

Larval rearing of orange mud crab, *Scylla olivacea*: improving survival rate and shortening metamorphosis period

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Abstract. Orange mud crab (Scylla olivacea) is an important aquaculture species in Southeast Asian countries, especially in the Southwest costal region of Bangladesh. Due to unavailability of hatchery produced seeds the farming of S. olivacea in exclusively depends on the wild for small juveniles resulting in exploitation of this species in Bangladesh. The development of proper hatchery techniques considering better understandings related to larval development are needed to increase the survival and growth rate of S. olivacea in hatchery conditions. Therefore, this study aimed to optimize the water treatment strategy for the upgrading of water quality, minimizing mortality and shortening the metamorphosis duration during larval rearing of mud crab. Newly hatched larvae were reared in three different rearing conditions where the water was treated with both prebiotic (Super-TCT-Biotic, 5 ppm) and probiotics (Fish probiotic, 0.5 ppm) in treatment-1 (T1) but only prophylaxis (mixure of 0.025 ppm Teflan and 0.3 ppm Furazolidone) in treatment-2 (T2). In treatment-3 (T3), prebiotic (5 ppm) and probiotics (0.5 ppm) were combinedly used during first 10 days and prophylaxis (0.3 ppm) was also used from day 14 to day 25. The stocking density was maintained as 50 zoea.L⁻¹ and rotifer, liquid rotifer, enriched Artemia and liquid Artemia were used as feed for the larval growth. The findings revealed that hydrological parameters viz., temperature, salinity, pH, dissolved oxygen and ammonia in all the treatments were similar and found within the acceptable ranges for mud crab larvae rearing. The value of larval stage index (LSI) was found significantly higher (p < 0.05) on 21st day of rearing in T3 (6.87 ± 0.06) than T1 (6.74 ± 0.04) and T2 (6.61 ± 0.07). Between, zoeal and crablet phase, the significantly higher survival rate was observed in T3 (7.00 ± 1.00) than T1 (5.00 ± 0.00) and T2 (4.00 ± 1.00) . The result of the present study suggested that combined water treatment with prebiotic, probiotic and prophylaxis can shorten the metamorphosis period and enhance the survival rate of S. olivacea. Keywords: Orange mud crab, Prebiotic, Probiotic, Prophylaxis, Survival

Introduction

The Orange mud crab, *Scylla olivacea* is one of the major species that contributes significantly to the world's aquaculture production from the genus *Scylla* (FAO 1999). Moreover, there has been a common trend of increasing the exploitation rate in the recent years as mud crabs belong to lucrative seafood business items to the small-scaled coastal fisheries of many countries in tropical and sub-tropical Asia (Keenan 1999a). Generally, mud crabs have high demand in the international market as a luxury food product (Azra and Ikhwanuddin 2016) and its popularity is mounting rapidly as an alternative species for aquaculture (Abol-Munafi and Azra 2018) since the coastal, brackishwater and marine shrimp aquaculture industry started to faces inevitable threats from disease outbreaks and raised environmental crisis (Holme *et al.* 2006).

In Bangladesh, brackishwater farms, tide-fed shallow lagoons, estuaries and mangrove swamps are the major habitat for mud crabs (Shelley, 2008). At present, mud crab farming exclusively relies on the wild for small juvenile crabs in aquaculture farms and the main revealed issues which are provoking wild exploitation due to unavailability of hatchery produced seeds of mud crab (Keenan 1999a). Therefore, the development of proper hatchery techniques is

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prerequisite in order to support the development of the mud crab aquaculture industry (Gunarto et al. 2016).

Larvae rearing techniques, disease outbreak and nutritional composition of larvae feed are the major three areas of research which support the commercial production of marine finfish and crustacean larvae (Sorgeloos and Leger 1992). Thus, it is not possible to establish a complete hatchery protocol unless these three predominantly and interconnected areas are addressed and optimized properly. Unfit rearing conditions such as lack of oxygen or sub-optimal water quality parameters mostly imposes physical stress, greatly affect the larval health and causes massive mortality due to out breaking of diseases (Nghia et al. 2007). Since the beginning of marine larval rearing technology in the 1960s, there has been a considerable progress (Howell et al., 1998) with time passed. Many technical improvements were developed over the past years those could be useful for mud crab larvae rearing with several modifications. Moreover, the upgraded strategies should try for every single species of the genus Scylla (Keenan 1999b) in terms of the local environmental conditions like the seawater source, water treatment strategy, hatchery management status and available local resources. Although much knowledge and skills have been gained from this system but there is still a necessity for further improvement of the rearing protocol to maximize the larval survival rate and quicker production. Considering the facts, this investigation aimed to integrate the existing rearing guidelines for producing larvae of mud crab species (S. olivacea) and to develop another rearing method for reducing metamorphosis period and to maximize survival rate of crab larvae.

Materials and Methods

To evaluate the impact of different water treatment protocols for water quality improvement and to reduce disease occurrences in orange mud crab (*S. olivacea*) larvae rearing, an experiment was conducted in 2019-2020 fiscal year at crab hatchery complex in Brackishwater Station of Bangladesh Fisheries Research Institute (BFRI), Paikgacha, Khulna-9280, Bangladesh.

Experimental animal: Orange mud crab (*S. olivacea*) larvae were collected from the crab hatchery of Brackishwater Station of BFRI and transferred to the larval rearing tanks (LRT). The crab larvae of Zoea1 stage, were chosen for the experiment in accordance with the objectives.

Prebiotic, probiotic and prophylaxis: Super-TCT-Biotic (PVS lab Ltd., India) was given into the water as a prebiotic for the treatment during larval rearing. This prebiotic was made up of *Bacillus subtilis*, oligofructose, mannan-oligosaccharides, organic acids, acetic acid, propionic acid, lactic acid, butyric acid, and digestive enzymes like: protease, lipase, amylase and cellulose. Besides, FISH PROBIOTIC (Shijiazhuang Shiwei Pharmaceutical Co. Ltd., China) was used as a probiotic in this study. *Bacillus subtilis, Nitrobacterium, Nitrococcus,* and Photosynthetic bacteria were combined to make that probiotic. Similarly, a mixtures of Furazolidone (0.25-0.30 ppm) and Teflon (0.020-0.025 ppm) was prepared to make prophylaxis.

Preparation of larvae rearing cemented tanks (LRT): The larvae rearing cemented tanks of one ton capacity were used for this research. All the tanks were initially cleaned with chlorine at the rate of 150 ppm and then neutralized with sodium thiosulfate (Na₂S₂O₃) at the dose of 25

ppm, just before of rinsing with clean freshwater. Afterwards, all the tanks were connected with aerator for ensuring sufficient oxygen supply and water circulation.

Experimental design: The study was designed with three different treatments (T1, T2, and T3) and each treatment was conducted with three replications to optimize the water treatment plan for the improvement of water quality and to reduce disease outbreak during the larval rearing of *S. olivacea.* In treatment-1 (T1) water was treated with both prebiotic (5 ppm) and probiotics (0.5ppm), whereas, water in treatment-2 (T2) was treated with prophylaxis at the rate of 0.3 ppm until 25^{th} day. On the contrary, for treatment-3 (T3) the water was treated with both prebiotic (5 ppm) and probiotics (0.5 ppm) up to 10 days and with prophylaxis at the rate of 0.3 ppm from day 14 to day 25 for the larvae rearing of mud crab. Stocking density of larvae in all the treatments were maintained at the rate of 50 zoea.L⁻¹. The larvae were reared until they reached the crablet (C1) stage in different experimental tanks.

Water quality management: The water used in the experiment was firstly chlorinated with 150 ppm calcium hypochloride, vigorously aerated and left for overnight. Then aerated properly and neutralized with sodium thiosulfate (Na₂S₂O₃) at the dose of 25 ppm. Water was finally treated with biological (sand bio-filter) and physical filter (cartridge and UV filter) before using for larvae rearing. Immediately after using prebiotic, both probiotic and prophylaxis were applied at the same rate of doses and continued with an interval of 3-4 days. About 50-70% of water was exchanged before application of prebiotic, probiotic and prophylaxis.

Feeding: Rotifer, liquid rotifer, enriched *Artemia* and liquid *Artemia* were used as feed for the larval rearing of the experimental organism (*S. olivacea*). Rotifer and liquid rotifer was applied for zoea1 to zoea2 stage at the density of 30 individuals.ml⁻¹ and 1 ml.ton⁻¹, respectively). Thereafter, the larvae were fed with live feed combination of enriched *Artemia* nauplii and liquid *Artemia* at the density of 5 individuals.ml⁻¹ and 0.5 ml.ton⁻¹, respectively until the end of the study (from zoea3 to megalopa stage). Feeding was administered three times a day in the morning (07:00 am), afternoon (03:00 pm) and night (11:00 pm).

Data collection: Daily sampling of *S. olivacea* larvae was performed to assess the survival, larval development and any fouling on the body of larvae up to metamorphosis into megalopa. Sampling for testing water quality parameters *viz.* temperature, salinity, pH, dissolved oxygen and ammonia were done twice daily during the larval rearing period. Parameters were monitored regularly in the early morning at 7:00 am and in the evening at 07.00 pm by following the methods of AOAC (1990) and APHA (1992). Survival rate of mud crab (*S. olivacea*) larvae was calculated with formula as mentioned by Sangand Fotedar (2004): $S = N_t/N_0 \times 100$

Where, S = Survival rate (%), N_0 = initial number of larvae, N_t = final number of larvae.

The metamorphosis period was determined based on the length of time required from zoeal stage to the megalopa stage.

The Larval Stage Index (LSI) value was calculated using the standard protocol described by Syafaat et al. (2019):

 $LSI = \{(A1 \times A2) + (B1 \times B2)\}/C$

Where A1 = number of previous stage larvae, A2 = previous stage, B1 = number of highest stage larvae, B2 = highest stage, C = total number of sample.

To calculate the LSI in each treatment, the scoring technique was applied to each larvae stage, namely; Zoea1 = 1, Zoea2 = 2, Zoea3 = 3, Zoea4 = 4, Zoea5 = 5, Megalopa (M) = 6. For example, from the 10 larvae sampled, three larvae in Zoea1 stage; rest seven larvae in Zoea2 stage are found. The LSI value was then calculated using the following equation: LSI = $\{(3 \times 1) + (2 \times 7)\}/10 = 1.7$

Data analysis: The collected data on crab larvae rearing such as LSI value, survival rates and water quality parameters were compiled, categorized, computed and tabulated using a computer program, Microsoft Office Professional Plus 2019. Moreover, some statistical tests were executed by using another computer program termed, Statistical Product and Service Solutions (SPSS) ver. 25. Here, one-way ANOVA and Duncan's Multiple Range Test were employed to observe the differences in LSI and survival rates of crab larvae among the treatments.

Results

LRT water quality: Temperature and salinity are the crucial physical regulators that affect the growth, survival and other biological activity of mud crabs. Water temperature in this research ranged between 29.1°C to 31.0°C among the treatments whereas the salinity from 27.0 ppt to 30.0 ppt. Moreover, the pH value ranged from 7.7 to 8.8, dissolved oxygen from 5.1 ppm to 6.7 ppm and total ammonia ranged between 0.00 ppm and 0.22 ppm, respectively in all the treatments (Table I). Water quality variables in all the treatments were found similar and within standard crustacean larvae rearing ranges.

	_		Treat	ments			Ontimum
Parameters	r	Г1	Т	2	Т	3	- Optimum level
	Lower	Upper	Lower	Upper	Lower	Upper	level
Temperature (°C	29.3	31.0	29.3	30.8	29.1	29.9	25-32
Salinity (ppt)	27.0	30.0	27.0	30.0	27.0	30.0	27-31
Water pH	7.9	8.3	7.8	8.6	7.7	8.8	7.5-9.0
Dissolved oxygen (ppm)	5.5	6.6	5.5	6.7	5.1	6.5	>5.0
Total ammonia (ppm)	0.00	0.20	0.00	0.21	0.00	0.22	< 0.5

Table I. Water quality variables recorded during the study period

Larval stage index (LSI): Larval growth was monitored as larval stage index (LSI) and it was observed that for the first four days, LSI was similar for all the treatments and then started to differ among the treatments due to a-synchronized metamorphosis to the next stage (Table II) and such variation continued for the remaining rearing period. Between fourth and twenty-first day, the larval stage indices were observed always significantly higher (p < 0.05) in T3 than T1 and T2. On the 21st day of larval rearing, the LSI value was calculated as 6.87 ± 0.06 , 6.61 ± 0.07 , and 6.74 ± 0.04 in T3, T2, and T1, respectively.

Survival rate and crablet production: Survival of larvae in all the treatments sharply decreased until first six days of rearing Thereafter, a gradual decline was noticed in all three treatments (Table III). In general, survival rates were found always significantly higher (p < 0.05) in T3 than T1 and T2 from 7th day to 21st days. On 21st day, the highest survival rate was 7.00 ± 1.00 in T3.

Discussion

The lower survival rates of mud crab (S. olivacea) larvae has been a major problem commonly encountered in the hatchery (Williams et al. 1999). Like other crustacean species, mud crab also grows through moulting and this process might be affected by many factors viz. temperature, stress and scares from predator, lack of shedding/hiding places, improper nutritional feeding, hydrology etc. Any interruption in moulting might slower the growth of mud crab larvae (Kulmiye and Mavuti 2005. Thus longer time is needed to attain desirable size and even cause death to the victim. The present study revealed the advantages of the proper water treatment during mud crab larvae rearing. Water quality parameters can play a vital role in regard to all aquaculture practices especially to maintain the healthy larvae (Gunarto and Sulaeman 2017). Suitable water quality variables ensure higher growth rate, survivability and production in mud crab larvae rearing (Ong et al. 2019). In the present study, the temperature of the rearing tank varied within the range of $29.1 \sim 31.0^{\circ}$ C. Hereafter, the water salinity and pH values in this study were recorded within the range of $27.0 \sim 30.0$ and $7.7 \sim 8.8$ ppm, respectively. The range of dissolved oxygen levels fluctuated between $5.1 \sim 6.7$ and was always above the sub lethal level. Furthermore, total ammonia $(0.0 \sim 0.22 \text{ppm})$ were also observed within the acceptable level in different treatments during the entire larvae rearing period. Water quality variables in all the treatments were almost similar and found within the acceptable ranges for the larvae rearing of S. olivacea (Table 1). The water quality parameters of this study during seed production and larval rearing of mud crab were very consistent as reported by some other researchers (Zeng and Li 1992, Dat 1999, Gunarto and Sulaeman 2017). Optimum water parameters provide all necessary environments for the seed production and larvae rearing of Scylla sp. which ensures high survival and quick metamorphosis (Thach 2009).

Days		Treatments					
	T1	T2	Т3				
0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00				
1	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00				
2	1.47 ± 0.06^{a}	$1.30 \pm 0.02^{\circ}$	1.37 ± 0.01^{b}				
3	1.93 ± 0.12	1.90 ± 0.01	2.00 ± 0.01				
4	2.00 ± 0.01^{b}	2.00 ± 0.02^{b}	2.07 ± 0.01^{a}				
5	2.43 ± 0.06^{b}	$2.17 \pm 0.07^{\circ}$	2.60 ± 0.05^{a}				
6	2.80 ± 0.12^{a}	2.50 ± 0.10^{b}	3.00 ± 0.03^{a}				
7	3.00 ± 0.03^{b}	$2.87 \pm 0.01^{\circ}$	3.13 ± 0.01^{a}				
8	3.33 ± 0.12^{b}	3.27 ± 0.05^{b}	3.67 ± 0.07^{a}				
9	3.87 ± 0.12^{b}	$3.63 \pm 0.03^{\circ}$	4.10 ± 0.05^{a}				
10	4.10 ± 0.10^{b}	$3.93 \pm 0.06^{\circ}$	4.37 ± 0.07^{a}				
11	4.33 ± 0.12^{b}	$4.13 \pm 0.04^{\circ}$	4.67 ± 0.07^{a}				
12	4.67 ± 0.12^{b}	$4.37 \pm 0.06^{\circ}$	5.00 ± 0.05^{a}				
13	4.87 ± 0.12^{b}	$4.53 \pm 0.08^{\circ}$	5.17 ± 0.09^{a}				
14	5.27 ± 0.12^{b}	$4.93 \pm 0.13^{\circ}$	5.60 ± 0.12^{a}				
15	5.50 ± 0.07^{b}	$5.33 \pm 0.05^{\circ}$	5.89 ± 0.10^{a}				
16	5.80 ± 0.10^{b}	$5.66 \pm 0.03^{\circ}$	6.01 ± 0.03^{a}				
17	6.00 ± 0.04^{b}	$5.80 \pm 0.05^{\circ}$	6.10 ± 0.04^{a}				
18	6.00 ± 0.05^{b}	5.98 ± 0.11^{b}	6.20 ± 0.06^{a}				
19	6.07 ± 0.12^{b}	6.02 ± 0.10^{b}	6.47 ± 0.09^{a}				
20	6.47 ± 0.12^{b}	6.38 ± 0.08^{b}	6.67 ± 0.07^{a}				
21	6.74 ± 0.04^{b}	$6.61 \pm 0.07^{\circ}$	6.87 ± 0.06^{a}				

Table II. Larval stage index (LSI) of larvae under different water treatments

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The development of metamorphosis in crustacean can be started by a variety of factors such as the availability of the proper water quality, nutrient and energy capable of accelerating the change of metamorphosis from zoea into megalopa (Tahya et al. 2016). In the present study, LSI values were almost equal up to four days for all the treatments then started to vary due to the asynchronous metamorphosis in later phases and this variation retained for the entire larvae rearing period. The LSI value was observed always greater for the treatment-3 where the water was treated with prebiotic, probiotic and prophylaxis than the other two treatments indicated suitable condition for faster metamorphosis. Similarly, LSI values during 20 days larvae rearing of purple mud crab showed an increasing trend until last day of culture (Syafaat et al. 2019). In the present study, the stage change from zoeal to megalopa required 18 days and megalopa to crablet took seven days for all the treatments. Therefore, the development of S. olivacea larvae from the zoea1 to crablet stage needs only 25 days. The larvae rearing of S. olivacea faces many problems resulted in low survival rate for severe cannibalism and prolonged larval rearing process (Gunarto and Sulaeman 2017). They also reported that, mud crab larvae normally required six days to develop from megalopa to crablet. Hence, the development of larvae from zoeal to the crablet stage needs 28 days. In our study, the metamorphosis period required only 25 days to transform into crablet which indicates that proper water treatment had a positive impact on larval development of crab.

Days		Treatments (survival)					
	T1 (%)	T2 (%)	T3 (%)				
0	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00				
1	93.33 ± 1.91^{b}	96.67 ± 1.91^{ab}	97.08 ± 1.44^{a}				
2	80.83 ± 2.50^{b}	80.83 ± 1.25^{b}	87.50 ± 1.25^{a}				
3	74.00 ± 4.39^{ab}	71.00 ± 1.25^{b}	78.00 ± 1.91^{a}				
4	60.42 ± 1.44^{b}	$56.00 \pm 1.25^{\circ}$	71.25 ± 1.91^{a}				
5	57.92 ± 2.17^{ab}	55.00 ± 3.82^{b}	64.58 ± 5.00^{a}				
6	54.50 ± 3.15^{ab}	52.00 ± 3.31^{b}	62.00 ± 6.29^{a}				
7	48.75 ± 1.91^{b}	47.00 ± 1.44^{b}	60.00 ± 4.02^{a}				
8	46.67 ± 2.05^{b}	44.00 ± 4.51^{b}	56.00 ± 3.75^{a}				
9	37.92 ± 3.75^{b}	37.00 ± 2.60^{b}	52.00 ± 3.82^{a}				
10	28.92 ± 3.15^{b}	27.00 ± 2.50^{b}	48.00 ± 3.82^{a}				
11	24.00 ± 5.05^{b}	24.00 ± 5.20^{b}	44.00 ± 6.41^{a}				
12	19.58 ± 3.91^{b}	21.00 ± 3.75^{b}	38.00 ± 5.91^{a}				
13	16.00 ± 5.02^{b}	14.00 ± 3.31^{b}	34.00 ± 3.31^{a}				
14	14.00 ± 4.73^{b}	12.00 ± 5.05^{b}	28.00 ± 4.51^{a}				
15	12.00 ± 3.15^{b}	11.00 ± 4.39^{b}	25.00 ± 4.73^{a}				
16	10.00 ± 1.91^{b}	10.00 ± 2.50^{b}	21.00 ± 2.08^{a}				
17	8.00 ± 2.52^{b}	8.00 ± 1.00^{b}	16.00 ± 1.53^{a}				
18	7.00 ± 1.53^{b}	7.00 ± 1.00^{b}	14.00 ± 1.00^{a}				
19	7.00 ± 1.53^{b}	7.00 ± 1.00^{b}	12.00 ± 1.00^{a}				
20	6.00 ± 1.00^{b}	6.00 ± 1.00^{b}	11.00 ± 1.00^{a}				
21	5.00 ± 0.50^{b}	5.00 ± 1.00^{b}	10.00 ± 1.00^{a}				
25	5.00 ± 0.00^{b}	4.00 ± 1.00^{b}	7.00 ± 1.00^{a}				

Table III. Survival of larvae at different rearing days under different water treatments

Survival rate of larvae is the key factor that ensures quick and mass production of larvae (Gunarto and Sulaeman 2017). There remain several factors that may affect the survival rate in the larvae rearing process of mud crab such as cannibalism, shedding, temperature and salinity in stabilities, feed, shelter and stocking density (Quinitio and Estepa 2011). Rotifers and *Artemia*

enriched with probiotics improved the survival and speeded up the metamorphosis rate of megalopa (Quy *et al.* 2018). As usual, crab survival improves with the treatment of probiotics and prebiotics mixture during larval rearing (Ayisi *et al.* 2017). Also, prophylaxis plays significant role for survival of crab larvae culture (De Pedro *et al.* 2007). The finding of enhanced survival was also confirmed in this study for mud crab larvae rearing under different experimental treatments. Except for the first two days, survival rate of larvae to crablet stage in T3 was always higher (7%) for rest of the days with a significant difference to the other treatments. The survival of zoeal developed to megalopa stage at 28 ppt salinity was 13.16% (Jantrarotai *et al.* 2002). Similarly, in the present study the survival rate up to this developmental stage was 14% in T3.

Optimizing the water treatment strategy for the improvement of water quality and minimize mortality and shorten metamorphosis period during the larval rearing of mud crab were the major goals of the study. However, the survival rate was higher in T3 (14%) than T1 (7%) and T2 (7%) up to megalopa. Moreover, the highest crab instar (C1) survival (7%) was also observed in T3. Based on obtained results, the higher larval stage index was always found in T3. The zoeal turns into megalopa stage at 18 days and crablet stage at 25 days after hatching. Therefore, the study concluded that, water treated with prebiotic, probiotics and prophylaxis had a positive response to improve the larval quality, enhancement of survival and promoting of metamorphosis in *S. olivacea* larvae rearing.

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