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Seasonal Variation in Biochemical Composition of Edible Oyster (*Saccostrea cucullata*) from Indian Sundarbans

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Protein, lipid, glycogen, moisture and ash content of the edible oyster species were analysed on monthly basis during 2004 and 2005 from the cultured site at Chotomollakhali in eastern sector of Indian Sundarbans. The culture of edible oyster was initiated in this part of the country in order to provide alternative livelihood to the poverty stricken population in the Indian Sundarbans, which may otherwise, lead to destruction of natural resources of this mangrove ecosystem. Simultaneous monitoring of hydrological parameters (surface water temperature, salinity, pH, nitrate, phosphate and silicate) and phytopigment level of the ambient water was also carried out in this cultured site to investigate the interrelationship between the hydrological parameters and biochemical composition of the oyster tissue. The two year study indicates significant seasonal oscillation of the major biochemical constituents of the oyster.

Key words: Indian Sundarbans, mangrove, phytopigment, *Saccostrea cucullata.*

Indian Sundarbans is one of the most biologically productive and taxonomically diversified, low-lying, mangrove detritus based, open, dynamic, heterogeneous coastal ecotone situated at the apex of the Bay of Bengal (between 21°132 to 22°402 N latitude and 88°032 to 89°072 E longitude). The entire forest of this unique ecosystem acts as a potential reservoir of marine biotic resources. The lower stretch of the estuarine complex and high saline zones are extremely favourable for the survival and growth of edible oyster (Mitra & Banerjee, 2005). *Saccostrea cucullata* is the dominant oyster species in Indian Sundarbans although *Crassostrea gryphoides and Crassostrea madrasensis* are also reported in the basal part of hard substrata (like sluice gates, jetties, pillars of fish landing stations, light house *etc.*). Oyster is a good source of protein, vitamins, minerals and trace elements. Researchers have collected detailed information on biochemical composition of edible oysters, largely because they can be valuable food (Tack *et al.*, 1992; Ruwa & Polk, 1994). However, many internal and environmental factors including pollutants can affect the growth and reproductive success of marine bivalves and can also affect biochemical composition. (Mac Donald & Thompson, 1985; Steelk & Mulcahy, 1999). The present investigation highlights the seasonal variation of biochemical composition (% lipid, % protein, % glycogen, % moisture and % ash) in the edible oyster (*Saccostrea cucullata*) sampled from the eastern sector of Indian Sundarbans with respect to hydrobiological parameters.

Materials and Methods

The entire network of the present programme comprised of the monthly sampling and collection of oysters (*Saccostrea cucullata*) from Chotomollakhali island in the eastern sectors of Indian Sundarbans for a period of two years (2004-2005) along with

simultaneous monitoring of hydrobiological parameters (surface water temperature, salinity, pH, nitrate, phosphate, silicate and phytopigment concentration *i.e.* Chl *a*, Chl *b* and Chl *c*). The different phases of the programme are discussed separately.

Edible oyster species, *Saccostrea cucullata* (Born) were collected from the cultured site of Chotomollakhali island in the eastern sector of Indian Sundarbans. These species are highly variable in shape growing in clusters on rocks, bricks, wooden piles or jetties and also settle on the stems of mangrove plants and on molluscan shells. Twenty samples of almost uniform size (mean length 8.0 cm) were collected at monthly interval from the cultured site during January, 2004 to December, 2005. They were transported live to the laboratory after proper washing and removal of fouling organisms from the outer surface of the shells for further biochemical analysis.

Hydrological parameters around the oyster culture site like surface water temperature, salinity, pH, nitrate, phosphate, silicate and phytopigment concentration of the ambient aquatic phase were analyzed on monthly basis as per the standard methodology outlined in Strickland & Parsons (1968) and APHA (1998). Surface water temperature of the aquatic medium in the sampling station was measured by a Celsius thermometer (scale ranging from 0 to 100°C). The salinity of the surface water was measured by means of refractometer and cross-checked in the laboratory by employing 'Mohr-Knudsen' method as outlined by Strickland & Parsons (1968). The correction factor was found out by titrating the silver nitrate solution against standard seawater (I.A.P.O. standard seawater service, Charlottenland, Slot Denmark, chlorinity = 19.376 ppt.). pH of the ambient water was determined by a portable pH meter (sensitivity = ±0.02).

Surface water for nutrient analysis were collected in clean bottles (Tarsons) and transported to the laboratory in ice-freezed condition. Triplicate samples were collected from the same culture site to maintain the quality of the data. The standard spectrophotometric method of Strickland & Parsons (1968) was adopted to determine the nutrient concentrations in the surface water. Nitrate was analyzed by reducing it to nitrite which was determined by treating the samples with a solution of sulphanilamide and the resultant diazonium ion was coupled with N-(1-napthyl)-ethylene diamine to give an intensely pink azodye. The reduction was then carried out by treating the sample with ammonium chloride and passing it through a glass column packed with amalgamated cadmium fillings. The determination of phosphate was carried out by treatment of an aliquot of the sample with acidic molybdate reagent containing ascorbic acid and a small quantity of potassium antimony tartarate. Dissolved silicate was determined by treating the sample with acidic molybdate reagent. The resultant silico-molybdic acid was reduced to molybdenum blue complex by ascorbic acid and incorporating oxalic acid prevented formation of similar blue complex by phosphate. Systronics Digital Spectrophotometer (Type-16S) was used for nutrient (nitrate, phosphate and silicate) analysis at their respective wavelengths.

Phytopigment concentration of the ambient aquatic phase was analyzed in order to monitor the food reservoir of the cultured species. For pigment analysis, 1 litre of surface water collected in black bottles from sampling station was filtered through a 0.45 μm Millipore membrane fitted with a vacuum pump. The filter paper was transferred to a homogenizer containing acetone. The contents were ground thoroughly and

placed in refrigerator for 24 hours in order to facilitate the complete extraction of phytopigment. Finally the chlorophyll density was estimated spectrophotometrically (Shimadsu UV 2100) as per the procedure of Jeffrey & Humphrey (1975).

The biochemical analyses were done on tissue samples pooled from 10 individual oysters. The samples (average length of 8.0 cm) were collected from the cultured site at monthly intervals. They were washed with double-distilled water and processed for biochemical analysis.

Total soluble protein was estimated using Lowry's (1951) method. The assay used 50 mg of the dried sample homogenized in 10 ml phosphate buffer followed by collection of supernatant after centrifuge. The supernatant was treated with complex forming reagent (2% Na_2CO_3 : 1% $CuSO_4$, $5H_2O$: 2% sodium potassium tartrate = 100 : 1 : 1) followed by addition of Folin's reagent. The optical density was determined at 750 nm using a spectrophotometer (Systronics Digital Spectrophotometer; Type-16S). BSA (Bovine Serum Albumin) was used as standard for the preparation of calibration curve.

Gravimetric method of Barnes & Blackstock (1973) was used for the estimation of lipid concentration. 0.5 g sample was homogenized in 5 ml of double-distilled water and allowed to stand for overnight in the refrigerator. For the gravimetric determination of lipid, aliquots of the homogenate were extracted in 5 ml of 1:2 (v/v) methanol:chloroform (Folch *et al.*, 1957). Lipid residues were weighed using a Mettler AB 204-S microbalance after evaporation of the chloroform using liquid nitrogen.

Methodology of Hewitt (1958) was used for the determination of glycogen content. 100 mg sample was homogenized in 5 ml sulphuric acid reagent and extracted overnight at 5°C. Then the homogenate solution was centrifuged and subdivided into two portions. One portion was incubated in a water-bath at 95°C for 4 hours and the other portion was stored at 5°C. The optical density of both portions was determined at 340 nm using a spectrophotometer (Systronics Digital Spectrophotometer; Type-16S). D-glucose was used as standard solution for the preparation of calibration curve.

The excess water of the oyster tissue was soaked using a Whatman filter paper (No.1). The meat was then homogenized by a tissue homogenizer. A part of the homogenized tissue was oven dried at 100°C to determine the moisture content. The ash content in oyster tissue was determined by placing the oyster sample in the Muffle Furnace overnight at 400°C. The ash was then weighed and expressed in percentage.

Results and Discussion

In Indian Sundarbans region, edible oyster (*Saccostrea cucullata*) exhibit a unique seasonal cycle with respect to their biology and biochemical composition.

The physico-chemical variables showed significant seasonal variations in the sampling station during the study period. Surface water temperature was high during pre-monsoon (March to June) and monsoon (July to October) and low during post monsoon (November to February). The surface water salinity and pH were highest in the season of pre-monsoon and lowest in monsoon that might be due to excessive evaporation in pre-monsoon and heavy precipitation and subsequent discharge of freshwater run-off from the adjacent city of Kolkata in monsoon. The quantum of discharge in the form of sewage also increases the nutrient load in the aquatic

Table 1. Hydrobiological parameters of the selected sampling station during Jan '04 to Dec '05.

Month	Salinity (‰)	pH	Water Temp. (°C)	Chl *a* (mg/m³)	Chl *b* (mg/m³)	Chl *c* (mg/m³)	NO_3 (µg at/l)	PO_4 (µg at/l)	SiO_3 (µg at/l)
Jan (2004)	21.44	7.68	28.6	2.22	1.35	1.42	21.14	2.12	63.18
Feb	21.69	7.80	29.8	2.53	1.47	1.55	18.63	2.08	49.22
Mar	22.37	7.82	29.9	2.65	1.66	1.70	16.82	1.54	51.49
April	22.99	7.84	32.8	2.88	1.71	1.84	15.03	1.38	46.15
May	25.86	7.86	33.3	2.91	1.66	1.73	12.44	1.21	46.30
June	27.04	8.00	33.7	3.02	1.78	1.85	10.52	1.08	38.66
July	19.45	7.79	34.1	2.37	1.55	1.62	19.65	2.14	52.20
Aug	16.98	7.40	33.6	2.29	1.46	1.51	23.71	2.48	57.15
Sept	13.72	7.36	33.0	1.79	1.27	1.34	25.82	2.50	60.30
Oct	17.88	7.60	32.1	2.72	1.33	1.49	20.99	2.36	52.37
Nov	19.59	7.62	30.8	2.61	1.69	1.80	17.16	2.22	46.14
Dec (2004)	20.36	7.65	27.2	2.89	1.48	1.65	22.05	2.09	40.24
Jan (2005)	22.30	7.67	28.7	2.09	1.23	1.37	19.65	2.19	55.72
Feb	23.18	7.76	29.7	2.42	1.36	1.48	18.70	2.10	53.00
Mar	23.17	7.79	29.9	2.55	1.53	1.61	18.56	1.67	48.06
April	24.46	7.80	32.9	2.79	1.68	1.77	17.11	1.56	43.21
May	24.80	7.87	33.4	2.80	1.70	1.80	14.09	1.33	37.89
June	26.17	8.01	34.0	2.92	1.77	1.82	13.46	1.27	35.01
July	20.19	7.77	34.2	2.29	1.44	1.50	19.05	2.29	57.99
Aug	18.76	7.40	33.8	2.20	1.30	1.42	24.15	2.50	60.03
Sept	14.50	7.36	33.3	1.50	1.16	1.30	27.38	2.61	71.47
Oct	18.71	7.55	32.9	2.64	1.20	1.29	23.39	2.45	62.83
Nov	21.60	7.60	30.9	2.50	1.38	1.41	19.42	2.39	58.20
Dec (2005)	21.42	7.64	27.4	2.33	1.40	1.48	17.89	2.31	52.76

phase during monsoon (Table 1). Report states that, 1125 millions lit. of wastewater is discharged per day through Hooghly estuary. The lower stretch receives waste and wastewater load of 396×10^8 km³ per hour along with the annual run-off of 493 km³. The total volume of sewage discharge from the environment of Kolkata has been estimated to be 350 mt. (Anon, 2003). The minimum nutrient load in the water of Indian Sundarbans during pre-monsoon may be attributed to their uptake by the phytoplankton community that propagates in March/April in the present geographical locale. The average composition of phytoplankton is $(CH_2O)_{108}(NH_3)_{16}H_3PO_4$ and in case of siliceous diatom it is slightly modified as $(CH_2O)_{108}(NH_3)_{16}H_3PO_4(SiO_4)_{40}$ as stated by (Riley & Chester, 1971), which justifies the incorporation of nutrients in cell system of phytoplankton leading to the reduction in nutrient concentration of ambient water.

The biochemical composition of the oyster tissue showed significant seasonal variation of protein (5.49% to 11.87%), lipid (6.11% to 10.76%), glycogen (1.02% to 7.30%),

Table 2. Biochemical composition (in %) of edible oyster (*Saccostrea cucullata*) collected from the selected sampling station during Jan '04 to Dec '05.

Month	Protein	Lipid	Glycogen	Moisture	Ash
Jan (2004)	11.60	8.49	2.29	74.60	3.02
Feb	11.87	9.92	1.90	74.70	1.61
Mar	10.31	9.75	2.63	75.10	2.21
April	8.12	8.93	4.59	75.80	2.56
May	8.47	9.15	6.28	75.90	0.20
June	5.73	10.09	7.30	76.20	0.68
July	10.58	7.95	2.15	76.80	2.52
Aug	10.16	8.10	1.82	77.10	2.82
Sept	10.39	6.43	3.20	77.00	2.98
Oct	10.09	6.92	4.15	76.50	2.34
Nov	10.29	6.33	5.42	76.30	1.66
Dec (2004)	9.49	6.51	5.95	75.40	2.65
Jan (2005)	11.15	10.11	2.42	75.10	1.22
Feb	11.01	10.01	1.86	74.80	2.32
Mar	10.36	10.32	2.59	75.10	1.63
April	8.00	9.12	4.72	75.30	2.86
May	8.33	8.80	6.95	74.83	1.09
June	5.49	10.76	7.23	76.10	0.42
July	10.46	9.03	1.02	76.40	3.09
Aug	10.21	8.98	1.99	76.80	2.02
Sept	10.43	7.12	3.01	76.70	2.74
Oct	10.46	6.57	4.56	76.40	2.01
Nov	10.13	6.11	5.55	76.20	2.01
Dec (2005)	9.65	6.11	5.92	76.30	2.02

moisture (74.60% to 77.10%) and ash (0.20% to 3.09%) content during the two years study period (Table 2).

The biochemical composition of oysters showed significant seasonal variation. It was seen that reproductive cycle greatly influences the protein-lipid-glycogen content of the tissue. Protein value reached maximum in the months of January-February *i.e.* in the pre-spawning period. In the month of June and April-May it showed minimum value *i.e.* immediately after spawning, the protein value sharply decreased. During other times of the year, protein showed average value of 10.22%. This was confirmed by significant negative correlation values between salinity and pH ($r_{\text{salinity x protein}}$ = -0.59349, $p<0.01$ and $r_{\text{pH x protein}}$ = -0.56255, $p<0.01$). Significant positive correlation between increased value of protein level with increase of nutrients ($r_{\text{nitrate x protein}}$ = 0.66204, $p<0.01$; $r_{\text{phosphate x protein}}$ = 0.73621, $p<0.01$; $r_{\text{silicate x protein}}$ = 0.70513, $p<0.01$) also proves that more amino acids are synthesized as more NO_3-N is being absorbed by the oysters (Table 3).

Considerable seasonal variations of glycogen and lipid content in oyster tissues

were also observed during the study period. There were significant positive correlations at 5% level ($r_{salinity \times glycogen}$ = 0.49416, $p<0.05$) of glycogen level in the oyster tissue with seawater salinity, which confirms that glycogen content in oyster hiked up before spawning during the period of high salinity (Table 3). No significant correlations were found in case of water temperature and pH.

In the post-spawning phase (June) when lipid and glycogen content reached maximum value, protein content exhibited minimum value indicating possible interconversion between them. This was confirmed by significant negative correlation between Chl *a*, Chl *b* and Chl *c* with protein ($r_{chl\ a \times protein}$ = -0.61533, $p<0.01$; $r_{chl\ b \times protein}$ = -0.70343, $p<0.01$; $r_{chl\ c \times protein}$ = -0.70514, $p<0.01$) and significant positive correlation between Chl *a*, Chl *b* and Chl *c* with glycogen ($r_{chl\ a \times glycogen}$ = 0.62197, $p<0.01$; $r_{chl\ b \times glycogen}$ = 0.52870, $p<0.05$; $r_{chl\ c \times glycogen}$ = 0.55211, $p<0.01$). Significant negative correlation between glycogen and nutrients may be possibly due to the transformation of glucose to glycogen. As carbohydrates are recognized as the major energy source in bivalves, (Gabbott, 1975) lower value of lipid concentration during pre-spawning period indicates possible mobilization of lipid towards glycogen to provide energy necessary for the spawning process. Significant positive correlation values between lipid with salinity and pH ($r_{salinity \times lipid}$ = 0.64504, $p<0.01$; $r_{pH \times lipid}$ = 0.64288, $p<0.01$) also conforms the increase of lipid level during post spawning period. Significant negative correlation between nutrient (NO_3 & PO_4) and lipid level ($r_{nitrate \times lipid}$ = -0.51341, $p<0.05$; $r_{phosphate \times lipid}$ = -0.62491, $p<0.01$) also confirms that there is inverse relationship between increase of nutrient and lipid level.

Salinity and pH has a negative role with moisture and ash content of the oyster tissue

Table 3. Inter-relationship between the relevant hydrobiological parameters and biochemical composition of edible oyster (*Saccostrea cucullata*) in the selected sampling station during Jan '04 to Dec '05.

combination	r-value	p-value
Salinity × protein	-0.59349	<0.01
pH × protein	-0.56255	<0.01
Water temp. × protein	-0.42872	IS
Chl *a* × protein	-0.61533	<0.01
Chl *b* × protein	-0.70343	<0.01
Chl *c* × protein	-0.70514	<0.01
NO_3 × protein	0.66204	<0.01
PO_4 × protein	0.73621	<0.01
SiO_3 × protein	0.70513	<0.01
Salinity × glycogen	0.49416	<0.05
pH × glycogen	0.40240	IS
Water temp. × glycogen	0.05704	IS
Chl *a* × glycogen	0.62197	<0.01
Chl *b* × glycogen	0.52870	<0.05
Chl *c* × glycogen	0.55211	<0.01
NO_3 × glycogen	-0.57905	<0.01
PO_4 × glycogen	-0.54597	<0.05
SiO_3 × glycogen	-0.64550	<0.01
Salinity × lipid	0.64504	<0.01
pH × lipid	0.64288	<0.01
Water temp. × lipid	0.13326	IS
Chl *a* × lipid	0.27067	IS
Chl *b* × lipid	0.41367	IS
Chl *c* × lipid	0.39518	IS
NO_3 × lipid	-0.51341	<0.05
PO_4 × lipid	-0.62491	<0.01
SiO_3 × lipid	-0.38423	IS
Salinity × moisture	-0.57976	<0.01
pH × moisture	-0.52594	<0.05
Water temp. × moisture	0.56999	<0.01
Chl *a* × moisture	-0.30426	IS
Chl *b* × moisture	-0.21510	IS
Chl *c* × moisture	-0.24338	IS
NO_3 × moisture	-0.37683	IS
PO_4 × moisture	0.43740	IS
SiO_3 × moisture	0.33197	IS
Salinity × ash	-0.66357	<0.01
pH × ash	-0.55235	<0.01
Water temp. × ash	-0.09356	IS
Chl *a* × ash	-0.49678	IS
Chl *b* × ash	-0.45275	IS
Chl *c* × ash	-0.44405	IS
NO_3 × ash	0.68315	<0.01
PO_4 × ash	0.60846	<0.01
SiO_3 × ash	0.56185	<0.01

IS = insignificant

which is evident from the significant negative correlation values ($r_{salinity \times moisture}$ = -0.57976, $p<0.01$; $r_{pH \times moisture}$ = -0.52594, $p<0.05$; $r_{salinity \times ash}$ = -0.66357, $p<0.01$; $r_{pH \times ash}$ = -0.55235, $p<0.01$).

The present study reveals that biochemical composition of edible oyster (*Saccostrea cucullata*) in Indian Sundarbans is controlled by the seasonal influence of hydrobiological parameters, which also governs the reproductive cycle in oysters. A long term monitoring of relevant hydrobiological parameters is therefore needed to explore the environmental potential of the region to develop large-scale oyster culture practice.

Sundarbans, being the only mangrove dominated tiger land in India is presently under severe stress due to natural calamities, erosion, shrimp culture related problems and lack of proper planning in resource management. Although the region is flooded with several species of seaweeds, edible molluscs and several organisms with biomedical values, hardly no initiative has been taken to link these untapped biological resources with the economics of the state. Under such circumstances, promotion of edible oyster culture may be an alternative livelihood scheme for the local population.

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