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The effect of seasonality on oxidative metabolism in the sea urchin *Loxechinus albus*

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Abstract The objective of the present study was to investigate seasonal variations in the oxidative metabolism of the sea urchin *Loxechinus albus* gonads. The reported spawning period for this species is from September to November. Lipid radical content showed non-significant changes upon the seasons. Ascorbyl radical content and the content of α-tocopherol were lower in samples collected in spring and summer as compared to the values in winter-collected animals. Ascorbate content decreased in samples collected in fall as compared to those collected in winter. For the lipophilic compartment, the lipid radical content/α-tocopherol content ratio is an indicator of oxidative stress. This index increased significantly in tissues during spawning as compared to the values in samples collected during winter. The ascorbyl content/ascorbate content ratio is an indicator of oxidative stress for the hydrophilic milieu. A significant decrease by 66% was determined in tissues from gonads of animals collected in summer as compared to values in animals collected in winter. The data reported here suggest a different profile of response against oxidative stress at the lipophilic and hydrophilic milieus in *L. albus* gonads.

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Introduction

Many benthic marine invertebrates show seasonal variation in feeding, growth, and oxygen consumption (Siikavuopio et al. 2007). In ectothermal animals from temperate areas, annual fluctuations in metabolic rates, locomotory activity, and growth rates are induced by environmental parameters such as photoperiod, temperature, and feed availability. In addition to fluctuations mediated by external factors, physiological events such as reproduction may influence feeding and growth cycles (Schöttler 1989; Heilmayer et al. 2005). The seasonal variations in metabolic rate are assumed to entail corresponding alterations in reactive oxygen species (ROS) formation (Abele and Puntarulo 2004). The generation of ROS, such as superoxide anion (O_2^{-}), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\bullet}OH$), takes place continuously in living cells, mainly as by-products of respiration. Indeed, seasonal changes in free radical metabolism have frequently been reported in marine ectotherms, including cichlid fish (Wilhelm Filho et al. 2001a), estuarine polychaetes (Abele-Oeschger et al. 1994; Geracitano et al. 2004; Keller et al. 2004), and in various mussel species (Viarengo et al. 1991; Power and Sheedan 1996; Wilhelm Filho et al. 2001b; Malanga et al. 2007).

However, little is known about the oxidative metabolism in sea urchins (Wong and Wessel 2005). This is a species with a wide latitudinal distribution in the Pacific Ocean, from Ecuador (6°S) to the Beagle Channel, in the south of Tierra del Fuego (54°S) (Dayton 1985; Bernasconi 1947; Bernasconi 1953; Vásquez and Buschmann 1997). Natural

stocks on Chilean coasts have been reduced or depleted due to excessive harvest for commercial purposes (Olave et al. 1997). In the Beagle Channel, *Loxechinus albus* population is exposed to strong variations of the photoperiod, temperature, and marked seasonal fluctuations in primary productivity due to the geographical distribution (Hernando 2006). Increasing demand for sea urchins' gonads has led to over-fishing of natural populations in many countries during the last decades (Siikavuopio et al. 2007). Many of the sea urchin fisheries show yield oscillations following the decline and recuperation of natural stocks (Keesing and Hall 1998; Andrew et al. 2002; Vadas et al. 2000). Integrative physiological and biochemical studies on the reproductive cycles, and growth rates will contribute both to the management of wild populations and to the knowledge of invertebrate physiology. Such information is unavailable for many species, including *L. albus* (Molina 1782). The objective of this study was to analyze the hypothesis that seasonal factors could influence the oxidative metabolism in the sea urchin *L. albus* gonads. Since cellular oxidative status could show a different profile in the hydrophilic and the lipophilic milieus, oxidative indices in both environments were studied and compared over the year.

Materials and methods

Sea urchin collection

SCUBA divers collected a total of 50 individuals of adult *L. albus*, with size ranging from 70 to 80 mm test diameter. Sampling was carried out from August 2005 to August 2006 off Bridges Islands, Ushuaia, Tierra del Fuego, Argentina (54°50′S, Beagle Channel) on a rocky cobble bottom. Sea urchins were transported to the laboratory in plastic boxes containing natural sea water within 2 h of collection. Even though handling was carefully done to avoid stress, manipulation could affect oxidative parameters. However, comparisons are valid since they were established between samples that afforded identical preparation procedures. Each sea urchin was weighed, and maximum diameter (through the madreporic plate) and height were measured using an electronic calliper (0.1 mm). Samples were stored at −80°C until analyzed.

Gonad index (GI)

Sea urchins were allowed to drip on a paper towel for approximately 1 min before the whole animal wet weight was recorded. Animals were dissected and gonads were weighed separately. The GI was calculated according to Eq. 1

$$\mathrm{GI} = \frac{\text{organ wet weight (g)} \times 100}{\text{total wet weight (g)}} \quad (1)$$

α-Tocopherol (α-TH) content

The content of α-TH in the gonads was quantified by reverse-phase HPLC with electrochemical detection using a Bioanalytical Systems LC-4C amperometric detector with a glass carbon working electrode at an applied oxidation potential of 0.6 V. Samples (0.5 g) 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). D,L-α-Tocopherol (Sigma, St Louis, MO) was used as standard.

Lipid radical content

Lipid radical content was detected by electron paramagnetic resonance (EPR) employing a spin trapping technique using *N-t*-butyl-α-phenyl nitrone (PBN). A 40 mM PBN stock solution was prepared in DMSO immediately prior to use. The homogenates (15 mg/ml) were prepared in DMSO–PBN (stock solution). EPR spectra were obtained at room temperature using a Bruker ECS 106 spectrometer, operating at 9.81 GHz with 50 kHz modulation frequency. EPR instrument settings for the spin trapping experiments were as follows: microwave power, 20 mW; modulation amplitude, 1.194 G; time constant, 81.92 ms; scans number, 5; center fields, 3,480 G; modulation frequency, 50 kHz; and receiver gain, 2×10^4 (Lai et al. 1986). Quantification was performed according to Kotake et al. (1996).

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

A Bruker ECS 106 spectrometer was used for $A^{\bullet}$ measurements. Homogenates from gonads were prepared in DMSO (1:3) and the spectra were scanned at room temperature under the following conditions: field modulation, 50 kHz; microwave power, 20 mW; modulation amplitude, 1 G; time constant, 655 ms; receiver gain, 1×10^5; microwave frequency, 9.81 GHz; and scan rate, 0.18 G/s (Giulivi and Cadenas 1993). Quantification was performed according to Kotake et al. (1996).

Ascorbate content (AH^-)

The content of AH^- was measured according to Foyer et al. (1983). The acid extracts were neutralized with 1.25 M K_2CO_3 and the amounts of AH^- were determined by addition of 5 U/ml ascorbate oxidase. AH^- was used as standard.

Statistical analyses

Data in the text and tables are expressed as mean ± SEM. Numbers of replicates (*N*) for each variable analyzed were between 20 and 4. Individuals were not differentiated by sex. Seasonality on GI and lipid radical content were analyzed using an analysis of variance (one-way ANOVA). The assumptions of normality (Kolmogorov–Smirnov test) and homogeneity of variances (Levene's test) were tested. Significant differences were analyzed by unplanned Tukey–Kramer multiple comparisons test. Seasonality on $A^{\bullet}$ content, AH^{-} content, α-TH content, the lipid radical content/α-TH content ratio and the $A^{\bullet}$ content/AH^{-} content ratio were analyzed using a non-parametric test (Kruskal–Wallis), and differences were analyzed using unplanned Dunn's multiple comparisons test (Zar 1984; Sokal and Rohlf 1995). Statistical analyses were performed with Statistica 6.0 and GraphPad Instat packages.

Results

In agreement with the great seasonal fluctuations in environmental parameters, such as productivity, temperature and photoperiod, a seasonal variation in the GI was observed in the *L. albus* population analyzed for this study (Table 1). The changes in GI could be interpreted as due to the variation in the amount of both nutritive phagocytes and gametes in different stages of development (Walker et al. 2007). The reported spawning period for this species is from September to November (Pérez et al. 2008). The decrease in the GI in the samples collected in spring and summer is consistent with the observations of Perez et al. (unpublished results), where preliminary histological studies showed a period of absence of maturing oocytes from October to February.

As a measurement of oxidative stress in the lipid phase, lipid peroxidation in the gonads was assessed by EPR as the tissue content of lipid radicals. Lipid radicals in the samples combined with the spin trap PBN resulted in adducts that gave a characteristic EPR spectrum with hyperfine coupling constants of $a_N = 15.56$ G and $a_H = 2.79$ G, in concordance with computer spectral simulated signals obtained using the overall mentioned parameters (Fig. 1). PBN itself was examined and no PBN spin adduct was observed. Bulk lipid radical content in the sea urchin gonads was not significantly changed over the studied period (Table 2).

The concentration of the non-enzymatic antioxidant α-TH is considered as one of the most critical factors to control lipid peroxidation in biological membranes (Yamamoto 2001). α-TH concentration was assessed in the sea urchin gonads over the year, and the data shown in Table 2 indicate that its concentration was significantly lower in the gonads from the animals collected during spring and summer, as compared to values obtained in gonads from animals collected in winter.

For the lipophilic compartment, the lipid radical content/α-TH content ratio can be understood as an indicator of the balance between free radical-dependent damage and antioxidant protection. The value of this index over the year is shown in Fig. 2. During spawning, this index increased significantly as compared to the values recorded in the samples collected during winter time, suggesting either the onset of an active oxidative metabolism preceding spawning in the lipid phase of the gonads or an increased metabolism due to the increasing water temperature.

A typical EPR spectrum of $A^{\bullet}$ in gonads from *L. albus* was observed with the characteristic two lines at $g = 2.005$ and $a_H = 1.8$ G (Fig. 3). DMSO itself was examined and no DMSO spin adduct was observed. EPR spectrum of $A^{\bullet}$ is significantly lower in gonads isolated from sea urchins collected in summer as compared to values in gonads from animals collected in winter (Fig. 3). $A^{\bullet}$ content, assessed by quantification of EPR signals, was decreased by 64% in samples from animals collected in summer as compared to the values in gonads from sea urchins collected in winter. It is important to point out that besides individual variation

Table 1 Physiological characteristics of body and gonads of *L. albus* over the year period

Season	Animal fresh weight (g)	Diameter (mm)	Height (mm)	Gonad weight (g)	GI
Winter (August 2005)	182 ± 5	76.7 ± 0.7	39.3 ± 0.8	25 ± 2	13.6 ± 0.9
Spring (October 2005)	182 ± 4	78.2 ± 0.6	39.5 ± 0.7	16 ± 2*	8.6 ± 0.8*
Summer (December 2006)	170 ± 6	75.6 ± 0.8	40.6 ± 0.7	16 ± 2*	9.0 ± 1.0*
Fall (April 2006)	157 ± 6	73 ± 1	37.2 ± 0.7	21 ± 1	13.6 ± 0.9
Winter (July 2006)	191 ± 8	78 ± 1	40.0 ± 1.0	26 ± 2	13.7 ± 0.9

$N = 40$, 10 individuals per seasonal group

* Values were significantly different (ANOVA, $P < 0.05$) from values in winter samples-August 2005. Multiple comparisons by Tukey–Kramer: the values in animals collected in summer and spring were significantly lower than the values registered in the gonads from the animals collected in both winters. The values in gonads from *L. albus* collected in summer were significantly lower than the values in the gonads from the animals collected in the fall

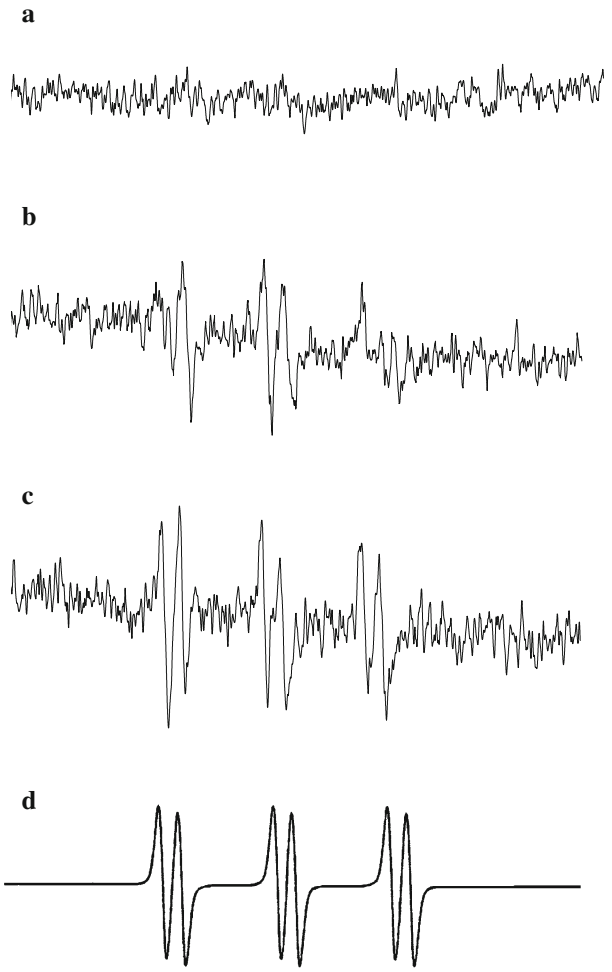

Fig. 1 EPR detection of lipid radicals in gonads from *L. albus*. The following spectra are shown: **a** *N*-*t*-butyl-α-phenyl nitrone (PBN), **b** gonads collected in winter-August 2005, **c** gonads collected in summer (December 2005), and **d** computer-simulated EPR spectra exhibiting hyperfine splittings ($a_N = 15.56$ G and $a_H = 2.79$ G)

Table 2 Lipid radical and α-TH content in *L. albus* over the year period

Season	Lipid radical content (pmol/mg FW)	α-TH content (nmol/mg FW)
Winter (August 2005)	0.7 ± 0.2	0.8 ± 0.3
Spring (October 2005)	0.5 ± 0.1	0.25 ± 0.03*
Summer (December 2006)	1.0 ± 0.3	0.17 ± 0.07*
Fall (April 2006)	1.0 ± 0.3	0.6 ± 0.2
Winter (July 2006)	0.9 ± 0.2	1.4 ± 0.4

$N = 35$, 5–10 individuals per seasonal group

* Values were significantly different (Kruskal–Wallis, non-parametric ANOVA, $P < 0.05$) from values in winter samples-August 2005. Dunn analysis: the values in the gonads of animals collected in summer and spring were significantly lower than the values registered in the gonads from the animals collected in winter 2006 ($P < 0.05$)

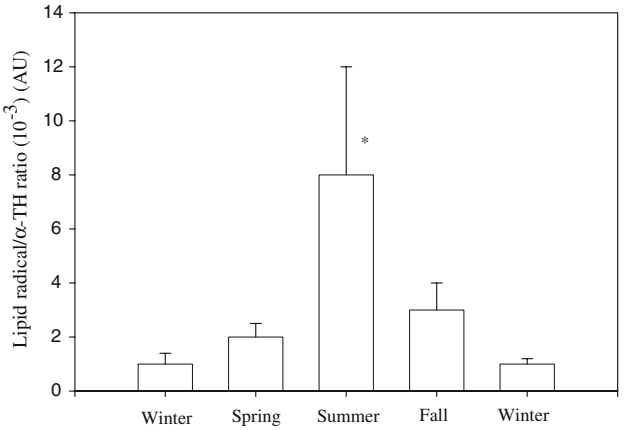

Fig. 2 The lipid radical content/α-TH content ratio in gonads from *L. albus* upon the year. *Values were significantly different (Kruskal–Wallis, non-parametric ANOVA, $P < 0.05$) from values in gonads from animals collected in winter (2005 and 2006). $N = 25$, 4–7 individuals per seasonal group

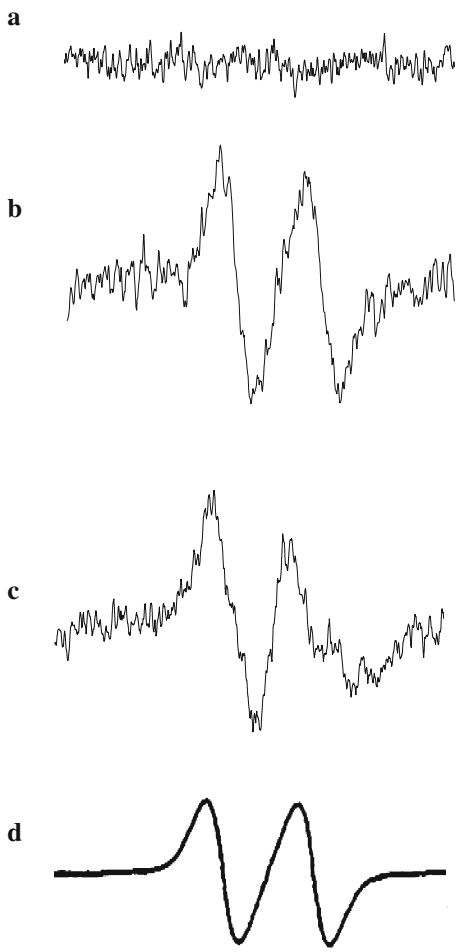

Fig. 3 EPR detection of $A^{\bullet}$ in gonads from *L. albus*. The following spectra are shown: **a** DMSO, **b** gonads collected in winter-August 2005, **c** gonads collected in summer (December 2005), and **d** computer-simulated EPR spectra exhibiting hyperfine splittings ($g = 2.005$ and $a_H = 1.8$ G)

Table 3 $A^{\bullet}$ and AH^{-} content in *L. albus* over the year period

Season	$A^{\bullet}$ content (pmol/mg FW)	AH^{-} content (nmol/mg FW)
Winter (August 2005)	0.3 ± 0.1	16 ± 2
Spring (October 2005)	0.12 ± 0.04	17 ± 3
Summer (December 2006)	0.09 ± 0.02*	17 ± 3
Fall (April 2006)	0.16 ± 0.05	4 ± 1*
Winter (July 2006)	0.4 ± 0.1	11 ± 2

$N = 24$, 4–6 individuals per seasonal group

Ascorbyl content (Dunn analysis): the values in the gonads of animals collected in summer were significantly lower than the values registered in the gonads from the animals collected in winter 2006 ($P < 0.05$). *Ascorbate content* (Dunn analysis): the values in the gonads of animals collected in fall were significantly lower than the values registered in the gonads from the animals collected in winter 2005, spring and summer ($P < 0.05$)

* Values were significantly different (Kruskal–Wallis, non-parametric ANOVA, $P < 0.05$) from values in winter samples-August 2005

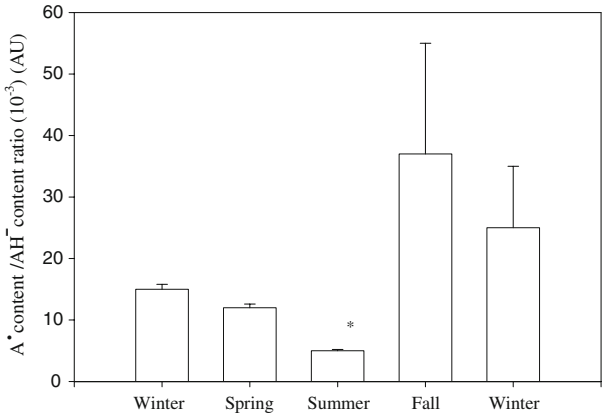

Fig. 4 The $A^{\bullet}$ content/AH^{-} content ratio in gonads from *L. albus* upon the year. *Values were significantly different (Kruskal–Wallis, non-parametric ANOVA, $P < 0.05$) from values in gonads from animals collected in winter (2005 and 2006) and fall. $N = 23$, 4–6 individuals per seasonal group

between samples isolated from the gonads of animals collected in both the winter seasons, average values were not statistically different among both groups of animals. All comparisons were established against values obtained in samples collected in winter-August 2005, and data collected in July 2006 were included to show reproducibility upon seasons.

The data shown in Table 3 indicate that AH^{-} content remained unchanged in tissues from gonads of *L. albus* collected in winter, spring and summer, but significantly decreased in gonads from sea urchins collected in fall.

For the hydrophilic milieu, the $A^{\bullet}/AH^{-}$ ratio was reported as an appropriate and accurate indicator of oxidative stress (Galleano et al. 2002). The calculated values for this ratio in the gonads of the *L. albus* collected upon the year are shown in Fig. 4.

Discussion

Reactive oxygen species react by removing a proton from the conjugated double bond system which then initiates lipid peroxidation chain reactions (Porter 1984; Halliwell and Gutteridge 1985). Many reports indicate that lipid peroxidation is affected by seasonality. An increase in thiobarbituric acid reactive species (TBARS) has been reported in digestive glands of *Mytilus edulis* in winter (Viarengo et al. 1991) and in brown mussels (*Perna perna*) from southern Brazil in summer (Wilhelm Filho et al. 2001b), among others. Moreover, Malanga et al. (2007) reported that both oxygen and nitrogen radical generation rates in *Nacella* (*P.*) *magellanica* were elevated in summer, when water temperatures and the metabolic activity of the limpets were high, although long daytime light duration and higher food availability in early spring may also trigger spawning. Thus, the activity increase corresponded to the active metabolic rates of whole animals (Schöttler 1989) and mitochondria during the summer reproductive season.

On the other hand, it is known that all synthetic processes slow down in the pre-spawning stage and this include protein synthesis (Lukyanova and Khotimchenko 1995), and accordingly a decrease in lipid peroxidation in this period has been previously described in *M. galloprovincialis* digestive organs (Viarengo et al. 1991). After spawning, a period characterized by the absence of gametogenetic activity, resorption of unshed gametes and membrane lysis, along with reparation of tissue damage and formation of new membranes structures has been described (Lukyanova and Khotimchenko 1995). Moreover, the environmental conditions of exposure of *L. albus*, in particular the diet, could alter their lipid composition and subsequently the nature of lipid radical generation (Brazao et al. 2003) and, thus the effectiveness of the lipid soluble antioxidants could be affected. Changes in saturation of fatty acids seem to depend also on temperature and many authors consider them to be adaptive reactions supporting membrane fluidity (Lukyanova and Khotimchenko 1995). An increase oxidative condition in the lipophilic phase could be predicted at the end of the spawning stage, related to an increased metabolic activity in association with various adaptive reactions and changes in physiological status. Thus, it could be postulated that lipid peroxidation is a required physiological event for the spawning process, e.g. separation of eggs and sperm from the tissue. In this regard, making the lipids available by oxidation could contribute to the process. This hypothesis contrast the model previously postulated were low temperatures lead to higher degrees of unsaturation in fatty acids that is thought to increase the levels of fatty acids

(Abele and Puntarulo 2004). The difference between these hypotheses could be related to the nature of the considered process. The requirement of triggering of specific signaling factors to avoid the general response to low temperature could be derived from the fact that lipid availability seems as important factor for a very specific process such as spawing.

However, to have a clear picture of the situation in the lipophilic environment, the contribution of lipid soluble antioxidants should be considered. Previously, it was reported that the content of carotenoids (lipophilic antioxidants) depends on the season for *Strongylocentrotus intemedius* and *S. nudus*. For *S. intemedius*, the content of carotenoids is the highest at the spawing gametogenic stage of gonad maturation for both sexes, meanwhile for *S. nudus*, the content of these pigments is the highest at stages of active gametogenesis and spawing for males and at growth stage for females. However, α-TH is the most powerful lipid soluble antioxidant which scavenges lipid radicals and thereby prevents initiation of radical chain reactions (Yamamoto et al. 2001). The results presented here strongly suggest that lipid radical content is kept constant over the year by a more active consumption rate of α-TH over spring and summer. This situation leads to a lesser degree of protection at the lipophilic cellular environment, in summer as compared to winter, assessed as a significant increase in the lipid radical content/α-TH content ratio.

AH^- has a central metabolic role since it can act as an antioxidant and a pro-oxidant leading to $A^\bullet$ generation (Sadrzadeh and Eaton 1988; Arrigoni 1994). AH^- pro-oxidant activity is a result of its ability to reduce transition metals (especially Fe) causing them to react with oxygen and subsequently initiating lipid radical reactions (Wills 1966). Since, in *L. albus* gonads, the $A^\bullet$ content is significantly lower in tissues from animals collected in summer than in winter, with a constant content of AH^- in the gonads from animals collected in both seasons, a lower $A^\bullet$ content/AH^- content ratio was observed in gonads from animals collected in summer as compared to values in animals collected in winter. This result shows a complex scenario where different profiles of action are followed by the critical factors involved in oxidative control in the cell.

AH^- antioxidant activity consists both in its ability to reduce various types of radicals, including peroxyl radicals that propagate lipid peroxidation, and to regenerate the antioxidant α-TH from the oxidized form (Doba et al. 1985). The reaction of AH^- with α-tocopheroxyl radical (α-$T^\bullet$) results in recycling of α-TH molecules (Buettner 1993), demonstrating close association between lipid and water soluble antioxidants (reaction 2):

$$AH^- + \alpha\text{-}T^\bullet \rightarrow A^\bullet + \alpha\text{-}TH \quad (2)$$

The fact that AH^- content was maintained over the spawning period meanwhile α-TH was decreased strongly suggests that either regeneration of AH^- occurs via controlling specific pathways or via dietary supplementation. The nature of food intake could vary during the year increasing AH^- content over the spawning period to preserve the tissue from oxidative damage in the hydrophilic milieu.

Previous data from Malanga et al. (2007) reported that, in digestive glands of *N.* (*P.*) *magellanica*, the $A^\bullet$ content/AH^- content ratio was elevated in tissues from animals collected in summer, as compared to the values obtained in samples collected over the rest of the year. The profile reported here in *L. albus* gonads is drastically different from the one reported in digestive glands of *N.* (*P.*) *magellanica* since this ratio is significantly lower in samples from animals collected in summer as compared to the values in tissues from animals collected in winter. This low value for the oxidative index in the hydrophilic domain could be related to the fact that the gonads do not produce gametes over the season and the majority of the individuals did not get to a maturate stage during summer, suggesting a low metabolic activity. Over this period, storage substances are accumulated for being used for gametogenesis in the fall, where indeed the studied ratio is significantly increased.

Taken as whole, the data reported here strongly suggest a differential behavior between the oxidative-dependent pathways triggered at the lipophilic and hydrophilic milieus in *L. albus* gonads at the last stages of the spawning period. Moreover, the AH^- content seems to be critical, over this period, to maintain an adequate protection in the soluble cellular fraction to avoid further damages.

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References

Abele D, Puntarulo S (2004) Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish. Comp Biol Physiol A 138:405–415. doi:10.1016/j.cbpb.2004.05.013

Abele-Oeschger D, Oeschger R, Theede H (1994) Biochemical adaptations of *Nereis diversicolor* (Polychaeta) to temporarily increased hydrogen peroxide levels in intertidal sandflats. Mar Ecol Prog Ser 106:101–110. doi:10.3354/meps106101

Andrew NY, Agatsuma A, Bazhin E, Creaser D, Barnes L, Botsford A, Bradbury A, Campbell S, Einnarsson P, Gerring K, Hebert M, Hunter SB, Hur C, Johnson MA, Juinio-Meñez P, Kalvass R, Miller C, Vadas D, Woobdy Z, Xiaoqui A (2002) Status and management of world sea urchins fisheries. Oceanogr Mar Biol Annu Rev 40:343–425

Arrigoni OJ (1994) Ascorbate system in plant development. J Bioenerg Biomembr 26:407–419. doi:10.1007/BF00762782

Bernasconi I (1947) Distribución geográfica de los equinoideos argentinos. Soc Arg Est Geog 6:97–114

Bernasconi I (1953) Monografía de los Equinoideos argentinos. Anales del Museo Hist Nat 2a Ser 6:23–25

Brazao S, Morais S, Boaventura D, Ré P, Narciso L, Hawkins SJ (2003) Spatial and temporal variation of the fatty acid composition of *Patella* spp. (Gastropoda: Prosobranchia) soft bodies and gonads. Comp Biochem Physiol B 136:425–441. doi:10.1016/S1096-4959(03)00243-4

Buettner GR (1993) The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol, and ascorbate. Arch Biochem Biophys 300:535–543. doi:10.1006/abbi.1993.1074

Dayton PK (1985) The structure and regulation of some South American kelp communities. Ecol Monogr 55:447–468. doi:10.2307/2937131

Desai I (1984) Vitamin E analysis methods for animal tissues. Methods Enzymol 105:138–146. doi:10.1016/S0076-6879(84)05019-9

Doba T, Burton GW, Ingold KU (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 multilamellar phospholipid liposomes. Biochim Biophys Acta 835:298–303

Foyer C, Rowell J, Walker D (1983) Measurement of the ascorbate content of spinach leaf protoplasts and chloroplasts during illumination. Planta 157:239–244. doi:10.1007/BF00405188

Galleano M, Aimo L, Puntarulo S (2002) Ascorbyl radical/ascorbate ratio in plasma from iron overloaded rats as oxidative stress indicator. Toxicol Lett 133:193–201. doi:10.1016/S0378-4274(02)00196-0

Geracitano LA, Monserrat JM, Bianchini A (2004) Oxidative stress in *Laeonereis acuta* (Polychaeta Nereididae): environmental and seasonal effects. Mar Environ Res 58:625–630. doi:10.1016/j.marenvres.2004.03.053

Giulivi C, Cadenas E (1993) The reaction of ascorbic acid with different heme iron redox states of myoglobin. FEBS Lett 332:287–290. doi:10.1016/0014-5793(93)80651-A

Halliwell B, Gutteridge JMC (1985) Lipid peroxidation: a radical chain reaction. In: Halliwell B, Gutteridge JMC (eds) Free radicals in biology and medicine, 2nd edn. Clarendon Press, Oxford, pp 188–276

Heilmayer O, Brey T, Storch D, Mackensen A, Arntz WE (2005) Population dynamics and metabolism of *Aequipecten opercularis* (L.) from the western English Channel (Roscoff, France). J Sea Res 52:33–44. doi:10.1016/j.seares.2003.07.005

Hernando MP (2006) Efectos de la radiación solar sobre el fitoplancton de aguas Antárticas y sub-Antárticas. PhD Thesis, University of Buenos Aires, School of Natural Sciences, Buenos Aires

Keesing JK, Hall KC (1998) Review of harvests of status of world sea urchins fisheries points to opportunities of aquaculture. J Shellfish Res 17:1597–1604

Keller M, Sommer A, Pörtner HO, Abele D (2004) Seasonality of energetic functioning and reactive oxygen species production by mitochondria of the lugworm *Arenicola marina*, exposed to acute temperature changes. J Exp Biol 207:2529–2538. doi:10.1242/jeb.01050

Kotake Y, Tanigawa T, Tanigawa M, Ueno I, Allen DR, Lai C (1996) Continuous monitoring of cellular nitric oxide generation by spin trapping with an iron–dithiocarbamate complex. Biochim Biophys Acta 1289:362–368

Lai EK, Crossley C, Sridhar R, Misra HP, Janzen EG, McCay PB (1986) In vivo spin trapping of free radicals generated in brain, spleen, and liver during γ radiation of mice. Arch Biochem Biophys 244:156–160. doi:10.1016/0003-9861(86)90104-9

Lukyanova ON, Khotimchenko YS (1995) Lipid peroxidation in organs of the scallop *Mizuhopecten yessoensis* and sea-urchin *Strongylocentrotus intermedius* during the reproductive cycle. Comp Biochem Physiol B 110:371–377. doi:10.1016/0305-0491(94)00154-M

Malanga G, Estevez MS, Calvo J, Abele D, Puntarulo S (2007) The effect of seasonality on oxidative metabolism in *Nacella* (*Patinigera*) *magellanica*. Comp Biochem Physiol A 146:551–558

Olave S, Bustos E, Lawrence JM, Cárcamo P (1997) The effect of size and diet on gonad production by the Chilean sea urchin *Loxechinus albus*. J World Aquacult Soc 32:210–214

Pérez AF, Morriconi E, Boy C, Calvo J (2008) Seasonal changes in energy allocation to somatic and reproductive body components of the common cold temperature sea urchin *Loxechinus albus* in a sub-Antarctic environment. Polar Biol 31:443–449. doi:10.1007/s00300-007-0370-3

Porter NA (1984) Chemistry of lipid peroxidation. Methods Enzymol 105:273–282. doi:10.1016/S0076-6879(84)05035-7

Power A, Sheedan D (1996) Seasonal variations in the antioxidant defense systems of gills and digestive gland of the blue mussel, *Mytilus edulis*. Comp Biochem Physiol C 114:99–103. doi:10.1016/0300-9629(95)02098-5

Sadrzadeh SMH, Eaton JW (1988) Hemoglobin-mediated oxidant damage to the central nervous system requires endogenous ascorbate. J Clin Invest 82:1510–1515. doi:10.1172/JCI113759

Schöttler U (1989) Anaerobic metabolism in the lugworm *Arenicola marina* during low tide: the influence of developing reproductive cells. Comp Biochem Physiol A 92:1–7. doi:10.1016/0300-9629(89)90731-7

Siikavuopio ST, Christiansen JS, Sæther B-J, Dale T (2007) Seasonal variation in feed intake under constant temperature and natural photoperiod in the green sea urchin (*Strongylocentrotus droebachiensis*). Aquaculture 272:328–334. doi:10.1016/j.aquaculture.2007.09.003

Sokal RR, Rohlf FJ (1995) Biometry: the principles and practice of statistics in biological research. W.H. Freeman, New York

Vadas RL, Beal B, Dowling T, Fegley JC (2000) Experimental field tests of natural algal diets on gonad index and quality in the green sea urchin, *Strongylocentrotus droebachiensis*: a case for rapid summer production in post-spawned animals. Aquaculture 182:115–135. doi:10.1016/S0044-8486(99)00254-9

Vásquez JA, Buschmann AH (1997) Herbivore–kelp interactions in Chilean subtidal communities: a review. Rev Chil Hist Nat 70:41–52

Viarengo A, Canesi L, Livingstone DR (1991) Seasonal variations in the antioxidant defence enzymes and lipid peroxidation of the digestive gland of mussels. Comp Biochem Physiol C 100:187–190. doi:10.1016/0742-8413(91)90151-I

Walker CW, Unuma T, Lesser MP (2007) Chapter 2. Gametogenesis and reproduction of sea urchins. Dev Aquac Fish Sci 37:11–33

Wilhelm Filho D, Torres MA, Tribess TB, Pedrosa RC, Soares CHL (2001a) Influence of season and pollution on the antioxidant defenses of the cichlid fish acará (*Geophagus brasiliensis*). Braz J Med Biol Res 34:719–726. doi:10.1590/S0100-879X2001000600004

Wilhelm Filho D, Tribess TB, Gáspari C, Claudio FD, Torres MA, Magalhaes ARM (2001b) Seasonal changes in antioxidant defenses of the digestive gland of the brown mussel (*Perna perna*). Aquaculture 203:149–158. doi:10.1016/S0044-8486(01)00599-3

Wills ED (1966) Mechanisms of lipid peroxide formation in animal tissue. Biochem J 99:667–676

Wong JL, Wessel GM (2005) Reactive oxygen species and Udx1 during early sea urchin development. Dev Biol 288:317–333. doi:10.1016/j.ydbio.2005.07.004

Yamamoto Y (2001) Role of oxygen species and antioxidants in photoaging. J Dermatol Sci 27:1–4. doi:10.1016/S0923-1811(01)00120-7

Yamamoto Y, Fujisawa A, Hara A, Dunlap WC (2001) An unusual vitamin E constituent (α-tocomonoenol) provides enhanced antioxidant protection in marine organisms adapted to cold-water environments. Proc Natl Acad Sci USA 98:13144–13148. doi:10.1073/pnas.241024298

Zar JH (1984) Biostatistical analysis. Prentice-Hall, New Jersey