RESEARCH ARTICLE

Baseline levels of biomarkers of oxidative damage in *Odontesthes nigricans* (Pisces, Atherinopsidae) from two coastal areas of the Beagle Channel, Argentina

Niveles basales de biomarcadores de daño oxidativo en *Odontesthes nigricans* (Pisces, Atherinopsidae) en dos áreas costeras del Canal Beagle, Argentina

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ABSTRACT

We assessed several biomarkers of oxidative damage on silversides *Odontesthes nigricans* from Varela and Golondrina bays, located on the North shore of the Beagle Channel (Tierra del Fuego, Argentina) and we tried to relate those responses to environmental data. Varela bay is visited mostly by local fishermen and tourism during the spring-summer season; on the other hand Golondrina bay is a low urban impacted area, despite the population's growth around it. We employed the condition factor as general biomarker of fish health and quantified biomarkers of oxidative damage in gills and liver. Fishes from Golondrina bay showed a better condition as compared to those from Varela bay. The activity of enzymatic antioxidants and the content of non-enzymatic antioxidants were lower in gills than in the liver, independently of the sampling site. No significant differences were found between sites in the gills. However, an increase in catalase and superoxide dismutase activities and a decrease in GSH/GSSG ratio were observed in the liver of fishes from Varela bay, possibly being due to higher levels of environmental natural stresses, mainly metals in sediments. This study indicates an organ-dependent response and establishes different baseline levels of biochemical parameters for each bay that could be considered as values of normal homeostasis for wild silversides.

Key words: Beagle Channel, *Odontesthes nigricans,* oxidative stress biomarkers.

RESUMEN

Se evaluaron diversos biomarcadores de daño oxidativo en el pejerrey *Odontesthes nigricans* proveniente de las bahías Varela y Golondrina, ubicadas sobre la costa norte del Canal Beagle (Tierra del Fuego, Argentina), y se trataron de relacionar las respuestas observadas a los datos ambientales. Bahía Varela es principalmente visitada por pescadores locales y turistas durante la temporada de primavera-verano, por otro lado Bahía Golondrina es un área de escaso impacto humano, a pesar del crecimiento poblacional registrado en sus inmediaciones. Los peces de Bahía Golondrina mostraron una mejor condición en comparación a aquéllos provenientes de Bahía Varela. La actividad de los antioxidantes enzimáticos y el contenido de los antioxidantes no enzimáticos fueron más bajos en las branquias que en el hígado, independientemente del sitio de muestreo. No se encontraron diferencias significativas en las branquias entre sitios de muestreo. Sin embargo, se observó un aumento en la actividad de la catalasa y la superóxido dismutasa y una disminución en la relación GSH/GSSG en el hígado de los peces de Bahía Varela, posiblemente debido a mayores niveles de estresores ambientales naturales, principalmente metales en los sedimentos. Este estudio indica una respuesta órgano-dependiente y establece diferentes niveles basales de parámetros bioquímicos para cada bahía que pueden ser considerados como valores normales de homeostasis de pejerreyes salvajes.

Palabras clave: Biomarcadores de estrés oxidativo, Canal Beagle, *Odontesthes nigricans*.

INTRODUCTION

Aquatic ecosystems are the main recipients of pollutants, which, over time, can cause serious consequences for biota that may not become apparent until changes occur at the population or ecosystem level, a point at which it may be too late to take

effective countermeasures (Linde-Arias et al. 2008). The need to detect and assess the impact of pollutants, particularly at sublethal concentrations, on environmental quality has led to measure a range of biological responses in different species (Alves de Almeida et al. 2007, Viarengo et al. 2007, Vlahogianni & Valavanidis 2007, Ahmad et al. 2008). The use of biological responses to different environmental conditions, defined as biomarkers, has become a useful tool in environmental quality evaluation and risk assessment. Among the numerous ecotoxicological biomarkers, those based on responses at the molecular and cellular levels represent the earliest signals of environmental disturbance and they are commonly used for biomonitoring (Moore et al. 2004, Viarengo et al. 2007). 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 pollutants (Giarratano et al. 2010). Although biomarkers can be helpful for gaining insight regarding the mechanisms causing observed effects of chemicals on whole-organism performance and may, in some cases, provide useful indicators of exposure, individual biomarker responses should not be expected to provide useful predictions of relevant ecological effects and probably not even predictions of whole-organism effects (Forbes et al. 2006).

Fishes are generally considered feasible organisms for pollution monitoring because they can be found virtually everywhere in the aquatic environment and they play a major ecological role in aquatic food-webs because of their function as carriers of energy from lower to higher trophic levels (Beyer 1996). The use of fish in environmental monitoring has become increasingly important in recent years in the investigation of natural variability, as well as anthropogenic substances, many of which function as prooxidants, accumulating in aquatic environments (Almroth et al. 2008). Prooxidants can induce morphological and physiological alterations in fish tissues (Bainy et al. 1996, Varanka et al. 2001), such as oxidative damage to lipids (lipid peroxidation). To counteract tissue damage, fishes as well as other organisms have developed different enzymatic and non-enzymatic defensive mechanisms to protect themselves against reactive oxygen species (ROS) production

(Winston & Di Giulio 1991). The enzymes superoxide dismutase (SOD) and catalase (CAT) are among the enzymatic antioxidants. Superoxide anions are dismutated by SOD to hydrogen peroxide (H_2O_2), which are subsequently detoxified by CAT present in peroxisomes. Numerous non-enzymatic low-molecular-weight antioxidants, such as ascorbate, β-carotene, α-tocopherol and GSH, have also been described (Stegeman et al. 1992, Lopez-Torres et al. 1993). Ascorbate is an important water-soluble antioxidant which is known to directly scavenge ROS (Halliwell & Gutteridge 1999), acting also as a crucial micronutrient and performing various vital physiological functions such as collagen synthesis and connective tissue formation (Wilson & Poe 1973). The α-tocopherol and β-carotene are lipid-soluble antioxidants which play a major role in protecting membranes from lipid peroxidation (van der Oost et al. 2003). Glutatione reduced (GSH) is the most abundant cellular thiol and it is involved in several metabolic processes (DeLeve & Kaplowitz 1991), playing a central role in the detoxification of ROS. As consequence of oxidizing conditions, two molecules of GSH are linked by a disulfide bond to comprise one molecule of oxidized glutathione (GSSG). Both total GSH and the ratio of GSH content/GSSG content can be used as biomarkers for oxidative stress (Koivula & Eeva 2010). Swiergosz-Kowalewska et al. (2006) concluded that the GSH content/GSSG content ratio may be the best and the most sensitive indicator of metal toxicity for animals living in chronically contaminated environments.

The silverside *Odontesthes nigricans* (Richardson, 1848) is a native fish species in Tierra del Fuego (southernmost limit of its distribution) that can be found by following the tides in the coastal waters and estuaries of the Atlantic Ocean and Beagle Channel. It is a species of a regional socioeconomic relevance due to the sport and artisanal fishing developed upon it. The oxidative metabolism of *O. nigricans* was previously characterized by Lattuca et al. (2009). *O. nigricans* was also chosen because another species of the same genus, *Odontesthes bonariensis* (Valenciennes, 1835), has been recently promoted as test species in a standardized acute toxicity test method in Argentina (IRAM 2007), due

to its relatively high sensitivity to metals (Carriquiriborde & Ronco 2002, 2008).

The aim of this work was to determine baseline levels of a condition factor, as general biomarker of fish health, and of several biomarkers of oxidative damage, such as lipid radical content, enzymatic antioxidants (activities of superoxide dismutase and catalase), non-enzymatic antioxidants (α-tocopherol, β-carotene, ascorbate and glutathione contents) and general indexes of oxidative stress (ascorbyl/ascorbate and reduced glutathione/oxidized glutathione ratios) in gills and liver of *O. nigricans* from two coastal areas within Beagle Channel. Present study represents the first comprehensive investigation that set oxidative stress responses in fishes from clean coastal waters of the Beagle Channel, essential information for a proper interpretation of future ecotoxicological data and environmental monitoring programs in this austral region.

METHODS

Authors wish to declare that this study was conducted in accordance with Guidelines on the care and use of animals for scientific purposes established by the National Advisory Committee for Laboratory Animal Research (2004).

Sampling sites

Fishes were collected in spring (October) of 2007 at Golondrina and Varela bays, located on the North coast of the Beagle Channel (Tierra del Fuego, Argentina) (Fig. 1). Golondrina bay (54°50'S, 68°21'W) is located
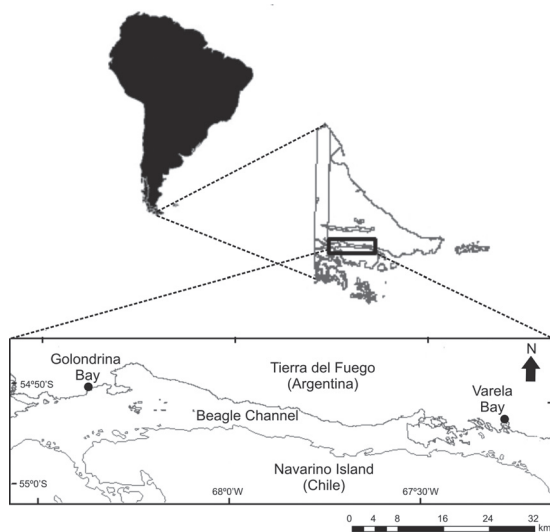

Fig. 1: Location of sampling sites.

Localización de los sitios de muestreo.

near Ushuaia city and has been recently considered by Duarte et al. (2011) as low urban impacted area, despite the population's growth around it. Varela bay (54°52'S, 67°16'W), located 70 km East from Golondrina bay, is considered a low impacted area, being mostly visited by local fishermen and tourists during the spring-summer season. The nearest place where environmental data has been reported is Brown bay (54°52' S, 67°34'W) (Giarratano & Amin 2010), 20 km west of Varela bay. At that place, only a commercial production of mussels is being developed since 2001 (Hernando et al. 2008[1]).

Fish collection and processing

Ten adult specimens (185 - 210 mm total length, TL; 35 - 55 g body weight, BW) were collected at both sampling sites by means of a seine net (60 m long, 1.5 m high and 12 mm stretched mesh) covering ca. 200 m, from 1 m depth to the shallower littoral zone. Fishes were carried alive to the laboratory into 100 L tanks containing aerated seawater. In order to minimize stress generated during capture and transportation, fishes were held in the laboratory during 24 h under similar field conditions (8 ± 0.5 °C; light:dark photoperiod of 14:10 h). According to Cazenave et al. (2009) and Nahrgang et al. (2010), twenty-four hours after catch fishes from both sampling sites were sacrificed by a blow to the head. TL of each individual was measured with a digital calliper (± 0.1 mm) and BW was recorded to the nearest 0.01 g. Condition of individual fish was indexed by Fulton's condition factor calculated as $K = [BW \times [TL^3]^{-1}] \times 100$ (Bagenal & Tesch 1978). Considering that Lattuca et al. (2009) found no significant differences between sexes in oxidative stress biomarkers for *O. nigricans*, sex differentiation was not made in this sudy. Gills and liver were dissected, weighed (± 10^{-5} g) and stored at -80 ºC for 2 weeks, until analysis.

Environmental physicochemical parameters

At each sampling site, surface water temperature, pH, conductivity and dissolved oxygen were recorded once in situ by means of an HORIBA U-10 multiparameter device. Concentration of chlorophyll-a was measured according to Holm-Hansen & Riemann (1978) using a Sequoia Turner (model 450) fluorometer.

Biochemical measurements

Quantification of lipid radicals by electron paramagnetic resonance (EPR)-spin trapping

Lipid radicals were detected by a spin trapping technique using N-t-butyl-α-phenyl nitrone (PBN). A 40 mM PBN stock solution was prepared in dimethyl sulfoxide (DMSO) immediately prior to use. The homogenates were prepared by adding DMSO-PBN (stock solution) in frozen tissue (10 to 20 mg). EPR spectra were obtained at room temperature using a Bruker spectrometer ECS 106, operating at 9.81 GHz with 50 kHz modulation frequency. EPR instrument settings for the spin trapping experiments were: microwave power, 20 mW;

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modulation amplitude, 1.194G; time constant, 81.92 ms; scan numbers, 5; center fields, 3480 G; modulation frequency, 50 kHz; and receiver gain, 2·104 (Lai et al. 1986). Quantification was performed according to Kotake et al. (1996).

Content of lipid soluble antioxidants

The contents of α-tocopherol (α-TH) and β-carotene in homogenates from gills and liver were quantified by reverse-phase HPLC with electrochemical detection using a Bioanalytical Systems LC-4C amperometric detector with a glassy carbon working electrode at an applied oxidation potential of 0.6 V. Samples were extracted with 1 mL of ethanol and 4 mL of hexane. After centrifugation at 600 g for 10 min, the hexane phase was removed and evaporated to dryness under N_2. Extracts were dissolved in methanol/ethanol (1:1) and injected for HPLC analysis (Desai 1984). The equipment employed was a Perkin Elmer 250 LC bomb, a fixed phase: Supelcosil LC-8 column (3 µm size particle) 4.6 x 330 mm, a mobile phase: 20 mM lithium perchlorate in methanol/water 99:1 (v/v), and a 1 mL min⁻¹ flow. D,L-α-tocopherol (Sigma) and β-carotene (Sigma) were used as standards (Fig. 2).
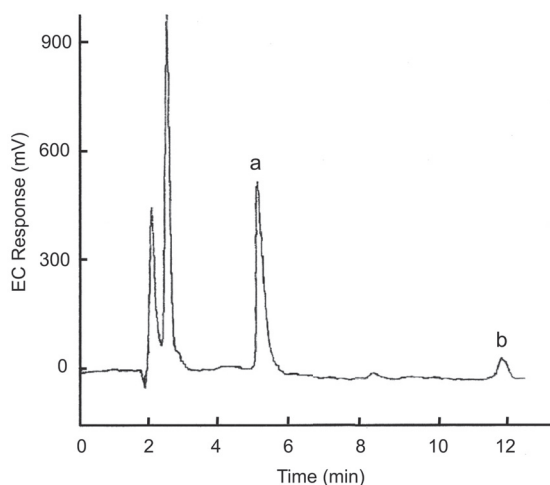

Fig. 2: Determination of lipid soluble antioxidants content by the high-performance liquid chromato-graphy (HPLC) method. Elution time for the tested standards were: 5.5 min for α-TH (a) and 11.8 min for β-carotene (b).

Determinación del contenido de antioxidantes liposolubles por cromatografía líquida de alta resolución (HPLC). Los tiempos de elución para los estándares fueron para: α-TH 5.5 min (a) y β-caroteno 11.8 min (b).

Enzymatic assays

Homogenates from gills and liver were prepared in 50 mM potassium phosphate 120 mM KCl (pH = 7.4). Catalase activity was assayed spectrophotometrically by the decomposition of H_2O_2 at λ = 240 nm in a reaction mixture consisting of 50 mM potassium phosphate buffer (pH 7.0) containing 1% Triton-X100, 1:9 (w/v) and 12.5 mM H_2O_2 (Aebi 1984). Total superoxide

dismutase activity was determined by the epinephrine method (Misra & Fridovich 1972), based on the capacity of SOD to inhibit the autooxidation of epinephrine to adrenochrome at 480 nm.

Ascorbyl radical content (A·)

A• measurements were done using a Bruker ECS 106 spectrometer. Gills and liver homogenates were prepared by adding 100-150 mg of tissue to pure DMSO (1:3) (w/v). The spectra were scanned at room temperature under the following conditions: 50 kHz field modulation, microwave power 20 mW, modulation amplitude 1G, time constant 655 ms, receiver gain 1 x 10⁵, microwave frequency 9.81 GHz, and scan rate 0.18 G/s (Giulivi & Cadenas 1993). The amount of spin adduct was calibrated using an aqueous solution of TEMPOL, introduced into the same cell used for spin trapping. EPR spectra of spin adduct and TEMPOL solutions were recorded at exactly the same spectrometer settings. The first-derivative EPR spectra were doubly integrated to obtain the area intensity, and then the concentration of spin adduct was calculated using the ratio of these areas. Quantification was performed according to Kotake et al. (1996).

Ascorbate content (AH⁻)

The content of AH⁻ was measured by reverse-phase HPLC with electrochemical detection using a LC-4C amperometric detector with a carbon working electrode at an applied oxidation potential of 0.6 V. The samples were homogenized in metaphosphoric acid (10 %, w/v) according to Kutnink et al. (1987). A Supelcosil LC-18 column was stabilized with metaphosphoric acid (0.8 %, w/v) and a freshly prepared solution of ascorbic acid in metaphosphoric acid (10 %, w/v) (1 µg mL⁻¹) was used as standard.

Reduced glutathione (GSH) and oxidized glutathione (GSSG) contents

In order to assess GSH and GSSG contents, homogenates from gills and liver were prepared in 1.0 M $HClO_4$ and 2 mM EDTA (1:5) (w/v). After centrifugation at 29000 g and 4 °C for 20 min, the lipid layer was discarded. Meanwhile, the protein free supernatant was filtered with a 0.22 µm nylon membrane and immediately analyzed. The contents of GSH and GSSG were quantified by reverse-phase HPLC analysis (isocratic modality) following the method described by Rodriguez-Ariza et al. (1994). The equipment employed was: a Perkin Elmer 250 LC bomb, a fixed phase: Supelcosil LC-18 column (5 µm size particle) 4.6 x 250 mm, a mobile phase: 20 mM NaH_2PO_4, pH 2.7, and an electrochemical detector: ESA Coulochem II with an analytical cell ESA 5011, at an applied potential of +0.45 V and 0.80 V and a 1.2 mL min⁻¹ flow. Quantification of GSH and GSSG was performed through a standard curve with a linear relationship between 5-200 ng GSH or GSSG, respectively.

Statistical analyses

Data in the text and tables are expressed as mean ± SE for three replicates of each of the five individuals from each sampling site. Statistical test of Mann-Whitney was carried out using GraphPad InStat, version 3.01. When the two-tailed P value was < 0.05, it was considered significant.

TABLE 1

Physicochemical parameters of water from the different sampling sites. Data are expressed as mean ± S.E. for three replicates of each of the five individuals from each sampling site.

Parámetros fisicoquímicos del agua de los distintos sitios de muestreo. Los datos están expresados como la media ± EE de tres réplicas para cada uno de los cinco individuos de cada sitio de muestreo.

	Varela bay	Golondrina bay
pH	7.9 ± 0.3	8.2 ± 0.3
Conductivity (mS cm^{-1})	40 ± 3	46 ± 3
Dissolved oxygen (mg L^{-1})	9.2 ± 0.8	10.2 ± 0.2
Temperature (°C)	7.7 ± 0.4	8.4 ± 0.6
Salinity (ppm)	28.1 ± 0.9	25 ± 2
Chlorophyll-a (µg L^{-1})	0.29 ± 0.07	0.22 ± 0.07

RESULTS

Physicochemical parameters of seawater were similar in both bays (Table 1).

The condition of the whole body was used as an initial screening biomarker, measured through K condition factor. Differences in K values were observed between sampling sites where fishes from Golondrina bay had significant higher K factor than those from Varela bay (Fig. 3, Mann-Whitney test; U = 2.000; n = 10; P = 0.032).
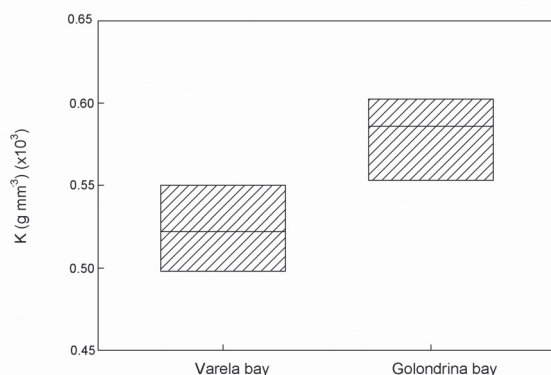

Fig. 3: Condition factor (K) of ten *O. nigricans* collected from different sampling sites. Median, quartiles and 5th and 95th percentiles are indicated.

Factor de condición (K) de diez *O. nigricans* capturados en los distintos sitios de muestreo. Se indican mediana, cuartiles y percentilos 5° y 95°.

Oxidative damage to lipid in the gills and the liver of silversides was estimated

in the present study as lipid radical content assessed by EPR. Lipid radicals in both organs combined with the spin trap PBN resulted in adducts that gave a characteristic EPR spectrum with hyperfine coupling constants of a_N = 15.56G and a_H = 2.79G, in concordance with computer spectral simulated signals obtained using the overall mentioned parameters. PBN itself was examined and no PBN spin adduct was observed. Spectra showed a signal from a second radical species with hyperfine coupling constants a_N = 15.91 and a_H = 3.19, corresponding to a carbon-centered endogenous radical of unknown origin. However, even though the signal was not fully identified, it may reflect the formation of a PBN-CH_3 adduct. Since hydroxyl radical formation in vivo may occur under these experimental conditions, its reaction with DMSO could result in the generation of methyl radical that could be trapped by PBN, forming the adduct PBN-CH_3 detected by EPR (Mason et al. 1994, Dikolova et al. 2001). Neither lipid radical content in gills (Golondrina bay: 3.7 ± 0.7 pmol mg^{-1} FW; Varela bay: 2.5 ± 0.9 pmol mg^{-1} FW; Mann-Whitney test; U = 7.000; n = 10; P = 0.309) nor in the liver of *O. nigricans* (Golondrina bay: 0.8 ± 0.2 pmol mg^{-1} FW; Varela bay: 0.99 ± 0.08 pmol mg^{-1} FW; Mann-Whitney test; U = 7.000; n = 10; P = 0.309) showed significant differences between sampling sites. When comparing the lipid radical content between organs of fishes from the same sampling site, only those from Golondrina bay showed significant differences, with higher values in gills (Mann-Whitney test; U = 0.000; n = 10; P = 0.008).

The content of non-enzymatic antioxidants and the activity of the antioxidant enzymes were also evaluated in both organs assayed. Independently of the sampling site, all of them were lower in gills than in the liver.

Regarding the non-enzymatic lipid antioxidants, the contents of α-tocopherol (Mann-Whitney test; U = 2.000; n = 10; P = 0.063) and β-carotene (Mann-Whitney test; U = 3.000; n = 10; P = 0.25) in the gills did not showed significant differences between sampling sites (Table 2). In the liver (Table 2), the content of α-tocopherol was significantly higher in fishes from Varela bay than in those from Golondrina bay (Mann-Whitney test; U = 0.000; n = 10; P = 0.029), whereas the content of β-carotene did not show significant differences between sampling sites (Mann-Whitney test; U = 8.000; n = 10; P = 0.421). The contents of both non-enzymatic lipid antioxidants were significantly higher in the liver than in the gills of fishes from the same sampling site (α-tocopherol for Varela bay: Mann-Whitney test; U = 0.000; n = 10; P = 0.016; α-tocopherol for Golondrina bay: Mann-Whitney test; U = 0.000; n = 10; P = 0.008; β-carotene for Varela bay: Mann-Whitney test; U = 0.000; n = 10; P = 0.036 and β-carotene for Golondrina bay: Mann-Whitney test; U = 1.000; n = 10; P = 0.015).

A high activity of CAT was also registered in the liver, being 53 % higher in fishes from Varela bay than in those from Golondrina bay (Table 2, Mann-Whitney test, U = 27.000; n = 10; P = 0.050). The activity of CAT could not be determined in the gills due to the lack of sample. The activity of SOD in the gills did not differ significantly between sampling sites (Table 2, Mann-Whitney test; U = 10.000; n = 10; P = 0.429), but it did in the liver, being 44 % higher in Varela bay than in Golondrina bay (Table 2, Mann-Whitney test; U = 0.000; n = 10; P = 0.009). No significant differences were observed in the activity of SOD between organs at each sampling site (Table 2, Varela bay: Mann-Whitney test; U = 14.000; n = 10; P = 0.931; Golondrina bay: Mann-Whitney test; U = 10.000; n = 10; P = 0.240).

Typical EPR spectra of A· in the gills and in the liver of fishes collected at the two sampling sites were observed with the characteristic two lines at g = 2.005 and a_H = 1.8 G, in accordance to computer spectral simulated signals obtained with the parameters given in the Material and Methods section. Neither the content of A· (Mann-Whitney test; U = 11.000; n = 10; P = 0.841) and AH· (Mann-Whitney test; U = 9.000; n = 10; P = 0.548) nor the A· content/AH·content ratio in gills (Mann-Whitney test; U = 11.000; n = 10; P = 0.841) did differ significantly between Varela and Golondrina bays (Fig. 4). Typical EPR spectra of A· in the liver are shown in Fig. 5A. The contents of A· (Mann-Whitney test; U = 0.000; n = 10; P = 0.050), AH· (Mann-Whitney test; U = 0.000; n = 10; P = 0.036) and the A· / AH· ratio (Mann-Whitney test; U = 0.000; n = 10; P = 0.050) assessed in the liver (Fig. 5B) were significantly different between both sampling sites. Significant higher values of A· content in gills than in liver were measured at Varela (Mann-Whitney test; U = 1.000; n = 10; P = 0.016) and Golondrina (Mann-Whitney test; U = 1.000; n = 10; P = 0.016) bays. The A· /AH·

TABLE 2

Antioxidant capacity of *O. nigricans* from the different sampling sites. Data are expressed as mean ± S.E. for three replicates of each of the five individuals from each sampling site. ND: not determined.

Capacidad antioxidante de *O. nigricans* de los distintos sitios de muestreo. Los datos están expresados como la media ± EE de tres réplicas para cada uno de los cinco individuos de cada sitio de muestreo. ND: no determinado.

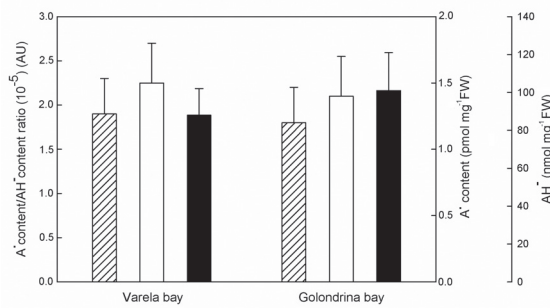|	Varela bay		Golondrina bay	
	Gills	Liver	Gills	Liver
α-tocopherol (nmol mg^{-1}FW)	3 ± 1	405 ± 77	7 ± 2	194 ± 49
β-carotene (nmol mg^{-1} FW)	0.1 ± 0.05	1.4 ± 0.3	0.3 ± 0.06	0.9 ± 0.3
CAT (U mg^{-1} FW)	ND	7570 ± 1368	ND	3560 ± 1149
SOD (U mg^{-1} FW)	991 ± 142	1086 ± 284	799 ± 118	605 ± 65

Fig 4: A· (☐) and AH· (■) contents and A·content /AH· content ratio (▨) in gills of *O. nigricans* from different sampling sites.

Contenidos de A· (☐) y AH· (■) y la relación de contenido de A· /contenido de AH· (▨) en branquias de *O. nigricans* capturados en los distintos sitios de muestreo.

ratio was also significantly higher in the gills of fishes from Golondrina bay than in those from Varela bay (Mann-Whitney test; U = 2.000; n = 10; P = 0.05; Figs. 4 and 5B).

The contents of GSH, GSSG and the GSH/GSSG ratio, used as markers of the redox status, are shown in Table 3. The contents of GSH and GSSG in the liver were 50 % and 86 % higher at Varela bay respectively, meanwhile the GSH/GSSG ratio was 85 % higher at Golondrina bay (Table 3, Mann-Whitney test; U = 3.000; n = 10; P = 0.050). No significant differences in the contents of GSH (Golondrina bay: Mann-Whitney test; U = 7.000; n = 10; P = 0.309 and Varela bay: Mann-Whitney test; U = 7.000; n = 10; P = 0.555), GSSG (Golondrina bay: Mann-Whitney test; U = 11.000; n = 10; P = 0.841 and Varela bay: Mann-Whitney test; U = 8.000; n = 10; P = 0.730) and the GSH/GSSG ratio (Golondrina bay: Mann-Whitney test; U = 5.000; n = 10; P = 0.151 and Varela bay: Mann-Whitney test; U = 5.000; n = 10; P = 0.286) were found between organs at each sampling site.

DISCUSSION

In the present study, oxidative stress responses in fishes from Golondrina and Varela bays were employed to evaluate the usefulness of a multibiomarker approach in environmental monitoring programs in the Beagle Channel region. Golondrina bay is located near Ushuaia city, the southernmost location on Earth, and has been recently considered by Duarte et al. (2011) as low urban impacted area, despite the
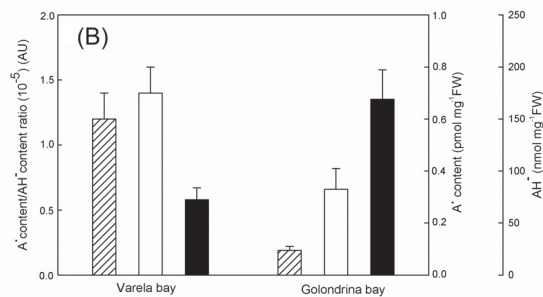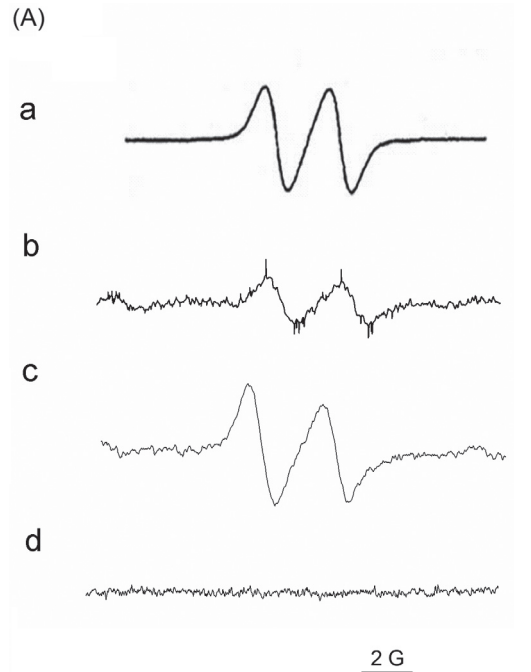

Fig. 5: A: EPR detection of ascorbyl radical in liver of *O. nigricans* from different sampling sites: (a) computer-simulated EPR spectra employing the following spectral parameters g = 2.005 and a_H = 1.8 G, (b) typical EPR spectra from Golondrina bay, (c) typical EPR spectra from Varela bay and (d) EPR spectra of dimethyl sulfoxide (DMSO). B: A· (☐) and AH· (■) contents and A·/AH· ratio (▨) in liver of *O. nigricans* from different sampling sites.

A: Detección del radical ascorbilo por EPR en el hígado de *O. nigricans* capturados en los distintos sitios de muestreo: (a) espectro de EPR simulado por computadora empleando los siguientes parámetros espectrales g = 2.005 y a_H = 1.8 G, (b) espectro típico de EPR para Bahía Golondrina, (c) espectro típico de EPR para Bahía Varela y (d) espectro de EPR de dimetilsulfóxido (DMSO). B: Contenido de A· (☐) y de AH· (■) y relación A·/AH· (▨) en el hígado de *O. nigricans* de distintos sitios de muestreo.

population's growth around it. Varela bay does not receive anthropogenic inputs, been only visited by local fishermen and tourists during

TABLE 3

Reduced and oxidized glutathione and GSH / GSSG ratio in the gills and the liver of *O. nigricans* from the different sampling sites. Data are expressed as mean ± S.E. for three replicates of each of the five individuals from each sampling site.

Glutatión reducido y oxidado y relación contenido de GSH / contenido de GSSG en las branquias y el hígado de *O. nigricans* de los distintos sitios de muestreo. Los datos están expresados como la media ± EE de tres réplicas para cada uno de los cinco individuos de cada sitio de muestreo.

	Varela bay		Golondrina bay	
	Gills	Liver	Gills	Liver
GSH (ng mg^{-1} FW)	10 ± 3	6 ± 1	6 ± 4	3 ± 1
GSSG (ng mg^{-1} FW)	3 ± 1	37 ± 9	3.3 ± 0.9	5 ± 2
GSH/GSSG	3.8 ± 0.9	0.2 ± 0.1	2.5 ± 0.8	1.3 ± 0.3

a short part of the year. In order to assess the link between spatial and temporal changes in quality of coastal waters of the Beagle Channel and antioxidant responses, different works have been previously carried out in native (Duarte et al. 2011, 2012) and transplanted (Giarratano et al. 2010, 2011) mussels *Mytilus edulis chilensis* (D'Orbigny, 1847) in the Beagle Channel. They found significant differences in oxidative stress responses analyzed in relation to dissolved nutrients in seawater and trace metals in the finest sediment fraction (< 62 μm). Sediments from Brown bay, which is only 20 km West of Varela bay, showed higher levels of Fe and Zn than those from Golondrina bay (Giarratano & Amin 2010, Duarte et al. 2012). Even though fishermen's activities could be contributing to the trace metal enrichment of sediments from Brown bay, Giarratano & Amin (2010) believe that most of the concentrations measured derive from the Andean belt itself, since spurts of variable flow reach this coast associated to thawing and drift of materials contributed by Almanza river (Hernando et al. 2008).

The xenobiotics such as metals are known to induce ROS, which are responsible for oxidative stress (Viarengo et al. 1990, Almroth et al. 2005, Gonzalez & Puntarulo 2011). Assuming that the higher content of Fe and Zn found in fine sediments from Brown bay could be similar in Varela bay due the its proximity, these metals could be resuspended in the water column by wave action and/or by physico-chemical changes. That fact could be responsible of the increase of the lipid radical content and the antioxidant activities in the liver, affecting *O. nigricans* from Varela bay

through the decrease of the condition factor. However, measurements of trace metals in sediments from Varela bay should be carry out to test this hypothesis.

In several works the K condition factor has proven to be a useful biomarker to indicate different environmental stress conditions among sites with different characteristics (van der Oost et al. 2003, Linde-Arias et al. 2008). However, this parameter is not sensitive enough to give information of specific responses to the toxic substances in the environment. 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). Fishes from Golondrina bay showed K values significantly higher than those from Varela bay making evident that the former place is a site with less stressful environmental conditions.

The degree of oxidative damage on the fish *O. nigricans* was assessed through the estimation of lipid peroxidation products in the gills and in the liver. Gills were chosen for being the first organ which gets in contact with the environment becoming a potential target for oxidative disruption. Liver was chosen because it possesses high potential for ROS generation, which seems to be efficiently counterbalanced by powerful protective mechanisms to detoxify and repair damaged lipid and proteins (Oliveira et al. 2008, Lattuca et al. 2009, Nahrgang et al. 2010). Endogenous lipid radical products have extremely short half-lives and are present in low concentrations, making difficult their detection. Spin trapping analysis followed

by EPR overcomes the limit of sensitivity of endogenous radicals in biological systems, and it has proved to be the least ambiguous method to detect short-lived and reactive free radicals generated in low concentrations in biological systems (Luo et al. 2006). Lipid peroxidation has been conveniently used as an effect biomarker by pollution (Ahmad et al. 2008). Particularly, Luo et al. (2006) detected ROS generation induced by 2-chlorophenol in fish *Carassius auratus* (Linnaeus, 1758) based on the EPR method. Ahmad et al. (2008) observed an increased lipid peroxidation in the gills of the European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) from a contaminated coastal lagoon in Ria de Aveiro of Portugal. However, lipid radical content neither in gills nor in the liver of *O. nigricans* showed significant differences between Varela and Golondrina bays. The same response was found by Pandey et al. (2003) in the fish *Wallago attu* (Bloch & Schneider, 1801) and they argued that induction of antioxidants might have contributed in subsiding the peroxidative effect. It is also possible that this parameter was not sensitive enough to detect differences between the selected areas. Measurement of lipid peroxidation provided no indication of a differential pollution level between sampling sites.

Fishes tend to adapt to oxidative conditions by mobilizing enzymatic as well as non-enzymatic antioxidant defences (Ahmad et al. 2008, van der Oost et al. 2003). SOD and CAT activities, the α-tocopherol content and the high A•/AH⁻ ratio found at Varela bay suggest that the natural environmental stress at this site is higher than at Golondrina bay. The enzymatic antioxidants SOD and CAT provide the first defence against oxygen toxicity. A simultaneous induction of the activities of SOD and CAT has been observed in organisms exposed to pollutants (Dimitrova et al. 1994). In that sense, such relationship found in liver in this study could be attributable to higher natural levels of some trace metals in Varela bay. Jena et al. (2009) proposed the use of ascorbic acid as a biomarker for environmental monitoring. The content of AH⁻ in Golondrina bay was higher than in Varela bay. This may be either due to dietary deficiency or the utilization of ascorbic acid in response to elevated ROS generation (Oakes et al. 2004). The pattern of variation in ascorbic acid content in *O. nigricans* is comparable to that reported by Jena et al. (2009) for *Perna viridis* (Linnaeus, 1758) from the coast of India. Further studies would fully describe the use of ascorbic acid as a biomarker for environmental monitoring in the studied areas.

GSH is a co-factor for GPx and GST enzymes and is also an effective protection capable of quenching oxyradicals (Ross 1988). It may be either a result of the novo synthesis as previously demonstrated in fish or a transfer from other organs (Deneke & Fanburg 1989, Oliveira et al. 2009). An increment of GSH was observed in the liver of fishes from Varela bay, which could be due to the fact that most GSH is synthesized in liver and transported to the bloodstream for supply to other tissues. The same explanation was given by Almroth et al. (2008) for rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792). Similarly, DiGiulio et al. (1993) observed high levels of GSH in catfish exposed to polluted sediment. The ratio between the reduced GSH and oxidized forms of GSH (GSSG) plays an important role in intercellular signalling and gene regulation (Almroth et al. 2008) and may be a potential biomarker for oxidative stress (van der Oost et al., 2003; Jena et al., 2009). In the current study, the lowest hepatic GSH/GSSG ratio was found at Varela bay indicating a more oxidized redox status. Similar responses of decreased GSH/GSSG ratio have been shown in *Perna perna* (Linnaeus, 1758) exposed to lead and paraquat (Dafre et al. 2004) and in *Mytilus galloprovincialis* (Lamarck, 1819) in response to an oil spill in Spain (Sureda et al. 2011).

Taken as a whole, the antioxidant responses were higher in the liver making it an ideal tissue for biomarker studies in *O. nigricans*. These results are in concordance with those found by Lattuca et al. (2009) who showed that the liver of *O. nigricans* exhibited a better control of the oxidative damage than the gills, allowing minimization of intracellular damage when it is exposed to environmental stressful conditions. The current study indicates that this species also displays an antioxidant response spatially differentiated, in order to counteract pro-oxidant natural factors present in these marine habitats.

We studied silversides from two coastal environments from Beagle Channel and

we determined the background levels of biochemical parameters that can be considered values of normal homeostasis for wild silversides. The activity of antioxidant defence enzymes investigated in this work should be taken into account in biomonitoring studies with fish species and adequately considered when biomarker responses are interpreted to detect anthropogenic disturbances. Our results support the suitability of employing *O. nigricans* as a sentinel species for assessing the health of coastal areas of the Beagle Channel, as well as in future ecotoxicological field studies.

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