EFFECT OF SALINITY ON EMBRYONIC DEVELOPMENT OF *Macrobrachium rosenbergii* (DE MAN)

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ABSTRACT

This experiment aimed to study the effect of various salinities (5, 15 and 25 ppt) using artificial sea water on the embryonic development and hatching percentages of unripe berried female (*M. 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 15ppt for the second part of the study. After hatching, the healthy larvae from the brooders which were previously incubated in 3 salinities 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 Sodium, Magnesium, Potassium, Calcium and Chlorine determination.

The percentage of ripe berried females (heart beating stage embryos) were not significantly different ($p>0.05$) between 5 and 15ppt salinities but their values were significantly higher ($p<0.05$) than that of 25ppt salinity. The hatching rate of berried female incubated in 5ppt was significantly higher ($p< 0.05$) than those of 15 and 25ppt while the hatching rate in 15ppt was significantly higher ($p< 0.05$) than that of 25ppt. There were no significant differences ($p>0.05$) for survival rate of post larvae and metmorphosis period among the treatments. The first post larvae stage occurred on the 26th day. During 30 days of larviculture, the survival rate of all treatments was 100% until the 19th day after which, their survival rate suddenly decreased (9th to post larval stage). When we determined the ions in the rearing water in all treatments, we found out that the Magnesium concentration rapidly declined ($p<0.01$) while Sodium and Potassium concentrations decreased gradually ($p<0.05$). No change was observed in the Calcium and Chloride concentrations 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 elements especially Magnesium.

INTRODUCTION

The giant freshwater prawn, *Macrobrachium rosenbergii* (de Man) is a highly esteemed food by Thais. In the former time, this prawn was highly abundant in rivers, lagoons, freshwater reservoirs and brackish water area (Ling, 1961; Singh, 1980). However, a very drastic reduction of 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* (Ling, 1962) numerous hatcheries have developed techniques to mass produce 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 (FAO, 2000). Thailand's production in 2002 was approximately 15,000 MT (DOF, 2004) or an estimated 12.6% of world production. The giant freshwater prawns are an important economic aquatic animal that can be cultured in all freshwater areas throughout Thailand. The central area is the most prominent for hatcheries and grow out but because of the high demand, production is still not enough. This 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 elsewhere because of low transportation cost. Unfortunately, because of the continuing increase in the transportation cost for saline water and shrimp larvae, growout production is becoming less feasible. Furthermore, lower survival rate of larvae can occur due to stress from long hours of travel , thus the need for local hatcheries was realized. 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 for lower cost operation. Artificial seawater could be the best choice but information in its use is lacking. To address this problem, the use of commercial artificial seawater under closed circulatory saline water system with trickling filter unit packed with fiberglass and bioballs was investigated. The major ions were also examined throughout culture period. Furthermore, the embryonic development and hatching of berried female (*M. rosenbergii* were carried out under different salinities using artificial sea water to observe its effect to the larval production.

MATERIALS AND METHODS

The experiment was divided into two parts. The first part studied the effect of artificial sea water (at 5, 15 and 25 ppt salinities) on the embryonic development and hatching percentage of berried female (*M. rosenbergii*). After incubation until 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 salinities were collected for the larviculture experiment. A closed recirculating water system using artificial sea water was applied for shrimp larviculture. The metamorphosis period and survival rate were examined.

Source of Water and Brooder

Artificial sea salt powder (Marinium®) was used. The 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±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 using glass aquariums with trickling filter unit packed with fiberglass and bioballs was used for incubation and larviculture system All aquariums were rectangular with 30.5 cm x 61.0 cm x 38.0 cm in size and covered tightly with plastic lids. The three compartments were included in each aquarium. The first compartment (29.3 cm x 49.0 cm x 38.0 cm in size) with fine mesh net cage was provided for larval rearing area. The fine mesh net cages at size 15 cm x15 cmx13 cm was used for 1-10 days old larvae while 20 cm x 20 cm x 20 cm was used for ≥ 10 days-old larva The second compartment (10.0 cm x 18.7 cm x 27.8 cm) was provided for trickling filter unit. The internal compartment contained 2 layers of fiberglass at the top and 720 bioballs at bottom. The last compartment (10.0 cm x 10.0 cm x 27.8 cm) was installed with the water pump (600l/hr) for water circulation

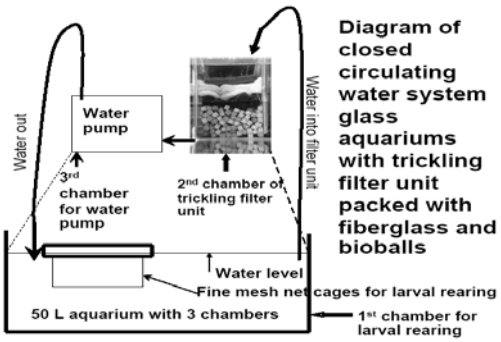


Figure 1. Diagram of the closed recirculating water system aquarium.

Egg Incubation

The berried females at gastrula stage were held in 50-l closed recirculating water system aquariums at a density of six females/aquarium (system detailed item 2.2) replicated three times per salinity. Acclimatization to 5, 15 and 25ppt artificial seawater was done prior to the experiment proper. During incubation they were fed with sliced fresh squid.

Ten berried eggs from each female were sampled daily to examine the embryonic stage. The developmental period from gastrula stage to heart beating stage (grayish black color) was individually recorded. The major ions Sodium, Magnesium, Potassium, Calcium and Chlorine from the incubation water among 3 salinities were determined.

Preparation of larvae and Larviculture

The ripe berried females from different salinities (item 2.4) were separately stocked in 100 L fiberglass tanks which contained 15ppt artificial seawater until hatching. The healthy larvae from the brooders were then randomly sampled (80 larvae/l) to each 50L aquarium at 15 ppt for larviculture experiment. The closed recirculating water system with trickling filter unit packed with fiberglass and bioballs (item 2.3) was used. They were fed with newly hatched *Artemia nauplii* at a density of five individuals/ml twice a day throughout the experiment. There was no water exchange but the debris was removed regularly. The larval development was checked daily at 8.00 am.

Sampling of Rearing Water and Elements 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 the determination of Sodium, Magnesium, Potassium, Calcium and Chlorine through high performance energy dispersive X- ray fluorescence spectrophotometry (Oxford ED^{2000}) (Pratoomchat et al., 2002a ; Pratoomchat et al., 2003)

Water Quality

Temperature and pH were checked daily using Horiba model D-21. Nitrite and Ammonia were checked every two days using Hanna C203 Multi-parameter specific ion meter. D.O was checked daily using Jenway 9002 model.

Data Analysis

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

RESULTS

1. Embryonic Development

After the unripe berried females were incubated in various salinities for 10 days, we observed that the percentage of the ripe females between 5 ppt and 15 ppt during 7-10 days were not significantly different ($p>0.05$), but their values were significantly higher than that of 25 ppt.($p< 0.05$) (Fig. 2). Calcium, Magnesium, Sodium, Potassium and Chlorine among 3 salinities are shown in table 1. It was clear that the concentrations of those elements were positively correlated to higher salinity.

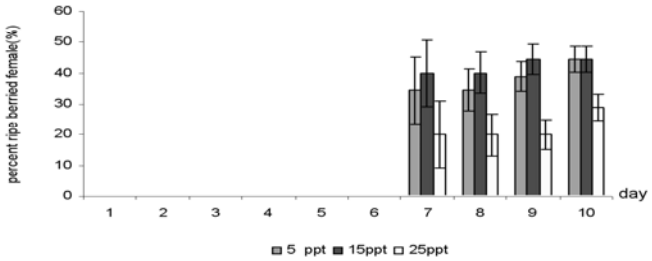


Figure 2. Percentage of the ripe berried females after incubation in various salinities during 10 days.

Table 1 Concentration of Cl, Na, Mg, Ca and K in 5, 15 and 25 ppt artificial seawater.

Elements	5ppt (mM/l)	15ppt (mM/l)	25ppt (mM/l)
Cl	83.31±0.98	252.64±0.99	344.02±0.36
Na	72.49±0.80	207.58±1.14	300.61±0.52
Mg	8.74±0.17	21.36±1.27	45.95±0.47
Ca	1.74±0.10	4.97±0.10	8.37±0.10
K	1.53±0.03	4.72±0.12	8.05±0.94

2. Hatching Rate

The hatching rate of berried female incubated in 5ppt was significantly higher ($p< 0.05$) than those of 15ppt and 25ppt while and hatching rate of 15ppt was significantly higher ($p< 0.05$) than that of 25ppt (Fig. 3).

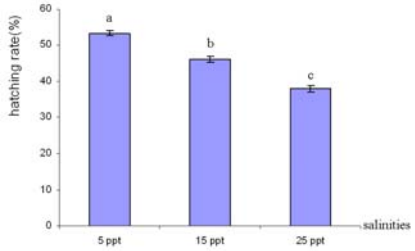


Figure. 3 Hatching rate of berried females incubated in 5, 15 and 25ppt.

3. Survival and Metamorphosis

The hatched larvae from the ripe berried females from different salinities were collected for nursery trial. The closed recirculating water system was operated at 15 ppt. During the 30 days larviculture period, the survival rate of among all treatments was 100% until the 19th day. Thereafter, their survival rate suddenly decreased and a drastic significant mortality was observed on days 26-30 (Fig. 4a). The survival rate averaged 20-26% at day 30 (100% post larvae). The metamorphosis period was not significantly different ($p>0.05$) among 3 salinities (Fig. 4b).

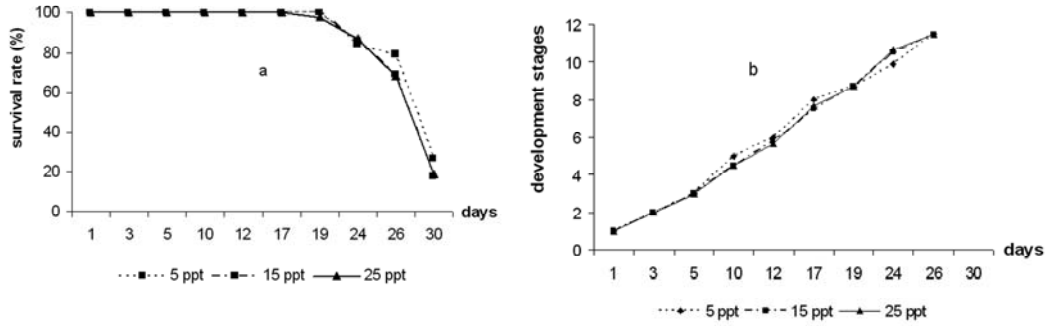
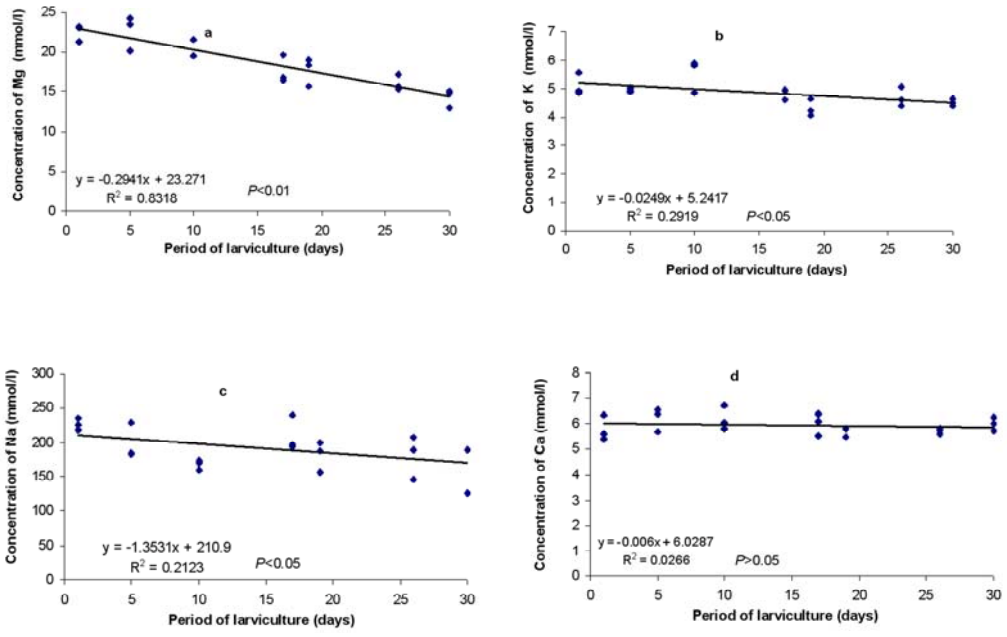


Figure 4. Survival rate (a), Development stages (b) of the larvae produced from brooder which had incubated their eggs in 5ppt, 15ppt and 25 ppt during 30 days experiment.

The water quality of all treatments was acceptable, the ammonia and nitrite level of all treatments were 0.08±0.03 ppm and 0.03+0.005 ppm respectively, while dissolved oxygen was ≥ 7.0 ppm.

4. Element fluctuation during Larviculture

Magnesium concentration (Fig.55a) rapidly declined (*p<0.01*) while Potassium (Fig.5b) and Sodium (Fig.5c) concentrations decreased gradually (*p<0.05*) during the 30 days of larviculture. Calcium concentration (Fig. 5d) throughout the experiment did not change (*p>0.05*). In contrast, Chlorine concentration (Fig.5d) increased but not significantly (*p>0.05*).



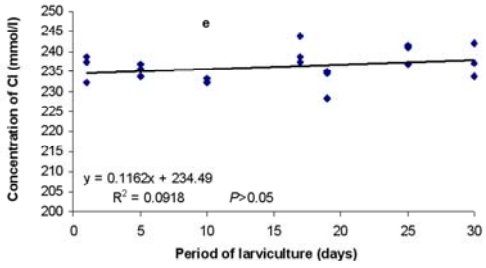


Figure 7. Concentration of Magnesium (a), Potassium (b), Sodium(c), Calcium (d) and Chlorine (e) in rearing water during 30 days of the closed recirculating system.

DISCUSSION

The embryonic development of berried females was significantly affected by the salinity. A significantly higher percentage of ripe berried females were observed in 5 and 15ppt salinities. New (1990) reported that the suitable salinity for incubation was ≤15ppt. Damrongphol et al. (2001) 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 or a bit lower than in the 15ppt salinity in our experiment. This phenomenon further supported Ling's (1969) findings that the berried females have to migrate to brackish water area to release the larvae. Furthermore, Singh (1980) reported that the isosmotic point for this prawn was 17.5-18 ppt. It is indicated that the embryos should need isosmotic balance for optimum development. The 15 ppt in this experiment was the most suitable. However, high percentage of berried females also occurred at 5 ppt in our experiment. It means that it is also possible to incubate eggs at 5 ppt salinity although it is considered hyposmotic of major elements. This agreed with Brohmanonda and Sahavacharin (1970) who earlier reported that the wild berried females were consistently caught in 3-6 ppt salinities. These salinities were also used for their incubation. Furthermore, New and Singholka (1995) reported that some hatcheries placed the berried females in 0-5 ppt during egg incubation prior to transfer to 12 ppt. for hatching without osmotic shock. Currently, some hatcheries in Thailand observed that keeping berried females in 3 to 5ppt water salinities will stimulate and improve embryonic development. This clearly indicates that the isosmotic point concept was not a limitation for *M. rosenbergii* but it still needs some ions especially Na, K and Cl for development. Egg development and hatching was not possible with deionized water (Damrongphol et al., 1990). Also, hyperosmotic medium was not suitable for egg development which was negatively affected by extremely high concentration of ions.

After 10 days egg incubation, all ripe berried females were separately transferred to 15 ppt salinity for hatching. The hatching rate of females incubated in 5ppt was significantly highest. It agreed with New (1990) that higher hatchability occurred in females incubated in ≤15ppt. This result is supported by Brohmanonda and Sahavacharin (1970) who also reported a 58% hatching rate at 3-6 ppt. Therefore, the concentration of major elements at low salinity (3-5ppt) improved egg incubation. The survival rate and metamorphosis period of larvae among the 3 salinities of egg incubation was not significantly different ($p>0.05$) when cultured in 15 ppt. It indicated that the influence of different salinities during egg incubation was not a limiting factor for nursery in artificial sea water at 15ppt under the closed recirculating system. It did not agree with the practice of commercial hatcheries in Thailand who believed that higher production occurs when they use low salinity (3-5 ppt) for egg incubation.

The experiment tried to reduce the utilization of saline water in *M. rosenbergii* larviculture by using artificial seawater instead of concentrated seawater under the closed recirculating water system. The survival rate in our experiment was 18-27% (14-22 PL/l) which was higher than the findings of Tansakul (1983) who got 15% from nursing in a static water system using artificial seawater at 12 ppt under density of 10 larvae/ and 12.5% water exchange every five days. This was comparable to Ang (1996) who had 17-50 PL/l in a recirculation system. This result was also comparable with the report of Menasveta and Piyatiratitivorakul (1980) who investigated the closed recirculating system with a separate sub-sand filter unit and with sub-sand filter in side the rearing tank. They could produce 15.9-18.7% survival rate with a density of 20 larvae/l. Thapa (2002) observed a 23% survival rate in an open circulating water system using rock salt source with a density of 50 larvae/l. However, it was noted that the initial stocking density of larvae of these experiments were significantly lower than that of our experiment. Our survival rate was lower than the commercial farms in Thailand (35-40%) with a culturing density of 80-100 larvae/L using an open system. It indicated that the system in this experiment was acceptable for *M. rosenbergii* larviculture but needs more improvement.

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 concentrations in the culture medium. The Magnesium decreased more than 2 folds during the study. It should be noted that Magnesium is an essential element for cuticle formation and for the neurosystem (Visudtibhan, 1993; Pratoomchat, 2002a, b) while Potassium is necessary for osmoregulatory system and membrane potential (Visudtibhan, 1993; Pratoomchat, 2003). As with the previous studies it showed that the optimum level of magnesium and potassium for *M. rosenbergii* larvae were 16.45 mM/l of magnesium and 7.67 mM/l of potassium (Visudtibhan, 1993). Furthermore, Zang et al. (1995) 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 and 2.0. It is clear that Mg/Ca ratio of this experiment was 1.51±0.16 at start and decreased to 0.61±0.09 during 20-30 days. It means

that the low survival rate of larvae in our experiment may have been affected by this condition. Sodium, Magnesium and Calcium are also necessary for supporting normal embryonic development, survival, hatching or survival of newly hatched larvae *M. rosenbergii* (Damrongphol et al., 2001). With these findings, the supplementation of Magnesium, Sodium and Potassium is a must in the culture medium to maintain the suitable concentration throughout culture. Calcium and Calcium/Magnesium ratio should also be maintained although it is of minor effect to the survival rate.

The average concentration of ammonia and nitrite during 30 days experiment was 0.08ppm in ammonia and 0.03ppm in nitrite which were very low. The recommended range of total ammonia and nitrite are less than 1.0ppm and less than 0.25ppm respectively. (Jayachandran, 2001). Therefore, the mortality was not caused by ammonia and nitrite which was produced from the larvae and live feeds.

The closed recirculating system in this experiment was not an impact to metamorphosis of the larvae because the first day of post larvae stage occurred at 26 days in this experiment which was similar to that of commercial production (Pitipornchai, personal comm). Furthermore, this closed recirculating system used only 50 l. of saline water throughout the experiment, while water exchange system used 300 l. or 6 times of this system.

This experiment concludes that artificial seawater from commercial sea salt powder can be used as an alternative saline water source instead of concentrated seawater from salt farms. The closed recirculating water system with trickling filter unit packed with fiberglass and bioballs can be efficiently used to reduce saline water usage. There is a high possibility to apply this system to commercial production of *M. rosenbergii* larvae in the future. However, maintenance of Magnesium and Potassium concentrations in culture medium during larval rearing process should be addressed.

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REFERENCES

Ang, K.J. 1996. A recirculated biostream hatchery system for larval culture of *Macrobrachium rosenbergii* (De Man). In : Cresswell, L.R. (Ed), Abstract of World Aquaculture1996, 29 January-2 February 1996, Baton Rouge, Bangkok, Thailand. *World Aquaculture Society*, pp. 202-203.

Brohmanonda, P. and Sahavacharin, S. 1970. 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*. 23 pp.

DOF. 2004. Fisheries Statistic of Thailand 2002. Fishery information tecnology center. Department of fisheries. Ministry of agriculture and cooperatives, 91 pp.

Damrongphol, P., Engchuan, N. and Poolsanguan, B. 1990. Simple in *vitro* culture of embryos of the giant freshwater prawn(*Macrobrachium rosenbergii*).*Journal of Scence Society. Thailand.*16, 17-24.

Damrongphol. P., Jaroensastraraks. P. and Poolsanguan. B. 2001. Effect of various medium compositions on survival and hatching rates of embryos of the giant freshwater prawn (*Macrobrachium rosenbergii*) cultured *in vitro* . *Fisheries Science.* 67,64-70.

FAO. 2000. FAO Year Book Fisheries Statistic: *Aquaculture production*.90(2) , 78 pp.

Ling, S.W. and Merican, A.B.O. 1961. Notes on the life and habitats of the adults and Larval stages of *Macrobrachium rosenbergii* (de Man). *Indo-Pacific fisheries council proceeding 9th session , Karacchi , Pakistan 6-23 Jan 1961. Sect II & III IPFC. Secretariat. FAO. Regional office for Asia & Far East Bangkok.* pp, 55-60.

Ling. S.W. 1962. Studies on the rearing of larvae and culturing of adults of *M.rosenbergii* (de Man). FAO Indo-Pac. *Fisheries Council Current Affair*., 11 pp., mimeographed.

Ling, S.W. 1969. The general biology and development of *Macrobrachium rosenbergii* (De Man) . *FAO Fisheries Report* 57(3). pp, 589-606.

Menasveta, P. and Piyatiratitivorakul, S. 1980. A comparative study on larviculture techniques for the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man)*Aquaculture.* 20,139-249.

New, M.B. 1990. Freshwater prawn culture:a review. *Aquaculture* 88, 99-143.

New, M.B. and Singholka, S. 1995. Freshwater prawn farming. A manual for the culture Of *Macrobrachium rosenbergii*. FAO Fisheries Technical Paper 225 (Rev 1).FAO, Rome.

Pratoomchat, B., Sawangwong, P. and Machado, J. 2002a. Organic and inorganic compound variations in haemolymph, epidermal tissue and cuticle over the molt cycle in *Scylla serrata* (Decapoda). *Comparative Biochemistry and Physiology.*131A, 243-255.

Pratoomchat. B., Sawangwong. P., Guedes. R., Reis. M. L., and Machado. J. 2002. Cuticle ultrasructure changes in the crab *Scylla serrata* over the molt cycle. *Journal of Experimental Zoology*. 293, 414-426.

Pratoomchat, B., Sawangwong, P. and Machado, J. 2003. Effects of controlled pH on organic and inorganic composition in haemolymph,epidermal tissue and cuticle of mud crab *Scylla serrata*. *Journal of Experimental Zooloy.* 295A, 47-56.

Qureshi, T.A., Basha. S.M. and Biwas. R. 1993. Larval rearing of Indian river prawn *Macrobrachium malcolmsomii* through synthetic seawater. Limnol. Barkatullah Univ., Bhopoal 462026, India. From discovery to commercialization. *Oostende Bellgium European Aquaculture Society.* 19,158.

Singh,T. 1980. The iso osmotic concept in relation to the aquaculture of the giant prawn, *Macrobrachium rosenbergii*. *Aquaculture.* 20, 251-256.

Tansakul.R. 1983. Progress in Thailand rearing larvae of the giant prawn *Macrobrachium rosenbergii*. *Aquaculture*. 31, 95-98.

Thapa, A,B. 2002. Effect of different sources of salt water and ionic concentrations on larval nursing of giant freshwater prawn (*Macrobrachium rosenbergii*) de Man.*Master of Science Thesis. Kasetsart University Thailand.* 59 pp.

Visudtibhan. C. 1993. Effect of different levels of magnesium ions and potassium ion on The survival *Macrobrachium rosenbergii* (de Man) post larvae in the rock salt water. *Master of Science Thesis. Kasetsart University Thailand.* 61 pp.

Zang. Z., Dai.X., Zhang.J. and Zhu. Z. 1995. Effect of Mg^{2+}, Ca^{2+} and Mg^{2+}/Ca^{2+} Contents on survival retes of *Macrobrachium rosenbergii* larvae reared in mixed water. *Oceanologia et Limnologia Sinica*. 26,552-557.