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Biochemical response of amphipods (*Gammarid: Paramorea*) in a sediment laboratory exposure from Ushuaia Bay, Beagle Channel

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ABSTRACT

A coastal system (Ushuaia Bay, Argentina) impacted by anthropogenic activities was studied by the response of local amphipods (*Parmorea sp.*, *Gammaridae*) to the exposure of coastal sediments in a laboratory assay. Four coastal areas with different loadings of contaminants and one considered as reference were studied. Organic matter, carbohydrates, proteins and heavy metals were measured in sediment samples. Organisms were exposed to sediments for seven days and catalase (CAT), glutathione S-transferase (GST), acetylcholinesterase (AChE) and lipid peroxidation (LPO) were measured afterward. Amphipods exhibited an activation of GST and inhibition of AChE in most impacted areas. Principal Component Analysis (PCA) was conducted in order to associate the biological responses with sediment metal concentration and its eutrophicated status. Levels of Cd and Cr were associated with the inhibition of AChE and with the enhancement of GST. CAT and LPO were enhanced in most areas, but no link was found with the contaminants studied by PCA, suggesting that other parameters present in sediments not included in the PCA affect the amphipods. The most impacted area corresponds to Nautical Club station, with a highly eutrophicated status and high content of metals, where amphipods after the exposure were affected in a biochemical level.

1. Introduction

Sediments act as integrators and amplifiers of the concentrations of anthropogenic chemicals in the waters which pass over and transport them, and play an important role in the shallow water areas. For this reason, sediments have been widely used to identify sources of contamination, to measure their extent, and to diagnose the environmental quality of aquatic systems (Del Valls et al., 1998). Sediment bioassays are relatively simple tests that evaluate the responses of the selected organism to contaminated sediments under controlled conditions to establish certain biological effects (Martín-Díaz et al., 2004).

Crustaceans species used for marine toxicity assessments in Argentina include *Chasmagnathus granulata* (Lavarías et al., 2009) and *Macrobrachium borellii* (Ferrer et al., 2006) among others, mostly from the northern part of the country in template climates. In the last years, there has been a growing interest in animals permanently inhabiting cold marine environments such as polar oceans (Regoli et al., 1997, 2000a, 2000b; Viarengo et al., 1998, Heise et al., 2003). In Argentina, exists the possibility of analyzing the effect towards sediments of species inhabiting colder climates, such as subantarctic amphipods that habit in Beagle Channel.

Marine and estuarine amphipods have been extensively used worldwide for sediment contamination evaluation, due to their sensitivity to contaminants and changes in the benthic environment. Amphipods are also an important source of energy for higher aquatic life, serving as prey to larger crustaceans and birds (Melo and Nipper, 2007). Studies on Gammarid amphipods were done mainly in the Arctic and Antarctic zones (Duquesne et al., 2000; Duquesne and Liess, 2003; Camus and Gullisken, 2005; Obermüller et al., 2005). And the Gammarid amphipod *Paramorea* sp. is a representative species of the shoreline waters in Beagle Channel as well and, therefore, suitable for the study of biological impacts of land-derived contaminants.

Finally, previous works about contamination in Beagle Channel have been focused mainly in chemical analysis of sediments (Amin et al., 1996a, 1996b), in the use of bivalve molluscs such as *Mytilus edulis chilensis* in experimental field studies (Giarratano et al., 2010) and response to water exposure of selected heavy metals in isopods *Exosphaeroma gigas* (Giarratano et al., 2007). However studies on sediment toxicity using a local organism have not been done before in the area.

During the past years, many studies focusing on the biological effects of contaminants have been conducted on several aquatic species. Indeed, several contaminant effect indicators have been developed (Van der Oost et al., 2003), and a number of promising and sensitive early warning signals, or biomarkers, that reflect adverse biological responses consecutive to anthropogenic contaminants and environmental toxin exposure and stress, have been identified (McCarthy and Shugart, 1990). A wide range of biomarkers and particularly enzymatic activities, that provide information on uptake, biotransformation and detoxification patterns (Livingstone,

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1993; Snyder, 2000), has been developed, tested and proposed for application in monitoring and assessing deleterious effects of chemical stress on organisms (Handy et al.,, 2003; Lam and Gray, 2003). Besides, the use of a battery of biomarkers in combination with chemical analyses is recommended (Cajaraville et al., 2000).

The objective of this work was to try to determine the quality of coastal sediments of Ushuaia and Golondrina Bays (Beagle Channel, Tierra del Fuego, Argentina) and to evaluate the impact on amphipods exposed to those sediments in laboratory assay measuring conventional biomarkers. From among the possible biomarkers we selected the antioxidant enzymes catalase (CAT), which transforms H_2O_2 to H_2O and O_2, and glutathione-S-transferase (GST). GSTs are important components of various detoxification, antioxidant and stress-tolerance pathways. Since they protect against injury induced by environmental chemicals, they have been used as biomarkers to estimate exposure in aquatic organisms (Van der Oost et al., 2003).

We also considered acetylcholinesterase (AChE), commonly used to detect environmental pollution caused by neurotoxic compounds (Cailleaud et al., 2007). Acetylcholinesterase is an important enzyme that hydrolyzes the neurotransmitter acetylcholine (ACh). The inhibition of AChE leads to an accumulation of ACh which, in turn, overstimulates sensitive neurons at the neuromuscular junction resulting in tonic spasm and tremors. In invertebrates, the accumulation of ACh can induce a pattern of nerve poisoning with hyper-activity, tremors, convulsions and paralysis, which may finally lead to death (Hoguet and Key, 2008). Finally, we determined lipid peroxidation (LPO) since it indicates lipid membrane damage as a consequence of exposure to reactive oxygen species (ROS) and insufficient antioxidant defences.

2. Materials and methods

2.1. Study site

Ushuaia city (54°49′ S , 68°19′ W, Tierra del Fuego, Argentina), located along the coasts of Encerrada Bay (EB), Ushuaia Bay (UB), and Golondrina Bay (GB), has increased severely in the past years its urban waste waters, industries, shipping, tourism and general urban influences (Fig. 1). Encerrada Bay is a semi-closed system that is connected to Ushuaia Bay by an artificial pathway, and communicates through two vents hence allowing water exchange in each tide cycle (Torres et al., 2009). This Bay receives three sewages and the discharge of Buena Esperanza Stream, which transports thaw water from Martial glacier as well as runoff and waste water from Ushuaia city. Ushuaia Bay can be divided in two areas: one principally impacted by urban waste waters and untreated sewage, and the other one, influenced by the commercial port, different industries along the coast and the Fuel Dock (FD), which is a fuel loading dock for boats. In the first area we found principally the connection of Encerrada Bay with Ushuaia Bay (CE) and the Nautical Club (NC). This last one was a recreational wharf and is influenced by diffuse urban input and storm water runoff. Golondrina Bay, which is located SW to the city receives the discharge of the raw sewage network that collects domestic water of the city through underwater pipeline. Larga Beach (LB) located at 20 km north from the city in the Natural Reserve of Larga Beach is considered as a reference site due to the distance from the impacted areas and the influence of seawater currents.

2.2. Sediments

Sediments were collected with a Birge-Ekman grab in three sites within Ushuaia Bay: FD, NC and CE, and with a plastic spoon in the intertidal coast in GB and LB in April 2009. All samples were transported to the laboratory and kept in plastic bags protected from light, at 4 °C until use for the experiments (less than 1 week).

Sub-samples were taken for physical–chemical characterization. Water content (*wc*) and porosity were determined as described by Danovaro et al. (1999). Organic matter (OM) was determined by weight loss on ignition at 450 °C for 5 h. Grain size was analyzed by dry sieving, proteins (PRT) were determined by Lowry et al.'s method (1951), and carbohydrates (CH) by the method of Dubois et al. (1956), modified by Gerchakov and Hatcher (1972). Total metal concentration in sediments were measured following the method described by Marcovecchio et al. (1988), treated with analytical-grade HNO_3 and $HClO_4$ for mineralization, as well as for blanks and calibration curve standards build ups. Concentrations of Cu, Cr, Cd, Pb, Fe, Mn, Ni and Zn were measured using a PerkinElmerAA-2380 atomic absorption spectrophotometer with air–acetylene flame and deuterium background correction (D2BGC).

2.3. Laboratory assay

Two assays were done, the first one with sediments from GB and FD and the second one with sediments from NC and CE, using sediments from LB as reference in both assays, named LB1 and LB2 for each assay, respectively. Test organisms were handpicked at low tide from LB in April 2009 before the assays. In the laboratory, organisms were separated and acclimated in beakers (30 organisms each) containing filtered and UV sterilized natural seawater (salinity 35), at a temperature of 8 ± 1 °C and a photoperiod of 12 h-light:12 h-dark cycle until the assay. During acclimation period organisms were deprived of food. The mean $\pm$ standard deviation of wet weight of the organisms used for the assays was 23 ± 3 mg.

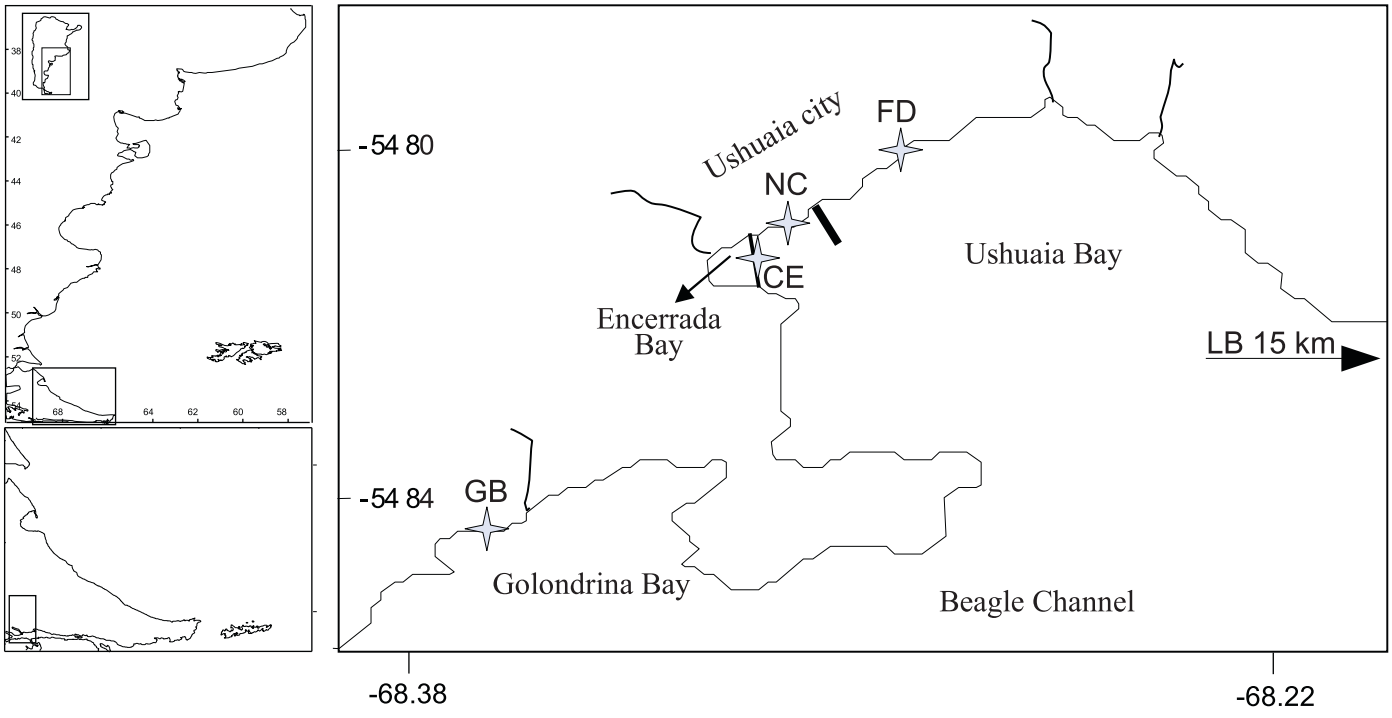

Fig. 1. Map of Ushuaia with location of the sampling sites: GB, Golondrina Bay; FD, Fuel Dock; CE; Connection Encerrada; NC, Nautical Club; LB, Larga Beach.

Since sexual identification needs extra manipulation that could produce stress and body damage, the sampling was made randomly. Thus we assumed that the experimental group used is representative of the natural population and it was under similar physiological condition based on size parameter.

Organisms were exposed for 7 days to sediments in plastic containers of 7 cm × 20 cm × 27 cm. One day before the beginning of the assay, a layer of sediment of approximately 1 cm was carefully placed at the bottom and filtered seawater was added up to 5 cm. At the beginning and at the end of the experiment, quality of water was measured through pH, dissolved oxygen (DO), temperature (T) and ammonia concentration (NH_4^+). Six replicates per sediment were considered in the experiment; using 30 organisms in each. Water replacement was performed continuously using a peristaltic pump (10 mL/min) during the exposure period constant aeration was supplied. Temperature and photoperiod were the same as in the acclimatization process, and no food was provided during the exposure. At the end of the experiment, sediment was sieved through a 1000 μm mesh and the living organisms were separated. The criterion of mortality was established as total absence of movement and no response after repeated mechanical stimulus. All living organisms were rinsed with distilled water and dried with paper towel, freezed at −20 °C and lyophilized for biochemical parameters determination.

2.4. Enzymatic assays and lipid peroxidation

Samples to determine CAT and GST activities were constituted by pools of 10 organisms, homogenized in 1:4 (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), centrifuged at 8000 rpm for 30 min and supernatant was removed for analysis.

The activity of CAT was determined according to the decrease in the concentration of hydrogen peroxide, measuring the reduced absorbance at 240 nm (Beutler, 1982). Values are expressed as UCAT/mg prot, where 1 UCAT is equivalent to 1 μmol of H_2O_2 consumed per minute. GST was determined by monitoring the rate of conjugation of glutathione (GSH) to 1-chloro-2.4-dinitrobenzene (CDNB) at 340 nm (Kleen et al., 1976), and was expressed as UGST/mg prot, where 1 UGST is the quantity of enzyme that conjugates 1 mmol of CDNB per minute.

AChE activity was determined according to Ellman et al. (1961) adapted to microplate (Guilhermino et al., 1996) in samples constituted by pools of 10 organisms, homogenized in 0.1 M phosphate buffer pH 7.2, centrifuged at 8500 rpm for 30 min and expressed as nmol/min mg prot .

LPO was measured by the method proposed by Buege and Aust (1978), measuring production thiobarbituric acid reactive substances (TBARS) at 535 nm. 0.1 M Tris buffer pH 7.8 was used in relation 1:4 (w/v) for homogenization in pools of 10 organisms, and centrifuged for 10 min at 9000 rpm and supernatant was kept for analysis. Data is expressed as TBARS nmol/mg prot.

The protein content was determined in triplicate by the method of Markwell et al. (1978), using bovine albumin as standard.

2.5. Statistical analysis

Data was analyzed by one-way analysis of variance (ANOVA). Previous to ANOVA analysis, data was tested for normality by Shapiro–Wilk's "goodness-of-fit-test" and by Levene's test for homogeneity of variance. Tukey post-hoc test was used to find significant differences between treatments. If data did not fit normality, they were log transformed. These analyses were done using the R commander program (Rcmdr: R commander, R package version 1-5-3, 2009). Statistical differences were considered when $p < 0.05$.

PCA was performed to evaluate possible relations between environmental and biochemical variables. In this case, the variable CH was omitted because of the lack of data for the site LB.

3. Results

3.1. Sediments

Physical–chemical parameters determined in sediments are shown in Table 1. Differences on grain size composition were encountered between sediments. LB presented a high percentage of coarser sand (51.8%), and GB of medium sand (40.8%). All other sediments presented high percentages of silt, ranging from 34.13% to 52.95%.

Differences between LB1 and LB2 were found in wc and porosity, with values lower than those found in other stations. Organic matter content was higher in sediments from NC and CE (20%), and the lowest was in LB (6%). High concentrations of proteins were encountered in NC (10.9 mg g^{-1}), which was statistically different from LB1 and LB2 (0.11 and 0.12 mg g^{-1}, respectively). Carbohydrate concentration was also higher in NC (9.01 mg g^{-1}), and was non-detectable in LB. The highest PRT:CH ratio was found in FD (3.9), and the lowest in GB (0.4).

Metal analysis in sediments showed variations among the different sites studied. LB1 and LB2 presented slight differences between them; however, metal content was lower compared to sediments from NC.

Fe was predominant in all sites, with values ranging from 15.5 mg g^{-1} in NC to 34.2 mg g^{-1} in GB. Cr concentrations were between 4.1 and 9.1 μg g^{-1}, with the highest value found in NC and the lowest in CE. Pb had similar concentrations in sediments from GB, FD, CE and NC, from 19 to 21 μg g^{-1}, being in LB1 and LB2 lower (11–12 μg g^{-1}). CE presented the highest value of Cd (1.23 μg g^{-1}). This metal was between 0.8 and 1 μg g^{-1} in the sediments of the other sites studied. Cu showed the lowest values in LB (11.74–15.1 μg g^{-1}) and an increment was measured in the rest of the sediments analyzed, with values varying between 27 μg g^{-1} in GB and 36 μg g^{-1} in NC. Ni showed a similar pattern, with lower values in LB (18–16 μg g^{-1}) and higher in the other sediments studied (21–31 μg g^{-1}). Zn, on the other hand, was lower in CE and NC stations (58 and 59 μg g^{-1}, respectively) and higher in FD (100 μg g^{-1}) and GB (97 μg g^{-1}). LB1 and LB2 were between 73 and 69 μg g^{-1}, respectively. Mn was present in all sediments, with values ranging from 417 μg g^{-1} in NC to 520 μg g^{-1} in CE.

Table 1
Values of coarse sand (> 1000 μm, %), medium sand (1000–250 μm, %), fine sand (250–62 μm, %), silt (< 62 μm, %), water content (wc, %), porosity, organic matter (OM, %), proteins (PRT, mg g^{-1}), carbohydrates (CH, mg g^{-1}), protein:carbohydrate ratio (PRT:CH) and 8 metals (μg g^{-1} dry sediment, Fe is in mg/g) analyzed in sediments from the selected stations in Ushuaia and Golondrina Bays. *nd* means non-detectable data.

	LB1	LB2	GB	CE	NC	FD
Coarse sand	51.81	64.81	17.73	10.03	1.92	0.94
Medium sand	42.37	32.27	40.78	35.80	28.06	30.34
Fine sand	3.72	2.08	27.47	20.04	17.07	23.25
Silt	2.10	0.83	14.02	34.13	52.95	45.47
wc	0.058 ± 0.002	0.21 ± 0.01	0.27 ± 0.02	0.65 ± 0.02	0.668 ± 0.005	0.62 ± 0.01
Porosity	0.137 ± 0.005	0.40 ± 0.02	0.48 ± 0.02	0.83 ± 0.01	0.839 ± 0.003	0.811 ± 0.004
OM	6.3 ± 0.6	6 ± 1	7 ± 2	20 ± 6	20 ± 5	16 ± 2
CH	*nd*	*nd*	1.5 ± 0.1	5.1 ± 0.3	9.0 ± 1.1	1.8 ± 0.4
PRT	0.11 ± 0.05	0.12 ± 0.02	0.59 ± 0.05	9.1 ± 0.4	10.9 ± 0.3	6.8 ± 0.1
PRT:CH	*nd*	*nd*	0.4 ± 0.2	1.78 ± 0.09	1.2 ± 0.2	3.9 ± 0.3
Cd	0.79 ± 0.12	0.90 ± 0.04	1.07 ± 0.13	1.14 ± 0.19	0.80 ± 0.12	0.91 ± 0.04
Pb	11.47 ± 1.66	12.18 ± 1.59	21.01 ± 1.59	19.34 ± 0.83	19.35 ± 1.59	20.18 ± 1.59
Cu	11.74 ± 0.83	15.12 ± 0.94	26.51 ± 2.73	32.14 ± 3.04	35.60 ± 3.77	27.61 ± 1.65
Zn	73.22 ± 8.52	68.89 ± 6.02	96.7 ± 9.3	58.3 ± 6.1	58.67 ± 878	100.24 ± 7.42
Cr	6.68 ± 0.32	6.6 ± 0.2	6.18 ± 1.08	4.14 ± 0.27	9.1 ± 0.9	5.52 ± 0.74
Ni	18.4 ± 1.4	16.89 ± 3.15	31.19 ± 4.54	25.06 ± 1.66	21.19 ± 1.71	22.34 ± 1.97
Mn	463.85 ± 24.85	438.7 ± 66.48	444.95 ± 64.17	520.81 ± 44.32	417.3 ± 33.2	455.89 ± 54.39
Fe	20.1 ± 2.7	20.3 ± 2.3	34.2 ± 3.2	19 ± 1	15.5 ± 0.9	20 ± 4

3.2. Laboratory assay

Water quality in the test chambers were within acceptable levels according to USEPA (1994) throughout all treatments, with pH between 7.6 and 8.02, and DO > 10 mg L^{-1} at all times. Ammonia concentration differed between treatments, presenting in the FD treatment the highest concentration of 169.71 μM.

Mean amphipod survival in sediment established *a priori* as reference was 93.3% in LB2 and 87.8% in LB1. Amphipod survival was not significantly reduced relative to those placed in LB sediments, although, a lower survival was recorded in amphipods exposed to FD sediments (84%) (Table 2).

3.3. Enzymatic activities and lipid peroxidation

Mean CAT values among amphipods exposed to LB1 and LB2 sediments (0.68 and 1.07 UCAT/mg prot., respectively) were significantly different (Fig. 2A). Activity of CAT in organisms exposed to sediments from FD (1.02 UCAT/mg prot) and CE (0.97 UCAT/mg prot) were significantly higher than those of LB1. NC presented a CAT activity similar to LB1 of 0.69 UCAT/mg prot, and was found to be significantly different with those exposed to sediments from LB2 and FD. Furthermore, organisms exposed to sediment from GB had a low activity, 0.79 UCAT/mg prot, and was significantly different to LB2 (Fig. 2A). GST activities were comparable among amphipods from all sites except for NC. GST activity was significantly elevated among amphipods exposed to NC sediments (Fig. 2B). AChE activities were significantly elevated among amphipods exposed to LB1 sediments in comparison to activities measured from all other treatments (Fig. 2C). AChE activities among amphipods exposed to GB, FD CE and NC were comparable to activity measured among amphipods exposed to LB2 sediments. LPO activities among amphipods exposed to GB, FD, CE, and NC sediments were comparable to activities measured among amphipods exposed to LB1 and/or LB2 sediments (Fig. 2D).

Table 2
Values of *T* (°C), DO (mg mL^{-1}), pH, ammonia (NH_4^+, μM) and survival (%) of amphipods in the bioassay.

	T	DO	pH	[NH_4^+]	Survival
LB1	8.67	11.45	7.93	*nd*	87.78
LB2	8.65	10.41	8.02	*nd*	93.33
GB	7.88	12.97	7.90	49.72	88.89
FD	7.70	13.29	7.79	169.71	84.00
CE	8.19	11.26	7.85	112.28	87.78
NC	7.92	10.90	7.63	124.49	90.56

3.4. Principal component analysis

Application of PCA to the variables described indicated that 15 variables can be represented by three new variables or principal components. These new components explain 73.71% of the variance in the new data set. In the present study, we selected to interpret a group of variables as those associated with a particular component where the loadings were 0.6 or greater, corresponding to an associated explained variance of more than 40%. This improves the approximation of Comrey's cut-off of 0.55 for a good association between an original variable and a component, and also takes into account discontinuities in the magnitudes of the loadings of the original variables (Del Valls et al., 1998).

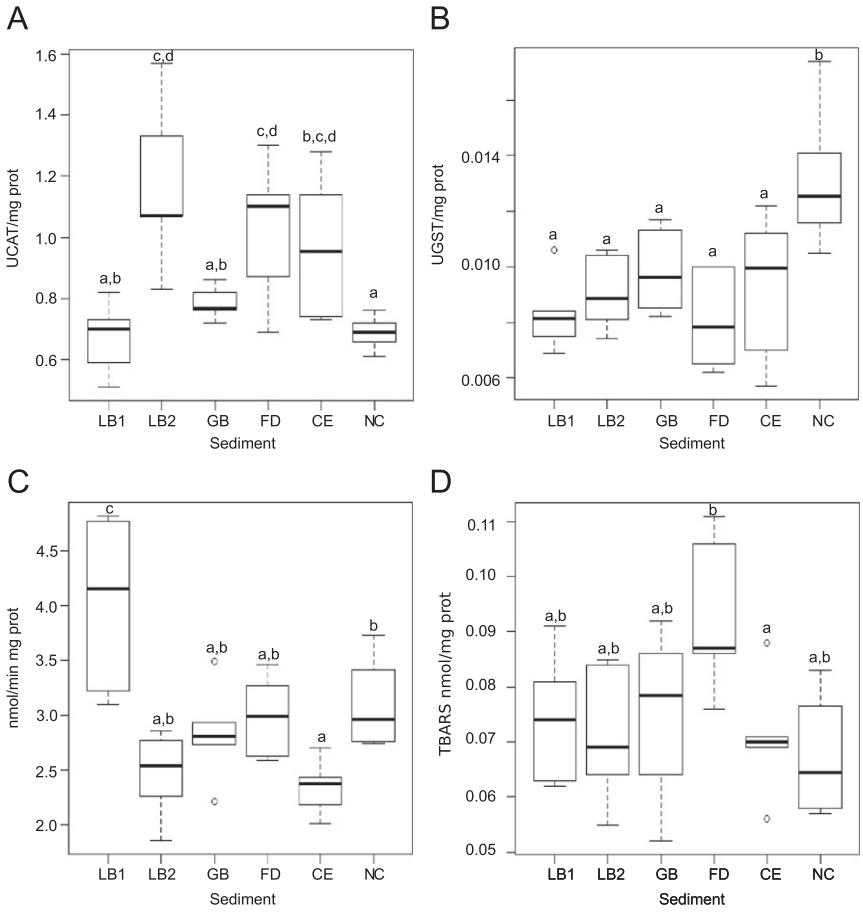

Fig. 2. Boxplots of enzymatic activities and oxidative stress of amphipod *Paramorea* sp. exposed to sediments from Ushuaia city. (A) Acetylcholinesterase, nmol/min mg prot. (B) Catalase UCAT/mg prot. (C) Glutathione S-transferase, UGST/mg prot. (D) Lipid peroxidation. TBARS nmol/mg prot. Values accompanied by same letter are not statistically different ($p < 0.05$).

The first principal component (PC1) explained 35.92% of the variance and combines the chemical concentrations of Pb, Cu, Ni, PRT, OM and porosity. This component, with negative loadings, identifies marine contamination phenomena with no associated adverse effects studied. The second component PC2 accounts for 21.61% and combines the enzymatic activities of AChE and GST with the presence of Cr and absence of Cd. The third component PC3 (16.18%) is a combination of Fe and Zn which represents the natural variability due to processes in sediments. CAT and LPO were not linked to any PC (Table 3).

Table 3
PCA: correlations between physical and chemical variables. Correlation coefficients are significant when they are higher than 0.6 (bold coefficients).

	PC1	**PC2**	**PC3**
Eigenvalue	*5.38736962*	*3.24129711*	*2.42772233*
Variance (%)	*35.9157974*	*21.6086474*	*16.1848156*
CAT	0.37	0.41	−0.30
GST	−0.26	**−0.75**	0.12
AChE	0.14	**−0.70**	0.34
LPO	−0.37	0.36	0.43
Cd	−0.51	**0.74**	−0.23
Pb	**−0.89**	−0.05	0.30
Cu	**−0.94**	−0.21	0.05
Zn	−0.10	0.08	**0.80**
Cr	0.28	**−0.82**	0.16
Ni	**−0.67**	0.19	0.54
Mn	−0.34	0.58	0.02
Fe	0.19	0.31	**0.83**
PRT	**−0.85**	−0.36	−0.36
OM	**−0.90**	−0.11	−0.22
Porosity	**−0.93**	−0.11	−0.21

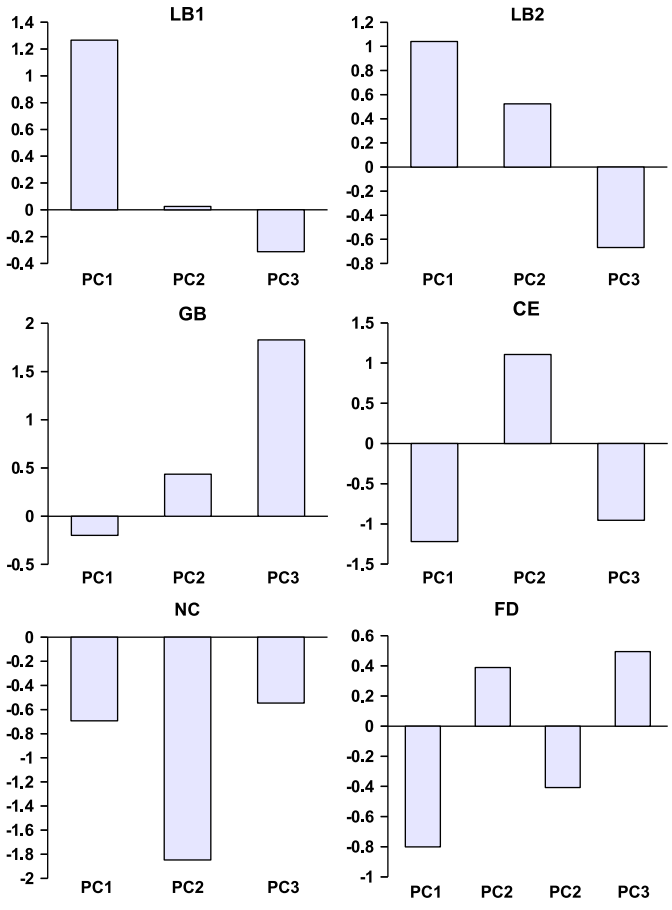

Fig. 3. Factor scores for each of the areas studied in the new principal components.

In order to confirm these factor descriptions and to establish the relationship between components to define environmental degradation at each of the studied treatments, we propose a representation of estimated factor scores from each case (Fig. 3).

LB1 and LB2 had a positive loading in PC1, indicating a low content of the contaminants analyzed. LB also has positive loadings in PC2, indicating a low response of the amphipods exposed to this sediment. GB is principally represented by PC3, due to the high content of Fe found in this site, it also presents negative loadings in PC1 and positive loadings in PC2. FD may be described as a moderately impacted area, with negative loadings in PC1 and positive in PC3. PC2 presented variability which may be due to the response of biomarkers by other variables not included in the analysis. CE is represented with negative loadings in PC1 and PC3, and positive in PC2, due to the highly contaminated parameters found, that did not affect negatively on the biomarkers. NC has negative loadings in all principal components, considered as a highly contaminated place, resulting in negative effects on the amphipods exposed.

The distribution of the sites studied in the space defined by the first three factors are represented in Fig. 4. LB1 and LB2 were in the same area, separated from all others, and NC opposite to PL in Fig. 4A, presenting a highly contaminated status. On the other hand, CE and FD can be considered as moderately contaminated, with some variations due to the effects the contaminants had on the biomarkers studied, and GB can be considered as a slightly contaminated area.

4. Discussion

The composition and concentration of sedimentary organic matter are important indicators of the trophic state of the marine environments (Fabiano et al., 1995). In this study, high OM content found in CE could be due to the influence of Encerrada Bay, which has been characterized as a highly impacted area due to four discharges with typical features of sewage (Torres et al., 2009). NC presented a high OM content as well, since there is a pipeline that discharges mixed effluents, i.e. pluvial and sewage waters. This area has been described as highly influenced by anthropogenic activities in the past, with low content of dissolved oxygen, high concentrations of nutrients (ammonium, nitrite and phosphate) and an elevated presence of fecal coliform and *E.coli* (Esteves et al., 2001).

Due to the conservative composition of the sedimentary OM, variations in the trophic state of the sediments are more evident in terms of organic matter composition (e.g., ratio of proteins to carbohydrates) rather than in quantity. This is reflected by the tendency of eutrophic systems to accumulate large amounts of low-quality organic matter as opposed to oligotrophic systems, which tend to have much less sedimentary OM of a much higher quality (Fabiano et al., 1995; Fabiano and Danovaro, 1998).

According to levels of CH and PRT proposed by Danovaro et al. (1999) to determine trophic status (Table 4), the sediments from NC and CE can be described as hypertrophic–eutrophic, FD as eutrophic–meso-oligotrophic, GB and LB as meso-oligotrophic. High concentrations of PRT and CH in NC may be due to the higher inputs of anthropogenic wastes described above. Since proteins are more utilized by bacteria than CH, and are rapidly bound into refractory compounds (Cividanes et al., 2002), low values of PRT:CH ratio, such as those found in NC, CE and GB, suggest the presence of aged OM and a role of labile proteins as a potentially limiting factor for benthic consumers. The high value of PRT:CH ratio in sediment of FD suggests the presence of a more newly produced matter than those in the other stations.

Fe was the most abundant metal in sediment at all stations, and is the metal with the highest concentration under natural conditions in

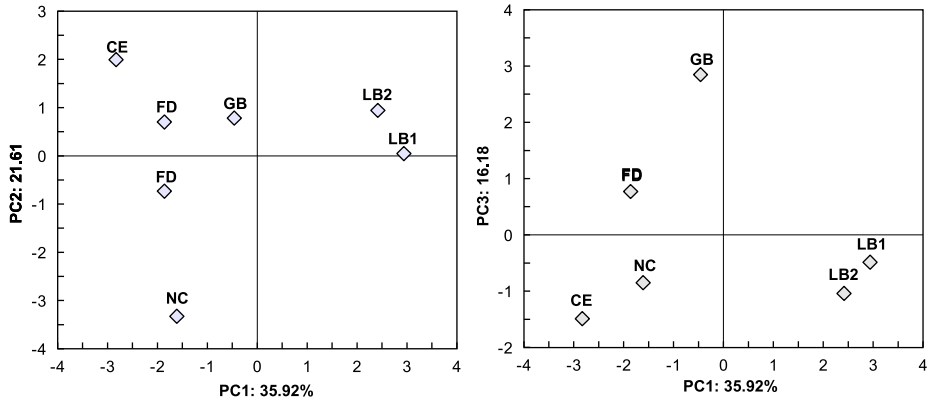

Fig. 4. Graphical representation of studied sites in the space defined by the three Principal Components: PC1 versus the other Principal Components, PC2 and PC3. The explained variance for each factor is reflected in each axis.

Table 4
Protein (PRT) and carbohydrates (CH) limits of three trophic states adapted from Danovaro et al. (1999).

Trophic state	PRT mg g^{-1}	CH mg g^{-1}
Hypertrophic	>4	>7
Eutrophic	1.5–4	5–7
Meso-oligotrophic	<1.5	<5

this area, which is in accordance with previous data (Amin et al., 1996a; Giarratano et al., 2010). Changes in the Fe concentrations are therefore related to a varying contribution of Andean (Fe-rich) versus Coastal Range (Fe-poor) source rocks ultimately controlled by continental rainfall changes (Dezileau et al., 2007). Therefore, slight anthropogenic input may not affect significantly the content of this metal in sediment.

Concentrations of Zn, Cu, Cd and Fe in FD were increased in the last years, and Pb diminished, in comparison to data taken by Giarratano et al. (2010), due to the increment of using unleaded fuel. Amin and collaborators (personal communication) have found an increment in Cu and a diminishment of Cd, Fe, Ni and Zn in NC; meanwhile, we found in GB increments in Cd, Fe, Ni, Cu and Zn. The increment of metal concentration in GB may be due to the population growth in the last 3 years that has occurred suddenly in the area. Metal concentrations and physical–chemical parameters of sediment from LB constitute baseline data since there is no previous information of this kind from LB.

Values of Cr, Cu, Pb, Ni and Zn obtained in this work are within the ranges reported as background values for uncontaminated coastal and marine sediments (Cobelo-Garcia and Prego, 2003). The comparison of metal concentrations in sediment with values proposed by Long et al. (1995) for Effects Range-Low (ERL) (150, 34, 81, 21, 47, 1.2 μg g^{-1}, respectively, for Zn, Cu, Cr, Ni, Pb, Cd) within the sediment quality guidelines indicates that NC presented values above the ERL for Cu and Ni. All other metals, except for Ni, presented values below ERL in all sediments.

A common pathway of toxicity induced by several stressful conditions is the imbalance between reactive oxygen species (ROS) generation and the efficiency of antioxidant defenses (Frenzilli et al., 2001). Negative effects due to ROS accumulation include an increment of free-radical oxidation reactions with oxidative damages of important polymers and an increment of lipid peroxidation, which may lead to dysfunctions of cells, tissues, organs and, ultimately, mortality of the organism (Livingstone, 2001).

Catalase is often one of the earliest antioxidant enzymes to be induced. The enhancement of this enzyme in amphipods exposed to sediments from FD, CE and GB (Fig. 2A) was not linked to any of the parameters included in the PCA, and could be related to another factor that affects the enzymatic activity. Increased activities of CAT have been described in several other aquatic species, particularly from impacted sites by organic contaminants (Di Giulio et al., 1993; Regoli et al., 2002; Livingstone et al., 1993; Rodriguez-Ariza et al., 1993). On the other hand, factors other than pollutants, such as environmental parameters, influence these enzyme activities, which result in transient variations (Cossu et al., 1997; Stohs and Bagchi, 1995).

Glutathione S-transferases are a family of enzymes that utilize glutathione (GSH) as a substrate in reactions which permit the biotransformation and disposal of a wide range of exogenous compounds (Contreras-Vergara et al., 2004). An induction of GST was observed principally in NC (Fig. 2B), which presents a highly eutrophicated status and high content of Cr. PCA linked the enhancement of the activity of this enzyme with the presence of Cr. In this sense, Martín-Díaz et al. (2007, 2008) has found that the induction of the activity of GST was correlated with Cr in *C. maenas*, which coincides with our results. Although a direct relationship has not been observed, PCA related a decrease of GST activity with Cd. Inhibition of GST by Cd has been observed in previous works in vertebrates (Serafini and Romeu, 1991; Dierickx, 1982; Sen and Semiz, 2007) and invertebrates (Salazar-Medina et al., 2010; Alves de Almeida et al., 2004). In the literature, there is speculation on the effect of metals on GST activities. Some researchers have speculated that metals exert their effects by depleting GSH concentrations (Stacey and Klassen, 1981), while others have suggested that they display their effects by direct binding of GST proteins (Iscan et al., 1995; Coban et al., 1996; Gate et al., 1999).

Although GST activity was related to presence of metals by PCA, the presence of other contaminants influencing its activity cannot be discarded. The increase in GST activity in organisms exposed to sediments from Beagle Channel is in accordance with other studies suggesting that GST, which is known to display a distinct GPx activity, may play a protective role against oxidative stress when the activity of antioxidant enzyme is lowered (Power and Sheehan, 1996; Sheehan and Power, 1999).

Oxidative damage can occur when antioxidant and detoxifying systems are deficient and not able to neutralize the active intermediates produced by toxics and their metabolites (Frenzilli et al., 2001). The high induction of GST found in amphipods exposed to sediments from NC have prevented ROS to react with membrane and other lipids, thus minimizing lipid peroxidation. On the contrary, despite the activation of CAT, there was a low activity of GST on organisms exposed to sediments from FD, and could not cope efficiently with ROS production and oxidation of polyunsaturated fatty acids occurred, leading to LPO (Fig. 2). Although LPO was not related to any of the parameters included in the PCA,

its enhancement cannot be discarded as a consequence of exposure to contaminants in coastal sediments. Several studies have shown enhanced lipid peroxidation in aquatic organisms exposed to high concentrations of xenobiotics (Viarengo et al., 1990; Wenning et al., 1988; Doyotte et al., 1997; Funes et al., 2006) and of pollutants in contaminated sediments (Di Giulio et al., 1993; Livingstone et al., 1993; Sole et al., 1996).

The activity of AChE can be inhibited by various contaminants, among them, metals present in urban waste waters (Lionetto et al., 2003). In this study, there was an inhibition of the enzymatic activity in all treatments, compared to LB1. A response towards Cd and Cr was observed, as in GST. CE presented the lowest activity of AChE, which had the highest content of Cd and the lowest of Cr, which is seen in PCA, where we found that the activity of AChE had an inverse relation with Cd and a direct relation with Cr. Inhibition of AChE activity by Cd have been presented previously in *Daphnia magna* (Guilhermino et al., 1996), *Gambusia affinis* (Mohamed et al., 2008) and brown trout *Salmo trutta* (Payne et al., 1996). Although, an inhibition caused by organophosphates and polyaromatic hydrocarbons (PAH) cannot be discarded (Fulton and Key, 2001; Gagnaire et al., 2008), since high contents of hydrocarbons were measured along the coast of Ushuaia city in a previous study (Esteves et al., 2006).

Few studies have reported stimulation of AChE in organisms exposed to metals, it has been seen principally in mammals (Kaizer et al., 2005, 2008; Kouniniotou-Krontiri and Tsakiris, 1989), and in the scorpion *Heterometrus bengalensis* (Datta et al., 1988). In aquatic organisms, there is considerable diversity in the biochemical properties and distribution of cholinesterases, as well as in their sensitivity to anticholinesterase agents (Habig et al., 1988; Bocquené et al., 1990). The mechanism by which metals affect AChE activity is not fully known yet (Frasco et al., 2005; Datta et al., 1988; Parveen and Kumar, 2005), some have suggested that most heavy metals would have little potential for enzyme inhibition (Payne et al., 1996), while other studies do not support this statement (Oliveira Ribeiro et al., 2005; Rabitto et al., 2005). Despite the controversies, in this study it showed to be a sensitive biomarker in amphipods exposed to sediments and believe to be a potential biomarker, as proposed by Bocquené and Galgani (1991) and Gagnaire et al. (2008) before.

Cd and Cr have been studied extensively as causes of oxidative stress in mammals and invertebrates (Stohs and Bagchi, 1995; Sen and Semiz, 2007; Iscan et al., 1995; Kakkar and Jaffrey, 2005). Cr is a highly carcinogenic metal that undergoes redox cycling, and produces DNA cleavage in presence of H_2O_2, and ROS, such as hydroxyl radicals, through a catalyzed Fenton-like reaction (Stohs and Bagchi, 1995; O'Brien et al., 2003). Cd is known to generate ROS due to an inhibitory effect on mitochondria electron transport (Stohs et al., 2000). But it has been seen that a binding to –SH groups in proteins is also possible, producing an inhibitory effect on enzymatic activities (Viarengo et al., 1997; Salazar-Medina et al., 2010).

The slight enhancement of Cd in LB2, compared to LB1, could have caused the inhibition of AChE in the organisms exposed to this sediment. Moreover, the content of Cd in LB2 did not differ much from other stations (e.g. Cd in LB2 and FD), but LB presents a lower content of OM and fine fraction, which may increase the bioavailability of metals. Casado-Martínez et al. (2009) have reported a strong complexation of metals by organic ligands, particularly Cu, Ni, Cd and Zn. This seems to be related to the fact that silt sediment has a large surface area per unit mass and therefore binds metals more efficiently than do coarse-grained, sandy sediments (Luoma, 1989). Correia and Costa (2000) have found lower toxicity in amphipods *Gammarus locusta* exposed to metals in fine-grained sediments than in coarse grain. On the other hand, studies with amphipod crustaceans have shown that the toxicity and metal concentrations in interstitial water were reduced when metal-spiked sandy sediments were modified by the addition of fine-grained particles or sewage sludge (Swartz et al., 1985; DeWitt et al., 1988).

In this study measurement of CAT, GST AChE and LPO allow monitoring of biological effects that can be due to a variety of compounds, although CAT and LPO were not specifically linked to any of the contaminants analyzed, an effect was observed in the organisms exposed to coastal sediments. AChE and GST in amphipods showed sensitive responses against the exposure to metals in sediments, and can be considered as useful tools for studying the environmental pollution along the coast of Beagle Channel. They provide results complementary to the chemical analysis as they integrate potential multiple mechanisms of action of compounds that are present as complex mixtures.

By PCA we were able to classify the sites indicating different conditions on each study area. LB was separated from all other sites with a low response of the biomarkers analyzed, proving to be a good reference site, although more studies must be done on its biogeochemical characteristics. NC showed to be the most affected area, followed by CE, which are near to high anthropogenic activity. FD and GB were the least affected area, although attention must be taken since there was a response from the amphipods that was not related to the contaminants considered in the PCA.

5. Conclusions

A relation between chemical parameters of sediments and biomarkers in organisms was able to explain and determine the status of the marine coast of Ushuaia city. The results clearly pointed out that Nautical Club (NC) is a critically impacted area in Ushuaia Bay and could represent an ecological risk for native populations in the future. Overall data suggest that the biochemical changes measured in amphipods exposed to sediments from NC are linked to the presence of metals, especially Cd and Cr, although other contaminants cannot be discarded.

The amphipods (*Gammarid: Paramorea*) used in the sediments assays resulted to be sensitive organisms, to identify a biological response related to the sediment contamination, and permitted to establish differences in the biomarkers measured, validating the use of this species in sediment assessments.

The activation of GST and the inhibition in AChE showed sensitivity towards metals present in sediments. CAT and LPO could not be related to any of the contaminants studied. Future studies need to be done to clarify the variations found in these biomarkers, since other factors, such as time of exposure, e.g., could be affecting the organisms and must be considered.

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