Stocking density optimization of juvenile mud crab (*Scylla olivacea*) cultivation in bamboo fetched earthen ponds

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Abstract. Mud crab (*Scylla olivacea*) has become one of the most popular and lucrative farming ventures on the south-west coast of Bangladesh. Due to rapid and increasing demand on the world market, a sustainable and scientific management-based culture system is urgently required. Therefore, this study aimed to find out a suitable stocking density (SD) of juvenile mud crab considering an economically viable cultural approach. Three stocking densities were tested for a period of four months in brackish water ponds (500^2 m each): 5000 crabs/ha, 10000 crabs/ha and 20000 crabs/ha under three treatments T-1, T-2, and T-3, respectively. At stocking, the average body weight of juvenile crab was 34.0±2.55 g and they were fed with chopped tilapia (*Oreochromis niloticus*), consisting of 6-8% of standing biomass once a day. The water quality parameters fluctuated but were at the congenial state across the culturing period. The regression analysis revealed that, pH, dissolved oxygen, temperature, and salinity were vigorously interrelated (R^2=0.71-0.88) with the growth performance of *S. olivacea*. However, treatment T-1 gave the significantly ($p<0.05$) best production performance with 75.31±1.14 % survival rate, 1.77±0.015 % specific growth rate, 2.11±0.81 g/ind/day absolute growth rate and food conversion ratio of 2.21±0.51 followed by T-2 and T-3. In fact, total production was significantly ($p<0.05$) higher in T-3 (1994.12± 7.24 kg ha^{-1}) at a SD of 2 crab m^{-2} than that of T-1 (1078.12±5.0 kg ha^{-1}) at a SD of 0.5 crab m^{-2} and T-2 (1546.84±6.54 kg ha^{-1}) at a SD of 1 crab m^{-2} and even highest net benefit was generated from T-3 (BDT 386888±10130) also. But still, benefit cost ration (BCR) was significantly ($p<0.05$) higher in T-2 (0.96±0.02), followed by T-1 (0.84±0.03) and T-3 (0.63±0.02). Therefore, considering a smaller food conversion rate (FCR) along with bigger final weight and SR, the SD of 5000 crablets ha^{-1} is advisable while occupying a higher BCR, and the SD of 10000 crablets ha^{-1} would be economically perfect for monoculture of mud crab in earthen pond.

Key Words: brackish water pond, crablet, economic return, production, specific growth rate.

Introduction. Currently, mud crab (*Scylla olivacea*) has emerged among the top most non-conventional export-oriented fishery item of Bangladesh receiving high economic value and contributing to achieve sustainable development goals. In recent times, mud crab aquaculture has increased promptly, as well as its commercial exploitation, trying to meet global demand and it is predicted to continue to increase (Azra & Ikhwanuddin 2016; Hungria et al 2017). In Bangladesh, crab farming in terms of 'crab fattening' was initiated since the mid-1990s (Azam et al 1998) throughout the south-west coastline following traditional methods innovated by the farmers themselves whilst other farming categories commenced in the 2000s (Khatun et al 2009). Farming practices of mud crab in Bangladesh consists of three strategies: fattening, grow-out and soft-shell production (Rahman et al 2017). However, mud crab aquaculture has been sharply soaring the export earnings of Bangladesh constantly (Ali et al 2004) and executing as an indispensable employment generation sector (Jahan & Islam 2016; Salam et al 2003). In 2018-2019 Bangladesh exported 11435.33 metric tons of mud crab to the international market both in frozen and live state and earned US$ 42.93 million (EPB 2019). In addition, mud crab culture and fattening are sustainable and have promising prospects for the marginal and poor coastal peoples in many Southeast Asian countries (Patterson & Samuel 2005; Rahman et al 2020).

In recent years, Bangladesh shrimp farmers are being dramatically biased to shift to crab farming because of its reduced vulnerability to disease outbreak, simple culture system, and the fact that mud crabs are extremely resistant to hostile culture environments (Zafar et al 2004; Paul & Vogl 2001; Salam et al 2012; Islam et al 2015; Akber et al 2017). Hitherto, no scientifically standard pond management systems are developed for the grow-out of crabs. Consequently, crab farmers use the conventional approach to increase growth and survival without maintaining any definite stocking density (Rahman et al 2017). Alongside, a considerable amount of mortality occurs during stocking due to quality seeds, handling stress and deficiency of best management practices (Rahman et al 2020) thereby hampering production and income in crab farming systems. Productive, efficient, and cost-effective technology are prerequisite for sustainable and feasible mud crab culture. Adequate and timely supply of production inputs such as crablets optimum stocking density, feed and feeding technology, sound health and water quality management protocols are also needed for standardization of crab farming approach. Most of these requirements can be made available through research (Cholik 1999). Other than this, Bangladesh Fisheries Research Institute, Brackishwater Station have recently artificial bred *S. olivacea* with a satisfactory level of survival (7.5%) in hatchery condition (unpublished data), and this will certainly disclose a new dimension to expand mud crab aquaculture in Bangladesh (Ahmed 2019). In this backdrop, this experiment was conducted to know the productive and reasonable stocking density for cultivation of mud crab (*S. olivacea*) and their economic feasibility in brackish water pond rearing.

Material and Method

Experimental venue and duration. The study was executed in twelve on-station ponds of Brackishwater Station, Bangladesh Fisheries Research Institute (BFRI) for a period of four months from February to May 2020.

Research design. Monoculture of mud crab comprising of three treatments (T-1, T-2, and T-3) were tested with the stocking density (SD) of 5000 crablets/ha, 10000 crablets/ha and 20000 crablets/ha, respectively. Each treatment had three replications where pond size was maintained at 0.05 ha each.

Pond preparation. Initially, after renovating the dyke and monk, the ponds were sun dried till the bottom soil cracks. Each pond was prepared by encircling it with split bamboo slits, locally called "bana", and nylon net. The bamboo slits were pressed into the mud up to 50-60 cm of depth to prevent the escape of crabs. The inner part of each pond embankment was enclosed with bamboo fence covered with nylon net, one meter apart from the periphery of water. The bottom of all the experimental ponds were treated with 250kg/ha of lime (Quicklime: Dolomite 1:1) then filled with water from the adjacent river Kapataksha during high tide up to 1.0 m of depth. The pond's unwanted biota was removed by applying phostoxin, using 1 tablet/20 tons water. After 10 days of phostoxin applications, the ponds were fertilized with urea and triple superphosphate (TSP) using 25 and 30 kg/ha, respectively for natural productivity. Over the culture period, pond water depth was maintained at least one-meter level.

Stocking of crab and feeding. Required quantities of seed crab weighing approximately 34g/ind was collected from crab catcher of Sundarban mangrove area. They were then acclimatized with pond water in a plastic bowl to avoid physico-chemical stress. Crabs were unbiasedly selected to avoid stocking size variation among the treatments and were stocked after 3 days of pond fertilization according to the design above. The young crab was fed with low cost chopped tilapia (*Oreochromis niloticus*) consisting of 6-8% of the biomass daily (Wedjatmiko & Dharmadi 1994) and feed quantities were adjusted at each 15 days interval, considering growth performance.

Monitoring and sampling. Growth of crabs along with any other external fouling/abnormalities was monitored on a fortnightly basis. Carapace length and width were measured by slide calipers. Water quality parameters like, temperature, salinity, pH, dissolved oxygen (DO), transparency and alkalinity were periodically observed and recorded at every 15 days interval according to the standard methods of APHA (1992).

Harvesting and data collection. After four months of grow-out period, all crabs were harvested through scoop net, collecting through "thopa" (line with baits) and finally by hand picking (Christensen et al 2004) after pond drying. The production performance data such as total production, stocking and harvesting weight, net weight gain (NWG), absolute growth rate (AGR), specific growth rate (SGR), survival rate (SR) and feed conversion ratio (FCR) were computed following the equations suggested by Castell and Tiews (1979), Hopkins (1992) and Goddard (1996). Total operational cost and farm gate price including total return, net benefit, and benefit cost ratio (BCR) from each treatment were calculated and analyzed to compare profitability and economic feasibility following the method stated by Shang (1990).

Statistical analysis. After compilation, the final data were statistically analyzed by Microsoft Excel-2010 and SPSS Statistics version-20 and data was expressed as mean ± standard deviation (SD). Linear regression analysis was performed to know the relationship among hydro-biological parameters and growth. One-way analysis of variance (ANOVA) and Duncan multiple range test, DMRT (Duncan 1955) was carried out to compare the treatments and significance level was assigned at 5% ($p>0.05$).

Results

Water quality. The fluctuation of different hydro-biological parameters over the 120 days of culture period are presented in Figure 1. However, the recorded temperature in all the treatment ponds ranged from 28.2±0.12°C to 35.2±0.52°C. Water pH was nearly alkaline and basically similar and satisfactory throughout the experiment ranging from 7.9±0.14 to 8.64±0.81. A sufficient range of DO levels from 4.53±1.3 ppm to 6.79±1.32 ppm was marked beyond any significant variation among treatments. The mean water transparency in all the treatments was noted in the range between 30±7.0 cm and 83±8.5 cm. The recorded alkalinity varied between 122±6.43 ppm and 265±9.0 ppm in different treatments which was also fitted with convenient alkalinity range for culturing of crustaceans like *S. olivacea*. A wide range of salinity was observed (4.5-15 ppt) over the experimental period with its lowermost in March and higher margin in late June.

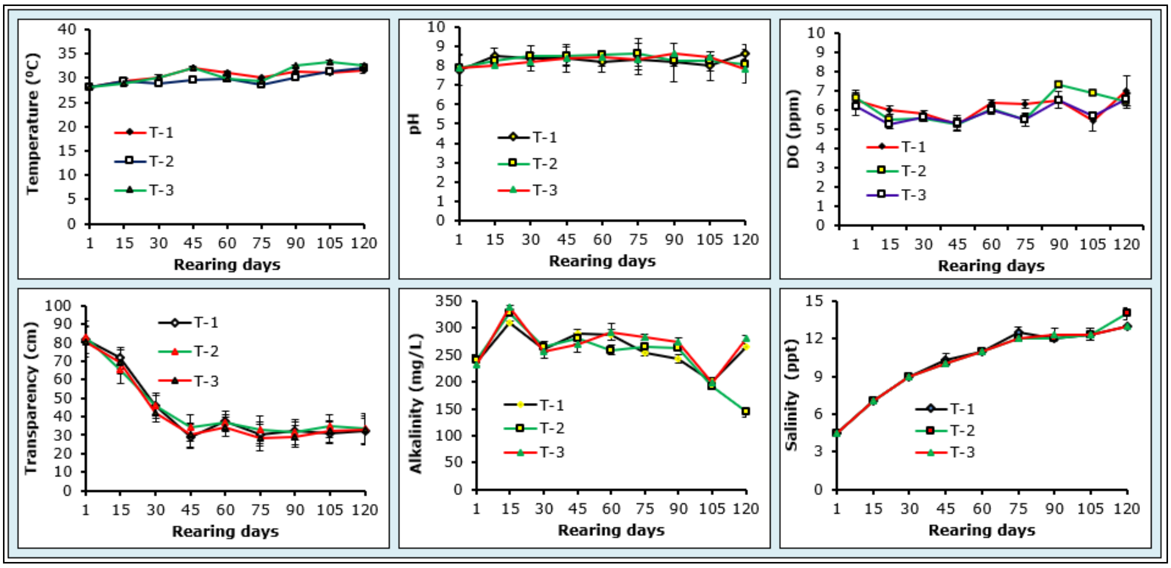

Figure 1. Trends of water quality variables during the culture period under different treatments.

Growth and production performance of mud crab (Scylla olivacea). The growth progression of crab in term of ABW (Average Body Weight) at each fortnightly sampling under different stocking densities are plotted to show the growth curves in Figure 2. Up till 45 days, growth increment was similar in all treatments after that a faster growth was observed which was significantly ($p<0.05$) higher in T-1 followed by T-2 and T-3 (Figure 2). The different production parameters are furnished in Table 1. The results of the ANOVA test revealed that stocking density significantly influenced the production parameters like final body weight, NWG, AGR, SGR, SR and FCR ($p<0.05$). The highest increment of final weight was observed in T-1 (287.50±5.10 g) bearing the stocking density of 5000 crabs/ha and was the lowest in T-3 (247.41±2.57 g) and significant differences ($p<0.05$) were found amidst all treatment. No significant differences were found between T-1 and T-2 in case of final carapace length, but it differed significantly ($p<0.05$) when compared to T-3. However, juvenile crabs in T-1 under a SD of 5000 crabs/ha showed the highest final carapace width (94.30±1.09 mm) along with maximum weight gain and was significantly different ($p<0.05$) from T-3, but indifferent with T-2.

In terms of AGR, T-1 was quite different from T-3 but indifferent with T-2. Similarly, no significant ($p>0.05$) difference was marked within T-2 and T-3 but apparently difference was seen in size and individual weight among the treatments. Apart from AGR, NWG, SGR, SR and total production were significantly different ($p<0.05$) from each other across the treatments. SGR in T-1 (1.77±0.015 %) was remarkably higher compared to other two treatments and displayed the lowest FCR (2.21±0.51). After all, best NWG (250.32±4.32 g), AGR (2.11±0.81 g), SGR (1.77±0.015) and FCR (2.21±0.51) were also recorded in T-1 where the smallest number of crabs was reared. In line with this, highest survival rate (75.31±1.14 %) was also recorded in T-1 followed by T-2 (55.97±1.98 %) and T-3 (40.31±1.23 %). Though, highest total production was achieved in T-3 (1994.12±7.24 kg/ha), but literally production of T-1 (1078.12±5.0 kg/ha) at 5000 crabs/ha SD seems to be more viable due to its lower FCR value than that of T-2 and T-3.

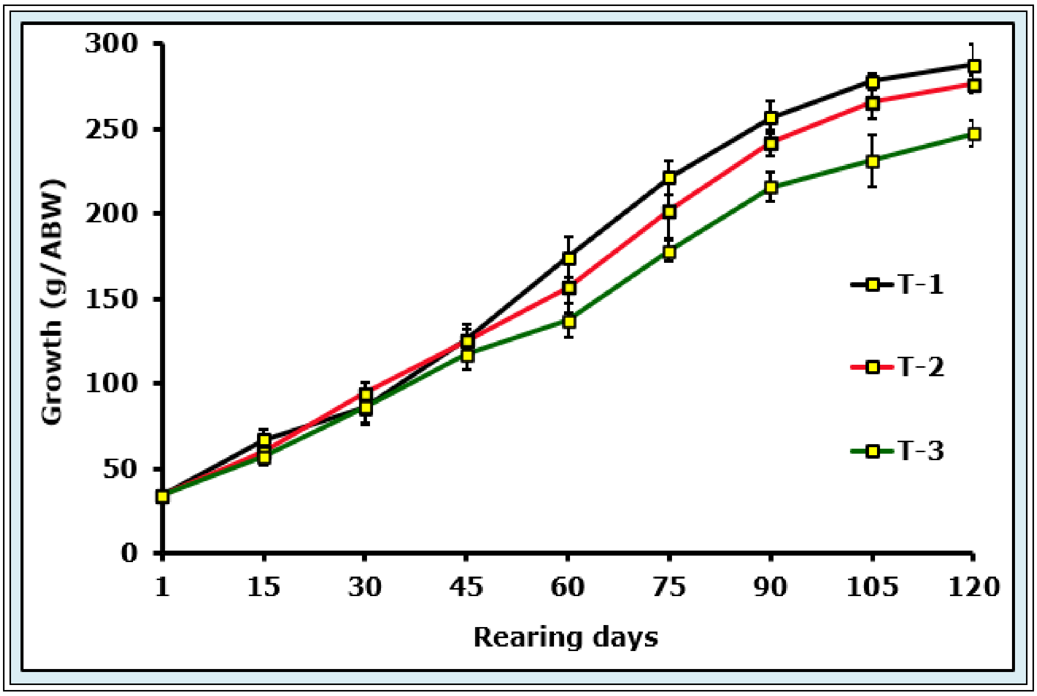

Figure 2. Growth curves of mud crab (*S. olivacea*) grown in pond culture system under different stocking densities.

Table 1
Production parameters of *S. olivacea* after 120 days of culture under different stocking densities

Parameter	*Treatment*		
	T-1	*T-2*	*T-3*
Initial carapace length (mm)	38.00±1.60	39.1±2.05	38.66±2.61
Final carapace length (mm)	71.40±2.00[a]	67.35±2.74[a]	61.41±3.49[b]
Initial carapace width (mm)	53.70±3.20	53.73±2.47	54.10±2.07
Final carapace width (mm)	94.30±1.09[a]	91.00±1.39[ab]	88.00±2.25[b]
Initial body weight (g)	34.00±2.55	34.00±2.55	34.06±2.55
Final body weight (g)	287.50±5.10[a]	276.37 ± 3.41[b]	247.41±2.57[c]
NWG (g)	250.32±4.32[a]	240.20±3.98 [b]	211.35±2.08[c]
AGR (g/crab/day)	2.11±.81[a]	2.01±0.98 [ab]	1.76±0.53[b]
SGR (%)	1.77±0.015[a]	1.69±0.013[b]	1.60±0.014[c]
SR (%)	75.31±1.14[a]	55.97±1.98[b]	40.31±1.23[c]
FCR	2.21±0.51[a]	2.97±0.75[b]	4.69±0.56[c]
Production (kg/ha)	1078.12±5.0[c]	1546.84±6.54[b]	1994.12± 7.24[a]

Note: numbers on the superscript followed by the same letter are not significantly different ($p>0.05$) and a>b>c>d.

Relationship of Scylla olivacea growth with different physico-chemical parameters. The relationship of growth over the culturing time with pH, DO, temperature, transparency, and salinity fluctuation are presented in Figure 3 (A, B, C, D, E and F). The regression analysis represents that, strong relation was existing between growth and pH where $R^2=0.78$ (Figure 3-A) thereby implies that pH may have significant effect on mud crab growth. Similarly DO level also actively impacted on the growth of mud crab ($R^2=0.83$) suggesting a very high positive correlation (Figure 3-B). Beside this, a highly positive correlation ($R^2=0.71$) was observed between temperature and growth (Figure 3-C). Since transparency is a measuring tool of primary productivity and inversely related to each other, in Figure 3-D, transparency showed moderate and inversely positive correlation ($R^2=0.62$) with the growth of *S. olivacea*. More importantly, a vigorous positive relationship was viewed among wide fluctuations in salinity and growth of mud crab with a R^2 value of 0.88 (Figure 3-E). As a result, it can be said that growth of cultured shellfish continuously increased in the same rhythm with the increasing effect of salinity level. On the contrary, a negligible correlation ($R^2=0.22$) was observed between alkalinity and growth of *S. olivacea* (Figure 3-F).

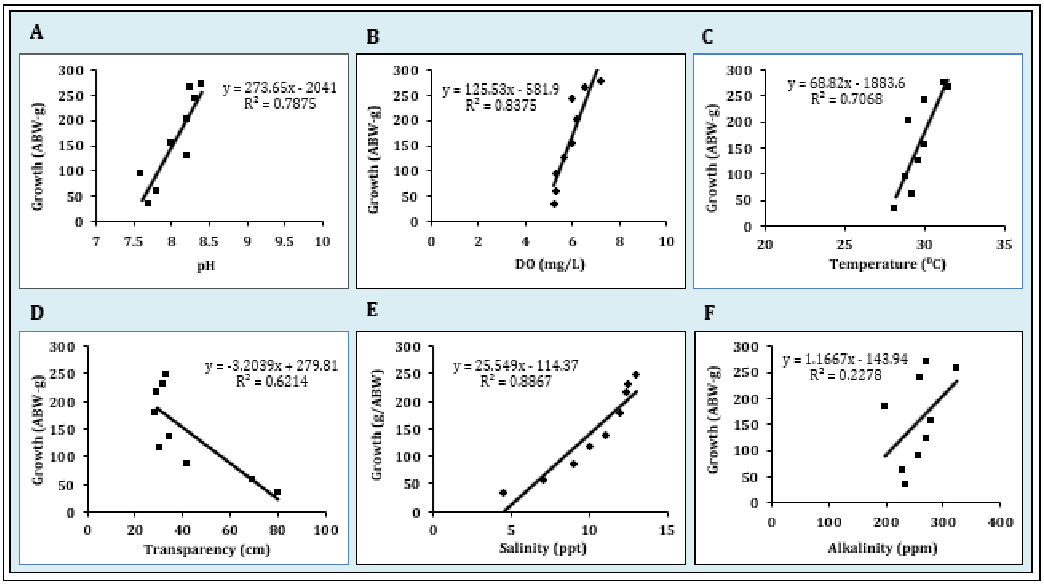

Figure 3. Scatter plot for relationship of mud crab growth with pH (A), DO (B), temperature (C), transparency (D), salinity (E) and (F) alkalinity in different treatment.

Investment, net return, and benefit. The economics of mud crab culture after 120 days are furnished in Table 2. Total cost of production in T-1, T-2 and T-3 was estimated in Bangladesh Taka (BDT) at 292029, 394693 and 610172, respectively. However, highest net return (BDT 997060) and benefit (BDT 386888±10130) was obtained in T-3 at SD of 20000 crabs/ha followed by T-2 (BDT 773420 and 378727±8450) at a SD of 10000 crabs/ha and T-1 (BDT 539060 and 247031±8550) at a SD of 5000 crabs/ha and they were significantly ($p<0.05$) different from each other. Interestingly, higher BCR value was found in T-2 (0.96±0.02) and T-1 (0.84±0.03) which carried a lower stocking density, though net benefit was lower than T-3. Therefore, considering economic point of view T-2 SD then T-1 SD would be more feasible to practice the culture of mud crab in brackish water ponds. It is important to note that, total cost of production is inferring a bit high in all the treatments due to the big amount of investment for fencing, which generally lasts for 4-5 years. As a result, net benefit must be much higher in crop after first cultural cycle and following years since total cost of production will be extensively decreased.

Table 2

Costs and returns from the culture of *S. olivacea* in 1ha earthen ponds for a period of 120 days

Line item	*Treatment*		
	T-1	*T-2*	*T-3*
a. Variable cost (BDT):			
Fencing	77000	77000	77000
Price of juveniles (230/kg)	36400	72720	145450
Feed (30.00/kg)	71479	137823	280572
Fertilizer, lime etc.	4150	4150	4150
Labor cost (400/day)	28000	28000	28000
Chemicals (Kit)	3000	3000	3000
Total variable cost (TVC)	220029	322693	538172
b. Fixed cost (BDT):			
Two caretakers wage (300/day)	72000	72000	72000
Total fixed cost (TFC)	72000	72000	72000
Total cost (TC=TVC+TFC)/ha	292029	394693	610172
c. Total return (TR)/ha	539060^{c}	773420b	997060^{a}
d. Net Benefit (TR-TC)/ha	247031 ± 8550^{c}	378727 ± 8450^{b}	386888 ± 10130^{a}
e. BCR	0.84 ± 0.03^{b}	0.96 ± 0.02^{a}	0.64 ± 0.02^{c}

Note: numbers on the same row followed by the same letter are not significantly different ($p>0.05$) and a>b.

Discussion. This study attempted to optimize stocking density of *S. olivacea* cultured in brackish water ponds and to assess their economic feasibility. It is evident that, poor technical knowledge, and management strategy of crab farmers (e.g., stocking of heterogeneous sizes of crabs, mixed-sex culture, evasion from ponds, cannibalism) often resulted in overall production loss in crab farming (Sulaeman & Hanafi 1993; Shelley 2008). Indeed, water quality parameters are immensely important for the well-being of aquatic organism such as-oxygen ingestion, absorption, reproduction, development, moulting, hormone production, phagocytosis and salt water balance that exert optimum growth, survival and production of fish and shellfish, including crab (Handeland et al 2008; Abbink et al 2012; Gao et al 2011; Chakraborty 2018).

However, physico-chemical parameters of all experimental ponds water were found within the acceptable level preferred for grow-out of mud crab in brackish water ponds. In this research, the pH was found to be (7.9±0.14 - 8.64±0.81) tending to an acid environment favorable for mud crab growth which coincided with the research findings demonstrated by Cholik and Hanafi (1988) and Mia et al (2007). DO level (4.53±1.3 - 6.79±1.32) in the rearing ponds during the 120 days culture period were similar with the FAO (2011) standards (>5 mg/L) for crabs. The optimum alkalinity for the growth performance of mud crab is above 80 ppm and the best suggested was 120 ppm (FAO 2011). Meanwhile, the alkalinity range was recorded in the present study from

122±6.43 to 265±9.0 ppm which was close to the results of Kurnia et al (2017). The recorded water temperature in the experimental pond was from 28.2±0.12°C to 35.2±0.52°C and are believed to be conducive for the growth of crab as it is very much identical with the reported range (5-30°C and 28-33.5°C) by Cholik and Hanafi (1992) and Saha et al (2000). Regarding the salinity results, a vast fluctuation was seen over the cultural duration (4.5 to 15 ppt) which is thoroughly harmonious as mentioned at 3 to 16 ppt by Mia et al (2007) whereas Hastuti et al (2015) suggested an optimum salinity o 25 ppt for the good growth performance of mud crab. Furthermore, Bhuiyan and Islam (1981) and Le Vay (2001) narrated those crabs (*Scylla* sp.) are remarkably climate adjusted and extremely tolerant of salinity as well as temperature deviation, capable of persisting in salinities from 2-50 ppt and temperatures from 12°C to 35°C.

Determination of stocking density is the capital importance in mud crab production, as it is interrelated to the competition for both feed and space, which may lead to cannibalism and escapement that significantly affects the survival rate (Hastuti et al 2017; Liong 1992). In the present study, significant differences were observed among treatments in terms of survival percentage ($p<0.05$). The mean survival was marked lowest (40.31±1.23%) in T-3 at a SD of 2 ind/m^2 followed by T-1 (75.31±1.14%) at 0.5 ind/m^2 and T-2 (55.97±1.98%) at 1 ind/m^{-2}, that could be a consequence of the highly cannibalistic nature of crabs. As a strategy, farmers are commonly adapting the stocking density, feeding ratio, or the rear crabs individually, to overcome cannibalism (Laranja et al 2010; Zhao et al 2015). More importantly, stocking densities (0.5 crabs/m^2, 1 crab/m^2 and 2 crabs/m^2) specified for this study were within the range recorded by Chong (1993), Trino et al (1999) and Mia et al (2007).

According to Sartje (2010) growth is regulated by both internal factors (i.e., genetics and immunity), as well as external factors (environment and availability of food in the habitat). Based on Table 1, T-1 was significantly ($p<0.05$) different from T-3 but indifferent with T-2 in AGR where the best one was 2.01±0.98 g/crab/day in T-1. It is plausible that, growth increment in crustaceans/shellfish should be asynchronized as stated by Latiful (2020) and that might be the probable reason for significantly ($p<0.05$) different SGR among the treatments. Our ANOVA results revealed that stocking density had significant effect ($p<0.05$) on the FCR, and T-1 had the best FCR (2.21±0.51), than that of T-2 (2.97±0.75) and T-3 (4.69±0.56), also indicating that the given feed was efficient in generating higher body mass in T-1 and T-2. In such a way, lower FCR value is collectively supporting an elevated survival rate (75.31±1.14 %) and final body weight (287.50±5.10 g) in T-1 (Table-1). Though highest production was obtained in T-3 (1994.12±7.24 kg/ha), considering smaller FCR of T-1 along with its highest survival and growth rate, the SD of 5000 crabs/ha would be more efficient for the monoculture of *S. olivacea* in brackish water ponds and these results are strongly coincided with findings of Trino et al (1999) and Shelley and Lovatelli (2011). Again, average final weight gain in T-2 (276.37±3.41 g) of this study is a bit higher that obtained in Indonesia (146 g) with same culture period and stocking density (Allan & Fielder 2004). A former study (Chakraborty et al 2018) observed highest growth (252.70±3.01 g), survival rate (64.10±2.14%) and production (4783.44±6.94 kg/ha) of mud crab at a SD of 2.5 crabs/m^2 after culturing for 135 days in the southwest coastal region of Bangladesh and these higher productions could be due to higher SD and longer cultural extent of time. On the contrary, Baliao et al (1981) reported a production of 891.12 kg/ha with a SR of 52% and 947.67 kg/ha with a SR of 31% at a SD of 1 crab/m^2 and 2 crabs/m^2, covering a 90-day culture period which is showing lower results than the obtained results of this study. Moreover, when AGR trends (2.28 g, 1.89 g, and 1.69 g) with their respective stocking densities are compared it seems very much consistent with our downward AGR trends (2.11±0.81 g, 2.01±0.98 g and 1.765±0.53 g) which mean that if they could extend the culture period for 120 days and the total production might be closely similar with the present study. Meanwhile, production of mud crab in monoculture of the present experiment was higher compared to average production (1196 Kg crabs/ha) revealed by FRSS (2018) in Bangladesh.

The growth had no strict interrelation with all physico-chemical parameters. However, the pH, dissolved oxygen level and temperature had highly strong influence on growth holding the R^2 value of 0.78, 0.83 and 0.71 (Figure 3, A, B & C) respectively by acting as a catalyst for shell formation and moulting. Again, a moderately positive relation (R^2=0.62) was found among growth and transparency (Figure 3-D). More importantly, increasing salinity trends had extensively affected the growth in a rhythmical way with a R^2 value of 0.88 (Figure 3-E). Regarding alkalinity, negligible correlation (R^2=0.22) was observed with increasing growth trends of mud crab (Figure 3-F).

Regarding return and net benefit analysis (Table 2), consistently higher net benefits were achieved from T-3 (BDT 386888±10130) than T-1 (247031±8550) and T-2 (BDT 378727±8450) and was also significantly different ($p<0.05$). Nevertheless, BCR of T-2 (0.81±0.02) with a stocking density of 10000 crabs/ha are significantly higher ($p<0.05$) than the BCR value of T-1 (0.84±0.03) at a SD of 5000 crabs/ha and T-3 (0.64±0.02) at 20000 crabs/ha are vividly implying that, lower stocking density would be economically viable for crab farmers.

By and large, depending on higher growth, survival and smaller FCR in treatment T-1, with a SD of 5000 crabs/ha can be appreciated as viable for the marginal farmer whose investment capacity is limited. Furthermore, a superior BCR at 10000 crabs/ha is generating a robust net benefit compared to other two stocking densities. In that sense it can fairly be suggested that lower SD, particularly 1 crab/m^2, would be suitable and economically viable for the cultivation of *Scylla olivacea* in brackish water pond systems.

Conclusions. Obviously, culturing mud crab in ponds is difficult due to their highly cannibalistic nature. However, this study was performed based on the current need of Bangladesh and the overall findings conclude that a lower stocking density of 10000 crabs/ha might be practiced efficiently to culture mud crab (*Scylla olivacea*) in earthen ponds to maximize profitability. It is obvious that, advanced culture technology should be addressed in the future i.e., culturing crabs in individual containers, or various forms of recirculation systems, are promising ways to abate excessive cannibalism, which will certainly lead to increased production.

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Conflict of Interest. The authors declare no conflict of interest.

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