World Applied Sciences Journal 18 (3): 379-384, 2012
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2012.18.03.62156

Effects of Stocking Density on the Survival, Growth and Development Rate of Early Stages Blue Swimming Crab, *Portunus pelagicus* (Linnaeus, 1758) Larvae

[1]M. Ikhwanuddin, [1,3]A.D. Talpur, [1]M.N. Azra,
[2]B. Mohd Azlie, [2]Y.S. Hii and [2]A.B. Abol-Munafi

[1]Institute of Tropical Aquaculture, Universiti Malaysia Terengganu,
Kuala Terengganu, Terengganu, Malaysia
[2]Faculty of Fisheries and Aqua-Industries, Universiti Malaysia Terengganu,
Kuala Terengganu, Terengganu, Malaysia
[3]Department of Fisheries, Government of Sindh, Pakistan

Abstract: The study was aimed to determine the effects of high stocking densities on the larval survival, growth and development rate of blue swimming crab, *Portunus pelagicus*. The study was carried out with four different stocking densities of 50, 200, 300 and 400 larvae L^{-1} in five liters aquaria filled with disinfected seawater and control against each 50 larvae L^{-1}. The results shows that the 200 larvae L^{-1} treatment did produce highest survival over the others and was significantly different ($p<0.05$). However, lowest survival was achieved 50 larvae L^{-1}. The mean mortality was highest during Zoea 1 (Z1) to Zoea 2 stages (Z2) and lowest in Zoea 4 (Z4) stage to Megalopa stage. Higher development was achieved in low density trials. It can be concluded that the high stocking density affected the survival rate, growth and development of larvae.

Key words: Megalopa · Mortality · *Portunus pelagicus* · Stocking Density · Zoea

INTRODUCTION

Crabs cultures are not distributed widely, still considered as a secondary species to shrimp and finfish [1, 2]. However, blue swimming crab, *Portunus pelagicus* culture gained its important from the beginning of last decade owing to great demand of live crabs and crab products in the export market [3]. Currently, *P. pelagicus* are cultured for the production of the lucrative soft-shell crab market on recirculating and lined pond systems in Australia [4, 5]. However, in most of the countries to date, hatchery seed production of *P. pelagicus* has been experimental and commercial seed production technology has not been developed so far.

P. pelagicus is one of important Malaysian marine species [6] that played vital role in domestic consumption but also for recreation fisheries. In Malaysia, the landings of *P. pelagicus* was 3514 tons in 2007 and increased to 4427 tons in 2008 but it was decreased to 3057 tons in 2009 [7]. The decline in nature stock of *P. pelagicus* due to over-exploitation has an impact on natural ecology particularly in Asian countries [8]. *P. pelagicus* is widely liked in Southeast Asia particularly in Singapore, Malaysia, Hong Kong, Thailand, Vietnam and other parts of the world India, South Korea, Taiwan, Japan and U.S.A etc. Presently, the crab culture operations mainly depend on juvenile collected from the wild, which are vary in size, age with the seasons [9]. Development of the seed production technology for *P. pelagicus* is needed to reduce the pressure on the wild crab seed resources to ensure environment sustainability and establishment of crab farming on commercial scale.

The stocking density is considered one of the most important factors that affect the survival rate and specific growth rate of the red King crab, *Paralithodes camtschaticus* [10] and *Labeo rohita* [11]. The significant of this study was to determine the effects of high stocking densities of crab larvae on survival rate, specific growth rate and growth development and to make the effort to establish the seed production technology for commercial seed production of *P. pelagicus* larvae.

Corresponding Author: M. Ikhwanuddin, Institute of Tropical Aquaculture, Universitiy Malaysia Terengganu, 21030, Kuala Terengganu, Malaysia. Tel: +609-6683638, Fax: +609-6683390.

MATERIALS AND METHODS

Seawater for Broodstock and Larviculture: Seawater for crab culture was treated according to talpur *et al.* [12]. Uv treated seawater was filtered through a 10 µm net and then disinfected with active chlorine for 24 h. This procedure, which eliminated almost all naturally occurring bacteria, treated water was supplemented EDTA (after 24 h of chlorine treatment) to settle down the heavy metals was followed by neutralization with sodium thiosulphate (same concentration of chlorine) at the beginning of the experiment. The culture water exchange began from the day second, using disinfected seawater.

Water Parameter: The water parameters for broodstock and larviculture were maintained such as salinity 30-35 ppt, pH 7.5-8.0, temperature 30°C and dissolved oxygen > 5 mg L^{-1} during the trials. All the water parameters were measured daily on site with YSI 556 Multi-probe (USA).

Brood Stock Maintenance and Hatching: Berried females were collected from Strait of Tebrau, Johor, Malaysia, (1° 22' N and 103° 38' E) and were transported to marine hatchery of Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Malaysia. Berried female was disinfected according to Talpur *et al.* [12, 13] and one berried crab was placed per (300 L) capacity hatching tank with 3 cm substrate of thick sand tray with adequate aeration and 100% water exchanged daily. Hatching tank filled with disinfected water and was equipped with heater in order to maintain the temperature constant at 30°C. Berried females were not fed until hatch. The berried crabs were monitored daily for hatching and on spawning the water in incubation tank reduced to 50 L. After hatching, the larvae were transferred to 15 L plastic aquarium filled with disinfected water. Energetically moving larvae were collected and used for stocking in rearing tanks.

Experimental Design: 7 L capacity plastic aquaria were filled with 5 L of disinfected seawater. Larvae were stocked at density of 50, 200, 300 and 400 larvae L^{-1} with 50 larvae L^{-1} against each as the control. Each experiment had three replicates. The treatment was conducted in a water bath and the water bath half filled with tap water and two water heaters (50 Watt) were provided to maintain the constant temperature (30°C). The larviculture started on first day after hatching until the Megalopa stage. The sampling of larvae was done on day 1, day 4, day 7, day 10 and day 13. The experiment was stopped immediately when the larvae reached to Megalopa stage.

All the larval stages were fed according to Hassan *et al.* [14] with rotifers, *Branchionus* sp. and *Artemia* sp. nauplii. The larvae fed with rotifer at the rate of 30 individual mL^{-1} and *Nannochlropsis* sp. ($1\text{-}10\times10^5$ cell mL^{-1}) to Zoea 1 to Zoea 4 stages. The addition of rotifers was stopped once the zoea reached megalopa stage. *Artemia* sp. nauplii at the rate of 20 individual mL^{-1} were provided from Zoea 4 until Megalopa stage. Each tank was supplied with gentle aeration. A volume of 10-20% of water was changed daily from Zoea 1 to Zoea 4 stages and 50% water changed daily for Megalopa stage. To remove left over feed, detritus and dead larvae each day, the aeration was stopped temporarily and settled particles were removed from tank bottom by siphoning. 12 h light and 12 h dark photoperiod was maintained during the trials. Soon after appearance of first crab stage they were transferred to new rearing tanks.

Larval Stage Determination: The larval stage was determined using dissecting microscope at 10 magnifications to ensure that the larvae reached the stage in the time period. The larval stage were differentiated by identify its telson, eye-stalked, the abdomen segmented, spine and setae. Larval morphology used in the present study as described by Arshad *et al*. [15].

Data Collection: Sampling of 12 larvae from each treatment including control (3 larvae per replicate) were collected for each crab larvae stage on day 1, 4, 7, 10 and day 13 to determine growth and development rates. The larval growth rate was calculated as Specific Growth Rate (SGR). The SGR was measured in every larval stage with the following formula:

$$\text{SGR (\%)} = \frac{\text{Final body weight (mg)} - \text{Initial body weight (mg)}}{\text{Culture period (day)}} \times 100\%$$

The larvae were weighed using microbalance to measure the initial and final body weight (BW) and the mean BW increment was calculated within the culture period (13 days).

The larval development rate was calculated using Larval Stage Index (LSI). To calculate the LSI, the larval stages were index as Z1=1, Z2=2, Z3=3, Z4=4 and megalopa=5. The LSI was determined through following formula:

$$\text{LSI} = \frac{[(\text{Zoea later stage} \times \text{Later stage Zoea No.}) + (\text{Zoea earlier stage} \times \text{Earlier stage Zoea No.})]}{\text{Total number of larvae in sample}}$$

Note: For Zoea later stage means next stage Zoea for example Z2, Later Zoea stage No. means number in sample and earlier stage Zoea means Zoea before later stage for example Z1 and so on. If 7 larvae were sampled, where Z2 were 4 in sample and Z1 were 3 then it would be (2x4) + (1x3)/7. This can be used as Z3 later stage Zoea and Z2 as earlier stage Zoea and so on.

The survival rate from the sampling was determined by counting the mortality of the larvae daily using the following formula:

$$\text{Survival rate Day x (\%)} = \frac{\text{Survival on day 1} - \text{Survival on day } x}{\text{Survival on day 1}} \text{ x } 100$$

At the end of the experiment percent survival of larvae was calculated using the formula by Talpur *et al.* [16]:

$$\text{Survival rate (\%)} = \frac{\text{Total number of Survived larvae}}{\text{Initial number of Stocked larvae}} \text{ x } 100$$

Data Analysis: The data was analyzed using SPSS version 16.0 software for Windows. One-way ANOVA was used to determine whether the significant level of $p< 0.05$ between treatments, while Tukey's test was used for multiple comparisons between the different treatments.

RESULTS

Survival Rate: The mean survival rate of larvae for all treatments observed decreasing every day until day 13. The highest mean survival rate was achieved in treatment 200 larvae L^{-1}, 6.8% followed by 4.9% in treatment 300 larvae L^{-1} and 4.2% in treatment 400 larvae L^{-1} and lowest survival was seen in treatment 50 larvae L^{-1}, 2.7%.

The results shows that survival in treatment 200 larvae L^{-1} treatment and control was significantly different ($p<0.05$) and rest of treatments were not significantly different ($p>0.05$).

The highest mean mortality was observed in treatments on day 4 during the moulting transition from Zoea 1 to Zoea 2 stages and lowest was seen on day 7 during the moulting transition from Zoea 4 stage to Megalopa stage Table 1.

The result indicates that the highest mortality rate (previous sub stage to the next sub stage (Z1-Z2)) was observed in treatment 50 larvae L^{-1} and the lowest in 200 larvae L^{-1} treatment. Mortality among the treatment was not significant ($p>0.05$) Figure 1.

Table 1: Mean mortality rate (%) from the previous sub stage to the next sub stage

Larval stages	Mean mortality rate (%)
Zoea 1-Zoea 2	32.51±9.88
Zoea 2-Zoea 3	22.87±6.90
Zoea 3-Zoea 4	19.43±1.82
Zoea 4-Megalopa	18.88±2.40

Table 2: Mean body weight (BW) and mean Specific Growth Rate (SGR) of crab larvae

Stocking density (larvae L^{-1})	Final mean BW on day 13 (mg)	Mean SGR (%)
50	1.67±0.17[a]	12.58±0.91[a]
200	0.98±0.03[b]	7.27±0.91[b]
300	0.96±0.13[c]	7.14±0.84[c]
400	0.90±0.09[d]	6.68±0.78[d]

Note: Values showing different letters in columns are not statistically significant ($p>0.05$).

Specific Growth Rate: The highest larval mean body weight (BW) and SGR was observed in 50 larvae L^{-1} treatment followed by 200 larvae L^{-1}, 300 larvae L^{-1} and lowest in 400 larvae L^{-1} treatments. All treatments did not show the significant different ($p>0.05$) Table 2.

The 50 larvae L^{-1} treatment showed the higher SGR for Z1-Z2, Z2-Z3 and Z4-M, however, 200 larvae L^{-1} did show better SGR in Z3-Z4. Moreover, higher densities 300 larvae L^{-1} and 400 larvae L^{-1} did demonstrate lower SGR compared to others.

Larval Development: The results demonstrates that in all the treatment zoea developed to Zoea 2 on day 4, Zoea 3 on day 7, Zoea 4 on day 10 and Megalopa stages on day 13 but in 400 larvae L^{-1} not all population turn to Megalopa on day 13 (Table 3). Larval development between treated groups was not significant ($p>0.05$). In different treatments, zoea showed different LSI, details are shown in Table 3.

Water Parameter: The mean water quality parameters recorded throughout the study. Temperature ranged between 29.7-30.4°C, salinity 34.1-34.4 ppt, pH 6.4-8.1 and dissolved oxygen (DO) 5.5±0.3 mg L^{-1} Table 4.

DISCUSSION

Present study shows the prospect of stocking densities over a 13 days culture period and their subsequent effects on early stages larval survival, growth and development. In the present study higher survival was achieved in treatment 200 larvae L^{-1} followed by

Table 3: Mean Larval Stage Index (LSI) of *P. pelagicus* throughout the larvae stages at different stocking densities

	50 larvae L^{-1}		200 larvae L^{-1}		300 larvae L^{-1}		400 larvae L^{-1}	
Day	LSI	Larval stage	LSI	Larval stage	LSI	Larval stage	LSI	Larval stage
1	1.0	Zoea 1	1.0	Zoea 1	1.0	Zoea 1	1.0	Zoea 1
4	1.6	Zoea 2	1.6	Zoea 2	1.6	Zoea 2	1.5	Zoea 2
7	2.8	Zoea 3	2.7	Zoea 3	2.6	Zoea 3	2.5	Zoea 3
10	3.7	Zoea 4	3.7	Zoea 4	3.6	Zoea 4	3.5	Zoea 4
13	4.9	Megalopa	4.6	Megalopa	4.6	Megalopa	4.4	Megalopa and Zoea 4

Note: Zoea 1, LSI=1.0; Zoea 2, LSI=2.0; Zoea 3, LSI=3.0; Zoea 4, LSI 4; Megalopa, LSI=5.0

Table 4: The mean daily water parameters readings throughout the study period

Water Parameter	Temperature (°C)	Salinity (ppt)	pH	Dissolved Oxygen (mg/L)
Mean	29.6	34.1	7.4	5.5
Max.	30.4	34.4	8.1	6.8
Min.	29.7	32.4	6.4	4.7
SD	0.3	0.3	0.2	0.3
N	12.0	12.0	12.0	12.0

SD= standard deviation, N= number of replicates

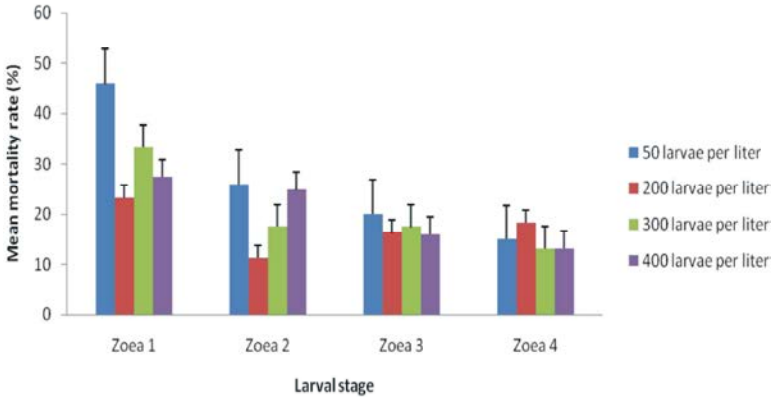

Fig. 1: The mean mortality rate from the previous sub stage to the next sub stage during the larvae stage from different stocking densities

300 larvae L^{-1} and 400 larvae L^{-1} however, the lowest survival was determined in treatment 50 larvae L^{-1}. According to Trino *et al*. [17], the survival rate of mud crabs, *S. serrata* significantly increased with lower stocking density. However, in present study, the larval survival was better in 200 larvae L^{-1}, even though the survival was not much higher compared to stocked density but in all groups, it reached at higher mark. Survival in 300 larvae L^{-1} and 400 larvae L^{-1} treatments was lower because of high number of cannibalism, competition of food and space. Cannibalism affects survival and appears to be partly dependent on stocking density [18]. The survival rate of 50 larvae L^{-1} was recorded the lowest compared to other treatments by unknown reasons.

Hai and Nhut [19] in their study stated that rearing density of 100 larvae L^{-1} of *P. pelagicus* gave the best results. It can be explained that the results of present study are in match of previously research and the higher density can affect the survival of larvae. Therefore, 200 larvae mL^{-1} did afford best survival rate among all treatments. The survival in treatment 200 larvae L^{-1} treatments and control was significantly different ($p<0.05$) however, in rest of treatments it was not significantly different ($p>0.05$). The mortality of larvae in first stage to megalopa is very critical. Maximum survival was reported in Zoea 1 (Z1) and minimum was in Megalopa [20]. The survival rate decreases with increasing larval stage and, larval duration increases with increasing larval stage [20]. In the present study the highest mortality was observed

during the moulting transition from Zoea 1 (Z1) to Zoea 2 (Z2) stages while the lowest was seen in the moulting transition from Zoea 4 (Z4) stage to Megalopa stage. It can be explained that earlier moulting in early stages Zoea is one of the factors of mortality and low survival of larvae owing to moulting death. Next Zoea stage (Z-3-Z4) become healthier and resulted in low mortality. The present study's results are similar as described by Soundarapandian *et al*. [20].

Study by Daly *et al.* [10] reported that low to intermediate stocking densities provides good survival and growth. Heasman and Fielder [21] reported that the *Scylla* spp. larvae stocking rates within the wide range of 10-150 larvae L^{-1} do not appear to have a critical effect on larval growth. *S. serrata* growth rates in different stocking densities were not significantly different [17, 22]. The highest SGR was observed in 50 larvae L^{-1}, followed by 200 larvae L^{-1}, lowering with the increase in stocking density such as in 300 larvae L^{-1} and 400 larvae L^{-1} treatment. All treatments did not show the significant different ($p>0.05$). It can be reported that growth rate was directly proportioned with the stocking density. Higher densities directly affected the growth rate and resulted in low growth. This might be happened probably due to plenty of food and enough space.

Soundarapandian *et al*. [20] reported that the optimum water parameters for the larval rearing of *P. pelagicus* were pH 7.5-8.0, temperature 28-31°C, DO 5.0-6.0 mgL^{-1} and the water salinity 33-35 ppt. In the present study water parameters such as temperature, salinity, pH and dissolved oxygen (DO) were in accordance to Soundarapandian *et al*. [20]. It is necessary the important water parameters should be maintained within desired range, any fluctuation could led to higher mortality among larvae. Ikhwanuddin *et al*. [9] found out that the treatment with stocking density 50 larval L^{-1} of *P. pelagicus* reached to Zoea 2 on day 4, Zoea 3 on day 7, Zoea 4 on day 10 and Megalopa stage on day 13. The results of present study demonstrates that larvae developed to Zoea 2 on day 4, Zoea 3 on day 7, Zoea 4 on day 10 and Megalopa stages on day 13 but in 400 larvae L^{-1} not all population turn to megalopa on day 13. It can be illustrated that high density particularly 400 larvae L^{-1} affected the development of larvae and not all Zoea were turned to megalopa this was owing to food competition and space requirement. The results of Arshad *et al*. [15] stated that the duration of each of the first two zoeal stages were 3-4 days, the following two stages 2-3 days and the megalopa 3-4 days with stocking density of 30 larvae L^{-1}. The growth development of larvae obtained during the present study was similar to Ikhwanuddin *et al*. [9] except for 400 larval per liter treatment that all population did not reach the Megalopa stage on day 13 because of high stocking density, competition of food and space. The controls and treated 50 larvae L^{-1} showed very fast growth development compared to other treatments, which caused by more space and enough food.

It can be concluded that higher/lower stocking density were affect the survival, growth and development of *P. pelagicus* larvae. Higher densities other than 200 larvae L^{-1} are not suitable for culture conditions.

ACKNOWLEDGEMENTS

This study was financially supported by (Science Fund), Ministry of Science, Technology and Innovation (MOSTI), Government of Malaysia under grant Vot. No 52042. Researchers also express the sincere thanks to the staff of Institute of Tropical Aquaculture (AKUATROP), Universiti, Malaysia Terengganu for their help.

REFERENCES

1. Ikhwanuddin, M., M.N. Azra, A.D. Talpur, A.B. Abol-Munafi and M.L. Shabdin, 2012. Optimal water temperature and salinity for production of blue swimming crab, *Portunus pelagicus* 1st Day Juvenile crab. Aquaculture, Aquarium, Conservation and Legislation, 5(1): 4-8.
2. Ikhwanuddin, M., M.N. Azra, Y.S. Yeong, A.B. Abol-Munafi and M.L. Shabdin, 2012. Live foods for Juveniles' production of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1766). Journal of Fisheries and Aquatic Science, 7(4): 266-278.
3. Josileen, J. and N.G. Menon, 2005. Growth of the blue swimmer crab, *Portunus pelagicus*, in captivity, Crustaceana, 78: 1-18.
4. O'Neill, H., 2003. Do the soft shell shuffle, The Weekend Australia Magazine, 34: 5-6.
5. Walker, T., 2006. Coral coast Mariculture claws a market for softshell, Austrasia Aquaculture, pp: 3-5.
6. Ikhwanuddin, M., T. Nor Adila, M.N. Azra, Y.S. Hii, A.D. Talpur and A.B. Abol-Munafi, 2011. Determination of live prey ingestion capability of blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) Larvae. World Journal of Fish and Marine Sciences, 3(6): 570-575.

7. Department of Fisheries Malaysia, 2010. Malaysia Fisheries statistics.
8. Ikhwanuddin, M., M.L. Shabdin and A.B. Abol-Munafi, 2009. Catch information of blue swimming crab (*Portunus pelagicus*) from Sarawak coastal water of South China Sea. Journal of Sustainability Science and Management, 4(1): 93-103.
9. Ikhwanuddin, M., M.L. Shabidin and B. Khairulhayadi, 2005. The development of seed production technology of blue swimming crab, *Portunus pelagicus* in Sarawak. Proceeding of the Tropical Fish Aquaculture Conference, Kuala Terengganu, pp: 1-21.
10. Daly, B., J.S. Swingle and G.L. Eckert, 2009. Effects of diet, stocking density and substrate on survival and growth of hatchery-cultured Red King Crab (*Paralithodes camtschaticus*) Juveniles in Alaska, USA, pp: 68-73.
11. Kabir, K.A., R.L. Baby, I. Hasan, N. Naser and S. Ali, 2010. Observations on Larval Growth and Survival of *Labeo rohita* in Response to Different Diets and Stocking Densities. World Journal of Fish and Marine Sciences, 2(1): 37-39.
12. Talpur, A.D., A.J. Memon, M.I. Khan, M. Ikhwanuddin, M. Danish Daniel and A.B. Abol-Munafi, 2011. Supplementation of indigenous Lactobacillus bacteria in live prey and as water additive to larviculture of *Portunus pelagicus* (Linnaeus, 1758), Advance Journal of Food Science and Technology, 3(5): 390-398.
13. Talpur, A.D., A.J. Memon, M.I. Khan, M. Ikhwanuddin, M. Danish Daniel and A.B. Abol-Munafi, 2011. A novel of gut pathogenic bacteria of blue swimming crab *Portunus pelagicus* (linneaus, 1758) and pathogenicity of *Vibrio harveyi*-A transmission agent in larval culture under hatchery conditions, Research Jounal of Applied Sciences, 6(2): 116-127.
14. Hassan, A., T.N. Hai, A. Chatterji and M. Sukumaran, 2011. Preliminary Study on the Feeding Regime of Laboratory Reared Mud Crab Larva, *Scylla serrata* (Forsskal, 1775). World Applied Sciences Journal 14(11): 1651-1654.
15. Arshad, A., Efrizal, M.S. Kamarudin and C.R. Saad, 2006. Study of fecundity, embryology and larval development of blue swimming crab, *Portunus pelagicus* (Linneus, 1758) under laboratory conditions. Research Journal of Fisheries and Hydrobiology, 1(1): 35-44.
16. Talpur, A.D., A.J. Memon, M.I. Khan, M. Ikhwanuddin, M.M. Danish Daniel and A.B. Abol-Munafi, 2012. Effects of *Lactobacillus* Probiotics on the Enhancement of Larval Survival of *Portunus pelagicus* (Linnaeus, 1758) Fed Via Bioencapsulated in Live Feed. World Journal of Fish and Marine Sciences, 4(1): 42-49.
17. Trino, A.P., O.M. Millamena and C. Keenan, 1998. Commercial evaluation of monosex pond culture of the mud crab Scylla species at three stocking densities in the Philippines, pp: 109-118.
18. Baliao, D.D., E.M. Rodriguez and D.D. Gerochi, 1981. Culture of the mud crab *Scylla serrata* (Forskal) at different stocking densities in brackish water ponds. SEAFDEC/AQD Q. Res. Rep., 5: 10-14.
19. Hai, T.N. and T.M. Nhut, 2008. Effects of rearing densities, Artemia densities and substrates on the growth and survival rates of blue swimming crab (*Portunus pelagicus*) larvae. Scientific Journal of Can Tho University, (2): 124-132. Specific Issue on Aquaculture and Fisheries.
20. Soundapandian, P., E. Thamizhazhagan and N.J. Samuel, 2007. Seed production of commercially important blue swimming crab.CAS in Marine Biology, Annamalai University, pp: 302-309.
21. Heasman, M.P. and D.R. Fielder, 1983. Laboratory spawning and mass rearing of the Mangrove crab, *Scylla serrata* (Forskal), from first zoea to the first crab stage. Aquaculture, 34: 303-316.
22. Le Vay, L., V.N. Ut, T.T. Nghia and T.T. Hong Hanh, 2007. Development of nursery culture techniques for the mud crab *Scylla paramamosain* (Estampador) Scientific Journal of Can Tho University, Aquaculture and Fisheries, pp: 1563-1568.