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Larval Biology and Seed Production of Ganga River Prawn *Macrobrachium gangeticum* (Bate)

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Abstract

Larval rearing trials for *Macrobrachium gangeticum* (Ganga River prawn) were carried out during 2003-2004 under hatchery conditions using 10-19 ppt brackish water. Ten thousand larvae were stocked in 300 L tank and fed with live *Artemia* naulpii, egg custard and mussel meat. The water quality parameters viz. water temperature, pH, dissolved oxygen, total hardness, total alkalinity and ammonia were found within suitable range. First occurrence of post larvae (PL) has been recorded within 20 days and trials were concluded on the 40th day. Production of PL in three trials during the first year was 4,748, 5,170 and 5,070 with 15.28, 17.30 and 16.9 $PL \cdot L^{-1}$ in trials 1, 2 and 3, respectively. Post larval production in trials 1, 2 and 3 during the second year was recorded at 6,388, 5,399 and 5,047 with 21.29, 17.66 and 16.82 $PL \cdot L^{-1}$ respectively. The results of the present trials indicated comparatively earlier occurrence, higher survival and production of PL than those of *Macrobrachium rosenbergii* and *Macrobrachium malcolmsonii* and this could be a potential species for commercial aquaculture.

Introduction

Macrobrachium gangeticum (Bate), the third largest Indian river prawn inhabits river Ganga and Brahmaputra which drain into the Bay of Bengal. Being migratory in nature and migrating about 1,300 km from estuary to different riverine systems, they need brackish water for completion of their larval phase of life (Tiwari, 1955; Tiwari and Holthuis, 1996; Kanaujia, 2003; Kanaujia et al. 2000, 2005). About 5 decades earlier, the availability of four prawn species was recorded in the river Ganga covering a distance of 1,300 km from estuary to Kanpur, among them *M. gangeticum* was one of the major prawn species (Jhingran, 1956). The major river Ganga flowing through the Indian states of Uttar Pradesh, Bihar, Jharkhand and West Bengal and Bangladesh, and the river Brahmaputra passing through the Indian state of Assam, Arunachal Pradesh, Tripura, Meghalaya and Nagaland and also Bangladesh, have great potential for rearing *M. gangeticum*. They grow to marketable size (50-250 g weight and 200-250 mm length), and contribute to capture fishery in considerable quantities. The development of hatchery technology of this species provides ample scope for its grow out culture for commercial farming (Tiwari and Holthuis, 1996; Kanaujia et al. 2001, 2005). Freshwater prawn species are gaining popularity for developing farming industry in the

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country with the local species available in the river system. It is, therefore, necessary to provide systematic information on the larval biology of *M. gangeticum*, which will be of great help in developing the farming industry of this species. The present trials on larval biology were planned to produce the seed of Gangetic prawn, *M. gangeticum* under laboratory condition.

Materials and Methods

The experimental trials were carried out during 2003 and 2004 using brackish water of 10-19 ppt salinity. The berried females, carrying grey eggs, were collected from the prawn hatchery and reared under captivity in 5 ppt brackish water maintained with proper aeration and food. Soon after hatching, salinity of the medium was increased up to 12 ppt gradually by adding hypo-saline water required for the rearing of *M. gangeticum* larvae. The larvae present in the 300 L tank were assessed through 10 randomized samples taken in 100 ml beaker from different parts of the tank. Before taking samples, some more air diffusion stones were used for uniform distribution of the larvae. The larvae in the samples were counted one by one by pouring them from the beaker of water and mean number of larvae computed to find out the larvae present in the tank during larval rearing cycle. The same technique was adopted to assess the larval density. Larvae were fed with the freshly hatched *Artemia* nauplii twice daily during morning between 6-7 am and evening between 5-6 pm for a week. Thereafter, the feed was supplemented with mussel meat and egg custard at an interval of 6 hr. The live feed *Artemia* nauplii was fed only during the night between 11pm and 12 midnight. A few PL appeared in the tank within 19-22 days, and a small amount of mussel meat and/or egg custard was provided at every 2 hours interval till the rearing was completed. Larval rearing tanks were cleaned daily during morning hours by siphoning out the water along with the metabolites, molted shells and leftover food found at the bottom of the tanks. Thereafter, water exchange was done to maintain good water quality and water level, and food was provided to the larvae. To maintain an optimum rearing environment and to maximize PL production, water quality parameters *viz.* temperature, salinity, pH, ammonia, total hardness, total alkalinity and dissolved oxygen (DO) were analyzed at regular intervals following standard methods (APHA, 1985). The distinguishing characteristic features *i.e.* progressive increase in size and instars duration between two consecutive larval stages, were observed and studied following the guidelines of Kanaujia (1999). As soon as the first appearance of PL, strings of shells designed at the Central Institute of Freshwater Aquaculture (Kanaujia et al. 2002) were hung into the larval rearing tanks. The strings of shells were lifted out carefully and kept in a tub containing 6 L of water from the same tank. The PL hidden in between the shells came out from the shell bed and started moving actively. In this way, newly metamorphosed PL were removed daily. Since the PL were developed and removed from higher salinity they were acclimatized gradually in freshwater in 1 hr till the salinity became zero. The PL were counted and released in a freshwater tank for juvenile production.

Result

Water quality

The different water quality parameters of the larval rearing trials recorded during 2 years are shown in Fig. 1 and Fig. 2.

Salinity

The salinity of larval rearing medium during the first year ranged from 12-19 ppt. in all the three trials conducted (Fig. 1). Lower salinity ranging from 10-15, 10-16 and 12-16 ppt were recorded in trials 1, 2 and 3, respectively during the second year (Fig. 2).

Temperature

The variation in ambient water temperature during the first year recorded in three experimental trials ranged between 29.2-30.0 °C in trial 1, 29.2-30.2 °C in trial 2 and 29.0-30.0 °C in trial 3 (Fig.1). Water temperature during the second year was almost similar to the first year, which varied from 29.6-30.1°C in trial 1, 29.5-30.2 °C in trial 2 and 29.6-30.1°C in trial 3 (Fig. 2).

pH

The pH in larval rearing medium of different trials was maintained through the installation of airlift bio-filter re-circulatory system, exchange of water and weekly application of calcium sulphate. However, the variation in pH was recorded in different trials during the two years. The variation in pH during the first year ranged from 7.5-7.8 in trial 1, 7.6-7.8 in trial 2 and 7.7-7.9 in trial 3 (Fig. 1). The pH value during second year ranged from 7.5 -7.8 in trial 1, 7.6-7.8 in trial 2 and 7.6-7.8 in trial 3 (Fig. 2).

Dissolved oxygen (DO)

The DO in water medium is an important factor that directly affects the growth and survival of the larvae. The oxygen contents of the three larval trials during the first year were almost similar, and ranged from 4.0-4.4 $mg \cdot L^{-1}$ (Fig. 1). During the second year, the DO ranged from 4.2-4.8 $mg \cdot L^{-1}$ in trial 1, 4.2-4.4 $mg \cdot L^{-1}$ in trial 2 and 4.0- 4.4 $mg \cdot L^{-1}$ in trial 3 (Fig. 2).

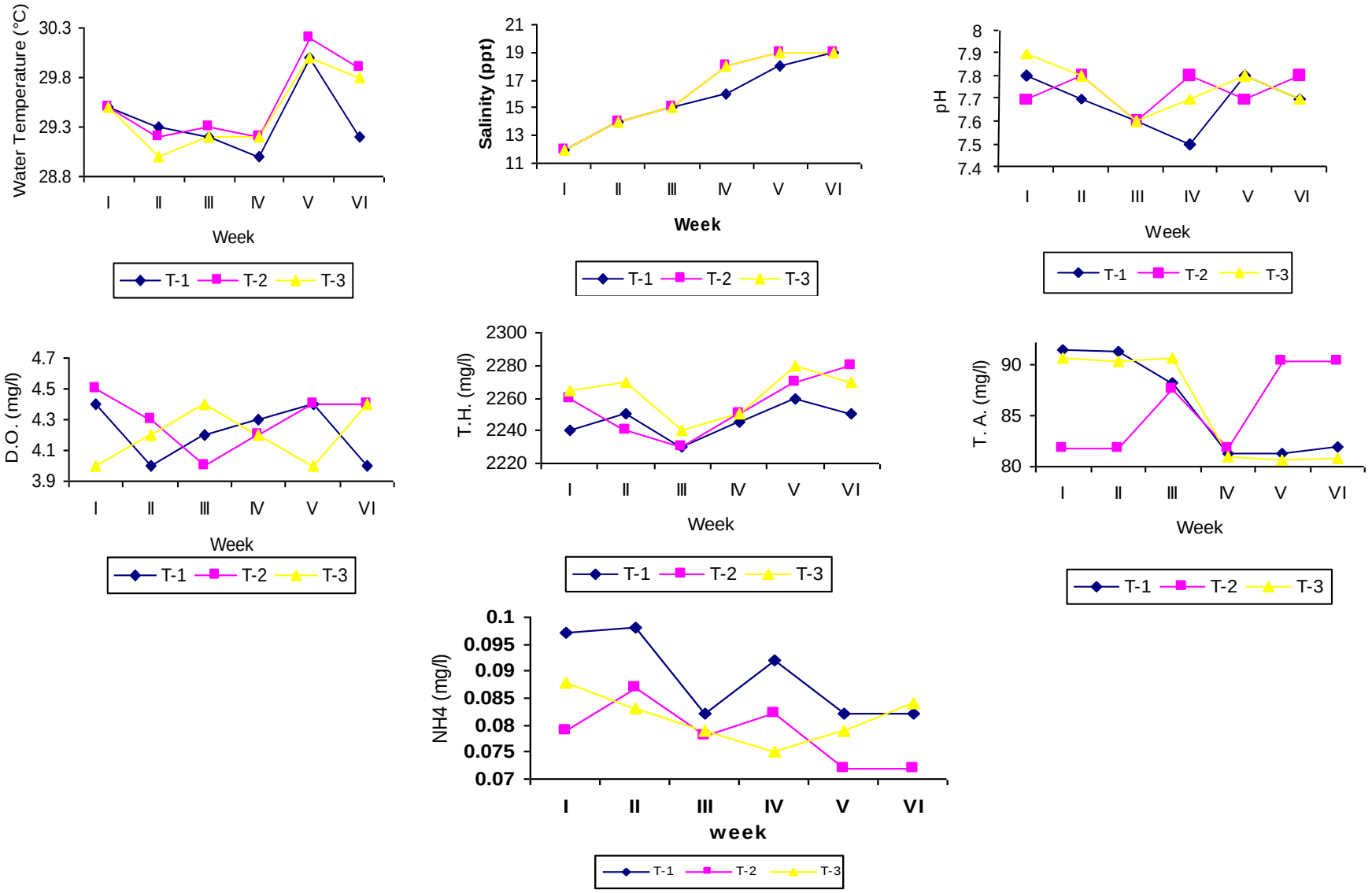


Fig. 1. Average weekly water quality parameters of three larval rearing trials of *M. gangeticum* during first year (2003).

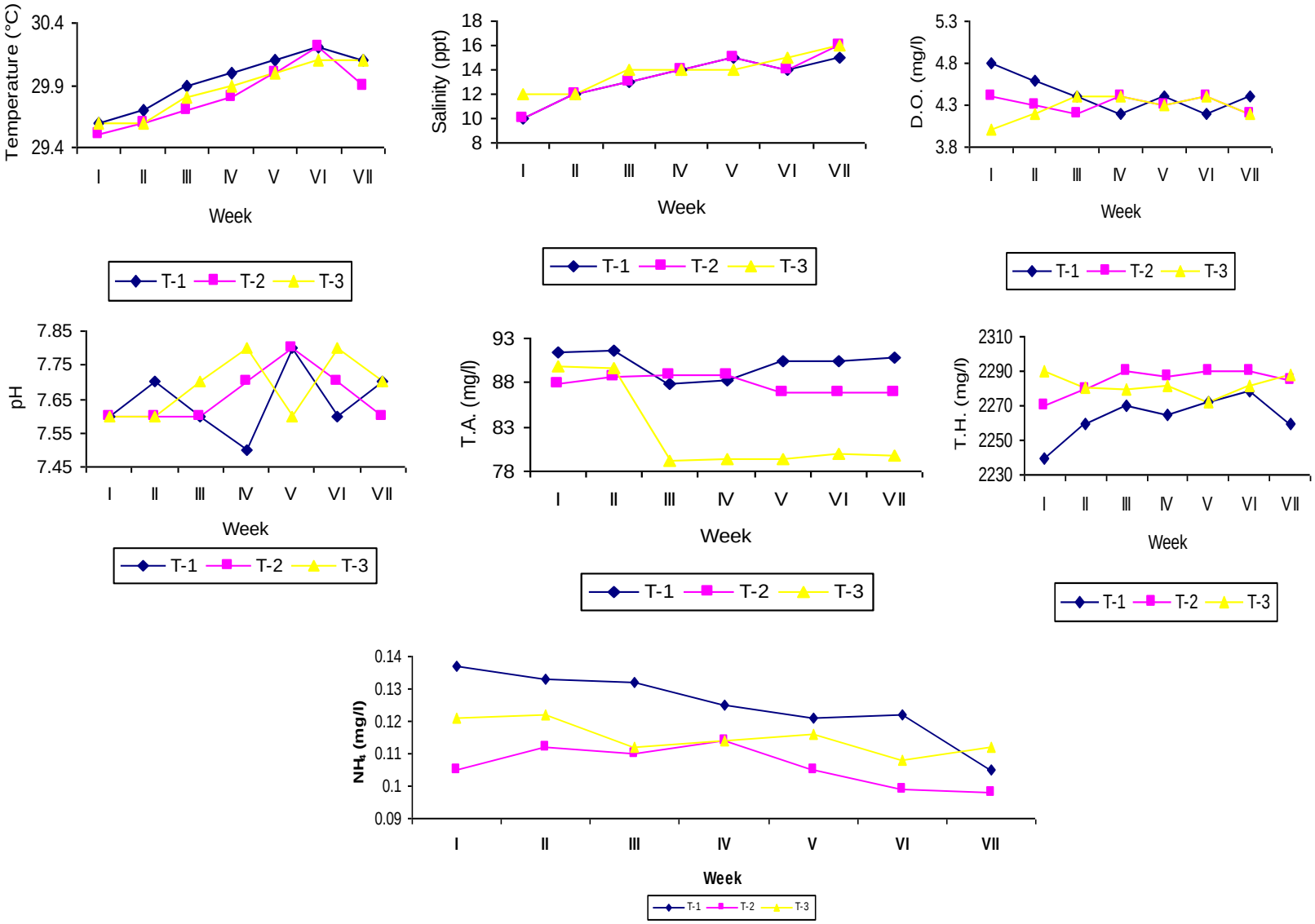


Fig. 2. Average weekly water quality parameters of three larval rearing trials of *M. gangeticum* during second year (2004).

Total hardness (TH)

The variations in TH in the larval rearing medium during the first year ranged from 2,230-2,260 mg· L^{-1} in trial 1, 2,230-2,280 mg· L^{-1} in trial 2 and 2,240-2,280 mg· L^{-1} in trial 3 (Fig. 1). More or less similar range of TH was recorded during the second year (Fig. 2).

Total alkalinity (TA)

The TA recorded during the two years in all the trials are depicted in Figs. 1 and 2. During the first year, the average TA ranged from 85.6-86.3 mg· L^{-1} while it ranged from 82.44-90.1 mg·L^{-1} during the second year.

Ammoniacal nitrogen (NH_4)

The ammoniacal nitrogen in the water medium is an important factor which directly influences the life of aquatic organisms present in the system. The dissolved ammonia in the three trials during the first year ranged from 0.082-0.097 mg· L^{-1} in trial 1, slightly less (0.072 -0.087 mg· L^{-1}) in trial 2 and 0.075-0.088 mg· L^{-1} in trial 3 (Fig. 1). However, the values were found to be much higher in all the three trials during the second year (0.125 ± 0.01 mg· L^{-1} in trial 1, 0.106 ± 0.005 mg· L^{-1} in trial 2, 0.115±0.005 mg·L^{-1} in trial 3) (Fig. 2).

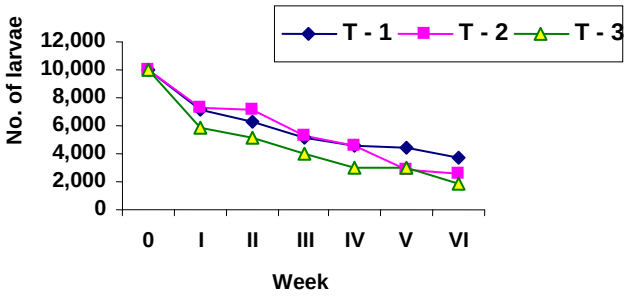
Table 1. Size and duration of various larval stages of *M. gangeticum* during culture.

Stages	*M. gangeticum*			
	Size (mm)	Growth increment (mm)	Duration (day)	Time consumed (day)
I	1.75	0.0	1.5	0.0
II	2.05	0.76	3.0	1.5
III	2.51	0.46	4.5	1.5
IV	3.03	0.52	5.5	1.0
V	3.57	0.54	6.5	1.0
VI	4.08	0.51	10.5	4.0
VII	5.06	0.98	12.5	2.0
VIII	5.47	0.41	14.5	2.0
IX	5.95	0.48	16.5	2.0
X	6.35	0.40	17.0	0.5
XI	7.00	0.65	18.5	0.5
PL	8.05	1.08	20.0	1.5

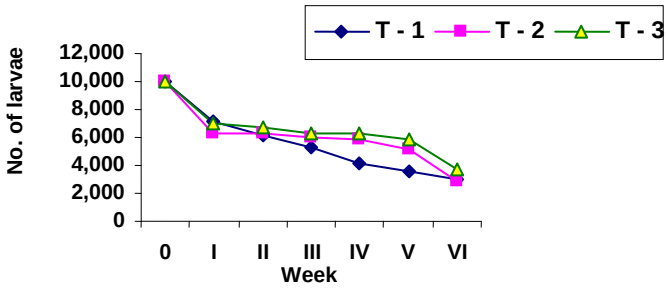
Larval growth and metamorphosis

The newly hatched zoea I stage larvae appeared transparent and translucent in color with red and blue chromatophore spots during the early stages. However, the color disappeared and reappeared on some other parts of the body following different developmental stages. The

characteristics of different larval stages *viz.* progressive increase in size, duration of each larval stage in the larval cycle in six trials are shown in Table 1. The size of newly hatched larvae (zoea I) was recorded as 1.75 mm. The duration for attainment of 5th larval stage was found 6.5 days and the size recorded was 3.57 mm. The stage V was found to pass through further 5 more molts within 4 days to attain stage VI. Thereafter, it took 2 days to attain subsequent stages viz. VII, VIII and IX and 2.5 days to reach the post larval stage. Thus, *M. gangeticum* took 20 days to attain post larval stage (Table 1). The size at post larval stage was 8.05 mm. The maximum duration was needed to attain stage VI from stage V. Therefore, the larval growth from zoea stage I to XI and subsequent attainment of post larval stage was not synchronous during all stages.



2003



2004

Fig. 3. Initial stocking in 300 l water and estimated weekly survival of larvae of *M. gangeticum* in different trials during first and second years (2003 -2004).

Larval survival

Initial stocking quantity of the larvae in all the six trials during two years were kept uniform (10,000 per tank) but started reducing gradually with the progress of the cycle (Fig. 3). During the

first year, the average survival rate at the end of first week was recorded at 67.83% where, the minimum (59.28%) was observed in trial 3 and maximum (72.56%) in trial 2. During the second week, the average survival rate of three trials was further reduced and reached to 61.84%. Similarly, gradual reductions of survival rate up to 48.19%, 40.90%, 34.27% and 27.08% were recorded during third, fourth, fifth and sixth weeks, respectively during the first year (Fig. 3). The gradual reductions in average survival also were observed during second year's trials, which were recorded as 68.51%, 64.09%, 58.37%, 54.48%, 48.56% and 31.81% in first, second, third, fourth, fifth and sixth weeks, respectively.

Post larval harvest and production

Daily harvesting of PL (Table 2, 3) indicated an increasing trend in post-larval production from the first occurrence to its peak in the middle of the cycle but thereafter, it reduced gradually till the end of the trial. The number of PL harvested daily from different trials during first and second year, varied significantly at 5% level.

Occurrence of first PL as observed on 20^{th} day ranged between 6 and 11 in number in three trials during first year. On 25^{th} day, it reached to 351, 259 and 221 in trials 1, 2 and 3 respectively. Maximum amount of PL were recorded on 29^{th} and 30^{th} day, which ranged from 500-671 and 521-659, respectively. On 35^{th} day, it reduced gradually and ranged from 90 to 161. Post larval metamorphosis was further reduced to the number ranged from 39 to 45 PL along with few advanced larvae on the closure of experimental trials on 40^{th} day. Thus, the total production of PL during first year (2003) was recorded 4748, 5170 and 5070 with 15.82, 17.30 and 16.9 PL/L in trials 1, 2 and 3, respectively. A similar trend in post larval metamorphosis also was recorded during the second year (2004). Total post larval production in trials 1, 2 and 3 during second year was recorded as 6388, 5399 and 5047 with 21.29, 17.66 and 16.82 $PL \cdot L^{-1}$ respectively.

Table 2. Trends of daily post larval metamorphosis in *M. gangeticum* recorded in larval rearing trials during first year (2003).

Days	T-1	T-2	T-3	Mean	± SD
20	8	11	6	8.33	2.55
21	30	51	60	47.00	15.39
22	70	81	78	76.33	5.68
23	95	78	95	89.33	9.81
24	200	169	171	180.00	17.35
25	351	259	221	277.00	66.84
26	325	391	321	345.67	39.31
27	400	321	291	337.33	56.30
28	325	452	431	402.67	68.07
29	500	561	671	577.33	86.66
30	651	659	521	610.33	77.46
31	500	491	345	445.33	87.01

Table 2 (contd.)

32	450	451	459	453.33	4.93
33	230	365	391	328.67	86.43
34	160	251	261	224.00	55.65
35	90	161	151	134.00	38.43
36	75	111	195	127.00	61.57
37	100	95	121	105.33	13.79
38	80	79	141	100.00	35.51
39	63	89	101	84.33	19.42
40	45	41	39	41.67	3.05
Total PL	**4748**	**5170**	**5070**	**4996.00**	**220.52**
PL· L^{-1}	**15.82**	**17.30**	**16.90**	**16.67**	**0.76**

Table 3. Trends of daily post larval metamorphosis in *M. gangeticum* recorded in three larval rearing trials during second year (2004).

Days	T-1	T-2	T-3	Mean	±SD
20	5	7	9	7.00	2.00
21	21	21	29	23.67	4.62
22	68	56	79	67.67	11.50
23	151	78	100	109.67	37.45
24	221	151	161	177.67	37.85
25	345	345	200	296.67	83.71
26	591	571	511	557.67	41.63
27	399	201	345	315.00	102.35
28	415	467	456	446.00	27.40
29	591	321	345	419.00	149.43
30	751	589	371	570.33	190.68
31	671	725	625	673.67	50.05
32	591	463	325	459.67	133.03
33	341	145	298	261.33	103.02
34	291	191	201	227.67	55.07
35	361	345	159	288.33	112.29
36	219	191	201	203.67	14.19
37	101	151	171	141.00	36.05
38	85	101	200	128.67	62.29
39	96	85	195	125.33	60.58
40	74	95	75	81.33	11.85
Total PL	**6,388**	**5,299**	**5,047**	5,578.00	712.71
PL· L^{-1}	**21.29**	**17.66**	**16.82**	18.59	2.37

Discussion

Water quality

Salinity

Salinity indicates the total concentration of all ions in brackish water medium. In the present study, salinity varied from 10-19 ppt in six trials showing relatively higher variation as the optimum range for this species is reported to be 12-16 ppt (Kanaujia et al. 2001). The variation in salinity was however, statistically significant in different trials. Kanaujia and Mohanty (1992) and Kanaujia et al. (2001) have reported that the salinity range between 18-20 ppt was optimum for the better growth and post larval production of *M. malcolmsonii* and 12-16 ppt for *M. gangeticum*. Lower salinity range may prolong the duration of metamorphosis with a poor survival rate (Rao, 1991; Kanaujia and Mohanty, 1992; Mitra, 2001).

Temperature

Prawns are temperature dependent cold-blooded animals. The temperature of water regulates the metabolism and growth of various larval stages of prawn (Rao, 1991). The variations in temperature are found to be of very narrow range and were recorded twice daily (morning and evening) to know the maximum and minimum temperature during the rearing trials for effective management. Gibson (1975) reported optimum temperature range of 28-31°C for *M. rosenbergii* during the larval rearing stage.

pH

In the present study, the increase in pH during the larval rearing period was observed and maintained within the range of 7.5 to 7.9 by periodic application of calculated amount of calcium sulphate and calcium hydrogen phosphate in biological filter tank of the rearing trials as has also been reported by Kanaujia and Mohanty (1993) in larval rearing trials of *M. malcolmsonii*. The total dissolved ammonia affects the survival particularly during molting when the pH of the water is high. Therefore, to avoid possible toxicity of ammonia in prawn hatchery Kanaujia and Mohanty (1992) suggested maintaining the water pH within the range of 7.5 to 8.5. New and Singholka (1985) have also reported a suitable range of pH between 7.5- 8.5 during larval rearing of *M. rosenbergii*. In the present study, however, the pH in all the six trials for seed production of *M. gangeticum* was slightly lower in comparison to other reports.

Dissolved oxygen

Dissolved oxygen has been reported to be the most important physico-chemical parameter of the rearing medium influencing the growth and production of the larvae through its indirect impact

on optimum feed consumption and metabolism. In the present study, the oxygen ranged between 4.0-4.8 mg· L^{-1} with a very narrow range of variation. The wide variations in DO were reported by Mohapatra (2001) as a result of the wide variation in climatic temperature and disruption of power. Since temperature and salinity both influence the oxygen solubility in water, the use of airlift bio-filter re-circulatory system maintained the DO within range in all the trials. However, the mean value recorded at weekly intervals was not significantly different.

Total hardness

Total hardness affects the growth of the larvae and mineralization of carapace. Its optimum level needed for larval metamorphosis was reported within a range of 3,800-5,200 mg· L^{-1} in *M. malcolmsonii* (Kanaujia and Mohanty, 1992). In the present study, the total hardness ranged from 2,230-2,290 mg· L^{-1} in all the trials during two years which is found similar to the report of Mohapatra (2001) in the trials of *M. malcolmsonii* and *M. rosenbergii*. However, it differed from the report of Kanaujia and Mohanty (1992) who recorded higher hardness level between 3,800-5,200 mg· L^{-1} in *M. malcolmsonii*. Thus, it is concluded that *M. gangeticum* shows better efficiency in terms of growth, reproduction, larval development, salinity requirement, duration of larval cycle etc. than *M. malcolmsonii* under similar environmental conditions. Mohapatra (2001) recorded it within 2,020-2,220 mg· L^{-1} as calcium carbonate in comparative larval rearing trials of *M. malcolmsonii* and *M. rosenbergii* and found it within the desired level.

Total alkalinity

It denotes the quantity of acid consuming constituents present in water. In natural water, bicarbonates and carbonates are the main alkaline sources which determine the pH of water. Water with low alkalinity recorded with low buffering action leads to a wide range of fluctuation in pH value. High alkalinity increases the pH and causes larval mortality. In the present study, alkalinity ranged from 79.4-90.8 mg· L^{-1} in all the trials while ranges from 50-100 mg· L^{-1} have been reported desirable for *M. rosenbergii* larvae (Chandraprakash and Reddy, 1993)

Ammonical nitrogen

Ammonia is the second most important water quality parameter after oxygen as it is one of the most toxic factors to the culture organisms. In the present study, the initial accumulation of excretory ammonia was found in all the trials during first week of rearing. However, it has been controlled effectively through the operation of airlift re-circulatory system with the provision of bio-filter and kept within 0.072-0.137 mg· L^{-1} which was much below the 'safe level'. Such a trend of ammonia level in the rearing trials suggests the improper conditioning of the biological filter that has accumulated total ammonia in the rearing medium initially, but thereafter, the level was reduced due to the conditioning of the bio-filter unit thereby stimulating the nitrification rate which corroborates with the views of Kanaujia and Mohanty (1992) and Mohapatra (2001). Interestingly, the average ammonia content was significantly lower in some of the trials which might be due to the

ineffective nitrification of chelated ammonia in the rearing trials in the presence of Na-EDTA (The disodium salt of ethylene dinitro tetra acetic acid).

Larval behavior

Initially, the movement of *M. gangeticum* larvae in the tanks was found active like *M. rosenbergii* as reported by Fujimura (1974), Kanaujia et al. (2001) and Kanaujia (2003). The larvae of *M. gangeticum* during early stages appeared with churning movement but in later stage, they moved along the side of the tank as well as in the water column. The movement of the advanced stages of larvae of *M. malcolmsonii* appeared to be moderate (Rao, 1991; Kanaujia and Mohanty, 1992). The number of larval stages in *Macrobrachium* species is non-synchronous in nature and depends on several factors (Sandifer and Smith, 1977). In *M. gangeticum*, several moults with eleven distinct larval stages were recorded to attain post larval stages which are more or less similar to *M. malcolmsonii* as reported by Rao (1991), Kanaujia and Mohanty (1992) and Mohapatra (2001). The number of larval stages in *M. malcolmsonii* was reported to be 16 by Kewalramani et al. (1971) and eleven stages by several other different in References workers (Rao, 1991; Kanaujia, 1998, 1999; Yadav, 1993; Kanaujia and Mohanty, 1992; Mitra, 2001; Mohapatra, 2001). The stocking density, food and water qualities are important factors which influence the larval growth and post larval production under hatchery conditions.

In the present study, it has been observed that the acceptance of *Artemia* nauplii was better than the other two supplementary diets (egg custard and mussel meat). In this study, the feed was prepared by passing the fine minced materials through appropriate sieves. This study clearly indicated that the appropriate size of food particles and strict schedule of feeding 4 times·day^{-1} were proved to be useful for good survival, uniform growth and development of the larvae which corroborate with the observations of Kanaujia and Mohanty (1992) and Raje and Joshi (1992).

Growth and development

There are several factors which directly or indirectly affect the growth and development of prawn larvae under culture system. In *M. gangeticum*, the growth of the larvae was more or less similar in size from stage I onward but it varied during development to post larval stage at the same pace. The progressive growth increment from stage I to stage VI is rather slow but increases rapidly from stage VI onward. Stage V takes 5-6 days to attain stage VI. Thereafter, the growth rate and time taken by each subsequent stage is quite short and more or less similar except stage XI which takes a little longer time to attain post larval stage. However, it is similar to *M. rosenbergii* which takes 23-32 days to attain the post larval stage but was different from that of *M. malcolmsonii* where subsequent development of larval stages from stage I to post larvae recorded 40 -60 days (Kanaujia and Mohanty, 1992). The observations made in the present study indicated that the transition of individual larvae from stage I to next stage occurred synchronously up to stage IV. Overlapping of stages was recorded during later stages (after stage V and above). The overlapping stage was more

significant in *M. malcolmsonii* than *M. gangeticum* and *M. rosenbergii* (New and Singholka, 1985; Kanaujia and Mohanty, 1992; Rao and Tripathi, 1993; Mishra et al. 2009).

Post larval production

The present study revealed that most of the larvae in *M. gangeticum* metamorphosed into PL within 40 days of larval cycle. Total production of PL in three trials during the first year (2003) was 4,748, 5,170 and 5,070 @15.82, 17.30 and 16.9 $PL \cdot L^{-1}$ in trials 1, 2 and 3, respectively. A similar trend in post larval metamorphosis was recorded in initial, middle and later stages during the second year (2004) where the total PL production was 6,388, 5,399 and 5,047 @ 21.29, 17.66 and 16.82 $PL \cdot L^{-1}$, respectively recorded in trials 1, 2 and 3, respectively. As described above, the occurrence of first PL was observed on 20^{th} day in all the six trials which ended on the 40th day. This duration of cycle was much shorter than that of *M. malcolmsonii*, which have been recoded more than 40-60 days (Rao, 1991; Kanaujia and Mohanty, 1992).

In conclusion, it is suggested that a suitable and effective larval rearing medium is the key to the success of large-scale seed production of freshwater prawn. Six trials undertaken during two years in the present study resulted in successful metamorphosis of the PL. The appearance of first PL was noticed earlier and total number of PL was also recorded maximum in *M. gangeticum* in all the six trials as compared to PL produced in *M. rosenbergii* and *M. malcolmsonii*. The post-larval production per liter ($PL \cdot L^{-1}$) and survival percentage also followed the same trend. The trials of *M. gangeticum* gave better results in every respect as compared to *M. rosenbergii* and *M. malcolmsonii.* However, the major differences among the three species with regard to larval survival, post larval production and duration for the larval cycle are perhaps due to genetical differences among them. Further, the mode of preparation of larval food and rearing medium may also have a bearing on the larval growth. It needs further investigation on the ingredient composition for various larval stages for better growth and survival rate of *M. gangeticum* larvae so that salinity requirement and duration of larval cycle may further be reduced to produce seed in mass scale.

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