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CELLULAR AND MOLECULAR BIOLOGY

Effect of heat stress on the antioxidant defense system and erythrocyte morphology of Antarctic fishes

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Abstract: This study analyzed the effect of thermal stress on erythrocytes of *Notothenia rossii* and *Notothenia coriiceps*, abundant notothenioids in Admiralty Bay, Antarctic Peninsula. In both species, the antioxidant defense system enzymes, superoxide dismutase, catalase, glutathione peroxidase, glutathione-S transferase, glutathione reductase were punctually altered (8° C for 1, 3 and 6 days) in erythrocytes, indicating that these markers are not ideal for termal stress. However, under the influence of thermal stress, morphological changes in *Notothenia coriiceps* erythrocytes were observed at all exposure times (1, 3 and 6 days at 8° C), and in *Notothenia rossii* occurred in 6 days. These results suggest that *Notothenia corriceps* presents a lower tolerance to thermal stress at 8° C for up to 6 days, since the cellular and nuclear alterations recorded are pathological and may be deleterious to the cells. Among the morphological markers analyzed in this work, we believe that the shape change and nuclear bubble formation may be good stress biomarkers in erythrocytes of *Notothenia rossii* and *Notothenia coriiceps*.

Key words: Admiralty bay, nototheniidae, oxidative stress, red blood cells, temperature.

INTRODUCTION

Erythrocytes have gas exchange functions (Li et al. 2016), therefore, they are sensitive to oxidative stress because their cytoplasm has high concentrations of iron and oxygen, which can generate reactive oxygen species (ROS) by the Fenton reaction (Li et al. 2013). Furthermore, their membranes have a high polyunsaturated fatty acid content, making them thus more susceptible to ROS (Gwozdzinski et al. 2017). Some studies regarding marine fish have shown that short term exposure to high temperature, especially in an acute way, results in oxidative damage (Grim et al. 2013, Mueller et al. 2012), as a result of an interruption of electron transfer within the respiratory chain (Mueller et al. 2014). When the enzymatic and non-enzymatic components of the antioxidant defense system, are not able to eliminate or neutralize the ROS occurs oxidative stress, able to oxidize lipids and proteins and in more serious cases lead the cell to apoptosis (Li et al. 2013).

The Antarctic ichthyofauna has adapted to the Antarctic climate and temperature conditions, and changes in the thermal stability of the environment can be lethal (Bilyk & DeVries 2011, Crockett 2011, Brodte et al. 2008, di Prisco et al. 2007, Somero & DeVries 1967). The phase of Antarctic fish acclimation to heat can affect oxygen supply to tissues (Pörtner 2002), and heat stress can cause hyperglycemia,

slow cortisol release in plasma and increased hematocrit in notothenioids (Heise & Abele 2008). Furthermore, increasing the temperature at 4°C for 6 days may increase lipoperoxidation in the erythrocytes of these animals (Klein et al. 2017). Studies have investigated the antioxidant defense system, of several tissues, of the Antarctic fish, especialty *Notothenia rossii* and *Notothenia coriiceps*, submitted to thermal stress, and were demonstrated that the responses may be species-specific and tissue-specific, making it difficult to establish biomarkers (Mueller et al. 2012, 2014, Machado et al. 2014, Sattin et al. 2015, Forgati et al. 2017, Klein et al. 2017, Souza et al. 2018)

Other responses may be associated with oxidative stress and thermal stress in erythrocytes, as is the case with cellular alterations, since ROS may affect membrane permeability, cause nuclear changes (Nagasaka et al. 2004, Zafalon-Silva et al. 2017), lead to dephosphorylation of membrane proteins and lipids due to oxidation of hemoglobin, and cause formation of vesicles on the plasma membrane of erythrocytes (Vodyanoy 2015).

N. rossii and *N. coriiceps* were chosen in the present study, because they are phylogenetic species and environmentally similar (Near & Cheng 2008, Raga et al. 2015). In addition, these two species are abundant in biomass and are important for the Antarctic trophic chain, including the Admiralty Bay, a Special Site of Scientific Interest (SSSI No. 8) and an Antarctic Specially Managed Area (ASMA No. 1) (Arigony-Neto et al. 2004, Braun et al. 2001). *N. rossii* has slightly compressed head and body, with an obliquely arranged mouth in adulthood, light brown in color, with darker back, with black spots along the body. *N. coriiceps* has a broad body with a slightly depressed head, adult coloration is approaching dark brown and may have light brown spots (Fisher & Hureau 1985). Studies on climate change in this region have reported observations of accelerated warming. Over the past 60 years, the seawater temperature of that region at depths between 700-1100 m (between 35-65°S) has increased by 0.2°C (Beers & Sidell 2011), and recently, a similar increase has also been recorded in shallow waters (Turner et al. 2014, Oliva et al. 2016).

This paper was carried out to answer a question raised by Machado et al. (2014) who evaluated the effect of temperature acclimation on the liver antioxidant defence system of the *N. coriiceps* and *N. rossii*. The authors suggest, in addition to existing literature (Cassini et al. 1993, Ansaldo et al. 2000), erythrocytes may be important cells for antioxidant defense, especially in neutralizing of superoxides. Besides that, the use of fishes erythrocytes as a biomarker becomes interesting mainly because the analysis of the material is possible without the need of animal sacrifice. Thus, this work proposes to evaluate the effect of thermal stress on the antioxidant defence system, changes in cellular and nuclear morphology in erythrocytes of two endemic species of Antarctic fish, *N. coriiceps* and *N. rossii*. No progressive acclimation was use, and the animals were place directly into water at the experimental temperatures used (0°C to 8°C). Although the thermal shock exposes the animals to sharply higher temperatures than their natural environment, this approach is important for reveal physiological responses to high temperatures. Exposure at 8°C is close to the thermal maximum tolerated by Antarctic fishes (Strobel et al. 2013, Somero & DeVries 1967), to justify using the 8°C temperature in the study.

MATERIALS AND METHODS

Experimental design

Notothenia rossii (n = 60; total length = 37 ± 3 cm; weight= 791 ± 145 g) and *Notothenia coriiceps* (n = 60; total length = 35 ± 3 cm; weight= 775 ± 182 g) were caught by hook and line at Admiralty Bay (61ºS – 63º30'S, 53º55'W – 62º50'W) and transported to Brazilian Antarctica Station Comandante Ferraz (EACF), located on King George Island in the South Shetlands Archipelago. After collection, fish were kept for 3 days in the laboratory under controlled conditions of temperature (-0.5º to 1.0ºC), 12-hours light and 12-hours dark photoperiod (Donatti & Fanta 2002), salinity (35 ± 1.5) and constant aeration, aiming to reduce collection stress. Next, fish were acclimated in the experimental laboratory for 3 days (Machado et al. 2014, Ryan 1995) under the same controlled abiotic conditions, except that the temperature was maintained at 0 ± 0.5ºC.

Subsequently, fishes were fish were marked with a coloured line and acutely transferred to experimental tanks (1000 L of seawater) under the same abiotic acclimation conditions at two different temperature conditions, control (0±0.5° C) and experimental (8±0.5° C), for 1, 3 and 6 days using a total of 10 fish per experiment. The water temperature of the tanks was strictly maintain by thermostats coupled to heaters, and tanks were cleaned every two days by exchanging 50% of the water under the same experimental conditions.

Fish were fed individually every other day with muscle of Antarctic fish (1% of their body mass) (Crawford 1979, Egginton et al. 1991, Coggan 1997) hooked to a long rigid wire. The animals were observed to verify no regurgitation of the food (Donatti & Fanta 2002). Fish that failed to were excluded from the experiments. In all experiments, the first day of acclimation was also the first day of feeding, and both feeding and sacrifice occurred at the same time for the experimental groups and their respective controls. The last offer of food occurred 12 hours before the animal was sacrificed.

Fish were anaesthetized with 20 $mg.L^{-1}$ benzocaine (from a stock solution of 0.1% ($w.v^{-1}$) in 95% ethanol), and blood samples were obtained using heparinized syringes by caudal vein puncture and killed by spinal transection. Erythrocytes were obtain by centrifuging whole blood at 2,000g for 10 minutes at 4° C. After plasma and white cell removal, a portion of the erythrocytes were fixed in modified Karnovsky fixative (2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, at 4° C) (Karnovsky 1965) for morphological analysis. A portion of the erythrocytes was placed in 0.9% NaCl, centrifuged at 5000 x g for 10 min at 4° C. Then, 20 mM Tris-HCl buffer pH 8.0 was added (3: 1 v/v) to obtain hemolysate, which was centrifuged at 12,000 x g for 15 min at 4° C, and the supernatant was frozen in liquid nitrogen to perform enzymatic analysis. The Environmental Assessment Group of the Brazilian Ministry of Environment granted the environmental licenses and the Ethics Committee of the Federal University of Paraná (UFPR) under number 496 approved the experiments.

Analytical methods

The total protein concentration was determine by the Bradford method (1976) using bovine serum albumin (BSA) to establish the standard curve, and the absorbance of the samples was determined at 595 nm.

The readings of superoxide dismutase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase, concentration of glutathione and lipid peroxidation were performed in triplicate in an microplate spectrophotometer (Epoch Microplate Spectrophotometer, BioTek, Winooski, VT, USA),

with the exception of catalase (CAT), for which a spectrophotometer with quartz cuvette was employed.

Antioxidant enzyme activity assays

Levels of superoxide dismutase (SOD, EC 1.15.1.1) activity were measured by inhibiting nitrobluetetrazolium (NBT) reduction to blue formazan by the O_2^- generated by hydroxylamine (Crouchet al. 1981), and the absorbance was determined at 560 nm.

The catalase (CAT, EC 1.11.1.6) activity levels were measured by the consumption of hydrogen peroxide (H_2O_2) using a spectrophotometer at 240 nm following the method of Beutler (1975).

Levels of glutathione peroxidase (GPx, EC 1.11.1.9) activity were determine by NADPH oxidation (Wendel 1981). The reaction occurred at 25°C was initiate by the addition of 0.5 mM hydrogen peroxide and was monitored for 3 min at 340 nm.

Glutathione-S-transferase (GST, EC 2.5.1.18) activity levels were determined according to the method of Keen et al. (1976) using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. CDNB-glutathione was evaluate spectrophotometrically at 340 nm.

Glutathione reductase (GR, EC 1.8.1.7) activity levels were evaluate spectrophotometrically by measuring the changes in absorbance at 340 nm due to the oxidation of NADPH concomitantly with the reduction of GSSG according to the method of Carlberg & Mannervik (1985). The reaction was initiate by the addition of GSSG and monitored for 10 min.

The antioxidant enzyme activities are expressed as units (U)/mg of protein. One unit of SOD activity was define as the amount of enzyme that inhibits the NBT oxidation reaction by 50% of the maximal inhibition. One unit of CAT activity is defined as the amount of enzyme consuming one mmol of substrate per minute. One unit of GPx, GST and GR activity is defined as the amount of enzyme generating one µmol of product per minute. All enzyme activities were determined at 20°C (Jayasundara et al. 2013, Machado et al. 2014, Rodrigues et al. 2015, Almeida et al. 2015, Forgati et al. 2017).

Oxidative stress indices

The concentration of glutathione (GSH) and other thiols was determined using the method described by Sedlak & Lindsay (1968). The method is based on protein precipitation and subsequent reaction of non-protein thiols with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), generating a product that absorbs light at 415 nm. The GSH values are expresse as nmol of thiols per mg of protein^{-1}.

The lipid peroxidation (LPO) index was determined via the levels of malondialdehyde (MDA) through the reaction of TBARS in a spectrophotometer at 535 nm using malondialdehyde-(bis)-acetate as a standard (Uchiyama & Mihara 1978). MDA values are express as nmol of MDA per mg of protein^{-1}.

Morphological analysis

For light microscopy (LM), sample smear glass slides stained with May-Grunwald (Rios 2010) were use. The images were obtained using a Zeiss Imager.Z2 scanner microscope via the MetaSystems/VSViewer program.

For the morphometric analysis of cellular changes obtained in LM, 1,000 erythrocytes per individual were classified as erythrocytes with abnormal cell shape, erythrocytes with vesicle formation in the plasma membrane, erythrocytes with micronuclei, erythrocytes with blebbed nuclei, or erythrocytes with notched nuclei according to methodology adapted from Grisolia et al. (2009) and Souza & Fontanetti (2006).

To confirm the analyzes performed in LM, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) were realized. For TEM analysis, erythrocytes were post-fixed in 2% osmium tetroxide, 0.1 M cacodylate buffer, pH 7.2, contrasted en-bloc with 2% uranyl acetate and embedded in Epon-812 resin (Luft 1961). The ultra-thin sections were contrasted in aqueous 2% uranyl acetate (Watson 1958), lead nitrate (Reynolds 1963) and analyzed in a JEOL 1200EX II transmission electron microscope. For SEM, erythrocytes were submitted to critical point drying in a Bal-Tec CPD - 030, metalized in gold in a Balzers SCD - 030 and analyzed using a JEOL JSM-6360 LV scanning electron microscope. The analyzes were performed at the Electron Microscopy Center of Federal University of Paraná.

Statistical analysis

Morphometric data, oxidative stress enzyme levels (SOD, CAT, GPx, GST and GR) and non-enzymatic parameters (MDA and GSH) were initially tested for normal distribution using the Kolmogorov-Smirnov test and for variance homogeneity by Levene's test. If the data were homogeneous, they were subject to two-factor analysis of variance (two-way ANOVA) (F) in which the exposure time (TI) (1, 3 and 6 days) and temperature (TE) (0 and 8°C) were categorical variables. In the case of non-parametric data, non-parametric Kruskal-Wallis analysis (H) was use. In the case of significant variation the data were subjected to Tukey's post-test and multiple comparisons. To evaluate the relationship between levels of oxidative stress markers and morphological analysis variables were used correlation and linear regressions. All statistical analyzes were performed with $P<0.05$ as the level of significance.

RESULTS

All animals in this study survived to effect of independent variables, temperature (0 to 8 ° C) and exposure time (1, 3 and 6 days) no death being recorded. The results presented below indicate the differences between temperatures (0-8ºC). The differences between the exposure times at the same temperature can be seen in detail in Figures 1, 2 and 3. To both species and temperatures tested there were no correlation between oxidative stress markers and morphological changes (Table II). Also none of these variables included in the linear regression model were significant (Table I).

Antioxidant enzyme activity

The SOD levels were modulated by increasing temperature, only in *N. rossii* and displayed an up regulation at 8°C relative to 0°C over 6 days of exposure (Figure 1a and 1c). In *N. rossii*, CAT activity was modulate positively on day 3 and negatively on day 6 at 8°C. As for *N. coriiceps*, CAT activity was negatively modulated as the temperature increased on days 1 and 3 (Figure 1b and 1d).

Regarding the GPx activity, there was no modulation in relation to the temperature in either species (Figure 2a and 2d). In *N. rossii*, the GST activity was reduced on day 6 at 8°C with respect to temperature of 8°C. As for GST activity at 8°C, in *N. coriiceps*, there was an up regulation on day 3 at 0°C and an increase in activity after 6 days (Figure 2b and 2e). The GR levels were modulated by increasing temperature, only in *N. coriiceps*, GR activity showed an up regulation on days 1 and 3 at 8°C (Figure 2c and 2f). All results of twoway ANOVA test are represents at Table II.

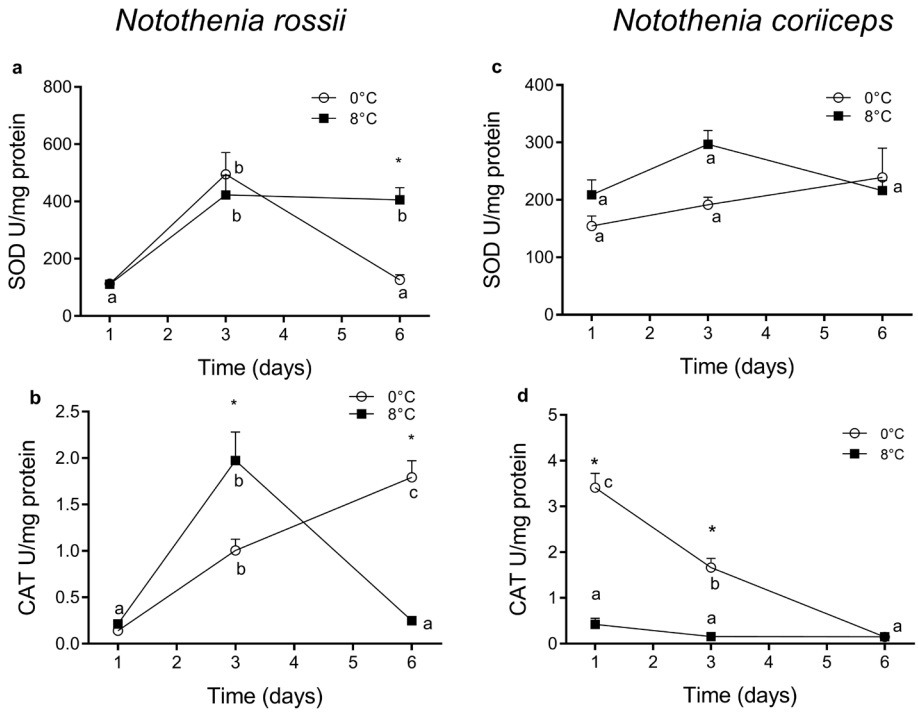

Figure 1. Antioxidant enzyme activity. Levels of superoxide dismutase (SOD), catalase (CAT), activity in *N. rossii* (a-b) and *N. coriiceps* (c-d) erythrocytes exposed to 0°C or 8°C (empty circles and solid squares, respectively) for 1, 3 or 6 days. The vertical lines indicate mean + SE. Different lowercase letters indicate differences among the exposure times (1, 3 and 6 days) at each temperature. Asterisks indicate differences (P≤0.05) between temperatures (0°C and 8°C).

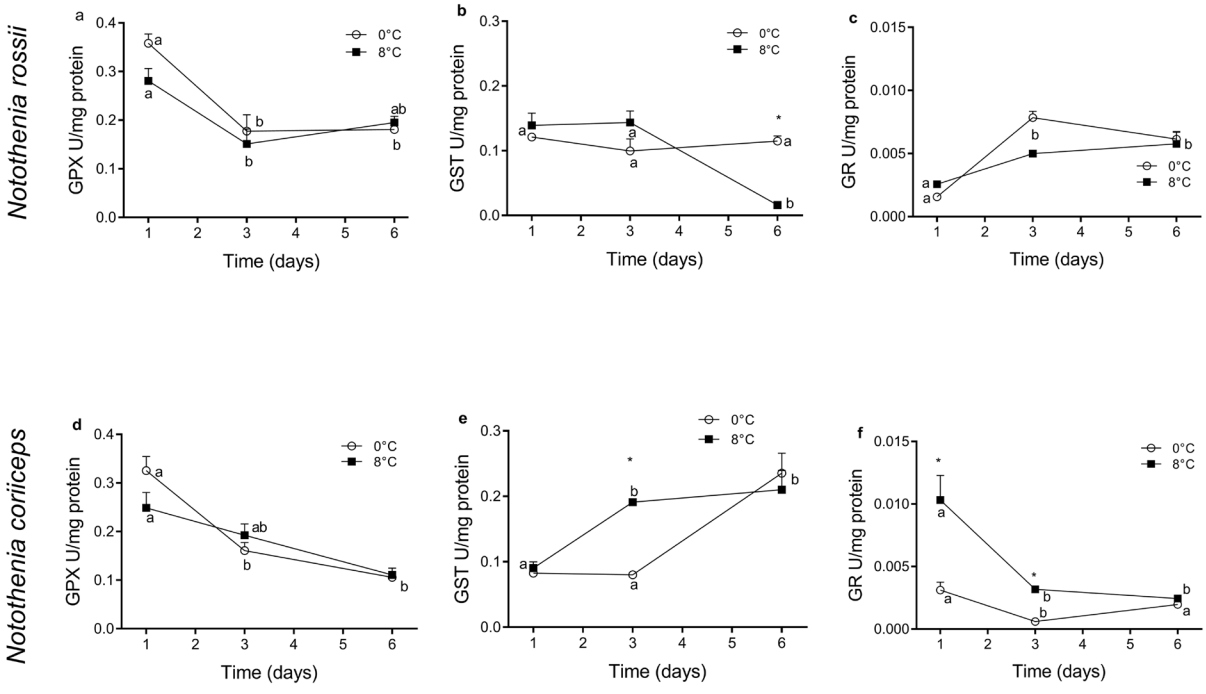

Figure 2. Antioxidant enzyme activity. Levels of glutathione S-transferase (GST), glutathione reductase (GR) and glutathione peroxidase (GPx) activity in *N. rossii* (a-b-c) and *N. coriiceps* (d-e-f) erythrocytes exposed to 0°C or 8°C (empty circles and solid squares, respectively) for 1, 3 or 6 days. The vertical lines indicate mean + SE. Different lowercase letters indicate differences among the exposure times (1, 3 and 6 days) at each temperature. Asterisks indicate differences (P≤0.05) between temperatures (0°C and 8°C).

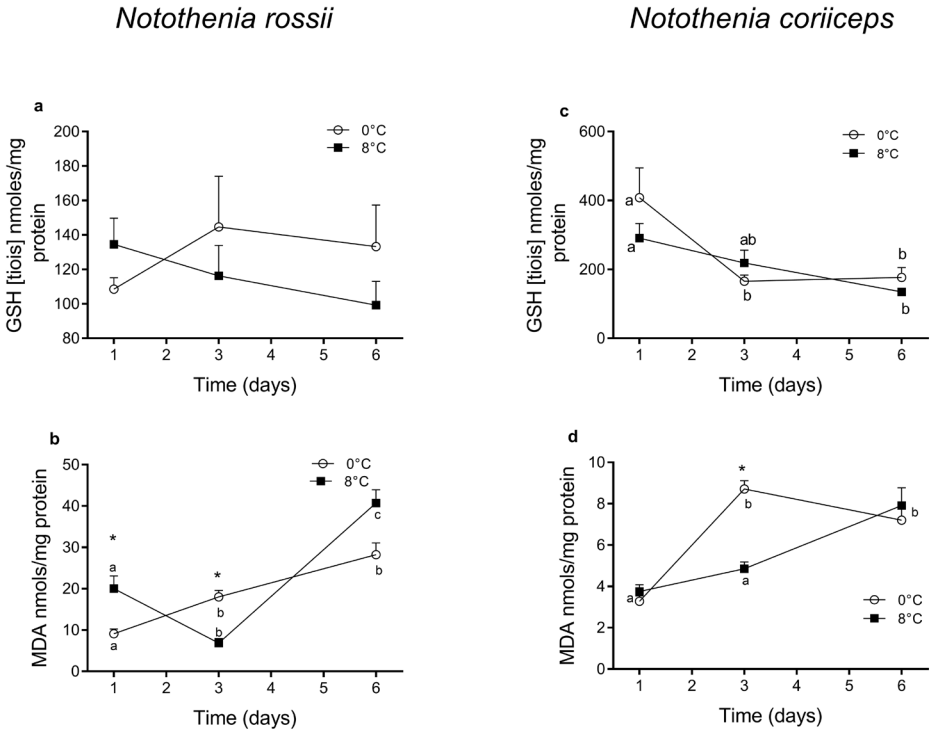

Figure 3. Oxidative stress indices. Concentration of reduced glutathione (GSH) and (MDA). *N. rossii* (a and b) and *N. coriiceps* (c and d) erythrocytes exposed to 0°C or 8°C (solid squares and empty circles, respectively) for 1, 3 or 6 days. The vertical lines indicate mean + SE. Different lowercase letters indicate differences between the exposure times at each temperature (0°C and 8°C).

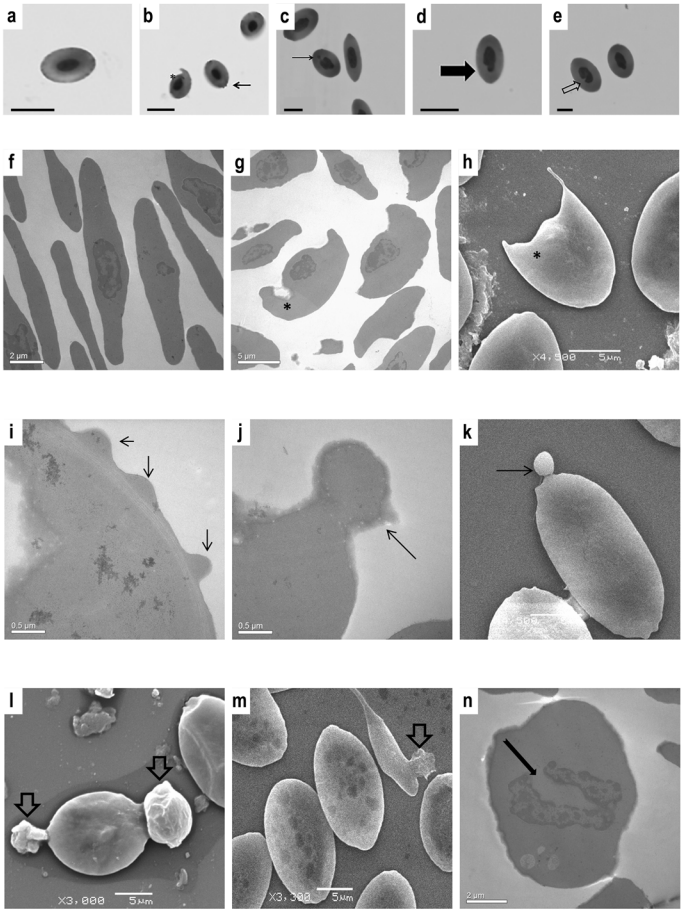

Figure 4. Erythrocytes fish. Morphological changes in *N. rossii* and *N. coriiceps* erythrocytes subjected to warming, optical microscopy (a-e) and electron microscopy (f-n). a and f: Erythrocytes with usual cellular and nuclear morphology. b, g and h: Erythrocytes with changes in cell morphology (*). c: Erythrocytes with micronuclei (➔) d: Erythrocytes with blebbed nuclei (è). b, i, j and k: Protrusions or vesicles in formation or released of the cell membrane (➔). l and m: Cytoplasm leakage (_). n: Notched nuclei (°).

Table I. Correlation and coefficients of the linear regression model of oxidative stress and morphological alterations of *N. rossii* and *N. coriiceps* at 0°C and 8 ° C, for 1, 3 and 6 days.

	Temperature	Time	correlation	Multiple R-squared	P-value	DF	F	Intercept (b)	a
Notothenia rossii	0°C	1 day	0.64	0.41	0.16	1 and 4	2.86	0.12	0.63
		3 days	0.38	0.14	0.31	1 and 7	1.18	1.82	0.03
		6 days	0.14	0.02	0.71	1 and 7	0.14	1.85	0.01
	8°C	1 day	0.38	0.15	0.30	1 and 7	1.24	1.85	0.20
		3 days	-0.25	0.06	0.48	1 and 8	0.54	2.36	-0.02
		6 days	-0.32	0.10	0.38	1 and 7	0.84	3.14	0.09
Notothenia coriiceps	0°C	1 day	0.33	0.11	0.42	1 and 6	0.74	2.04	0.04
		3 days	-0.44	0.19	0.23	1 and 7	1.75	2.01	-0.05
		6 days	-0.53	0.28	0.11	1 and 8	3.15	3.23	-0.27
	8°C	1 day	0.41	0.17	0.23	1 and 8	1.67	2.75	0.07
		3 days	-0.55	0.30	0.12	1 and 7	3.13	4.65	-0.44
		6 days	-0.23	0.05	0.54	1 and 7	0.40	2.69	-0.01

Oxidative stress indices

The GSH concentration was not modulated at 8°C for either species (Figure 3a and 3c). But the MDA content was modulated at 8°C in both species. In *N. coriiceps*, there was down regulation on day 3 at 8°C (Figure 3d). As for *N. rossii*, there was up regulation on day 1 followed by negative modulation on day 3 (Figure 3b).

Morphological analysis

The morphological changes found in the erythrocytes of *N. coriiceps* and *N. rossii* are show in Figure 4b-n and Table III.

In both species, mature erythrocytes showed oval to ellipsoid shapes. The central electron-dense nucleus is due to the predominance of heterochromatin that follows the cell

shape, and the cytoplasm has compartments delimited by membranes (Figure 4a). The temperature increase influenced the frequency of erythrocytes with altered shapes (Figure 4b-c) with cases of cell lysis being observed by EM (Figure 4g-h). At 8°C, *N. coriiceps* showed more cell shape changes on days 1 and 3, whereas *N. rossii* changes were observe mainly on day 6.

The 8°C, in *N. coriiceps* at 1 day of exposure the temperature increased the rate of erythrocytes presenting cell membrane vesicle formation (Figure 4d-e-f). The frequency of micronuclei was unaffected by the increased temperature or the exposure time in both *N. coriiceps* and *N. rossii*. The frequency of blebbed nuclei was influence by the 8°C temperature in *N. coriiceps* on days 3 and 6 and in *N. rossii* on day 6. The frequency of notched nuclei (Figure 4i) was influence by temperature and was higher at 8°C only for *N. coriiceps* on day 6.

DISCUSSION AND CONCLUSION

Studies have found that Antarctic fishes have higher antioxidant defence activity than tropical fishes (Coppe et al. 2013, Chen et al. 2008). This response appears to be related to the adaptation of these organisms to the low temperature conditions of the Antarctic environment, to the high dissolved oxygen amount and to the high concentration of intracellular lipids (Abele & Puntarulo 2004). These characteristics have led to significant specialization of the enzymes related to oxidative stress, thereby preventing them from being elevated as the temperature increases. And in *N. rossii* and *N. coriiceps*, the absence or weak modulation of antioxidant defenses is the more widespread behavior seen in the literature, as reported in several tissues (Kandalski et al. 2018, Souza et al. 2018, Forgati

Table II. Significance levels of the two-way ANOVA testing effects of temperature (TE) (0 and 8°C) and time of exposition (TI) (1, 3 and 6 days) of the erythrocytes of the *Notothenia rossii* and *Notothenia coriiceps* for SOD, CAT, GST, GR, GPx,GSH, MDA. * Indicated significant difference.

		SOD		CAT		GST		GR		GPx		GSH		MDA	
		F	P	F	P	F	P	F	P	F	P	F	P	F	P
Notothenia rossii	TE	7.95	0.01*	10.97	<0.01*	22.56	<0.01*	0.19	0.67	1.51	0.22	0.60	0.44	2.80	0.10
	TI	46.68	<0.01*	175.00	<0.01*	40.62	<0.01*	47.24	<0.01*	17.60	<0.01*	0.28	0.76	38.26	<0.01*
	TExTI	15.27	<0.01*	85.23	<0.01*	49.97	<0.01*	7.11	<0.01*	1.05	0.36	1.48	0.24	10.30	<0.01*
Notothenia coriiceps	TE	6.62	0.01*	139.000	<0.01*	12.09	0.01*	47.62	<0.01*	0.51	0.48	0.35	0.55	0.064	0.80
	TI	3.17	0.051	72.00	<0.01*	43.28	<0.01*	25.33	<0.01*	31.10	<0.01*	12.95	<0.01*	43.33	<0.01*
	TExTI	1.03	0.37	37.50	<0.01*	14.47	<0.01*	6.21	<0.01*	3.10	0.05	1.68	0.20	27.91	<0.01*

et al. 2017, Machado et al. 2014, Mueller et al. 2014, 2012).

The response patterns of the activity of antioxidant enzymes and morphology of erythrocyte relative to increased temperature were diferente between *N. rossii* and *N. coriiceps*. In both species, it were observed changes in the levels of enzymes of the antioxidant defense during at 0°C, we believe that such variations can be explained by operational reasons intrinsic to the experimental activity such as confinement and social isolation, since these factors can cause stress in fish, leading to physiological changes (Zuberi et al. 2014). However, to ensure that these factors did not influence the markers evaluated, the experiments at 8°C and their respective controls were carried out simultaneously and their experimental conditions, and abiotic factors strictly controlled, ensuring that differences observed between the experimental and control groups were due to temperature, the only variable tested in this study.

SOD is responsible for the conversion of the superoxide anion to H2O2, the latter being decomposed into O2 and H2O by CAT (Vasconcelos et al. 2007). These two enzymes are fundamental in antioxidant defense processes. Machado et al. (2014) analyzing the hepatic cells of *N. rossii* and *N. coriiceps* found no alterations in the activities of SOD and CAT and suggest that the modulation of these enzymes under thermal stress can be dependent on other cells, among them the erythrocytes, which have mechanisms for the removal of superoxide anions, since they carry high concentrations of oxygen. In *N. rossii* collected in the wild, SOD values were approximately 200-fold higher in erythrocytes than in liver (Ansaldo et al. 2000) and corroborate the high levels of SOD found in this work. In this work the temperature of 8°C was not able to modulate the erythrocyte SOD activity in *N. coriiceps* and similar results were observed by Klein et al. (2017). However, in *N. rossii* there was an increase in SOD activity in 6 days at 8 °C, this alteration may be a response to an increase in the production of superoxide radicals, resulting in a higher production of hydrogen peroxide, to which erythrocytes can be susceptible, since there was a decrease in CAT activity and absence in GPx modulation. The damage to hydrogen peroxide degradation may also have occurred in *N. coriiceps*, since there was a decrease in CAT activity in up to 3 days and no modulation of GPx.

The GST, GR and GPx enzymes integrate the antioxidant defense system and are markers used to detect oxidative stress against thermal stress in fish (Bagnyukova et al. 2007). In non-Antarctic marine fishes, the induction of heat stress may lead to an increase in GST activity to remove products of lipid peroxidation (Madeira et al. 2013). This may have occurred with *N. coriiceps*, since there was a decrease in the LPO levels in 3 days which was accompanied by increased GST at the same time, conversely, the decrease in MDA levels in 3 days may have influenced the reduction of GST levels in 6 days. Madeira et al. (2013) related that exposure to CTMax promotes protein denaturation, reducing antioxidant defenses and increasing LPO, indicating that in the case of strong oxidative stress, the activity of GST can be suppressed (Li et al. 2010).

The GR activity is involved in metabolism and the reduction of GSSG to GSH (Halliwell & Gutteridge 1999). However, studies warn that the action of multiple alternative systems present in the cytosol of erythrocytes, are able to reduce GSSG and maintain sufficiently high levels of GSH against oxidative stress (Morgan et al. 2013). In *N. rossii*, the absence of modulation of GR did not affect GSH levels, because this levels was not modulate by the temperature

Table III. Morphological analysis in *Notothenia coriiceps* and *N. rossii* erythrocytes (Shape, vesicular formation, micronuclei, blebbed and notched: number of cells means ± SE for each 1000 erythrocytes).

		0°C			8°C				
Notothenia rossii		**1 day**	**3 days**	**6 days**	**1 day**	**3 days**	**6 days**	**Kruskal-Wallis**	**P**
Cell morphology	Shape	4.17 ± 0.48 [a]	0.89 ± 0.20 [ab]	0.33 ± 0.24 [b] *	5.11 ± 1.65	3.40 ± 0.79	3.11 ± 0.68	26.62	<0.01*
	Vesicular formation	28.83 ± 3.96	22.56 ± 2.76	18.89 ± 1.79	35.78 ± 3.90	26.10 ± 0.98	43.33 ± 4.11	26.71	<0.01*
Nuclear morphology	Micronuclei	0.33 ± 0.21	0.00	0.22 ± 0.22	1.22 ± 0.43	0.40 ± 0.16	1.44 ± 0.85	12.98	0.02*
	Blebbed	13.17 ± 3.66	10.67 ± 1.24	12.33 ± 1.55*	18.33 ± 2.40	15.40 ± 1.84	27.22 ± 1.85	23.66	<0.01*
	Notched	2.83 ± 0.95	2.44 ± 0.77	2.67 ± 0.83	7.22 ± 2.40	4.00 ± 0.67	2.44 ± 1.43	13.12	0.02*
Notothenia coriiceps									
Cell morphology	Shape	1.75 ± 0.94*	6.11 ± 1.55*	4.90 ± 0.57	16.30 ± 4.63	14.56 ± 2.01	5.89 ± 0.56	28.02	<0.01*
	Vesicular formation	19.38 ± 3.35*	14.56 ± 2.05	27.80 ± 3.14	63.20 ± 7.38	28.44 ± 5.31	25.56 ± 1.93	30.89	<0.01*
Nuclear morphology	Micronuclei	1.00 ± 0.38	0.67 ± 0.29	0.20 ± 0.13	1.10 ± 0.43	0.75 ± 0.30	0.63 ± 0.35	4.51	0.48
	Blebbed	20.63 ± 2.85	7.22 ± 1.27*	9.90 ± 1.79*	21.70 ± 2.44	27.78 ± 5.49	29.78 ± 5.02	27.36	<0.01*
	Notched	5.38 ± 0.53	2.67 ± 0.87	2.40 ± 0.62*	5.80 ± 1.12	7.67 ± 1.86	8.78 ± 1.66	17.10	<0.01*

The asterisks indicate differences between the temperatures, and lowercase letters indicate differences between the times. The results of the Kruskal-Wallis statistical test and respective P are presented.

increase, thus supporting the theory that these oxidative stress markers may only be modestly affect by increased water temperature (Morgan et al. 2013). The GPx activity was unaffected by the elevated temperature in both species, this can be explained by microarray analysis reveals that the constitutive positive regulation of GPx in tissues of Antarctic notothenioids relative to that of temperate fishes is an evolutionary adaptation to an oxygen-rich environment (Chen et al. 2008).

Changes in erythrocyte cell and nuclear morphology are related to disorders that affect homeostasis (Tavares-Dias & Moraes 2004), including excess free radicals (Cimen 2008) and temperature variations (Qiang et al. 2013, de Pedro et al. 2005). Although the erythrocyte morphological changes are widely used to indicate environmental genotoxicity (Bhattacharjee & Das 2017, Maheshwari & Dua 2016, Grisolia et al. 2009), this methodology was pointed out as an efficient tool in the biomonitoring of thermal stress in notothenids (Zafalon-Silva et al. 2017). And this study attempted to ensure the quality and control of the experimental conditions, for both the animals submitted to the increased

temperature (8°C) and control animals (0°C). Then, the increase registered in the number of erythrocytes with altered shapes and nuclear alterations, was due because of heat stress.

The frequency of changes in erythrocyte shape was influence by theincrease in temperature, occurring more frequently in the early days for *N. coriiceps* and later for *N. rossii*. These changes in erythrocyte shape are related to cellular macromolecules that make up the cytoskeleton, including actin filaments, which are abundant in these cells and may lead to the loss of cytoplasmic contents as well as to apoptosis (Anbumani & Mohankumar 2012). Vesicle formation, occurs by the weakening of certain cytoskeletal areas (Wong et al. 2012), and cytoplasmic flow in these regions would have formed the protrusions (Taylor et al. 2008) observed in this work in *N. coriiceps*.

Changes in erythrocyte cell and nuclear morphology are related to disorders that affect homeostasis and may indicate an environmental impact on live organisms (Souza & Fontanetti 2006, Tavares-Dias & Moraes 2004, Talykina et al. 2003). It is believe that mutations in nuclear lamina could be responsible for the loss of the oval shape and the stability of the nuclear envelope (Anbumani & Mohankumar 2012, Strunjak-Perovic et al. 2009). In the present study, the temperature of 8°C did not influence the frequency of micronuclei, but it increased the frequency of blebbed e notched nuclei. Studies have indicated that these nuclear changes possess a similar origin to the micronucleus and allow for the removal and inactivation of damaged genetic material from the cell (Souza & Fontanetti 2006, Cavas & Ergene-Gozukara 2003). In addition, excess free radicals are capable of promoting DNA degradation and LPO in fish erythrocytes that damage cell membranes, causing irreversible damage that can lead to apoptosis (Cimen 2008).

Finally, in this study, statistical analysis showed that there was no correlation between oxidative stress markers and morphological changes indicating that it is not possible to relate the occurrence of oxidative stress to morphological changes. Point changes found in oxidative stress, indicating that these markers are not ideal for the thermal stress analysis in *N. rossii* and *N. coriiceps* erythrocytes. However, these results in conjunction with the Kandalski et al. (2018, 2019) studies, which involved plasma analysis, showed that markers such as cortisol and catalase are good blood indicators of heat stress (Kandalski et al. 2018), and are more suitable for biomonitoring than markers of stress oxidative in erythrocytes.

Among the morphological markers analyzed in this work, the shape change and nuclear bubble formation may be good stress biomarkers in erythrocytes of *N. rossii* and *N. coriiceps*. The highest occurrence of cellular and nuclear alterations due to thermal stress was observed in *N. coriiceps*, being observed at all exposure times (1, 3 and 6 days), while in *N. rossii* the highest number of alterations occurred in 6 days. These results suggest that *N. coriiceps* presents a lower tolerance to thermal stress at 8°C for up to 6 days, since the cellular and nuclear alterations recorded are pathological and may be deleterious to the cells. Thus, in order to elucidate which metabolic pathways or cellular components were responsible for the cellular alterations observed in this work, it is necessary to continue investigating the physiology of these species under thermal stress.

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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 Biochem Physiol A Mol Integr Physio 138: 405-415. doi:10.1016/j.cbpb.2004.05.013.

ALMEIDA JR, GRAVATO C & GUILHERMINO L. 2015. Effects of temperature in juvenile seabass (*Dicentrarchus labrax* L.*)* biomarker responses and behaviour: implications for environmental monitoring. Estuar Coast 38: 45-55. doi.org/10.1007/s12237-014-9792-7.

ANBUMANI SE & MOHANKUMAR MN. 2012. Gamma radiation induced micronuclei and erythrocyte cellular abnormalities in the fish *Catla catla.* Aquat Toxicol 122-123: 125-132. doi: 10.1016/j.aquatox.2012.06.00.

ANSALDO M, LUQUET CM, EVELSON PA, POLO JM & LLESUY S. 2000. Antioxidant levels from different Antarctic fish caught around South Georgia Island and Shag Rocks. Polar Biol 23: 160-165. doi: 10.1007/s003000050022.

ARIGONY-NETO J, SIMÕES JC & BREMER UF. 2004. Implementation of the Admiralty Bay Geographic Information System, King George Island, Antarctica. Rio de Janeiro: Pesquisa Antártica Brasileira, p. 187-190. ISSN 0103-4049.

BAGNYUKOVA TV, LUSHCHAK OV, STOREY KB & LUSHCHAK VI. 2007. Oxidative stress and antioxidant defense responses by goldfish tissues to acute change of temperature from 3 to 23° C. J Therm Biol 32: 227-234.

BEERS JM & SIDELL BD. 2011. Thermal tolerance of Antarctic notothenioid fishes correlates with level of circulating hemoglobin. Physiol Biochem Zool 84: 353-362. doi.org/10.1086/660191.

BEUTLER E. 1975. Red cell metabolism: a manual of biochemical methods. New York: Grune and Stratton, 160 p. doi:10.7326/0003-4819-83-6-9191.

BHATTACHARJEE D & DAS S. 2017. Microscopic Studies on Erythrocytes of *Channa punctata* Exposed to Commercial Grade Lindane. Braz Arch Biol Technol 60: 1-10. doi:10.1590/1678-4324-2017160341.

BILYK KT & DEVRIES AL. 2011. Heat tolerance and its plasticity in Antarctic fishes. Comp Biochem Physiol - Part A 158: 382-390. doi:10.1016/j.cbpa.2010.12.010.

BRADFORD MM. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248-254. doi:10.1016/0003-2697(76)90527-3.

BRAUN M, SIMÕES JC, VOGT S, BREMER UF, BLINDOW N, PFENDER M, SAURER H, AQUINO FE & ANDFERRON FA. 2001. An improved topographic database for King George Island: compilation, application and outlook. Antarct Sci 13(1): 41-52. doi:10.1017/S0954102001000074.

BRODTE E, GRAEVE M, JACOB U, KNUST R & PÖRTNER, HO. 2008. Temperature-dependent lipid levels and components in polar and temperate eelpout (Zoarcidae). Fish Physiol Biochem 34(3): 261-274. doi: 10.1007/s10695-007-9185-y.

CARLBERG I & MANNERVIK B. 1985. Glutathione-Reductase. Methods Enzymol 113: 484-490. PMID: 3003504.

CASSINI A, FAVERO M & ALBERGONI V. 1993. Comparative studies of antioxidant enzymes in red-blooded and white-blooded Antarctic teleost fish *Pagothenia bernacchii* and *Chionodraco hamatus*. Comp Biochem Physiol - Part C 106(2): 333-336. doi:10.1016/0742-8413(93)90142-8.

CAVAS T & ERGENE-GOZUKARA S. 2003. Micronuclei, nuclear lesions and interphase silver stained nucleolar organizer regions (AgNORs) as cytogenotoxicity indicators in *Oreochromis niloticus* exposed to textile mill effluent. Mutat Res 538: 81-91. doi:10.1016/S1383-5718(03)00091-3.

CHEN Z ET AL. 2008. Transcriptomic and genomic evolution under constant cold in Antarctic notothenioids fish. Proc Natl Acad Sci USA 105: 12944-12949. doi:10.1073/pnas.0802432105.

CIMEN MYB. 2008. Free radical metabolism in human erythrocytes. Clin Chim Acta 390: 1-11. doi:10.1016/j.cca.2007.12.025.

COPPE A, AGOSTINI C, MARINO IA, ZANE L, BARGELLONI L, BORTOLUZZI S & PATARNELLO T. 2013. Genome evolution in the cold: Antarctic icefish muscle transcriptome reveals selective duplications increasing mitochondrial function. Genome Biol Evol 5: 45-60. doi:10.1093/gbe/evs108.

CROCKETT EL. 2011. Antioxidant potential is positively correlated with mitochondrial enzyme activity in Antarctic and non-Antarctic notothenioid fishes. Polar Biol 34: 113-118. doi:10.1007/s00300-010-0864-2.

CROUCH RK, GANDY SC, KIMSEY G, GALBRAITH RA, GALBRAITH GMP & BUSE MG. 1981. The inhibition of islet superoxide dismutase by diabetogenic drugs. Diabetes 30: 235-241. doi: 10.2337/diab.30.3.235.

DE PEDRO N, GUIJARRO AI, LÓPEZ-PATIÑO MA, MARTÍNEZ-ÁLVAREZ R & DELGADO MJ. 2005. Daily and seasonal variations in hematological and biochemical parameters in the tench, *Tinca tinca* Linnaeus, 1758. Aquac Res 36(12): 1185-1196. doi:10.1111/j.1365-2109.2005.01338.x.

DI PRISCO G, EASTMAN JT, GIORDANO D, PARISI E & VERDE C. 2007. Biogeography and adaptation of Notothenioid fish: Hemoglobin function and globin–gene evolution. Gene 398: 143-155. doi:10.1016/j.gene.2007.02.047.

DONATTI L & FANTA E. 2002. Influence of photo period on visual prey detection in the Antarctic fish *Notothenia neglecta* Nybelin. Antarct Sci 14: 146-150.

FISHER W & HUREAU JC. 1985. FAO Species identification sheets for fishery purposes: Southern Ocean. FAO 2: 233-471.

FORGATI M, KANDALSKI PK, HERRERIAS T, ZALESKI T, MACHADO C, SOUZA MRDP & DONATTI L. 2017. Effects of heat stress on the renal and branchial carbohydrate metabolism and antioxidant system of Antarctic fish. J Comp Physiol B 187: 1137-1154. doi:/10.1007/s00360-017-1088-3.

GRIM JM, SIMONIK EA, SEMONES MC, KUHN DE & CROCKETT EL. 2013. The glutathione-dependent system of antioxidant defence is not modulated bytemperature acclimation in muscle tissues from striped bass, *Morone saxatilis*. Comp Biochem Physiol - Part A 164: 383-390.

GRISOLIA CK, RIVERO CLG, STARLING FLRM, SILVA ICR, BARBOSA AC & DOREA JG. 2009. Profile of micronucleus frequencies and DNA 67 damage in different species of fish in a eutrophic tropical lake. Genet Mol Biol 32: 138-143. doi:10.1590/S1415-47572009005000009.

GWOZDZINSKI K, PIENIAZEK A, TABACZAR S, JEGIER A & BRZESZCZYNSKA J. 2017. Investigation of oxidative stress parameters in differentlifespan erythrocyte fractions in young untrained menafter acute exercise. Exp Physiol 102(2): 190-201.

HALLIWELL B & GUTTERIDGE JMC. 1999. Free Radicals in Biology and Medicine. Oxford. OUP 3: 1-25.

HEISE K & ABELE D. 2008. Responde of blood parameters of the Antarctic fish *Notothenia coriiceps* (Richardson, 1844) to warming and hypoxia. Berichte zur Polarforschu 1: 10. hdl:10013/epic.30247.d001.

JAYASUNDARA N, HEALY TM & SOMERO GN. 2013. Effects of temperature acclimation on cardiorespiratory performance of the Antarctic notothenioid *Trematomus bernacchii*. Polar Biol 36: 1047-1057. doi: 10.1007/s00300-013-1327-3.

KANDALSKI PK, SOUZA MRDP, HERRERIAS T, MACHADO C, ZALESKI T, FORGATI M, GUILLEN AC, VIANA D, MOURA MO & DONATTI L. 2018. Effects of short-term thermal stress on the plasma biochemical profiles of two Antarctic nototheniid species. Rev Fish Biol Fisheries 28: 925-940. doi:10.1007/s11160-018-9535-0.

KANDALSKI PK, ZALESKI T, FORGATI M, BADUY F, EUGÊNIO DS, MACHADO C, SOUZA MRDP, PIECHNIK CA, FÁVARO LF & DONATTI L. 2019. Effect of long-term thermal challenge on the Antarctic notothenioid *Notothenia rossii*. Fish Physiol Biochem 45: 1445-1461.

KARNOVSKY MJA. 1965. Formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J Cell Biol 27: 137-138.

KEEN JH, HABIG WH & JAKOBY WB. 1976. Mechanism for several activities of the gluthatione Stransferases. J Biol Chem 251: 6183-6188.

KLEIN RD, BORGES VD, ROSA CE, COLARES EP, ROBALDO RB, MARTINEZ PE & BIANCHINI A. 2017. Effects of increasing temperature on antioxidant defense system and oxidative stress parameters in the Antarctic fish *Notothenia coriiceps* and *Notothenia rossii*. J Therm Biol 68: 110-118. doi.org/10.1016/j.jtherbio. 2017.02.016.

LI H, ZHOU X, WU M, DENG M, WANG C, HOU J & MOU P. 2016. The cytotoxicity and protective effects of Astragalus membranaceus extracts and butylated hydroxyanisole on hydroxyl radical-induced apoptosis in fish erythrocytes. Anim Nutri 2(4): 376-382.

LI HT, FENG L, JIANG WD, LIU Y, JIANG J, LI SH & ZHOU XQ. 2013. Oxidative stress parameters and anti-apoptotic response to hydroxyl radicals in fish erythrocytes: Protective effects of glutamine, alanine, citrulline and proline. Aquat Toxicol 126: 169-179. doi: 10.1016/j.aquatox.2012.11.005.

LI ZH, ZLABEK V, GRABIC R, LI P & RANDAK T. 2010. Modulation of glutathione-related antioxidant defence system of fish chronically treated by the fungicidepropiconazole. Comp Biochem Physiol Part C 152: 392-398. doi: 10.1016/j.cbpc.2010.06.006.

LUFT JA. 1961. Improvements in an epoxy resin embedding methods. J Biophys Biochem Cytol 9: 409-414.

MACHADO C, ZALESKI T, RODRIGUES E, CARVALHO CDS, CADENA SMSC, GOZZI GJ, KREBSBACH P, RIOS FSA & DONATTI L. 2014. Effect of temperature acclimation on the liver antioxidante defence system of the Antarctic nototheniids *Notothenia coriiceps* and *Notothenia rossii*. Comp Biochem Physiol Part B 172-173: 21-28. doi: 10.1016/j.cbpb.2014.02.003.

MADEIRA D, NARCISO L, CABRAL HN, VINAGRE C & DINIZ MS. 2013. Influence of temperature in thermal and oxidative stress responses in estuarine fish. Comp Biochem Physiol Part A 166: 237-243. doi: 10.1016/j.cbpa.2013.06.008.

MAHESHWARI S & DUA A. 2016. Structural Analysis of the erythrocytes of *Channa punctatus* (Bloch) exposed to

Mercuric Chloride using Scanning Electron Microscopy. J Fish Aquat Sci 16: 865-871. doi: 10.4194/1303-2712-v16_4_13.

MORGAN B, EZERIŅA D, AMOAKO TNE, RIEMER J, SEEDORF M & DICK TP. 2013. Multiple glutathione disulfide removal pathways mediate cytosolic redox homeostasis. Nat Chem Biol 9(2): 119-125. doi:10.1038/nchembio.1142.

MUELLER I, HOFFMAN M, DULLEN K & O'BRIEN K. 2014. Moderate elevations in temperature do not increase oxidative stress in oxidative muscles of Antarctic notothenioid fishes. Polar Biol 37: 311-320. doi: 10.1007/s00300- 013-1432-3.

MUELLER IA, DEVOR DP, GRIM JM, BEERS JM, CROCKETT EL & O'BRIEN KM. 2012. Exposure to critical thermal maxima increases oxidative. J Exp Biol 215: 3655-3664. doi:10.1242/jeb.071811.

MORGAN B, EZERIŅA D, AMOAKO TNE, RIEMER J, SEEDORF M & DICK TP. 2013. Multiple glutathione disulfide removal pathways mediate cytosolic redox homeostasis. Nat Chem Biol 9(2): 119-125. doi:10.1038/nchembio.1142.

NAGASAKA R, OKAMOTO N & USHIO H. 2004. Partial oxidative-stress perturbs membrane permeability and fluidity of fish nucleated red blood cells. Comp biochem physiol 139: 259-266.

NEAR TJ & CHENG CHC. 2008. Phylogenetics of notothenioid fishes (Teleostei: Acanthomorpha): Inferences from mitochondrial and nuclear gene sequences. Mol Phylogenetics Evol 47: 832-840. doi:10.1016/j.ympev.2007.11.027.

OLIVA M, NAVARRO F, HRBÁČEK F, HERNÁNDEZ A, NÝVLT D, PEREIRA P, RUIZ-FERNÁNDEZ J & TRIGOD R. 2016. Recent regional climate cooling on the Antarctic Peninsula and associated impacts on the cryosphere. Scien Total Environ 580: 210-223. doi.org/10.1016/j.scitotenv.2016.12.030.

PÖRTNER HO. 2002. Climate variations and the physiological basis of temperature dependent biogeography: systemic to molecular hierarchy of thermal tolerance in animals. Comp Biochem Physiol - Part A 132: 739-761. hdl:10013/epic.15426.d001.

QIANG J, YANG H, WANG H, KPUNDEH MD & PAO XU P. 2013. Interacting effects of water temperature and dietary protein level on hematological parameters in Nile tilapia juveniles, *Oreochromis niloticus* (L.) and mortality under *Streptococcus iniae* infection. Fish Shellfish Immunol 34: 8-16. doi:10.1016/j.fsi.2012.09.003.

RAGA G, PICHLER HA, ZALESKI T, SILVA FBV, MACHADO C, RODRIGUES E, KAWALL HG, RIOS FS & DONATTI L. 2015 Ecological and physiological aspects of the antarctic fishes *Notothenia rossii* and *Notothenia coriiceps* in Admiralty Bay, Antarctic Peninsula. Environ Biol Fishes 97: 1-14. doi: 10.1007/s10641-014-0311-2.

REYNOLDS ES. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17: 208-212.

RIOS FS. 2010. Hematologia. In: Oliveira-Ribeiro CA, Herculano SRF and Grotzner SR (Org). Técnicas e métodos para utilização prática de microscopia. Rio de Janeiro: Guanabara-Koogan, 440 p.

RODRIGUES JRE, FEIJO-OLIVEIRA M, NOHOMEKAWAGOE C, GANNABATHULA SV, DONATTI L, RODRIGUES E & LAVRADO HP. 2015. Metabolic responses of the Antarctic fishes *Notothenia rossii* and *Notothenia coriiceps* to sewage pollution. Fish Physiol Biochem 41(5): 1205-1220. doi:10.1007/s10695-015-0080-7.

RYAN SN. 1995. The effect of chronic heat stress on cortisol levels in the Antarctic fish *Pagothenia borchgrevinki*. Experientia 51: 768-774.

SATTIN G, BAKIU R, TOLOMEO AM, CARRARO A, COPPOLA D, FERRO D & SANTOVITO G. 2015. Characterization and expression of a new cytoplasmic glutathione peroxidase 1 gene in the Antarctic fish *Trematomus bernacchii*. Hydrobiologia 761(1): 363-372.

SEDLAK J & LINDSAY RHC. 1968. Estimation of total, protein bound and nonprotein sulfhydryl groups in tissue with Ellmann's reagent. Anal Biochem 25: 192-205. PMID: 4973948.

SOMERO GN & DEVRIES AL. 1967. Temperature tolerance of some Antarctic fishes. Science 156(772): 257. doi:10.1126/science.156.3772.257.

SOUZA MRDP, HERRERIAS T, ZALESKI T, FORGATI M, KANDALSKI PK, MACHADO C, SILVA DT, PIECHNIK CA, MOURA MO & DONATTI L. 2018. Heat stress in the heart and muscle of the Antarctic fishes *Notothenia rossii* and *Notothenia coriiceps*: carbohydrate metabolism and antioxidante defence. Biochimie 146: 43-55. doi.org/ 10.1016/j.biochi.2017.11.010.

SOUZA TS & FONTANETTI CS. 2006. Micronucleus test and observation of nuclear alterations in erythrocytes of Nile tilapia exposed to waters affected by refinery effluent. Mutat Res 605: 87-93. doi:10.1016/j.mrgentox.2006.02.010.

STROBEL A, LEO E, PÖRTNER HO & MARK FC. 2013. Elevated temperature and PCO_2 shift metabolic pathways in differentially oxidative tissues of *Notothenia rossii*. Comp Biochem Physiol - Part B 166(1): 48-57. doi: 10.1016/j.cbpb.2013.06.006.

STRUNJAK-PEROVIC I, POPOVIC NT, COZ-RAKOVAC R & JADAN M. 2009. Nuclear abnormalities of marine fish erythrocytes. J Fish Biol 74: 2239-2249. doi: 10.1111/j.1095-8649.2009.02232.x.

TALYKINA MG, PAPOULIAS DM, ALLERT JA, IZYUOV Y, VILLALOBOS SA, GIESY JP & TILLITT DE. 2003. The effect of polychlorinated naphthalenes and tributyltin on the occurrence of aberrant nuclei in erythroid cells of medaka. Environ Sci 10(6): 337-348.

TAVARES-DIAS M & MORAES FR. 2004. Hematologia de peixes teleósteos. Ribeirão Preto. Villimpress 1: 1-144. ISSN 178-7573.

TAYLOR RC, CULLEN SP & MARTIN SJ. 2008. Apoptosis: controlled demolition at the cellular level. Nat Rev Mol Cell Biol 9: 231-241. doi:10.1038/nrm2312.

TURNER J ET AL. 2014. Antarctic climate change and the environment – anupdate. The Pol Recor 50(3): 237-259.

UCHIYAMA M & MIHARA M. 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 86: 271-278. doi:10.1016/0003-2697(78)90342-1.

VASCONCELOS RP, REIS-SANTOS P, FONSECA V, MAIA A, RUANO M, FRANÇA S, VINAGRE C, COSTA MJ & CABRAL H. 2007. Assessing anthropogenic pressures on estuarine fish nurseries along the Portuguese coast: a multi-metric index and ceptual approach. Sci Total Environ 374: 199-215.

VODYANOY V. 2015. Thermodynamic evaluation of vesicles shed by erythrocytes at elevated temperatures. Colloid Surface B 133: 231-238.

WATSON ML. 1958. Staining of tissue sections for electron microscopy with heavy metals. J Biophys Biochem Cytol 4: 75-478.

WENDEL A. 1981. Glutathione peroxidase. Methods Enzymol 77: 325-333.

WONG SK, ZHANG XH & WOO NYS. 2012. Vibrio alginolyticus thermolabile hemolysin (TLH) induces apoptosis, membrane vesiculation and necrosis in sea bream erythrocytes. Aquaculture 330-333: 29-36. doi:10.1016/j.aquaculture.2011.12.012.

ZAFALON-SILVA B, ZEBRAL YD, BIANCHINI A, ROSA CE, MARINS LF, COLARES EP, MARTINEZ PE, BOBROWSKI VL & ROBALDO RB. 2017. Erythrocyte nuclear abnormalities and leukocyte profile in the Antarctic fish *Notothenia coriiceps* after exposure to shortand long-term heat stress. Pol Biol 40(9): 1755-1760.

ZUBERI A, BROWN C & ALI S. 2014. Effect of confinement on water-borne and whole body cortisol in wild and captive-reared rainbowfish (*Melanoteaniaduboulayi*). Int J Agric Biol 16(1): 183-188.

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