

Integrative biomarker approach to decode seasonal variation in biomarker responses of *Scylla serrata* and *Penaeus monodon* from Sundarbans estuarine system

Sritama Baag, Sumit Mandal*

Abstract

Sundarbans Estuarine System is a highly productive estuary and is considered the most important spawning and nursery ground for various commercial fish and shellfish species. Estuarine organisms are frequently exposed to a wide variety of pollutants due to their vicinity to human habitation. Marine organisms residing in this area are also exposed to extensive fluctuations of environmental factors which vary with season. In the present study, effects of seasonal variation on oxidative stress biomarkers such as superoxide dismutase, catalase, glutathione-S-transferase, and lipid peroxidation in the hepatopancreas of mud crab *Scylla serrata* and shrimp *Penaeus monodon*, were studied during monsoon, winter, spring and summer seasons. The integrated biomarker response (IBR) was assessed with the biomarker scores for all four seasons in both species. Our results suggested seasonal discrepancies as the governing factor behind biomarkers' variability. The breeding period of the animals also seems to play a significant role in their oxidative stress physiology. The IBR results indicated that moderately high temperatures and low salinity in the monsoon season are the most stressful for crabs. This stress might also be ascribed to the breeding period of these crabs which exacerbates the stress level during this season. However, in the case of shrimps, the highest IBR value was observed in the winter season due to impaired ROS elimination at low temperatures. This study also offers baseline values in various seasons that would be beneficial to be considered in environmental monitoring programs to avoid the misinterpretation of environmental factors, which change seasonally.

Keywords

Seasonal variation; Biomarkers; Antioxidant enzymes; Estuary; Integrated biomarker approach

Marine Ecology Laboratory, Department of Life Sciences, Presidency University, 86/1, College Street, Kolkata – 700073, India.

*Correspondence: sumit.dbs@presiuniv.ac.in (S. Mandal)

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1. Introduction

Estuary is regarded as one of the most prolific ecotones on this planet (Prandle, 2009). It supports numerous essential ecological functions and services (Costanza et al., 1997). It plays a pivotal role in various biogeochemical cycles, nutrient cycling, water purification, water flux regulation and shoreline protection (Kennish, 2002; Alves et al., 2015). Estuaries are dynamic environments and owing to their connections with conterminous freshwater and marine ecosystems they exhibit rapid variations in temperature, salinity, turbidity, dissolved oxygen and nutrient concentrations (Semprucci et al., 2014; Ghosh et al., 2018; Nandy and Mandal, 2020). Estuarine organisms are frequently exposed to a wide variety of pollution owing to their vicinity to human habitation (Kennish, 2002).

Sundarbans Estuarine System is the largest monsoonal micro-tidal deltaic front comprising hundreds of estuar-

ies located alongside the Indian coast across India and Bangladesh at the estuarine phase of the Ganga-Brahmaputra-Meghna River (Ghosh and Mandal, 2019). It is a highly productive estuary and is considered the most important spawning zone and nursery ground for a wide variety of commercial fish and shellfish communities. It is a world heritage site and Ramsar site (Bhowmik and Mandal, 2021). The climatic pattern of this mangrove ecosystem is dominated primarily by the southwest monsoon (June–September) with an average annual rainfall between 1500 to 2500 mm year⁻¹ (Attri and Tyagi, 2010). The monsoon season accounts for the major percentage of the annual precipitation (Ghosh et al., 2018). Moreover, monsoonal rain is regarded as the key factor behind the fluctuations in physicochemical variables in the SES (Nandy et al., 2018). Thus, marine organisms residing in this area are also exposed to extensive fluctuations of environmental factors which vary according to season. Moreover, for the

past few decades, the Sundarbans have been under threat due to natural and anthropogenic disturbances like surface water warming, increased absorption of CO₂, and oil spills (Baag and Mandal, 2023a). The local socio-economic development is entirely reliant on wild catch, fishery and various brackish water prawn cultures. Hence, proper identification of a suitable time frame to collect seeds from this region is crucial for sustainable aquaculture practices (Nandy et al., 2021). Proper knowledge about the seasonal variation in the health status of shrimp larvae and crabs would be helpful for formulating better sustainable fishery development in SES.

Marine shellfish especially intertidal organisms have been extensively used in stress and climate change research as their physiology is governed by the surrounding environment. The shifting environmental conditions play a vital role in shaping their ecology and evolution (Madeira et al., 2016). Shellfishes hold an imperative role in the country's economic status by generating foreign revenue and livelihood opportunities. Decapod crustaceans also regulate the trophic dynamics and nutrient cycling of coastal ecosystems (Grilo et al., 2011; Madeira et al., 2018). They are also excellent sentinel species and reliable environmental bio-monitors (Zheng et al., 2019). The mud crab (*Scylla serrata*) and tiger prawn (*Penaeus monodon*) are species of ecological importance because of their commercial significance for aquaculture in coastal areas of the Indian sub-continent, particularly Sundarbans. Total crustacean landings contributed about 16% of the total marine fish landings for the state of West Bengal in 2019 (CMFRI, 2020).

Reactive oxygen species (ROS) are generated at a basic level in the course of essential cellular metabolic pathways. Oxidative stress occurs when ROS generation outcompetes its neutralization rate (Halliwell and Gutteridge, 2007). Underneath oxidative stress, organisms elicit an antioxidant and detoxification response producing enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), and glutathione S-transferase (GST). SOD and CAT provide the first line of protection to the cells by scavenging ROS and attenuating damages caused due to their highly reactive nature (Hu et al., 2015). GST aids in the detoxification mechanism by scavenging free radicals. These defence mechanisms might occasionally fail to counteract the stress and lead to elevated lipid peroxidation levels, which also serve as a good indicator of oxidative stress (Regoli and Giuliani, 2014; Na et al., 2021). Environmental variations might alter the generation of free radicals (Paschke et al., 2010), which would lead to oxidative damage and may eventually cause a change in the quality and taste of meat (Romero et al., 2011). Biochemical biomarkers are recently gaining attention as early-warning monitoring tools, as they can detect stress at subcellular levels even before visual or lethal alterations are established (Marques et al., 2019). Using biomarkers from various levels

of biological strata enables an improved understanding of stress response in organisms. Integrated Biomarker Response (IBR) evaluates the cumulative response obtained from individual biomarker scores (Samanta et al., 2018). This index is well established to provide a more holistic interpretation of organisms' health status (Brooks et al., 2011). Many previous studies have pointed out that this approach is one of the best tools available for monitoring marine pollution (Dagnino et al., 2007; Brooks et al., 2009). Furthermore, as biomarkers serve as an excellent tool for early warning signals in detecting environmental perturbation, they are useful in formulating robust public policies intent to identify alternatives capable of minimizing the effects of anthropogenic pollutants before the cascading effect jeopardize the entire estuarine ecosystem (Amaral et al., 2020). Biochemical biomarkers have a direct relationship with environmental stress. They may be influenced by abiotic factors of water (like temperature, pH, salinity, dissolved oxygen, and nutrients), which vary considerably according to seasons inducing stress (Van Der Oost et al., 2003). Temperature itself regulates the physiology of ectothermic animals like shellfishes (Stoliar and Lushchak, 2012).

In situ, observations on shellfish physiology on a temporal scale are meagre (Paital et al., 2013; Madeira et al., 2016; Vinagre et al., 2021), but are important to serve as baseline information for future biomonitoring studies. The prime objective of the present study was to elucidate the sensitivity and resilience of two economically significant crustacean species from SES – *S. serrata* and *P. monodon* in different seasons (summer, monsoon, winter, and spring). We evaluated multiple biomarkers (antioxidant and detoxification defence mechanisms and lipid peroxidation levels) of both species. The general stress level was emphasized and the individual biomarker results were integrated using an “Integrated Biomarker Response (IBR)” approach. To unravel the influence of seasonal factors on the antioxidant defence mechanism of any species is crucial while considering them as bioindicators for future monitoring programmes.

2. Material and methods

2.1 Species collection

Adult *Scylla serrata* (length: 63.41 ± 4.08 mm, weight: 48.35 ± 4.79 g) and *Penaeus monodon* postlarvae (carapace length: 13.25 ± 0.5 mm, weight: 14.5 ± 0.5 mg) were collected in monsoon (August), winter (December), spring (March), and summer (May) from the tidal creek of river Matla in Sundarbans. The hepatopancreatic tissues were dissected out, packed in cryovials and frozen immediately in liquid nitrogen in the field. The tissues were then transported to the laboratory and stored at -80°C for further analyses. Physio-chemical parameters (temperature, salinity, pH, dissolved oxygen, nitrate, nitrite, phosphate, silicate, ammonia) of the estuarine water were measured

during each sampling period following standard protocol (Grasshoff et al., 1999).

2.2 Biochemical assays

For analysing the biochemical biomarkers, the hepatopancreatic tissues were homogenised in a cold tissue homogenizer containing phosphate buffer (pH 7.2, 0.1 M, 4°C) at a concentration of 50 mg ml⁻¹. It was centrifuged at 10,000 rpm (9391 g) at 4°C for 20 min and collected supernatant (2 ml) was used for the spectrophotometric assays. The results were normalized as per the total protein content estimated by the Bradford method (Bradford, 1976) using Bovine serum albumin as the standard.

SOD activity assessment was done by studying the rate of haematoxylin autooxidation inhibition according to Martin et al. (1987). SOD was measured at 560 nm and expressed as units (U)/mg of protein. One unit of enzyme activity is equivalent to the enzyme concentration requisite to inhibit haematoxylin autooxidation by 50%. CAT activity was determined by measuring the rate of degradation of H₂O₂ the substrate of the enzyme (Aebi, 1984). The activity of CAT was measured at 240 nm and expressed as μmol of H₂O₂ consumed/min/mg protein. GST activity was measured following Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene as a substrate. GST activity was measured at 340 nm and expressed as nM/min/mg of protein. LPO level was determined by measuring the formation of thiobarbituric acid reactive substances (TBARs) by the method of Ohkawa et al. (1979). The amount of TBARs was measured in the spectrophotometer at 530 nm. LPO activity was expressed as *n* moles of TBARs/mg of protein.

2.3 Integrated biomarker response

Integrated biomarker response (IBR) was calculated following Beliaeff and Burgeot (2002) with modifications by Devin et al. (2014) following Bhagat et al. (2016). Every individual biomarker response data was standardized using the formulae $Y = \frac{(X-m)}{s}$, where *Y* is the standardized biomarker response, *X* is the response value of each biomarker at a given time; *m* and *s* are mean values and standard deviation, respectively. *Z* was then calculated using $Z = -Y$ or $Z = Y$, responding to a biological effect respectively for an inhibition or an induction. The scores (*S*) for the biomarker were computed as $S = Z + |\text{Min}|$, where *Min* is the minimum value for each biomarker calculated from the standardized biomarker response. Star plots were then used to display Score results (*S*) and to calculate the integrated biomarker response (*IBR*) as:

$$IBR = \sum_{i=1}^n A_i$$

$$A_i = S_i \times S_{i+1} \times \frac{\sin\left(\frac{2\pi}{k}\right)}{2}$$

where *A_i* is the triangular area represented by two consecutive biomarker scores on the star plot, *S_i* and *S_{i+1}* represent the individual biomarker scores (calculated from standardized data) and their successive star plot radius coordinates, and *k* represents the number of radii corresponding to the biomarkers used in the survey.

2.4 Statistical analysis

Permutational analysis of variance (PERMANOVA) was applied to investigate the effect of various seasons on biochemical biomarkers of both the species *S. serrata* and *P. monodon* individually. It tests differences between the groups with many variables and permutations to avoid possible biases. PRIMER 6 (Clarke and Gorley, 2006; Clarke et al., 2008) with PERMANOVA + add-on (Anderson et al., 2008) was used for the investigations.

3. Results

3.1 Physio-chemical parameters

The results of physio-chemical parameters (temperature, salinity, pH, dissolved oxygen, nitrate, nitrite, phosphate, silicate, ammonia) of the estuarine water analysed during each sampling period are presented in Table 1. The temperature of the estuary was recorded highest in summer with very low dissolved oxygen which was contrasting to the winters with low temperature and high dissolved oxygen. In monsoon, the temperature of the waters was quite high with the lowest salinity.

3.2 Biomarkers

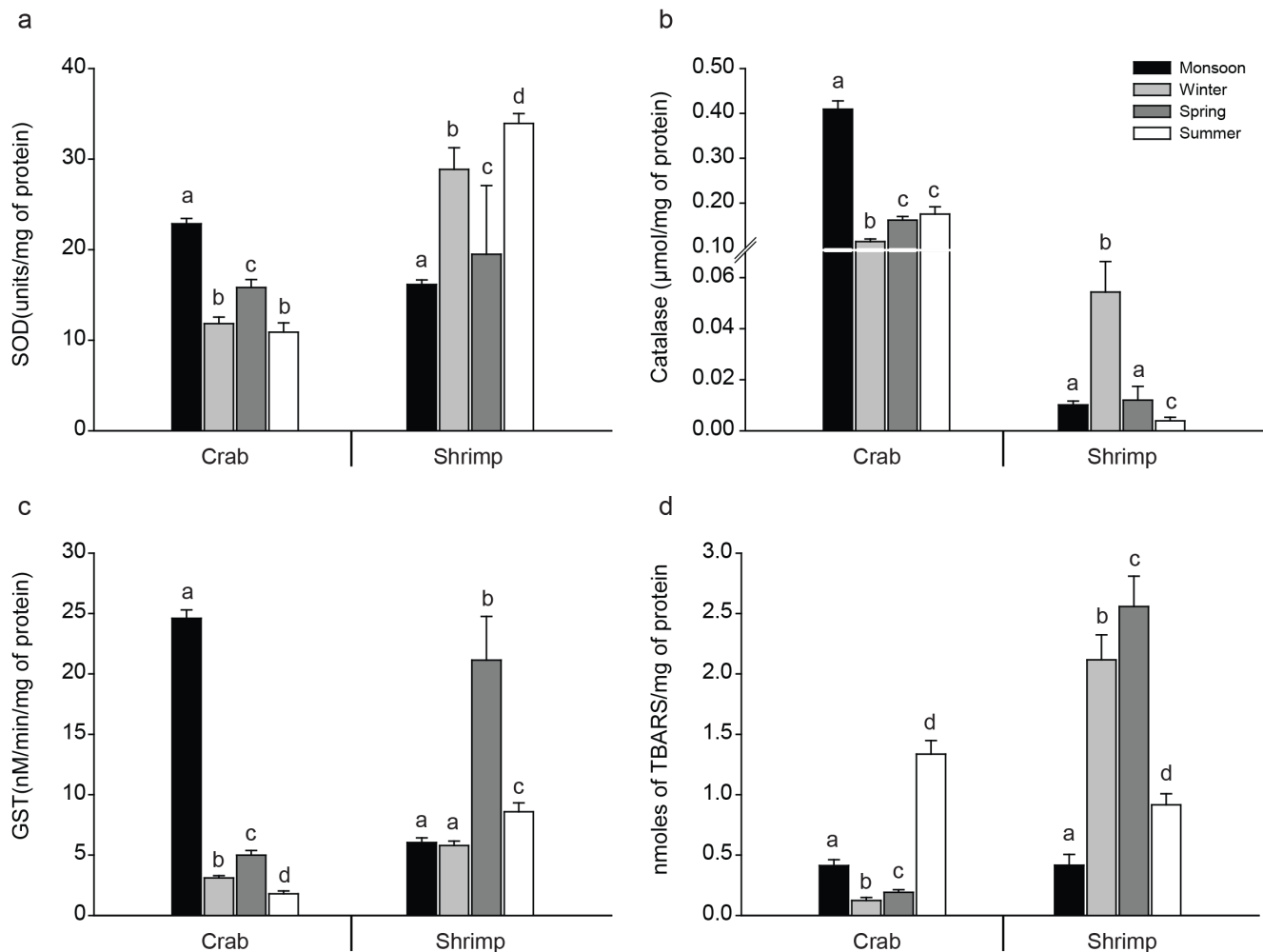
A significant variation was recorded in SOD activity in crabs among seasons except between summer and winter (Figure 1a, Table S1) ($p < 0.05$). The highest SOD activity was found during the monsoon season in crabs. In shrimps, significantly different SOD activity was noted throughout all seasons ($p < 0.05$), the highest being in summer (Figure 1a, Table S1).

CAT activity significantly varied among all the seasons in crabs ($p < 0.05$) with the highest activity observed in monsoon (Figure 1b, Table S1). The CAT activity also varied in shrimps significantly among the seasons ($p < 0.05$) except between monsoon and spring (Figure 1b, Table S1). The highest CAT activity in shrimp was noted in winter.

Significantly different GST activity was noted among all the seasons in crabs ($p < 0.05$) with the highest activity detected during monsoon (Figure 1c, Table S1). The GST activity was also found to vary significantly in shrimps among the seasons ($p < 0.05$) except between monsoon and winter (Figure 1c, Table S1). The highest GST activity in shrimp was noted in spring. LPO levels also varied significantly in crabs among all seasons ($p < 0.05$) with the highest found in summer (Figure 1d, Table S1). In shrimp, also a significant variation in LPO levels was noted ($p < 0.05$) among all seasons with the highest observed in spring (Figure 1d, Table S1).

Table 1. Physio-chemical parameters (temperature, salinity, pH, dissolved oxygen, nitrate, nitrite, phosphate, silicate, ammonia) of the estuarine water measured during each sampling season.

	Temperature (°C)	Salinity (psu)	pH	DO (mg L ⁻¹)	Nitrate (μM)	Nitrite (μM)	Phosphate (μM)	Silicate (μM)	Ammonia (μM)
Monsoon	29.5 ± 0.28	18.5 ± 0.70	8.05 ± 0.02	6.55 ± 0.49	13.49 ± 4.4	0.06 ± 0.04	0.98 ± 0.19	59.47 ± 28.23	0.05 ± 0.07
Winter	23.8 ± 0.42	19 ± 1.4	7.7 ± 0.5	8.15 ± 0.35	2.32 ± 0.94	0.22 ± 0.10	0.87 ± 0.075	30.37 ± 24.82	0.08 ± 0.007
Spring	29.05 ± 0.63	27.96 ± 0.05	8.6 ± 0.66	7.71 ± 0.16	6.34 ± 1.32	0.99 ± 0.97	0.64 ± 0.26	15.82 ± 1.39	0.45 ± 0.10
Summer	33.1 ± 0.42	32.82 ± 1.15	8.09 ± 0.06	5.25 ± 0.48	11.68 ± 2.55	0.46 ± 0.09	1.61 ± 0.38	15.51 ± 20.04	1.60 ± 0.75

**Figure 1.** Changes in superoxide dismutase (SOD) activity (a), catalase (CAT) activity (b), glutathione-s-transferase (GST) activity (c), lipid peroxidation (LPO) levels (d) in crab *Scylla serrata* and shrimp *Penaeus monodon*, at different seasons. Values represent mean ± standard deviation (n = 6). Lowercase letters indicate significant differences ($p < 0.05$) among the different groups by permutational analysis.

3.3 Integrative biomarker response

The IBR was assessed with four biomarkers (SOD, CAT, GST, LPO) for all the seasons in both species. The standardized biomarker responses used in the IBR calculation are projected in star plots for all the seasons (Figure 2) and all individual biomarkers (Figure 3). The IBR results confirmed that CAT and SOD were the most responsive biomarkers for crabs and shrimp respectively. The IBR var-

ied significantly in crabs among all seasons ($p < 0.05$). In shrimp also a significant variation in IBR was noted among all seasons ($p < 0.05$) except between monsoon and spring. The IBR results are represented in Figure 4 where the highest IBR value was found in the monsoon season for the crab and in the winter season for shrimp.

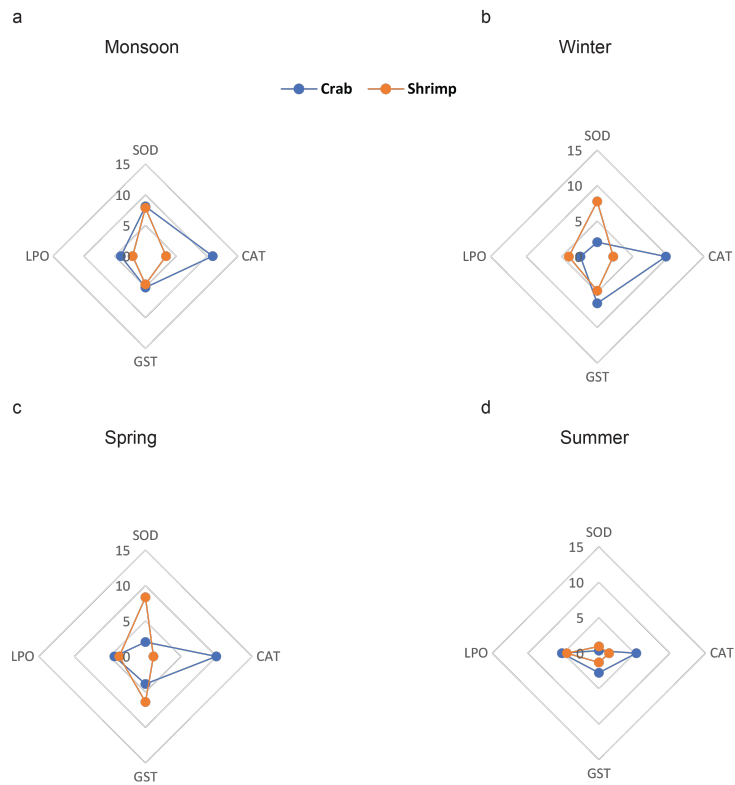


Figure 2. Star plots representing standardized biomarker response (SOD, CAT, GST and LPO) at monsoon (a), winter (b), spring (c), and summer (d) in crab *Scylla serrata* and shrimp *Penaeus monodon*.

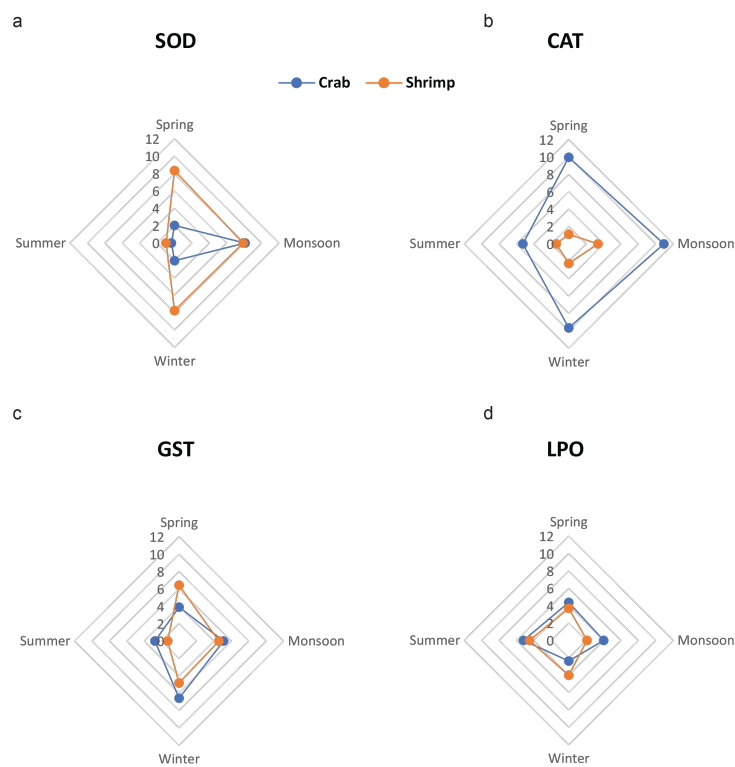


Figure 3. Star plots representing the contribution of various seasons (monsoon, winter, spring, summer) in oxidative stress response of various biomarkers SOD (a), CAT (b), GST (c), and LPO (d) in crab *Scylla serrata* and shrimp *Penaeus monodon*.

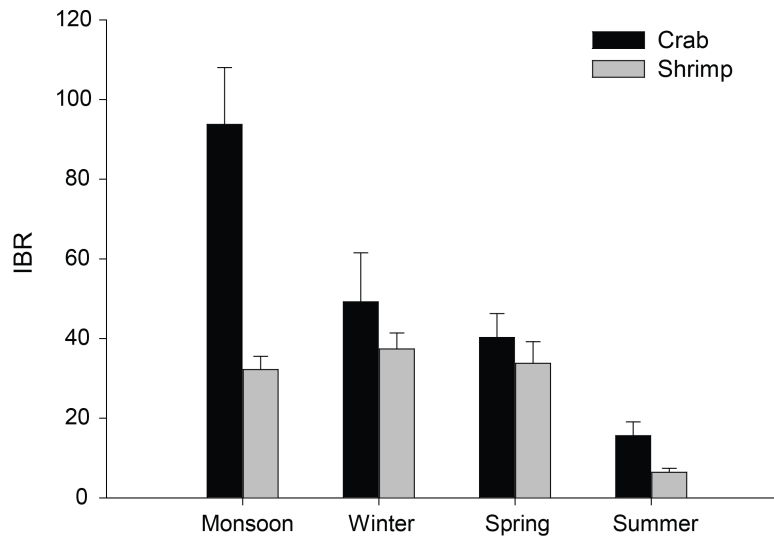


Figure 4. Integrated biomarker response (IBR) score for different seasons in crab *Scylla serrata* and shrimp *Penaeus monodon*.

4. Discussion

Seasonal fluctuation of the cellular stress responses is important which allows organisms to adjust with the environmental variations. The present study puts forward a comparatively large dataset on the baseline levels of frequently used biomarkers (SOD, CAT, GST, LPO) in *Scylla serrata* and *Penaeus monodon*, also their variations across seasons from Sundarbans estuarine system. The obtained information is significant for the determination of the usual range of baseline biomarker value and its variability at different seasons. The variation might be a result of several environmental parameters' fluctuations like temperature, pH, dissolved oxygen and salinity as well as the nutrient content of the water like nitrate, nitrite, phosphate, silicate, and ammonia.

In monsoon, the antioxidant response of crab was highly active. Among all seasons, SOD and CAT activity were highest in monsoon. This enhanced activity might be a reflection of the comparatively elevated water temperature of the estuary during this season (Nandy and Mandal, 2020). Augmented water temperature has been previously reported to induce oxidative stress in crabs and other shellfish (Kong et al., 2008; Madeira et al., 2018; Baag et al., 2021; Baag and Mandal, 2023b). Furthermore, in monsoon, the salinity of the estuarine waters is low which might cause stress in crabs. Regulation of osmotic concentration requires a significant amount of energy in decapod crustaceans resulting in metabolic alterations (Chen and Chia, 1997; Luvizotto-Santose et al., 2003). This might increase energy expenditure that is directly associated with the generation of reactive oxygen species (Gutteridge and Halliwell, 2018). A similar phenomenon was reported in the mangrove crab *Ucides cordatus* during the rainy season (dos Santos et al., 2019). An induction of antioxidant

defences and of oxidative damage was noticed suggesting that lower salinities generate an increase in metabolic demand. It has been earlier demonstrated that changes in salinity can cause oxidative stress and disturb the antioxidant defence mechanism in crab *Scylla serrata* (Paital and Chainy, 2010). The elevated antioxidant activity might be attributed to the breeding period of mud crabs in this season. Physiological changes like increased production of ROS, can be induced during the reproductive period of species which was previously reported in other shellfishes (Paital and Chainy, 2013; Schvezov et al., 2015; Sardi et al., 2020). The high GST activity during monsoon also corresponds to a significant amount of detoxification mechanism in crabs which finally prevents lipid peroxidation. Thus, it can be concluded that increased activity of SOD, CAT and GST were sufficient to prevent membrane damage in the case of *Scylla serrata*. Contrastingly, in the shrimp species, monsoon seemed to be a favourable time exhibiting low antioxidant response and lipid peroxidation levels. This might be due to the favourable abiotic factors especially high temperature (Nandy et al., 2021) and low salinity (Rahi et al., 2021) in this season. In general, the capacity of organisms to endure and alleviate the impact of stressful environmental conditions has two necessary adaptive components, genetic and physiological (Madeira et al., 2018). Intertidal shrimps generally tend to inhabit extremely warm and variable environments (Madeira et al., 2016). The thermal acclimation potential differs among species and studies have shown shrimps are able to maintain a relatively high baseline level of heat shock proteins (Hsps) with no seasonal variation in Hsp levels (Madeira et al., 2015). Furthermore, penaeid shrimps are also quite tolerant to salinity variations (Jackson and Burford, 2003). Previous studies have also revealed that salinity had no sig-

nificant effect on oxygen conductance in shrimps (Ern et al., 2012). Thus the energy required to fuel basal physiological processes like ventilation, circulation and osmoregulation were unaffected by salinity.

Winter was the most favourable time for the crabs as mirrored by the low antioxidant response of SOD, CAT, GST and correspondingly attenuated LPO levels. Low temperature and high dissolved oxygen of the estuarine waters during this season favours this organism. Due to lower temperatures, metabolic rates were also lowered in winter and the reproductive processes were at rest. The balance between energy demand and supply was maintained due to lower temperature and moderate salinity levels that did not affect the dissolved oxygen levels in water. In summer, the collective role of high temperature and salinity with low oxygen content in the habitat appears to be responsible for high ROS levels and oxidative stress in crabs which is not present in winter. In other shellfishes too, a decreased antioxidant activity was noted in winter (Malanga et al., 2007; Feidantsis et al., 2021). Contrastingly, winter was one of the most stressful seasons for the shrimp species. The SOD and CAT activity were significantly high in winter demonstrating higher antioxidant response. The GST activity was significantly lower in winter consistent with poor ROS scavenging and detoxification potential by this animal as mirrored by high LPO levels. This might be due to the low temperature of the estuary during this season. Oxidative stress was also induced in shrimps *Macrobrachium nipponense* (Wang et al., 2006) and *Litopenaeus vannamei* (Qiu et al., 2011) due to low water temperature. A drop in temperature weakens the ROS elimination mechanism in crustaceans. Temperature can directly impact the activity of enzymes by altering their catalytic efficiency or binding capacity (Cailleaud et al., 2007) and enhancing ROS production (Lushchak, 2011).

During spring, high SOD activity and decreased CAT activity was recorded howbeit, moderate GST levels were noted in crabs. This season was found to be quite favourable to crabs as reflected by low LPO levels. The SOD is the first enzyme to react with oxyradicals to counter ROS. Thus, it can be assumed that the increased level of SOD is due to enhanced intracellular ROS production. It has increased as a defence mechanism to encounter oxidative stress-induced damage to the cell. Lipid peroxidation was prevented by the high activities of SOD. Similar phenomenon was also noted in crabs previously by researchers (Schvezov et al., 2015; Rangunathan, 2016) where the antioxidant defence was sufficient to prevent cellular damage. Late winter and spring are the breeding period of *Penaeus monodon* in this part of the world (Kannan et al., 2014). Shrimp SOD activity was found to be highly active in spring but the CAT and GST activity were quite low during this season. The high SOD activity was not sufficient to prevent oxidative stress as noticed by high levels of LPO in spring. Thus, the spring season was the most stressful season for the

shrimp species that corresponds to this species' breeding period.

In summer, the antioxidant and detoxification mechanisms of crabs were not responsive enough. Amplified intracellular ROS generation led to reduced SOD activity, which further led to decreased CAT activity as a chain reaction. The SOD and CAT could not counterbalance the excessive ROS production because of continuous stress induced by high temperature and salinity during the summer season. Due to high temperature a hypoxic condition was also created in the estuarine waters which might have caused additional stress in the crabs. A similar observation was made for this species from the Ennore estuary (Tamil Nadu, India) in summer which corroborates our findings (Rangunathan, 2016). Prolonged exposure to stress leads to enhancement in oxidative stress thus causing loss of antioxidant activities. The observed diminution in the SOD activity directly influences the CAT activity and probably their combined reduction in crab might be the reason for the increased LPO levels. This might be attributed to the inadequate ability of ROS removal by the crabs as reflected by lower levels of GST. A similarly high level of LPO was also observed in summer for the same species collected from Xiamen Island (Southeast China) (Kong et al., 2008). At higher temperatures, excessive ROS is produced and accumulated due to accelerated metabolic rates, without being removed on time is the cause behind elevated LPO levels. However, in shrimps, the high SOD activity in summer is sufficient to prevent damage as expressed by the moderately lower LPO levels. The lower CAT activity and moderate GST activity depict moderately favourable conditions during this season. Since low temperature causes oxidative stress in shrimps (Wang et al., 2006; Qiu et al., 2011) they prefer higher temperatures reflected in summer.

The IBR index is a valuable tool to evaluate stress responses and environmental risks (Beliaeff and Burgeot, 2002). High IBR scores indicate intensified stress in test species. The IBR results affirmed monsoon to be the most stressful season for crabs. Moderately high temperatures and low salinity prevailed during this season and might have caused stress in crabs. The elevated IBR value might also be attributed to the breeding period of these crabs during this season which exacerbates the stress level alongside unfavourable environmental conditions. However, in the case of shrimps, the highest IBR value was observed in the winter season. Low temperature has been proven to induce oxidative stress in shrimps with impaired ROS elimination. Although the highest LPO level was obtained in summer for both the species however, the combined IBR value indicated monsoon and winter to be the most stressful for crab and shrimp respectively. While the LPO level denotes the degree of cellular injury howbeit, other biomarker levels also denote oxidative stress. IBR score provides an integrated view of stress response mechanisms in the species.

In the present study monsoon and winter triggered the maximum amount of stress response. This might be due to the overall stress and energy expenditure to activate defence mechanisms.

5. Conclusions

In this study, seasonal variations of biomarker activities were investigated for *Scylla serrata* and *Penaeus monodon* from the Sundarbans Estuarine System. Biomarker levels clearly varied seasonally according to species-specific physiological adjustments and surrounding environmental conditions. Temperature, salinity as well as dissolved oxygen levels appeared to play a significant role in modulating oxidative stress status in both species. Besides environmental factors, the breeding period of both species also seems to have a determining role in their stress physiology. Overall, the IBR index presented a cohesive picture of biological changes taking place due to seasonal fluctuations that might be beneficial to evaluate the health status of coastal organisms. The present results suggest that antioxidant and detoxifying enzymes can also be used as potential biomarkers to monitor estuarine environments as they are substantially influenced by seasonal changes. Since crustaceans have vital roles in the aquaculture industry hence, any seasonal perturbation may affect their physiological processes and taste. Furthermore, this would consequently disturb other organisms at various trophic levels by altering food web dynamics. Climate change-induced additional seasonal fluctuations will impose extensive socio-economic consequences on the commercially pertinent aquaculture sector which might cause reduced food security, livelihood opportunities and employment. The adverse effects might result in a restricted geographical distribution of these species in impending future climate change scenarios. The populations might face local extinction in the absence of long-term adaptation and tolerance. This would significantly jeopardize the rural economy as well as the ecological balance of the largest mangrove ecosystem in the world. Thus, strong background knowledge about the antioxidant defence strategies of shellfish is need of the hour to balance sustainable exploration of these resources and the economic prospect associated with it. Furthermore, it is also important to identify the most suitable season for aquaculture of these species, in order to preserve their natural population and avoid the breakdown of the country's economy.

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Conflict of interest

None declared.

Supplementary materials

Please follow this [link](#) to see the supplementary material associated with this article.

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