DOI : 10.35124/bca.2019.19.2.3477

Biochem. Cell. Arch. Vol. 19, No. 2, pp. 3477-3484, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

RECYCLING OF HATCHERY-USED WASTEWATER FOR SEED PRODUCTION OF GANGETIC PRAWN, *MACROBRACHIUM GANGETICUM* (BATE, 1868)

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(Received 12 March 2019, Revised 30 May 2019, Accepted 12 June 2019)

ABSTRACT : Seed production of freshwater prawns requires brackishwater of varying salinity to complete their larval cycle in river mouths (estuaries). Though, the hatchery technology for seed production of freshwater prawn *Macrobrachium gangeticum* has been developed and standardized, the activity is mostly concentrated in coastal regions as huge quantity of seawater is required for larval development. Attempts were made to operate the hatcheries for seed production of the freshwater prawn in interior area with the chemically reconstituted seawater as well as natural seawater transported from the sea, the procedure is complicated and involves high cost. An attempt has, therefore, been made to reuse the hatchery wastewater for the seed production of *M. gangeticum* after proper treatment and aging. PL production was documented during the two years which was 5,577, 5,299 and 5,056 with PL/L 21.29, 17.66 and 16.82 in trials 1, 2 and 3, respectively during the first year. PL production recorded during second year was 5,167, 5,070 and 4,748 with PL/L 17.3, 16.9 and 15.8 in trials 1, 2 and 3, respectively. The average post-larvae production of three trials was 5,367 during the first year and 4,995 the second year.

Key words : Hatchery used seawater, post-larvae, production, *Macrobrachium gangeticum.*

INTRODUCTION

Macrobrachium rosenbergii farming is practiced in many countries of the world (New and Valenti, 2000). *M. acanthurus* (Wiegmamm), *M. amazonicum* (Heller), *M. americanum* (Bate), *M. carcinus* (Linnaeus), *M. formosense* (Bate), *M. lar* (Fabricius) and *M. ohione* (Smith) are also of commercial value in some other countries which could develop better markets (New and Valenti, 2000; New and Nair, 2012). The freshwater prawn farming is now on a commercial scale in India as it has a high foreign exchange trade (Kutty, 2005; New, 2005; Kutty *et al*, 2009; Nair and Salin, 2012; New and Nair, 2012). In India, some of the work on freshwater prawns have been done which includes *M. rosenbergii*, *M. malcolmsonii*, *M. nobilli*, *M. equidens*, *M. idae*, *M. idella* and *M. lanchesterii* (New and Valenti, 2000; Kanaujia and Mohanty, 1992, 2001; Kanaujia, 1998, 1999; New, 2002; Nair and Salin, 2012; Mishra *et al*, 2011, 2014). Of the approximately 40 cultivable species of prawns reported from India, 15 are considered important for cultivation in commercial-scale to augment production (Tiwari, 1949, 1975; Tiwari and Holthuis, 1996; Kutty *et al*, 2009; Pandey *et al*, 2010; Nair and Salin, 2012). *Macrobrachium gangeticum* is also a potential candidate for commercial farming with the development of seed production and grow-out culture technologies (New and Valenti, 2000; Kanaujia *et al*, 200l, 2005; Mishra *et al*, 2011). It occurs in river Ganga and Brahmaputra draining through West Bengal, Bihar, Uttar Pradesh and Assam (Tiwari, 1949, 1955; Tiwari and Holthuis, 1996). The survey report indicated its migration up to Kanpur about 1300 km away from the estuary of Bay of Bengal (Tiwari, 1949, 1955; Jhingran, 1956; Prasad and Kanaujia, 2006). The total length and weight of the species ranged between 200-250 mm and 50-100 g in males and 150-200 mm and 35-75 g in females (Tiwari and Holthuis, 1996; Kanaujia *et al*, 2001; Mishra and Dash, 2019). Larval development of the species occurs in natural brackishwater in the estuary (Kanaujia *et al*, 2001, 2005; Mishra *et al*, 2011). The development of *M. gangeticum* under hatchery conditions needs a large amount of brackishwater for commercial seed production which is labour intensive, expensive and only viable in coastal regions. Some studies have reported the development of post-larvae production of prawns in synthetic seawater (Aquacop, 1977; Tunsutapanich, 1980; Nair and Hameed; 1990; Kanaujia, 1998, 1999, 2006;

Sounarapandian and Kannupandi, 2000; Brock, 2003; Jhingran, 2003; Kanaujia *et al*, 2007; Thongroad *et al*, 2007; Mishra, 2015). An attempt has, therefore, been made to develop the hatchery technology for seed production of *M. gangeticum* through re-circulating hatchery-used wastewater.

MATERIALS AND METHODS

The larval rearing experiments were conducted (in triplicate) at Prawn Hatchery Complex, ICAR-Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar (India). Each rearing unit consisted of one circular plastic tank 2'h x 3' dia of 450 l water holding capacity. It was attached with a biofilter plastic drum of 80 l water capacity housed for the airlift re-circulatory system for larval rearing.

Preparation of hatchery-used wastewater

Hatchery-used wastewater was stored in plastic pools and treated with Ca SO_4, $CaHPO_4$ and NaOCl as disinfectant measure. The water was agitated with a powerful agitator and kept under sunlight for algal growth and stored for proper aging. The filtered water free from nitrogenous compounds was stored, aerated and used for larval rearing for seed production of *M. gangeticum.*

Collection and rearing of berried females

The berried females carrying gray eggs were collected from the broodstock ponds. The healthy berried prawns were selected and given a bath in 0.3 ppm $KMnO_4$ solution for about 2 minutes as a prophylactic measure and released into hatching tanks. The berried prawns were fed daily with egg custard *ad libitum* during the morning (6 am) and evening (6 pm).

Water quality parameters

To maintain an optimum rearing environment and maximizing the post-larvae production, water quality parameters such as temperature, salinity, pH, ammonia, total hardness, total alkalinity and dissolved oxygen were analyzed at regular intervals following standard methods of APHA (1985).

Assessment of post-larvae

Daily harvested post-larvae were counted one-by-one by pouring the tub water along with post-larvae in a nursery tank or in a tub. In this way, the number of post-larvae harvested daily were recorded and finally calculated for total production.

RESULTS AND DISCUSSION

Larvae of *M. gangeticum* were transparent, translucent and in the early phases showed red and blue chromatophores. During later stages, the colour deepens and observed only on some portion of the body. Larvae in all the stages were found active swimmers, planktonic in nature,photopositive, attracted to the light and displayed churning movement during early stages and moved along the side of the tank and water column at later stages. Initially, the larvae readily accepted *Artemia nauplii* but showed feeding propensity towards the supplementary feed like egg custard and mussel meat from 8th day onwards The first stage zoea completed eleven zoeal stages to metamorphose into post-larvae (PL).

Water quality

Water quality in a hatchery system is the most important factor which plays an important role in the growth, metamorphosis and survival of the larvae. The various water quality parameters such as temperature, salinity, pH, dissolved oxygen, total hardness, total alkalinity and ammoniacal nitrogen required to be maintained within the desired level for effective larval rearing.

Temperature

Water temperatures in different experimental units recorded during two years are presented in Tables 1, 2 and Fig. 1a. The ambient water temperature in hatchery-used wastewater in all the three trials recorded during the first year ranged from 29.0-30.0°C with an average of 29.4±0.33°C (Fig. 2a) which was 29.9-30.2°C with an average of 29.9±0.22°C during second year (Fig. 1a). The ambient temperature was recorded slightly higher during the second year as compared to the first year.

Salinity

The salinity of hatchery-used wastewater recorded in three trials during the first year ranged from 12-16 ppt with an average of (14.2±1.48 ppt) (Table 1, Fig. 1b) and salinity ranged 12-16 ppt with an average of 14.4±1.42 ppt during the second year (Table 2, Fig. 1b). The mean value of salinity recorded during different weeks did not show any significant variations for the two years.

pH

The pH of larval rearing medium in different trials was maintained through the adoption of the airlift bio-filter recirculatory system along with the management of water exchange and application of calcium sulfate and Na-EDTA. The pH of three larval trials in hatchery used wastewater in the first year ranged from 7.3-7.8 with an average of 7.5±0.14 (Table1, Fig. 1c). It was recorded 7.5-7.8 with an average of 7.6±0.09 during the second year (Table 2, Fig. 1c). The variation in pH was increased initially and maintained lower after the fourth week onwards as compared to the initial three weeks.

Table 1 : Weekly variations in water quality parameters of three larval rearing trials in hatchery used wastewater during first year.

Week	Temp. (°C)	Salinity (ppt)	pH	D O (mg/l)	T. H. (mg/l)	T. A. (mg/l)	NH_4 (mg/l)
Trial 1							
I	29.5	12	7.6	4.8	2264	94.8	0.088
II	29.3	15	7.5	4.7	2266	93.8	0.085
III	29.2	16	7.7	4.7	2269	87.3	0.085
IV	29.0	13	7.5	4.6	2277	86.9	0.077
V	30.0	14	7.5	4.8	2278	85.6	0.072
VI	29.2	16	7.3	4.3	2283	90.2	0.073
Average	29.4	14.3	7.5	4.6	2272	89.7	0.08
Trial 2							
I	29.5	12	7.6	4.1	2265	87.6	0.111
II	29.3	14	7.8	4.4	2265	87.8	0.105
III	29.2	13	7.7	4.3	2270	87.7	0.107
IV	29.0	15	7.5	4.2	2268	88.2	0.102
V	30.0	16	7.7	4.2	2274	86.9	0.105
VI	29.2	14	7.4	4.4	2286	90.2	0.110
Average	29.4	14	7.6	4.2	2271	88.06	0.106
Trial 3							
I	29.5	13	7.4	4.6	2265	90.8	0.085
II	29.3	15	7.6	4.4	2267	92.7	0.085
III	29.2	16	7.3	4.5	2264	87.3	0.087
IV	29.0	12	7.5	4.4	2269	84.8	0.078
V	30.0	14	7.7	4.6	2273	87.6	0.072
VI	29.2	16	7.6	4.3	2278	92.2	0.073
Average	29.4	14.33	7.5	4.4	2269	89.2	0.08
Mean	29.4	14.2	7.5	4.5	2271.2	89.0	0.1
± SD	0.33	1.48	0.14	0.21	6.77	2.85	0.01

Dissolved oxygen

The dissolved oxygen in water medium reported during the first year varied from 4.1-4.8 mg/l with an average of 4.5±0.21 mg/l (Table 1, Fig. 1d), however, the value recorded in the second year ranged between 4.0-4.6 mg/l with an average of 4.3±0.158 mg/l (Table 2, Fig. 1d). Due to the brackish water environment, the value of dissolved oxygen recorded in two media in a different week was over 4.0 mg/l during the entire cycle in spite of continuous aeration. The mean values of DO differed significantly between the first and second years in all test media.

Total hardness

The total hardness ranged between 2264-2283mg/l with an average 2271.2±6.77 mg/l during the first year (Table 1, Fig. 1e). During the second year, it was recorded slightly higher range from 2274-2290 mg/l with an average of 2282.4±8.45 mg/l (Table 2, Fig. 1e). Significant variations ($p<0.05$) in the total hardness were recorded during the first and second years of operation. However, higher values have been well registered during the second year than the first year.

Total alkalinity

Total alkalinity was recorded between 84.8-94.8 mg/l with an average 89.0±2.85 mg/l during first year (Table 1, Fig. 1f). It ranged between 79.3-97.2 mg/l with an average 89.6±5.03 mg/l in the second year (Table 2, Fig. 1f). The variations in total alkalinity levels were recorded to be trace and negligible. Total alkalinity levels declined gradually during the first year. A similar trend was also recorded in both years of the experiment.

Ammonical nitrogen

The value of dissolved ammonia in hatchery-used wastewater during the first year ranged between 0.072-0.111 mg/l with an average of 0.08±0.01 mg/l (Table 1, Fig. 1g). It was recorded 0.016-0.110 mg/l with an average 0.095±0.020 mg/l in second year (Table 2, Fig. 1g).

Post-larvae production

PL production was documented during the two years which was 5,577, 5,299 and 5,056 with PL/L 21.29, 17.66 and 16.82 in trials 1, 2 and 3, respectively during the first year (Fig. 2). PL production recorded during second year was 5,167, 5,070 and 4,748 with PL/L 17.3, 16.9

Table 2 : Weekly variations in water quality parameters of three larval rearing trials in hatchery used waste-water during second year.

Week	Temp. (°C)	Salinity (ppt)	pH	D O (mg/l)	T. H. (mg/l)	T. A. (mg/l)	NH_4 (mg/l)
Trial 1							
I	29.6	12	7.6	4.4	2274	93.6	0.090
II	29.7	15	7.7	4.5	2285	94.7	0.091
III	29.9	14	7.6	4.6	2286	96.6	0.093
IV	30.0	16	7.5	4.5	2288	89.6	0.092
V	30.1	15	7.6	4.4	2287	90.7	0.103
VI	30.2	16	7.7	4.3	2290	92.7	0.104
Average	29.9	14.6	7.6	4.4	2285	92.9	0.095
Trial 2							
I	29.6	12	7.6	4.2	2265	87.6	0.110
II	29.7	13	7.7	4.4	2268	87.8	0.105
III	29.9	14	7.6	4.3	2269	87.7	0.107
IV	30.0	15	7.8	4.2	2278	97.2	0.102
V	30.1	16	7.6	4.3	2290	86.9	0.103
VI	30.2	14	7.7	4.4	2289	94.2	0.106
Average	29.9	14	7.6	4.3	2276	90.2	0.105
Trial 3							
I	29.6	12	7.6	4.0	2278	87.2	0.108
II	29.7	14	7.8	4.3	2282	88.3	0.097
III	29.9	15	7.6	4.2	2285	86.3	0.016
IV	30.0	16	7.5	4.0	2289	79.3	0.087
V	30.1	16	7.8	4.3	2293	92.4	0.105
VI	30.2	15	7.6	4.4	2287	79.6	0.094
Average	29.9	14.6	7.6	4.2	2285.6	85.51	0.0845
Mean	29.9	14.4	7.6	4.3	2282.4	89.6	0.1
± SD	0.22	1.42	0.09	0.16	8.45	5.03	0.02

and 15.8 in trials 1, 2 and 3, respectively (Fig. 3). The average post-larvae production of three trials was 5,367 during the first year and 4,995 in second year.

All the 11 larval stages of *M. malcolmsonii* showed the propensity to feed on the egg custard, mussel meat and *Artemia* nauplii, therefore, supplementary feed provided on 8th day onwards found most acceptable. Feed quality, quantity and feeding schedules are very important to achieve successful post-larval production (Sounarapandian and Kannupandi, 2000). The exact quantity of food required at each meal depends upon the utilization of food by the larvae which need to be judged visually (Aquacop, 1977; New and Singholka, 1985; Sounarapandian and Kannupandi, 2000). In the present study, it has been observed that the acceptance of *Artemia* nauplii was better than that of the supplementary diet (egg custard and mussel meat). The suitable size of food particles for different stages of the larvae of *M. malcolmsonii* has been suggested by Kanaujia and Mohanty (1992) and Kanaujia (1998, 1999). The present study indicated that the appropriate size of food particles and the schedule of (feeding 4 times/day) was useful for better production of post-larvae of *M. gangeticum*, which corroborates the findings in *M. rosenbergii* (Raje and Joshi, 1992; Joshi and Raje, 1993).

Water quality in a hatchery system is the most important factor, which plays an important role in the growth, metamorphosis and survival of the larvae (Boyd, 1990; Mitra, 2001; Mohanty, 2003). Since this study has been carried out under similar conditions, the variations in ambient temperature in the rearing medium was minimum and insignificant among the treatments. The ambient temperature recorded during the second year was slightly higher as compared to the first year in natural seawater. Water temperature regulates the metabolism and growth of various larval stages of prawn and has been recommended a favourable temperature range 28-31^{0}C for optimum larval growth and development of *M. malcolmsonii* (Kanaujia and Mohanty, 1992; Mohapatra, 2001; Mukherjee *et al*, 2013, 2015) and *M. rosenbergii* (Mohapatra, 2001; Prasad and Singh, 2006). New and Singholka (1985) and Diaz and Ohno (1986) have reported that the temperature above 35^{0}C and less than 24^{0}C may result into retarded growth and mortality of the larvae of *M. rosenbergii*. More or less similar observations have also been reported in *M. malcolmsonii*

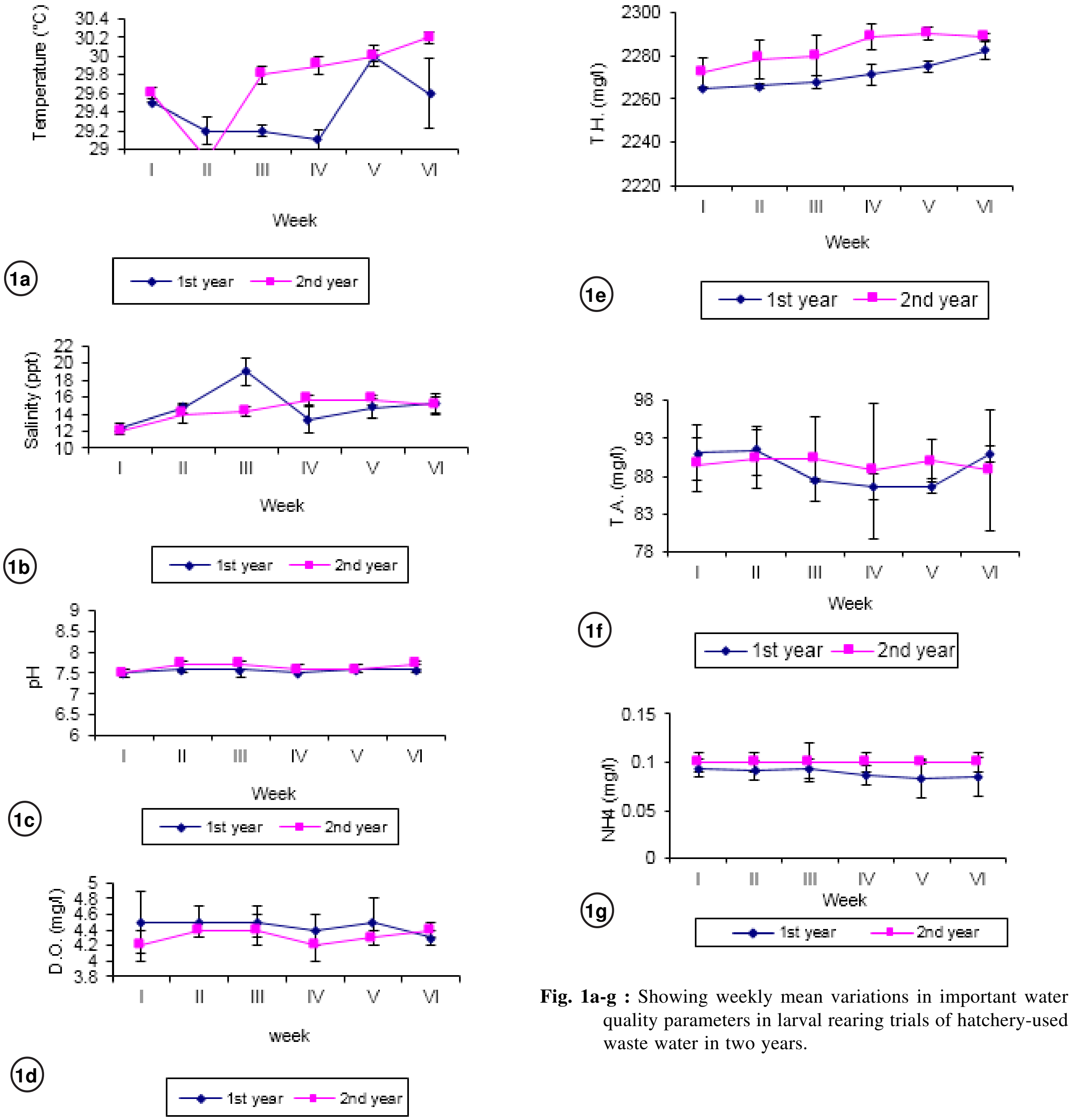
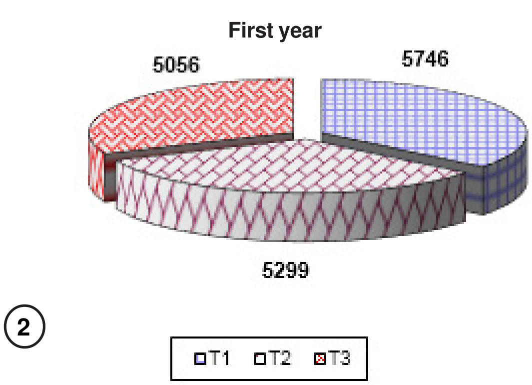
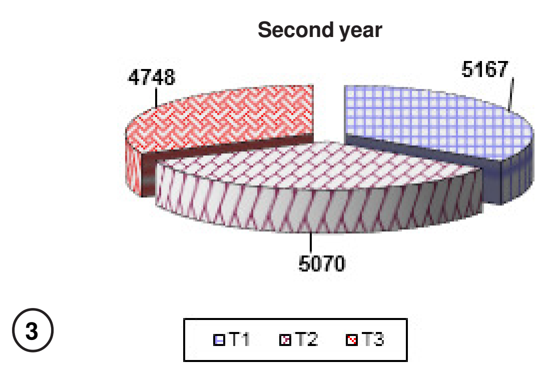


Fig. 1a-g : Showing weekly mean variations in important water quality parameters in larval rearing trials of hatchery-used waste water in two years.





Figs. 2, 3 : Post-larval production of *M. gangeticum* recorded in three trails during two years.

(Kanaujia *et al,* 2001, 2005; Mohapatra, 2001). pH is an important factor in determining the productivity in aquaculture (Boyd, 1990). In the present study, an increase in pH during the larval rearing period was observed and maintained within the range of 7.5-7.7. The total dissolved ammonia affects the survival particularly during moulting when the pH of the water is high. Therefore, to avoid possible toxicity of ammonia in prawn hatchery, New and Singholka (1985) have reported a suitable range of pH between 7.5-8.5 in water during larval rearing of *M. rosenbergii*. The dissolved oxygen (DO) in the medium reported as an important factor which directly affects the growth and survival of the larvae. Due to the brackishwater environment, DO level in medium was maintained over 4 mg/l during the entire cycle of larval production of *M. gangeticum.* In spite of continuous aeration, DO value was recorded lower (4.2 mg/l) during the first year. The mean values of DO differed significantly between the first and second years. In the present study, the mean value recorded was not significantly different in the trials. The wide variations in dissolved oxygen during larvae culture of *M. malcolmsonii* were reported by Mohapatra (2001). DO is important not only for respiration but also for maintenance of most favourable chemical and hygienic environmental conditions of the larval rearing medium (Mohanty, 2003). In the test media, significant variations ($p<0.05$) in the total hardness were recorded during the first and second years of operation. However, higher values have been registered in the second year than the first year.

Freshwater prawns, as well as most of the crustaceans, require high calcium concentrations for the enzymatic processes involved in moulting and there is also a relationship between magnesium and neural muscular energy transmission. Total hardness of water affects the growth of the larvae and mineralization of carapace (Brown *et al*, 1991). The total hardness for larval metamorphosis was reported within the range of 3,800-5,200 mg/l in *M. malcolmsonii* (Kanaujia and Mohanty, 1992). Mohapatra (2001) recorded a total hardness level within 2,020-2,220 mg/l as calcium carbonate in the comparative larval rearing study of *M. malcolmsonii* and *M. rosenbergii* and found within the desired level. Brown *et al* (1991) found that growth of *M. rosenbergii* was maximum at hardness levels below 53 mg/l as $CaCO_3$ and survival were at higher rates. The total alkalinity of water is mainly caused by the contents of Ca, Mg, Na, K, NH_4 and Fe combined either with carbonates, bicarbonates or occasionally by hydroxide (Jhingran, 2003). In the present observations, total alkalinity in two years ranged between 79.2-91.7 mg/l. Water with low alkalinity recorded with low buffering action which leads to a too wide range of fluctuation in pH value. High alkalinity increases the pH and leads to larval mortality. Alkalinity range from 50-100 mg/l have been reported as the desirable level for *M. rosenbergii* larvae (Chandraprakash and Reddy, 1993). Ammonical nitrogen in the water medium is important factor which directly influence the life of aquatic organisms. The maximum value of ammonical nitrogen levels during the second year was significantly higher than the first year. Further, it was found that there was no accumulation of ammonia in the test media in first and second year. Ammonia exists in water in two forms, namely un-ionized ammonia (NH_3^+) and ammonium ion (NH_4^+) (Mohanty, 2003). In the present study, it ranged between 0.072- 0.137 mg/l in both the years which was much below the 'safe level'. In this study, the initial accumulation of excretory ammonia was found in all the trials during rearing. A suitable and effective larval rearing medium is the key to success in large-scale prawn seed production. All the rearing trials of the experiment resulted in successful metamorphosis to the post-larvae of *M. gangeticum*.

CONCLUSION

Successful hatchery operation for large-scale seed production of freshwater prawn requires a suitable and effective larval-rearing medium. In the present study, all trials carried out in hatchery-used wastewater for two years resulted in better production of the post-larvae. Thus, it can be concluded that larval rearing trials in hatchery-used wastewater gave better efficacy in terms of growth, reproduction, larval development, salinity requirement, duration of the larval cycle *etc.* Therefore, the study demonstrates the possible use of hatchery-used wastewater for the seed production of *M.gangeticum* to establish hatcheries in inland areas far away from the coastal regions.

ACKNOWLEDGMENTS

We are gratitude to Director, ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar for providing the laboratory facilities to carry out the work and encouragement. Help extended by the staff of Prawn Hatchery Complex during the study is acknowledged.

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