Mj. Int. J. Sci. Tech. **2008**, *2*(03), 471-482

Maejo International

Journal of Science and Technology

ISSN 1905-7873

Available online at www.mijst.mju.ac.th

Full Paper

Embryonic development, hatching, mineral consumption, and survival of *Macrobrachium rosenbergii* (de Man) reared in artificial seawater in closed recirculating water system at different levels of salinity

Krasindh Hangsapreurke[1, 6,*], Thon Thamrongnawasawat [2], Sorawit Powtongsook[4], Pratak Tabthipwon[5], Prajuab Lumubol[2], and Boonyarath Pratoomchat [3]

[1] Faculty of Fisheries Technology and Aquatic Resources, Maejo University, Sansai, Chiangmai, Thailand

[2] Graduate School, Kasetsart University, Bangkok, Thailand

[3] Department of Marine Science, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand

[4] Marine Biotechnology Research Unit, c/o Department of Marine Science, Chulalongkorn University, Bangkok, Thailand

[5] Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand.

[6] Department of Aquatic Science, Faculty of Science, Burapha University, Bangsaen, Chonburi, Thailand

Corresponding author, e-mail : krasindh@yahoo.com

Received: 22 January 2008 / Accepted: 29 July 2008 / Published: 20 August 2008

Abstract: This experiment aims to study the effect of different levels of salinity (5, 15 and 25 ppt) using artificial sea water on the embryonic development and hatching percentage of the eggs of unripe berried female giant freshwater prawns (*Macrobrachium rosenbergii*) with an average size of 14.3±0.6 cm TL. After incubation through the heart beating stage (grayish black eggs), the brooders in each salinity were separately transferred to the hatching tank with 15 ppt saline water for the second part of the study. After hatching, the healthy larvae from the brooders which were previously incubated in 3 levels of salinity were collected for the larviculture experiment. The closed recirculating water system with trickling filter unit packed with fiberglass and bioballs was used as incubation and larviculture units. The metamorphosis period and survival rate were examined. The rearing water from each larviculture aquarium was collected for determination of sodium, magnesium, potassium, calcium and chloride ions. The result showed that the percentage of ripe berried females (with heart beating

Mj. Int. J. Sci. Tech. **2008**, *2*(03), 471-482

stage embryos) were not significantly different ($p>0.05$) between 5 and 15 ppt salinity but their values were significantly higher ($p<0.05$) than that obtained in 25 ppt salinity. The hatching rate of eggs from berried females incubated in 5 ppt salinity was significantly higher ($p< 0.05$) than those obtained in 15 and 25 ppt salinity while the hatching rate in 15 ppt salinity was also significantly higher ($p< 0.05$) than that in 25 ppt salinity. There were no significant differences ($p>0.05$) in the survival rate of post larvae and metamorphosis period among the treatments. The first post larvae stage occurred on the 26^{th} day. During 30 days of larviculture, the survival rate of all treatments was 100% until the 19^{th} day, after which it suddenly decreased. When the concentrations of the ions in the rearing water were determined in all treatments, it was found that the concentration of magnesium ions rapidly declined ($p<0.01$) while those of sodium and potassium ions decreased gradually ($p<0.05$). No change was observed in the calcium and chloride ion concentration throughout the experiment ($p>0.05$). The low survival rate during the final stage of larviculture might be due to the depletion of the previously mentioned ions especially that of magnesium.

Keywords: *Macrobrachium rosenbergii*, giant freshwater prawns, mineral consumption, artificial sea water, salinity, larval production

---

Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii* (de Man) is a highly esteemed food for Thais. In former time, these prawns were highly abundant in rivers, lagoons, freshwater reservoirs and brackish water areas [1-2] . However, a very drastic reduction of the wild stock 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. To provide for the global market demand many giant freshwater prawn farms have been established. Since the discovery of the importance of salinity as a basic requirement for the larval survival of *M. rosenbergii* [1] numerous hatcheries have developed techniques to mass-produce the post-larvae at a commercial scale.

In 2000, the world production of giant freshwater prawns was estimated at 118,501 MT and was valued at $ 410,001,000 [3]. Thailand's production in 2002 was approximately 15,000 MT [4] or an estimated 12.6% of world production. The giant freshwater prawn is an important economic aquatic animal that can be cultured in all freshwater areas throughout Thailand. The central area is the most prominent for hatcheries which have been expanding to other areas. However, because of the high demand, production is still not enough. Although some areas are not suitable for culture because of low temperatures in winter time, some studies have reported success by increasing the temperature of the water by 3-4° C through covering the earthen ponds with plastic film [5]. Furthermore, some researchers have studied the effect of culture season and stocking density for giant freshwater prawn culture in the northern part of Thailand and found out the best conditions for the area where temperature decreases in the winter time [6]. These findings led to the expansion of culture areas in the North and Northeast which are far from the source of larvae production. In the past, it was possible to transport larvae from the source to anywhere because of low transportation cost. Unfortunately,

Mj. Int. J. Sci. Tech. **2008**, *2*(03), 471-482

because of the continuing increase in the transportation cost for saline water and shrimp larvae, increased 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, these 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. Artificial sea water should be the best choice but information in its use is lacking. To address this problem, the use of commercial artificial sea water under closed circulating saline water system with trickling filter unit packed with fiberglass and bioballs has been investigated in this study. The major ions were also examined throughout culture period. Furthermore, the embryonic development and hatching of the berried females eggs were monitored under different levels of salinity using artificial sea water to observe its effect on the larval production.

Materials and Methods

The experiment was divided into two parts. The first part involved the effect of artificial sea water at 5, 15 and 25 ppt (parts per thousand) salinity on the embryonic development and hatching percentage of the eggs of berried females (*M. rosenbergii*). After incubation until the heart beating stage (grayish black eggs), the brooders in each salinity were separately transferred to the hatching tank which had a salinity of 15 ppt. After hatching, the healthy larvae from each brooder assigned in three different salinity levels were collected for the larviculture experiment. The second part focused on studying the mineral consumption of the prawn larviculture in a closed recirculating water system using artificial sea water. The metamorphosis period and survival rate were examined.

Source of water and brooders

Artificial sea salt powder (Marinium®) from Mariscience International Co. Ltd. was used. Salinity was adjusted to desirable levels using Jenway conductivity meter (model 4200). The unripe berried females (embryonic stage of gastrula) at an average size of 14.3$\pm$0.6 cm in total length (TL) from a commercial farm in Chiangrai province were used.

Incubation and larviculture system

A closed recirculating water system employing glass aquariums with trickling filter unit packed with fibreglass and bioballs was used for incubation and larviculture. All aquariums were rectangular with a dimension of 29.5 x 60.0 x 38.0 cm and covered tightly with a plastic lid. Three compartments were included in each aquarium. The first compartment (29.5 x 44.5 x 38.0 cm) with a fine mesh net cage was provided for larval rearing area. The fine mesh net cage of 15 x15 x13 cm was used for 1- to 10- day-old larvae, while the cage of 20 x 20 x 20 cm was used for >10-day-old larvae. The second compartment (14.3 x 17.5 x 38.0 cm) was provided with a trickling filter unit. The inside of the compartment contained 2 layers of fibreglass at the top and 720 bioballs at the bottom. The last

Mj. Int. J. Sci. Tech. **2008**, *2*(03), 471-482

compartment (11.7 x 14.3 x 38.0 cm) was installed with a water pump (capacity 600 L/hr) for water circulation (Figure 1).

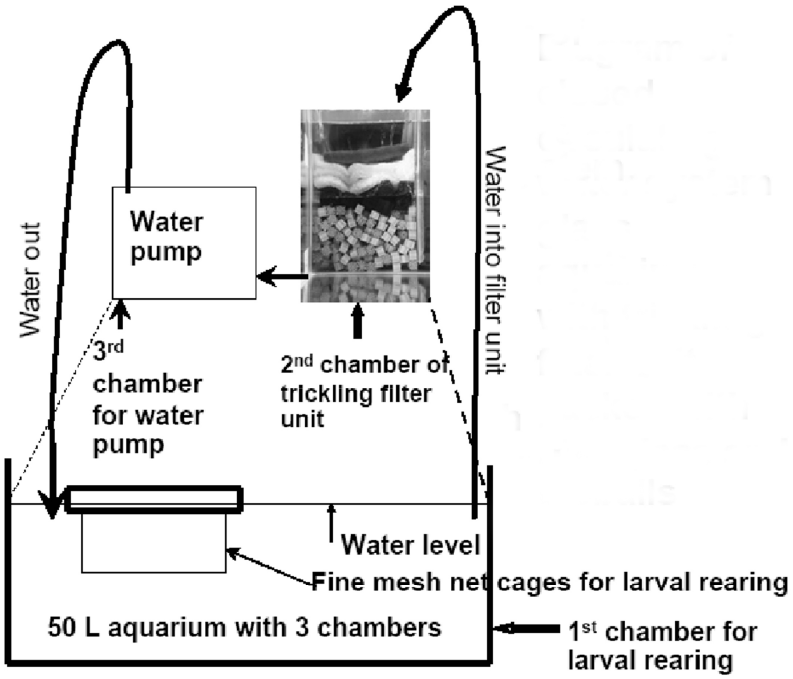

Figure 1. Diagram of the closed recirculating water system aquarium

Egg incubation

The berried females at gastrula stage were held in the 50-L closed recirculating water system aquariums at a density of six females/aquarium. The experiment was performed in triplicate per salinity. Acclimatisation to 5, 15 and 25 ppt artificial sea water was done prior to the experiment proper. During incubation they were fed with sliced fresh squid.

Ten eggs from each female were sampled daily to examine the embryonic stage. The developmental period from gastrula stage to heart beating stage (grayish black colour) was individually recorded. The major ions, viz. sodium, magnesium, potassium, calcium and chloride from the incubation water of each salinity values were determined.

The water in each incubation aquarium was sampled at the beginning and the 10th day of incubation.

Preparation of larvae and larviculture

The ripe berried females from different salinity levels were separately stocked in 100-L fibreglass tanks which contained 15 ppt artificial sea water until hatching. The healthy larvae from the brooders

Mj. Int. J. Sci. Tech. **2008**, *2*(03), 471-482

were then randomly sampled (80 larvae/L.) and put in each 50-L aquarium containing artificial sea water at 15 ppt salinity for the larviculture experiment. The closed recirculating water system with trickling filter unit packed with fibreglass and bioballs was used. The lavae were fed with newly-hatched *Artemia nauplii* at a density of five individuals/ml twice a day throughout the experiment. There was no water change, but the debris was removed regularly. The larval development was checked daily at 8.00 am.

Sampling of rearing water and mineral determination

The rearing water from each larviculture aquarium was collected at the start and three times a week thereafter throughout the experiment. One ml from each water sample was drawn (using an automatic pipette) for determination of sodium, magnesium, potassium, calcium and chloride ions by high performance energy dispersive X- ray fluorescence spectrophotometry (Oxford ED^{2000}) [7-8]

Water quality

Water temperature and pH were checked daily using a Horiba (Model D-21) pH-metre. Nitrite and ammonia were checked every two days using a Hanna C203 multi-parameter specific ion metre. DO was checked daily using a Jenway (9002 Model) DO metre.

Data analysis

All data were analysed by regression analysis, ANOVA and Tukey's using SPSS program.

Results and Discussion

Embryonic development

After the unripe berried females were incubated in various levels of salinity for 10 days, it was observed that the percentages of the ripe berried females in 5 ppt and 15 ppt salinity during 7-10 days were not significantly different ($p>0.05$), but their values were significantly higher than those in 25 ppt salinity ($p< 0.05$) (Figure 2). Sodium, magnesium, potassium, calcium, and chloride ions in the 3 levels of salinity are shown in Table 1. It was clear that the concentrations of those ions were positively correlated with the degree of salinity.

Mj. Int. J. Sci. Tech. **2008**, *2*(03), 471-482

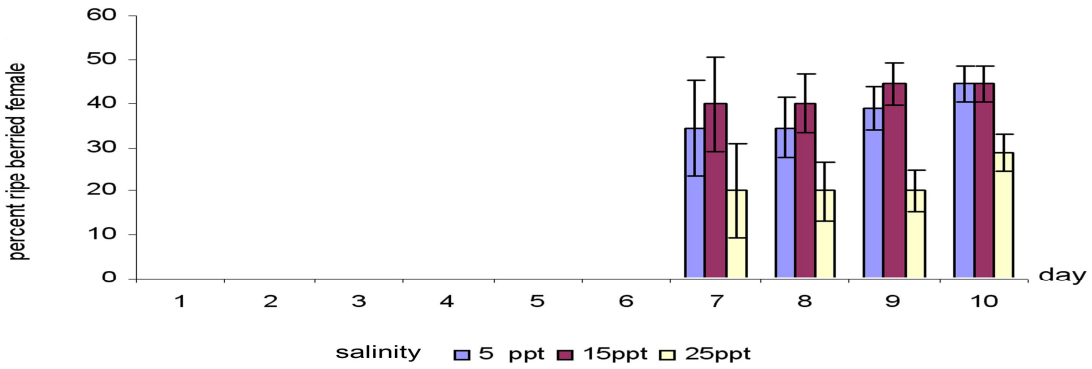

Figure 2. Percentage of ripe berried females after incubation in different levels of salinity during 7-10 days

Table 1. Concentration (mM/L) of Na, Mg, K, Ca and Cl ions in artificial sea water (5, 15 and 25 ppt salinity) at the 1st and 10th day of incubation

Ions	5 ppt (mM/L)		15 ppt (mM/L)		25 ppt (mM/L)	
	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10
Na	64.26±8.45[a]	46.02±2.11[b]	199.36±4.03[a]	155.07±16.95[b]	287.21±7.22[a]	243.22±6.7[b]
Mg	6.95±0.45[a]	3.66±0.44[b]	23.93±1.29[a]	18.95±0.72[b]	36.57±0.47[a]	36.61±0.33[a]
K	2.43±0.19[a]	2.35±0.10[a]	5.24±0.45[a]	5.00±0.40[a]	7.73±0.56[a]	7.39±0.17[a]
Ca	1.96±0.04[a]	1.94±0.16[a]	4.82±0.83[a]	5.13±0.81[a]	7.82±0.64[a]	7.95±0.25[a]
Cl	2.43±0.19[a]	2.35±0.10[a]	5.24±0.45[a]	5.00±0.40[a]	7.73±0.56[a]	7.39±0.17[a]

Note: Means in the same row with different superscripts differ significantly ($p<0.05$).

The embryonic development of berried females was significantly affected by salinity. Significantly higher percentages of ripe berried females were observed in 5 and 15 ppt salinity. New [9] reported that the suitable salinity for egg incubation was ≤15 ppt. Damrongphol et al. [10] also reported that the embryonic development in vitro was dramatically altered by the concentrations of Na, K and Cl ions in the medium during egg incubation. Their findings (169.2 mM of NaCl and 3.6 mM of KCl) were comparable to, though somewhat lower than ours in the 15 ppt salinity. This phenomenon further supports Ling's findings [11] that the berried females have to migrate to a brackish water area to release the larvae. Furthermore, Singh [12] reported that the isosmotic point for this prawn is 17.5-18.0 ppt. It was indicated that the embryos should need an isosmotic balance for optimum development. The 15 ppt in this experiment seemed to be the most suitable. However, a high percentage of ripe berried females also occurred at 5 ppt, which means that it is also possible to incubate the eggs at 5 ppt salinity although it is considered hyposmotic for the major minerals. This agrees with the findings of Brohmanonda and Sahavacharin [13] who earlier reported that the wild berried females were consistently caught in waters with 3-6 ppt salinity, which was also used for their incubation. Furthermore, New and Singholka [14] reported that some hatcheries placed the berried females in 0-5 ppt saline water during egg incubation prior to transfer to 12 ppt saline water for hatching without osmotic shock. Currently, some hatcheries in Thailand observe that keeping berried females in water of 3-5 ppt salinity can stimulate and improve embryonic development. This clearly indicates that the

Mj. Int. J. Sci. Tech. **2008**, *2*(03), 471-482

isosmotic point concept is not a limitation for *M. rosenbergii*, although it still needs some ions especially Na^+, K^+ and Cl^- for development. Egg development and hatching was not possible with deionised water [15]. Also, a hyperosmotic medium was not suitable for the egg development which was negatively affected by an extremely high concentration of ions.

During the 10-day incubation period, it was apparent that sodium ions declined at all salinity levels, but magnesium ions declined only at 5 and 15 ppt salinity. It is possible that the embryos need sodium and magnesium from the environment for their normal development [10]. This experiment has shown that the salinity range of 5-15 ppt was suitable for egg incubation and larval rearing, and that the salinity at 5 ppt should be the most optimum for *M. rosenbergii* egg incubation. A salinity of 15 ppt has also been used commercially by some Thai farmers for a long time. The salinity at 25 ppt is apparently too high for the incubation of *M. rosenbergii* eggs since the isosmotic point of the prawn is between 17.0-17.5 ppt [12]. Sandifer et al. [16] reported that the prawn could not survive longer than 24 hours if introduced abruptly into sea water of salinity greater than 18 ppt.

Hatching rate

The hatching rate from berried females incubated in water of 5 ppt salinity was significantly higher ($p< 0.05$) than that in 15 ppt and 25 ppt salinity while the hatching rate in 15 ppt salinity in turn was significantly higher ($p< 0.05$) than that in 25 ppt salinity (Figure 3).

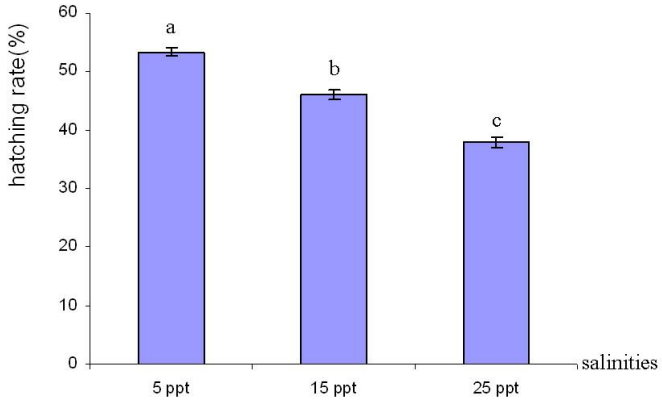

Figure. 3 Hatching rate from berried females incubated in 5, 15 and 25 ppt salinity (± SE)

After 10 days of incubation, all ripe berried females were separately transferred to 15 ppt saline water for hatching. The hatching rate of females incubated in 5 ppt salinity was significantly highest. This agrees with the findings of New [9] that higher hatchability occurs in females incubated in ≤15 ppt salinity. This result is further supported by Brohmanonda and Sahavacharin [13] who also reported a 58% hatching rate at 3-6 ppt salinity. The concentration of the major ions at low salinity (3-5 ppt) apparently improved egg incubation. The survival rate and metamorphosis period of the larvae during the egg incubation at 3 different levels of salinity were not significantly different ($p>0.05$) when cultured in 15 ppt salinity. This indicates that the influence of different salinity during the egg incubation is not the limiting factor for the nursery in artificial sea water at 15 ppt salinity under the closed recirculating

Mj. Int. J. Sci. Tech. **2008**, *2*(03), 471-482

system. The result does not agree, however, with the practice of commercial hatcheries in Thailand who believe that higher production occurs when low salinity (3-5 ppt) was used for the egg incubation.

Ion fluctuation during larviculture

As illustrated in Figure 4, during the 30 days of larviculture, magnesium ion concentration rapidly declined ($p<0.01$) while potassium and sodium ion concentration decreased gradually ($p<0.05$). Calcium ion concentration did not change throughout the experiment ($p>0.05$). On the other hand, chloride ion concentration increased but not significantly ($p>0.05$).

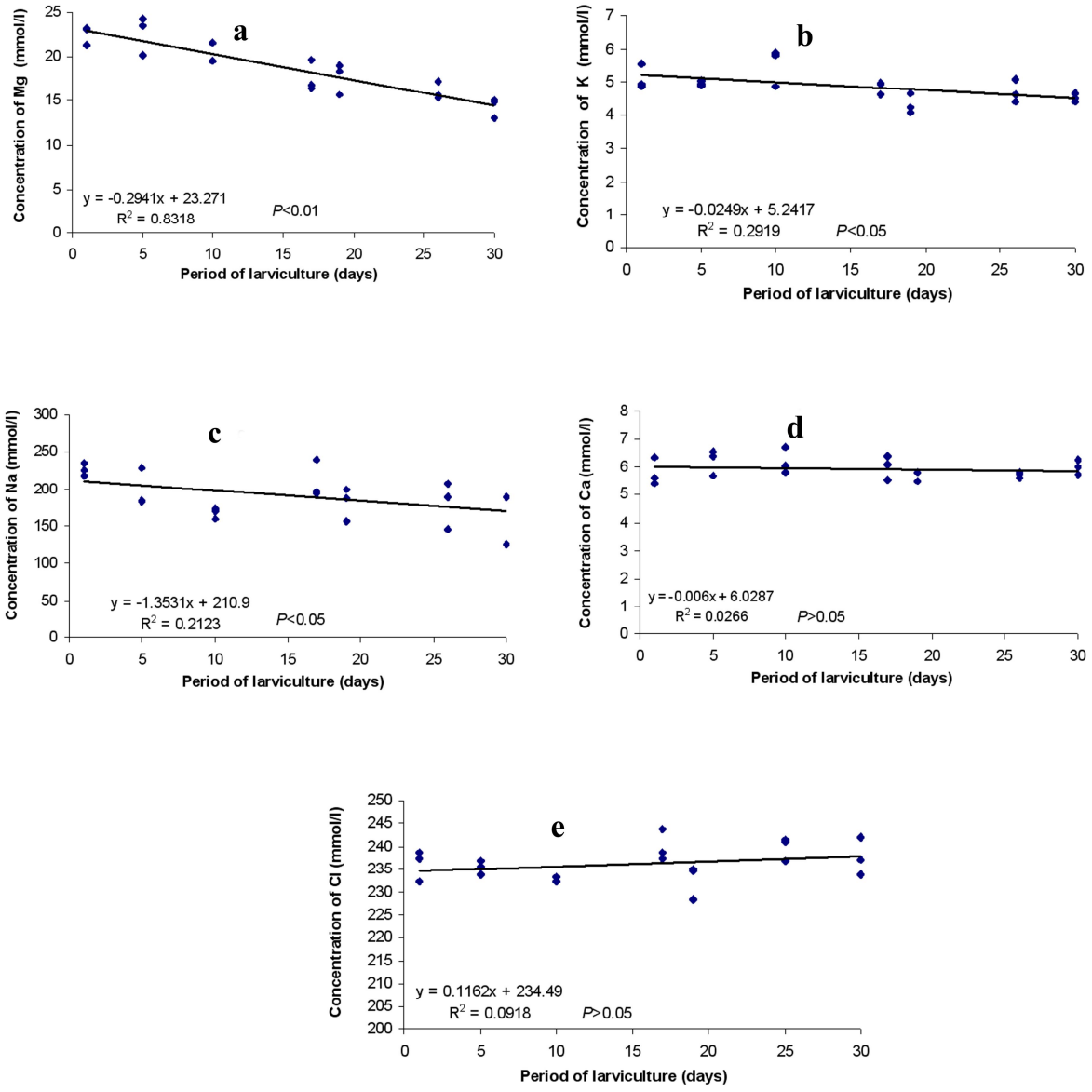

Figure 4. Ion concentrations of magnesium (a), potassium (b), sodium(c), calcium (d) and chlorine (e) in rearing water during 30 days in the closed recirculating system

Mj. Int. J. Sci. Tech. **2008**, *2*(03), 471-482

Survival rate

In trying to reduce the utilisation of saline water in *M. rosenbergii* larviculture by using artificial sea water in place of concentrated sea water under the closed recirculating water system, the survival rate (Figure 5) in our experiment was 18-27% (14-22 PL/L), which was higher than the findings of Tansakul [17] who obtained 15% from nursing in a static water system using artificial sea water at 12 ppt salinity and a density of 10 larvae/L and 12.5% water changing every five days. This is comparable to the result of Ang [18], who obtained 17-50 PL/L in a recirculation system, and also comparable with the report of Menasveta and Piyatiratitivorakul [19] who investigated the closed recirculating system with a separate sub-sand filter unit and a sub-sand filter inside the rearing tank. They could achieve 15.9-18.7% survival rate with a density of 20 larvae/L. Thapa [20] observed a 23% survival rate in an open circulating water system containing rock salt with a density of 50 larvae/L. However, it was noted that the initial stocking density of larvae was significantly lower than that used in our experiment. The survival rate obtained from this study, however, was still lower than that from the commercial farms in Thailand (35-40%) with a culturing density of 80-100 larvae/L using an open system. This indicates that though the system in this experiment is acceptable for *M. rosenbergii* larviculture it seems to need further improvement.

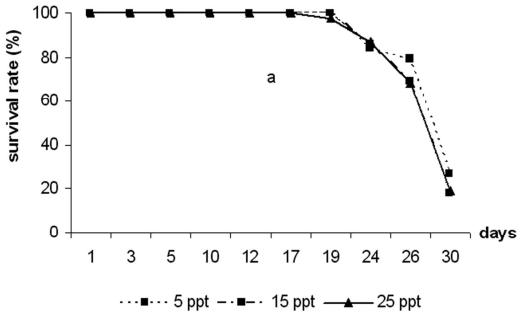

Figure 5. Survival rates of the larvae produced from brooders incubating their eggs in 5 ppt, 15 ppt and 25 ppt salinity during 30-day experiment

The survival rate decreased rapidly after 19 days of culture in this experiment. It might have been caused by the negative effect of continuous depletion of magnesium, potassium and sodium ions in the culture medium. Magnesium ions decreased more than twofold during the study. It should be noted that magnesium is an essential element for cuticle formation and for the neurosystem [7,21,22] while potassium is necessary for the osmoregulatory system and membrane potential [18,22]. The optimum levels of magnesium and potassium ions for *M. rosenbergii* larvae are 16.45 and 7.67 mM/L respectively [22]. Zang et al. [23] reported that ions such as Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, Br^-, HCO_3^-, and possibly SO_4^{2-} are essential for *M. rosenbergii* rearing and suggested that the Mg^{2+}/Ca^{2+} ratio should be between 1.8-2.0. It is clear that Mg^{2+}/Ca^{2+} ratio in this experiment was 1.51 ± 0.16 from the start and decreased to 0.61 ± 0.09 after 20 days. This means that the survival rate of the larvae in our experiment might have been affected by this condition. Sodium, magnesium and calcium are also necessary for

Mj. Int. J. Sci. Tech. **2008**, *2*(03), 471-482

supporting the normal embryonic development, hatching, and survival of the newly hatched larvae [10]. With these findings, the supplementation of magnesium, sodium and potassium is needed in the culture medium to maintain a suitable concentration throughout the culture period. Calcium ions and calcium/magnesium ion ratio should also be maintained although they are of minor factor for the survival rate.

The average concentrations of ammonia and nitrite during the 30-day experiment were 0.08 and 0.03 ppm respectively, which were very low. The recommended range of total ammonia and nitrite are less than 1.0 and 0.25 ppm respectively [24]. The mortality was therefore not caused by ammonia and nitrite which were produced by the larvae and live feed. The closed recirculating system in our experiment had no impact on the metamorphosis of the larvae because the first day of the post larval stage occurred at the 26th day, which was similar to the result from the commercial production [25].

Conclusions

Unripe females of *Macrobrachium rosenbergii* incubated in saline water with 5 ppt salinity produced the highest hatching compared to those obtained with 15 and 25 ppt salinity. Embryonic and larval development of the freshwater prawns consume minerals from the rearing water. The larvae consume magnesium, sodium and potassium ions for their metamorphosis during the rearing process.

It was found that artificial sea water from commercial sea salt powder can be used to prepare artificial saline water in place of concentrated sea water from salt farms. Moreover, the use of closed recirculating water system with trickling filter unit packed with fibreglass and bioballs can efficiently reduce saline water usage. This system should therefore be suitable for hatcheries in the northern or north-eastern part of Thailand or in areas that are far away from the coast. There is thus a high possibility to apply this system to the commercial production of *M. rosenbergii* larvae in the future. However, maintenance of magnesium and potassium concentrations in the culture medium during larval rearing process should be addressed.

Acknowledgements

This research was financially supported by the National Research Council of Thailand (NRCT). We acknowledge the Mariscience International Company Limited for providing commercial sea-salt powder (Marinium®), and Kasetsart University (Sriracha Campus) for allowing us to use the X-ray fluorescence spectrophotometer. We appreciate the assistance of Mr. Krirk Wongsontam for operating the X-ray fluorescence spectrophotometer.

References

1. S. W. Ling and A. B. O. Merican, “Notes on the life and habitats of the adults and larval stages of *Macrobrachium rosenbergii* (de Man)”, *Indo-Pacific Fisheries Council Proceedings*, Karachi, Pakistan, 6-23 Jan.**1961**, pp. 55-60.

Mj. Int. J. Sci. Tech. **2008**, *2*(03), 471-482

2. T. A. Qureshi, S. M. Basha, and R. Biwas, "Larval rearing of Indian river prawn *Macrobrachium malcolmsomii* through synthetic seawater", *Oostende Bellgium European Aquaculture Society*, **1993***, 19*, 158-224.
3. FAO, "FAO Year Book fisheries statistic", *Aquaculture Production,* **2000**, *90*, 1-78.
4. Department of Fisheries, "Fisheries Statistic of Thailand 2002", Fishery Information Technology Center, Department of Fisheries, Ministry of Agriculture and Cooperatives, **2004**, 91 pp.
5. N. Whangchai, T. Ungsethaphand, C. Chimanat, K. Mengumphan, and S. Uraiwan, "Performance of giant freshwater prawn *Macrobrachium rosenbergii* (de Man) reared in earthen ponds beneath plastic film shelters", *Chiang Mai J. Sci.* , **2007**, *34*, 89-96.
6. R. D. Baysa and N. Whangchai, "Effect of culture season and stocking density on growth and production of giant freshwater prawn *Macrobrachium rosenbergii* (de Man) raised in northern Thailand", *Mj. Int. J. Sci. Tech*., **2007**, *1*, 216-221.
7. B. Pratoomchat, P. Sawangwong, and J. Machado, "Organic and inorganic compound variations in haemolymph, epidermal tissue and cuticle over the molt cycle in *Scylla serrata* (Decapoda)", *Comp. Biochem. Physiol.,* **2002***, 131*, 243-255.
8. B. Pratoomchat, P. Sawangwong, and J. Machado, "Effects of controlled pH on organic and inorganic composition in haemolymph, epidermal tissue and cuticle of mud crab *Scylla serrata*", *J. Exp. Zool*, **2003**, *295*, 47-56.
9. M. B. New, "Freshwater prawn culture : a review", *Aquaculture,* **1990**, *88*, 99-143.
10. P. Damrongphol, P. Jaroensastraraks, and B. Poolsanguan, "Effect of various medium compositions on survival and hatching rates of embryos of the giant freshwater prawn *Macrobrachium rosenbergii* cultured *in vitro*", *Fish. Sci.,* **2001**, *67*, 64-70.
11. S. W. Ling, "The general biology and development of *Macrobrachium rosenbergii* (de Man)", *FAO Fisheries Report,* **1969**, *57*, 589-606.
12. T. Singh, "The isosmotic concept in relation to the aquaculture of the giant prawn *Macrobrachium rosenbergii*," *Aquaculture,* **1980**, *20*, 251-256.
13. P. Brohmanonda and S. Sahavacharin, "Results of experimental culture of giant freshwater prawn larvae *Macrobrachium rosenbergii* (de Man) at Songkhla Marine Fisheries Station", Division of Research and Investigations, Department of Fisheries, **1970***,* 23 pp.
14. M. B. New and S. Singholka, "Freshwater prawn farming", A manual for the culture of *Macrobrachium rosenbergii*, FAO Fisheries Technical Paper 225 (Rev .1), FAO Rome, **1995**.
15. P. Damrongphol, N. Engchuan, and B. Poolsanguan, "Simple in vitro culture of embryos of the giant freshwater prawn *Macrobrachium rosenbergii*", *J. Sci. Soc. Thailand,* **1990,** *16*, 17-24.
16. P. A. Sandifer, J. S. Hopkins, and T. I. J. Smith, "Observations on salinity tolerance and osmoregulation in laboratory-reared *Macrobrachium rosenbergii* postlarvae (Crustacea: Caridea)", *Aquaculture*, **1975**, *6*, 103-114.
17. R. Tansakul, "Progress in Thailand rearing larvae of the giant prawn *Macrobrachium rosenbergii*", *Aquaculture,* **1983**, *31*, 95-98.
18. K. J. Ang, "A recirculated biostream hatchery system for larval culture of *Macrobrachium rosenbergii* (de Man)", in "Abstract of World Aquaculture1996" (Ed. L. R. Cresswell), World Aquaculture Society, **1996**, 202-203.

Mj. Int. J. Sci. Tech. **2008**, *2*(03), 471-482

19. P. Menasveta and S. Piyatiratitivorakul, "A comparative study on larviculture techniques for the giant freshwater prawn *Macrobrachium rosenbergii* (de Man)", *Aquaculture,* **1980**, *20*,139-249.
20. A. B. Thapa, "Effect of different sources of salt water and ionic concentrations on larval nursing of giant freshwater prawn *Macrobrachium rosenbergii* (de Man)", *MSc. Thesis*, **2002**, Kasetsart University, Thailand.
21. B. Pratoomchat, P. Sawangwong, R. Guedes, M. L. Reis, and J. Machado, "Cuticle ultrastructure changes in the crab *Scylla serrata* over the molt cycle", *J. Exp. Zool.*, **2002**, *293*, 414-426.
22. C. Visudtibhan, "Effect of different levels of magnesium ion and potassium ion on the survival *Macrobrachium rosenbergii* (de Man) post larvae in the rock salt water", *MSc. Thesis*, **1993**, Kasetsart University, Thailand.
23. Z. Zang, X. Dai, J. Zhang, and Z. Zhu, "Effect of Mg^{2+}, Ca^{2+} and Mg^{2+}/Ca^{2+} contents on survival rates of *Macrobrachium rosenbergii* larvae reared in mixed water", *Oceanologia et Limnologia Sinica*, **1995**, *26*, 552-557.
24. K. V. Jayachandran, "Palaemonid Prawns: Biodiversity, Taxonomy, Biology and Management", Science Publishers Inc., Cochin (India), **2001**.
25. S. Pitipornchai, Personal communication, **2007**.