**Research Article****Effects of temperature, feeds and feeding frequency on larval survivability and crablet production of mud crab (*Scylla Olivacea*)**

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ABSTRACT

Limited known about the impact of temperature and feed on the survivability of different larval development stages at crab hatchery. This study was conducted to investigate the hatchery room temperatures and different feeds and feeding frequency impact on berried crab production, hatching rate, larval survival and crablet production. An experiment in the hatchery tank was set up in April'24 (30-43°C), May'24 (27-42°C), and June'24 (32-39°C). Another individual experiment was conducted with three Treatments: T1 (artemia; four times daily), T2 (artemia + formulated feed; six times daily), and T3 (artemia; six times daily), each in triplicate, and was cultured for 26 to 32 days. Results of the temperature indicated that the highest berried crab production rate ($20.83 \pm 2.89\%$), hatching success rate ($64.55 \pm 8.14\%$), and crablet production rate ($2.52 \pm 0.13\%$) were observed in June (32-39°C) compared to the room temperature of April (30-43°C) and May (27-42°C). The T2 treatment resulted in the significantly highest Larval Stage Index (LSI) and survival rates across all larval stages, leading to the highest crablet production rate ($2.90 \pm 2.03\%$). The study suggests that maintaining a room temperature of 32–39°C and feeding six times daily with a combination of Artemia and formulated feed may improve zoeal survival and crablet production in mud crab hatcheries.

Introduction

The farming of mud crab, belonging to the genus *Scylla*, is rapidly growing in the Asia-Pacific area due to strong consumer demand, high market prices, and the species' adaptability to diverse environmental conditions (Murak et al., 2026). Since the 1970s, there has been increasing interest in cultivating *Scylla* species in tropical Asia, such as China and Bangladesh (FAO, 2010). A decline in shrimp production in 1994-1995, caused by falling international shrimp prices, led farmers to seek alternative aquaculture options beyond shrimp (Molla et al., 2009; FAO, 2010; Salam et al., 2012). Consequently, both live and soft-shell

crab farming have become popular in many Asian nations. The soft-shell crab farming sector has recently emerged in Bangladesh, with significant demand in global markets and rising prices both locally and internationally (FAO, 2018). Bangladesh's coastal areas present opportunities for the capture, cultivation, and trade of mud crabs (Islam et al., 2015; Hussain et al., 2018). Currently, crabs are raised in 27,010 hectares of coastal ponds in Bangladesh (FRSS 2017). Crabs have long been harvested from the Sundarbans and adjacent areas for export and juvenile crabs are raised in brackish water ponds until they

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reach marketable size (Rahman et al., 2020). Crab exports from Bangladesh to international markets began in 1977–1978. Since 1982, crab exports have relied solely on wild harvests (Rahman et al., 2017). The most common crab species in Bangladesh is *Scylla olivacea* (Rouf et al., 2016), found along the entire coastline (Bhuyan et al., 2021), particularly in the study area near the mangrove forest.

Increasing fishing pressure (Worm et al., 2009), habitat degradation (Alleway et al. 2023; Richards and Friess, 2016), and mortality (Lafferty et al., 2004) of wild crab are concerns for the sustainability of wild crab stocks as well as crab farming industries. In Bangladesh, exploitation of the mud crab population has increased over the last three decades (Baki et al., 2025). Similar trends are also observed for many Asian countries (Shelley, 2008; Ikhwanuddin et al., 2012). More than 73.5% of crab farming depends on the fingerlings from wild caught (FAO, 2016). Therefore, a large variation in seasonal supply and individual specimen size is observed for farming (Azra and Ikhwanuddin, 2015). Mud crabs are harvested mainly from channels, tidal rivers, and coastal creeks of mangrove forests in Bangladesh (Rahman et al., 2017) to supply the crab farms. There is no well-sustained mud crab hatchery in Bangladesh till now. Since crab farming started to date, this industry has depended on seeds from wild sources, but nowadays, crab farmers are facing scarcity of wild mud crab seeds for running the crab farming (Shelley, 2008; Quintio and Lwin, 2009). Therefore, the crab farmer keeps empty cage for a period of time. In these situations, mud crab farmers are searching for an alternative source of crab seed in Bangladesh. So, a well-established hatchery is needed to sustain the mud crab farming in Bangladesh.

The growth, development, and reproductive capabilities of crustaceans are significantly influenced by temperature, nutrition, and surroundings. The impact of temperature and feed on the survivability of different larval growth/ development stages at hatcheries is limited by our current knowledge (Hamasaki et al., 2002a; Holme et al., 2006a; Kuhn, 2017; Affandi et al., 2019). The survival & development of crustaceans, including intermolt

periods, can be affected by temperature (Ruscoe et al., 2004; Kuhn, 2017). Numerous studies have shown the effect of temperature on the development and survival of *Scylla serrata* and *S. paramamosain* (Nurdiani and Zeng, 2007; Do et al., 2020). Phuong et al. (2021) recommended that mud crab larvae should be cultivated within the temperature span of 27–30°C to optimize their yield. Win et al. (2021) noted that 33°C is the appropriate temperature for nurturing mud crabs from megalopa to juvenile stages. Furthermore, temperature range of 27°C to 30°C is ideal for juveniles of mud crab (Do et al., 2020). However, little is known about the temperature effect on different larval development stages of the mud crab hatchery in Bangladesh.

In general, mud crab larvae are highly sensitive, which can result in significant mortality during the early zoea stages (Davis, 2004). Globally, the average survival rate of mud crab larvae is only 3% (Misbah, 2020). A significant challenge in developing suitable hatchery methods for portunids crabs is the limited knowledge about what nutrients larvae need (Hamasaki et al., 2002a; Holme et al., 2006a; Affandi et al., 2019). High dietary lipids, fatty acids, and protein in crab feed for mud crab brood stock management, during the breeding and maturation stage, are required (Azra and Ikhwanuddin, 2016). Mass mortality during the Zoea stage (Z1 to Z2) and metamorphosis in the late zoeal stage (Z5 to megalopa) is linked to insufficient nutrition in the diet given (Hamasaki et al., 2002b; Suprayudi, 2002; Islam and Yahya, 2016). Several studies showed that mixture diet can support crablet phases (Haryati et al., 2014; Permadi and Juwana, 2015; Gunarto et al., 2018). Therefore, sufficient nutritional ingredients with continuous feeding are essential. Furthermore, to improve the success of artificial/processed feed used in mud crab larval rearing, attention must be paid to the feed ingredients, regularity, and suitable dosage at each stage. So, this study aims to observe the impact of variable room temperatures and with & without the processed feed with feed distribution frequency on survival, growth and production at different stages of crab (*S. olivacea*) larvae development.

Methodology

This experiment was conducted in concrete-made larvae rearing tanks filled with 4.5 tons of water volume at NGF-Crab Hatchery, Kalbari, Shyamnagar, Satkhira. Three experimental trials were conducted in April'24 (30-43°C), May'24 (27-42°C), and June'24 (32-39°C) to find out the effects of room temperature on the growth and survivability of larvae.

On the other hand, to investigate the effects of feed and feeding frequency on larval growth and survivability, two feed types, herein referred to as artemia and processed feed, were combined with varying feed frequencies. This resulted in three treatments, namely T1 (Only Artemia 4 Times), T2 (Artemia 4 times + processed food 2 times), and T3 (Only Artemia 6 times), and each treatment was carried out in triplicate. In this experiment, larvae rearing took 15-17 days (Zoea-1 to Zoea-5), metamorphosis from Zoea-5 to megalopa took 5-7 days, and from megalopa to crablet production took 6-8 days. So, the total culture period at the hatchery was 26-32 days.

Water sources and treatments in reservoir tanks

Seawater (salinity 30 ppt) was used in this experiment. It was collected from the sea and transported to the site via a water vessel. From the vessel, the water was pumped through a pipeline into the hatchery water reservoir. Before use, several treatment steps were executed. First, it was stored in a concrete tank reservoir. The water was then chlorinated with calcium hypochlorite powder (available chlorine

65%) with a concentration of 20 ppm and left overnight. The chlorination process was aided by providing gentle aeration to the reservoir. 10 ppm of sodium thiosulfate was used to neutralize the chlorine residue. Once all the chlorine was neutralized, the water was moved to a second reservoir through a sand and charcoal filter tank. After passing through a cartridge filter (0.01 µm), the water in the second reservoir was drained into a third reservoir. The water in the third reservoir served as the final stage of treatment before being passed through a 36 W ultraviolet light (Phillips®).

Live feed culture

The Artemia cyst of Vin Chau was used in this study. Salt water (30 ppt) was filled into the hatching tank (conical form, 40 L) with an air stone before stocking of Artemia cysts. The hatching process was accelerated by placing a 5 W LED bulb 20 cm above the hatching tank. The Artemia umbrella was harvested after 12 h, which was fed until the Zoea-3 (Z3) stage. Artemia nauplii were harvested after 24 h, which were fed from Zoea-4 (Z4) to Megalopa stage. The water valve at the bottom of the conical tank was opened slowly to begin the harvesting process, and a mesh filter (100 µm) was used to filter Artemia umbrella and nauplii. Harvested Artemia umbrella and nauplii were then washed with freshwater before being supplied into the rearing tank. Artemia culture has been done according to the previous methods by Ikhwanuddin et al. (2012; 2013) and Gunarto et al. (2014).

Table 1. Daily room temperature.

Months	Days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
April '24	Temp. (°C)	39	36	38	37	38	38	30	33	36	35	33	36	38	39	40	
May '24		42	40	39	38	39	36	34	32	33	33	35	34	36	36	37	
June '24		35	37	36	37	37	38	39	38	39	39	37	37	37	38	38	
	Days	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
April '24	Temp. (°C)	40	40	40	41	42	41	39	39	41	41	40	41	42	42	43	
May '24		38	40	40	37	35	36	36	37	38	38	32	27	35	36	36	31
June '24		37	38	35	36	37	32	35	35	36	37	37	34	32	34	34	

Collection & management of female crab

Gonad development stage

S. Olivacea mother crabs (weight ranged from 180 - 200 g; n = 60) were obtained from the depots of Cox's Bazar. The live specimens were transported to the hatchery in plastic cages and acclimated to the hatchery conditions by placing them in aerated seawater (30 ppt) in a plastic bowl for 30 minutes. The broods were then placed in another plastic bucket filled with treated seawater (30 ppt) and sterilized with 150 ppm formalin (37% formaldehyde) for a period of 30 minutes with aeration, followed by repeated washing with filtered seawater (30 ppt) to remove residual formalin. They were then placed into individual buckets of 60 L, filled half with filtered seawater (30 ppt) supplied with continuous aeration. Oyster meat as feed was provided twice daily, morning and evening, by hand at approximately 5 to 8% body weight. The water of the buckets was exchanged with clean, filtered seawater in the morning and evening hours routinely. In addition, the uneaten food and excreta were also removed by the time of water exchange. Each of the mother crabs was checked under light for gonadal development; it took 3 to 30 days for gonadal development.

Berried crabs' management and egg maturation

After spawning, the newly berried crabs were disinfected with 150 ppm formaldehyde for 30 minutes. The female was then relocated to a plastic bucket filled with 50 liters of clean, filtered seawater for egg incubation and hatching. Cooked oyster meat was supplied to the berried crabs for feeding in the evening daily. The berried crab-rearing bucket was cleaned every day and exchanged fully with filtered seawater in the evening. Continuous oxygen supply was ensured throughout the incubation period. The embryonic development of the egg was closely monitored visually (Fig. 1) and under microscope to determine the hatching time. Water quality parameters (Temperature: 29.5 -31°C, Salinity: 30 ppt, Dissolved oxygen: 5-6 ppm, and pH: 8.0 – 8.3, Alkalinity: 150 -180 ppm) were monitored regularly before the water exchange following standard methods (APHA, 1992). Hatching generally happens early in the morning (6 to 8 am). After completing hatching, aeration was stopped to turn

off the water flow and allow the larvae to aggregate on the water surface. The successful hatching of berried crabs required 10-12 days after spawning.

Larvae collection & management and stocking

Larvae were collected with a scoop net (0.1 mm mesh size) and placed in a bowl (25 L) filled with seawater, treated with 150-ppm formalin (soaked for 5 minutes with gentle aeration). After disinfection, the larvae were stocked into concrete tanks with 5000L volume of water at a density of 100 larva/L. Different water treatment was done before stocking the larvae in the rearing concrete tank. Erythromycin (3 ppm) was applied as an antimicrobial treatment to the rearing water. Additionally, as an antifungal treatment, Treflan® was used at a dose of 0.05 ppm, which was applied once a week into the rearing water. When the larvae entered the Zoea-5 (Z5) stage, the larvae were transferred to another rearing tank with a density of 10 in./ L by thinning. *S. olivacea* larvae rearing begins at Z1 to the 1st crablet stage (C1). Water exchange was performed every two days starting on the seventh day of the culture period, at a rate of 20%. Electric light was used for the photoperiod system under indoor conditions.

Feed and feeding frequency

Feed was provided manually at different times, following Table 2a, and the feeding rate is given in Table 2b. Live Artemia and two types of processed feed were used in this experiment. The composition of the processed diet is given in Table 2c. Processed food -1 was prepared by measuring the exact weight of the ingredients (Table 2c) and placing them in a can. Then shaken to mix properly and preserved for use. Processed food -2 was prepared by measuring the ingredients with appropriate weights (Table 2c) and placing them in a blender. The food ingredients were then blended properly until it prepared like a solution of condensed milk. The mixture was then warmed for 15 minutes. The mixture was then poured into an Artemia collection net to extract water. After water extraction, the food was preserved in a plastic can and kept in the refrigerator for use. Processed feed-1 was used only from Zoea 1 to Zoea 5 stage, and Processed feed-2 was applied for megalopa and crablet stage.

Table 2a. Feed and feeding frequency of orange mud crab, *S. olivacea* larvae rearing.

Treatment	Feed & Feeding Frequency	
	6.00 & 12.00 am and 6.00 & 12.00 pm	9.00 am and 3.00 pm
01	Artemia	-
02	Artemia	Processed food
03	Artemia	Artemia

Table 2b. Feeding dose in different stages of *S. olivacea* larvae rearing.

Stages	Artemia		Process Food (ppm)	
	Umbrella (Ind/ml)	Nauplii (Ind/ml)	1	2
Zoea-1	1		1	
Zoea- 2	1		1	
Zoea-3	1		1	
Zoea- 4		1	2	
Zoea-5		2	2	
Megalopa		3-5		1-5
Crablet		1-5		1-5

Table 2c. Compositions of processed food.

SL no.	Ingredients	Process Food (%)	
		1	2
1	FriPACK Fresh 1 CAR	30	0.5
2	FriPACK Fresh 2 CD	30	
3	Lancy Shrimp ZM	20	0.5
4	Vitamin BK 505 (Multivitamin)	20	
5	Oyster meat	-	72
6	Shrimp flesh	-	24
7	Egg yolk	-	3
Total:		100	100

Parameters

(1) Larval Stage Index (LSI) LSI is calculated daily as $LSI = ((A1 \times A2) + (B1 \times B2)) / C$

where A1 = amount of previous stage larvae, A2 = previous stage, B1 = amount of highest stage larvae, B2 = highest stage, C = total amount of sample. The larval population was observed every day until the 20 DAH (Days After Hatching) by taking samples of the larvae tank⁻¹. To calculate the LSI in each treatment, the scoring technique is applied to each larval stage,

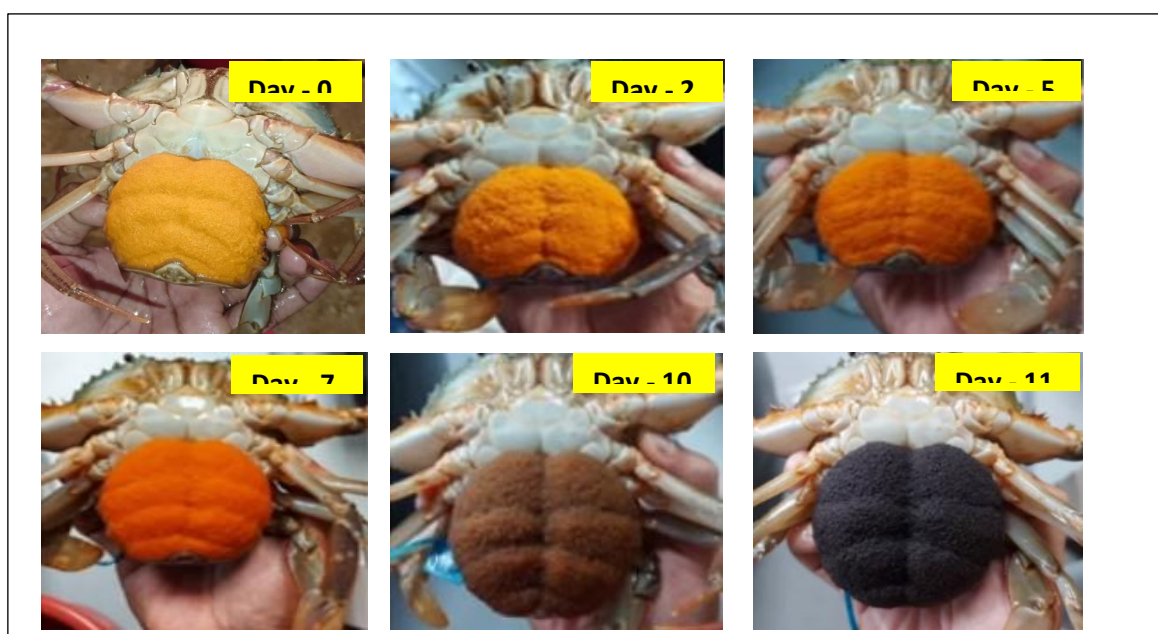


Fig. 1. Visual observation of egg maturation during berried crab rearing.

as follows: Zoea-1 = 1, Zoea-2 = 2, Zoea-3 = 3, Zoea-4 = 4, Zoea-5 = 5, and Megalopa (M) = 6. For example, from the 10 larvae sampled, three larvae were in the Zoea-1 stage; two larvae in the Zoea-2 stage, and five larvae in the Zoea-3 stage.

The LSI value is then calculated using the following equation: $LSI = (3 \times 1 + 2 \times 2 + 5 \times 3) / 10 = 2.2$

(2) Percentage of Megalopa Occurrence Index (MOI) The MOI value was estimated based on the occurrences of Megalopa per 100 individuals of Zoea-5 (Gunarto et al., 2016a; Gunarto et al., 2018). For instance, when there are 10 M in 100 larvae (Zoea-5), then the MOI value is 0.10 (10%). The MOI value was calculated by taking a scoop of 1 L of larvae rearing water from the tank at 20 DAH, then the total number of larvae and Megalopa was counted. The observation was repeated as many as 3 times for each tank.

(3) Daily population density (ind/L) Population density was determined by counting the number of larvae found in 500 ml of larval rearing water. The larvae rearing culture media were sampled using a bowl scoop. At least 3 scoops were done to calculate the mean daily population density.

(4) Survival rate (%) = $\frac{(\text{Number of larvae per liter}) \times (\text{Total volume of water})}{\text{Total number of larvae stocked}} \times 100$

(5) Crablet roduction rate (%) = $\frac{(\text{Number of crablets produced})}{\text{Total number of larvae stocked}} \times 100$

(6) Larvae ize: The size of larvae was measured in mm under a microscope with an eyepiece micrometer.

Data analysis

The descriptive statistical method was used to perform the Analysis of Variance (ANOVA). Data were tabulated and analyzed using the statistical software STAR 2.0.1, while graphs were created using Microsoft Excel version 2010.

Result

Berried and Hatching performance in different room temperatures

Temperature is a crucial abiotic factor for mud crab hatchery operations. The study revealed that room temperature variations substantially influence berried crab production reared from mother crabs. The highest percentage (20.83 ± 2.89%) of berried

crabs is produced at room temperature of 32–39°C in June, followed by 17.50 ± 2.50% in May (27–42°C) and 14.16 ± 1.44% in April (30–43°C) (Fig. 2). Similarly, room temperature variation also influenced the hatching rate of berried crabs. In June, when the temperature range was 32–39°C, the berried crabs performed better in successful hatching, which was 64.55 ± 8.14% (Fig. 2).

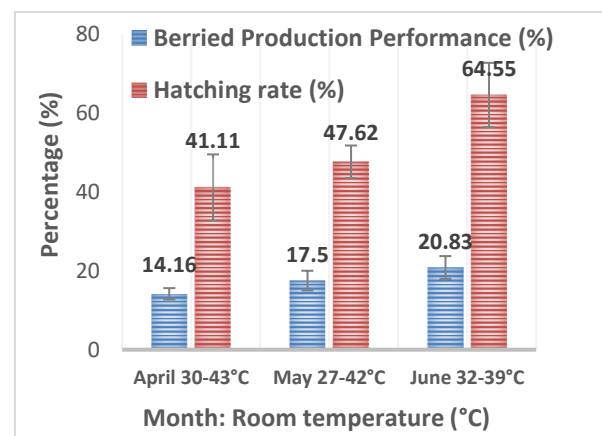


Fig. 2. Berried production and hatching performance in different room temperature.

Zoeal Survivability in different temperatures

The bar diagram represented that the highest survival rate occurred at the room temperature of 32–39°C in June for all the development stages of larvae (Fig. 3). The highest survival rate of Z1 was observed at 69.80 ± 5.17% at the temperature of 32–39°C in June, while the survival rate at room temperatures of 27–42°C and 30–43°C were 63.40 ± 5.68% and 65.20 ± 5.22%, respectively. The survival rate for Z2 was 41.40 ± 4.77% in April (30–43°C), 46.00 ± 5.96% in May (27–42°C), and 49.20 ± 5.45% in June (32–39°C). Z3 survivability was found to be more or less similar in all room temperature variations, but the highest survivability rate (33.40 ± 3.65%) was found in June (32–39°C). The highest survival rate (25.20 ± 3.77%) for Z4 was produced at 32–39°C room temperature, followed by 23.60 ± 4.39% and 19.40 ± 4.39% at the room temperatures of 27–42°C and 30–43°C, respectively. The room temperature in June (32–39°C) produced the highest larval survival rate at the Z5 development stage, which was 19.30 ± 3.11%, whereas room temperatures of 27–42°C and 30–43°C had the survival rate of 16.80 ± 2.77% and 10.20 ± 2.17%, respectively.

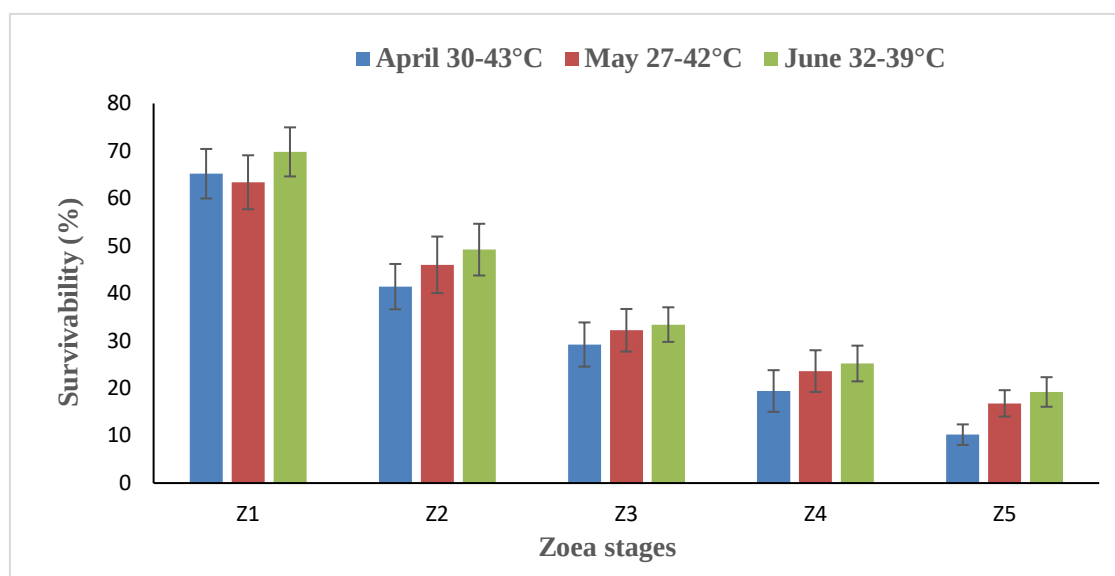


Fig. 3. Zoal survivability rate in different room temperatures.

Survivability at Megalopa and crablet stages

Room temperature variation affected the survivability of the megalopa and crablet development stages. The study found that the highest survival rate at the megalopa stage was $5.20 \pm 1.30\%$ at a room temperature of $32-39^{\circ}\text{C}$. In May, when the room temperature was $27-42^{\circ}\text{C}$, the survivability of megalopa dropped to $4.60 \pm 1.14\%$, and in April ($30-43^{\circ}\text{C}$), the survivability percentage dropped to $3.80 \pm 0.84\%$. Crablets' production rate is also affected by

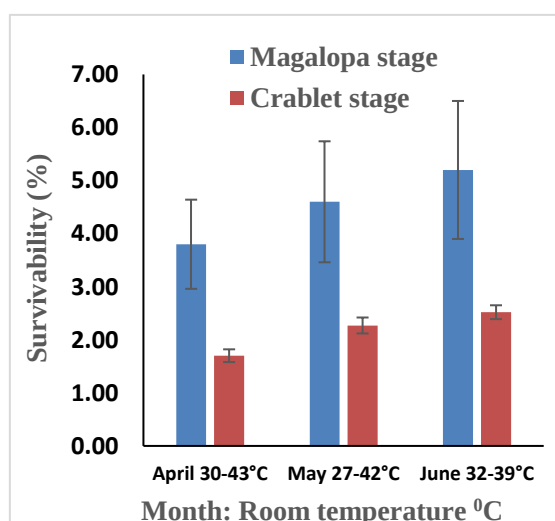


Fig. 4. Survivability at megalopa and crablet stages in different room temperatures.

room temperature variation, and the highest ($2.52 \pm 0.13\%$) percentage of crablets was produced at the room temperature of $32-39^{\circ}\text{C}$ in June. However, in May ($27-42^{\circ}\text{C}$), the crablet production rate was ($2.27 \pm 0.15\%$), and in April ($30-43^{\circ}\text{C}$), the rate was ($1.70 \pm 0.12\%$) (Fig. 4).

Daily survivability pattern and survival rate of larvae in different feeds and feeding frequency

At the first three days, the survival rate was higher in T2 and T3 treatments (Fig. 5) when larvae were fed six times a day. However, when the larvae were fed four times a day, an early mortality was observed in T1 within 24 hours. In the T1 and T3 treatments, where larvae were fed with only artemia, the survival rate suddenly decreased on the fourth day of rearing. Survival rate was observed below 50% in T1 on the fourth day when larvae were fed only four times in a diurnal period. After the fourth rearing day, the mortality was steady for all three treatments, and the highest survival rate was found in the T2 treatment, where larvae were fed six times a day with a combination of artemia and processed food. The survival rate of larvae at different developmental stages under various feeding regimes is shown in Table 3. The survival rate for Zoea-1 varied significantly ($p < 0.05$) among all feeding regimes,

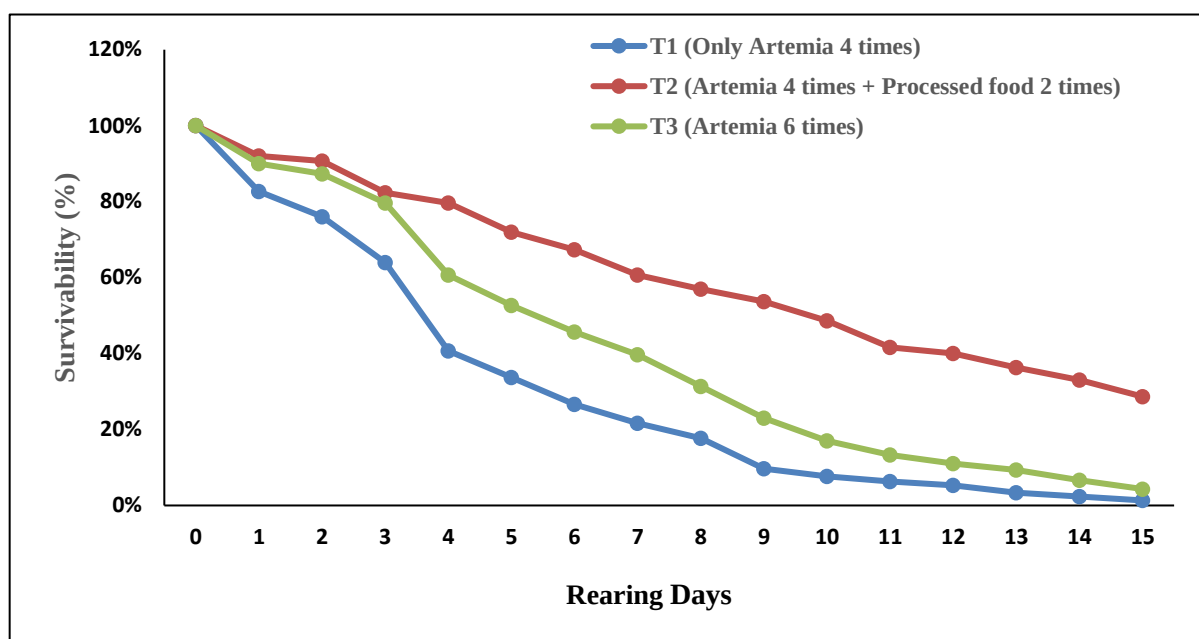


Fig. 5. Daily pattern of survivability (%) of mud crab larvae.

Table. 3 Survivability rate at different larva stages with varying feed and feeding frequency.

Treatments	Zoea Stages					
	Zoea-1	Zoea-2	Zoea-3	Zoea-4	Zoea-5	MOI (%)
T1	76.33 ± 1.52 ^c	40.67 ± 1.52 ^c	17.67 ± 2.52 ^c	5.33 ± 2.08 ^c	1.33 ± 0.58 ^c	3.33 ± 1.52 ^b
T2	90.67 ± 2.08 ^a	79.67 ± 0.58 ^a	60.67 ± 3.21 ^a	41.67 ± 1.52 ^a	33.00 ± 1.00 ^a	15.67 ± 2.08 ^a
T3	87.33 ± 0.58 ^b	60.67 ± 2.52 ^b	31.33 ± 3.05 ^b	11.00 ± 1.00 ^b	4.33 ± 2.31 ^b	4.67 ± 1.15 ^b

MOI = Megalopa Occurrence Index: Superscripts in the results indicate a statistically significant difference ($p < 0.05$).

with the highest survival rate (90.67%) in T2. The survival rates from Zoea-2 to Zoea-5 stages varied significantly ($p < 0.05$) among treatments, with T2 showing the highest values, followed by T3 and T1 (Table 3). Additionally, the percentage of megalopa appearance on day 20 varied significantly ($p < 0.05$) among treatments, with the highest percentage (15.67 ± 2.08) in T2. In contrast, the percentage of megalopa appearances in T1 and T3 were found 3.33 ± 1.52 and 4.67 ± 1.15 .

Daily pattern of Larval Stage Index (LSI)

Trends in the larval stage index (LSI) under different

Treatments are presented in Fig. 6. It is evident that the LSI of larvae was similar up to day two. The LSI then started to differ among the treatments and continued until the fifteenth day. T2 produced the highest LSI values starting on day three, followed by T3 and T1 (Fig. 6). The larval stage index (LSI) at three-day intervals is presented in Table 3. On day three, the LSI values varied significantly ($p > 0.05$) among the treatments, with the T2 treatment having the highest estimated LSI value of 1.67 ± 0.00 , indicating synchronized growth in all replicates. On the sixth day of rearing, the highest LSI value (2.60 ± 0.01)

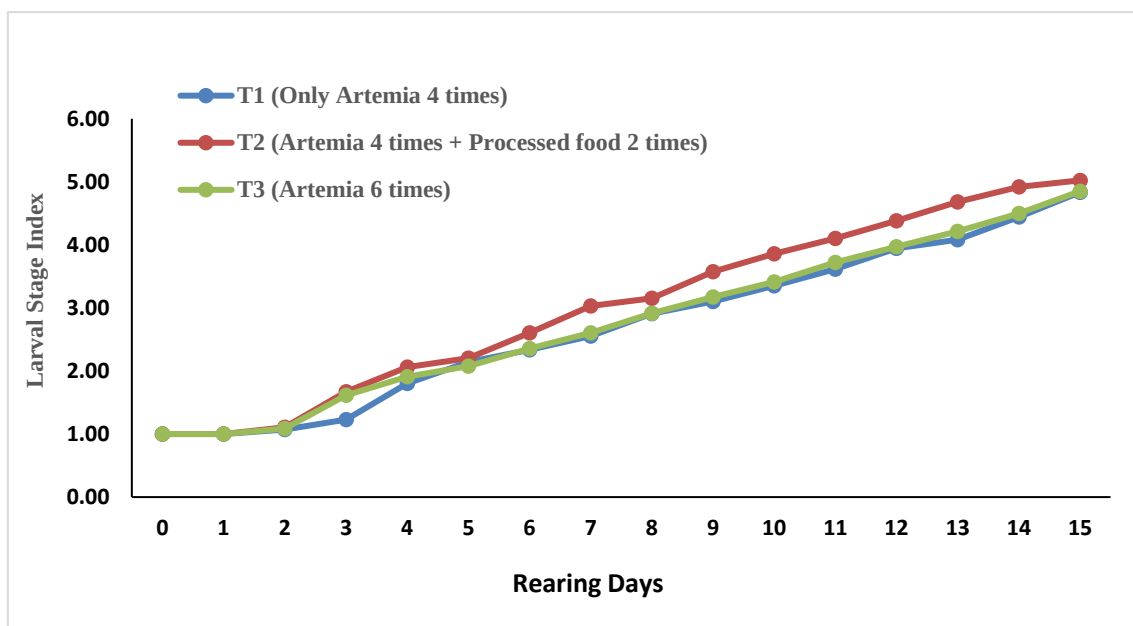


Fig. 6. Daily pattern of Larval Stage Index (LSI) of orange mud crab.

Table 4. Larval Stage Index (LSI) on each 3-day interval.

Treatments	Larval Stage Index (LSI) at each of three-day intervals				
	Day 3	Day 6	Day 9	Day 12	Day 15
T1	1.45 ± 0.03 ^c	2.34 ± 0.02 ^b	3.10 ± 0.10 ^b	3.94 ± 0.09 ^b	4.83 ± 0.28
T2	1.67 ± 0.00 ^a	2.60 ± 0.01 ^a	3.57 ± 0.02 ^a	4.38 ± 0.03 ^a	5.02 ± 0.02
T3	1.62 ± 0.01 ^b	2.36 ± 0.02 ^b	3.17 ± 0.03 ^b	3.97 ± 0.04 ^b	4.85 ± 0.13

Superscripts in the results indicate a statistically significant difference ($p < 0.05$).

was achieved with T2, which was significantly ($p < 0.05$) higher than that of T1 and T3. On the ninth day of rearing, the larvae were metamorphosed to the Z3 stage, and the T2 treatment had the highest LSI value (3.57 ± 0.02), which was substantially ($p < 0.05$) greater than the T1 and T3 treatments. On the twelfth day of culture, the T2 treatment likewise yielded a considerably ($p < 0.05$) higher LSI value (4.38 ± 0.03) than the T1 (3.94 ± 0.09) and T3 (3.97 ± 0.04), indicating faster larval growth. The LSI values were found to be insignificant ($p < 0.05$) among the feeding treatments on the fifteenth day of culture, and the highest LSI value, 5.02 ± 0.02 (Z5 stage), was obtained in T2 (Table 4).

Crablet production rate and crablet size variation

The crablet production rate was varied significantly ($p > 0.05$) among the feeding treatments, and the highest production ($2.90 \pm 2.03\%$) rate was obtained in Treatment T2. However, T1 and T3 treatment produced $0.06 \pm 0.31\%$ and $0.11 \pm 0.01\%$ crablets (Fig. 7). Insignificant variation in larval size from the zoea-1 to zoea-3 stage was observed among treatment but significant variation in larval size in the zoea-4 to zoea-5 stages was recorded. The highest larval size in the zoea-4 stage was found in T2 treatment, and the lowest size was found in T1 treatment. At the Zoea-5

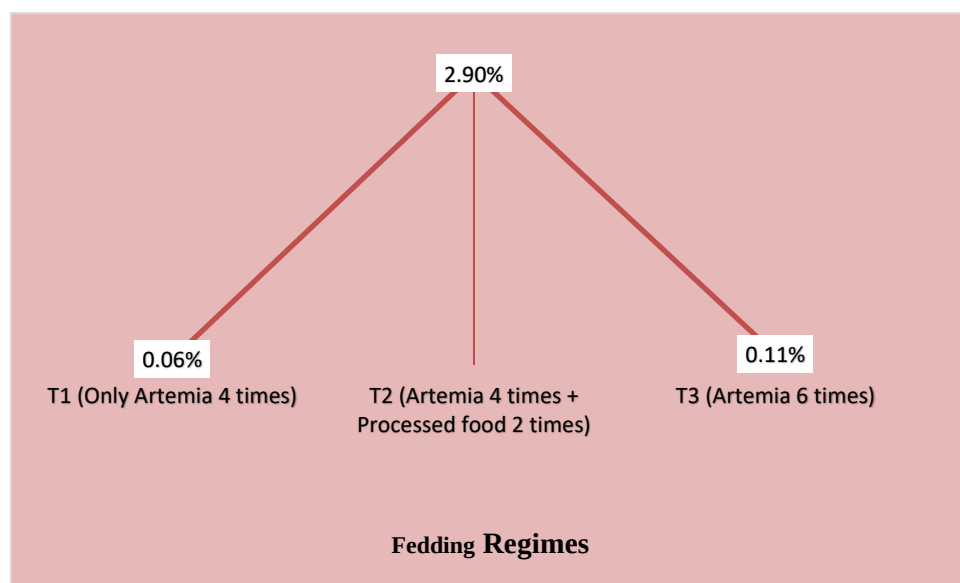


Fig. 7. Crablet production rate in different feeding regimes.

Table 5. Size variation of orange mud crab larvae in different feed and feeding frequencies.

Treatments	Zoeal Size (mm)				
	Zoea-1	Zoea-2	Zoea-3	Zoea-4	Zoea-5
T1	1.30 ± 0.10	1.60 ± 0.10	2.23 ± 0.21	2.44 ± 0.17 ^b	3.40 ± 0.06 ^b
T2	1.47 ± 0.15	1.80 ± 0.26	2.42 ± 0.10	2.94 ± 0.20 ^a	3.69 ± 0.15 ^a
T3	1.37 ± 0.06	1.66 ± 0.15	2.25 ± 0.06	2.51 ± 0.11 ^b	3.46 ± 0.10 ^b

Superscripts in the results indicate a statistically significant difference ($p < 0.05$).

stage, T2 treatment also produced the significantly highest larval size, and T1 produced the lowest larval size. However, in general, T2 treatment performed significantly better in larval size ($p < 0.05$) observed than T1 and T3 treatments (Table 5).

Discussion

Impact of room temperature

Temperature variations substantially influence the development stage of berried crabs and larvae. Room Temperature 32–39°C in June had the maximum development of berried crabs. In contrast, the room temperature of 30–43°C had developed the minimum

number of berried crabs. This means room temperature less than 32°C and greater than 39°C reduces the development of berried crabs reared from mother crabs. Yao (1991) reported that the fastest ovarian development occurs at temperatures ranging from 27.9 to 31.2°C, but ovarian development is inhibited when the temperature is either higher or lower than this range. Likewise, the maximum number of successful hatches was observed at the room temperature of 32–39°C, followed by 27–42°C and 30–43°C. The significantly highest survival rate for all development stages (zoea) of *S. olivacea* larvae was recorded at room temperatures of 32–39°C while the survival rate declined at temperatures of 27–42°C and

30–43°C. There are several works stating that the ideal temperature for survival was 26–30°C (Chen and Cheng, 1985), 25 to 35°C (Zeng and Li, 1992), and 27–30°C (Phuong et al., 2021), respectively. In the current work, the survival rates of megalopa and crablet stages of *S. olivacea* showed the best results at room temperature of 32–39°C. Azra et al. (2018) mentioned that higher temperatures promote active swimming, which leads to lower mortality due to reduced cannibalism impact. Furthermore, the temperature fluctuation was 16 °C, 14 °C and 8 °C in month of April, May and June. These results also indicate that may be low fluctuation of temperature has impact on survival rate at different phase and production of mud crab larvae.

Impact of processed feed

The initial development phases of aquatic faunas' nutritional requirements are essential (Marshall et al., 2010; Hamre et al., 2013; Wang and Jeffs, 2014). Recently hatched mud crab larvae are hungry due to lack of yolk sac; therefore, immediate feeding is required with good nutritional value feed (Li et al., 1999; Djunaidah et al., 2003). Therefore, initial nutritional feeding is crucial to reduce mass mortality and improve effective metamorphosis in different larval phases. Some studies mentioned that live feed with artificial/processed feed consumed by crab larvae (Quinito et al., 1999; Haryati et al., 2014; Gunarto et al., 2018). In these larvae rearing experiments, T2 treatment showed significantly higher survival rate at all developmental stages of *S. olivacea* larvae with the processed feed-1 and feeding frequency compared to T1 and T2 Treatments. These results indicated that feeding artemia with processed feed-1 meets the nutritional demands for survivability and effective metamorphosis to every development phase. A higher survival rate was observed from Zoea-1 to Zoea-3 fed rotifers with artemia in an experiment conducted by Baylon (2009). Furthermore, T2 treatment showed the highest survivability at the megalopa stage, as well as at other larval stages of the mud crab. This result indicates that the processed

feed-2, along with artemia in T2 treatment, may supplemented better nutrition at the Megalopa stage. Syafaat et al. (2019) found a higher percentage of Megalopa when artificial feed was used as a co-feeding. During the metamorphosis from megalopa to crablet, the highest production rate was observed in Treatment T2. This result indicated that the processed food, supplemented with additional nutritional requirements, enhances the growth and survival of larvae. Although the mortality rate is high in this stage compared to Zoea (Z1 to Z5) development phases, which is detected as a vital warning of the total larval achievement. Further research is needed to identify the cause of high mortality in this phase because success of final larval production depends on reducing the mortality rate. Syaffat et al. (2019) emphasized additional feed supply from megalopa to crablet stages. Dan (2015) reported that the poor nutritional status of Artemia was also linked to larval mortality during the transition from megalopa to first crab stages in *P. trituberculatus*. Newly hatched Artemia and starved Artemia had low eicosapentaenoic acid (EPA) levels. Larvae that fed on these Artemia during the late zoeal period (third and fourth zoeal stages) died during the megalopa stage or moulted into unviable first-stage crabs. Yusneri et al. (2021) highlighted that the highest survivability of megalopa was obtained with the feeding frequency of 3 times/day (41.56%) compared to 4 times/day, followed by 2 times/day. However, this study found that the highest survivability of megalopa occurred with the feeding combination of Artemia and processed food, at a frequency of 6 times/day. Effendi et al. (2005) reported that sufficient feed supply should be provided to maintain high survival rate. Gunarto et al. (2018) noticed that artificial commercial feed supplementation at the rate of 1.25 mg/L/2 days caused high vitality of megalopa and more megalopa successfully metamorphosed to the crablet stage. They also reported that the crablet production of *S. tranquebarica* was significantly higher with the supplementation of artificial commercial feed. They

concluded that supplementation of artificial commercial feed could be applied at the dosage range of 0.75-1.25 mg/L/2-days from zoea-3 until crablet (C-7) stage. Furthermore, Holme et al. (2006b) mentioned that nutritional requirements at mud crab hatchery operations are still inadequate. Differences in larval size up to the zoea-3 stage were observed among the treatments. Larval size varied significantly at the zoea-4 and zoea-5 stages (Table 5). The largest size of larvae was recorded in treatment T2. Visual observations indicated sluggish larval swimming in the T1 and T3 treatments. Fungal and protozoan infestations were observed through microscopic examination of samples collected from the T1 and T3 treatments. The microbial infestation may weaken the larvae, hinder normal growth, and cause smaller sizes. However, more research is needed on this topic.

Conclusion

A well developed *S. olivacea* seed production procedure/protocol at hatchery is highly limited in Bangladesh. So, we need to overcome this limitation for the success of newly developed mud crab farming. The results of this experiment revealed that the temperature range (32-39)^oC provided better performance in terms of berried crab production, successful hatching, megalopa, and crablet production. In addition, the supplementation of processed feed supply with artemia and six times of feeding a day (T2) produced the highest larval survivability, megalopa, and crablet. So, mud crab hatcheries are recommended to maintain a temperature of 32–39^oC and provide a combination of processed feed and Artemia six times daily to achieve the highest zoeal survival, megalopa, and crablet production.

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Authors contribution

Md. Masudul Haque: Data Collection, experimental design, data analysis, draft manuscript writing, Anirban Sarker: Research design, data analysis, editing manuscript, Shilpi Saha: Experimental design, data analysis, editing manuscript, Prianka Kundu: Data collection and analysis, Akond Mohammad Rafiqul Islam: Manuscript editing, Shaikh Nazrul Islam: Manuscript editing, and Mohammad Abdul Baki: Research design, data analysis, draft manuscript writing and editing.

Conflicts of interest

The authors declare no conflicts of interest.

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