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Edible oyster (Saccostera cucullata) from Indian Sundarbons: a study on its variation of nutrients-composition in relation to the microbial load

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Abstract : *Oysters are important delicious and nutritious seafood for coastal people in all over the world, apart from shrimp and crabs. Amount of bacterial load (total bacterial count, total coliform and fecal coliform) and the major nutrients (glycogen and protein) present in oyster (Saccostrea cucullata) tissue were measured in different seasons (pre-monsoon, monsoon and post-monsoon) from three different stations of Indian Sundarbans like Namkhana (S-I), Frazergaunge(S-II) and Sajnekhali(S-III). In pre-monsoon, both protein (6.13% at S-I, 6.44% at S-II, and 6.50% at S-III) and glycogen content (5.66 at S-I, 7.68% at S-II, and 7.55% at S-III) of the oyster found maximum in comparison to other seasons. Again the amount of glycogen and protein content in oyster are inversely varied with its microbial load. Microbial count of the oyster was found maximum in monsoon season (15×10^8, 10×10^8 and 6.6×10^8 cfu gm^{-1} for TBC, 18×10^4, 16×10^4 and 9.1×10^4 MPN $100gm^{-1}$ for TC and 18×10^4, 9.1×10^4 and 3.5×10^4 MPN $100gm^{-1}$ for FC at S-I, S-II and S-III respectively) compared to other season. ANOVA results indicate there is significant (<0.001) difference in bacterial consortia and major nutrients in oyster tissue between months and also between all the three stations. Therefore, oysters collected during pre monsoon are nutritionally more enrich and safe for consumer.*

Keywords *: Saccostrea cucullata. Total bacterial count. Total coliform. Fecal coliform. Glycogen. protein. Premonsoon. Monsoon. Postmonsoon.*

Introduction

Oysters are bivalve soft bodied molluscan shellfish (under class Bivalve), mostly marine or estuarine in habit. The edible oysters comprise of about 100 species are distributed in all

tropical coasts mainly between tidal levels or in shallow water near estuaries. These are good source of essential nutrients like protein, glycogen, fat, calcium, copper, phosphorus, iron, vitamin like A, B, B_{12} and mineral like Na, K, Ca etc (Ponis et al., 2003). Oysters have proved highly amenable to aquaculture and exploitation of wild population that contribute little to worldwide oyster production (FAO 2002). As a sea food, oysters have been introduced and established in food permanently in at least 24 countries (Jennifer et al., 2005). James Hornell (1910) was pioneer in developing oyster culture in India and scientific investigations on it were undertaken in 1970 by the central marine fisheries Research institute at Tuticorin. At present, it is now cultured at Tuticorine, Andhra pradesh, Goa, Cochin backwaters and Sundarbans along the Indian coast.

The present study was conducted in the Indian Sundarbans which is a large mangrove ecosystem with an area of 9630Km^2, is located at the apex of the Bay of Bengal (between 21º40´N to 22º40´N latitude and 88º03´to 89º07´E longitude). The southern part of the maritime state of West Bengal encompasses the mangrove dominated deltaic lobe, which covers the major portion of the north and south 24 Parganas districts. The presence of 34 true mangrove species and some 62 mangrove associate species (Mitra *et al*. 2011) in the entire area makes the home ground of Royal Bengal tiger (*Panthera tigris tigris*) in the planet. The deltaic complex composed of 102 islands, out of which 48 are inhabited and 54 are uninhabited. The flow of the Ganges river through Hooghly estuary in the western sector of Indian Sundarbans to end up at Bay of Bengal has made the geographical situation totally different from the central sector, where five major rivers (Saptamukhi, Thakuran, Matla, Gosaba, and Harinbhanga) have lost their roots due to heavy siltation and solid waste disposal (Mitra et al., 2009). UNESCO has declared the Sundarbans area as the World's Heritage site in 1987 on the basis of its taxonomic diversity and unique gene pool.

It is however, a tragedy that such a rich reservoir of natural resources is encircled by poverty strikers, island dwellers, most of who are living below poverty line. Man-resource conflict is thus inevitable in this zone. In recent times, thrust has been given to the culture of edible oysters, *S.cucullata* as an alternetive livelihood option for the local people (Mitra & Banerjee, 2005) with the aim to divert them from illegal intrusion into the forest area in search of wood, honey, fish, shrimp seeds etc. In recent years Government of India is planning to initiate oyster culture in the deltaic region as alternative livelihood generation scheme, but there are no information about the variation of nutrient in relation to microbial load of oysters.

The Indian Sundarbans is presently under stress due to discharge of untreated sewage and industrial wastes from the highly urbanized and industrialized city of Kolkata, Howrah and

the newly emerging Haldia port- cum- industrial complex. According to UNEP report (1985), 1125 millions lit. of waste water is discharged per day through Hooghly estuary. The lower stretches receive waste and wastewater load of 396 $\times 10^8$ km^3 per hour along with the annual run off 493km^3. The total volume of sewage discharge from the city of Kolkata has been estimated 350 million tons (Mukherjee, 2007). Along with this, a large number of shrimp culture firms situated at the out skirt of the Kolkata city area (like Malancha, Minakhan block etc) discharge huge organic load and trigger the microbial growth. A number of studies revealed that the sources of fecal pollution mainly include municipal sewage system, on-site sewage systems, storm water runoff, marines and boaters, recreationalists, farm animals, pets and wild life (Lipp et al., 2002).

In India, the common oyster species are *Saccostrea cucullata, Crassostrea madrasensis, Crassostrea gryphoides, Crassostrea rivulasis* and *Crassostrea discoidea*. Out of these five dominant species, the first three species are very common in the intertidal zones of the Indian Sundarbans. *S. cucullata* is the most abundant bivalve in the Hoogly estuary and normaly found attached to rock , boulders and several under water structures, submerged branches and trunks of mangroves, concretes, embankments and piles and even on light house bases of Indian Sundarbans ecosystem (Mitra et al., 1995).

In this context, it is extremely important to evaluate the seasonal variation of major nutrients in oyster (*Saccostrea cucullata*). The present paper also highlights the variation of nutrients in relation to microbial load in oyster (*Saccostrea cucullata*) tissue.

Materials and Methods

Study area

The present investigation was carried out for two consecutive years (2011-12) at three different stations(Fig.1) namely Namkhana(S-I), Frazergaunge(S-II) and Sajnekhali (S-III) . The sampling stations were selected considering the magnitude of anthropogenic pressure and degree of water pollution. Station-I [(Namkhana-21º45´48.54´´ (N) and 88º 13´52.55´´(E)] is situated in the western sector of Sundarbans, which is not only an important fish landing station, but also receives directly the wastewater from the Kolkata city and nearby other towns and industrial complex. Station -II [(Frasergaunge-21º36´55.72´´(N), 88º12´33.15´´(E)] is also an official fish landing station of the state of West Bengal, it is comparatively less polluting area than the former one as it receive the discharge of several hotels and tourism units only. Station -III [(Sajnekhali- 22º07´36.21´´ (N) and 88º49´50.60´´ (E)] is situated in the eastern sector of the Indian Sundarbans, which is noted for its wilderness.

Sample collection

Healthy oyster were collected from the intertidal zone of the selected sampling stations and were scrubbed, rinsed with distilled water (several times) and the meat was aseptically extracted using a sterile knife (Cleseri et al.,1998). The tissue were homogenated with PBS (phosphate buffer saline, 0.2M, pH-7.4) and the cell free supernatant after centrifugation (1000g for 30 min at 4ºC) were used for further analysis.

Estimation of protein

Total soluble protein was estimated following Lowry's (1951) method. The supernatant (100 µl) was treated with (5ml) complex forming reagent (2% Na_2CO3: 1% $CuSO_4$, $5H_2O$: 2% Sodium potassium tartarate=100:1:1) followed by addition of folin's reagent (0.5 ml). The optical density was determined at 750 nm using BSA (Bovine serum albumin) as standard.

Estimation of glycogen

200 mg. of each oyster tissue was weighed on a torsion balance and finely ground with 20 ml. of 5% TCA (trichloroacitic acid) in a tissue homogenizer (Potter & Elvehjem 1936).The tissue homogenate was centrifuged and clear supernatant was analyzed for glycogen content. Briefly, 2 ml. of filtrate was added to 3 ml. of Lugol's iodine (1%) reagent. After mixing, the optical density was measured at 650 nm against a blank, which contain 2 ml. of 5%TCA instead of tissue homogenate (Wagtendonk et al.,1946). Commercial glycogen was used for preparation of standard curve and tissue glycogen was expressed as g % (w/w).

Bacteriological Analysis

10 g oyster sample (meat) was blended with 90 ml of sterile 0.5% (w/v) peptone-phosphate buffer (p^H -7.0) and different dilutions (10^{-1} to 10^{-2}) were made. For enumeration of total coliform (TC) and fecal coliform(FC), the standard MPN (Most Probable Number) procedure (Cleseri et al., 1998) was adopted using LTB (lowryl tryptose broth) and EC (Escherichia coli) culture broth, respectively. Briefly, 10 ml of 10^{-1}dilution was added in five test tube containing 10 ml volume of double strength and 1ml of each dilution (10^{-1} and 10^{-2} dilution) was added separately in five test tube containing 10 ml volume of single strength of LTB broth. The total sets were incubated at 35±0.50C for 24h and examined for the presence of growth accompanied by gas production. The MPN was calculated and results were expressed as "presumptive coliform MPN $100g^{-1}$." Then the positive culture was inoculated in to brilliant green lactose bile broth and the tubes were incubated at 35±0.5ºC for 24h and examined for growth with gas production. The MPN of total coliform (TC) was calculated and results were expressed as "confirmed coliform MPN $100g^{-1}$". To enumerate fecal coliform (FC), inocula

from 24 h positive presumptive tubes were aseptically transferred to tubes of EC medium. These tubes were incubated at 44.0±0.5 ºC for 24h and examined for the presence of growth with gas production. Results were expressed as "fecal coliform MPN $100g^{-1}$". The total bacterial count (TBC) in oysters samples was done by standard plate counting method using tryptose glucose beef extract agar (TGBEA) media(Hunt et al.,1984).

Statistical Analysis

Each sample was analyzed in triplicate and data were represented as mean ±SD (standard deviation). The relationship among the parameters was analyzed statistically using analysis of variance (ANOVA) at 5% level. Analysis was done by using SPSS-9.0 software.

Result

Seasonal variation of microbial load in the oyster flesh

It was found that relatively higher levels of total bacterial count (TBC), total coliform (TC) and fecal coliform (FC) in oyster tissue were observed during the monsoon (July-October) season and lowest level during the pre-monsoon (March-June) season. The pattern of variation of the studied indicator microbial groups in oyster tissue was more or less similar among the three different stations. But, collectively the microbial load was much higher in the oyster tissue collected from Namkhana, followed by Frasergaunge and least amount in Sajnekhali (Fig. 2a, 2b and 2c). The quantity of TBC in oyster during monsoon and pre monsoon was recorded in Namkhana as $15x10^8$ cfu g^{-1} and $1.1x10^8$ cfu g^{-1} , in Fresergaunge $10x10^8$cfu g^{-1} and $8x10^7$cfu g^{-1} and in Sajnekhali $6.6x10^8$cfu g^{-1} and $10x10^6$cfu g^{-1} , respectively (Fig .2a).

The maximum density of total coliform (TC) in oyster tissue was recorded in monsoon period as in Namkhana 180000MPN $100g^{-1}$, in Fresergaunge 160000MPN $100g^{-1}$ and in Sajnekhali 91000MPN $100g^{-1}$, respectively (Fig. 2b). During pre monsoon season, a comparatively low amount of TC was enumerated in Namkhana 2400MPN $100g^{-1}$, in Fresergaunge 4140MPN $100g^{-1}$ and in Sajnekhali 2000MPN $100g^{-1}$ (Fig.2b) in the oyster tissue respectively.

In oyster tissue, the fecal coliform (FC) recorded during monsoon and pre monsoon season recorded as in Namkhana 180000MPN $100g^{-1}$ and 2400MPN $100g^{-1}$, in Fresergaunge 91000MPN $100g^{-1}$ and 2140MPN $100g^{-1}$ and in Sajnekhali 35000MPN $100g^{-1}$ and 1400MPN $100g^{-1}$ (Fig. 2c). Analysis of variance (ANOVA) results indicate a significant monthly difference of microbial load (F_{obs}=3.164658>F_{cri} =1.766805 for TBC, F_{obs} = 5.942346>F_{cri}= 1.766805 for TC and F_{obs}=3.522432>F_{cri}=1.766805 for FC) in oyster tissue and also station

base oscillation (F_{obs}=3.485787>F_{cri} =3.199582 for TBC, F_{obs} = 7.690461>F_{cri}= 3.199582 for TC and F_{obs}=5.226358>F_{cri}=3.199582 for FC) was observed (Annexure 3 and 4).

Seasonal variation of major nutrients like glycogen and protein in oyster

There are great variations in the amount of glycogen and protein percentage of oyster in all the sampling station during the study period. The glycogen content in the tissue of oyster was higher in pre monsoon (March-June) in comparison to monsoon (F_{obs} =24.46703>F_{cri} =1.766805of glycogen percentage). Similarly, the protein content reached to its peak during premonsoon months (March-June) (F_{obs} =12.70867>F_{cri} = 1.766 of protein percentage) and minimum during monsoon season.

The range of glycogen contents of *S.cucullata* in S-1, II and III are varied from 0.42% to 5.66%, 0.42% to 7.68% and 0.86% to 7.55% respectively (Fig-3a).

The range of protein contents of *S.cucullata* in S-1, II and III are varied from 1.13% to 6.13%, 2.73% to 6.44% and 2.34% to 6.50% respectively (Fig-3b). ANOVA results also indicate significant differences in protein (F_{ob}s =13.01874>F_{cri}=3.199582 for protein) and glycogen content (F_{obs} =7.382291> F_{cri} =3.199582 for glycogen) of oyster among the three sites (Annexure 1 and 2).

Interrelation between nutritional value and microbial load in the tissue of oyster

The nutrient content (glycogen and protein percentage) and microbial load (TBC, TC and FC) in the tissue of Saccostrea cucullata at the three sampling station exhibited a seasonal and station base oscillation. The glycogen value of edible oysters showed a significant negative correlation ((r = -0.417, p<0.05 at S-I and r = -0.438, p<0.05 at S-III respectively) in the oyster flesh [Except at S-II oysters sample (Table-1)]. The glycogen value exhibited significant negative correlation (r = -0.473, p<0.05 at S-I, r = -0.482, p<0.05 at S-II and r = -0.447, p<0.05 at S-III, respectively) with total coliform in the oyster flesh. The glycogen value also showed significant negative correlation (r = -0.413, p<0.05 at S-I, r = -0.477, p<0.05 at S-II and r = -0.545, p<0.01 at S-III, respectively) with fecal coliform in the oyster flesh (Table-1). The protein value showed an insignificant negative correlation (r = -0.042) with total bacterial count in the oyster flesh from S- II, while it has significant negative correlation with total bacterial count at S-I (r = -0.786, p<0.01) and at S-III (r = -0.531, p<0.01) (Table 1).The protein value exhibited significant negative correlation (r = -0.764, p<0.01 at S-I, r = -0.427, p<0.05 at St-II and r = -0.750, p<0.01 at St-III, respectively) with total coliform in the oyster flesh. The protein value also showed significant negative correlation (r = -0.611, p<0.01 at S-I, r = -0.459, p<0.05 at S-II and r = -0.758, p<0.01 at S-III, respectively) with fecal coliform in the oyster flesh (Table-1).

Discussion

Oyster is an important sea food for the coastal people of India. The whole body of the oyster is consumed either cooked (backed, boiled, steamed and fried) or raw. Apart from their own consumption, they sell the excess into the local market and finally it reached to the cities and big hotel and restaurant, and some portion also exported to the other country. Oysters help to boosts mental energy and also served as mood elevators-they really are a "brain food". The protein in oyster is rich in the amino acid tyrosine, which can convert into mentally energing chemicals through brain. Raw pacific oysters are a good source of protein and would supply about 20% of the daily requirements for an adult instead of milk (Anon 2005). Oysters are now recommended in daily diet allowance for iron and protein. The protein in oysters contains an essential amino acid taurine which may reduce serum cholesterol by altering the activity of certain enzymes that either influence cholesterol metabolism through bile acid synthesis or inhibit its absorption from the intestine (Dragnes & Larsen et al., 2009). Cats are unable to synthesis taurine and therefore need dietary sources of this essential amino acid from oyster protein (Kube & Gerber et al., 2006). Oyster also contains a major nutrient as glycogen which also has bioactive properties. Proteoglycan are heterogeneous group of complex polysaccharides found in oysters that have been shown to have anti-inflammatory properties and have been used in the treatment of osteoarthritis (Volpi & Maccari 2005). The nutritive value of mollusks is governed by the various ecological and environmental parameters in ambience (Ruiz et al.,1992). In the present study, biochemical composition of edible oyster (*S.cucullata*) of Indian sundarbans in relation to seasonal variation and microbial load for the first time has been evaluated. A significant seasonal change ($p<0.001$) in the nutrient value like glycogen and protein in the oyster tissue of three sampling stations was observed. Glycogen and protein content gradually increases and recorded to the highest level during pre monsoon season but sharply declined during monsoon where as the microbial load (TBC, TC and FC) in oyster tissue also significantly ($p<0.001$) varied and their quantity reached maximum during monsoon. This is may be due to stormed water runoff and increased runoff from adjacent landmasses. Actually, in the Sundarbans estuary water budget is regulated by fresh water discharge that bring huge microbial load in the system. The annual fresh water discharge through the estuary accounts for 67200, 16200 and 62100 million ft3 from the main channel of the River Ganga, Damodar and Roopnarayan covering an aggregate of about 11900 km2 of catchment area. Significant fresh water volume during monsoon in the present study area increases the microbial load in the tissue of the oyster due to their filter feeding behavior.

Present study found that the station-wise order of microbial load (TBC, TC and FC) in the tissue of oyster is S-I > S-II > S-III (Fig. 2a, 2b and 2c). This spatial variation may be attributed

to the different degree of anthropogenic stress in three different sampling stations. Namkhana and Frazergaunge, being the fish landing sites are constantly exposed to wastes of complex nature. In addition to decomposed fish products, these sampling stations are also contaminated with wastes released from fishing vessels and trawlers. The wastes generated from the city of Kolkata, Howrah and Haldia industrial belt also find their way to the Bay of Bengal through Namkhana and Frazergaunge (Barua et al., 2011). Sajenkhali, on the other hand is a wildlife sanctuary in the eastern sector of Indian Sundarbans with minimum environmental stress. Similar results were also obtained in coastal South Carolina, where scientists have employed a variety of technique to monitor and compare land uses and ecosystem responses in highly urbanized Murrells Inlet and relatively undeveloped North Inlet (Scott et al., 1996; White et al., 2004). Murrells Inlet also had higher occurrences of E. coli bacteria, fewer coliform free stations, and fewer bacterial species comprising the coliform group-findings that the researchers attributed to urban influences and higher densities of on- site sewage systems in the Murrells Inlet water shed (Booth & Jackson 1997). Subsequent analysis of Murrells Inlet by Kelsey (Kelsey et al., 2004, 2003) identified concentrations of on- site sewage systems, a rain fall events, and runoff from urban areas as key predictors of fecal coliform levels. Therefore, oyster harvested from sajnekhali are nutritionaly high and microbialy safe than other two stations. Present result also revealed that there is a strong correlation exists in between microbial load and nutrient content (table-1). The glycogen content in the oyster tissue reached to the lowest level during monsoon season which could be attributed to richness of microbes in tissue during monsoon than other season because higher level of microflora initiate glycogen breakdown (Feiger & Novak 1996). Similar result also was observed by Miceli *et al* (1993), they observed that the glycogen content of oyster has been shown to be seasonal, with a larger concentration during the summer and fall and lower concentration in early summer and again in the winter. Scott & Lawrence (1982) postulated that at high coliform concentrations (> 100/100ml in water), the oysters would expend considerable energy in particle expulsion through pseudofaeces, thereby making additional meat production difficult. Hence, the observed decline in glycogen in oyster may be attributable during monsoon season compare to other season. Similarly, protein value of the oyster tissue also changes seasonally. Therefore, variation of nutrient in relation to microbial load of this important sea food are more important to convey this information among the rural flock and Govt. sectors. Oyster, being an edible product needs continuous monitoring with respect to coliform load to overcome the barrier of consume.

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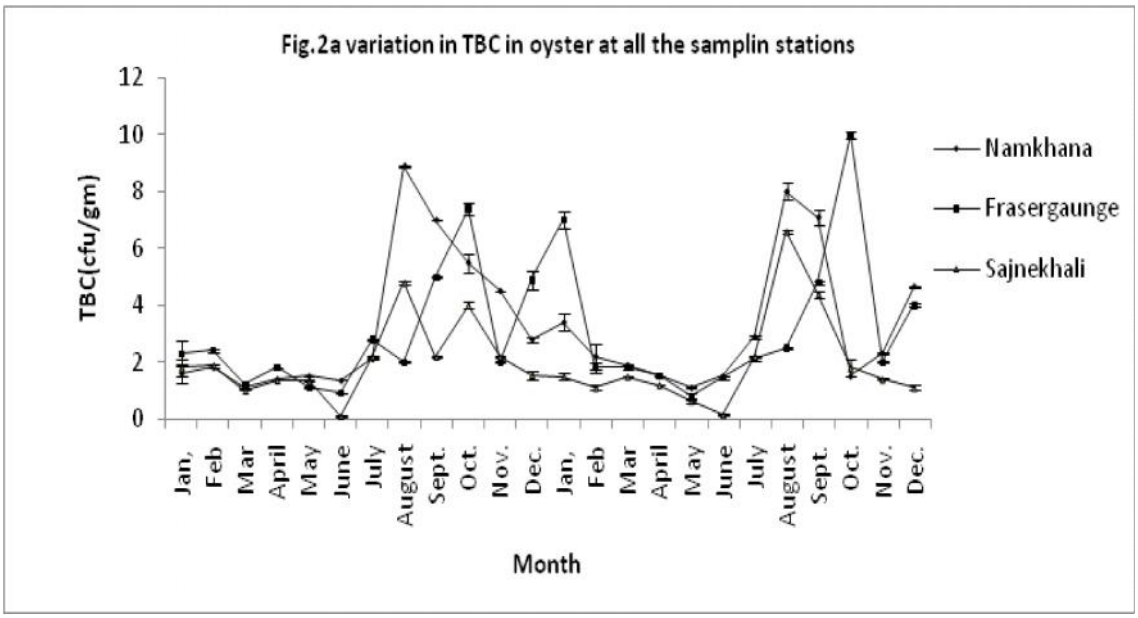


Fig.2a variation in TBC in oyster at all the samplin stations
TBC(cfu/gm)
12
10
8
6
4
2
0
Jan,
Feb
Mar
April
May
June
July
August
Sept.
Oct.
Nov.
Dec.
Jan,
Feb
Mar
April
May
June
July
August
Sept.
Oct.
Nov.
Dec.
Month
Namkhana
Frasergaunge
Sajnekhali

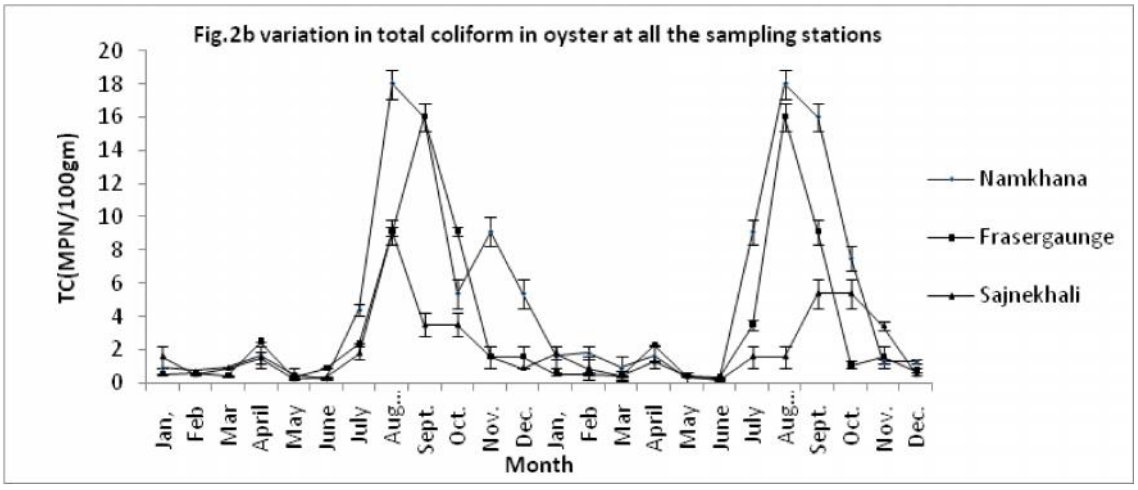


Fig.2b variation in total coliform in oyster at all the sampling stations
TC(MPN/100gm)
20
18
16
14
12
10
8
6
4
2
0
Jan,
Feb
Mar
April
May
June
July
Aug...
Sept.
Oct.
Nov.
Dec.
Jan,
Feb
Mar
April
May
June
July
Aug...
Sept.
Oct.
Nov.
Dec.
Month
Namkhana
Frasergaunge
Sajnekhali

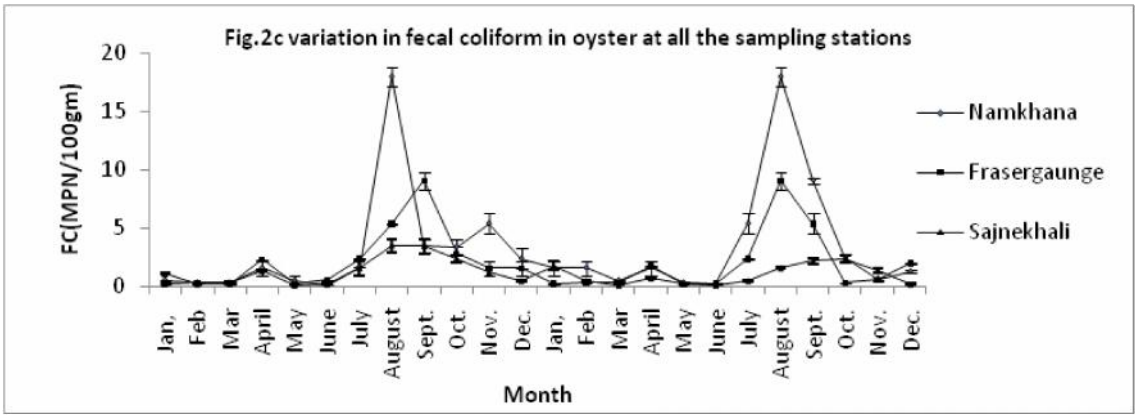
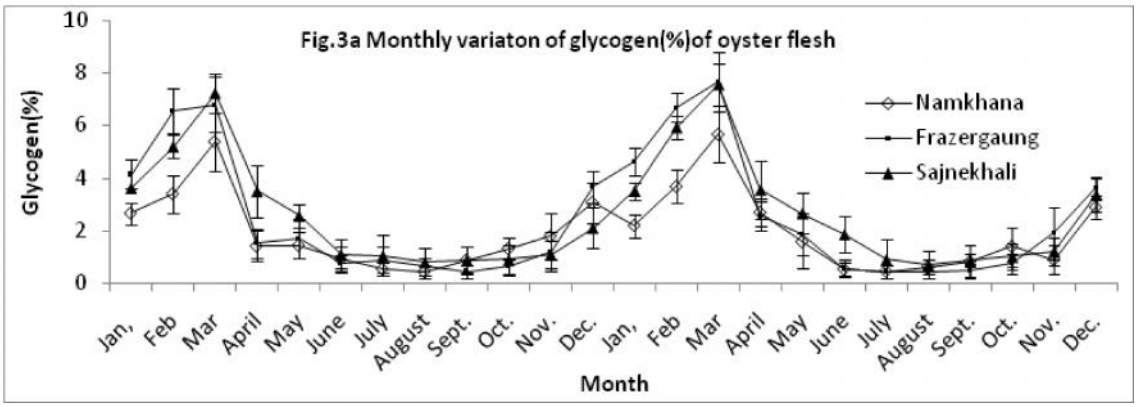


Fig.2c variation in fecal coliform in oyster at all the sampling stations
FC(MPN/100gm)
20
15
10
5
0
Jan,
Feb
Mar
April
May
June
July
August
Sept.
Oct.
Nov.
Dec.
Jan,
Feb
Mar
April
May
June
July
August
Sept.
Oct.
Nov.
Dec.
Month
Namkhana
Frasergaunge
Sajnekhali



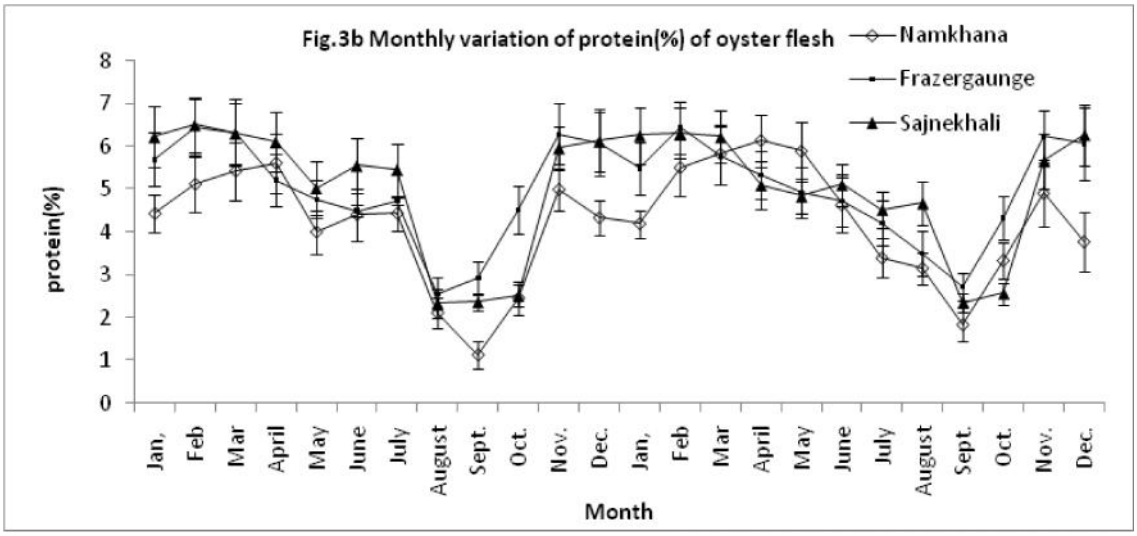


Table 1: Inter-relation between different variables at three different stations viz., Namkhana, Fresergaunge and Sajnekhali.

CHARACTER	NAMKHANA		FRESERGAUNGE		SAJNEKHALI	
	'r'-value	'p'-value	'r'-value	'p'-value	'r'-value	'p'-value
Glycogen of oyster × TBC of oyster	-0.417	<0.05	-0.155	IS	-0.438	<0.05
Glycogen of oyster× TC of oyster	-0.473	<0.05	-0.482	<0.05	-0.447	<0.05
Glycogen of oyster ×FC of oyster	-0.413	<0.05	-0.477	<0.05	-0.545	<0.01
Protein of oyster × TBC of oyster	-0.786	<0.01	-0.042	IS	-0.531	<0.01
Protein of oyster× TC of oyster	-0.764	<0.01	-0.427	<0.05	-0.750	<0.01
Protein of oyster× FC of oyster	-0.611	<0.01	-0.459	<0.05	-0.758	<0.01

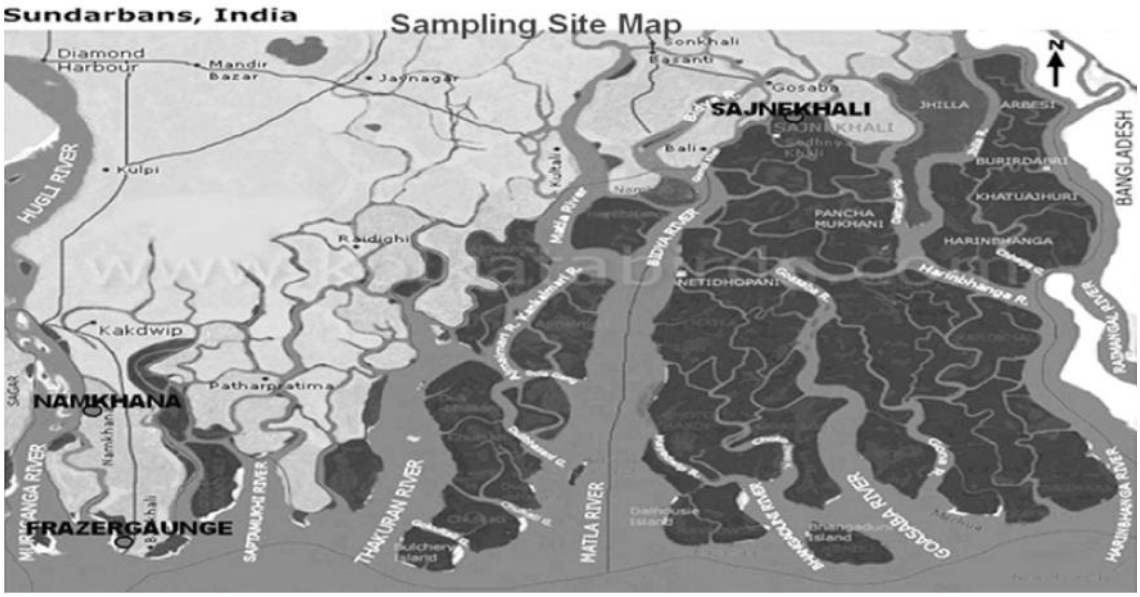


Fig. 1 Map of Indian Sundarbans showing the location of sampling stations. Three stations viz., Namkhana, Frazergaunge and Sajnekhali.

Annexer-1 : Two-way ANOVA analysis of glycogen in oyster flesh between months and stations

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Months	257.3996	23	11.19129	24.46703	1.18E-18	1.766805
Stations	6.75336	2	3.37668	7.382291	0.001658	3.199582
Error	21.04053	46	0.457403			
Total	285.1935	71				

Annexer-2 : Two-way ANOVA analysis of protein in oyster fleshes between months and stations

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Months	103.4667	23	4.498553	12.70867	4.45E-13	1.766805
Stations	9.216623	2	4.608311	13.01874	3.31E-05	3.199582
Error	16.28286	46	0.353975			
Total	128.9662	71				

Annexer-3 : Two-way ANOVA analysis of total bacterial load in flesh between months and stations

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Months	1.76E+18	23	7.65E+16	3.164658	0.000435	1.766805
Stations	1.68E+17	2	8.42E+16	3.485787	0.038944	3.199582
Error	1.11E+18	46	2.42E+16			
Total	3.04E+18	71				

Annexer-4 : Two-way ANOVA analysis of total coliform in oyster flesh between months and stations

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Months	1.12E+11	23	4.86E+09	5.942346	1.53E-07	1.766805
Stations	1.26E+10	2	6.29E+09	7.690461	0.001314	3.199582
Error	3.76E+10	46	8.18E+08			
Total	1.62E+11	71				

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