

CHAPTER-I

INTRODUCTION

Aquaculture is a vibrant sector throughout the world as it produces high quality protein, nutritious and delicious food. Its impact on the human nutrition has been fully estimated and the demand for other aquatic food is expecting to grow even higher in the upcoming years (FAO, 2017). In 2014, fish harvested from aquaculture amounted to 73.8 million tons and the world capture fisheries production has been relatively stable for the past decade. Recent challenges in aquaculture like pollution, spread of disease, antibiotic use, shortage of quality fish seed, quality feed and irregular environmental events makes a threat to the aquaculture industry. On the other hand, changes in the human taste and demand for non-conventional aquatic food products are increasing day by day as the world fish stock is declining continuously. Climate change due to global warming increases the mean sea level consequently intrusion of saline waters in the freshwater regions is one of the key environmental issues throughout the world (Coll, 2008 and Worm, 2006). Effect of sea level rise, salinity intrusion, changing temperature and precipitation regimes, more frequent weather events and coastal erosion is experienced all around the world (IPCC, 2007).

Salinity intrusion is one of the key environmental issues in Bangladesh as the coastal area covers 32% of the country's total and is a habitat for 35 million people (Huq and Rabbani, 2011). Bangladesh coastal aquaculture is based on shrimp and prawn. Shrimp production in inland and marine water bodies of Bangladesh is 2, 34,188 MT which consists of 6.04 percent of the total fish production (Yearbook of Fisheries Statistics of Bangladesh, 2015-2016). However, decreased price, international market competition and impact of climate change have reduced production of shrimp (Chowdhury and Muniruzzaman, 2003; Karthik *et al.*, 2005; Nuruzzaman, 1996 and Primavera, 1995). Recent outbreaks of shrimp diseases resulted in the dramatic decline in the production and subsequently affect the livelihood of coastal communities (Faruque *et al.*, 2008). Besides, crop production has also been negatively affected across the coastal belt for many decades due to salinity problems (Clarke *et al.*, 2015). So, it is high time to find an alternative livelihood options for the coastal community and crab farming could be the prime candidate for the coastal community of Bangladesh.

Shrimp farmers are now changing to crab farming (Hussain, 2014; Islam *et al.*, 2005; Uddin *et al.*, 2013) as it is less susceptible to disease, easier to culture, more resistant to adverse environmental conditions and has a high commercial value and market price both locally and internationally. Under these circumstances, mud crab production is triggered as the first choice of that people because they can easily grow in wide range of salinity level (Chandra *et al.*, 2012; Zafar and Siddiqui, 2000). Crab farming can provide a new source of income for coastal fisher folk (Khan and Alam, 1992) and may become the second-most exported crustacean from Bangladesh. According to Export Promotion Bureau (EPB), Bangladesh started exporting crab in 1977 and the first consignment was worth \$2,000 only. Crab exports grew over the years to 2,973 tons in 2008-09 Fiscal Year, 4,416 tons in 2011-12 FY and 8,520 tons in 2013-14 FY. Crab exports fetched \$22.91 million in 2013-14 FY (Export promotion bureau, 2001, 2014). Mud crab production appears to have a bright future both in creating employment opportunity and foreign exchange earnings (Jahan and Islam, 2016).

Mud crab (*Scylla sp.*) is one of the most popular sea-food species in many parts of the world particularly in the south-east Asian countries. 90-99% of the total mud crab is produced in Asia from the year 1974 to 2005 (FAO, 2006). So, it has become a great challenge for the Asian countries like Bangladesh, India, Thailand and Philippine along with China to regain the past glory (Islam *et al.*, 2017). The mud crab is widely distributed throughout the coastal zones of the Indo-Pacific region (MacNae, 1968). They represent a valuable component of traditional, small scale coastal fisheries in several tropical and subtropical Southeast Asian countries which stands as a significant commodity that fetches a high price in the international seafood market (BOBP, 1992). There are thirteen marine and three fresh water species of crabs reported from the coastal areas of Bangladesh. Among them, six important genera that are used as food crabs are *Scylla*, *Portunus*, *Charybdis*, *Matuta*, *Varuna* and *Sartorina*. Generally, mud crab (*Scylla olivacea*) is a commonly cultured species in Bangladesh whereas some other species such as *Scylla tranquebarica*; *Scylla paramamosain* and *Scylla serrata* has also the culture potentiality (Jahan and Islam, 2016). Moreover, mud crab is a promising aquaculture species due to its fast growth and good market acceptability and price worldwide due to its low fat, high protein, vitamin and mineral content and taste.

Soft shell crab is a culinary term that is used for crab that have recently shed their old skeleton and are still soft. As like shrimp and other crustaceans, crabs grow by shedding their old skeleton and replacing with a new one through a periodic manner (Chang and Mykles, 2011). A crab usually molts 20-25 times in its life span (Gaude and Anderson, 2011) and in each molting it increases about 30-40% of its body weight. Traditionally crabs are sold in whole pieces without extracting their meat and usually eaten with or without extracting their exoskeleton (Oesterling, 1988). However, if captured soon after molting, as they remain soft without their exoskeleton, the entire body can be consumed without extracting meat which makes the soft-shell crab production as a more profitable business (FAO 2015; He, 2015; Perry *et al.*, 2010). The crabs are harvested before they start to make exoskeleton hardening and are sold at higher prices (Mwaluma, 2002; Primavera *et al.*, 2010).

Moulting is a systematic physiological process of crab, through which it enhances the physical growth. Before molting crab takes shelter to protect itself from the predatory attack. On that moment of moulting crabs remain the most vulnerable. Generally, moulting depends upon the lunar cycle and crabs usually moult during the full moon or new moon.

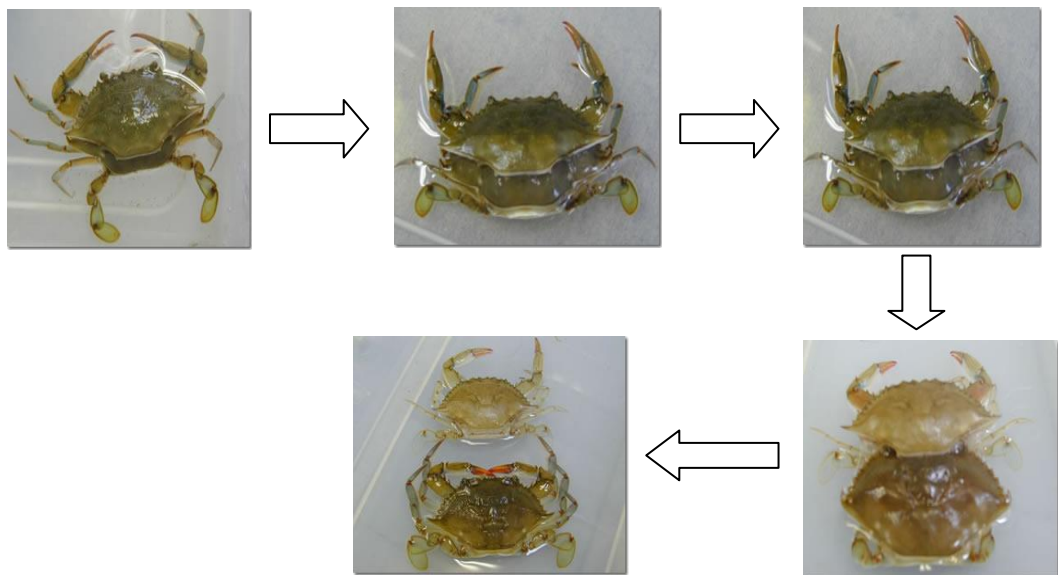


Figure 1: Moulting steps of crab

Soft shell crab farming is totally a new technology. This culture techniques is currently practiced in countries like China, Thailand, Philippine, Myanmar, India and Vietnam. Recently, this culture technique has been introduced commercially in small

scale in Khulna and Cox's Bazar regions of Bangladesh. Technologies regarding the soft shell crab culture system are still very poor and research about production strategies would increase the rate of production of soft shell mud crab over the time. As an influencing factor, claw removal of crab would be a possible strategy to increase the production of soft-shell mud crab. It is reported that, eye stack and claw ablation in several crustacean species gives better production and growth (Majid *et al.*, 2008; Shivakumar, 2008; Sagi *et al.*, 1997; Tamon *et al.*, 2005; Venkitraman *et al.*, 2004). This claw ablation technique is already reported as an effective way to reduce the molting duration in Freshwater Rice Field Crab (*Oziothelphusa senex senex*) (Hosamani *et al.*, 2016). There have been several studies where it shows that, claw removal from crabs makes it molt faster and more frequently (FAO, 2015). This phenomenon helps the crab to gain weight within very short period of time.

Most of the crab studies in Bangladesh were dealing with biological and physical aspects of crab production but a few is known about the claw ablation on the molting duration and their economic effects in soft-shell mud crab farming. Therefore, the objectives of the present study were:

1. To evaluate the effects of claw ablation on molting, growth performance and mortality of mud crab
2. To shorten the molting duration through the removal of single or double chelipeds of crab in soft-shell mud crab farming process; and
3. To peruse the economic feasibility of chilepeds removal in soft-shell crab farming

CHAPTER-II

REVIEW OF LITERATURE

Claw ablation is the process whereby one or both claws of a live crab are manually pulled off and the animal is then returned to the water. It is practiced worldwide in different fisheries.

Florida Fish and Wildlife Conservation Commission, (2012) experimented about claw ablation and found that, it occurs in several fisheries worldwide, such as in the Florida stone crab (*Menippe mercenaria*) fishery, the north-east Atlantic deep-water red crab (*Chaceon affinis*) fishery and in southern Iberia, where the major claws of the fiddler crab *Uca tangeri* are harvested. Around Northern Europe, an extensive fishery exists for claws of the edible crab (*Cancer pagurus*). The practice is defended because some crabs can naturally autotomise (shed) limbs and then about a year later after a series of moults, regenerate these limbs. It is argued that declawing therefore provides a sustainable fishery. One crab that is subject to widespread declawing is the Florida stone crab, for which tourist information is provided.

Lynsey *et al.*, (2009) found that claw removal is also promoted to assist in handling of animals and to decrease losses through entanglement in fishing nets and cannibalism. Declawing crabs is legal in the UK since revocation in 2000 of the Crab Claws (Prohibition of Landing) Order 1986.

Quinitio and Estepa, (2011) experimented about the survival and growth pattern of juvenile of *Scylla serrata* by removing chelipeds at different molting stages. They showed that, trimming of chelipeds at different molts stages effects on the regeneration, molt interval and specific growth rate (SGR) in crabs. They found that, autotomy and not trimmed crabs showed significant decrease in the SGR but when trimming was done at the post-molt or inter-molt stages the SGR remains same at the end of their experiment. They also demonstrate a higher survival rate in trimmed crabs by controlling the feeds and shelter in the treatment. They conclude that, trimming reduces the cannibalism effect of crab, thus better performance is observed in trimming of chelipeds.

Viswanathan and Raffi, (2015) found that the natural diet of mud crab *Scylla olivacea* in Pichavaram mangroves of India and found that crustaceans form the

predominant food item of mud crab while molluscs showed a preference in few size groups. The other dietary item includes fishes, detritus, mud, sand and miscellaneous. Gut content analysis of both male and female mud crab revealed no significant variation between the quantities of food consumed by both sexes. Feeding intensity was higher in juveniles and sub-adults of both sexes than that of adults, revealing a greater preference to feed on fast moving prey such as crustaceans and fishes.

Jahan and Islam, (2016) researched on Economic performance of live crab (*Scylla serrata*) business in the southwest coastal region of Bangladesh. They found that live crab business has proven as a promising fisheries/aquaculture practice in Bangladesh. The average benefit-cost ratio (BCR) of crab collecting (1.5) and crab fattening (1.18) revealed that there is a great business potentiality in mud crab collection and fattening. Considering the total export earnings from mud crabs, this sector shows future potential and for achieving this development and support should come from government and different non-government organizations to improve the sustainability of this sector in Bangladesh.

Islam et al., (2017) experimented on “Challenges in mud crab production and marketing: A study on Khulna and Satkhira District”. They found that Crab cultivation has been treated as one of the dependable sources of livelihood for many years. A majority number of families in that area have chosen crab cultivation as their exclusive source of income. Analysis of data shows that mud crab production challenges come from natural disaster, virus attack, lack of training, lack of government loan, low quality crab seeds etc. On the other hand crab marketing fall into challenges of not following proper marketing system, instability in channel and pricing system, unfavorable relationship between middlemen and crab cultivators, unhealthy competition etc. Based on the analysis, some recommendations have been suggest that would be helpful to the people and organization who are involved with mud crab production and marketing.

Shivakumar et al., (2012) stated that sexual dimorphism and differential growth rate is evident in *Macrobrachium rosenbergii* and one of the major hurdles in the uptake of commercial farming. Two of the main difficulties for communal culture of many crustaceans are cannibalism and the social suppression of the growth. But crustaceans are blessed with ‘Autotomy’; regeneration of organs.

Ilan Karplus and Gideon, (1995) worked on the effect of claw ablation on the growth and survival of juvenile *Macrobrachium rosenbergii* was tested; two sets of controls were used consisting of a group of intact prawns, and an additional group whose first pair of walking legs had been ablated so as to serve as controls for injury and regeneration. He found that the biomass of the claw-ablated group doubled within the test period while that of the controls was stable because the gain in mean weight and the mortalities counter balanced each other.

Banu et al., (2014) found the freshwater prawn *Macrobrachium rosenbergii* exhibits three male morphotypes: Blue Claw (BC) male, Orange Claw (OC) male and Small Male (SM). Effects of bilateral eyestalk ablation on growth and survival of SM freshwater prawn were determined in monosex culture. The survival of bilaterally ablated prawn was significantly low 50%. The potency of bilateral eyestalk ablation in enhancing growth rates may be limited due to high mortalities of ablated prawn.

Venkitraman et al., (2010) worked on penaeid prawns where two different size ranges were ablated both unilaterally and bilaterally. Growth was estimated as length and weight changes and significant variation was observed. The unilateral eyestalk ablated prawns exhibited highest conversion efficiency. The production rate of unilateral eyestalk ablated prawns were two to three times more than control where as bilateral eyestalk ablated prawns indicated negative production.

Radhakrishnan and Vuayakumara, (1984) worked on bilateral eyestalk ablation accelerated moulting frequency and weight gain in juvenile, maturing and mature *Panulirus homarus*, irrespective of their reproductive status. Three to seven fold increases in weight gain was obtained on eyestalk ablation. Ablation also did not incapacitate the lobsters in locating food, nor did it interfere with regeneration of autotomised limbs. The study indicates the presence in *P. homarus* of the Moulting Inhibiting Hormone (MIH) and the Gonad Inhibiting Hormone (GIF) factors in the eyestalk.

Quackenbush and Herrnkind, (1981) recommended that eyestalk-ablated spiny lobster, *Panulirus argus* develop gonads larger than their intact controls. He also suggested that eyestalk-ablated lobsters molt faster than intact controls and both groups survive ecdysis without mortality.

Meñez and Ruinata, (1996) experimented on the viability of eyestalk ablation as a means to enhance the growth of juvenile spiny lobsters in commercial grow-out cultures in a four month experiment. Where they found the high survivorship and generally higher growth rates of unilaterally ablated lobsters compared with unablated lobsters resulted in the highest gross yield among the three treatments. The results indicated that unilateral ablation may be a viable option to consider in accelerating the growth of small juvenile *P. ornatus* to a marketable size of 200–300 g.

Hesni et al., (2008) found that molting, as an important function, plays an important role for the growth and reproduction in crustacean, so by increasing water temperature and performing eyestalk ablation, the rate of molting and then the growth and reproduction was improved. Since there were not any significant differences between the temperatures 24°C and 32°C for the molting and a simultaneous lower mortality in 24°C, then the temperature 24°C determined a suitable temperature to induce molting in ablated crayfish *A. leptodactylus*.

Freeman and Costlow, (1980) stated that stages of the zoeal and megalopal molt cycles of *Rhithropanopeus harrisi* were characterized by the appearance of epidermal cells in the spines and antennae. Eyestalk removal at the beginning of the last zoeal instar slightly accelerated the molt cycle. Fourth-instar larvae which had undergone eyestalk ablation during the third instar progressed through the molt cycle significantly faster than did control larvae.

Hopkins, (1982) recommended that eyestalk removal forces crabs into proecdysis. If crabs are in early proecdysis (stage D0) at eyestalk removal, the proecdysial period is accelerated. Eyestalk removal results in large increases in size at ecdysis which can be blocked by multiple autotomy. Ecdysis does not always result in growth. Molting in *Uca* sp. may result only in regeneration of missing limbs. Crabs regenerating a number of limbs may actually become smaller at molt.

Amer et al., (2015) used eyestalk ablation for inducing molting in the freshwater crayfish, *Procambarus clarkii* at the laboratory conditions. The results exhibited that, both of unilateral and bilateral eyestalk removal accelerated molting rate than in non-ablated or intact individuals of this species. In his experiment he found the use of unilateral eyestalk removal has low effect where the bilateral ablation has high effects and induced molting rapidly at the first and second week.

Shivakumar, (2008) found the mechanism of social control of growth in claw-ablated *M. rosenbergii* is also attributable to reduced aggression, uniform growth rate and increased survival rate. Though, the claws are essential for catching food item and for defense mechanism in natural waters, in feed-based culture systems, they do not have much role. Since they have regeneration capacity their removal will support the somatic growth positively thereby increasing net biomass.

Karplus and Hulata, (1995) stated that eyestalk ablation had no effect on growth of male jumpers. Ablation had a highly significant effect on growth of male and female laggards, manifested in both increase in size increment per molt and shortening of the molt interval. The effects of unilateral eyestalk ablation on *Macrobrachium rosenbergii* juveniles classified according to their relative rank were investigated. Jumper and laggard prawns were separately tested for the effect of ablation and sex on survival, daily weight gain, final weight, size increment per molt, molt interval and egg production.

Karplus et al., (1992) stated that claw ablation and dactyli immobilization in bulls resulted in a complete removal of growth suppression in runts which grew similarly to the control. The growth rate of runts raised with bulls seems to be positively related to their social status.

Mariappan et al., (2000) stated that the regeneration of the autotomized chelipeds imposes an additional energy demand called “regeneration load” on the incumbent, altering energy allocation for somatic and/or reproductive processes. A partial withdrawal of chelae also found leading to incomplete exuviation.

Juanes and Smith, (1995) recommended that an autotomy response may provide immediate survival benefits, the loss of one or more appendages can result in long-term functional and energetic costs.

Karplus et al., (1989) found the biomass of the claw-ablated group doubled within the test period while that of the controls was stable because the gain in mean weight and the mortalities counterbalanced each other.

CHAPTER-III

MATERIALS AND METHODS

3.1. Site and period of study

This study was conducted in the wet lab of Institute of Coastal Biodiversity, Marine Fisheries and wildlife conservation in Cox's Bazar of Chittagong Veterinary and Animal Sciences University under the Department of Aquaculture, Faculty of Fisheries. Each experimental duration was at least 120 days. The study was conducted from 1st February 2017 to 30th January 2018.

3.2. Experimental species:

For this experiment mud crab (*Scylla sp.*) (Figure 2) was chosen due to its availability in the coastal regions of Bangladesh, high disease resistant, and can tolerate a wide range of environmental fluctuations. This species is also called the mangrove crab and locally they are known as “habba kankra” or “khosha kankra”.



Figure 2: Mud crab (*Scylla sp.*)

3.3. Systemic position of Mud crab:

Kingdom: Animalia

Phylum: Arthropoda

Sub phylum: Crustacea

Class: Malacostraca

Order: Decapoda

Infra order: Brachyura

Family: Portunidae

Genus: *Scylla*

Species: *Scylla olivacea* (Herbst, 1796)

3.4. Experimental design:

To observe the culture performance of mud crabs under different claw ablation conditions, this experiment was grouped into four categories. In Treatment 1 (T1), individual crabs were stocked in without removing their chelipeds. In treatment two and three one and both chelipeds were removed and treated as T2 and T3 respectively. In treatment four (T4) two crabs were stocked in one box by removing both chelipeds. Each treatment was consists of twenty individuals and repeated two times. The same experimental designs with thirty replication for each treatment were also followed in the farm level.

3.5. Experimental set up:

A continuous recirculating system was applied to conduct this experiment (Figure 3). A total of 80 plastic buckets and an iron frame were used for this experiment. Individual bucket serves as a separate chamber for each crab. Each chamber was supplied with an inlet and outlet for water flow and also an oxygen pipe for continuous aeration. All drained water was collected in a reserve tank facilitated with a graded purification system. Purified waters were then sent to an overhead tank by a motor and then recirculated again in the similar process.

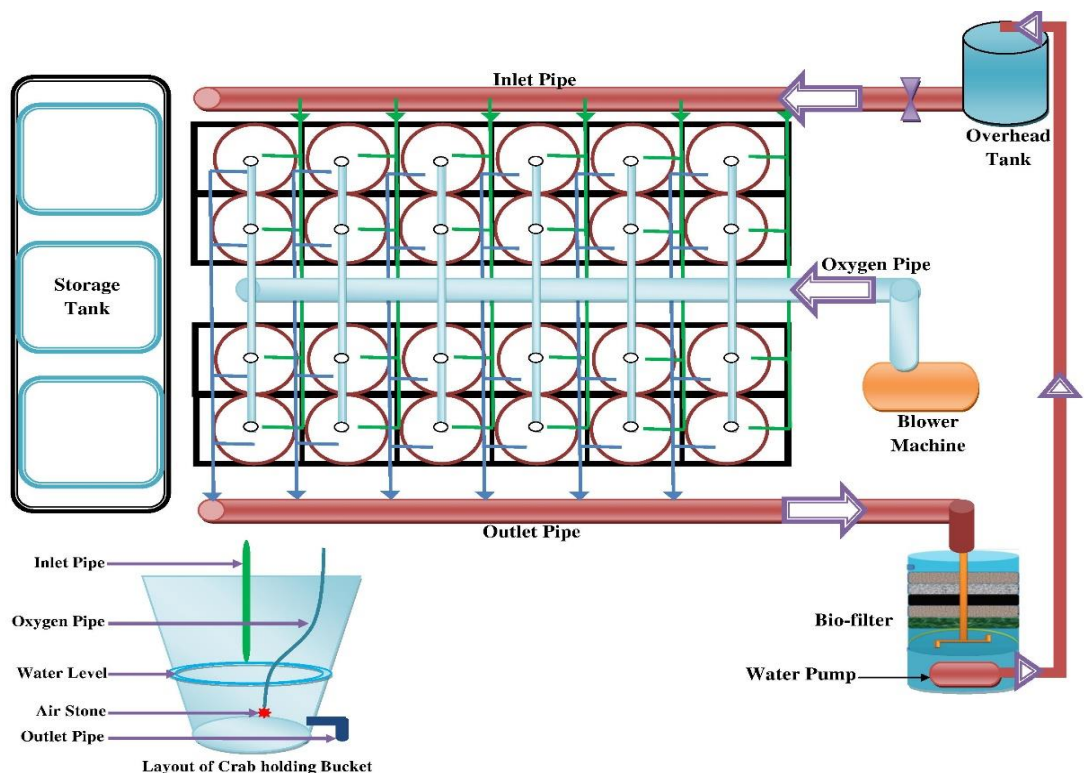


Figure 3: Experimental layout of the Laboratory

3.6. Collection of crabs:

Wild Crabs of same size of 50-60g were collected from the Moheshkhali channel, Kutubdia and Teknaf regions of Cox's Bazar districts of Bangladesh from the local crab catchers (Figure 4). Chelipeds of the harvested crabs were gently tightened with thin plastic rope to avoid movement and escape of crab. Collected crabs were brought to the laboratory as soon as possible after capture with minimal transportation and heat stress. Transportation was done with utmost care and usually done in the morning or in evening.

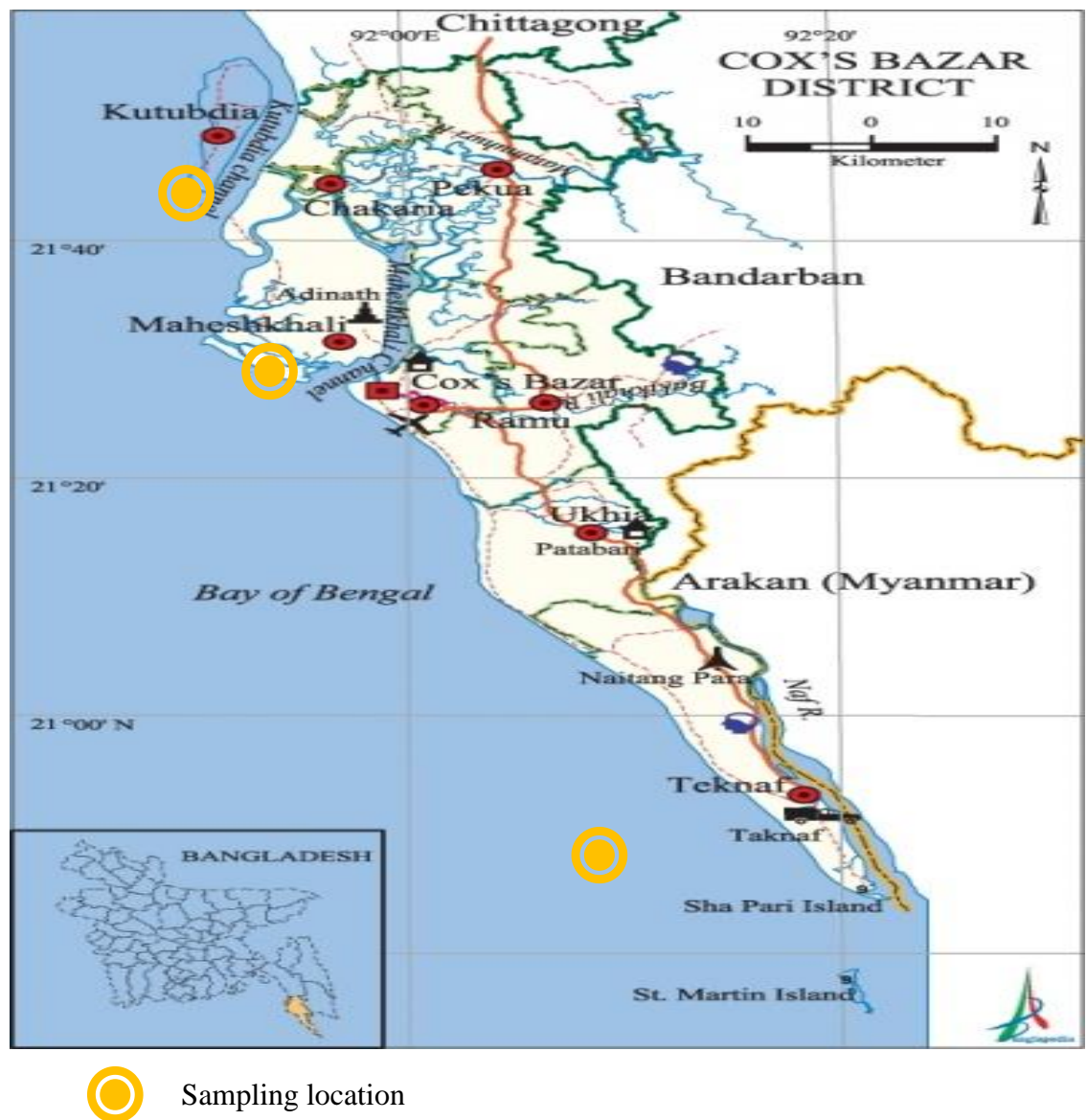


Figure 4: Crab sampling areas in Cox's Bazar district

3.7. Conditioning of crabs:

Before starting experiment, crabs were conditioned for a period of at least 15 days in a large stocking tank with continuous aeration. Regular water exchange, balanced feeding, continuous monitoring, sufficient shelter was maintained during the conditioning periods to prevent cannibalism and fighting.

3.8. Chelipeds removal and stocking:

Chelipeds were ablated in crabs by imposing manual stress to force it to autotomize. Autotomy is the spontaneous casting off a limb or other body part by an animal when injured. Like other animals, mud crab also poses same characteristics of autotomy. Claw was removed by mechanical device in a safe manner that it could not harm the physical condition of crab and there was no infection at the ablated site due to claw removal (Figure 5). Then the crab was stocked according to the experimental design.

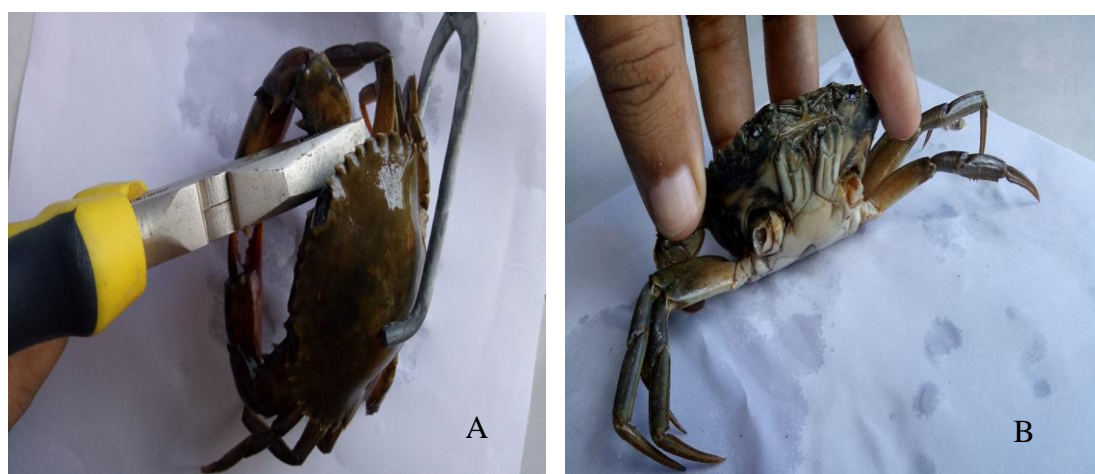


Figure 5: Removal of chelipeds from crabs

Figure (A) shows the way of giving mechanical pressure to crabs, crabs are then release their claw by the process of autotomy and figure (B) shows an ablated crab

3.9. Water quality monitoring:

Water quality was monitored regularly during the experiment. Water temperature, pH, salinity and Alkalinity was recorded and maintained in an optimum level. The water temperature was measured by using a portable thermometer and was 28-32°C during the experimental time. Salinity was measured twice in a day by using a refractometer (Sp20S, Switzerland) and maintained in between 12 to 18ppt by adding sea water or freshwater. pH of the experimental system was recorded in a range of 6.5 to 8.0 and

checked daily using a portable pH meter (HANNA 8I96107, Romania) and the alkalinity was above 80 in the experimental system and measured using a test kit.

3.10. Feeds and feeding:

Individual crabs were fed with trash fish (Tilapia, Olua, Poa, Chamili) at two days interval (Figure 6). Trash fish were chopped and provided to the crabs at 10g per crab at the evening. Unused and left over feeds were removed carefully from the boxes for giving a better environment to the crabs.



Figure 6: Chopped trash fish

3.11. Behavior of crabs:

Behavior of individual crabs was also monitored during the experiment in the laboratory condition. During behavioral study individual crabs are allowed to move freely and a stress free environment was also ensured. Behaviors of the crabs were monitored after claw ablation and also during their molting time.

3.12. Parameters to be study:

Different parameters such as weight gain, percent weight gain, carapace length, yield etc was examined in regular basis to collect data of the experiment.

3.12.1. Weight gain: It was calculated by the following formula-

$$\text{Mean weight gain} = \text{Mean final weight} - \text{Mean initial weight}$$

3.12.2. Percent weight gain: It was calculated by the following formula-

$$\text{Percent weight gain} = \frac{\text{Mean final weight} - \text{Mean initial weight}}{\text{Mean initial weight}} \times 100$$

3.12.3. Carapace length: Carapace Length (CL) is the distance between the tips of the posterior most lateral carapace spines which was measured by slide calipers (Figure 7). Increase in the carapace length indicates the increase of growth.



Figure 7: Measuring of carapace length of crab

3.12.4. Calculation of yield: The crabs were stocked initially at the boxes after taking the weight and at the end of the study the final weight of the remaining crabs were taken. That means the actual amount produced when an experiment was carried out from which the gross yield of each treatment was measured and compared according to different treatments.

3.13. Data collection and analysis:

Carapace length, survival and body weight was recorded. Molting duration was recorded for individual crab. Finally, the health condition (infection due to removal of chilepeds) and market demand and price were recorded for further analysis. Collected data was subjected to an analysis of variance (ANOVA) followed by comparison of means using Tukey's HSD test to determine significance of each data treatment. Significant differences were indicated by p-values <0.001 and <0.05 . All statistical analysis were performed using Microsoft excel worksheet and SPSS16.0.

CHAPTER-IV

RESULTS

From the present experiment, detailed information about behavior of crabs after claw ablation, % weight gain at different claw ablation methods, individual growth of crab, molting duration, mortality of crabs in culture periods and also the cost-benefit ratio were presented in this chapter.

4.1. Behavior study:

In the wild, crabs have to compete for food, mates, shelter and want to avoid predators. After declawing they remain quite most of the time. Their inactive behavior after claw ablation mainly reduces their movement and they accumulate more energy to molt quickly. Crab molts earlier than the natural crab after declawing. The quicker their molting, the better their growth performance is. It indicates that claw ablated crab grows faster than the natural crab. Declaw crabs significantly eat more mass of feed and especially when they need. Claw ablated crabs survive by switching their behavior from predation to scavenging. Their cannibalistic nature is also reduced by claw ablation process. Declawed crabs can be cultivated more than one in the same box. This is the result of no cannibalistic nature after removal of claw.

4.2. Weight gain (%):

In this experiment, percentage of weight gain was calculated after every 15 days as well as immediate after each molting. To clarify the difference among each treatment, the experiment was run until 80% of the crabs have completed the second molting. After 1st molting, significant difference in the weight was observed at 0.001% level of significance in the ablated crabs compared to non-ablated crabs. Percentage weight gain was maximum in the both claw ablated crabs and minimum at the no ablation groups. Here the variation is evident due to the claw ablation which is responsible to reduce cannibalistic nature and sluggish movement which saves the waste of metabolic energy. In the 2nd molting, significant difference was found at 0.005 levels in the ablated crabs rather than no ablated groups. But no significant differences were found in claw ablated groups (T2, T3, and T4) during 2nd molting. Weight gained by the individual crabs of both claw ablated group (T3) and the both claw kept two crabs in same box (T4) were statistically are not significant.

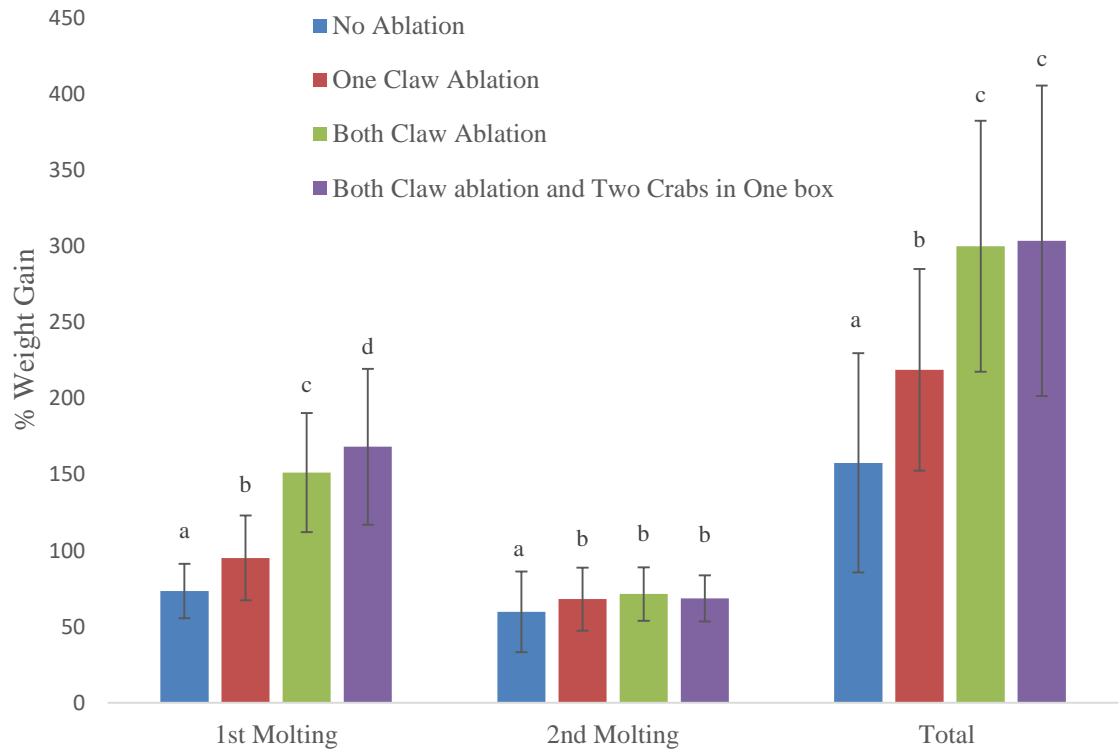


Figure 8: Weight gain (%) of crabs at experimental conditions in the soft shell farming system. Weight of individual crabs was recorded at the end of 1st and 2nd molting in different claw ablation procedures. Data are presented as mean \pm standard deviations.

4.3. Yield:

Crabs were cultivated in re-circulatory system in the laboratory allowing individual crabs to molts 2nd times. Total yield at the end of the experiment was 2052g, 2436g, 2876g and 3942g from the four treatments respectively. Comparing the yield, it was found that there were significant differences present among all the four treatment at 0.001 significant levels. In T1, no claw ablated crabs are naturally molt but process was comparatively slow than others. Their growth and percentage weight gain increased in a conventional process. In T2 the one claw ablated crabs molted faster than the controlled one. For the regeneration of the ablated limb crabs molt faster. T3 have higher yield than the other two treatments. The removal of two chelipeds makes the production better. It shows that T4 have the highest yield than the other treatments as because two crabs were kept in one box. But in one sense it says that T4 doesn't have the highest yield. Because there were two crabs in a single box which tends to double the yield in the experimental sense.

4.4. Carapace Length:

The carapace length in crabs as well as in many crustaceans indicates an important tool to study biology, physiology and ecology. In this experiment, the carapace length of crabs was measured throughout the experimental periods at 15 days interval. Data showed that, increased carapace length was observed in between and within groups after 1st molting and 2nd molting at 0.001% and 0.005% level of significance respectively in four treatments. In contrast, significant differences was also observed in both claw ablated crabs (T3) at 0.005% level and at 0.001% level in T4 compared with no ablated and one claw ablated groups at 1st molting, 15 days after 1st molting and after 2nd molting time. But no significant differences were observed at 30 days after 1st molting and no ablated groups with one claw ablated crabs. The following table indicates that the differences in the carapace length related with the ablation in different treatments in the soft shell farming system.

Table 1: Carapace length of crabs at different treatments of claw ablation of crabs in the soft shell farming system. Data values are presented as mean \pm standard deviations. Carapace length was measured by Vernier calipers at 15 days interval and also after 1st and 2nd molting time. Asterisk (*) marks indicates the significance ($p < 0.005$) from T1 and T2 and double asterisk (**) indicated significant difference ($p < 0.001$).

Carapace length/Days	No Ablation of claw cm	One Claw ablation cm	Two claw ablation cm	Two claw ablation in two crabs in one box cm
0 Days	5.75 \pm 0.50	5.20 \pm 0.22	5.48 \pm 0.31	5.58 \pm 0.37
1 st Molting	7.21 \pm 0.70	7.41 \pm 0.90	7.80 \pm 0.86 *	7.91 \pm 0.53 **
15 days after 1 st molting	7.22 \pm 0.70	7.41 \pm 0.90	7.84 \pm 0.86 *	7.98 \pm 0.53 **
30 days after 1 st Molting	8.20 \pm 1.41	8.77 \pm 1.62	9.27 \pm 1.47	9.93 \pm 1.53
2 nd Molting	8.57 \pm 1.47	8.86 \pm 1.60	9.33 \pm 1.46 *	9.96 \pm 1.54 **

4.5. Moulting duration:

In this experiment, successful reduction of the moulting duration was done by applying claw ablation techniques. Significant decrease in the duration of moulting was found in the ablated groups rather than non-ablated crabs. In the non - ablated crabs the average time required for 1st moulting is 33.95 ± 11.34 days while in the one claw ablated crabs it requires about 28.22 ± 12.64 days (Figure 9). Moulting duration was also shortened in the both claw ablated crabs compared with no ablation or one claw ablated crabs. In the both claw ablated crabs the molting duration was 26.34 ± 8.96 days for the first molting. But no significant differences were observed in the T4 compared with T3. Similar molting duration patterns were also observed after the second moulting of crabs. After 2nd moulting, the mean moulting days required for non-ablated crabs were 40.28 ± 7.21 days. By contrast, the moulting duration was compacted within 34.92 ± 7.05 days in the one claw ablated groups. Moulting time was also less in the double claw ablated crabs in both T3 and T4. In these two treatments the moulting duration was 30.62 ± 10.30 days and 29.88 ± 6.59 days respectively after the 2nd moulting. Data value of day's required for moulting in both 1st and 2nd moulting time was statistically significant ($p < 0.005$) in both T2, T3 and T4 compared with T1. But, there were no significant differences found between the both claw ablation (T3) and one claw + two crab in a box (T4) groups.

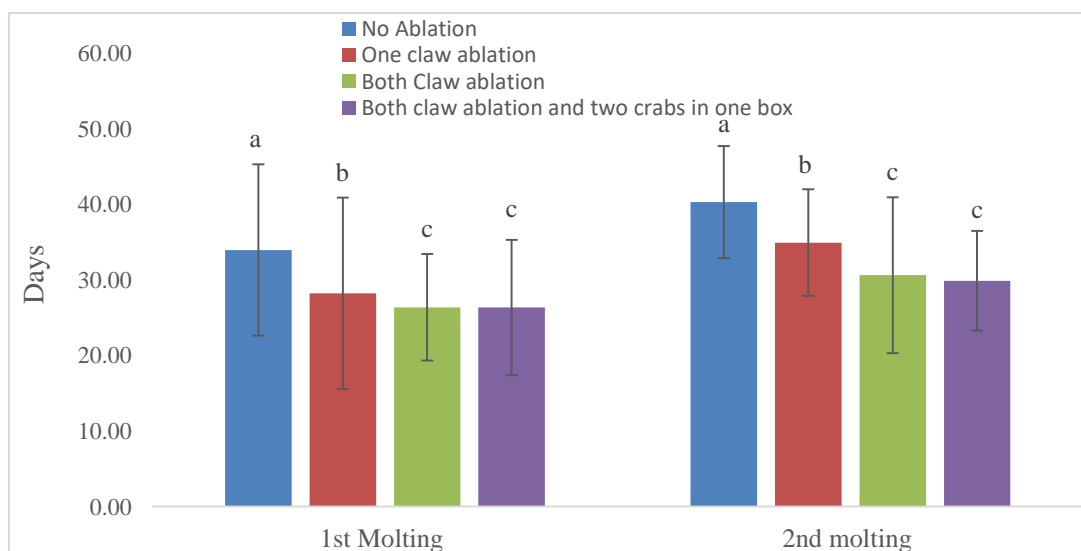


Figure 9: Moulting duration of crabs in the soft shell farming system. Data are presented as mean \pm standard deviation. Significant differences ($p < 0.005$) are found in T2, T3 and T4 compared with T1 in both 1st and 2nd moulting time. T3 and T4 are statistically very similar in both times.

4.6. Mortality:

Mortality is one of the crucial issues in any culture system. In this experiment highest mortality was found in the T4 after 1st moulting where two crabs were kept by removing two claws. In T4, 30% of the crabs were died after 1st moulting which is significantly higher ($p < 0.001$) compared to other treatments due to predatory behavior of another crabs in the same box as because soft shelled crabs were not separated from the hard one. In other treatments the mortality was less than 20% and there were no significant variations among three treatments.

4.7. Cost Benefit Analysis:

Table 2 shows a comparison of income and expenditure of the traditional farming and claw ablated crab (calculation of one month) farming in a one acre pond is given below-

Traditional crab farming		Claw ablated crab farming (both claw)	
Expenses of expenditure	Amount (Taka)	Expenses of expenditure	Amount (Taka)
Cage setup and pond preparation	25,000	Cage setup and pond preparation	25,000
Fry (Stocking 30,000/ acre and 150 taka/ kg)	2,50,000	Fry (2 crab per box, in a total 60,000 fry)	5,00,000
Feed cost	1,00,000	Feed cost	2,00,000
Workers' salary (One manager and 8 labor)	80,000	Workers' salary (One manager and 11 labor)	1,20,000
Other miscellaneous	15,000	Other miscellaneous	30,000
Exporting cost (packaging, processing, license etc)	1,30,000	Exporting cost (packaging, processing, license etc)	2,80,000
Total expenditure	6,00,000	Total expenditure	11,55,000

Traditional crab farming		Claw ablated crab farming (both claw)	
Income sector	Amount (Taka)	Income sector	Amount (Taka)
Soft shell crab; total production 1,200 kg (30% mortality and as per moulting in 35 days, the price of each kg is 600 taka) [total moulting 50%]	1200×600 = 7,20,000	Soft shell crab; total production 2600 kg (25% mortality and as per moulting in 28 days, the price of each kg is 600 taka) [total moulting 60%]	2600×600 = 15,60,000
Total income	7,20,000	Total income	15,60,000
Total expenditure	6,00,000	Total expenditure	11,55,000
Real income	1,20,000	Real income	4,05,000
The ratio of Income expenditure	1 : 0.83	The ratio of Income expenditure	1 : 0.74

Reviewing the above income expenditure, it is clearly seen that, cultivating crab by improved cultivation of equivalent water bodies, one can be benefitted 3-4 times if did it in high stocking densities.

CHAPTER-V

DISCUSSION

Autotomy is the ability of certain animals to release part of the body that has been grasped by an external agent. It refers to a reflex severance of one or more limbs in response to injury or its threat, which occurs always in a predetermined breakage plane. In this experiment, claw of crabs were ablated by the process of autotomy which has resultant in reduced cannibalism, less mortality of the mud crab. Further, declawing acted as an inducing activity which stunted all other normal activity of crab such as excess movement or to run away from the culture box, and their first and most important concern becomes the molting. For this, they saved energy and molted as soon as possible. Crustaceans widely practice self-amputation of one or more limbs during inter and intra-specific competition for limited resource like food, shelter, mate and also has a strategy to avoid predation and wound limitation (McVean, 1982). Apart from such biological reasons some accidental events may also occurring during the arthropod life cycle causes damage their limbs by fishing gear (Kirkwood and Brown, 1998) and culling of undesirable individuals (Kennelly *et al.*, 1990). Trimming appears to be less stressful than autotomy. It has been hypothesized that the effects that follow autotomy in crustaceans are due to the severance of a critical number of leg nerves (Skinner and Graham, 1972).

After the first molting of the ablated crabs, complete regeneration of their ablated chelipeds was found in this study. Crustaceans have the ability to regenerate lost limbs. The regeneration of lost limbs to its original size depends on the age/ size (Mariappan *et al.*, 2000), the moult stage in which the limbs were lost (Hopkins, 1982), and the number of limbs removed (Fingerman and Fingerman, 1974; Skinner and Graham, 1972; Smith, 1990). Limbs can be replaced only by moulting. The loss of limbs hastens the onset of the next moult (Hopkins, 1982).

In this study, after the ablation of single and both chelipeds, the experimental crabs showed normal feeding behavior. Ablated crabs consumer feed as like as the normal crab and no problem was observed in crab to uptake feed without their chelipeds. Chelipeds are important for crabs resulting in the immediate acquisition of a food and shelter as well as in the establishment and maintenance of dominance relationships.

This has been demonstrated in several species of crustaceans where intact and claw ablated individuals tested (Berzins and Caldwell, 1983; Conover and Miller, 1978; O'Neil and Cobb, 1979; Lee and Fielder, 1983; Lowe, 1956). Claw seems to be essential not only for winning contests but also in triggering aggression. Clawless individuals become handicapped and were tolerated far more readily as was evident in a lower attack rate in *Munida sarsi* (Berril, 1970) and in the higher frequency of shelter-sharing in *Homarus americanus* (O'Neil and Cobb, 1979) and *Alpheus heterochaelis* (Conover and Miller, 1978).

Claw ablated individuals showed lower aggressiveness in this experiment which helped them to save more energy for their molting and growth in comparison to normal group of crabs. Groups of claw-ablated crustaceans of other species, including crabs and *Macrobrachium rosenbergii*, undoubtedly differ from their intact conspecifics in both type and intensity of aggressive interactions. Karplus *et al.*, (1989) observed the results similar to that of reports for *Homarus americanus* (Aiken and Young-Lai, 1979, 1981; Kendall *et al.*, 1982). In all the species, the claws are prominent and essential in aggressive interactions. Death in claw-ablated animals might be due to stress, dominance and cannibalism by early recovered ones and hence it is inferred that claw-ablation does not preclude cannibalism. Segal and Roe (1975) have observed cannibalism among claw-ablated *Macrobrachium rosenbergii*. The relative impact of either cannibalism or claw ablation on survival can only be evaluated by comparing survival in isolated and group raised individuals. In claw-ablated *Homarus americanus* mortality due to cannibalism was minimal in isolation compared to group raised (Kendall *et al.*, 1982). The marked effect of claw-ablation on the survival of crabs, as observed in this study is similar to that of other studies.

Here in this study, it was found that the moulting and growth fastens in the claw ablated crabs where the moulting and growth found better in the double claw ablated crabs than the single claw ablation. Amer *et al.*, (2015) found that, the hypotheses that tested in these experiments were accelerating molting by eyestalk ablation to reduce intermolt period and fasting growth. The effects of these treatments differ significantly according to the type of eyestalk ablation. He found the unilateral eyestalk removal has low effect where the bilateral ablation has high effects and induced molting rapidly at the first and second week. Banu *et al.*, (2014) found that the growth increments of bilaterally ablated prawns were significantly higher than the

control prawns. Although final individual weight of bilaterally ablated prawns was still higher, yield was not significantly different from either ablated or unablated prawns. However, survival of bilaterally ablated prawns (50%) was significantly lower than the control (100%) prawns. There were no signs of infection of the ablated eyestalks but death occurred while moulting.

The result from the present study noted that the production of double claw ablated group is better than the control group. This may be the result of cheliped removal. Similar result was found that the biomass of the claw-ablated group doubled within the test period while that of the controls was stable because the gain in mean weight and the mortalities counterbalanced each other. Appendage ablation in crustaceans has a profound effect on growth by shortening the molt cycle and decreasing the carapace increment per molt (Hopkins, 1982). These effects are positively correlated with the regeneration load. The more and large appendages removed, the stronger these effects are (Fingerman and Fingerman, 1974). The relative impact of claw and walking leg ablation on growth was similar in *Gecarcinus lateralis* (Skinner and Graham, 1972). The overall net effect of appendage ablation may be attributed to increased density, due to higher survival of claw-ablated animals, or to regeneration. It is clear from the present study that claw-ablation has effect on growth and survival of crabs when they are reared commercially in the separate boxes. This study shows a significant production increase in terms of growth, survival, and yield.

It was found that, the claw ablation reduces the moulting duration of the mud crab. This might be attributable to diversion of energy, less aggressiveness, reduce cannibalistic nature, movement, predation and increased food conversion efficiency in claw-ablated crabs. Chelotomy, dactylotomy and immobilization of the dactyls have been shown to reduce the degree of cannibalism in *H. americanus* (Karplus *et al.*, 1982) and in *M. rosenbergii* (Diaz *et al.*, 1990; Karplus *et al.*, 1989) which ultimately shortened the periods of molting. In *C. spidus*, the loss of chelipeds was shown to have not only a negative effect on foraging ability and also channelize more metabolic energy for regeneration of chelipeds. Thus in species like *Calinectes sapidus* (Ary *et al.*, 1987; Smith, 1990b), the loss of chelipeds leads to a reduction in moult increment due to energy diversion; such energy demand is called regeneration load (Skinner, 1985), which may reduce reproductive output (Luppi *et al.*, 1997; Norman and Jones, 1993). Moulting is one of the most important physiological processes for all

arthropods. The moulting process is the most difficult and stressful time in the life of the crab and the time it is most vulnerable to cannibalization from other crabs. Even small mechanical, chemical or physiological problems during this time will result in near-certain death for a crab, so it is absolutely necessary to give crabs the highest level of care before they are placed in the culture system and throughout the culture duration (Gaude and Anderson 2011). In this experiment, the stress of crabs was reduced by providing sufficient nutritious protein enriched food to crabs immediate after claw ablation. Fermented chopped trash fish was provided that was easily taken by the crabs. According to Aflalo *et al.* (2006) supplementation of high dietary protein and vitamin C ameliorate stress. From the finding of this experiment, it was clearly observed that, claw ablation successfully reduced the moulting duration of soft-shell mud crab in compare to the no ablated group of mud crab where all the parameters were same except the claw was removed in some groups.

In this experiment a significant survival rate was found in the claw ablated crabs. Though two ablated crabs in one box showed higher mortalities. This is due to the molted soft-shell crab was not harvested from the box after 1st molting. So, non-molted crabs tend to fight with soft one and causing the increase of mortality. Higher percent survival in claw-ablated animals is attributable to less cannibalism, less aggressiveness and increased feed efficiency. The marked effect of ablation on the survival of group of crabs as observed in the present study which was similar to that reported for *Homarus americanus* (Aiken and Young-Lai, 1979; Kendall *et al.*, 1982) and *M. rosenbergii* (Shivakumar, 2008; Karplus *et al.*, 1989). In both these species, the claws are prominent and essential in aggressive interactions (Peebles, 1979b; Atema and Cobb, 1980). The low survival of crabs with intact legs could be due to the fact that the claws eventually aid cannibalism and aggression behavior and suppress the growth of subordinates. The relative impact of either cannibalism or claw removal on survival can only be evaluated by comparing survival in isolated and group raised claw-ablated individuals. In *Homarus americanus*, mortality due to cannibalism was minimal as isolated, claw-ablated, controls had a survival rate of 62% and mass reared ones 67% (Kendall *et al.*, 1982). In this study, declawing showed reduction of the normal rate of mortality from 35-40% to nearly 25% through reducing excess movement, cannibalism and predation in culture environment which makes this technique more profitable in farm level with the easy and safe handling.

Claw of crabs is an important body parts constitute about 10-30% of the total body weight depending on the species. In commercial culture of crustaceans, the position of large crusher chelae triggers aggression between individuals leading to physical damage of body parts (especially chelipeds) increasing the rate of limb loss and mortality. Indeed chelipeds constitute 10-26% of the body weight in *M. nobilii* (Mariappan and Balasundaram, 1999a), 20% in *Carcinus maenas* and *Liocarcinus holsatus* and 50% in *Menippe mercenaria* (Simonson and Steele, 1981). Removal of the chelipeds from the crab body is a simple technique and no need of much expertization which makes it a commercially viable technique. Workers can be easily trained and better profit margin can be gained applying claw ablation technique in comparison to the traditional culture practice. Applying claw ablation technique, it was found that two crabs can be effectively cultured within a single box which opened the door to earn better benefit in soft-shell crab business. This technology also proven to reduce mortality and the management cost.

CHAPTER-VI

CONCLUSION

Recently the mud crab culture is considered as a profitable and potential business in our country. A considerable scope apparently exists in the expansion of soft-shell mud crab farming in Bangladesh. Due to certain constraints such as lack of available seeds and technical personnel mud crab farming in the country has not spread to the extent it deserves. Technologies regarding the soft shell crab culture system are still very poor. As an influencing factor, claw removal of crab found to be a possible strategy to increase the production and to be economically more benefitted from soft-shell mud crab farming. From different previous study it is reported that, eye stack and claw ablation in several crustacean species gives better production and growth. The present study was the first approach in Bangladesh to develop a sustainable culture technique for soft-shell crab farming by applying claw ablation. This study successfully reduced the moulting duration of crabs by declawing and the production of crabs was significantly higher than non-ablated crabs. It is obvious from different analysis that fattening, grow-out and soft shell culture practices give better profits than penaeid shrimp culture practices. Soft shell crab farming is commercially held very small at the Cox's Bazar and Khulna region. This improved farming practice of *Scylla sp.* through cheliped ablation reduces the molting duration around two weeks which will help a crab farmer to be economically more benefitted. The simultaneous culture of more than one crab within a box is done by the removal of both chelipeds that can improve the profitability of soft-shell crab farming and would help long term sustenance of soft-shell crab business. But it is necessary to produce crab seed in hatchery and to improve the culture technique of the mud crab in a sustainable and profitable manner. It can be concluded that government, NGOs, researchers should come forward to make this sector more sustainable and profitable. There also present many further scope of research on soft-shell mud crab such as through removal of all walking legs along with chelipeds which could trigger their molting process and to develop commercial formulated feed for the mud crab cultivation.

CHAPTER-VII

FUTURE PROSPECTS AND RECOMMENDATIONS

Mud crab is a fast growing and highly salinity tolerant species. The only problem reported in the grow-out phase of mud crabs is mortality due to cannibalism. Chelipeds are the major responsible organ for cannibalism of crab. The chelipeds removal significantly improves the growth performance and reduces the molting duration around two weeks. This will help to reduce the production cost. The most important fact is, in this condition, two crabs can be cultured simultaneously within a box that can improve the profitability of soft-shell crab farming and would help long term sustenance of soft-shell crab business. Increasing prices, outbreaks of diseases specially viral diseases, impacts of climate change make obstruction of shrimp culture and farmers are moving towards the crab culture because the crabs are less susceptible to the climate change and disease. Availability of brackish water and optimal salinity and temperature conditions will make mud crab farming easier in the coastal districts of Bangladesh. The great demand and high price of mud crabs in the international markets will motivate the farmers to expand mud crab culture. Mud crab farming can serve employment opportunity to the coastal poor communities in Bangladesh. For the future sustainable development of the mud crab business in Bangladesh, following recommendations need to be fulfilled-

- Reduce the dependency on wild stock seeds and commercial production of crablet in hatchery
- Crabs should be kept moist during transport. It will reduce the stress on crab
- Effective monitoring and proper checking system should be ensured
- Maintaining of good water quality parameters in the culture pond
- Government agencies and relevant NGOs should run a simplified consultation process and workshops to review the management arrangements for the country's mud crab stocks and how they can be improved to sustainably manage the resource
- Training on soft shell crab production should be provided
- Technological support should be developed for crab production and marketing

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Appendix A: Soft-shell mud crab *Scylla sp.* of Bangladesh



Soft-shell mud crab (*Scylla sp.*)

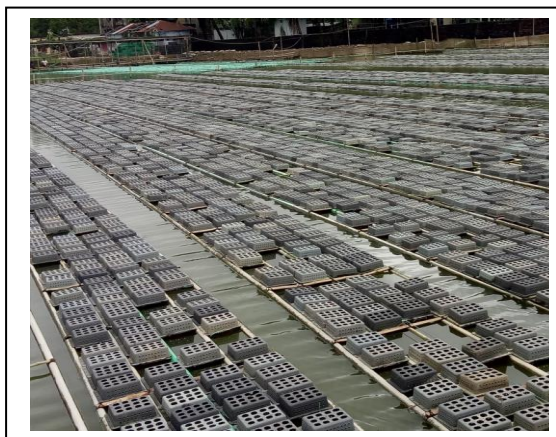
Appendix B: Scenario of a soft-shell mud crab farm



Irawan crab farm, Cox's Bazar



Research project in farm level



Floating cages with crab box



Bridge for operation

Appendix C: Scenario of the experimental setup in Laboratory



Shade of simple recirculatory system as a laboratory for crab culture



Experiment setup in laboratory condition



Soft-shell crab

Empty shell after molting

Appendix D: Scenario of experiment setup in farm



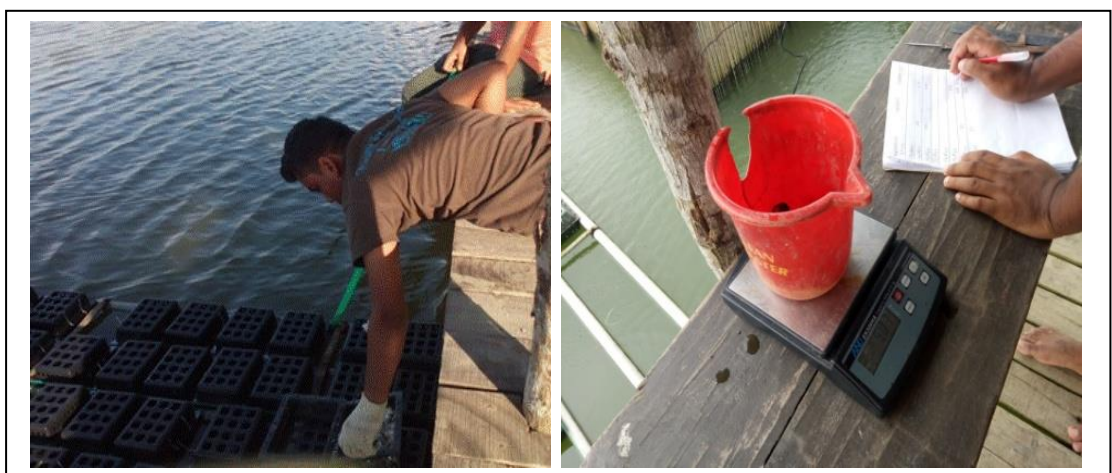
Experiment setup in farm

Claw ablation of crab



Single claw ablation

Double claw ablation



Feeding of crab

Weighing of crab

Appendix E: Scenario of system management activity



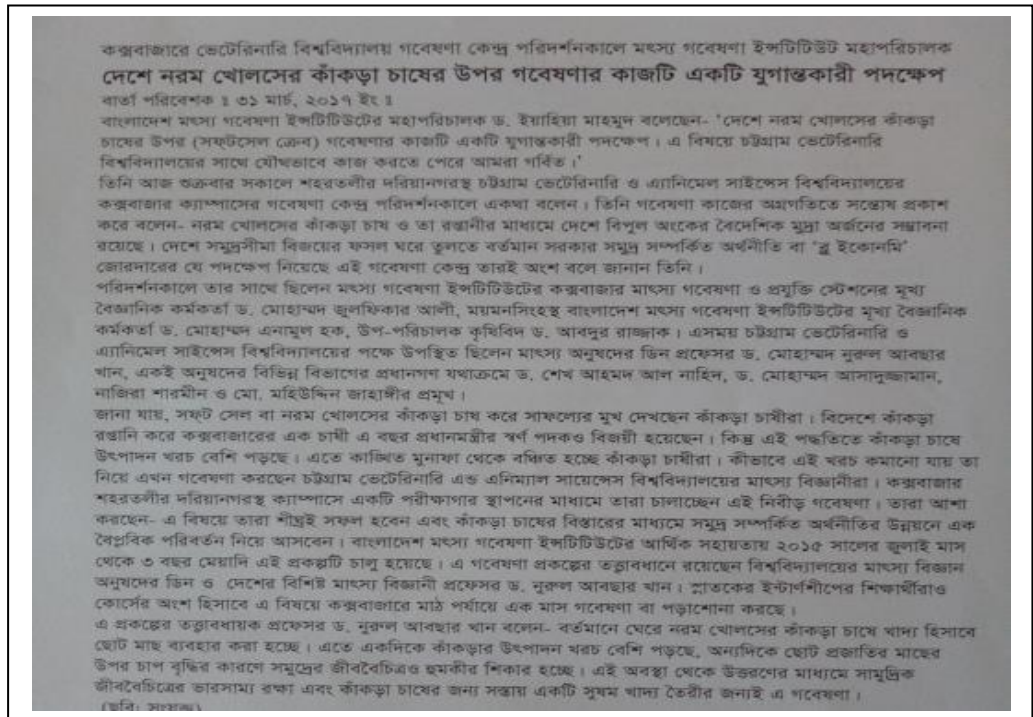
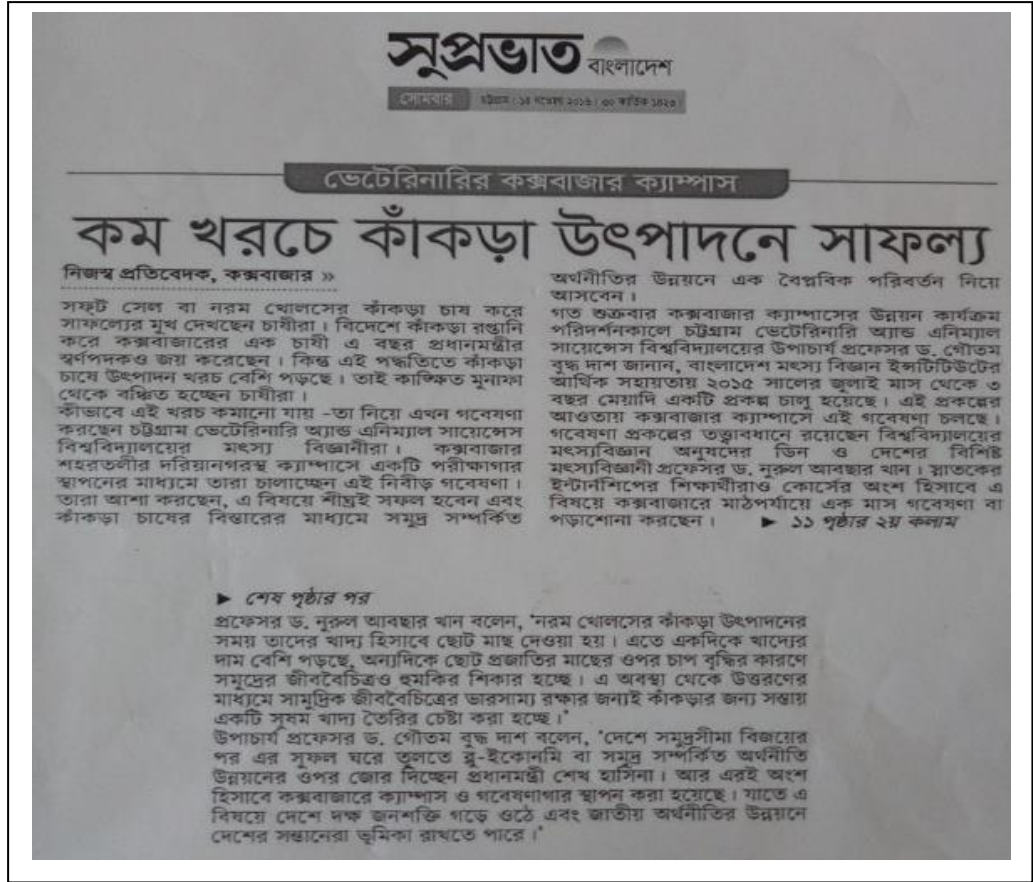
Work activity to manage the system



Checking of moulting

Moulted soft-shell crab with the old shell

Appendix F: News coverage in Paper of the success of the Research



Brief Biography

Mohammad Redwanur Rahman completed B.Sc. in Fisheries (Hon's) from the Faculty of Fisheries of Chittagong Veterinary and Animal Sciences University (CVASU), Chittagong, Bangladesh with CGPA 3.97 out of 4.00 and secured the second position in merit. He joined as a Lecturer in the Department of Aquaculture of Chittagong Veterinary and Animal Sciences University (CVASU) in 2017. Now, he is a candidate for the degree of MS in Aquaculture under the Department of Aquaculture, Chittagong Veterinary and Animal Sciences University (CVASU). He has strong passionate in research of Fisheries and published his undergraduate's research project entitled "Preparation of fish peptide powder through enzymatic hydrolysis of white croaker (*Otolithoides pama*)" in international Journal. His research interests are on marine crustaceans such as soft-shell mud crab, shrimp, lobster and mollusks such as oyster, green mussel culture, integrated aquafarming, organic fish culture, ornamental fish culture, fish nutrition, fish feed formulation, live food culture, aquafarm designing, aquaculture engineering etc. He has already participated in different national scientific conference as a presenter of the research project. From the last two years, he also working as the project implementer of soft-shell crab research project titled "Improvement of the traditional culture techniques of soft-shell mud crab *Scylla* sp. in Bangladesh" from which he has earned tremendous experience of research and project running. His soul focus is now to develop modern culture techniques of the commercially important marine fin fishes as well as nontraditional but highly demandable saline water crustaceans and mollusks.