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Effect of temperature acclimation on the liver antioxidant defence system of the Antarctic notothenioids *Notothenia coriiceps* and *Notothenia rossii*

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ABSTRACT

The aim of this study was to determine whether endemic Antarctic notothenioid fish are able to adjust their liver antioxidant defence system in response to the temperature increase. The activity of the superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reductase (GR) enzymes as well as the content of non-enzymatic oxidative stress markers such as reduced glutathione (GSH), lipid peroxidation (LPO) and protein carbonyl (PC) were measured in the liver of two Antarctic fish species, *Notothenia rossii* and *Notothenia coriiceps* after 1, 3 and 6 days of exposure to temperatures of 0 °C and 8 °C. The GST activity showed a downregulation in *N. rossii* after 6 days of exposure to the increased temperature. The activity profiles of GST and GR in *N. rossii* and of GPx in *N. coriiceps* also changed as a consequence of heating to 8 °C. The GSH content increased by heating to 8 °C after 3 days in *N. coriiceps* and after 6 days in *N. rossii*. The content of malondialdehyde (MDA), a LPO marker, showed a negative modulation by the heating to 8 °C in *N. rossii* after 3 days of exposure to temperatures. Present results show that heating to 8 °C influenced the levels and profiles of the antioxidant enzymes and defences over time in the notothenioid fish *N. rossii* and *N. coriiceps*.

1. Introduction

Temperature is an abiotic factor that influences many biological processes and can determine and delimit the geographic distribution of living organisms (Windisch et al., 2011). In fish, the temperature can function as a stressing agent (Wedemeyer et al., 1990), and the metabolic rate of an ectothermic organism is strongly related to environmental temperature (Clarke and Johnston, 1996; Clarke and Fraser, 2004). Studies indicate that the thermal tolerance of an organism is closely related to its aerobic ability because both cooling and heating may alter oxygen balance in the tissues, promoting the generation of reactive oxygen species (ROS). These oxygen species react with lipids and nucleic acids, causing oxidative stress and damage to the cells (Abele et al., 1998; Stadtman and Levine, 2000; Martínez-Álvarez et al., 2005; Halliwell and Gutteridge, 2007). To prevent the increase of ROS levels cells use their antioxidant defences (Abele et al., 1998, 2001; Martínez-Álvarez et al., 2005; Heise et al., 2006), which can be either enzymatic or non-enzymatic (Sies, 1985; Martínez-Álvarez et al., 2005; Yilmaz et al., 2006).

The Southern Ocean is considered to be the coldest and most thermally stable marine environment on Earth. Sea temperature remains constant at approximately −1.9 °C in the areas close to the continent and can vary from +1.5 °C in summer to −1.8 °C in the winter in the Antarctic Peninsula and islands north of it (Sidell, 2000). The current environmental pressure on Antarctica (Clarke et al., 2007; Hellmer et al., 2012; Ross et al., 2012) indicates that the water temperature of the Austral Ocean has increased approximately 0.2 °C since 1950 at depths of 700 to 1100 m, between 35 and 65°S. Studies show that this increase in temperature extends to superficial waters (King, 1994; King and Harangozo, 1998; Renwick, 2002; Turner et al., 2005).

The fish that inhabit the Southern Ocean have developed adaptation mechanisms to extremely low temperatures that are responsible for their survival in this environment (Crossley, 1995; Somero et al., 1996; Chen et al., 1997; Jin and DeVries, 2006; diPrisco et al., 2007; Brodte et al., 2008; Crockett, 2011). Attributed to the higher oxygen

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solubility in cold sea water, a high concentration of oxygen in tissues of polar fish and consequently high levels of ROS can be expected (Ansaldo et al., 2000; Abele and Puntarulo, 2004). Fish species in the Antarctic, the high concentration of solutes in the aqueous cytosol, and increased surface area and density of mitochondria may facilitate the adaptation of these animals to such changes in tissue oxygen conductance influenced by low temperatures (Sidell and Hazel, 1987; Sidell, 1998; Guderley and St-Pierre, 2002; Abele and Puntarulo, 2004). Although the polar animals are adapted to cold and balanced environment in relation to antioxidant processes resulting in this adaptation, these animals are sensitive to oxidative stress caused by other stressors such as increased temperature, intoxication radiation, starvation, etc. (Abele and Puntarulo, 2004; Abele, 2012).

The acclimation and thermal tolerance of Antarctic organisms to high temperatures have been studied, and studies on this topic are increasing due to global climate change (Somero and DeVries, 1967; Brauer et al., 2005; Lannig et al., 2005; Lowe and Davison, 2005; Jin and DeVries, 2006; Clarke et al., 2007; Franklin et al., 2007; Brodte et al., 2008; Robinson and Davison, 2008; Clark and Peck, 2009; Bilyk and DeVries, 2011; Windisch et al., 2011; Jayasundara et al., 2013). The activity and expression of antioxidant enzymes and other oxidative stress markers have been used for the evaluation of Antarctic organisms subjected to thermal stress. Mueller et al. (2012) tested the hypothesis that the lower thermal tolerance of icefishes from the family Channichthyidae (white-blooded) compared with those from the family Nototheniidae (red-blooded) (Bilyk and DeVries, 2011) may stem from a larger vulnerability of the white-blooded fish to oxidative damage as a result of the exposure to a maximum critical temperature (CTMax). Thorne et al., 2010 evaluated, among other markers, the expression profiles of some antioxidant enzymes in the liver of *Harpagifer antarcticus* (the Antarctic plunderfish) exposed to a temperature of 6 °C in 48 h and no differences were observed in gene regulation of antioxidants "classics".

The present study aimed at investigating whether the hepatic tissue of the notothenids (*Notothenia rossii* and *Notothenia coriiceps*) adapts its oxidative metabolism and its antioxidant defence in response to an increase of environmental temperature despite the fish live in a cold and thermally stable environment. These two species are phylogenetically very close (Near and Cheng, 2008) and found at the same places in many Antarctic regions (Bellisio and Tomo, 1974), but they have different geographical distributions (Gon and Heemstra, 1990) and are, therefore, subjected to different environmental temperatures.

2. Materials and methods

The experiments were performed at the Brazilian Antarctica Station Comandante Ferraz (EACF) located at Admiralty Bay (61°S and 63°30′S and 53°55′W and 62°50′W), King George Island, in the South Shetland Islands, Antarctic Peninsula. The environmental licences were granted by the Environmental Assessment Group of the Brazilian Ministry of Environment. The experiments were approved by the Ethics Committee of the Federal University of Paraná (UFPR) under the number 496.

2.1. Temperature bioassays

N. coriiceps (n = 60; TL = 35 ± 3 cm; W = 775 ± 182 g) and *N. rossii* (n = 60; TL = 37 ± 3 cm; W = 791 ± 145 g) were collected with hook and line at 10–25 m depth. After collection, fish were maintained for 3 days, according to Ryan (1995), in 1000-L tanks with controlled abiotic conditions, a salinity of 35 ± 1.0, a temperature of 0 ± 0.5 °C and a photoperiod of 12 h of light and 12 h of dark. The fish were fed with epaxial muscle every two days, alternating with the change of water in the tanks.

For each species, the fish were randomly selected and transferred to 1000-L tanks containing sea water with a temperature of 8 ± 0.5 °C (experimental) for 1, 3 or 6 days or 0 ± 0.5 °C (control) for 1, 3 or 6 days. Ten individuals were used for each experiment. The water temperature of the tanks was rigorously maintained with Aquatherm 09-01T-11457 thermostats (Full Gauge) coupled to heaters (Altman).

The temperature of 0 °C was chosen as the control and is within the thermal variation range found in the areas close to the Antarctic Peninsula and South Shetland Islands (Sidell, 2000), where the fish were captured. The experimental temperature of 8 °C approaches the thermal tolerance of Antarctic fish as reported by Somero and DeVries (1967), Pörtner et al. (2004), Lowe and Davison (2005), Franklin et al. (2007) and Strobel et al. (2013).

Behavioural observations were made during the experiments based on previous studies of Antarctic fishes (Fanta et al., 2001a, 2001b; Fanta et al., 2002; Donatti and Fanta, 2007, 2002). Qualitatively, the motility of animals (resting or swimming), the agonistic interactions between specimens and food consumption were observed. *N. rossii* and *N. coriiceps* survived the times (1, 3 and 6 days) and temperatures (0 °C and 8 °C). Higher swimming speed and greater competition for food in *N. rossii* were observed to 8 °C.

After the bioassays were performed, the animals were anesthetised with 1% (p/v) benzocaine and killed by spinal transection. Subsequently, liver samples were removed, frozen in liquid nitrogen and stored in a freezer at −80 °C.

2.2. Analytical methods

The liver samples from *N. coriiceps* and *N. rossii* were homogenised in phosphate-buffered saline (PBS), pH 7.2, and then centrifuged at 12,000 g at 4 °C for 20 min. The supernatant was separated for the determination of protein concentration, the activity of antioxidant enzymes and the non-enzymatic marker levels.

Total protein concentration was determined according to the Bradford method (1976), using bovine serum albumin (BSA) as the standard. The absorbance of the samples was measured at 545 nm.

2.3. Assay of antioxidant enzyme activities

CAT (EC 1.11.1.6) activity was evaluated by measuring the consumption of hydrogen peroxide using a spectrophotometer at 240 nm (Beutler, 1975). The reaction medium contained potassium phosphate buffer (50 mM, pH 7.0) and 1.0 mg/mL of protein. The reaction was initiated by the addition of 10 mM H_2O_2 and monitored for 60 s at 25 °C.

The activity of SOD (EC 1.15.1.1) was determined according to the methodology described by Crouch et al. (1981), which is based on the ability of this enzyme to inhibit the reduction of nitroblue tetrazolium (NBT) into blue formazan by the O_2^- generated by the hydroxylamine in the alkaline solution. The reaction system was maintained at 25 °C and consisted of NBT solution (100 μM) and hydroxylamine in alkaline solution (36.85 mM). This inhibition was spectrophotometrically measured at 560 nm.

The activity of GR (EC 1.8.1.7) was evaluated spectrophotometrically by measuring the oxidation of NADPH and the concomitant reduction of glutathione disulphide (GSSG) at 340 nm (Carlberg and Mannervik, 1985). The reaction medium contained potassium phosphate buffer (100 mM, pH 7.0), 5.0 mM EDTA, 0.5 mM NADPH and 5 mM GSSG. The reaction was started by the addition of GSSG and monitored for 10 min.

The activity of GPx (EC 1.11.1.9) was determined by measuring the oxidation of NADPH at 340 nm. The enzyme uses GSH to reduce organic peroxide, resulting in GSSG. GSSG is reduced by the enzyme GR using electrons donated by NADPH (Wendel, 1981). The reaction system consisted of 100 mM phosphate buffer (pH 7.0), 2 mM reduced glutathione (GSH), 0.2 mM NADPH, 0.2 U of purified glutathione reductase (GR), 0.5 mM *t*-butyl hydroperoxide and 1 mg/mL of protein. The reaction occurred at 25 °C and was initiated by the addition of 0.5 mM hydrogen peroxide and monitored for 3 min at 340 nm.

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