of egg custard and decreased due to water exchange. The mean values of NO_2 in T_1, T_2 and T_3 were 0.63±0.06, 0.67±0.03, and 0.65±0.03 ppm, respectively. Nitrite concentration of larvae rearing tanks gradually increased in all treatment due to metabolic activity of larvae and decomposition of egg custard. Salinity range from 11-12.50 which was suitable for prawn larvae culture. The physico-chemical parameters of water in the experimental tanks were not significantly ($p<0.05$) different. The water quality parameters observed during the experimental period were within the acceptable range for prawn larval rearing (New and Singholka 1985, New 2002).

Table VI.Variations in physico-chemical parameters of water in Jononi hatchery during April - June 2015

Parameters	T_1 (mean ± SE)	T_2 (mean ± SE)	T_3 (mean ± SE)
Water Temperature (°C)	31.18 ± 0.95 °C[a]	31.10 ± 0.42°C[a]	31.52 ± 0.41°C[a]
Dissolved Oxygen (mg/L)	6.16 ±0.12[a]	6.24 ±0.13[a]	6.18 ±0.21[a]
pH	8.16 ± 0.37[a]	8.12 ± 0.26[a]	8.35± 0.14[a]
Alkalinity (mg/L)	147.5±2.12[a]	155.3±2.61[a]	143.7±2.15[a]
Ammonia (mg/L)	0.25 ± 0.02[a]	0.21 ± 0.03[a]	0.24 ± 0.03[a]
Nitrite (NO_2) (mg/L)	0.63 ± 0.06[a]	0.67 ± 0.03[a]	0.65 ±0.03[a]
Salinity (ppt)	11.56± 0.08[a]	11.66± 0.08[a]	11.62± 0.07[a]

(T_1 = Brine solution, T_2 = artificial sea water-1 and in T_3 = artificial sea water-2).

Survival rate: Production of *M. rosenbergii*post larvae (PL) under various treatments is presented in Table VII. The production rate of 45.16± 3.25PL/l with a corresponding survival rate of 56.4±1.80[a] % was obtained for larvae reared in brine solution (T_1), the production rate of 44.51±2.61PL/l with a corresponding survival rate of 55.63±1.34% obtained for larvae reared in artificial sea water-1 (T_2) and the production rate of 44.63±3.89 PL/l with a corresponding survival rate of 55.78 ±3.45% was obtained for larvae reared in the artificial sea water-1 (T_3). There were no significant differences ($p>0.05$) among the survival rates of different treatments. Metamorphosis started at 21st day and completed on 33th day in T_1, started at 22th day and completed on 32th day in T_2 and started at 22th day and completed on 33th day in T_3. No significant differences ($p>0.05$) were observed among the metamorphosis time of different treatments. Significantly lower ($p<0.05$) production cost (1000PL) was observed in T_1 (US$ 8.97) followed by T_2 (US$ 13.42) and T_3 (US$ 11.53). Significantly higher ($p<0.05$) production cost (1000PL) was observed in T_2 (US$ 13.42) due to higher cost of artificial sea water.

Table VII. Production of *M. rosenbergii* post larvae reared under different treatments in Jononi hatchery during April - June 2015

Treatment	Larvae stocked/l	Days of metamorphosis		Post-larvae/l	Average survival rate (%)	Production Cost/1000 PL (USD)
		Start	Complete			
T_1	80	21	33	45.16± 3.25	56.45± 1.80[a]	8.97±0.23 [a]
T_2	80	22	32	44.51 ± 2.61	55.63± 1.34[a]	13.42±0.32 [c]
T_3	80	22	33	44.63 ± 3.89	55.78 ±3.45[a]	11.53±0.29 [b]

(Figure in the same row with different superscript are significantly different ($p<0.05$)

Discussion

Ali *et al.* (2004) found the average survival rate of larvae as 39.44%, 38.88% and 38.33% in different treatments having same stocking densities (60/L) using brine solution but the survival rate of *M. rosenbergii* larvae were found as 57.91%, 56.81% and 22.87%, using of probiotic as a substitute of antibiotic for post larval production of giant freshwater prawn (Ali and Mahmud, 2012). Ali and Sattar (2012) found the survival rate rates of prawn larvae was 50.92 and 48.88 in sea water and brine solution respectively. The survival rate rates of prawn larvae of this experiment were more or less similar with the above findings.

Commercial production of *M. malcolmsonii* seeds using synthetic water and crude common salt was tested by Soundarapandian*et al*. (1994). They found that there was no significant difference in the post-larval production, percentage of survival and post larvae/L in both the media used for the study. They stated that artificial seawater will be useful not only in the production of seeds of *M. malcolmsonii* round the year but also in establishing hatcheries in inland regions. Kanaujia and Mohanty (1996) established seed production of *M. malcolmsonii* in synthetic seawater and found the post-larval production in synthetic and natural sea water did not show any significant differences. The first few post-larvae were observed within 39-45 d and yielded 5,750-10,450 post-larvae at 12-23 PL/l. Mean duration of the hatchery period was 28 days in natural brackish water and 31 days in artificial brackish water. The metamorphosis rate and the average productivity for the natural brackish water treatment were 74% and 60 post-larvae/L, respectively, and values obtained with artificial brackish water were 55% and 44 post-larvae/L. Reddy *et al*.(1991) observe the survival rate of prawn larvae from 5 to 52% in their initial experiment on larval rearing of *M.rosenbergii*in artificial sea water. Hangsapreurke*et al*. (2008) conducted an experiment on *M. rosenbergii*larviculture by using artificial sea water in place of concentrated sea water under the closed recirculating water system, the survival rate in their experiment was 18-27% (14-22 PL/L). Thapa (2002) observed a 23% survival rate in an open circulating water system containing rock salt with a density of 50 larvae/L. The average metamorphosis rate for the artificial brackish water treatment was higher than those observed by Yasharian*et al*.(2005) who used artificial brackish water in the hatcheries of *M. rosenbergii*; these investigators obtained metamorphosis rates of 44.1% and 45.0% and produced, on average, 32 and 23postlarvae/L, respectively. These productivities are lower than that obtained in our investigations with the artificial brackish water treatment. In addition, the mean duration of larval period observed in these other studies was approximately 40 d, higher than larval period obtained in our investigation. Differences may be the result of variations in management, feeding, water quality parameters or stability of the medium arising from the culture system that was used. However, according to New (2002) artificial seawater has been used in some recirculation system, especially in research and the stimulus for such work is that its use may reduce the problems caused by water pollution, parasites and the presence of prawn competitors and predators in larval rearing tanks.

In our experiment the costof 12 ppt brine water was 12 US\$/m^3, artificial seawater-1 (complex formula) was 95 US\$/m^3 and artificial seawater-2 (simple formula) was 71 US\$/m^3. Mallas andValenti (1998) observed that the cost of artificial seawater about US\$50.00/m^3 in Brazil. New (2002) stated that the unit cost of artificial seawater for such a simple formula, is.US\$ 75/m^3. Difference the cost of artificial sea water due to time, ingredients and price of chemicals which varies from country to country. The cost of brine solution will vary according to the distance between the hatchery and a source of brine water. The results of our study revealed that brine solution is the best media for *M. rosenbergii*postlarvae production in the hatchery far from sea water sources because of low production cost US\$ 8.97/1000 PL. Between two, artificial seawater-2 (simple formula) is better than artificial seawater-1 (complex formula) for giant freshwater prawn PL production because the complex formula required higher production cost (US\$ 13.42/1000 PL) than simple formula (US\$ 11.53/1000 PL), whereas the survival rate of PL more or less similar. Moreover the artificial seawater-2 is simple, well defined and may be easily replicated by farmers for practical commercial use.

The cost of brine solution will vary according to the distance between the hatchery and a source of brine water. So the cost of brine solution was very lower than the cost of artificial seawater. The cost of brine solution can be higher than the artificial seawater if the distance is very far due to high transportation cost. According to Mallas andValenti (1998) the cost of natural seawater will vary according to the distance between the hatchery and a source, reaching approximately US$70.00/m3, US$ 130.00/m3 and US$200.00/m3 at 500, 1,000 and 1,500 km from the sea, respectively. Brine solution is the best larvae culture media for an inland hatchery if the location of the hatchery not very far from the brine source. Use of artificial seawater permits location of hatcheries in areas far away from the coast and close to grow out farms and consumer markets. These results will play an important role to establish prawn hatchery at inland areas and also contribute to an increase of commercial freshwater prawn culture. This result can be used in to produce prawn PL in inland area far from sea to mitigate the present PL crisis for prawn culture.

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(Manuscript received 4 December 2019)