Chapter 1
Introduction

Blue economy is an emerging issue all over the world that may support growing needs of the world population. The more a country has its natural resources in sea, and has technologies to harness it the better it has the possibility to lead this era. The Bay of Bengal large marine ecosystem is an ecological gift from nature to Bangladesh that offers rich and diverse marine productivity (654687 tons/year) (Maruf 2004; DoF 2018). Plenty of aquatic resources of the Bay of Bengal bolster the need of millions of coastal people who live hand to mouth depending on this resources. Moreover, a great percentage of foreign earnings regarding frozen food products comes from marine aquatic resources (DoF 2018).

Oyster is one of the bivalve molluscs, observed in temperate, tropical, and subtropical seas, worldwide. Most of the commercially important oysters inhabit the coastal waters, comprising lagoons, estuaries, and coastal backwaters. They possess a pelagic larval life which facilitates wider distribution, and undergo metamorphosis prior to the start of sedentary life. The soft body parts remain enclosed within the shells which protect the internal organs from wild predators. The metamorphosed larvae use to settle on hard rocks, molluscs shells, hard bottom, and on any kind of hard structures. Being a filter feeder, oysters play a critical role in coastal ecosystem.

Out of all resources, marine fish and shellfishes are not only satisfying hunger of people but also significantly providing nutraceutical value to the consumers containing comparatively higher amount of fatty acids (Aziz et al. 2013). Oyster can be eaten both raw and cooked. During cooking process meat textural degradation occurs due to loosing water from low protein seafood (Økland et al. 2005). The quality, and texture of seafood mainly depends on the quality of protein. The highest concentration of protein and glycogen are found in oysters out of any other animal species (Sizaret and Jardin 1985).

Although, oysters have been eaten by humans for centuries, but they have been cognizant of other beneficial services of oysters (Coen and Grizzle 2007; Grabowski and Peterson 2007). Suspension filter feeding of oyster refine water quality as well as clarity (Kirby 2004), and increase nitrogen removal (Grabowski and Peterson 2007). Vertical oyster reef structures provide harbor for resident macro fauna, raise larval

retention, augment foraging, abridge competition, and facilitate shoreline protection (Peterson et al. 2003; Soniat et al. 2004; Grabowski et al. 2012; Humphries and La Peyre 2015).

The cupped oyster, *Crassostrea* spp. is the most commercially cultivable oyster species around the world that contributes 28% of world total molluscs production (FAO 2018). World trade of oyster was worthy of $301M in 2018. In 2018, top exporters of oysters were France ($109M), Ireland ($49.6M), Canada ($33.5M), United States ($22.7M), and Netherlands ($20.2M); top importers of oysters were France ($40.7M), Hong Kong ($34.6M), China ($33.8M), United States ($31.1M), and Italy ($30.2M). Fortunately, one species under the genus *Saccostrea* and three species under the genus *Crassostrea* are available in the coastal waters of Bangladesh (Pagcatipunan 1984). There was several oyster reefs along the coast; however, indiscriminate wild oyster harvesting resulted in declined oyster abundance. However, Bangladesh has a 710 km long coastline, endowed with lagoons, estuaries, and coastal backwaters which can be utilized for oyster farming. Appropriate planning may offer us to contribute in world oyster trade, regional food supply, and restoration of oyster reefs.

Notwithstanding, Bangladesh has just taken footsteps in culturing marine fish and shellfish species where Norway started Salmon farming in 1970, and thus developed countries having marine water resources didn't neglected that opportunity. Oyster farming is a growing aquaculture sector as well as the most significant bivalve industry around the world (FAO 2006). Japan, China, European countries, and USA started oyster farming several decades ago. Even, India also started oyster farming few decades ago. Nonetheless, commercial oyster farming has not been practiced yet in Bangladesh. FAO executed a pilot project in 1983–1984 to initiate oyster farming in Bangladesh (Pagcatipunan 1984). But it didn't sustained in the long run due to lack of investments and appropriate approaches. Withal, investors didn't come forward due to several reasons. Firstly, due to information gap in economic viability of the enterprise. Secondly, as people do not know the nutritional value of oyster, local market demand of oyster was not established and government approaches weren't sound enough to initiate oyster farming. Finally, unavailability of seeds and lack of farming associated technical knowledge discouraged investors to come forward, resulting in dependence on natural resources and harvesting year after year for

satisfying the existing demands. Along with other shellfishes, oysters are harvested indiscriminately from natural stock in Cox's Bazar coast. Mostly, Rakhain and Chakma tribes, lived in Cox's Bazar, are the primary harvesters as well as consumers of oysters.

Major oyster farming countries throughout the world are harnessing economy from oyster production as well as oyster farm oriented tourism. Thus this single group of species is assisting a lot to earn blue economy. As Bangladesh is also planning for harnessing blue economy, oyster farming can be an appropriate option for this. Considering this opportunity, study on spat settlement pattern, nutritional composition of oyster, and economic viability of an oyster farm were necessary. This will help to burgeon awareness on oyster nutritive value, and to encourage investors to commercial oyster farming. Hence, the aims of this study include –

i. Identification of the potentiality of study sites for allocation for spat collection, based on oyster spat settlement pattern, to foster commercial farming as well as restoration efforts
ii. Determination of nutritional value of farmed oyster in Bangladesh
iii. Evaluation of economic viability of oyster farming in Bangladesh

Chapter 2

Review of Literature

Ahmed et al. (1978) observed *Crassostrea gryphoides* (Schlotheim), *C. belcheri* (Sowerby), and *C. madrasensis* (Preston) in the coastal waters of Bangladesh (Pagcatipunan 1984); these oysters could also be commercially exploited (Shahabuddin et al. 2010). However, global assessment of oyster reefs has shown that existing natural oyster reefs are the most jeopardized habitat on earth, while 85 – 91% of oyster habitats have already been lost (Jackson 2008; Beck et al. 2011), despite restoration attempts that have been widespread for centuries (MacKenzie 1997; Banks et al. 2007).

2.1. Spat settlement

Primarily, exacerbation in overharvesting, disease outbreak, and alteration in coastal hydrology have resulted in declined oyster habitats (Rothschild et al. 1994; Kirby 2004). Similarly, the overharvesting and consumption of oysters by local tribal communities at Cox's Bazar coast, Bangladesh could have reduced the oyster population (Shahabuddin et al. 2010). Withal, ecosystem disturbances and region-wide decline of oysters have also been stimulated by sedimentation, pollution, habitat degradation or loss from dredging/coastal development, and introduced diseases (Beck et al. 2011; Wilberg et al. 2011). Oyster fisheries, ecosystem services as well as local ecology could be affected by a sharp decline in the oyster population, which could be prevented by oyster farming or applying other restoration processes (Grabowski and Peterson 2007). Although Pagcatipunan (1984) reported that FAO executed a pilot project during 1983–1984 to initiate oyster farming in Bangladesh, it didn't result in the initiation of sustainable commercial oyster farming due to lack of adequate data on spat settlement pattern.

The term 'Settlement' is used when sessile existence is committed by any organism (Connell 1985). The settlement and growth of diversified fouling organisms can be fostered by large arrays of aquaculture structures (Milne 1975; Hodson et al. 1997; Hossain et al. 2013). However, differential settlement patterns could take place due to planktonic zonation, physical environment, and influence of existing inhabitants (Bushek 1988). Withal, Connell (1961), Dayton (1971), Menge and Sutherland (1976) observed intertidal communities structuring was remarkably

influenced by competition and predation. Most of the successful oyster restoration projects employed oyster shell as substrate for oyster spat settlement from existing natural oyster reefs (Bartol and Mann 1997; Blomberg et al. 2018). Even though shellstring fail to provide an accurate assessment of oyster settlement on actual reef topography (Baker 1994), they are efficient and reliable predictors of the presence of late-stage pediveligers at a given site (Bartol and Mann 1997; Southworth 1998; Metz et al. 2015). Metz et al. (2015) also found the highest mean spat densities (2,040.9 spat/m^2) on oyster shells in shellstring method using a comparative study of substrates in the Loxahatchee river estuary, Florida. A chemical cue could come from the calcium content of oyster shell that can lure spat to settle (Tanyaros 2011). Nonetheless, the settlement pattern is affected negatively by fouling (Sutherland and Karlson 1977; Nalesso et al. 2008). The concentration of waste products, food, and oxygen supply could be influenced negatively in the farming environment due to the water flow reduction caused by biofouling (Mohammad 1976; Lodeiros et al. 2002). However, phytoplankton community could also increase as a beneficial effect of biofouling (Kaehler 1999). The bivalve shells could experience extensive damage (i.e., cavities, burrows, and tunnels deep within the nacreous layer) caused by photosynthetic endoliths (Cobb 1969; Cronin et al. 1999; Braithwaite and McEvoy 2005).

2.2. Nutritional composition

Highest concentration of Glycogen is found in oysters rather than other animal species (Sizaret and Jardin 1985). Though being a seafood item oyster contains good quality protein but their standard quality depends on high level of n-3 (EPA, DHA, and n-3 HUFA) fatty acids (Sargent and Tacon 1999).

Evidences prove that n-3 HUFA ($C \geq 20$), EPA, and DHA have remarkable significance in human disease prevention. Moreover, imbalance in n-6/n-3 fatty acids ratio may contribute to coronary heart disease with increased risk (Simopoulos 1990). It is also proved that regular EPA and DHA intake with diet significantly prevents inflammatory, cardiovascular, and neural disorders (Casula et al. 2013). Absence of these fatty acids in diet may also cause immune disorders, hypertension, depression, inflammatory disorders, and neurological disorders. However, certain functions in retina and in brain cannot be performed by n-6 series which can successfully be carried out by DHA (Neuringer et al. 1988).

Being a bivalve, oyster is filter-feeding animal which accumulate elements from water, inorganic particulate, and food that may also result in bioaccumulation of toxic substances (Liao and Ling 2003; Amiard et al. 2008). But it can only be potentially hazardous if the concentration level of these substances exceed the maximum residue limits (Liao and Ling 2003; Amiard et al. 2008).

2.3. Economic viability

According to Cheremisinoff (1995), any enterprise can be marked as economically viable if the revenue exceeds the production cost. It can be figured out by using payback period method or by net present value method or even by using internal rate of return method. But this is applicable for a medium to large scale enterprises. Payback period method may potentially be good enough for short-duration small-scale enterprises or farm. In the last few years, different institution have developed several budgeting tools to assist bivalve producers in budgeting (Adams et al. 2001; Hudson et al. 2012a). Most of the tools are used for cultchless method but there is no specific tools for shellstring method.

It is expected that in near future the consumer demand of bivalves will increase greatly and the worldwide production has consistently increased from 7.1 million to 16.1 million over the years 1995 to 2014 (FAO 2016) which may help to sustain oyster farming. However, billions of dollars can be wasted in freshwater infrastructures including aquaculture due to biofouling (Abbott et al. 2000; Champ 2000), which is difficult to estimate in budgeting.

Chapter 3

Materials and Methods

3.1. Study Sites

Cox's Bazar coast is prevailed by a subtropical monsoonal climate. From winter to summer, air temperature varies from 10 °C to 38 °C. In early June, heavy southwest monsoon rains begin, and continue to mid-October. During the monsoon months (i.e., June to September), 80% of the total rainfall occurs with the annual rainfall ranging 2320 – 5447 mm (BMD 2017). Typically, a semi-diurnal tidal pattern is observed in these coastal waters. Seasonal variations in Mean Tide Level is 50 – 80 cm with approximately 3.5 m tidal range (BIWTA 2017). Three different sites: (a) Nunia Chara (NC – 21°28ˈ19.5" N, 91°57ˈ42.7" E), (b) Chowfoldandy (CD – 21°30ˈ44.1" N, 92°01ˈ00.1" E), and (c) Sonadia Island (SI – 21°30ˈ18.7" N, 91°53ˈ43.3" E) were chosen to establish experimental units (see Figure 1).

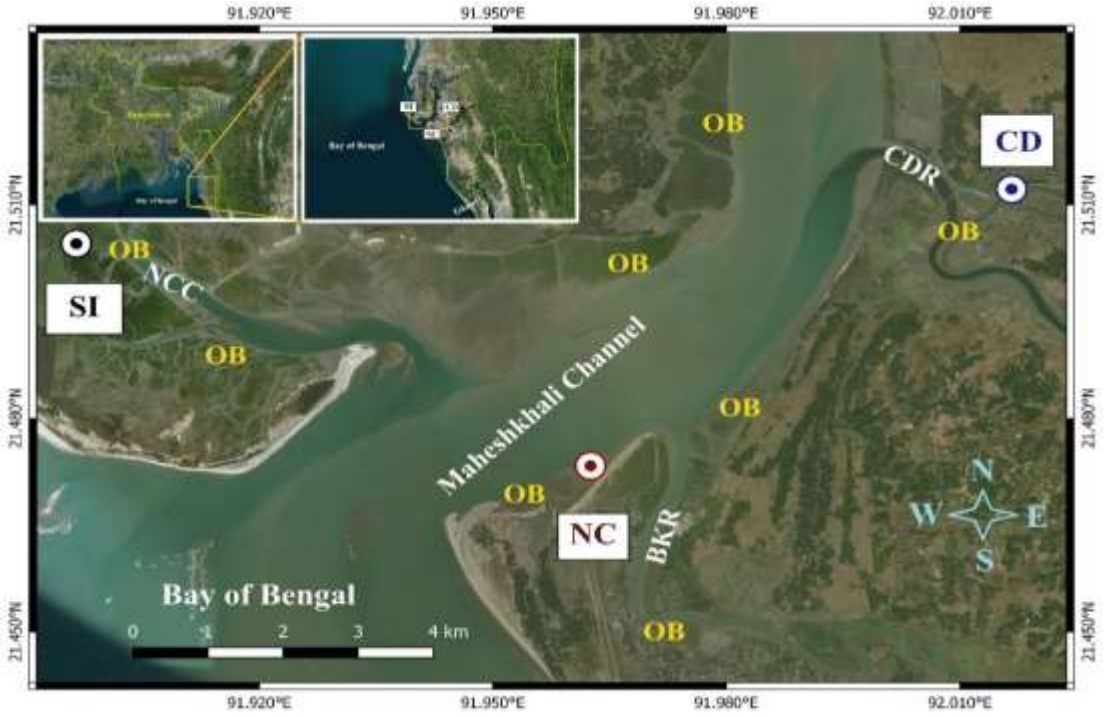

Figure 1 Map of the study sites. NC, CD, and SI represents Nunia Chara, Chowfoldandy, and Sonadia Island study sites, respectively. NC site is situated beside Maheshkhali channel; CD site is situated in Chowfoldandy River (CDR); SI site is situated in Noa Chira Canal (NCC). OB represents the sources of natural oyster brood, identified by visual inspection.

NC is an inter-tidal zone characterized by a muddy bottom, and becomes dry during low tide throughout the neap tide. It is moderately influenced by surface runoff

carried through the Maheshkhali channel. CD is a sub-tidal zone characterized by a rocky and muddy bottom. It is strongly influenced by surface runoff and river discharge. SI is also a subtidal zone characterized by a muddy bottom, and surrounded by mangroves. It is slightly influenced by surface runoff. Random dispersion of *Crassostrea* spp. is observed in NC, whereas clumped dispersion is observed in CD and SI sites.

3.2. Experimental unit

Triplicates of experimental unit were constructed in all the three study sites maintaining 1 m distance between two units. Shellstring arrays were deployed (modified from Haven and Fritz 1985) in each experimental unit that contained 12 strings placed in a pattern as showed in Figure 2. Each of the strings was tagged with a unique identification. Each string contained 5 oyster shells at 20 cm distance from each other, and the first one was placed at 20 cm water depth from the surface. Thus, each experimental unit was consisted of 60 oyster shells. Each shell surface area was measured using digital planimeter, and the sum of the surface area of both sides of 60 shells was the total surface area of an experimental unit**.** Mean shell surface area of three experimental units at NC, CD, and SI study sites were 5889.9 ± 265.9 cm^2, 4865.0 ± 100.6 cm^2, and 5095.5 ± 357.2 cm^2, respectively. The Floating bamboo raft was used to set the experimental unit, and was anchored to the bottom mud in such a way that it could easily move up and down along with tidal fluctuation.

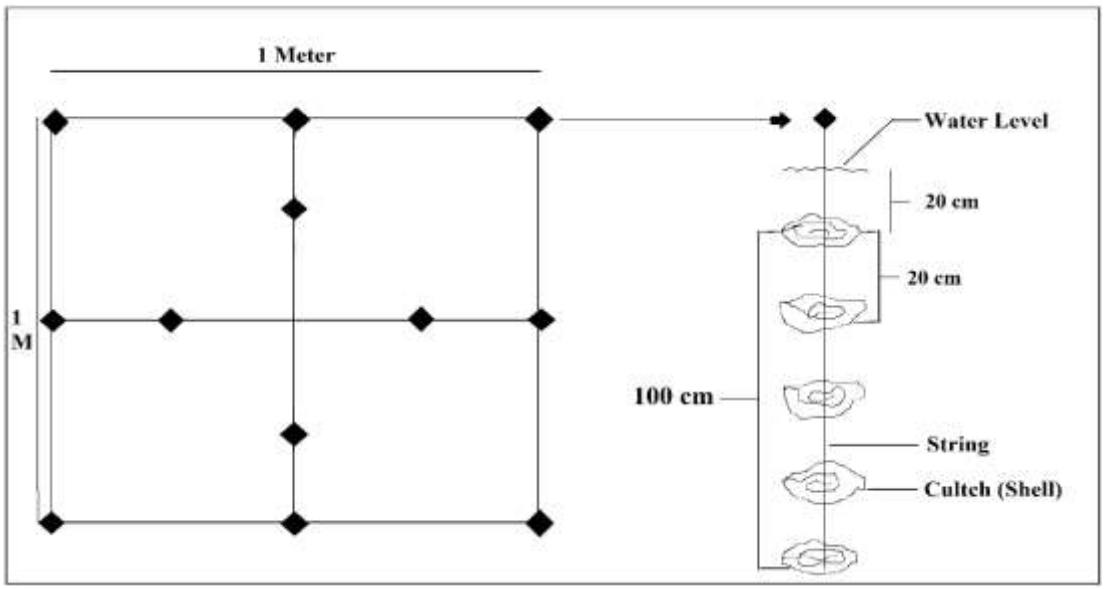

Figure 2 Pattern of a substrate unit holding 12 shellstrings and hanged from bamboo raft.

3.3. Spat count

Live and dead spat were counted in situ following the non-destructive and destructive method, respectively, in 15 days interval from February 2019 to January 2020. The spats of more than 2 mm in length were counted by naked eyes, and then returned to the water in all the three study sites. The spat of *Crassostrea* spp. was identified by local oyster harvesters by observing their color, shape, and structure. Live oyster spats were identified by closed valves, while dead spats were recognized either by observing a left shell or by observing opened immobilized valves.

3.4. Determination of environmental variables

High and low tide water depth, water salinity, temperature, and pH were measured in situ during every sampling. During the water depth measurement, either high tide or low tide water depth was measured manually using lead line (i.e. a long rope with a lead weight at one end). The tidal range of sampling day was taken from the real–time tide chart available at https://www.tide-forecast.com/, and then either added with low tide water depth to get high tide water depth or subtracted from high tide water depth to get low tide water depth. The water temperature, water pH, and water salinity was measured from surface water by using a glass thermometer, a handheld pH meter (pHep-HI98107, HANNA), and a handheld ATC refractometer (YEGREN), respectively. All the instruments were calibrated before use.

3.5. Biofouling observation

Eight major groups of fouling organisms (seaweed, sponges, marine macrophytes and bush like organisms, mussels, barnacles, other oysters, polychaetes, and oyster drills) were monitored throughout the study period across all the study sites. The observations were made based on absence or presence of organisms on oyster shell substrates. Both sides of all the 60 shells of each experimental unit were observed to identify the fouled shells. Then, the number of affected shells was converted into percentage. All the fouling organisms were cleaned using brush during each sampling to clearly define the temporal variation as well.

3.6. Oyster collection for nutritional composition analysis

A number of 50 live oysters were collected randomly from each oyster farm on January 2020. The collected oysters were 36.5 ± 2.4 mm in shell length, 30.3 ± 1.9

mm in shell width, and 20.0 ± 1.9 mm in shell thickness at NC farm; 37.1 ± 0.9 mm in shell length, 36.1 ± 1.2 mm in shell width, and 18.7 ± 0.7 mm in shell thickness at CD farm; 34.3 ± 1.2 mm in shell length, 28.6 ± 1.9 mm in shell width, and 18.6 ± 1.1 mm in shell thickness at SI farm. Oysters were stored in ice after collection. Within 12 hours of collection, oysters were taken to the laboratory, and fresh meat (whole body) was collected. Oyster meat was then dried using hot air oven.

3.7. Nutritional composition analysis

Nutritional composition of oyster varies with season (Martino and Cruz 2004). That's why oysters were collected at late winter, and that were settled at the beginning of winter. Winter season was selected for oyster collection because late winter to early summer are preferably the most suitable time for commercial harvesting of oyster in Bangladesh. Oyster samples (whole body) were dried firstly. All the samples were blended into fine powder. Then proximate and fatty acid content were analyzed.

3.7.1. Proximate analysis

Oyster samples (whole body) for protein, lipid, and carbohydrate were dried firstly. All the samples were blended into fine powder. Moisture, protein, lipid, ash, and crude fiber were determined according to the standard methods of AOAC (2000). Wet oyster samples were dried at 105 °C temperature in hot air oven until reaching to a constant weight. Protein content of dry oyster sample was determined by Kjeldahl method (N × 6.25) using Kjeldahl apparatus and manual titration. Soxhlet apparatus was used to determine lipid at 100 °C, and using diethyl ether as solvent. Ash content was determined by using muffle furnace at 550 °C temperature for 6 hours. Crude fiber was determined by using fiber extraction apparatus and muffle furnace. Samples were first acid boiled and then alkali boiled at 100 °C, and then filtered with acetone. Then the residue was ignited at 600 °C for 3 hours in muffle furnace. Carbohydrate analysis was conducted based on the method (Dubios et al. 1956). For each sample, 5 mg dried powder was taken, and made into 25 ml solution by mixing with distilled water. Tissue homogenizer was used for homogenous mixing. Prior to the analysis, 5% phenol solution and concentrated sulphuric acid was prepared. Samples were analyzed by adding 1 ml of 5 % phenolic solution and 5 ml of concentrated sulphuric acid. The standard was prepared using glucose. The optical density was measured at 488 nm using a spectrophotometer (UV-VIS Double beam, Model-T80, HANNA).

3.7.2. Fatty Acids

Fatty acids were determined according to Prato et al. (2017). At first, lipid was extracted from the dried (60 °C) sample using Soxhlet apparatus. Diethyl ether was used as solvent during lipid extraction. At the final stage of lipid extraction 60 °C temperature was maintained. This lipid sample was used to analyze fatty acid methyl esters. Analysis of Fatty acids methyl esters (FAMEs) were conducted by gas chromatography mass spectrophotometry using a GCMS-QP2020 (Shimadzu, Japan), equipped with flame ionization detector. FAMEs were separated with a capillary column (Length 30 m, internal diameter 0.25 mm, film thickness 0.15 μm, and phase ratio 250). Helium was used as carrier gas at a flow rate of 1.42 ml/min. The column temperature program was as follows: 180 to 280 °C at 5 °C /min , and then held at 280 °C. FAMEs were identified by comparing retention times with a standard (FAME mix C8-C24; Sigma-Aldrich, Germany). Quantities were expressed in ppm. Then it was converted into % of total fatty acids.

3.8. Data collection for economic evaluation

Cost and estimated sell data of the three pilot oyster farm, situated at NC, CD, and SI, were collected from District Fisheries Officer (Project Director– "Introduction of Oyster (*Crassostrea* spp.) in Bangladesh", funded by IORA, and implemented by Department of Fisheries, Bangladesh), Cox's Bazar, Bangladesh. This data included the actual expenditures, based on local product prices required for oyster farming. Sell prices were based on survey data from local restaurants, Rakhain, and Chakma communities, where oyster can be sold. There were usually 2 grades of oyster during selling in local market. Prices varied according to the grade and the demand varied according to consumer preferences.

3.9. Economic viability

Income–Expenditure data (Appendix A and B) of the three oyster farms were collected from District Fisheries Office, Cox's Bazar. Economic viability was estimated from the net profitability and payback period of the farms.

3.10. Calculations

Spat settlement determinant was calculated as follows:

Spat density (spat/m^2) $= \frac{\text{Sum of spat count}}{\text{n} \times \text{Total shell surface area}} \times 10000$; ($n$ = number of observations)

Recruitment rate (spat m^{-2} $week^{-1}$) $= \frac{\Sigma(\text{Spat count} - (\text{Previous spat count} - \text{Dead spat}))}{\text{n} \times \text{Month days} \times \text{Total substrate surface area}} \times 7 \times 10000$; ($n$ = number of observations)

Mortality (%) $= \frac{\Sigma\ (\text{Dead spat} \div \text{Spat count})}{n} \times 100$; ($n$ = number of observations)

Biofouling was calculated as follows:

% of shell affected = (Number of shells affected by fouling organism$\div$60) $\times$ 100; (60 is the total number of shells in an experimental unit)

Following calculations were used to estimate net profit and payback period:

Net profit = Annual income – (Depreciation cost + recurring cost)

Where, Depreciation cost is the 33.33% of fixed cost.

Payback period (years) = Initial investment / net profit

Where, Initial investment = Fixed cost + recurring cost

3.11. Statistical Analysis

Mean and standard error of the mean (SE = $\sigma/\sqrt{n}$) were calculated by using MS Excel. All the data, except the data of high tide water depth, were found non-normally distributed. Spat density and mortality data were transformed into $\log_{10}$ (x+1); recruitment and salinity was transformed into sqrt ((xMax+1)-x) (negative reflection); low tide water depth data was transformed into square root; pH, temperature, and total suspended solids data were transformed into $\log_{10}$ (x).

An ANOVA (two factor) was performed to test spatial and temporal variability in spat density, recruitment, and mortality rate with the transformed data (Appendix C). Normality and hereoscedasticity of residuals were checked visually. Box and whisker plots were used to display the temporal and spatial variation in spat density, recruitment, and mortality. Tukey's honestly significant difference test was applied to

differentiate the spat density, recruitment, and mortality rate among different months as well as study sites.

ANCOVA (two factor) was used to compare different spat density, recruitment, and mortality regression model with regard to the application conditions (linearity, homoscedasticity, and independence) (Appendix D). The relationship of spat density, recruitment, and mortality rate were further investigated using a linear multiple regression analysis with the transformed data. Collinearity was not minimized to maximize the adjusted R-squared (Appendix E, F, and G).

One way ANOVA was applied for proximate and fatty acids (Appendix H, I, and J). When assumptions were met, Tukey's honestly significant difference test was applied to differentiate the proximate and fatty acids among the three oyster farms.

The level of significance was set as 0.05. These tests were performed using SPSS (IBM v. 25.0) statistical software.

Chapter 4

Results

4.1. Spatial variability of spat density, recruitment, and mortality

Spat density, recruitment, and mortality significantly ($p < 0.05$) varied in accordance with the study sites. The median value of spat density was the highest at NC (375 spat/m^2, n = 36), and was the lowest at CD (70 spat/m^2, n = 36) (Figure 3A). The median value of spat recruitment was the highest at NC (29 spat m^{-2} $week^{-1}$, n = 36), and was the lowest at CD (10 spat m^{-2} $week^{-1}$, n = 36) (Figure 3B). The median value of spat mortality was the highest at SI (20.8%, n = 36), and was the lowest at CD (10.8 %, n = 36) (Figure 3C). However, the mean value of spat density after $\log_{10}$ (x+1) transformation was the highest at NC, and was the lowest at CD ($p < 0.05$, Figure 3A). The mean value of spat recruitment after square root transformation was the highest at NC, and were the lowest at both CD and SI ($p < 0.05$, Figure 3B). The mean values of mortality after $\log_{10}$ (x+1) transformation were the highest at both NC and SI, and was the lowest at CD ($p < 0.05$, Figure 3C). The mean values of spat density, recruitment and mortality were more inconsistent at CD than NC and SI sites.

4.2. Temporal variability of spat density, recruitment, and mortality

Spat density, recruitment, and mortality significantly ($p < 0.05$) varied in accordance with the study period. The median value of spat density was the highest in January 2020 (382 spat/m^2, n = 9), and was the lowest in July 2019 (35 spat/m^2, n = 9) (Figure 4A). The median value of spat recruitment was the highest in March 2019 (93 spat m^{-2} $week^{-1}$, n = 9), and was the lowest in July 2019 (2 spat m^{-2} $week^{-1}$, n = 9) (Figure 4B). The median value of spat mortality was the highest in April 2019 (38.6 %, n = 9), and was the lowest in August 2019 (0 %, n = 36) (Figure 4C). However, the mean values of spat density after $\log_{10}$ (x+1) transformation were the highest in March 2019, April 2019, November 2019, December 2019, and January 2020, and was the lowest in August 2019 ($p < 0.05$, Figure 4A). The mean value of spat recruitment after square root transformation was the highest in March 2019, and were the lowest in June 2019, July 2019, August 2019, September 2019, October 2019, and January 2020 ($p < 0.05$, Figure 4B). The mean values of mortality after $\log_{10}$ (x+1) transformation were the highest in April 2019, May 2019, Jun 2019, July 2029, and January 2020, and was the lowest in August 2019 ($p < 0.05$, Figure 4C). The mean values of spat density were

more inconsistent in April 2019 to September 2019; the mean values of spat recruitment were more inconsistent in April to May 2019, July to August 2019, and January 2020; the mean values of mortality were more inconsistent in August to September 2019 than other months.

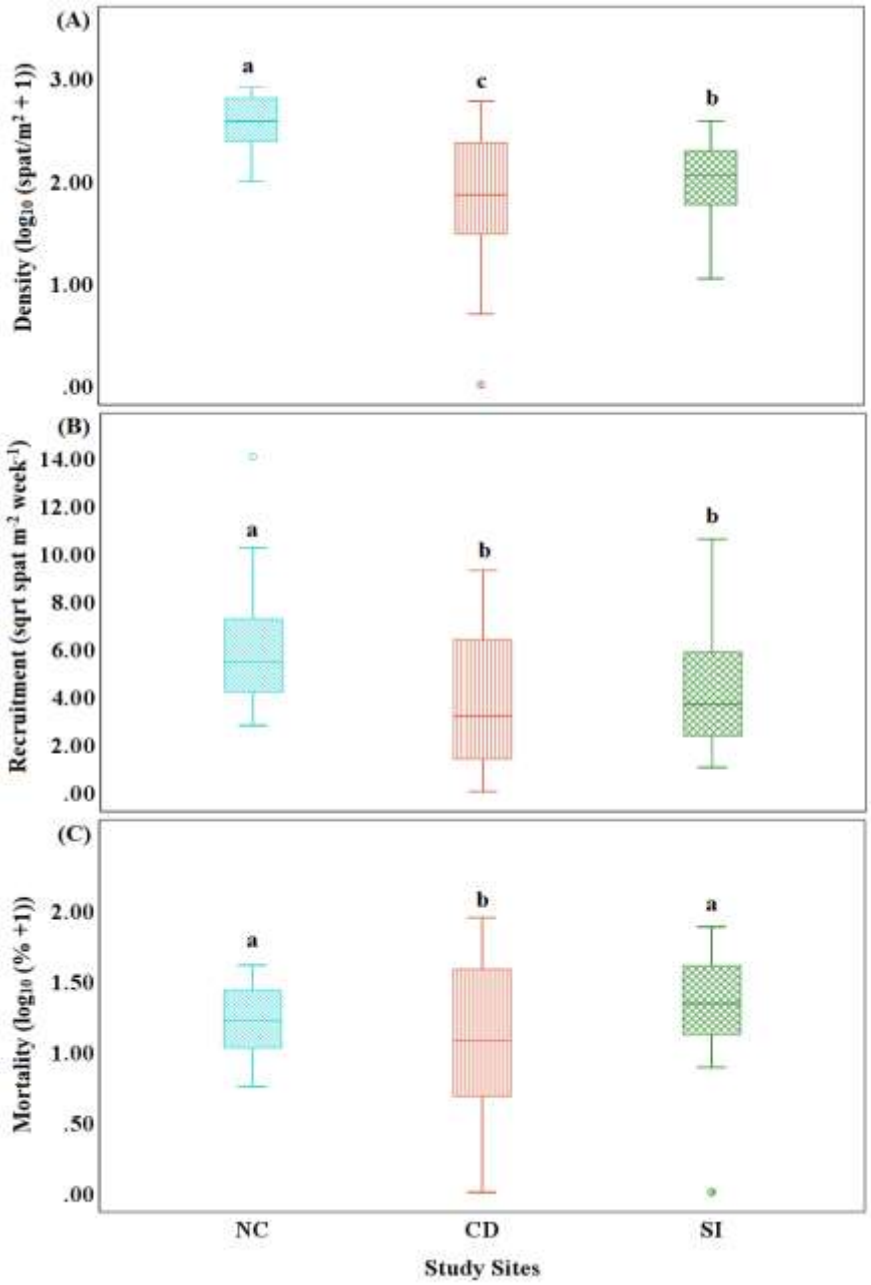

Figure 3 Boxplot of (A) spat density (log_{10} (spat/m^2 + 1)), (B) spat recruitment (sqrt spat m^{-2} $week^{-1}$), and (C) spat mortality (log_{10} (% +1)) at the three study sites (NC– Nunia Chara, CD– Chowfoldandy, and SI– Sonadia Island; n=36). Different letters indicate significant differences among groups resulting from a Tukey's Honestly Significant Difference test ($p \leq 0.05$). Box: 25^{th} and 75^{th} percentiles; whiskers: 1.5 × the interquartile range; mid-line: median; circle: outliers.

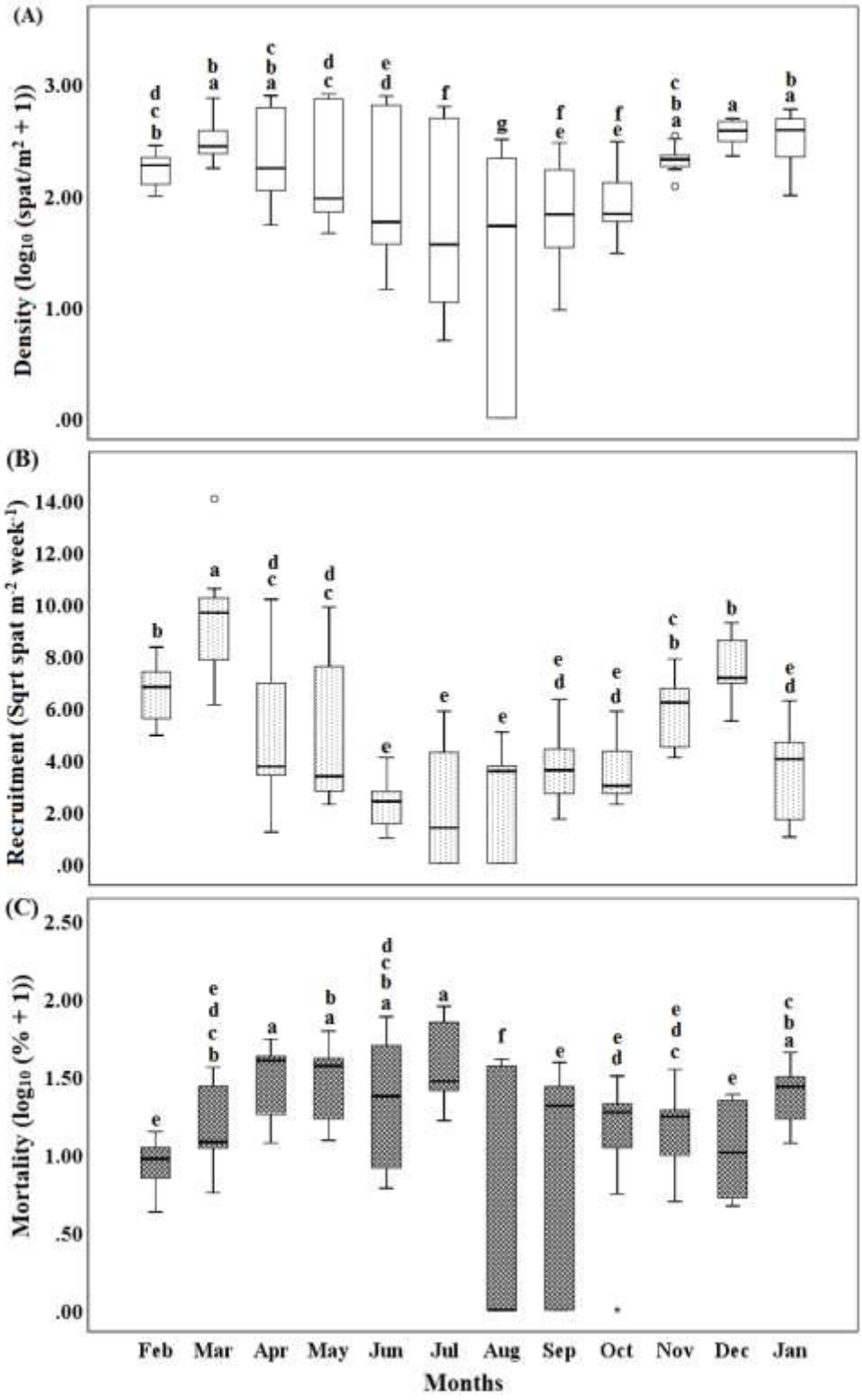

Figure 4 Boxplot of (A) spat density ($\log_{10}$ (spat/m^2 + 1)), (B) spat recruitment (sqrt spat m^{-2} $week^{-1}$), and (C) spat mortality ($\log_{10}$ (% +1)) during the study period (n=9). Different letters indicate significant differences among groups resulting from a Tukey's Honestly Significant Difference test ($p \leq 0.05$). Box: 25th and 75th percentiles; whiskers: 1.5 × the interquartile range; mid-line: median; circle: outliers.

4.3. Variability of spat density, recruitment, and mortality with the interaction effect (study sites:months)

Spat density (log_{10} (x+1) transformed), recruitment (sqrt transformed), and mortality (log_{10} (x+1) transformed) significantly ($p < 0.05$) varied in accordance with the interaction of study sites and months (study site:months). The mean value of spat density was the highest at NC in May 2019 (761 spat/m^2, n = 6), and was the lowest at CD in August 2019 (0 spat/m^2, n = 6) (Figure 5A). The mean value of spat recruitment was the highest at NC in March 2019 (131 spat m^{-2} $week^{-1}$, n = 6), and was the lowest at CD in July to August 2019 (0 spat m^{-2} $week^{-1}$, n = 6) (Figure 5B). The mean value of mortality was the highest at CD in July 2019 (100 %, n = 6), and was the lowest at CD in August to September 2019 (0 %, n = 6) and at SI in August 2019 (0 %, n = 6) (Figure 5C).

4.4. Relationship of spat density, recruitment, and mortality with environmental variables

When spat density (log_{10} (x+1) transformed), recruitment (sqrt transformed), and mortality (log_{10} (x+1) transformed) were linked with study sites and months. Considering environmental variables as covariates, significant relationships of study sites and months were found with spat density, recruitment, and mortality ($p < 0.05$). Further investigation were carried out through linear multiple regression analysis which could significantly ($p < 0.05$) represent 70.5% spat density, 40.3% of spat recruitment, and only 7.6% of spat mortality. However, spat density (log_{10} (x+1) transformed) was significantly related to salinity (sqrt ((xMax+1)-x) transformed), water temperature (log_{10} transformed), pH (log_{10} transformed), high tide water depth, low tide water depth (sqrt transformed), and total suspended solids (log_{10} transformed). The relationships of spat density (log_{10} (x+1) transformed) with salinity (sqrt ((xMax+1)-x) transformed), high tide water depth, and low tide water depth (sqrt transformed) were significantly negative ($p < 0.05$, Figure 6A, D, E), while the relationships of spat density (log_{10} (x+1) transformed) with water temperature (log_{10} transformed), pH (log_{10} transformed), and total suspended solids (log_{10} transformed) were significantly positive ($p < 0.05$, Figure 6B, C, F). However, the data of salinity was negatively reflected, and thus the relationship between spat density and salinity was positive (Figure 6A).

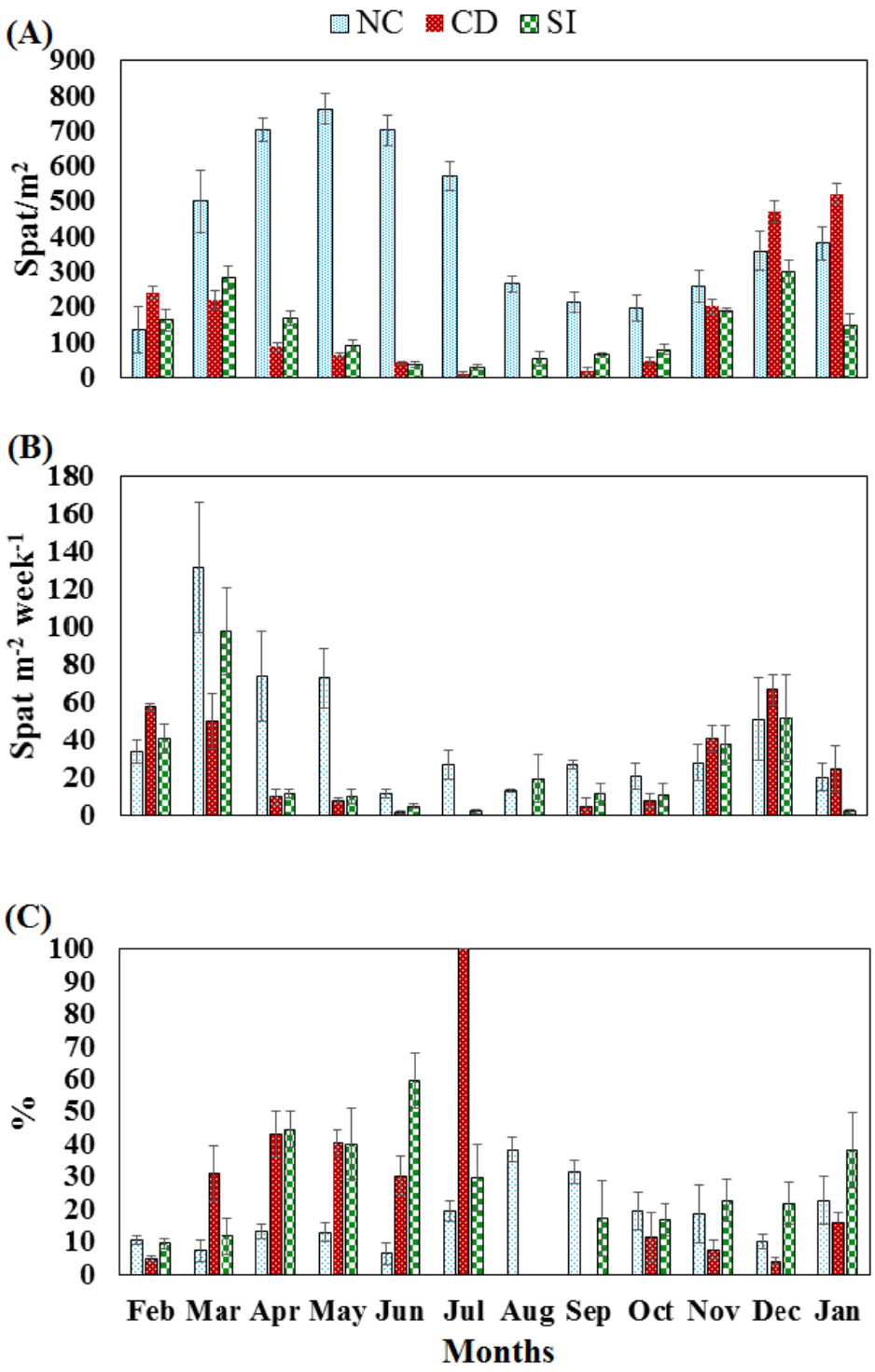

Figure 5 Mean (± SE, SE= σ/√n) spat settlement determinants observed across the three study sites (NC– Nunia Chara, CD– Chowfoldandy, and SI– Sonadia Island) along the Cox's Bazar coast from February 2019 to January 2020. (A) Spat density (spat/m^2), (B) Spat recruitment (spat m^{-2} $week^{-1}$), and (C) Spat mortality (%) were observed at 15 days interval, and converted into monthly data. 60 shells were observed on both sides of all the three replicates in all the three study sites each time.

Spat recruitment (sqrt transformed) was significantly related to salinity (sqrt ((xMax+1)-x) transformed) and low tide water depth (sqrt transformed), while both relationships were significantly negative ($p < 0.05$, Figure 7). Nonetheless, the data of salinity was negatively reflected, and thus the relationship between spat recruitment and salinity was positive (Figure 7A). Withal, relationships of water temperature (log_{10} transformed), pH (log_{10} transformed), high tide water depth, and total suspended solids (log_{10} transformed) with spat recruitment (sqrt transformed) were non-significant ($p > 0.05$). Spat mortality (log_{10} (x+1) transformed) was significantly related to high tide water depth and low tide water depth (sqrt transformed), while both relationships were significantly negative ($p < 0.05$, Figure 8). However, relationships of salinity (sqrt ((xMax+1)-x) transformed), temperature (log_{10} transformed), pH (log_{10} transformed), and total suspended solids (log_{10} transformed) with spat mortality (log_{10} (x+1) transformed) were non-significant ($p > 0.05$).

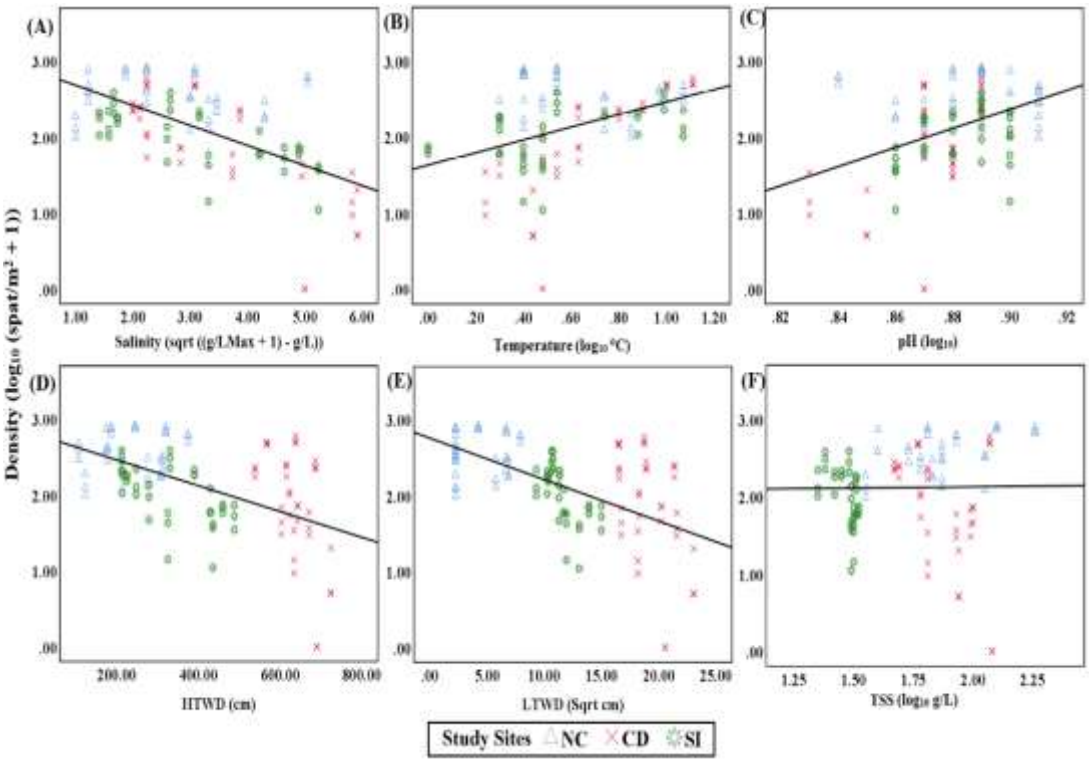

Figure 6 Significant relationship ($p < 0.05$) of spat density with (A) salinity, (B) temperature, (C) pH, (D) HTWD (High tide water depth), (E) LTWD (Low tide water depth), and (F) TSS (Total suspended solids). Different colored symbols represent the values of the three different study sites on plots. The data of density are log_{10} (x+1) transformed; salinity is sqrt ((xMax+1)-x) transformed (negatively reflected); LTWD is square root transformed; temperature, pH, and TSS are log_{10} (x) transformed.

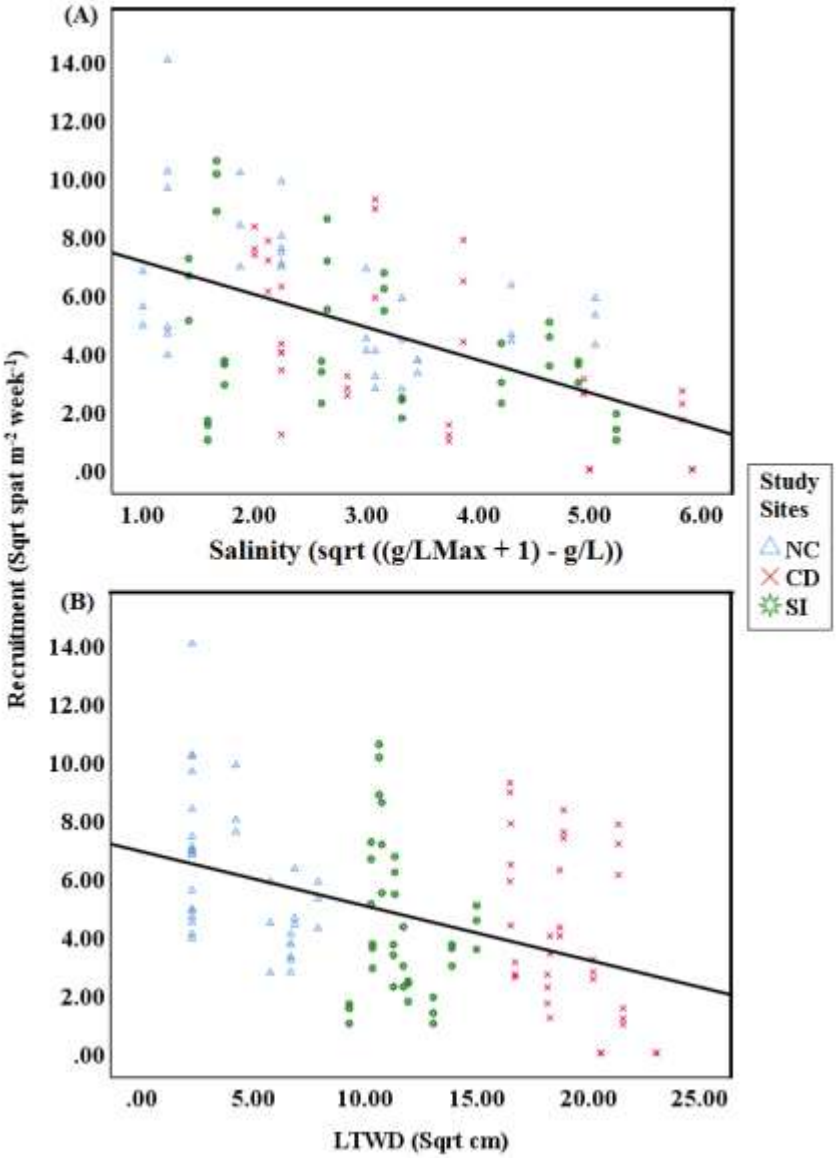

Figure 7 Significant relationship ($p < 0.05$) of spat recruitment with (A) salinity and (B) LTWD (Low tide water depth). Different colored symbols represent the values of the three different study sites on plots. Salinity is sqrt ((xMax+1)-x) transformed (negatively reflected), and LTWD is square root transformed.

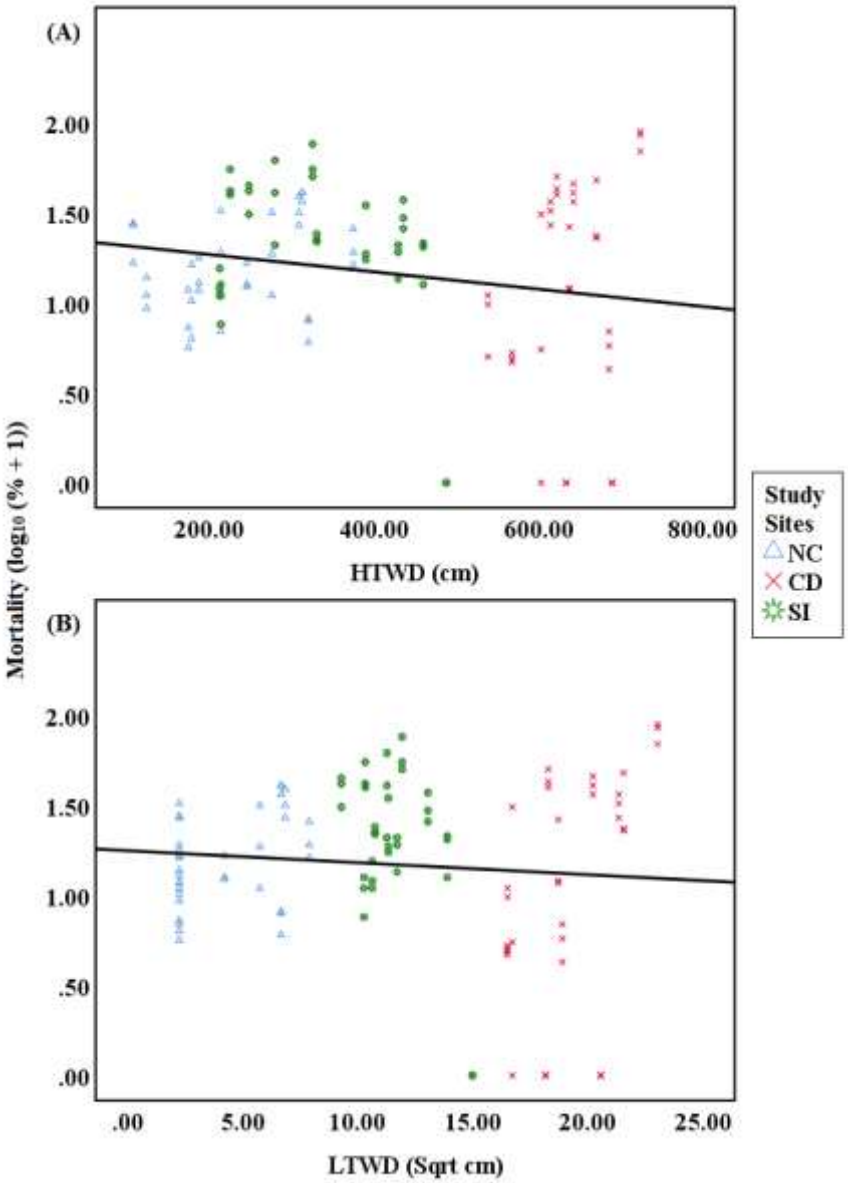

Figure 8 Significant relationship ($p < 0.05$) of spat mortality with (A) HTWD (High tide water depth) and (B) LTWD (Low tide water depth). Different colored symbols represent the values of the three different study sites on plots. The data of mortality and LTWD are $\log_{10}$ (x+1) and square root transformed, respectively.

4.5. Biofouling

Seaweeds, sponges, marine macrophytes and bush like organisms, mussels, barnacles, other oysters, polychaetes, and oyster drills (sea snails) were observed with different fouling pattern across the three study sites during the study period (Figure 9). Seaweeds affected the highest at NC (25 % substrate shells) in December 2019, while no evidence of seaweeds was found at both CD and SI (Figure 9A); however, during May to September 2019 no seaweeds fouling was observed at NC. Sponges affected the highest at NC (13.33 % substrate shells) in June 2019, while no evidence of sponges was found during February to March, July to September 2019, and January 2020 at any of the three sites (Figure 9B). Marine macrophytes and bush like organisms affected highest at CD (75 % substrates shells) in August, while no evidence of marine macrophytes and bush like organisms was observed in February 2019 at any of the three sites; additionally, no evidence was also found at NC site throughout the study period (Figure 9C). Mussels affected the highest at CD in November (51.11 % substrate shells), while no evidence of mussels was found at any of the sites during February to March and June to August 2019 (Figure 9D). Barnacles affected the highest at CD in June (24.44 % substrate shells), while no evidence of barnacles was found at any of the three sites during July to August (Figure 9E). Other oysters affected the highest at NC in October 2019 (23.89 % substrate shells), while no evidence of other oysters was found at any of the three sites during June to August 2019 (Figure 9F). Polychaetes affected the highest at CD in April 2019 (20.56 % substrate shells), while no evidence of polychaetes was found at any of the three sites during June to September 2019 and in January 2020 (Figure 9G). Oyster drills affected the highest at NC in July to August 2019 (23.33 % substrate shells), while no evidence of oyster drills was found at any of the three sites during February to April and November 2019; additionally, no evidence of oyster drills was also observed at CD throughout the study period (Figure 9H).

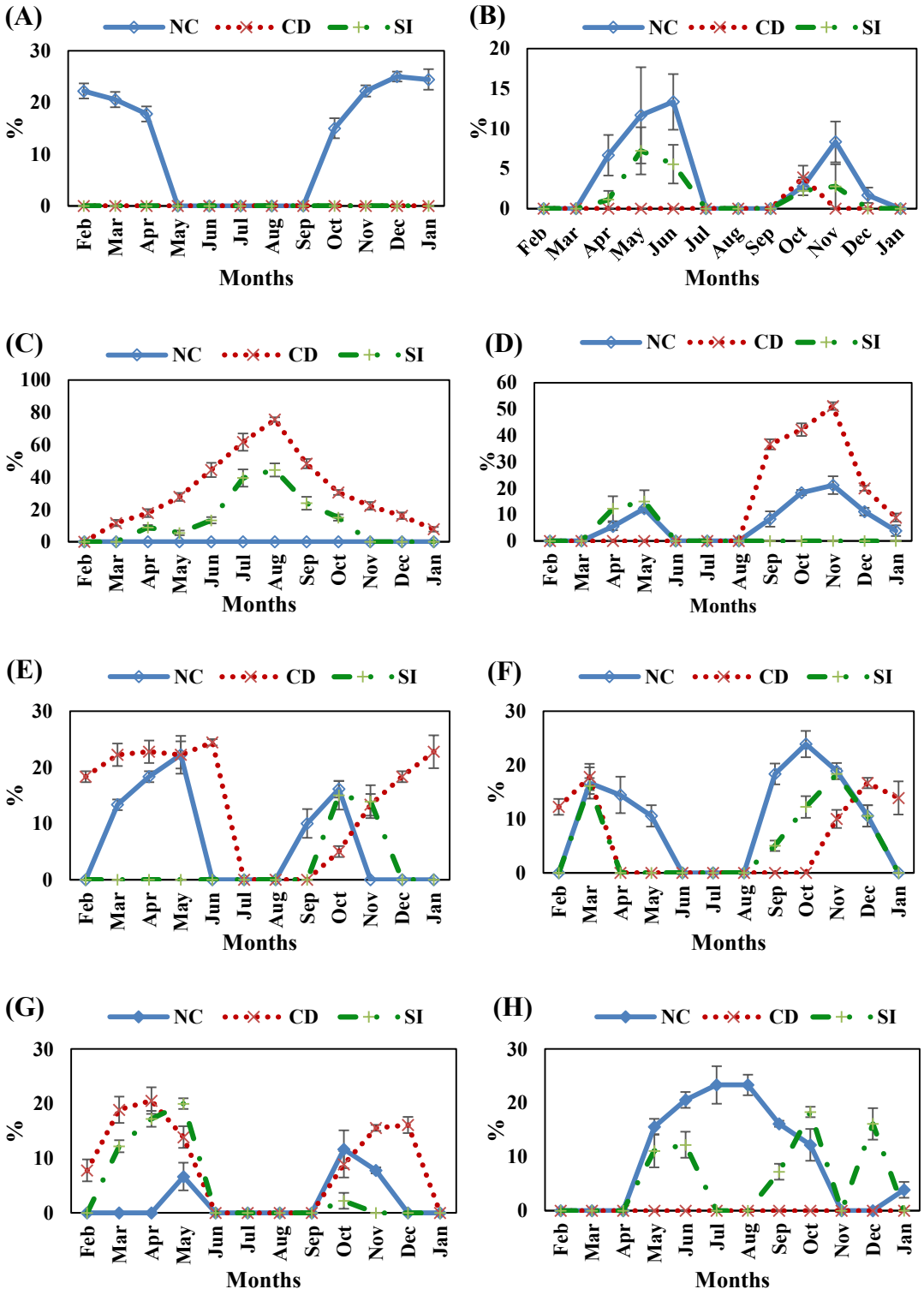

Figure 9 Major fouling organisms observed across the three study sites (NC– Nunia Chara, CD– Chowfoldandy, and SI– Sonadia Island). (A) Seaweed, (B) Sponges, (C) Marine macrophytes and bush like organisms, (D) Mussels, (E) Barnacles, (F) Other oysters, (G) Polychaetes, and (H) Oyster drills are represented in % of substrate shells affected during study period from February 2019 to January 2020.

4.6. Proximate composition

This study determined moisture content on wet weight basis, while protein, lipid, carbohydrate, ash, and fiber content on dry weight basis from the three pilot oyster farms. Moisture (78.8–79.6%, wet weight basis), ash (11.1–13.5%, dry weight basis), lipid (9.3–11.5%, dry weight basis), and fiber content (0.3–0.4%, dry weight basis) of oyster were not significantly ($p < 0.05$) different among the three farms. Contrarily, protein and carbohydrate content were significantly ($p < 0.05$) different among the three sites. The highest protein (61.6 ± 0.7%, dry weight basis) and carbohydrate content (16.1 ± 0.2, dry weight basis) were found in SI and CD farm, respectively, while the lowest protein (54.4 ± 0.3%, dry weight basis) and carbohydrate content (11.3 ± 0.2, dry weight basis) was found in NC and SI farm, respectively (Figure 10).

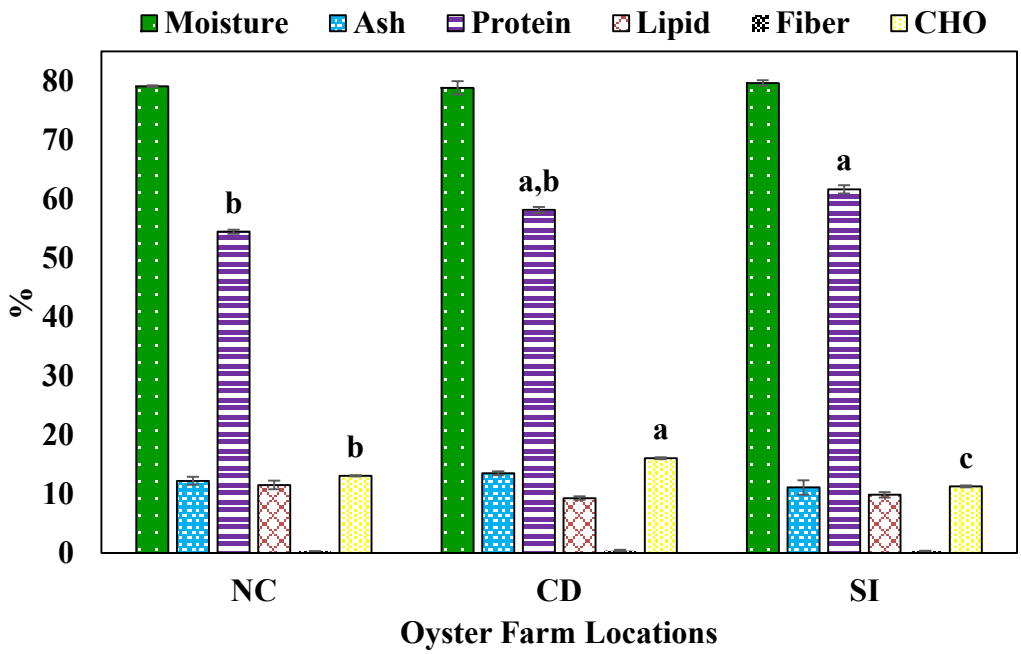

Figure 10 Proximate composition of oyster from the three pilot oyster farms (NC– Nunia Chara, CD– Chowfoldandy, and SI– Sonadia Island). Moisture content was represented on wet weight basis, and rests were represented on dry weight basis. Values are means of three replicates with error bar (standard error; $SE = \sigma/\sqrt{n}$). Values with different letters within each series are significantly different ($p < 0.05$).

4.7. Fatty Acids

Fatty acid content of oyster significantly varied among the three farming sites ($p < 0.05$). Variance of lauric acid, tridecanoic acid, myristic acid, stearic acid, heptadecanoic acid, behenic acid, tricosanoic acid, palmitoleic acid, cis-11-eicosenoic

acid, linoleic acid, eicosatrienoic acid, arachidonic acid, linolenic acid, eicosapentanoic acid, and docosapentaenoic acid differed significantly; however, variance of octanoic acid, decanoic acid, palmitic acid, arachidic acid, heneicosanoic acid, lignoceric acid, oleic acid, erucic acid, nervonic acid, and docosahexaenoic acid did not differ significantly ($p > 0.05$) (see Table 1).

Table 1 Fatty acid content of oyster (% of total fatty acids) from the three pilot oyster farms. Significantly different values are in bold.

Carbon	Fatty Acids	NC	CD	SI
C8:0	Octanoic acid	1.14 ± 0.04	1.30 ± 0.09	1.08 ± 0.03
C10:0	Decanoic acid	1.01 ± 0.03	1.16 ± 0.08	1.02 ± 0.02
C12:0	Lauric acid	**3.63 ± 0.06^{b}**	**5.89 ± 0.43^{a}**	**3.83 ± 0.10^{b}**
C13:0	Tridecanoic acid	**0.66 ± 0.03^{b}**	**1.00 ± 0.06^{a}**	**1.23 ± 0.03^{a}**
C14:0	Myristic acid	**7.69 ± 0.16^{b}**	**20.90 ± 1.53^{a}**	**8.31 ± 2.22^{b}**
C16:0	Palmitic acid	4.21 ± 0.08	13.23 ± 5.03	5.36 ± 1.41
C18:0	Stearic acid	**0.83 ± 0.01^{b}**	**2.97 ± 0.18^{a}**	**0.46 ± 0.08^{b}**
C20:0	Arachidic acid	1.90 ± 0.02	1.73 ± 0.20	1.33 ± 0.07
C17:0	Heptadecanoic acid	**0.02 ± 0.00^{b}**	**3.63 ± 0.26^{a}**	**0.01 ± 0.00^{b}**
C21:0	Heneicosanoic acid	0.03 ± 0.03	0.02 ± 0.00	0.06 ± 0.00
C22:0	Behenic acid	**0.85 ± 0.01^{b}**	**2.62 ± 0.16^{a}**	**1.04 ± 0.03^{b}**
C23:0	Tricosanoic acid	**0.36 ± 0.02^{b}**	**0.47 ± 0.02^{a}**	**0.41 ± 0.00^{a,b}**
C24:0	Lignoceric acid	1.01 ± 0.48	2.09 ± 0.33	1.18 ± 0.03
C16:1	Palmitoleic acid	**0.98 ± 0.01^{c}**	**15.88 ± 1.17^{a}**	**7.24 ± 0.35^{b}**
C18:1	Oleic acid	0.69 ± 0.03	0.75 ± 0.04	0.39 ± 0.11
C20:1	cis-11-Eicosenoic acid	**4.72 ± 0.37^{a}**	**3.54 ± 0.06^{a}**	**1.80 ± 0.13^{c}**
C22:1	Erucic acid	1.47 ± 0.39	1.43 ± 0.59	1.40 ± 0.51
C24:1	Nervonic acid	0.10 ± 0.01	0.70 ± 0.65	0.22 ± 0.20
C18:2n-6	Linoleic acid	**62.66 ± 0.83^{a}**	**0.32 ± 0.02^{b}**	**57.85 ± 2.60^{a}**
C20:3n-6	Eicosatrienoic acid	**0.54 ± 0.07^{b}**	**1.53 ± 0.13^{a}**	**0.78 ± 0.02^{b}**

C20:4n-6	Arachidonic acid	**1.98 ± 0.17^{b}**	**3.45 ± 0.11^{a}**	**1.75 ± 0.00^{b}**
C18:3n-3	Linolenic acid	**0.41 ± 0.01^{b}**	**2.67 ± 0.48^{a}**	**0.45 ± 0.21^{b}**
C20:5n-3	Eicosapentanoic acid	**2.10 ± 0.09^{b}**	**11.06 ± 0.73^{a}**	**2.29 ± 0.19^{b}**
C22:5n-3	Docosapentaenoic acid	**0.57 ± 0.17^{b}**	**0.98 ± 0.09^{a}**	**0.08 ± 0.05^{b}**
C22:6n-3	Docosahexaenoic acid	0.46 ± 0.00	0.68 ± 0.09	0.44 ± 0.04

Values are means of duplicates with standard error (SE = σ/√n). (NC–Nunia Chara, CD–Chowfoldandy and SI– Sonadia Island).

Withal, different groups of fatty acids also varied significantly among different farming sites ($p < 0.05$, Figure 11). Highest amount of SAFA was 57% at CD farm, MUFA was 22.3 % at CD farm, n6-PUFA was 65.2% at NC farm, n3-PUFA was 15.4% at CD farm, and total PUFA was 68.7% at NC farm. On the other hand, different fatty acid ratios varied significantly among different oyster farms ($p < 0.05$, Figure 12). The highest n3/n6 PUFA was 2.919 at CD farm, DHA/EPA was 0.219 at NC farm, SAFA/TUFA was 1.33 at CD farm, SAFA/ TFA was 0.57 at CD farm, and TUFA/TFA was 0.767 at NC farm.

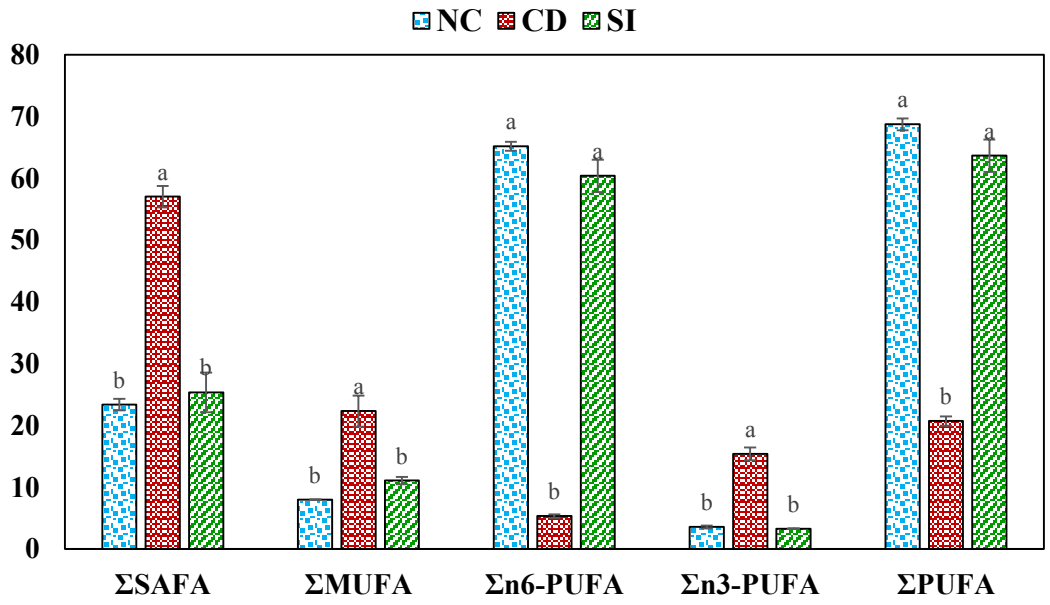

Figure 11 Fatty acid content (% of total fatty acids) of oyster in groups from the three oyster farms (NC– Nunia Chara, CD– Chowfoldandy and SI– Sonadia Island). Values are means of duplicates with error bar (standard error; SE = σ/√n). Values with different letters within each category are significantly different ($p < 0.05$). SAFA– Saturated Fatty Acids, MUFA– Mono Unsaturated Fatty Acids, and PUFA– Poly Unsaturated Fatty Acids.

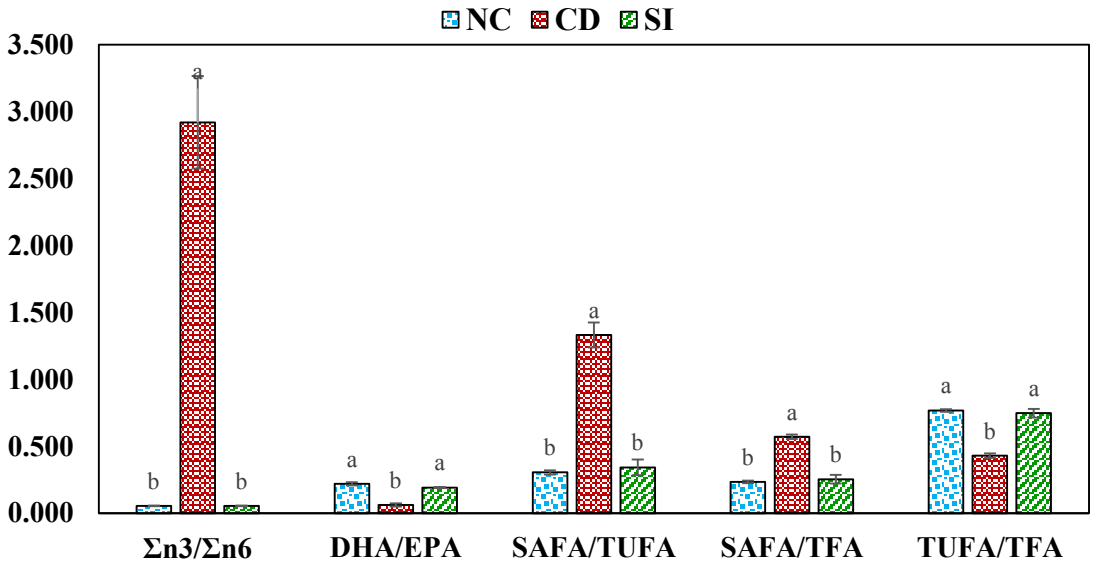

Figure 12 Fatty acid ratios of oyster from the three oyster farms (NC– Nunia Chara, CD– Chowfoldandy and SI– Sonadia Island). Values are means of duplicates with error bar (standard error; SE = σ/√n). Values with different letters within each category are significantly different ($p < 0.05$). SAFA– Saturated Fatty Acids, DHA– Docosahexaenoic Acid, EPA– Eicosapentaenoic Acid, TUFA– Total Unsaturated Fatty Acids, TFA– Total Fatty Acids, n3– Omega 3 fatty acids, and n6– Omega 6 fatty acids.

4.8. Economic viability

The payback period of the three oyster farms, driven by the net profitability, showed that the fastest recovery of the investment can be obtained from CD farm, while the slowest recovery can be obtained from SI farm (Table 2).

Table 2 Projected Income (BDT), net profit (BDT), and payback period (years) of the pilot oyster farms developed by Department of Fisheries at Cox's Bazar coast, Bangladesh.

Farms	Investment (BDT)	Income (BDT)/ year	Net profit (BDT)/ year	Payback period (years)
NC	101650	125000	45728	2.22
CD	63900	80000	30974	2.06
SI	94050	100000	27256	3.45

Chapter 5
Discussion

5.1. Spat Settlement Pattern

Significant ($p < 0.05$) spatial and temporal variability of spat density, recruitment, and mortality as well as the variability with the interaction effect of study sites and months (study sites:months) were observed in this study (Figure 3 – 5). Similarly, spatial and temporal variability of oyster spat density in France, USA and New Zealand (Bartol and Mann 1997; Wilson et al. 2005; Metz et al. 2015; Lagarde et al. 2019), recruitment in France and USA (Bartol and Mann 1997; Metz et al. 2015; Lagarde et al. 2017), and mortality in USA (Pollack et al. 2011; Parker et al. 2013) were also observed from different studies. The highest spat density and recruitment were observed at NC, while lowest mortality was observed at CD (Figure 3–5); however, mean differences of mortality at CD varied the most, and up to 100% mortality was observed at CD in July (Figure 3C, 5C). Between NC and SI, NC was observed with the lower mortality (Figure 3C). These observations of NC sites could be due to its geographical location at downstream inter-tidally, while CD and SI were located comparatively at upstream sub-tidally (Figure 1). According to Hidu and Haskin (1971), although great settlement was observed at offshore sub-tidal zone where tidal flats merge with deep water at a transitional slope, the settlement was found the greatest inter-tidally near the shore in shallow water. Similarly, settlement or early recruitment, using the bag method, was also found higher in the inter-tidal zone than in the sub-tidal zone (McNulty 1953). Figure 4 shows that spat density were lower during May to October, recruitment was lower during April to October and January, and mortality was higher during April to July and January. Withal, mortality values of August did not represent the actual situation as there was no spat at CD on August (Figure 4C, 5C). Monsoon was observed during late May to mid-October, and tropical storms were observed in October 2019 and January 2020, which altered the environmental variables majorly.

Different studies found that spat density, recruitment, and mortality varied due to the alteration of environmental variables (Rothschild et al. 1994; Bartol and Mann 1997; Wilson et al. 2005; McLeod and Wing 2008; Jordan-Cooley et al. 2011; Pollack et al. 2011; Parker et al. 2013; Metz et al. 2015; La Peyre et al. 2016; Lagarde et al.

2017; Lagarde et al. 2019; Marshall et al. 2019). In this study, we also found that environmental variables varied across study sites with the temporal variation (Figure 13). ANCOVA also showed significant influence of environmental variables in spat settlement ($p < 0.05$). Further analysis showed that spat density was positively related to salinity (negative relation with negatively reflected salinity represents the positive relation with original salinity data), temperature, pH, and TSS (Figure 6A–C, F). Studies from New Zealand and Texas shows that the rate and duration of salinity alteration have considerable influence on oyster abundance (McLeod and Wing 2008; Pollack et al. 2011); withal, evidences indicate that episodic pulses of freshwater for short duration enhance oyster population (Marshall et al. 2019). However, comparatively higher spat densities were observed in an intertidal zone with increased salinity (Metz et al. 2015). This study found that spat densities increased with the increasing temperature (21 – 32 °C, Figure 6B, 13A). Notwithstanding, it was found that for a comparatively extended period (> 2 months) of time, the eastern oyster can survive under low salinity (<5 g/L) but at low temperature (<11 °C); however, at elevated temperature (11 – 32 °C), the oyster cannot survive at low salinity (Powell et al. 1996; La Peyre et al. 2016), which was also observed in NC, CD, and SI sites (Figure 5C, 13A-B). Though spat density was positively related to TSS, but the correlation was poor between them (Figure 6F). Contrarily, different studies from North Carolina and Chesapeake Bay show that oyster abundance decreases and mortality increases with the increase of sedimentation (Lenihan 1999; Jordan-Cooley et al. 2011). Spat density was negatively related to HTWD and LTWD. Likewise, Bartol and Mann (1997) observed significant negative relationship of spat density with tidal height in a shallow intertidal area in the Piankatank river in Virginia. Effect of water depths and subsequently, negative phototropism was also observed by oyster spat during settlement (Baker and Mann 1998).

Recruitment was positively related to salinity (negative relation with negatively reflected salinity represents the positive relation with original salinity data) and negatively related to LTWD (Figure 7). Hence, spat recruitment and subsequently, the density was observed higher at NC due to its higher salinity and lower low tide water depth (Figure 3 A-B, 13B, D). Marshall et al. (2019) found that low salinity due to precipitation negatively reflected the spat recruitment in an artificially constructed oyster reef in an inter-tidal zone of Matagorda Bay in Texas. Similarly, Butler (1949)

and Loosanoff (1953) observed delayed spawning in low salinity conditions. Moreover, Loosanoff (1953) and La Peyre et al. (2009) observed that natural fluctuation in oyster reproduction could influence reflected oyster seed variability over time and subsequently, the recruitment of wild oysters could fluctuate considerably.

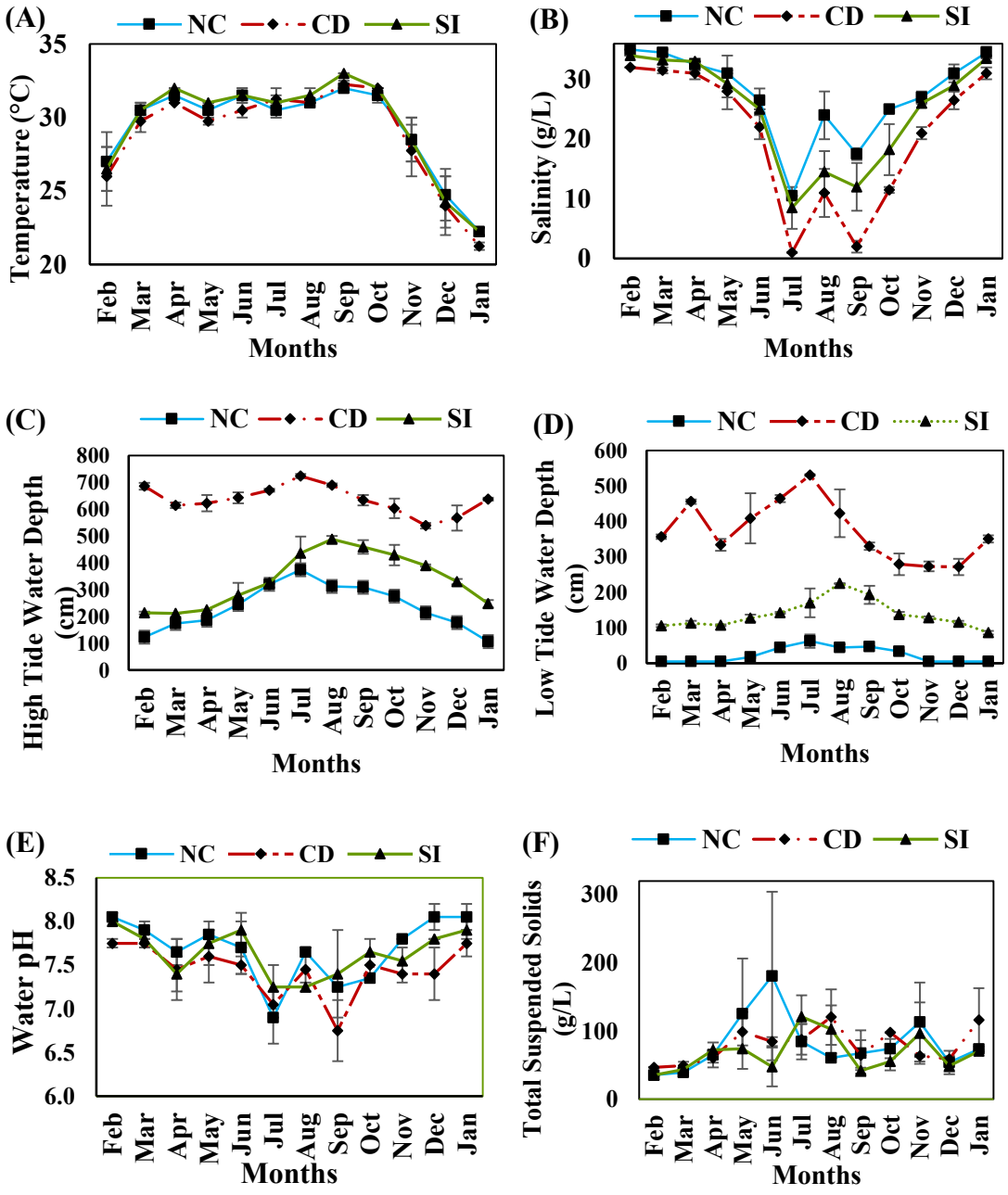

Figure 13 Environmental variables (A) temperature, (B) salinity, (C) high tide water depth, (D) low tide water depth, (E) water pH, and (F) total suspended solids, recorded in the three study sites along Cox's Bazar coast throughout the study period from February 2019 to January 2020. Values are means of two replicates with error bar (standard error; SE = $\sigma/\sqrt{n}$).

Mortality was negatively related to HTWD and LTWD (Figure 8); therefore, up to 100% mortality was observed at CD due to the highest HTWD and LTWD prevailed by it (Figure 5C, 13C–D). Although relationship of mortality with salinity was found non-significant in our study, but HTWD and LTWD was higher during monsoon as well as with low salinities. Nonetheless, 7.6% mortality can be defined by the linear regression model analyzed in this study, which may not represent the actual relationship. Previous studies in Florida also shows that, during rainy season increased precipitation decreases salinity (Metz et al. 2015) and subsequently, strong decrease in survival of oyster spat due to physiological constraints in low salinity (<10 g/L) areas was also observed (Wilson et al. 2005; Parker et al. 2013).

Different fouling organisms that were observed throughout the study in different level of fouling could have a major influence in the recruitment and the mortality (Figure 9). Oyster drills at NC during monsoon, and marine macrophytes and bush like organisms at CD and SI could contribute to high mortality during monsoon. Although fouling organisms were cleaned in every 15 days, they had sufficient time to negatively influence spat recruitment and to positively influence spat mortality (Figure 5C, 9C, H). Likewise, Lodeiros et al. (2002) found that fouling organisms could directly induce oyster mortality. Marine macrophytes and bush like organisms, seaweeds, and sponges made the substrate soft and unavailable to oyster larvae to settle that could cause decreased recruitment. Tanyaros (2011) found that oyster shells send chemical cue from shellstring to oyster spat for settlement. Nonetheless, marine macrophytes and bush like organisms also hindered the process of chemical cue secretion into water which may cause decreased recruitment rate. Cobb (1969) and Thomas (1979) found oyster shell was penetrated and excavated by boring sponges resulting in oyster mortality. Mussels, barnacles, other oysters, and polychaetes made the substrate unavailable for target oyster spat to settle and competed for food. Dharmaraj and Chellam (1982) also addressed boring polychaetes and barnacles as significant reason of oyster mortality. Oyster drills (sea snails), another boring organism that caused direct death of spats by boring the shell of spats, and eating its internal organs. Similarly, La Peyre et al. (2016) and Munroe et al. (2013) also reported unpredictable mortalities affected by predation and diseases. During the monsoon, heavy attachment of marine macrophytes and bush like organisms were attached with substrate in the CD and the SI sites, while oyster drills fouled the

substrates in the NC site; this could contribute to a decreased recruitment and increased mortality rate during this period in all three study sites (Figure 5B–C, 9C).

Finally, this study showed that none of the three sites would be potential for spat collection or oyster culture on shellstring method during the monsoon period; NC has the higher potentiality of allocation for spat collection than others. However, spat density was in increasing trend during November to May, recruitment was comparatively higher during November to May, and mortality was higher during July to September. Thus, NC has higher potentiality during late October to mid-May for spat collection both for commercial oyster farming and for restoration to enhance coastal resilience. Contrarily, NC offers comparatively lower spat/m^2 than observed by Metz et al. (2015) but similar to the observation of Bartol and Mann (1997); notwithstanding, the settlement greatly depends on the broodstock population and larval abundance in water column (Loosanoff 1953; La Peyre et al. 2009; Lagarde et al. 2017).

5.2. Nutritional composition of oyster

Although, the protein and carbohydrate contents varied among different oyster farms, the proximate composition was similar to a previous study on *Crassostrea rhizophorae* (Martino and Cruz 2004) (Figure 10). Withal, proximate composition varies from the findings of Prato et al. (2019) in *Ostrea edulis*. This variation could be due to the variation in species as well as the variation in plankton diversity. However, according to Martino and Cruz (2004), proximate composition of *Crassostrea* spp. from all the three farms are nutritionally good for human health.

High palmitic acid was observed in farmed oyster from this study, while the highest was in oysters from CD farm (Table 1). According to Ackman and Eaton (1966), palmitic acid plays key role in many metabolic processes in a lot of fish and other aquatic animals. Martino and Cruz (2004) and Prato et al. (2019) found higher EPA and DHA in other oysters; however, EPA was the highest in oysters from CD farm, while DHA didn't vary among the oysters from different farms (Table 1). Long-chain omega-3 PUFAs must be taken through diet by human as they cannot synthesize those fatty acids (Alasalvar et al. 2002). This study revealed that, oysters from NC and SI farms offered higher n6-PUFA than n3-PUFA, while the oysters from CD farm offered higher n3-PUFA than n6-PUFA (Figure 11). Martino and Cruz

(2004) and Prato et al. (2019) found higher omega-3 fatty acids than omega-6 fatty acids, which supports the findings of the CD farm but varies from the findings of NC and SI farms. For similar reasons, n-3/n-6 PUFA and DHA/EPA ratios varied from Martino and Cruz (2004) and Prato et al. (2019). However, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, and other fatty acid ratios are similar to their findings.

Fatty acids compositions varies with different intrinsic factors (age, sex, size, and way of life) as well as extrinsic factors (diet, temperature, and salinity). Among these factors, temperature has remarkable influence on fatty acids composition such as decreased level of temperature stimulates unsaturation of fatty acids thus to ensure body flexibility and membrane fluidity through maintaining freezing point below the temperature of surrounding water (Eastman 1990; Martino et al. 2002). However, increased temperature also triggers raising phospholipids thus to counteract excessive membrane fluidity (Martino et al. 2002). On the other hand, with the increased concentration of phytoplankton in water, Bachok et al. (2003) observed energetically important fatty acids at higher levels. Furthermore, phytoplankton availability varies seasonally and spatially in coastal areas which are preferably consumed by oysters (Mehedi et al. 2017). In the tissue of marine primary producers, Dalsgaard et al. (2003) discovered unique fatty acid patterns that can be unchangeably passed to species with a higher trophic level. Availability of 20:1 And C18:2n-6 in marine bivalves indicates the presence of herbivore zooplankton, algae, and fungi as a dietary source in their habitat (Erwin 1973; Kayama et al. 1989; Auel et al. 2002). Withal, dinoflagellates as a major food source is reflected by higher level of C22:6n-3 in tissues (Joseph 1975; Sargent et al. 1977). On the other hand, presence of C20:5n-3 and C16:1 intimate the dominance of diatoms (Graeve et al. 1997), whereas the presence of C22:6n-3, C20:1, and C14:0 reflect the abundance of dinoflagellates, herbivorous zooplankton and diatoms (Joseph 1975; Sargent et al. 1977; Graeve et al. 1997; Auel et al. 2002). Abundance of bacteria, algae, fungi, and diatoms are reflected in the concentration of C20:4n-6, C18:2n-6, and C17:0 in marine bivalves (Erwin 1973; Ackman 1989; Kayama et al. 1989; Kharlamenko et al. 2001).

Being a bivalve oyster is filter-feeding animal which accumulate elements from water, inorganic particulate, and food that may also result in bioaccumulation of toxic substances (Liao and Ling 2003; Amiard et al. 2008). But it can only be potentially

hazardous if the concentration level of these substances exceed the maximum residue limits (Liao and Ling 2003; Amiard et al. 2008). Though this farmed oyster have good nutritional value, but still it can't be declared as health safe before heavy metals and other persistent organic pollutants analysis.

5.3. Economic viability

Net profit of all the farms showed that all the farms will be viable as the revenue exceeds the production cost (Cheremisinoff 1995) (Table 2). But the payback period data showed that SI farm needed 3.45 years to recover the initial investment, while the longevity of the farm infrastructures was estimated as 3 year (Table 2). Therefore, SI farm will not be a commercially sustainable farm at all. Contrarily, the most viable farm will be CD farm and then the NC farm. Nonetheless, it is expected that in near future the consumer demand of bivalves will increase greatly, and the worldwide production has consistently increased from 7.1 million to 16.1 million over the years 1995 to 2014 (FAO 2016), which may enhance the price of oysters as well as affect the economic viability.

Chapter 6
Conclusions

This study was conducted to compare spat settlement pattern of *Crassostrea* spp., nutritional value, and economic viability of oyster farming among different sites. This study revealed that oyster spat (1) settlement (spat density and recruitment rate) could be the highest with comparatively low mortality rate (consistent low mean difference) on shells at NC sites; (2) spat could potentially be settled largely at the true intertidal zone; (3) spat mortality increased largely during the monsoon period in all the three sites, but comparatively less at the NC site; (4) heavy fouling was observed during monsoon at the CD and the SI sites. Subsequently, from an oyster farmer or oyster spat collector perspective, these data attest to the suitability of NC site during late October to mid-May for spat collection both for commercial oyster farming as well as for restoration to enhance coastal resilience.

This study also revealed that the farmed oyster had high nutritional value with high protein, carbohydrate, and lipid content consisting good quantity of fatty acids. Although the omega-3 PUFA values were below recommended level at NC and SI farm, but other fatty acids were found in good quantity. Contrarily, all the fatty acids were found at satisfactory level in the oysters of CD farm. Burgeoning awareness among local consumers about the food value of this seafood would make it more sustainable.

On the other hand, oyster farming will not be viable at SI. Contrarily, NC and CD will be economically viable for oyster farming. Therefore, this findings will encourage the investors to come forward to initiate commercial oyster farming in Bangladesh.

Chapter 7

Recommendations and Future Perspectives

Observation of a consecutive twelve months could not represent inter-annual variability in spat density, recruitment rate, mortality rate, environmental variables, biofouling, and their relationships. Thus spat settlement patterns may deviate from our findings in the coming years. Besides, this study couldn't represent the seasonal variability, for which the observations should be extended at least 2-3 consecutive years. Additionally, broodstock assessment and larval availability in water column are necessary to define the variability of recruitment as well as to determine the present condition of wild oyster reef population. Besides, Growth study and juvenile mortality will also evaluate the potentiality of sites for farming practices. On the other hand, plankton study is necessary to identify the variation in fatty acids. Large scale oyster farm will require a huge amount of seeds, which may not be satisfied from natural sources. Therefore, future research attempts may include the followings:

i. Stock assessment of oysters and identification of oyster reefs in Bangladesh coast;
ii. Characterization of inter-annual variability of spat settlement and factors of recruitment;
iii. Duality of trophic supply and hydrodynamic drivers in oyster recruitment;
iv. Comparative growth study of oyster in different growing methods both indoor and in open marine environment;
v. Artificial propagation of oysters;
vi. Larval response to different microalgal feeding (growth, survival, and immunostimulation);
vii. Amino acid profiling of the cultured oyster;
viii. Assessment of water quality, and pollutants (POPs, micro-plastics, and heavy metals) in the farming sites; and
ix. Heavy metal, bio-toxins, and persistent organic pollutants assay of farmed oyster before consumption.

References

Abbott A, Abel PD, Arnold DW, Milne A. 2000. Cost-benefit analysis of the use of TBT: The case for a treatment approach. The Science of the Total Environment. 258: 5-19.

Ackman R, Eaton CA. 1966. Some commercial Atlantic herring oils; fatty acid composition. Journal of the Fisheries Research Board of Canada. 23: 991-1006.

Ackman RG. 1989. Fatty acids, marine biogenic lipids, fats and oils. CRC Press, Boca Raton, Florida. 2: 145-178.

Adams C, Sturmer L, Sweat D, Blake N, Degner B. 2001. The economic feasibility of small-scale, commercial culture of the southern bay scallop (*Argopecten irradians concentricus*). Aquaculture Economics & Management. 5: 81-97.

Ahmed ATA, Islam R, Sanaullah M. 1978. A preliminary report on the molluscan fauna of the Bay of Bengal. Journal of the Asiatic Society of Bangladesh. 3(2): 59-82.

Alasalvar C, Shahidi F, Quantick P. 2002. Food and health applications of marine nutraceuticals: A review. In: Alasalvar C, Taylor T, editors. Seafoods Quality, Technology and Nutraceutical Applications. New York: Springer. pp. 175-204.

Amiard JC, Amiard TC, Charbonnier L, Mesnil A, Rainbow PS, Wang WX. 2008. Bioaccessibility of essential and non-essential metals in commercial shellfish from Western Europe and Asia. Food and Chemical Toxicology. 46(6): 2010-2022.

AOAC. 2000. Official methods of analysis. Gaithersburg, MD, USA: Association of Official Analytical Chemists.

Auel H, Harjes M, Rocha R, Stübing D, Hagen W. 2002. Lipid biomarkers indicate different ecological niches and trophic relationships of the Arctic hyperid amphipods *Themisto abyssorum* and *T. libellula*. Polar Biology. 25: 374-383.

Aziz N, Azlan A, Ismail A, Muhammad Alinafiah S, Razman M. 2013. Quantitative determination of fatty acids in marine fish and shellfish from warm water of straits of malacca for nutraceutical purposes. BioMed Research International. pp. 284-329.

Bachok Z, Mfilinge P, Tsuchiya M. 2003. The diet of the mud clam *Geloina coaxans* (Mollusca, Bivalvia) as indicated by fatty acid markers in a subtropical mangrove forest of Okinawa, Japan. Journal of Experimental Marine Biology and Ecology. 292: 187-197.

Baker P, Mann R. 1998. Response of settling oyster larvae, *Crassostrea virginica*, to specific portions of the visible light spectrum. Journal of Shellfish Research. 17: 1081-1083.

Baker PK. 1994. Quantification of settlement and recruitment processes in bivalve mollusks. Virginia: College of William and Mary. p. 381.

Banks P, Berrigan M, Choudhury A, Craig L, Diaz D, Kern F, King J, Marshall M, Robinson L, Steimle F. 2007. Status review of the eastern oyster (*Crassostrea virginica*). Report to the National Marine Fisheries Service, Northeast Regional Office, Gloucester, MA, USA.

Bartol I, Mann R. 1997. Small-scale settlement patterns of the oyster *Crassostrea virginica* on a constructed intertidal reef. Bulletin of Marine Science. 61: 881-897.

Beck MW, Brumbaugh RD, Airoldi L, Carranza A, Coen LD, Crawford C, Defeo O, Edgar GJ, Hancock B, Kay MC and others. 2011. Oyster reefs at risk and recommendations for conservation, restoration, and management. BioScience. 61(2): 107-116, 110.

BIWTA. 2017. Bangladesh tide tables. Dhaka, Bangladesh: Department of Hydrology, Bangladesh Inland Water Transport Authority.

Blomberg BN, Palmer TA, Montagna PA, Beseres Pollack J. 2018. Habitat assessment of a restored oyster reef in South Texas. Ecological Engineering. 122: 48-61.

BMD. 2017. Record book of meteorological data, Kutubdia station. Bangladesh: Bangladesh Meteorological Department.

Braithwaite RA, McEvoy L. 2005. Marine biofouling on fish farms and its remediation. Advances in Marine Biology. 47: 215-252.

Bushek D. 1988. Settlement as a major determinant of intertidal oyster and barnacle distributions along a horizontal gradient. Journal of Experimental Marine Biology and Ecology. 122: 1-18.

Butler PA. 1949. Gametogenesis in the oyster under conditions of depressed salinity. The Biological Bulletin. 96(3): 263-269.

Casula M, Soranna D, Catapano AL, Corrao G. 2013. Long-term effect of high dose omega-3 fatty acid supplementation for secondary prevention of cardiovascular outcomes: A meta-analysis of randomized, double blind, placebo controlled trials. Atherosclerosis Supplements. 14(2): 243-251.

Champ MA. 2000. A review of organotin regulatory strategies, pending actions, related costs and benefits. Science of The Total Environment. 258(1): 21-71.

Cheremisinoff PN. 1995. Chapter 3 - Waste minimization data/information requirements—A general approach for manufacturing. In: Cheremisinoff PN, editor. Waste Minimization and Cost Reduction for the Process Industries. Park Ridge, NJ: William Andrew Publishing. pp. 73-103.

Cobb WR. 1969. Penetration of calcium carbonate substrates by the boring sponge, *Cliona*. American Zoologist. 9(3): 783-790.

Coen L, Grizzle R. 2007. The importance of habitat created by molluscan shellfish to managed species along the Atlantic Coast of the United States. Habitat Management Series. 8: 1-108.

Connell J. 1961. Influence of interspecific competition and other factors on distribution of barnacle *Chthamalus stellatus*. Ecology. 42: 710-723.

Connell J. 1985. The consequences of variation in initial settlement vs. post-settlement mortality in rocky intertidal communities. Journal of Experimental Marine Biology and Ecology. 93: 11-45.

Cronin E, Cheshire A, Clarke S, Melville AJ. 1999. An investigation into the composition, biomass and oxygen budget of the fouling community on a tuna aquaculture farm. Biofouling. 13: 279-299.

Dalsgaard J, John M, Kattner G, Mueller-Navarra D, Hagen W. 2003. Fatty acid trophic markers in the pelagic marine environment. Advances in Marine Biology. 46: 225-340.

Dayton P. 1971. Competition, disturbance, and community organization: the provision and subsequent utilization of space in a rocky intertidal community. Ecological Monographs. 41: 351-389.

Dharmaraj S, Chellam A. 1982. Settlement and growth of barnacle and associated fouling organisms in pearl culture farm in the Gulf of Mannar. Mumbai. pp. 608-613.

DoF. 2018. Yearbook of fisheries statistics of Bangladesh, 2017-18. Dhaka, Bangladesh: Fisheries Resources Survey System (FRSS), Department of Fisheries : Ministry of Fisheries & Livestock. p. 129.

Dubios M, Gilles KA, Hamilton JK, Rebers PA, Smith F. 1956. Colorimetric method for determination of sugar and related substances. Analytical Chemistry. 28: 250-256.

Eastman JT. 1990. The biology and physiological ecology of notothenoid fishes. In: Dewitt HH, Heemstra PC, Gon O, editors. Fishes of the Southern Ocean. Grahamstown: JLB Smith Institute of Ichthyology. pp. 34-51.

Erwin JA. 1973. Comparative biochemistry of fattya cids in eukaryotic microorganism In: Erwin JA, editor. Lipids and Biomembranes of Eukaryotic Microorganisms. New York: Academic Press. pp. 42-143.

FAO. 2006. The state of food and agriculture. Rome, Italy: Publishing Management Service, Information Division : Food and Agriculture Organization of the United Nations. p. 183.

FAO. 2016. The state of world fisheries and aquaculture 2016: Contributing to food security and nutrition for all. Rome. p. 220.

FAO. 2018. The state of world fisheries and aquaculture 2018: Meting the sustainable development goals. Rome. p. 227.

Grabowski J, Brumbaugh R, Conrad R, Keeler A, Opaluch J, Peterson C, Piehler M, Powers S, Smyth A. 2012. Economic valuation of ecosystem services provided by oyster reefs. BioScience. 632: 900-909.

Grabowski JH, Peterson CH. 2007. Restoring oyster reefs to recover ecosystem services. In: Cuddington K, Byers JE, Wilson WG, Hastings A, editors. Theoretical Ecology Series: Academic Press. pp. 281-298.

Graeve M, Kattner G, Piepenburg D. 1997. Lipids in Arctic benthos: Does the fatty acid and alcohol composition reflect feeding and trophic interactions? Polar Biology. 18: 53-61.

Haven D, Fritz L. 1985. Setting of the American oyster *Crassostrea virginica* in the James river, Virginia, USA: Temporal and spatial distribution. Marine Biology. 86: 271-282.

Hidu H, Haskin HH. 1971. Setting of the American oyster related to environmental factors and larval behavior. Proceedings of the National Shellfisheries Association. 61: 35-50.

Hodson S, Lewis T, Burke C. 1997. Biofouling of fish-cage netting: Efficacy and problems of in situ cleaning. Aquaculture. 152: 77-90.

Hossain MS, Rothuis A, Chowdhury SR, Smaal A, Ysebaert T, Sharifuzzaman SM, Van Sluis C, Hellegers P, Van Dujin A, Dankers P and others. 2013. Oyster aquaculture for coastal defense with food production in Bangladesh. Aquaculture Asia. 18(1): 15-24.

Hudson K, Kauffman D, Solomon A. 2012a. Cultchless (single-seed) oyster crop budgets for Virginia: User manual. Virginia: Virginia Institute of Marine Science. p. 11.

Humphries A, La Peyre M. 2015. Oyster reef restoration supports increased nekton biomass and potential commercial fishery value. Peer Journal. 3: 19.

Jackson J. 2008. Ecological extinction and evolution in the brave new ocean. Proceedings of the National Academy of Sciences of the United States of America. 105: 11458-11465.

Jordan-Cooley W, Lipcius R, Shaw L, Shen J, Shi J. 2011. Bistability in a differential equation model of oyster reef height and sediment accumulation. Journal of Theoretical Biology. 289: 1-11.

Joseph J. 1975. Identification of 3,6,9,12,15-octadecapentaenoic acid in laboratory-cultured dinoflagellates. Lipids. 11: 250-250.

Kaehler S. 1999. Incidence and distribution of phototrophic shell-degrading endoliths of the brown mussel *Perna perna*. Marine Biology. 135: 505-514.

Kayama M, Araki S, Sato S. 1989. Lipids of marine plants. In: Ackman RG, editor. Marine Biogenic Lipids, Fats and Oils. Boca Raton, Florida: CRC Press. pp. 3-48.

Kharlamenko V, Kiyashko S, Imbs AB, Vyshkvartzev DI. 2001. Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. Marine Ecology-Progress Series. 220: 103-117.

Kirby M. 2004. Fishing down the coast: Historical expansion and collapse of oyster fisheries along continental margins. Proceedings of the National Academy of Sciences of the United States of America. 101: 13096-13099.

La Peyre MK, Geaghan J, Decossas G, La Peyre JF. 2016. Analysis of environmental factors influencing salinity patterns, oyster growth, and mortality in lower

Breton sound estuary, Louisiana, using 20 years of data. Journal of Coastal Research. 32(3): 519-530.

La Peyre MK, Gossman B, La Peyre JF. 2009. Defining optimal freshwater flow for oyster production: Effects of freshet rate and magnitude of change and duration on Eastern oysters and *Perkinsus marinus* infection. Estuaries and Coasts. 32(3): 522-534.

Lagarde F, d'Orbcastel E, Ubertini M, Mortreux S, Bernard I, Fiandrino A, Chiantella C, Bec B, Roques C, Bonnet D and others. 2017. Recruitment of the Pacific oyster Crassostrea gigas in a shellfish-exploited Mediterranean lagoon: Discovery, driving factors and a favorable environmental window. Marine Ecology Progress Series. 578: 1-17.

Lagarde F, Fiandrino A, Ubertini M, d'Orbcastel E, Mortreux S, Chiantella C, Bec B, Bonnet D, Roques C, Bernard I and others. 2019. Duality of trophic supply and hydrodynamic connectivity drives spatial patterns of Pacific oyster recruitment. Endangered Species Research. 632: 81-100.

Lenihan H. 1999. Physical-biological coupling on oyster reefs: How habitat structure influences individual performance. Ecological Monographs. 69: 251-275.

Liao CM, Ling MP. 2003. Assessment of human health risks for arsenic bioaccumulation in tilapia (*Oreochromis mossambicus*) and large-scale mullet (*Liza macrolepis*) from blackfoot disease area in Taiwan. Archives of Environmental Contamination and Toxicology. 45(2): 264-272.

Lodeiros C, Pico D, Prieto A, Narváez N, Guerra A. 2002. Growth and survival of the pearl oyster *Pinctada imbricata* (Röding 1758) in supended and bottom culture in the Golfo de Cariaco, Venezuela. Aquaculture International. 10: 327-338.

Loosanoff V. 1953. Behavior of oysters in waters of low salinity. Proceeding of National Shellfish Association.

MacKenzie CL. 1997. The history, present condition, and future of the molluscan fisheries of North and Central America and Europe. Volume 1: Atlantic and Gulf coasts. Washington: NOAA. p. 244.

Marshall DA, Lebreton B, Palmer T, De Santiago K, Beseres Pollack J. 2019. Salinity disturbance affects faunal community composition and organic matter on a restored Crassostrea virginica oyster reef. Estuarine, Coastal and Shelf Science. 226: 106267.

Martino R, Cruz G. 2004. Proximate composition and fatty acid content of the mangrove oyster. Brazilian Archives of Biology and Technology. 47(6): 955-960.

Martino R, Cyrino JE, Portz L, Trugo L. 2002. Performance and fatty acid composition of surubim (*Pseudoplatystom coruscans*) fed diets with animal and plant lipids. Aquaculture. 209(1-4): 233-246.

Maruf H. 2004. National report of Bangladesh on sustainable management of the Bay of Bengal Large Marine Ecosystem (BOBLME). BOBLME programme in Bangladesh: FAO. p. 4-9.

McLeod R, Wing S. 2008. Influence of an altered salinity regime on the population structure of two infaunal bivalve species. Estuarine, Coastal and Shelf Science. 78: 529-540.

McNulty JK. 1953. Seasonal and vertical patterns of oyster setting off Wadmalaw Island. S.C: Bears Bluff Laboratories. p. 17.

Mehedi I, Billah M, Nurul H, Islam M, Payel H, Bhuiyan MKA, Dawood M. 2017. Seasonal distribution of phytoplankton community in a subtropical estuary of the south-eastern coast of Bangladesh. Zoology and Ecology. 27(3-4): 304-310.

Menge B, Sutherland J. 1976. Species diversity gradients: Synthesis of the roles of predation, competition, and temporal heterogeneity. American Naturalist. 110(973): 351-369.

Metz J, Stoner E, Arrington D. 2015. Comparison of substrates for Eastern oyster (*Crassostrea virginica*) spat settlement in the Loxahatchee River Estuary, Florida. Journal of Shellfish Research. 34: 861-865.

Milne PH. 1975. Fouling of marine cages. Fish Farming International. 2: 15-19.

Mohammad MB. 1976. Relationship between biofouling and growth of the pearl oyster *Pinctada fucata* (Gould) in Kuwait, Arabian Gulf. Hydrobiologia. 51: 129-138.

Munroe D, Tabatabai A, Burt I, Bushek D, Powell EN, Wilkin J. 2013. Oyster mortality in Delaware Bay: Impacts and recovery from Hurricane Irene and Tropical Storm Lee. Estuarine, Coastal and Shelf Science. 135: 209-219.

Nalesso R, Paresque K, Piumbini P, Tonini J, Galletti de Almeida L, Níckel V. 2008. Oyster spat recruitment in Espírito Santo State, Brazil, using recycled materials. Brazilian Journal of Oceanography. 56(4): 281-288.

Neuringer M, Anderson G, Connor W. 1988. The essentiality of n-3 fatty acids for the development and function of the retina and brain. Annual Review of Nutrition. 8: 517-541.

Økland HM, Stoknes IS, Remme JF, Kjerstad M, Synnes M. 2005. Proximate composition, fatty acid and lipid class composition of the muscle from deep-sea teleosts and elasmobranchs. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 140(3): 437-443.

Pagcatipunan R. 1984. Technological assistance on oyster and pearl culture in Bangladesh. FAO.

Parker ML, Arnold WS, Geiger SP, Gorman P, Leone EH. 2013. Impacts of freshwater management activities on Eastern oyster (*Crassostrea virginica*) density and recruitment: Recovery and long-term stability in Seven Florida Estuaries. Journal of Shellfish Research. 32(3): 695-708.

Peterson CH, Grabowski J, Powers SP. 2003. Estimated enhancement of fish production resulting from restoring oyster reef habitat: Quantitative valuation. Marine Ecology-Progress Series. 264: 249-264.

Pollack J, Kim H-C, Morgan E, Montagna P. 2011. Role of flood disturbance in natural oyster (*Crassostrea virginica*) population maintenance in an estuary in South Texas, USA. Estuaries and Coasts. 34: 187-197.

Powell EN, Klinck J, Hofmann EE. 1996. Modeling diseased oyster populations. II. Triggering mechanisms for *Perkinsus marinus* epizootics. Journal of Shellfish Research. 15: 141-165.

Prato E, Biandolino F, Parlapiano I, Giandomenico S, Denti G, Calò M, Spada L, Di Leo A. 2019. Proximate, fatty acids and metals in edible marine bivalves from Italian market: Beneficial and risk for consumers health. Science of The Total Environment. 648: 153-163.

Prato E, Chiantore M, Kelly M, Hughes A, James P, Ferranti M, Biandolino F, Parlapiano I, Sicuro B, Fanelli G. 2017. Effect of formulated diets on the proximate composition and fatty acid profiles of sea urchin *Paracentrotus lividus* gonad. Aquaculture International. 26(2): 1-18.

Rothschild B, Ault J, Philippe G, Maurice H. 1994. Decline of Chesapeake Bay oyster population: a century of habitat destruction and overfishing. Marine Ecology Progress Series (Inter-Research). 111: 29-39.

Sargent J, Tacon A. 1999. Development of farmed fish: A nutritionally necessary alternative to meat. The Proceedings of the Nutrition Society. 58: 377-383.

Sargent JR, Gatten RR, McIntosh R. 1977. Wax esters in the marine environment — their occurrence, formation, transformation and ultimate fates. Marine Chemistry. 5: 573-584.

Shahabuddin AM, Salam M, Wahab M, Miah M. 2010. Abundance, distribution and culture potentials of three commercially important mollusks species along the coast of Bay of Bengal. Research Journal of Agriculture and Biological Sciences. 6: 754-762.

Simopoulos A. 1990. Omega-3 fatty acids in health and disease. Progress in Clinical and Biological Research. 326: 129-156.

Sizaret F, Jardin C. 1985. Estudo nacional de despesa familiar (ENDEF). Tabelas de composicao dos alimentos. Rio de Janeiro: IBGE.

Soniat T, Finelli C, Ruiz J. 2004. Vertical structure and predator refuge mediate oyster reef development and community dynamics. Journal of Experimental Marine Biology and Ecology. 310: 163-182.

Southworth M. 1998. Oyster reef broodstock enhancement in the Great Wicomico River, Virginia. Journal of Shellfish Research. 17: 1101-1114.

Sutherland J, Karlson R. 1977. Development and stability of the fouling community at Beaufort, North Carolina. Ecological Monographs. 48: 248.

Tanyaros S. 2011. The effect of substrate conditioning on larval settlement and spat growth of the cupped oyster, *Crassostrea belcheri* (Sowerby), in a hatchery. Kasetsart Journal - Natural Science. 45: 629-635.

Thomas PA. 1979. Boring sponges destructive to economically important molluscan beds and coral reefs in indian seas. Indian Journal of Fisheries. 26(1&2): 163-200.

Wilberg MJ, Livings ME, Barkman JS, Morris BT, Robinson J. 2011. Overfishing, disease, habitat loss, and potential extirpation of oysters in upper Chesapeake Bay. Marine Ecology Progress Series. 436: 131-144.

Wilson P, Scotto L, Scarpa J, Volety A, Laramore S, Haunert D. 2005. Survey of water quality, oyster reproduction and oyster health status in the St. Lucie Estuary. Journal of Shellfish Research. 24: 157-165.

Appendix A: Expenditures of the three commercial oyster farms at Cox's Bazar, developed by Department of Fisheries, Bangladesh

Farm	Categories	Item	Unit Price (BDT)	Units	Amount (BDT)
NC	Fixed	Anchor	2500	4	10000
		Floats	1200	12	14400
		Drums	500	8	4000
		Rope	500	10	5000
			Sub total		33400
	Recurring cost	Bamboo	400	20	8000
		Rope	370	25	9250
		Cultch	25	100	2500
		Labor	500	20	10000
		Security	4000	8	32000
		Oyster cleaning	10	400	4000
		Transport	2000	1	2000
		Trade license	500	1	500
			Sub total		68250
	Total				**101650**
CD	Fixed	Anchor	2500	4	10000
		Floats	1200	4	4800
		Drums	600	4	2400
		Rope	500	10	5000
			Sub total		22200
	Recurring cost	Bamboo	400	10	4000
		Rope	370	10	3700
		Cultch	25	60	1500
		Labor	500	10	5000
		Security	3000	8	24000

		Oyster cleaning	10	200	2000
		Transport	1000	1	1000
		Trade license	500	1	500
			Sub total		41700
	Total				**63900**
SI	Fixed	Anchor	2500	4	10000
		Floats	1200	10	12000
		Drums	600	8	4800
		Rope	500	10	5000
			Sub total		31800
	Recurring cost	Bamboo	400	20	8000
		Rope	370	25	9250
		Cultch	25	100	2500
		Labor	500	24	12000
		Security	3000	8	24000
		Oyster cleaning	10	300	3000
		Transport	3000	1	3000
		Trade license	500	1	500
			Sub total		62250
	Total				**94050**

Appendix B: Income of the three commercial oyster farms at Cox's Bazar, developed by Department of Fisheries, Bangladesh

Farm	Grade	Yield (Kg)	Unit Price (BDT)	Total Price
NC	A	400	250	100000
	B (off-shell)	50	500	25000
	B (on-shell)	300	–	–
CD	A	200	250	50000
	B (off-shell)	60	500	30000

	B (on-shell)	300	–	–
SI	A	300	250	75000
	B (off-shell)	50	500	25000
	B (on-shell)	280	–	–

Appendix C: Analysis of variance examining the effect of study sites, months and their interaction (study sites:months) on oyster (*Crassostrea* spp.) spat density (log_{10} (x+1) transformed), recruitment (square root transformed), and mortality (log_{10} (x+1) transformed). Significant values ($p < 0.05$) are in bold.

ANOVA Table

Source	Dependent Variable	Sum Sq.	df	Mean Sq.	F	Sig.
Study sites	Density log_{10}(x+1)	11.105	2	5.553	216.178	**.000**
	Recruitment sqrt	95.948	2	47.974	46.028	**.000**
	Mortality log_{10}(x+1)	1.134	2	.567	17.967	**.000**
Months	Density log_{10}(x+1)	13.220	11	1.202	46.789	**.000**
	Recruitment sqrt	493.233	11	44.839	43.021	**.000**
	Mortality log_{10}(x+1)	8.679	11	.789	25.002	**.000**
Study sites:Months	Density log_{10}(x+1)	13.767	22	.626	24.363	**.000**
	Recruitment sqrt	180.116	22	8.187	7.855	**.000**
	Mortality log_{10}(x+1)	13.099	22	.595	18.868	**.000**
Error	Density log_{10}(x+1)	1.849	72	.026		
	Recruitment sqrt	75.044	72	1.042		
	Mortality log_{10}(x+1)	2.272	72	.032		
Total	Density log_{10}(x+1)	522.833	108			
	Recruitment sqrt	3253.740	108			
	Mortality log_{10}(x+1)	173.135	108			
Corrected Total	Density log_{10}(x+1)	39.941	107			
	Recruitment sqrt	844.341	107			

	Mortality $log_{10}(x+1)$	25.184	107

Appendix D: Analysis of covariance examining the effect of study sites, months and their interaction (Study sites:months) on oyster (*Crassostrea* spp.) spat density (log_{10} (x+1) transformed), recruitment (square root transformed), and mortality (log_{10} (x+1) transformed) considering environmental variables (water salinity (sqrt ((xMax+1)-x) transformed), water temperature (log_{10} (x) transformed), water pH (log_{10} (x) transformed), high tide water depth, low tide water depth (square root transformed), and total suspended solids (log_{10} (x) transformed) as covariates. Significant values ($p < 0.05$) are in bold.

ANCOVA Table

Source	Dependent Variable	Sum Sq.	df	Mean Sq.	F	Sig.	Partial Eta Sq.
Intercept	Density $log_{10}(x+1)$	2.236	1	2.236	87.044	**.000**	.547
	Recruitment sqrt	39.577	1	39.577	37.972	**.000**	.345
	Mortality $log_{10}(x+1)$	3.503	1	3.503	111.010	**.000**	.607
Study sites	Density $log_{10}(x+1)$	1.693	2	.846	32.954	**.000**	.478
	Recruitment sqrt	18.185	2	9.093	8.724	**.000**	.195
	Mortality $log_{10}(x+1)$	.479	2	.240	7.597	**.001**	.174
Months	Density $log_{10}(x+1)$	4.837	11	.440	17.118	**.000**	.723
	Recruitment sqrt	288.892	11	26.263	25.198	**.000**	.794
	Mortality $log_{10}(x+1)$	15.897	11	1.445	45.796	**.000**	.875
Study sites:Months	Density $log_{10}(x+1)$	8.063	16	.504	19.620	**.000**	.813
	Recruitment sqrt	109.000	16	6.812	6.536	**.000**	.592
	Mortality $log_{10}(x+1)$	2.131	16	.133	4.220	**.000**	.484
Error	Density $log_{10}(x+1)$	1.849	72	.026			
	Recruitment sqrt	75.044	72	1.042			
	Mortality $log_{10}(x+1)$	2.272	72	.032			
Total	Density $log_{10}(x+1)$	522.833	108				
	Recruitment sqrt	3253.740	108				

Corrected Total	Mortality $\log_{10}(x+1)$	173.135	108
	Density $\log_{10}(x+1)$	39.941	107
	Recruitment sqrt	844.341	107
	Mortality $\log_{10}(x+1)$	25.184	107

a. R Squared = .954 (Adjusted R Squared = .931)

b. R Squared = .911 (Adjusted R Squared = .868)

c. R Squared = .910 (Adjusted R Squared = .866)

Appendix E: Analysis of the linear multiple regression between the oyster spat density ($\log_{10}$ (x+1) transformed) and environmental variables. Significant values ($p < 0.05$) are in bold.

Linear model: Density ($\log_{10}$ (spat/m^2 + 1)) ~ Salinity (sqrt ((xMax+1)-x) transformed) + Temperature ($\log_{10}$ (x) transformed) + pH ($\log_{10}$ (x) transformed) + High tide water depth + Low tide water depth (square root transformed) + Total suspended solids ($\log_{10}$ (x) transformed)

					Correlations		
Coefficients	**Estimate**	**Std. Error**	**t**	**Sig.**	**Zero-order**	**Partial**	**Part**
(Constant)	-2.382	.828	-2.877	**.005**			
Sqrt_Salinity	-.388	.044	-8.756	**.000**	-.596	-.659	-.460
Log_Temperature	.545	.158	3.456	**.001**	.372	.327	.181
Log_pH	2.596	.658	3.944	**.000**	-.386	.367	.207
HTWD	-.011	.002	-5.295	**.000**	-.523	-.468	-.278
Sqrt_LTWD	-.085	.019	-4.364	**.000**	-.574	-.400	-.229
Log_TSS	.406	.190	2.140	**.035**	.011	.209	.112

Std. error of the estimate: .33174 on 7 and 100 degrees of freedom

Multiple R^2	.724
Adjusted R^2	.705
F(df= 7, 100)	37.562

p	**.000**

*HTWD: High Tide Water Depth, LTWD: Low Tide Water Depth. TSS: Total Suspended Solids

Appendix F: Analysis of the linear multiple regression between the oyster spat recruitment (Square root transformed) and environmental variables. Significant values ($p < 0.05$) are in bold.

Linear model: Recruitment (square root transformed) ~ Salinity (sqrt ((xMax+1)-x) transformed) + Temperature (log_{10} (x) transformed) + pH (log_{10} (x) transformed) + High tide water depth + Low tide water depth (square root transformed) + Total suspended solids (log_{10} (x) transformed)

					Correlations		
Coefficients	**Estimate**	**Std. Error**	**t**	**Sig.**	**Zero-order**	**Partial**	**Part**
(Constant)	5.879	5.417	1.085	.280			
Sqrt_Salinity	-1.560	.290	-5.380	**.000**	-.551	-.474	-.402
Log_Temperature	.611	1.032	.592	.556	.312	.059	.044
Log_pH	7.120	4.306	1.653	.101	-.385	.163	.124
HTWD	-.001	.013	-.090	.928	-.403	-.009	-.007
Sqrt_LTWD	-.499	.127	-3.927	**.000**	-.425	-.366	-.293
Log_TSS	-2.385	1.242	-1.920	.058	-.149	-.189	-.143
Std. error of the estimate: 2.17091 on 7 and 100 degrees of freedom							
Multiple R^2	.442						
Adjusted R^2	.403						
F(df= 7, 100)	11.308						
p	**.000**						

*HTWD: High Tide Water Depth, LTWD: Low Tide Water Depth. TSS: Total Suspended Solids

Appendix G: Analysis of the linear multiple regression between the oyster spat mortality (log_{10} (x+1) transformed) and environmental variables. Significant values ($p < 0.05$) are in bold.

Linear model: Mortality (log_{10} (% + 1)) ~ Salinity (sqrt ((xMax+1)-x) transformed) + Temperature (log_{10} (x) transformed) + pH (log_{10} (x) transformed) + High tide water depth + Low tide water depth (square root transformed) + Total suspended solids (log_{10} (x) transformed)

					Correlations		
Coefficients	**Estimate**	**Std. Error**	**t**	**Sig.**	**Zero-order**	**Partial**	**Part**
(Constant)	-.478	1.164	-.411	.682			
Sqrt_Salinity	-.072	.062	-1.162	.248	-.212	-.115	-.108
Log_Temperature	-.075	.222	-.336	.737	.029	-.034	-.031
Log_pH	.004	.925	.005	.996	-.160	.000	.000
HTWD	-.007	.003	-2.411	**.018**	-.187	-.234	-.224
Sqrt_LTWD	.063	.027	2.306	**.023**	-.089	.225	.214
Log_TSS	.280	.267	1.050	.296	-.158	.104	.098
Std. error of the estimate: .46641 on 7 and 100 degrees of freedom							
Multiple R^2	.136						
Adjusted R^2	.076						
F(df= 7, 100)	2.253						
p	**.036**						

*HTWD: High Tide Water Depth, LTWD: Low Tide Water Depth. TSS: Total Suspended Solids

Appendix H: Analysis of variance examining the effect of different oyster farms on the proximate composition of oyster. Significant values ($p < 0.05$) are in bold.

ANOVA Table						
Source	**Dependent Variables**	**Sum of Sq.**	**df**	**Mean Sq.**	**F**	**Sig.**
Oyster	Moisture	1.921	2	.960	.365	.721

Farms	Ash	9.469	2	4.735	1.329	.386
	Protein	41.287	2	20.644	19.158	**.020**
	Lipid	5.452	2	2.726	7.911	.064
	Fiber	.007	2	.003	.233	.805
	CHO	22.521	2	11.261	108.645	**.002**
Error	Moisture	7.895	3	2.632		
	Ash	10.689	3	3.563		
	Protein	3.233	3	1.078		
	Lipid	1.034	3	.345		
	Fiber	.045	3	.015		
	CHO	.311	3	.104		
Total	Moisture	37771.895	6			
	Ash	855.468	6			
	Protein	20122.326	6			
	Lipid	639.531	6			
	Fiber	.818	6			
	CHO	1119.888	6			
Corrected	Moisture	9.816	5			
Total	Ash	20.158	5			
	Protein	44.520	5			
	Lipid	6.486	5			
	Fiber	.052	5			
	CHO	22.832	5			

Appendix I: Analysis of variance examining the effect of different oyster farms on the fatty acid contents of oyster. Significant values ($p < 0.05$) are in bold.

ANOVA Table

Source	Dependent Variables	Sum of Sq.	df	Mean Sq.	F	Sig.
Oyster	Octanoic acid	.050	2	.025	3.382	.170
Farms	Decanoic acid	.028	2	.014	2.810	.205
	Lauric acid	6.265	2	3.133	23.578	**.015**
	Tridecanoic acid	.329	2	.164	49.542	**.005**
	Myristic acid	222.092	2	111.046	22.837	**.015**
	Palmitic acid	96.510	2	48.255	2.648	.217
	Stearic acid	7.346	2	3.673	135.681	**.001**
	Arachidic acid	.343	2	.171	5.731	.094
	Heptadecanoic acid	17.475	2	8.737	192.028	**.001**
	Heneicosanoic acid	.002	2	.001	1.648	.329
	Behenic acid	3.810	2	1.905	111.009	**.002**
	Tricosanoic acid	.012	2	.006	9.142	.053

	Lignoceric acid	1.358	2	.679	2.969	.194
	Palmitoleic acid	223.800	2	111.900	111.742	**.002**
	Oleic acid	.147	2	.073	8.200	.061
	cis-11-Eicosenoic acid	8.599	2	4.300	41.077	**.007**
	Erucic acid	.006	2	.003	.006	.994
	Nervonic acid	.399	2	.199	.654	.581
	Linoleic acid	4812.572	2	2406.286	485.510	**.000**
	Eicosatrienoic acid	1.077	2	.539	34.927	**.008**
	Arachidonic acid	3.404	2	1.702	63.030	**.004**
	Linolenic acid	6.713	2	3.357	18.257	**.021**
	Eicosapentanoic acid	104.748	2	52.374	135.591	**.001**
	Docosapentaenoic acid	.807	2	.404	16.099	**.025**
	Docosahexaenoic acid	.071	2	.035	5.930	.091
Error	Octanoic acid	.022	3	.007		
	Decanoic acid	.015	3	.005		
	Lauric acid	.399	3	.133		
	Tridecanoic acid	.010	3	.003		
	Myristic acid	14.587	3	4.862		
	Palmitic acid	54.665	3	18.222		
	Stearic acid	.081	3	.027		
	Arachidic acid	.090	3	.030		
	Heptadecanoic acid	.137	3	.046		
	Heneicosanoic acid	.002	3	.001		
	Behenic acid	.051	3	.017		
	Tricosanoic acid	.002	3	.001		
	Lignoceric acid	.686	3	.229		
	Palmitoleic acid	3.004	3	1.001		
	Oleic acid	.027	3	.009		
	cis-11-Eicosenoic acid	.314	3	.105		
	Erucic acid	1.510	3	.503		
	Nervonic acid	.915	3	.305		
	Linoleic acid	14.869	3	4.956		
	Eicosatrienoic acid	.046	3	.015		
	Arachidonic acid	.081	3	.027		
	Linolenic acid	.552	3	.184		
	Eicosapentanoic acid	1.159	3	.386		
	Docosapentaenoic acid	.075	3	.025		
	Docosahexaenoic	.018	3	.006		

	acid		
Total	Octanoic acid	8.302	6
	Decanoic acid	6.864	6
	Lauric acid	125.590	6
	Tridecanoic acid	5.873	6
	Myristic acid	1144.180	6
	Palmitic acid	497.710	6
	Stearic acid	19.567	6
	Arachidic acid	16.785	6
	Heptadecanoic acid	26.560	6
	Heneicosanoic acid	.012	6
	Behenic acid	17.400	6
	Tricosanoic acid	1.051	6
	Lignoceric acid	14.214	6
	Palmitoleic acid	614.131	6
	Oleic acid	2.406	6
	cis-11-Eicosenoic acid	76.446	6
	Erucic acid	13.835	6
	Nervonic acid	2.005	6
	Linoleic acid	14559.405	6
	Eicosatrienoic acid	6.537	6
	Arachidonic acid	37.856	6
	Linolenic acid	15.546	6
	Eicosapentanoic acid	264.958	6
	Docosapentaenoic acid	2.646	6
	Docosahexaenoic acid	1.740	6
Corrected Total	Octanoic acid	.072	5
	Decanoic acid	.044	5
	Lauric acid	6.664	5
	Tridecanoic acid	.339	5
	Myristic acid	236.679	5
	Palmitic acid	151.175	5
	Stearic acid	7.427	5
	Arachidic acid	.432	5
	Heptadecanoic acid	17.611	5
	Heneicosanoic acid	.004	5
	Behenic acid	3.862	5
	Tricosanoic acid	.014	5
	Lignoceric acid	2.044	5
	Palmitoleic acid	226.804	5

	Oleic acid	.174	5
	cis-11-Eicosenoic acid	8.913	5
	Erucic acid	1.516	5
	Nervonic acid	1.314	5
	Linoleic acid	4827.440	5
	Eicosatrienoic acid	1.124	5
	Arachidonic acid	3.485	5
	Linolenic acid	7.265	5
	Eicosapentanoic acid	105.907	5
	Docosapentaenoic acid	.883	5
	Docosahexaenoic acid	.089	5

Appendix J: Analysis of variance examining the effect of different oyster farms on the fatty acid groups and fatty acid ratios in oyster. Significant values ($p < 0.05$) are in bold.

ANOVA Table

Source	Dependent Variables	Sum of Sq.	df	Mean Sq.	F	Sig.
Oyster Farms	ΣSAFA	1429.028	2	714.514	74.902	**.003**
	ΣMUFA	227.718	2	113.859	25.859	**.013**
	Σn3-PUFA	191.756	2	95.878	125.245	**.001**
	Σn6-PUFA	4427.315	2	2213.657	444.376	**.000**
	ΣPUFA	2784.153	2	1392.077	244.620	**.000**
	Σn3/Σn6	10.946	2	5.473	68.917	**.003**
	DHA/EPA	.028	2	.014	76.635	**.003**
	SAFA/TUFA	1.354	2	.677	81.936	**.002**
	SAFA/TFA	.143	2	.071	74.902	**.003**
	UNS/TFA	.143	2	.071	74.902	**.003**
Error	ΣSAFA	28.618	3	9.539		
	ΣMUFA	13.209	3	4.403		
	Σn3-PUFA	2.297	3	.766		
	Σn6-PUFA	14.944	3	4.981		
	ΣPUFA	17.072	3	5.691		
	Σn3/Σn6	.238	3	.079		
	DHA/EPA	.001	3	.000		
	SAFA/TUFA	.025	3	.008		
	SAFA/TFA	.003	3	.001		
	UNS/TFA	.003	3	.001		

Total	ΣSAFA	8900.642	6
	ΣMUFA	1378.919	6
	Σn3-PUFA	521.733	6
	Σn6-PUFA	15857.064	6
	ΣPUFA	18411.739	6
	Σn3/Σn6	17.294	6
	DHA/EPA	.177	6
	SAFA/TUFA	3.982	6
	SAFA/TFA	.890	6
	UNS/TFA	2.664	6
Corrected	ΣSAFA	1457.646	5
Total	ΣMUFA	240.927	5
	Σn3-PUFA	194.052	5
	Σn6-PUFA	4442.259	5
	ΣPUFA	2801.226	5
	Σn3/Σn6	11.184	5
	DHA/EPA	.029	5
	SAFA/TUFA	1.379	5
	SAFA/TFA	.146	5
	UNS/TFA	.146	5

Brief Biography of the Author

Tashrif Mahmud Minhaz is the second son of Md. Shaiful Islam and Momtaz Begum, was born and grown up in Mirsarai, Chattogram. He has achieved Secondary School Certificate from Moliaish High School and Higher Secondary Certificate from Govt. Haji Mohammad Mohsin College. He has also achieved B.Sc. Fisheries (Hons) from Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University. He is now a candidate of Master of Science in Aquaculture under the Department of Aquaculture, Chattogram Veterinary and Animal Sciences University. He was employed as a research assistant since February 2019 to July 2020, under the project "Introduction of Oyster Farming in Bangladesh", implemented by Department of Fisheries and Funded by Indian Ocean Rim Association through Ministry of Foreign Affairs, Bangladesh. He was trained on freshwater pearl production by Bangladesh Fisheries Research Institute. He has already published two scientific papers in well reputed national and international journals. His research interest includes oyster (stock assessment, biology, farming, breeding, disease, trophic and hydrodynamic drivers, pearl production etc.), microalgae culture, Recirculatory aquaculture system, and biofloc technology. He is passionate to qualify himself as a competent researcher, and thus to develop the aquaculture sector of Bangladesh.