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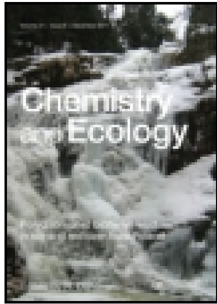

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Variation of antioxidant biomarkers in the edible oyster Saccostrea cucullata collected from three different water bodies of Sundarbans

Harekrishna Jana[a], Keshab Chandra Mondal[a], Chiranjit Maity[a], Kuntal Ghosh[a], Abhijit Mitra[b], Kakoli Banerjee[c], Sankar Dey[d] & Bikas Ranjan Pati[a]

[a] Department of Microbiology, Vidyasagar University, Midnapore, India

[b] Department of Marine Science, University of Calcutta, Kolkata, India

[c] School for Biodiversity and Conservation of Natural Resources, Central University of Orissa, Koraput, India

[d] Department of Physiology, Santal Bidraha Sardha Satabarsiki Mahavidyalaya, Goaltore, India
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Variation of antioxidant biomarkers in the edible oyster *Saccostrea cucullata* collected from three different water bodies of Sundarbans

Harekrishna Jana[a], Keshab Chandra Mondal[a]*, Chiranjit Maity[a], Kuntal Ghosh[a], Abhijit Mitra[b], Kakoli Banerjee[c], Sankar Dey[d] and Bikas Ranjan Pati[a]

[a]*Department of Microbiology, Vidyasagar University, Midnapore, India;* [b]*Department of Marine Science, University of Calcutta, Kolkata, India;* [c]*School for Biodiversity and Conservation of Natural Resources, Central University of Orissa, Koraput, India;* [d]*Department of Physiology, Santal Bidraha Sardha Satabarsiki Mahavidyalaya, Goaltore, India*

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Variations of antioxidant biomarkers such as catalase (CAT), superoxide dismutase (SOD) activities and lipid peroxidation (LPO) were studied in edible part of mangrove oyster *Saccostrea cucullata,* collected from three different water bodies, such as Namkhana (S-I), Frazergaunge (S-II) and Sajnekhali (S-III) of Indian Sundarbans which are exposed to different degrees of anthropogenic activity. The study was conducted for consecutive two years (2010 and 2011) in the respective water bodies. Characteristics of biomarkers in oyster from the two polluted water bodies, i.e. S-I and S-II, are similar in nature in comparison to less polluted site (S-III). The catalase, superoxide dismutase activity and lipid peroxidation in oyster flesh exhibit significant ($p < 0.01$) spatial and temporal variation among the three stations. Oysters from S-I were significantly higher ($p < 0.05$) in antioxidant enzyme activities than oysters from S-III, which differ in their amount of pollution sources. Maximum antioxidant enzymes activity of all collected samples were recorded in pre-monsoon time and decreased in monsoon season. But maximum lipid peroxidation was noted during monsoon followed by post-monsoon and pre-monsoon.

Keywords: antioxidant biomarkers; catalase; Indian Sundarbans; lipid peroxidation; *Saccostrea cucullata*; superoxide dismutase

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1. Introduction

The Indian Sundarbans biosphere reserve with an area of 9630 km^2 is located at the apex of the Bay of Bengal (between 21°40′N to 22°40′N latitude and 88°03′to 89°07′E longitude). The deltaic complex is composed of 102 islands, out of which 48 are inhabited and 54 are uninhabited. The western sector of the deltaic lobs receives the snowmelt water of Himalayan glaciers, after it has been regulated through several barrages on the way. It also receives wastes and effluent of a complex nature from multifarious industries concentrated mainly in the upstream zone. The eastern sector on the other hand is fully deprived from such a supply owing to heavy siltation and clogging of the Bidyadhari channel since the late fifteenth century.[1] According to a UNEP report, 1125 million litres of waste water are discharged per day through the Hooghly estuary. The lower stretches receive waste and wastewater load of 396×10^8 km^3 per hour along with the

*Corresponding author. Email: mondalkc@gmail.com

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annual run off 493 km^3. The total volume of sewage discharge from the city of Kolkata has been estimated at 350 million tons.[1,2]

The Gangetic delta, at the apex of Bay of Bengal is recognised as one of the most diversified and productive ecosystem of the tropics. The deltaic lobe is unique for its wilderness, mangrove gene pool and tiger habitat. However, owing to intense industrial activities in the upstream zone, and several anthropogenic factors, the western part of the deltaic complex is exposed to pollution from domestic sewage and industrial effluent, which has a serious impact on the biota.[3]

Pollution causes an imbalance of the redox-status, damages in biomolecules such as DNA, lipids and proteins, as well as being responsible for mutagenicity and carcinogenicity.[4] Detection of the sub-lethal effect of pollutants on marine and estuarine organisms at population and community levels of biological organisation often becomes problematic.[5] This has led to suggestions that changes in various physiological and biochemical parameters at the individual species level of sentinel organisms may be useful for identifying and delineating the impact of pollutants, thereby, developing the concept of biomarkers.[6] Biochemical biomarkers can be broadly classified into two categories: one that measures only exposure to a pollutant and another that measures both exposure and toxic effect.[7] The antioxidant defenses include both enzymatic (e.g., catalase, glutathione peroxidase and superoxide dismutase) and non-enzymatic [8] ones (e.g., reduced glutathione, ascorbic acid, uric acid and vitamin E).

Environmental pollution monitoring studies employing biochemical biomarkers in bivalves such as oyster and mussels have been widely reported in literature.[9] The mangrove oyster *Saccostrea Cucullata* (Bivalvia: Ostreidae) is a euryhaline species predominantly distributed in east to west Sundarbans. Like other mollusc bivalves, *Saccostrea cucullata* has important characteristics as a sentinel species in pollution biomonitoring. Only a few studies have focused on the seasonal change in biomarker with respect to PAH (polycyclic aromatic hydrocarbons) concentration [5] in oyster tissue collected from Sundarbans area, but no study have been done on the temporal and spatial variation of biomarker responses with respect to varying levels of pollution in Sundarban areas.

In light of the above, the aim of the present study was to evaluate the response of several biochemical biomarkers such as catalase, superoxide dismutase and lipid peroxidation in the mangrove oyster, *Saccostrea cucullata* collected from three different water bodies with varying degrees of anthropogenic pollution.

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2. Material and methods

2.1. *Sampling sites*

The present investigation was carried for consecutive two years (2010–2011) in the three different stations - Namkhana, Frazergaunge and Sajnekhali of Indian Sundarbans - which are exposed to varying degrees of anthropogenic pollution (Figure 1). Station-I {(Namkhana − 21°45′48.54″ (N) and 88°13′52.55″(E)} is situated in the western sector of Sundarbans, where a high concentration of heavy metals was observed in water as well as in the oyster.[10] Station-II {(Frazergaunge − 21°36′55.72″(N), 88°12′33.15″(E)} is located further from pollution sources than station I. Station-III {(Sajnekhali − 22°07′36.21″ (N) and 88°49′50.60″(E)} is situated in the eastern sector of Indian Sundarbans, which is considered to be relatively low in sources of pollution.[10]

2.2. *Sampling protocol*

Six healthy oysters (4–5 cm) were collected in each month from the intertidal zone of the each selected sampling stations. Each oyster was scrubbed, rinsed with distilled water (several times)

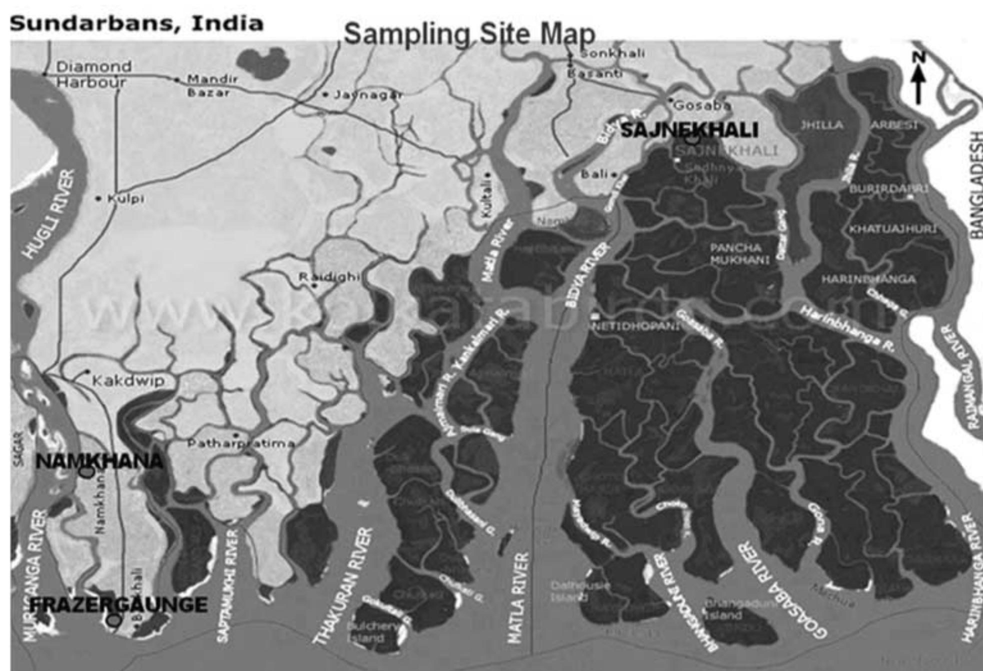

Figure 1. Map of Indian Sundarbans showing the location of sampling stations. Namkhana (S-I), Frazergaunge (S-II) are located in the western sector and Sajnekhali (S-III) in the eastern sector of Indian Sundarbans.

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and stored in a container, preserved in crushed ice and brought to the laboratory. The meat was aseptically extracted using a sterile knife and immediately used for the biochemical analysis of oyster flesh.[11]

2.3. *Biochemical analysis*

2.3.1. *Assay of catalase (CAT) activity*

Catalase activity (CAT) was measured by a spectrophotometric method [12] at $25°C \pm 0.5°$C. In this method, the oyster's tissues were homogenised in ice cold 50 mM phosphate buffer solution (pH−7.0). The homogenates were centrifuged at 10,000 *g* at 4°C for 10 min. The reaction mixture contained 50 mM ice cold phosphate buffer (pH−7.0), 50 mM H_2O_2 and supernatant of tissue homogenate. The reaction rate was determined by measuring the decrease of H_2O_2 concentration at 240 nm and the subsequent six reading were noted at 30 seconds interval against a blank, containing 2 mL phosphate buffers. The unit of catalase activity is (unit/mg tissue).

2.3.2. *Assay of superoxide dismutase (SOD) activity*

Oyster tissues were homogenised in ice-cold 100 mM tris- cocodylate buffer (Loba chem., India) to give a tissue concentration of 50 mg/mL and centrifuged at 10,000 *g* for 20 min at 4°C. The SOD activity of these supernatant was estimated by measuring the percentage of inhibition of the pyrogallol (HIMEDIA, India) autooxidation by SOD.[13] The buffer was 50 mM tris (pH−8.2) containing, 50 mM cocodylic acid (PH-8.2), 1 mM ethylene diamine tetra acetic acid (EDTA-HIMEDIA, India) and 10 mM hydrochloric acid (HCl). The reaction mixtures containing 2 mL of buffer, 20 μl of 10 mM pyrogallol and 20 μl of supernatant were taken in a spectrophotometric

cuvette and the absorbance was noted at 420 nm for 3 minutes against a blank containing 2.04 mL buffer. One unit of SOD was defined as the enzyme activity that inhibited the autooxidation of pyrogallol by 50%. The unit of superoxide dismutase activity is expressed by (unit/mg tissue).

2.3.3. *Measurement of lipid peroxidation from the level of Malondialdehyde (MDA)*

The oyster tissues were homogenised separately at the tissue concentration of 50 mg/mL in 0.1 M of ice-cold phosphate buffer (pH-7.4) and the homogenates were centrifuged at 10,000 *g* at 4°C for 5 min. Supernatants were used for the estimation of MDA. For the measurement of MDA, 0.5 mL homogenate was mixed separately with 0.5 mL normal saline(0.9 g% Nacl) and 2 mL of TBA-TCA mixture(0.392 g of TBA in 75 mL of 0.25 N Hcl with 15 g of TCA, the volume of the mixture was made up to 100 mL by 95% ethanol) and boiled at 100°C for 10 min. The mixture was then cooled at room temperature and centrifuged at 4000 *g* for 10 min. The whole supernatant was transferred in spectrophotometer cuvette and absorbance was read at 535 nm against a blank, containing 2 mL TBA mixture. The MDA in the sample was calculated by using the extinction coefficient of 1.56×10^5 M/cm and lipid peroxidation was measured in the supernatant fraction as malondialdehyde equivalent using thiobarbuteric acid.[14] The unit of lipid peroxidation is expressed by (*n* moles of malondialdehyde formed/mg tissue).

2.4. *Physico- chemical parameters*

All physico- chemical parameters were assessed during each sampling. Surface water temperature was measured with a Celsius thermometer (ranging from 0 to 100°C). The surface water salinity around the sampling stations was estimated by employing the 'Mohr-Knudsen method'.[15] The correction factor was determined by titrating silver nitrate solution against standard seawater [I.A.P.O. standard sea- water service, charlottenlund, Stot, Denmark (Salinity 19.376)]. The pHs of the water of all stations were determined on the spot by a portable pH metre (sensitivity = ±0.02 units).

2.5. *Statistical analysis*

To assess whether biomarker responses varied between sites and time of sampling a t factor ANOVA was performed, with factor site (3 level: S-I, S-II and S-III) and month (24 levels). Possibilities less than 0.01 ($p < 0.01$) were consider statistically significant. Post-Hock test was analysed by SIGMA PLOT. Analysis was done by using SPSS-10.0.

3. Results and discussion

3.1. *Pollution- related changes in biomarkers*

Pollution-related responses of antioxidant biomarkers such as CAT, SOD and LPO were noticed in oyster tissue collected from three different stations of Indian Sundarbans. The catalase activity (unit/mg tissue) of the oyster flesh ranged from 1025 ± 222 U to 15500 ± 537 U during 2010 and 986.26 ± 215 U to 15715.3 ± 500 U during 2011 at station-I, 712.21 ± 202 U to 15005.5 ± 525 U during 2010 and 786.26 ± 212 U to 15000.5 ± 501 U during 2011 at station-II, and 402.12 ± 212 U to 11800.4 ± 501 U during 2010 and 436.48 ± 211 U to 11948.5 ± 500 U during 2011 at station-III as shown in Figure 2(a). Significant ($p < 0.01$, Table 1) differences in catalase activity in oyster flesh between all the three stations were observed. The station-wise order of catalase

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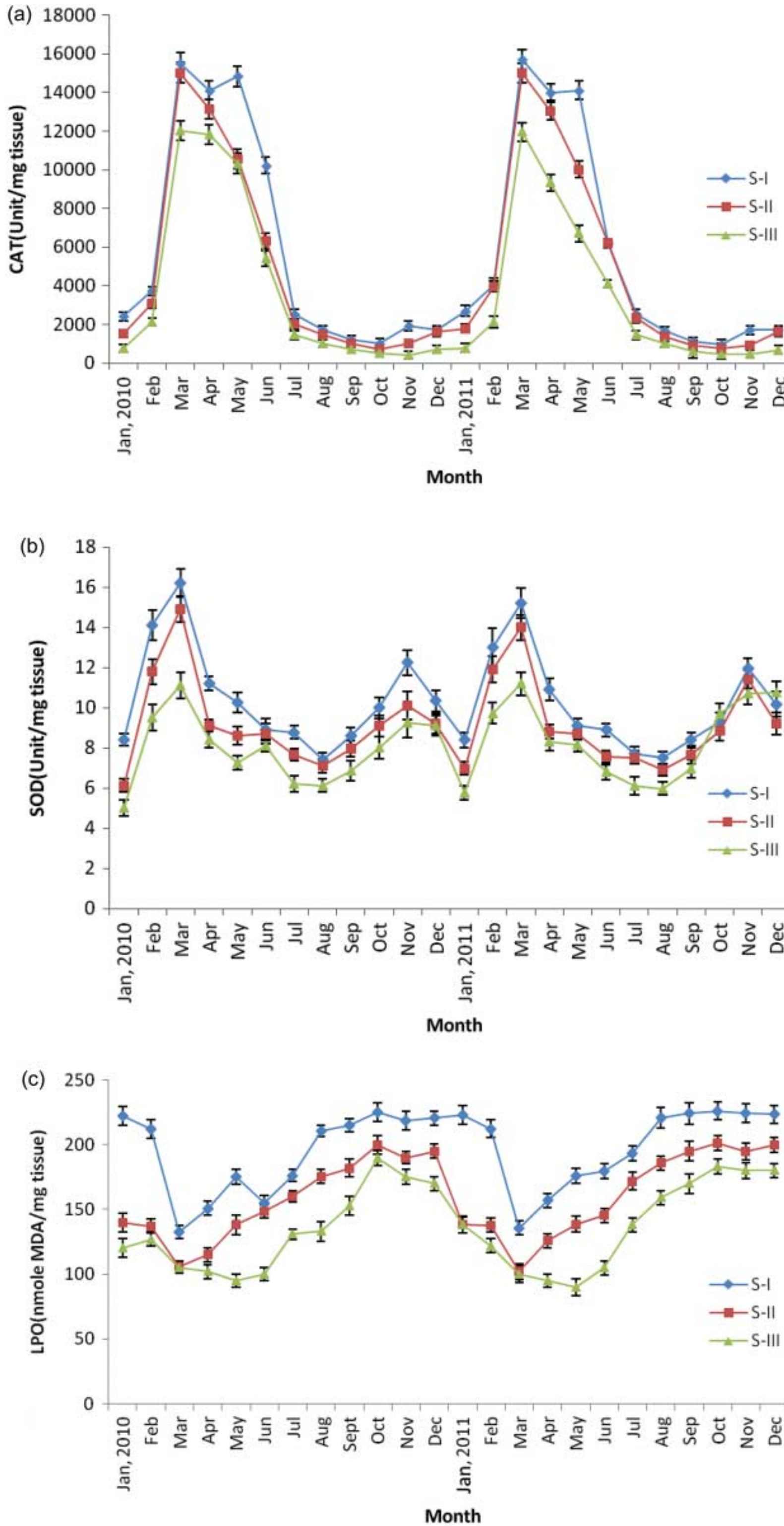

Figure 2. Monthly variations in Catalase activity (a), Superoxide dismutase (b) and lipid peroxidation (c) at all the stations in oyster tissue during 2010–2011.

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Table 1. Two-way ANOVA analysis of catalase activity of oysters between months and stations.

Source of Variation	SS	df	MS	F	*P*-value	F crit
Months	1.69E + 09	23	73652877	73.01198	7.81E-29	1.766805
Stations	50808172	2	25404086	25.18303	4.1E-08	3.199582
Error	46403788	46	1008778			
Total	1.79E + 09	71				

Table 2. Post- hoc test by Tukey method for catalase, superoxide dismutase and lipid peroxidation.

All pairwise multiple comparison procedures for catalase (Tukey test):			
Comparison	**Diff of Ranks**	***q***	***P* < 0.05**
S-I vs S-III	382.000	3.726	Yes
S-I vs S-II	152.000	1.483	No
S-II vs S-III	230.000	2.243	No
All pairwise multiple comparison procedures for superoxide dismutase (Tukey test):			
Comparison	**Diff of Ranks**	***q***	***P* < 0.05**
S-I vs S-III	446.500	4.355	Yes
S-I vs S-II	249.500	2.433	No
S-II vs S-III	197.000	1.921	No
All pairwise multiple comparison procedures for lipid peroxidation (Tukey Test):			
Comparison	**Diff of Ranks**	***q***	***P* < 0.05**
S-I vs S-III	741.000	7.227	Yes
S-I vs S-II	438.000	4.272	Yes
S-II vs S-III	303.000	2.955	No

Table 3. Two-way ANOVA analysis of superoxide dismutase of oysters between months and stations.

Source of Variation	SS	df	MS	F	*P*-value	F crit
Months	299.0302	23	13.00131	21.72771	1.33E-17	1.766805
Stations	56.4639	2	28.23195	47.18107	7.19E-12	3.199582
Error	27.52523	46	0.598375			
Total	383.0193	71				

activity in the study area is Namkhana (S-I) > Frazergaunge (S-II) > Sajnekhali (S-III). The catalase activity of the oyster was significantly ($q = 3.726$, $p < 0.05$) higher at S-I than S-III as shown in Table 2.

The superoxide dismutase activity (unit/mg tissue) of the oyster flesh ranged from 7.4 ± 0.34 U to 16.21 ± 0.69 U during 2010 and 7.5 ± 0.32 U to 15.24 ± 0.75 U during 2011 at station-I, 7.12 ± 0.35 U to 14.92 ± 0.66 U during 2010 and 7.525 ± 0.32 U to 14.0 ± 0.64 U during 2011 at station-II, and 6.12 ± 0.32 U to 11.1 ± 0.65 U during 2010 and 5.986 ± 0.32 U to 11.21 ± 0.58 U during 2011 at station -III as shown in Figure 2(b). Significant ($p < 0.01$, Table 3) differences of superoxide dismutase in oyster flesh between all the three stations were observed. The comparatively higher superoxide dismutase activity ($q = 4.355, p < 0.05$) of the oyster flesh was observed at station-I as shown in Table 2.

The lipid peroxidation (*n* moles of malonaldehyde (MDA) formed/mg tissue) of the oyster flesh ranged from 132.51 ± 5.32 U to 225.5 ± 7.2 U during 2010 and 135.71 ± 5.35 U to 224.51 ± 7.12 U during 2011 at station-I, 115.0 ± 5.38 U to 200.0 ± 7.2 U during 2010 and 102.0 ± 5.3 U to 201.3 ± 5.75 U during 2011 at station-II, and 95.0 ± 5.35 U to 190.0 ± 5.76 U during 2010 and 90.0 ± 6.45 U to 180.0 ± 5.33 U during 2011 at station-III as shown in Figure 2(c). Significant ($p < 0.01$, Table 4) differences of lipid peroxidation in oyster flesh between all the three stations

Table 4. Two-way ANOVA analysis of lipid peroxidation of oysters between months and stations.

Source of Variation	SS	df	MS	F	P-value	F crit
Months	62726.05	23	2727.22	15.74799	7.72E-15	1.766805
Stations	44834.17	2	22417.08	129.4446	1.28E-19	3.199582
Error	7966.232	46	173.179			
Total	115526.5	71				

were observed. The comparatively higher lipid peroxidation in the oyster flesh was also observed at station-I ($q = 7.227$, $p < 0.05$) than station-III.

The station wise order of enzyme activity (CAT and SOD) and lipid peroxidation (LPO) in oyster tissue in the study area is Namkhana (S-I) > Frazergaunge (S-II) > Sajnekhali (S-III). This spatial variation may be attributed to the degree of anthropogenic stress; level of LPO and biomarker is a reflection of environmental stress that is highest in station- I owing to fish landing and marketing activities. The high enzyme activity and lipid peroxidation levels in oysters at station I is likely to be the higher presence of metal at this station.[10] Similarly the lower enzyme activity at station III is reflective of the minimal pollution sources in this area. The lowest catalase activity at station-III is mainly because of the non-existence of industries and anthropogenic activities in the Reserved Forest area. Namkhana station receives the wastewater from Kolkata and nearby Haldia port cum industrial complex but Frazergaunge station receives the discharge of several hotels and tourism units located at Bakkhali, on the other hand, Sajnekhali station is situated in eastern sector of Indian Sundarbans, which is noted for its wilderness, anthropogenic stress is minimum in this sampling station owing to reverse Mangrove forest. Higher enzyme activity and LPO level was observed in Namkhana than other sampling stations, suggesting a compensatory increase of this antioxidant to cope with higher H_2O_2 directly or indirectly induced by contaminants present at this polluted site. In agreement with this idea, other studies also have shown that higher CAT activity and LPO level can also indicate an increased peroxisomal proliferation induced by organic xenobiotics such as PAHs.[16]

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3.2. *Month-related changes in biomarkers*

Significant ($p < 0.01$, Table 1) differences in catalase activity in oyster flesh between months of the three stations were observed. The catalase activity in oyster was highest during the months of March to June (pre-monsoon) and lowest during July to October (monsoon) as recorded in Namkhana 15500.50 ± 537 U and 1025 ± 222 U, in Frazergaunge 15005.50 ± 525 U and 712.21 ± 202 U and in Sajnekhali 11800.40 ± 501 U and 436.48 ± 211 U as shown in Figure 2(a).

Significant ($p < 0.01$. Table 3) differences in activity of superoxide dismutase in oyster flesh between months of the three stations were observed. The superoxide dismutase activity was highest in oyster tissue during the months of March to June (pre-monsoon) and lowest during July to October (monsoon) as recorded in Namkhana 16.20 ± 0.69 U and 7.40 ± 0.37 U, in Frazergaunge 14.92 ± 0.66 U and 6.91 ± 0.30 U and in Sajnekhali 11.21 ± 0.58 U and 6.12 ± 0.32 U as shown in Figure 2(b).

Significant ($p < 0.01$, Table 4) differences in lipid peroxidation in oyster flesh between months of the three stations were observed. The lipid peroxidation in oyster tissue was highest during the months of July to October (monsoon) and lowest during March to June (pre-monsoon) as recorded in Namkhana 226.20 ± 7.2 U and 132.51 ± 5.32 U, in Frazergaunge 201.30 ± 5.75 U and 115 ± 5.38 U and in Sajnekhali 190 ± 5.76 U and 95 ± 5.35 U as shown in Figure 2(c).

Levels of oxidative stress may also fluctuate throughout the year owing to seasonal change in reproductive status, growth, water temperature, salinity and nutrient availability.[17]

Table 5. Seasonal variation of physico-chemical parameters of surface waters in three sites.

	Pre-monsoon			Monsoon			Post-monsoon		
Parameter	S- I	S- II	S- III	S- I	S- II	S- III	S- I	S- II	S- III
Temperature (°C)	35.5 ± 0.69	35.0 ± 0.60	34.5 ± 0.61	30.0 ± 0.55	29.5 ± 0.59	28.5 ± 0.52	22.0 ± 0.72	20.0 ± 0.69	20.5 ± 0.70
Salinity(ppt)	17.5 ± 0.34	26.0 ± 0.29	28.0 ± 0.33	5.9 ± 0.39	6.9 ± 0.38	10.0 ± 0.37	14.5 ± 0.74	17.5 ± 0.77	18.0 ± 0.75
p^H	8.3 ± 0.04	8.3 ± 0.05	8.3 ± 0.05	7.8 ± 0.03	7.9 ± 0.04	8.0 ± 0.03	8.1 ± 0.05	8.2 ± 0.04	8.2 ± 0.04

The biomarker level in natural population of organism can be expected to fluctuate due to physiological adaptation caused by seasonality.[18] In the present study, CAT, SOD activity and LPO of oyster showed a significant ($P < 0.01$) monthly variation. This monthly variation of antioxidant enzyme activity in oyster tissue may be attributable to gametogenic cycle of *Saccostrea cucullata*. CAT and SOD activity showed highest value in the month of March–April, i.e. in the pre-spawning period and lowest value after spawning i.e. during gametogenesis (September to October). Some researchers also found that the levels of antioxidant enzyme activity also fluctuated throughout the year owing to seasonal change in reproduction status.[17,19] The change of biomarker in oyster tissue are in accordance with the change of physico-chemical parameters of water. All the physico-chemical parameters show maximum value during pre-monsoon period, followed by minimum values during the monsoon except temperature (Table 5). Oysters from three sampling sites showed a higher CAT and SOD activity in summer (pre-monsoon) than in winter (post- monsoon) and monsoon season, but in case of lipid peroxidation, the higher LPO level in oyster tissue was observed during winters period than summer. This may be attributed to the fact that higher levels of PUFA (polyunsaturated fatty acid) during winter periods could contribute to increased LPO levels in oyster tissue, as these unsaturated fatty acids are more easily prone to peroxidation reaction in the presence of reactive oxygen species.[5,20] However, data from the present study suggest that the time of sampling and level of pollution can be important factors for the influence of biomarker response in oysters.

4. Conclusions

Results from this work demonstrate the need for future monitoring of the biological effects in the western sector of Sundarbans areas. Data from the present study also conclude that spatial and temporal changes in biomarkers of oysters have been associated with the changes in the level of water pollution and the month of sampling.

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