Comparative Biochemistry and Physiology, Part C xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Seasonal and ontogenetic changes modulate oxygen consumption and antioxidant defenses in the cutlassfish *Trichiurus lepturus* (Pisces, Trichiuridae)

Danilo Wilhelm-Filho[a,*], César G. Fraga[b], Alberto Boveris[b]

[a] Departamento de Ecologia e Zoologia, CCB, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil
[b] Laboratório de Radicales Libres, Facultad de Farmacía y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

ARTICLE INFO

Keywords:
Fish
Oxygen consumption
Antioxidants
Ontogeny
Seasonal variation
Adaptation

ABSTRACT

Several oxidative stress markers and liver oxygen consumption were measured in different tissues of the marine fish *Trichiurus lepturus* in late summer and late winter, as well as in juveniles and adult females. Oxygen consumption in liver, superoxide dismutase (SOD) and catalase (CAT) activity in liver, red cells, lens and roe, vitamin E, ubiquinol$_{10}$, β-carotene in liver, red cells, and roe, as well as contents of reduced glutathione (GSH) and lipoperoxidation (TBARS) in red cells were evaluated. Regarding ontogeny, compared to adult fish, juveniles showed significant higher SOD activity in liver and lens, as well as higher liver contents of vitamin E. In contrast, adult females showed higher contents of vitamin E in roe, ubiquinol$_{10}$ in liver and roe, and higher GSH levels in red cells, while the other markers remained unchanged. Regarding seasonal changes, no differences were detected in adult females for liver CAT and ubiquinol$_{10}$, CAT in roe, vitamin E in roe and in red cells, liver and red cell ubiquinol$_{10}$, and in GSH in red cells. However, and coinciding with the spawning period of late summer, liver oxygen consumption, SOD and CAT activity and ubiquinol$_{10}$ contents in roe and SOD activity in red cells, and red cell TBARS contents were higher compared to late winter. These temporal antioxidant adjustments of *Trichiurus lepturus* seem to be parallel to the higher oxygen consumption typical of juvenile forms and also to the intense spawning and foraging activities of adult females in late summer.

1. Introduction

Fish often must cope with marked seasonal and even daily changes in temperature, oxygen availability and pH, and must also cope with the continuous generation of reactive oxygen species (ROS) to avoid oxidative stress (Wilhelm-Filho et al., 2000). Mitochondrial ROS generation is directly related to oxygen availability (Boveris, 1977; Wilhelm-Filho, 2007) as well as to activity levels in most fish species (Janssens et al., 2000; López-Cruz et al., 2012; Ross et al., 2001; Wilhelm-Filho and Boveris, 1993; Wilhelm-Filho et al., 1993, 2000). In addition, the abiotic factors such as environmental physico-chemical changes as well as biotic factors such as metabolic changes also induce adaptative responses in fish antioxidant defenses (e.g. Härdig and Höglund, 1983; Jones, 1985; Martínez-Álvarez et al., 2005; Nunes et al., 2015; Palace and Klaverkamp, 1993; Rudneva, 1997; Ronisz et al., 1999; Rudneva et al., 2010; Wilhelm-Filho and Boveris, 1993; Wilhelm-Filho et al., 1993). When ROS generation exceeds the normal physiological antioxidant defenses a condition called oxidative stress (OS) occurs (Sies, 1985), damaging membranes and important biomolecules, such as proteins, lipids and DNA (Halliwell and Gutteridge, 2007). ROS overgeneration can also be associated with routine physiological functions such as growth and reproductive cycle, as well as seasonal changes in temperature and oxygen availability (e.g. Jones, 1985; Nunes et al., 2015; Wilhelm-Filho et al., 2000; Wilhelm-Filho et al., 2001a).

The marine cutlassfish *Trichiurus lepturus* is a cosmopolitan fish species found either in tropical or temperate waters of different oceans, being an important fishery representative species in many countries (Portsev, 1980) and is an active and voracious predator that faces daily and seasonal environmental changes in temperature and oxygen availability (Lee, 1978; Munekiyo and Kuwahara, 1984). Adults are found in shallow inshore waters during the reproductive period (personal observations) as well as in offshore waters in depths between 200 and 300 m, therefore showing a high capability of daily vertical migrations (Lee, 1978). Furthermore, the feeding habits of adults and young are characterized by substantial differences (Portsev, 1980), making this species a good natural model to assess fish antioxidants with respect to ontogenetic changes.

* Corresponding author.
E-mail address: dawifi@ccb.ufsc.br (D. Wilhelm-Filho).

http://dx.doi.org/10.1016/j.cbpc.2017.03.013
Received 28 November 2016; Received in revised form 15 March 2017; Accepted 21 March 2017

Please cite this article as: Filho, D.W., Comparative Biochemistry and Physiology, Part C (2017), http://dx.doi.org/10.1016/j.cbpc.2017.03.013

Because fish exhibit high oxygen consumption and antioxidant protection in blood and liver, both tissues were used in the present study (Aksnes and Njaa, 1981; Wilhelm-Filho et al., 1993). In addition, the importance of lens in relation to the feeding habits of the cutlassfish (Harrison, 1981), and the roe related to seasonal reproductive changes, was also considered in the present study.

There are several studies on antioxidant defenses dealing with ontogenetic and seasonal changes reported in fish (e.g. Härdig and Höglund, 1983; Gabryelak et al., 1983; Martínez-Álvarez et al., 2005; Nunes et al., 2015; Ronisz et al., 1999; Rudneva et al., 2010; Wdzieczak et al., 1981, 1982). Nevertheless, these previous studies did not correlate such ontogenetic and seasonal changes in antioxidant defenses directly to fish oxygen consumption. In addition, obligatory water-breathing active fish seem to avoid the potential oxidative stress associated with routine changes in O_2 tensions in the environment and their tissues, by maintaining relatively high constitutive antioxidant levels (Wilhelm-Filho et al., 1993, 2000). Therefore, the main goal of the present paper was to evaluate the relationship among tissue oxygen consumption and markers of oxidative stress present in different tissues of the cutlassfish, especially focusing the influences promoted by ontogeny and season on such changes.

2. Material and methods

2.1. Fish capture and sample harvesting

Forty two ($n = 42$) female specimens of *T. lepturus* were used for the ontogenetic study, and collected in August (late winter in the South Hemisphere). The weight of juvenile fish ($n = 20$) was in the range of 200 to 600 g, while adult ($n = 22$) specimens weighed 900 to 2000 g, according to the weight categories defined by Portsev (1980) for this species. For the seasonal study further adult females were considered: twenty six ($n = 26$) specimens of *T. lepturus* were caught in late summer (March, in the Southern Hemisphere) and twenty three ($n = 23$) specimens were caught in late winter (August), together with the fish used for the ontogenetic study. All the specimens used in the present study for both and independent sampling campaigns were collected at down (06:00–07:00 a.m.) at the near and shallow coast of the “Praia da Armação”, Santa Catarina Island, southern Brazil (latitude 27° 35′ 48" S; longitude 48° 32′ 57″ W).

Fish were caught by fishermen especially for the purpose of the present study through purse seiners and were immediately killed through cerebral inactivation with a stylet introduced quickly in the upper part of the cranium according to specific international ethical procedures for fish and by the local Ethical Committee of our institution (CCB, UFSC) for animal research. Fish were then bled by dorsal aorta puncture, and blood samples were kept on ice. Fish were also kept on ice and rapidly transported to the laboratory for organ dissection. The use of anesthesia was avoided because of interference on the evaluation of the biochemical markers (Halliwell and Gutteridge, 2007). Blood samples for GSH analysis were rapidly precipitated in acid and kept on ice (see below). Choosing of the different tissues was mainly due to the relatively high oxygen consumption and antioxidant protection found in blood and liver (e.g. Aksnes and Njaa, 1981; Wilhelm-Filho et al., 1993), and also due to the importance of lens related to the feeding habits of the species (Harrison, 1981), and the roe due to seasonal reproductive changes. Lens was evaluated only regarding ontogenetic changes considering the difficulty to obtain the corresponding homogenates of fish crystalline.

2.2. Preparation of blood samples

Blood samples were obtained using minute amounts of heparin. Blood samples for GSH analysis were immediately after blood harvesting diluted in three volumes (1:3, *v:v*) of trichloroacetic acid (12%) and kept on ice. After plasma removal, red cells were washed three times in saline solution. Hemolysates were obtained after addition of three volumes of 20 mM Tris-HCl, pH 8.0, and centrifuged at 5000*g* for 10 min. Hemolysate supernatants were used for enzymatic analysis. Regarding CAT activity the hemolysates were further diluted 500 times to measure its activity (Aebi, 1984). SOD was measured after treating the hemolysates with a mixture of chloroform/ethanol (3:5, *v:v*). All samples were kept frozen in liquid nitrogen (≤ 170 °C) for subsequent determinations.

2.3. Preparation of tissue and organ extracts

At the laboratory homogenates were immediately obtained after being washed in ice-cold saline solution to remove blood excess. Organs were surface-dried with filter paper, weighed, exhaustively washed in ice-cold saline solution, and homogenenized in a buffer comprising 0.1% Triton X-100, 0.12 M NaCl, 30 mM Na_2PO_4, pH 7.4 (1:9 w:v) containing also freshly prepared protease inhibitors (0.3 mM PMSF and 0.05 mM trypsin inhibitor). Homogenizations were carried out on ice (4 °C) employing 20 strokes in a Potter-Elvehjem homogeneizer, followed by centrifugation at 10,000*g* for 10 min. The supernatants were used for enzymatic assays. All samples were kept frozen in liquid nitrogen (≤ 170 °C) for subsequent determinations.

2.4. Antioxidant enzyme assays

Aliquots of the corresponding extracts were stored in liquid nitrogen and examined separately for each enzyme to minimize loss in activity due to the freezing/thawing procedures. Superoxide dismutase (SOD; EC 1.15.1.1) activity was measured in a GBC UV/VIS 916 spectrophotometer (Sydney, NSW, Australia), according to the adrenochrome method of Misra and Fridovich (1972), and modified by Boveris et al. (1983). Aliquots of supernatant were added to a cuvette containing a 1.95 ml of 50 mM glycine pH 10.2, and 50 µl of 60 mM epinephrine pH 2.0, and the absorbance of the solutions read at 480 nm. Values were expressed in USOD g^{-1} of tissue or USOD ml^{-1} blood, considering that one arbitrary unit of SOD corresponds to the amount of sample able to inhibit 50% of the rate of adrenochrome formation in the cuvette. Catalase (CAT; EC 1.11.1.6) activity was evaluated by measuring the decrease in hydrogen peroxide concentration at 240 nm (Aebi, 1984) promoted by the enzyme present in the sample. Decays in A_{240} were registered in a GBC UV/VIS 916 spectrophotometer (Sydney, NSW, Australia) during the first minute, in a cuvette containing 50 mM Na-phosphate, pH 7.0, and a freshly prepared 10 mM hydrogen peroxide solution. Hydrogen peroxide stock solution was previously titrated to ascertain the concentration. Values were expressed in mmol min^{-1} g^{-1} of tissue or in mmol min^{-1} ml^{-1} hemolysate.

2.5. Glutathione assay

Reduced glutathione (GSH, considering that is the major small thiol present in whole blood) was measured in whole blood according to Beutler et al. (1963), using the Elmann's reagent (DTNB). Blood acid extracts were obtained by the addition of one third of the blood volume of a solution containing metaphosphoric acid 1.67% (*w/v*), 5% NaCl (*w/v*), and 0.2% EDTA (*w/v*), and then centrifuged at 5000*g* for 5 min. Supernatants from the acid extracts were added with 0.25 mM DTNB in 0.1 M Na phosphate, pH 8.0, and the formation of thiolate anion determined at 412 nm in a GBC UV/VIS 916 spectrophotometer (Sydney, NSW, Australia). Total glutathione was measured according to the enzymatic method of Tietze (1969). Values were expressed in µmol g^{-1} of tissue or in µmol ml^{-1} blood, using $\varepsilon = 14.1\ mM^{-1}\ cm^{-1}$.

2.6. Vitamin E, ubiquinol 10 and β-carotene assays

Vitamin E, ubiquinol$_{10}$, and β-carotene were analyzed in liver and

roe homogenates and pellets of red cells by reverse phase HPLC, using a C8 column and an elution mixture of methanol and ethanol (22.5:77.5%, *v:v*) (ethanol with isopropane 9.5:5.0, *v:v*), containing 20 mM LiCl (Buttriss and Diplock, 1984; Lang et al., 1986; Lucesoli and Fraga, 1995). Flow rate was 1 ml min^{-1}, and electrochemical detection was used. Values were expressed in μmol g^{-1} of tissue or in μmol ml^{-1} red cells, comparing to the calibration curve of each standard (Sigma Co, Ohio US).

2.7. Lipid oxidation

Determination of thiobarbituric acid-reactive substances (TBARS) was used for assessing endogenous lipid oxidation in tissue essentially according to Ohkawa (1979) and Bird and Draper (1984). Homogenates and red cells were previously treated with butilhydroxitoluen (BHT) 0.2 mM (10 μl/ml of sample) to avoid further artificial lipoperoxidation in the samples (Bird and Draper, 1984). The samples were then added with TCA 30% (1:4 *v:v*) immediately after being obtained, centrifuged at 5000*g* for 3 min, and the supernatants were added with 1 ml of 0.67% (*w/v*) 2-thiobarbituric acid, maintained in boiling water for 45 min, cooled at 5 °C for 30 min, and then measured in triplicate at 535 nm in a GBC UV/VIS 916 spectrophotometer (Sydney, NSW, Australia). Absorbances were expressed as equivalent to nmol T-BARS g^{-1} tissue or ml^{-1} red cells using $E_{535} = 153\ mM^{-1}\ cm^{-1}$.

2.8. Hemoglonin concentrations

Hemoglobin (Hb) concentrations were measured spectrophotemetrically in lysates, in duplicate, at 540 nm, by the conversion of hemoglobin to cyanomethemoglobin, using the Drabkin reagent, and were expressed in mmol l^{-1} (Wilhelm-Filho et al., 2001a).

2.9. Tissue oxygen consumption

Liver oxygen consumption was evaluated in fresh samples at the same day of each sampling campaign in triplicate using an oxygraph (Vanderkooi et al., 1991), containing a Clark electrode in a 2 ml Tucker chamber, where the small tissue slices (≈ 10 to 50 mg) were maintained in a Ringer solution (pH 7.4 glicose 5 mM, for teleost fish) during ca. 5–10 min, under gentle stirrer and at controlled temperature (20 °C) (Estabrook, 1984). Oxygen consumption was calculated after the formula: VO_2 (μmol $O_2\ min^{-1}\ g^{-1}$) = Δ% $O_2\ min^{-1}$ 2.64/tissue weight [2.64 correspond to O_2 concentration (nM) at 1% saturation (starting after calibrating at 100% of saturation) inside the chamber (sea level) at the temperature of 20 °C].

2.10. Statistics

Statistical analysis was performed using the unpaired *t* test of Student (Tukey, 1977) to compare the different sub-groups studied, i.e. adults vs. juveniles, summer vs. winter, using a minimal level of significance of 5% ($p < 0.05$). The main purpose of the study was to verify the influence of age/size and seasonality on several oxidative stress parameters present in different tissues of the fish species, in an independent way. Therefore, no interrelationships or interactions between the biochemical parameters and the variables ontogeny and season were considered in the present study.

3. Results

3.1. Ontogenetic changes

A very significant ($p = 0.006$) higher oxygen consumption was found in the liver of juvenile compared with adult cutlassfish (2.97 ± 0.51 and 0.94 ± 0.48 mmol $min^{-1}\ g^{-1}$, respectively; Fig. 1A).

In the present study and irrespective to the size of the cutlassfish, lens and roe showed the highest SOD activity, while the highest CAT activity was found in the liver (Table 1). The high SOD activity measured in the lens of *T. lepturus* is in the range of those found in different tissues of mammals and birds (Wilhelm-Filho et al., 2000). The SOD (273.6 ± 20.4 and 206.4 ± 23.6 USOD g^{-1}, respectively; $p = 0.0390$) and CAT (11.91 ± 0.69 and 9.08 ± 0.87 mmol $min^{-1}\ g^{-1}$, respectively; $p = 0.0185$) activity found in liver, and the SOD activity found in lens (511.8 ± 31.3 and 430.0 ± 34.5, respectively; $p = 0.0018$) of juvenile cutlassfish were significantly higher compared to those found in adults (Table 1). Furthermore, comparing the roe of adult females with juveniles, a higher non-enzymatic antioxidant protection involving vitamin E (16.7 ± 2.3; 10.0 ± 1.8, respectively; $p = 0.0292$) and $ubiquinol_{10}$ contents (23.0 ± 4.2; 11.7 ± 1.8, respectively; $p = 0.0260$) were also detected in the roe of mature fish (Table 1). Also, the liver vitamin E mean concentration found in juveniles compared to the value found in adults (15.8 ± 4.2; 36.2 ± 8.1, respectively; $p = 0.0270$), was approximately two-fold higher, which is a significant difference. Conversely, the levels of UQ_{10} found in the liver of juveniles was less than half those found in the liver of adults, which is a very significant difference (10.9 ± 1.8; 33.0 ± 5.2**, respectively; $p = 0.0004$) (Table 1).

However, no significant differences were found between adults and juveniles in SOD (300.1 ± 51.8 and 290.9 ± 45.4, respectively; $p = 0.8953$) and CAT activity measured in the red cells (0.55 ± 0.52; 0.35 ± 0.17, respectively; $p = 0.6212$), roe (1.09 ± 0.25; 0.96 ± 0.17, respectively; $p = 0.0676$) and lens (1.75 ± 0.20; 1.45 ± 0.26, respectively; $p = 0.4261$) (Table 1). Similarly, no significant differences were found between adults and juveniles regarding the SOD activity found in red cells (300.1 ± 51.8; 290.9 ± 45.4, respectively; $p = 0.2504$), in vitamin E (16.7 ± 2.8; 10.0 ± 1.8 nmol g^{-1}, respectively; $p = 0.8210$) and $ubiquinol_{10}$ (22.2 ± 8.9; 11.7 ± 3.6, respectively; $p = 0.2978$) concentrations in the roe, as well as in the contents of vitamin E found in red cells (0.9 ± 0.2; 1.1 ± 0.5 nmol g^{-1}, respectively; $p = 0.7028$), and UQ_{10} found in red cells (1.1 ± 0.4; 1.9 ± 0.5 nmol g^{-1}, respectively; $p = 0.2510$) (Table 1).

No differences were found regarding lipid oxidation measured as TBARS levels comparing the different tissues examined in adult and young fish (Table 1). The values found in liver (172.7 ± 30.1 and 171.1 ± 25.1 nmol g^{-1}, respectively; $p = 0.9720$), red cells (179.6 ± 13.4; 176.9 ± 17.0, respectively; $p = 0.0905$), and roe (71.7 ± 28.9; 63.4 ± 18.6, respectively; $p = 0.8145$), showed very similar TBARS concentrations. On the other hand, the intraerythrocytic GSH levels (0.81 ± 0.07; 0.43 ± 0.11 mM, respectively; $p = 0.0045$) and the GSH/Hb ratio (0.28 ± 0.02; 0.18 ± 0.11, respectively; $p = 0.0068$) were higher in adults than in juveniles (Fig. 2). Interestingly, juveniles showed approximately half of the total glutathione content in the oxidized form (GSSG), whereas adults showed less than one third of GSSG compared to GSH (depicted from Fig. 2). No differences between adult and young fish were obtained for total glutathione levels (1.11 ± 0.08 and 0.99 ± 0.10 mM, respectively; $p = 0.3487$), the ratio TG/Hb (0.38 ± 0.09; 0.39 ± 0.04, respectively; $p = 0.9230$) and also for the ratio GSH/TG (0.70 ± 0.21; 0.60 ± 0.11, respectively; $p = 0.6664$).

3.2. Seasonal changes

The oxygen consumption found in the liver of the cutlassfish during late summer was significantly higher ($p = 0.020$) compared to fish collected during late winter (2.71 ± 0.48 and 1.01 ± 0.60 mmol $min^{-1}\ g^{-1}$, respectively; Fig. 1B). Similarly, the activities of SOD measured in red cells and in roe almost doubled in summer compared with winter (Table 2). The erythrocytic CAT activity in the cutlassfish increased approximately two-fold in winter compared to summer (Table 2). Also, the activity of SOD and CAT (472.7 ± 23.3 and

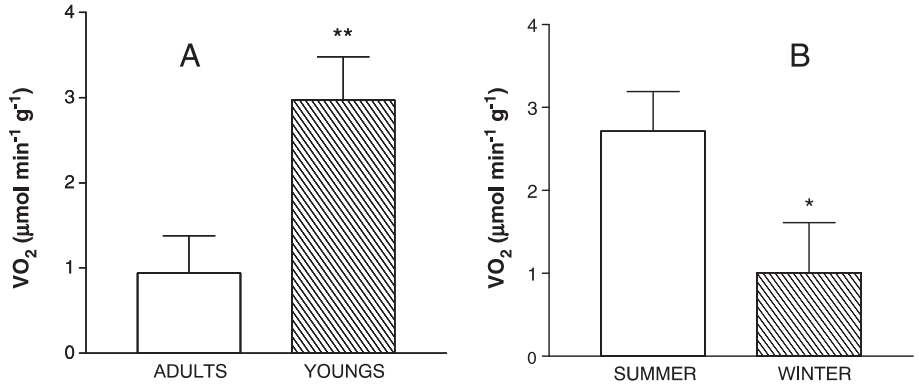

Fig. 1. Oxygen consumption measured in liver (µmol min^{-1} g^{-1} tissue) of young and adult specimens (A; n = 20 and 22 respectively), and adult specimens collected in late summer and late winter (B; n = 26 and 23, respectively) of *Trichiurus lepturus*. Asterisk denotes significant differences between the groups: *($p < 0.05$) and ** ($p < 0.01$).

Table 1
Activity of superoxide dismutase (SOD) and catalase (CAT), contents of non-enzymatic antioxidants (β-carotene, vitamin E, ubiquinol$_{10}$, and reduced glutathione, GSH), and lipid oxidation (TBARS contents) in different tissues (liver, red cells and roe) of *Trichiurus lepturus* examined in young (n = 20) and adult females (n = 22). Asterisk denotes significant differences *($p < 0.05$) comparing both ontogenetic forms.

Tissue	SOD (USOD g^{-1})	CAT (mmol min^{-1} g^{-1})	VIT E (nmol g^{-1})	UQ10 (nmol g^{-1})	TBARS (nmol g^{-1})
Liver (A)	206.4 ± 23.6	9.08 ± 0.87	15.8 ± 4.2	33.0 ± 5.2*	172.7 ± 30.1
Liver (J)	273.6 ± 20.4*	11.91 ± 0.69*	36.2 ± 8.1*	0.9 ± 1.8	171.3 ± 25.1
Blood (A)	300.1 ± 51.8	0.55 ± 0.52	0.9 ± 0.2	1.1 ± 0.4	179.6 ± 13.4
Blood (J)	290.9 ± 45.4	0.35 ± 0.17	1.1 ± 0.5	1.9 ± 0.5	176.9 ± 17.0
Roe (A)	400.9 ± 47.3	1.09 ± 0.25	16.7 ± 2.3*	23.2 ± 4.2*	71.7 ± 28.9
Roe (J)	370.9 ± 36.8	0.96 ± 0.17	10.0 ± 1.8	10.9 ± 3.0	63.4 ± 18.6
Lens (A)	330.0 ± 34.5	1.75 ± 0.30	n.e.	n.e.	n.e.
Lens (J)	511.8 ± 31.3**	1.45 ± 0.21	n.e.	n.e.	n.e.

In parenthesis after each tissue: A = adults; J = juveniles; n.e. = means not examined; n = (in parenthesis) means number of specimens examined.

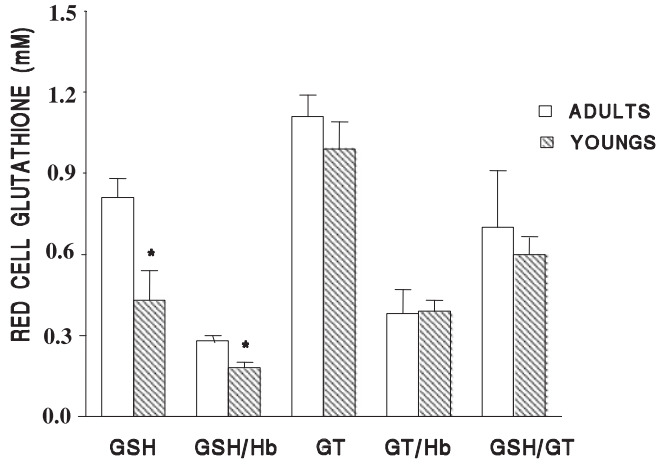

Fig. 2. Blood glutathione concentrations (mM): GSH = reduced form; GSH/Hb = molar ratio related to hemoglobin; GT = total glutathione (GSH + GSSG); GT/Hb = molar ratio related to hemoglobin; GSH/GT molar ratio between reduced glutathione and total glutathione, of young (n = 20) and adults (n = 22) of *Trichiurus lepturus*. Asterisk denotes significant differences *($p < 0.05$) comparing both ontogenetic forms.

290.9 ± 15.6 USOD g^{-1}, respectively; p = 0.0002; 1.65 ± 0.12 and 0.99 ± 0.31 mmol min^{-1} g^{-1}; p = 0.0434, respectively) and the levels of ubiquinol$_{10}$ (36.0 ± 9.9 and 2.4 ± 0.7, 1.1 ± 0.4; 1.9 ± 0.5 nmol g^{-1}, respectively; p = 0.0026) in the roe were increased in late summer compared to late winter (Table 2). The increases detected in the activity of both antioxidant enzymes was ca. 1.6 higher, while in the levels of ubiquinol$_{10}$ was very pronounced, ca.15 times higher. Furthermore, red cell contents (238.2 ± 25.9 and 79.2 ± 15.3, respectively; $p \leq 0.0001$) of TBARS showed increased concentrations in late summer compared to late winter, while no differences were detected for liver (242.0 ± 27.0; 171.3 ± 25.1 nmol g^{-1}, respectively; p = 0.0627) and roe (172.7 ± 30.1; 171.1 ± 25.1 nmol g^{-1}, respectively; p = 0.8706) (Table 2).

No significant seasonal differences were also found in fish collected in late summer compared to late winter for CAT activity (10.86 ± 1.02 and 10.50 ± 0.43 mmol min^{-1} g^{-1}, respectively; p = 0.7574) and ubiquinol$_{10}$ contents (19.04 ± 4.2; 10.9 ± 1.8 nmol g^{-1}, respectively; p = 2.252) in liver, vitamin E contents in roe (11.7 ± 2.4 and 14.5 ± 1.9 nmol g^{-1}, respectively; p = 0.3735), and red cells (3.8 ± 1.1 and 2.7 ± 0.7 nmol g^{-1}, respectively; p = 0.4169). Contrary to changes detected in the ontogenetic study, red cell glutathione concentrations and the ratio between glutathione and hemoglobin contents did not change in blood collected in late summer compared to samples obtained in late winter (Fig. 3). Therefore, no statistical differences were detected in reduced (GSH; 0.54 ± 0.11 and 0.50 ± 0.13 mM, respectively; p = 0.8178) in total glutathione (TG; 1.05 ± 0.06 and 1.07 ± 0.07 mM, respectively; p = 0.8282) contents found in red cells, nor in the corresponding ratios GSH/Hb (0.19 ± 0.04 and 0.28 ± 0.02, respectively; p = 0.0592), TG/Hb (0.35 ± 0.10 and 0.44 ± 0.03, respectively; p = 0.4179) or GSH/TG (0.71 ± 0.06 and 0.76 ± 0.05, respectively; p = 0.5313) (Fig. 3).

4. Discussion

4.1. Ontogenetic changes

The higher oxygen consumption was found in the liver of juvenile compared with adult cutlassfish might be associated with the inverse relationship between body weight and specific metabolic rates and therefore ontogeny, a characteristic shared in general by all vertebrates (Prosser, 1991). Such elevated oxygen consumption might reflect the

Table 2
Activity of superoxide dismutase (SOD) and catalase (CAT), contents of non-enzymatic antioxidants (β-carotene, vitamin E, ubiquinol$_{10}$, and reduced glutathione, GSH), and lipid oxidation (TBARS contents) in different tissues (liver, red cells and roe)of adult females of *Trichiurus lepturus* examined in late summer and late winter (n = 26 and 23, respectively). Asterisk denotes significant differences *($p < 0.05$) comparing both seasons.

Tissue	SOD (USOD g^{-1})	CAT (mmol min^{-1} g^{-1})	VIT E (nmol ml^{-1})	UQ10 (nmol g^{-1})	TBARS (nmol g^{-1})
Liver (S)	258.1 ± 17.1	10.86 ± 1.02	13.2 ± 6.4	19.4 ± 4.1*	242.0 ± 27.0
Liver (W)	294.6 ± 20.6*	10.50 ± 0.43	36.2 ± 8.1*	10.9 ± 1.8	171.3 ± 25.1
Blood (S)	709.2 ± 26.9*	0.66 ± 0.21	3.8 ± 1.1	1.2 ± 0.3	238.2 ± 25.9**
Blood (W)	463.6 ± 19.8	1.32 ± 0.40	2.7 ± 0.7	1.8 ± 0.9	79.2 ± 15.3
Roe (S)	472.7 ± 23.3**	1.65 ± 0.12* 0.52	11.7 ± 2.4	6.0 ± 9.9	68.8 ± 13.8
Roe (W)	290.9 ± 15.6	0.99 ± 0.31	14.5 ± 1.9	2.4 ± 0.7	65.7 ± 12.7

In parenthesis after each tissue: S = summer (n = 26), W = winter (n = 23); n = (in parenthesis) means number of specimens examined.

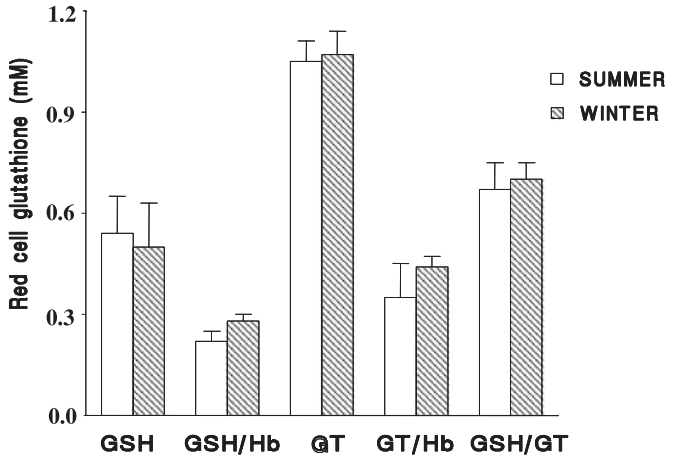

Fig. 3. Blood glutathione concentrations (mM): GSH = reduced form; GSH/Hb = molar ratio related to hemoglobin; GT = total glutathione (GSH + GSSG); GT/Hb = molar ratio related to hemoglobin; GSH/GT molar ratio between reduced glutathione and total glutathione of *Trichiurus lepturus* examined in late summer (n = 26) and late winter (n = 23).

higher hepatic antioxidant levels (higher SOD and CAT activity and higher contents of vitamin E) found in young compared to adult fish. This relationship was already described in other marine and freshwater fish species (e.g. Wdzieczak et al., 1982), while the antioxidant enzyme activity found in different tissues of more active fish are also higher when compared to sedentary or bottom-dwelling species (López-Cruz et al., 2012; Wilhelm-Filho and Boveris, 1993; Wilhelm-Filho et al., 1993) or to deep-sea species (Janssens et al., 2000). Furthermore, during the first and early development stages fish generally exhibit higher metabolic rates, higher ROS generation and therefore higher antioxidant levels to avoid a potential related oxidative stress (Rudneva, 1997; Rudneva et al., 2010; Wilhelm-Filho et al., 2000; this study). Accordingly, a similar indirect relationship to oxygen consumption was also found between more active fish and hematological parameters such as the hemoglobin content and hematocrit (Wilhelm-Filho et al., 1992a), as well as the contents of erythrocytic nucleoside triphosphates (ATP and GTP) (Wilhelm-Filho et al., 1992b), when compared to less active or sluggish marine fish species.

In the present study and irrespective to the size of the cutlassfish, lens and roe showed the highest SOD activity, while the highest CAT activity was found in the liver, a general feature displayed by fish (Wilhelm-Filho et al., 1993; Wilhelm-Filho and Boveris, 1993), as well as by other vertebrates (e.g. Pérez-Campo et al., 1993; Wilhelm-Filho et al., 2000). In this regard, the high SOD activity measured in the lens of *T. lepturus*, which falls in the range of those found in different tissues of mammals and birds (Wilhelm-Filho et al., 2000), may reflect the high oxygen consumption that characterizes the retina metabolism of vertebrates in general (Prosser, 1991). In addition, the cutlassfish depends highly on vision to pray (Harrison, 1981), as some other fish species do (Morris and Albright, 1984). Accordingly, a high SOD activity may account for retina protection in fish against hyperoxia when compared to other vertebrates (Desrochers and Hoffert, 1983), and this seems also true for the lens (this study).

The SOD and CAT activity found in liver and the SOD activity found in lens of juvenile cutlassfish were higher compared to those found in adults. As mentioned before, the higher enzymatic antioxidant activity displayed by juvenile cutlassfish in these tissues might be related to their specific high metabolic rates. This is not surprising, considering that superoxide anion generation has an almost linear relationship with the oxygen consumption (Boveris, 1977). For instance, deep-sea fishes, which are exposed to permanent environmental low O_2 tensions, possess low antioxidant defenses capability (Janssens et al., 2000). However, no apparent correlation was found in adult teleosts from the Black Sea regarding age and activity of blood antioxidant enzymes (Rudneva et al., 2010).

No significant difference was found in SOD and CAT activity measured in the roe, while in adult fish the vitamin E and ubiquinol$_{10}$ concentrations found in the roe were higher than those found in juveniles. These higher non-enzymatic antioxidant protection involving vitamin E and ubiquinol$_{10}$ contents found in the roe may safeguard the eggs from a sudden change in oxygen availability because after fertilization eggs are rapidly exposed to a normoxic external environment, contrasting with the previous anoxic celomic environment (Hoar, 1957). Therefore, a combination of an environment rich in oxygen as well as in lipids (Ando and Hatano, 1991) will favor pro-oxidant conditions in the roe after fertilization. This is in agreement with the fact that fat increases with age in fish, and that fat storage occurs just to supply energy during reproduction, then decreasing and showing lowest levels of lipids at the end of the spawning period (Hoar, 1957). Accordingly, the activity of SOD and CAT and levels of ubiquinol$_{10}$ in the roe were all increased in late summer, coinciding with the intense activity related to spawning of the cutlassfish in this period (Lee, 1978), probably to avoid a related oxidative stress.

Irrespective of the seasonal contribution, the overall SOD activity found in all tissues examined prevailed over the corresponding CAT activity (Tables 1, 2). These differences were approximately 10 times for liver and roughly 100 times for red cells and roe. The SOD/CAT ratio found in the liver of the cutlassfish was fairly constant, in agreement with our previous studies on other marine (Wilhelm-Filho et al., 1993; Wilhelm-Filho and Boveris, 1993) and on freshwater fish species (Wilhelm-Filho and Marcon, 1996). The cellular prevalence of higher SOD in relation to CAT concentrations seems to fairly reflect the concentrations of their corresponding ROS substrates, superoxide anion ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2), which are continuously generated even under physiological conditions (Boveris, 1977). Such ratio calculations are possible assuming that essentially all the isoforms of both antioxidant enzymes found in different cells and tissues of all vertebrates so far evaluated, share the same molecular weight (Boveris, 1977; Wilhelm-Filho et al., 2000).

The liver vitamin E mean concentration found in juveniles was

approximately two-fold higher compared to those values found in adults, while no significant differences were detected for vitamin E and ubiquinol$_{10}$ contents in the erythrocytes. Vitamin E is a very important exogeneous nutritional antioxidant that acts mainly as a chain-breaker in membranes, among other functions (Burton and Ingold, 1981). Its presence in the cutlassfish as well as in other marine animals depends on the phytoplankton and zooplankton ingestion (Russell-Hunter, 1970). In this regard, food composition changes in relation to body weight in the cutlassfish, thus juveniles prefer euphasiids while adults prefer fish and squid in their diet (Portsev, 1980). Euphasiids are the second group in importance following the copepods through the marine zooplankton web and, in general, they are consumed by squids and small fish (McConnaughey, 1974), while both groups are rich in polyunsaturated fatty acids as well as in vitamin E (Russell-Hunter, 1970). As a consequence, the higher liver and erythrocytic vitamin E contents found in juvenile cutlassfish compared to adults are probably due to the dietary cumulative ingestion of vitamin E in the marine food-chain in this stage of development. Interestingly and in accordance to this, the other main antioxidant vitamin C also showed declined concentrations in adult fish (Dabrowski et al., 1996).

The intraerythrocytic GSH levels and the GSH/Hb ratio were higher in adults than in juveniles, while juveniles showed approximately half of the total glutathione content in the oxidized form (GSSG), whereas adults showed about one third of GSSG compared to GSH. A study carried out on a freshwater species from the Amazon basin (*Astronotus ocellatus*), which was submitted to either severe hypoxia or hyperoxia, showed a depletion in the original high content of erythrocytic GSH, which was probably driven to counteract this oxidative stress condition (Marcon, 1996). The relatively high amount of oxidized form of glutathione present in the juveniles of cutlassfish suggests an important antioxidant role of this ubiquitous tripeptide in fish red cells (Wilhelm-Filho and Marcon, 1996), as well as in other tissues of vertebrates and also of invertebrates, where concentrations in the mM range are usually found (Wilhelm-Filho et al., 2000).

On the other hand, it also indicates an unusual tolerance to relatively high GSSG contents found in different tissues of fish compared to mammals, usually being in the range of 1–2% of total gluthatione contents (Wilhelm-Filho et al., 2000). In this regard, erythrocytes are continuously exposed to oxidative damage and thereby exhibit a relatively high constitutive antioxidant protection (Stern, 1985; Godin and Garnett, 1992; Kurata et al., 1993). Accordingly, fish red cells have a much higher longevity compared to mammals (Clark, 1988; Kurata et al., 1993), and some fish and reptiles keep the same red cells during all their ontogeny (Prosser, 1991). Among other aspects, the high antioxidant protection of fish erythrocytes, particularly the levels of GSH, may account for such longevity (Wilhelm-Filho et al., 2000).

4.2. Seasonal changes

The significantly higher oxygen consumption found in the liver of the cutlassfish during late summer compared to fish collected during late winter could be attributable to the increased activity during the spawning period of this species (Lee, 1978; Portsev, 1980). Increased values of SOD in red cells, SOD and CAT activity and ubiquinol$_{10}$ concentrations were also found in the roe, which were concomitant to the increased red cell lipoperoxidation contents also detected in late summer. In contrast, the erythrocytic and hepatic CAT activity in the cutlassfish remained seasonally unchanged, while liver SOD activity, together with contents of vitamin E, was enhanced in late winter, a period of high foraging activity, compared to late summer.

This higher antioxidant levels found in the roe in late summer for the cutlassfish might be associated with its intense reproductive period. An antioxidant adaptative interpretation might be as follows. Mature ovaries are exposed to a sudden increase in oxygen availability when they switch from the almost anoxic celomic microenvironment to the normoxic condition, together with the burst of cellular activity upon fertilization (Hoar, 1957; Vershinin and Lukyanova, 1993). The increase of roe antioxidants such as SOD and CAT activity and contents of ubiquinol$_{10}$ in adults compared to young fish would minimize the oxidative insult related to lipid oxidation processes, especially considering the high content of polyinsaturated fatty acids that follows fish vitelogenesis (Hoar, 1957; Love, 1970).

Seasonal changes were already reported in three freshwater species (Gabryelak et al., 1983) where the activities of SOD, CAT and glutathione peroxidase in red cells were increased in spring compared to autumn. In addition, SOD and CAT activities were increased in the liver of freshwater fish species during the warm months compared to the cold months (Palace and Klaverkamp, 1993). Similar seasonal antioxidant adjustments were also found in the brown mussel *Perna perna*, which were well correlated with high temperatures and oxygen consumption, and also coinciding with the highest reproduction activity of gonad maturation and gamete emission (Wilhelm-Filho et al., 2001a).

Examples of coupling of antioxidant adaptations to physiological conditions involving foraging and reproductive cycles were also found in other two pelagic species of the Northern Hemisphere, the sea bass *Dicentrarchus labrax* (Vinagre et al., 2012) and the European sardine *Sardina pilchardus* (Nunes et al., 2015). In the European sardine, antioxidants were enhanced during the foraging period while declining during the reproduction period. In this regard, the striped bass *Morone saxatilis* displayed the lowest level of polyunsaturated fatty acids during late summer compared to other seasonal periods (Gallagher et al., 1989).

As already stressed out before, high levels of vitamin E were found in the liver of the cutlassfish in late winter, which are probably derived from euphasiids and squids, the main food preferred by adult cutlassfish (Portsev, 1980). In late winter the higher indirect ingestion of vitamin E seems to be important also to counteract the high ROS generation probably related to the intense activity of this fish during the reproductive cycle (Lee, 1978; Munekiyo and Kuwahara, 1984). This finding is well in line with several fish species from the Amazon basin that change drastically the hepatic vitamin E contents according to the great seasonal changes in food availability (Marx and Maia, 1985).

Interestingly, many fish lack erythrocytic CAT (Wilhelm-Filho et al., 1993, 1994; Wilhelm-Filho and Marcon, 1996), including some few cutlassfish specimens examined in the present study. Considering that the passive hydrogen peroxide elimination through the gills is probably a widespread adjuvant mechanism in water-breathing organisms (Wilhelm-Filho et al., 1994), makes such seasonal adaptations even more difficult to ascertain.

Concerning the non-enzymatic antioxidants, liver and roe displayed the highest mean concentrations of vitamin E and ubiquinol$_{10}$. Moreover, values for SOD activity as well as for the concentrations of vitamin E, GSH and ubiquinol$_{10}$ found in liver, roe and in red cells of the cutlassfish were in the same order of magnitude of those generally reported for mammals (e.g. López-Torres et al., 1993a,b; Wilhelm-Filho et al., 2000). In contrast to the α-tocopherol concentrations usually found in the liver of other fish, which are relatively lower than those observed in mammalian liver (Wilhelm-Filho and Marcon, 1996; Wilhelm-Filho et al., 2000), very active freshwater fish species from the Amazon basin also exhibited high concentrations (≈ 1–40 μM range) of this important nutritional antioxidant (Wilhelm-Filho and Marcon, 1996). However, a wide range of α-tocoferol content, also around the μM level, was reported in the liver of some herbivorous fish species from the Amazon basin (Marx and Maia, 1985).

Interestingly, β-carotene was not detected in all tissues of the cutlassfish during both seasons, an absence also revealed in juveniles of this fish species. Other studies carried out by our laboratory also failed to detect the presence or significant amounts of β-carotene in liver and erythrocytes of both freshwater (Wilhelm-Filho and Marcon, 1996) as well as marine fish species (Wilhelm-Filho et al., 1993). The

same applies to δ-tocoferol, which is sometimes present in fish (Wilhelm-Filho et al., 2000).

Contrary to changes detected in the ontogenetic study, whole blood glutathione concentrations and the ratio between glutathione and hemoglobin contents did not change in blood collected in late summer and in late winter, contrasting with the seasonal GSH variation found in the nursery reared Baltic salmon (Härdig and Höglund, 1983). High intraerythrocytic concentrations of GSH are commonly found in fish (Härdig and Höglund, 1983; Braddon et al., 1985) as well as in mammals (Wilhelm-Filho et al., 2000).

Lipid oxidation seems to remain seasonally unchanged in liver and roe of the cutlassfish, but higher TBARS levels were found in red cells in late summer compared to late winter. This increase may reflect the higher oxygen consumption detected in liver during late summer together with a general decrease in antioxidants, which is in accordance with the enhanced lipid utilization and an overall metabolic increase during the spawning period in fish (Hoar, 1957). As already mentioned, a similar seasonal antioxidant adjustment was detected in the digestive gland of the brown mussel *Perna perna*, which was correlated with high reproduction activity and oxygen consumption associated with gonad maturation and gamete emission (Wilhelm-Filho et al., 2001a).

Fish are characterized by metabolic rates approximately one order of magnitude lower than those described for birds and mammals, but fish in general possess lower antioxidant defenses compared to those reported for mammals (Wilhelm-Filho et al., 2000). In addition to this metabolic difference, fish seem to be more tolerant to the potential tissue damage that frequently accompany high levels of lipoperoxidation (TBARS) and oxidized glutathione (GSSG) compared to birds and mammals (Wilhelm-Filho et al., 2000).

As a very active fish, the cutlassfish possesses a relatively high constitutive antioxidant capacity in the tissues when generally compared to other fish species (Wilhelm-Filho et al., 1993; this study). The maintenance of such high antioxidant capacity might be associated with its daily vertical migrations to depths around 300 m (Portsev, 1980). In such way, it would be able to face either the sudden shifts in temperature and oxygen availability associated with such behavior, or the relatively slow ontogenetic and seasonal changes regarding foraging and reproductive cycles. Such antioxidant adaptation seems also to include the high tolerance regarding products derived from the oxidative insult mentioned above, thereby safeguarding very active and obligatory water-breathing fish species from the potential systemic oxidative stress (Wilhelm-Filho et al., 1993, 2000). This strategy resembles the long-term transitions of arrested states described in other vertebrates (Storey, 1996; Wilhelm-Filho et al., 2000). Irrespective of the time duration considered, all these antioxidant strategies were classified by Fridovich (1989) as biochemical adaptations comprising a second line of antioxidant defense to avoid oxidative stress.

The present results reinforce the concerns previously published (Wilhelm-Filho et al., 2000, 2001b; Torres et al., 2002; Vinagre et al., 2012; Nunes et al., 2015), showing the necessity of taking into consideration, among other interfering factors, the seasonal and reproductive influences on oxidative stress biomarkers on fish (as well as in other aerobic organisms), including evaluations of environmental contamination. In this regard, Harshman and Zera (2007) state that "one of the proximate mechanisms underlying the cost of reproduction includes defenses against stress and toxicity", which certainly includes oxidative stress.

5. Conclusions

Seasonal and ontogenetic antioxidant adjustments in different tissues compatible to liver oxygen consumption seem to be biochemical adaptations present in *Trichiurus lepturus* to avoid systemic oxidative stress related to these changes. Such antioxidant adjustments present in most tissues seem to be parallel to the higher oxygen consumption of juveniles compared to adults, as well as during high summer, coinciding with the intense activity during foraging and spawning periods of adult females compared to young fish.

Conflict of interest

The authors declared no conflict of interest related to the present work.

Acknowledgements

The authors are grateful to all fishermen from "Praia da Armação", Santa Catarina Island, who gently participated in catching the fish for this study. We thank Dr. Gareth Cuttle for English revision. This work was conducted at the Laboratório de Radicales Libres, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. DWF thank this institution for its hospitality and use of laboratory facilities, as well as all the Argentinian friends who helped in the analysis to obtain different data of the present study. This work was partially supported by grants from the University of Buenos Aires (FA089 to CGF and FA088 to AB), and DWF is recipient of a Research Fellowship from CNPq (Proc. 303234/2015-6). We are also grateful to Dr. Marcelo Farina and Dr. Andre Wüst Zibetti from UFSC, for advisement related to statistic methodology.

References

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121–126.
Aksnes, A., Njaa, L.R., 1981. Catalase, glutathione peroxidase, and superoxide dismutase in different fish species. Comp. Biochem. Physiol. 69B, 893–896.
Ando, S., Hatano, M., 1991. Distribution of carotenoids in the eggs from four species of salmonids. Comp. Biochem. Physiol. 99B, 341–344.
Beutler, E., Duron, O., Kelly, B.M., 1963. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 61, 882–890.
Bird, R.P., Draper, A.H., 1984. Comparative studies on different methods of malondyhaldehyde determination. Methods Enzymol. 90, 105–110.
Boveris, A., 1977. Mitochondrial production of superoxide radical and hydrogen peroxide. Adv. Exp. Med. Biol. 78, 67–82.
Boveris, A., Fraga, C.G., Varsavsky, A.I., Hoch, O.R., 1983. Increased chemiluminescence and superoxide production in the liver of chronically ethanol-treated rats. Arch. Biochem. Biophys. 227, 534–541.
Braddon, S.A., McIlvaine, C.M., Balthrop, J.E., 1985. Distribution of GSH and GSH cycle enzymes in black sea bass (*Centropistis striata*). Comp. Biochem. Physiol. 80B, 213–216.
Burton, G.W., Ingold, K.U., 1981. Autooxidant of biological molecules. I—the antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. J. Am. Chem. Soc. 103, 6472–6477.
Buttriss, J.L., Diplock, A.J., 1984. High-performance liquid chromatography methods for vitamin E in tissues. Methods Enzymol. 105, 131–138.
Clark, M.R., 1988. Senescence of red blood cells: progress and problems. Physiol. Rev. 68, 503–554.
Dabrowski, K., Moreau, R., El-Saidy, D., Ebeling, J., 1996. Ontogenetic sensitivity of channel catfish to ascorbic acid deficiency. J. Aquat. Anim. Health 8, 22–27.
Desrochers, P.E., Hoffert, J.R., 1983. Superoxide dismutase provides protection against the hyperoxia in the retina of the rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 76B (2), 241–247.
Estabrook, R.W., 1984. Mitochondrial respiratory control and the polarographic measurement of ADP-O ratios. Methods Enzymol. 10, 41–47.
Fridovich, I., 1989. Superoxide dismutase—an adaptation to a paramagnetic gas. J. Biol. Chem. 264, 7761–7764.
Gabryelak, T., Piatkowska, M., Leyko, W., Pérès, G., 1983. Seasonal variations in the activities of peroxide metabolism enzymes, in erythrocytes of freshwater fish species. Comp. Biochem. Physiol. 75C, 383–385.
Gallagher, M.L., McLeod, S.H., Rulifson, R., 1989. Seasonal variation in fatty acids of striped bass *Morone saxatilis*. J. World Aquacult. Soc. 20, 38–45.
Godin, D.V., Garnett, M.E., 1992. Species-related variations in tissue antioxidant status—II. Differences in susceptibility to oxidative challenge. Comp. Biochem. Physiol. 103B, 743–748.
Halliwell, B., Gutteridge, J.M.C., 2007. Free Radicals in Biology and Medicine, third ed. Oxford University Press, London.
Härdig, J., Höglund, L.B., 1983. Seasonal and ontogenetic effects on methaemoglobin and reduced glutathione content in the blood of reared baltic salmon. Comp. Biochem. Physiol. 75A (1), 27–34.
Harrison, G., 1981. The cranial nerves of the teleost *Trichiurus lepturus*. J. Morphol. 167, 119–134.
Harshman, L.G., Zera, A.J., 2007. The cost of reproduction: the devil in the details. Anthony Zera Publications. In: Papers in the Biological Sciences, Paper 6. University of Nebraska, Lincoln. (http://digitalcommons.unl.edu/bioscizera/6).
Hoar, W.S., 1957. The gonads and reproduction. In: Brown, M.E. (Ed.), The Physiology of

Fishes. Academic Press, New York, pp. 287–322.
Janssens, B.J., Childress, J.J., Baguet, F., Rees, J.F., 2000. Reduced enzymatic antioxidative defense in deep-sea fish. J. Exp. Biol. 203 (24), 3717–3725.
Jones, D.P., 1985. The role of oxygen concentration in oxidative stress: hypoxic and hyperoxic models. In: Sies, H. (Ed.), Oxidative Stress. Academic Press, London, pp. 151–195.
Kurata, M., Suzuki, M., Agar, N.S., 1993. Antioxidant systems and erythrocyte lifespan in mammals. Comp. Biochem. Physiol. 106B, 477–487.
Lang, J.K., Gohil, R., Packer, L., 1986. Simultaneous determination of tocopherols, ubiquinols, and ubiquinones in blood, plasma, tissue homogenates, and subcellular fractions. Anal. Biochem. 157, 106–116.
Lee, S.-C., 1978. Food and feeding habits of ribbonfishes *Trichiurus japonicus* and *T. lepturus*. Bull. Inst. Zool. Acad. Dermatol. Sin. 17 (2), 117–124.
López-Cruz, R.I., Dafré, A.L., Wilhelm-Filho, D., 2012. Oxidative stress in sharks and rays. In: Zenteno-Savin, T., Vázquez-Medina, J.P., Abele, D. (Eds.), Oxidative Stress in Aquatic Ecosystems. Willey-Blackwell, London, pp. 23–29.
López-Torres, M., Pérez-Campo, R., Cadenas, S., Rojas, C., Barja, G., 1993a. A comparative study of free radicals in vertebrates. II—non-enzymatic antioxidants and oxidative stress. Comp. Biochem. Physiol. 105B, 757–763.
López-Torres, M., Pérez-Campo, R., Rojas, C., Cadenas, S., Barja, G., 1993b. Maximum life span in vertebrates: relationship with liver antioxidant enzymes, glutathione system, ascorbate, urate, sensitivity to peroxidation, true malondialdehyde, in vivo H_2O_2, and basal and maximum aerobic capacity. Mech. Ageing Dev. 70, 177–199.
Love, R.M., 1970. The Chemical Biology of Fish. Academic Press, London.
Lucesoli, F., Fraga, C.G., 1995. Oxidative damage to lipids and DNA concurrent with decrease of antioxidants in rat testes after acute iron intoxication. Arch. Biochem. Biophys. 316, 567–571.
Marcon, J.L., 1996. Estresse oxidativo em duas espécies de teleósteos amazônicos, *Astronotus ocellatus* e *Colossoma macropomum*, expostos a diferentes tensões de oxigênio: uma abordagem comparativa. (PhD.Thesis) Instituto Nacional de Pesquisas da Amazônia, INPA/FUA.
Martínez-Álvarez, R.M., Morales, A.E., Sanz, A., 2005. Antioxidant defenses in fish: biotic and abiotic factors. Rev. Fish Biol. Fish. 15 (1–2), 75–88.
Marx, F., Maia, J.G.S., 1985. Determination of fat-soluble vitamins from Amazonian freshwater fishes. Acta Amazon. 15 (1–2), 185–192.
McConnaughey, B.H., 1974. Introduction to Marine Biology. Mosby, Saint Louis.
Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247, 3170–3174.
Morris, S.M., Albright, J.T., 1984. Catalase, glutathione peroxide, and superoxide dismutase in the *rete mirabile* and gas gland epithelium of six species of marine fishes. J. Exp. Zool. 232, 29–39.
Munekiyo, M., Kuwahara, A., 1984. Spawning ground, mating systems and distribution pattern of ribbonfish. Bull. Jpn. Soc. Sci. Fish. 50 (9), l527–l533.
Nunes, B.S., Travasso, R., Gonçalves, F., Castro, B.B., 2015. Biochemical and physiological modifications in tissues of *Sardina pilchardus*: spatial and temporal patterns as a baseline for biomonitoring studies. Front. Environ. Sci. 3 (7), 1–14.
Ohkawa, H., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358.
Palace, U.P., Klaverkamp, J.F., 1993. Variation of hepatic enzymes in three species of freshwater fish from precambrian shield lakes and the effect of cadmium exposure. Comp. Biochem. Physiol. 104C, 147–154.
Pérez-Campo, R., López-Torres, M., Rojas, C., Cadenas, S., Barja, G., 1993. A comparative study of free radicals in vertebrates—I. Antioxidant enzymes. Comp. Biochem. Physiol. 105B, 749–755.
Portsev, P.I., 1980. Feeding behaviour of the saberfish, *Trichiurus lepturus* (Trichiuridae), of the west coast of India. Vopr. Ikhtiol. 20, 836–841.
Prosser, C.L., 1991. Environmental and metabolic animal physiology. In: Prosser, C.L. (Ed.), Comparative Animal Physiology. Wiley-Liss, New York, pp. 45–64.
Ronisz, D., Larsson, D.G.J., Förlin, L., 1999. Seasonal variations in the activities of selected hepatic biotransformation and antioxidant enzymes in eelpout (*Zoarces viviparus*). Comp. Biochem. Physiol. 124C, 271–279.
Ross, S.W., Dalton, D.A., Kramer, S., Christensen, B.L., 2001. Physiological (antioxidant) responses of estuarine fishes to variability in dissolved oxygen. Comp. Biochem. Physiol. C130, 289–303.
Rudneva, I., 1997. Blood antioxidant system of Black Sea elasmobranch and teleosts. Comp. Biochem. Physiol. 118, 255–260.
Rudneva, I., Skuratovskaya, E.N., Kuzminova, N.S., Kovyrshina, T.B., 2010. Age composition and antioxidant enzyme activities in blood of Black Sea teleosts. Comp. Biochem. Physiol. C Toxicol. Pharmacol. 151 (2), 229–239.
Russell-Hunter, W.D., 1970. Aquatic Productivity. McMillan, New York.
Sies, H., 1985. Oxidative stress: introductory remarks. In: Sies, H. (Ed.), Oxidative Stress. Academic, London, pp. 1–8.
Stern, A., 1985. Red cell oxidative damage. In: Sies, H. (Ed.), Oxidative Stress. Academic Press, London, pp. 331–349.
Storey, K.B., 1996. Oxidative stress: animal adaptation in nature. Braz. J. Med. Biol. Res. 29, 1715–1733.
Tietze, F., 1969. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione. Anal. Biochem. 27, 502–522.
Torres, M.A., Testa, C.P., Gaspari, C., Masutti, M.B., Panitz, C.M., Pedrosa, R.C., Almeida, E.A., Di Mascio, P.D., Wilhelm-Filho, D., 2002. Oxidative stress in the mussel *Mytella guyanensis* from polluted mangroves on Santa Catarina Island, Brazil. Mar. Pollut. Bull. 44, 923–932.
Tukey, J.W., 1977. Exploratory Data Analysis. Addison-Wesley, London.
Vanderkooi, J.M., Erecinska, M., Silver, I.A., 1991. Oxygen in mammalian tissue: methods of measurement and affinities of various reactions. Am. J. Physiol. 260C, 1131–C1150.
Vershinin, A., Lukyanova, O.N., 1993. Carotenoids in the developing embryos of sea urchin *Strongylocentrotus intermedius*. Comp. Biochem. Physiol. 104B, 371–373.
Vinagre, C., Madeira, D., Narciso, L., Cabral, H.N., Diniz, M., 2012. Effect of temperature on oxidative stress in fish: lipid peroxidation and catalase activity in the muscle of juvenile seabass, *Dicentrarchus labrax*. Ecol. Indic. 23, 274–279.
Wdzieczak, J., Zalésna, G., Bartkowiak, A., Witas, H., Leyko, W., 1981. Comparative studies of superoxide dismutase, catalase, and peroxide levels in erythrocytes of different fish species. Comp. Biochem. Physiol. 68B, 357–358.
Wdzieczak, J., Zalésna, G., Wujec, E., Pérès, G., 1982. Comparative studies on superoxide dismutase, catalase and peroxidase levels in erythrocytes and livers of different freshwater and marine fish species. Comp. Biochem. Physiol. 73B, 361–365.
Wilhelm-Filho, D., 2007. Reactive oxygen species, antioxidants and fish mitochondria. Front. Biosci. 12, 1229–1237.
Wilhelm-Filho, D., Boveris, A., 1993. Antioxidant defenses in marine fish. II—elasmobranchs. Comp. Biochem. Physiol. 106C, 415–418.
Wilhelm-Filho, D., Marcon, J.L., 1996. Antioxidant defenses in fish of the Amazon. In: Val, A.L., Almeida-Val, V.M.L., Randal, D.J. (Eds.), Physiology and Biochemistry of the Fishes of the Amazon. INPA, Manaus, pp. 299–312.
Wilhelm-Filho, D., Eble, G.J., Kassner, G., Caprario, F.X., Dafré, A.L., Ohira, M., 1992a. Comparative hematology in marine fish. Comp. Biochem. Physiol. 102A, 311–321.
Wilhelm-Filho, D., Marcon, J.L., Caprario, F.X., Nollis, A.C., 1992b. Erythrocytic nucleoside triphosphates in marine fish. Comp. Biochem. Physiol. 102A (2), 323–331.
Wilhelm-Filho, D., Giulivi, C., Boveris, A., 1993. Antioxidant defences in marine fish. I—teleosts. Comp. Biochem. Physiol. 106C, 409–413.
Wilhelm-Filho, D., González-Flecha, B., Boveris, A., 1994. Gill diffusion as a physiological mechanism for hydrogen peroxide elimination by fish. Braz. J. Biol. Med. Res. 27, 2879–2882.
Wilhelm-Filho, D., Torres, M.A., Marcon, J.L., Fraga, C.G., Boveris, A., 2000. Antioxidant defenses in vertebrates—emphasis on fish and mammals. Trends Comp. Biochem. Physiol. 7, 37–45.
Wilhelm-Filho, D., Tribess, T.A., Gáspari, C., Claudio, F.D., Torres, M.A., Magalhães, A.R.M., 2001a. Seasonal changes in antioxidant defenses in the digestive gland of the brown mussel (*Perna perna*). Aquaculture 203, 149–158.
Wilhelm-Filho, D., Torres, M.A., Tribess, T.B., Pedrosa, R.C., Soares, C.H., 2001b. Influence of season and pollution on the antioxidant defenses of the cichlid fih acará (*Geophagus brasiliensis*). Braz. J. Med. Biol. Res. 34, 719–726.