Natural adaptation to the environmental conditions affects the oxidation-dependent processes in limpets

Paula Mariela González, Susana Puntarulo and Gabriela Malanga*

Facultad de Farmacia y Bioquímica,
Universidad de Buenos Aires,
Fisicoquímica, Buenos Aires, Argentina

and

CONICET-Universidad de Buenos Aires,
Instituto de Bioquímica y Medicina Molecular (IBIMOL),
Buenos Aires, C1113AAD, Argentina
Email: paulag@ffyb.uba.ar
Email: susanap@ffyb.uba.ar
Email: gmalanga@ffyb.uba.ar
*Corresponding author

Abstract: Exploitation of benthonic resources is an important fraction within the craft catch. The main objective of this work was to obtain information on the oxidative condition and tissue damage in gills of the limpets *Nacella deaurata* and *N. magellanica* with subtidal and intertidal natural habitats, respectively. No significant differences were observed between the two species in the carbohydrates, lipids, proteins and ashes content. Ascorbyl radical and lipid radical contents were higher in *N. deaurata* compared to *N. magellanica* (224% and 3.5-fold, respectively). However, catalase and superoxide dismutase activities were significantly higher in *N. magellanica* than in *N. deaurata*. These data suggested that in the intertidal species, which is exposed to extreme conditions, antioxidant activity is responsible for preventing lipid damage. Thus, organoleptic characteristics are improved, and conservation and handling stress could be prevented in intertidal compared to subtidal species. These studies would contribute to the sustainable management of valuable native species.

Keywords: limpets; oxidative damage; ascorbyl radical; lipid radicals; antioxidants; catalase; superoxide dismutase; biochemical composition; natural marine environments; craft catch; electron paramagnetic resonance.

Reference to this paper should be made as follows: González, P.M., Puntarulo, S. and Malanga, G. (2017) 'Natural adaptation to the environmental conditions affects the oxidation-dependent processes in limpets', *Int. J. Environment and Health*, Vol. 8, No. 4, pp.302–315.

Biographical notes: Paula Mariela González is a career investigator at the National Council of Science and Technology (CONICET) and teacher from the University of Buenos Aires, School of Pharmacy and Biochemistry, Argentina, in the subject Physical Chemistry and in post graduate courses. Her specialty is oxidative stress in molluscs and algae. She has been working in Antarctic and Germany institutions (Instituto Antártico Argentino and Alfred Wegener Institute, respectively). She has published 18 papers in peer review journals,

Copyright © 2017 Inderscience Enterprises Ltd.

and six book chapters. She has participated and published in more than 20 national and international meetings. She has also been a reviewer of several international journals.

Susana Puntarulo is a Professor of Physical Chemistry, at the School of Pharmacy and Biochemistry, University of Buenos Aires, and a Senior Researcher at the National Council of Science and Technology (CONICET), Argentina. She is studying the role of free radicals in biological systems, mainly focused on the participation of iron in the generation and metabolism of active oxygen and nitrogen species. She was director of 11 PhD thesis, and mentor of magister dissertations. Up to now, she is author of over 150 scientific papers, 26 book chapters, and participated in web publications, and national and international meetings.

Gabriela Malanga is a career investigator at the National Council of Science and Technology (CONICET) and teacher from the University of Buenos Aires, School of Pharmacy and Biochemistry, Argentina, in the subject Physical Chemistry and in post graduate courses. Her specialty is oxidative stress in fish, invertebrates and algae. She has been working in Antarctic (Instituto Antártico Argentino). She has published 42 papers in peer review journals, and 16 book chapters. She has participated and published in more than 100 national and international meetings. She has also been a reviewer of several international journals. She had directed doctoral students and obtained research supports.

This paper is a revised and expanded version of a book chapter entitled 'Preliminary studies of Subantarctic limpets metabolism as a possible resource' presented at *II Congreso Internacional de Ciencia y Tecnología Ambiental, y II Congreso Nacional de la Sociedad Argentina de Ciencia y Tecnología Ambiental*, FCEyN, UBA, Ciudad de Buenos Aires, Argentina, 1–4 December, 2015.

1 Introduction

Limpets, in their natural habitats, are exposed to physicochemical unstable intertidal/subtidal rocky shores owing to a broad variety of stressors such as tidal immersion/emersion cycles, desiccation and hypoxia, sharp salinity variations, extreme temperature, seasonality effects and anthropogenic influences (González et al., 2013a). These ecological changes generate misbalances between the production and elimination of reactive O_2 species (ROS) and reactive N_2 species (RNS). ROS and RNS are related to cellular oxidative damage of DNA, proteins and lipid membranes that, eventually, could affect the survival of these organisms (Lu et al., 2014). Thus, to maintain hazardous reactive species at low steady-state concentrations an efficient antioxidant defence system is required in all living organisms (González et al., 2015).

Exploitation of benthonic components (Concholepas, limptes, sea urchins, razor clams, clams, etc.) is a part of the craft catch, since these invertebrates have been historically known as an important potential food resource (Claassen, 1998). Limpets of the *Nacella* genus are exploited in Chile and in other Asian and European countries (Gutiérrez Gregoric et al., 2015). However, marine gastropod catching is very low in Argentina sea shore and the consumption and capture is limited to restaurants or families

(Gutiérrez Gregoric et al., 2015). The interest for these products in markets is constantly increasing even though an official fishery regulation is still missing in Argentina. This fact may lead to an increasing use of natural banks that would result in the decrease of the population densities and change the specific biodiversity turning these organisms in threatened species. The good quality of the limpet flesh is often acknowledged; however, neither the basic nutritional contribution nor the food safety is adequately registered and controlled. Content of water, protein, lipid, mineral and glycogen in the meat, together with the availability of minor components of hydrophilic or lipophilic nature, contribute to the nutritional value and the organoleptic characteristics of molluscs (Orban et al., 2002). It is widely recognised that water temperature, food availability and reproductive cycle of animals may influence the eat yield and biochemical composition of molluscs (Fernandez-Reiriz et al., 1996; Okumus and Stirling, 1998).

Limpets are linked to temperate or relatively cold seas, where they reach the higher specific biodiversity. *Nacella deaurata* (Gmelin 1971) and *N. magellanica* (Gmelin 1971) are the most conspicuous limpet species in the Beagle Channel, Tierra del Fuego, Argentina. Even though they inhabit the same region, the variation in their shore level distribution affects the animals contact to aerial or marine environmental conditions. Owing to tidal characteristics of the Beagle Channel, *N. magellanica* are daily exposed to air twice for 3–5 h each time, but *N. deaurata* are daily exposed to air for 3 h, only during spring tides. The dissimilar regime of exposition refers to extreme temperatures (under 0°C and more than 20°C during winter and summer time, respectively) for *N. magellanica*; meanwhile, *N. deaurata* is regularly covered by more than 0.3 m water with a temperature of 4°C in winter and 11°C in summer (Malanga et al., 2004). Gills are the first tissue in contact with the surrounding water and may properly reflect the environmental changing effects. The high content of polyunsaturated fatty acids (Joseph, 1982) of the membrane lipids in marine organisms implies a special susceptibility to lipid peroxidation (Halliwell and Gutteridge, 1984), and the particular response of their antioxidant system may reflect an adaptation of these species to their vastly changeable environment (Abele et al., 2002).

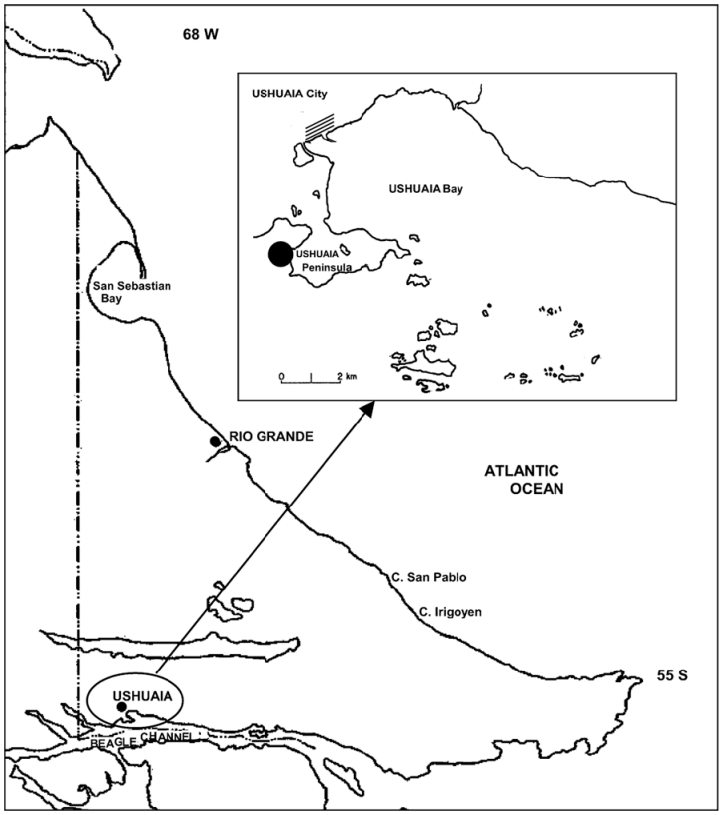
The main objective of this work was to obtain information on the metabolism, biochemical composition, oxidative stress and damage, and antioxidant activity in the gills of two Sub-Antarctic limpets *N. deaurata* and *N. magellanica*, collected during summer time. The knowledge of these features will allow the characterisation of the quality of the products to be used for human consumption.

2 Materials and methods

2.1 Animal collection and maintenance

The limpets *N. deaurata* and *N. magellanica* were collected from an intertidal area at Punta Occidental (54°50′S, 68°20′W) in the Beagle Channel, during summer time (Figure 1). At the sampling site, environmental physical parameters such as surface water temperature, dissolved O_2, pH, conductivity, salinity, turbidity and low tide duration were recorded simultaneously with the collection of the limpets by means of an HORIBA U-10 multi-parameter device.

Figure 1 Sampling area. Map of the south region of South America indicating the Beagle Channel. Insert: enlarged map showing the location of the sampling area (●)



Source: Modified from Malanga et al. (2004)

N. deaurata limpets were sampled at 0.3–0.5 m water depth, whereas *N. magellanica* limpets were sampled at the intertidal area. Only adult animals were sampled and they were maintained without feeding in natural seawater at 31.2 PSU, at constant water temperature for 24 h, in a light:dark cycle 12:12 h in the aquaria. Then, the animals were dissected and the gills were separated and frozen at –80°C until analyses.

Organisms were measured in length, width and height; and body parameters (circumference, volume and roundness) were calculated according to Wallace (1972) and Lowell (1984) (equations (1)–(4)).

$$\text{Circumference} = 2 \times (\text{legth}^2/8 + \text{width}^2/8)^{0.5} \quad (1)$$

$$\text{Volume} = (\text{length} \times \text{width} \times \text{height})/12 \quad (2)$$

$$\text{Slope} = (2 \times \text{height})/(\text{length} + \text{width}) \quad (3)$$

$$\text{Roundness} = \text{width}/\text{length} \quad (4)$$

Total weight (valve plus soft tissues), valves, soft tissues and separated gills were also weighted and three indices were calculated employing equations (5)–(7), according to Malanga et al. (2007) and Orban et al. (2002).

Condition index = soft tissue weight/valve weight (5)

Gill index = (total weight × 100)/gill weight (6)

Body index = soft tissue weight/length. (7)

2.2 Oxygen consumption

Whole animal respiratory rate was measured using the method of Peck and Veal (2001) using a Rank Brothers oxymeter (Cambridge, Great Britain) equipped with a closed chamber of 370 ml volume. Prior to respiration measurements the shells were cleaned with alcohol to remove epibenthos. Animals were left undisturbed for 24 h within the respirometer, after which oxygen consumption was recorded over 6 h. Respirometer oxygen partial pressure was maintained between 75% and 100% saturation over the measurement period.

2.3 Biochemical composition

The water content of the gills was measured after drying the flesh in an oven at 60°C until constant weight. Glycogen content was extracted by boiling the tissues with 30% (w/v) KOH and was determined as total carbohydrates using the Anthrone reagent (Carroll et al., 1956). Quantification was performed after precipitation with 99% (v/v) ethanol, using a 20 μg/ml glucose solution as standard. Lipids content was measured by a gravimetric method (Bligh and Dyer, 1959). Total protein content was assessed after hydrolysis with 0.5N NaOH, using 1 mg/ml bovine serum albumin as a standard according to Lowry et al. (1951). The results were expressed as mg biochemical component per gram of tissue ash-free dry weight (AFDW). Ash content was measured by burning the tissues in an oven at 450°C for 36 h. The ashes were weighed and the content in whole gills was calculated.

2.4 Ascorbyl radical ($A^{\bullet}$) content

Gills homogenates were prepared in pure dimethyl sulfoxide (DMSO) (1 : 3, w/v). Measurements were performed by electron paramagnetic resonance (EPR). Spectra were obtained at room temperature using a Bruker spectrometer ECS 106 using the following conditions: 50 kHz filed modulation, 20 mW microwave power, 1 G modulation amplitude, 655 ms time constant, 1×10^5 receiver gain, 9.81 GHz microwave frequency and 0.18 G/s scan rate (Giulivi and Cadenas, 1993). Quantification was performed according to Kotake et al. (1996).

2.5 Lipid radicals ($LR^{\bullet}$) content

The homogenates were prepared in potassium phosphate buffer (pH 7.4) containing 50 mM α-(4-pyridyl 1-oxide)-*N*-*t*-butyl nitrone (POBN). Measurements were performed by an EPR-spin trapping technique (Jurkiewicz and Buettner, 1994). EPR spectra were obtained at room temperature using: 9.81 GHz with 50 kHz modulation frequency, 20 mW microwave power, 1.194G modulation amplitude, 81.92 ms time constant and 2×10^4 receiver gain. Quantification was performed according to Kotake et al. (1996).

2.6 Antioxidant enzymatic activities

Gills were homogenised in 30 mM potassium phosphate buffer, 120 mM KCl, pH 7.4 (1 : 9, w/v), and centrifuged at $600 \times g$ for 10 min at 4°C. Catalase (CAT) activity was determined according to Aebi (1984). The supernatant was added to 50 mM potassium phosphate buffer, pH 7.0 and 15 mM H_2O_2. The H_2O_2 consumption was measured spectrophotometrically at $\lambda = 240$ nm during 30 s ($\varepsilon = 40\ M^{-1}\ cm^{-1}$) at 20°C. Superoxide dismutase (SOD) activity was analysed spectrophotometrically measuring the cytochrome *c* reduction by the superoxide anion (O_2^-) generated by the xanthine/xanthine oxidase system. Measurements were performed in 50 mM potassium phosphate buffer, 100 µM EDTA, pH 7.8, adding 21 µM cytochrome *c* and 10 µM xanthine. Absorbance was recorded at $\lambda = 550$ nm, at 20°C. One unit of SOD was defined as the amount of enzyme able to inhibit the cytochrome *c* reduction rate by 50% (McCord and Fridovich, 1969). Protein content was calculated according to Lowry et al. (1951).

2.7 Total fe content

Isolated gills from limpets were digested with an HNO_3 solution and after heating to dryness, the digests were dissolved in 2 ml 5% (v/v) HCl (Lawrie et al., 1991). Concentrations of Fe in the extracts were measured spectrophotometrically after reduction with thioglycolic acid, followed by the addition of bathophenanthroline (Brumby and Massey, 1967).

2.8 Statistical analyses

Data are expressed as mean ± SEM of three to six independent experiments, with two replicates in each experiment. Statistical tests were carried out using Statview for Windows, ANOVA, SAS Institute Inc., version 5.0.

3 Results and discussion

The sampled area is located near Ushuaia city, the southernmost location on Earth, and has been recently considered by Duarte et al. (2011) as a low urban impacted area, despite the population's growth around it. Data in Table 1 show the environmental physical parameters recorded at the sampling site. These parameters reflect the environmental conditions of the limpets in their natural habitats, which are in agreement with the values previously recorded by Duarte et al. (2012) and Lattuca et al. (2013).

The size of the organisms was analysed and was shown to be similar in both species (Figure 2). The valve measurements, except for slope and roundness, were similar between both species (Table 2). The body higher slope and roundness found in the valves of the intertidal specie, with respect to the subtidal one, might account for a phenotypic adaptation that may help to avoid desiccation during low tide hours supported by intertidal molluscs. Comparable results were found in intertidal and subtidal populations of *Nacella concinna* limpets from Antarctic (González and Puntarulo, 2016). No significant changes in tissues and valve weights were observed between the species (Table 2). The condition index is a calculated parameter of ecophysiological and economic relevance, especially in assessment of the industrial processing, since

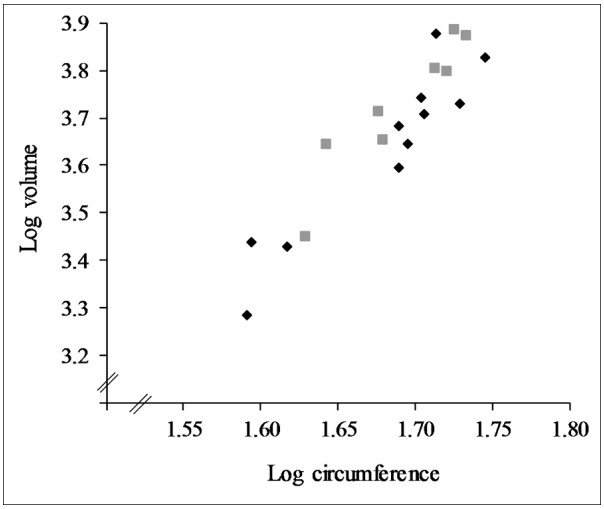
it represents a measurement of the apparent health and commercial quality of molluscs (Orban et al., 2002). Gill index reflects the tissue status as an indicator of its general condition. Neither the organisms condition nor the gill indexes showed any significant difference between the studies species. The body index is a general biomarker of the limpet health. This parameter was significantly higher in *N. magellanica* compared to *N. deaurata*, suggesting a general deterioration in the subtidal organisms (Table 2).

Table 1 Environmental parameters measured in the area of the study

Environmental parameters	
Temperature (°C)	10
Dissolved O_2 (mg/l)	12
pH	7.3
Conductivity (mS/cm)	44
Salinity (‰)	28
Turbidity (ntu)	190
Low tide duration (h)	9

ntu = nephelometric turbidity unit.

Figure 2 Internal volume as a function of the circumference for *N. deaurata* (■) and *N. magellanica* (■). The function volume = *f* (circumference) for *N. deaurata* followed the lineal equation: log (volume) = 3.4607 (log circumference) – 2.1184, being $R^2 = 0.8951$. For *N. magellanica* the function was: log (volume) = 3.1565 (log circumference) – 1.6692, being $R^2 = 0.8765$



Intertidal limpets tolerate fluctuations of environmental factors such as temperature, salinity and oxygen availability, and are therefore regarded as comparatively stress resistant (Peck, 2005). In addition to the conspicuous differences in shell morphology between the two species, behavioural traits may differ such as the capacity to regulate the oxygen partial pressure in shell water (Weihe and Abele, 2008). Oxygen consumption was significantly higher in the subtidal than in the intertidal species (0.8 ± 0.1 and

0.30 ± 0.06 µmol O_2/g fresh weight (FW) h, respectively). This observation fits with data from *N. concinna* populations were intertidal limpets maintained shell water oxygen partial pressure at low levels independently of submergence state remaining aerobic. Whereas sub-littoral limpets induce anaerobic metabolism when facing air exposure and hypoxia suggesting insufficient adaptation for life under intertidal exposure conditions (Weihe and Abele, 2008; Weihe et al., 2010). In the Beagle channel, an important increase of phytoplankton biomass occurs at the end of September/beginning of October increasing the metabolism (Malanga et al., 2007). Even more, during summer the main spawning event takes place, and water temperatures reach their yearly maximum and a more rapid increase in day length and radiation intensity at Ushuaia may affect feeding activity of the limpets.

Table 2 Morphological features of the sub-antarctic limpets

		N. deaurata	*N. magellanica*
Valve measurements	Length (mm)	55 ± 2	54 ± 2
	Width (mm)	41 ± 1	44 ± 2
	Height (mm)	24 ± 2	28 ± 2
	Slope	0.50 ± 0.03	0.58 ± 0.02*
	Roundness	0.75 ± 0.01	0.835 ± 0.008**
Weights	Total weight (g)	25 ± 3	33 ± 4
	Valve weight (g)	11 ± 2	15 ± 2
	Soft tissues weight (g)	12 ± 2	17 ± 2
	Gill weight (g)	0.66 ± 0.07	0.7 ± 0.1
Indexes	Condition index	1.16 ± 0.05	1.4 ± 0.3
	Gill index	2.2 ± 0.3	1.9 ± 0.3
	Body index (g/mm)	0.22 ± 0.02	0.31 ± 0.03*

*Significantly different to *N. deaurata* (ANOVA, $p < 0.05$).

**Significantly different to *N. deaurata* (ANOVA, $p < 0.0001$).

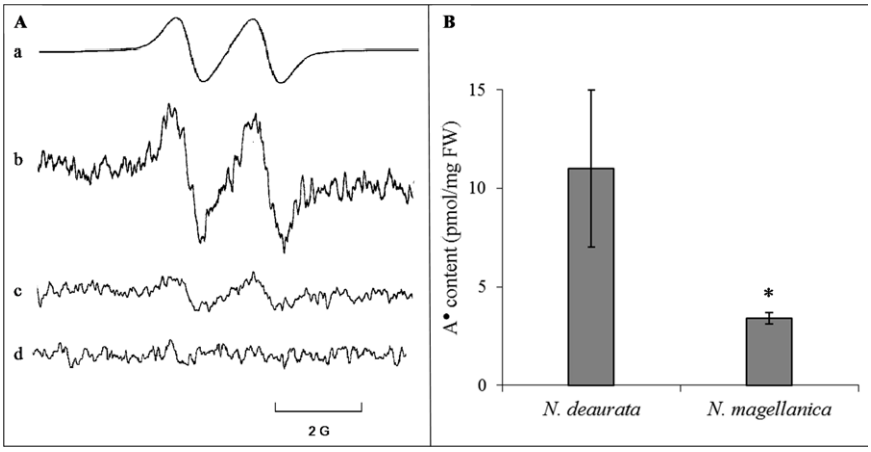
Biochemical composition, including carbohydrates, lipids, proteins and ashes content, showed no significant differences between both species (Table 3). This fact suggests a similar nutritional input in these limpets, mainly in terms of the high protein content. The elevated protein content was a characteristic feature previously reported for other gastropod, *Babylonia spirata* (Periyasamy et al., 2011).

Table 3 Biochemical composition in gills of the limpets

	N. deaurata	*N. magellanica*
Carbohydrates (mg/g AFDW)	1.1 ± 0.1	0.80 ± 0.07
Lipids (mg/g AFDW)	8 ± 2	11 ± 4
Proteins (mg/g AFDW)	567 ± 71	498 ± 19
Ashes (mg/g AFDW)	13 ± 2	6.0 ± 0.3

Ascorbate (AH^-) plays a central metabolic role since it can act as an antioxidant and a pro-oxidant and its oxidation leads to $A^\bullet$ generation (Malanga el al., 2005). AH^- pro-oxidant activity is the result of its ability to reduce transition metals (especially Fe) causing them to react with oxygen and initiators of $LR^\bullet$ reactions (Wills, 1966). AH^- antioxidant activity consists in the ability to reduce various types of radicals, including peroxyl radicals and propagate lipid peroxidation, and to regenerate the antioxidant α-tocopherol from the oxidised form (Doba et al., 1985). The ratio $A^\bullet/AH^-$ was considered to serve as an appropriate and accurate indicator of oxidative stress in the hydrophilic medium (Galleano et al., 2002). However, in biological systems where AH^- content is not affected by the studied condition, the $A^\bullet$ content by itself could be considered as an oxidative stress indicator in the hydrophilic medium of the tissue (González et al., 2013b). Previous data from studies performed in samples from *N. magellanica* and *N. deaurata* collected in the Sub-Antarctic Beagle Channel during July, have shown that AH^- content was not different neither in gills (Malanga el al., 2005) nor in digestive glands (Malanga et al., 2004) when comparing both species. Thus, the $A^\bullet$ content was assessed as an oxidative parameter in this work. A typical EPR spectrum of $A^\bullet$ in gills from both limpets, with the characteristic two lines at $g = 2.005$ and $a_H = 1.8$G, was observed (Figure 3(A) b, c), in accordance with computer spectral simulated signals obtained using the parameters previously described (Figure 3(A) a). DMSO itself was examined and no DMSO spin adduct was observed (Figure 3(A) d). The $A^\bullet$ content was 224% higher in *N. deaurata* than in *N. magellanica* gills (Figure 3(B)). Malanga et al. (2005) reported that $A^\bullet$ content in gills from *N. deaurata* and *N. magellanica* collected during winter time was 6 ± 2 and 3.6 ± 0.2 pmol/mg FW, respectively. The data shown here on $A^\bullet$ content in gills from animals collected during summer are in the same order of magnitude, suggesting that seasonality does not significantly affect oxidative conditions in the hydrophilic medium in both species.

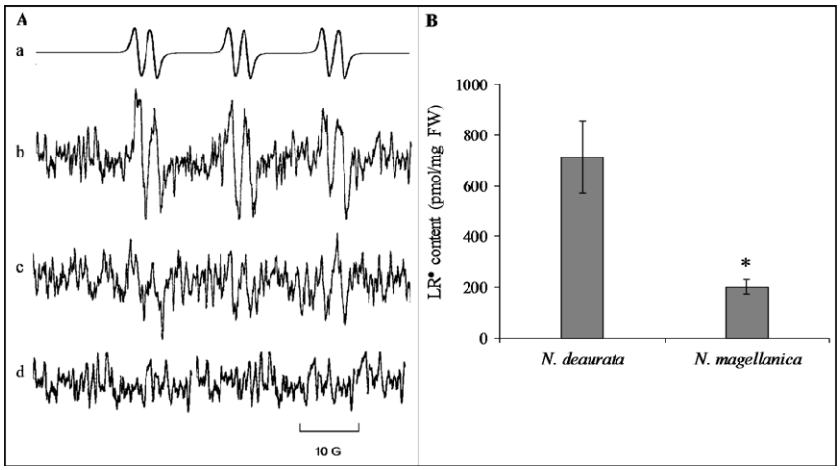
Figure 3 (A) Detection of $A^\bullet$ measured by EPR, spectra from: (a) computer-simulated employing the following spectral parameters $g = 2.005$ and $a_H = 1.8$ G,(b) gills homogenates from *N. deaurata*, (c) gills homogenates from *N. magellanica* and (d) DMSO without tissue. (B) $A^\bullet$ content in limpets



*Significantly different to *N. deaurata* (ANOVA, $p < 0.05$). FW stands for fresh weight.

$LR^{\bullet}$ content was analysed as an oxidative damage indicator to lipids (González et al., 2013b). Lipid peroxidation in the gills from both species was analysed as the $LR^{\bullet}$ content assessed by EPR. $LR^{\bullet}$ combined with the spin trap POBN resulted in adducts of characteristic EPR spectrum with hyperfine coupling constants of $a_N = 15.56$G and $a_H = 2.79$G (Figure 4(A) b, c), consistent with computer spectral simulated signals acquired using the overall mentioned parameters (Figure 4(A) a). POBN itself was examined and no spin adduct was observed (Figure 4(A) d). Spin trapping studies cannot discriminate between peroxyl ($ROO^{\bullet}$), alcohoxyl ($RO^{\bullet}$) and alkyl ($R^{\bullet}$) adducts, due to the similarity of the corresponding coupling constants; however, these constants could be assigned to $LR^{\bullet}$ in general (Jurkiewicz and Buettner, 1994). This parameter resulted 3.5-fold higher in *N. deaurata* as compared to *N. magellanica* (Figure 4(B)). However, $LR^{\bullet}$ content in gills from *N. deaurata* and *N. magellanica* collected during winter time was 412 ± 98 and 80 ± 36 pmol/mg FW, respectively (Malanga et al., 2005). Thus, a significant increase (2-fold) was observed in gills from animals collected over summer as compared to the $LR^{\bullet}$ content in gills from limpets studied in winter. Taken into consideration that the increase in lipid peroxidation would be associated to deterioration in the organoleptic characteristics (palatability) (Cowey et al., 1984), these data strongly suggest that animals collected during winter time could be more appreciated for human consumption than the summer-collected molluscs. Even more, frequent occurrence of harmful algae blooms during spring in the Beagle channel (Arzula et al., 1999), makes molluscs from this area hard to sell (Lomovasky et al., 2002) owing to the potential danger to human health. In this regard, catch during winter can improve safety commercialisation.

Figure 4 (A) Detection of $LR^{\bullet}$ measured by EPR, spectra from: (a) computer-simulated employing the following spectral parameters $a_N = 15.56$ G and $a_H = 2.79$ G, (b) gills homogenates from *N. deaurata*, (c) gills homogenates from *N. magellanica* and (d) POBN without tissue and (B) $LR^{\bullet}$ content in limpets



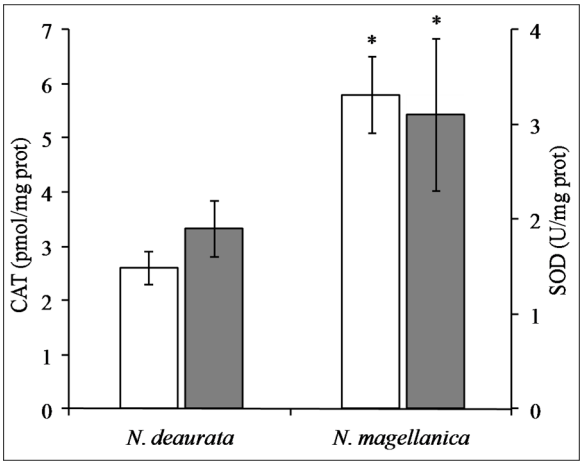
*Significantly different to *N. deaurata* (ANOVA, $p < 0.05$). FW stands for fresh weight.

Since high environmental Fe content in the studied area was found (35 ± 12 mg/g DW, Duarte et al., 2011), it is feasible that the higher levels of lipid peroxide formation in the gills from the subtidal *N. deaurata* as compared to the intertidal *N. magellanica* may

result from increased accumulation of transition metals such as Fe in these organisms as compared to molluscs from other regions. The Fe content in the gills of both molluscs was examined and the overall content of Fe was found to be not significantly different between the two species (3.2 ± 0.5 and 3.7 ± 0.9 nmol/mg FW in *N. deaurata* and *N. magellanica*, respectively). Even though the actual nature of the intracellular ligands participating in the labile iron pool (LIP) formation remains obscure, it is accepted that the affinity of a chelator for Fe, its ability to permeate different cell compartments as well as the steric accessibility of specific metal binding sites on proteins could all influence the LIP (González et al., 2008). Quantification of the LIP in digestive glands from *N. deaurata* and *N. magellanica*, did not show any significant difference between both species, thus the amount of Fe available for catalysis did not differ in both limpets in these tissues (González et al., 2008). In agreement with this observation, since total Fe content was not different in gills from these species, it could be postulated that Fe capacity of catalysing lipid peroxidation, through the LIP, could not be considered as a key factor in the difference in the $LR^{\bullet}$ content observed.

Antioxidant capacity was studied in both limpets by assaying the activity of the antioxidant enzymes CAT and SOD. Both enzymes activities were significantly higher in *N. magellanica* as compared to *N. deaurata* (Figure 5). The activity of these enzymes could be an important factor to keep the lipid damage under control and to contribute to a successful adaptation of *N. magellanica* to the stressful intertidal conditions.

Figure 5 Antioxidant enzymes activity in gills of the limpets



*Significantly different to *N. deaurata* (ANOVA, $p < 0.05$).

4 Conclusions

Oxidative stress may arise from the changing oxygen availability during tidal cycles. Especially during low tide aerial exposure, gills are a main target for oxidative injury (Malanga et al., 2005), and consequently gills from intertidal limpets must be better protected by antioxidant enzymes against oxidative stress than gills from the subtidal animals. The oxidative stress during low tides can be generated either from breathing air in the intertidal organisms, or from hypoxia during shell contraction and subsequent

reoxygenation. The antioxidant protection under such variable conditions is afforded through the activities of the enzymes SOD and CAT. Data presented here for *N. magellanica*, an intertidal species which is exposed to extreme environmental conditions, showed a more elevated antioxidant activity than for *N. deaurata*, a subtidal species. This higher antioxidant protection would be a contributing factor to prevent lipid damage, essential to improve organoleptic characteristics and to control stress during the processes of conservation and commercialisation.

Acknowledging the nutritional, economic, social, cultural and environmental fishing importance (FAO, 1995), it is needed to point out that the use of responsible catching practices would improve the conservation, the management and the development of aquatic resources, preserving the ecosystem and the biodiversity. These studies would contribute to find a suitable strategy that would make possible a progress in the subexploitation of a promising food resource, by improving the sustainable management opportunities of native species in a growing market, adequately selecting the more suitable organism for human consumption.

Acknowledgements

This study was supported by grants from the University of Buenos Aires (20020130100383BA), National Agency of Science and Scientific Promotion (ANPCYT PICT 00845 and PICT 1414-2014) and National Council for Science and Technology (CONICET PIP 0697 and PIP 0635). Susana Puntarulo, Gabriela Malanga and Paula Mariela González are career investigators from CONICET.

References

Abele, D., Heise, K., Pörtner, H.O. and Puntarulo, S. (2002) 'Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya Arenaria*', *Journal of Experimental Biology*, Vol. 205, pp.1831–1841.

Aebi, H. (1984) 'Catalase in vitro', *Methods in Enzymology*, Vol. 105, pp.121–126.

Arzula, G., Seguel, M., Guzman, L. and Erard-Lea Denn, E. (1999) 'Comparison of allelopathic properties in three toxic *Alexandrium* species', *Journal of Experimental Marine Biology and Ecology*, Vol. 232, pp.285–295.

Bligh, E.G. and Dyer, W.J. (1959) 'A rapid method of total lipid extraction and purification', *Canadian Journal of Physiology and Pharmacology*, Vol. 37, pp.911–917.

Brumby, P.E. and Massey, V.(1967) 'Determination of nonheme iron, total iron and copper', *Methods in Enzymology*, Vol. 10, pp.463–472.

Carroll, N.V., Longley, R.W. and Roe, J.H. (1956) 'The determination of glycogen in liver and muscle by use of anthrone reagent', *The Journal of Biological Chemistry*, Vol. 220, pp.583–593.

Claassen, C. (1998) *Shells*, Cambridge University Press, Cambridge.

Cowey, C.B., Degener, E., Tacon, A.G.J., Youngston, A. and Bell, J.G. (1984) 'Effect of vitamin E and oxidised fish oil on the nutrition of rainbow trout (*Salmo Gairdneri*), growth at natural varying water temperature', *British Journal of Nutrition*, Vol. 51, pp.443–451.

Doba, T., Burton, G.W. and Ingold, K.U. (1985) 'Antioxidant and co-antioxidant activity of vitamin, C. The effect of vitamin C., either alone or in the presence of Vitamin E or a water-soluble vitamin E analogue, upon the peroxidation of aqueous multi-lamellar phospholipid liposomes', *Biochimica et Biophysica Acta*, Vol. 835, pp.298–303.

Duarte, C.A., Giarratano, E. and Gil, M.N. (2012) 'Trace metal content in sediments and autochthonous intertidal organisms from two adjacent bays near ushuaia, beagle channel (Argentina)', *Marine Environmental Research*, Vol. 79, pp.55–62.

Duarte, C.A., Giarratano, E., Amin, O.A. and Comoglio, L.I. (2011) 'Heavy metal concentrations and biomarkers of oxidative stress in native mussels (*Mytilus Edulis Chilensis*) from beagle channel coast (Tierra del fuego, Argentina)', *Marine Pollution Bulletin*, Vol. 62, No. 8, pp.1895–1904.

FAO (1995) *Código De Conducta Para La Pesca Responsable*, Roma.

Fernandez-Reiriz, M.J., Labarta, U. and Babarro, J.M.F. (1996) 'Comparative allometries in growth and chemical composition of mussel (*Mytilus Galloprovincialis LMK*) cultured in two zones in the Ria Sada (Galicia, NW Spain)', *Journal of Shellfish Research*, Vol. 15, No. 2, pp.349–353.

Galleano, M., Aimo, L. and Puntarulo, S. (2002) 'Ascorbyl radical/ascorbate ratio in plasma from iron overloaded rats as oxidative stress indicator', *Toxicology Letters*, Vol. 133, pp.193–201.

Giulivi, C. and Cadenas, E. (1993) 'The reaction of ascorbic acid with different heme iron redox states of myoglobin', *FEBS Letters*, Vol. 332, pp.287–290.

González, P.M. and Puntarulo, S. (2016) 'Fe effects on the oxidtive and nitrosative metabolism in the antarctic limpet *Nacella Concinna*', *Comparative Biochemistry and Physiology–Part A: Molecular and Integrative Physiology*, Vol. 200, pp.56–63.

González, P.M., Malanga, G. and Puntarulo, S. (2008) 'Ferritin and labile iron pool in limpets from the beagle channel', in Svensson, E.P. (Ed.): *Aquatic Toxicology Research Focus*, Nova Science Publishers Inc. New York, Chapter 9, pp.177–188.

González, P.M., Puntarulo, S. Malanga, G. (2013a) 'Oxidative stress and antioxidant capacity in different species of limpets', *Trends in Comparative Biochemistry and Physiology*, Vol. 17, pp.1–12.

González, P.M., Aguiar, M.B., Malanga, G. and Puntarulo, S. (2013b) 'Electronic paramagnetic resonance (EPR) 'for the study of ascorbyl radical and lipid radicals in marine organisms'', *Comparative Biochemistry and Physiology–Part A: Molecular and Integrative Physiology*, Vol. 165, pp.439–447.

González, P.M., Malanga, G. and Puntarulo, S. (2015) 'Cellular oxidant/antioxidant network: update on the environmental effects over marine organisms', *The Open Marine Biology Journal*, Vol. 9, pp.1–13.

Gutiérrez Gregoric, D., Darrigran, G. and Damborenea, C. (2015) *Los Moluscos Marinos De Argentina y Su Consumo*, División Zoología Invertebrados, Sección Malacología, Serie Didáctica N° 2.

Halliwell, B. and Gutteridge, J.M.C. (1984) 'Oxygen toxicity, oxygen radicals, transition metals and disease', *Biochemical Journal*, Vol. 219, pp.1–14.

Joseph, J.D. (1982) 'Lipid composition of marine and estuarine invertebrates. Part II. Mollusca', *Progress in Lipid Research*, Vol. 21, pp.109–153.

Jurkiewicz, B.A. and Buettner, G.R. (1994) 'Ultraviolet light-induced free radical formation in skin: an electron paramagnetic resonance study', *Photochemistry and Photobiology*, Vol. 59, pp.1–4.

Kotake, Y., Tanigawa, T., Tanigawa, M., Ueno, I., Randel Allen, D. and Lai, C-S. (1996) 'Continuous monitoring of cellular nitric oxide generation by spin trapping with an iron-dithiocarbamate complex', *Biochimica* et *Biophysica Acta*, Vol. 1289, pp.362–368.

Lattuca, M.E., Perez, A., Giarratano, E. and Malanga, G. (2013) 'Biomarkers of oxidative damage and environmental impact assessment in *Odontesthes Nigricans* Richardson (Pisces, atherinopsidae) from two coastal areas of the beagle channel, Argentina', *Revista Chilena De Historia Natural*, Vol. 86, pp.453–464.

Lawrie, S., Tancock, N., Mc Growth, S. and Roger, J. (1991)'Influence of complexation on the uptake by plants of iron, manganese, copper and zinc.I. Effect of EDTA in a multimetal and computer simulation study', *Journal of Experimental Botany*, Vol. 42, pp.509–515.

Lomovasky, B.J., Brey, T., Morriconi, E. and Calvo, J. (2002) 'Growth and production of the venerid bivalve *Eurhomalea Exalbida* in the beagle channel, tierra del fuego', *Journal of Sea Research*, Vol. 48, pp.209–216.

Lowell, R.B. (1984) 'Desiccation of intertidal limpets: effects of shell size, fit to substratum and shape', *Journal of Experimental Marine Biology and Ecology*, Vol. 77, pp.197–207.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) 'Protein measurement with the folin phenol reagent', *The Journal of Biological Chemistry*, Vol. 193, pp.265–275.

Lu, T.H., Tseng, T.J., Su, C.C., Tang, F.C., Yen, C.C., Liu, Y.Y., Yang, C.Y., Wu, C.C., Chen, K.L., Hung, D.Z. and Chen, Y.W. (2014) 'Arsenic induces reactive oxygen species-caused neuronal cell apoptosis through JNK/ERK-mediated mitochondria-dependent and GRP.78/CHOP-regulated pathways', *Toxicology Letters*, Vol. 224, pp.130–140.

Malanga, G., Estevez, M.S., Calvo, J and Puntarulo, S. (2004) 'Oxidative stress in limpets exposed to different environmental conditions in the beagle channel', *Aquatic Toxicology*, Vol. 69, pp.299–309.

Malanga, G., Estévez, M.S., Calvo, J., Abele, D. and Puntarulo, S. (2005) 'Oxidative stress in gills of limpets from the beagle channel: comparison with limpets from the antarctic', *Scientia Marina*, Vol. 69, No. 2, pp.297–304.

Malanga, G., Estevez, S., Calvo, J., Abele, D. and Puntarulo, S. (2007) 'Seasonality effect on oxidative metabolism in *Nacella* (*P.*) *Magellanica*', *Comparative Biochemistry and Physiology–Part A: Molecular and Integrative Physiology*, Vol. 146, pp.551–558.

McCord, J.M. and Fridovich, I. (1969) 'Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein)', *The Journal of Biological Chemistry*, Vol. 244, pp.6049–6055.

Okumus, I. and Stirling, A.P. (1998) 'Seasonal variations in the meat weight, conditionindex and biochemical composition of mussels (*Mytilus Edulis,* L.) in suspended culture in two Scottish sea lochs', *Aquaculture*, Vol. 159, Nos. 3–4, pp.249–261.

Orban, E., Di Lena, G., Nevigato, T., Casini, I., Marzetti, A. and Caproni, R. (2002) 'Seasonal changes in meat content, condition index and chemical composition of mussels (*Mytilus Galloprovincialis*) 'cultured in two different Italian sites', *Food Chemistry*, Vol. 77, pp.57–65.

Peck, L. and Veal, R. (2001) 'Feeding, metabolism and growth in the antarctic limpet', *Nacella Concinna* (Strebel 1908)', *Marine Biology*, Vol. 138, pp.553–560.

Peck, L.S. (2005) 'Prospects for survival in the southern ocean: vulnerability of benthic species to temperature change', *Antarctic Science*, Vol. 17, No. 4, pp.497–507.

Periyasamy, N., Srinivasan, M., Devanathan, K. and Balakrishnan, S. (2011) 'Nutritional value of gastropod *Babylonia Spirata* (Linnaeus, 1758) 'from Thazhanguda, Southeast Coast of India', *Asian Pacific Journal of Tropical Biomedicine*, Vol. 1, No. 2, pp.249–252.

Wallace, L.R. (1972)'Some factors affecting vertical distribution and resistance to desiccationin the limpet', *Acmaea Testudinalis* (Muller)', *The Biological Bulletin*, Vol. 142, pp.186–193.

Weihe, E. and Abele, D. (2008) 'Differences in the physiological response of inter- and subtidal antarctic limpets *Nacella Concinna* to aerial exposure', *Aquatic Biology*, Vol. 4, pp.155–166.

Weihe, E., Kriews, M. and Abele, D. (2010) 'Differences in heavy metal concentrations and in the response of the antioxidant system to hypoxia and air exposure in the antarctic limpet *Nacella Concinna*', *Marine Environmental Research*, Vol. 69, No. 3, pp.127–135.

Wills, E.D. (1966) 'Mechanisms of lipid peroxide formation in animal tissue', *Biochemistry Journal*, Vol. 99, pp.667–676.