



Evaluation of synthetic sea water for larvae culture of gangetic prawn *Macrobrachium gangeticum* (Bate)

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Abstract

Commercial freshwater prawn farming has gained great momentum all over the Indo-Pacific region. Seed production of freshwater prawns requires brackish water of varying salinity, which is reported to complete their larval cycle in river mouths (estuaries). The establishment of hatcheries for seed production of these species may be possible in coastal regions. The freshwater prawn farming is now in a commercial scale in India, as it has high foreign exchange trade. However the activity is mostly concentrated in coastal regions, as huge quantity of seawater is required. Persons involved in this field have tried to operate the hatcheries for seed production of the freshwater prawn in inland area with the synthetic (chemically prepared) seawater. Natural seawater constitutes different salt components out of which nine major components i.e. common salt, Magnesium chloride, sodium sulphate, calcium chloride, potassium chloride, sodium bicarbonate, potassium bromide, boric acid and strontium chloride used to prepare synthetic seawater. *Macrobrachium gangeticum* larvae were reared in 300l tanks adopting an air lift biofilter recirculatory system. Larvae were fed with nauplii of *Artemia salina* twice day for one week thereafter food was supplemented with egg custard and mussel meat. Occurrence of first few post larvae observed on 19th day and trials continued for 40 days. The post larvae production during first year of three trials ranged from 4858 to 6388 @ 16.19 -21.29pl/l more or less same trends was found during second year which varied from 4562-5790 @ 15.2-19.3pl/l. The production of post larvae in synthetic seawater and natural seawater did not show any significant variations.

Keywords: Freshwater prawns, *Macrobrachium gangeticum*, synthetic seawater, *Artemia salina*.

Introduction

Aquaculture is the fastest growing activity in the food sector. From aquaculture point of view, among the crustaceans; shrimps, prawns, lobsters and squids are considered important for culture. The crustaceans possess a commendable position in view of their culture potential as well as the economic value. Among crustaceans, prawn contributes to major share of the cultured crustacean production being over to 94.6%. Freshwater prawn farming has been gaining prominence globally ever since the life cycle of the giant prawn *Macrobrachium rosenbergii* was closed in Malaysia (Ling, 1969). While certain countries like

Thailand and Taiwan, a province of China, took advantage of this breakthrough and established prawn farming early, India and Bangladesh seriously initiated the prawn farming (MPEDA, 2001; FAO, 2002). An “International symposium on Freshwater prawns 2003” held at Cochin, Kerala, India, strongly emphasized the vital need to augment quality seed production to improve and sustain freshwater prawn culture in the coming years. For instance the freshwater prawn production potential of India is 1, 50,000 tones worth Rs 3000 cores @ Rs.200/kg (US\$ 65 million). Among the nine coastal states of India,

Andhra Pradesh contributes the lion share of 88.6 % in production of freshwater prawns. In this state the current production is about 27,020 tones, is expected to increase threefold to reach 75,000 tones, accounting to 50 % of the total Indian production within the next 5 years (MPEDA, 2004).

There are some drastic changes in water qualities occur with the progresses of larval rearing and its maintenance is the key for the successful seed production of prawns under hatchery condition. Brackish water of desired salinity used for hatchery operation, is prepared by the dilution of seawater with fresh water, though it contains varying amounts of impurities such as colloidal suspensions and dissolved organic matters (New and Singholka, 1985). Therefore chemical treatments followed by aging are essential for purification before its use in prawn hatchery. *Macrobrachium gangeticum* is the third largest freshwater prawn of the Indian continents recoded migration about 1300 km from estuary to freshwater riverine system, where they grow, mature, breed and spawn. Larval development occurs in natural brackish water in estuary (Prasanti Mishra *et al* 2011) (Kanaujia *et al.*, 2001). The development of hatchery needs large amount of brackish water for commercial seed production, which is labour intensive, expensive and only viable in costal regions. Therefore this study was made to evaluate the possible use of synthetic seawater for seed production of *M.gangeticum* to establish inland hatcheries far away from the coastal regions.

Materials and Methods

Experimental design

The experimental trials were carried out at the Central Institute of Freshwater Aquaculture (CIFA), Kausalyaganga, Bhubaneswar (Orissa) (Lat. 20° 11' 6" – 20° 11' 45" N; Long. 85° 50' 52" – 85° 51' 35" E) during two successive years. The experiment was set up in *Macrobrachium malcolmsonii* hatchery located in CIFA. The larval rearing experiment was conducted in triplicate. Each rearing unit consisted with one circular plastic tank 2'h x 3' dia of 450 l water holding capacity. Inner surface of the larval rearing tank was smooth and sky blue in colour. It was attached with a biofilter plastic drum of 80 l water capacity, which has housed for the airlift recirculatory system for larval rearing. The larval rearing units were provided with artificial light through fluorescent tubes during morning and evening hours to provide sufficient light and maintain desired water temperature.

Rearing medium

Synthetic seawater (Artificial seawater) was prepared with nine salt components mixed together in the proportion of common salt (NaCl) 12.53 kg, magnesium chloride (Mg Cl₂) 2.53 kg, sodium sulphate (Na SO₄) 2.08 kg, calcium chloride (CaCl₂) 0.59 kg, potassium chloride (KCl) 0.35 kg, sodium bicarbonate (NaHCO₃) 0.11 kg, potassium bromide (KBr) 0.05 kg, boric acid (H₃BO₃) 0.013 kg and strontium chloride (SrCl₂) 0.007 kg dissolved in 1000 l freshwater and properly mixed with the help of an agitator for 2-3 days. In tank individual salt was dissolved in water properly, and then mixed together in freshwater under vigorous aeration to obtain 12 ppt salinity. The prepared medium was exposed to sunlight under aeration for 2-3 days and allowed the suspended particles to be settled at the bottom and it was further kept for one week for proper aging. The prepared artificial seawater medium was mixed with 30 % natural seawater of same salinity (12 ppt) and mixed properly using agitator. The medium was sand filtered, aerated and used for hatchery operation.

Berried females and larval rearing

The berried females carrying gray eggs were collected from the prawn hatchery reared under captivity using 5ppt brackish water maintaining with proper aeration and food. Soon after hatching, salinity of the medium was increased gradually by adding hypo-saline water medium to increase the salinity level 5 to 12 ppt required for the rearing of *M. gangeticum* larvae. Larvae fed with the freshly hatched *Artemia* nauplii twice daily between 6-7 AM and 5-6 PM for one week. Thereafter the feed was supplemented with mussel meat and egg custard at an interval of six hours using *Artemia* nauplii between 23 – 24 hr. Once few post larvae appeared in the tank within 19-22 days, small amount of mussel meat and/or egg custard was provided at every two hours interval till the rearing was completed. Tanks were cleaned daily during morning hours by siphoning out of the water along the metabolites, molted shells and left-out feed. Thereafter water exchange was done to maintain good water quality and water level and then feed was provided to the larvae.

Water quality parameters

Water quality parameters such as temperature, salinity, pH, ammonia, total hardness, total alkalinity and dissolved oxygen were analyzed at regular intervals following standard methods (APHA 1985).

Results

Macrobrachium gangeticum larvae observed in transparent, translucent with red and blue chromatophores during early stages. The color deepens at later stages and only observed on some portion of the body. All the eleven larval stages found active swimmers, planktonic in nature, photopositive and attracted towards the light and displayed churning movement during early stages and moved along the side of the tank and water column at later stages. They were active and moved up side down with their tail up and head down obliquely into the water column in the tank. Initially larvae readily accepted *Artemia* nauplii have also shown feeding propensity towards egg custard and mussel meat.

Water Quality Parameters

Temperature

The water temperature in all the three trials of synthetic seawater recorded during first year ranged from 29.0 - 30.0°C with an average of $29.4 \pm 0.33^\circ\text{C}$. (Table 1 and Fig.1a) whereas during second year it ranged from 29.9 - 30.2°C with an average of $29.9 \pm 0.22^\circ\text{C}$ (Table 2 and Fig.1a).

Since the study has been carried out under similar condition the variations in ambient temperature in rearing medium was minimum and insignificant among the treatments. The ambient temperature was recorded slightly higher during second year as compared to first year.

Salinity

The salinity of synthetic seawater in three trials ranged from 12-16 ppt with an average 14.4 ± 1.50 ppt recorded during first year (Table 1 and Fig.1b) and more or less similar salinity range (12 - 16 ppt) with an average of 14.4 ± 1.42 ppt was recorded during second year (Table 2 and Fig.1b). The mean value of salinity recorded during different weeks did not show any significant variations during two years.

pH

The pH of larval rearing medium in different trials maintained through the installation of airlift bio-filter re-circulatory system along with water exchange and also with the application of calcium sulphate (CaSO_4)

@ 5ppm weekly. However the variations in pH recorded in different trials during two years.

The pH values of three trials in this Medium during first year ranged from 7.5 -7.9 with an average of 7.7 ± 0.11 (Table 1 and Fig. 1c) and in second year it varied from 7.6 -7.8 with an average of 7.7 ± 0.10 (Table 2 and Fig. 1c). It was also found that the variation in pH was remarkably lower after 4th week onwards as compared to initial three weeks.

Dissolved oxygen

The dissolved oxygen in water medium reported to be an important factor, which directly affects the growth and survival of the larvae. DO ranged from 4.4 - 4.9 mg/l with an average of 4.6 ± 0.17 mg/l during first year (Table 1 and Fig. 1d). However slightly higher DO 4.0 - 4.5 mg/l with an average of 4.3 ± 0.14 mg/l was recorded during second year (Table 2 and Fig. 1d). The mean values of DO differed significantly between first and second year of test media.

Total hardness

Total hardness in three trials observed 2255-2296 mg/l with an average 2276 ± 12.35 mg/l during first year (Table 1 and Fig.1e). However, it varied from 2244 - 2299 mg/l with an average 2275.4 ± 14.03 mg/l during second year (Table 2 and Fig.1e).

The total hardness level increased slowly with the progress of the larval cycle. In the test media, significant variations ($p < 0.05$) in the total hardness were recorded during first and second year of operation. However, higher values have been well registered in during second year than first year.

Total alkalinity

The total alkalinity ranged from 83.4-98.2 mg/l with an average of 92.7 ± 4.21 mg/l during first year (Table 1 and Fig.1f). Slightly lower value 78.2 - 94.2 with an average of 87.2 ± 4.25 mg/l was recorded during second year (Table 2 and Fig. 1f).

The variations in total alkalinity in the level was recorded to be trace and negligible. Total alkalinity level declined gradually during first year. Similar trend was recorded in second also in both the years of experimentation. Maximum and minimum values were recorded in Ist year.

Table 1- Weekly variations in water quality parameters of three larval rearing trials in synthetic seawater during first year

Trial 1							
Week	Temp. (°C)	Salinity (ppt)	pH	D. O. (mg/l)	T. H. (mg/l)	T. A. (mg/l)	NH₄ (mg/l)
I	29.5	12	7.9	4.9	2260	93.7	0.082
II	29.3	14	7.8	4.8	2266	93.4	0.088
III	29.2	16	7.6	4.7	2268	93.6	0.089
IV	29.0	13	7.5	4.5	2266	98.2	0.078
V	30.0	14	7.8	4.6	2255	88.2	0.092
VI	29.2	16	7.6	4.4	2269	83.4	0.079
Average	29.4	14.1	7.7	4.6	2264	91.7	0.084
Trial 2							
I	29.5	13	7.8	4.8	2268	94.8	0.088
II	29.3	15	7.9	4.7	2269	95.8	0.087
III	29.2	16	7.7	4.8	2266	89.3	0.088
IV	29.0	12	7.6	4.6	2277	88.9	0.079
V	30.0	14	7.8	4.8	2278	87.6	0.073
VI	29.2	16	7.7	4.4	2286	92.2	0.074
Average	29.4	14.3	7.7	4.6	2278	91.62	0.082
Trial 3							
I	29.5	12	7.8	4.4	2290	98.6	0.092
II	29.3	15	7.7	4.6	2296	97.8	0.093
III	29.2	14	7.6	4.8	2286	97.7	0.094
IV	29.0	16	7.8	4.6	2288	89.6	0.092
V	30.0	15	7.7	4.4	2289	92.5	0.105
VI	29.2	16	7.8	4.5	2292	92.7	0.108
Average	29.4	14.6	7.7	4.5	2290.1	94.8	0.097
Mean	29.4	14.4	7.7	4.6	2276.0	92.7	0.1
± SD	0.33	1.50	0.11	0.17	12.35	4.21	0.01

Ammonical nitrogen

The ammonical nitrogen in water medium reported to be important factors, which directly influence the life of aquatic organisms present in the aquatic ecosystem. Total dissolved ammonia during first year was recorded 0.073-0.108 mg/l with an average of 0.087 ± 0.009 mg/l (Table 1 and Fig.1g). During second year it ranged from 0.072-0.111 mg/l with an average of 0.1 ± 0.006 mg/l (Table 2 and Fig. 1g).

Maximum values of ammonical nitrogen level during second year was significantly higher than Ist year. It was further found that there was no accumulation of ammonia in all the test media displaying similar trend in both first and second year.

Post larvae production

The production of post larvae during first year in Trial 1 (6388) was maximum followed by Trial 2 (5106) and Trial 3 (4858) (Fig 2). More or less same trend was recorded during second year; it was (5790), (4853) and (4562) with PL/L 19.3, 16.17 & 15.2 in Trials 1, 2 & 3 respectively (Fig.2).

The PL production during second year in Trial 1 was recorded 5790, which has followed the same trend as recorded during first year. As indicated in (Fig 2) the average PL production of three trials during first year (5450.6) was comparatively more than that of second year (5068.3).

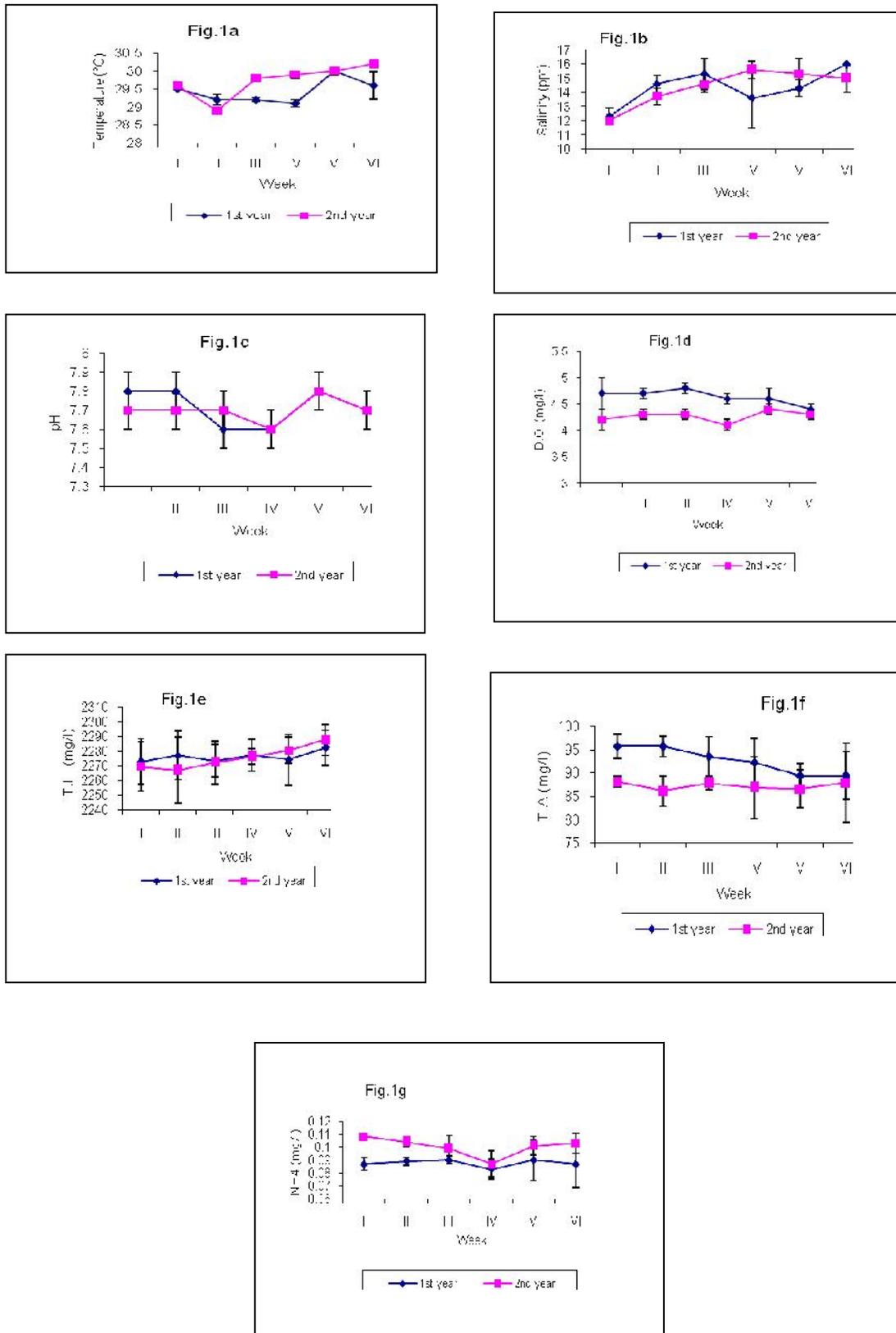


Fig 1: Showing weekly mean variations of water quality parameters in larval rearing medium

Table 2- Weekly variations in water quality parameters of three larval rearing trials in synthetic seawater during second year

Trial 1							
Week	Temp. (°C)	Salinity (ppt)	pH	D. O. (mg/l)	T. H. (mg/l)	T. A. (mg/l)	NH ₄ (mg/l)
I	29.6	12	7.8	4.4	2255	89.4	0.105
II	29.7	14	7.6	4.2	2244	82.3	0.108
III	29.9	15	7.8	4.4	2259	89.4	0.094
IV	30.0	16	7.6	4.2	2270	92.1	0.072
V	30.1	14	7.8	4.4	2272	90.4	0.092
VI	30.2	16	7.7	4.2	2278	94.2	0.105
Average	29.9	14.5	7.7	4.3	2263	89.6	0.096
Trial 2							
I	29.6	12	7.7	4.2	2265	87.6	0.111
II	29.8	13	7.8	4.4	2268	87.8	0.105
III	29.9	14	7.7	4.3	2269	87.7	0.110
IV	30.2	15	7.6	4.2	2278	89.2	0.102
V	30.0	16	7.7	4.3	2290	86.9	0.105
VI	30.2	14	7.8	4.4	2299	91.2	0.110
Average	29.9	14	7.7	4.3	2278	88.4	0.107
Trial 3							
I	29.6	12	7.6	4.0	2288	87.2	0.108
II	29.7	14	7.8	4.4	2289	88.3	0.099
III	29.9	15	7.6	4.2	2288	86.3	0.092
IV	30.0	16	7.5	4.0	2280	79.3	0.089
V	30.1	16	7.8	4.5	2279	82.3	0.105
VI	30.2	15	7.6	4.4	2286	78.2	0.094
Average	29.9	14.6	7.6	4.2	2285	83.6	0.097
Mean	29.9	14.4	7.7	4.3	2275.4	87.2	0.1
± SD	0.22	1.42	0.10	0.14	14.03	4.25	0.01

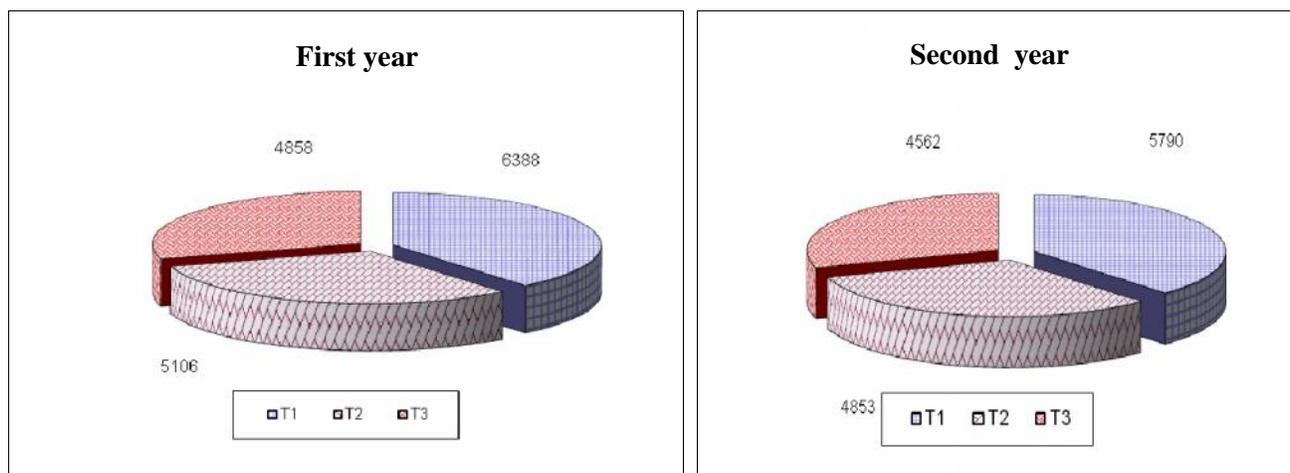


Fig 2 Post larvae production in synthetic sea water during 1st and 2nd Year

Discussion

All the 11 larval stages have shown the propensity to feed the *Artemia* nauplii, egg custard and mussel meat. Therefore supplementary feed provided on 8th day onwards found most acceptable. Feed quality, quantity and feed schedules are very important to achieve successful post larval production (Sounarapandian and Kannupand, 2000). The phase of larval rearing is critical step in prawn culture. The important factors that determine the seed output are the water quality, temperature and larval food (Sandifer and Smith, 1974; Dugan *et al.*, 1975; Smith *et al.*, 1976; Aquacop, 1977; Rao, 1991; Kanaujia and Mohanty, 1992; Prasad, 2005; Kanaujia *et al.*, 2005). The freshwater prawn culture has taken the shape of an industry. However, the major constraints encountered for its development in the country, is inadequate quantity and quality of seed. Although small quantity of prawn seed of some species are available in natural environment, which are being utilized for stocking in ponds. But it has got greater variations in size (Suhartro *et al.*, 1980). Recent attempts have been made to rear the *M. gangeticum* larvae in artificial sea water to develop hatchery technology and implement it in inland as well as coastal regions.

Several larval rearing media have been used for larval rearing of freshwater prawn. Yambot and Cruz (1986) used brine solution, sea salt and seawater for larvae culture medium of *M. rosenbergii* and found highest survival rate of post larvae in brine solution (25.7 %) followed by seawater (17.9 %) and sea salt (6.7 %). Tunsutapanich (1980, 1986) used rock salt, seawater, rock salt brine and salt stock solution for larval culture of *M. rosenbergii* and found more or less similar growth rate and survival as compared to diluted seawater. In this mediums, although first few post larvae occurred on the 18th day and full batch attained PL within 31 days with an average survival of 42% and at a production rate of 13PL/l. Kanaujia *et al.* (1998) reared *M. malcolmsonii* larvae in salt stock solution and first post larvae occurred on 25th day and a total of 14,721 PL with a production rate of 29.5 PL/l was achieved in 60 days.

Water temperature regulates the metabolism and growth of various larval stages of prawn and have been recommended a favorable temperature range 28-31°C for optimum larval growth and development of *M. malcolmsonii* (Kanaujia and Mohanty, 1992). New and Singholika (1982) and Diaz and Ohno (1986) have reported that the temperature above 35°C and less than 24°C may result retarded growth and mortality of the larvae of *M. rosenbergii*. In the present study, water

temperature was recorded twice daily during morning and evening hrs to study the maximum and minimum temperature during the rearing trials for effective management. Kanaujia *et al.* (2001, 2005) recorded suitable water temperature (27-31°C) for better PL production of *M. gangeticum*.

Salinity refers to the total concentration of dissolved salts in water and is defined as the weight of dissolved solids (g) in 1 liter of water, when all bromides and iodides are replaced by equivalent amounts of chloride. The salinity is mainly due to carbonates and sulphate of the sodium and potassium (Boyd, 1984). It ranged from 12-16 during first year and second year. optimum range of salinity for this species was reported 12 – 16 ppt (Kanaujia *et al.*, 2001, 2005). Whereas salinity range in *M. rosenbergii* recorded 10-16 ppt (New and Singholika, 1985; New and Valenti 2000). Ling and Merican (1961) and Fujimura (1966) reported that the larval rearing of *M. rosenbergii* occurs in brackish water since the larvae are euryhaline. Hence salinity reflects the rate of growth. It indicates the total concentration of all the ions in brackish water medium. The quality and quantity of salts significantly influenced the growth and development of the larvae. Adults *M. gangeticum* are hypertonic of environment as they inhabit in freshwater but the osmoregulation particularly during the larval stages takes ions from the environment and restricts ions loss from their body.

Most of the biological parameters of aquatic water bodies are influenced by pH. It measures the hydrogen ion concentration in the water and therefore, serves as indicator of acidity and alkalinity (Mohanty, 2005). The pH is defined as the negative logarithm of the hydrogen ion concentration and it shows acidic or basic nature of water (Boyd, 1982). Kanaujia and Mohanty (1992) suggested for maintaining the water pH within the range of 7.5 – 8.5. New and Singholika (1985) have reported a suitable range of pH between 7.5 and 8.5 in the water during larval rearing of giant freshwater prawn *M. rosenbergii*. In the present study, pH was maintained 7.5 -7.7 in all the trials for larval rearing.

Dissolved oxygen is the most critical water quality variable in aquaculture. It plays an important role to assess the water quality of the aquatic system. In the present study in the oxygen varied between 4.0 - 4.9mg/l, which is found a very narrow range. The wide variations in dissolved oxygen during larvae culture of *M. malcolmsonii* were reported by Mohapatra (2001), which was a variation in climatic

temperature and disruption of power failure during hatchery operation. Dissolved oxygen is important not only for respiration but also for maintenance of most favorable chemical and hygienic environmental conditions of the larval rearing medium.

Hardness of water caused due to the carbonate, bicarbonate, chloride and sulphate ions in association with calcium and magnesium. Sawyer and Mc Carty (1978) have categorized water into four types, as soft water (0 - 75 mg/l as CaCO₃), moderate hard water (75 -100 mg/l as CaCO₃), hard water (150 -300 mg/l as CaCO₃) and very hard water (over 300 mg/l as CaCO₃). In the present study, the total hardness ranged from 2230- 2292 mg/l during two years. The optimum total hardness level needed for larval metamorphosis was reported within the range of 3800 – 5200 mg/l in *M. malcolmsonii* (Kanaujia and Mohanty, 1992). Mohapatra (2001) recorded total hardness level within 2020 - 2220 mg/l as calcium carbonate in the comparative larval rearing study of *M. malcolmsonii* and *M. rosenbergii* and found within the desired level. However, it differ from those of Kanaujia and Mohanty (1992), they have recorded higher-level of hardness 3800 – 5200 mg/l in *M. malcolmsonii*.

The total alkalinity of water is mainly caused by the contents of Ca, Mg, Na, K, NH₄ and Fe combined either with carbonates and or bicarbonates or occasionally by hydroxide (Jhingran, 2003). In the present observations total alkalinity ranged from 78.2 - 98.6 mg/l which was maximum during first year and minimum in second year. This level is found to be a desirable as reported above in *M. rosenbergii*. Total alkalinity denotes the quantity of acid consuming constituents present in the water. In natural water bicarbonates and carbonates are the main alkaline sources, which determines the pH of water. Ammonia although toxic to the prawn larvae, originates in larval rearing medium through excretion by the larvae and presence of algal blooms as well (Chin and Chen, 1987). Ammonia exists in water in two forms, namely un-ionized ammonia (NH₃⁺) and ammonium ion (NH₄⁺). Which was much below the 'safe level'. The proportion of ammonia as unionized ammonia increases with increasing pH, salinity and temperature (Emerson *et al.*, 1975).

Post larval production

Commercially important larger *Macrobrachium* species or mostly estuary bound and need brackish water for completion of their larval phase (Rajyalaxmi, 1980). Some of the species like *M.malcolmsonii* and *M.gangeticum* migrate long

distance from estuary to inland region where climatic conditions are suitable for their growth. Collection and transportation of their seed from natural resources is only alternative to use for culture. However it is uncertain and irregular. Establishment of hatchery for seed production necessitates a larger amount of seawater, the transportation among which is costly. Some of the workers have experimented the seed production of *Macrobrachium* species using brine, salt stock solution and artificial seawater. (Tunsutapanich, 1980; Kanaujia *et al.*, 1992).

The average PL production of three trials during first year (5450.6) was comparatively more than that of second year (5068.3). In the present study perhaps mixing of 30 % natural seawater. Imbalance in different salt composition and its concentration and proper ageing during preparation of artificial seawater may also restrict the molting and cause lower survival. Smith *et al.* (1976) gave various formulations for preparation of artificial seawater, which have been tried in the present study for larval culture of *M.gangeticum*. Proper composition, dilution and ageing of artificial seawater is most important for preparation and its use for seed production

Conclusion

Successful hatchery operation for large-scale seed production of freshwater prawn require suitable and effective larval rearing medium. In present study all the trials carried during two years resulted better production of the post larvae. Although the occurrence of first few post larvae in *M. gangeticum* in all the trails was observed earlier than those of other commercially cultured prawns *M. rosenbergii* and *M. malcolmsonii*. The provision for larval food and effectiveness of rearing medium and technique may also have a bearing on the larval growth and survival of post larvae. Further the post larval production and shorter larval duration (40 days) in *M. gangeticum* indicated the suitability of the medium of synthetic sea water for hatchery operation in inland as well as coastal regions. It also suggest for the inclusion of one more candidates of *Macrobrachium* species i.e. *M. gangeticum* in freshwater prawn farming. It also needs further investigations on the management of husbandry and composition of feed ingredient for various larval stages, so that survival rate, salinity requirement and duration of larval cycle may further reduce to produce post larvae in mass scale to develop the farming of another new fresh water prawn species.

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