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Trace metals and oxidative status in soft tissues of caged mussels (*Aulacomya atra*) on the North Patagonian coastline

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ABSTRACT

This study investigated metal accumulation and oxidative effects in mantle, gill and digestive gland of the ribbed mussel *Aulacomya atra* from the Argentinean North Patagonian coastline. Mussels were transplanted over an 18-month period from a site with low anthropogenic impact to a harbor site with higher seawater concentration of aluminum, chromium, copper, manganese, nickel and zinc. Total trace metal concentration in seawater did not change throughout the 18-month transplant in either site. *A. atra* bioaccumulated metals in digestive gland, gills and mantle at different levels. Digestive gland had the highest concentration of metals, especially towards the end of the transplant experiment in the harbor area. Mussels transplanted to the harbor site experienced an upregulation in their antioxidant system, which likely explains the lack of oxidative damage to lipids despite higher metal accumulation. These results demonstrate that *A. atra* selectively accumulates metals from the water column and their prooxidant effects depend on the tissue antioxidant defenses and the exposure time.

1. Introduction

Changes in physicochemical variables and biological parameters due to pollution have been recorded in most of the world's coastal zones. High concentrations of pollutants in closed seas and coastal waters are a major environmental concern because these areas have high biological productivity and human activities (Cohen et al., 1997; Muniz et al., 2015). Metal pollution is one of the most severe anthropogenic disturbances affecting marine organisms because these are unable to degrade metals. As a consequence, metals may be bioaccumulated and biomagnificated throughout food chains and result in several toxic effects (Macfarlane and Burchett, 2000; Miller et al., 2002; Censi et al., 2006). Metal uptake into the body of aquatic animals occurs through the permeable surfaces of the body, mainly the gills and mantle, or through injection of contaminated food particles (Wang and Fisher, 1996). Determination of pollutants in tissues of aquatic organisms is a suitable indicator of its presence in the marine environment (Baqueiro-Cárdenas et al., 2007), especially for those xenobiotics that

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are not metabolized, like metals and metalloids (Luoma and Rainbow, 2005).

Mussels filter relatively high volumes of water for feeding, and as a result typically take up large amounts of trace metals from the surrounding environment, which they accumulate in their soft tissues (Andral et al., 2004; Attig et al., 2010; Ciacci et al., 2012). Transplanting caged mussels' from a site with low anthropogenic impact to polluted sites is widely applied in ecotoxicological studies for monitoring coastal environments (Box et al., 2007; Fasulo et al., 2012; Cappello et al., 2015). The caging approach allows analyzing the harmful effects of aquatic pollutants for a certain time period reducing the effects on the measured biomarkers due to the genetic variability and physiological status (growth and reproduction, between others) of the studied population (Cappello et al., 2013; Marigómez et al., 2013). *Aulacomya atra* is an epifaunal bivalve mollusk that inhabits rocky or mixed bottoms in temperate-cold coastal waters, from shallow depth up to 40–50 m, on hard and soft substrates (Guzmán et al., 1998; Zaixso, 1999). This species is a resource exploited by multispecies artisanal fisheries in several rural and urban locations in Argentinean Patagonia and it constitutes an important economic support for family and regional economies (Narvarte et al., 2007; Orensanz et al., 2007).

Metals can cause oxidative damage to cellular components by increasing the levels of reactive oxygen species (ROS) (Livingstone, 2001; Lesser, 2006; Sheehan and McDonagh, 2008; Tsangaris et al., 2010; Jaishankar et al., 2014) through Haber-Weiss and Fenton-like reactions (Lloyd and Phillips, 1999; Eberhardt, 2001). Aquatic organisms have a ROS scavenging antioxidant defense system that protects against oxidative damage (Gorinstein et al., 2003; Valavanidis et al., 2006; Troschinski et al., 2014; Banni et al., 2015). The antioxidant system comprises enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, and non-enzymatic agents like reduced glutathione and vitamin E, among others. Oxidative stress parameters such as changes in antioxidant levels and oxidative damage to different cell components are frequently used as biomarkers to quantify effects induced by trace metals, test their toxicity and assess the health of aquatic life (de Almeida et al., 2004; Führer et al., 2012; Kumari et al., 2014).

Along the ~3000 km long Argentinean Patagonian coastline, trace metals toxicity is associated to harbor areas, which generally have the highest pollutantion levels (Esteves, 2008). The Almirante Storni pier (Puerto Madryn, North Patagonia) was used by the aluminum industry for importing raw materials (aluminum oxide, aluminum fluoride, silicon metal and steel) and for exporting manufactured aluminum products for approximately 50 years, with a maximum metal production capacity of 460,000 t of metal per year (Di Salvatore et al., 2013). Previous studies reveal higher levels of metal and polycyclic aromatic hydrocarbons (PAHs) concentrations in the Almirante Storni pier (Gil et al., 1999; Commendatore and Esteves, 2007; Massara Paletto et al., 2008; Di Salvatore et al., 2013).

The aim of this study was to investigate metal accumulation and their oxidative effects on soft tissues of the native ribbed mussel *A. atra*. To achieve this goal, mussels were transplanted from a site with low anthropogenic impact to a site near the Almirante Storni pier, and oxidative stress parameters were measured over an 18-month period.

2. Materials and methods

2.1. Sampling area and experimental design

96 mussels of similar size (7.75 ± 0.48 cm shell length) were sampled at Punta Cuevas (PC) (42° 46 ´28 ´´S; 64° 56 ´54 ´´W) (Fig. 1) by diving at a depth of 9–10 m during March (summer) 2012, on the southern edge of Puerto Madryn city. This site experiences low anthropogenic impact (Di Salvatore et al., 2013). After collection, mussels were randomly sorted and placed inside 16 plastic (PVS) cages with mortar bottoms as substrate (6 mussels per cage, similar density per square meter that registered in the sampling site). The cages were placed in the collection site for a two-week acclimation period. After the acclimation period, all cages were recovered by diving and mussels from four cages were sampled (PC, initial time: it), while the others 12 cages were transported to the harbor site at a depth of 9–10 m (Almirante Storni pier, AS) (42° 44 ´14 ´´S; 65° 1 ´43 ´´W). Four cages were collected at random after 6, 12 and 18 months. After collection, mussels were immediately anesthetized by placing on ice before they were killed. The digestive gland, gills and mantle were dissected and weighted and immediately frozen at − 80 °C during three days. After that, samples were transported frozen (− 4 °C) to the University of Buenos Aires where metals levels and oxidative stress biomarkers were measured. In addition, 500 mL seawater samples were collected at the beginning and the end of the experiment to determine the total concentration of metals in each sampling site. Water samples were collected and stored in sterile plastic bottles previously washed with 2 M nitric acid, and acidified to pH < 2 with (1:1) nitric acid (Martin et al., 1991),

2.2. Metals measurements

Aluminum (Al), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni) and zinc (Zn) concentrations were measured by ICP-OES (Perkin Elmer, Optima DV 2000, USA) equipped with a sample introduction unit consisting of a Scott chamber and a flow GemCone™ nebulizer. Perkin Elmer quality control standards N9300281 and N9300280 were used as the stock standards for preparing working solutions of Al, Cr, Cu, Mn, Ni and Zn. In order to validate the method for accuracy and precision, certified reference materials ERMCE278K (SIGMA-ALDRICH) and the Fish Protein DORM-4 NRC were analyzed. In whole set of measurements recovery (%) were around 85–110% (data not shown).

Water samples were analyzed directly, while mussels' soft tissues were weighed, washed in ice-cold saline, homogenized individually in 0.134 M KCl (1:5, w/v) as described by Türkmen and Ciminli (2007) and Di Salvatore et al. (2013). Solid particles were removed using a cellulose nitrate filter (0.45 μm) coupled to a syringe prior to measuring metal concentrations.

Each sample was analyzed by triplicate (standard deviation less than 4%) and a blank was run to correct the intensity emission values. Additionally, for every ten water or tissue samples, a procedure blank and a spike sample containing all reagents were run to check for interference and cross-contamination. The water used throughout the present study was obtained from a Milli-Q water purification system (Millipore GmbH, France) with a resistivity of 18.2 MOhm cm^{-1}.

Data is expressed as μg metal per L water and μg metal per g wet tissue, respectively.

2.3. Sample preparation for biochemical measurements

Digestive gland, gills and mantle tissues from each animal were homogenized on ice with 0.134 M KCl (1:5, w/v) containing protease inhibitors (phenylmethylsulfonil fluoride 0.5 mM and benzamidine 10 mM). The homogenates were centrifuged 15 min at 11,000 × *g* and the resulting supernatants were used for the assays described below.

2.4. Protein content

Total soluble protein content was measured by the method of Bradford (1976), using bovine serum albumin as standard. Results are expressed as mg protein per mL.

2.5. Reduced glutathione (GSH) content

Reduced glutathione (GSH) content was determined in the supernatants as described by Anderson (1985) in presence of 5,5-dithiobis-(2- nitrobenzoic) acid (DTNB). A freshly prepared solution of

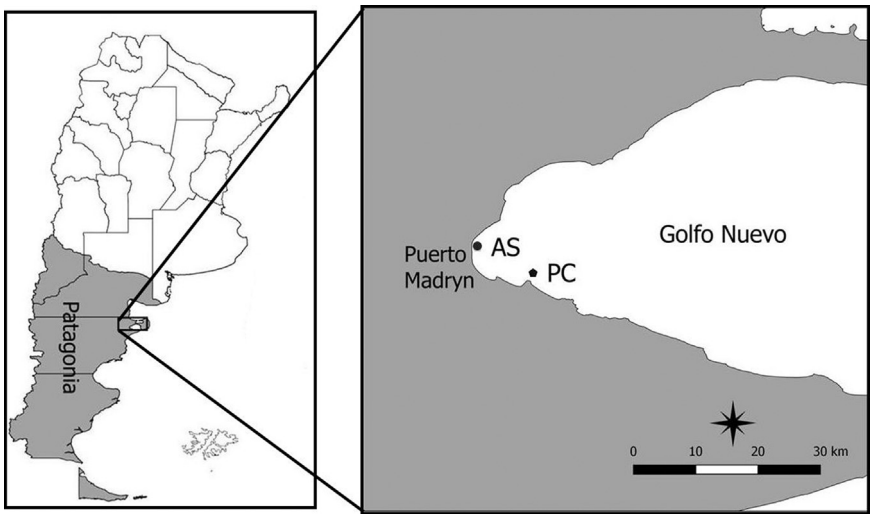

Fig. 1. Locations of Punta Cuevas (PC) and Almirante Storni pier (AS).

glutathione was used to generate a standard curve. Results are expressed as nmol GSH per mg proteins.

2.6. Enzyme assays

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined by measuring the inhibition of the reduction of nitroblue tetrazolium (NBT) by the light activated generation of O_2^- by riboflavin in the presence of methionine at 560 nm (Beauchamp and Fridovich, 1971). The reaction was carried out in 50 mM potassium phosphate buffer (pH 7.4). Results are expressed as SOD units per mg of proteins. One SOD unit was defined as the enzyme amount necessary to inhibit 50% NBT reduction rate.

Glutathione-S-transferase (GST, EC1.11.1.9) activity was measured by the technique of Habig et al. (1974) in presence of 1chloro-2,4dinitro- benzene (CDNB) at 340 nm during 60 s. One GST Unit was defined as the amount of enzyme needed to catalyze the formation of 1 μmol of GS-DNB per min at 25 °C.

2.7. ROS and TOSC

Digestive gland, gill and mantle supernatants were used to measure Reactive Oxygen Species (ROS) production by a fluorometric method using H_2DCF-DA (2,7 dichlorofluorescein diacetate, 0.8 mM, Sigma) (Amado et al., 2009). H_2DCF-DA is cleaved by esterases in the reaction buffer (30 mM HEPES, 200 mM KCL, 1 mM $MgCl_2$, pH 7.2) and the resulting non-fluorescent compound (H_2DCF) is oxidized by ROS. The fluorescent compound (DCF) was detected at 488 and 525 nm, for excitation and emission, respectively and ROS content was referred to a H_2O_2 standard curve with H_2DCF-DA. Results are expressed as nmol H_2O_2 per mg protein.

Total Oxyradical Scavenging Capacity (TOSC) was measured in the supernatant fraction of digestive gland, gill and mantle as described by Amado et al. (2009). Half-aliquot of sample (50 μL) was mixed with ABAP solution (2,2-Azobis 2-methylpropionamidine dihydrochloride 4 mM, Sigma) and the other one without ABAP solution. Samples were kept at 35 °C inside a microplate reader (FLUOstar OPTIMA BMG Labtech) during 5 min to produce peroxyl radicals by thermal decomposition of ABAP. H_2DCF-DA was added in all the wells and florescence was read every 5 min for 30 min. The difference of the relative area below the curves obtained with and without ABAP (background)/ without ABAP was calculated and TOSC was considered as follows: TOSC = 1/ [(ROS area$_{ABAP}$ – ROS area $_{background}$)/ ROS area $_{background}$]. Results are expressed as arbitrary units (a. u.).

2.8. Lipid peroxidation

Quantification of lipid peroxides through dosage of thiobarbituric acid reactive substances (TBARS) was carried out according to Beuge and Aust (1978). Briefly, the 11,000 ×*g* supernatant from total homogenate was mixed with thiobarbituric acid (TBA) solution and incubated at 95–100 °C for 45 min. After cooling, the reaction mixture was centrifuged and the supernatant absorbance was determined at 535 nm. Results are expressed as micromoles of TBARS per mg proteins.

2.9. Statistical analyses

Metal content in seawater at the begging and end of the experiment in both sites were compared statistically by one-way analysis of variance (ANOVA) followed by a Tukey's test. Metal accumulation and biochemical variables measured in each tissue of *A. atra* among exposure times at the harbor site were compared using one-way ANOVA followed by Dunnett's post hoc test. Differences were considered significant when $p < 0.05$. The assumptions of normality and homogeneity of variances were tested with Lilliefors` and Bartlett tests, respectively (Sokal and Rohlf, 1999) and in all cases, these assumptions were achieved. Graph Pad Prism 6 software was used for statistical analysis.

3. Results

3.1. Water temperature

Temperatures ranged from an average temperature ~ 18 °C in the summer (March) to ~10 °C in the winter (July). Seawater temperatures during the transplant experiment are summarized in Table 1.

Table 1
Water temperature at Punta Cuevas (PC) and Almirante Storni pier (AS) from March 2012 to July 2013.

	March 2012		*July 2012*	*March 2013*	*July 2013*	
	PC	**AS**	**AS**	**AS**	**PC**	**AS**
Average temperature (°C)	17.8	17.9	10.5	17.6	10.8	10.7
Maximum temperature (°C)	20.3	20.1	11.4	19.3	11.6	11.3
Minimum temperature (°C)	15.4	15.9	9.6	16.0	9.9	10.1

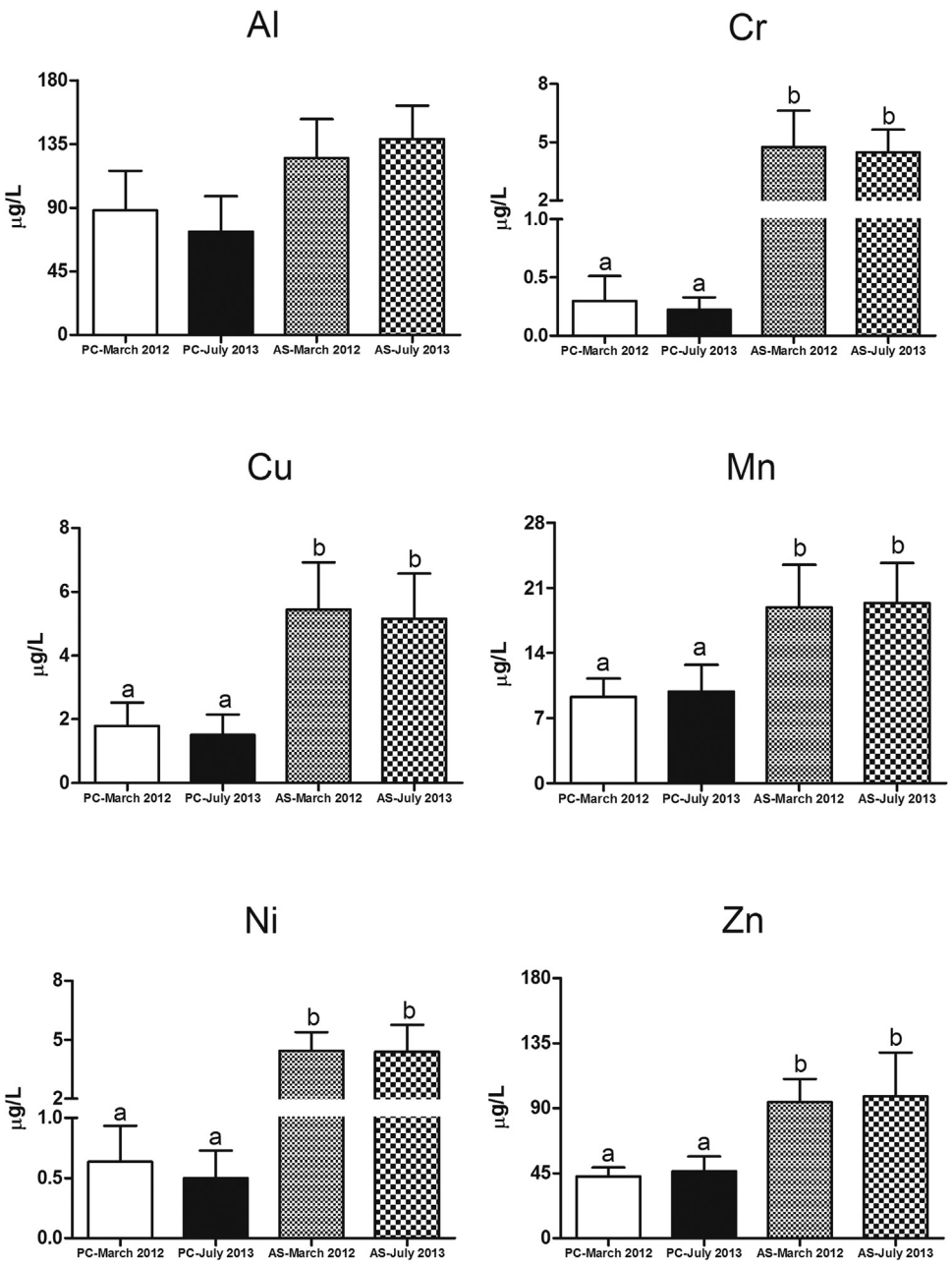

Fig. 2. Concentration of metals (µg/L) in seawater from Punta Cuevas (PC) Almirante Storni pier (AS) at March 2012 (initial time: **it**) and July 2013 (final time: **ft).** Results are expressed as means ± S.D. (n = 4). Different letters indicate significant differences s ($p < 0.05$).

3.2. Metal concentration in seawater

There were no significant differences in total trace metals in seawater in neither site between the beginning and the end of the transplant experiment ($p > 0.05$) (Fig. 2). However, with the exception of aluminum, the concentrations of the other 5 metals were significantly higher ($p < 0.05$) in the harbor site compared to the reference site (PC), reaching values ranging from 2 to 18 times higher for manganese and chromium, respectively.

3.3. Metal concentration in tissues

Variations of metals accumulation in harbor-caged mussels in the different tissues and transplant periods are summarized in Figs. 3–5. In digestive gland (Fig. 3), most metals (with the exception of Ni) significantly increased ($p < 0.05$ or $p < 0.001$). In addition, significantly higher Cu levels were recorded starting in July 2012, Cr and Zn from March 2013, and Mn in July 2013.

In gills (Fig. 4), Al accumulation was significantly higher from March 2013 ($p < 0.05$) and Cu accumulation was significantly higher in July 2013. The others metals did not show significant differences during the experimental period ($p > 0.05$).

In mantle (Fig. 5) Zn accumulation significantly increased ($p < 0.05$) during the transplant periods, the other metals did not change.

3.4. Protein content

Protein content was not affected during the transplant period in the harbor site ($p > 0.05$) in any of the three analyzed soft tissues (Table 2).

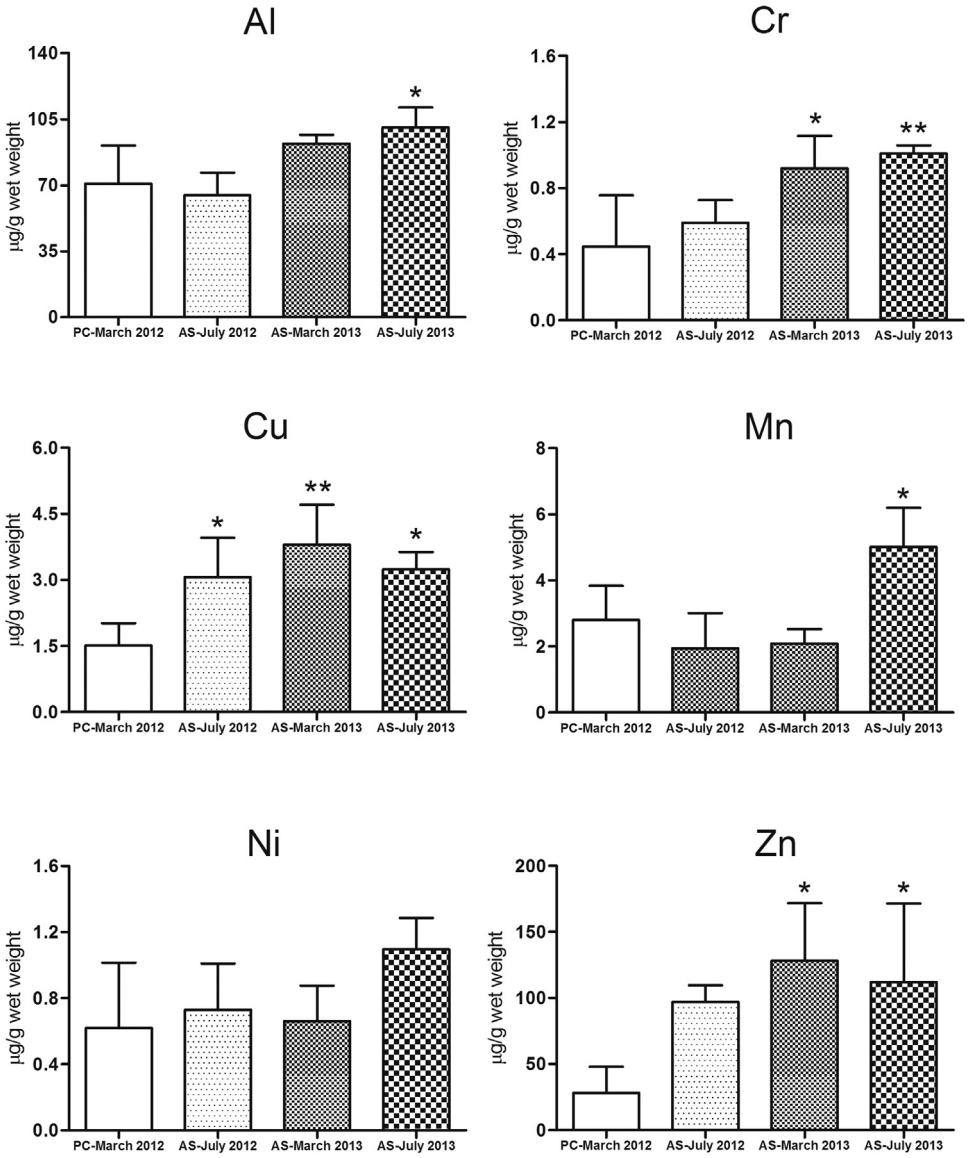

Fig. 3. Concentration of metals (µg/g wet weight) in digestive gland of *A. atra* from Punta Cuevas (PC) in March 2012 and transplanted to Almirante Storni pier (AS) until July 2013. Results are expressed as means ± S.D. (n = 4). Significant differences with respect to the reference site (PC) in March 2012 are indicated by * ($p < 0.05$) or * * ($p < 0.001$).

3.5. Antioxidant defenses

SOD activity significantly increased ($p < 0.05$) in digestive gland and gills (Table 2). In digestive gland, a significant increase was detected from July 2012, while in gills it was from March 2013. However, SOD activity in mantle did not change throughout the transplant experiment ($p > 0.05$).

GST activity also showed tissue-specific responses. Whereas GST activity in digestive gland and gills (Table 2) significantly increased ($p < 0.05$), mantle did not show any differences throughout the transplant experiment ($p > 0.05$). The increase in GST activity in the digestive gland was significant ($p < 0.05$) starting in March 2013, whereas in gills it was from July of the same year ($p < 0.05$) (Table 2).

GSH content (Table 2) significantly decreased ($p < 0.05$) in all tissues during the transplant periods. In digestive gland and gills, GSH levels were significantly decreased starting in March 2013, while in mantle it was four months later.

3.6. Lipid peroxidation

Harbor-caged mussels did not show any significant alterations in TBARs levels in any tissue ($p > 0.05$) (Table 2), suggesting that oxidative damage to lipids would not be occurring.

3.7. Reactive oxygen species (ROS) and total oxyradical scavenging capacity (TOSC)

Digestive gland samples from transplanted mussels had significantly lower ROS levels compared to the reference site starting in March 2013 ($p < 0.05$). An increase in TOSC levels was recorded from July 2012 until March 2013 ($p < 0.05$) (Table 2). Neither ROS nor TOSC showed significantly differences during the transplant period in gill samples ($p > 0.05$). Mantle samples showed significant differences in caged-mussels at the harbor area in ROS levels ($p < 0.05$). The lower values were recorded since March 2013. At beginning of the transplant period, the highest values in TOSC were recorded with significant differences compared to the reference site until March 2013 ($p < 0.05$) (Table 2).

4. Discussion

The present study shows that total trace metals levels in seawater are generally higher in the harbor site (Almirante Storni pier, AS)

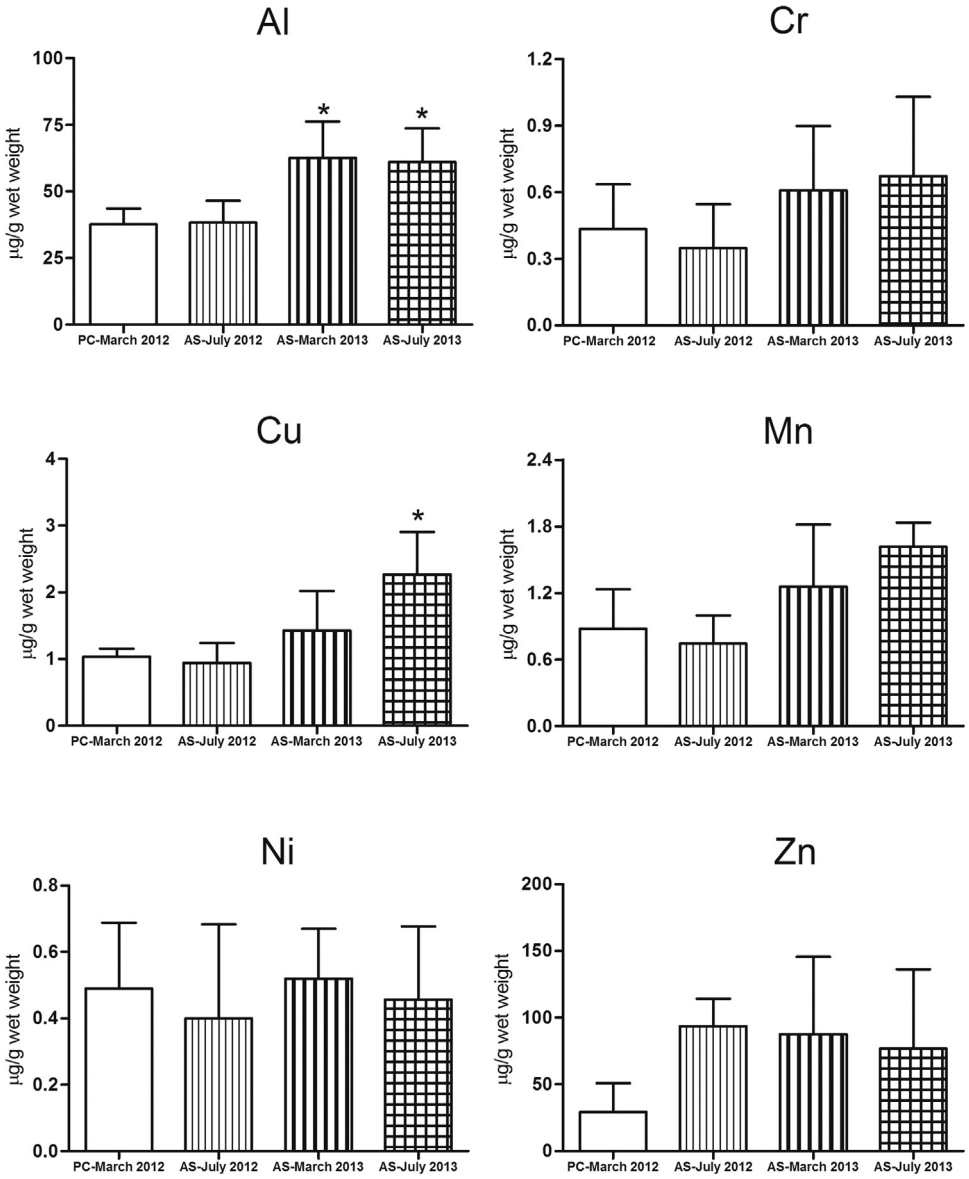

Fig. 4. Concentration of metals (µg/g wet weight) in gills of *A. atra* collected from Punta Cuevas (PC) in March 2012 and transplanted to Almirante Storni pier (AS) until July 2013. Results are expressed as means ± S.D. (n = 4).). Significant differences with respect to the reference site (PC) in March 2012 are indicated by * ($p < 0.05$).

compared to the reference site (Punta Cuevas, PC). In addition, total trace metals in seawater did not show significant differences between the beginning and the end of the transplant experiment, in either site. Thus, seawater metal concentrations likely remained constant during the 18 month transplant experiment.

The pathways for metal uptake depend on the specific diet and ecological lifestyle of an organism (Livingstone, 2001). Metal bioaccumulation is influenced by exposure time, availability of each metal in the aquatic environment, and excretion and detoxification mechanisms (Rainbow et al., 2009; de Souza Machado et al., 2016; Pan et al., 2016; Pilote et al., 2017). Moreover, due to differential metal binding affinities, different metals accumulate to greater extent in certain organs within the same organism (Rainbow, 2002). Our results provide evidence of unequal accumulation of metals in digestive gland, gills, and mantle from caged mussel *A. atra*. We observed that five of the six metals analyzed in this work (aluminum, chromium, copper, manganese, nickel and zinc) significantly accumulated in digestive gland after a year and a half of exposure in Almirante Storni pier (AS). On the other hand, gills preferentially accumulated aluminum and chromium, whereas in mantle only zinc showed differences between beginning and the end of the transplant experiment. Several previous studies in marine bivalves (*Mytilus edulis* and *M. galloprovincialis*) have reported that the digestive gland is the organ where higher metal accumulation occurs (Marigómez et al., 2002; Raspor et al., 2005; Strižak et al., 2014). Accordingly, digestive gland had the highest concentrations of aluminum, chromium, copper and manganese, especially towards the end of the transplant experiment. The differences in metal bioaccumulation in the three soft tissues of *A. atra* could be explained by metal bioavailability in seawater and by different metal uptake mechanisms: while the digestive gland assimilates metals from the food, gills and mantle take up metals directly from the aquatic environment. Also, trace metal concentrations in soft tissues of *A. atra* could show seasonal variability (Di Salvatore et al., 2013) probably due to seasonal physiological changes for example related to reproduction, and due to environmental factors, especially temperature that affects solution chemistry and physical kinetics, as well as metal levels in seawater and its bioavailability (Mubiana and Blust, 2007; Rouane-Hacene et al., 2015).

Oxidative responses take place in the early phases of environmentally induced stress and therefore are excellent biomarkers to assess metal pollution in aquatic ecosystems (van der Oost et al., 2003;

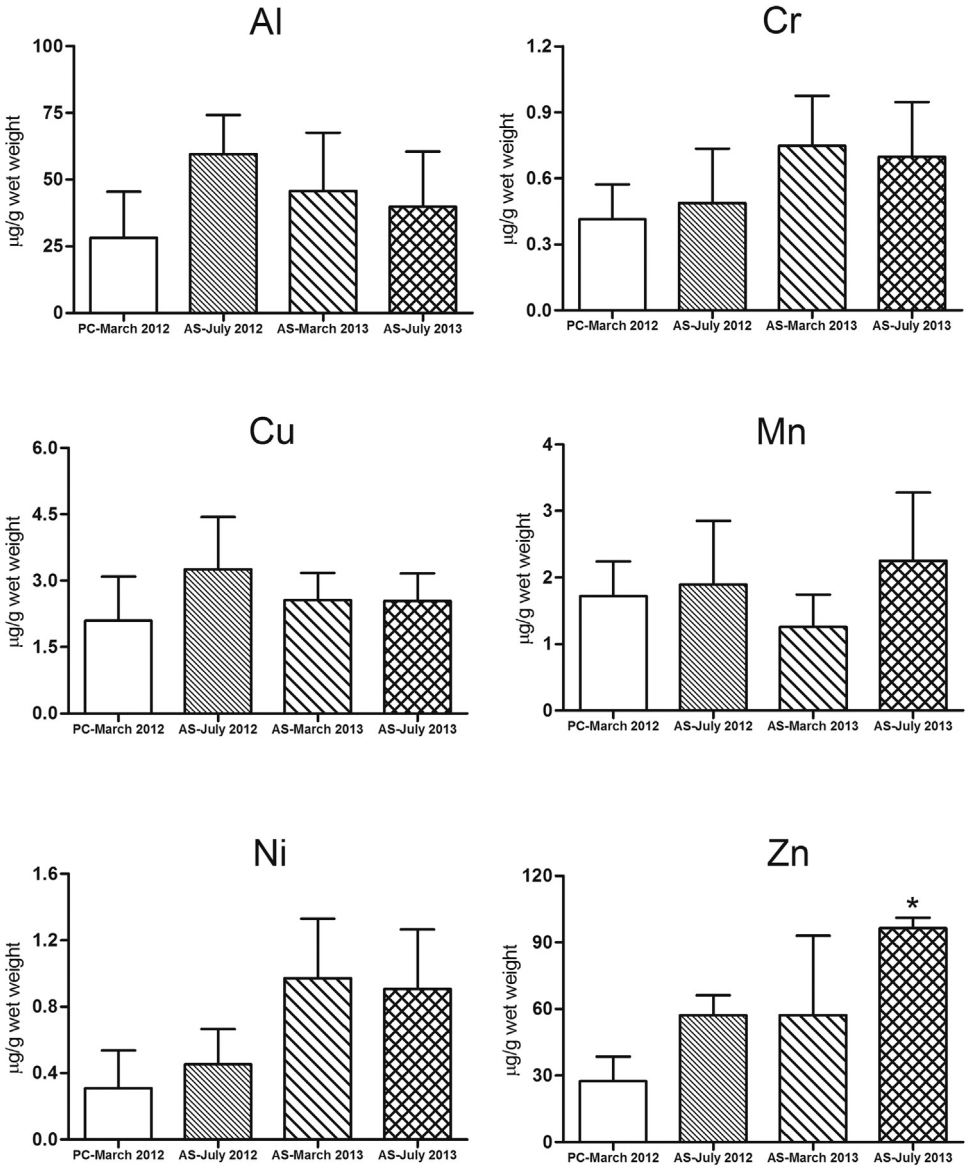

Fig. 5. Concentration of metals (µg/g wet weight) in mantle of *A. atra* collected from Punta Cuevas (PC) in March 2012 and transplanted to Almirante Storni pier (AS) until July 2013. Results are expressed as means ± S.D. (n = 4).). Significant differences with respect to the reference site (PC) in March 2012 are indicated by * ($p < 0.05$).

Dailianis et al., 2005; Lushchak, 2011; Benedetti et al., 2015; Chandurvelan et al., 2015). Previous studies in bivalves have reported that exposure to trace metals can stimulate the production of reactive oxygen species (ROS) and induce oxidative stress (Frenzilli et al., 2004; Vlahogianni et al., 2007; Sabatini et al., 2011; Taylor et al., 2016). It was expected that mussels transplanted to the harbor site would experience increased ROS production due to the metal pollution observed in the area. Our results show a trend to increased in ROS production in digestive gland and mantle at the beginning of the transplant (March 2012); however ROS significantly dropped at the two subsequent sampling times (March 2013 and July 2013) both in digestive gland and mantle. On the other hand, ROS production in gills did not change significantly during the transplant. In addition, we observed both an increase in total oxyradical scavenging capacity (TOSC) levels and an absence of lipid oxidative damage at the beginning of the transplanted period in digestive gland and mantle. We conclude the antioxidant response measured in this study counteracted H_2O_2 and $HOO^{\cdot}$, and therefore ROS did not induce cellular toxicity. The overall scavenger capacity measured in this work is a holistic approach to understand the antioxidant response against peroxyl radicals, particularly. Total oxyradical scavenging capacity (TOSC) mainly measures non-enzymatic low-molecular-weight scavengers such as reduced glutathione (GSH), vitamin E and ascorbic acid which represent the 70% of the total scavenging capacity against peroxyl radicals (Regoli and Winston, 1999; Regoli, 2000). In addition, thermal decomposition of ABAP could generate enzymatic inhibition (Amado et al., 2009). The decrease in TOSC reported in long- term transplanted bivalves to the harbor site is consistent with the markedly decrease in GSH levels in the three soft tissues of *A. atra* analyzed at the end of the transplant experiment. These results are in agreement with those reported by us and other authors (Dafre et al., 2004; Sabatini et al., 2011; Marasinghe Wadige et al., 2017). The possible binding of metals to GSH and the increase in activity of several glutathione-dependent and antioxidant enzymes, using GSH as cofactor or substrate (glutathione S-transferases or glutathione peroxidases, between others), can reduce the amount of GSH (Regoli, 1998). Glutathione S-transferase (GST) is a phase II detoxifying enzyme involved in conjugation reactions of GSH with several xenobiotic compounds. In agreement with the observed decrease of GSH levels, our results indicate an increase of GST activity in digestive gland and gills of *A. atra* starting at 12 and 18 months of exposure in the

Table 2
Protein content (mg/mL homogenate); Superoxide Dismutase (SOD) activity (U/mg prot); Glutathione-S-transferase (GST) activity (U/mg prot); Reduced glutathione content (nmol/mg prot) Lipid peroxidation (μmol TBARS/mg prot; ROS (nmol H_2O_2/ mg prot) and TOSC levels (relative area) in digestive gland, gills and mantle of *A. atra* collected from Punta Cuevas (PC) in March 2012 and transplanted to Almirante Storni pier (AS) until July 2013. Results are expressed as means ± S.D. (n = 6). Significant differences with respect to the reference site (PC) in March 2012 are indicated by * ($p < 0.05$) or * * ($p < 0.001$).

	Tissue	*PC- March 2012*	*AS-July 2012*	*AS-March 2013*	*AS-July 2013*
Protein content (mg/mL)	**Digestive gland**	2.615 ± 0.943	2.605 ± 0.276	2.892 ± 0.232	2.475 ± 0.339
	Gills	0.993 ± 0.145	1.121 ± 0.119	1.152 ± 0.136	1.041 ± 0.114
	Mantle	1.112 ± 0.223	1.185 ± 0.112	1.262 ± 0.281	1.211 ± 0.152
Superoxide Dismutase (SOD) activity (U/mg protein)	**Digestive gland**	22.736 ± 4.783	33.415 ± 1.497 **	28.708 ± 3.449 *	28.999 ± 3.085 *
	Gills	53.475 ± 14.537	75.397 ± 6.564	90.447 ± 24.212 *	81.835 ± 6.516 *
	Mantle	63.190 ± 4.196	61.797 ± 7.860	55.886 ± 6.931	55.820 ± 13.298
Glutathione-S-transferase (GST) activity (U/mg protein)	**Digestive gland**	0.032 ± 0.008	0.037 ± 0.004	0.049 ± 0.003 *	0.058 ± 0.009 *
	Gills	0.347 ± 0.064	0.345 ± 0.054	0.319 ± 0.085	0.513 ± 0.115 *
	Mantle	0.152 ± 0010	0.118 ± 0055	0.160 ± 0.050	0.148 ± 0.059
Reduced glutathione (GSH) content (nmol/mg protein)	**Digestive gland**	40.430 ± 8.955	34.795 ± 3.038	17.878 ± 2.667 *	15.333 ± 3.082 *
	Gills	55.787 ± 8.955	75.833 ± 22.415	20.257 ± 0.977 **	27.980 ± 12.620 *
	Mantle	33.037 ± 7226	21.684 ± 13,769	21.618 ± 9144	12.837 ± 4296 *
Lipid peroxidation (μmol TBARS/mg protein)	**Digestive gland**	10.657 ± 1.743	10.681 ± 1.244	9.055 ± 2.523	10.375 ± 0.939
	Gills	7.372 ± 1.263	9.453 ± 1.120	6.887 ± 1.566	8.255 ± 1.507
	Mantle	8.089 ± 0962	6.438 ± 1558	7.089 ± 1.486	7.766 ± 0.695
Reactive Oxygen Species (ROS) (nmol H_2O_2 /mg protein)	**Digestive gland**	22.051 ± 3.595	24.940 ± 5.189	12.231 ± 3.332 *	13.611 ± 4.132 *
	Gills	10.012 ± 6.370	14.454 ± 4.453	9.520 ± 4.452	22.590 ± 8.521
	Mantle	13.442 ± 4508	24.340 ± 10,471	4.403 ± 2.011 *	8.834 ± 3.534 *
Total Oxyradical Scavenging Capacity (TOSC) (arbitrary units)	**Digestive gland**	3.334 ± 1.559	9.254 ± 4.495 *	12.011 ± 5.962 *	5.907 ± 2.577
	Gills	1.038 ± 0.223	0.929 ± 0.201	0.732 ± 0.133	0.818 ± 0.162
	Mantle	0.865 ± 0.088	2.705 ± 1474 *	1.123 ± 0.379 *	1.130 ± 0.147

harbor site, respectively, while this enzymatic activity remained constant in mantle during this experiment. Previous studies in our laboratory had shown higher GST activity in digestive gland and gills in individuals of *A. atra* from Almirante Storni pier compared to those from the reference site (Di Salvatore et al., 2013). Canesi et al. (1999) observed a possible relation among Cu levels and an increase in GST activity in digestive gland and gills of the mussel *M. galloprovincialis*. Furthermore, it has been observed a positive correlation between aluminum concentrations and GST activity in *A. atra* (Giarratano et al., 2014), and also an increase in GST activity was observed in the digestive gland of *M. galloprovincialis* transplanting caged to a petrochemical polluted area (Cappello et al., 2013). Superoxyde dismutase (SOD) is an antioxidant enzyme which either dismutate $O_2^{\bullet-}$, generated under metabolic pathways and several abiotic stresses, to uncharged H_2O_2 (Bocchetti et al., 2008; Winterbourn and Hampton, 2008; Zhao et al., 2017). Several studies have reported increased SOD activity in marine bivalves exposed to trace metals` pollution in coastal waters (Nasci et al., 2002; Vlahogianni et al., 2007; Belcheva et al., 2011; Breitwieser et al., 2016). In our study, SOD activity in soft tissues of *A. atra* was increase in both digestive gland and gills in animals from Almirante Storni pier, while in mantle it remained constant revealing a relationship between increased SOD activity and the pro-oxidant effects of accumulated metals.

In aquatic organisms, the most commonly used approach to measuring oxidative damage is lipid peroxidation due to the high content of lipids with polyunsaturated fatty acid residues (Lushchak, 2011). Various studies reported that metals induce oxidative damage to lipids in mussels, (Torres et al., 2002; Rajkumar and John Milton, 2011; Mejdoub et al., 2017). These results are in agreement with those reported in a previous studies where mussels collected from the harbor site (AS) revealed higher lipid peroxidation levels compared with those collected from the reference site (PC) (Di Salvatore et al., 2013). In contrast to those results, lipid peroxidation levels remind constant in any of the soft tissues of *A. atra* between the beginning and the end of the transplant experiment. In addition, many others studies have shown an activation of the antioxidant system in mussels exposed to metals without observing oxidative damage to lipids (Viarengo et al., 1990; Cossu et al., 2000; Perić et al., 2017). We conclude that a longer chronic exposure time at harbor area would be necessary than those performed in the present study to evidence the existence of oxidative damage to lipids in *A. atra*.

5. Conclusions

The results obtained in this work reveal that mussels transplanted to a harbor site for 1.5 years experienced changes in tissue metal levels as well as pro-oxidant effects. *A. atra* bioaccumulated metals in digestive gland, gills and mantle at different levels. The experimental period and water metal concentration at harbor area induced an activation of antioxidant systems, which was effective in preventing oxidative damage to lipids. We conclude *A. atra* can selectively accumulate metals from the water column, and that their pro-oxidant effects depend on tissue-specific antioxidant defenses and the time of exposure to anthropogenic pollution. These results provide relevant information about the biological effects of the environmentally metal concentration on the physiology of filter feeders. Additionally, because *A.atra* is a species of commercial interest, our results are a potential biomarker of its health status. Finally, this study contributes to the knowledge on the environmental condition of the Argentinean Patagonian coastal waters and the effects of human activity.

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