CHAPTER ONE
INTRODUCTION

Bangladesh is blessed with vast marine resources in the Bay of Bengal, which is a part of the largest Malacological province of the world. This Bay is relatively shallow with tidal inundation and salinity is two main features that make this region distinctly different from other parts of the country. Though the coastal resources are very rich with a wide diversity of the mollusks species, which has economic importance as food [Hossain, M.A., 1999]. The high tidal amplitude, sufficient tidal current, less pollutants and high phytoplankton-zooplankton abundance may offer a suitable environment for the development of mollusk culture fishery in some area of the coastal zone of Bangladesh [Hossain, M.A., 1999]. Fisheries resources are only dependable means for reducing the animal protein gap for the people of the country. This is because a bright scope to increase in aquaculture production in the form of fishes, shellfishes, arthropods etc. Even the organized cultivation of sea based mariculture, coastal aquaculture, now looms as an inevitable as well as fascinating task for future generations.

The mollusks species constitute a significant part of the world fishery today. They are of major interest to man and many species can play a great role in the national economy. From the standpoint of food alone mollusks have been the tremendous importance to man since prehistoric times. Mollusks meat is extensively used as human food. Some bivalves e.g. pearl oyster, mussels produce natural pearls having values ranging up to several hundred dollars each. Mollusks also use for the preparation of lime, poultry, shrimp and fish feed. Mollusks species in the Bay of Bengal can be an important part of our economy. Three commercially important mollusks species are available along the coastal belt of Bangladesh with their abundance, distribution and culture potentials investigated by Shahabuddin, A. M., 2010. Cultivation and export of *Crassostrea* sp. could enrich the country's economy immensely. The production of aquaculture in our country can be increased to a certain extent by diversifying from only fish culture to shrimps, crabs, mollusk etc. In coastal area of Bangladesh, suitable mollusks habitats are sandy and rocky ground, mangrove and coral reefs exists; and thus are suitable for development of shellfish culture. The edible oysters are particularly exploited and underutilized; as vast majority of population do not consider shellfish suitable for eating. They however are popular food items in many others

developed as well as developing neighboring countries. However, there was little attempt made for mollusk like edible oyster culture in Bangladesh due to lack of proper knowledge on distribution and abundance of mollusks populations in our coast and ignorance of mollusk as food value etc.

Mollusks fishery in Bangladesh is still a virgin field in respect of exploitation. It is not yet realized that there are about two million tribal people who regularly take mollusks meat in their daily diet. They even use the meat in different forms such as cooked meat, fried or boiled meat, pastes, powder etc. the coastal people even market the excess products to the other tribal communities living in the hill districts. Since the distribution of mollusks is patchy and labour intensive collection procedure, and supply is dependent on seasons therefore, culture of mollusks especially mussels and oysters may be considered an urgent option of coastal aquaculture. Side by side an export market in the neighboring Southeast Asian countries needs to be explored so that excess product can be sold at a higher price.

Ahmad, 1990 stated that the coastal water of Bangladesh is one of the most productive zones in the world and rich in fish and shellfishes including mollusks. Oyster (*Crassostrea* sp.), is also found around different coastal areas in Bangladesh. I choose coastal region on Moheshkhali Channel as my study area because of there are varieties of marine habitats such as sandy, muddy and rocky grounds, mangrove areas and coral reefs are inhabited by the bivalves, and thus are potentially viable for the development of shellfish fishery. The high tidal amplitude, sufficient tidal current, absence of pollutants and high phytoplankton abundance offer an ideal environment for the development of edible oyster *(Crassostrea* sp*).*

The present study was undertaken to make a systematic survey on oyster (*C. madrasensis*) in the coastal zone of Bangladesh considering its economic as well as biological importance and culture potentials. Precise and detailed information particularly on oyster from Bangladesh is scanty. Though these studies comprehend the seasonal variation of proximate composition & heavy metal content of edible oyster (*Crassostrea madrasesnsis)* and the results of these studies are different in many ways and no studies till now have been studied about the seasonal variation of proximate composition of edible oyster (*C. madrasensis*) mainly for the coast of Bangladesh. The general

features of the channel, distribution and magnitude of standing stocks of oysters and seasonal changes in meat of oyster have been studied and the results are presented here.

1.1 Specific Aims & Objectives of this study: The specific objectives of the proposed project are as follows:

- To observe the seasonal growth rate and predicting the total production of edible oyster (*Crassostrea madrasesnsis)* in terms of weight
- To comprehend the seasonal variation of proximate composition & heavy metal content
- To understand the abundance, distribution, exploitation of edible oyster

1.2 Significance of the Project:

Marine fin fishes occupy an important place in human diet followed by crustaceans and mollusks to a much smaller extent. Consumption of shellfish is considerably low but shellfish contain significant amounts of omega-3 fatty acids and high quality protein. Government has taken initiative to ensure coastal and marine-based economic activities and its resources management through “Blue Economy”. In support of this Blue Economy, Government focusing on expansion of coastal aquaculture of non-traditional species such as edible oysters *(Crassostrea* sp., *Saccostrea* sp.).This research will be useful for the development of aquaculture technology of the edible oyster which ultimately will contribute in future employment opportunities, ensure economic growth and create a new horizon for coastal aquaculture of Bangladesh.

CHAPTER TWO
REVIEW OF LITERATURE

Oysters are a marine animals belonging to the family Ostreidae. They are one of the best known and most widely cultivated marine animals. Its dramatic growth and natural abundance have encouraged efforts to culture this species as a cheap protein source in this region which includes China (Menzel, 1988), Singapore (Cheong and Lee, 1981), the Philippines (Walter, 1982), Thailand (Chalermwat and Lutz, 1989) and India (Rajagopal et al., 1998).

Chemical composition and fatty acid profile of Pacific oysters are varies from season to season (Linehan et al., 1998). Bangladesh has six seasons in each year. Scientific study on the edible oyster in Bangladesh is insignificant. The high tidal amplitude, sufficient tidal current, absence of pollutants and high phytoplankton abundance offer an ideal environment for the development of mollusc's culture around coastal waters of Bangladesh (Ahmad, 1990). Information on marine mollusks from Bangladesh were provided by Ali (1975), Ali and Aziz (1976), Ahmed et al. (1978) and Ahmed (1990) which are confined mainly into systematic account with some notes on ecology of the overall mollusks fauna. Shahabuddin et al (2010) reported that three species of mollusks namely green mussel (*Perna viridis*), clam (*Meretrix meretrix*) and oyster (*Crassostrea madrasensis*) are naturally abundant in coastal areas of Bangladesh which are commercially very important in all over the world. Potential local markets have been identified for these mollusks by Sahadat (2004) and Ghose (2004) among the people of Rakhain community at Cox's Bazar and tribal people in Hill Tracts regions.

Inspite of considerable economic importance (Parulekar & Qasim, 1981), only a little information is available on the growth of Giant Oyster (Durve & Bal, 1962). Observations on the length-weight relationship of an organism are important in predicting the total production of the said organism in terms of weight (Durve & Shrikhande, 1976). The chemical composition of the oysters grown in Cork Harbour was similar to literature values for the same species grown in Pacific waters. Ranges for the chemical composition (dry flesh weight basis) were: fat (7.8±8.7%), protein (39.1±53.1%), glycogen (21.6±38.9%) and ash (4.0±12.1%) (Linehan et al. 1998). The n-3/n-6

index was high indicating a predominance of n-3 fatty acids in the species (Asha et al. 2014). Variability of the nutritional quality of the oysters was generally good, especially just before gamete release (premonsoon) when the concentration of nutrients was at its maximum. A low level of fat was detected in the edible meat of oysters and mussels (G.M. Salaskar & V.N. Nayak, 2011). Scientific study on the edible oyster in Bangladesh is insignificant. Data on its biochemical composition may also prove important for future policy formulation for sustainable exploitation of this species (Asha et. al., 2014). In the whole world, shell fish aquaculture represents 14–16% of the average per capita animal protein for 1.5 billion people and support over 2,00,000 livelihoods, mostly in developing countries (Tidwell and Allan, 2002; FAO, 2010).

CHAPTER THREE
MATERIALS AND METHOD

3.1 Abundance and distribution of oysters:-

The abundance and distribution of oyster was understand based on the field visits in Cox's Bazar; telephone communication, responses of mailed questionnaire to district and upazilla fisheries officers, secondary literatures and local people who are involved directly or indirectly in the mollusk fishery. The study was conducted from March 2018 to February 2019. Physical visits were mainly conducted in Chittagong-Cox's Bazar region and the distribution and abundance of oysters to other regions were confirmed mainly by e-mailed questionnaires, secondary information from the published materials. According to Shahabuddin et al. (2010), coastal belt was divided into four regions i.e. Khulna, Barishal, Noakhali and Chittagong-Cox's Bazar (Figure 1).

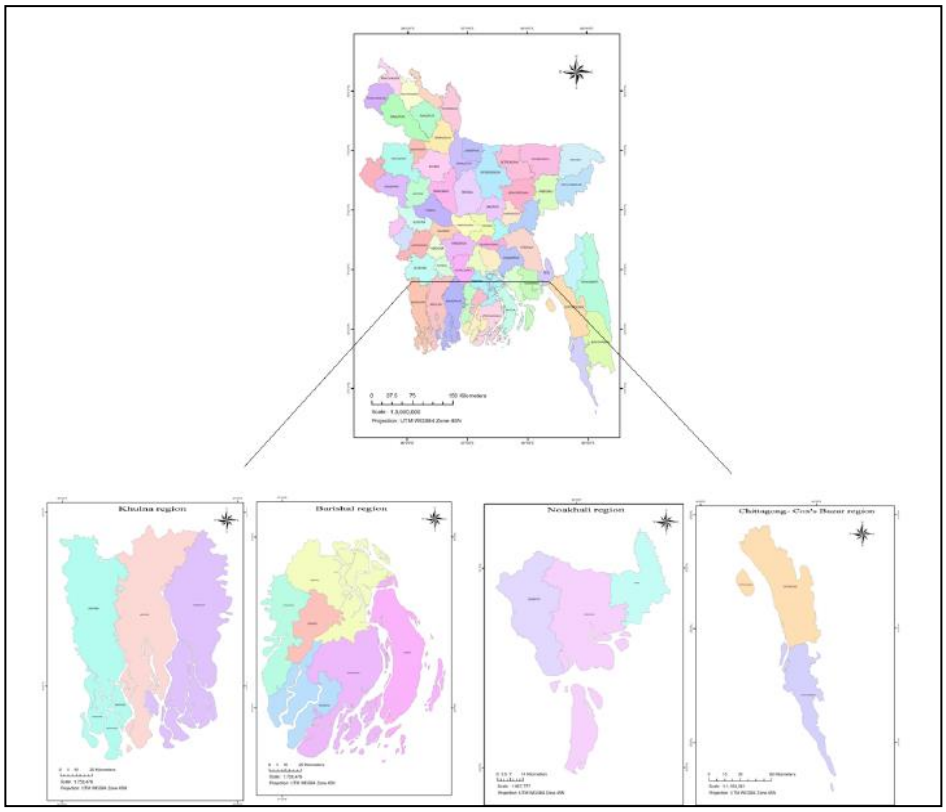

Figure 1: Map of Bangladesh with showing the studied area (Adapted from Shahabuddin et al. 2010)

Among these regions eleven coastal districts along the Bay of Bengal were selected for the study. The districts including Shatkhira, Khulna, Bagharhat, Borguna, Patuakhali, Barishal, Bhola, Noakhali, Feni, Laxmipur, Chittagong and Cox's Bazar, Sandwip, Shitakunda, Kutubdia, Maheskhali, Cox's Bazar sadar, Teknaf and Saint Martin Island were selected for direct interviews, participatory reflection and action (PRA) and direct field visits in Chittagong-Cox's Bazar region. Primary data were collected through direct interviews with shellfish collectors, traders, fishermen, boatmen, and fisheries officer in the coastal zone. Samples of oysters were directly collected from the field for identification (Figure 2).

Figure 2: Interview and PRA discussion with the oyster collectors about the abundance and distribution of oyster in Cox's Bazar area

3.2 Study Area:

Sampling was conducted indifferent areas of Moheshkhali channel (Figure 3c&3d). Moheshkhali Channel located in between 21°32' and 6°32'Nlatitudes and in between 91°58' and 47°57'Elongitudes (Fig. 3a). It is bounded by Chakaria Upazilla on the north, Cox's Bazar Sadar Upazilla and Bay of Bengal on the south, Chakaria and Cox's Bazar Sadar Upazilla on the east, Kutubdia Upazilla and Bay of Bengal on the west.

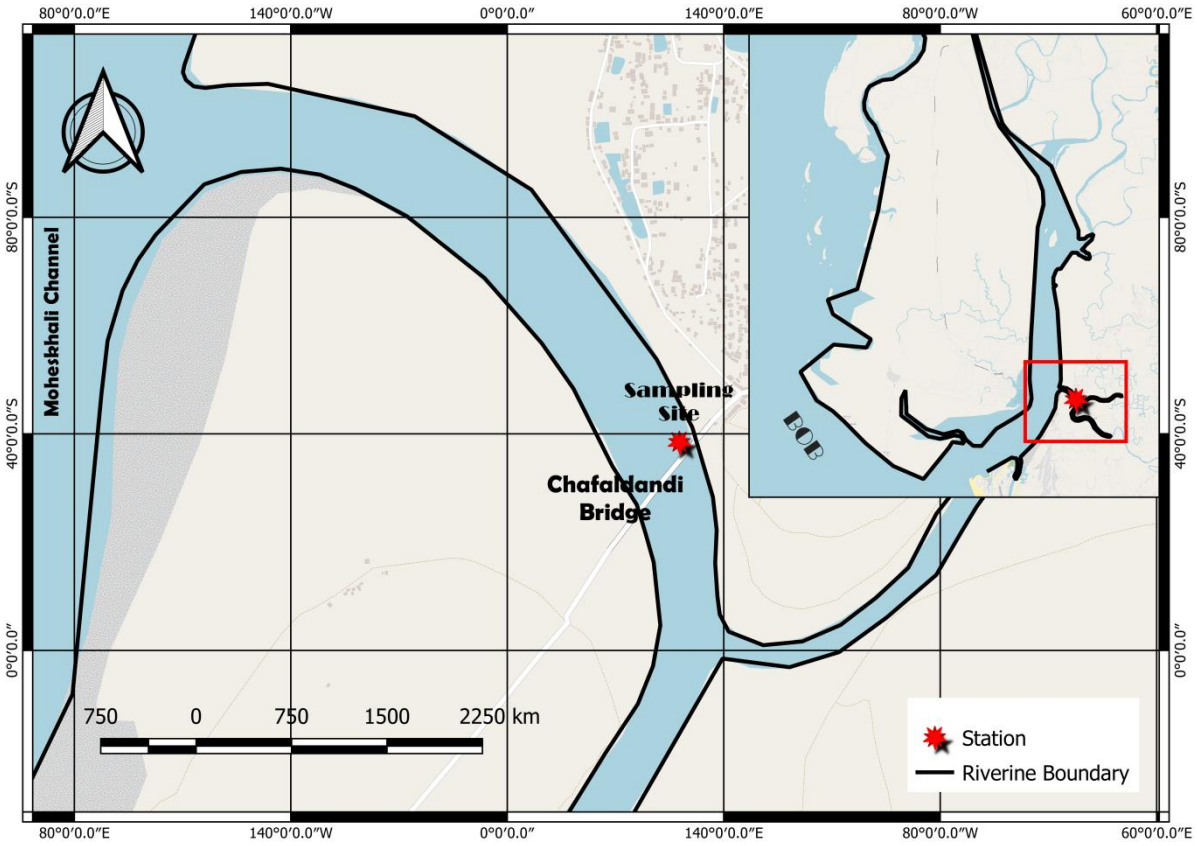

Figure 3: Latitudinal and Longitudinal direction of Moheskhali Channel (study area)

3.3 Methods of exploitations

Generally, oysters are found in the bottom attached with different types of substrates. Therefore, the collector must go to the bottom of the water-body to collect the samples. Considering the depth of water and tidal fluctuation, the collectors follow two different types of diving procedures. In case of high tide as well as high water depth, it takes too much time to collect the oyster from the bottom. In this situation the diver dives into the water having oxygen support. Usually an oxygen cylinder, an oxygen tube and a pumper used to give oxygen support to the diver. One opening of the oxygen tube is in the mouth of diver and another opening is attached to the cylinder. Then the cylinder and the pumper are connecting via a tube. After that a person continuously pumps the pumper until the diver back into the boat. In case of low tide and less water depth, collector easily collects the oyster from the bottom without any oxygen support.

Figure 4: Exploitation procedure of oysters by the local communities at Moheshkhali channel.

3.4 Length-weight relationship of oysters

The study was conducted to accomplish the length-weight data for twelve months from March 2018 to February 2019. Samples for this study were collected from the Maheshkhali Channel and Kutubdia Island, Cox's bazaar district, Bangladesh. During the study period about 20-30 oysters were collected in every month. After collecting, they were kept in ice and brought to the "Marine Lab" of the 'Faculty of Fisheries', 'Chittagong Veterinary and Animal Sciences University, Chittagong', for further research.

Figure 5. Collected oysters from Maheskhali Channel and cleaning at the laboratory for further analysis at CVASU

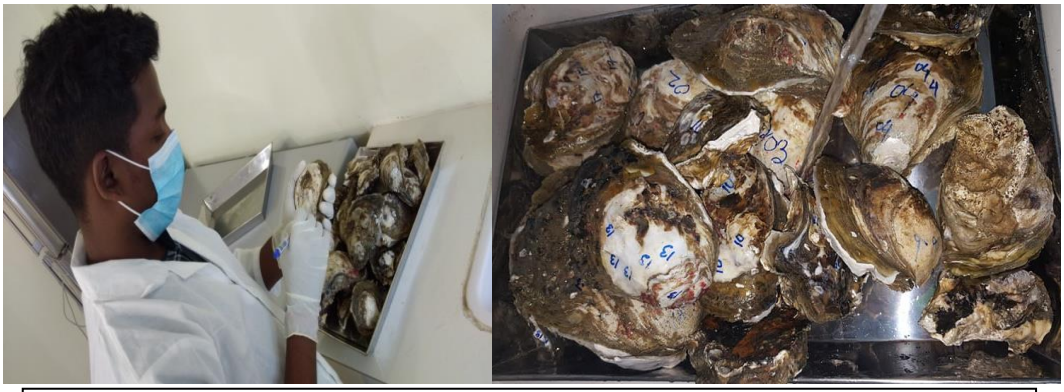

Figure 6. Marking and tagging of oyster before length-weight measurement

3.5 Recording of length-weight data

Total length of each oyster was measured by using a slide caliper. Data of length were recorded in centimeter and the body weight of each mussel was measured by using electrical balance and the data was recorded as gram (g) unit.

Figure 7: Measuring length and weight of oyster at laboratory

3.6 Determination of length-weight relationships:

The relationship between length (L) and weight (W) of mollusk species are represented by using "length-weight relationship" formula. The length-weight relationship between male and female specimen were taken separately and also for combined records, sexes were calculated using the following equation (Le Cren, C.D. (1951) The Length-Weight Relationship and Seasonal Cycle in Gonad Weight and Condition in Perch, *Perca fluviatilis*.)

$TW = qTL^{b}$

Where,

TW is the total weight (expressed in g),

TL is the total length (expressed in cm),

"q" is a coefficient related to body form and

"b" is an exponent indicating isometric growth when equal to "3".

In this equation the parameters q and b are constant, where the parameter b (also known as the allometry coefficient indicates the rate of weight gain relative to length. This power curve equation was converted into linear form by the use of natural logarithms as (Le Cren, C.D. (1951) The Length-Weight Relationship and Seasonal Cycle in Gonad Weight and Condition in Perch, *Perca fluviatilis*). Length-weight relationship is curvilinear functions often are transformed into linear functions by taking the logarithm or the natural logarithms of both sides:

$$\text{Ln } TW = \text{Ln } q + b \text{ Ln } TL$$

This equation is equivalent to regression equation:

$Y = a+bX$

This mean that; y is equivalent to lnW, 'a'represents the y-intercept (the point where the line crosses the y axis) of the regression line is equivalent to lnq, b is the slope of the line, and x is equivalent to lnL.

In excel by putting the length on X axis and weight on Y axis a scatter diagram was drawn. After that the length-weight data was converted to natural logarithms where,

Y-axis = Ln weight (g)

X-axis = Ln length (cm)

By using the converted length-weight data again a scatter diagram was plot. From that diagram the value of 'a' and 'b' was calculated.

3.7 Calculating condition factor

Condition factor is considered one of the important factors influencing the body composition of mollusc. It was calculated by using the following equation:

$$CF=W/L^b$$

Where,

CF= condition factor,

W= weight,

L=length.

In every month CF was calculated from the monthly samples, which helps to detect seasonal variations of mussel.

3.8 Calculation of Condition Index

Condition index (CI) is generally defined as a measure of the meat content relatively to total size of the organism. Its concept was developed primarily for the examination of oyster populations (Hawkins and Rowell, 1987). Grave (1912) was the first to express the quality of oyster meat by such an index by using the volume of the meat divided by the volume of the shell cavity. Monthly samples of oysters were collected from March 2018 to February 2019 from the Maheskhali Channel and Kutubdia Channel. Oysters were initially cleaned from all the encrusting organisms. Biometric measurements such as length (maximum length along the anterior-posterior axis), height

(maximum length along the dorsal-ventral axis), and width (maximum length through both valves) of each individual were measured using a Vernier caliper (Figure 7). The total weight of each individual was recorded after the inter valval (or mantle) fluid was drained. They were then dissected and the wet soft-tissue weight and the shell weight were accordingly measured. Dry tissue weight was measured after drying them individually for at least 72 hours at 105^{o}C to a constant weight and was cooled in a desiccator. To ensure that the 72 hours were sufficient to completely dry up the tissue, the tissue was weighed every 24 hours until the difference between consecutive weights were less than 5%. Generally, the constant weight was reached in about 72 hours. A CI value was calculated according to the following formula.

$$CI\ (g/cm^3) = \frac{\text{total soft tissue dry weight (g)}}{\text{shell volume } (cm^3)} \times 1000$$

Figure 8. Oysters ready for measuring soft-tissue weight and other parameters

Figure 9. Measuring the oyster shell volume

3.9 Biochemical analysis

Moisture:

All analyses (n = 6) were carried out in triplicate process. Moisture of the fish samples (10 g) were determined according to the AOAC (2000)/APHA 2005 method by drying in an oven at 105°C (n = 6). Results were expressed as percentage (%) of wet weight.

Ash:

Ash content was determined by heating the sample (5 g) for 12 h in a silica crucible in a furnace at 525 °C (n = 6) according to the AOAC (2000) method. Results were expressed as percentage of wet weight. Minerals were assayed using the AOAC method. Macro elements were determined by flame photometry using working standards in the range of 10–40 ppm for each element (Na, K and Ca). Trace metals were determined by Varian Spectra-220 AA atomic absorption spectrophotometer. Samples were aspirated into the flame and the corresponding absorption of the characteristic radiation by each element was recorded. Values are expressed in ppm.

Protein:

Total protein content in the homogenized samples (5 g) was determined using the Kjeldahl method. Results were expressed as percentage of wet weight (n = 6) basis.

Amino acids analysis:

Total amino acid composition was determined following the method of Ishida et al. (1981) using a Shimadzu chromatograph LC-10AT high performance liquid chromatography (HPLC) equipped with an ion exchange column, quaternary pump, a 20 µl injection valve and a fluorescence detector. Mobile phase A contained sodium citrate and ethanol (pH 3.5) and B had sodium citrate and NaOH (pH 9.8). The flow rate was constant at 0.4 ml/min, and the column temperature was set at 60 °C. The fluorescence excitation and emission wavelengths were 340 and 450 nm, respectively. Samples were hydrolyzed in 6 N HCl in evacuated sealed tubes at 110 °C for 24 h. After derivatisation by O-phthalaldehyde, amino acids were identified and quantified by comparison of their retention times with those of standards (Sigma). The results were expressed in terms of gram amino acid per 100 g of crude protein.

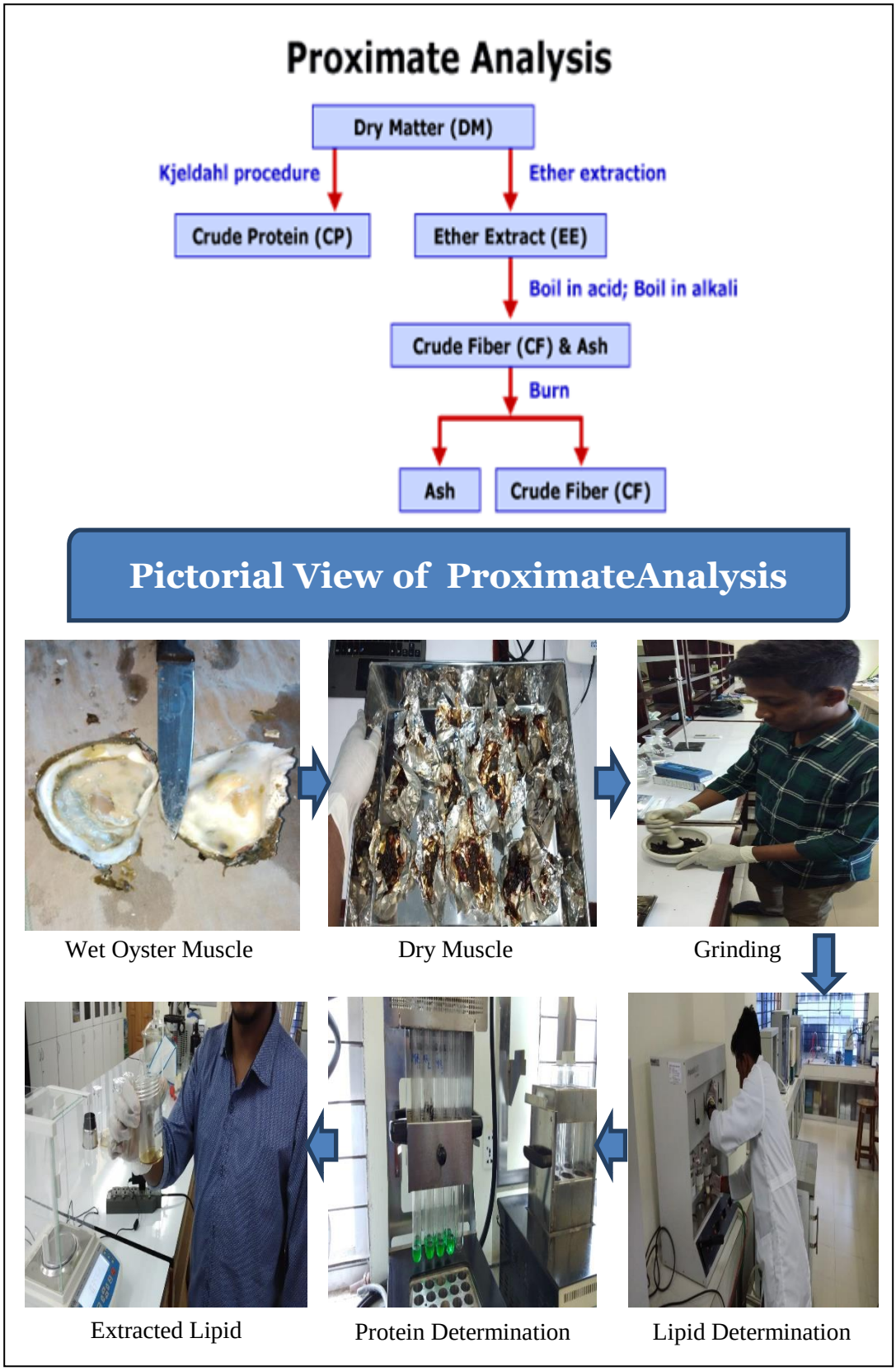

Figure 10: Analysis of Proximate Composition in laboratory

Lipid extraction:

The estimation of crude fat content was done by continuous extraction of fat with petroleum ether according to AOAC method (2000). Total lipids were extracted according to the method of Folch et al. (1957), using chloroform/methanol (2:1). Aliquots of the chloroform layer extract were evaporated to dryness under nitrogen and the lipids were quantified gravimetrically.

Fatty acid analysis:

Fatty acids methyl esters (FAMEs) were obtained by the method described by Metcalfe et al. (1966). A fraction of the lipid extract was saponified with 0.5 N NaOH in methanol followed by methylation in 14% boron trifluoride in methanol (BF_3/MeOH). The methylated sample was then extracted with 8 ml*n*-hexane. All of these reactions were performed in quadruplet for each sample. The resulting methyl esters were analysed using an Agilent Gas chromatograph system 6890 N equipped with a Flame Ionisation Detector (FID), a splitless injector and a polar fused silica capillary column (30 m * 0.25 mm i.d. * 0.25 μm film thickness). The temperature of the injector and the detector were 250 and 275 °C, respectively. Helium was used as a carrier gas with a flow rate of 1.5 ml/min. Peaks were identified by comparison of their retention times with FAMEs standards (Supelco).

Heavy Metal Analysis:

Heavy metals (Mercury ‘Hg’, Lead ‘Pb’, Cadmium ‘Cd’, Cromium ‘Cr’) will be examined from oven-dried sample which will be sent to BCSIR (Bangladesh Council of Scientific and Industrial Research) seasonally where the analysis will be done following standard methods.

Amino acids analysis:

Total amino acid composition was determined following the method of Ishida et al. (1981)using a Shimadzu chromatograph LC-10AT High Performance Liquid Chromatography (HPLC) equipped with an ion exchange column, quaternary pump, a 20 ll injection valve and a fluorescence detector. Mobile phase A contained sodium citrate and ethanol (pH 3.5) and B had sodium citrate and NaOH (pH 9.8). The flow rate was constant at 0.4 ml/min, and the column temperature was set at 60°C. The fluorescence excitation and emission wave lengths were 340 and 450 nm, respectively. Samples were hydrolysed in 6 N HCl in evacuated sealed tubes at 110 °C for 24 h. After

derivatisation by O-phthalaldehyde, amino acids were identified and quantified by comparison of their retention times with those of standards (Sigma). The results were expressed in terms of g amino acid per 100 g of crude protein.

3.10 Statistical analysis:

All the data were analyzed by Microsoft office Excel program and SPSS version 21.0.

CHAPTER FOUR

RESULT

The outcomes of a yearlong study made through questionnaire survey, several field visits and interviewing the coastal communities including fisher flocks have been conducted. The summarization of this questionnaire survey and presents in the Appendix A.

4.1 Survey on availability:

Direct field visits were done in Sandwip Island and Shitakunda region of Chittagong district, but there was no evidence of oysters in these places. However, large amounts of oyster were found in Kutubdia, Moheskhali Island under Cox's Bazar district as well as in Cox's Bazar Sadar. The direct field survey also indicated that oysters are also present in large quantities in Saint Martin's Island. The outcomes of the mailed questionnaires, telephone communication and field visits of Teknaf had provided firm indication that there was no evidence of oyster in this region. The findings of the study also indicated that considerable quantities of oyster are present in the region of Sundarban Mangrove Forest, Khulna covering three coastal districts; Bagherhat, Khulna and Shatkhira.

The level of oyster availability incoastal regions includes Khulna, Kutubdia, Moheskhali, Saint Martin and Teknaf are significant. High availability occurred in Kutubdia, Saint Martin and Teknaf following the medium abundance in Khulna and Moheshkhali whereas low availability occurred in other investigated sites. The details information regarding the distribution of oyster along the coastal belt of Bangladesh is in the Appendix A.

4.2 Exploitation:

Diversity of oyster exploitation was observed in the studied regions. Recent survey results indicate that the level of exploitation is high in most of these regions of availability. About 30-40 families in Khulna district was dependent on collection of oysters for their livelihood. In Cox's Bazar region, tribal communities used oysters' meat as food and sold within their communities. According to the collectors there was about 50-60 ton mollusks were collected from this region (Appendix B).

4.3 Monthly variation in proximate analysis:

The bio-chemical analysis of moisture, protein, crude fat and ash content of *C. madrasensis* are recorded as 10.41%, 9.25%, 76.64%and 1.01% respectively. The details on the chemical composition are given in Appendix C. The protein percentage varies from 32% to 43%where highest amount of protein recorded during May, August and November and lowest observed in March. The mean value of fat investigated from 5% to 11%. The ash content observed highest in July, August and September and lowest in other month. On the other hand, the protein percentage varies from 56% to 42%. (Figure 11)

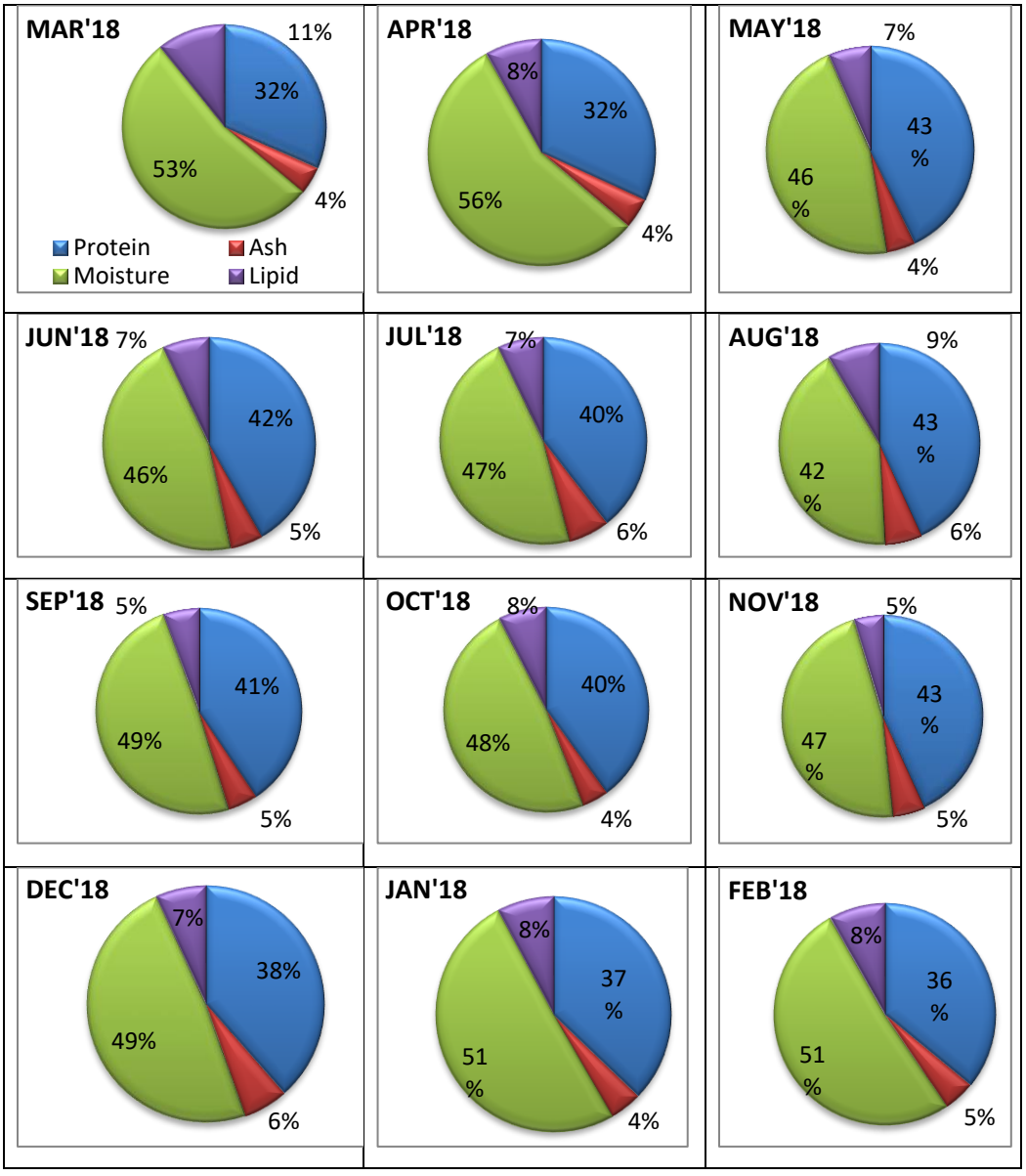

Figure 11: Biochemical content in Oyster flesh in dry basis

4.4 Seasonal variation in biochemical composition

4.4.1. Heavy Metal Content:

The average heavy metal content was found 0.2330 ppm (dry flesh weight basis) with a maximum of 0.36275 ppm observed in rainy season and minimum 0.152 ppm observed in winter (Figure 12). The selected heavy metals' concentration show the seasonal trend as: Rainy Season > Winter Season >Spring> Autumn > Summer > Late Autumn irrespective of all the heavy metals.

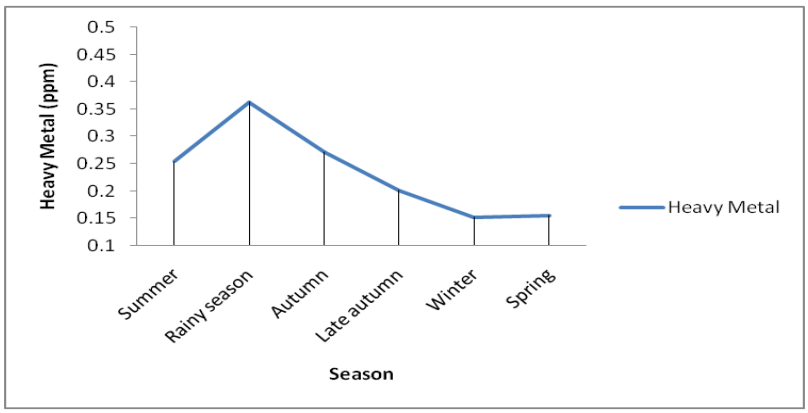

Figure 12: Seasonal Variation of Heavy metal

The concentrations of selected heavy metals in the tissue of *Crassosstrea madrasensis* was observed to occur in order as Pb>Cr>Cd>Hg irrespective of all seasons. The Pb concentration in the oyster muscle ranged from 0.56 ppm dry wt. (during rainy season) to 0.19 ppm dry wt. (during spring) whereas, Cr concentration reached 0.621 ppm dry wt. (during rainy season) from0.218 ppm dry wt. (during winter). On the other hand, Cd concentration was found 0.17 ppm dry wt. (during rainy season) to 0.08 ppm dry wt. (during winter) while, Hg concentration was found in constant around 0.1 ppm around the year (Figure 13).

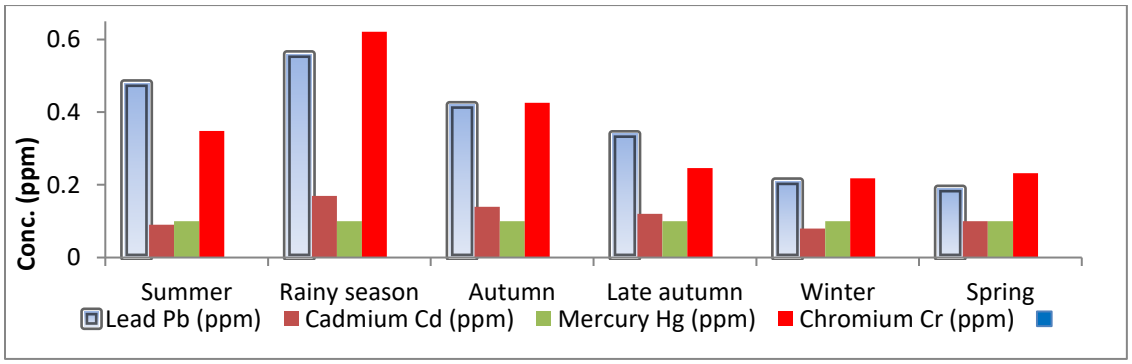

Figure 13: Concentration of heavy metals (in ppm dry wt.) in *Crassostrea madrasensis* collected from Moheshkhali Channel during the study period.

4.4.2 Fatty Acid Content

The level of different fatty acids content ranged from myristic (14:0) to docosahexaenoic (22:6) was observed during this research. The findings revealed that the fatty acid profile remained relatively constant during the study period with high concentrations of omega-3 fatty acids (18:3, 20:5 and 22:6). The overall lower concentrations of saturated fatty acids (14:0, 16:0 and 18:0) observed during this study. Palmitic acid (16:0) and oleic acid (18:1) were the main saturated and monounsaturated fatty acids, respectively among the Fatty acids content. The seasonal variation in fatty acid composition of *C. madrasensis* is shown in details at Appendix F.

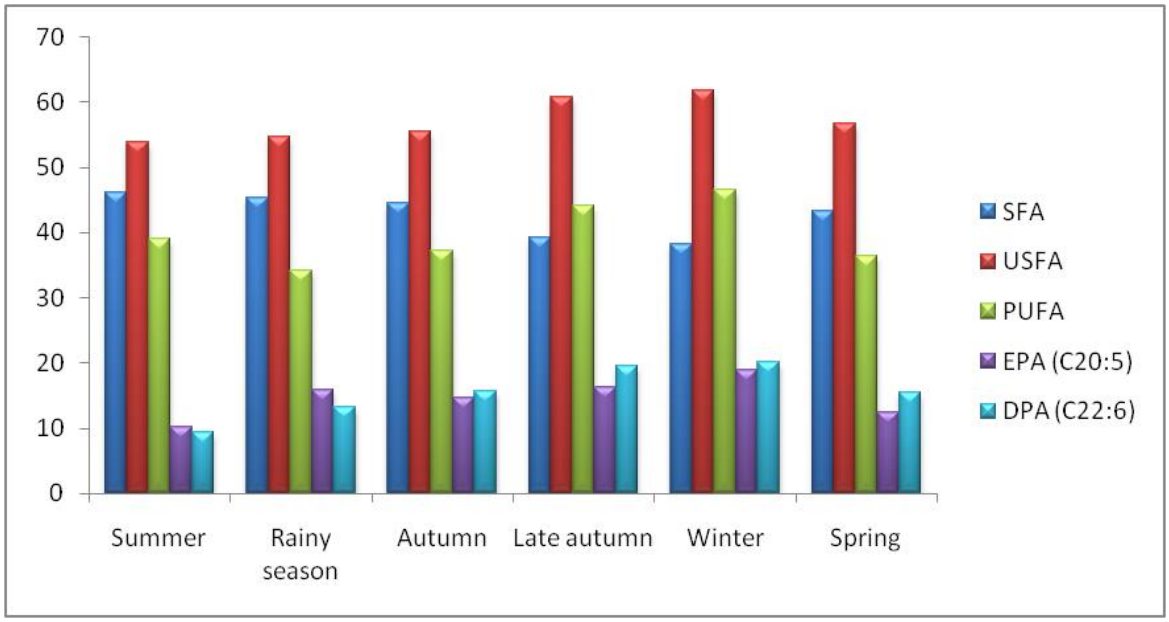

Figure 14: Variation of Fatty acid content of Oyster flesh during study period

4.4.3 Amino Acid Profile:

Among essential amino acids, lysine (5.23 g %) content was the highest followed by aspartic acid (3.79 g %), limiting amino acid leucine (2.99 g%); and threonine (2.69 g%) were also present in high concentration among the non-essential amino acid (Figure 15).The details on amino acid profile of *C. madrasensis* are shown in Appendix E.

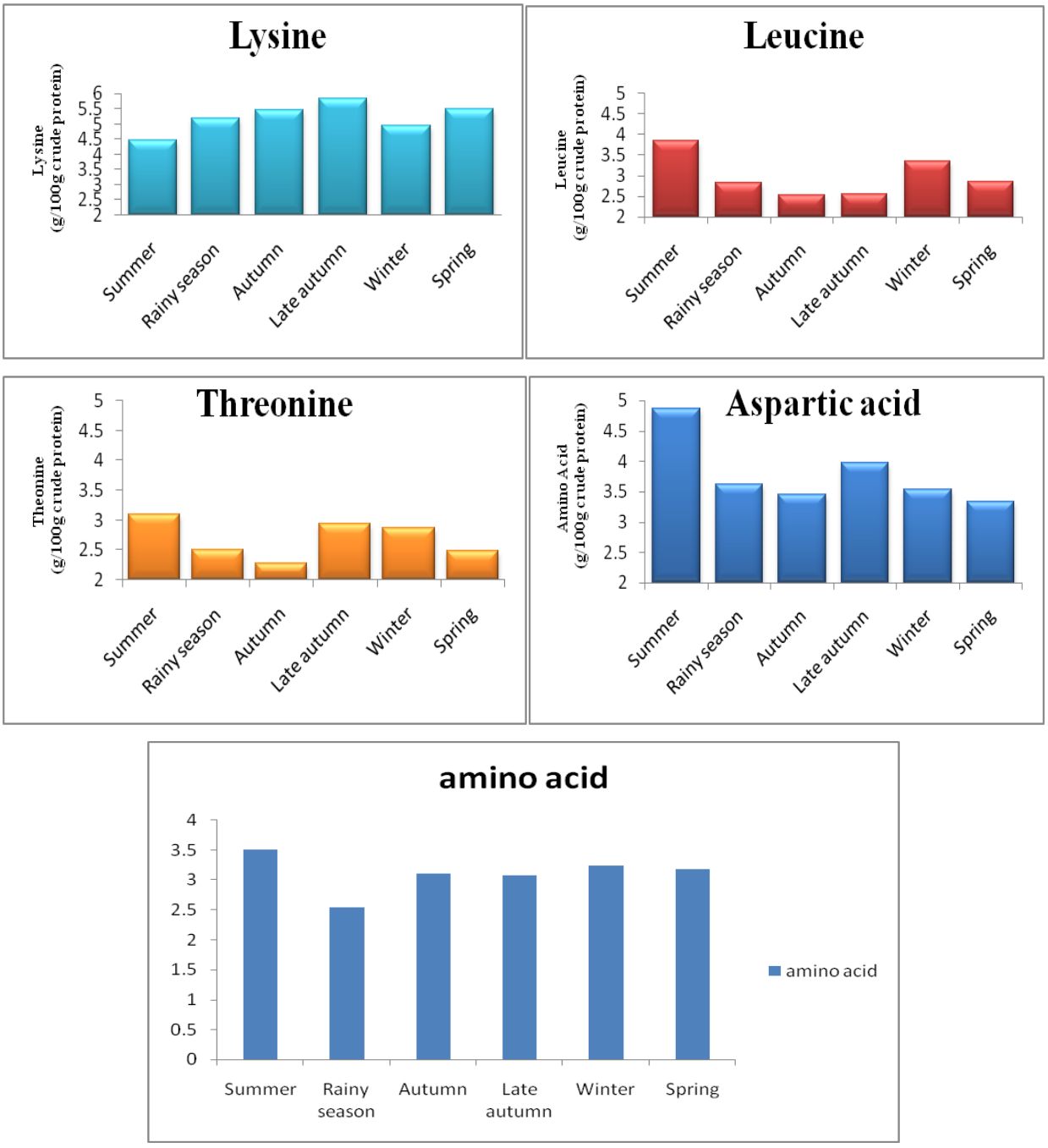

Figure 15: Amino Acid Profiling of *C. madrasensis*

4.4.4 Protein Content:

The protein content of the oysters fluctuated throughout the study period, ranging from maximum66.92±2.65 % (dry flesh weight basis) in rainy and autumn seasons to a minimum of 45.27±2.55% in spring season (Figure 16). Seasonal protein content in oyster flesh was significantly different $p<0.05$ with 95% level of significance (Appendix H).

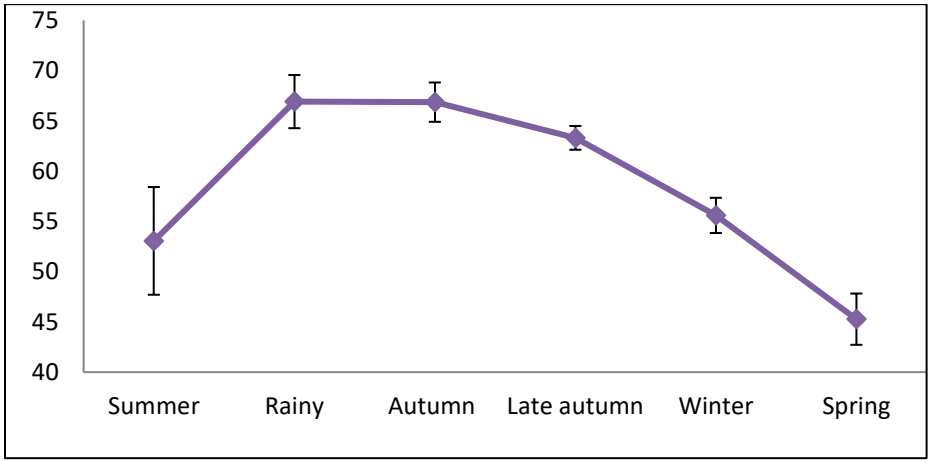

Figure 16: Variation of Protein content season to season

4.3.5 Lipid Content:

The Lipid content of the oysters varied throughout the seasons, fluctuating from 9.38±0.75% (dry flesh weight basis) in rainy season to 6.16±0.26% in summer (Figure 17). Lipid content was significantly different from season to season ($p<0.05$) with 95% level of significance (Appendix H).

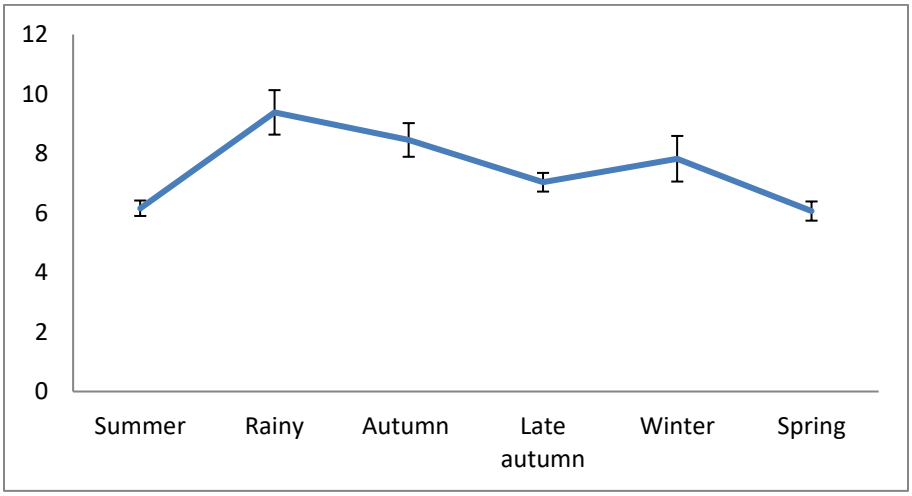

Figure 17: Seasonal Lipid Content in oyster flesh in dry weight

4.3.6 Ash Content:

The ash content varied significantly throughout the study period with highest during spring 12.61±0.61% (dry flesh weight basis) and lowest value 9.33±1.21% in late autumn (Figure 18). No seasonal significant difference were shown by ash content regarding with 95% significance level ($p<0.05$)(Appendix H).

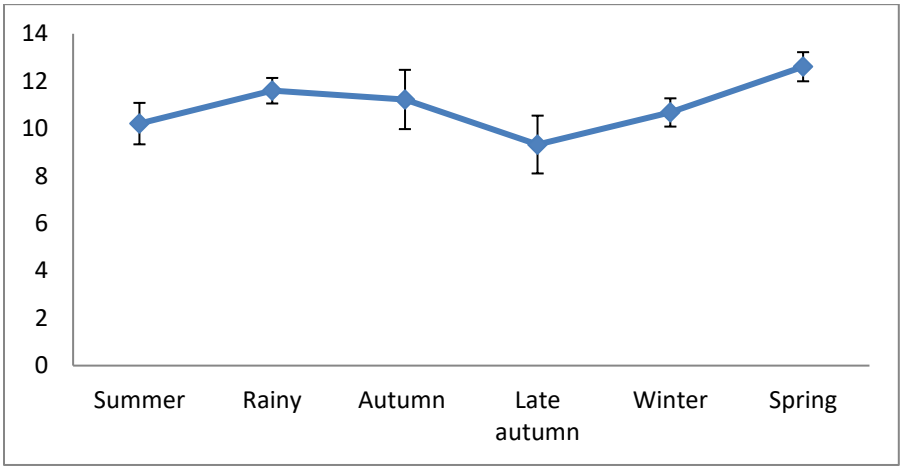

Figure 18: Ash Content of Oyster in Dry weight during study period

4.3.7 Moisture Content:

Moisture content also fluctuated from 76.96±1.41% (dry flesh weight basis) during rainy season, following 69.85±1.36% in spring (Figure 19).There was no seasonal difference present in moisture content regarding with 95% significance level ($p<0.05$)(Appendix H).

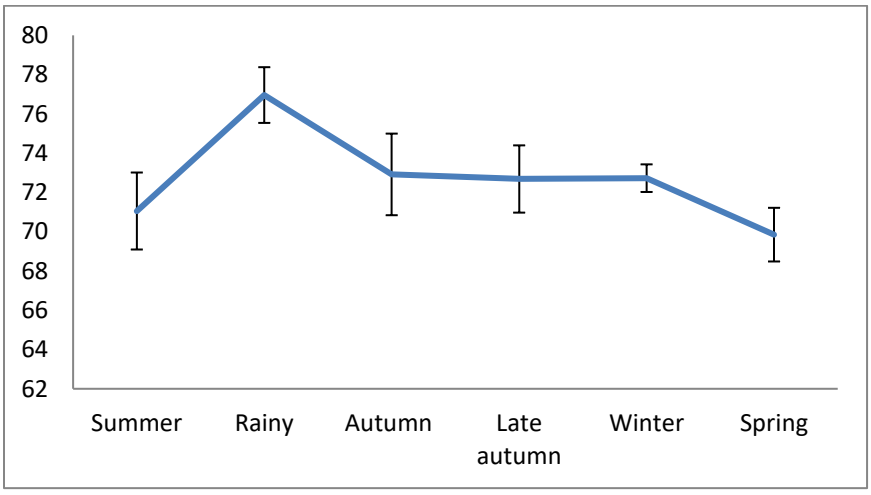

Figure 19: Moisture content variation from season to season

CHAPTER FIVE

DISCUSSION

5.1 Distribution and abundance of oysters:

The distribution and abundance of oyster related to salinity content of water bodies, where salinity level need optimum and constant all the year round. The coastal areas where fresh water runoff comes through different rivers into the estuaries and results in low salinity and high turbidity of silt, sand and clay particles, are not suitable habitats these three species of mollusks. As oysters are filter feeders, therefore, a non-turbid environment is also a prerequisite for their survival.

5.1.1. Barisal Region:

The outcomes of the mailed questionnaires, telephone communication and field visits had provided firm indication that there was no evidence of oyster in this region. According to the fisheries officer and secondary data analysis it was observed that low salinity, high sediment transportation with silt content as well as absence of suitable substrates might be responsible for the absence of oysters' population in the region.

5.1.2 Khulna Region:

The findings of the study indicated that considerable quantities of oyster are available in Sundarban Mangrove forest covering three districts of Bagerhat, Khulna and Shatkhira. Oyster are distributed all the channels criss-crossed, mud flats and the entire forests bottoms of Sundarban region. They were found to attach in hard substrate, trunk of mangrove trees, roots or even on old oyster shells and around the channel in cluster as a pile and also in the sluice gates, bridges adjacent to this reserve forest. Huge amount of larger size oysters is found in the deep forest along the sea. According to the shell collectors and fishermen of this mangrove forest, a huge amount of oysters' availability is mentioned. Moreover, oyster was found in the islands which are situated in southern tip of the Sundarbans, such as Dublar char, Alorkoal, Meheralir char, Haldir char, Moger char, Dhansatur char and Hiron point. A considerable number of families (about 50-60) in the village Pollimongal at Koira in Khulna district was dependent on collection of mollusks for their livelihood. They informed that the abundance of oysters was higher in winter season.

5.1.3 Noakhali Region:

The interviewee informed that oysters are not found in Noakhali region. The secondary water quality data clearly provide an indication that low salinity along with high siltation from the upstream runoff are responsible for non-existence of these three species of mollusks in the Noakhali region. Through interviewing the coastal communities in these areas on the uses of mollusks as food or for making lime or poultry feed were not traced out, which further strengthened the assumption that no oyster population of exists in these locality.

5.1.4 Chittagong-Cox's Bazar Region:

Direct field visits were done in the area of Sandwip Island and Shitakunda of Chittagong district, but there was also no evidence of oysters. The main reason of absence of these species in these areas includes low salinity, turbidity and lack of suitable substrates. According to the survey it was found that in the rainy season, the enormous water along with the silt and sand particles come from the upstream, which makes the region very turbid and muddy and thus mollusks population cannot survive in that unfavorable environment. However, large amounts of oyster were found in Kutubdia upazilla under Cox's Bazar district. Large amount of oyster also presents in Maheskhali Island along the channel. The direct field survey also indicated that oysters are also present in large quantities in Saint Martin Island. Oysters are found attached on the hard substrates. According to the collectors there was about 50-60 ton mollusks were collected from this region per year. The salinity at Chaufoldandi in Maheskhali channel was higher all the year round and the channel water was less turbid, which offered a suitable habitat for this species. In Cox's Bazar sadar Upazilla, large amount of oysters are present.

5.2 Exploitation:

Diversity of oyster exploitation was observed in the studied regions. Recent survey results indicated that level of exploitation is high in most of the investigated regions. Therefore, abundance of oysters are decreasing day by days. About 30-40 families in Khulna district was dependent on collection of oysters for their livelihood. According to the shellfish collectors in the Khulna region, about thirty boats each were having five persons collected mollusks from different places in the Sundarban area. Each boat was collected about 8 to 10 tons of oysters in each time. When they came back to the shore muscle parts were separated and the meat mainly sold to the shrimp and fish farms. According the middlemen about 500-550 tons of shells sold to the industry each year from this region. Higher amount of shells were found in the month of October to December.

In Cox's Bazar region, tribal communities used oysters' meat as food for them and sold within communities. They collected large amounts of oysters from the hard substrates in the channel. The shells of oyster are also sold to the poultry and tiger shrimp farming industries for making poultry and shrimp feed. According to the collectors there was about 50-60 ton mollusks were collected from this region (Appendix B).

5.3 Biochemical analysis of oyster samples:

Biochemical composition of a species helps to assess its nutritional and edible value in terms of energy units.

5.3.1. Heavy Metal Content:

This unique seasonal variation of selected heavy metals may be attributed to several factors like precipitation, evaporation, dilution etc. The sources of Zn and Cd in this present area from the galvanization units, painting industries and pharmaceautical processing industries. Among the main sources of Cr and Hg, anti-fouling paint is most important whereas, Pb finds its way to the aquatic system via. Painting and dyeing industries, battery manufacturing units etc. The significant levels of accumulated heavy metals in the muscle tissue of *C. madrasensis*areattribute to large amount of industrial discharges from highly industrial belt areas. Pb is a toxic and biologically non-essential element. The permissible limits of oyster muscle Pb, Cd, Hg and Cr are 1, 1, 0.5 and 0.5~0.8 ppm dry wt. respectively (WHO, 1989). Concentrations of heavy metals found in this study are much below from the permissible limit (Table 01).

Table 01: Comparison between permissible limit of heavy metals by WHO, 1989 and metal level found in the present study.

Heavy Metal	Permissible limit (in ppm dry wt.) (as per WHO, 1989)	Range of metal concentrations found in oyster muscle in the present study (in ppm dry wt.)
Lead (Pb)	1	0.19-0.48
Cadmium (Cd)	1	0.08-0.17
Mercury (Hg)	0.5	0.1
Chromium (Cr)	0.5-1	0.22-0.62

So, it is safe for consumption. Heavy metal accumulation in oyster tissue and shell is influenced by oyster metabolism and availability of the different metals from environment. A few metals in shells like Zn and Cu are derived from tissues because the mantle of oysters secretes a viscid

organic film at the edge of the valves where mineralization process occurs (Carriker et al. 1980).

5.3.2 Fatty Acid Content:

Significant monthly variations were seen in all fatty acids. Significantly higher concentrations of saturated fatty acid, unsaturated fatty acid and polyunsaturated fatty acids were recorded during summer, Late Autumn and winter season respectively. Significantly higher concentrations of omega 3 fatty acids were recorded during winter while significantly higher omega 6 concentrations were recorded during spring (Appendix F). This variation was done due to the changes of environmental parameters such as salinity of the water, availability of food and temperature. Sidwell et al., 1979 reported that, the variations of fatty acid concentrations in each site may be due to the changes of environmental parameters.

There were a significant differences between omega 3 and omega 6 fatty acids in wild oysters and cultured oysters. An appropriate ratio of n-3 to n-6 is very important to the production of eicosanoids at a balanced level. Omega 3 to omega 6 ratios of 3:1-6:1 have been recommended by some authors, to enable greater conversion of a linolenic acid into DHA (Krauss, 2000; Simopoulos, 2003; Lira et al., 2013). In the present study ratios between n-3 to n-6 were within this permissible range. The high ratio of n-3/n-6 ratio in oysters shows an occurrence of a high proportion of n-3 polyunsaturated fatty acids over n-6 polyunsaturated fatty acids. This ratio is very useful for comparing the nutritional value of fish lipids due to their human health effects in coronary heart diseases, cancer and autoimmune diseases (Wang et al., 1990; Simopoulos, 2002; Asha et al., 2014).

5.3.3 Amino Acid Profile:

Fifteen amino acids were identified and quantified in the wild and cultured samples of *C. madrasensis* (Appendix E). Table 6 shows the amino acid profile of *C. madrasensis*. Among essential amino acids, lysine (5.23 g%) content was the highest followed by aspartic acid (3.79 g%) , threonine (2.69 g%); and histidine (1.90 g%) were present in high concentration among the non-essential amino acid. The concentration of lysine in oyster protein is 5.23 g per 100 g crude protein which is significantly similar with the FAO/WHO recommended reference lysine standard value of 5.8 g per 100 g of dietary protein for a 2–5 year child. An abnormally high content of leucine in a protein interferes with the balance of two amino acids namely, isoleucine and threonine and additionally hinders the absorption of isoleucine and tryptophan. Grain proteins like those of sorghum and maize contain a high proportion of leucine that becomes a precipitating factor for the manifestation of pellagra in nutritionally challenged subjects.

Interestingly, in oyster meat, leucine is present at a low concentration of 2.99 g per 100 g crude protein.

The richness of amino acids in wild oysters has also been related to the maximum ripeness (Dridi et al., 2007). This demonstrates the potential capability of *C. madrasensis*, growing in wild condition, to withstand salinity and adverse stress conditions during summer, because glycine or its conjugate (glycine betaine) was earlier reported to have unique osmolytic property (Eklund et al., 2005) and helps to protect the cells during summer against osmotic injury.

5.3.4 Protein Content:

The protein content of the oysters fluctuated throughout the study period. Thus content peak in rainy & autumn seasons and minimum in amount during spring season. In rainy season they went to their reproductive phase that's why they need more protein in their flesh. The main constituent of oyster flesh is water, which is tightly bound to the proteins in the structure in such a way that it cannot readily be expelled even under high pressure and is an index of freshness. The high protein content and the less than average lipid levels are similar to that found in species of fish. Oyster meat contains good amount of carbohydrate unlike in fin fish in which it is negligible. Jeng et al. (1979) observed a similar cycle but reported a maximum protein content of 65%. Linehan et al. (1998) reported then protein content of *C. gigas* from 53.1% to 39.1%. Whyte and Englar (1982) also reported peak protein content throughout the month of August.

5.3.5 Lipid Content:

Lipid concentration in the oysters remained relatively constant when compared to other constituents. The Lipid content of the oysters fluctuated throughout the study duration, ranging from a maximum in rainy season to a minimum in summer season. Whyte and Englar (1982) reported an average lipid content of 7.35% for tray-cultured oysters while Jeng et al. (1979) found an average of 8.7% with a maximum of 12.9% in February and a minimum of 7.15% in June. Linehan et al. (1998) reported Lipid concentration in the oysters remained relatively constant when compared to other constituents. The average lipid content was 8.2% (dry flesh weight basis) with a maximum of 8.7% observed in December and a minimum of 7.8% observed in September. This research finding agrees with the previous research on the same field of biochemical analysis.

5.3.6 Ash Content:

Ash content varied significantly over the study period with highest in spring season and lowest in late autumn for hydrological factor and feeding habit. Jeng et al. (1979) reported ash content varied from approximately 10 to 20% due to their feeding habit while Whyte and Englar (1982) reported concentrations from approximately 9 to 14%. Linehan et al. (1998) reported Ash content varied significantly over the 13 month study period with a maximum in October of 12.1% (dry flesh weight basis) and a minimum of 4% in May due to their muscle content.

5.3.7 Moisture Content:

Moisture content varied significantly over the study period with a peak in rainy season and lowest in spring. In the rainy season moisture content high in amount due to the higher intake of water for osmoregulation. Linehan et al. (1998) reported variations in Moisture content of oysters in this study fluctuated from a maximum value of 79.5% in January to a minimum of 73.0% in August.

Table 02: Seasonal variation of Biochemical content of oyster with 95% level of significance

Parameters	**Df**	**F**	**sig**	**comment**
Protein	35	8.952	0	p***
Lipid	35	5.957	0.001	p**
Moisture	35	2.252	0.075	NS
Ash	35	1.615	0.186	NS

Here, the mean values and Standard errors followed by the different superscript letter in each factor indicate significant difference at 0.05. If the seasonal variation of proximate composition were Significant, ANOVA was followed by HSD. *p<0.05; **p<0.01; ***$p<0.001;$ NS, Not Significant.

CHAPTER SIX
CONCLUSION

The oyster *Crassosstrea madrasensis* is distributed at several places along the east and southwest coasts of Bay of Bengal with its good economic potential. As the population of developing countries are increasing day by day, the demand of protein rich food is the utmost important. Cyclical changes in biochemical composition of animal tissue are mainly studied to assess the nutritive status of an organism. Seafood is an important contributor to the diets of many individuals because of their unique nutritional composition. The shellfishes are known to be high in protein, low in fat and low in calories. *C. madrasensis* is comparable to fin fish with respect to its nutritional attributes with its protein being of high quality and its lipids being a good source of omega 3 and omega 6 fatty acids. Their high utilizable energy due to protein will prevent protein-energy malnutrition in their consumers. A high monounsaturated and polyunsaturated fat ratio compared to saturated fatty acids revealed a possibility to market *C. madrasensis* as a food ideal for coronary heart diseases, cancer, autoimmune diseases and hypertension etc. The omega 3 to omega 6 ratios also confirms the importance of *C. madrasensis* as an ideal source of omega 3.Thus it might be considered as a kind of aquatic food with high protein and low healthy fat. Biochemical composition and nutritional attributes of *C. madrasensis* may prove important for formulations of nutraceuticals and future policy regarding exploitation of this species. So, a comprehensive knowledge of their biochemical constituents during different seasons of the year are valuable for large-scale exploitation from natural resources and to promote culture techniques.

RECOMMENDATIONS AND FUTURE PERSPECTIVES

As a developing country, Bangladesh needs the protein for its increasing population. Fish is a source of protein in here in Bangladesh but sometimes it can be difficult to afford fish protein for some of the infra dignitatem population. Local people for food exploit these highly nutritious shellfish resources. And shells are transferred to small-scale industries for preparation of lime. As oyster (*C. madrasensis*) is useful for making palatable dishes throughout the world, the toxicological study like this is very much necessary for health perspectives. Moreover, this study is also ecologically significant as oyster belongs to a particular trophic level in complex food-web in the estuarine ecosystem.

Diets that are high in MUFAs and PUFAs are associated with reduced risk of cardiovascular disease and atherogenesis. The atherogenic and thrombogenic indices were found to be higher in the cultured samples compared with wild samples, which gives an indication of the attitude of a composite diet or a single food to protect from atherosclerosis and platelets aggregation. The higher omega 3 fatty acid content and consequently the higher omega 3/omega 6 fatty acid ratio in the wild samples apparently contributed to lower atherogenic and thrombogenic indices. It has been reported that due to the antiatherogenic and antithrombogenic properties, the n-3 PUFAs play a major role to protect human beings from atherosclerosis and platelet. The ideal omega 3 and omega 6 ratio noted in the oysters also contributed toward its qualities to be judged as desirable from the consumer health perspective.

If the fish protein can be replaced by the shellfish protein like Oyster, *Crassostrea madrasensis* then the demand of the protein may be fulfilled. For this purpose, culture of oyster can be the possible solution. From this study the seasonal variation of proximate composition of edible oyster can be helpful for the farmers and consumers. But the study would be more perfect if it was done for comparing the food availability under different environmental conditions and water quality parameters. Future studies are necessary in this sector to confirm the pollution status in this species of dominant edible oyster.

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APPENDICES

Appendix A: Abundance and distribution of oysters along the coastal belt of Bangladesh

Districts	Upazilla	Oyster (P/A)	Level of abundance
Shatkhira	Shamnagar	P	Medium
Khulna	Paikgacha	P	Medium
	Koira	P	High
	Dakop	P	Medium
Bagherhat	Sharankhola	P	Low
	Mongla port	P	Medium
Laxmipur	Ramgoti	A	-
	Raipur	A	-
	SadarUpazilla	A	-
Noakhali	SadarUpazilla	A	-
	Hatia	A	-
Chattogram	Sandwip	A	-
	Shitakundaw	A	-
Cox's Bazar	Kutubdia	P	High
	Maheskhali	P	Medium
	Saint Martin	P	High
	Teknaf	P	High

**Here, P expressed as present & A expressed as absent

Appendix B: Present status of exploitation of oyster in different regions

Region	**Location**	**Harvest**		**Amount**	**Uses**		**Beneficiaries**
		Commercial	Subsistence	**(Ton/year)**	Meat	Shell	
Khulna	Shamnagar	Yes	-	200-250	Yes	Yes	Poor people Business men Poultry Industry Lime maker
	Koira	Yes	-		Yes	Yes	
	Paikgacha	Yes	-		Yes	Yes	
	Dacopes	Yes	-		Yes	Yes	
	Mongla	Yes	-		Yes	Yes	
	Sahrankhola	Yes	-		Yes	Yes	
Cox's Bazar	Kutubdia	-	Yes	50-60	Yes	Yes	Tribal Communities Lime maker Poultry Industry
	Maheskhali	Yes	Yes		Yes	Yes	
	Choufaldandi	Yes	Yes		Yes	Yes	
	Teknaf	Yes	Yes		Yes	Yes	
	Saint's Martin	No	Yes		No	No	

Appendix C: Biochemical data of Oyster during Study period

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Protein	1	6	53.0633	13.12994	5.36027	39.2843	66.8424	38.5	69.22
	2	6	66.9217	6.481	2.64586	60.1203	73.7231	60	75
	3	6	66.8683	4.79488	1.9575	61.8364	71.9003	62.32	75
	4	6	63.315	2.89796	1.18309	60.2738	66.3562	59.76	67.57
	5	6	55.5983	4.29268	1.75248	51.0934	60.1032	50.06	61.3
	6	6	45.2733	6.24995	2.55153	38.7144	51.8322	35	52.66
	Total	36	58.5067	10.39057	1.73176	54.991	62.0223	35	75
Lipid	1	6	6.16	0.63567	0.25951	5.4929	6.8271	5.25	6.88
	2	6	9.3817	1.83211	0.74795	7.459	11.3043	7.2	12.06
	3	6	8.455	1.38714	0.5663	6.9993	9.9107	6.67	10.15
	4	6	7.0333	0.76912	0.31399	6.2262	7.8405	6.01	8.34
	5	6	7.8233	1.87872	0.76698	5.8517	9.7949	5.98	10.35
	6	6	6.065	0.7897	0.32239	5.2363	6.8937	5.2	7.11
	Total	36	7.4864	1.72312	0.28719	6.9034	8.0694	5.2	12.06
Moisture	1	6	71.05467	4.801964	1.960394	66.01531	76.09402	65.318	78.12
	2	6	76.96	3.477073	1.419509	73.31104	80.60896	72.55	81.26
	3	6	72.91833	5.09064	2.078245	67.57603	78.26063	64.59	78.33
	4	6	72.685	4.195077	1.712633	68.28254	77.08746	66.67	78.11
	5	6	72.72667	1.721716	0.702888	70.91984	74.5335	70.3	75.3
	6	6	69.85	3.345451	1.365775	66.33916	73.36084	63.56	72.35
	Total	36	72.69911	4.270321	0.71172	71.25424	74.14398	63.56	81.26
Ash	1	6	10.21	2.141345	0.874201	7.9628	12.4572	6.8	12.72
	2	6	11.5975	1.318134	0.538126	10.2142	12.9808	9.88	13.65
	3	6	11.22833	3.061368	1.249798	8.01562	14.44104	7.22	14.87
	4	6	9.32667	2.987472	1.21963	6.19151	12.46183	6.25	13.52
	5	6	10.68	1.460822	0.596378	9.14696	12.21304	8.95	12.79
	6	6	12.61	1.505138	0.61447	11.03045	14.18955	11.05	14.64
	Total	36	10.94208	2.293732	0.382289	10.166	11.71817	6.25	14.87

Appendix D: Heavy metals analysis of oyster samples

Parameters	Summer	Rainy season	Autumn	Late autumn	Winter	Spring
Lead Pb (ppm)	0.48	0.56	0.42	0.34	0.21	0.19
Cadmium Cd (ppm)	0.09	0.17	0.14	0.12	0.08	0.1
Mercury Hg (ppm)	<0.10	<0.10	<0.10	<0.100	<0.10	<0.10
Chromium Cr (ppm)	0.348	0.621	0.426	0.246	0.218	0.232

Appendix E: Seasonal variation of amino acid content of oyster (g/100 g of crude protein)

Parameters	Summer	Rainy season	Autumn	Late autumn	Winter	Spring
Aspartic acid	4.86	3.62	3.45	3.98	3.54	3.34
Threonine	3.1	2.5	2.28	2.94	2.86	2.48
Serine	3.85	2.66	3.94	3.68	3.12	3.48
Glutamic acid	7.54	5.47	8.54	6.78	5.46	7.94
Glycine	3.75	2.69	2.86	2.54	3.46	3.86
Alanine	3.32	2.53	3.82	2.24	3.56	3.12
Valine	2.79	1.98	1.98	2.34	2.88	2.94
Methionine	1.05	0.8	1.24	1.84	1.86	1.68
Isoleucine	2.22	1.59	1.25	2.32	2.46	2.12
Leucine	3.84	2.83	2.54	2.56	3.36	2.86
Tyrosine	2.59	1.75	2.43	2.54	2.95	2.04
Histidine	2.05	1.22	1.82	1.88	2.42	2.02
Lysine	4.46	5.19	5.46	5.84	4.96	5.48
Arginine	3.68	2.88	2.86	2.56	3.46	3.24
Average	3.507143	2.550714286	3.105	3.074285714	3.239286	3.185714

Appendix F: Variation of fatty acid content in different seasons

Parameters	Summer	Rainy season	Autumn	Late autumn	Winter	Spring
Saturated fatty acids	46.21±3.21	45.35±2.86	44.45±2.54	39.25±3.42	38.24±3.24	43.24±3.44
Myristic acids (C14:0)	--	8.88±1.15	6.23±0.87	5.88±1.12	6.12±0.78	8.21±1.24
Palmitic acids (C16:0)	39.17±2.84	30.35±2.38	31.64±2.52	30.63±3.12	28.12±2.65	29.94±3.14
Stearic acid (C18:0)	7.04±.0.89	6.12±0.54	6.58±0.46	2.74±0.24	4.01±0.46	5.09±0.86
Unsaturated fatty acids	53.79±4.21	54.65±3.64	55.55±4.86	60.75±4.22	61.76±4.22	56.76±3.84
Monounsaturated fatty acids	14.75±1.12	20.48±1.26	18.24±0.86	16.68±0.96	15.24±0.84	20.26±1.34
Myristoleic acid (C14:1)	0.72±0.06	0.96±0.12	1.14±0.16	0.88±0.12	1.24±0.14	1.36±0.22
Palmitoleic acid (C16:1)	14.02±1.12	14.62±1.06	12.24±1.24	10.26±1.46	8.9±0.84	12.98±1.34
Oleic acid (C18:1)	-	4.89±0.22	4.76±0.46	5.54±0.24	5.02±0.42	5.92±0.24
Polyunsaturated fatty acids	39.03±3.48	34.17±2.88	37.31±3.24	44.07±4.12	46.52±4.20	36.5±3.12
Linoleic acid (C18:2)	2.20±0.14	1.22±0.12	2.24±0.22	1.88±0.08	2.54±0.12	2.12±0.42
Linolenic acid (C18:3)	4.00±0.48	1.04±0.08	2.58±0.44	3.24±0.48	2.34±0.12	4.44±0.48
Arachidonic acid (C20:4)	13.32±1.24	2.84±0.84	2.12±0.26	3.12±0.38	2.36±0.24	1.96±0.24
Eicosapentaenoic acid (C20:5)	10.18±2.12	15.80±2.14	14.68±2.24	16.24±3.82	19.04±4.08	12.54±2.44
Docosahexaenoic acid (C22:6)	9.33±1.14	13.27±1.68	15.69±3.12	19.59±4.06	20.24±3.94	15.44±3.14

**Results are presented as mean concentration (mg/100g) ± SE

Appendix G: Multiple Comparison of proximate content in different seasons

Multiple Comparisons								
Dependent Variable		(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Protein	Tukey HSD	1	2	-13.85833*	4.10472	0.023	-26.3432	-1.3735
			3	-13.80500*	4.10472	0.023	-26.2899	-1.3201
			4	-10.2517	4.10472	0.157	-22.7365	2.2332
			5	-2.535	4.10472	0.989	-15.0199	9.9499
			6	7.79	4.10472	0.423	-4.6949	20.2749
		2	1	13.85833*	4.10472	0.023	1.3735	26.3432
			3	0.05333	4.10472	1	-12.4315	12.5382
			4	3.60667	4.10472	0.949	-8.8782	16.0915
			5	11.32333	4.10472	0.093	-1.1615	23.8082
			6	21.64833*	4.10472	0	9.1635	34.1332
		3	1	13.80500*	4.10472	0.023	1.3201	26.2899
			2	-0.05333	4.10472	1	-12.5382	12.4315
			4	3.55333	4.10472	0.952	-8.9315	16.0382
			5	11.27	4.10472	0.095	-1.2149	23.7549
			6	21.59500*	4.10472	0	9.1101	34.0799
		4	1	10.25167	4.10472	0.157	-2.2332	22.7365
			2	-3.60667	4.10472	0.949	-16.0915	8.8782
			3	-3.55333	4.10472	0.952	-16.0382	8.9315
			5	7.71667	4.10472	0.433	-4.7682	20.2015
			6	18.04167*	4.10472	0.002	5.5568	30.5265
		5	1	2.535	4.10472	0.989	-9.9499	15.0199
			2	-11.3233	4.10472	0.093	-23.8082	1.1615
			3	-11.27	4.10472	0.095	-23.7549	1.2149
			4	-7.71667	4.10472	0.433	-20.2015	4.7682
			6	10.325	4.10472	0.152	-2.1599	22.8099
		6	1	-7.79	4.10472	0.423	-20.2749	4.6949
			2	-21.64833*	4.10472	0	-34.1332	-9.1635
			3	-21.59500*	4.10472	0	-34.0799	-9.1101
			4	-18.04167*	4.10472	0.002	-30.5265	-5.5568
			5	-10.325	4.10472	0.152	-22.8099	2.1599
Lipid	Tukey HSD	1	2	-3.22167*	0.7612	0.003	-5.5369	-0.9064
			3	-2.295	0.7612	0.053	-4.6103	0.0203
			4	-0.87333	0.7612	0.857	-3.1886	1.4419
			5	-1.66333	0.7612	0.274	-3.9786	0.6519

			6	0.095	0.7612	1	-2.2203	2.4103
		2	1	3.22167*	0.7612	0.003	0.9064	5.5369
			3	0.92667	0.7612	0.825	-1.3886	3.2419
			4	2.34833*	0.7612	0.045	0.0331	4.6636
			5	1.55833	0.7612	0.341	-0.7569	3.8736
			6	3.31667*	0.7612	0.002	1.0014	5.6319
		3	1	2.295	0.7612	0.053	-0.0203	4.6103
			2	-0.92667	0.7612	0.825	-3.2419	1.3886
			4	1.42167	0.7612	0.44	-0.8936	3.7369
			5	0.63167	0.7612	0.96	-1.6836	2.9469
			6	2.39000*	0.7612	0.04	0.0747	4.7053
		4	1	0.87333	0.7612	0.857	-1.4419	3.1886
			2	-2.34833*	0.7612	0.045	-4.6636	-0.0331
			3	-1.42167	0.7612	0.44	-3.7369	0.8936
			5	-0.79	0.7612	0.901	-3.1053	1.5253
			6	0.96833	0.7612	0.797	-1.3469	3.2836
		5	1	1.66333	0.7612	0.274	-0.6519	3.9786
			2	-1.55833	0.7612	0.341	-3.8736	0.7569
			3	-0.63167	0.7612	0.96	-2.9469	1.6836
			4	0.79	0.7612	0.901	-1.5253	3.1053
			6	1.75833	0.7612	0.222	-0.5569	4.0736
		6	1	-0.095	0.7612	1	-2.4103	2.2203
			2	-3.31667*	0.7612	0.002	-5.6319	-1.0014
			3	-2.39000*	0.7612	0.04	-4.7053	-0.0747
			4	-0.96833	0.7612	0.797	-3.2836	1.3469
			5	-1.75833	0.7612	0.222	-4.0736	0.5569
Moisture	Tukey HSD	1	2	-5.90533	2.270812	0.128	-12.8122	1.00155
			3	-1.86367	2.270812	0.961	-8.77055	5.04322
			4	-1.63033	2.270812	0.978	-8.53722	5.27655
			5	-1.672	2.270812	0.976	-8.57889	5.23489
			6	1.204667	2.270812	0.994	-5.70222	8.11155
		2	1	5.905333	2.270812	0.128	-1.00155	12.81222
			3	4.041667	2.270812	0.493	-2.86522	10.94855
			4	4.275	2.270812	0.432	-2.63189	11.18189
			5	4.233333	2.270812	0.442	-2.67355	11.14022
			6	7.110000*	2.270812	0.041	0.20311	14.01689
		3	1	1.863667	2.270812	0.961	-5.04322	8.77055
			2	-4.04167	2.270812	0.493	-10.9486	2.86522
			4	0.233333	2.270812	1	-6.67355	7.14022
			5	0.191667	2.270812	1	-6.71522	7.09855
			6	3.068333	2.270812	0.755	-3.83855	9.97522
		4	1	1.630333	2.270812	0.978	-5.27655	8.53722
			2	-4.275	2.270812	0.432	-11.1819	2.63189
			3	-0.23333	2.270812	1	-7.14022	6.67355

			5	-0.04167	2.270812	1	-6.94855	6.86522
			6	2.835	2.270812	0.81	-4.07189	9.74189
		5	1	1.672	2.270812	0.976	-5.23489	8.57889
			2	-4.23333	2.270812	0.442	-11.1402	2.67355
			3	-0.19167	2.270812	1	-7.09855	6.71522
			4	0.041667	2.270812	1	-6.86522	6.94855
			6	2.876667	2.270812	0.8	-4.03022	9.78355
		6	1	-1.20467	2.270812	0.994	-8.11155	5.70222
			2	-7.110000*	2.270812	0.041	-14.0169	-0.20311
			3	-3.06833	2.270812	0.755	-9.97522	3.83855
			4	-2.835	2.270812	0.81	-9.74189	4.07189
			5	-2.87667	2.270812	0.8	-9.78355	4.03022
Ash	Tukey HSD	1	2	-1.3875	1.269717	0.88	-5.24946	2.47446
			3	-1.01833	1.269717	0.965	-4.8803	2.84363
			4	0.883333	1.269717	0.981	-2.97863	4.7453
			5	-0.47	1.269717	0.999	-4.33196	3.39196
			6	-2.4	1.269717	0.427	-6.26196	1.46196
		2	1	1.3875	1.269717	0.88	-2.47446	5.24946
			3	0.369167	1.269717	1	-3.4928	4.23113
			4	2.270833	1.269717	0.488	-1.59113	6.1328
			5	0.9175	1.269717	0.978	-2.94446	4.77946
			6	-1.0125	1.269717	0.966	-4.87446	2.84946
		3	1	1.018333	1.269717	0.965	-2.84363	4.8803
			2	-0.36917	1.269717	1	-4.23113	3.4928
			4	1.901667	1.269717	0.668	-1.9603	5.76363
			5	0.548333	1.269717	0.998	-3.31363	4.4103
			6	-1.38167	1.269717	0.882	-5.24363	2.4803
		4	1	-0.88333	1.269717	0.981	-4.7453	2.97863
			2	-2.27083	1.269717	0.488	-6.1328	1.59113
			3	-1.90167	1.269717	0.668	-5.76363	1.9603
			5	-1.35333	1.269717	0.891	-5.2153	2.50863
			6	-3.28333	1.269717	0.132	-7.1453	0.57863
		5	1	0.47	1.269717	0.999	-3.39196	4.33196
			2	-0.9175	1.269717	0.978	-4.77946	2.94446
			3	-0.54833	1.269717	0.998	-4.4103	3.31363
			4	1.353333	1.269717	0.891	-2.50863	5.2153
			6	-1.93	1.269717	0.655	-5.79196	1.93196
		6	1	2.4	1.269717	0.427	-1.46196	6.26196
			2	1.0125	1.269717	0.966	-2.84946	4.87446
			3	1.381667	1.269717	0.882	-2.4803	5.24363
			4	3.283333	1.269717	0.132	-0.57863	7.1453
			5	1.93	1.269717	0.655	-1.93196	5.79196

*. The mean difference is significant at the 0.05 level.

Appendix H:One-way ANOVA for proximate content in different seasons

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Protein	Between Groups	2262.355	5	452.471	8.952	0
	Within Groups	1516.383	30	50.546		
	Total	3778.738	35			
Lipid	Between Groups	51.772	5	10.354	5.957	0.001
	Within Groups	52.148	30	1.738		
	Total	103.92	35			
Moisture	Between Groups	174.155	5	34.831	2.252	0.075
	Within Groups	464.093	30	15.47		
	Total	638.248	35			
Ash	Between Groups	39.046	5	7.809	1.615	0.186
	Within Groups	145.096	30	4.837		
	Total	184.142	35			

BRIEF BIOGRAPHY OF THE AUTHOR

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