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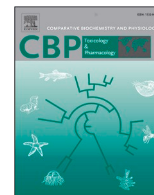

Research article

Heatwaves beneath the ice: organ-specific physiological responses in the Antarctic emerald rockcod, *Trematomus bernacchii* (Boulenger, 1902)

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ABSTRACT

Over the past few decades, the climate has undergone significant transformations, with increasing temperatures emerging as one of the most critical threats to marine ecosystems. Mounting evidence of marine heatwaves (MHWs) events in the Southern Ocean and Antarctic region has intensified scientific efforts, revealing that these fragile ecosystems may act as crucial early indicators of wider climate-driven changes in marine environments' biodiversity. This study explores the transcriptional responses of *Trematomus bernacchii* to thermal stress, focusing on the liver and spleen, key organs in immune defence and metabolic regulation. Adult specimens (total $n = 30$) were collected from Terra Nova Bay (Ross Sea) during the austral summer (late October 2022) and exposed to a temperature gradient, mimicking MHWs, ranging from 0 °C to +3 °C; a separate control group was maintained at 0 °C. Expression levels of antioxidant-related genes (*prdx3*, *prdx5*, *cat*, *sod1*, *sod2*, *gpx1*, *gpx3*, *gpx4*) along with two innate immunity genes (*tlr2* and *tlr9*) were analysed in liver and spleen tissues from five biological replicates. The liver showed an early and dynamic response: *gpxs*, *sod2*, *Prdxs*, and *tlrs* were upregulated at +1 °C and +2 °C but downregulated at +3 °C, suggesting early mitochondrial antioxidant response and immune activation. In contrast, the spleen remained largely unresponsive at mild stress levels, with marked activation of *gpx1*, *sods*, *Prdxs*, and *tlrs* only at +3 °C. *Cat* remained unresponsive to thermal stress. These results indicate a delayed but coordinated response, suggesting an energy-conservation mechanism under increasing thermal stress. They underscore the limited plasticity of polar fish physiology and offer molecular insights into the vulnerability of Antarctic marine species in a warming climate.

1. Introduction

Anthropogenic climate change is causing rapid and profound transformations in marine ecosystems worldwide, with polar regions experiencing some of the fastest and most unpredictable shifts. Among these shifts, Antarctica stands out not only for its climatic extremes but also for the increasing frequency of unusual warming events, challenging its historic reputation for thermal isolation (Ducklow et al., 2013). This warming has been particularly pronounced along the western coast of the Peninsula, during the summer months, with significant

Abbreviations: CAT, catalase; DAMP, damage-associated molecular pattern; GPX, glutathione peroxidase; HIS, hepato-somatic index; MHW, marine heatwave; PRDX, peroxiredoxin; PRR, pattern recognition receptor; qRT-PCR, quantitative Real time PCR; ROS, reactive oxygen species; SEM, standard error of the mean; SOD, superoxide dismutase; SSI, spleen-somatic index; TLR, toll-like receptor.

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consequences for the cryosphere, contributing to glacier recession and a reduction in sea ice (Ding and Steig, 2013).

In parallel, increased freshwater input into coastal systems is leading to ocean freshening, which, as demonstrated by recent studies, may further challenge ectothermic organisms by altering osmotic balance, metabolic processes, and thermal sensitivity (Pörtner et al., 2004; Vargas-Chacoff et al., 2021).

Previous studies have shown that *T. bernacchii* exhibits limited upper thermal tolerance and lacks an inducible heat shock protein response, indicating constrained physiological capacity to cope with acute warming (Hofmann et al., 2000). Nonetheless, transcriptomic and physiological analyses have revealed that *T. bernacchii* can mount a coordinated cellular stress response to longer-term temperature increases, involving metabolic and immune pathways, even if classical heat-shock responses remain muted (Dowd and Kueltz, 2023). Oxidative stress in fish affects both immune regulation as well as cellular integrity. Reduced immune competence can result from elevated ROS levels that alter cytokine release, reduce phagocytic activity, and disrupt the balance between pro- and anti-inflammatory responses (Gao et al., 2024). The impacts are particularly pronounced in cold-adapted Antarctic species, where low constitutive antioxidant capacity and limited metabolic flexibility may aggravate oxidative–immune interactions in increasing temperatures (Saravia et al., 2022; Scharsack and Franke, 2022). This interaction indicates the heat-induced oxidative imbalance could damage the innate immune systems of polar teleosts, further reducing their capacity to endure marine heatwaves.

Episodic marine heatwave (MHW) events, in the Southern Ocean, can last from a few days to several weeks (Samuels et al., 2021), a concerning trend given its high biodiversity and the presence of species already living near their thermal tolerance limits (Smale et al., 2019). In the Southern Ocean, these events are becoming more frequent and intense, with significant impacts on marine ecosystems (Smith et al., 2023), as they challenge the survival and resilience of endemic Antarctic fish, such as the emerald rockcod *T. bernacchii*, a stenothermal species highly adapted to survive extreme thermal constancy, with physiological systems finely tuned to function within a narrow temperature range (Dowd and Kueltz, 2023).

Even modest increases in environmental temperature can cause wide-ranging disruptions in cellular homeostasis. MHWs events may trigger both direct and indirect effects on the body and on many biological functions, including heart rate, metabolism, reproduction, behaviour, and immune responses, leading to mass mortality events (Villeneuve and White, 2024). These disruptions could overwhelm the organism's capacity to maintain redox balance and immune integrity, compromising the organism's resilience to stress.

One of the primary consequences of this imbalance induced by thermal stress is the overproduction of reactive oxygen species (ROS), driven by enhanced mitochondrial respiration and electron transport chain leakage (Banh et al., 2016; Filho, 2007). Although ROS play signalling roles at physiological levels, their accumulation under stress conditions can result in oxidative damage to lipids, proteins, and nucleic acids (Madkour, 2020; Pacchini et al., 2023). To counteract this, organisms rely on a complex network of antioxidant defences. In teleost fish, the glutathione peroxidases (GPXs), superoxide dismutases (SODs), catalase (CAT), and peroxiredoxins (PRDXs) represent core components of this defence system (Nualart et al., 2025; Pacchini et al., 2025a; Schumann et al., 2025). There are also proteins and molecules rich in cysteine, such as glutathione and metallothioneins, which play a fundamental role as a non-enzymatic scavengers system (Bakiu et al., 2022; Espírito-Santo et al., 2025). These proteins and molecules act in a combined manner to neutralise superoxide radicals and hydrogen peroxide, preserving cellular integrity and maintaining redox homeostasis. The transcriptional regulation of these genes in response to temperature elevation provides insight into how Antarctic fish attempt to mitigate oxidative damage during heat stress (Bakiu et al., 2024; Rizzotti et al., 2022).

These thermal fluctuations not only affect oxidative pathways but has been shown to modulate neuroendocrine regulation through the hypothalamic-pituitary-interrenal (HPI) axis (Faught and Schaaf, 2024; Schumann et al., 2024). In teleost fish, heat stress primarily stimulates cortisol synthesis, which in turn drives systemic physiological adjustments, including changes in intermediary metabolism and osmoregulation (Farrell, 2011). Because ion balance and osmotic homeostasis rely on membrane fluidity, membrane-associated proteins, and enzymatic function, these processes are highly temperature sensitive and can be altered under thermal stress (Vargas-Chacoff et al., 2021, 2020). In addition to these physiological effects, cortisol-mediated signalling influences cytokine networks, innate immune activity, and overall stress resilience. At the cellular level, thermal stress can induce protein damage, triggering the activation of heat-responsive genes, proteasomal degradation of irreversibly damaged proteins, and, under severe conditions, programmed cell death pathways (Saravia et al., 2022).

In teleosts, temperature modulates immune activity, with innate immune responses predominating at low temperatures, while acquired immunity operate more effectively at intermediate temperatures (Scharsack and Franke, 2022; Xv et al., 2024). Stress can markedly affect the number and distribution of leucocytes in fish blood and tissues; acute stress rapidly leads to a transient increase in circulating leukocytes, typically involving a reduction in lymphocytes and monocytes, together with an elevation in neutrophils (Dhabhar, 2002; Wojtaszek and Adamowicz, 2003). Chronic stress results in a decline in overall leucocyte numbers in circulation. Among immune effector molecules, immunoglobulins are a key group. In teleosts, immunoglobulins are classified mainly as IgM, IgD, and IgT/IgZ (species-dependent). IgM is the predominant serum isotype, whereas IgT/IgZ plays a key role at mucosal surfaces (Bilal et al., 2021; Watts et al., 2001).

In addition, key elements of the innate immune system are toll-like receptors (TLRs), a class of proteins that belong to the pattern recognition receptors (PRRs) family that recognize pathogen-associated molecular patterns (PAMPs) and are key elements of the innate immune response against pathogens (Akira et al., 2001). To date, 21 TLRs have been identified in multiple teleost species and are categorised into six families according to their genetic nature and the specific pathogens that they can identify. TLR2 and TLR9 are especially important in fish immunity, recognising bacterial lipoproteins and unmethylated CpG DNA motifs, respectively.

Stress-induced changes in the expression of these receptors may reflect immune shifts or systemic inflammation, highlighting their value as molecular markers of immune reactivity (Zhang et al., 2014).

In Antarctic and sub-antarctic fish (like *Notothenia rossii*, *N. coriiceps*, and *Eleginops maclovinus*), thermal stress can trigger vertebrate-like inflammatory responses. For instance, results obtained in *Harpagifer antarcticus* highlight transcriptional shifts in energy metabolism and oxidative stress pathways despite lacking a classical heat shock response (Saravia et al., 2022; Guillen et al., 2022; Martínez et al., 2020; Thorne et al., 2010; Zafalon-Silva et al., 2017). Furthermore, high temperature increases expression of several genes involved in the innate immune response, among others, *tlr1* and *tlr3*. Molecular analysis of the *tlr2* gene of the Antarctic species *T. bernacchii* and *Chionodraco hamatus* highlights specific codons under significant Darwinian selection and negative selection. This adaptive process involves changes in the molecular structure to enhance flexibility, allowing it to function under extremely cold conditions (Varriale et al., 2012).

To comprehensively understand the physiological impact of thermal stress, it is essential to investigate not only gene expression patterns but also organ-specific responses. The liver supports detoxification, metabolism, and the production of antioxidant enzymes, whereas the spleen is a critical organ for haematopoiesis and the activation of immune cells (Piva et al., 2022). Their sensitivity to environmental stressors makes them useful indicators of systemic physiological status. Furthermore, the relative size of organs to body mass can reflect energy allocation strategies under stress. Hepato-somatic index (HSI) and spleno-somatic

index (SSI) are common proxies to assess potential shifts in metabolic activity, immune investment, or organ atrophy in response to environmental challenges (Morado et al., 2017).

The aim of this research was to explore how temperature variations, ranging from 0 °C, typical of the Southern Ocean coastal areas, to +3 °C, affect the oxidative and immune responses in *T. bernacchii*. These temperature regimes simulate present-day and short-term marine heatwave projections, thereby allowing a comprehensive analysis of the potential future impacts on the species. Specifically, we quantified the expression of key antioxidant genes (*gpx1*, *gpx3*, *gpx4*, *sod1*, *sod2*, *cat*, *prdx3*, *prdx5*) and innate immunity genes (*tlr2, tlr9*) in liver and spleen, two organs pivotal to oxidative balance and immune defence. Comparing gene expression across different temperature conditions in Antarctic fish enables us to identify potential shifts in immune function that may reflect either adaptive capacity or thermal sensitivity. This integrative approach offers new insights into the susceptibility and resilience of Southern Ocean endemics under short-term, yet ecologically significant, thermal perturbations.

2. Materials and methods

2.1. Ethical procedures

Sample collection and animal research were conducted in compliance with regulations set by the Italian Ministry of Education, University, and Research regarding activities and environmental protection in Antarctica, as well as with the Protocol on Environmental Protection to the Antarctic Treaty (Annex II, Article 3). All animal-related procedures carried out during the XXXIII Italian Antarctic Expedition were overseen by the PNRA Ethics Referent, representing the Italian Ministry of Foreign Affairs. The project identification code is PNRA16_00099. The ethics committee or institutional review board responsible for oversight is the Italian Ministry of Foreign Affairs. The designated PNRA Ethics Referent is Dr. Carla Ubaldi from ENEA Antarctica, Technical Unit. Additionally, all experiments were performed in accordance with the U.K. Animals (Scientific Procedures) Act 1986 and related guidelines, the EU Directive 2010/63/EU, and the Italian Legislative Decree 2014/26 governing animal experimentation.

2.2. Fish sampling and maintenance

A total of 30 adult females of *T. bernacchii* (Boulenger, 1902; mean length: 23.76 ± 2.99 cm; mean weight: 211.92 ± 80.40 g) were captured in late October 2022 from Baia Terra Nova in the Ross Sea (74°42′S, 164°7′E), at depths ranging between 60 and 100 m, using hook-and-line methods with artificial lures. Following capture, the fish were temporarily maintained in containers and immediately transferred to the aquarium facility at the Mario Zucchelli Station, where they were housed in 100-L aerated tanks, continuously supplied with running seawater pumped from a depth of 5 m, closely replicating their natural environmental conditions. The water temperature was maintained at 0 ± 0.02 °C to promote acclimation and recovery from capture-related stress over a period of five days (Guillen et al., 2022). To further mimic natural conditions at their typical living depth and minimise stress from human activity, tanks were covered to simulate low-light conditions. During this acclimation phase, fish were not fed, consistent with established protocols for short-term thermal experiments (Guerreiro et al., 2022; Johnston and Battram, 1993; Peter et al., 2020; Stepanowska and Nędzarek, 2020). Both water quality and temperature were regularly monitored to ensure a stable environment. Additionally, behavioural observations, including activity levels and body posture, were used to assess the animals' recovery status (Martins et al., 2012). Following acclimation, fish were gradually exposed to increasing temperatures (from 0 °C up to +3 °C) according to the experimental protocol described below (Section 2.3), with continuous monitoring to prevent thermal shock.

2.3. Experimental set-up

After acclimation, the fish were randomly divided into control ($n = 15$) and experimental ($n = 15$) groups and housed in two separate 100-L tanks. The control group was maintained in a tank at the same conditions used during the acclimation step (described in Section 2.2), and the experimental group was housed in the second tank equipped with a thermostat system to simulate MHWs-like thermal stress. Fish density and husbandry conditions were identical in control and experimental tanks throughout the experiment. The experimental design involved sequential sampling over time, with the same number of individuals removed at each temperature step, thereby progressively reducing fish density in both tanks in parallel. This approach ensured that temperature was the only experimental variable and that potential density-related stress was constant across treatments. The thermal stress for the experimental group began with a gradual temperature increase from 0 °C to +1 °C over 24 h, to prevent thermal shock, followed by four days of exposure at +1 °C. At the end of this first phase, five experimental fish (+1 °C) and five control fish (0 °C) were randomly selected and euthanised. Afterward, the temperature in the experimental tank was raised slowly to +2 °C over 24 h, followed by four additional days of exposure. Upon completion of this second phase, five fish were sampled from both the experimental group and the control group. In the final phase, the temperature in the experimental tank was further increased to +3 °C with the same timing as previously described. After fifteen days, the remaining fish from each group were sampled: five experimental fish (exposed to +3 °C) and five control fish (always at 0 °C). The temperature ramping protocol followed established procedures for Antarctic fish (Tolomeo et al., 2019, 2016). Temperature was increased gradually by +1 °C every four days to reduce mortality risk, approximate natural thermal fluctuations, and accommodate logistical limitations of conducting experiments in Antarctic field conditions. As only one tank per temperature treatment was available, replication at the tank level was not feasible. Consequently, individual fish were considered the experimental units, and inferences should be interpreted as reflecting individual responses under the specific tank conditions rather than fully replicated system-level effects.

Efforts were made to reduce artificial stressors, such as light and noise, to mimic natural environmental conditions. Key water quality parameters, including pH (8.03 ± 0.01), salinity (34.79 ± 0.01 PSU), and dissolved oxygen (pO_2, 6.82 ± 0.04 mL/L), were continuously monitored using calibrated multiparameters sensors and maintained at stable levels to ensure that temperature remained the sole experimental variable.

Before tissue collection, the fish were anaesthetised with a clove oil solution (50 mg/L) to minimise handling stress. Once entirely unresponsive, fish were humanely euthanised via spinal cord severance, in accordance with ethical standards for animal experimentation. Each specimen was measured (total length and total weight), and the liver and spleen were immediately dissected, flash-frozen in liquid nitrogen, within one minute to prevent RNA degradation, and stored at −80 °C for subsequent analyses.

2.4. Primer design, total RNA extraction, cDNA synthesis, PCR amplification, and sequencing

The coding sequences of *cat*, *sod1*, *sod2*, *gpx1*, *gpx3*, *gpx4*, *prdx3*, *prdx5*, *tlr2* and *tlr9* genes were designed on the genome of *T. bernacchii* (Bista et al., 2023). Primers for quantitative real-time PCR (qRT-PCR; see Table S1) were designed based on these coding regions and evaluated for quality and performance using the IDT Oligo Analyzer tool (https://eu.idtdna.com/calc/analyzer). The specificity and amplification efficiency of each primer pair were verified by running the PCR products on 2% agarose gels.

Total RNA was extracted from tissue samples (65 ± 10 mg of liver and 51 ± 12 mg of spleen) using Trizol® reagent (Invitrogen), following

the manufacturer's instructions with reagent volumes adjusted to sample weight. To eliminate carbohydrate contaminants, RNA was further purified via overnight precipitation with 8 M Lithium Chloride (LiCl). A DNase treatment (Promega) step was included to remove residual genomic DNA. RNA concentration and purity were assessed using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific), based on the A_{260}/A_{280} and A_{260}/A_{230} absorbance ratios. RNA integrity was also checked by electrophoresis on a 1% agarose gel.

cDNA synthesis was carried out in a single-step reaction. Each reaction contained 2 μl of 10 mM dNTP mix, 0.5 μl of RNase inhibitor (40 U/μl), 0.5 μl of Oligo(dT)12–18 (10 μM), 4 μl of 5× reverse transcriptase buffer (final concentration 1×), 1 μl of RNA template (1000 ng/μl), 1 μl of RevertUP™ II Reverse Transcriptase, and 11 μl of nuclease-free water. PCR amplifications were performed on 50 ng of cDNA using 2× YourTaq™ PCR Master Mix and the specific primers listed in Table S1. The thermocycling conditions included an initial denaturation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 30 s, annealing at gene-specific temperatures for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 5 min.

2.5. Quantitative real-time PCR (qRT-PCR)

Gene expression levels were assessed via quantitative real-time PCR (qRT-PCR) using the qPCR BIO SyGreen Mix Separate-ROX kit (PCR Biosystems) in a total reaction volume of 10 μl, which included a 1:10 dilution of cDNA. Reactions were performed on an Applied Biosystems 7500 Real-Time PCR System. The thermal cycling protocol consisted of an initial denaturation step at 95 °C for 2 min, followed by 38 cycles of 95 °C for 20 s and 60 °C for 1 min. A melting curve analysis was subsequently conducted to detect potential genomic DNA contamination, with the following conditions: 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s, and 60 °C for 15 s. Each reaction was carried out in technical triplicate to ensure reproducibility. Relative expression levels were calculated using the Pfaffl method ($2^{-\Delta\Delta Ct}$) (Pfaffl, 2001). Transcript levels were normalised to the housekeeping gene β-actin to account for variations in cDNA quantity. Gene expression in fish exposed to each temperature increment was compared with that of the respective control group, which was maintained at 0 °C to identify temperature-dependent changes in gene expression.

2.6. Morphological indexes

After each exposure step, five fish were randomly sampled from the control and experimental groups. The fish were weighed and measured in total length. Subsequently, the liver and spleen were excised and weighed.

Hepato-somatic index (HIS) and spleno-somatic index (SSI) were thus calculated using the following formulas:

$$SSI = ((\text{spleen weight}(g)/(\text{total body weight}(g) - (\text{gonads weight}(g))*100$$

$$HSI = ((\text{liver weight}(g)/(\text{total body weight}(g) - (\text{gonads weight}(g))*100$$

Gonad weight was removed from total body weight calculations to exclude possible bias due to differences in the maturity stage of the female gonads (Pham and Nguyen, 2019).

2.7. Statistical analysis

All statistical analyses were conducted using GraphPad Prism (version 10.4.1, build 532; December 3, 2024). The Shapiro–Wilk test was employed to assess data normality, which informed the selection of appropriate statistical tests. To investigate the physiological responses of *T. bernacchii* under heatwave-like conditions, a two-way ANOVA was performed on morphological indices across three temperature treatments, with Tukey's post hoc test applied for multiple comparisons. For gene expression data, unpaired *t*-tests with Welch's correction were used, followed by Holm–Šidák post hoc tests to adjust for multiple comparisons.

Statistical significance was considered at a *p*-value cutoff of 0.05. Results are reported as mean ($n = 5$) ± standard error of the mean (SEM). Significance levels are indicated as follows: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and not significant (ns) for $p > 0.05$.

3. Results

3.1. Liver gene expression

The relative transcript levels of *cat*, *gpx1*, *gpx3*, and *gpx4* in the liver were analysed following exposure to increasing temperatures (Fig. 1). All four genes are key representatives of hydrogen peroxide-scavenging enzymes. The *cat* expression exhibited a significant reduction at +1 °C (−40.5% relative to control; $p < 0.01$) and + 2 °C (−44.5% vs. control; $p < 0.05$), while no statistically significant change was observed at +3 °C, the highest temperature tested.

By contrast, *gpx1* displayed a stress-dependent expression pattern. Its mRNA levels were significantly elevated at +1 °C (+122% compared to the control; $p < 0.01$), returned to baseline at +2 °C (with no significant difference from the control), and were then significantly downregulated at +3 °C (−37.3% vs. the control; $p < 0.01$). The *gpx3* transcription showed no difference from control at +1 °C, but was strongly induced at +2 °C, reaching a 13-fold increase (+1196.8% vs. control; $p < 0.01$). However, expression levels sharply declined at +3 °C, falling significantly below control values (−37% vs. control; $p < 0.05$). Another gene that was markedly upregulated under moderate thermal stress was *gpx4*, with significant transcript induction at both +1 °C and + 2 °C (+188.2% and + 173.6% vs. control, respectively; $p < 0.001$ for both). At +3 °C, this trend reversed, and expression was significantly suppressed by 54% compared to the control ($p < 0.01$).

The expression dynamics of *sod1* and *sod2* (Fig. 2), which encode cytosolic and mitochondrial SOD isoforms, revealed a precise temperature-specific modulation.

The gene *sod2* exhibited a transient yet significant increase in transcript abundance at +1 °C (+180% vs. control; $p < 0.05$). On the other hand, only at +2 °C, there is evidence of a minimal but still significant activation of the cytoplasmic isoform *sod1* (+32.6% vs. control; $p < 0.05$). In the other exposure conditions, the transcript levels of *sod2* do not exhibit different patterns between the control and exposed groups. Conversely, *sod1* exhibits a downregulation in its expression profile when exposed to +3 °C, with a 54% decrease compared to the control ($p < 0.01$).

The expression of *prdx3* and *prdx5* in the liver is shown in Fig. 3. Both genes encode peroxiredoxin enzymes that scavenge hydrogen peroxide, with PRDX3 primarily localized in mitochondria, and PRDX5 distributed across mitochondria, cytoplasm, and peroxisomes. A significant upregulation of both genes was observed only at +1 °C. Specifically, *prdx3* showed a 3-fold induction (+179% vs. control; $p < 0.05$), while *prdx5* exhibited the highest induction among all genes analysed in the liver, with a 62-fold increase (+6123% vs. control; $p < 0.01$). No statistically significant changes were detected at temperatures of +2 °C and + 3 °C for both genes.

Gene expression analysis of the immune-related genes reveals that *tlr2* is overexpressed 14-fold at +1 °C (+1296% vs. control; $p < 0.001$), whereas it is only 4-fold overexpressed at +2 °C (+330% vs. control; $p < 0.05$) (Fig. 4). Similarly to peroxiredoxins, *tlr2* mRNA levels return to baseline at +3 °C. Conversely, qPCR analysis of *tlr9* shows a first expression at +1 °C (+244% vs. control; $p < 0.001$), reaching the highest level of mRNA at +2 °C (+3143% vs. control; $p < 0.001$). Like *tlr2*, *tlr9* expression also declines (−30% vs. control; $p < 0.05$) at the last temperature trigger, even reaching lower levels compared to the controls.

Overall trends in liver antioxidant gene expression across temperature treatments are summarized in Table S2 in the Supplementary

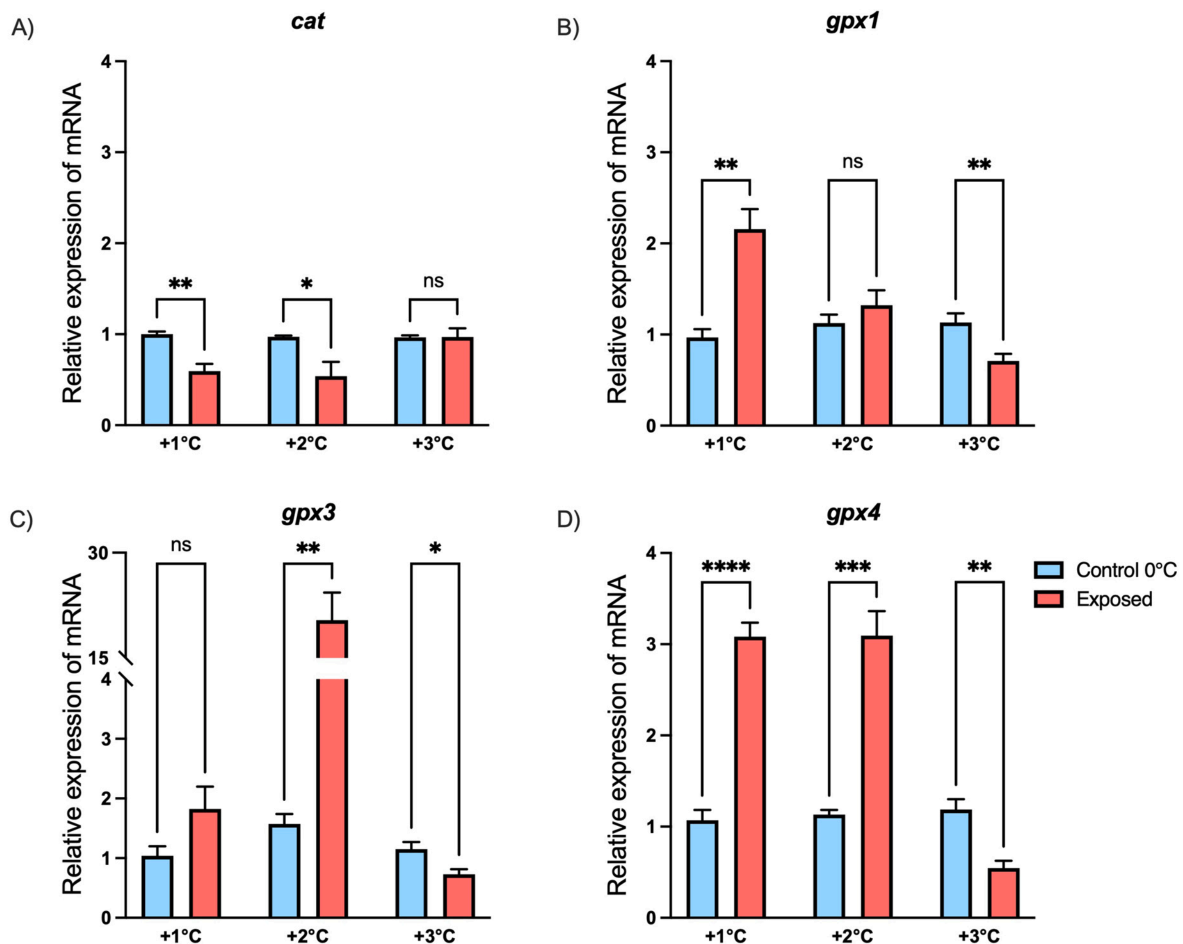

Fig. 1. Gene expression levels of *cat* (A), *gpx1* (B), *gpx3* (C), and *gpx4* (D) in liver tissue across temperature treatments (+1 °C, +2 °C, and +3 °C; red bars) and comparison with the control groups (0 °C; light blue bars). Gene expressions are normalised to β-actin and are presented as mean ± standard error of the mean (SEM). Means obtained with five fish each. Asterisks indicate statistical significance (unpaired *t*-tests with Welch's correction followed by Holm–Šidák) * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and ns ($p > 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

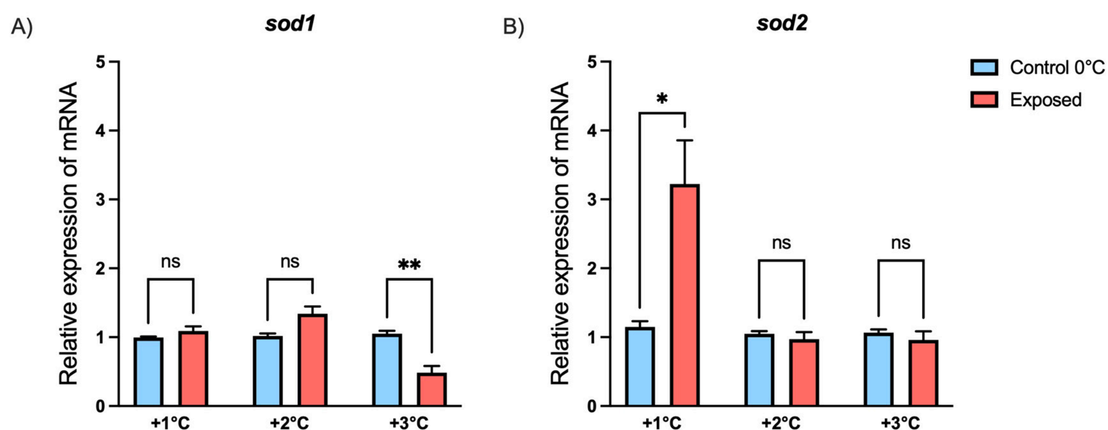

Fig. 2. Gene expression levels of *sod1* (A), *sod2* (B), in liver tissue across temperature treatments (+1 °C, +2 °C, and +3 °C; red bars) and comparison with the control groups (0 °C; light blue bars). Gene expressions are normalised to β-actin and are presented as mean ± standard error of the mean (SEM). Means obtained with five fish each. Asterisks indicate statistical significance (unpaired *t*-tests with Welch's correction followed by Holm–Šidák) * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and ns ($p > 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

section.

3.2. Spleen gene expression

The relative mRNA expression levels of *cat*, *gpx1*, *gpx3*, and *gpx4* in the spleen were analysed following exposure to increasing temperatures (Fig. 5). First, similarly to the liver, *cat* expression doesn't show significant changes, except for a decrease at a temperature of +3 °C (−77% vs. control; $p < 0.001$). On the other hand, *gpx1* shows a stress-dependent modulation, with a marked repression at +1 °C (−84% vs. control, p

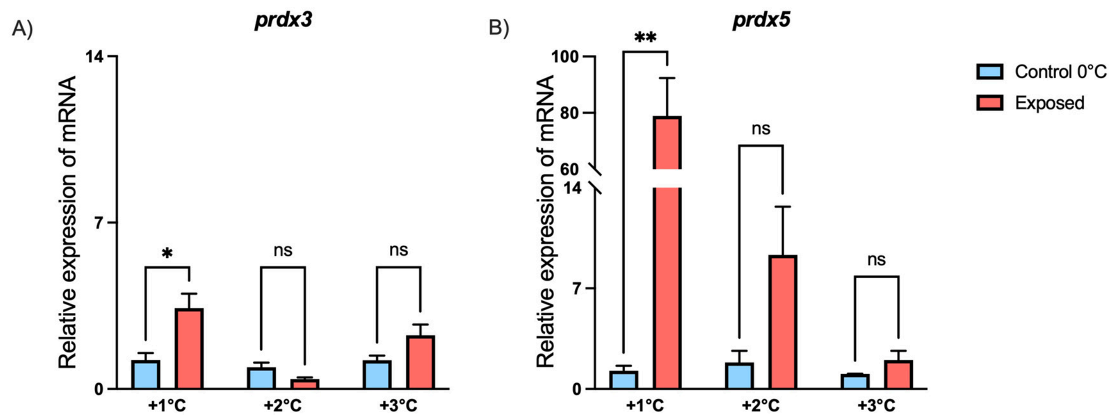

Fig. 3. Gene expression levels of *prdx3* (A), *prdx5* (B), in liver tissue across temperature treatments (+1 °C, +2 °C, and +3 °C; red bars) and comparison with the control groups (0 °C; light blue bars). Gene expressions are normalised to β-actin and are presented as mean ± standard error of the mean (SEM). Means obtained with five fish each. Asterisks indicate statistical significance (unpaired *t*-tests with Welch's correction followed by Holm–Šidák) * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and ns ($p > 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

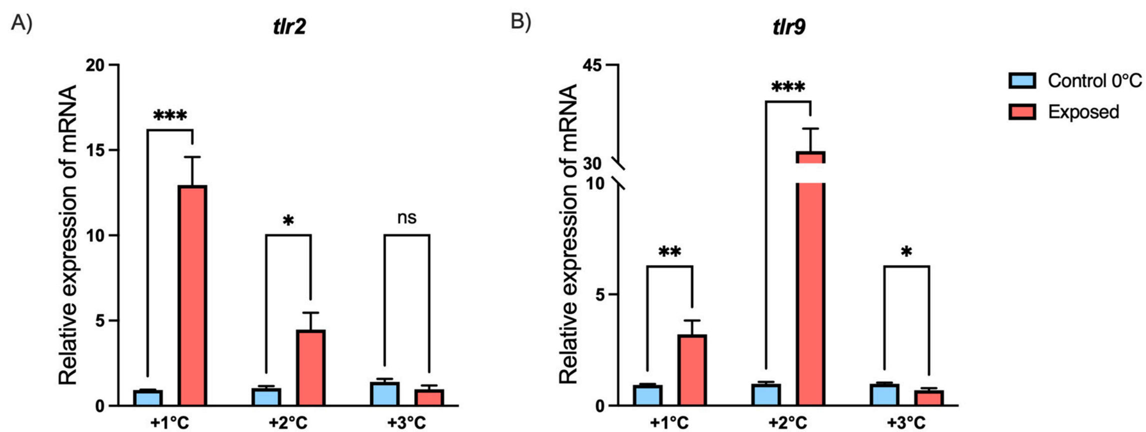

Fig. 4. Gene expression levels of *tlr2* (A), *tlr9* (B), in liver tissue across temperature treatments (+1 °C, +2 °C, and +3 °C; red bars) and comparison with the control groups (0 °C; light blue bars). Gene expressions are normalised to β-actin and are presented as mean ± standard error of the mean (SEM). Means obtained with five fish each. Asterisks indicate statistical significance (unpaired *t*-tests with Welch's correction followed by Holm–Šidák) * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and ns ($p > 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

< 0.001), which remains slightly significant even at +2 °C (−30% vs. control; $p < 0.05$). However, at the end, its expression significantly increases at +3 °C (+435% vs. control; $p < 0.01$). Similarly to *gpx1*, the transcription of the *gpx3* gene also shows a notable decline in gene expression compared to control levels when first triggered by +1 °C ($p < 0.001$). Whereas both at +2 °C and + 3 °C, *gpx3* mRNA levels do not differ significantly from physiological control levels. As regards *gpx4*, transcript levels underwent significant declines at +1 °C (−46%; $p < 0.01$) and + 2 °C (−35%; $p < 0.05$), while they reached control levels at +3 °C.

Moving on to superoxide anion scavengers, our results reveal a similar pattern for both *sod1* and *sod2* (Fig. 6). Neither gene activates significantly compared to the control in the first two MHW-like events. Instead, they are both triggered only when facing the last temperature ramp (+3 °C). In particular, *sod1* shows a 15-fold change (+1390% vs. control; $p < 0.001$) whereas *sod2* exhibits only a weaker induction (+97% vs. control; $p < 0.05$).

The qPCR analysis of the key antioxidant genes, *prdx3* and *prdx5*, reveals an opposite pattern compared to the liver (Fig. 7). Notably, *prdx3* shows a 33-fold induction (+3224% vs. control; $p < 0.01$) at +3 °C, which is the highest mRNA induction, compared to the control, among the investigated gene expression in the spleen. The second in terms of activation is *prdx5* with a 19-fold change compared to the control group (+1796% vs. control; $p < 0.001$), always at +3 °C.

On the contrary, a downregulation when the temperature began to rise was observed for both *prdx3* (−82% vs. control; $p < 0.001$) and *prdx5* (−65% vs. control; $p < 0.001$), with no significant changes at the second temperature trigger.

Gene expression analysis of the immune-related genes reveals that *tlr2* is under-expressed at +1 °C (−74% vs. control; $p < 0.001$) and at +2 °C (−78% vs. control; $p < 0.001$) (Fig. 8). Real time PCR analysis of *tlr9* shows a significantly reduced expression at +1 °C (−69% vs. control; $p < 0.001$) and a slight increase at +2 °C (−33% vs. control; $p < 0.01$). The gene activation is triggered only at +3 °C (+256% vs. control; $p < 0.05$), similarly to *tlr2*. The results from the spleen regarding toll-like receptors show a high degree of similarity with the expression of peroxiredoxins, with *tlr2* mRNA levels also increasing at +3 °C (+155% vs. control; $p < 0.01$). Overall trend in spleen antioxidant gene expression across temperature treatments is summarized in Table S3 in the Supplementary section.

3.3. Somatic indexes

The HSI and SSI indices were calculated for *T. bernacchii* during the experimental temperature increase (Fig. 9).

The HSI showed statistically significant increases (+72% vs. control;

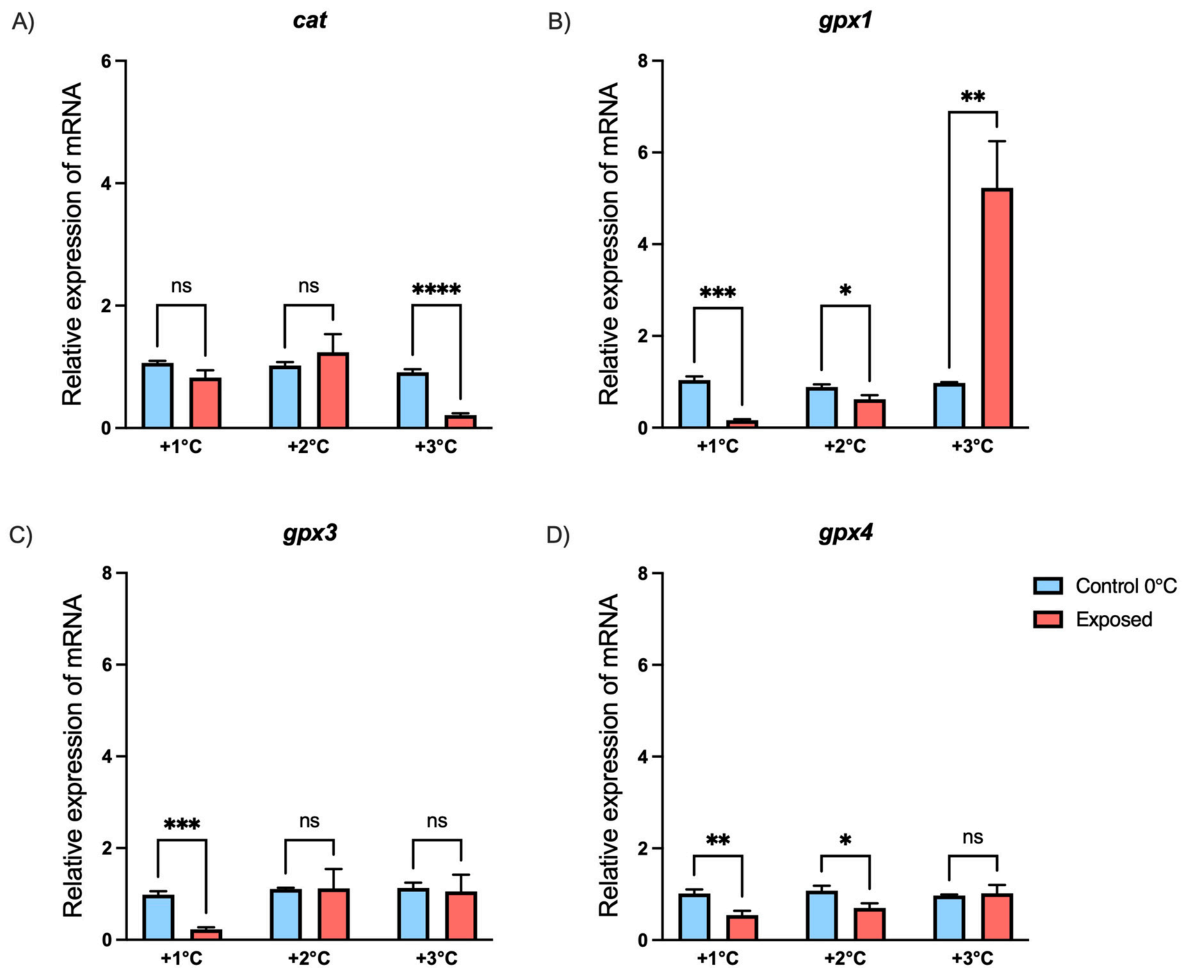

Fig. 5. Gene expression levels of *cat* (A), *gpx1* (B), *gpx3* (C), and *gpx4* (D) in spleen tissue across temperature treatments (+1 °C, +2 °C, and +3 °C; red bars) and comparison with the control groups (0 °C; light blue bars). Gene expressions are normalised to β-actin and are presented as mean ± standard error of the mean (SEM). Means obtained with five fish each. Asterisks indicate statistical significance (unpaired *t*-tests with Welch's correction followed by Holm–Šidák) * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and ns ($p > 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

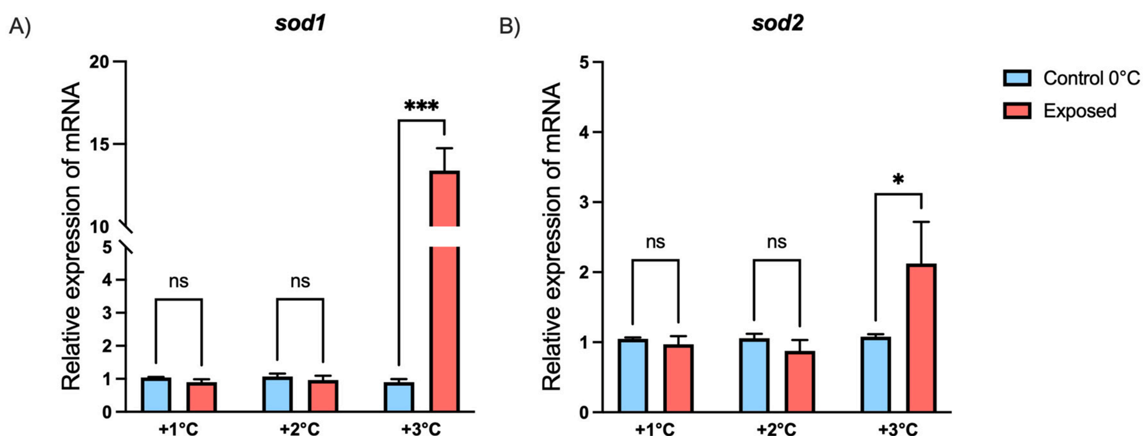

Fig. 6. Gene expression levels of *sod1* (A), *sod2* (B), in spleen tissue across temperature treatments (+1 °C, +2 °C, and +3 °C; red bars) and comparison with the control groups (0 °C; light blue bars). Gene expressions are normalised to β-actin and are presented as mean ± standard error of the mean (SEM). Means obtained with five fish each. Asterisks indicate statistical significance (unpaired *t*-tests with Welch's correction followed by Holm–Šidák) * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and ns ($p > 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

$p < 0.01$) between the control group and fish exposed to +1 °C, while at the end of the experiment, a clear reduction in liver size was observed in the exposed group at +3 °C compared to that exposed at +1 °C (−43% vs. +1; $p < 0.01$).

For the SSI, the two-way ANOVA analysis revealed no statistically significant differences between the groups.

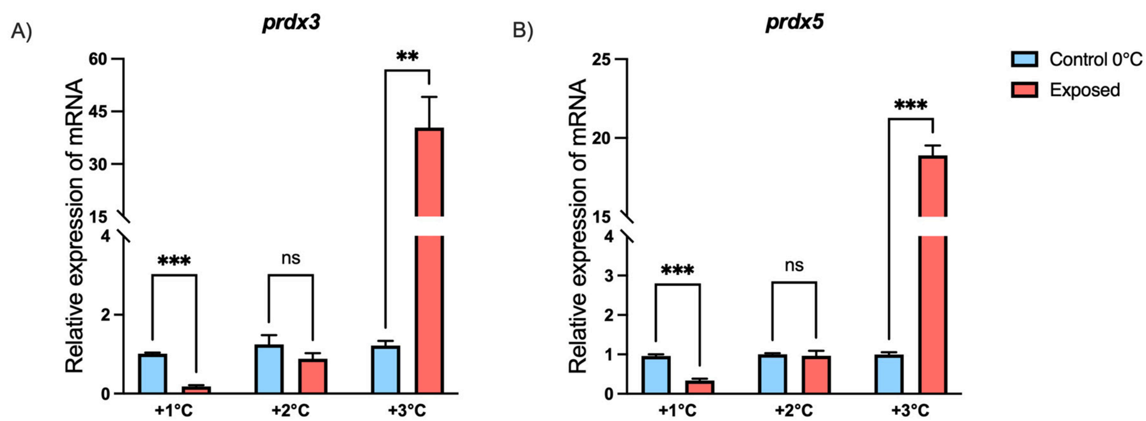

Fig. 7. Gene expression levels of *prdx3* (A), *prdx5* (B), in spleen tissue across temperature treatments (+1 °C, +2 °C, and +3 °C; red bars) and comparison with the control groups (0 °C; light blue bars). Gene expressions are normalised to β-actin and are presented as mean ± standard error of the mean (SEM). Means obtained with five fish each. Asterisks indicate statistical significance (unpaired *t*-tests with Welch's correction followed by Holm–Šidák) * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and ns ($p > 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

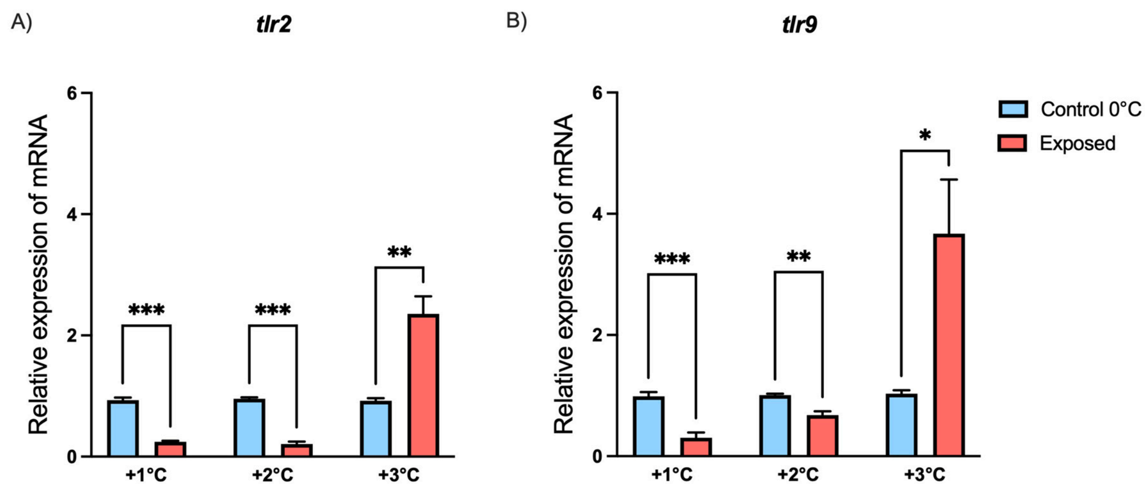

Fig. 8. Gene expression levels of *tlr2* (A), *tlr9* (B), in spleen tissue across temperature treatments (+1 °C, +2 °C, and +3 °C; red bars) and comparison with the control groups (0 °C; light blue bars). Gene expressions are normalised to β-actin and are presented as mean ± standard error of the mean (SEM). Means obtained with five fish each. Asterisks indicate statistical significance (unpaired *t*-tests with Welch's correction followed by Holm–Šidák) * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and ns ($p > 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

This study sheds light on the transcriptional regulation of antioxidant pathways and innate immunity genes in *Trematomus bernacchii*. It proposes a robust organ-specific dynamic of physiological modulation in *T. bernacchii* under projected marine heatwave scenarios. Notably, the liver of *T. bernacchii* responded to simulated MHWs with a temporal transcriptional modulation of genes related to antioxidant enzymes and immunity. At mild to moderate thermal stress levels (+1 °C and + 2 °C), antioxidant responses were rapidly activated, with several genes peaking during the early or intermediate phases. However, at +3 °C, this activation sharply declined, suggesting a threshold beyond which the liver's compensatory mechanisms begin to fail. This trajectory likely reflects a dose-dependent physiological adjustment, balancing initial acclimation with signs of functional overload.

Building upon the observed interplay among antioxidant mechanisms, notably in the liver, several genes involved in oxidative stress response shared a similar temporal pattern of expression, indicating a possible coordination across different key antioxidant factors in response to heat stress. Among these, all *gpx* isoforms exhibited an initial upregulation, although their expression peak occurred at different time points. *gpx1*, *gpx3*, and *gpx4* exhibited a biphasic pattern, with early induction at lower or intermediate stress levels, followed by marked downregulation at +3 °C. The instant upregulation against hydrogen peroxide (H_2O_2), a crucial ROS generated during increased metabolic activity, suggests that these enzymes may participate in an early yet transient oxidative defence mechanism, with an early boost at the onset of stress (Esposito et al., 2000). Found primarily in the cytoplasm, GPX1 includes all five residues involved in the GSH-dependent reduction of peroxides. It functions in close cooperation with glutathione reductase (GR), and its peaking gene expression could pose a generalized oxidative stress risk to the cell.

The *gpx3* gene encodes an enzyme secreted extracellularly that may respond to an increase in the formation of systemic ROS rather than local intracellular damage (Lee et al., 2008). The *gpx4*, a mitochondrial isoform, on the other hand, plays a unique role in neutralising lipid hydroperoxides and protecting cellular membranes from peroxidation, a form of damage that may take longer to develop (Li et al., 2018). Its strong and consistent expression up to +2 °C indicates its essential role in mitochondrial ROS detoxification during early stress exposure and the prolonged need for membrane protection (Piva et al., 2022).

The behaviour of *sod* genes further supports the presence of this

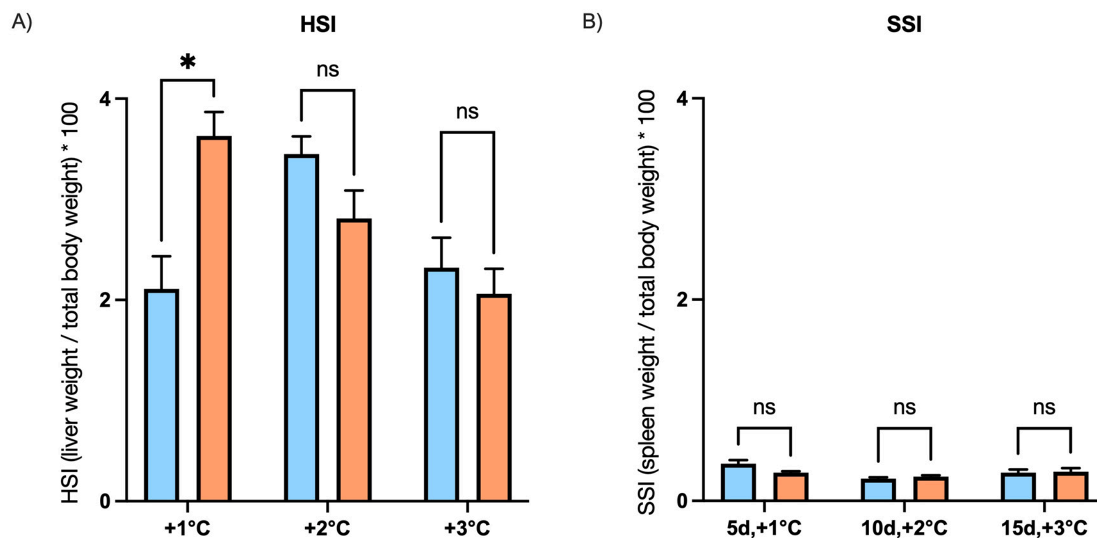

Fig. 9. HSI (A) and SSI (B) mean values referred to *T. bernacchii* specimens from the control groups (0 °C; light blue bars) and the exposed groups (+1 °C and +3 °C; orange bars). Both HSI and SSI are expressed as ((liver weight (g) - gonad weight (g))/total body weight (g)) and ((spleen weight (g) - gonad weight (g))/total body weight (g)), respectively. Data are presented as mean ± standard error of the mean (SEM). Means obtained with five fish each. Asterisks indicate statistical significance (unpaired *t*-tests with Welch's correction followed by Holm–Šidák) * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$), and *ns* ($p > 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

temporal specificity in antioxidant responses. The *sod1* gene, encoding the cytosolic isoform, displayed delayed upregulation, peaking at +2 °C, while *sod2* (mitochondrial) responded earlier, at +1 °C. This complementary timing between cytosolic (*gpx1* and *sod1*) and mitochondrial (*gpx4* and *sod2*) antioxidant defences activation aligns with the idea of a compartmentalised oxidative defence. A possible explanation for the earlier induction of *sod2* is that mitochondria represent a primary source of reactive oxygen species during rapid increases in metabolic rate under thermal stress. In support of this hypothesis, in vitro assays simulating acute heat stress have shown a pronounced increase in mitochondrial superoxide and H_2O_2 production in several teleost species, including rainbow trout, carp, and lake sturgeon, suggesting that fish mitochondria are particularly sensitive to rapid temperature elevations (Banh et al., 2016). These observations support the hypothesis that early mitochondrial ROS production may drive the earlier activation of mitochondrial antioxidant defences.

In contrast, *cat* was significantly downregulated at +1 °C and + 2 °C, and showed no significant change at +3 °C. This pattern suggests that *cat* may not play a major role in the early antioxidant response to thermal stress in this species, possibly due to limited H_2O_2 accumulation, or more likely, as indicated by our results, due to a preferential reliance on the GPX pathway (Pacchini et al., 2025b). Previous studies have shown that this gene is highly sensitive to ROS overproduction and is susceptible to thermal inactivation (Kazmi et al., 2022; Mieiro et al., 2011). In Antarctic fish, the generally low activity of CAT is often attributed to the compensatory action of other antioxidant enzymes (Bagnyukova et al., 2005). GPXs, which also neutralise H_2O_2, are considered the primary compensatory mechanism when CAT activity decreases (Mieiro et al., 2011). However, it is also true that CAT is specifically expressed in peroxisomes, and the lack of upregulation might suggest that these organelles are not adversely affected by heat stress.

Peroxiredoxins also exhibited distinct temporal expression profiles. Our results indicate that *prdx3* and *prdx5* responded with transient upregulation at +1 °C, like *sod2* and *gpx4*, reinforcing their role in early mitochondrial antioxidant defence. This exclusive upregulation at +1 °C could represent a first threshold-dependent line of defence under mild oxidative conditions in the liver. Among them, *prdx5* exhibits the most pronounced response with respect to the control among all the genes analysed, which may reflect its wider subcellular distribution, including the cytoplasm, mitochondria, nucleus, and peroxisomes, enabling it to engage more actively in oxidative stress conditions (Knoops et al., 2011). Instead, as *prdx3* is exclusively mitochondrial, it may have a more targeted scope and localized function (Rebelo et al., 2021).

Beyond antioxidant regulation, we aimed to investigate a possible link between redox homeostasis responses and immunological responses to broaden our knowledge related to Antarctic fish physiological responses.

In mammals, members of the peroxiredoxin family, particularly PRDX1, PRDX2, and PRDX5, can function as DAMPs by directly binding to Toll-like receptors like TLR2, which activate pro-inflammatory signalling cascades via NF-κB and MAPK pathways. Although the precise molecular interactions in Antarctic teleosts remain unexplored, our observed parallel upregulation of *prdxs* and *tlrs* suggests that a similar redox–immune coupling may exist in *T. bernacchii* (Shichita et al., 2012). Mitochondrial oxidation and the subsequent release of PRDXs could function as endogenous signals that stimulate TLRs during heat stress, increasing immune capacity and coordinating inflammatory and antioxidant responses. In the absence of typical heat shock reactions, this mechanism would serve as an adaptive way to identify and react to cellular damage, presenting another stress-sensing route that links redox imbalance to innate immunity.

Our data reveal the early transcriptional activation of innate immune sensors in the liver, suggesting preliminary crosstalk between antioxidant and immune systems. From the first temperature trigger (+1 °C), the mRNA expression of both *tlr2* and *tlr9* started to increase, returning to the control levels only at the final temperature point (+3 °C).

Significantly, *tlr2* peaked earlier than *tlr9* (at +1 °C and + 2 °C, respectively), suggesting a more immediate immune alert mechanism, while *tlr9* was possibly engaged in more sustained or downstream immune signalling. This difference could be explained by their subcellular localization TLR2, a surface receptor, responded earlier, maybe requiring less sustained stimuli for peak activation, while TLR9, which resides in endosomal compartments, peaked later, likely reflecting differences in ligand access and activation kinetics (Medvedev and Vogel, 2008).

Interestingly, the expression profiles of *tlr2* and *tlr9* partially mirror those of *prdx3* and *prdx5*, supporting the idea that the liver initiates a short-term, broad-spectrum innate immune response that diminishes as stress persists (Khansari et al., 2019). It is well recognised that ROS can act as signalling molecules modulating innate immune activation,

including the TLRs signalling route (Yang et al., 2013). Conversely, TLRs activation can promote oxidative bursts via NADPH oxidases and mitochondrial dysfunction (Manoharan et al., 2024). Although speculative, our findings raise the possibility that *T. bernacchii* engages a ROS-TLRs axis in the liver tissue under thermal stress, coordinating an integrated response that couples environmental sensing, redox detoxification, and immune activation.

Remarkably, at +3 °C, the highest temperature tested, almost all antioxidant and immune genes were downregulated or returned to basal levels. This consistent pattern suggests that the liver undergoes a plastic regulatory shift during prolonged thermal stress (Shi et al., 2020), reflecting multiple, non-exclusive mechanisms. These may include metabolic overload, where increased energy demand limits the capacity to sustain antioxidant activity that leads to antioxidant system fatigue and a shift toward energy conservation (Helguera et al., 2013; Rani et al., 2016). Moreover, this pattern is consistent with cellular stress models in which oxidative damage to key metabolic regulators leads to a strategic downregulation of energy-demanding processes in order to limit further ROS production and preserve cellular integrity (Bigelow and Squier, 2005). Together, these processes suggest a coordinated but transient response of the liver antioxidant system under extreme thermal stress. Rather than reflecting a simple failure of antioxidant capacity, the downregulation of antioxidant and immune genes at higher temperatures likely arises from integrated redox and metabolic feedback mechanisms potentially involving mitochondrial dysfunction or systemic stress-induced reprioritisation of energy allocation. Within this framework, the liver initially mounts a coordinated antioxidant and immune response, but subsequently suppresses these pathways under extreme thermal stress to preserve energy and maintain essential cellular functions, resulting in a coordinated but transient regulatory response (Shi et al., 2020; Mooli et al., 2022). Prolonged ROS exposure may also activate negative feedback mechanisms, in which redox-sensitive pathways such as Nrf2 modulate antioxidant transcription to prevent excessive or energetically costly responses (Schieber and Chandel, 2014).

However, the observed decrease in HSI at the end of the experiment could indeed indicate the massive reduction in liver size with respect to the beginning of the experiment, thus suggesting a marked reallocation of energy resources away from hepatic functions (Faught and Vijayan, 2016). The reduction may stem from cellular atrophy and metabolic downscaling, consistent with the observed decline in gene expression across both antioxidant and immune markers (Parra et al., 1995). Together, these molecular and physiological responses underscore the limited plasticity of Antarctic fish to cope with prolonged and intensifying marine heatwave conditions.

In contrast to the liver, the spleen of *T. bernacchii* exhibited a markedly different transcriptional response to thermal stress, characterized by a delayed transcriptional response, with most antioxidant and immune-related genes, primarily at the highest temperature exposure (+3 °C). This organ-level specificity highlights the importance of considering tissue context when investigating physiological strategies to environmental change. It suggests a threshold-dependent activation in the spleen, reserved for more extreme or prolonged stress conditions.

The overall expression pattern of antioxidant genes supports a tiered activation model, in which specific enzymes are recruited depending on stress intensity, tissue redox state, subcellular localization, or a combination of these factors. Among the key antioxidant enzymes, *prdx3*, *prdx5*, *gpx1*, *sod1*, and *sod2* were significantly upregulated at +3 °C. Notably, *gpx1* showed a biphasic pathway: it was significantly downregulated at +1 °C, moderately increased at +2 °C, and sharply upregulated at +3 °C. This trend suggests that *gpx1* is excluded from the early antioxidant response in the spleen, but is recruited later, likely in response to accumulated oxidative damage and peak immune activity (Sun et al., 2024). The induction of *sod1* and *sod2* at the highest temperature may reflect enhanced production of superoxide anions, requiring increased dismutase activity. The initial downregulation of *gpx1* may instead represent a physiological strategy to modulate ROS levels, thereby facilitating redox-sensitive immune signalling through pathways such as NF-κB and MAPKs (Gao et al., 2024). The final upregulation of *gpx1* coincided with the peak expression of *tlr2*, *tlr9*, *prdx3*, *prdx5*, *sod1*, and *sod2*, indicating a late-stage antioxidant increase likely aimed at containing oxidative stress during immune activation. This observation is consistent with previous studies linking GPX1 to the maintenance of the redox balance in immune tissues and the resolution of inflammation (Burk and Hill, 2015).

Notably, *gpx3* and *gpx4* were either consistently downregulated or unchanged, suggesting a minimal contribution of these isoforms to spleen antioxidant protection. Given its extracellular localisation, GPX3 primarily contributes to systemic redox buffering and may not play a central role in intracellular oxidative regulation in spleen-resident immune cells (Pei et al., 2023). Its muted activity suggests that *gpx3* is either non-essential under local thermal stress or possibly regulated by systemic signals that were not strongly triggered under the conditions of this experiment.

The expression of *gpx4* followed a distinct pattern as it was significantly downregulated at the first time point. It partially recovered at +2 °C, and by +3 °C, its expression returned to control levels. This trend suggests that *gpx4* is tightly regulated and mobilized only under sustained or more severe oxidative conditions in the spleen. As GPX4 is crucial for preventing lipid peroxidation and ferroptosis (He et al., 2025), its modest activation in the spleen, compared to the liver, may reflect compensatory *prdx3* upregulation and the organ's lower mitochondrial density (Norin and Malte, 2012). This may result in reduced oxidative burden and diminished demand for *gpx4* expression. The synchronised recovery to control levels, with increased *prdxs* and *tlrs* expressions, supports the idea that GPX4 could contribute to stabilising membrane integrity as immune signalling intensifies (Jia et al., 2020). Similarly to the liver, *cat* showed no significant regulation at each temperature step, further reinforcing the idea that this hydrogen peroxide scavenger is not engaged in heat stress responses in either of the tissues.

Equally, both *prdx3* and *prdx5* in the spleen reached their peak expression at the final time point (+3 °C), with *prdx3*, as previously said, showing the highest induction with respect to control. This response was mirrored by *sod2*, representing a model of coordinated mitochondrial engagement under extreme oxidative pressure.

The expression of the immune-related genes *tlr2* and *tlr9* further supports this model of late-phase activation in the spleen. Both toll-like receptors showed significant upregulation only at +3 °C, perfectly reflecting the expression patterns of *prdx3* and *prdx5*, in contrast to their early activation in the liver. This parallel suggests a coordinated immune-antioxidant response, likely driven by heat-induced cellular stress and the release of DAMPs that engage both TLR signalling and redox-sensitive pathways (Kono and Rock, 2008). The observed synchronisation of TLR and peroxiredoxin responses, both in spleen and liver, is consistent with previous studies in teleosts, which have demonstrated crosstalk between innate immunity and oxidative stress pathways, particularly under intense or prolonged environmental stress (Biller and Takahashi, 2018). Studies on murine models revealed that TLR agonists can also act as activators of the *Nrf2* pathway, increasing the expression of antioxidant molecules and linking the organism's immune responses to its antioxidant system (Mohan and Gupta, 2018).

Considered together, the spleen's transcriptional profile under thermal stress supports a model in which physiological responses are coordinated as part of a strategy to conserve energy as much as possible, engaging each defence only when stress intensity reaches a critical point, after which innate immune signalling and antioxidant enzymes are both activated simultaneously (Mariana and Badr, 2019). This behaviour may reflect a strategic energy conservation mechanism, with the spleen functioning as a backup immune-metabolic organ, engaging only when needed. Such a delay in activation, while potentially protective against unnecessary inflammation or energy loss under mild

conditions, may also suggest a limited plasticity in this organ, raising concerns about its capacity to cope with sustained or recurrent MHWs events. The SSI shows no significant differences in spleen size between the control and the exposed group at the start and end of the experiment, indeed, suggesting that the organ did not undergo major structural changes despite the transcriptional activation observed at the highest temperature. This stability may indicate that the spleen's response is predominantly functional and transient rather than morphological, further supporting the idea of a threshold-dependent, energy-efficient activation (Udroiu and Sgura, 2017). With due caution, we are confident that the observed results were minimally influenced by housing conditions during the acclimation period. However, it must be acknowledged that the lack of feeding may have influenced the results. Antarctic fish have exceptionally low metabolic rates compared to temperate and tropical species (Johnston and Battram, 1993), and although they are known to feed infrequently during summer and may completely cease feeding during winter, we cannot rule out that hunger may still influence specific physiological processes. Stepanowska and Nędzarek (2020) have reported that up to 50 days of starvation periods caused no significant changes in body weight or chemical composition in two Antarctic species, *Notothenia coriiceps* and *N. rossii*. The same authors reported a marked reduction in excretion rates in starved fish, indicating changes in metabolic activity after the first day of starvation, with limited changes (non-statistically significant) thereafter. This can only provide a conceptual example of metabolic adjustment in different Antarctic species under energetic challenge, which should be specifically investigated in *T. bernacchii* to determine whether the fasting period used in our experiment contributed to some of the temporal differences observed, particularly in the initial phase. To our knowledge, no studies have specifically examined the physiological effects of starvation in *T. bernacchii*, highlighting an important knowledge gap in our understanding of species- specific energetic responses. Future studies should focus specifically on the broader physiological adjustments to warming, integrating metabolic, endocrine, and oxidative parameters to provide a more comprehensive understanding of thermal resilience. However, it should be considered that ensuring frequent nutrition can also increase metabolism at feeding time, temporarily increasing oxygen consumption. It is known that this factor increases the production of reactive oxygen species, leading to the activation of the antioxidant system, which in these fish is very efficient (Bakiu et al., 2024) and to post-transcriptional regulation (Piva et al., 2024) that may affect the results.

5. Conclusion

This study reveals that *T. bernacchii* mounts distinct, tissue-specific responses to simulated marine heatwave stress, with the liver acting as an early, metabolically driven responder and the spleen remaining largely inactive until late-stage exposure. While the liver promptly activated antioxidant and immune gene expression at moderate temperature increases, this response diminished at +3 °C, indicating a possible tipping point beyond which physiological defences collapse. In contrast, the spleen initiated its response only under the most extreme thermal condition, suggesting a threshold-based activation strategy possibly aimed at conserving energy under mild stress. These patterns highlight a precise temporal and functional partitioning between the two organs: the liver, rich in mitochondria and central to metabolic regulation, rapidly senses and reacts to thermal perturbations, while the spleen, an immune-hematopoietic organ, delays its engagement until damage signals accumulate. This delayed activation may reduce unnecessary immune expenditure but could also limit resilience to prolonged or recurrent thermal stress. Crucially, the coordinated transcription of antioxidant and toll-like receptor genes in both tissues supports the hypothesis of crosstalk between redox homeostasis and innate immunity, offering new insights into how Antarctic fish integrate multiple stress pathways. This interaction may represent a conserved physiological strategy but also a potential point of vulnerability if energy demands exceed available resources. Altogether, our findings underscore the limited plasticity of *T. bernacchii* in coping with sustained warming and raise concerns about its ability to withstand increasingly frequent MHW events. As climate change accelerates, understanding the thresholds and coordination of physiological responses across organ systems will be essential for predicting species survival in polar environments. Future studies should explore how thermal stress influences immune performance by combining similar experimental approaches based on treatment with pathogens or immunostimulants mimicking bacterial or viral infections, and by assessing how these biological challenges interact with thermal stress to modulate antioxidant capacity, immune responses, and energy allocation. Moreover, future work should also investigate the effects of combined thermal and chemical stressors, such as environmental pollutants, to better reflect realistic climate change scenarios. Additionally, further research is needed to investigate antioxidant enzyme activities in the same organs, which would provide evidence for potential post- and pre-transcriptional regulation of gene expression (Pacchini et al., 2023; Piva et al., 2024; Tittarelli et al., 2026).

CRediT authorship contribution statement

Elisabetta Piva: Writing – original draft, Validation, Supervision, Investigation, Formal analysis, Data curation. **Shaghayegh Kholdihaghighi:** Writing – original draft, Methodology, Formal analysis, Data curation. **Alessia Ametrano:** Writing – review & editing, Data curation. **Sophia Schumann:** Writing – review & editing. **Sara Pacchini:** Writing – review & editing, Methodology. **Martina Cortese:** Writing – review & editing. **Laura Drago:** Writing – review & editing. **Fabio Baroni:** Writing – review & editing. **Chiara Fogliano:** Writing – review & editing. **Maria Rosaria Coscia:** Writing – review & editing, Data curation. **Paola Irato:** Writing – review & editing, Resources. **Gianfranco Santovito:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors have declared no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpc.2026.110509.

Data availability

The data used in this study are openly available at the Research Data Unipd repository (DOI: 10.25430/researchdata.cab.unipd.it.00001675; URI: https://researchdata.cab.unipd.it/1675/).

References

Akira, S., Takeda, K., Kaisho, T., 2001. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat. Immunol. 2, 675–680. https://doi.org/10.1038/90609.

Bagnyukova, T.V., Storey, K.B., Lushchak, V.I., 2005. Adaptive response of antioxidant enzymes to catalase inhibition by aminotriazole in goldfish liver and kidney. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 142, 335–341. https://doi.org/10.1016/j.cbpb.2005.08.003.

Bakiu, R., Pacchini, S., Piva, E., Schumann, S., Tolomeo, A.M., Ferro, D., Irato, P., Santovito, G., 2022. Metallothionein expression as a physiological response against metal toxicity in the striped rockcod *Trematomus hansoni*. Int. J. Mol. Sci. 23, 12799. https://doi.org/10.3390/ijms232112799.

Bakiu, R., Piva, E., Pacchini, S., Santovito, G., 2024. Antioxidant systems in extremophile marine fish species. J. Mar. Sci. Eng. 12, 1280. https://doi.org/10.3390/jmse12081280.

Banh, S., Wiens, L., Sotiri, E., Treberg, J.R., 2016. Mitochondrial reactive oxygen species production by fish muscle mitochondria: potential role in acute heat-induced

oxidative stress. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 191, 99–107. https://doi.org/10.1016/j.cbpb.2015.10.001.

Bigelow, D.J., Squier, T.C., 2005. Redox modulation of cellular signaling and metabolism through reversible oxidation of methionine sensors in calcium regulatory proteins. Biochim. Biophys. Acta, Proteins Proteomics 1703, 121–134. https://doi.org/10.1016/j.bbapap.2004.09.012.

Bilal, S., Etayo, A., Hordvik, I., 2021. Immunoglobulins in teleosts. Immunogenetics 73, 65–77. https://doi.org/10.1007/s00251-020-01195-1.

Biller, J.D., Takahashi, L.S., 2018. Oxidative stress and fish immune system: phagocytosis and leukocyte respiratory burst activity. An. Acad. Bras. Ciênc. 90, 3403–3414. https://doi.org/10.1590/0001-3765201820170730.

Bista, I., Wood, J.M.D., Desvignes, T., McCarthy, S.A., Matschiner, M., Ning, Z., Tracey, A., Torrance, J., Sims, Y., Chow, W., Smith, M., Oliver, K., Haggerty, L., Salzburger, W., Postlethwait, J.H., Howe, K., Clark, M.S., William Detrich, H., Christina Cheng, C.-H., Miska, E.A., Durbin, R., 2023. Genomics of cold adaptations in the Antarctic notothenioid fish radiation. Nat. Commun. 14, 3412. https://doi.org/10.1038/s41467-023-38567-6.

Burk, R.F., Hill, K.E., 2015. Regulation of selenium metabolism and transport. Annu. Rev. Nutr. 35, 109–134. https://doi.org/10.1146/annurev-nutr-071714-034250.

Dhabhar, F.S., 2002. Stress-induced augmentation of immune function—the role of stress hormones, leukocyte trafficking, and cytokines. Brain Behav. Immun. 16, 785–798. https://doi.org/10.1016/S0889-1591(02)00036-3.

Ding, Q., Steig, E.J., 2013. Temperature change on the Antarctic Peninsula linked to the tropical Pacific. J. Clim. 26, 7570–7585. https://doi.org/10.1175/JCLI-D-12-00729.1.

Dowd, W., Kueltz, D., 2023. Muted molecular responses to thermal stress in the Antarctic emerald rockcod (*Trematomus bernacchii*): proteomic evidence and possible evolutionary mechanisms. Physiol 38, 5731667. https://doi.org/10.1152/physiol.2023.38.S1.5731667.

Ducklow, H., Fraser, W., Meredith, M., Stammerjohn, S., Doney, S., Martinson, D., Sailley, S., Schofield, O., Steinberg, D., Venables, H., Amsler, C., 2013. West Antarctic Peninsula: an ice-dependent coastal marine ecosystem in transition. Oceanog 26, 190–203. https://doi.org/10.5670/oceanog.2013.62.

Espírito-Santo, C., Alburquerque, C., Cavalheri, T., Guardiola, F.A., Ozório, R.O.A., Magnoni, L.J., 2025. Induced swimming reduced stress and modulated immune response and antioxidant status in juvenile rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem. 51, 151. https://doi.org/10.1007/s10695-025-01569-w.

Esposito, L.A., Kokoszka, J.E., Waymire, K.G., Cottrell, B., MacGregor, G.R., Wallace, D. C., 2000. Mitochondrial oxidative stress in mice lacking the glutathione peroxidase-1 gene. Free Radic. Biol. Med. 28, 754–766. https://doi.org/10.1016/S0891-5849(00)00161-1.

Farrell, A., 2011. Encyclopedia of Fish Physiology: From Genome to Environment. Academic Press.

Faught, E., Schaaf, M.J.M., 2024. Molecular mechanisms of the stress-induced regulation of the inflammatory response in fish. Gen. Comp. Endocrinol. 345, 114387. https://doi.org/10.1016/j.ygcen.2023.114387.

Faught, E., Vijayan, M.M., 2016. Mechanisms of cortisol action in fish hepatocytes. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 199, 136–145. https://doi.org/10.1016/j.cbpb.2016.06.012.

Filho, D.W., 2007. Reactive oxygen species, antioxidants and fish mitochondria. Front. Biosci. 12, 1229. https://doi.org/10.2741/2141.

Gao, F., Zhao, Y., Shi, X., Qiao, D., Pei, C., Kong, X., 2024. Signalling regulation of reactive oxygen species in fish inflammation. Rev. Aquac. 16, 1266–1285. https://doi.org/10.1111/raq.12895.

Guerreiro, P.M., Silva, S., Louro, B., Alves, A., Couto, E., Canário, A.V.M., 2022. The stress response in Antarctic fish: HPI modulation, cortisol profiles, interrenal sensitivity, and gene expression of *Notothenia rossii* acclimated to temperature challenges. Biol. Life Sci. Forum 13. https://doi.org/10.3390/blsf2022013058.

Guillen, A.C., Borges, M.E., Herrerias, T., Kandalski, P.K., De Souza, M.R.D.P., Donatti, L., 2022. Gradual increase of temperature trigger metabolic and oxidative responses in plasma and body tissues in the Antarctic fish *Notothenia rossii*. Fish Physiol. Biochem. 48, 337–354. https://doi.org/10.1007/s10695-021-01044-2.

He, M., Wang, H., Liu, H., 2025. Glutathione peroxidase 4 in blunt snout bream (*Megalobrama amblycephala*) regulates ferroptosis and inflammation in response to *Aeromonas hydrophila* infection. Curr. Issues Mol. Biol. 47. https://doi.org/10.3390/cimb47050326.

Helguera, P., Seiglie, J., Rodriguez, J., Hanna, M., Helguera, G., Busciglio, J., 2013. Adaptive downregulation of mitochondrial function in down syndrome. Cell Metab. 17, 132–140. https://doi.org/10.1016/j.cmet.2012.12.005.

Hofmann, G.E., Buckley, B.A., Airaksinen, S., Keen, J.E., Somero, G.N., 2000. Heat-shock protein expression is absent in the Antarctic fish *Trematomus Bernacchii* (family Nototheniidae). J. Exp. Biol. 203, 2331–2339. https://doi.org/10.1242/jeb.203.15.2331.

Jia, M., Qin, D., Zhao, C., Chai, L., Yu, Z., Wang, W., Tong, L., Lv, L., Wang, Y., Rehwinkel, J., Yu, J., Zhao, W., 2020. Redox homeostasis maintained by GPX4 facilitates STING activation. Nat. Immunol. 21, 727–735. https://doi.org/10.1038/s41590-020-0699-0.

Johnston, I.A., Battram, J., 1993. Feeding energetics and metabolism in demersal fish species from Antarctic, temperate and tropical environments. Mar. Biol. 115, 7–14. https://doi.org/10.1007/BF00349380.

Kazmi, S.S.U.H., Wang, Y.Y.L., Cai, Y.-E., Wang, Z., 2022. Temperature effects in single or combined with chemicals to the aquatic organisms: an overview of thermo-chemical stress. Ecol. Indic. 143, 109354. https://doi.org/10.1016/j.ecolind.2022.109354.

Khansari, A.R., Balasch, J.C., Vallejos-Vidal, E., Teles, M., Fierro-Castro, C., Tort, L., Reyes-López, F.E., 2019. Comparative study of stress and immune-related transcript outcomes triggered by *Vibrio anguillarum* bacterin and air exposure stress in liver and spleen of gilthead seabream (*Sparus aurata*), zebrafish (*Danio rerio*) and rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol. 86, 436–448. https://doi.org/10.1016/j.fsi.2018.11.063.

Knoops, B., Goemaere, J., Van der Eecken, V., Declercq, J.-P., 2011. Peroxiredoxin 5: structure, mechanism, and function of the mammalian atypical 2-cys peroxiredoxin. Antioxid. Redox Signal. 15, 817–829. https://doi.org/10.1089/ars.2010.3584.

Kono, H., Rock, K.L., 2008. How dying cells alert the immune system to danger. Nat. Rev. Immunol. 8, 279–289. https://doi.org/10.1038/nri2215.

Lee, Y.S., Kim, A.Y., Choi, J.W., Kim, M., Yasue, S., Son, H.J., Masuzaki, H., Park, K.S., Kim, J.B., 2008. Dysregulation of adipose glutathione peroxidase 3 in obesity contributes to local and systemic oxidative stress. Mol. Endocrinol. 22, 2176–2189. https://doi.org/10.1210/me.2008-0023.

Li, C., Deng, X., Xie, X., Liu, Y., Friedmann Angeli, J.P., Lai, L., 2018. Activation of glutathione peroxidase 4 as a novel anti-inflammatory strategy. Front. Pharmacol. 9. https://doi.org/10.3389/fphar.2018.01120.

Madkour, L.H., 2020. The roles and mechanisms of ROS, oxidative stress, and oxidative damage. In: Madkour, L.H. (Ed.), Nanoparticles Induce Oxidative and Endoplasmic Reticulum Stresses: Antioxidant Therapeutic Defenses. Springer International Publishing, Cham, pp. 139–192. https://doi.org/10.1007/978-3-030-37297-2_4.

Manoharan, R.R., Prasad, A., Pospíšil, P., Kzhyshkowska, J., 2024. ROS signaling in innate immunity via oxidative protein modifications. Front. Immunol. 15. https://doi.org/10.3389/fimmu.2024.1359600.

Mariana, S., Badr, G., 2019. Impact of heat stress on the immune response of fishes. J. Surv. Fish. Sci. 5. https://doi.org/10.18331/SFS2019.5.2.14.

Martínez, D., Vargas-Lagos, C., Saravia, J., Oyarzún, R., Loncoman, C., Pontigo, J.P., Vargas-Chacoff, L., 2020. Cellular stress responses of *Eleginops maclovinus* fish injected with *Piscirickettsia salmonis* and submitted to thermal stress. Cell Stress Chaperones 25, 93–104. https://doi.org/10.1007/s12192-019-01051-6.

Martins, C.I.M., Galhardo, L., Noble, C., Damsgård, B., Spedicato, M.T., Zupa, W., Beauchaud, M., Kulczykowska, E., Massabuau, J.-C., Carter, T., Planellas, S.R., Kristiansen, T., 2012. Behavioural indicators of welfare in farmed fish. Fish Physiol. Biochem. 38, 17–41. https://doi.org/10.1007/s10695-011-9518-8.

Medvedev, A.E., Vogel, S.N., 2008. Toll-like receptors in the mammalian innate immune system. In: Innate Immunity of Plants, Animals, and Humans, pp. 135–167.

Mieiro, C.L., Pereira, M.E., Duarte, A.C., Pacheco, M., 2011. Brain as a critical target of mercury in environmentally exposed fish (*Dicentrarchus labrax*)—bioaccumulation and oxidative stress profiles. Aquat. Toxicol. 103, 233–240. https://doi.org/10.1016/j.aquatox.2011.03.006.

Mohan, S., Gupta, D., 2018. Crosstalk of toll-like receptors signaling and Nrf2 pathway for regulation of inflammation. Biomed. Pharmacother. 108, 1866–1878. https://doi.org/10.1016/j.biopha.2018.10.019.

Mooli, R.G.R., Mukhi, D., Ramakrishnan, S.K., 2022. Oxidative stress and redox signaling in the pathophysiology of liver diseases. In: Prakash, Y.S. (Ed.), Comprehensive Physiology, 1st ed. Wiley, pp. 3167–3192. https://doi.org/10.1002/cphy.c200021.

Morado, C.N., Araújo, F.G., Gomes, I.D., 2017. The use of biomarkers for assessing effects of pollutant stress on fish species from a tropical river in Southeastern Brazil. Acta Sci. Biol. Sci. 39, 431. https://doi.org/10.4025/actascibiolsci.v39i4.34293.

Norin, T., Malte, H., 2012. Intraspecific variation in aerobic metabolic rate of fish: relations with organ size and enzyme activity in brown trout. Physiol. Biochem. Zool. 85, 645–656. https://doi.org/10.1086/665982.

Nualart, D.P., Paschke, K., Guerreiro, P.M., McCormick, S.D., González-Wevar, C., Cheng, C.-H.C., Chacoff, L.V., 2025. Combined effects of PVC microplastics and thermal rise alter the oxidative stress response in Antarctic fish *Harpagifer antarcticus* and Sub-Antarctic *Harpagifer bispinis*. Mar. Pollut. Bull. 220, 118438. https://doi.org/10.1016/j.marpolbul.2025.118438.

Pacchini, S., Piva, E., Schumann, S., Irato, P., Pellegrino, D., Santovito, G., 2023. An experimental study on antioxidant enzyme gene expression in *Trematomus newnesi* (Boulenger, 1902) experimentally exposed to perfluoro-octanoic acid. Antioxidants 12, 352. https://doi.org/10.3390/antiox12020352.

Pacchini, S., Drago, L., Cortese, M., Vanzan, G., Piva, E., Kholdihaghighi, S., Barbarossa, A., Bardhi, A., Schumann, S., Fogliano, C., Bottacin-Busolin, A., Irato, P., Marion, A., Santovito, G., 2025a. From genes to organs: physiological responses of European chub (*Squalius cephalus*) to chronic PFAS pollution. Front. Toxicol. 7, 1654272. https://doi.org/10.3389/ftox.2025.1654272.

Pacchini, S., Vanzan, G., Piva, E., Schumann, S., Cortese, M., Drago, L., Kholdihaghighi, S., Fogliano, C., Bertotto, D., Bottacin-Busolin, A., Irato, P., Marion, A., Santovito, G., 2025b. Chronic PFAS exposure induces the activation of selenium-dependent glutathione peroxidases and catalase as antioxidant defences in the European chub (*Squalius cephalus*) (Linnaeus, 1758) kidney. Aquat. Toxicol. 287, 107524. https://doi.org/10.1016/j.aquatox.2025.107524.

Parra, O.M., Hernandez-Blasquez, J., Silva, R.A. de S. e, Silva, J.R. da, Peduto, L., Soares, M.A.M., Saad, W.A., Saad, W.A., 1995. Reduction of liver mass due to malnutrition in rats. Correlation with emaciation of animals and size of organs not inserted in the portal system. Sao Paulo Med. J. 113 (3), 903–909. https://doi.org/10.1590/S1516-31801995000300004.

Pei, J., Pan, X., Wei, G., Hua, Y., 2023. Research progress of glutathione peroxidase family (GPX) in redoxidation. Front. Pharmacol. 14. https://doi.org/10.3389/fphar.2023.1147414.

Peter, M., Lv, H., Jiang, X., Liu, Y., Hur, J., Esbon, M., Samwel, C., Superius, E., Khalfan, Z., Gao, Y., Chu, Z., 2020. Effects of starvation on enzyme activities and intestinal microflora composition in loach (*Paramisgurnus dabryanus*). Aquacult. Rep. 18, 100467. https://doi.org/10.1016/j.aqrep.2020.100467.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, 45e–45. https://doi.org/10.1093/nar/29.9.e45.

Pham, H.Q., Nguyen, A.V., 2019. Seasonal changes in hepatosomatic index, gonadosomatic index and plasma estradiol-17β level in captively reared female rabbit fish (*Siganus guttatus*). Aquac. Res. 50, 2191–2199. https://doi.org/10.1111/are.14100.

Piva, E., Schumann, S., Dotteschini, S., Brocca, G., Radaelli, G., Marion, A., Irato, P., Bertotto, D., Santovito, G., 2022. Antioxidant responses induced by PFAS exposure in freshwater fish in the Veneto Region. Antioxidants 11, 1115. https://doi.org/10.3390/antiox11061115.

Piva, E., Nicorelli, E., Pacchini, S., Schumann, S., Drago, L., Vanzan, G., Tolomeo, A.M., Irato, P., Bakiu, R., Gerdol, M., Santovito, G., 2024. Unravelling stress granules in the deep cold: characterisation of TIA-1 gene sequence in Antarctic fish species. Fish Shellfish Immunol. 154, 109903. https://doi.org/10.1016/j.fsi.2024.109903.

Pörtner, H.O., Mark, F.C., Bock, C., 2004. Oxygen limited thermal tolerance in fish? Respir. Physiol. Neurobiol. 141, 243–260. https://doi.org/10.1016/j.resp.2004.03.011.

Rani, V., Deep, G., Singh, R.K., Palle, K., Yadav, U.C.S., 2016. Oxidative stress and metabolic disorders: pathogenesis and therapeutic strategies. Life Sci. 148, 183–193. https://doi.org/10.1016/j.lfs.2016.02.002.

Rebelo, A.P., Eidhof, I., Cintra, V.P., Guillot-Noel, L., Pereira, C.V., Timmann, D., Traschütz, A., Schöls, L., Coarelli, G., Durr, A., Anheim, M., Tranchant, C., van de Warrenburg, B., Guissart, C., Koenig, M., Howell, J., Moraes, C.T., Schenck, A., Stevanin, G., Züchner, S., Synofzik, M., the PREPARE network, 2021. Biallelic loss-of-function variations in PRDX3 cause cerebellar ataxia. Brain 144, 1467–1481. https://doi.org/10.1093/brain/awab071.

Rizzotti, D., Manfrin, C., Gerdol, M., Greco, S., Santovito, G., Giulianini, P.G., 2022. Morphological analysis of erythrocytes of an Antarctic teleost under heat stress: Bias of the stabling effect. J. Therm. Biol. 103, 103139. https://doi.org/10.1016/j.jtherbio.2021.103139.

Samuels, T., Rynearson, T.A., Collins, S., 2021. Surviving heatwaves: thermal experience predicts life and death in a southern ocean diatom. Front. Mar. Sci. 8, 600343. https://doi.org/10.3389/fmars.2021.600343.

Saravia, J., Paschke, K., Pontigo, J.P., Nualart, D., Navarro, J.M., Vargas-Chacoff, L., 2022. Effects of temperature on the innate immune response on Antarctic and sub-Antarctic fish *Harpagifer antarcticus* and *Harpagifer bispinis* challenged with two immunostimulants, LPS and Poly I:C: in vivo and in vitro approach. Fish Shellfish Immunol. 130, 391–408. https://doi.org/10.1016/j.fsi.2022.09.025.

Scharsack, J.P., Franke, F., 2022. Temperature effects on teleost immunity in the light of climate change. J. Fish Biol. 101, 780–796. https://doi.org/10.1111/jfb.15163.

Schieber, M., Chandel, N.S., 2014. ROS function in redox signaling and oxidative stress. Curr. Biol. 24, R453–R462. https://doi.org/10.1016/j.cub.2014.03.034.

Schumann, S., Negrato, E., Piva, E., Pietropoli, E., Bonato, M., Irato, P., Marion, A., Santovito, G., Bertotto, D., 2024. Cortisol levels reveal species-specific stress condition in fish from PFAS polluted rivers. Chemosphere 363, 142925. https://doi.org/10.1016/j.chemosphere.2024.142925.

Schumann, S., Stoilova, V., Bortoletto, E., Piva, E., Pacchini, S., Mozzi, G., Jechow, A., Marion, A., Bertotto, D., Erlandson, A., Hölker, F., Greenberg, L., Santovito, G., 2025. Impact of artificial light at night (ALAN) on cardiac function and acute oxidative stress physiology in migratory brown trout smolts. Environ. Pollut. 381, 126593. https://doi.org/10.1016/j.envpol.2025.126593.

Shi, L., Xu, Y., Mao, C., Wang, Z., Guo, S., Jin, X., Yan, S., Shi, B., 2020. Effects of heat stress on antioxidant status and immune function and expression of related genes in lambs. Int. J. Biometeorol. 64, 2093–2104. https://doi.org/10.1007/s00484-020-02000-0.

Shichita, T., Hasegawa, E., Kimura, A., Morita, R., Sakaguchi, R., Takada, I., Sekiya, T., Ooboshi, H., Kitazono, T., Yanagawa, T., Ishii, T., Takahashi, H., Mori, S., Nishibori, M., Kuroda, K., Akira, S., Miyake, K., Yoshimura, A., 2012. Peroxiredoxin family proteins are key initiators of post-ischemic inflammation in the brain. Nat. Med. 18, 911–917. https://doi.org/10.1038/nm.2749.

Smale, D.A., Wernberg, T., Oliver, E.C.J., Thomsen, M., Harvey, B.P., Straub, S.C., Burrows, M.T., Alexander, L.V., Benthuysen, J.A., Donat, M.G., Feng, M., Hobday, A.J., Holbrook, N.J., Perkins-Kirkpatrick, S.E., Scannell, H.A., Sen Gupta, A., Payne, B.L., Moore, P.J., 2019. Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nat. Clim. Chang. 9, 306–312. https://doi.org/10.1038/s41558-019-0412-1.

Smith, K.E., Burrows, M.T., Hobday, A.J., King, N.G., Moore, P.J., Sen Gupta, A., Thomsen, M.S., Wernberg, T., Smale, D.A., 2023. Biological impacts of marine heatwaves. Annu. Rev. Mar. Sci. 15, 119–145. https://doi.org/10.1146/annurev-marine-032122-121437.

Stepanowska, K., Nędzarek, A., 2020. Changes in the body chemical composition and the excretion of nitrogen and phosphorus during long-term starvation of Antarctic fish *Notothenia coriiceps* and *Notothenia rossii*. Eur. Zool. J. 87, 571–579. https://doi.org/10.1080/24750263.2020.1822451.

Sun, Y., Fu, Z., Ma, Z., 2024. The impact of acute ammonia nitrogen stress on serum biochemical indicators and spleen gene expression in juvenile yellowfin tuna (*Thunnus albacares*). Animals 14. https://doi.org/10.3390/ani14213090.

Thorne, M.A.S., Burns, G., Fraser, K.P.P., Hillyard, G., Clark, M.S., 2010. Transcription profiling of acute temperature stress in the Antarctic plunderfish *Harpagifer antarcticus*. Mar. Genomics 3, 35–44. https://doi.org/10.1016/j.margen.2010.02.002.

Tittarelli, E., Carotti, E., Palladinelli, C., Barucca, M., Carducci, F., Santovito, G., Piva, E., Canapa, A., Biscotti, M.A., 2026. Transcriptional response of transposable elements to thermal stress in the Antarctic fish *Trematomus bernacchii*. Sci. Rep. https://doi.org/10.1038/s41598-025-33487-5.

Tolomeo, A.M., Carraro, A., Bakiu, R., Toppo, S., Place, S.P., Ferro, D., Santovito, G., 2016. Peroxiredoxin 6 from the Antarctic emerald rockcod: molecular characterization of its response to warming. J. Comp. Physiol. B. 186, 59–71. https://doi.org/10.1007/s00360-015-0935-3.

Tolomeo, A.M., Carraro, A., Bakiu, R., Toppo, S., Garofalo, F., Pellegrino, D., Gerdol, M., Ferro, D., Place, S.P., Santovito, G., 2019. Molecular characterization of novel mitochondrial peroxiredoxins from the Antarctic emerald rockcod and their gene expression in response to environmental warming. Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 225, 108580. https://doi.org/10.1016/j.cbpc.2019.108580.

Udroiu, I., Sgura, A., 2017. The phylogeny of the spleen. Q. Rev. Biol. 92, 411–443. https://doi.org/10.1086/695327.

Vargas-Chacoff, L., Arjona, F.J., Ruiz-Jarabo, I., García-Lopez, A., Flik, G., Mancera, J.M., 2020. Water temperature affects osmoregulatory responses in gilthead sea bream (*Sparus aurata* L.). J. Therm. Biol. 88, 102526. https://doi.org/10.1016/j.jtherbio.2020.102526.

Vargas-Chacoff, L., Dann, F., Paschke, K., Oyarzún-Salazar, R., Nualart, D., Martínez, D., Wilson, J.M., Guerreiro, P.M., Navarro, J.M., 2021. Freshening effect on the osmotic response of the A ntarctic spiny plunderfish *Harpagifer antarcticus*. J. Fish Biol. 98, 1558–1571. https://doi.org/10.1111/jfb.14676.

Varriale, S., Ferraresso, S., Giacomelli, S., Coscia, M.R., Bargelloni, L., Oreste, U., 2012. Evolutionary analysis of Antarctic teleost Toll-like receptor 2. Fish Shellfish Immunol. 33, 1076–1085. https://doi.org/10.1016/j.fsi.2012.07.010.

Villeneuve, A.R., White, E.R., 2024. Predicting organismal response to marine heatwaves using dynamic thermal tolerance landscape models. J. Anim. Ecol., 1365-2656.14120 https://doi.org/10.1111/1365-2656.14120.

Watts, M., Munday, B.L., Burke, C.M., 2001. Immune responses of teleost fish. Aust. Vet. J. 79, 570–574. https://doi.org/10.1111/j.1751-0813.2001.tb10753.x.

Wojtaszek, J., Adamowicz, A., 2003. Haematology of the fire-bellied toad, *Bombina bombina* L. Comp. Clin. Pathol. 12, 129–134. https://doi.org/10.1007/s00580-003-0482-2.

Xv, Z., Chen, S., Song, G., Hu, H., Lin, S., Long, Y., 2024. Biochemical, histological and transcriptomic analyses for the immunological organs provide insights into heat stress-induced disease susceptibility in Largemouth Bass. Sci. Total Environ. 912, 168758. https://doi.org/10.1016/j.scitotenv.2023.168758.

Yang, C.-S., Kim, J.-J., Lee, S.J., Hwang, J.H., Lee, C.-H., Lee, M.-S., Jo, E.-K., 2013. TLR3-triggered reactive oxygen species contribute to inflammatory responses by activating signal transducer and activator of transcription-1. J. Immunol. 190, 6368–6377. https://doi.org/10.4049/jimmunol.1202574.

Zafalon-Silva, B., Zebral, Y.D., Bianchini, A., da Rosa, C.E., Marins, L.F., Colares, E.P., Martinez, P.E., Bobrowski, V.L., Robaldo, R.B., 2017. Erythrocyte nuclear abnormalities and leukocyte profile in the Antarctic fish *Notothenia coriiceps* after exposure to short- and long-term heat stress. Polar Biol. 40, 1755–1760. https://doi.org/10.1007/s00300-017-2099-y.

Zhang, J., Kong, X., Zhou, C., Li, L., Nie, G., Li, X., 2014. Toll-like receptor recognition of bacteria in fish: ligand specificity and signal pathways. Fish Shellfish Immunol. 41, 380–388. https://doi.org/10.1016/j.fsi.2014.09.022.