Antioxidant Defenses and Oxidative Stress Parameters in Tissues of *Penaeus monodon* Acclimated to Different Salinities

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Abstract: *P. monodon* juveniles were subjected to decreasing salinities (30 to 20, 10, 5ppt) and its effects on the antioxidant defence system, O_2 consumption, CO_2 release and NH_3 excretion were studied. An decrease in salinity caused an increase in O_2 consumption, CO_2 release and decrease in NH_3 excretion by *penaeus monodon*. Lipid peroxidation, protein carbonyl, H_2O_2 levels and total antioxidant capacity of the tissues decreased significantly at 5ppt salinity except in abdominal muscle where H_2O_2 content was high. With decreasing salinity, a gradual increase in SOD, an decrease in catalase, no change in GPx and a increase followed by an decrease in GR activities were recorded for abdominal muscle. While for hepatopancreas, an decrease followed by a increase in SOD and catalase, increase in GPx and GR activities were noticed with decreasing salinity. In the case of gills, a increase followed by an decrease in SOD, a increase in catalase and GPx and an decrease in GR activities were noted when the salinity decreased from 30ppt to 5ppt. These results suggest that salinity modulation of oxidative stress and antioxidant defenses in *P.monodon* is tissue specific.

Key words: *P. monodon* % Salinity stress % Oxidative stress % O_2 consumption% NH_3 excretion

INTRODUCTION

Salinity is a important abiotic factor affecting the metabolism, growth, survival and osmotic capacity and possibly, the immune system of penaeid shrimp [1-3]. Although juvenile are competent hyper- hypo osmoregulators [4], salinity changes may cause stress to the animal, especially when associated with inadequate handling procedures and poor water quality [5] and thus facilitate infections, Molecular and biochemical indicators are potentially suitable for evaluating stress, because stress classically leads to a rapid onset and a cascade of molecular and physiological response [6]. Various stress have been associated with enhanced free radical generation causing oxidative damage [7]. Free radicals, including reactive oxygen species (ROS) such as the superoxide anion (O_2-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen ($^1O_2$) are produced during normal aerobic metabolism. In normal physiology conditions there is continuous production of ROS which induces oxidative stress and can result in damage to cell membranes, inactivation of enzymes and damage to genetic material and other vital cell

components. Radical damage can be significant because it can proceed as a chain reaction [8]. Consequently, mortality can occur due to severe destruction by massive radicals generated from acute stresses or long- term chronic stresses. The effective control and rapid elimination of ROS is essential to the proper functioning and survival of organisms. This is performed by antioxidant defense system, which combat in vivo oxidation, maintains health and prevents oxidation - induced lesions.

The health of aquatic organisms is linked to overproduction of reactive species and antioxidants protect cell membranes against the production of free radicals. Thus, normal metabolism depends upon the ratio of free radical production and the activity of lipid peroxidation protection factor [9]. Recently, several investigators reported the effect of a variety of putative stressors on antioxidant defense system in an attempt to obtain simple and reliable biomarkers of oxidative stress for aquatic organisms[10,11,12]. These system include enzymatic components. The enzymes include radial scavenging such as catalase (CAT) and superoxide dismutase (SOD) acting on H_2O_2 and O_2. respectively and

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glutathione peroxidase (GPX), which scavenges H_2O_2 and lipid hydroperoxides [13,14]. Antioxidant enzymes are interdependent in nature and subject to variations due to intrinsic biological cycles, ambient physico-chemical environment and anthropogenic pollutants [15]. There has been much interest in recent years in enzyme activities involved in metabolism of ROS in crustaceans such as prawns and crabs [16-21]. There are reports that describe about the manifestation of physiological and biochemical variations especially that of the O_2 consumption of euryhaline prawn at altered salinity conditions [22,23]. Since O_2 uptake is directly related with the oxidative metabolism of animals, therefore, we hypothesize that changing salinity may channelize with altered oxidative stress and antioxidant defence of euryhaline prawn *P.monodon*. In this context, it is note worthy to mention that information on responses of antioxidant defense of *P.monodon* to changing salinity is lacking. Therefore, the present study was undertaken to investigate about the effect of salinity on (i) levels of activities of enzymes of antioxidant defense system (such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase: GR); (ii) levels of total antioxidant capacity and (iii) levels of oxidative stress parameters (such as lipid peroxidation: LPx, protein carbonylation: PC and hydrogen peroxide: H_2O_2) in tissues of prawn under laboratory conditions. Also rate of oxygen consumption, rate of release of carbon dioxide and ammonia by prawns were determined at different salinity points. Results of the present study were not only expected to provide additional contribution to the emerging field of invertebrate oxidative stress but also will help in understanding the physiological and biochemical basis of adaptation of *P.monodon* to changing salinity.

MATERIALS AND METHODS

Experimental Animals and Design: Juvenile white shrimp (*P.monodon*) were obtained from a commercial farm in Muttembaka village near Nellore andra Pradesh and were transferred to the laboratory in aerated plastic containers and maintained in laboratory holding tanks for a week in continuously aerated and filtered marine water (30ppt) with a 12hr light-dark cycle. During this period the prawns were fed ad libitum with commercial pelleted feed and then acclimated to the desired salinity by changing 5per day. After the shrimp 12g were separately acclimated from 30% to three salinity levels, i.e., 20, 10 and 5ppt, the animals were randomly stocked into 6 tubes a density of 10 shrimp per tube with four replicates. During the

periods of acclimation and 7days experiment All shrimp used in this study were individually examined for molt stage and only those at intermolt were used for biochemical analyses to minimize internal variations. once a day changing at least 25% of the ambient medium and tap water was aerated before being added to the tube to adjust salinity. Water quality parameters including pH, salinity, temperature, dissolved oxygen and ammonia were monitored throughout the Experimental period.

Sample Collection: Animals were sacrificed by removing their carapace from their abdomen with a forcipes and the hepatopancreas, gills and abdominal muscle tissues were dissected out quickly. Tissues were washed in ice-cold normal saline (0.67%, w/v), blotted, flash frozen in liquid nitrogen and stored at -80°C. A 10% (w/v) homogenate of tissues were prepared at 4°C in the homogenizing buffer (50 mM Tris-Cl, 1 mM EDTA, I mM DTT, 0.5 mM sucrose, 150 mM KCl and 1 mM PMSF, pH 7.8). Tissues were homogenized in pre-cooled mortar and pestle. The crude homogenates were centrifuged at 1,000g for 10min at 4°C in a cooling centrifuge to sediment nuclei and tissue debris. Again the supernatant fractions were centrifuged at 10,000g for 10min at 4°C to obtain the clear supernatant which was referred as sample.

Measurement of O_2 Consumption, CO_2 Release and NH_3 Excretion: A respiratory chamber (RC) was designed with a cylindrical glass jar (volume 3.121 L) fitted with a hard plastic net at 7cm height at its bottom. The RC was placed on a magnetic stirrer and a magnetic bid was kept under the plastic net to circulate the water within the jar. The upper side of the RC was equipped with an O_2 electrode connected with a monitor (µP based soil and water analysis kit, Esico. Co., New Delhi, India). One prawn was introduced into the cambered filled with freshly O_2 saturated artificial saline water of required salinity. Prior to releasing the prawns into the RC each was acclimated in a similar chamber for 10 min with the water of the same salinity. During experiments, the RC was sealed to prevent the diffusion of atmospheric O_2 into the chamber. Fall of O_2 concentration in the chamber was recorded at 15min interval up to 60min and the result was expressed as mg O_2 consumed/100g body mass/h. At the end of 1h, a 500ml water sample was drawn into an air tight dark bottle for CO_2 measurement and 4ml water into the RC, water samples were collected to assess initial O_2, CO_2 and NH_3 present before the experimental and the values were deduced from the respective resulted values after the end of the experiment. Free CO_2 was measured

by titrating 50ml of water sample with 0.1ml of phenolphthalein indicator against N/10 NaoH according to [24]. The end point of titration was achieved when a pink color was developed in the solution. The result was expressed as mg of CO_2 released /100g body mass/h. NH_3 in the water sample 0.1 ml of manganous salt was added followed by 1ml of 25% alkaline phenol and 0.5 ml of hypochlorite solution. The mixture was gently rotated and boiled for 5min, cooled and was centrifuged at 200g for 1min at 25°C. Absorbance of the color of solution was measured at 625nm. NH_3 concentration was calculated from its standard curve and the results were expressed as mg of NH_3 excreted/100g body mass of prawn/h)

Biochemical Determinations: Lipid peroxidase assay: The lipid peroxidase content in tissues was determined using thiobarbituric acid reaction substances (TBARS) as described by Ohkawa [25]. To mixture 5ml of tissue extract was added 1.5ml of 20% acetic acid, 0.2 ml of 8% SDS and 1.5ml of 0.8 TBA. The mixture was made up to 4.ml with distilled water and then heated for 60 min at 95°C using a water both. After cooling 4 ml of butanol- pyridine mixture was added and the mixture was shaken well. After centrifugation at 4000 rpm for 10min, the organic layer was collected and its absorbance was read at 532nm in a UV spectrophotometer. 1,1', 3.3'-tetramethoxy propane was used as standard and was treated in a similar way to the test mixture. The lipid peroxide concentration was expressed as nmoles TBARS released/mg protein.

Protein Carbonylation Assay: Protein carbonylation content was measured in post-nuclear fractions of tissue samples according to the method of Levine *et al.* [26] Carbonyl content was calculated from its molar absorption coefficient as 22,000 MG^1cmG^1 and results were expressed as nmol protein carbonyl per mg protein.

Hydrogen Peroxide Hydrogen Peroxide (H_2O_2) Assay: Content in tissue samples were measured Spectrofluorimetrically according to Anguelov and Chichovska *et al.* [27].

Determination of Antioxidant Enzyme Activities: Superoxide dismutase assay: Superoxide dismutase activity was assayed according to the method of Misra and Fridovich *et al.* [28], which is based on the oxidation of epinephrine to adrenochrome by the enzyme. 0.1 ml of tissue homogenate was added to the tubes containing 0.75ml of ethanol and 0.15 ml of chloroform (chilled in ice) and centrifuged. To 0.5 ml of supernatant were added 0.5 ml of EDTA solution and 1 ml of buffer. The reaction was initiated by the addition of 0.5 ml of epinephrine and the increase in absorbance at 480 nm was monitored at 30-s intervals for 3 min. The enzyme activity was expressed as 50% inhibition of epinephrine auto-oxidation/ min/mg protein.

Catalase Assay: Catalase activity was assayed according to the method of Takahara *et al.* [29]. To 1.2 ml of phosphate buffer, 0.5 ml of tissue homogenate was added. The enzyme reaction was started by the addition of 1.0 ml of hydrogen peroxide solution. The decrease in absorbance was monitored at 240nm every 30 s up to 3 min. the enzyme activity was expressed as μ moles of hydrogen peroxidase decomposed/min/mg protein.

Glutathione Peroxidase: Glutathione peroxidase activity was assayed by method of Rotruck [30]. The reaction consisting of 0.2 ml of EDTA, 0.1 ml sodium azide, 0.1 ml of H_2O_2, 0.2 ml of GSH, 0.4 ml of phosphate buffer and 0.2 ml of homogenate was incubated at 37°C for 10 min. the reaction of arrested by the addition of 0.5 ml of TCA and the tubes were centrifuged at 2000 rpm. To the supernatant 3 ml of disodium hydrogen phosphate and 1.0 ml of DTNB were added and the colour was read at 420 nm immediately. The activity of GPx was expressed as μmoles of glutathione oxidize/min/ mg protein.

Glutathione-S-Transferase: Glutathione-S-transferase activity was assayed by the method of Habig [31]. The reaction mixture containing 1.0 ml buffer, 0.1 ml of CDNB and 0.1 ml of tissue homogenate was made up to 2.5 ml with water. The reaction mixture was pre incubated at 37°C for 5 min. 0.1 ml of GSH was added and the change in OD at 340nm was monitored at 30-s intervals for 3 min. The activity of glutathione-S-transferase was expressed as nmoles of 1-chloro-2, 4-dinitrobenzene (CDNB) conjugate formed/min/mg protein.

Glutathione Reductase: Glutathione reductase activity was assayed by the method of Dubler and Anderson [32]. The reaction mixture containing 50mM phosphate buffer, 1mM NADPH was made up to 3 ml with water. After the addition of 0.1ml of tissue homogenate, the change in OD at 340 nm was monitored at 30-s intervals for 3 min. The activity of glutathione reductase was expressed as nmoles of NADPH oxidizes/min/mg protein.

Total Antioxidant Capacity: Tissue sample were homogenized in 50mM phosphate buffer containing 2 mM EDTA, pH 7.6 (PBE) for measurement of total antioxidant capacity of tissues in the form of DPPH (2,2-diphenyl-1-

Table 1: Effect of salinity on O_2 consumption, CO_2 release and NH_3 excretion of *P.monodon*.

Parameters	Salinity (ppt)			
	30	20	10	5
O_2 consumption (mg/100 g body mass/h)	10.26 ± 0.01	15.45 ± 0.02	18.64 ± 0.01	20.13 ± 0.01
CO_2 released (mg/100 g body mass/h)	15.35 ± 0.009	25.15 ± 0.01	65.45 ± 0.01	70.54 ± 0.01
NH_3 excreted (mg/100 g body mass/h)	7.25 ± 0.01	5.67 ± 0.01	1.36 ± 0.01	0.26 ± 0.01

Each value represents the mean±SD of 6 individuals. Individual data for each parameter for different salinities were subjected to multiple comparison (ANOVA) P<0.001 level

picryl hydrazyl) scavenging activity. The crude homogenate (10% w/v) was centrifuged at 1000g for 10min to obtain PNF. DPPH scavenging assay was performed according to Marxen *et al.* [33] in the aqueous phase of PNF. The results were expressed as % of decrease in absorbance by 100µl of PNF from a control set (without sample) at 515nm. The calculation was performed as % of inhibition = (Abs control- Abs of sample)/ Abs control x100.

Statistical Analysis: A multiple comparisons (Duncan's) test was conducted to compare significant differences among treatments using the SPSS software and differences were considered significant when P<0.001.

RESULTS

Oxygen Consumption, Carbon Dioxide Release and Ammonia Excretion: Table 1 show changes in oxygen consumption, Carbon dioxide release and ammonia excretion by the shrimps when they were acclimated to 30 ppt to 20, 10, 5ppt salinity levels. Significant increase (96%) in oxygen consumption by the prawns were recorded when the prawns were transformed from 30 to 5ppt salinity levels, respectively. Although release of Carbon dioxide by the prawns increased (359%) when they were transformed 30ppt to 5ppt salinity levels. Similarly ammonia excretion was decreased (96%) in 5ppt salinity groups in compared with 30ppt groups.

Lipid Peroxidation: A gradual reduction in LPx value of abdominal muscle of the prawn was recorded in repose to decreasing salinities. Lipid peroxidation level in abdominal muscle of the prawns was decreased 92% at 5 ppt salinity in comparison to 30 ppt salinity, respectively. Although the LPx value of hepatopancreas of the prawns was 32% lower in 5ppt compared to 30ppt salinity . In case of gills of the prawns, LPx value was 64% decrease at 5ppt salinity levels.
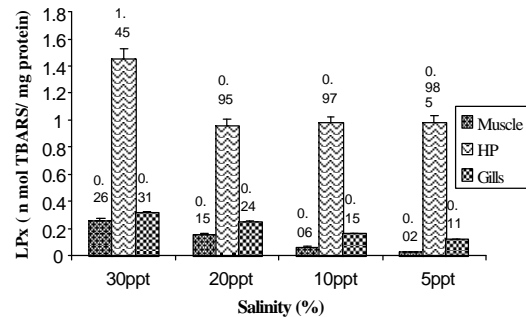

Fig. 1: Effect of salinity on activities of lipid peroxidation (LPx) on abdominal muscle (M), hepatopancreas (HP) and gills (G) of *P.monodon*.
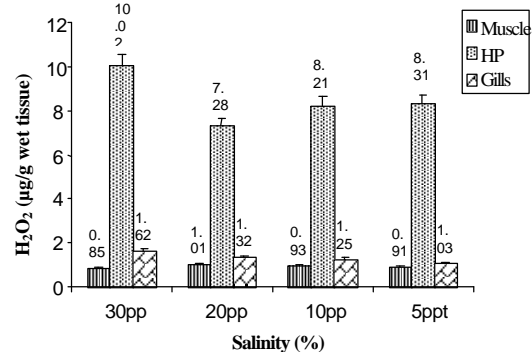

Fig. 2: Effect of salinity on activities of hydrogen peroxide (H_2O_2)on abdominal muscle (M), hepatopancreas (HP) and gills (G) of *P.monodon*.

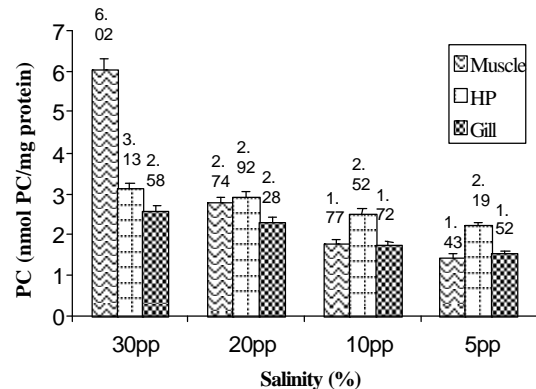Fig. 3: Effect of salinity on activities of protein carbonyl (PC)on abdominal muscle (M), hepatopancreas (HP) and gills (G) of *P.monodon*.
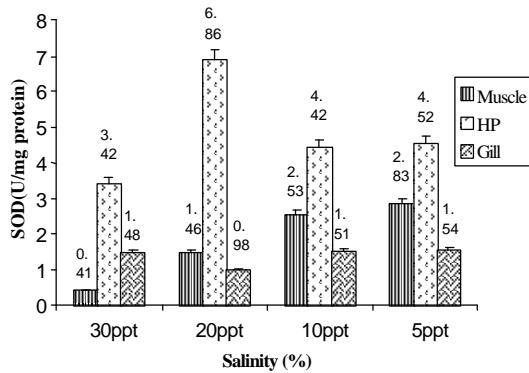
Fig. 4: Effect of salinity on activities of superoxide dismutase (SOD)on abdominal muscle (M), hepatopancreas (HP) and gills (G) of *P.monodon*.
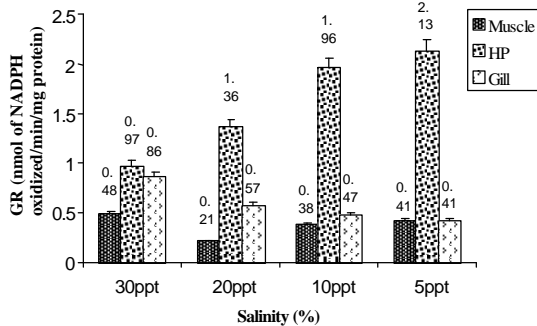

Fig. 6: Effect of salinity on activities of catalase (CAT)on abdominal muscle (M), hepatopancreas (HP) and gills (G) of *P.monodon*.

Fig. 5: Effect of salinity on activities of glutathione reductase (GR)on abdominal muscle (M), hepatopancreas (HP) and gills (G) of *P.monodon*.

Fig. 7: Effect of salinity on activities of glutathione peroxidase (GPx) on abdominal muscle (M), hepatopancreas (HP) and gills (G) of *P.monodon*.

Protein Carbonyl Content: In all the three tissues viz. abdominal muscle, hepatopancreas and gills of the prawns, protein carbonyl content was significantly lower at 5ppt salinity in comparison with 30ppt salinity. However, the magnitude of change in protein carbonyl content was different from one tissue to the other. Protein carbonyl content of abdominal muscle of the prawns at 5ppt was 76% lower than 30ppt salinity groups. It was further noticed that the protein carbonyl content of hepatopancreas of prawns of the 5ppt group was 30% lower than that of 30ppt groups. Protein carbonyl content of gills of the prawns was 41% decreased at 5ppt in comparison with 30ppt salinity groups, respectively.

Hydrogen Peroxide Content: A small but a significant increase (7%) in hydrogen peroxide content of abdominal muscle of the prawns of 5ppt salinity groups was recorded in comparison with 30ppt salinity group. On the other hand, the enzyme activity decrease (16%) in hepatopancreas of the prawns of 5ppt group were
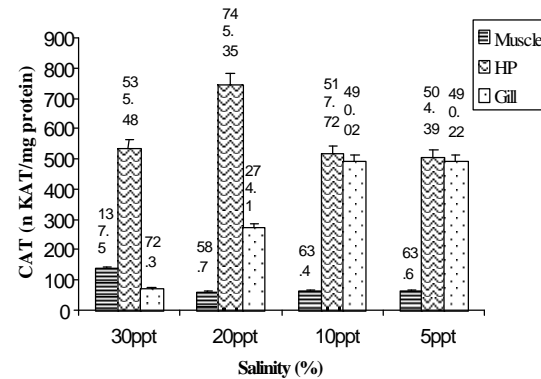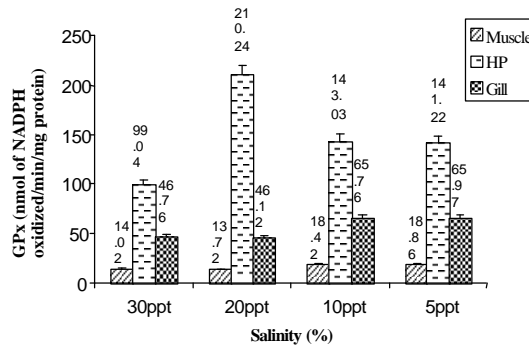
recorded in comparison with 30ppt groups. Similarly 36% decrease in gills of the prawns at 5ppt groups was noted in comparison with 30ppt groups, respectively.

Antioxidant Enzymes

Superoxide Dismutase: It was noticed that the level of SOD in abdominal muscle of the prawns increased gradually as the level of salinity decreased. The activity of the enzyme in abdominal muscle tissue of the prawns increased 5% in 5ppt groups in comparison with 30ppt group. In case of hepatopancreas, the enzyme activity of the prawns at 5ppt salinity was observed to be higher (32%) than that of 30ppt group. On the other hand, the level of the enzyme was 4% higher in gills of the prawn of 5ppt groups in comparison with 30ppt groups, respectively.

Catalase: The response of catalase to different salinities varied from one tissue to another. Significant 53% decrease in abdominal muscle of the pawns at 5ppt group

were noted comparison with 30ppt group. In case of hepatopancreas, 5% decrease in enzyme activity was recorded for the prawns of 5ppt in comparison with 30ppt group. The enzyme activity in gills of the prawns gradually increased as the level of salinity decreased. It was 578% higher in gills of the prawns of 5ppt group in comparison with 30 ppt and groups, respectively.

Glutathione Peroxidase: Glutathione peroxidase activity was 31% higher in abdominal muscle of the prawns of 5ppt in comparison with 30ppt groups. A significant increase (42%) in the enzyme activity of hepatopancreas was recorded in 5ppt group than that of prawns of 30ppt group. It was observed that the enzyme level in gills of the prawns increased to 41% in 5ppt group in comparison with 30ppt group, respectively.

Glutathione Reductase: The response of glutathione reductase to changing salinity varies from one tissue to another. Although the activity of the enzyme was lower (14%) in abdominal muscle of the prawns of 5ppt salinity group comparison with 30ppt salinity groups. It was noticed that the enzyme activity was 119% lower in hepatopancreas of the prawns of 5ppt in comparison with 30ppt groups, respectively. On the other hand, the enzyme activity was 52% lower in gills of the prawns of 5ppt in comparison with 30ppt groups.

DISCUSSION

The optimal salinity for growth of *P.monodon* should be around 20- 30ppt and much deviation from this salinity will adversely affect shrimp growth and survival due to the energy expenditure for osmoregulation. But till now, due to poor understanding on *P.monodon* physiology, it was not clear how and where the extra energy for osmoregulation. Therefore, with the aim to get a relatively integrated understanding on physiological adaptation of *P.monodon* to different salinities, further analyzed the sampled and carried out the study using the sample of the shrimp in the growth trial for which the growth performance and survival rate have been observed and the healthy status of these shrimp ensured the objectivity and authenticity of the results in the present study. Similar tissue variation regarding activities of antioxidant enzymes was reported for several other species belonging to phylum Arthropoda: *M. rosenbergi* [34], *Charybdis japonica* [35], *M. malcolmconi* [36], *Chasmagnathus*

granulate (now *Neohelice granulata*) [37] and *Orconectus limosus* [38]. In the present study, prawns were acclimated to 30ppt to 20, 10, 5ppt of salinity in the laboratory. It was observed that oxygen consumption by the prawns increased at 5ppt comparison with 30ppt (Table 1). From above results the possibility of experiencing a transition state of internal organs of prawns from hyperoxia to hypoxia or 30 to 5ppt increase in oxygen consumption rate, respectively. Our results are in good agreement with earlier results for other euryhaline crabs species which exhibited elevated [39,40] or reduced [22,23] oxygen consumption rate when they were acclimated to low or high salinities. Increased consumption of oxygen by juveniles *P.monodon* at low salinity suggest that a higher energy demand by the organism may be due to active pumping of ions from environment to hemolymph. It is now a well established fact that most euryhaline prawns species are hyperosmoregulators at low salinities, actively pumping ions from sea water into hemolymph [41]. Induced rate of oxygen consumption and CO_2 production by prawns at low salinity indicate high oxidative metabolism of the organism to regulate is hypoionic state. Ammonia, amino acids and urea are the three principal end products of protein metabolism that are released to the environment through gills in decapods [42]. Chen and Chia [23] reported that a shift in nitrogen excretion pattern from ureotelism to ammoniotelism takes place in *Scylla serrata* when crabs were transformed from higher salinity to lower salinity. Chen and Chia [23] observed a decrease in ammonia-N release at low salinity. On the contrary, we have noted that low percentage of ammonia release by prawns at low salinity. The low percentage of ammonia at lower salinity may be attributed to low protein catabolism.

The hepatopancrease in crustaceans is a major organ which is mainly associated with diverse metabolic activities ranging from supply of nutrition to ovary, to digestion and absorption [43,44,45]. It can be considered metabolically more active than gills and muscle tissue. It is difficult to explain the physiological basis for the observed increase in activities of antioxidant enzymes in hepatopancreas of prawn at 5ppt without any change in oxidative stress markers. It is possible that production of ROS in hepatopancreas is increased at 5ppt salinity. Which might have caused increase in synthesis of antioxidant enzymes. It is interesting to note that a significant decrease in lipid peroxidation in abdominal muscle and gills and protein carbonylation in gills was

recorded at 5ppt salinity. Observed increase in SOD activity in abdominal muscle and SOD, catalase and GPx in gill may be responsible for lowering LPx values in low salinity. It contributes to the mechanism to understand that due to higher oxygen consumption by the organism at low salinity, these two tissues might have experienced higher oxygen tension and consumption which might have resulted in enhanced production of ROS. Consequently, levels of antioxidant enzyme were enhanced to counteract the ROS. In prawns, gills are primarily responsible for respiration, acid - base balance and osmotic and ionic regulation [46,47]. Furthermore it has been noted that a battery of biochemical and physiological changes takes place in gills of euryhaline prawns in response to low salinity stress in order to maintain osmotic and ionic homeostasis [48]. It has been reported that exposure of cell to hypoxia resulted in increase generation of ROS by enzymatic mechanisms [49]. The increase consumption of oxygen by gills in prawns at low salinity would have enhanced production of superoxide radicals, tissue level SOD activity might have induced. The consequence of increased SOD activity would have resulted in more production of hydrogen peroxide. Hydrogen peroxide is highly toxic to cell and subsequently generates highly toxic and reactive hydroxyl radicals by classical Fenton reactions. Consequently, activities of both the enzymes elevated in gills of prawns to keep the hydrogen peroxide level at basal level. Generally, in almost all animals hydrogen peroxide is neutralized by catalase and GPx enzyme. Nevertheless, neutralization of hydrogen peroxide by muscles and gills of tiger prawn was quit distinct. In the former tissue, where only GPx plays an active role in neutralizing hydrogen peroxide, both the enzymes are required for the later tissue. It is evident from the present study that enzymatic neutralization mechanism of ROS at low salinity is different among the tissues of prawns. It is well known that the rate of production of ROS at the mitochondrial level in many biological systems is proportional to oxygen consumption and to mitochondrial metabolic rate. But in our experiment the increase in oxygen consumption in prawn at 5ppt salinity have resulted in higher production of ROS. Several studies have clearly established that deprivation of oxygen to cells or tissues has resulted in generation of ROS [50,51,52]. Several reasons have been described as the cause for increased generation of SOD during hypoxia such as residual oxygen level in tissues [53] and switch

over of biochemical reacion [54,55]. Limiting oxygen supply to prawns during high salinity will slow down the electron transport in the lower part of the ETC propagating ROS formation from downward electron carriers such as complex III ubiquinone [56]. There several reports that conformed increased production of ROS during hypoxia condition in marine invertebrates [57]. Among the three organs, hepatopancrease is one most subjected to oxidative stress during transition of prawns from 30 ppt to 5ppt salinity as evident by augmentation of LPx, PC and hydrogen peroxide levels. Lipid peroxidation and protein carbonylation are considered as consequence of oxidation of lipids and proteins by ROS. Therefore, both parameters i.e lipid peroxidation and protein carbonylation are considered as indices of tissue oxidative stress in almost all animals including that of estuarine invertebrates [58]. In the present study, decreased levels of TBARS and PC contents in tissues of *P.monodon* at low salinity suggested reduction of oxidative stress.

Not much information is available in the literature on biochemical changes taking place in hepatopancrease of *P.monodon* in response to changing environmental factors in general and salinity in particular. A significant increase in SOD activity was observed in hepatopancreas of prawns at low salinity which is accompanied with induced catalase and GPx activities may explain for the observed low hydrogen peroxidase level in the tissue. The physiological significance of induced activities of antioxidant enzymes at low salinity is difficult to explain at present. However, it may be attributed to low oxidative metabolism of the *P.monodon* as consumption of oxygen by prawns increased during its exposure to low salinity. SOD activity of gills of *P.monodon* induced when the prawns were subjected to low salinity condition. Low salinity stress might have produced a large amount of ROS in gills which could have induced SOD to scavenger superoxide radicals. However, increase in catalase activity along with GPx at low salinity may be able to add hydrogen peroxide causing reduction of lipid peroxide level in the tissue. Gills, the principal organ for respiration in *P.monodon* are directly expose to the surrounding environment. It also maintains the osmotic balance of body fluid. Several biochemical adaptations in gills of prawns to changing salinity are reported. For Example, Na$^+$- K$^+$ATPase (*Crytograpus angulatus*) [59] and Carbonic anhydrase were reported to be influenced by environmental salinity. In comparison with

hepatopancrease and gills, abdominal muscle is metabolically less active. SOD activity of the abdominal muscle increased with low salinity (Figure 4). The present results proved an idea to support the fact that at low salinity, the metabolic activity of the abdominal muscle may be very low. Consequently, less amount of superoxide radicals are generated. Hence, the tissue needs low SOD activity. Increased level of SOD activity along with enhanced level of catalase in abdominal muscle at low salinity may be the reason for induced hydrogen peroxide level in tissue. It is here to note that GP activity of the muscle increased in response to low salinity. SOD and CAT are the two primary enzymes for radical scavenging, which are involved in protective mechanisms within tissue injury following oxidative process and phagocytosis. And their activities are related to the status of the organisms affected by different factors including dietary nutrition, environmental factors etc . Usually, higher SOD and CAT activities indicate there are more radicals need to be reacted. Therefore, significantly higher SOD and CAT activities in shrimp at lower salinity might indicate that the stress of low salinity resulted in an accumulation of radicals to a higher level in shrimp. If these radicals induced by low salinity were not scavenged, the organisms would suffer from serious oxidative damage [13]. Therefore, the enhanced activities of both SOD and CAT at low salinity may enable shrimp to maintain health by scavenging the radicals produced. However, in a long term culture study similar to commercial farming, 70% of shrimp survived and maintained adequate growth at 30% suggesting that there must be some adaptive mechanism ensuring the organism survival. The adaptive mechanism may be partially explained by the increasing activities of SOD and CAT for scavenging the radicals produced in a certain extent.

In conclusion, result of the present study suggest that the magnitude of oxidative stress (measured in terms of lipid peroxidation and protein carbonylation) and status of antioxidant defense complexes of *P.monodon* are tissue specific and their responses to changing salinity vary from one tissue to the other, However, low salinity (5ppt) induces more oxidative stress in the tissues of *P.monodon* in comparing with high salinities.

ACKNOWLEDGEMENTS

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