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Research Article

Seasonal Dynamics in Gross Biochemical Composition of Asiatic Hard Clam, *Meretrix meretrix* Collected from the West Coast of Cox's Bazar, Bangladesh

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ABSTRACT

The study aimed to evaluate the seasonal dynamics in gross biochemical compositions of Asiatic hard clam (*Meretrix meretrix*) collected monthly from the west coast of Cox's Bazar, Bangladesh from July 2018 to June 2019. The proportion of crude protein, crude lipids, carbohydrates, ash, crude fiber and moisture contents of the clam varied from 10.81 to 15.56%, 0.45 to 3.99%, 0.26 to 5.88%, 0.88 to 2.85%, 0.26 to 1.96% and 72.63 to 82.70% respectively on wet weight basis. Biochemical analysis revealed remarkable temporal variations in the percentage of biochemical components of the species. Pearson's product-moment correlation coefficient indicated that water quality parameters (temperature, salinity and pH) and biochemical compositions of *M. meretrix* had different levels of correlations among themselves. Protein and fibre contents were moderately positively correlated with pH ($r = 0.515$ and 0.495, respectively) but moisture content was moderately negatively correlated with pH ($r = -0.484$). Protein exhibited a strong positive correlation with ash content ($r = 0.856$) and a strong negative correlation with carbohydrates ($r = -0.800$). Moderate positive correlation was found between protein and lipid ($r = 0.596$). Protein, lipid, ash and fiber contents had moderate negative correlations with moisture content ($r = -0.542$, -0.641, -0.503 and -0.423, respectively). Overall, the study revealed that Asiatic hard clam is an excellent source of several nutrients and the information furnished could be useful to the clam collectors and fishery managers for harvesting and marketing of *M. meretrix* in a particular season of the year based on the consumers' specifications.

Introduction

Mollusks are an important fishery contributing 20.23% (17.7 million tonnes) of the global aquatic animal production from the aquaculture sector (87.5 million tonnes) in 2020 (FAO, 2022). Bivalves are environment-friendly and commercially important edible aquatic animal group due to their short food chain, high nutritive value and ability to reduce harmful microbes (Shah et al., 2022). Its demand is increasing in some parts of the world considering the presence of specific proteins, vitamins and minerals essential for human body not generally found in other aquatic animal groups (Karakoltsidis et al., 1995; Espana et al., 2007). Bangladesh possesses rich aquatic biodiversity and habitat types due to its favorable geographic position. There are 6 freshwater and 142 marine and brackish water bivalve species so far reported from Bangladesh (Siddiqui et al., 2007). Although Asia is the main contributor to bivalve shellfish production (more than 80%) it's still a neglected sector in Bangladesh. Asiatic hard clam, *Meretrix meretrix* is distributed to Indo-West Pacific: from East Africa to Philippines; north to Japan and south to Indonesia. This clam is widely available along the coasts of Bangladesh (Chowdhury et al., 2019). Traditionally coastal tribal people of Bangladesh take it as food from the natural population and in some other countries; this species is used as a medicinal agent (Jia et al., 2018).

It is pertinent to evaluate the proximate composition of edible living organisms from nutritional and economic view point (Nagabhushanam and Mane, 1978). Previous

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studies showed that the biochemical composition of bivalves, particularly moisture, protein, lipid and carbohydrate exhibited clear seasonal variations. Besides, various factors like gametogenic cycle, food availability, meat content, spawning season, fecundity, and depth of culture area affect the proximate composition of bivalves (Ngo et al., 2006; Uddin et al., 2007) and are strongly related to water temperature, food availability and the gametogenic cycle (Baird, 1958; Uddin et al., 2013; Sawant and Mohite, 2013; Niogee et al., 2019; Siddique et al., 2020). The *M. meretrix* is edible to both human and animals and is also used as drugs in the treatment of extravasation agents, arthritis, ischemic heart disease and hyperlipidemia. It could be a good source of income for coastal people by developing a farming system or by exploiting *M. meretrix* from natural stock to meet the huge demand of nutrition in addition to export earnings. In recent past, the demand for protein-rich food has been increasing, especially in developing countries, which in turn stimulates the exploration of unexploited non-traditional resources. But for this which substrate are important, and how the timing of their consumption is related to a particular season is a major concern for both researchers and consumers. Although *M. meretrix* is available off the coast of Moheskhali Island, Cox's Bazar, Bangladesh but study on the seasonal variations in its biochemical composition of it have not been the subject of intense studies despite the presence of rich diversity of edible and commercial fish species in Bangladesh. Report on temporal changes in biochemical components of clams may provide vital information to the managers and collectors for determining the suitable time of harvesting nutritionally rich animals from the habitat from economic view point. Thus, a sampling program was undertaken to investigate the monthly changes in the biochemical composition of *M. meretrix* from Ghotibhanga, west coast off the Moheskhali Island, Cox's Bazar, Bangladesh, over a year to specify the optimum time of harvesting nutritionally rich animals for consumption.

Materials and Methods

Sampling activities

The study was carried out in the Ghotibhanga, west coast off the Moheskhali Island, Cox's Bazar, Bangladesh (21°29'10''N, 91°53'7''E) (Figure 1). Samples were collected monthly for a period of 12 months from July 2018 to June 2019. Samples of Asiatic hard Clam (*Meretrix meretrix*) were collected by hand-picking from the intertidal zone during low tide. After collection, *M. meretrix* samples were conveyed from sampling site to Wet Laboratory of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Then the samples were brushed and washed with running tap water to remove algal biomass, mud and other waste material. When completely washed, the cleaned samples were then kept for depuration for 12 hr in laboratory conditions under constant aeration. Finally, the shell was opened and soft body tissue was extracted. After weighing the soft tissue wet weight, the tissue was excised and stored at 4°C for biochemical analysis.

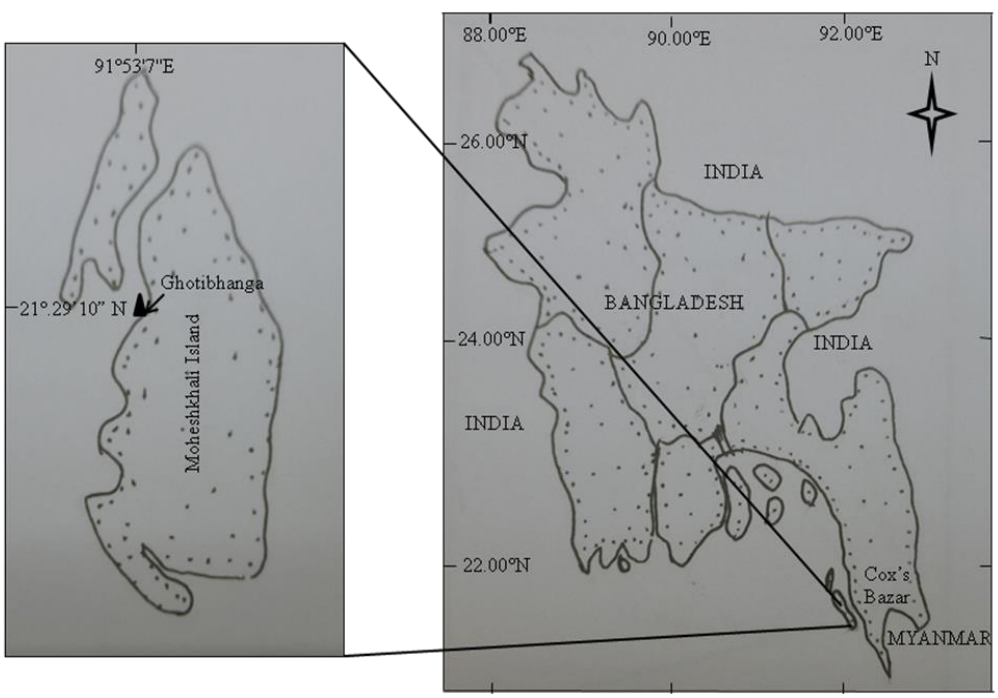

Figure 1. Map showing location of the sampling site indicated by triangle (▲).

Analysis of biochemical compositions

Proximate composition of *M. meretrix* such as moisture, crude protein, crude lipid, ash, crude fibre and carbohydrate were analyzed with certain modifications of the method of Association of Official Analytical Chemists (AOAC, 2000). Briefly, the moisture content of the analyzed clams was measured by weighing the differences of the sample (2 to 3 g) kept in porcelain crucible before and after drying in an oven at 105ºC for 24 h. Crude protein content in the homogenized sample (0.5 g) was determined using the Kjeldahl method (%N × 6.25) after acid digestion, distillation and titration of the samples. Crude lipid content of tissue samples was estimated gravimetrically after extraction with petroleum ether in a Soxhlet system. Total ash was estimated gravimetrically by ignition at 550°Cin muffle furnace. Crude fiber was estimated gravimetrically after acid and alkali digestion and loss in mass by combustion at 600°C for 3 h. Total carbohydrate content was determined by subtracting the sum of the percentage of moisture, ash, lipid, crude protein and crude fiber from 100%.

Monitoring water quality parameters

Water quality parameters of the sampling site such as temperature, salinity and pH were monitored monthly at each sampling date. Water temperature (°C) was taken from three different locations of the sampling site using a Celsius thermometer. The salinity of the sea water (‰) was recorded in triplicate at each sampling date using a portable salinity meter (Model HI 96822 seawater refractometer, Hanna Instruments). pH of the sea water was recorded by using a direct reading pH meter (pHep® pH meter, Hanna Instruments) in triplicate at every sampling date.

Statistical analysis

All the data recorded from the study were tabulated in the Microsoft Excel spreadsheet and expressed as mean (±SD). Temporal differences in the biochemical composition (crude protein, crude lipid, carbohydrate, ash, crude fiber and moisture) were compared by Duncan's multiple range test (DMRT; $p<0.05$) using SAS statistical package. Pearson product-moment correlation coefficient was tested among water quality parameters and biochemical compositions of the investigated species using SPSS version 25. Correlation coefficients were interpreted according to Schober et al. (2018) (0.10 to 0.39 as weak correlation; 0.40 to 0.69 as moderate correlation; 0.70 to 0.89 as strong correlation; and 0.90 to 1.00 as very strong correlation).

Results

Water quality parameters

Monthly changes in water temperature, salinity and pH of the sampling site monitored over 12-month are shown in Figure 2. The mean surface water temperature of the sampling site over the study period was 25.84 ± 3.07ºC. The surface water temperature was found to range from 19.9 to 29.0ºC. The highest surface water temperature was observed in May and the lowest temperature was noted in January. Salinity of the sea water varied from 23.1 to 35.2 ppt during the study period. The minimum salinity value was recorded in June, whereas the maximum value was recorded in January. The mean value of salinity was 30.35 ± 3.61 ppt over the study period. pH of the sampling site fluctuated from 7.5 to 8.6during the study period. The minimum pH was recorded in November and the maximum pH was found in February. The mean value of pH was 8.11 ± 0.29 during the study period.

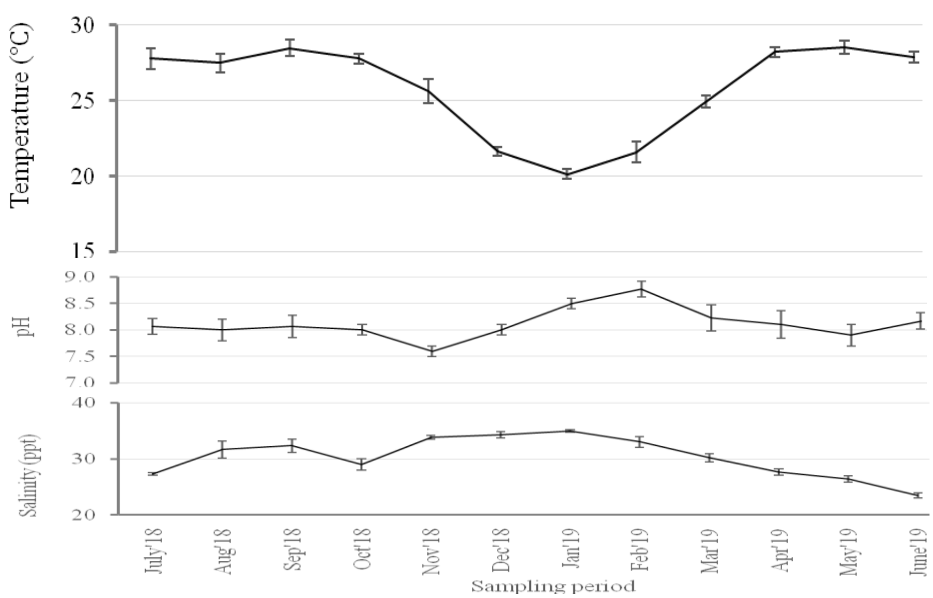

Figure 2. Monthly changes in mean water temperature, pH and salinity of the sampling site. Vertical bars indicate standard deviation (SD).

Biometric characteristics

Biometric measurements of the Asiatic hard clam, *Meretrix meretrix* such as shell length, shell width, shell height and wet tissue weight, are shown in Table 1. The shell length of the sampled clams ranged from 41.50 to 74.84 mm and the soft tissue wet weight varied from 2.82 to 16.59 g.

Table 1. Biometric parameters of *Meretrix meretrix* (mean ± SD) collected from Ghotibhanga, west coast of Moheshkhali, Cox's Bazar, Bangladesh

Month	Shell length (mm)	Shell height (mm)	Shell width (mm)	Wet tissue weight (g)
Jul '18	56.16±5.06	34.95±3.72	49.75±4.55	8.54±2.72
Aug '18	57.59±7.50	48.64±5.33	34.40±3.89	7.58±2.74
Sep '18	49.98±3.72	42.55±3.69	30.29±2.26	6.48±1.85
Oct '18	56.64±7.00	48.08±5.25	33.52±4.04	7.76±2.56
Nov '18	61.89±6.18	51.25±4.29	36.33±3.71	9.47±2.92
Dec '18	57.93±6.18	50.43±4.51	34.50±3.58	9.42±3.06
Jan '19	55.82±3.65	33.17±2.83	48.83±3.48	7.90±1.91
Feb '19	56.41±3.98	32.06±3.42	48.51±3.09	8.06±1.48
Mar '19	57.06±6.73	33.96±3.71	48.48±5.28	7.30±2.87
Apr '19	59.34±7.03	50.58±5.49	35.38±4.12	8.08±2.32
May '19	48.65±5.59	28.85±3.75	41.61±4.57	4.65±1.33
Jun '19	49.80±6.17	29.00±3.04	43.20±4.83	6.98±2.37

Temporal changes in proximate composition (wet weight)

Monthly changes in the values of protein, lipids, carbohydrate, ash, fibre and moisture contents are shown in Table 2 and Figures 3 to 8.

Table 2. Proximate composition (wet weight basis) of *Meretrix meretrix* collected from Ghotibhanga, west coast of Moheshkhali, Cox's Bazar, Bangladesh

Months	Protein (%)	Lipids (%)	Carbohydrate (%)	Ash (%)	Fibre (%)	Moisture (%)
Jul '18	11.39	3.27	4.49	1.05	0.67	79.45
Aug '18	12.14	3.01	3.56	2.00	0.77	78.05
Sep '18	13.33	2.37	2.35	2.15	0.81	78.59
Oct '18	12.91	2.65	5.89	2.35	0.79	76.50
Nov '18	12.37	2.45	5.54	2.10	0.87	77.98
Dec '18	11.26	2.01	6.85	2.15	0.87	76.61
Jan '19	14.08	1.25	0.89	2.13	1.11	78.60
Feb '19	15.11	2.40	5.71	2.42	1.76	72.58
Mar '19	14.57	4.47	3.51	2.07	0.88	75.09
Apr '19	15.17	4.68	3.16	2.69	0.91	74.73
May '19	14.12	4.85	4.26	3.00	1.00	73.44
Jun '19	12.65	3.96	5.39	3.35	1.07	73.96

Crude protein (%)

Protein values of *M. meretrix* varied from 10.81 to 15.56% on wet weight basis having an average of 13.26±0.57% over the study period. Remarkable temporal variations in the proportion of protein were observed in the clams throughout the study period. Protein percentage was significantly higher ($p<0.05$) from February to April when compared with other months of the investigated year except for January, May and September. Significantly lower proportion of protein ($p<0.05$) was noted in December than the other months except July and August when the values were statistically identical ($p>0.05$) with December (Figure 3).

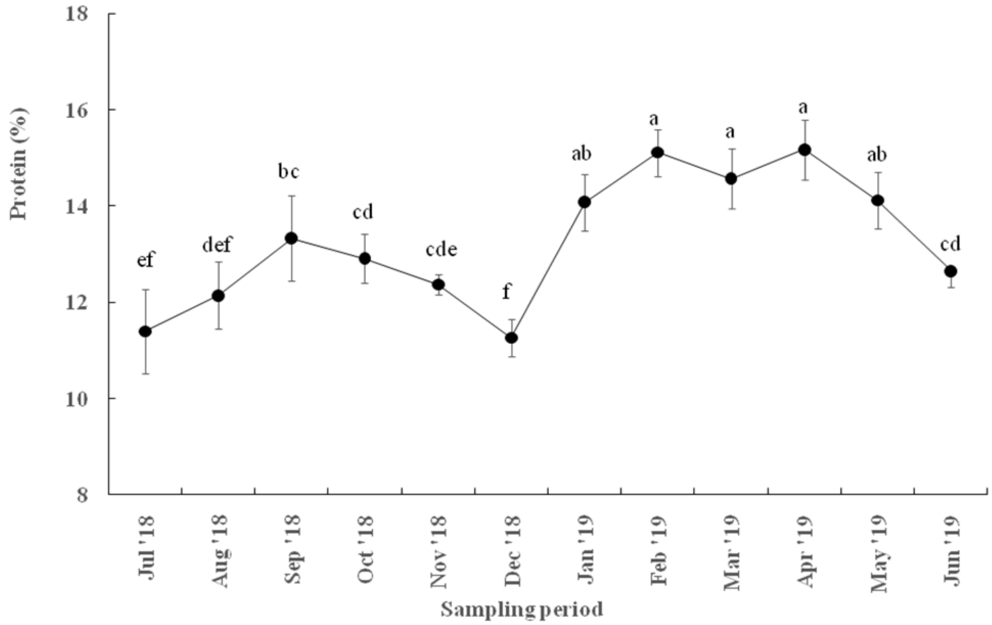

Figure 3. Monthly changes in crude protein (%) of Asiatic hard clam (*Meretrix meretrix*) collected from the west coast of Moheshkhali, Cox's Bazar, Bangladesh. Different letters indicate significant differences (p<0.05) among different months.

Crude lipids (%)

The proportion of crude lipids of *M. meretrix* was found to range between 0.45 to 3.99% with an average of 2.36±0.24% over the 12-month study period. Significant temporal differences were noted in the proportion of crude lipids of the clams. There was a significant increase (p<0.05) in the proportion of crude lipid from December onwards. Crude lipid percentage was significantly higher (p<0.05) from April to June than the other months except for March and August. The values remained significantly lower (p<0.05) and consistent from September to December than the other months except for July when the values were reported as the lowest (p<0.05) over the study period (Figure 4).

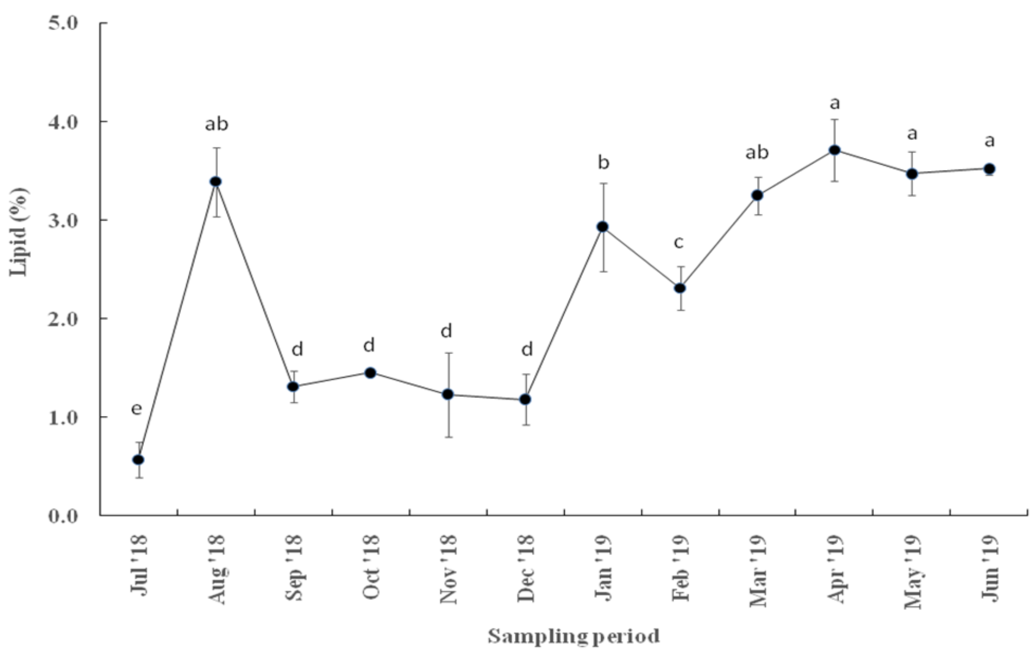

Figure 4. Monthly changes in crude lipid (%) of Asiatic hard clam (*Meretrix meretrix*) collected from the west coast of Moheshkhali, Cox's Bazar, Bangladesh. Different letters indicate significant differences (p<0.05) among different months.

Carbohydrate (%)

Biochemical analysis revealed that the proportion of carbohydrate of *M. meretrix* varied from 0.26 to 5.88% on wet weight basis over the 12-month study period. The overall mean value of carbohydrate of the investigated clams was 2.61±0.36%. Significant differences were also observed in the values of carbohydrates of the clams among different months. Carbohydrate values dropped suddenly after January and the proportion remained significantly lower ($p<0.05$) from February to May than the remainder of the study period. There was a sudden increase in carbohydrates from May to June and the values reported in June were significantly higher ($p<0.05$) than any other months of the year. Significantly higher values ($p<0.05$) in carbohydrates were also reported in July, August and October than in the other months except for June (Figure 5).

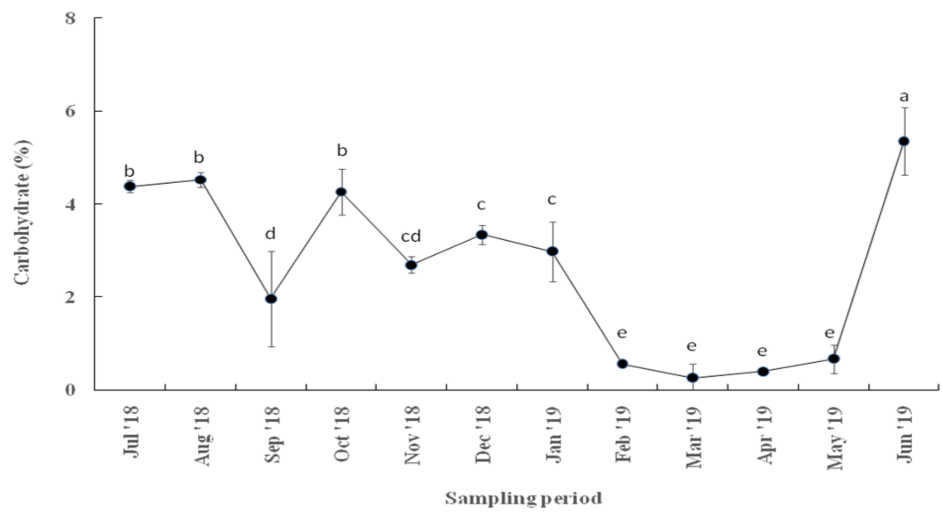

Figure 5. Monthly changes in carbohydrate (%) of Asiatic hard clam (*Meretrix meretrix*) collected from the west coast of Moheshkhali, Cox's Bazar, Bangladesh. Different letters indicate significant differences ($p<0.05$) among different months.

Ash (%)

Ash contents of *M. meretrix* ranged from 0.88 to 2.85% on wet weight basis with an average of 1.99±0.17% over the study period. Significant temporal variations ($p<0.05$) in the proportion of ash contents were observed in the clams throughout the study period. Ash contents increased significantly ($p<0.05$) from July to October consistently. The values remained statistically identical ($p>0.05$) during October and November and then decreased significantly ($p<0.05$) in December. Ash contents increased significantly again ($p<0.05$) from January and peaked in April. There was a decreasing tendency in its values after April during the remainder of the study period till June (Figure 6).

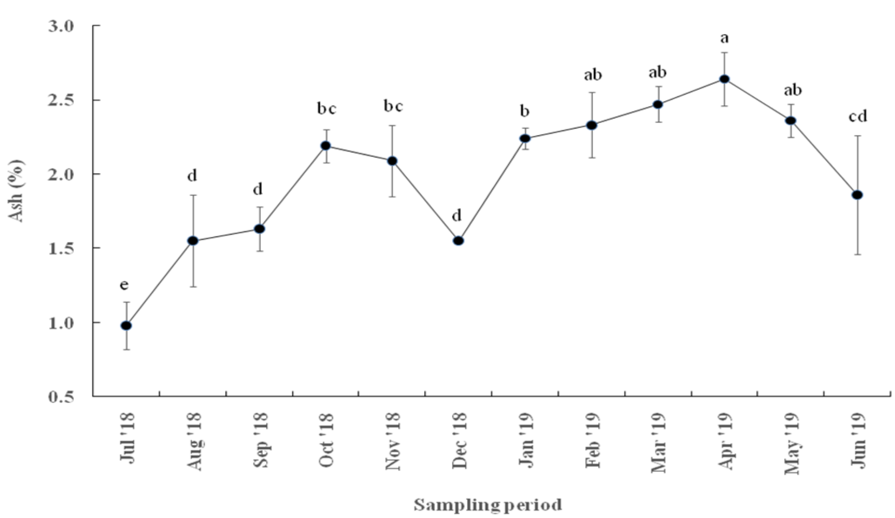

Figure 6. Monthly changes in ash content (%) of Asiatic hard clam (*Meretrix meretrix*) collected from the west coast of Moheshkhali, Cox's Bazar, Bangladesh. Different letters indicate significant differences ($p<0.05$) among different months.

Crude fibre (%)

The proportion of crude fibre of *M. meretrix* was found to range between 0.26 to 1.96% with an average of 0.88±0.13% over the 12-month study period. Significant temporal differences were noted in the proportion of crude fibre of the clams. Crude fibre contents remained consistently lower from July to December. The values increased significantly ($p<0.05$) from December to January and then decreased again with significantly lowest value (p<0.050 in March than the other months except July and April. Crude fibre contents increased again after April as shown in Figure 7.

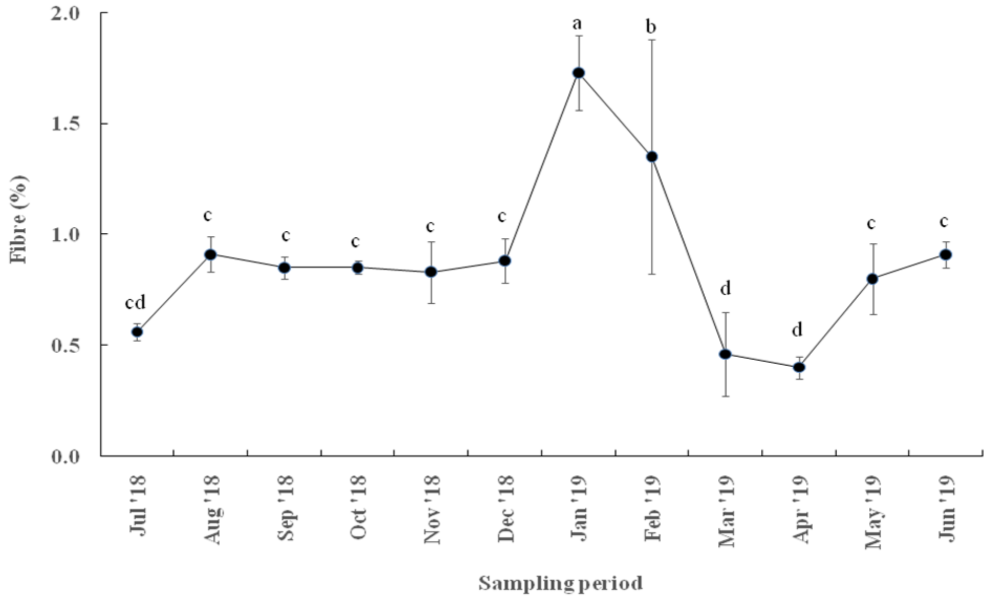

Figure 7. Monthly changes in crude fibre (%) of Asiatic hard clam (*Meretrix meretrix*) collected from the west coast of Moheshkhali, Cox's Bazar, Bangladesh. Different letters indicate significant differences ($p<0.05$) among different months.

Moisture (%)

The proportion of moisture was found to range from 72.63 to 82.70%, having an average of 78.26±0.85% over the study period. Significant temporal variations ($p<0.05$) in the proportion of moisture were observed in the clams throughout the study period. Moisture percentage decreased persistently from July to October and then increased significantly ($p<0.05$) till December. The values dropped suddenly after December with the significantly lowest value ($p<0.05$) in January when compared with the other months except for June. Significant increments ($p<0.05$) in the proportion of moisture were noted from January to February and the values remained more or less stable till May (Figure 8).

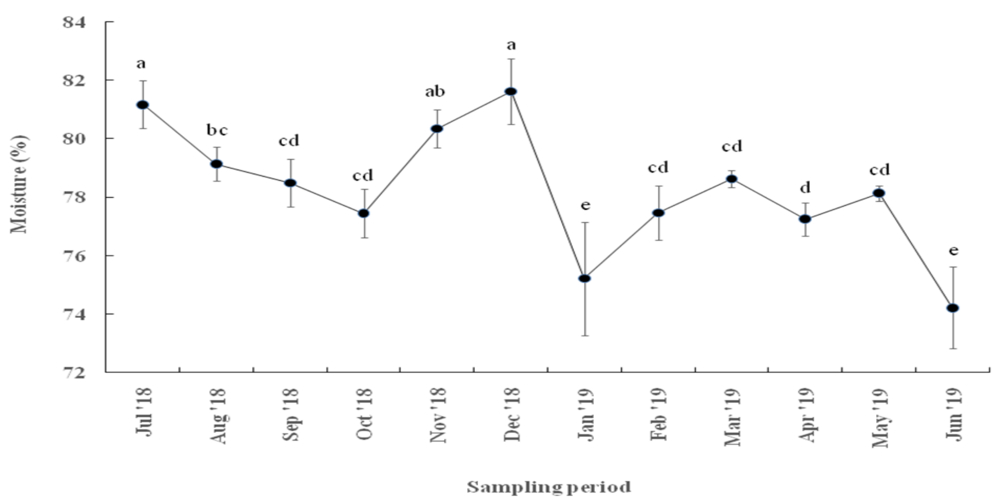

Figure 8. Monthly changes in moisture (%) of Asiatic hard clam (*Meretrix meretrix*) collected from the west coast of Moheshkhali, Cox's Bazar, Bangladesh. Different letters indicate significant differences ($p<0.05$) among different months.

Correlation among environmental parameters and biochemical composition of M. meretrix

Correlations among environmental parameters and various biochemical compositions of Asiatic hard clam are shown in Table 3.

Table 3. Correlation matrix among different environmental parameters and proximate composition of *Meretrix meretrix* collected from Ghotibhanga, west coast of Moheshkhali, Cox's Bazar, Bangladesh

	Temp.	Salinity	pH	Protein	Lipid	Carbo.	Ash	Fiber	Moisture
Temp.									
Salinity	-0.593*								
pH	-0.493*	0.457*							
Protein	-0.121	-0.302	0.515*						
Lipid	0.095	-0.313	0.281	0.596*					
Carbo.	0.146	0.321	-0.219	-0.800**	-0.293				
Ash	-0.151	-0.471*	0.193	0.856**	0.600*	-0.683*			
Fiber	-0.687*	0.698*	0.495*	0.116	0.023	0.150	0.070		
Moisture	0.012	0.010	-0.484*	-0.542*	-0.641*	-0.023	-0.503*	-0.423*	

* Indicates moderate correlation and ** indicates strong correlation.

Strong, moderate or weak correlations were evident among the variables. Salinity had a moderate negative correlation with the water temperature (r =-0.593), pH (r = -0.493). Fibre contents of *M. meretrix* were also negatively correlated with the water temperature (r = -0.687). Salinity was moderately positively correlated with pH (r = 0.457). Ash content was moderately positively correlated with salinity (r = -0.471) whereas, crude fibre was moderately positively correlated with salinity (r = 0.698). Protein and fibre contents were moderately positively correlated with pH (r = 0.515 and 0.495, respectively); in contrast, moisture content was negatively correlated with pH (r = -0.484). A moderate positive correlation was reported between protein and lipid (r = 0.596), whereas a strong positive correlation was noticed between protein and ash contents (r = 0.856). Nevertheless, a strong negative correlation was noticed between protein and carbohydrate (r = -0.800) and a moderate negative correlation was obtained between protein and moisture (r = -0.542). A moderate positive correlation existed between lipid and ash contents (r = 0.600) and a moderate negative correlation was reported between lipid and moisture (r = -0.641). Carbohydrate content was moderately negatively correlated with ash contents (r = -0.683), whereas ash content was moderately negatively correlated with moisture contents (r = -0.503).Crude fibre was also moderately negatively correlated with moisture contents (r = -0.423). Weak correlations were evident in other cases.

Discussion

The present study was undertaken to investigate and evaluate the seasonal changes in nutritional quality of Asiatic hard clam (*Meretrix meretrix*) that will be useful as a key ingredient for the development of functional foods or for pharmaceutical uses. From the study after monthly biochemical analysis for a period of 12 months, it was found that the proximate composition such as crude protein, crude lipid, carbohydrates, ash, crude fiber and moisture contents of *M. meretrix* collected from the west coast of Moheshkhali, Cox's Bazar, Bangladesh ranged from 10.81 to 15.56%, 0.45 to 3.99%, 0.26 to 5.88%, 0.88 to 2.85%, 0.26 to 1.96% and 72.63 to 82.70% respectively (Figures 3 to 8). Seasonal sampling appeared to be insufficient for clearly understanding the temporal biochemical changes of bivalves due to an excessively lengthy sampling interval (Celik et al., 2014; Chowdhury et al., 2019). Thus, in the current study, monthly sampling of *M. meretrix* was performed over a year. The proximate composition of different ingredients of this species reported by Chowdhury et al. (2019) is consistent with our findings, as the values are within the ranges obtained in the current study. The analysis of body parts is often more instructive than the analysis of the whole animal when studying biochemical composition in relation to growth and gametogenic cycles (Berthelin et al., 2000). In fact, the storage metabolism of the gonad-visceral mass components of the Asiatic hard clam revealed tissue-specific changes during gametogenic cycle. Elsewhere Mackie and Ansell (1993) reported that environmental factors apparently play a dominant role in determining events in the storage cycles.

In the current study the mean protein content of *M. meretrix* was13.26% whereas, the maximum protein content was recorded during April and the minimum was noticed in December. Protein is the major ingredient of bivalve eggs and main source of energy during gonadal development (Serdar and Lok, 2009). Protein concentrations in *M. meretrix* exhibited an increasing trend from July to September and December to February perhaps due to accumulation in the body and subsequent mobilization to gonads for their development during these periods. Highest protein content was reported in pre-spawning clams by Celik et al. (2014). According to the literature, the protein content in different bivalve species predominantly regulated by exogenous factors (Newell and Bayne, 1980). A sharp declining trend in protein content was observed after September till December and after April till June and perhaps due to release of gametes and energy loss for spawning activities during this time. Although it was hard to correlate the events without histology data, this assumption was supported by many reports (Galap et al., 1997; Uddin, 2008; Sarder and Lok, 2009).

After protein for the energy metabolism of bivalves, lipid is essential. In the present study the average lipid content of *M. meretrix* was 2.36% and the maximum *percentage* was found in April to June that could be correlated with primary breeding peak and show a large fluctuation within 12 months of sampling (Figure 4). This was corroborated by Balasubramanian and Natarajan (1988), as after an intense period of breeding the lipid values declined. Current study results are also in agreement with Kamble and Muley (2009), as lipid concentration was high in summer and monsoon while low during winter.

Along with protein and lipid average carbohydrate found in *M. meretrix* was 2.61% where highest percentage was found in June (Figure 5). A large fluctuation was found in carbohydrate content throughout the sampling period that show a strong negative correlation with protein ($r = -0.08$) might be related to the annual gametogenic cycle as reported by Celik et al. (2014).

During this research, average ash content was about 1.99% with the highest value during monsoon (Figure 6). Throughout the study period a moderately positive correlation was found between ash and protein content which was not in consistent with Eswar et al. (2016). Such discrepancy may be due to the selection of mature individuals in the current study as immature mussels were reported to contain lower ash contents than the mature ones by them.

In our research, the average crude fiber content was 0.88% with the highest percentage in January (Figure 7) but average moisture content was 78.26% while the concentration was high during monsoon and winter (Figure 8) was similar with Sunila (1998) may be due to an inverse relationship between protein, lipid and carbohydrates with moisture.

Conclusion

M. meretrix distributed along the coast off Moheskhali Island, Cox's Bazar, Bangladesh exhibited remarkable temporal variations in the proximate compositions at different magnitude for different components. The monthly variations in biochemical composition of *M. meretrix* could be due to endogenous and exogenous factors, including maturity stages, diets, seasons etc. The results revealed that Asiatic hard clam harvested from the coast off Moheskhali Island could be a rich source of nutrients, including proteins, fats and minerals and from the findings of this research, farmers or researchers can be able to know the peak timing of biochemical compositions and suitable time of harvesting of this species in different seasons of a year as per consumers' demand.

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