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#### **Research Article**

Biochemical composition of *Meretrix meretrix* in the Bakkhali river Estuary, Cox's Bazar, Bangladesh

#### Abstract

The present study was conducted in the Bakkhali river estuary, Cox's Bazar, Bangladesh from September 2012 to July 2013. Percentage of protein was the main component of the biochemical composition in the mussels followed by carbohydrate, ash, and lipid. The proximate composition of *Meretrix Meretrix revealed* that it contained protein (12.184-14.291%), lipid (0.721-0.922%), ash (2.435-3.201%), moisture (77.0-78.9%) and carbohydrate (4.914-5.907%). The mineral content of the *Meretrix Meretrix* were Ca (0.601-0.801 mg/g), Fe (0.070-0.099 mg/g) and phosphorus (0.300-0.794 mg/g). In the present investigation, the seasonal changes of protein and lipid were found to follow a similar pattern. Hence, a significant positive correlation was found between the percentage of protein and lipid in *Meretrix meretrix*. A positive relationship was found between the percentage of protein and lipid, protein and ash and, lipid and ash. A significant inverse correlation was found between protein and carbohydrates, carbohydrate and lipid and between carbohydrate and ash content of dry tissue of *Meretrix meretrix* (p<0.01). Further study on *Meretrix meretrix* should be carried out since their acceptability to the coastal dwelling community.

## Introduction

The clam, *Meretrix meretrix*, is a delicious, cheaper, and protein-rich food source for people residing along with the coast [1,2]. It has been traditionally used as medicine and raw material for village industries [3]. These edible bivalves are filter feeders with high conversion efficiency and high levels of biochemical constituents. Due to high protein content and the high nutritive value, several species of clams were studied [4-7]. Some important features like high protein content, low calorific values, low fat/ cholesterol profile and lower proportions of saturated fat, the presence of good lipids, significant amounts of omega-3-fatty acids, dietary essential amino acids, vitamin  $B_{12}$  and several important minerals such as iron, zinc and copper make the calm a good food [8,9].

The biochemical analysis is the percentage of water, protein, lipids, carbohydrate, and minerals required for human [10]. This nutritive value of edible living organisms is needed for consumption and marketing [11]. The proximate composition is an important tool to identify the quality of meat and to understand the changes in the nutritive value in the clams during the gametogenesis [12].

Various factors like spawning season, fecundity, and depth

of culture area affect the proximate composition of *Meretrix meretrix* [11,13–17]. Growth of body size, maturation of the clam increase in the protein, lipid, and carbohydrate (glycogen) contents in calm [18]. The season played a significant role in the lower concentration of fat and it happens during spawning just after the release of gametes [19–21]. Similar observations had been conducted by Pease [22] and Durve and Bal [20], in oysters and Joshi and Bal [19], in *K. Marmorata*. Giese et al. [23] observed that lipid levels were relatively stable for most of the year except for the small variation in the gonads; the lipid reached a peak value at the time of most active formation of gametes.

Biochemical composition of bivalves studied by various researchers [12,19,24-32].

*Meretrix meretrix* abounds along the coastal region from St. Martin's Island, Bakkhali river estuary, Sahaparirdip, Teknaf, Cox's Bazar, Moheskhali, Kutubdia in Cox's Bazar district. Galachipa, Kalapara in Patuakhali district. Amtoli, Borguna, and Paterghata in Borguna district. Mongla port, Saronkhola in Bagherhat district. Dakop, Koira, Paikgacha in Khulna and Satkhira district. The availability and high nutrients contents compelled us to know the biochemical composition of *Meretrix meretrix*.

## **Materials and Methods**

### Study area

The study was carried out in the Bakkhali river estuary, Cox's Bazar district (Figure 1). The global positions of four stations were between latitude (N) 21°26'50" to 21°32'50" and longitude (E) 91°65'30" to 91°90'30". Almost 2 sides of three stations were open to the Moheshkhali channel and another west side was surrounded by Cox's Bazar town. *Meretrix meretrix* is found as sub-soil animals. The species inhabits in the sand and muddy sand bottom in the intertidal and sub-tidal water to a depth of about 20m in the Indo-west pacific seas [33].

#### Sample collection and processing

*Meretrix meretrix* samples were collected from the Bakkhali river estuary. Immediately after collection, the clams were transferred to the laboratory of Faculty of Marine Sciences and Fisheries in live condition. Then the clams were acclimatized with filtered aerated estuarine water in the plastic containers. The clams were adopted for 48 hours and water was changed 3 times in a day during this period [26]. Then clams were washed completely and the shell was opened. Matured and immature clams were identified and weighed after removing the excess moisture. Finally, the meat was extracted and stored at 4°C for further analyses [27].

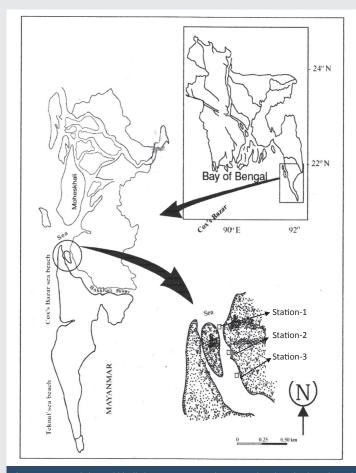


Figure 1: Map of the Bakkhali river estuary, Cox's Bazar showing the location of the sampling stations.

### **Protein determination**

The nitrogen was estimated by the micro-Kjeldahl method [34] and the amount of crude protein was calculated by multiplying nitrogen value by 6.25. The fat was extracted in solvent ether from samples of dried meat by using Soxhlet apparatus. Glycogen was estimated by the method of Kemp and Heijningen [35], using Engel's colorimeter. The amount of glycogen was calculated by multiplying the glucose value by factor 0.927. The results are expressed in percentage of dry weight.

The protein content of the feedstuff is obtained by estimating the nitrogen content of the materials and multiplying the nitrogen value by 6.25 this is referred to as crude protein content. According to Kjeldahl methods-proteins are hydrolyzed to amino and with  $H_2SO_4$ . Further heating decomposes the amino acid releasing-Ammonia which immediately trapped on  $(NH_4)_2SO_4$  and water. Micro Kjeldahl Method [36–38], was to determine the crude protein.

% of Nitrogen =V×N×0.014×100/wt of sample

Where, V= Volume of HCl

N= Normality of HCl

% of Crude protein = % of Nitrogen×Conversion factor

**Lipid determination:** Fat is examined with low boiling organic solvent (petroleum ether/ diethyl ether, xylene) by soxhlet extraction and the extract thus obtained weighed after recovery of the solvent. Crude fat was determined through Sox let Extraction Technique [39,40], using hexane (65°C-70°C) as the solvent.

% of crude fat = (Corrected weight of fat÷Weight of sample)×100

Ash determination: Ash is the residues of the inorganic matter (mineral) of the sample after burning. If the sample in a muffle furnace at 600°c the organic matter is evaporating and residues are called ash. Ash content of each feed was estimated by following incineration Method [39].

% of ash = (Weight of ash+Weight of sample)×100

**Moisture determination:** Moisture is an important, debutante of the nutrient of the feed or feed ingredients. It is necessary to know the moisture contents of the feed because it has an important function determines the form of the diet. Moisture contents in the feed were determined by the following oven method [41].

The percentage of the moisture content in the sample was calculated by the following formulae:

% of moisture = {(Weight of original sample–Weight of dried sample) /Weight of original sample}×100.

**Carbohydrate determination:** The percentage of carbohydrate was calculated as follows:

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% of Carbohydrate = 100-% of (protein+lipid+ash+moisture)

Calcium determination: The evaluation of calcium in feed is very important since imbalance with phosphorous or other minerals will lead to reducing growth.

% of Ca = V×N× use of diquot from mother solution/wt. of sample ×250

=0.5×7.4×100/2×250

=0.74

% of Ca (mg/100g) =740

Iron (Fe) determination: Fe (mg/100g) =Abs×0.49×100/wt. of sample

Phosphorus (P) determination: The percentage of Phosphorus =1.435 a/p

Where, a = The weight in gm of the ammonium phosphate molybdate precipitate

p = Weight in gm of the sample taken for analysis

#### Statistical analysis

One-Way Analysis of Variance (ANOVA) was executed to show the significant variations and relations among the concentrations (SPSS v.22). MS-Excel was used to produce graphs.

## **Results and Discussion**

Protein is the major biochemical constituent of wet mussel meat forming 12.184% to 14.291%. The average protein content of Meretrix meretrix is 13.26% (Table 1). In the case of clam, it was noticed that as temperature increases protein content also increases. Bivalves use reserve protein during harsh condition. This scenario was observed in Turbo sarmaticus and in Paphia malabarica [28,42]. During monsoon season gradual fall in protein content can be seen. While in the case of mussel two peaks can be seen during post-monsoon (14.291%) and premonsoon (13.321%). In the present study, seasonal changes of protein and lipid in Meretrix meretrix were found to follow a similar pattern. Kamble and Muley [26], reported that the highest protein content in Meretrix meretrix in winter and

monsoon season than the summer season. Nagabhushanam and Dhamane [43], recorded relatively high amount of protein in Papiha laterisulca all the year round. Durve and Bal [44], also reported higher concentrations of protein in *C. gryphoides*. Different Protein concentrations were found in A. ligamertina and A. plictata [45]. High protein concentration was found in adult stage since bivalves use protein to form oocytes [46-48].

Hence, a significant positive correlation was found between the percentage of protein and lipid. Similar results were reported by Fatima et al. [49], in the green mussel, Perna viridis.

Lipid is very important for energy metabolism of bivalves such as Patinopecten vessoensis [50]. Lipids are found basically in gonads of adult bivalves [51,52]. In the present study, the lipid content ranged between 0.721% to 0.922% in wet mussel (Figure 2). The average lipid content of mussel 0.86%. Like carbohydrate content lipids also exhibited large fluctuations in all months of the year. Lipid content in mussel was very high in post-monsoon.

During monsoon, temperature and salinity decreases sharply lipids also decreases sharply. Kamble and Muley [26], stated that the lipid concentration was high during summer and monsoon seasons and low during the winter season. In the case of mussel, lipids showed an inverse relation with carbohydrates. As lipid increases carbohydrate decreases.

In contrary to protein and lipid, seasonal changes in carbohydrate followed a different course. i.e. when carbohydrate content decreased, protein and lipid fractions were found to increase (Table 1). Thus, a reciprocal relationship between the percentage of protein and carbohydrate was found in Meretrix meretrix. A similar relationship was reported in case of Medulis by Dare and Edwards [53], and Drzycimski [54], and in the case of Meretrix meretrix by Fatima et al. [49]. But for all the oyster species, a positive correlation was reported between the carbohydrate and the lipid level [55]. This difference between mussel and oyster may be related to the form of the reproductive cycle [55].

Comparison of functional ingredients of M. meretrix with other studies presented in Table 2.

The ash content varied from 2.435% to 3.136% in wet mussel (Table 1). The average lipid content of clam is 2.844%. Ash

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Seasons	Station	Protein (%)	Lipid (%)	Ash (%)	Moisture (%)	Carbohydrate (%)	Ca (mg/100gm)	Fe (mg/100gm)	P (mg/100gm)
Pre-monsoon	Station 1	13.125	0.900	2.854	77.8	5.320	0.740	0.095	0.329
	Station 2	13.321	0.891	2.647	77.4	5.741	0.761	0.082	0.658
	Station 3	13.041	0.917	2.435	77.7	5.907	0.776	0.089	0.425
Monsoon	Station 1	12.653	0.721	3.136	78.4	5.090	0.601	0.070	0.300
	Station 2	12.482	0.792	3.012	78.8	4.914	0.653	0.072	0.302
	Station 3	12.184	0.775	3.201	78.9	4.940	0.613	0.075	0.321
Post- monsoon	Station 1	14.176	0.899	2.824	77.1	5.001	0.798	0.099	0.435
	Station 2	14.079	0.912	2.793	77.2	5.102	0.801	0.085	0.794
	Station 3	14.291	0.922	2.734	77.0	5.053	0.789	0.080	0.536

content in clam was very high in monsoon whereas in summer months it was low (Table 1). Eswar et al. [25], found 5.56% ash content in *G. divaricatum* collected from Mumbai, West Coast of India. Ash content showed an inverse relation with protein content. On the basis of yearly average, mature mussels contained higher ash content than the immature ones which was just an opposite trend found in the case of carbohydrate and lipid content but similar to the protein content.

The moisture content showed a range of 77.00% to 78.9% in wet mussel (Table 1). The average moisture content of 77.81%. The highest percentage of moisture was recorded in the monsoon while the lowest concentration was found in post-monsoon (Figure 3). Eswar et al. [25], recorded 6.11% ash content in *G. divaricatum* collected from Mumbai, West Coast of India. Moisture had an inverse relationship with salinity. Moisture contents in *Meretrix meretrix, Katelysia* 

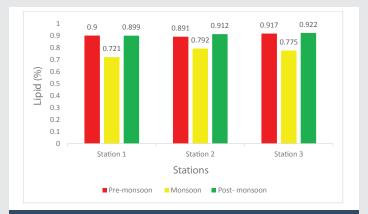


Figure 2: Concentrations of lipid (%) in *Meretrix meretrix* collected from the Bakkhali river estuary.

Table 2: Comparison of functional ingredients of *M. meretrix* (wet weight) with other results.

Chemical compositions	Present study (%)	Others study (%)	References	
Protein	12.184-14.291	10.5-15.54	[73,74]	
Lipid	0.721-0.922	1.07-6.78	[75-78]	
Ash	2.435-3.201	12.8-22.4	[72,75]	
Moisture	77.0-78.9	76.39-80.2	[73]	
Carbohydrate	4.914-5.907	4.14-8.3	[72-74]	

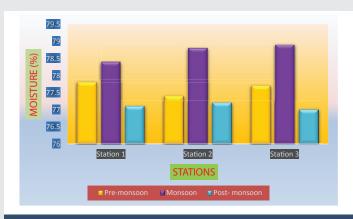


Figure 3: Concentrations of moisture (%) in *Meretrix meretrix* collected from the Bakkhali river estuary.

opima, Marcia opima, Lima trains, Nucula sulcate, Donax cuneatus, Donax incarnates increased with the decreased salinity during monsoon season [32,56–62]. Similar results were found in the present study.

Another major biochemical content was carbohydrate found in Meretrix meretrix. Carbohydrate exhibited large fluctuations in all months of the year. The concentrations varied from 4.914% to 5.907% in wet mussel (Table 1). The average carbohydrate content of clam was recorded 5.229%. Eswar et al. [25], found 11.23% ash content in G. divaricatum collected from Mumbai, West Coast of India. The trend of the percentage of carbohydrate was just opposite to that of protein. The higher percentage values of carbohydrate were observed during the monsoon period and lower during the post-monsoon and premonsoon period for both the mature and immature mussels (Table 1). Arun [24], recorded comparatively low carbohydrate concentration during pre-monsoon. Tivela stultorum, Meretrix meretrix, Donax cuneatus, Villorita cyprinoides var. cochinensis and Sunetta scripta converted carbohydrate to lipid and protein and stored in the gametes [32,43,63-65]. They used these reserve lipid and protein during stress environment [39,66].

Calcium concentrations ranged from 0.601–0.801mg/100g (Table 1). The calcium content an observed Maximum in the season of post-monsoon (0.801mg/100g) and minimum in monsoon (0.601mg/100g) in wet mussel and average 0.726 mg/100g (Figure 4). Ranjan and Babu [67], recorded 51.03±1.65gm and 909±45.3mg CaCO<sub>3</sub>/gm in *Meretrix meretrix* collected from Bhavanapadu mangroves, North East coastal Andhra Pradesh. High concentrations of Ca was found in the shells of *Achatinaa chatina*, *Turritella* sp., *Cardium edule*, *Ampullela* sp., and *Spondylus spinosus* [68]. Darwin and Padmavathi [69], recorded 912±31.21mg CaCO<sub>3</sub>/g in the shells of *Meretrix meretrix* collected from Tamilnadu coast, India.

The amount of Fe was found between 0.07-0.99mg/100g (Table 1). The Iron (Fe) content was found to be highest in post-monsoon (0.99mg/100g) and the lowest in monsoon season (0.070mg/100g). The average value was estimated 0.083 mg/100g for Fe (Table 1). Zakaria et al. [70], recorded a small amount of Iron in the hard clam shells collected from several stalls in the wet market located in, Selangor and Peninsular Malaysia. Böhlmark [71], reported elevated levels of Fe in *Meretrix meretrix* at Villa do Pescadores, Maputo Bay.

Phosphorus values fluctuated between 0.300-0.794 mg/100g (Table 1). The highest amount (0.794mg/100g) was found in the post-monsoon season while the minimum concentration (0.300mg/100g) was recorded in monsoon in wet mussel (Figure 5). The average 0.455mg/100g of phosphorus was documented in *Meretrix meretrix*. Zakaria et al. [70], reported 0.05% phosphorus in hard clam shells collected from several stalls in the wet market located in Selangor, Peninsular Malaysia.

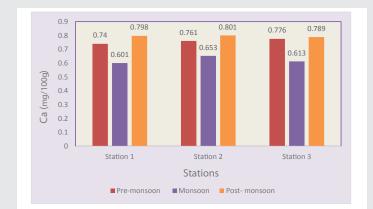
#### Conclusion

*Meretrix meretrix* known as Asiatic hard clam used mainly for marine food and a valuable source of traditional medicine.

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**Figure 4:** Concentrations of calcium (mg/100g) in *Meretrix meretrix* collected from the Bakkhali river estuary.

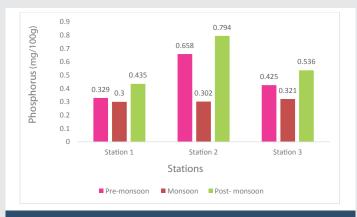


Figure 5: Concentrations of phosphorus (mg/100g) in *Meretrix meretrix* collected from the Bakkhali river estuary.

It is widely distributed in the estuarine and coastal ecosystems of South and Southeast Asia, including China, Korea, Japan, and India. In the present study, *Meretrix meretrix* was recorded in the Bakkhali river estuary. Biochemical compositions were found in good concentrations in *Meretrix meretrix*. The compositions were varied spatiotemporally in the present study. It is important to study further since it is traditionally used as food by the coastal community.

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