Biomarkers and heavy metal bioaccumulation in mussels transplanted to coastal waters of the Beagle Channel

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ABSTRACT

Mussels coming from a mussel farm at Brown Bay (Beagle Channel) were transplanted to four sites inside Ushuaia Bay for 2 and 4 weeks. The objective of this study was to assess the quality of coastal waters of Ushuaia Bay by measuring catalase activity, lipid peroxidation, total lipid content, bioaccumulation of heavy metals and condition index in transplanted mussel Mytilus edulis chilensis. Biomarkers except condition index showed significant differences among exposure times as well as among tissues. Digestive gland presented the highest catalase activity, malondialdehyde level and total lipid content. Digestive gland also was the main target tissue of accumulation of iron and copper, while gill accumulated the highest levels of zinc. A principal component analyzes with the whole set of data allowed to separate stations based on physicochemical conditions and biochemical responses of each studied area.

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1. Introduction

Coastal waters are exposed to several mechanisms of disturbance; among them, chemical pollution associated with industrial production and high urbanization are of major concern.

Ushuaia Bay (54°48′ S, 68°19′ W, Beagle Channel, Tierra del Fuego, Argentina) is not immune to decline of its environmental quality. This area has been receiving significant inputs of contaminants from the city of Ushuaia, the southernmost inhabited location on Earth, which has increased severely in the past years its urban wastewaters, industries, shipping, tourism and general urban influences. Relatively high concentrations of several metals have been found by Amin et al. (1996a, 1997, 2000) in sediments, mussels and water of Ushuaia Bay.

Bivalves, and particularly the genus Mytilus sp., are considered suitable bioindicators for biomonitoring studies and they are considered appropiate for caging experiments along coast lines (Viarengo et al., 2007). Transplanted mussels facilitate the investigation of areas where native specimens are absent, and/or compensatory adaptative mechanisms likely occur in native populations from chronically contaminated areas, which can attenuate the capacity of biomonitoring to discriminate different levels of environmental disturbance (Regoli and Principato, 1995; Nigro et al., 2006).

There are numerous studies showing that living organisms use free radicals and reactive oxygen species (ROS) for advantageous biological effects (Dröge, 2003). Regulated production of free radicals in higher organisms and maintenance of "redox homeostasis" are essential for the physiological health of organisms (Ames et al., 1993). Several toxic pollutants produce an imbalance in free radical reactions and the production of toxic ROS being responsible for a variety of oxidative damages that finally cause adverse health effects and diseases (Valavanidis et al., 2006). Organisms have developed mechanisms to protect themselves from the toxic effects of increased ROS production activating the antioxidant system (Young and Woodside, 2001; Sureda et al., 2006; Valavanidis et al., 2006).

The use of biological responses to contaminant exposure by sentinel species has become a useful tool in environmental quality evaluation and risk assessment. Among the numerous ecotoxicological biomarkers proposed in the last three decades, those based on responses at the molecular and cellular levels represent the earliest signals of environmental disturbance and they are commonly used for biomonitoring (Viarengo et al., 2007; Moore et al., 2004). Measuring the same biomarker in different locations simultaneously gives us information about the pollution status of the region and provides a better comprehension of the mechanisms of response of the organisms to the pollutants (Frenzilli et al., 2004). Besides, using caged mussels that belong to the same cohort, observed changes in organisms are believed to be related to the effects of toxic chemicals present in water (Box et al., 2007).

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Catalase activity (CAT), although not responding specifically to a group of contaminants but to oxidative stress, was measured during this work since it has been considered as the primary defense against oxidative damage and it has been studied in bivalve molluscs around the world (Frenzilli et al., 2004; Pellerin-Massicotte, 1997). CAT is an enzimatic intracellular antioxidant involved in different defense systems against the radicals generated by the environmental oxidative pollutants. It is a peroxisomal hydroperoxidase that degrades H_2O_2 to H_2O and O_2 (Baumard et al., 1999).

Malondialdehyde (MDA) is an intermediate product of lipid peroxidation and, as reactive toxic metabolite, it is usually rapidly degraded. However, measurements of MDA are still considered a relevant biomarker of lipid peroxidation in tissue sample preparations, especially for comparative purposes (Hermes-Lima, 2004). It is used as a non-enzimatic marker of oxidation of membrane phospholipids through lipid peroxidation and it has been considered as a relevant index of chemical damage induced by toxics in mussels (Box et al., 2007). An increment in MDA level in organisms can be related to degradation of an environmental site by the diminishment of the water quality (Charissou et al., 2004).

Presence of contaminants in the environment frequently leads to the depletion of energy reserves as a compensatory mechanism to the higher demand of energy required by detoxification processes (Guerlet et al., 2006). In this sense, body condition indexes, stress response and total lipid content have been frequently evaluated in biomonitoring programs relative to exposure conditions (Smolders et al., 2004; Mubiana et al., 2006; Valdez Domingos et al., 2007; Yeats et al., 2008). These biomarkers are mainly used as an ecophysiological measure of the health status of the animals.

The purpose of this study was to try to evaluate the quality of coastal waters of Ushuaia Bay (Beagle Channel, Tierra del Fuego, Argentina) measuring conventional biomarkers such as catalase activity, lipid peroxidation, total lipid content, condition index and bioaccumulation of heavy metals in mussel *Mytilus edulis chilensis* transplanted from a mussel farm to various sites of Ushuaia Bay. However, the main goal of the present study, besides biomonitoring coastal waters of Beagle Channel, was to detect possible relationships between biomarkers and environmental parameters in order to contribute to an overall understanding of their potential role in water quality assessment.

2. Materials and methods

The authors declare that the experiments described in this article were conducted in accordance with national and institutional guidelines for the protection of animal welfare.

2.1. Experimental design

In August 2006, mussels were brought from a mussel farm at Brown Bay (BB) located 24 nautical miles East of Ushuaia city and transported in ice-cold boxes to the laboratory located in Ushuaia city. Organisms used in the experiment belonged to the same cohort and had homogeneous shell length (61 ± 4 mm), which minimize the variability. Ushuaia Bay (UB) receives the impact of Ushuaia city through urban and industrial effluents that reaches the waterline of the bay by runoff, streams or rivers. High concentrations of nutrients and total coliforms have been registered at Industrial Zone and Fuel Dock (Esteves and Amin, 2004). Moreover, high quantities of heavy metals have been measured in sediments of the mentioned sites (Amin et al., 1996a) and in mussel tissues (Amin et al., 1996b, 2000). There is no previous published data of Aspirante Creek and Ushuaia Peninsula. Due to the long distance from Ushuaia city to these last two sites and the inexistent anthropogenic activity here (O. Amin, personal communication), Aspirante Creek and Ushuaia Peninsula were estimated as low impacted sites. Mussels were kept for 2 and 4 weeks in four sites inside Ushuaia Bay selected according the kind of anthropogenic source of contamination (Table 1). The location of the experimental sites is shown in Fig. 1.

Table 1
Sources of anthropogenic contamination at each sampling site.

Station	Human impacts	Estimated degree of impact
Industrial Zone (IZ)	Factories Population Urban wastes Industrial wastes	High
Fuel Dock (FD)	Fuel dock Military dock Intense maritime traffic	High
Aspirante Creek (AC)	Access only sailing No human use	Low
Ushuaia Peninsula (UP)	Access only sailing Diving area	Low
Brown Bay (BB)	Small fishermen community Mussel farm	Low
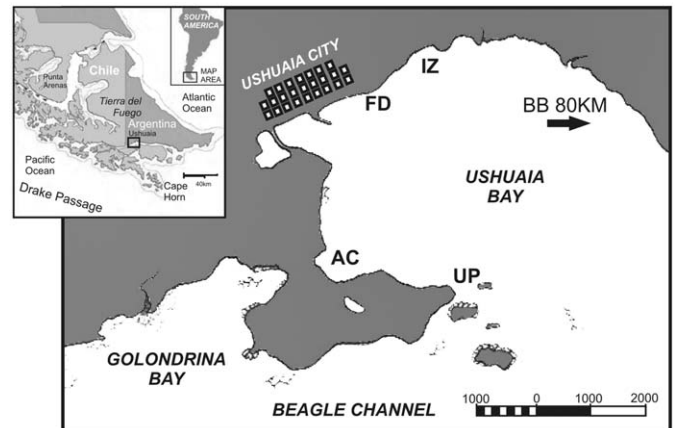
Fig. 1. Location of the cages containing the transplanted mussels inside Ushuaia Bay (Tierra del Fuego, Argentina).

After a first sorting, mussels were divided into sub-groups of 45 individuals and each sub-group was placed in a cage of $18 \times 12 \times 18$ cm built in polypropylene netting (1 cm^2 of mesh size) allowing free water circulation through it. Three cages were used per site, suspended in the water column at an average depth of 5 m using a rope and anchored to the bottom by a ballast weighing approximately 15 kg. The experimental devices were kept vertically straight by using a plastic buoy. Samples of mussels were taken at the beginning of the experiment ($t=0$), at weeks 2 ($t=2$) and 4 ($t=4$) of exposure, while water samples were taken at weeks 1 and 3.

2.2. Physical–chemical parameters

2.2.1. Water

Surface water temperature, pH, conductivity and dissolved oxygen were recorded *in situ* by means of an HORIBA U-10 multiparameter device. Water samples for the study of dissolved inorganic nutrients were filtered through Whatman GF/C filters and then were frozen (-20 °C) in plastic bottles until its analysis in the laboratory. Nitrates and nitrites were determined following the methods previously described by Treguer and Le Corre (1975) and Grasshoff (1983) respectively; while phosphates and silicates were measured following the methods described by Eberlein and Kattner (1987) and Technicon® (1973), respectively. A four channels automatic Technicon® AA-II autoanalyzer was used to perform the corresponding nutrient analyzes. Concentration of chlorophyll-*a* was measured according to Holm-Hansen et al. (1965) using a Sequoia Turner (model 450) fluorometer. Ammonia was analyzed by the indophenol method described by Strickland and Parsons (1972).

2.2.2. Sediment

At the beginning of the experiment, sediment samples at each site were obtained using a van Veen grab. These samples were stored in polyethylene bags and transported to the laboratory. Samples were dried until constant weight at $60 \pm 5\,°C$ and then were sieved through stainless steel meshes in order to separate sediment with grain size $< 62\,\mu m$. About $0.5\,g$ of that fraction was used to determine total metal concentrations (Cu, Cd, Pb, Zn and Fe) following the method described by Marcovecchio et al. (1988). Metal concentrations were measured using a Perkin Elmer AA-2380 atomic absorption spectrophotometer with air–acetylene flame and deuterium background correction (D_2BGC). Analytical-grade reagents were utilized for sediment mineralization as well as for blanks and calibration curve standards build ups. Each sample was run in duplicate obtaining a mean value for each site.

2.3. Preparation of tissue extracts for analysis of biomarkers

Even though we know that new and better techniques than those used in our experiments have been developed to evaluate cause–effect relationships in organisms exposed to contaminants, for technical reasons we were not able to use them in the present work. Nevertheless, we do think that those techniques are very useful and we are considering them for future studies in the area.

2.3.1. Catalase

Enzymatic activity was evaluated individually in gill, mantle and digestive gland of 5 mussels following the method described by Bainy et al. (1996). Catalase activity was measured in the resulting supernatant by the rate of hydrogen peroxide (H_2O_2) decomposition at $240\,nm$ (Beutler, 1982) with an UV-1203 Shimadzu spectrophotometer. Each sample was run by duplicate.

2.3.2. Lipoperoxidation

Gill, mantle and digestive gland of 5 mussels were individually analyzed according to Buege and Aust (1978). Lipoperoxidation (LPO) was measured by the generation of thiobarbituric acid reactive species and quantified in terms of MDA equivalents. Its absorbance was measured at $532\,nm$ with an UV-1203 Shimadzu spectrophotometer. Each sample was run by duplicate.

LPO level and CAT activity were quantified with soluble proteins content which was determined by the Markwell et al. (1978) method.

2.3.3. Total lipid content

Total lipid content was measured individually in mantle and digestive gland from 4 mussels of each station. Lipids were quantified using the gravimetric method of Bligh and Dyer (1959) as the difference between the weight of the extract placed in a pre-weighed bottle and the weight of the bottle once the solvent had evaporated.

2.3.4. Condition index

To examine the individual Condition Index (CI) of mussels, 4 specimens were taken at each exposure time. Soft tissues were separated from the shell and dried at $60\,°C$ until constant weight. Condition index of each mussel was calculated as the dry tissue weight/dry shell weight (Couillard et al., 1995).

2.4. Metal bioaccumulation in tissue mussels

At the beginning of the experiment, after 2 and 4 weeks of exposure samples of 10 mussels at each site were collected. Gill and digestive gland were dissected out and dried at $60\,°C$ until constant weight. Pooled samples of each tissue were homogenized with a porcelain mortar and stored in polyethylene bags until analysis. For each tissue, aliquots of about $0.5\,g$ were taken from the well-homogenized total sample to determine total metal concentrations (Cu, Cd, Pb, Zn and Fe) following the method described by Marcovecchio et al. (1988) in Section 2.2.2. Each sample was run by duplicate.

2.5. Statistical analysis

Comparisons among tissue and exposure time for each site were made by two-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons with unequal numbers of samples. Spearman's rank correlation analyzes were performed to determine the degree of relationship between heavy metals in sediments. The significance level of all analyzes was set at $p < 0.05$.

For heavy metal concentrations, measured values of each analyte were divided by the average value of that analyte from all survey sites. These unitless values were then combined in various groupings by adding analyte values then dividing by the number of analytes in the group (Fisher et al., 2000). This procedure was applied for concentrations in gill, in digestive gland and in sediment resulting three new variables 'total metal in gill', 'total metal in digestive gland' and 'total metal in sediment' that were used in principal component analysis (PCA).

A PCA was conducted with physical–chemical parameters to describe the studied sites. Variables with factor co-ordinates < 0.7 were not used to analyze the relationship between biomarker responses and environmental data by PCA. Separated PCA were run for Week 2 and Week 4 of exposure. All statistical tests were undertaken with Statistica software version 6.0.

3. Results

To summarize, we mention that higher levels of nutrients were found at Ushuaia Bay than at Brown Bay, excepting silicates. We recorded the highest concentrations of Fe, Cu and Zn in sediments of BB. We measured the maximum levels of Pb in sediments of IZ and FD, while at AC and UP we found the highest concentrations of Cd. Catalase activity, lipoperoxidation levels and total lipid content at all sites of UB showed increments respect to the initial conditions. No spatial or temporal changes were registered in condition index at all sites. Catalase activity was higher in digestive gland than in the other tissues, while lipid peroxidation and total lipid content did not show a defined trend according to the organ assayed. Bioaccumulation of heavy metals was variable, both in space and in time. Fe and Cu were most accumulated in digestive gland, Zn was mainly accumulated in gill and Cd and Pb did not show a special target organ. The integral assessment of biological and physical–chemical data revealed that biomarkers were directly correlated with nutrients and inversely correlated with dissolved oxygen and total metal in sediment. This global analysis allowed us to group the sites as follow: IZ and FD together while AC and UP formed another group. Both groups were separated from the reference site according to the overall information gathered.

3.1. Physical–chemical parameters

3.1.1. Water

Similar values for both sampling times (T_1 and T_3) at all stations were registered for conductivity, pH, dissolved oxygen and temperature; while in inorganic nutrients, ammonia and chlorophyll-a variations were detected (Table 2).

Nitrites and nitrates concentrations kept constant along exposure time at all experimental sites of UB (0.25–0.42 and 13.34–15.93 $\mu mol/L$, respectively). Considering all stations, levels of phosphates were between 0.57 and 1.59 $\mu mol/L$, although tending to be greater at T_1. Highest values of silicates were measured at both sampling times in IZ and FD (8.37–11.03 $\mu mol/L$). Concentration of chlorophyll-a was higher at T_1 than at T_3 except in AC where no change was observed. Contrarily, maximum values of ammonia were registered at T_3 (1.44–7.71 $\mu mol/L$) in all sites of UB. Except for phosphates, the others inorganic nutrients, ammonia and chlorophyll-a were higher in UB respect to those of BB (T_0).

3.1.2. Sediment

Heavy metal concentrations in the five studied sites are summarized in Table 3. Range concentrations expressed as $\mu g/g$ DW for each metal were as follow: Cu 5.50–36.25, Pb 9.39–25.31, Cd 0.25–1.80, Zn 51.34–105.63, while Fe ranged between 12.81 and 24.91 expressed as mg/g DW. BB was the station with the greatest values of Cu (36.25 ± 0.01 $\mu g/g$ DW), Zn (105.63 ± 2.94 $\mu g/g$ DW) and Fe (24.91 ± 0.02 mg/g DW). AC was the site with the highest level of Cd (2.31 ± 0.07 $\mu g/g$ DW); while FD showed the maximum value of Pb (28.76 ± 2.81 $\mu g/g$ DW). Fe was the most abundant metal at all sites. Strong significant positive correlations ($p < 0.01$) were found for pairs Cu–Zn ($r=0.97$), for Fe–Zn ($r=0.87$) and for Cu–Fe (0.78).

Table 2
Physical–chemical characteristics of the water samples at each sampling site.

Station	Week	pH	Cond. (mS/cm)	DO (mg/L)	Temp. (°C)	Nitrites (µmol/L)	Nitrates (µmol/L)	Phosphates (µmol/L)	Silicates (µmol/L)	Chl-a (µg/L)	Ammonia (µmol/L)
BB	0	7.93	49.7	9.97	4.8	0.17	12.39	0.91	4.07	0.024	0.411
IZ	1	7.83	49.6	8.93	5.1	0.40	15.93	1.12	10.65	0.216	6.533
FD	1	7.85	49.5	8.70	4.8	0.40	14.62	0.57	11.03	0.067	3.333
AC	1	7.87	49.7	9.17	4.6	0.37	14.24	1.10	6.08	0.106	0.700
UP	1	7.80	49.0	9.25	5.4	0.40	14.62	1.59	6.08	0.130	0.467
IZ	3	7.75	49.2	8.72	5.3	0.40	15.27	0.76	9.89	0.096	7.713
FD	3	7.82	48.5	8.54	4.3	0.42	14.44	0.73	8.37	0.038	7.484
AC	3	7.70	49.4	8.74	4.5	0.37	13.34	0.98	7.60	0.110	1.740
UP	3	7.70	49.0	8.39	5.7	0.25	14.03	1.06	8.37	0.082	1.444

Cond. – conductivity; DO – dissolved oxygen; Temp. – temperature; Chl-a – chlorophyll-a.

Table 3
Heavy metal concentrations in sediments expressed in µg/g of dry weight.

Station	Fe[a]	Cu	Zn	Cd	Pb
BB	24.91 ± 0.02 **c**	36.25 ± 0.01 **d**	105.63 ± 2.94 **b**	0.25 ± 0.01 **a**	12.12 ± 0.85 **a**
IZ	19.85 ± 3.22 **b**	7.54 ± 0.53 **a**	57.83 ± 5.60 **a**	0.55 ± 0.13 **b**	14.88 ± 0.21 **b**
FD	14.88 ± 3.37 **ab**	6.38 ± 1.29 **a**	57.93 ± 4.97 **a**	0.65 ± 0.01 **b**	28.76 ± 2.81 **c**
AC	13.27 ± 1.33 **a**	8.73 ± 0.18 **b**	50.59 ± 2.96 **a**	2.31 ± 0.07 **d**	11.19 ± 1.43 **a**
UP	15.19 ± 1.34 **a**	10.53 ± 0.34 **c**	58.86 ± 1.99 **a**	1.03 ± 0.07 **c**	10.19 ± 2.88 **a**

Data are expressed as mean values ± standard deviations. For each metal, stations sharing the same lower case letter are not significantly different.

[a] Concentration expressed in mg/g of dry weight.

3.2. Biomarkers

3.2.1. CAT activity

In IZ significant increment ($p < 0.05$) of activity was registered in digestive gland at T_2 (103.3%) and T_4 (93.9%) as well as in mantle at T_2 (64.8%) respect to the initial conditions. In this station there was no difference in gill at different exposure times. In FD, an increment in the activity was measured at T_2 and T_4 in digestive gland (126.7% and 140.8%, respectively) and mantle (68.1% and 183.8%, respectively), while gill activity did not show changes. Activity of CAT in AC at T_4 was significantly higher than that at the beginning of the experiment in the three organs assayed (33.8% in gill, 189.7% in mantle and 118.3% in digestive gland). However, a significant increment was also measured in digestive gland at T_2 (128.7%). In UP, activity in digestive gland was significantly higher in both sampling times than that at T_0 (69.6% at T_2 and 169.2% at T_4). In mantle an increment of 133% was registered at T_4; meanwhile, there were no changes in gill activity, comparing it to the initial time. At all sites, significant differences were found among tissues and exposure times. Digestive gland presented the highest catalase activity, followed by mantle and the lowest activity was registered in gill (two-way ANOVA, $p < 0.05$). Regarding the exposure time, the activity showed that $T_4 \geq T_2 \geq T_0$ except in IZ where $T_2 \geq T_4 \geq T_0$ (Fig. 2).

3.2.2. MDA

Initial levels of MDA in BB were 5.10 ± 0.50; 1.95 ± 0.58 and 4.30 ± 1.22 µmol MDA/mg protein in gill, mantle and digestive gland, respectively. Significant increments of MDA levels respect to T_0 were measured in IZ at T_4 in gill (74%) and mantle (350%); while in digestive gland, the highest increment was registered at T_2 (128.5%). In FD levels of MDA in mantle and digestive gland at T_2 and T_4 were significantly superior to those of T_0. Meanwhile, in gill the increment in MDA level was recorded at T_4 (63%). AC did not show differences in mantle and digestive gland along exposure time. In gill, a significant increment was measured at T_4 (38%). In the three organs of UP, MDA levels were higher at T_2
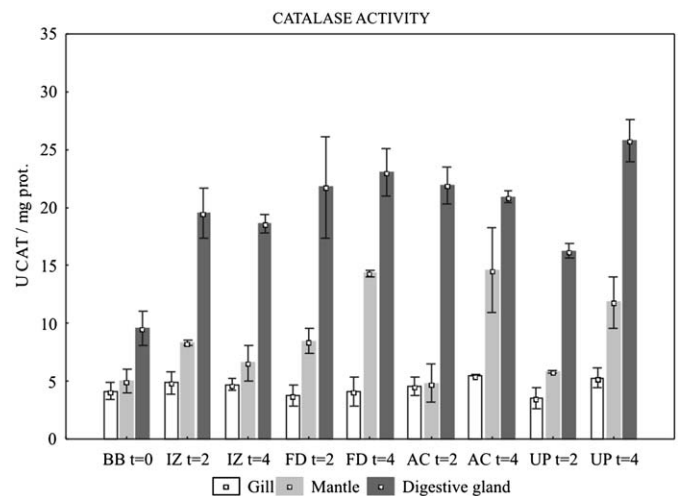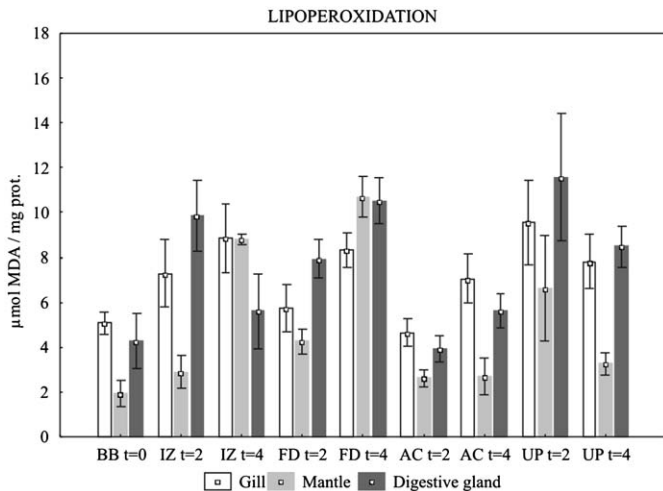

Fig. 2. Catalase activity in gill, mantle and digestive gland of *Mytilus edulis chilensis* from four sites inside Ushuaia Bay after 2 ($t=2$) and 4 ($t=4$) weeks of exposure and reference organisms ($t=0$), before transplantation. Values are expressed as U CAT/mg prot. (mean values ± standard deviations).

(between 87% and 239%) and T_4 (between 53% and 97%) respect to those of T_0. At all sites, significant differences were found among tissues and time of exposure. MDA in digestive gland and gill were higher than in mantle being greater at T_4 than at the other times (two-way ANOVA, $p < 0.05$), except in UP where the highest MDA level was registered at T_2 (Fig. 3).

3.2.3. Total lipid content

IZ and UP showed significant increments at T_2 and T_4 in mantle (between 5 and 9 fold higher) as well as in digestive gland (between 9 and 11 fold higher). The highest increments were

Fig. 3. Lipoperoxidation level in gill, mantle and digestive gland of *Mytilus edulis chilensis* from four sites inside Ushuaia Bay after 2 ($t=2$) and 4 ($t=4$) weeks of exposure and reference organisms ($t=0$), before transplantation. Values are expressed as μmol MDA/mg prot. (mean values ± standard deviations).
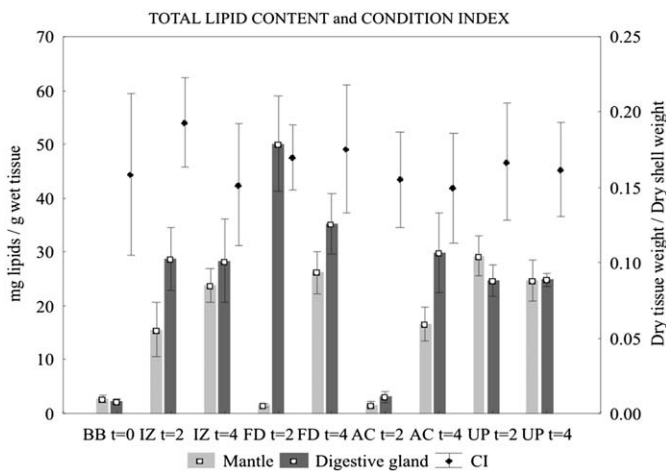
Fig. 4. Total lipid content in mantle and digestive gland and condition index of *Mytilus edulis chilensis* from four sites inside Ushuaia Bay after 2 ($t=2$) and 4 ($t=4$) weeks of exposure and reference organisms ($t=0$), before transplantation. Total lipid content is expressed as mg lipids/g wet tissue on left axis and condition index as dry tissue weight/dry shell weight on right axis (mean values ± standard deviations).

measured in FD at T_2 (20 fold bigger) and T_4 (14 fold bigger) in digestive gland; while in mantle was only at T_4 (8 times higher). Total lipid content increased at T_4 in both organs assayed in AC (5 times higher in mantle and 12 times in digestive gland). At all sites, significant differences were found among times of exposure ($T_4 \geq T_2$ and T_0), while in UP $T_2 \geq T_4 > T_0$. Significant differences among tissues (digestive gland > mantle) were found in IZ, FD and AC (two-way ANOVA, $p < 0.05$) (Fig. 4).

3.2.4. Condition index

To assess the general health of the transplanted mussels, the condition index was measured at each time of collection (T_0, T_2 and T_4) at all sites (Fig. 4). Results showed that the condition index remains stable with low variability (0.16 ± 0.01). No differences were found among sites nor among times (two-way ANOVA, $p < 0.05$).

3.3. Metal bioaccumulation in mussels

Accumulated metal concentrations in mussel's tissues are presented in Table 4. Digestive gland was the main target tissue of accumulation of Fe and Cu, while gill accumulated the highest levels of Zn (two-way ANOVA, $p < 0.05$). No significant differences were registered between tissues for Cd and Pb. These two metals showed high standard deviation. Concentrations of Fe, Cu and Zn in digestive gland as well as in gill were significantly different (two-way ANOVA, $p < 0.05$) among sites and times of exposure. Highest Cu and Zn concentrations were found at FD in both tissues, while Fe levels were higher at IZ, FD and UP especially in digestive gland. No significant correlation ($p > 0.05$) was found between metal concentrations in tissues and sediments.

3.4. Principal components analysis

PCA analysis with physical–chemical parameters revealed that 65.6% of the cumulative variance was explained by two components. PC1, which explained 45.5% of the variance, was negatively correlated with nitrites, nitrates, silicates and ammonia and positively correlated with total metal-in sediment and dissolved oxygen. PC2 explained 20.1% of the variance, represented mainly by phosphates and negatively correlated (Table 5). Stations from Ushuaia Bay were clearly different from the reference site (Fig. 5). Physical–chemical parameters seemed to be relatively stable at each station during the assayed period. Variations are given mainly by PC 2 except for AC where variation on PC1 was also noticed.

A second PCA analysis was done using significant physical–chemical parameters obtained in first PCA and biomarker data. The co-ordinates of PC1 and PC2 give the positions of the transplantation stations and the reference site at week 2 (Fig. 6A) and at week 4 (Fig. 6B). At both sampling times, PC1 explained more than 50% of the total variance. At week 2 and 4 dissolved oxygen and total metal in sediment had a positive correlation with PC1 while nutrients and biological responses showed a negative correlation for both periods of time. Total metal in digestive gland had a positive correlation with PC1 only at week 2. At week 2 PC2 showed phosphates, LPO in gill and Lipid in mantle on the positive axis and Total metal in gill on the negative axis. At week 4 PC2 had the highest loadings of phosphates and CAT in gill with positive signs and total metal in digestive gland with negative sign (Table 6). More defined positions of the sites were obtained for week 4 of exposure where FD and IZ were grouped together and AC and UP formed another group; both clearly separated from the reference site.

4. Discussion

In Ushuaia Bay a permanent strong current moves westward along the northern coast of the bay at 2 cm s^{-1} and then progresses to the southeast along the southern coast at 16.3 cm s^{-1} (Balestrini et al., 1998). At all studied sites, mussels increased their levels of the biomarkers analyzed. In general terms, PCA revealed that all biomarkers were directly related to nutrients and inversely related to dissolved oxygen and total metal in sediment. FD and IZ were the sites with the highest concentrations of ammonia and silicates due to the discharges of Este Stream and Grande Stream, respectively. Our findings are consistent with data reported by Esteves and Amin (2004), who have monitored these streams and considered them as "intermediate impacted" for their nutrient loadings. In concordance with findings made by Lau et al. (2004) on mussel *Perna viridis*, this study demonstrated the

Table 4
Heavy metal concentrations in gill and digestive gland of *Mytilus edulis chilensis* expressed in µg/g of dry weight.

Station	Week	Tissue	Fe	Cu	Zn	Cd	Pb
BB	0	Gill	80.14 ± 7.04	6.49 ± 0.09	270.63 ± 0.34	1.28 ± 0.14	7.11 ± 5.10
		Dig. gland	448.57 ± 9.10	12.07 ± 0.54	63.17 ± 7.40	2.03 ± 1.05	9.10 ± 3.33
IZ	2	Gill	110.71 ± 15.80	7.42 ± 1.65	83.97 ± 2.09	0.38 ± 0.54	15.57 ± 0.32
		Dig. gland	887.69 ± 184.01	17.68 ± 2.19	59.92 ± 5.68	0.30 ± 0.43	6.22 ± 2.99
FD	2	Gill	128.20 ± 12.72	11.98 ± 0.35	280.57 ± 32.60	1.25 ± 1.09	nd
		Dig. gland	760.95 ± 121.69	23.22 ± 0.97	102.54 ± 6.62	2.03 ± 0.17	7.99 ± 0.14
AC	2	Gill	109.69 ± 3.49	9.40 ± 1.15	222.27 ± 45.71	2.57 ± 1.15	9.17 ± 6.35
		Dig. gland	491.83 ± 14.40	16.14 ± 0.49	68.55 ± 3.98	1.46 ± 0.69	5.05 ± 2.38
UP	2	Gill	87.65 ± 2.93	9.31 ± 0.14	95.16 ± 7.13	1.46 ± 1.02	7.59 ± 3.47
		Dig. gland	726.73 ± 64.02	16.68 ± 0.38	77.31 ± 10.87	1.18 ± 0.25	6.99 ± 4.98
IZ	4	Gill	159.23 ± 6.91	8.58 ± 0.48	212.06 ± 15.85	0.98 ± 0	3.37 ± 0
		Dig. gland	611.72 ± 13.32	15.09 ± 0.95	60.96 ± 1.89	1.22 ± 0.57	6.73 ± 4.75
FD	4	Gill	87.75 ± 12.25	9.61 ± 0	92.36 ± 5.19	0.89 ± 0.80	5.05 ± 2.38
		Dig. gland	492.08 ± 71.01	25.76 ± 3.40	74.55 ± 2.21	1.38 ± 0.58	3.37 ± 4.77
AC	4	Gill	106.02 ± 2.50	6.42 ± 0.68	96.07 ± 1.08	1.16 ± 0.10	5.52 ± 2.49
		Dig. gland	381.39 ± 14.30	11.56 ± 0.49	67.58 ± 8.80	1.94 ± 0.51	5.49 ± 2.60
UP	4	Gill	158.77 ± 6.78	7.19 ± 0.48	244.70 ± 20.51	2.11 ± 0.92	5.04 ± 2.38
		Dig. gland	572.08 ± 42.74	12.02 ± 2.43	55.42 ± 5.95	3.25 ± 0.23	3.37 ± 0

Data are expressed as mean values ± standard deviations.

Table 5
PCA: correlations between physical–chemical variables and principal components (loadings).
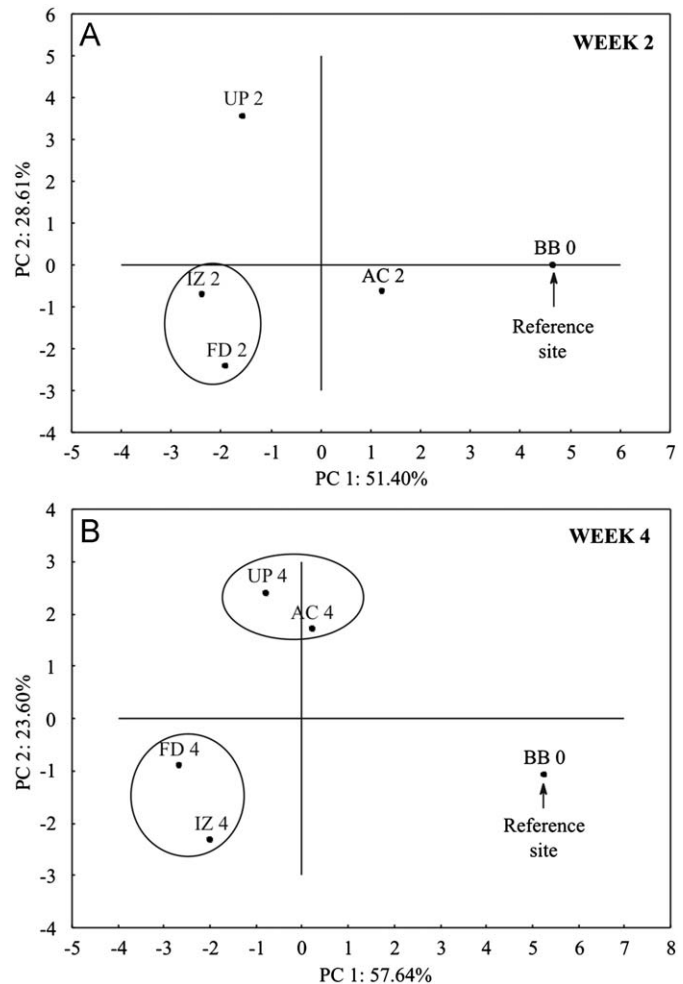
Variables	PC1	PC2
pH	0.57	0.18
Conductivity	0.44	−0.22
Dissolved oxygen	**0.82**	−0.24
Temperature	−0.25	−0.67
Chlorophyll-*a*	−0.52	−0.65
Ammonia	**−0.70**	0.47
Nitrites	**−0.76**	0.09
Nitrates	**−0.87**	−0.17
Phosphates	0.08	**−0.91**
Silicates	**−0.86**	0.24
Total metal sediment	**0.95**	0.24
Cumulative explained variance (%)	45.43	65.56

Correlation coefficients are significant when they are higher than 0.7 (bold coefficients).
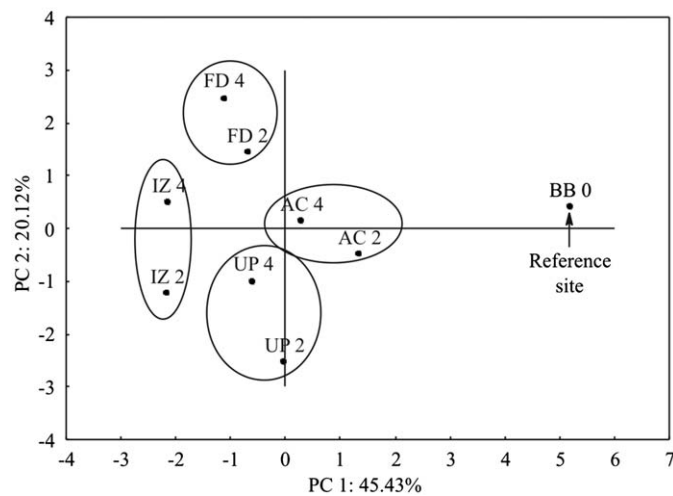
Fig. 5. Graphical representation of sampling sites in the first two axis of PCA derived from physical–chemical parameters.

Fig. 6. Graphical representation of sampling sites in the first two axis of PCA (**A**) derived from significant physical–chemical parameters and biomarker data at week 2 of exposure and (**B**) derived from significant physical–chemical parameters and biomarker data at week 4 of exposure.

importance of factors other than heavy metals on the bio-marker responses of mussels *Mytilus edulis chilensis*. Nutrient loadings and abiotic factors were shown to induce variations in the biomarker responses. Therefore, interpretation of the

biomarker responses in short time studies should be made with caution.

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

Table 6
PCA: correlations between significant environmental variables and biological variables.

Variables	Week 2		Week 4	
	PC1	PC2	PC1	PC2
Dissolved oxygen	**0.89**	0.38	**0.92**	−0.38
Ammonia	−0.63	−0.47	−**0.77**	−0.64
Nitrites	−**0.93**	0.01	−**0.84**	−0.24
Nitrates	−**0.93**	−0.07	−**0.90**	−0.31
Phosphates	−0.16	**0.92**	0.37	**0.89**
Silicates	−**0.81**	−0.55	−**0.94**	−0.02
Total metal sediment	**0.97**	−0.11	**0.94**	−0.14
Total metal gill	−0.06	−**0.85**	−0.07	0.22
Total metal Dig. gland	**0.93**	−0.19	0.05	−**0.89**
CAT gill	0.05	−0.44	−0.21	**0.75**
CAT mantle	−**0.76**	−0.47	−0.56	0.61
CAT Dig. gland	−**0.70**	−0.42	−**0.83**	0.54
LPO gill	−0.59	**0.76**	−**0.97**	−0.12
LPO mantle	−0.60	0.63	−**0.76**	−0.59
LPO Dig. gland	−**0.81**	0.47	−**0.70**	0.20
LIP mantle	−0.50	**0.84**	−**0.98**	0.12
LIP Dig. gland	−**0.80**	−0.29	−**0.95**	0.14
Cumulative explained variance (%)	51.40	80.01	57.64	81.24

Correlation coefficients are significant when they are higher than 0.7 (bold coefficients).

conditions in this area, which is in accordance with previous data (Amin et al., 1996a; Amin et al., 1997). 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. The highest concentrations of Fe, Cu and Zn at BB must be of natural origin since there would not be a human activity that could cause such heavy metal input into the system. Metal concentration of sediment from BB constitutes baseline data since there is no previous information of this kind from BB. The highest value of Pb found in FD probably is associated with the intense shipping activity although it is one order of magnitude below the value reported by Amin et al. (1997). The concentration of Pb found in present work in IZ was five times lower than the value registered by Amin et al. (1997). Sediments from AC and UP showed the highest concentrations of Cu and Cd. Unfortunately there are no existing records about heavy metal in these sites that allow us to make comparisons. In the case of Cu, values of AC and UP were below the levels measured by Amin et al. (1996a) in several sites within Ushuaia Bay. In the previously mentioned study, non-detectable levels of Cd were found, meaning that the concentrations of Cd found in the present work would be of anthropic origin. Further studies are necessary to establish the real source of Cd. The diminishment of Cu, Zn and Pb concentration in sediments of Ushuaia Bay could be related to a lower input coming from the industrial sewages as a consequence of the economic crisis in 2001 when most electronic industries closed down their factories and left the area (INDEC, 2005). By comparing metal concentrations in sediments determined in this study with the levels found in coastal sediments of other Argentine coasts (Commendatore et al., 1996; Vázquez, 2005) or even with other parts of the world (Muniz et al., 2004; Lee et al., 1998), it can be observed that concentrations are in the same order of magnitude or even below. Results found during this study for Cu, Zn and Pb are within the ranges reported as background values for uncontaminated coastal and marine sediments (Cobelo-García et al., 2005; Cobelo-García and Prego, 2003). The significant correlation coefficient between concentrations of Cu,

Zn and Fe in sediments indicated that these metals might have come from the same natural source.

Data presented in this paper demonstrate that mussel *Mytilus edulis chilensis* from Ushuaia Bay in the period assayed accumulated mainly Fe and Cu in digestive gland and Zn in gill. It has been suggested that metal concentration in gill and digestive gland can be used to estimate dissolved and particulate exposure of metals, respectively (Fisher et al., 1996). Then, Fe and Cu could be taken up bound to particulate material and Zn in soluble form. Pb and Cd concentrations were similar in both tissues. The same tissue distribution of Fe, Cu and Zn was found in *Mytilus galloprovincialis* by Regoli and Principato (1995), but they found higher concentrations of Pb in gill. Also in *M. galloprovincialis*, Irato et al. (2003) measured higher concentrations of Fe and Cd in digestive gland, while they did not find selective patterns of accumulation for Cu and Zn. Regoli (1998) found high Fe and Cu accumulation in digestive gland, meanwhile Pb and Zn did not show a specific target organ. The levels of metals accumulated by *M. edulis chilensis* were similar to those obtained by Gil et al. (2006) in mussel *Mytilus edulis* from various sites of the South of Argentina. Many researchers have evaluated bioaccumulation of heavy metals in various bivalves reaching diverse results in different organs and species. Results are difficult to compare because responses differ among species, threats differ among metals, and environmental influences are complex (Luoma and Rainbow, 2005).

Despite the variation found between the study sites, the overall pattern was constant at all sites. The order of magnitude of accumulation at all sites was Fe > Zn > Cu > Pb > Cd. Although BB showed the highest levels of Fe, Cu and Zn in fine sediment, except for Zn that was accumulated in gill, the other metals were not accumulated by mussels from BB. FD was the site with the top rates of incorporation of Cu and Zn in gill. We measured the highest accumulation of Fe in digestive gland at IZ, UP as well as at FD. Maximum values of Cd were found in mussels transplanted to AC and UP, precisely where we registered the highest concentration of Cd in sediment. Under oxic conditions, Cd is a labile competitor in adsorption on metallic oxyhydroxides. Therefore it is probable that dissolved forms are predominant and bioavailable to be incorporated by the biota (Bewers et al., 1987). Considering that there is no industrial or mineral source in the proximities of AC and UP, Cd would be of natural origin. Apart from possible natural differences such as geochemical compositions of sediment, the observed site-specific concentration differences may be caused by anthropogenic sources. One possible source of metal, besides the natural one, constitutes the several metallurgic industrial and urban sewages that reach the coastal waters without treatment (Esteves and Amin, 2004). Metals could be transported from IZ southeast along the southern coast by the counter-clockwise current arriving to AC and UP.

There were no significant correlations between total metal concentrations in sediment and mussel tissue levels in this study, as it has been shown in other works (Hickey et al., 1995; Gundacker, 1999; Beiras et al., 2003). The absence of relationships between mussel tissue and sediment concentrations suggests that differences in bioavailability may be influenced markedly by local site conditions (Hickey et al., 1995). Sediments are known to act as 'traps' for pollutants and to reflect rather long-term contamination, whereas suspended matter and filtrate concentrations vary due to local metal emissions, quality and quantity of suspended materials, as well as physicochemical parameters (Salomons et al., 1995).

CAT has biological importance due to the fact that H_2O_2 is the main cellular precursor of the hydroxyl radical (HO^-), which is a highly reactive and toxic form of ROS. The removal of H_2O_2 by this enzyme is an important strategy of marine organisms against oxidative stress (Regoli et al., 2002). The enhancement of the CAT

activity observed at all sites compared with reference site confirms the presence of an oxidative stress that may affect mussels throughout the Ushuaia Bay. The highest activity of CAT was measured in the digestive gland being the values similar at all stations within Ushuaia Bay. The higher response in digestive gland compared to that in gill and mantle tissues is in agreement with other studies on bivalves transplanted to coastal waters (De Luca-Abbott et al., 2005; Box et al., 2007). CAT activities measured in this work were higher than those reported by Giarratano and Amin, 2008 in mussels transplanted to the same sites within Ushuaia Bay but in summer season. The same seasonal differentiation was found in mussels *Perna viridis* (Lau et al., 2004) and in *Mytilus galloprovincialis* (Santovito et al., 2005). One possible explanation is that the water temperature is the lowest in winter and the solubility of oxygen increases. This condition implies a high probability of ROS formation in cells influencing the higher CAT activity (Santovito et al., 2005).

Several studies have evidenced that lipid peroxidation increases in tissues of different species of aquatic organisms, as a result of being exposed to environmental pollutants (Almeida et al., 2007). Lipid peroxidation corresponds to reactions in chain that can be induced by transition metals such as Cd, Cu, Fe and Pb in mussels (Almeida et al., 2004; Viarengo et al., 1990). During these reactions, various compounds are produced such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE), both able to bind to proteins and to form adducts. Indeed, these compounds react in a spontaneous way with cysteines of proteins and with glutathione (Manduzio et al., 2005). An increment in MDA concentration was found at all sites, being higher in gill and digestive gland than in mantle. Box et al. (2007) neither evidenced significant differences in MDA concentration between digestive gland and gill. We measured a progressive increment at IZ and FD, meanwhile a late and lower increment was registered at AC at week 4 of exposure. The depletion of MDA levels measured at week 4 of exposure in digestive gland at UP and at IZ could be related to the diminishment of Cu and Fe accumulated in the organ mentioned above in these two sites. The depletion of MDA levels could also be related to a faster transformation of MDA to other metabolites at UP and IZ rather than in the other three sites. Another possible explanation is that other antioxidant parameter than those measured in this study, such as the tripeptide GSH, could ensure protection and compensation for the above depletion (Santovito et al., 2005). Levels of MDA registered in this study are similar to those reported by Giarratano and Amin (2008) for summer season, who did not find a clear differentiation among tissues. There is an inverse correlation between MDA content and the activity of catalase suggesting its importance in protecting the cell from membrane damage. In fact an effective CAT control will end up with a low MDA level and vice versa (Lau and Wong, 2003). The enzyme efficiently prevented lipid peroxidation by neutralizing oxyradical leading to low TBARS concentration at AC and UP. However, in this study, correlation between MDA and CAT was not found.

Natural stress and stress related to pollution are often linked with reduced growth rates (Dame, 1996) and with changes in biochemical composition, specifically in fractions that involve changes in the cycle of accumulation and use of energy reserves (Smolders et al., 2004). Seasonal biochemical and energy storage cycles in marine bivalves are closely related to reproductive activity. Seasonal metabolic activity in molluscs results from complex interactions among food availability, environmental conditions and gametogenic cycle (Dridi et al., 2007). In this study, an increment in total lipid content was registered at all sites of Ushuaia Bay with respect to Brown Bay. Only in UP total lipid content kept constant with time, but increased along exposure time in the other sites. PCA results revealed that total lipid content was directly related to nutrients. Additionally, although chlorophyll-*a* concentrations at all sites of Ushuaia Bay were relatively low, they were greater than the value measured at Brown Bay. Levels of nutrients in Ushuaia Bay were higher than in Brown Bay too. When present study was carried out in august, mussels would be starting gametogenesis (Gray et al., 1997). Probably lipid content would continue increasing until late spring (November–December) when mussels reach their phase of highest ripeness (Gray et al., 1997) likely associated to a superior availability of phytoplankton and warmer temperature. In the same sense, Lomovasky et al. (2004) found in clam *Eurhomalea exalbida*, also from Beagle Channel, the highest lipid content in September (spring) and January (summer). *E. exalbida* was characterized by an important spawning event in November, followed by a quick recovering in summer with the presence of ripe gonads in the rest of the year (Morriconi et al., 2002). In Pacific oyster, *Crassostrea gigas*, lipids were also accumulated in gonads during the period of maximum ripeness (spring) and decrease in summer. During autumn, the increment of lipids appeared to be related to the available food in association with an increment in the chlorophyll-*a* concentrations (Dridi et al., 2007).

Mussels that experience a life period in urban influenced areas are exposed to a complex mixture of pollutants and they are forced to spend a great part of their energy budget in detoxification processes and maintain homeostasis to the detriment of body growth and gonad production (Pampanin et al., 2005). The condition index has been used as an estimation of the physiological condition in mussels (Smolders et al., 2004; Pampanin et al., 2005). It is a parameter strongly linked to the availability of food resources and the quality of the diet (Mourgaud et al., 2002). The low value of condition index is related to reproductive phase (mussels with empty gonads after the spawning) and/or to adverse environmental conditions like presence of pollutants, scarce food availability or changes in temperature (de Zwaan et al., 1995; Damiens et al., 2007). During the present work condition indexes of transplanted mussels were similar among stations, and compared to those belonging to the farm indicated similar water trophic conditions and reproductive phase. Perhaps the differences in the availability of nutrients found between Ushuaia Bay and Brown Bay are so small that cannot affect the condition index. It is also possible that a period of 4 weeks may not be enough time to appreciate an effect over the condition index of transplanted mussels.

Biological responses measured at stations IZ and FD were related with the proximity to discharge points of domestic and industrial sewages and contaminants (Esteves and Amin, 2004). Despite that AC and UP were considered to be relatively low contaminated sites due to the distance to local points of discharges from Ushuaia city, they also showed increments in the biochemical markers studied in concordance with previous researches made in the same area (Giarratano et al., 2006; Giarratano and Amin, 2008). This could be associated to the existing counter-clockwise water circulation within Ushuaia Bay (Balestrini et al., 1998) that could transport the urban and industrial sewages generated by Ushuaia city until far sites such as AC, UP or even farthest.

In this sense, the PCA proved an easy and useful tool to summarize the obtained results, also able to classify the sites indicating different physicochemical conditions on each study area. IZ and FD are located nearer to the urban influences and grouped together, while AC and UP are the most away sites from the city influence and constituted another group, both groups separated from the reference site.

5. Conclusions

As a whole, the biological and chemical results obtained by transplanting mussels from a reference site to coastal waters of

Ushuaia Bay suggest that transplanting can be a good strategy for biomonitoring environmental and urban effects in coastal zones.

An increment in CAT activity and MDA content at all studied sites in the period assayed confirm the use of these parameters as early warning biomarkers of oxidative stress, representing sensitive responses of the mussel antioxidant defense system. Our results suggest that antioxidant enzyme CAT, especially in digestive gland and mantle, and LPO levels in gill and in digestive gland may be considered as good markers of urban pollution, with a relatively short time response to environmental stressors, as suggested by the transplantation experiment.

We believe the progressive increment in total lipid content measured in our experiment represents mainly the reproductive phase of mussels before spawning and the improvement in available food supply of Ushuaia Bay. For a better understanding we suggest that studies in much longer period of time should be carried out in order to be able to distinguish physiological variations from natural and anthropogenic stress at least during a whole year.

Metal concentrations in tissue of M. edulis chilensis were not significantly correlated to sediment concentrations, at least for the studied areas. Mussels resulted poorly suitable as a heavy metal indicator tool in this short experiment, may be because the analysis of total metal concentrations without consideration of chemical speciation and specific bioavailability. Probably further investigations that include changes in the distribution of metals between the dissolved and particulate phases are crucial to understand the importance of waterborne and dietary exposure routes and their environmental relevance to metal accumulation by these filter-feeding invertebrates.

The whole set of data allowed to group the stations as a function of the chemical data and biochemical responses of transplanted mussels. These responses suggested the potential presence of different stressors.

Acknowledgments

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