Growth, Development and Survival Rate of The Blue Swimming Crab (*Portunus pelagicus*) Cultured using Different Larvae Feeds

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Abstract

Blue swimming crab (Portunus pelagicus) is one of the most important commodities of softshell industry. Besides mud crab its demand as feed is increasing day by day. As a coastal dominant country, Bangladesh has a great feasibility of this species in culture, production, use and export. However, the production contribution from Bangladesh is still from natural sources and far behind in terms of farming, culture and production. Optimization of larval rearing techniques is therefore important to develop intensive hatchery rearing technique for this species. So, this study is aimed to develop larvae production technique of Portunus pelagicus with a better survival rate. Newly hatched first zoeal of Portunus pelagicus were reared using three different diets till second zoeal stage: (Treatment 1= Artemia franciscana umbrella + Rotifer (Brachionus rotundiformis); Treatment 2= Rotifer (B. rotundiformis) and Treatment 3= Artemia franciscana umbrella). Then, Artemia franciscana nauplii were used for all three treatments till they metamorphosed to crab instar. Regularly, water quality parameters were monitored and maintained and the survival and molting to next stages was observed. The result showed that, all the hatched zoea of P. pelagicus could successfully turns into crab instar stage under all the treatment applied, but the highest survival rate 6.08% was found in case of treatment 2 followed by 0.58%, 0.91%, respectively in treatment 1 and 3. In terms of metamorphosis, it took more than 15 and 14 days respectively from zoea 1 to metamorphose into megalopa for treatments 1 and 3, whereas metamorphosis from zoea 1 to megalopa in treatment 2 finished at the 12th day with a higher Larval Stage Index (LSI) value which was 4.9. Finally, at the 15th day larvae were metamorphosed into crablet for treatment 2 and 18 and 16 days required for metamorphosis into crablets for treatment 1 and 3, respectively. However, the larval feed showed a significant effect on growth and survival of P. pelagicus larvae, whereas the earlier larval stage of P. pelagicus rearing with Rotifer (B. rotundiformis) is found as the most suitable diet.

Keywords: *Blue swimming crab, larval rearing, feeding trial, survival rate*

Introduction

Crabs can be alternative to shrimp industries as shrimp is more prone to diseases (Salam *et al*., 2012). Sixteen shrimp diseases worldwide and eleven shrimp diseases have been reported from Bangladesh (Lee *et al*., 2022). The introduction of Vannamei shrimp in Bangladesh may also cause some other diseases to spread (Paul and Vogl, 2011). There are fewer diseases of crabs reported in Bangladesh (Hasanuzzaman *et al*., 2021). Though crabs (specially mud crab) are the most commonly cultured species in south-east Asian countries, the price of crabs (mud crab, blue swimming crab, Japanese spider crab, stone crab etc.) are in the hike in international markets, according to seafoodnews.com, the price of blue swimming crab increased by more than 10 dollars in a couple of months during the beginning of 2021, which is more than 34% more than some previous years. The increased demand of blue swimming crab in the international markets is well known to all. However, the larval rearing, culture and soft-shell production of this valuable species is yet to be commenced in Bangladesh.

Blue swimming crabs (*Portunus pelagicus*) easily spawn in captivity and have four zoeal stages (Z_1, Z_2, Z_3, Z_4), and one megalopa (M) stage (Shinkarenko, 1979; Arshad *et al*., 2006) and it takes only two weeks to develop into first crab stage (Romano and Zeng, 2008). Live feed is an important aspect for larval rearing, especially the marine larvae (Islam *et al*., 2022). Many types of research like effects of temperature, salinity (Ravi and Manisseri, 2012), stocking density (Ikhwanuddin *et al*., 2012),

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photoperiod (Andrés *et al*., 2010; Ravi and Manisseri, 2013), feed and feeding type (Ikhwanuddin *et al*., 2012) on the survival and development of larvae of blue swimming crab were being conducted in different countries but little known about this in Bangladesh. Bangladesh solely depends on the commercial catch of blue swimming crab which is much more common phenomenon for different countries (Otto and Jamieson, 2003), but the fast growth rate of this species in culture (Josileen and Menon, 2005) left everyone to think twice about its potentiality to be commercialized. The increased demand for this species has led to larval rearing for better production (Romano and Zeng, 2006).

This study is executed to develop larval rearing technique of blue swimming crab by using different feed and to determine the survival rate of the larvae for better seed production, ultimately leading better culture of this species and income generation.

Materials and methods

Brood-stock rearing

Local fishermen collected broods by locally known "Ber Jal" which was finally brought to the hatchery of Marine Fisheries and Technology Station (MFTS), Bangladesh Fisheries Research Institute (BFRI), Cox's Bazar, Bangladesh with maintaining standard operating protocol. All the broods were then weighed, and carapace length was measured. Broods were disinfected by formalin (100µlL^{-1}) for 6h and then transferred to a 500 L fiber tank. One brood was kept in one tank where temperature was maintained 28-30 ºC and salinity within 28-30 ppt. Gravid broods were fed daily with fresh bi-valve muscle and the unfed leftover muscles cleaned out on daily basis. Barried crabs were transferred to 100 L plastic bucket for smooth collection of zoea-1 of crab after hatching. Barried broods were reared in the plastic bucket and the water in the plastic bucket was exchanged daily until the crab spawned.

Experimental design

Larvae were transferred to 500 L circular concrete tank filled with treated 480 L seawater for larval rearing. About 30000 larvae were stocked in each concrete tank for the experiment. Larvae were fed four times a day from 9 am - 6 pm in 3 h intervals. A single factor experimental design was being used to determine the survival rate, growth and development of larvae of *P. pelagicus*. Three different feeding regimes were used: T_1= Use of both *A. franciscana* umbrella and *Rotifer (B. rotundiformis)* in Z_1-Z_2 and then shift to only *A. franciscana* nauplii from Z_3-Z_4 stage, T_2 = Use of only *Rotifer (B. rotundiformis)* whole day in Z_1-Z_2, T_3= Use of only *Artemia franciscana* umbrella whole day in Z_1-Z_2. From Z_3-Z_4 stage *Artemia franciscana* nauplii was being used for every treatment. From megalopa stage *Artemia franciscana* nauplii was replaced by shrimp egg custard. Temperature was maintained at 28-30ºC, salinity 28-30 ppt, ammonia <0.5 mgL^{-1} and photoperiod 12L:12D. Zoeal stage and developmental time was determined according to Josileen and Menon (2008) by using electronic light microscope (Leica DM1000).

Feeding of Rotifer (B. rotundiformis) and Artemia franciscana

Rotifer (*B. rotundiformis*) ranging from 100-150µm in size and *Artemia franciscana* (Vinh Chau-Bac Lieu) umbrella ranging from 400-500 µm are provided daily in the treatment tanks with a density of 15indv.mL^{-1} and 1 indv.2 mL^{-1} in the larval rearing tank respectively. Before next feeding the *Rotifer (B. rotundiformis)* and *Artemia franciscana* residue was counted. The larval feed regime in different treatments is shown in Table 1.

Data collection

Sampling of nine larval tanks for three treatments was collected for each crab larval stage to determine survival rate, development rates and developmental time. The larval survival rate was calculated by incorporating following formula:

$$\text{Survival rate} = \frac{\text{Total number of Survived larvae}}{\text{Initial number of Stocked larvae}} \times 100$$

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 (Ikhwanuddin *et al*., 2012a):

$$\text{LSI} = \frac{(Zoea\ Later\ Stage \times Later\ Stage\ Zoea\ No.) + (Zoea\ Earlier\ Stage \times Earlier\ Stage\ Zoea\ No.)}{Total\ Number\ of\ Larvae\ in\ Sample}$$

Water quality monitoring

Five parameters, DO, pH, temperature, salinity, and ammonia, were measured on three days intervals using HANNA HI-98194 multi-parameter.

Statistical analysis

Mean and standard error of mean for all the parameters were calculated using Microsoft excel (Version 2016). Post hoc test was performed using IBM SPSS (v.26.0), when the assumptions were met to determine the significance difference among the treatments.

Results and Discussion

Survival rate

Better survival rate was observed in all four (4) larval stages including megalopa and crablets stages for treatment 2 (Figure 1.). Larval density was decreased frequently in all three treatments. Survival rate of crablets were found maximum in case of treatments 2 (6.08 %), where 0.58% and 0.91% was observed in treatment 1 and 3, respectively. In the zoea 4 to megalopa stage drastic mortality occurred for all three treatments. Survival rates were found more or less similar in case of treatment 1 and 3 at Zoea 3, Megalopa and Crablet stages. However, the treatment 2 showed a better survival rate among all the treatments and a significance difference (P< 0.05) for all the stages among the treatments.

Larval Stage Index (LSI) of P. pelagicus

Mean larval stage is calculated for all the three treatments until megalopal stage and better metamorphosis was observed in treatment 2 (Table 2.) and in the 13th day 95% of the larvae were metamorphosed into megalopa. In treatment 1and 3 the metamorphosis was delayed and was in transition for all the stages.

Rotifer (B. rotundiformis) and Artemia franciscana intake

In the treatment 1 and 2 *Rotifer (B. rotundiformis)* was used (Figure 2.) in the Zoea 1-2 stage where most of the *Rotifer (B. rotundiformis)* was taken up by the larvae. Few *Rotifers (B. rotundiformis)* were left in the treatment 1 as *Artemia franciscana* was also provided in the larval rearing tank and few *Artemia franciscana* was also ingested as well in the treatment 1. In the treatment 3 only *Artemia franciscana* was used from very beginning of the larval rearing systems.

For *Artemia franciscana* ingestion (Figure 3.), low *Artemia franciscana* intake was observed for early larval stage specially in the treatment 1 and 3, but in the late zoea stage like 3 and 4, *Artemia franciscana* ingestion was increased which varied significantly (P<0.05). For treatment 2 better ingestion of the *Artemia franciscana* was observed. In the treatment 1 most of the *Artemia franciscana* left unfed as additional *Rotifer (B. rotundiformis)* was added in the larval rearing tank.

Larval developmental period

Treatment 2 resulted faster development of the larvae than the other treatments and within 12 days approximately 50% of the zoea 4 had metaphorically transformed into megalopa. However, treatment 3 showed bit slower growth of larvae than treatment 2. Lowest growth rate was observed for treatment 1. It took 15 days to metaphorize into megalopa. For treatment 2, megalopa molted into crablets on the 15 days, but for treatments 1 and 3, it took 18 and 16 days, respectively. In case of metamorphosis, there was significant difference (P<0.05) observed among the treatments (Figure 4.).

Table 1. Larval feed regime in different treatments and different larval stages of the blue swimming crab

Larval stage	Treatment 1	Treatment 2	Treatment 3	Water exchange
Zoea 1	*Artemia franciscana* umbrella, *Rotifer (B. rotundiformis)*	*Rotifer (B. rotundiformis)*	*Artemia franciscana* umbrella	10% a day
Zoea 2	*Artemia franciscana* umbrella, *Rotifer (B. rotundiformis)*	*Rotifer (B. rotundiformis)*	*Artemia franciscana* umbrella	10% a day
Zoea 3	*Artemia franciscana* nauplii	*Artemia franciscana* nauplii	*Artemia franciscana* nauplii	10% a day
Zoea 4	*Artemia franciscana* nauplii	*Artemia franciscana* nauplii	*Artemia franciscana* nauplii	10% a day
Megalopa	Shrimp egg custard	Shrimp egg custard	Shrimp egg custard	20% a day
Crablet	Shrimp egg custard	Shrimp egg custard	Shrimp egg custard	30% a day

Table 2. Mean Larval Stage Index (LSI) of blue swimming crab in different larval stages of blue swimming crab

Day	Treatment 1		Treatment 2		Treatment 3	
	LSI	Larval stage	LSI	Larval stage	LSI	Larval stage
1	1	Zoea 1	1	Zoea 1	1	Zoea 1
4	1.4	Zoea 2 and Zoea 1	1.6	Zoea 2	1.5	Zoea 2
7	2.6	Zoea 3 and Zoea 2	2.9	Zoea 3	2.7	Zoea 3
10	3.5	Zoea 4 and Zoea 3	3.8	Zoea 4	3.7	Zoea 4
13	4.4	Megalopa and Zoea 4	4.9	Megalopa	4.6	Megalopa

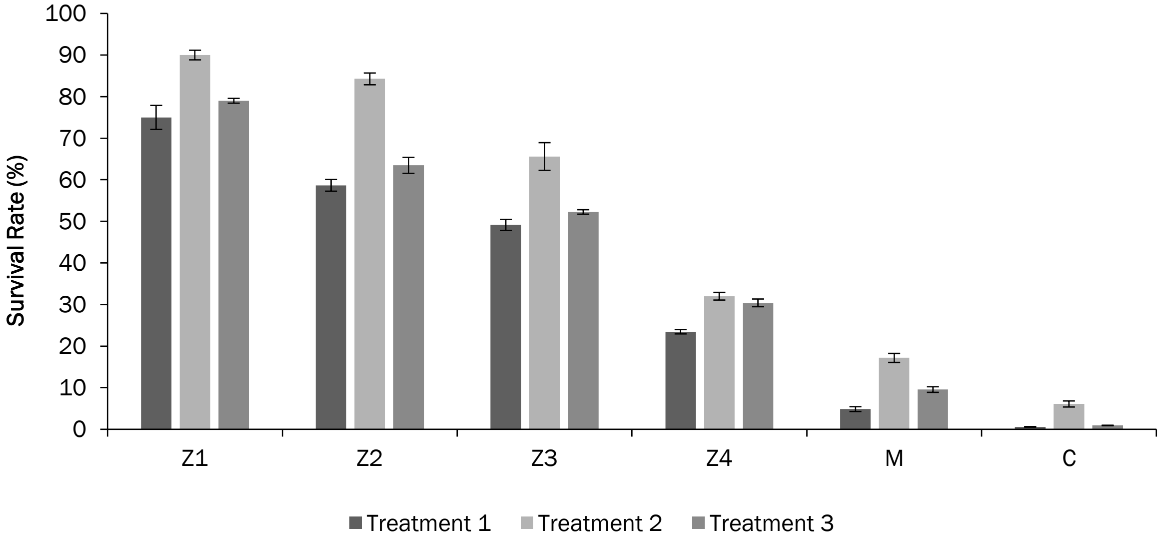


Figure 1. Survival rate of larvae in different larval stages

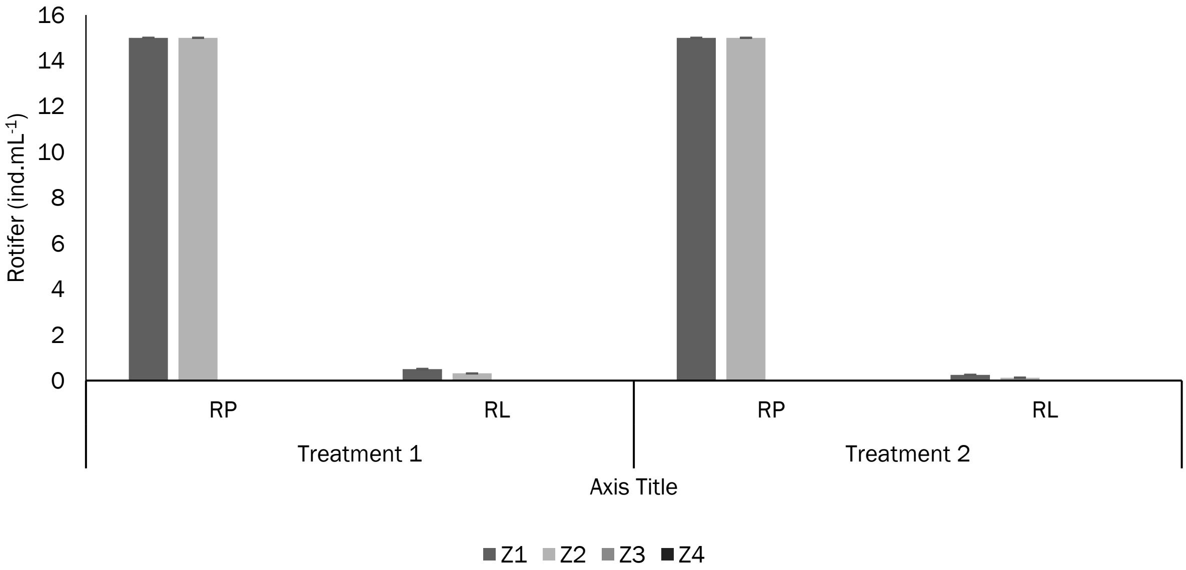


Figure 2. *Rotifer (B. rotundiformis)* intake in the larval rearing tank
Note: RP- *Rotifer (B. rotundiformis)* provided and RL- *Rotifer (B. rotundiformis)* left (Z= zoea)

Water quality monitoring data

Water quality monitoring data are shown in Figure 5 for different treatment tanks. Dissolved oxygen (DO) of all the three treatments were started to drop from 10th day and for treatment 1 DO dropped to 2.3 mg.L^{-1} at the 13th day whereas, DO was 4.8 mg.L^{-1} for treatment 2 at 13th day. Temperature was started to drop from 10th day for sudden continued rain in Cox's Bazar and lowest temperature was 25.3^{o}C recorded for treatment 3 during day 13th. Salinity and pH were between 30-28 ppt and 8.3-7.9 respectively for all three treatments. Ammonia had increased from day 10 in all three treatments and highest was recorded 1.9 mg.L^{-1} for treatment 1 at 13th day. Ammonia was 1.1 mg.L^{-1} and 1.7mg.L^{-1} for treatment 2 and 3, respectively (Figure 5.).

Larval stages are being affected by its feed type (Hasan *et al*., 2022). Treatment 2 represented better survival rate of the larvae other than two treatments. Better ingestion of *Rotifer (B. rotundiformis)* observed in the treatment 2 in the early zoea stage of 1-2. Similarly in the treatment 1, *Rotifer (B. rotundiformis)* ingestion was observed but lower than treatment 2. Ikhwanuddin *et al.* (2012b) stated, in the earlier larval stage of blue swimming crabs, the ingestion rate of *Artemia franciscana* was low, which was observed throughout the larval rearing period. Maurer *et al*., 2017 stated the zoeae raised under a high prey density had a shorter duration by having fewer zoeal stages and greater survival rate than those with a low prey density. In the treatment 3 *A. franciscana* umbrella was limited supplied 2indv.ml^{-1}. In the treatment 1 and 3 most of the *Artemia franciscana* was unfed because of the prey number in the tanks during zoea 1-2 stages and they got bigger throughout the rearing period of the larvae. *Artemia franciscana* biomass had increased for this, creating an imbalanced condition for the larvae in the tank. In treatment 2 *Artemia franciscana* nauplii was used from zoeal 3 stage, so the ingestion rate was better in contrary to other two treatments. Prey density, possibly affecting the nutritional status of larvae, was a critical factor that influences the zoeal development of C. sapidus (Maurer *et al*., 2017).

Research demonstrated that rotifers were the only food source required for appropriate growth and survival during the first zoeal stage (Z1), and that their use improved survival rates compared to co-feeding Artemia. It was also discovered that there was no negative impact on growth or survival when the rotifers were taken out of the co-feeding regime at Z3. When rotifers were fed individually up to zoea 2 and subsequently co-fed with Artemia through to megalopa, a final survival rate of 58.67±7.35% was attained. During the rearing period, crab larvae that were only fed Artemia experienced a prolonged time to reach the megalopa stage and a greater rate of mortality (Ruscoe *et al*., 2004). In that study in the second experiment, it was investigated the effects on survival, when rotifer feeding was discontinued at Z2, Z3, Z4, Z5 and when rotifers were fed up to the megalopa stage (M). Artemia were offered to these treatments from Z2 onwards. A no-rotifer (NR) control that was fed Artemia-only from stocking was also tested. Once more, it was demonstrated that rotifers encouraged respectable levels of development and survival, particularly in the early phases. Crab larvae's survival throughout the first two zoeal stages was greatly reduced and their moulting was delayed in the absence of rotifers. Additionally, there was a noteworthy distinction in megalopa survival as well as a noteworthy inverse correlation between megalopa survival and rotifer feeding length. Removal of the rotifers resulted in the highest survival rate of 78.00±5.54%.

Earlier larval stage has difficulties to catch *Artemia franciscana* nauplii and holding the prey against their mouthparts as their abdomen parts are not well developed (Ikhwanuddin *et al.,* 2012b).The abdomen part helps in catching prey and takes into the mouthparts by the zoea of *S. serrata* (Ong, 1964). Furthermore, the survival rate of Zoea 1 and 2 of *Thalamita crenata* was found higher when it was fed with *Rotifer (B. rotundiformis)* alone (Godfred *et al.,* 1997). Ikhwanuddin *et al.* (2012b) stated that between *Artemia franciscana* and *Rotifer (B. rotundiformis)*, larvae preferred *Rotifer (B. rotundiformis)* more in earlier developmental stage due to smaller size and lack of speed of *Rotifer (B. rotundiformis).*

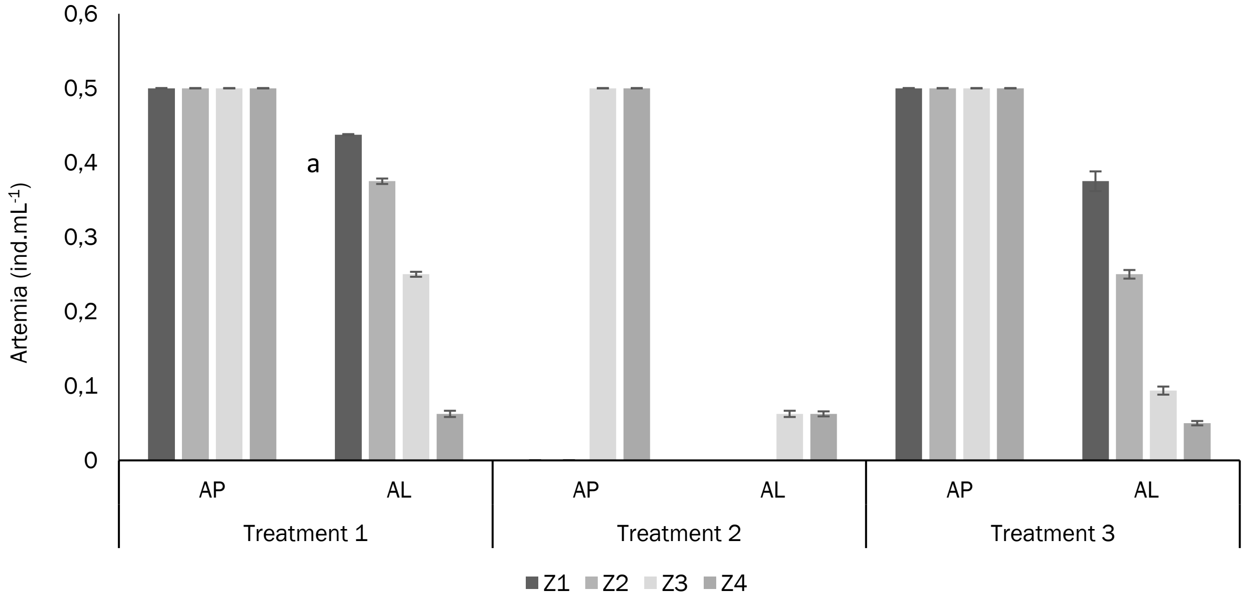


Figure 3. *Artemia franciscana* intake in the larval rearing tank
Note: AP- *Artemia franciscana* provided and AL- *Artemia franciscana* left (Z= zoea)

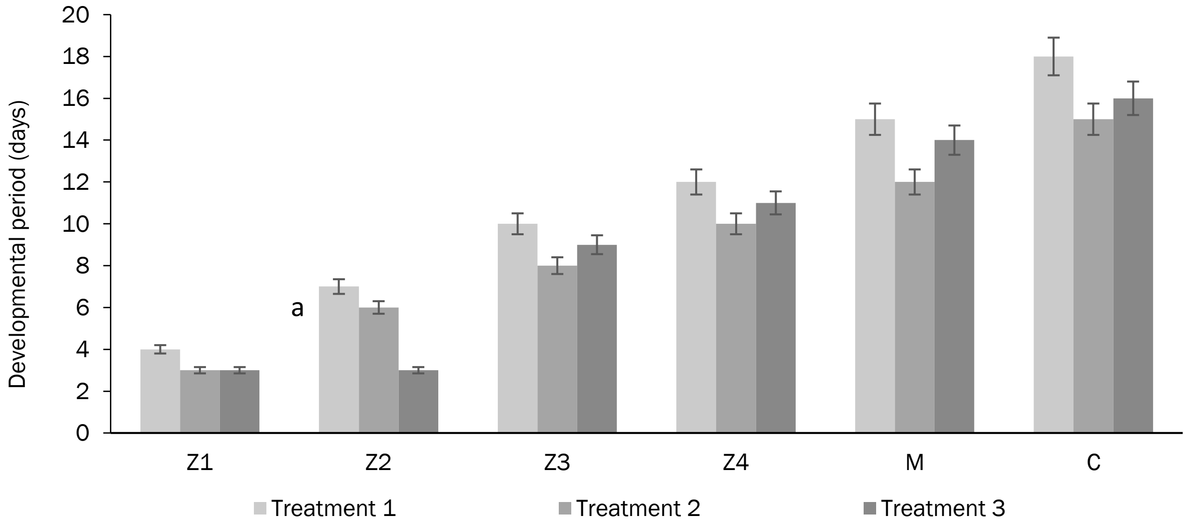


Figure 4. Larval developmental period (days) (Z= zoea; M= megalopa; C= crablets)

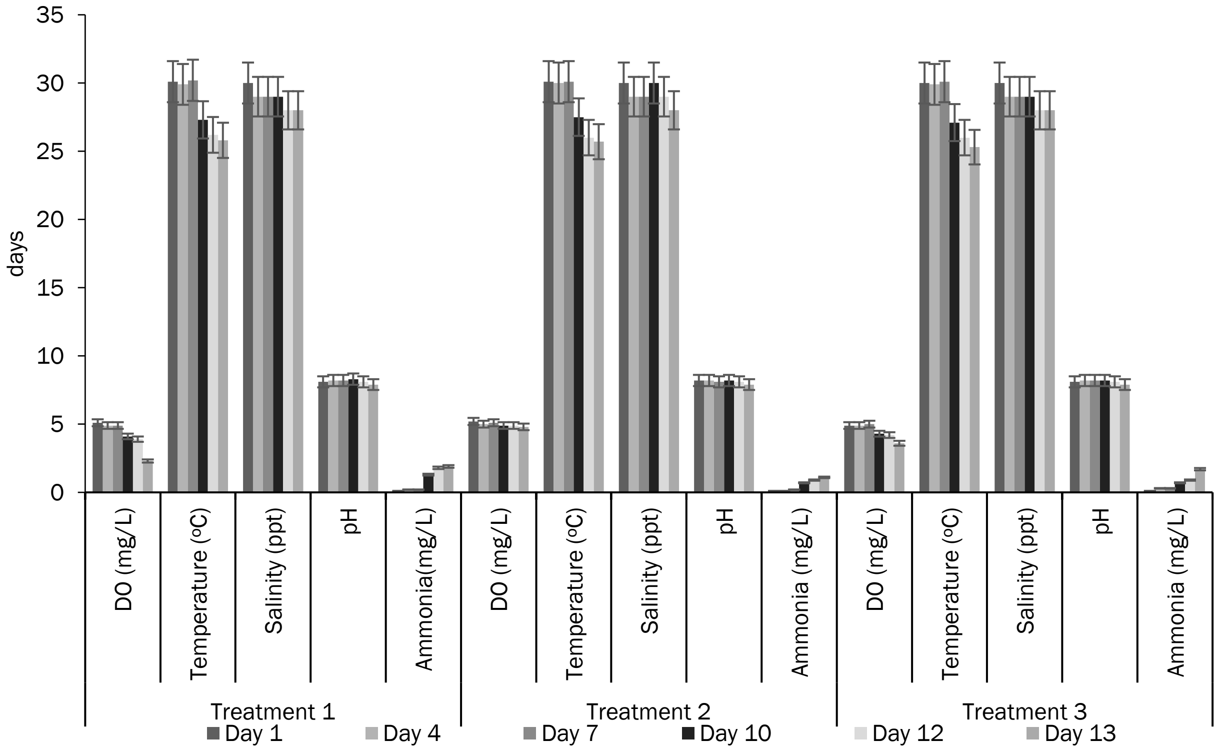


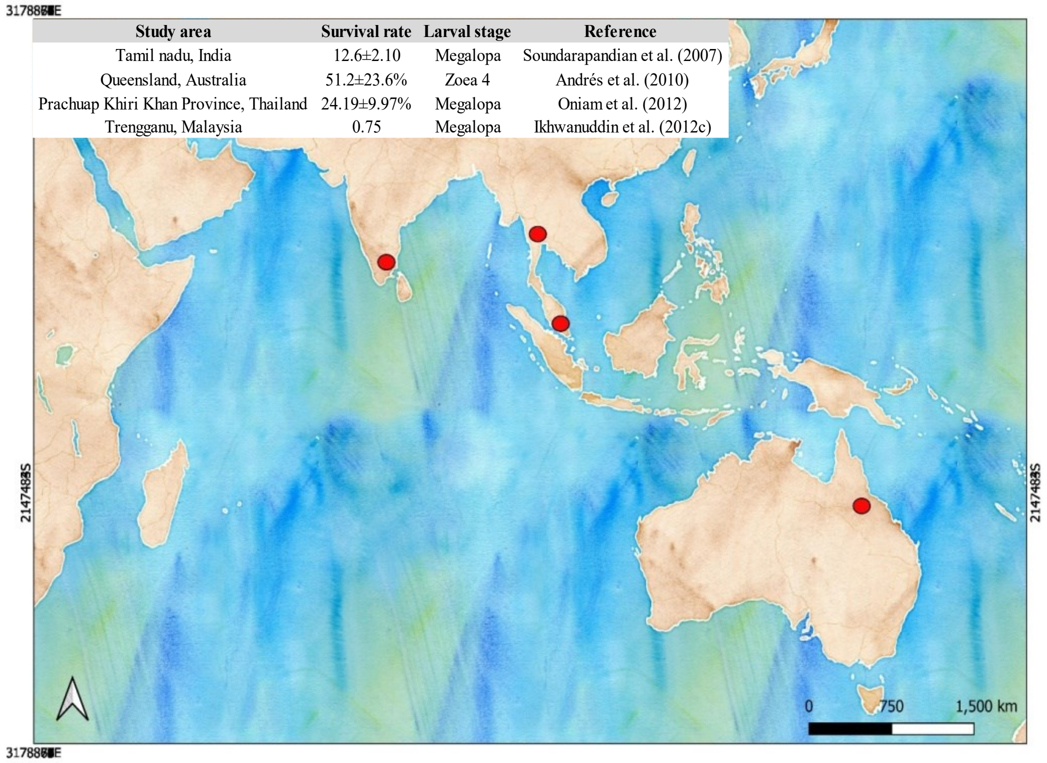
Figure 5. Mean water quality data of different treatment tanks

In all the treatments, in the later zoeal stages larvae were fed with *Artemia franciscana* nauplii as lower nutrition from *Rotifer (B. rotundiformis)* might hinder metamorphosis to megalopa from zoea 4 according to McConaugha (1982). High survival of the zoea larvae, fed Artemia nauplii, suggests that they were able to consume the nauplii, despite their large size and relatively high-speed swimming in their study on the ingestion of Artemia nauplii by *S. serrata* larvae described by Baylon *et al*. (2001). Although the zoea larvae of *S. serrata* were unable to consume the entire Artemia nauplius, they were able to ingest bits and pieces of the body, specifically the head and the appendages. The heavy mortalities that took place during metamorphosis of Z5 larvae to megalopa may indicate that the presence of both rotifers and Artemia nauplii in the diet in the early stages have provided the larvae with the nutrients required for postmolt, which a diet of Artemia nauplii alone could not provide (Baylon, 2009). However blue swimming crab larvae are about 1.5- 1.8 mm in size in Z_1 stage (Josileen and Menon, 2005) which is smaller than Z_1 of *S. serrata*. So, zoeal stages of blue swimming crab fed with *Artemia* nauplii had lower chance to feed on. And though higher density of *Artemia* nauplii assisted growth of zoea of treatment 3, most of them were unfed by the zoea, survived and grew bigger and fought for space and water quality deteriorated faster in treatment-3.

However, ingestion rate was not the only cause for low survival rate in treatment 1 and 3, but also sudden rainfall occurred in Cox's Bazar. Rainfall dropped the temperature and the metabolic rate was slowed. Larvae are much more sensitive to temperature changes described by (Ravi and Manisseri, 2012) and survival rate increased with increased temperature by Baylon *et al*. (2001) in *S. serrata* and Hoang (1999) in *S. paramamosain.* For low temperature feed intake was low and that changed water quality raucously. *Artemia franciscana* biomass also played a big role behind the water quality deterioration. Feeding Artemia from the early zoeal stage or at a high prey density (4 ind.ml^{-1}) accelerated metamorphosis and caused the acceleration of morphological characteristics, such as a greater chela length, carapace length and setae bearing pleopods at the Z5 stage of *S. serrata* described by Suprayudi *et al*., 2002 however, in this experiment, rainfall played a big role dropping the temperature which imperil the metabolism, growth and survival of the larvae.

Treatment 1 and showed lower LSI index from treatment 2. This could reflect that zoeal feed is important parameter for the development of the larvae. Better nutrition from the earlier stages of the larvae ensures better metamorphosis (Godfred *et al.,* 1997) thus resulting in lower LSI index for treatment 1 and higher LSI value for treatment 2. It is also larvae of treatment 2 grown faster than other two treatments. In treatment 3 it was close to treatment 2 in case of LSI index which shows better moulting in the zoeal stages which should be resulting in better survival rate though survival rate dropped due to poor water quality in the larval rearing tanks.

Blue Swimming Crab, this crustacean is getting a great popularity as food in the global market. Scientists around the earth are also getting conscious about the breeding and culture of this important species. Till today, a little work has been done on the growth and survival rate of this valued species (Figure 6.). However, this study also carried a result of growth and survival of blue swimming crabs (*Portunus pelagicus*) with different larval diets which could be helpful for future studies.



Study area	Survival rate	Larval stage	Reference
Tamil nadu, India	12.6±2.10	Megalopa	Soundarapandian et al. (2007)
Queensland, Australia	51.2±23.6%	Zoea 4	Andrés et al. (2010)
Prachuap Khiri Khan Province, Thailand	24.19±9.97%	Megalopa	Oniam et al. (2012)
Trengganu, Malaysia	0.75	Megalopa	Ikhwanuddin et al. (2012c)

Figure 6. Survival rate in different larval stages of blue swimming crab larvae around the world (QGIS.V. 3.24.1)

Conclusion

Type of larval feed had a significant role in terms of development and survival of blue swimming crab larvae. The findings indicate a significant role of *Rotifer* as early larval diet for better survival rate of blue swimming crab. Moreover, it also effects on early metamorphosis and growth. However, the environment, water quality, stocking density etc. are also important for better growth and survival of blue swimming crab larvae. Findings of the study will provide a better understanding on initial larval diet for blue swimming crab larvae. Further studies on integrated management with stocking density, water quality and feeds would promote better survival rate with good quality larvae.

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