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Use of biomarkers in resident organisms as a tool for environmental monitoring in a cold coastal system, Tierra del Fuego Island

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ABSTRACT

Antioxidant status of *Nacella (P) magellanica* and *Mytilus edulis* related with heavy metal in sediment and tissues were analysed in five stations close to Ushuaia city in winter and spring. The principal component analysis produced a two-dimensional pattern of the degree of similarity between sites. The Industrial–Urban Contamination Index (IUCI) showed that the Industrial Zone (IZ) and Oil Marine Station (OMS) represent areas with anthropic inputs. Heavy metals have differential association with biomarkers depending on the species. In limpets, digestive gland presented major activities of enzyme defence in winter and gonads have shown higher values of Catalase (CAT) during spring while lipid peroxidation (LPO) presented higher values in IZ. For mussels CAT and LPO increased in spring time. For superoxide dismutase (SOD) peaks have been detected in IZ and NW stations for winter. Differences in biomarker responses due to seasons did not influence the grouping of the sites into references and contaminated groups.

1. Introduction

The antioxidant status of an organism gives critical information about the capability to resist environmental stress. Aquatic ones, especially marine bivalves, exhibit variety of changes in enzymatic antioxidant defences due to exposure to pollutants with oxidative potential (Regoli et al., 2002a, b). Prominent, among others, these antioxidant defence systems are superoxide dismutase (SOD) converting the superoxide anion into hydrogen peroxide and catalase (CAT) converting H_2O_2 into water. On the other hand, membranes, lipoproteins and fatty acids contain double bonds, which can be peroxidised in the presence of reactive oxygen species (ROS). The concentration of the resulting lipid peroxidation (LPO) product is a common used measurement of oxidative damage (Solé et al., 2006; Bebianno et al., 2005).

Some molluscs, such as mussels and limpets, are sedentary species prone to the accumulation of pollutants. Particularly, population of these organisms are subjected to a wide variety of environmental stressors, diurnal immersion, emersion cycles; salinity variations; temperature effects; solar and UV irradiation; plus anthropogenic influences due to their location on an impacted area. These characteristics have made them suitable to use extensively in marine monitoring programs concerned with the impact of anthropogenic influences including xenobiotic compounds such as hydrocarbons and heavy metals among other environmental factors mentioned previously (Bocchetti et al., 2008; Gagné et al., 2008; Zanette et al., 2008).

Nacella (Patinigera) magellanica (Gmelin, 1971) and *Mytilus edulis chilensis* (Hupe, 1854) are two conspicuous species which inhabit the intertidal zone along the Beagle Channel, both species being present jointly in the study area. Biological aspects of both species, including reproductive cycles, were well known (see Morriconi, 2005; Tortorelli, 1987).

Both are suitable to use as sentinel organisms due to huge local distribution, easy manipulation, relatively large size, abundance in the study area and sessile habits.

Previous data about heavy metal concentration in sediments and mussels from Ushuaia Bay have been reported by Amin et al. (1996, 1997) and Delucchi (2007). These authors have mentioned the potential sources such as the lack of sewage effluent treatment plant, the discharges from electronic assembling factories in the area and the intensive maritime traffic. Heavy metals reported in the area, such as Cd, Ni, Cr, Pb and Sn are toxic in aquatic organisms mainly because of the oxidative potential, whereas other metals found, such as Fe, Zn and Cu, are essential for their activity in the organisms metabolism but become toxic when their concentrations are in excess.

Antioxidant defence enzymes come from a synergic effect of both, natural and anthropogenic changes and prolonged exposure leads to oxidative damage of basic biological molecules, such as lipid peroxidation (Bebianno et al., 2005).

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Biomarkers have been used extensively as indicators of biological response in laboratory studies in relation to individual contaminants or stressors (Comoglio et al., 2005; Gravato et al., 2005; Richardson et al., 2008). Their inclusion in field surveys of contaminated sites has been increased, where they offer the potential to assess the general health of organisms inhabiting impacted ecosystems (Watson et al., 2004; Pampanin et al., 2005; Flemming et al., 2008; Gagné et al., 2008; Yeats et al., 2008).

Consequently, in ecological quality monitoring programs, considering that organisms are currently being exposed to multiple chemical contaminants, the integration of chemical data with biological responses (biomarkers) is strongly recommended to characterize effects of contaminants to organisms, providing a better picture of the existence of stress situations (Solé, 2000; Barata et al., 2005; Faria et al., 2009).

The present study attempts, according to the knowledge of the environmental condition and species biological cycles, to analyse in two different periods of the year the antioxidant status of selected resident species related with heavy metal concentration in both sediment and tissue samples. The study includes the analysis of antioxidant enzymes involved in detoxifying reactive oxygen species as SOD and CAT and a marker of oxidative tissue damage as LPO in two conspicuous molluscs that inhabit the coastal zone close to Ushuaia city. The results obtained could be useful to describe not only the current status but also to identify areas with potential environmental risk.

2. Materials and methods

2.1. Sampling

Five sampling stations close to Ushuaia city were selected according to their characteristics (Table 1, Fig. 1). Adults of *M. edulis* (mean shell length 56.7 ± 4.6 mm; $N=200$) and *Nacella (P) magellanica* (mean shell length 47.7 ± 5.5 mm; $N=150$) were collected by hand from the intertidal zone of each station, in two seasons: winter (August) and last spring (November) of 2005, respectively. The temperature in the study area is of 5.0 ± 0.6 and 9.7 ± 1.2 °C and the salinity of 32.0 ± 0.2 and 23.0 ± 3.7 for winter and spring, respectively (Amín et al., 2010). On the other hand, notorious differences in daylight between sampling periods selected are evident; ranging between 8.5–10.5 h and 15–17 h in August and November, respectively (Dr. Deferrari, Laboratory of UV and Ozone-CADIC, pers. com.)

The organisms were taken regarding similar sampling conditions such as time and low tide, giving an equivalent situation between samples. The organisms were transported to the laboratory in ice cold containers, within half an hour of collection.

Simultaneously, intertidal sediment was sampled to determine total heavy metals concentrations.

2.2. Preparation of tissue extracts and biochemical determinations

For *N. magellanica* digestive gland and gonad were dissected while for *M. edulis* digestive gland, gills and mantle-gonad tissue were selected for study. It is important to remark that in *M. edulis* the gonads are largely located within the extensive folds of the mantle (Gabbott and Peek, 1991), so that are hereinafter mentioned as a mantle-gonad tissue.

For *N. magellanica* five organisms, for each season and time, were used for all biochemical determinations, taking subsamples for both LPO and enzymatic determinations. In *M. edulis* attempted to weigh of each tissue required, for each season and time, five organisms were used for LPO and another five for enzymatic determinations ($N=50$ and $N=100$ for *N. magellanica* and *M. edulis*, respectively). The organisms were immediately dissected and homogenised in cold conditions; the supernatants were removed and frozen until biochemical determination performed.

For enzymatic evaluation of Catalase activity (CAT, EC 1.11.1.6) and Superoxide dismutase (SOD, EC 1.15.1.1), samples were homogenised in 1:3 (w/v) Tris-buffer pH 7.6 (20 mM Tris, 1 mM EDTA, 1 mM DTT, 500 mM sucrose, 150 mM KCl and 0.1 mM PMSF). Later, homogenates were centrifuged at 8000 rpm for 30 min and the supernatants were removed for further analysis.

CAT activity was determined by the decrease in absorbance due to H_2O_2 consumption at 240 nm (Beutler, 1982). Activity was expressed as μmol of H_2O_2 per minute per milligram of protein, or U mg^{-1}. SOD activity was measured by the inhibition of the auto-oxidation of epinephrine at 480 nm (Misra and Fridovich, 1972).

Table 1
Brief description of sample sites.

Sample site	Reference map	Location	Description
Industrial Zone	IZ	54°47.715S 68°15.724W	Untreated domestic and industrial waste. Storm water runoff
Oil Marine Station	OMS	54°48.269S 68°17.514W	Next to oil gas marine station
Nautical Wharf	NW	54°48.879S 68°18.401W	Influenced by diffuse urban input and storm water runoff. Shipping area
Ushuaia Peninsula	UP	54°50.753S 68°19.347W	Next to marine pipeline, where wastewater is released untreated to the coast
Golondrina Bay	GB	54°50.542S 68°22.108W	Moderate–low urban influence

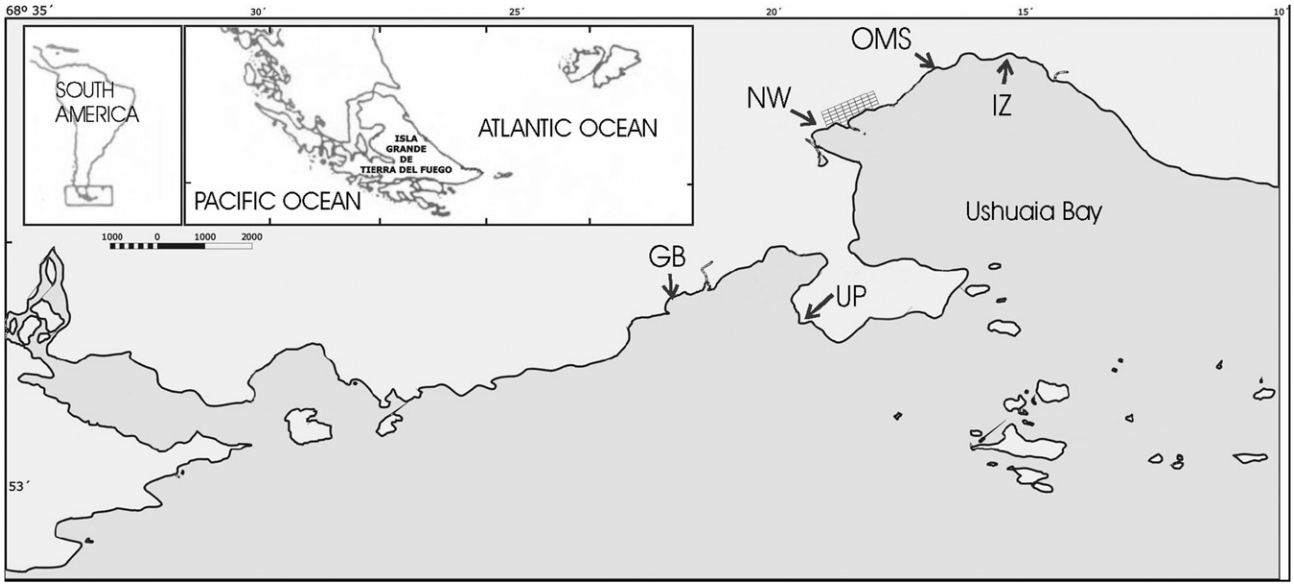

Fig. 1. Study area and sampling locations on Ushuaia city coastline. Refer to Table 1 for site descriptions.

One unit of SOD is defined as the amount of enzyme inhibiting the rate of by 50%. Results were expressed as units per milligram of proteins (U mg^{-1}).

For lipid peroxidation (LPO), 0.1 M Tris-buffer pH 7.8 was used in relation 1:3 (w/v) for homogenisation, adding BHT at a final concentration of 0.01% w/v and then samples were centrifuged for 10 min at 9000 rpm and the supernatants were kept for further analysis. LPO was estimated by the formation of thiobarbituric acid reactive species (TBARS) and quantified in terms of malondialdehyde (MDA) equivalents according to Beuge and Aust (1978) and expressed as nanomoles of MDA formed per mg protein.

In order to quantify SOD, CAT activity and LPO, soluble protein content was measured according to Markwell et al. (1978), reading the optical density at 750 nm in spectrophotometer (Lambda 25) using bovine albumin as standard.

2.3. Heavy metal analysis

For heavy metals determination, superficial sediments (taken by plastic spoon from approximately less than 5 cm depth) and pool of 10 organisms were obtained for each location and time. Once in the laboratory, samples were dried at approximately 50 °C until constant weight. Pooled samples of total body organisms were homogenised using a porcelain mortar and stored in polyethylene bags until analysis. Aliquots of about 0.5 g were taken from the well-homogenised sample for the analysis described below. On the other hand sediment was sieved using the smallest fraction (<62 μm) for analysis. For both types of sample total metal concentrations (Cd, Zn, Cu, Pb and Fe, plus Cr and Ni for sediments) were determined following the method described by Marcovecchio and Ferrer (2005). This technique includes a sediment and soft tissue mineralisation using a strong acid mixture ($HClO_4$:HNO_3, 1:3 v/v) under a controlled temperature glycerine bath (110 ± 5 °C). The residue, less than 1 ml, was diluted in 0.7% (v/v) HNO_3 up to 10 ml. Metal concentrations in this extract were measured using Atomic Absorption Spectroscopy (Perkin Elmer 2380) with air–acetylene flame. Analytical quality (AQ) was checked against certified reference materials from NIES, Tsukuba (Japan). In all cases each sample was run by duplicate. Results are expressed as micrograms per gram dry weight.

2.4. Statistical analyses

To detect significant differences among sites and periods, one-way analysis of variance (ANOVA) was employed after confirmation of normality and homogeneity of variance by Shapiro–Wilk *W* and Bartlett *Chi*-square test, respectively. When ANOVA revealed statistically significant differences among groups, a posteriori comparisons (least significant differences, LSD) were used to identify which groups differed from each other. Data that were not normally distributed were evaluated using a Kruskal–Wallis test and multiple comparisons of Dunn's test (Daniel, 1978). For all analyses p was set at 0.05 as detailed in Sokal and Rohlf (1981).

The relationship between biomarker responses and levels of trace metals measured in organisms and environment was examined by principal components analysis (PCA). All statistical analyses were performed using STATISTICA (Statsoft) software version 6.0.

3. Results

3.1. Heavy metals

Selected heavy metals (Cd, Zn, Cu, Cr, Pb, Ni and Fe) were found in all sediment samples from the studied area (Table 2). No differences were detected among sites for Cd, Fe, Cr and Ni concentrations, while for the others significant differences were found (ANOVA for Pb, Cu, Zn, Cr and Ni; Kruskal–Wallis for Fe and Cd; $p<0.05$) In order to describe the jointly behaviour of Cu, Zn and Pb, an Industrial–Urban Contamination Index (Amat Infante et al., 2006) was calculated as follows: IUCI=$\sum$[Cu]+[Zn]+[Pb], taking into account that these metals came from principally anthropic activities (Papakostidis et al., 1975). Stations IZ and OMS showed the higher values of Zn, Cu and Pb, which presented significant high correlations among them (Table 3), and consequently the higher IUCI values. No significant differences were detected in the other metals analysed among stations.

The principal component analysis (PCA) of metals contents in sediments identified two composite variables which explained 72.0% of the total variance (Table 4). The first factor with 44.09% of explained variance is represented by the presence of Pb, Cu, Zn and Fe. The second factor is assigned to Ni, Cr and Cd and explains the 27.9% of the total variance. The PCA produced a two-dimensional pattern of the degree of similarity between the five sites (Fig. 2). A first group conformed by stations IZ and OMS, characterised by the presence of Pb, Cu, Zn and Fe (Factor 1); a second group conformed by UP and GB and finally station NW that presented a different pattern in the two sampling times.

Concentrations of heavy metals in organisms are presented in Table 5. The concentrations of Cd, Cu and Pb for both studied species were in the same order of magnitude and ranged within values considered as low–moderate pollution. The Fe concentrations were 10 times higher in *N. magellanica* and Zn concentrations were 2 times higher in *M. edulis*.

Table 3
Pearson's correlation coefficients between metal concentrations in sediments.

	Cd	Pb	Cu	Zn	Fe	Cr	Ni
Cd	1.00						
Pb	−0.34	1.00					
Cu	0.17	***0.77***	1.00				
Zn	−0.36	***0.87***	***0.70***	1.00			
Fe	0.14	0.28	0.56	0.52	1.00		
Cr	0.18	−0.01	0.37	0.14	0.65	1.00	
Ni	0.20	−0.11	0.26	−0.02	0.32	0.43	1.00

Bold data indicate significance at $p<0.05$ level.

Table 4
Factor analysis for heavy metal concentrations in sediments, based on correlations (principal components analysis).

Heavy metal	Factor 1	Factor 2
Cd	−0.09	**0.68**
Pb	**0.79**	−0.55
Cu	**0.90**	0.05
Zn	**0.87**	−0.41
Fe	**0.75**	0.41
Cr	0.49	**0.66**
Ni	0.27	**0.66**
% of total variance	*44.09*	*27.93*
Eigenvalues	*3.09*	*1.96*

Bold data indicate significance at $p<0.05$ level.

Table 2
Heavy metal concentrations in sediments and Industrial and Urban Contamination Index (IUCI).

Stations	Cd (μg g^{-1})	Fe (mg g^{-1})	Cr (μg g^{-1})	Ni (μg g^{-1})	Pb (μg g^{-1})	Cu (μg g^{-1})	Zn (μg g^{-1})	IUCI
IZ	1.6 ± 1.17	26.19 ± 0.69	18.72 ± 1.64	20.79 ± 0.28	35.81 ± 3.00a	32.04 ± 2.03ab	96.22 ± 5.32a	**164.07**
OMS	1.17 ± 0.01	24.34 ± 0.04	20.29 ± 6.26	19.64 ± 0.68	34.31 ± 0.89a	33.32 ± 2.13a	90.47 ± 8.92ab	**158.09**
NW	2.14 ± 1.62	20.05 ± 5.79	14.11 ± 9.77	21.53 ± 9.06	28.48 ± 1.69ab	26.56 ± 10.79abc	64.33 ± 2.14b	**119.37**
UP	2.05 ± 1.96	21.57 ± 0.54	17.63 ± 2.62	22.62 ± 5.51	21.59 ± 6.29b	19.47 ± 2.09bc	65.18 ± 20.11b	**106.24**
GB	0.74 ± 0.07	23.80 ± 4.04	20.78 ± 1.28	21.22 ± 0.40	24.23 ± 1.77b	18.76 ± 18.76c	63.82 ± 63.82b	**106.81**

Values are expressed as mean ± S.D. of two samples periods. Stations with different letter for each metal indicate significant differences among them. ANOVA (for Cr, Ni, Pb, Cu and Zn) and Kruskal–Wallis (for Fe and Cd), $p<0.05$.

For *N. magellanica* significant differences in Cu, Pb and Fe concentrations were detected among sampling sites, OMS and NW being the stations with highest values of Cu and Pb, respectively ($p < 0.05$). On the other hand, for *M. edulis*, Zn, Cd and Cu concentrations presented significant differences among some sites. The highest value of Zn corresponded to organisms from NW and OMS, in this last station the highest value of Cu was present too (ANOVA, $p < 0.05$).

No correlation was present among metals in limpets. In mussels positive correlation between Cu and Zn in tissue was detected ($r = 0.86$) and negative between Cu and Pb ($r = -0.72$). Otherwise no correlation was noted among metals in sediments and tissues for both species (Pearson's correlation).

3.2. Biomarkers in limpets

The values determined of studied biomarkers in limpets are shown in Fig. 3. Significant differences among sampling sites are also included in the figure (ANOVA, LSD test, $p < 0.05$). For each biomarker the values obtained for both tissues studied were in the same order of magnitude.

The CAT activity in digestive gland resulted higher in OMS, NW and IZ stations, being major during winter than spring time for all stations (mean value = 21.6 and 12.9 U mg protein^{-1} for winter and spring, respectively). In gonads, the minor values of CAT activity corresponded to NW for both seasons studied (2.8 and 10.3 U mg protein^{-1} for winter and spring, respectively), while the higher values were detected in IZ, also for both periods (mean value 23.2 U mg protein^{-1}). As a tendency, higher values of CAT activity in gonad corresponded to spring time for all stations (Fig. 3a).

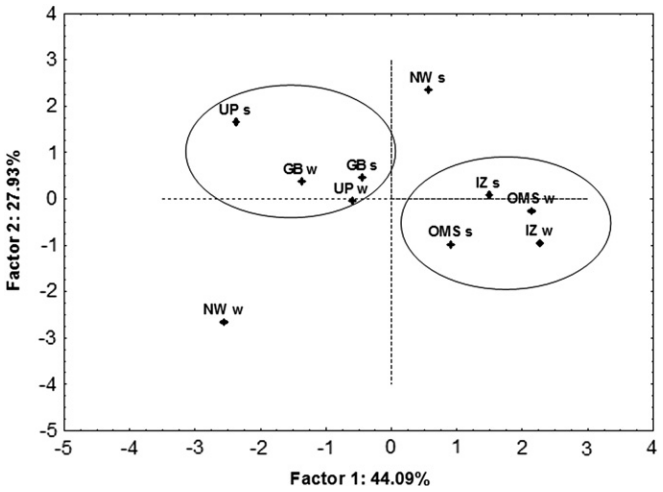

Fig. 2. Heavy metals in sediments. Plots of scores (mean values for each sampling site) from Factor Analysis (Factor 1 and Factor 2, principal components extraction, and % of explained variance). Lower cases **s** and **w** indicate spring and winter sampling, respectively, for each site.

The SOD activity in digestive gland tissue was higher in NW followed by OMS for both seasons (67.4 and 36.4 U mg protein^{-1} for winter and 30.5 and 22.0 U mg protein^{-1} for spring in NW and OMS, respectively), being, as a tendency, the values detected in winter higher than spring period. The station IZ showed the minimum values of SOD activity (1.3 and 8.3 U mg protein^{-1} for winter and spring, respectively). In gonad the activity presented a different pattern, the organisms from IZ and OMS being those which presented major values during spring (41.3 and 74.2 U mg protein^{-1}, respectively). In winter the higher values were detected in NW, UP and GB (mean value = 64.6 ± 3.0 U mg protein^{-1}) (Fig. 3b).

The LPO in limpets were in similar range for both seasons and tissues studied, with the exception of IZ station which showed the highest values during winter for both tissues (9.5 and 4.5 nmol MDA mg protein^{-1} for gonad and digestive gland, respectively). During spring the higher values detected correspond to UP for both tissues too (5.6 and 3.6 nmol MDA mg protein^{-1} for gonad and digestive gland, respectively) (Fig. 3c).

In general, for digestive gland tissue major activities of enzyme defence were detected during winter for all stations with slight differences. A different pattern was observed in gonad where CAT activity showed higher values during spring time, while SOD activity showed a response related to sample site. LPO had homogeneous values in all organs and period surveys, with the exception detected in IZ where the higher values were registered.

3.3. Biomarkers in mussels

In Fig. 4 the results of the biomarkers analysed in mussels are shown. Significant differences among sampling sites are also included in the figure (ANOVA and LSD test for CAT and LPO; Kruskal–Wallis and multiple comparisons of Dunn for SOD; $p < 0.05$). For each biomarker, the values obtained for the tissues studied were in the same order of magnitude.

The CAT activity for digestive gland and mantle-gonad was in the same order of magnitude, being higher during spring time. Particularly in mantle-gonad low activity was detected in UP during spring (5.6 U mg protein^{-1}) while similar low values were observed in all stations for winter season, with a minimum in GB (2.3 U mg protein^{-1}). In digestive gland no significant differences in CAT activity values were detected during spring

Table 5
Heavy metal concentrations in soft tissues of *N. magellanica* and *M. edulis* (μg g^{-1} dry weight). Values are expressed as mean ± S.D. of two samples periods. Stations with different letter for each metal and species indicate significant differences among them (ANOVA, followed by LSD test, $p < 0.05$).

	Cd	Cu	Pb	Zn	Fe
N. magellanica					
IZ	1.73 ± 0.44a	7.51 ± 1.48c	3.09 ± 0.01a	70.08 ± 5.76a	1934.47 ± 288.70ab
OMS	1.05 ± 0.17a	6.64 ± 0.31bc	5.91 ± 0.99b	102.63 ± 6.35a	2026.70 ± 793.52ab
NW	0.93 ± 0.43a	7.56 ± 0.61c	5.91 ± 0.99b	78.74 ± 6.09a	2879.33 ± 417.75bc
UP	2.71 ± 1.67a	3.93 ± 0.22a	3.79 ± 1.02ab	73.99 ± 22.40a	1636.28 ± 518.70a
GB	2.63 ± 1.28a	5.11 ± 0.61ab	5.20 ± 1.01ab	75.01 ± 24.80a	3822.41 ± 87.32c
M. edulis					
IZ	1.08 ± 0.40ab	3.50 ± 0.03a	5.97 ± 0.01a	172.08 ± 65.05a	251.93 ± 41.64a
OMS	1.27 ± 0.04ab	8.09 ± 1.77b	2.53 ± 1.61a	390.50 ± 36.87b	243.47 ± 29.85a
NW	0.51 ± 0.27a	4.14 ± 1.17a	3.81 ± 2.35a	236.82 ± 172.33ab	182.56 ± 153.80a
UP	1.15 ± 0.31ab	3.52 ± 1.18a	4.09 ± 1.58a	128.95 ± 3088a	133.29 ± 60.82a
GB	1.60 ± 0.60b	3.80 ± 0.19a	3.70 ± 1.11a	121.80 ± 1.41a	199.31 ± 145.13a

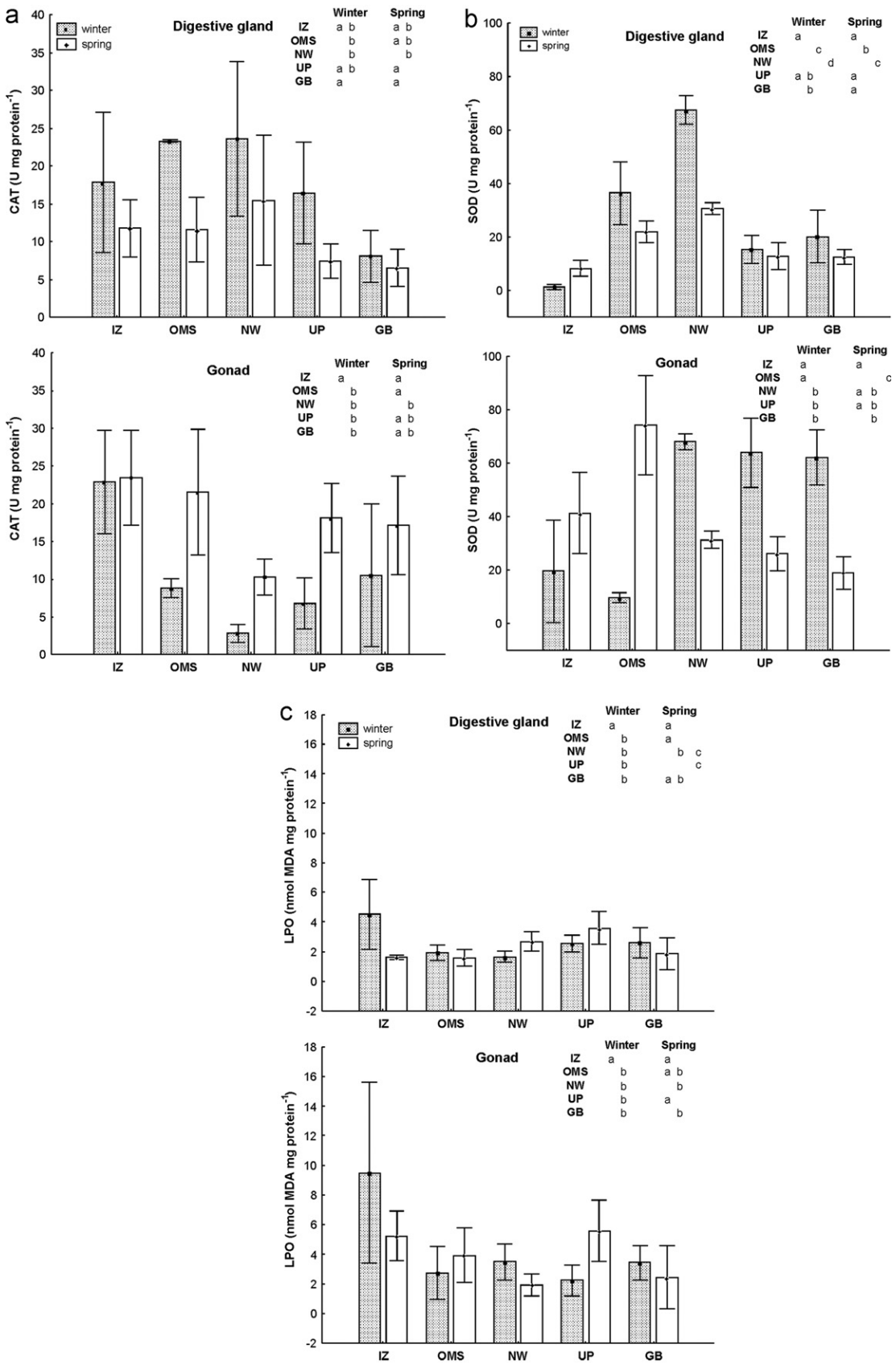

Fig. 3. Biomarkers in limpet's *N. magellanica* in digestive gland and gonad tissues (mean values ± standard deviations, $n=5$). Stations sharing different letters for each sampling period indicate significant differences (ANOVA and LSD test; $p<0.05$): (a) catalase activity (CAT); (b) superoxide dismutase activity (SOD) and (c) lipid peroxidation (LPO).

for all stations and for winter period a lower value was registered in UP (4.2 U mg protein^{-1}). On the other hand, a less defined tendency relating sampling-season in gills could be observed, presenting IZ the maximum values of CAT activity recorded for both sampling periods (26.4 and 15.6 U mg protein^{-1} for winter and spring, respectively), and the winter value being the highest recorded (Fig. 4a).

No significant differences were detected in SOD activity for both tissues analysed during spring time (mean value 3.6 and 2.4 U mg protein^{-1} for mantle-gonad and digestive gland, respectively).

In winter some maximum peaks were recorded for IZ in both tissues (18.0 and 6.3 U mg protein^{-1} for mantle-gonad and digestive gland, respectively) (Fig. 4b).

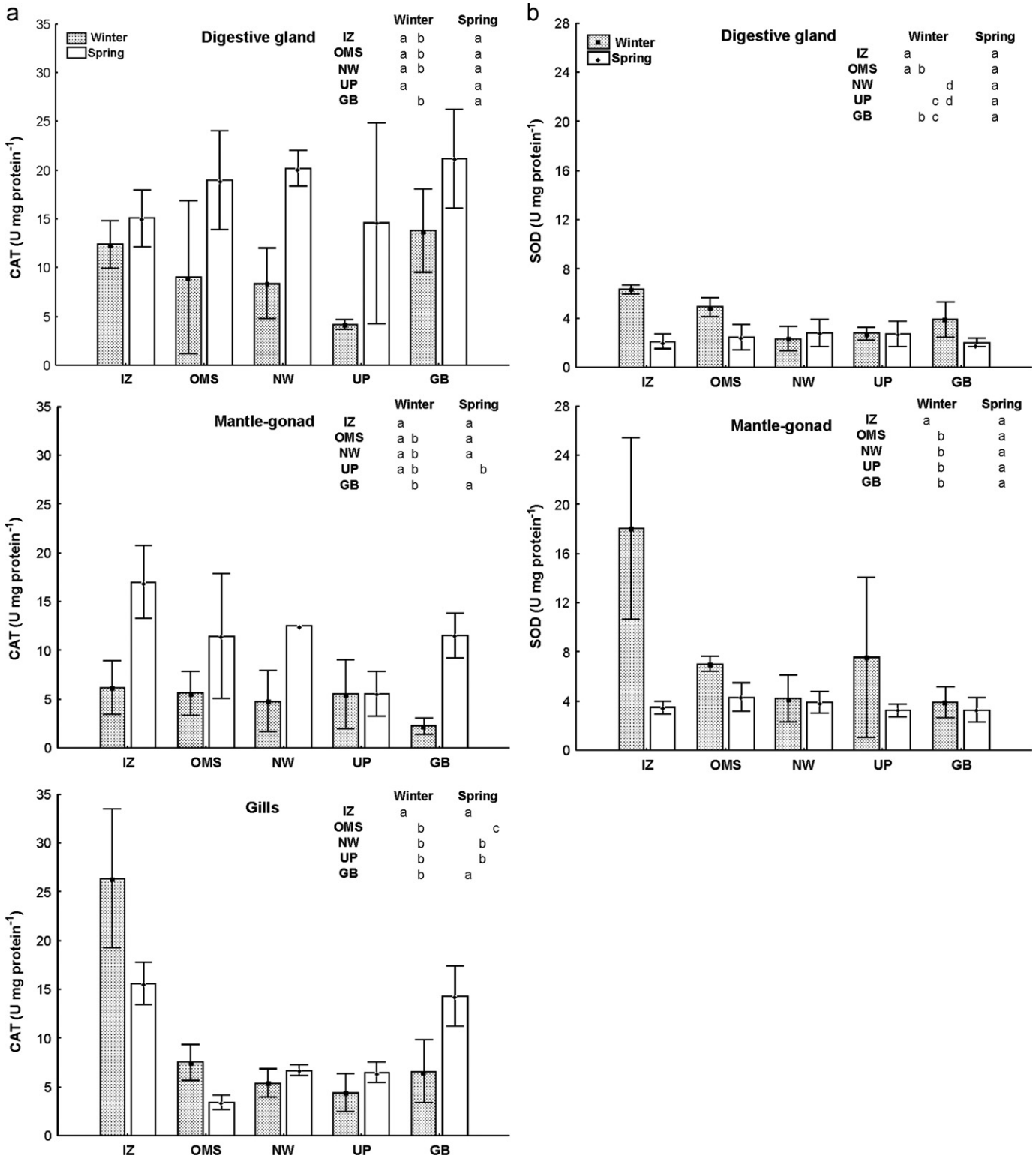

Fig. 4. Biomarkers in mussel's *M. edulis* in digestive gland, mantle-gonad and gills tissues (mean values ± standard deviations, $n=5$). Stations sharing different letters for each sampling period indicate significant differences (ANOVA and LSD test for CAT and LPO; Kruskal–Wallis and multiple comparisons of Dunn for SOD; $p<0.05$): (a) catalase activity (CAT); (b) superoxide dismutase activity (SOD) and (c) lipid peroxidation (LPO).

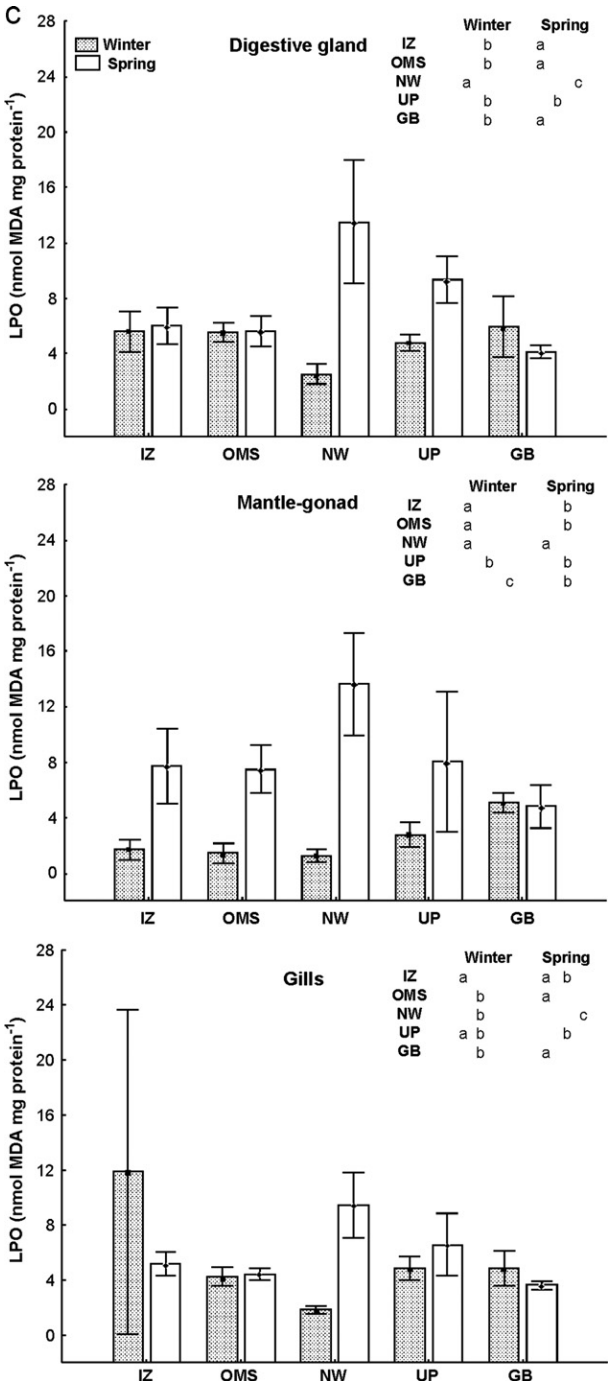

Fig. 4. (*Continued*)

LPO showed a trend of major activity in spring period for all tissues, NW being the highest one among them (13.6, 13.5 and 9.4 nmol MDA mg protein^{-1} for mantle-gonad, digestive gland and gills, respectively).

For winter, the data was slightly different but a major activity in mantle-gonad corresponding to UP and GB could be detected (2.8 and 5.1 nmol MDA mg protein^{-1}, respectively) and a peak for gills in organisms provided from IZ station (11.9 nmol MDA mg protein^{-1}) (Fig. 4c).

In the present study it could be observed for mussels that CAT and LPO values increased in spring time. For SOD a homogeneous response has been observed along sampling seasons for each organ. Furthermore, peaks, when these existed, were detected in IZ and NW stations for winter period.

3.4. Factorial PCA multivariate analysis

The extracted factors of PCA based on correlations for limpets are shown in Table 6. The first factor that accounts with 32.8% of the total variance is represented negatively by the biomarkers CAT and LPO in gonads with metals in sediments. The second factor that accounts with 24.5% of the total variance is represented by the enzyme defence system in digestive gland (positively for CAT and SOD) and Cu concentration in tissues. The third factor (14.9%) is represented by the damage in digestive gland (LPO) and negatively with concentration of Zn, PB and Fe in tissues.

The projection of the cases on the factor plane (Fig. 5) showed that stations OMS and IZ are plotted to the left side of this first axis, represented by negative coordinate values for the first factor. This

Table 6
Nacella magellanica. Factor analysis for heavy metal concentrations in sediments and tissues and biomarkers in tissues, based on correlations (principal components analysis).

	Factor 1	Factor 2	Factor 3
$SOD_{digestive\ gland}$	0.58	**0.77**	0.03
SOD_{gonad}	0.46	0.14	0.28
$LPO_{digestive\ gland}$	−0.38	−0.41	**0.51**
LPO_{gonad}	**−0.72**	−0.22	0.39
$CAT_{digestive\ gland}$	−0.13	**0.89**	0.36
CAT_{gonad}	**−0.72**	−0.49	−0.30
Cu_{tissue}	−0.48	**0.62**	−0.07
Pb_{tissue}	0.46	0.43	**−0.50**
Zn_{tissue}	−0.04	0.26	**−0.68**
Fe_{tissue}	0.46	−0.26	**−0.53**
$Pb_{sediment}$	**−0.69**	0.62	0.03
$Cu_{sediment}$	**−0.72**	0.46	−0.28
$Zn_{sediment}$	**−0.82**	0.38	0.01
$Fe_{sediment}$	**−0.71**	−0.33	−0.55
% Of total variance	32.79	24.54	14.94
Eigenvalues	4.59	3.4	2.09

Bold data indicate significance at $p < 0.05$ level.

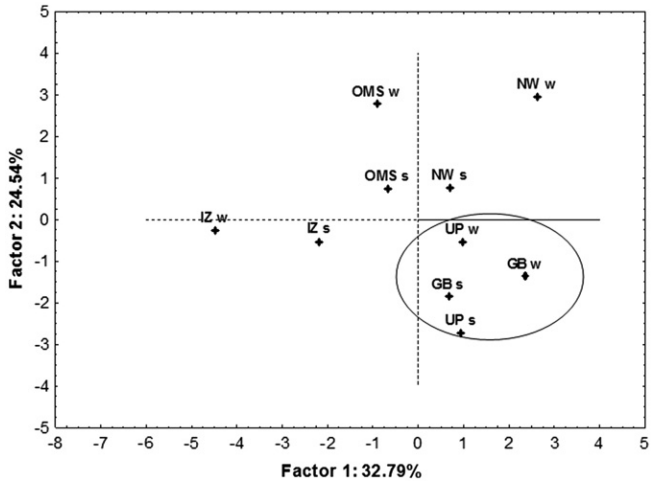

Fig. 5. Principal components analysis biplot of biomarkers in *N. magellanica* and heavy metal data in the coordinates of the first two principal components (Factor 1 and Factor 2, % of explained variance). Spatial and temporal separation of sampling sites is shown. Lower cases **s** and **w** indicate spring and winter, respectively, for each site.

Table 7
Mytilus edulis. Factor analysis for heavy metal concentrations in sediments and tissues and biomarkers in tissues, based on correlations (principal components analysis).

	Factor 1	Factor 2	Factor 3
$SOD_{mantle\text{-}gonad}$	**−0.85**	−0.06	0.40
$SOD_{digestive\ gland}$	**−0.76**	−0.09	0.39
$LPO_{mantle\text{-}gonad}$	0.36	**0.81**	−0.39
$LPO_{digestive\ gland}$	0.15	**0.82**	−0.08
LPO_{gills}	−0.56	**0.65**	0.24
$CAT_{mantle\text{-}gonad}$	−0.12	0.52	**−0.69**
CAT_{gland}	0.13	0.55	**−0.62**
CAT_{gills}	**−0.78**	0.25	0.14
Cu_{tissue}	−0.04	−0.51	**−0.71**
Pb_{tissue}	−0.47	**0.62**	0.48
Zn_{tissue}	−0.24	**−0.75**	−0.48
Fe_{tissue}	−0.42	−0.53	−0.33
$Pb_{sediment}$	**−0.83**	−0.23	−0.27
$Cu_{sediment}$	**−0.72**	0.26	−0.45
$Zn_{sediment}$	**−0.88**	−0.18	−0.24
$Fe_{sediment}$	−0.51	0.54	−0.41
% Of total variance	31.92	26.97	18.69
Eigenvalues	5.11	4.32	2.992

Bold data indicate significance at $p < 0.05$ level.

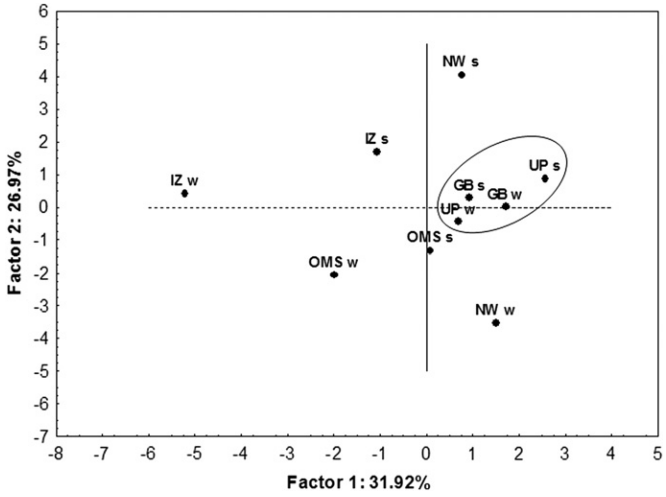

Fig. 6. Principal component analysis biplot of biomarkers in *M. edulis* and heavy metal data in the coordinates of the first two principal components (Factor 1 and Factor 2, % of explain variance). Spatial and temporal separation of sampling sites is shown. Lower cases **s** and **w** indicate spring and winter, respectively, for each site.

could be indicative that these stations can be characterised by the presence of heavy metals in sediments and the activity of CAT and LPO in gonads. Besides, OMS jointly to NW are positively correlated with factor 2, indicating the presence of Cu in tissues and enzymatic biomarkers in digestive gland. On the opposite side UP and GB stations have conformed another group representing stations to minor heavy metals concentrations in sediments.

On the other hand, Table 7 shows the extracted factors of PCA for mussels. The first factor that account with 31.9% of the total variance is represented by the SOD in mantle-gonad and digestive gland and CAT in gill (defence system) with the presence of Pb, Cu and Zn in sediments, all of them with negative correlation values. The second factor that accounts with 27.0% of the total variance is represented by LPO (damage system) in all tissues with Pb concentration (positively) and Zn concentration (negatively) both in tissues. The third factor (18.7% of the total variance) is represented by defence system CAT in digestive gland and gill with Cu concentration in tissues, all with negative correlation.

The projection of the cases on the factor plane showed that UP and GB have conformed group in the positive axis of factor 1, related with lower values of heavy metals in sediments, meanwhile the other stations are separated and disperse (Fig. 6).

4. Discussion

4.1. Metals in sediments and organisms

The levels of heavy metals in sediments in the present study were compared with those previously reported for other environments and/or baseline levels (Table 8). At this respect, comparing ours results with the elemental background compositions, surface sediments were observed to be not contaminated with Cr and Ni since its levels are comparable to unpolluted areas. The other elements, Cd, Cu, Pb and Zn, appear with a signal of anthropogenic

Table 8
Heavy metal concentrations in the present study area and other surrounding regions in sediment samples also including data from unpolluted sediments (< 62 μm fraction). Data represented minimum and maximum values, expressed in $\mu g\ g^{-1}$ dry weight.

Region	Cd	Cr	Cu	Pb	Zn	Ni	Reference
Ushuaia Bays (Argentina)	0.7–3.4	7–24	17.9–34.8	17.1–37.9	50.9–99.9	15.1–27.9	Present study
San Vicente Bay (Chile)	0.3	20	19.5	10	60	25	Aguirre-Martínez et al. (2009)
Florianopolis (Brazil)	0.07–0.11	11–25.1	22.7–28	42–43	94.2–114	NDA	Rivail Da Silva et al. (1996)
Black Sea Bays	0.02–0.93	10.8–115.5	4–95.5	0.05–31.1	33.9–267.4	13.5–65.2	Topcuoğlu et al. (2002)
Izmit Bay (Turkey)	2.5–9.5	38.9–112.4	24.5–102.4	55.2–172	440–1900	NDA	Pekey (2006)
Izmir Bay (Turkey)	0.22–0.42	208–308	32–70	36–62	99–260	NDA	Atgın (2000)
California (Mexico—USA)	0.08–0.64	55.9–802	4.88–17.2	5.96–21.3	39–188	15.5–44.4	Villaescusa-Celaya et al. (2000)
Yangtze Estuary (China)	NDA	NDA	9–84	7–38	41–550	21–133	Zhang et al. (2007)
Quanzhou Bay (China)	0.28–0.89	51.1–121.7	24.8–119.7	34.3–100.9	105.5–241.9	16.1–45.7	Yu et al. (2008)
Ria de Vigo (Spain)[a]	3.03 (± 1.74)	52.58 (± 29.51)	42.44 (± 17.07)	89.09 (± 40.95)	158.38 (± 86.34)	33.77 (± 5.86)	Rubio et al. (2000)
Continental rocks	0.2	71	34	19	127	49	Chester (1996)
Unpolluted sediments	0.01–0.2	30	5–25	5–25	20–100	10–20	Bryan and Langston (1992)

NDA: No data available.

[a] Average (± standard deviation).

inputs, even compared with other regions. Iron, as was reported previously (Amin et al., 1996), is the element with the highest concentration in all samples studied.

In spite of the variability of heavy metal concentrations in sediments, a spatial distribution draw was defined. At this respect the IUCI, which assumes that the urban and industrial contamination is referred to Cu, Pb, and Zn concentrations, showed that IZ and OMS stations are representing areas with this profile of inputs. In these stations the IUCI reveals that, the presence of metals were greater than the considered background levels; so these sites could be defined as moderately contaminated (see Table 1). This result is in agreement with the literature (Förstner and Whitman, 1979; Amat Infante et al., 2006) who reported that the inputs of these metals are associated with urban inputs. Additionally other contaminants such as PAH's and PCB's have been detected in OMS (Amin et al., in press), besides, hydrological conditions reflect that Ushuaia bay is influenced by mixed inputs coming from moderate urban impacts (Amín et al., 2010).

In the same context, the PCA confirms that there are two differential groups of stations, one with higher urban impact as OMS and IZ and the other with lower impact as UP and GB. Station NW presented differences between sampling periods probably due to be close to the denser and older urban emplacement, receiving a huge variety of types of inputs without treatment.

On the other hand the heavy metal concentrations in studied organisms are in the same range of magnitude with those reported by Gil et al. (2006) for molluscs from Patagonia region who describe this area as uncontaminated site by heavy metals. Particularly, levels on mussels from our results could be comparable with mean range of concentration data reported for the soft tissue of *Mytilus* species all over the world (see Szefer et al., 2004). The data are within the values corresponded to uncontaminated or moderately contaminated sites (Grimalt, 1989; Green and Knutzen, 2003), and according to WHO (1982) levels of Cd, Zn and Cu are closely to maximum permissible for food human consumption.

The lack of metal's correlation between sediments and organisms can be attributed to different reasons such as feeding habits, the wide variety of environmental stresses plus anthropogenic influences and/or the dynamics of metals in the sediment, which is influenced most notably by the pH and redox potential (Cacador et al., 2000; Sundareshwar et al., 2003). Previous studies have reported the presence of a differential geochemical distribution in trace metals along the present study area, which are strongly associated with pollution processes (Amin et al., 1997). As was pointed out by Lau et al. (1998) the accumulation of metals in molluscs would most likely be associated with the bioavailability of the metals in the sediment rather than with the total metal in the sediment. Regarding that *M. edulis* is a filter feeder and *N. magellanica* has a grazing habits, our results are in agree with those have been observed by Astorga España et al. (2007) for *M. chilensis* and *Perunytitus purpuratus*, who supported that the different feeding habits, abilities in the metal regulation of each species and seasonality could influence the metal contents in the organisms analysed.

4.2. Biomarkers in limpets

Morriconi (1999, 2005) reported that *N. magellanica* presented the maximum percentage of individuals in developing stage during winter, spawning in spring–summer period.

Some authors have established, for limpets species, that the storage of reserves substances (glycogen and lipid) in the digestive gland is specifically related to reproduction, being transfer from these sites to the gonads (Santini and Chelazzi, 1995; Simpson, 1982). So that, the higher activity of studied enzymes in digestive gland of limpets during winter, could be related to a major reserves mobilisation activity in this season.

For gonads, the enzymes activities could be linked to environmental conditions and/or the stage of gonad maturation. This is supported by the fact that major activity was generally detected in the stations with higher IUCI values and also because the principal spawning event takes place at the end of spring (November/December) (Morriconi, 2005), which coincides with the higher values for these biomarkers. In this sense, Viarengo et al. (1991) supported that seasonal variations of trace metals seems that do not influence directly to the same extent the antioxidant defence enzyme due to other biological factors (spawning, gametogenesis, growth) which regulates fluctuations of both defences.

Heavy metals, particularly Cu and Zn, are essential trace elements but in excess can be toxic and cause several biochemical effects (Funes et al., 2006). Besides, Kim et al. (2007) point out that copper has been detected as a decisive factor in controlling the SOD activity, showing inhibited or activity effects related to time and concentration of exposure. This relation has been observed in the present study, copper in tissue being positively associated with the activity of SOD and CAT in digestive gland. Besides, the digestive gland present a high sensitivity related to the bioavailability of the pollutants which may enter via the digestive pathway instead of entering through the gills (Cossu et al., 2000). For that it is assumed that this organ is the major target for oxidative disruption related to environmental stress (Livingstone, 1991).

The peaks of lipid peroxidation detected in IZ could be probably linked with the presence of heavy metals in this station, especially

Cu, Zn, Pb and Fe, in agreement with Stohs and Bagchi (1995) that support that Cu, similar to Fe, acts as a catalyst in the formation of reactive oxygen species and catalyzes peroxidation of membrane lipids. The absence of an increased LPO rate in the other stations during winter indicates that the adaptive antioxidant response of limpet living in these regions with different levels of pollution is enough to counteract the increased oxidative stress as has been observed by Box et al. (2007).

4.3. Biomarkers in mussels

The results obtained in the present study have a similar pattern to that observed by other authors (Viarengo et al., 1991; Solé et al., 1995; Cancio et al., 1999; Sheehan and Power, 1999). These authors have detected, in spite of some small differences, high antioxidant activities in spring–summer months and lower during autumn–winter.

Company et al. (2005)attributed these to a response that reflects a seasonal control due to variations in environmental conditions (i.e. temperature and food availability) and endogenous factors of the species (reproductive status and physiological condition). Hernando (2008) has reported high values of chlorophyll (6–11 μg/L) in the early spring (September–October) and a second peak during December (early summer) coincidently with a significant change in the hydrological conditions, especially in temperature, salinity and daylight as was pointed in Section 2.1. Besides, an intense reproductive activity occurs in spring–summer months (Tortorelli, 1987). In *M. edulis* gametogenesis takes place in the mantle at the expense of the connective storage tissue. One of the ways in which stored reserves are mobilised for gamete formation is by controlled autophagy (Gabbott and Peek, 1991).

So the present results, where CAT activity and LPO have generally increased in spring samples could be related to these events. Spring increases in antioxidant capacities have been linked to a need to cope with the excess of oxyradicals arising from an increase in the metabolic rates observed during this season (Cancio et al., 1999). The only exception to this behaviour has been the activity of SOD, where maximum activities in digestive gland were presented in IZ station which has a particular state.

Some authors (Viarengo et al., 1991; Power and Sheehan, 1996; Company et al., 2005) have indicated that during winter, as a result of a reduction in antioxidant enzymes activities, the LPO increase; this could not be observed in the present study where the LPO only increased in winter in IZ station.

Otherwise, low CAT activity in gills compared with digestive gland of mussels detected in the present study has been pointed out by other authors for similar species (Power and Sheehan, 1996; Cossu et al., 1997; Cheung et al., 2001; Gravato et al., 2005).

In the present study the increase in the activity of antioxidant enzymes in the polluted stations, accompanied with significant differences in MDA concentration for mussels may reflect a clear response to the chronic exposure to contaminants in accordance with other studies (Cheung et al., 2001; Lima et al., 2007).

4.4. Multivariate analysis

The PCA was a powerful tool to discriminate impacted sites, in spite that in the analysis with the limpet *N. magellanica* was more evident the separation of the study sites than the one produced with mussel's data. Probably, the most important cause of that is the major influence of sediments quality on *N. magellanica* than in *M. edulis*, principally related to the different feeding habits between these species.

The concentrations of heavy metals (in sediments and/or tissues) have differential association with biomarkers depending on the species under study. Briefly, heavy metals in sediments were linked in limpets with CAT_{gonad} and LPO_{gonad} and in mussels a high correlation with $SOD_{mantle\text{-}gonad}$, $SOD_{digestive\ gland}$ and CAT_{gill}. Besides, the principal heavy metal in organisms that have linked with biomarkers in digestive gland (CAT and SOD) is Cu in limpet and Pb with LPO in mussel.

In an integrative point of view, the present results suggest that the organisms from OMS, NW and IZ were more stressed than those from GB and UP.

5. Conclusions

As was pointed out by Bocchetti et al. (2008), our results indicate that some of the biological parameters used as biomarkers have presented a marked seasonality and species-specific differences in the studied organisms. These fluctuations and other injuries may be related to seasonal effects as temperature, salinity and sunlight exposure (Company et al., 2005; Letendre et al., 2009; Sheehan and Power, 1999). These factors, together with phenotypic and ontogenetic differences in susceptibility to stress, result in increased variability in biomarker responses (Depledge and Lundebye, 1996). Our results indicate that the differences in biomarker responses due to the sampling periods did not influence the grouping of the sites into references and contaminated groups after multivariate analysis.

Natural populations in polluted areas are possibly subjected to selective pressures for an increased resistance to toxics. This can result in the evolution of resistance, which may have important implications for decisions regarding safe ambient toxicant levels (Klerks and Weis, 1987). In agree with own data related to the status of environmental quality of the area (Amín et al., 2010, in press), in the present study differential responses can be detected, indicating that some sites produced higher pressure to resident organisms.

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