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# Effects of increasing temperature on antioxidant defense system and oxidative stress parameters in the Antarctic fish Notothenia coriiceps and Notothenia rossii

Roberta Daniele Klein[a], Vinicius Dias Borges[a], Carlos Eduardo Rosa[a,b], Elton Pinto Colares[a,b], Ricardo Berteaux Robaldo[c], Pablo Elias Martinez[a,b], Adalto Bianchini[a,b,*]

[a] *Programa de Pós-Graduação em Ciências Fisiológicas, Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Av. Itália, km 8, 96203-900, Rio Grande, RS, Brazil*
[b] *Instituto de Ciências Biológicas, Universidade Federal do Rio Grande, Av. Itália, km 8, 96203-900, Rio Grande, RS, Brazil*
[c] *Instituto de Biologia, Universidade Federal de Pelotas, Campus Universitário s/n, 96010-900, Pelotas, RS, Brazil*

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## ABSTRACT

Antioxidant defense system (ADS) and oxidative stress parameters were evaluated in the Antarctic fish *Notothenia rossii* and *N. coriiceps* exposed to increasing temperature. Acclimated fish were kept at 0 °C or exposed to 4 °C for 1 day (*N. rossii*) or to 2 and 4 °C for 1 and 6 days (*N. coriiceps*). Measurements were assessed in brain, gills, liver, white muscle and erythrocytes. Parameters analyzed included antioxidant capacity against peroxyl radicals (ACAP); reduced glutathione (GSH) and metallothionein-like proteins (MTLP) concentration; superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutamate-cysteine ligase (GCL) activity; lipid peroxidation (LPO) level and protein carbonyl (PC) concentration. Increased liver GST activity was observed in *N. rossii* exposed to 4 °C for 1 day. Increased muscle GPx activity was observed in *N. coriiceps* after exposure to 2 °C for 1 day. Reduced gill GPx activity and increased liver SOD activity were observed after exposure to 4 °C for 1 day. In *N. coriiceps*, increased gill GCL activity and reduced gill GPx activity, as well as reduced liver MTLP were observed after exposure to 2 °C for 6 days. Reduced brain SOD activity and increased brain LPO; reduced gill ACAP, GSH concentration and GPx activity, as well as increased gill GCL activity; reduced liver ACAP, MTLP, SOD activity, GST activity and increased liver and erythrocytes LPO were observed after exposure to 4 °C for 6 days. These findings indicate that ADS is more responsive to short-term increasing temperature in the sluggish *N. coriiceps* than in the active *N. rossii*. However, responses of *N. coriiceps* to long-term increasing temperature were transient and did not prevent tissue oxidative damage. Considering the predicted increase in temperature in the Southern Ocean over the next decades, our findings suggest that Antarctic fishes are sensitive to ocean warming, displaying tissue oxidative damage associated with the thermal stress.

## 1. Introduction

Organisms living in the Antarctic Ocean are continually exposed to environmental constraints (di Prisco and Verde, 2006; Peck et al., 2004). Antarctic waters are cold and there is only a narrow variation in temperature according to season (−1.86–1.5 °C) (Podrabsky and Somero, 2006). However, the Antarctic Peninsula is experiencing one of the strongest and quickly regional warming trend, which is more than twice of that observed for the global mean temperature (Mulvaney et al., 2012; Turner et al., 2014, 2005). In this region, the atmospheric temperature raised nearly 3 °C since 1950 (Vaughan, 2006), and in this same period, temperature of surface waters in the adjacent ocean raised more than 1 °C (Meredith and King, 2005). Furthermore, models predict that this trend of significant surface warming in Antarctica will continue during this century (Convey et al., 2009). Also, projections on climate change indicate that temperature on Earth will even rise between 2.6 and 4.8 °C until 2100 (IPCC, 2014).

The Antarctic coast is predominantly dominated by endemic fish species belonging to the suborder Notothenioidei (Eastman, 2005). The cold adaptation can be seen in several biochemical and physiological aspects in notothenioid fish. Some examples are the presence of higher density of mitochondria, presence of enzymes adapted to low tempera-

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* Correspondence to: Universidade Federal do Rio Grande–FURG, Instituto de Ciências Biológicas, Av. Itália km 8-Campus Carreiros, 96203-900 Rio Grande, RS, Brazil.
*E-mail address:* adaltobianchini@furg.br (A. Bianchini).

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tures, accumulation of lipid droplets in cytosol and higher percentage of polyunsaturated fatty acid (PUFA) in membranes (Beers and Jayasundara, 2015; Russo et al., 2010). Also, due the evolution occurred over 14 million years in thermally stable waters, there was no selective pressure for these fishes to maintain the heat shock response, that prevent thermal denaturation of proteins by the induction of heat shock proteins (Hofmann et al., 2000). Furthermore, these endemics fishes are extremely stenothermics. The upper incipient lethal temperature (UILT) for Antarctic notothenioid fishes was shown to be close to 5–6 °C (Somero and DeVries, 1967). Recent studies has demonstrated that several notothenioid fish show an increase in their UILT, survival time and critical thermal maximum (CTMax) when acclimated to a temperature higher than that observed in the natural environment before transfer to elevated temperatures (Bilyk and Devries, 2011; Podrabsky and Somero, 2006). Furthermore, Antarctic species from different biogeographic regions present differences in thermal tolerance limits. Nototheniid species endemic from the more thermally variable Seasonal Pack-ice Zone (SPZ), which include the Antarctic Peninsula region, showed a higher CTMax when compared with the species from McMurdo Sound, located in the High-Antarctic Zone (HAZ) region (Bilyk and Devries, 2011). However, the plasticity of thermal tolerance varies between species and the amplitude of such variation is lower than that observed for non-polar fishes (Beers and Jayasundara, 2015; Bilyk and Devries, 2011).

Considering the possible impact of climate changes on the Antarctic biota, the need for studies characterizing the ability of Antarctic organisms to deal with changes in water temperature is crucial. Indeed, these organisms can be vulnerable to long term changes in water temperature due their thermal specialization (di Prisco and Verde, 2006; Peck et al., 2004). Although Antarctic fishes are able to acclimate to increased temperatures, recent studies have demonstrated that exposure to elevated temperatures led to changes in metabolic processes that indicate a rise in anaerobic metabolism (Jayasundara et al., 2013; Van Dijk et al., 1999), as well as cell proliferation and apoptosis in hepatocytes of Antarctic fishes (Sleadd et al., 2014). Also, some fishes presented a limited cardiorespiratory capacity when exposed to higher temperatures (Egginton and Campbell, 2016; Jayasundara et al., 2013). In fact, this can contribute to the narrow window of thermal tolerance in Antarctic notothenioids. Moreover, as observed in Antarctic invertebrates (Heise et al., 2003) and fishes (Mueller et al., 2011), thermal stress increases oxygen consumption and consequently the reactive oxygen species (ROS) production (Heise et al., 2003; Mueller et al., 2011).

ROS generation can induce damage to biological molecules, with oxidative stress occurring when the antioxidant defense system (ADS) is overwhelmed by ROS production or when the redox signaling is disrupted, thus affecting cell functionality (Halliwell and Gutteridge, 2007). Considering the vulnerability of Antarctic fishes to oxidative stress due to the increased mitochondria volume density, which could raise the rate of ROS formation, as well as the increased risk of lipid peroxidation (LPO), as polyunsaturated fatty acids (PUFA) are easy target of oxidation (Abele and Puntarulo, 2004), a complete analysis of the antioxidant capacity can provide a better understanding of how Antarctic fish will respond to increase in temperature of natural sea water. However, only few studies were performed to evaluate the effects of temperature increase on oxidative stress parameters in Antarctic fishes. In the species evaluated up to now (*Gobionotothen gibberifrons*, *Notothenia coriiceps*, *N. rossii*, *Chaenocephalus aceratus* and *C. rastrospinosus*), increase in temperature was followed or not by changes in ROS generation, ADS response, and oxidative stress. Responses were dependent on species and tissue analyzed, as well as on the time and temperature of exposure tested (Machado et al., 2014; Mueller et al., 2013, 2012, 2011).

In the present study, we evaluated the effect of increasing sea water temperature on the response of a large suite of parameters related to ADS (enzymatic and non-enzymatic antioxidants) and oxidative damage (lipid and protein damage) in fish tissues or cells (brain, gills, liver, white muscle and erythrocytes). Experiments were performed on two of the most abundant notothenioids species at the Admiralty Bay (King George Island, Antarctica), the marbled notothen *N. rossii* and the rockcod *N. coriiceps*. The former is a benthopelagic fish and more active swimmer while the latter is a benthic and sluggish fish (Eastman et al., 2011; Hemmingsen et al., 1969; Morris and North, 1984). Some previous studies have demonstrated that both fishes can have different sensitivity to increasing temperature (Mark et al., 2012) and sewage pollution (Rodrigues Jr. et al., 2015). Also, we have demonstrated that ADS profile was different in several tissues of these two congener notothenioids fish. The marbled notothen *N. rossii* showed a higher antioxidant capacity in brain and peripheral tissues (gills, liver and white muscle) than the rockcod *N. coriiceps* (Klein et al., 2017). Therefore, it is expected that these species would also show a different sensitivity of the ADS in response to increasing sea water temperature. It is worth noting that the present study is first one to evaluate of a large suite of antioxidants, as well as, the oxidative status in several tissues of the marbled notothen *N. rossii* and the rockcod *N. coriiceps* in response to increased temperature.

## 2. Material and methods

### 2.1. Fish collection and acclimation

Notothenioids fish (*N. rossii* and *N. coriiceps*) were caught in December 2006 and January 2007 at the Admiralty Bay (King George Island, Antarctica; 62° 06´S and 58° 23´W). They were collected using nets onboard of vessels of the Brazilian Antarctic Station "Comandante Ferraz". The marbled notothen *N. rossii* (n =9; wet weight =311.3 ± 60.3 g; standard length =25.2 ± 1.6 cm; total length =29.8 ± 1.9 cm) and the rockcod *N. coriiceps* (n =27; wet weight =557.1 ± 47.8 g; standard length =30.5 ± 0.8 cm; total length =35.1 ± 0.8 cm) were collected and transferred to the Brazilian Antarctic Station.

In laboratory, fish were acclimated to 0 °C (−0.01 ± 0.05 °C) for 5 days. Natural daylight photoperiod was used. Every day, acclimation medium was renewed (90%). Nototheniids were fed daily with slices of other fish species resulting from the by-catch. Natural Antarctic sea water was employed for experimental medium during both acclimation and experimental periods.

After acclimation, the rockcod *N. coriiceps* was subjected to short-term (1 day) and long-term (6 days) increase in sea water temperature to reach 2 and 4 °C. Unfortunately, the marbled notothen *N. rossii* was subjected only to short-term experiments of increase in sea water temperature to reach 4 °C, since not enough fish was available in the collected site to run all set of experiments.

For short-term experiment, marble notothens (*N. rossii*) were randomly divided into two different groups while rockcods (*N. coriiceps*) were randomly divided into three different groups. In the control group, both *N. rossii* and *N. coriiceps* were kept at temperature 0 °C (n =4 for each species). In the first treatment (2 °C), only *N. coriiceps* was tested (n =4). In this case, the sea water temperature was raised up from 0 to 1 °C within 30 min, kept at 1 °C in the first 2 h of experiment, increased from 1 to 2 °C within 30 min, and then kept at 2 °C until the end of the trial (24 h). In the second treatment (4 °C), both *N. rossii* and *N. coriiceps* were tested (n =5 for each species). In this case, water temperature was raised up from 0 to 3 °C at the rate of 1 °C/30 min, kept at 3 °C in the first 2 h of experiment, increased from 3 to 4 °C within 30 min, and then kept at 4 °C until the end of the trial (24 h). Sea water in the experimental medium was then continuously heated to maintain the desired temperature. It was controlled using calibrated thermostatic heaters (TIC17RGTi, Full Gauge Controls, Canoas, RS, Brazil). Mean measured temperature of sea water corresponded to 0.11 ± 0.03 °C, 1.84 ± 0.09 °C, and 3.89 ± 0.07 °C for the desired temperatures of 0 (control), 2 and 4 °C, respectively. Dissolved oxygen content in sea water was measured using a hand oxymeter. No