Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man 1879) Larval Nursing in Relation to Chitosan Levels, Seawater, Artificial Seawater and Re-used Seawater

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Received: 16 June 2017 Accepted: 31 August 2017

ABSTRACT

The experiments were conducted at the Angthong Inland Fisheries Research and Development Center, Angthong Province (Experiments 1.1, 1.2 and 3) and at the Inland Aquaculture Research Institute, Phra Nakhon Si Ayutthaya Province (Experiment 2). These three experiments were carried out during February 2012 to April 2016 aiming to justify the effect due to different levels of supplementary chitosan in feed diet, artificial seawater formulae 1, 2 and the re-used of artificial seawater in nursing giant larval prawns (*Macrobrachium rosenbergii*). The work also aimed to examine larval prawn overturn postures of its bodies and the survival rate (%). For the Experiment 1.1, five chitosan treatments were used. They included chitosan levels of 0, 100, 200, 300 and 400 ppm for T1 up to T5, respectively. For the Experiment 1.2, the same rates of chitosan as that of Expt. 1.1 together with seawater formula 2 were used and again there were five treatments altogether. The Experiment 2 consisted of the re-used of seawater, artificial seawater formulae 1 and 2. It consisted of three treatments altogether. Experiment 3 consisted of nine treatments on ratio (proportion) between the re-used artificial seawater formula 2 and the unused artificial seawater formula 2 in relation to salinity levels of 9, 12 and 15 ppt. A Completely Randomized Design (CRD) with three replications was used for all of the three experiments.

The results showed that a temperature of the culture media of 19°C was not suitable for growth and overturn activity of body postures of the larval prawns. The lowest numbers of days to reach 100 % overturned of larval prawns was found with that of the artificial seawater formula 2 (39 days). A significant effect of chitosan was found with artificial seawater formula 2 at a chitosan level of 100 ppm. This was the most profitable chitosan level for use in culturing larval prawns. The best ratio between the re-used artificial seawater formula 2 and the unused artificial seawater formula 2 plus salinity level was found with T6, i.e., 50:50 plus 15 ppt salinity. A 100% overturned of larval prawns was found with artificial seawater formula 2 at 39 days of age. The ratio between the re-used artificial seawater formula 2 and the unused artificial seawater formula 2 plus 15 ppt salinity level of T6 was the most appropriate treatment.

Keywords: Artificial seawater formulae 1 and 2, chitosan levels, Giant Freshwater Prawns, turnover of its postures, seawater formula

Thai J. Agric. Sci. (2017) Vol. 50(2): 66−75

INTRODUCTION

Macrobrachium rosenbergii is recognized as one of the most important species of prawns being cultured in many tropical inland freshwater reservoirs (New, 1995). This prawn species is commonly inhibited in most tropical zone especially in the Southeast Asian countries, e.g. India, Thailand, Vietnam, Malaysia, Singapore and others (Holthuis, 1950; Holthuis and Rosa, 1965). In Thailand, this prawn species is commonly distributed in freshwater reservoirs those connected to the sea and this prawn species is largely used for aquaculture production annually since its flesh possesses a high palatability. It is worldwide accepted among the consumers all over the globe hence some considerable amount of production had been produced for a high demand of markets both domestic and overseas. In Thailand, the Ministry of Agriculture and Cooperatives reported that in the 2010−2014 the production ranged from 19,400 to 27,500 tons with valuable incomes ranged from 3,626.80 to 4,858.00 million Baht (1 US Dollar = 35 Baht) and the production tends to increase from time to time (Anonymous, 2016). In nursing larval prawns, it needs some essential practices, e.g. at larval growing stage, it requires some salinity water of 12−15 ppt and it takes approximately 28−30 days for larval prawns to overturn their bodies in order to be ready for a rapid growth and development. Thus in nursing of larval prawns, it requires seawater of the mentioned salinity level. If the place to be used for culturing the prawns is located far away from the sea then the cost of investment could be increased. To avoid an increase in the amount of capital investment, artificial seawater may be of a suitable choice for use in order to reduce investment expenditure.

The use of some aiding chemical has been utilized by many workers in order to achieve better growth and development of aquatic bodies, e.g. chitosan chemical has been used in culturing fish and other aquatic bodies as to improve its annual production. Chitosan is a linear homopolymer {b-(1,4)-2-amino-2-deoxy-D-glucose} and it is prepared by the alkaline deacetylation of chitin derived from shrimp, lobster, crabs and other crustacean shells. Its contents included some natural biological properties such as immunological function and bacterio-static activity and these properties are valuable for both plant and animal applications (Rinaudo, 2006). Chitosan has a variety of application such as in medicine, agriculture and aquaculture (Niu *et al.*, 2011). In aquaculture, it was used as an immunostimulants to protect salmonids against bacterial disease (Anderson and Siwicki, 1994; Siwicki *et al.*, 1994) and also in Gilthead Seabream, *Sparus aurata* (Esteban *et al.*, 2000; Esteban *et al.*, 2001; Ortuno *et al.*, 2000; Cuesta *et al.*, 2003). Nevertheless, they stated that the effects of dietary chitin and chitosan on crustacean are not well justified (Niu *et al.*, 2011). Little attention has been paid to the nutritive status of chitosan on larval or post larval shrimp development. Therefore, the objective in carrying out this work lies on (1) an evaluation of the effect of different levels of supplementary chitosan, artificial seawater formulae 1 and 2 and (2) the re-used of artificial seawater in nursing larval prawns in relation to their turnover of its postures and the survival rate (%) of the larval prawns. The obtained data may be of tangible value for growers in Thailand and overseas.

MATERIALS AND METHODS

Sites and Experimental Design

The experiments were carried out at the Angthong Inland Fisheries Research and Development Center, Angthong Province (for Experiments 1.1, 1.2 and 3) and at the Inland Aquaculture Research Institute, Phra Nakhon Si Ayutthaya Province (for Experiment 2). These two Thai government research stations are located in the Central Plane Region of the country. The experiments were conducted during February 2012 to April 2016 to search for numbers of days in overturning of their body postures of the larval prawns and also survival rate (%) with respect to chitosan levels and artificial seawater formula 1 for Experiment 1.1 where five chitosan treatments

were used, i.e. 0, 100, 200, 300 and 400 ppm for T1 up to T5, respectively. For Experiment 1.2, the same rates of chitosan levels as that of the Expt. 1.1 together with seawater formula 2 were used and again there were five treatments altogether. The Experiment 2 consisted of the re-used seawater, artificial seawater formulae 1 and 2. Thus there were three treatments altogether. The Experiment 3 consisted of nine treatments on ratio (proportion) between the re-used artificial seawater formula 2 and unused artificial seawater formula 2 in relation to salinity levels of 9, 12 and 15 ppt. They consisted of nine treatments, i.e., T1 = 0:100 + 9 ppt., T2 = 0:100 + 12 ppt., T3 = 0:100 +15 ppt., T4 = 50:50 + 9 ppt., T5 = 50:50 + 12 ppt., T6 = 50:50 + 15 ppt., T7 = 25:75 + 9 ppt., T8 = 25:75 + 12 ppt. and T9 = 25:75 + 15 ppt. A Completely Randomized Design (CRD) with three replications was used for all of the three experiments. Formulation of artificial seawater was carried out with different amounts of chemicals of commercial grade. They were mixed together to form artificial seawater of Formulae 1 and 2 (Table 1). Chemical components being used included sodium chloride (NaCl), magnesium chloride ($MgCl_2.6H_2O$), calcium chloride ($CaCl_2.6H_2O$), potassium chloride (KCl), sodium bicarbonate ($NaHCO_3$), strontium chloride ($SrCl_2$), sodium sulfate ($Na_2SO_4.2H_2O$), magnesium sulfate ($MgSO_4.7H_2O$) and potassium bromide (KBr). The two formulae were used and they were modified from that of the work reported by Menllen and Lanier (1985). All chemicals being used were collected from the local shop in Khon Kaen province.

Table 1 Formulation of chemical components being used in artificial seawater Formulae 1 and 2 (g 100^{-1} liters)

Chemical components	Formula 1	Formula 2
Sodium Chloride (NaCl)	2,347.7	2,700
Magnesium Chloride $MgCl_2.6H_2O$)	498.1	200
Calcium Chloride ($CaCl_2.6H_2O$)	110.2	100
Potassium Chloride (KCl)	64.4	40
Sodium Bicarbonate ($NaHCO_3$)	19.2	20
Strontium Chloride ($SrCl_2$)	2.4	-
Sodium Sulfate ($Na_2SO_4.2H_2O$)	150	-
Magnesium Sulfate ($MgSO_4.7H_2O$)	-	300
Potassium Bromide (KBr)	1	-

Source: Modified from Menllen and Lanier (1985) * Commercial grade

Larval Prawn Culture Technique

Fertilized female brood stock with gray-black eggs obtained from the Angthong Inland Fisheries Research and Development Center, Angthong Province, Thailand were used. The gray-black eggs were placed in clean water for 2−3 hrs and then soaked in to a 50 ml L^{-1} of formalin for an hour. This level of formalin solution was used for the removal of parasites and pathogens from the prawns then they were transferred to hatch in a plastic container of 15 ppt seawater and allowed them to remain in the plastic box for one night. At the following day some large numbers of larval prawns were attained. A thousand larval prawns of one day old larvae were used for each replication of all of the three experiments and they were allowed to thrive on in plastic containers of 30-L with a dimension of 52 x 39 x 15

cm in length, width and height, respectively. This volume of plastic container was used for each replication. Artificial seawater and normal seawater were filtered through plankton net of 69 µm as to remove sediment and the filtered water were stock in fiberglass tanks and then added alum (potassium alum; $KAl(SO_4)_2.12H_2O$) 10 mg L^{-1} and 10 mg L^{-1} of Calcium hypochlorite [$Ca(OCl)_2$] to precipitate the metal ions of iron and an air pump was used continuously for 7 days. Additional amount of lime to the medium water was carried out as to attain a pH range from 8.0−8.5 and adjusted salinity levels to a range from 10−15 ppt. This was done for all of the three experiments. An approximately 2,500 L of used seawater of formulae 1 and 2 and used normal seawater were collected and they were stored in cement tanks for further uses.

Feeding of Larval Prawns and Water Management

The larval prawns were fed with Artemia Nauplii from the first day up to day 7 for three times daily (8, 10 am and 3 pm) and after 7 days of age, a diet of egg custard was given twice daily (11 am and 2 pm) along with a certain amount of Artemia Nauplii where the process was taken place at 4 pm daily and this was done continuously throughout the experimental period until the larval prawns overturned its body postures more completely (100%). Early in the morning, an amount 10% of water in all replicated plastic containers was drained out then replaced with the same amount daily. After 7 days of age, plastic containers of each replication were exchanged to the new ones at two days interval as to assure the utmost sanitation. Water samples of each replication were collected to the depth of 6 cm. This was carried out to determine adequate amount of dissolved oxygen and temperature was determined with the use of a DO meter (Lutron, Model WA-2015SD) and pH values were determined with the use of a pH meter (Lutron, Model WA-2017SD). Alkalinity levels, hardness, ammonia and nitrite were determined once a week using standard procedures (APHA, 1989). The percentages of survival rate were determined using a formula: survival % = final prawn number/1000 x 100. The attained data were calculated using a computer programme (SAS, 1998).

RESULTS

With Experiment 1.1, the results due to the seawater showed that numbers of days to reach 50% overturned of its body postures due to levels of chitosan ranged from 26.67 to 27 days for T2 and T4, respectively (Table 2). There was no statistical difference due to chitosan levels found. A similar trend was found with artificial seawater formula 1, i.e. there was no significant difference due to chitosan levels found. For seawater formula 1, numbers of days to reach 100% overturned of its body postures ranged from 44.33 to 44.67 days for T3 and T4, respectively. There was no statistical difference found due to chitosan levels. With artificial seawater formula 1, numbers of days to reach 100% overturned were similar to those of the seawater and again there was no statistical difference due to chitosan levels found. For survival rate (%), the results showed that seawater gave percentages ranged from 77.17 to 87.87% for T5 and T1, respectively and again there was no statistical difference found. A similar trend as that of seawater was also found with that of the artificial seawater formula 1, i.e. there was no statistical difference found among the treatments due to chitosan levels.

Table 2 Numbers of days to reach 50 and 100% overturned and survival rate (%) of giant freshwater prawn larval of Experiment 1.1, cultured under seawater and artificial seawater Formula 1 in relation to different levels of supplementary chitosan

Levels of Chitosan	50% overturned		100% overturned		Survival rate (%)	
	Seawater	Artificial seawater formula 1	Seawater	Artificial seawater formula 1	Seawater	Artificial seawater formula 1
T1, 0 ppm (control)	27.00	27.67	44.67	44.67	87.87	87.03
T2, 100 ppm	26.67	25.33	44.67	44.67	78.57	70.43
T3, 200 ppm	26.67	25.67	44.33	44.33	79.90	84.40
T4, 300 ppm	27.00	26.33	44.67	44.67	78.40	79.80
T5, 400 ppm	26.67	26.00	44.67	42.00	77.17	72.50
F-test	ns	ns	ns	ns	ns	ns

Letters in each column indicated least significant differences (LSD) of means of Duncan's Multiple Range Test (DMRT) at probability (P) of 0.05, ns: Non significant

For the results of the Experiment 1.2, it revealed that numbers of days to reach 50% overturned of its body postures due to seawater ranged from 26.67 to 27.67 days for T5 and T3, respectively (Table 3). There was no statistical difference found among the treatments due to chitosan levels. With artificial seawater formula 2, numbers of days to reach 50% overturned of its body postures were similar in all chitosan levels and again there was no statistical difference found among the treatments used. Numbers of days to reach 100% overturned of its body postures when cultured under seawater, it ranged from 40.67 to 43.00 days for T4 and T3, respectively. There was no statistical difference found among the treatments due to chitosan levels. A similar trend as that of seawater was found with artificial seawater formula 2, i.e. there was no statistical difference found among the treatments used although numbers of days were much higher than that of the 50% overturned where it ranged from 42.67 to 43.00 days for T5 and T1, respectively. With survival rate (%), the results showed that survival percentages ranged from 76.83 to 89.40 for T5 and T1, respectively. There was no statistical difference found among the treatments due to chitosan levels. However, the results on artificial seawater formula 2 showed that the percentage of T1 was highest followed by T2, T4 and least with T5 with numbers of days ranged from 74.57 to 86.90 for T5 and T1, respectively. The difference was large and statistical significant ($P < 0.05$).

Table 3 Numbers of days to reach 50 and 100% overturned and survival rate (%) of giant freshwater prawn larval of Experiment 1.2, cultured under seawater and artificial seawater Formula 2 in relation to different levels of supplementary chitosan

Treatments/Levels of Chitosan	50% overturned		100% overturned		Survival rate (%)	
	Seawater	Artificial seawater formula 2	Seawater	Artificial seawater formula 2	Seawater	Artificial seawater formula 2
T1, 0 ppm (control)	27.00	28.33	43.00	43.00	89.40	86.90[a]
T2, 100 ppm	26.67	29.33	42.67	43.00	81.90	84.97[a]
T3, 200 ppm	27.67	28.33	43.00	43.00	80.53	76.50[b]
T4, 300 ppm	27.00	28.33	40.67	43.00	77.37	77.30[b]
T5, 400 ppm	26.67	28.00	42.67	42.67	76.83	74.57[c]
F-test	ns	ns	ns	ns	ns	*

Letters in each column indicated least significant differences (LSD) of means of Duncan's Multiple Range Test (DMRT) at probability (P) of 0.05, ns: Non significant

With the results of the Experiment 2, it showed that numbers of days to reach 50% overturned its body postures ranged from 26.20 to 28.20 days for T2 and T3, respectively. There was no statistical difference found (Table 4). At 100% overturned, the results showed that T3 was the lowest and highest with T2 with values of 39.00 and 41.40 days, respectively. The difference was large and statistically significant ($P < 0.05$). For the survival rate (%), it revealed that the lowest survival % was found with T2 and highest with T1 with values of 57.96 and 61.74, respectively.

Table 4 Numbers of days to reach 50 and 100% overturned of its body postures and survival rate (%) of giant freshwater prawn larval of Experiment 2 as influenced by re-used seawater and re-used artificial seawater formulae 1 and 2

Treatments	Numbers of days to reach		Survival rate (%)
	50% overturned	100% overturned	
T1, re-used seawater (control)	26.80	40.20[ab]	61.74
T2, re-used artificial seawater Formula 1	26.20	41.40[a]	57.96
T3, re-used artificial seawater Formula 2	28.20	39.00[b]	59.32
F-test	ns	*	ns

Letters in each column indicated least significant differences (LSD) of means of Duncan's Multiple Range Test (DMRT) at probability (P) of 0.05, NS: Non significant

For the Experiment 3, numbers of days to reach 50% overturned of its postures were lowest with T5 and highest with T9 with values of 29.00 and 31.33 days, respectively (Table 5). There was no statistical difference found among the three treatments. At 100% overturned of its postures, it was found that T7 was the lowest and the highest was with T9 with values of 37.33 and 40.00 days, respectively. The difference was large and statistically significant ($P < 0.05$). With survival rate (%), the results showed that T4 was lowest and T6 was highest with values of 68.50 and 89.13%, respectively. The difference was large and statistically significant ($P < 0.05$).

Table 5 Numbers of days to reach 50 and 100 % overturned of its body postures and survival rate (%) of giant freshwater prawn larval of Experiment 3 as influenced by ratio between the re-used artificial seawater Formula 2 and the unused artificial seawater Formula 2 plus salinity levels

	Treatments/Ratio between re-used artificial seawater formula 2 and unused artificial seawater formula 2 + salinity levels	Numbers of days		Survival rate (%)
		50% overturned	100% overturned	
T1	0:100 (9 ppt)	30.33	40.00^{a}	81.47^{b}
T2	0:100 (12 ppt)	30.00	39.33^{a}	77.63^{b}
T3	0:100 (15 ppt, control)	30.00	40.00^{a}	86.23^{ab}
T4	50:50 (9 ppt)	29.00	37.67^{b}	68.50^{d}
T5	50:50 (12 ppt)	29.00	40.00^{a}	75.13^{cd}
T6	50:50 (15 ppt)	29.67	40.00^{a}	89.13^{a}
T7	25:75 (9 ppt)	27.33	37.33^{b}	76.43^{bc}
T8	25:75 (12 ppt)	29.33	37.67^{b}	76.43^{bc}
T9	25:75 (15 ppt)	31.33	40.00^{b}	77.67^{bc}
F-test		ns	*	*

Letters in each column indicated least significant differences (LSD) of means of Duncan's Multiple Range Test (DMRT) at probability (P) of 0.05, NS: Non significant

DISCUSSION

Nowadays, it seems more likely that the need to culture more aquatic animals has been increasing more rapidly with time, particularly the production of giant freshwater prawns. This may be attributable to a high demand for edible freshwater prawn production as to meet the demand of the markets both domestic and overseas hence the production of giant freshwater prawns (*Macrobrachium rosenbergii* de Man, 1879) gains more interest from scientists and growers. Thus different techniques in culturing this type of animal had been searching by a number of workers such as Fujimaru (1966), New and Valenti (2000), Damrongphol *et al.* (2001), Chung (2006), Niu *et al.* (2011), Harikrishnana *et al.* (2012) and others. It was found within this present work that the nursing of larval freshwater prawns with the use of seawater and artificial seawater and chitosan levels in relation to numbers of days to reach 50 and 100% overturned of its body postures, it was found that there was no significant difference

found due to treatments. This may be attributable to the low environmental temperature of the media being used to culture the larval prawns which was approximately 19°C. It has been advocated that the most profitable temperature for this type of prawns should range from 28 to 31°C (Hanson and Goodwin, 1977; New, 1988; Lee and Wickin, 1992; Arrignon *et al.*, 1994; New and Valenti, 2000). Thus it is not advisable to culture this type of prawns at low temperature. It must be stated that a low temperature as low as that of 19°C was unusual for the Central Plane region of Thailand. Thus the changing of the environmental temperatures was unpredictable. It depends perhaps on a circumstance of high pressure from northern region of the China. However, it was found that the percentages of survival rate was statistically significant when larval prawns were cultured under artificial seawater formula 2 but only with a chitosan level of 100 ppm. The results suggested that chitosan chemical could possibly have manifested its significant effect at a low rate only, since the percentages of the control treatment were similar to that of the first level of chitosan being used and perhaps the larval prawns were too young to consume the given chitosan levels.

When it comes to the effects due to both the re-used seawater and the artificial seawater of the two formulae, it was found that the lowest numbers of days to reach 100% overturned of the larval prawns were found with that of the artificial seawater formula 2 which was taken only 39 days of age whilst other treatments were higher. The results indicated that seawater formula 2 can be the most profitable medium in culturing the larval prawns since there was no statistical difference found among the three treatments on survival percentages. Nevertheless, earlier numbers of days in overturning its body postures of larval prawns was reported by Uno and Soo (1969) when they carried out the experiment in a laboratory.

With the effect due to ratio between the re-used artificial seawater formula 2 and the unused artificial seawater formula 2 plus salinity levels, the results showed that the fastest number of days to reach 100% overturned was found with that of T7, i.e. with the ratio of 25:75 plus 9 ppt salinity level, yet survival rate was relatively lower (76.43%) when compared with that of T6 (89.13%) hence T6 should be the most profitable treatment since survival rate (%) was much higher and statistically significant ($P < 0.05$). This may be attributable to the ratio between the re-used artificial seawater formula 2 and the unused artificial seawater formula 2 when the ratio being used was a 50:50 plus 15 ppt. The results also indicated that a salinity level of 15 ppt may be of the most suitable concentration of salinity level in nursing larval prawns. Nonetheless, it was found in the literature that a wide range of salinity levels of 10 up to 18 ppt was considered to be suitable for larval prawn culture (New and Valenti, 2000; Jayachandran, 2001). Thus it may be of important value to maintain salinity concentration within a range of 12−15 ppt as stated by Ling (1969) for freshwater prawn culture.

CONCLUSION

Environmental temperature within the culture media of 19°C was not suitable for growth and overturned of the body postures of the larval prawns. The most appropriate temperature range should be at a range of 28 to 31°C. The lowest numbers of days to reach 100% overturned of larval prawns was found with that of the artificial seawater formula 2 (39 days). This artificial seawater formula 2 can be the most profitable formula in culturing larval prawns. The best ratio or proportion between the re-used artificial seawater formula 2 and the unused artificial seawater formula 2 plus salinity level was found with T6, i.e. a ratio of 50:50 plus 15 ppt.

ACKNOWLEDGEMENTS

The authors wish to thanks the Angthong Inland Fisheries Research and Development Center, Angthong Province and the Inland Aquaculture Research Institute, Phra Nakhon Si Ayutthaya Province, Thailand for research facilities provided. Thanks are also due to Graduate Schools of Khon Kaen University for financial assistance and staff members of the Faculty of Agriculture, Khon Kaen University for their kind assistance.

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