

**Seasonal variation of oxidative biomarkers in gills and digestive gland of  
green-lipped mussel *Perna viridis* from Arabian Sea**

**X. N. Verlecar<sup>a#</sup>, K. B. Jena<sup>a</sup>, G.B.N. Chainy<sup>\*</sup>**

<sup>a</sup>National Institute of Oceanography, Dona Paula, Goa, India

<sup>\*</sup>Department of Zoology & Biotechnology, Utkal University, Bhubaneswar, India

**# Corresponding Author:**

Dr. X. N. Verlecar

Environmental Laboratory, Biological Oceanography Division,

National Institute of Oceanography, Dona-Paula, Goa-403004

Phone:-0832-2450218

Fax:-0832-2450606

E-mail: [verlecar@nio.org](mailto:verlecar@nio.org)

## **Abstract**

Investigations on seasonal variation in oxidative stress biomarkers were carried on the natural population of green-lipped mussel *Perna viridis* collected from Bambolim beach area of Goa. Oxidative indices such as lipid peroxidation (LPX), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione transferase (GST), glutathione reductase (GR), reduced glutathione (GSH) and ascorbic acid (ASA) were measured in gills and digestive gland of *Perna viridis* during February, May, August and November. The present study reveals two important aspects regarding the antioxidant defence status of tissues of *P. viridis*. Firstly, antioxidant capacity of tissues of *P. viridis* exhibits seasonal variation. Secondly, various components of antioxidant capacity such as oxidative stress markers, levels of antioxidant enzymes and small antioxidant molecules vary differently in tissues with respect to different seasons. Although the oxidative stress status of gills and digestive gland of *P. viridis* expressed in terms of LPX and H<sub>2</sub>O<sub>2</sub> was the lowest in February, its level was maximal in gills and digestive gland during May and November, respectively. While activities of SOD and GPX of tissues of *P. viridis* were found to be low in August, activities of CAT and GR were recorded to be low in February. GST activity in gills although remained high in February, in digestive gland elevated values were recorded in August and November. A seasonal variation in the levels of small antioxidant molecules was also noticed. Among non enzymatic antioxidants ASA content of tissues was maximal in May and August in comparison to February and November, but GSH remained high in November. It therefore appears that environmental factors may play a crucial role in regulating the oxidative stress capacity of tissues of *P. viridis*.

**Key words:** Seasons; Biomarkers; Antioxidants defence system; Mussels.

## 1.1. Introduction

Bivalve molluscs, in particular mussels, are useful organisms to assess chemical pollution in aquatic environment. They are sedentary and sessile filter-feeders, having wide geographical distribution. Because of their abundance in coastal waters, they accumulate several classes of pollutants and hence are chosen as suitable organism for Mussel Watch monitoring programs (Goldberg, 1975). To understand the health of the aquatic environment, in recent years use of biochemical markers to evaluate molecular, biochemical and cellular effects induced by pollutants in bivalve mollusks have been studied. The metabolic activities of invertebrates are influenced to a large extent by changes in both biotic and abiotic factors, which depend on the season. Changes in environmental factors as a consequence of change in seasons may therefore influence normal metabolic activities of organisms and the induction of oxidative stress as a consequence of increased generation of reactive oxygen species (ROS). Antioxidant defences serve as an excellent scavenging potential against oxidative stress. Antioxidant defences constitute both enzymatic and non-enzymatic parameters which are both commonly in use as biomarkers in environmental monitoring studies. Antioxidant enzymes are interdependent in nature and subject to variations due to intrinsic biological cycles, ambient physico-chemical environment and anthropogenic pollutants (Sheehan and Power, 1999). The interpretation of these responses in an environmental context is very complex to account for all the possible causes (Sheehan and Power, 1999). The increasing awareness of mussels as sensitive bioindicators of environmental pollution and the need to evaluate seasonal variation of biomarker responses has initiated the present study. Investigations were designed to characterize the seasonal changes in oxidative stress biomarkers in a native population of mussels (*Perna viridis*) from an unpolluted reference site of the Arabian Sea. For this purpose the respiratory organ, the gills, and the digestive gland, which is the major site of xenobiotic and oxy-radical generating biotransformation enzymes (Livingstone et al., 1992) were chosen for analysis. The parameters measured include: (1) LPX and H<sub>2</sub>O<sub>2</sub> as oxidative stress markers; (2) activity of SOD, CAT, GPX and GR as antioxidant enzymes; (3) activity of GST as a biotransformation enzyme; (5) GSH as a conjugation substrate and free radical scavenger; and (6) ASA as a free radical scavenger.

## 2. Material and methods

### 2.1. Chemicals

Thiobarbituric acid (TBA), bovine serum albumin (BSA), GR, cumene-hydroperoxide, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) and horseradish peroxidase (HRP) were purchased from Sigma Chemical Co. USA. Oxidised glutathione (GSSG), NADPH, NADH, GSH, 1-chloro-2, 4-dinitrobenzene (CDNB), trichloro acetic acid (TCA), potassium chloride (KCl), ethylenediaminetetra acetic acid (EDTA) and phenylmethylsulphonylfluoride (PMSF) were obtained from SISCO Research laboratory, India. All other chemicals were analar grade.

### 2.2 Sample collection

Green-lipped mussels (*Perna viridis*), length 8-10 cm were collected in February, May, August and November from unpolluted sea beach at Bambolim (Lat-15° 27' N, Long- 73° 51' E), Goa, India. This area is relatively less polluted because of the absence of any industrial or major fishing activity around it. The annual variation of Hg, Cd, Pb and petroleum aromatic hydrocarbon in water near the sampling site ranged from 0.045 to 0.103, 0.02 to 0.1, 0.69 to 2.7 and 3.65 to 6.39 µg / l (Personal communication, NIO, 2004). The gills and digestive gland were rapidly dissected from 30-40 specimens, pooled in 10 samples, immediately frozen in liquid nitrogen and store at -20 °C for short-term storage.

### 2.3. Physico-chemical parameters

All the physico-chemical parameters were assayed on the spot during each sampling. Surface water temperature was measured with a thermometer. Surface water salinity around the sampling stations was measured with a portable Salinity Refractometer (Model: ATAGO, S/ Mill, Japan). The pH of surface water was determined by waterproof pH scan microprocessor-based pocket pH tester (Accuracy: ± 0.1, Model: pH Scan WP1, Malaysia).

### 2.4. Sample preparations

The gills and digestive gland was carefully excised, surface dried with tissue paper, thoroughly washed with potassium phosphate buffer (50 mM; pH 7.4) and homogenized with

50 mM potassium phosphate buffer pH 7.4 containing, 1 mM EDTA, 1 mM Dithiothreitol (DTT), 0.15 M KCl and 0.01 % (w / v) PMSF. Homogenization was carried out at 4 °C using 12-15 strokes of a motor driven Teflon Potter homogenizer and centrifuged at 10 000 x g. The supernatant was used for biochemical analysis.

### *2.5.Lipid peroxidation measurement*

LPX level was assayed by measurement of malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acids hydro peroxides were determined by the TBA reaction as described by Ohkawa et al. (1979). The absorbance was read at 532 nm after removal of any fluctuated material by centrifugation. The amount of thiobarbituric acid reactive substances (TBARS) formed was calculated by using an extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$  (Wills, 1969) and expressed as nmol TBARS formed per mg protein.

### *2.6.Antioxidant enzymes*

The activity of total SOD was determined by the method of Paoletti et al. (1990) by monitoring rate of oxidation of NADH. One unit of SOD activity was defined as 50 % inhibition of oxidation of NADH in the reaction. The absorbance was recorded at 340 nm and expressed as Unit / mg of protein. CAT activity was determined according to Aebi (1974) by monitoring decrease in absorbance of  $\text{H}_2\text{O}_2$  at 240 nm and enzyme activity was expressed as nkat / mg protein (1 katal =  $1 \text{ mol sec}^{-1}$ ). The activity of GPX was determined by the method of Paglia and Valentine (1967) using cumene-hydroperoxide as the substrate and enzyme activity was monitored by recording oxidation of NADPH at 340 nm. Enzyme activity was expressed as nmol NADPH oxidized / min / mg proteins using a molar extinction coefficient of  $6.22 \text{ mM}^{-1} \text{ cm}^{-1}$ . The activity of GR was assayed following the rate of NADPH oxidation at 340 nm in the presence of GSSG (Massy and Williams, 1965). Enzyme activity was expressed as nmol NADPH oxidized / min/ mg proteins using a molar extinction coefficient of  $6.22 \text{ mM}^{-1} \text{ cm}^{-1}$ . GST activity was measured according to Habig et al. (1974) using CDNB as a substrate. The change in the absorbance was recorded at 340 nm and enzyme activity was expressed as nmol CDNB conjugate formed / min / mg protein using a molar extinction coefficient of  $9.6 \text{ mM}^{-1} \text{ cm}^{-1}$ .

### *2.7.Measurement of small antioxidant molecules*

A 10 % tissue homogenate was prepared in 5 % TCA (w / v) and was centrifuged 1000g for 30 min at room temperature, the deproteinised supernatant was used for assay of GSH using DTNB (Ellman, 1959), and ascorbic acid following the stoichiometric reduction of phosphomolybdate by ascorbic acid (Mitusi and Ohata, 1961). ASA was taken as standard and results were expressed as  $\mu\text{g ASA} / \text{gm wt. tissues}$ .

### *2.8.Hydrogen peroxide*

$\text{H}_2\text{O}_2$  content in post-mitochondrial fractions was determined spectrophotometrically according to the methods of Pick and Keisari (1981) using HRP and phenol red. Protein content was estimated by the Folin-Phenol reaction as described by Lowry et al. (1951) using bovine serum albumin as standard.

### *2.9.Statistical analysis*

Significant variations of biological parameters were investigated by one-way ANOVA and post hoc tests (Newman-Keuls) were used to discriminate between means of values. Difference were considered statistically significant when  $p < 0.05$ .

## **3.Results**

### *3.1.Physico-chemical parameters*

Among the physico-chemical parameters, temperature and salinity exhibited distinct seasonal parameters (Table-1). Maximum values were recorded during May followed by November, February and August, respectively. Surface water pH showed minor decreased during August, otherwise approximately maintained a constant pattern throughout the year.

### *3.2.Lipid peroxidation*

*Perna viridis* sampled at Bambolim beach showed LPX level ( $1.9 \pm 0.3$ ,  $3.0 \pm 0.2$ ,  $2.4 \pm 0.3$  and  $2.5 \pm 0.4$  nmol TBARS / mg protein, Fig-1 A) in gills and ( $1.14 \pm 0.15$ ,  $2.7 \pm 0.2$ ,  $1.68 \pm$

0.3 and  $3.4 \pm 0.2$  nmol TBARS / mg protein, Fig-1 B) in digestive gland during February, May, August and November respectively.

### 3.3. Hydrogen peroxide

In gills during February, May, August and November H<sub>2</sub>O<sub>2</sub> levels were ( $3.45 \pm 0.37$ ,  $10.9 \pm 1.63$ ,  $8.72 \pm 0.97$  and  $16.51 \pm 1.9$  nmol / mg protein, Fig-1 C). In case of digestive gland H<sub>2</sub>O<sub>2</sub> levels were ( $3.3 \pm 0.81$ ,  $9.42 \pm 1.1$ ,  $8.57 \pm 1.8$  and  $10.98 \pm 0.9$  nmol / mg protein, Fig-1 D) during respective months.

### 3.4. Superoxide dismutase

SOD activities of  $7.3 \pm 0.8$ ,  $9.7 \pm 1.2$ ,  $2.9 \pm 0.6$  and  $15.7 \pm 2.4$  Unit / mg protein (Fig-1 E) were observed in gills during the month of February, May, August and November. In case of digestive gland SOD activities of  $2.5 \pm 0.6$ ,  $3.7 \pm 0.4$ ,  $1.5 \pm 0.4$  and  $4.3 \pm 0.5$  Unit / mg protein, (Fig-1 F) were detected in respective sampling periods.

### 3.5. Catalase

As with SOD activity, CAT was higher in May and November in both the tissues. In case of gills CAT activities were in February;  $82 \pm 11.5$ , May;  $190.5 \pm 30.5$ , August;  $144.8 \pm 29.4$  and November;  $264 \pm 36.5$  nkat / mg protein, observed in *P. viridis* collected from Bambolim beach, Goa (Fig-2 A). In digestive gland CAT activities were in February;  $174.3 \pm 16.5$ , May;  $358.3 \pm 40.9$ , August;  $334.8 \pm 49.1$  and November;  $602.8 \pm 54.3$  nkat / mg protein (Fig-2 B).

### 3.6. Glutathione peroxidase

This enzyme activity was observed  $55.4 \pm 6.3$  in February;  $65 \pm 9.8$  in May;  $37.9 \pm 3.8$  in August and  $75.9 \pm 7$  nmol NADPH oxidized / min / mg protein, in November in gills of *P. viridis* (Fig-2 C). Where as GPX activities were  $62.11 \pm 6.56$  in February;  $58.2 \pm 10.4$  in May;  $35.4 \pm 4.1$  in August and  $53.5 \pm 4.9$  nmol NADPH oxidized / min / mg protein in November observed in digestive gland of *P. viridis* (Fig-2 D).

### 3.7. *Glutathione transferase*

During seasonal study periods (February, May, August and November), GST activity was ( $94 \pm 6$ ,  $72 \pm 8$ ,  $93 \pm 14$  and  $85 \pm 8$  nmol CDNB conjugate formed / min / mg protein) in gills of *P. viridis* (Fig-2 E). In case of digestive gland enzyme activity was ( $127 \pm 24$ ,  $145 \pm 7$ ,  $182 \pm 17$  and  $168 \pm 23$  nmol CDNB conjugate formed / min / mg protein) during respective months (Fig-2 F).

### 3.8. *Glutathione reductase*

In gills GR activity was ( $8.8 \pm 0.9$ ,  $17.4 \pm 3.2$ ,  $12.3 \pm 1.3$  and  $56.6 \pm 7$  nmol NADPH oxidized / min / mg protein) during February, May, August and November (Fig-3 A). On the other hand in digestive gland enzyme activity was ( $11.8 \pm 2.3$ ,  $42.5 \pm 3.1$ ,  $15 \pm 0.8$  and  $70.6 \pm 6.8$  nmol NADPH oxidized / min / mg protein) observed respectively (Fig-3 B).

### 3.9. *Reduced glutathione*

GSH also function in the detoxification of electrophilic xenobiotics and in reduction of ROS. There appears to be a different pattern of seasonal variation in digestive gland and gill tissues investigated. In gills GSH level was  $0.93 \pm 0.1$ ,  $1.37 \pm 0.17$ ,  $1.24 \pm 0.24$  and  $3.1 \pm 0.24$   $\mu\text{mol} / \text{gm wt. tissue}$  in February, May, August and November (Fig-3 C). On the other hand in digestive gland GSH level was  $1.77 \pm 0.35$ ,  $3.41 \pm 0.3$ ,  $1.87 \pm 0.2$  and  $5.8 \pm 0.7$   $\mu\text{mol} / \text{gm wt. tissue}$  observed during respective months (Fig-3 D).

### 3.10. *Ascorbic acid*

ASA levels of  $26.55 \pm 3.27$ ,  $37.2 \pm 3.83$ ,  $35.2 \pm 7.19$  and  $26.79 \pm 3.55$   $\mu\text{g} / \text{gm wt. tissue}$  observed in gills during February, May, August and November (Fig-3 E). Where as in case of digestive gland ASA contents of  $33 \pm 4$ ,  $57 \pm 3$ ,  $77 \pm 8$  and  $40 \pm 3$   $\mu\text{g} / \text{gm wt. tissue}$  measured during respective sampling periods (Fig-3 F).



### 3.11. Correlation analysis

Among physico-chemical parameters, surface water temperature showed more significant correlation with biochemical parameters than followed by salinity and pH (Table-2). LPX showed a positive correlation with the H<sub>2</sub>O<sub>2</sub> level ( $r = 0.445, 0.832$ ; Fig-4 A & B). In addition to this in both the tissues a significant positive correlation was observed between H<sub>2</sub>O<sub>2</sub> and CAT+ GPX activity ( $r = 0.888, 0.816$ ; Fig-4 C & D).

## 4. Discussion

The seasonal changes in physico-chemical characters of aquatic environment, (particularly salinity and pH) in the estuarine and coastal waters in the Indian sub-continent are mainly derived by annual monsoon cycle (Verlecar, 1987). The present study also showed low salinity and pH during August as compared to the other period (Table-1). The decreased salinity is the result of heavy rainfall during monsoon.

Bivalve metabolism is known to vary with seasons in response to abiotic factors such as temperature, salinity and oxygen level etc and biotic features like food availability and gonadal cycles (Widdows, 1978; Livingstone, 1981). A close functional relationship between digestive system and gonadal cycle and periods of food availability has been reported in the molluscs (Mackie, 1984).

### 4.1. Oxidative stress marker

ROS can be highly toxic to aquatic organisms including mussels, often resulting in oxidation of lipids in membranes, protein oxidation and DNA damage (Almeida et al., 2007). LPX is well known mechanism of cellular injury in vertebrate and invertebrates, and it acts as an indicator of oxidative damage in cells and tissues (Pampanin et al., 2005). LPX product can react with amino acid groups of proteins and other molecules to form a variety of adducts (Esterbauer et al., 1991; Marnett, 1999). An increased level of LPX was observed in May in gills, and in case of digestive gland in May and November (Fig-1 A & B). Increased LPX level has also been reported in brown mussels *Perna perna* obtained from Grande Island coast in Brazil by Filho et al. (2001) during the same season. It has been reported that during May and November *P. viridis* usually displays a maximal annual gamete formation (Narasimham,

1980; Rajgopal et al., 1998). The increased metabolic rates during breeding season could possibly increase the rate of ROS formation, resulting in oxidative stress. Except H<sub>2</sub>O<sub>2</sub>, no measurement of O<sub>2</sub><sup>•-</sup>, OH<sup>•</sup> and other ROS markers were made in this study. According to this higher levels of H<sub>2</sub>O<sub>2</sub> were observed in May and November as compared to other seasons (Fig-1 C & D). H<sub>2</sub>O<sub>2</sub> can alter cell physiology through the formation of OH<sup>•</sup> by Fenton reaction (Cavelleto et al., 2002). This could be the possible reason for the high LPX observed in mussels during May and November (Fig-1 A & B), indicating a clear relationship between H<sub>2</sub>O<sub>2</sub> and LPX (Fig- 4 A & B).

#### 4.2. Antioxidant enzymes

Our data indicated that in *P.viridis*, SOD, CAT, GPX, GR and GST express both in gills as well as in digestive gland, with some tissue specific responses. SOD activity was higher in gills than digestive gland (Fig-1 E & F). This appears to be logical, because the gills are exposed to high oxygen because of its respiratory function (Santovito et al., 2005). SOD is a crucial antioxidant enzyme, which dismutate O<sub>2</sub><sup>•-</sup> to H<sub>2</sub>O<sub>2</sub>. In *P. viridis* the SOD activity was high in November and May in both the tissues (Fig- 1 E & F). This observation is in good agreement with an earlier report (Filho et al., 2001), which suggests that O<sub>2</sub><sup>•-</sup> radicals are produced during metabolic activities. The seasonal pattern of SOD activity found in the present study appears to be closely related to the reproductive cycles as well as physico-chemical parameters.

CAT unlike SOD was more active in digestive gland than gills (Fig-2 A & B). This enzyme catalyses the transformation of ROS, i.e. H<sub>2</sub>O<sub>2</sub> to water. The importance of this enzyme lies with Fenton reaction, which scavenges H<sub>2</sub>O<sub>2</sub> to generate highly reactive molecules such as OH<sup>•</sup> (Fe (II) / Cu (I) + H<sub>2</sub>O<sub>2</sub> → OH<sup>•</sup> + OH<sup>-</sup> + Fe (III) / Cu (II) (Halliwell and Gutteridge, 2001). In addition, GPX also catalyses H<sub>2</sub>O<sub>2</sub> and organic hydro peroxide to water and alcohol, using GSH as cosubstrate. In the present study, CAT activity significantly increased during May and November in both tissues (Fig-2 A & B). On the other hand GPX activity increased only during November (Fig-2 C & D), with almost similar response in both tissues (Fig-2 C & D). Correlation analysis performed with all H<sub>2</sub>O<sub>2</sub> and CAT + GPX data from the gills and digestive gland showed significant relationship between these parameters

(Fig-4 C & D). Consequently, for the purpose of H<sub>2</sub>O<sub>2</sub> detoxification, GPX and CAT may work more efficiently to protect cells from ROS toxicity.

GST catalyzes the conjugation of GSH with a variety of electrophilic metabolites resulting in detoxification of both reactive intermediates and oxygen radicals (Van der Oost et al., 2003). In the present study, increased GST activity was observed in August and November in digestive gland, with much lower values in gills (Fig-2 E & F). The seasonal variation of biotransformation enzymes including GST has been reported in *Perna perna* by Filho et al. (2001), while no seasonal effect was observed in case of *M. galloprovincialis* (Bocchetti and Regoli, 2006). Further studies would be needed to characterize the natural variability of GST activities in relation to environmental parameters and pollutants.

GR plays an important role in cellular antioxidant protection and adjustment process of metabolic pathway. GR catalyses the reduction of GSSG to GSH, in NADPH-dependent reaction. The elevated GR activity was observed during May and November (Fig- 3 A & B). The seasonal changes of GR activities have also been reported in *M.edulis*, collected from the industrial harbour complex of Le Havre, France (Manduzio et al., 2004). The elevated GR activity can be related to the thiol status of cells because it generates GSH from GSSG. In this regard, it is interesting to observe that the GSH concentrations (Fig-3 C & D) and the activity of GR showed a similar trend during the two months of May and November (Fig-3 C & D). The increased levels of the different antioxidants observed in *P.viridis* during May and November are probably of temporary nature having a direct linkage with environmental parameters as well as the reproductive cycle during the period.

#### 4.3. Non-enzymatic antioxidants

Increased GSH level seen presently in *P. viridis* during May and November in both the tissues (Fig-3 C & D). Such seasonal changes in GSH level has also been reported in bivalves by Power and Sheehan (1996) and Filho et al. (2001). Maximal gamete formation in *P.viridis* occurs during May and November (Narasimham, 1980; Rajgopal et al., 1998). There is known to be a close functional relationship between reproductive cycle and oxidative stress (Filho et al., 2001). It may be hypothesized that the increased level of GSH could be due to the ability of cells / tissues to cope with oxidative stress, by using GSH as a cosubstrate for antioxidant

enzymes or alternatively to bind with ROS. The other non-enzymatic antioxidant such as ASA showed a peak at August which later attains a basal level in digestive gland (Fig-3 F), whereas in the case of gills no significant variation was observed (Fig-3 E). There are not many reports available on seasonal variation of ASA in mussels. Hence it may be hypothesized that the changes in ASA level in digestive gland might be related to dietary intake of the animals.

Correlation of physico-chemical factors with biochemical parameters (Table-2) showed that the temperature had a significant relation with many of the biochemical compounds. This implies that there is a high probability of ROS formation in tissues. These observations are further confirmed by our recent laboratory experiments, which indicated that temperature significantly affects the antioxidant defences in *P.viridis* (Verlecar et al., 2007). It is well known that organic compounds and metal contaminants are possible sources of oxidative stress which can bring changes in antioxidant enzyme activities (Cavaletto et al., 2002; Cheung et al., 2004; Verlecar et al., 2007). Further studies would obviously help to characterize the natural variability of antioxidant responses or their changes due to pollution.

It could be concluded therefore that this study represents the first comprehensive report of antioxidant defence system in *P.viridis* collected from Goa coast along the Arabian Sea. The result obtained in this work indicates a significant influence of seasonal factors on both enzymatic and non-enzymatic antioxidants. Seasonal variation may be effective because of complex interactions between exogenous factors such as temperature (Verlecar et al., 2007; Kirchin et al., 1992), salinity (Prevodnik et al., 2007), pH (Lima et al., 2007) and food supply (Khessiba et al., 2005) and endogenous factors such as reproductive cycle (Filho et al., 2001). It is difficult therefore to draw exact conclusion as to which parameter is most effective to bring the changes in the antioxidant observed. However it is clear from the data that significant changes of antioxidant parameters in gills and digestive gland of *P.viridis* seems to be closely related with the seasonal variations of temperature (Table-2) and reproductive cycle. The observed correlation between ROS level such as H<sub>2</sub>O<sub>2</sub> with LPX, indicates that digestive gland has more significant contribution than gills (Fig-4 A & B). The digestive gland is the main metabolic organ in the body, which may be the consequence of physiological processes related to spawning during reproductive cycle. The above results

obviously bring out the importance of seasonal changes on the antioxidant status of *Perna viridis* in tissue specific manner and are valuable for interpretation of environmental monitoring data.

### **Acknowledgements**

The authors are grateful to the Director of NIO for help. This work is carried under the funds from NIO Project SSP1738. We are thankful to the Head, Department of Zoology and Biotechnology, Utkal University, for facilities.

### **References**

Aebi, H., 1974. Catalase In: Bergmeyer, H.U (Eds.), Methods of Enzymatic Analysis. vol.2. Academic Press, New York, pp. 673-678.

Almeida, E. A., Bainy, A. C. D., de Melo Loureiro, A. P., Martinez, G. R., Miyamoto, S., Onuki, J., Barbosa, L. F., Garcia C. C. M., Prado, F. M., Ronsein, G. E., Sigolo, C. A., Brochini, C. B., Martins, A. M. G., de Medeiros, M. H. G., Di Mascio, P., 2007. Oxidative stress in *Perna perna* and other bivalves as indicators of environmental stress in the Brazilian marine environment: Antioxidants, lipid peroxidation and DNA damage. Comparative Biochemistry and Physiology A 146, 588-600.

Bocchetti, R. and Regoli, F. 2006. Seasonal variability of oxidative biomarkers, lysosomal parameters, metallothioneins and peroxisomal enzymes in the Mediterranean mussel *Mytilus galloprovincialis* from Adriatic Sea. Chemosphere 65: 913-921.

Cavaletto, M., Ghezzi, A., Burlando, B., Evangelisti, V., Ceratto, N., Viarengo, A., 2002. Effect of hydrogen peroxide on antioxidant enzymes and metallothionein level in the digestive gland of *Mytilus galloprovincialis*. Comparative Biochemistry and Physiology C 131, 447-455.

Cheung, C.C.C., Siu, W.H.L., Richardson, B.J., DeLuca-Abbott, S.B., Lam, P.K.S., 2004. Antioxidant responses to benzopyrene and Aroclor1254 exposure in the green-lipped mussel, *Perna viridis*. Environmental Pollution 128, 393-403.

Ellman, G.L., 1959. Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics 82, 70-77.

Esterbauer, H., Schaur, R. J., Zollner, H., 1991. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biology and Medicine* 11, 81-128.

Filho, D. W., Tribess, T., Gaspari, C., Claudio, F.D., Torres, M.A., Magalhaes, A.R.M., 2001. Seasonal changes in antioxidant defenses of the digestive gland of the brown mussel *Perna perna*. *Aquaculture* 203, 149-158.

Goldberg, E. D., 1975. The mussel watch- a first step in global marine monitoring. *Marine Pollution Bulletin* 6, 111-132.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione-S-Transferases. *Journal of Biological Chemistry* 25, 7130-7139.

Halliwell, B., Gutteridge, J.M.C., 2001. *Free Radicals in Biology and Medicine*, Oxford-University Press, New York.

Khessiba, A., Roméo M., Aïssa P., 2005. Effects of some environmental parameters on catalase activity measured in the mussel (*Mytilus galloprovincialis*) exposed to lindane. *Environmental Pollution* 133, 275-281.

Kirchin, M.A., Wiseman, A., Livingstone, D.R., 1992. Seasonal and sex variation in the mixed function oxygenase system of digestive gland microsomes of the common mussel, *Mytilus edulis* L. *Comparative Biochemistry and Physiology* 101, 81-91.

Lima, I., Moreira, S. M., Osten, J. R., Soares A. M.V.M., Guilhermino, L., 2007. Biochemical responses of the marine mussel *Mytilus galloprovincialis* to petrochemical environmental contamination along the North-western coast of Portugal. *Chemosphere* 66, 1230-1242.

Livingstone, D.R., 1981. Induction of enzymes as a mechanism for the seasonal control of metabolism in marine invertebrates: glucose-6-phosphate dehydrogenases from the mantle and hepatopancreas of the common mussel *Mytilus edulis* (L). *Comparative Biochemistry and Physiology B* 69, 147-156.

Livingstone, D.R., Lips, F., Garcia, M. P., Pipe, R.K., 1992. Antioxidant enzymes in the digestive gland of the common mussel *Mytilus edulis*. *Marine Biology* 112, 265-276.

Lowry, O.H., Rosebrough, N.J., Farr, A L., Randall, R. J., 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 193, 265-275.

Mackie, G.M., 1984. Bivalves. In: Tompa AS, Verdonck NH, Van Den B, editors. *The mollusca*, 7. New York: Academic Press, pp 284-316.

Manduzio, H., Monsinjon, T., Galap, C., Leboulenger, F., Rocher, B., 2004. Seasonal variations in antioxidant defences in blue mussels *Mytilus edulis* collected from a polluted area: major contributions in gills of an inducible isoform of Cu/Zn-superoxide dismutase and of glutathione S-transferase. *Aquatic Toxicology* 70, 83-93.

Marnett, L. J., 1999. Lipid peroxidation DNA damage by malondi-aldehyde. *Mutation Research* 424, 83-95.

Massey, V., Williams, C.H., 1965. On the reaction mechanism of yeast glutathione reductase . *Journal of Biological Chemistry* 240, 4470-4481.

Mitusi, A., Ohata, T., 1961. Photooxidative consumption and photoreductive formation of ascorbic acid in green leaves. *Plant Cell Physiology* 2, 31-44.

Narasimham, K.A., 1980. Fishery and biology of the green mussels, *Perna viridis* (Linnaeus). *Central Marine Fisheries Research Institute Bulletin* 29, 10-17.

Ohkawa, H., Ohisi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* 95, 351-358.

Paglia, D.E, Valentine, W.N., 1967. Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine* 70, 158-169.

Paoletti, F., Mocali, A., Aldinucci, D., 1990. Superoxide-driven NAD(P)H oxidation induced by EDTA-Manganese complex and mercaptoethanol. *Chemico-Biological Interactions* 76, 3-18.

Pampanin, D. M., Camus, L., Gomiero, A., Marangon, I., Volpato, E., Nasci C., 2005. Susceptibility to oxidative stress of mussels (*Mytilus galloprovincialis*) in the Venice Lagoon (Italy). *Marine Pollution Bulletin* 50, 1548 -1557.

Pick, E., Keisari, Y., 1981. Superoxide anion and hydrogen peroxide production by chemically elicited peritoneal macrophages. Induction by multiple nonphagocytic stimuli. *Cell Immunology* 59, 301-318.

Power, A., Sheehan, D., 1995. Seasonal variations in the levels of antioxidant enzymes in *Mytilus edulis*. *Biochemical Society Transactions* 23, 354S-362S.

Prevodnik, A., Gardeström, J., Lilja, K., Elfving, T., McDonagh, B., Petrović, N., Tedengren, M., Sheehan D., and Bollner T., 2007. Oxidative stress in response to xenobiotics in the blue mussel *Mytilus edulis* L.: Evidence for variation along a natural salinity gradient of the Baltic Sea. *Aquatic Toxicology* 82, 63-71.

Rajgopal, S., Venugopalan, V.P., Nair, K.V.K., van der Velde, G., Jenner, H.A., den Hartog, C., 1998. Reproduction, growth rate and culture potential of the green mussels, *Perna viridis* (L.) in Edaiyur backwaters, east coast of India. *Aquaculture* 162, 187-202.

Santovito, G., Piccinni, E., Cassini, A., Irato, P., Albergoni, V., 2005. Antioxidant responses of the Mediterranean mussel, *Mytilus galloprovincialis*, to environmental variability of dissolved oxygen. *Comparative Biochemistry and Physiology* C140, 321-329.

Sheehan, D., Power, A., 1999. Effects of seasonality on xenobiotic and antioxidant defence mechanism of bivalve molluscs. *Comparative Biochemistry and Physiology* C123, 193-199.

Van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology* 13, 57-149.

Verlecar, X.N., 1987. Distribution of nutrients in the coastal and estuarine waters of Goa. *Mahasagar* 20, 205-215.

Verlecar, X.N., Jena, K.B., Chainy, G.B.N., 2007. Biochemical markers of oxidative stress in *Perna viridis* exposed to mercury and temperature, *Chemico-Biological Interactions* 167, 219-226.

Viarengo, A., Canesi, L., Pertica, M., Livingstone, D., 1991. Seasonal variations in the antioxidant defence enzymes and lipid peroxidation of the digestive gland of mussels. *Comparative Biochemistry and Physiology* C 100, 187-190.



Widdows, J., 1978. Combined effects of body size, food concentration and season on the physiology of *Mytilus edulis*. Journal of the Marine Biological Associations 58, 109-124.

Wills, E.D., 1969. Lipid peroxide formation in microsomes: General Considerations. Biochemical Journal 113, 315-324.

<b>Sampling seasons</b>				
	February	May	August	November
<b>Physico-chemical</b>				
<b>Parameters</b>				
pH	8.11 ± 0.19	8.1 ± 0.05	7.61 ± 0.22	7.97 ± 0.1
Salinity (%)	31.14± 0.66	33.79 ± 0.48	28.22 ± 0.52	30.92 ± 0.39
Temperature (°C)	26.52 ± 0.31	32.48 ± 0.32	26.96 ± 0.85	29.43 ± 0.59

**Table-1:** Physico-chemical parameters of water bodies in different seasons during sampling periods.

Biochemical parameters	Water temp.		Salinity		pH	
	GL	DG	GL	DG	GL	DG
<b>LPX</b>	c	b	x	x	x	x
<b>H<sub>2</sub>O<sub>2</sub></b>	c	a	a	x	x	x
<b>SOD</b>	a	b	a	b	a	a
<b>CAT</b>	x	c	x	a	x	x
<b>GPX</b>	x	a	b	x	a	x
<b>GR</b>	b	a	x	x	x	x
<b>GST</b>	x	a	x	a	b	x
<b>GSH</b>	a	c	x	x	x	x
<b>ASA</b>	x	x	x	a	b	c

**Table: - 2;** - Correlation coefficients (r) between physico-chemical parameters of the aquatic environment and biochemical parameters. LPX-Lipid peroxidation, H<sub>2</sub>O<sub>2</sub> - Hydrogen peroxide, SOD- Superoxide dismutase, CAT- Catalase, GPX- Glutathione peroxidase, GR- Glutathione reductase, GST- Glutathione- S-transferase, GSH- Reduced glutathione and ASA- Ascorbic acid. <sup>a</sup> p < 0.05 , <sup>b</sup> P < 0.01, <sup>c</sup> P < 0.001 and x- Not significant. GL-Gill, DG-Digestive gland.

### **Figure captions**

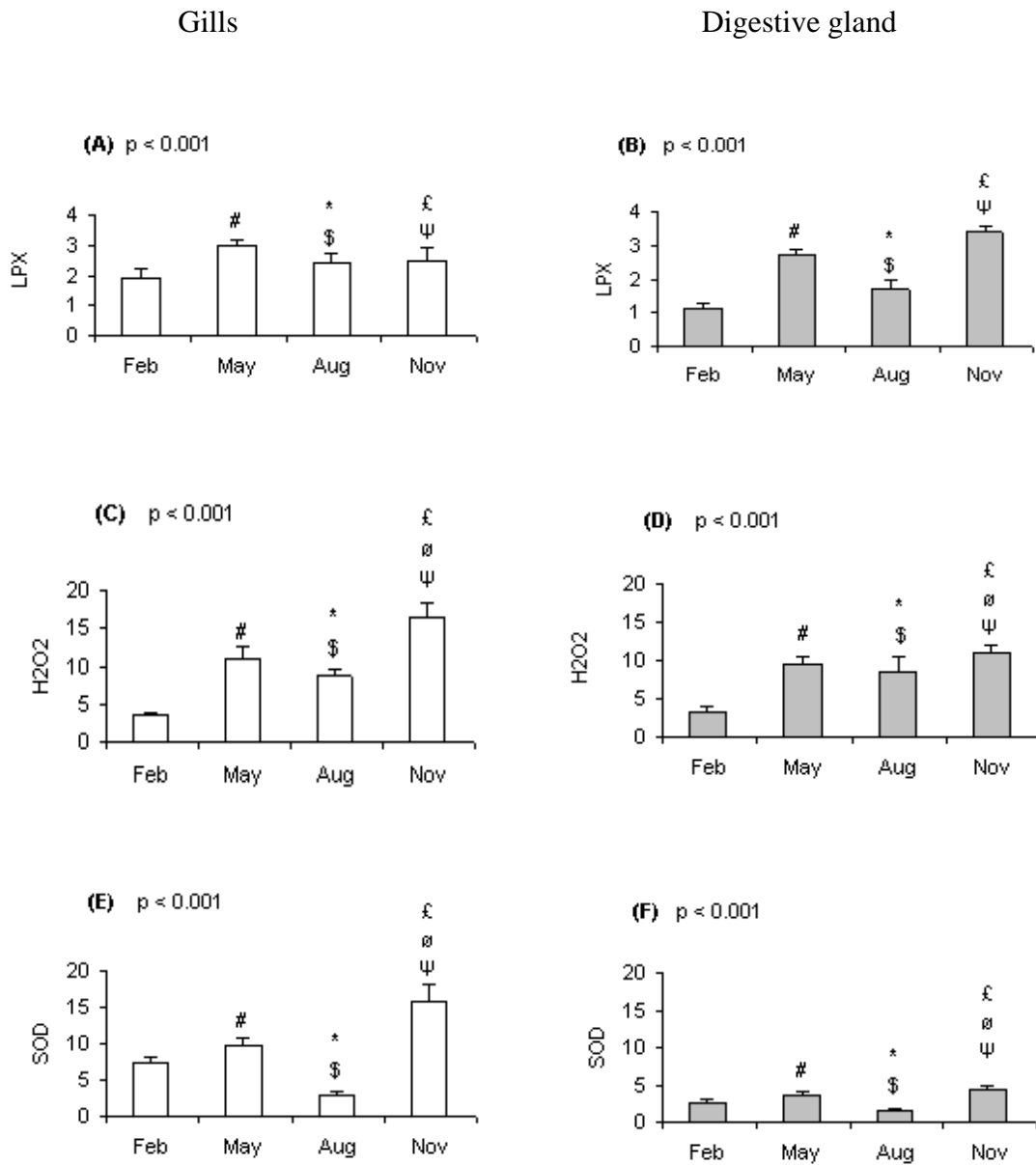
**Fig 1:** - Effect of season on (A & B) LPX (nmol TBARS/ mg protein), (C & D) Hydrogen peroxide content (nmol H<sub>2</sub>O<sub>2</sub>/ mg proteins) (E & F) Superoxide dismutase (Unit/mg protein). The p values are reported for significant variations (ANOVA). Superscript letter (#) denote February compared with May, (\*) denote February compared with August, (£) denote February compared with November, (\$) denote May compared with August, (Ψ) denote May compared with November and (ø) denote August compared with November. Data are expressed as mean  $\pm$  sd (n=5).

**Fig 2:** - Effect of season on (A & B) Catalase activity (nkat / mg protein), (C & D) Glutathione peroxidase (nmol NADPH oxidized / min / mg protein), (E & F) Glutathione -S-transferase (nmol CDNB conjugate formed / min / mg protein). Superscript letter (#) denote February compared with May, (\*) denote February compared with August, (£) denote February compared with November, (\$) denote May compared with August, (Ψ) denote May compared with November and (ø) denote August compared with November. Data are expressed as mean  $\pm$  sd (n=5).

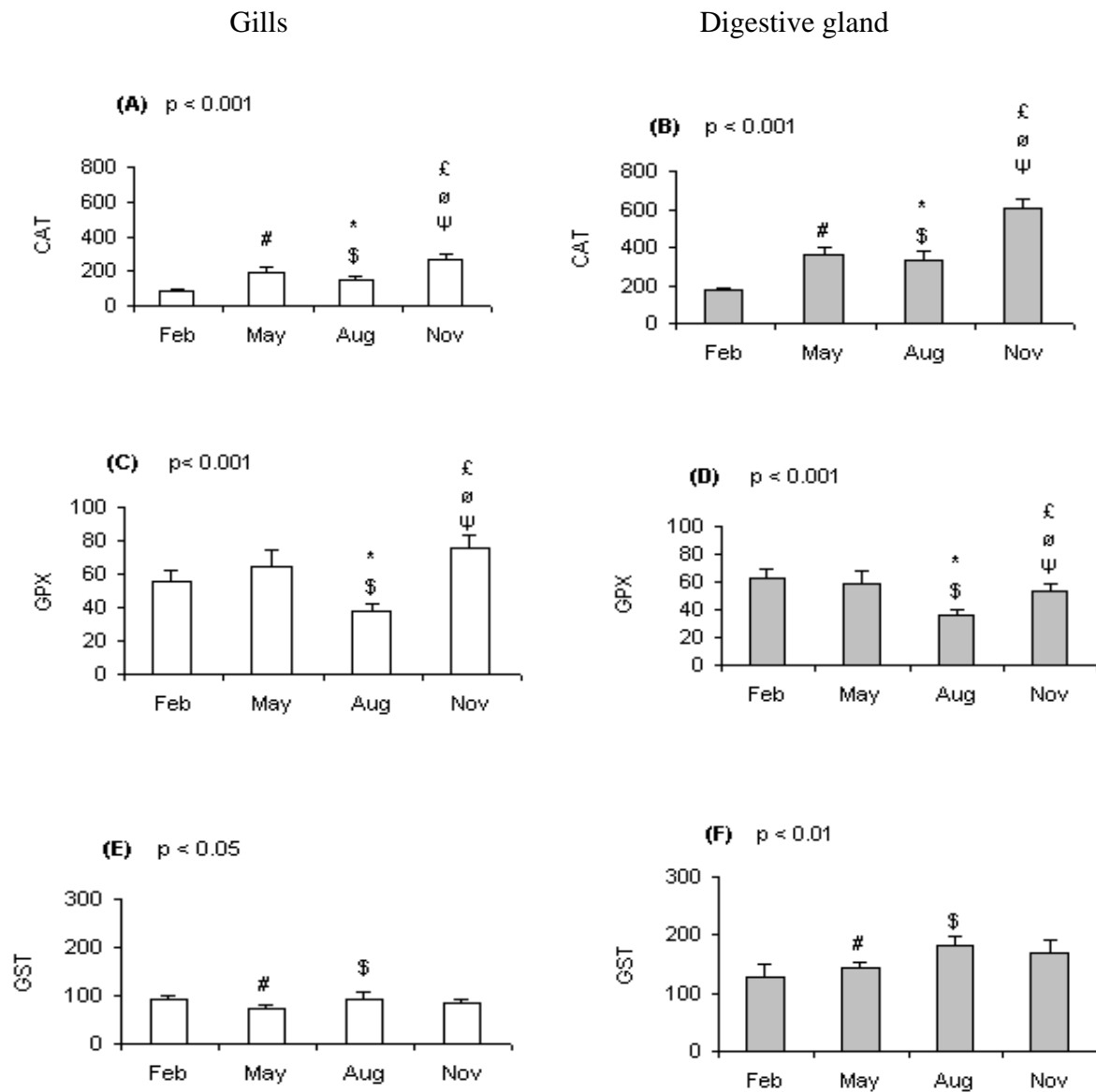
**Fig -3:** - Effect of season on (A & B) Glutathione reductase (nmol NADPH oxidized / min / mg protein) (C & D) Reduced glutathione level ( $\mu$ mol GSH / gm. wt. tissue), (E & F) Ascorbic acid content ( $\mu$ g / gm. wt. tissue). Superscript letter (#) denote February compared with May, (\*) denote February compared with August, (£) denote February compared with November, (\$) denote May compared with August, (Ψ) denote May compared with November and (ø) denote August compared with November. Data are expressed as mean  $\pm$  sd (n=5).

**Fig -4:** - (A & B) Correlation curves between LPX (nmol TBARS / mg protein) level vs H<sub>2</sub>O<sub>2</sub> (nmol / mg protein); (C & D) H<sub>2</sub>O<sub>2</sub> and CAT (nkat / mg protein) + GPX (nmol NADPH oxidized / min / mg protein) in gills and digestive gland of *P. viridis*.

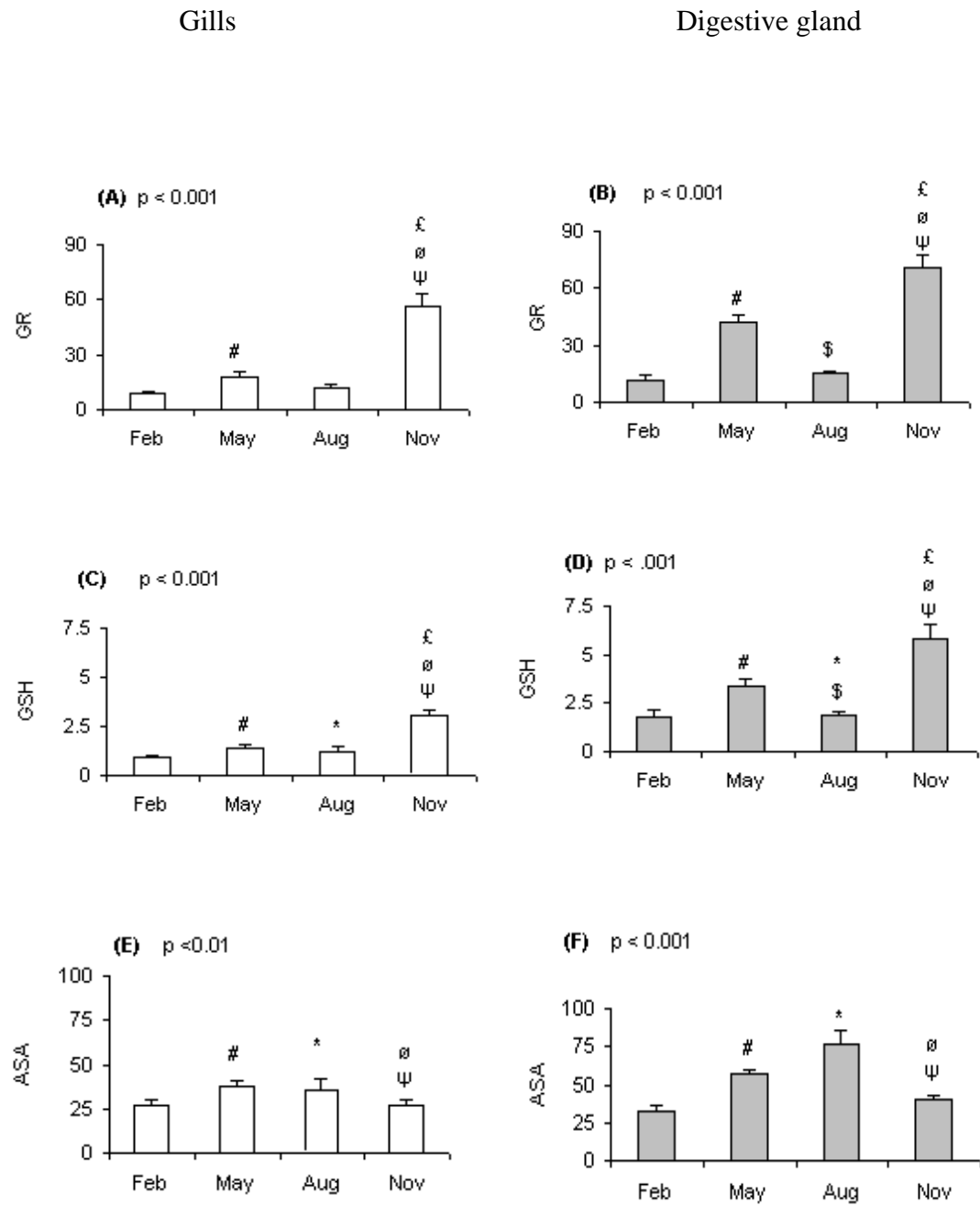
**Fig 1**



**Fig 2**



**Fig -3**



**Fig-4**

