Developmental and Comparative Immunology 164 (2025) 105332

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

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

High temperature induces oxidative damage, immune modulation, and atrophy in the gills and skeletal muscle of the teleost fish black cusk-eel (*Genypterus maculatus*)

Sofia Becerra [a], Marcia Arriagada-Solimano [b], Sebastian Escobar-Aguirre [c], Jaime Palomino [b], Jorge Aedo [d], Juan Manuel Estrada [e], Veronica Barra-Valdebenito [a], Rodrigo Zuloaga [f], Juan Antonio Valdes [f], Phillip Dettleff [a,*]

[a] *Escuela de Medicina Veterinaria, Facultad de Agronomía y Sistemas Naturales, Facultad de Ciencias Biológicas y Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, 7820436, Chile*
[b] *Escuela de Medicina Veterinaria, Centro de Estudios e Investigación en Salud y Sociedad, Facultad de Ciencias Médicas, Universidad Bernardo O'Higgins, Santiago, 8370993, Chile*
[c] *Facultad de Agronomía y Sistemas Naturales, Pontificia Universidad Católica de Chile, Santiago, 7820436, Chile*
[d] *Departamento de Biología y Química, Facultad de Ciencias Básicas, Universidad Católica del Maule, Talca, 3466706, Chile*
[e] *Centro de Investigación Marina Quintay (CIMARQ), Universidad Andrés Bello, Quintay, 2340000, Chile*
[f] *Facultad de Ciencias de la Vida, Universidad Andrés Bello, Santiago, 8370186, Chile*

ARTICLE INFO

Keywords:
Black cusk-eel
Thermal stress
Heat waves
Immune response
Oxidative stress
Atrophy

ABSTRACT

The high temperature associated with heat waves is a relevant abiotic factor that could impact the biology of teleost fish. The innate immune response, muscular growth, and oxidative stress status are relevant functions in fish tissues that could be affected by increased temperature. In this study, black cusk-eel (*Genypterus maculatus*) juveniles were subjected to increased temperature, to experimentally replicate heat waves registered from the South Pacific Ocean for five days. The results showed that thermal stress modulated the immune response in gills, with up-regulation of antibacterial peptides, pro-inflammatory cytokines, and Toll-like receptors genes, including *hepcidin, gzma, tnfa, cxcl8,* and *tlr5*, with no effect on complement system genes. In skeletal muscle, high temperature triggered atrophy-related gene expression, with up-regulation of *foxo1, foxo3, fbxo32, murf1,* and *atg16l.* Increased temperature also generated an up-regulation of transcripts encoding heat shock protein (*hsp60* and *hsp70*) in gills and skeletal muscle, generating oxidative stress in both tissues, with increased expression of the antioxidant genes *sod1* and *gpx1* in gills and skeletal muscle, respectively, with oxidative damage observed at the DNA level (AP sites), protein (carbonyl content), and lipoperoxidation (HNE content) in both tissues. The present study shows that short-term increases in temperature like those observed in heat waves could affect the immune response in gills, induced atrophy in skeletal muscle, and generate oxidative stress in a teleost species important for Chilean aquaculture diversification, information relevant under the context of climate change scenario.

1. Introduction

The temperature is an environmental factor that influences the metabolism of the poikilotherms fish. When the water temperature increases over the physiological range for one species, it could lead to a stress response and affect several fish functions (Dammark et al., 2018). Changes in water temperature could be relevant across seasons, (Abram et al., 2017), with increased frequency of extreme events such as heat waves in the ocean associated with climate changes (Montie et al., 2020). These extreme events in the South Pacific Ocean will increase related to El Niño-Southern Oscillation and the impact of climate change in its intensity (Wang et al., 2019). Marine fish could be affected by extreme heat events in the South Pacific Ocean, including wild and commercial populations associated with fisheries and aquaculture (Hsiung et al., 2018; Ong et al., 2015; Espinoza et al., 2023). Additionally, an increase in the water temperature of the ocean is expected to affect aquaculture species, generating stress in the fish and increasing disease outbreaks (Barange et al., 2018).

* Corresponding author.
E-mail address: phillip.dettleff@uc.cl (P. Dettleff).

https://doi.org/10.1016/j.dci.2025.105332
Received 3 October 2024; Received in revised form 29 January 2025; Accepted 29 January 2025
Available online 30 January 2025

The black cusk-eel (*Genypterus maculatus*) is a teleost fish from the *Genypterus* genus, which includes species that inhabit the Chilean, South African, New Zealand, Australian coast, and Atlantic coast of South America (Santaclara et al., 2014). The *G. maculatus* is an endemic species of the South Pacific coast, part of the native *Genypterus* species of Chilean fisheries (SERNAPESCA and SNdPy, 2023). Additionally, these species are relevant for aquaculture diversification (Gonzalez et al., 2019). Nevertheless, the biological knowledge is limited, without studies that evaluate the impact of high temperatures on the immune response of *G. maculatus* which could be relevant for the aquaculture development of this species.

An essential function in the teleost fish is the immune response, which allows response to infection and generates immunological memory to improve the response against new challenges with the same agent (Dezfuli et al., 2023). The immune system is formed by the innate immune system, which is the primary defense line with rapid response against pathogens; and the adaptive immune system, which is highly specific and could generate immunological memory against pathogens (Mokhtar et al., 2023). The normal function of the innate immune system is crucial to maintaining health and welfare in fish (Bjorgen et al., 2022). However, environmental stressors could affect the immune capacity of fish (Tort, 2011). Even more, a change in the temperature of the water could directly change the immune response of teleost, affecting the immune capacity of the fish, which could vary according to the temperature change and species affected (Abram et al., 2017). Additionally, stressors could affect the growth capacity in fish, modifying the protein composition of skeletal muscle in aquaculture species, and generating induction of atrophy (Bodine and Furlow, 2015; Molina et al., 2023a). These stressors also could generate cellular stress, modifying the oxidative status and generating oxidative stress in the cells in fish (Lesser, 2006). However, there is no information on how temperature affects *G. maculatus* response at immune response, atrophy, and oxidative stress. This study evaluated for the first time the impact of high temperatures associated with marine heat waves in the transcriptional regulation of immune response in gills, the induction of atrophy-associated biomarkers in skeletal muscle, and the oxidative stress induction in both tissues of *G. maculatus*.

2. Materials and methods

2.1. Ethics statement

All the procedures performed with black cusk-eel were approved by the bioethical committee of Andres Bello University (number 007/2018) according to animal welfare procedures and guidelines of the Chilean government's National Research and Development Agency (ANID).

2.2. Experimental procedure and fish sampling

To determine the effect of high temperature on immune response and oxidative stress in gills and skeletal muscle of black cusk-eel, juveniles of black cusk-eels of 1-year, reproductive immature, were used. Fishes were provided by the Center of Marine Research of Quintay (CIMARQ), with an average size of 600 ± 21 g and 50 ± 3 cm. The fish were maintained under natural photoperiod conditions (L:D 14:10), fed with 6 mm commercial pellet food (Skretting, Puerto Montt, Chile), and acclimatized for two weeks at 13 °C and constant air supply. Fish were separated in two replicated tanks per group with a recirculating system with UV and biological filters, and maintained for 5 days at the experimental temperature that simulated a heat wave (Dettleff et al., 2024) in each of the experimental groups, the control group (Control, n = 4) was maintained at 13 °C (±0.4 °C), and the stress by high-temperature group (Stress, n = 4) was maintained at 18 °C (±0.17 °C), after an initial increasing temperature protocol previously standardized for *Genypterus* species (0.5 °C/2.5 h) (Dettleff et al., 2020a). After 5 days, fish were euthanized with anesthetic overdose with tricaine (300 mg/L, Virbac, Puerto Montt, Chile). The gills and skeletal muscle tissue of the fish were sampled and stored on RNAsave solution (Biological Industries, Cromwell, CT, USA) and maintained at −80 °C for posterior RNA extraction. Additionally, gill and skeletal muscle tissue were frozen on liquid nitrogen and stored at −80 °C for oxidative stress analysis (AP sites, protein carbonylation, and lipoperoxidation analysis).

2.3. Oxidative stress evaluation

To evaluate the impact of temperature on oxidative status, we determined the effect at three levels of oxidative damage, including DNA, proteins, and lipids. For this, we determined the formation of apurinic/apyrimidinic sites or AP sites on genomic DNA from gills and skeletal muscle. Genomic DNA was obtained from samples using 20 mg of tissue with the DNAzol reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer protocol, and quantified by Epoch Spectrophotometer System (BioTek, Winooski, VT, USA). The AP sites were measured with the OxiSelect Oxidative DNA Damage Quantification Kit (Cell Biolabs, San Diego, CA, USA). To evaluate protein oxidation we measure protein carbonyl, generated by ROS interaction with proteins. For this, total proteins were extracted using 100 mg of tissue in 1 mL of lysis buffer containing 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, and 1% NP-40, with protease inhibitor cocktail (Calbiochem, San Diego, CA, USA), centrifugated at 14,000 rpm for 30 min at 4 °C. Total proteins were quantified by Pierce BCA Protein Assay Kit (Thermo Scientific, Rockford, IL, USA) and protein carbonyl content was determined with the OxiSelect Protein Carbonyl ELISA kit (Cell Biolabs, San Diego, CA, USA). To determine the effect of temperature on lipids, we evaluated the 4-hydroxynonenal (HNE) protein adducts, which is one of the quantitatively most important products of lipid peroxidation. For this, we use total proteins extracted from tissues as previously informed, measuring HNE with the OxiSelect HNE Adduct Competitive ELISA Kit (Cell Biolabs, San Diego, CA, USA). For AP sites, Protein Carbonyl, and HNE adducts determination, the Epoch Spectrophotometer System (BioTek, Winooski, VT, USA) was used.

2.4. Primer design, RNA purification and real-time quantitative PCR (qPCR)

Four genes were selected to determine the effect of temperature on oxidative stress and heat shock response in gills and skeletal muscle, including *hsp60, hsp70, gpx1, sod1*. Additionally, the gene *hif1a* was measured on gills, associated with oxygenation. To determine the effect of temperature on immune response in gills we selected 10 genes, including *hepcidin, gzma, tnfa, il1b, c3, c1qc, cxcl8, tlr2, tlr5,* and *tlr9*. Finally, to determine the effect of temperature on atrophy-associated biomarkers in skeletal muscle, we evaluated five genes, including *foxo1, foxo3, fbxo32, murf1,* and *atg16l*. Additionally, we used two reference genes (*actb* and *taf12*) previously validated for *Genypterus* species in several tissues (Dettleff et al., 2020a, 2020b, 2022). Primers were designed for each target gene using the Primer Quest™ Tool (Integrated DNA Technology, Inc.) using transcript sequences from a reference transcriptome previously generated in our group (DDBJ/EMBL/GenBank under the accession GKNI00000000, version GKNI01000000). The description of primers for *G. maculatus*, including sequence, size, Tm, and efficiency are in Table 1.

The total RNA was extracted from gills or skeletal muscle tissue stored in RNAsave solution (Biological Industries, Cromwell, CT, USA) using the TRIzol® reagent (Invitrogen, Carlsbad, CA, USA) protocol. The RNA quantification, the 260/280 ratio (above 2.0), and the 260/230 ratio (between 2.0 and 2.2) were determined with an Epoch Spectrophotometer system (BioTek, Winooski, VT, USA). The RNA integrity was evaluated using gel electrophoresis in a 1.2% formaldehyde agarose gel, where only samples with clear 28s and 18s bands were used. The RNA samples were treated with DNAse I and then reverse transcribed with the Affinity Script qPCR cDNA synthesis kit (Agilent, La Jolla, CA). The

Table 1
Primers sequences, amplicon size, Tm and efficiency of references and target genes of *Genypterus maculatus*.

Gene abbreviation	Gene name	Forward sequence (5′-3′)	Reverse sequence (5′-3′)	Amplicon size (bp)	Tm	Efficiency (%)
taf12	transcription initiation factor tfiid subunit 12	gatctgtaacgacgacgaagaa	caaatcagagggacgtcatgta	92	62	102
actb	Beta actin	tgtccctgtatgcttctggt	cccctctcagtcagaatcttcat	172	62	104
hif1a	hypoxia-inducible factor 1 alpha	ccaatctgtgggcagtagaa	gaccaacaagagcaggagaa	96	62	102
hsp60	Heat shock protein 60	gacggttccaatctctacatctc	cgctctccaaaccagttaca	86	62	100
hsp70	Heat shock protein 70	aagatcagcgacgacgataag	ctggtgctcatactcctctttc	105	62	96
gpx1	Glutathione Peroxidase 1	acctcagagagacgctacat	ttgaccgccaatctcctaac	93	62	100
sod1	Superoxide dismutase 1	agttctggtgctgctggttt	aaaaggcggtaacgacgag	100	62	103
hepcidin	Hepcidin	gaggagagagcccaagaaatc	gcagaggagcaccacaata	93	62	100
gzma	Granzyme A-like	acaggagatgtttgtcggtaag	tgtgaaggtgtgcttgtagg	100	62	103
tnfa	Tumor necrosis factor alpha	cagtgatgctggagactgatac	gcaaggcaaagggtgattg	97	62	102
il1b	Interleukin-1 beta-like	gggcgtacagaggagtataaag	ccagagcatcagacagatcaa	95	62	101
c3	Complement C3-like	ctgcttctggtgacctgttta	cttcgtgtcctctccatctttc	99	62	104
c1qc	Complement C1q 2-like	gatgtttgtggcgacgtatttg	gttgctttctcagcctctgta	99	62	99
cxcl8	C-X-C Motif Chemokine Ligand 8	cttgcgattggtctgctctc	cctggctttcgtggctattt	99	63	101
tlr2	Toll-like receptor 2	acacactcaacacccagaaata	aggaggaagactgagagaaaga	130	62	99
tlr5	Toll-like receptor 5	ggactcttggagtgaacagtag	tgtgctcggcttcaacat	103	62	100
tlr9	Toll-like receptor 9	cgatgctttcattgggctt	gccagactgaggacttctaaat	138	62	100
foxo1	Forkhead box O1-A-like	cagacgagacgtgacagattac	ctacggcgaaagacagaatca	98	62	99
foxo3	Forkhead box O3-like	gtgtgtcacgagtccaatga	cgtcgagatgaagaggaagaag	102	62	100
fbxo32	F-box only 32-like (atrogin-1)	gaagtgcagagtgtcattgtattg	cgcctgatggtgtcagataaa	105	62	101
murf1	E3 ubiquitin- ligase TRIM63-like	gacggtaacatcgaccttctaac	cagaggacgaagaggaggaata	94	62	99
Atg16l	Autophagy-related protein 16-1	taagaagcgagcagaca	caggaatcaccagcattg	152	62	104

qPCR was performed using a Rotor Gene Q qPCR platform (Qiagen, Hilden, Germany). The qPCR reaction contained 100 ng of total cDNA, 200 nM of each primer, 7.5 µl of 2x Brilliant® II SYBR® Green master mix (Agilent Technologies, Santa Clara, CA, USA) in a total volume of 15 µl. The qPCR amplification conditions were initial activation of 2 min at 95 °C, 40 cycles of 30 s of denaturation at 95 °C, 30 s of annealing at 62 °C, and 30 s of elongation at 72 °C. A melt curve was generated to confirm the presence of a single qPCR product and the absence of primer dimers. All samples were run on triplicates, with no template control and no RT control, following the MIQE guidelines (Bustin et al., 2009). The efficiency of each primer was determined with a standard curve using 2-fold dilutions with the $E = 10^{(-1/slope)}$-1 formula. The geometric means of *actb* and *taf12* were used to normalize the expression of target genes, according to the methodology described by (Vandesompele et al., 2002).

2.5. Statistical analysis

Significant differences between means of control and stress groups were determined using a multiple *t*-test analysis, with a threshold of $P < 0.05$, with correction by false discovery rate. All data are expressed as means ± standard errors of the means (SEM). Analyses and plots were performed with GraphPad Prism, v.5.00 (GraphPad Software, San Diego, CA, USA), and the free online platform SRplot (https://www.bioinformatics.com.cn/en).

3. Results

3.1. Oxidative stress response and heat shock protein under high temperature in gills and skeletal muscle

The effect of high temperature was determined in the oxidative stress response in gills and skeletal muscle, evaluating the antioxidant response and the oxidative damage in both tissues. The high temperature generated oxidative damage to DNA in gills observed as an increase in AP sites (Fig. 1A) ($P < 0.05$). This oxidative stress in gills was also observed in oxidative damage in proteins associated with increased carbonyl content (Fig. 1C) and lipid peroxidation (Fig. 1E) in response to temperature ($P < 0.05$). In the skeletal muscle, the effect of high temperature on oxidative stress was also observed, with a significant increase in DNA oxidative damage and protein oxidation (Fig. 1B and D) ($P < 0.05$). However, no significant difference was observed in lipid peroxidation in skeletal muscle in response to temperature (Fig. 1F).

At the transcriptional level, the increase in temperature associated with heat waves causes a significant up-regulation of transcripts encoding heat shock proteins *hsp60* and *hsp70* in the stress group (Fig. 2A and 2B) in the skeletal muscle ($P < 0.05$). Additionally, an antioxidant response was observed in this tissue, with the up-regulation of the antioxidant gene *gpx1* in the stress group (Fig. 2C) ($P < 0.05$), with no significant differences in the *sod1* in skeletal muscle between the groups (Fig. 2D).

In the gills, the transcriptional effect of the high temperature also generated a heat shock response, with a significant increase of *hsp60* and *hsp70* transcripts expression in the stress group (Fig. 3A and 3B) ($P < 0.05$). In terms of the antioxidant response in gills, the high temperature induces an up-regulation of *sod1* (Fig. 3E) ($P < 0.05$), with no significant differences in *gpx1* expression between the groups (Fig. 3D). Finally, we evaluated transcriptional response to hypoxia in the gills of stress group, measuring the hypoxia-inducible factor *hif1a* expression, with no significant differences observed due to temperature treatment (Fig. 3C).

3.2. Effect of high temperature on immune-related genes in gills of G. maculatus

To evaluate the effect of the high temperature on the immune response in gills, we determined the transcriptional response in several immune-related genes. The high temperature generates an up-regulation in the expression of the antibacterial peptide and iron regulator *hepcidin* (Fig. 4A) ($P < 0.05$). The cell-mediated cytotoxicity-related serine protease *gzma* was also increased in response to high temperature in the gills of the stress group (Fig. 4B) ($P < 0.05$). As part of the innate immune response, we evaluate two pro-inflammatory cytokines in gills, with a significant up-regulation of *tnfa* (Fig. 4C) ($P < 0.05$), with no differences in the pro-inflammatory cytokine *il1b* (Fig. 4D). An up-regulation was observed in response to high temperature in the acute-phase cytokine *cxcl8* (Fig. 4G) ($P < 0.05$). Related to the complement system, we evaluate the expression of *c3* and *c1qc*, with no significant effects of high temperature in these genes (Fig. 4E and F). To study the effect of temperature on receptors related to innate immunity defense, we determine the gene expression of three Toll-like receptors. The *tlr5* was up-regulated in the gills of the stress group (Fig. 4I) ($P < 0.05$), with no significant differences in the *tlr2* and *tlr9* expression (Fig. 4H and J).

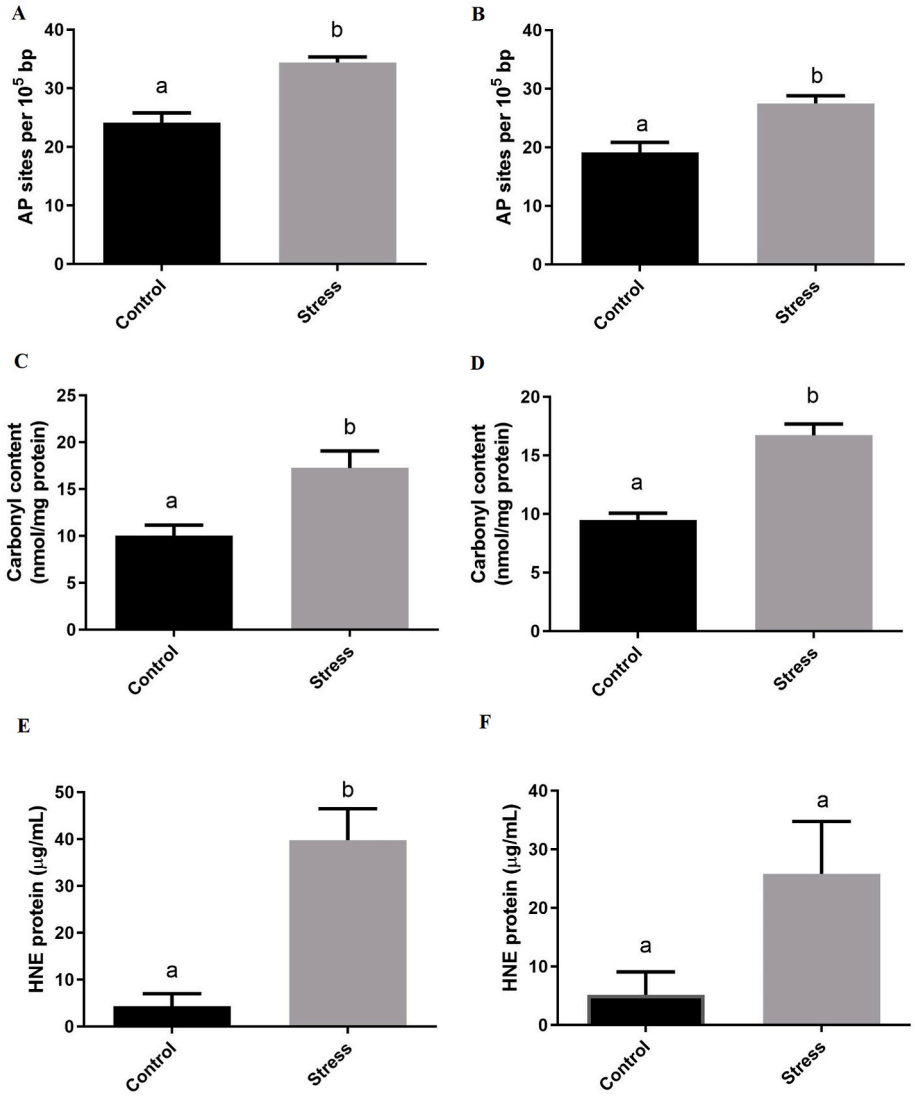

Fig. 1. Evaluation of oxidative damage in gills and skeletal muscle of *G. maculatus* under high temperature. Level of DNA damage as AP sites (A = gills; B = skeletal muscle), protein oxidation as carbonyl content (C = gills; D = skeletal muscle), and lipid peroxidation as HNE adducts (E = gills; F = skeletal muscle) in control and stress groups. Bars represent means ± SEM. Different letters indicate significant differences between the control and stress groups (p-value <0.05).

3.3. High temperature induces atrophy-related genes in the skeletal muscle of G. maculatus

To determine the effect of high temperature on skeletal muscle atrophy-associated biomarkers in black cusk-eel, we evaluate five atrophy and protein catabolism-related genes in *G. maculatus*. The high temperature generates an up-regulation of the atrophy-related transcription factor *foxo1* (Fig. 5A) ($P < 0.05$), but not in the *foxo3* transcription factor (Fig. 5B) in skeletal muscle. In the target atrogenes *fbxo32* and *murf1,* an up-regulation was observed in the stress group (Fig. 5C and D) ($P < 0.05$). Finally, in the *atg16l* gene associated with protein catabolism through autophagy, a significant increase was observed in response to high temperature in the skeletal muscle (Fig. 5E) ($P < 0.05$).

4. Discussion

The black cusk-eel is a relevant marine species for fisheries and aquaculture diversification in the Chilean industry. It is pertinent to understand the effect of heat waves associated with an increase in seawater temperature on the immune response in this species in relevant tissue such as gills, the impact on oxidative stress, and the effect on the protein catabolism of muscle. For this, we performed an experimental assay that simulated the marine heat waves in the black cusk-eel. Previous studies have evaluated the impact of high temperatures on other species of the *Genypterus* genus, determining the effect of temperature on liver metabolism, gills response, egg quality, and muscle atrophy in the red cusk-eel (*Genypterus chilensis*) (Dettleff et al., 2020a, 2021, 2022, 2024). However, the stress responses against different stressors, including temperature, could vary, according to the teleost species and their life environment (Dammark et al., 2018). In the black cusk-eel, no studies have been done evaluating the impact of temperature on the immune response, requiring more knowledge to understand the effect of environmental stressors in this species.

4.1. Temperature generates a moderated innate immune modulation in the gills of G. maculatus

In teleost fish, the immune response is a complex process, which involves two main components: innate immune response, associated with the response to pathogens via identifying pathogen-associated molecular patterns (PAMPs) (Dezfuli et al., 2023; Mahapatra et al.,

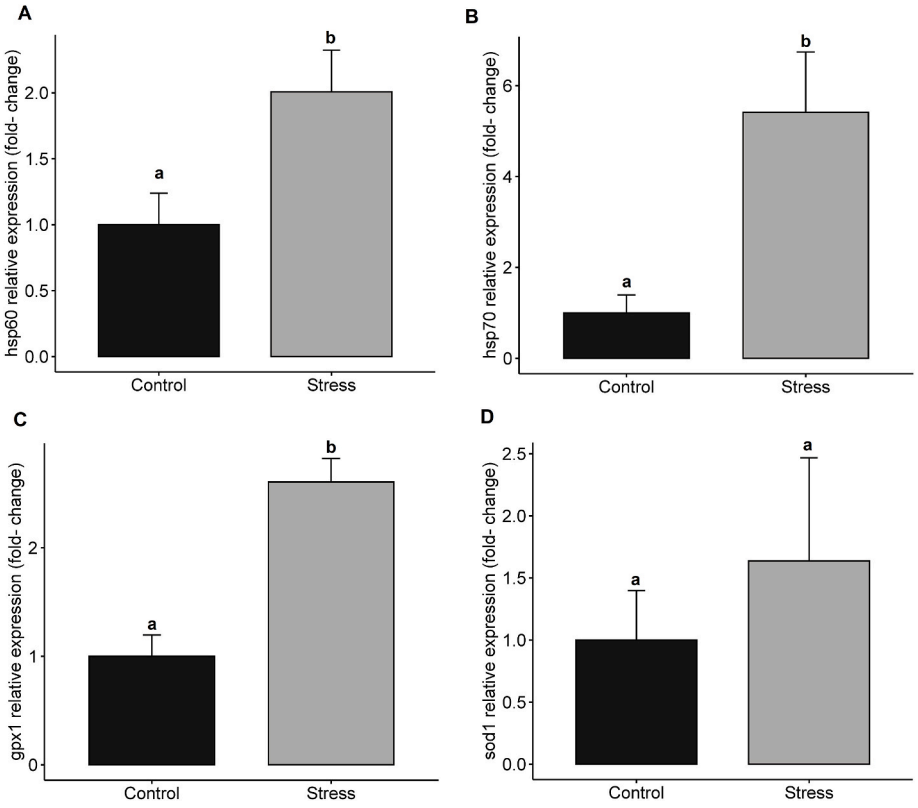

Fig. 2. Heat shock protein and antioxidant enzymes gene expression response to high temperature in skeletal muscle of *G. maculatus*. Relative gene expression of *hsp60* (A), *hsp70* (B), *gpx1* (C), and *sod1* (D) expressed as the fold-change relative to the control group. Values were normalized by the geometric mean of reference genes (*actb* and *taf12*). Bars represent means ± SEM. Different letters indicate significant differences between the control and stress groups (p-value <0.05).

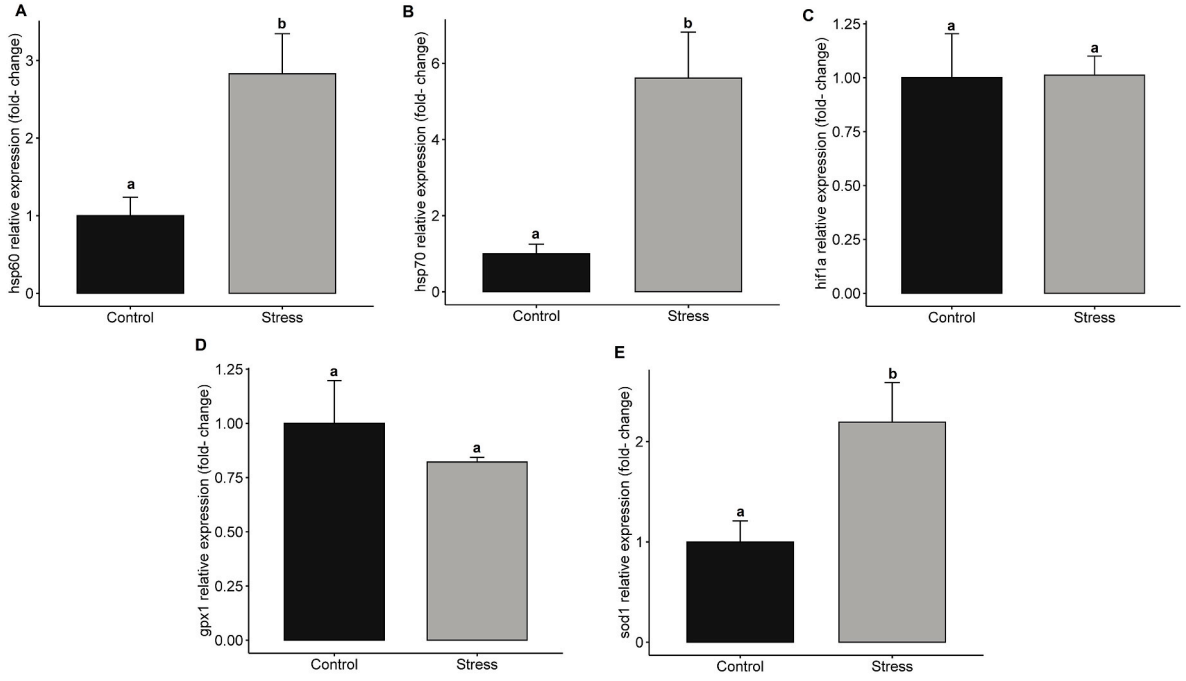

Fig. 3. Heat shock protein, antioxidant enzymes, and hypoxia gene expression response to high temperature in gills of *G. maculatus*. Relative gene expression of *hsp60* (A), *hsp70* (B), *hif1a* (C), *gpx1* (D), and *sod1* (E) expressed as the fold-change relative to the control group. Values were normalized by the geometric mean of reference genes (*actb* and *taf12*). Bars represent means ± SEM. Different letters indicate significant differences between the control and stress groups (p-value <0.05).

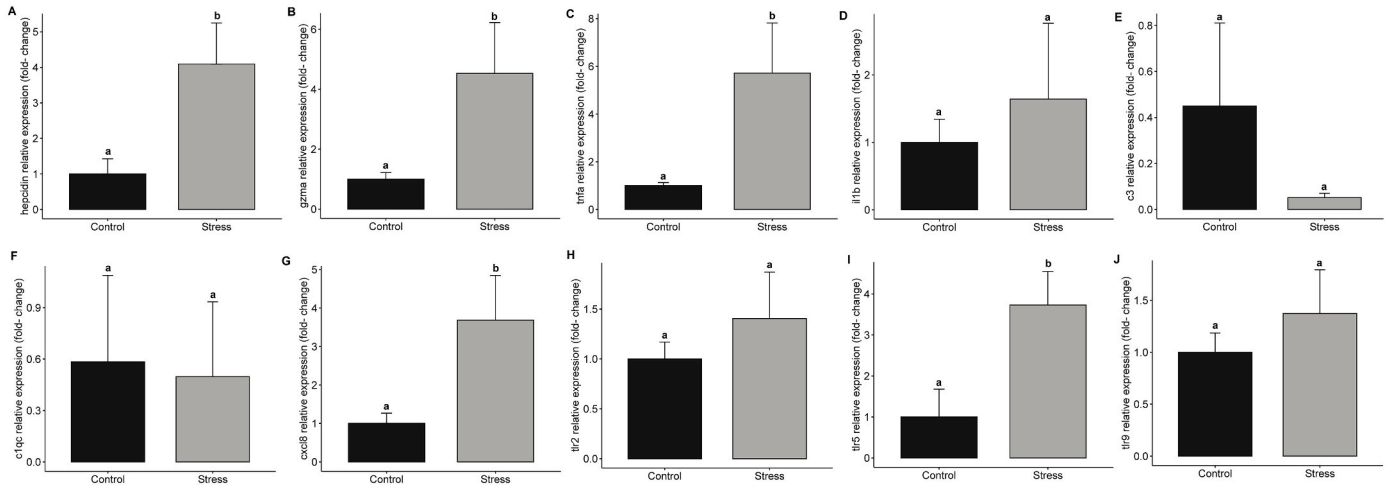

Fig. 4. Effect of high temperature on immune-related genes in gills of *G. maculatus*. Relative gene expression of *hepcidin* (A), *gzma* (B), *tnfa* (C), *il1b* (D), *c3* (E), *c1qc* (F), *cxcl8* (G), *tlr2* (H), *tlr5* (I), and *tlr9* (J) expressed as the fold-change relative to the control group. Values were normalized by the geometric mean of reference genes (*actb* and *taf12*). Bars represent means ± SEM. Different letters indicate significant differences between the control and stress groups (p-value <0.05).

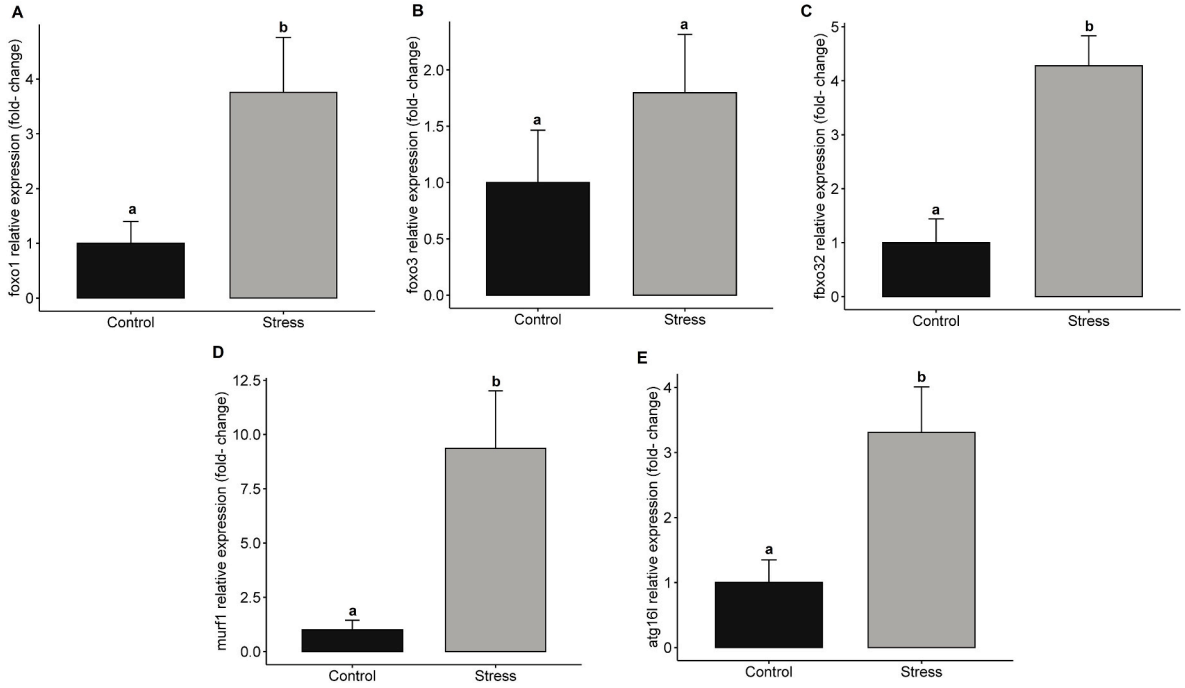

Fig. 5. Effect of high temperature on atrophy-related genes in skeletal muscle of *G. maculatus*. Relative gene expression of *foxo1* (A), *foxo3* (B), *fbxo32* (C), *murf1* (D), and *atg16l* (E) expressed as the fold-change relative to the control group. Values were normalized by the geometric mean of reference genes (*actb* and *taf12*). Bars represent means ± SEM. Different letters indicate significant differences between the control and stress groups (p-value <0.05).

2023); or adaptative immune response, related to specific antigen recognition and memory development, including B and T cells (Abos et al., 2022). This immune response is generated in the fish based on several organs and tissues with relevant immune functions, including the head kidney, spleen, bursa, nasopharynx-associated lymphoid tissue, thymus, and gills (Bjorgen et al., 2022; Das and Salinas, 2020). The gills represent one of the first physical barriers against infections with bacteria, viruses, and parasites. Additionally, this organ could participate actively in the immune response, through specialized immune components such as leukocytes, and cytokines, which are present in the gill-associated lymphoid tissue (GIALT) (Resseguier et al., 2020). In teleost fish, stressors could affect the gills response, as previously observed in mandarin fish (*Siniperca chuatsi*), where different salt concentrations affect inflammation and immune-related pathways, associated with down-regulation of immune genes (Zhao et al., 2023). The down-regulation of immune pathways was also observed in the gills of medaka (*Oryzias melastigma*) for osmotic stress (Li et al., 2022). Temperature induces an effect on the immune response of teleost, with modulation of the inflammatory and cellular response in fish under low temperatures (Abram et al., 2017; Wentworth et al., 2018). It has been observed that high temperature could modulate the immune response of gills in *G. chilensis*, with down-regulation of inflammatory cytokines and complement systems factors (Dettleff et al., 2024). However, the immune response to stressors is complex, with several studies showing an increase in the expression of immune-related genes in response to stress. This has been observed in hypoxia stress, with up-regulation of innate immune genes and down-regulation of adaptive immune genes in the gills of Nile tilapia (*Oreochromis niloticus*) (Ma et al., 2023), and stress by

air exposure in rainbow trout (*Oncorhynchus mykiss*), with increased *tnfa* and il1b expression (Khansari et al., 2018). In salinity stress was also observed an increased *tnfa* and *il1b* expression, with down-regulation of *tlr2* in gills of silvery pomfret (*Pampus argenteus*) (Li et al., 2020). Previous studies have shown that temperature in teleost could also induce the immune response, with an enrichment of process associated with the immune response in gills of sharp-snouted lenok (*Brachymystax lenok tsinlingensis*) under high temperature (Li et al., 2021), which is consistent with our results, where five of the ten immune-related genes evaluate were up-regulated in response to high temperature.

Regarding the proinflammatory response, we evaluated the expression of *tnfa* and *il1b* in gills, with an up-regulation of *tnfa* in response to high temperature. TNFa is a cytokine member of the TNF superfamily, that regulates the innate inflammatory response in fish, including systemic inflammation, apoptosis, cell proliferation, and regulation of immune cells (Mokhtar et al., 2023). Similar results have been previously observed in response to high temperature in other teleost fish, with increased expression of *tnfa* and *il1b* in gills of the *O. niloticus* (Esam et al., 2022), striped catfish (*Pangasianodon hypophthalmus)* (Kumar et al., 2022), as well as in the spleen of white sturgeon (*Acipenser transmontanus*) (Soto et al., 2022). This increase of *tnfa* expression in gills was also observed for other stressors, including hyposalinity in spotted scat (*Scatophagus argus*) (Zhong et al., 2021). The acute-phase cytokine *cxcl8,* was up-regulated in our study in response to high temperature. The CXCL8 is a cytokine with chemotactic activity for immune cells and participates in oxidative burst, inflammation, and angiogenesis (Umasuthan et al., 2020; van der Aa et al., 2010). The increased expression of *cxcl8* was also observed in response to other stressors, including restraint stress in common carp (*Cyprinus carpio*) (Klak et al., 2022). Other immune components that presented an increased response to temperature in *G. maculatus* were hepcidin, an antimicrobial peptide and iron regulator in fish (Alvarez et al., 2022; Liu et al., 2022), and *gzma*, a serine-protease that participates in the cell-mediated cytotoxicity in fish (Chaves-Pozo et al., 2019). Different responses have been observed for these molecules, with variable responses for hepcidin in largemouth bass (*Micropterus salmoides*) and Atlantic cod (*Gadus morhua*) (Sun et al., 2020; Hori et al., 2013). For *gzma*, no significant effect has been observed in the head kidney of olive flounder (*Paralichthys olivaceus*) (Avunje et al., 2012).

Another relevant factor in the innate immune response is the recognition of pathogens through the pattern recognition receptors (PRRs). The TLRs are a particular group of PRRs that recognize conserved pathogen molecules (Palti, 2011), with several TLR families, including three TLRs families associated mainly with prokaryote ligands recognition: TLR2 (recognition of lipoteichoic acid and peptidoglycan), TLR5 (recognition of flagellin in its heterodimer form and dsRNA in its homodimer form) and TLR9 (recognition of CpG DNA) (Palti, 2011; Pietretti and Wiegertjes, 2014; Zhang et al., 2014). The temperature could affect TLR expression, as observed in the liver of *P. hypophthalmus* (Kumar et al., 2022) and *B. lenok tsinlingensisas* (Li et al., 2021). In zebrafish (*Danio rerio*), increased temperature up-regulates the expression of TLR3, TLR5, and TLR21 in visceral mass after immunostimulation (Chen et al., 2021). Similarly, we observe an increase in the expression of *tlr5* in response to high temperature in gills, with no significant differences in the expression of *tlr2* and *tlr9*. In the gills of *P. olivaceus* opposite pattern of response according to temperature was observed in gills, with several down-regulated TLRs under low-temperature stress, and an up-regulation of some TLRs under high-temperature stress, with other TLRs without significant differences in this tissue (Yao et al., 2023). These results reveal that TLRs response could vary according to the specific TLR, temperature, and species, as was observed for *G. maculatus* in our study. Response of complement systems-related genes to temperature has been previously observed in gills, with down-regulation of *c3* in *O. niloticus* and several complement-related genes in *G. chilensis* (Dettleff et al., 2024; Esam et al., 2022). However, we did not observe an effect on complement system gene expression, showing that *G. maculatus* responds differently at the immune level to high temperature compared with *G. chilensis*.

4.2. Oxidative stress and heat shock response under high temperature

The oxidative stress is generated in the tissues when the balance between reactive oxygen species (ROS) production and the antioxidant capacity to detoxify these products is lost (Betteridge, 2000). The ROS plays relevant functions in fish, including cell signal transduction, as well as a role in the immune response through the respiratory burst associated with phagocytosis (Lesser, 2006; Biller and Takahashi, 2018). In our study, oxidative stress was evidenced by oxidative damage in DNA, protein, and lipoperoxidation, with an increased antioxidant expression of *sod1* in gills. This oxidative damage in gills generated by temperature has been previously observed in pacu (*Piaractus mesopotamicus*), pearlspot (*Etroplus suratensis),* and rock goby (*Gobius paganellus)* (Nitz et al., 2020; Joy et al., 2017; Vinagre et al., 2014). This response was also consistent with the heat wave effect observed in the gills of the related species *G. chilensis,* where oxidative damage was detected in response to temperature (Dettleff et al., 2024), supporting the susceptibility of gills to thermal stress. In the skeletal muscle oxidative damage and antioxidant activation also was observed in *G. maculatus*, with DNA damage, protein carbonylation, and *gpx1* up-regulation. Consistent results have been previously observed in oxidative stress generated by high temperature in muscle in seabass (*Dicentrarchus labrax*), *O. mykiss,* white seabream (*Diplodus sargus),* thinlip mullet (*Liza ramada),* and *G. chilensis* (Dettleff et al., 2020a; Madeira et al., 2013; Vinagre et al., 2012; Molina et al., 2023b).

The cellular stress response to temperature is executed by molecular chaperones known as heat shock proteins (HSP), which participate in the correct folding of the protein and preserve its integrity (Glickman and Ciechanover, 2002). A general up-regulation of transcripts encoding HSP was observed in the gills and skeletal muscle of *G. maculatus,* with increased expression of *hsp60* and *hsp70*. An up-regulation of these HSPs in response to temperature has been previously observed in the skeletal muscle, liver, and gills of *G. chilensis* (Dettleff et al., 2020a, 2022, 2024). This response has also been observed in skeletal muscle and gills of teleost fish in other genera, including O. *mykiss,* catfish (*Horabagrus brachysoma*), Atlantic salmon (*Salmo salar*), goby fish (*Gillichthys mirabilis*), clown anemonefish *(Amphiprion ocellaris),* and *G. morhua,* (Currie et al., 2000; Dalvi et al., 2012, 2017; Hori et al., 2010; Madeira et al., 2017; Vargas-Chacoff et al., 2018; Logan and Somero, 2011; Shi et al., 2015). However, the pattern of HSP response to temperature may vary according to tissue and species (Madeira et al., 2017), which is relevant to understanding the specific response in *G. maculatus*.

4.3. Transcriptional induction of muscle atrophy by stressors in fish

The skeletal muscle is the main commercial product of several aquaculture species, including *G. maculatus*. The growth in this tissue is regulated by myoblast proliferation, the hypertrophy of the muscular fiber by protein synthesis, and muscular protein degradation (known as muscular atrophy) (Johnston et al., 2011). The process of atrophy in fish is regulated by the ubiquitin-proteosome system, with two key ubiquitin ligases in fish, the FBXO32 and the MuRF1, which are regulated by the forkhead transcription factors FOXO1 and FOXO3 (Bonaldo and Sandri, 2013; Braun and Marks, 2015; Bodine and Baehr, 2014). In *G. maculatus* the high temperature induces atrophy-associated biomarkers of the skeletal muscle at the transcriptional level, observed by the increase in the transcription factors *foxo1* and *foxo3*, and its target genes *fbxo32* and *murf1*. This muscle atrophy induced by stressors has been observed in other teleost species, including the *O. mykiss, G. chilensis,* and fine flounder (*Paralichthys adspersus*) (Dettleff et al., 2020a; Molina et al., 2023b; Cleveland and Evenhuis, 2010; Fuentes et al., 2012). Our results evidence that stressors could affect muscle growth in fish, generating atrophy, HSP response, and oxidative stress, as immune modulation in

G. maculatus.

5. Conclusion

This study evaluated the effect of high temperature on the gills and skeletal muscle of *G. maculatus* for the first time. In conclusion, increased temperature could modulate the innate immune response in gills and induce atrophy-associated biomarkers response in skeletal muscle, generating oxidative stress and heat shock response in both tissues of *G. maculatus*. This information is relevant considering the increased frequency and intensity of heat waves in the South Pacific Ocean under the climate change scenario.

CRediT authorship contribution statement

Sofia Becerra: Formal analysis, Investigation, Methodology. **Marcia Arriagada-Solimano:** Formal analysis, Investigation, Writing – review & editing. **Sebastian Escobar-Aguirre:** Investigation, Writing – review & editing. **Jaime Palomino:** Investigation, Writing – review & editing. **Jorge Aedo:** Investigation, Methodology. **Juan Manuel Estrada:** Investigation, Methodology. **Veronica Barra-Valdebenito:** Investigation, Writing – review & editing. **Rodrigo Zuloaga:** Formal analysis, Methodology. **Juan Antonio Valdes:** Funding acquisition, Investigation, Project administration, Resources. **Phillip Dettleff:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft.

Funding

This study was supported by ANID FONDECYT Inicio [grant number 11230153], and ANID FONDECYT Postdoctorado [grant number 3180283] awarded to Phillip Dettleff, and ANID/FONDAP [grant number 15110027and 1522A0004] awarded to Juan Antonio Valdes. We are also grateful for the contribution of Italo Barrios of CIMARQ with fish assistance.

Declaration of competing interest

The authors declare that there are no financial or nonfinancial conflicts of interest.

Data availability

Data will be made available on request.

References

Abos, B., Bailey, C., Tafalla, C., 2022. Adaptive immunity. In: Buchmann, K., Secombes, C.J. (Eds.), Principles of Fish Immunology. Springer Nature, Switzerland, pp. 105–140.
Abram, Q.H., Dixon, B., Katzenback, B.A., 2017. Impacts of low temperature on the teleost immune system. Biology 6.
Alvarez, C.A., Santana, P.A., Salinas-Parra, N., Beltran, D., Guzman, F., Vega, B., et al., 2022. Immune modulation ability of hepcidin from teleost fish. Animals 12.
Avunje, S., Kim, W.S., Oh, M.J., Choi, I., Jung, S.J., 2012. Temperature-dependent viral replication and antiviral apoptotic response in viral haemorrhagic septicaemia virus (VHSV)-infected olive flounder (*Paralichthys olivaceus*). Fish Shellfish Immunol. 32, 1162–1170.
Barange, M., Bahri, T., Beveridge, M., Cochrane, K., Funge-Smith, S., Poulain, F., 2018. Impacts of climate change on fisheries and aquaculture. Synthesis of Current Knowledge, Adaptation, and Mitigation Options. FAO, Rome, pp. 1–654.
Betteridge, D.J., 2000. What is oxidative stress? Metab., Clin. Exp. 49, 3–8.
Biller, J.D., Takahashi, L.S., 2018. Oxidative stress and fish immune system: phagocytosis and leukocyte respiratory burst activity. An. Acad. Bras. Cienc. 90, 3403–3414.
Bjorgen, H., Koppang, E.O., 2022. Anatomy of teleost fish immune structures and organs. In: Buchmann, K., Secombes, C.J. (Eds.), Principles of Fish Immunology. Springer Nature, Switzerland, pp. 1–30.
Bodine, S.C., Baehr, L.M., 2014. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. Am. J. Physiol. Endocrinol. Metabol. 307, E469–E484.
Bodine, S.C., Furlow, J.D., 2015. Glucocorticoids and skeletal muscle. Adv. Exp. Med. Biol. 872, 145–176.
Bonaldo, P., Sandri, M., 2013. Cellular and molecular mechanisms of muscle atrophy. Disease Models & Mechanisms 6, 25–39.
Braun, T.P., Marks, D.L., 2015. The regulation of muscle mass by endogenous glucocorticoids. Front. Physiol. 6, 12.
Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., et al., 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 55, 611–622.
Chaves-Pozo, E., Valero, Y., Lozano, M.T., Rodríguez-Cerezo, P., Miao, L., Campo, V., et al., 2019. Fish granzyme A shows a greater role than granzyme B in fish innate cell-mediated cytotoxicity. Front. Immunol. 10.
Chen, H., Liang, Y., Han, Y., Liu, T., Chen, S., 2021. Genome-wide analysis of Toll-like receptors in zebrafish and the effect of rearing temperature on the receptors in response to stimulated pathogen infection. J. Fish. Dis. 44, 337–349.
Cleveland, B.M., Evenhuis, J.P., 2010. Molecular characterization of atrogin-1/F-box protein-32 (FBXO32) and F-box protein-25 (FBXO25) in rainbow trout (*Oncorhynchus mykiss*): expression across tissues in response to feed deprivation. Comparative Biochemistry and Physiology Part B. Biochemistry & Molecular Biology 157, 248–257.
Currie, S., Moyes, C.D., Tufts, B.L., 2000. The effects of heat shock and acclimation temperature on hsp70 and hsp30 mRNA expression in rainbow trout: in vivo and in vitro comparisons. J. Fish. Biol. 56, 398–408.
Dalvi, R.S., Pal, A.K., Tiwari, L.R., Baruah, K., 2012. Influence of acclimation temperature on the induction of heat-shock protein 70 in the catfish *Horabagrus brachysoma* (Gunther). Fish Physiol. Biochem. 38, 919–927.
Dalvi, R.S., Das, T., Debnath, D., Yengkokpam, S., Baruah, K., Tiwari, L.R., et al., 2017. Metabolic and cellular stress responses of catfish, *Horabagrus brachysoma* (Gunther) acclimated to increasing temperatures. J. Therm. Biol. 65, 32–40.
Dammark, K.B., Ferchaud, A.L., Hansen, M.M., Sorensen, J.G., 2018. Heat tolerance and gene expression responses to heat stress in threespine sticklebacks from ecologically divergent environments. J. Therm. Biol. 75, 88–96.
Das, P.K., Salinas, I., 2020. Fish nasal immunity: from mucosal vaccines to neuroimmunology. Fish Shellfish Immunol. 104, 165–171.
Dettleff, P., Zuloaga, R., Fuentes, M., González, J., Aedo, J., Estrada, J.M., et al., 2020a. Physiological and molecular responses to thermal stress in red cusk-eel (*Genypterus chilensis*) juveniles reveals atrophy and oxidative damage in skeletal muscle. J. Therm. Biol. 94, 102750.
Dettleff, P., Hormazabal, E., Aedo, J., Fuentes, M., Meneses, C., Molina, A., et al., 2020b. Identification and evaluation of long noncoding RNAs in response to handling stress in red cusk-eel (*Genypterus chilensis*) via RNA-seq. Mar. Biotechnol. 22, 94–108.
Dettleff, P., Zuloaga, R., Fuentes, M., Estrada, J.M., Molina, A., Valdes, J.A., 2021. Temperature effect on oxidative stress and egg quality-related genes on post-ovulatory eggs and ovary of red cusk-eel (*Genypterus chilensis*). J. Fish. Biol.
Dettleff, P., Zuloaga, R., Fuentes, M., Gonzalez, P., Aedo, J., Estrada, J.M., et al., 2022. High-temperature stress effect on the red cusk-eel (*Geypterus chilensis*) liver: transcriptional modulation and oxidative stress damage. Biology 11.
Dettleff, P., Toloza, C., Fuentes, M., Aedo, J., Zuloaga, R., Estrada, J.M., et al., 2024. Gills de novo assembly reveals oxidative stress, unfolded protein, and immune response on red cusk-eel (*Genypterus chilensis*) under thermal stress. Mar. Environ. Res. 196, 106440.
Dezfuli, B.S., Lorenzoni, M., Carosi, A., Giari, L., Bosi, G., 2023. Teleost innate immunity, an intricate game between immune cells and parasites of fish organs: who wins, who loses. Front. Immunol. 14.
Esam, F., Khalafalla, M.M., Gewaily, M.S., Abdo, S., Hassan, A.M., Dawood, M.A.O., 2022. Acute ammonia exposure combined with heat stress impaired the histological features of gills and liver tissues and the expression responses of immune and antioxidative related genes in Nile tilapia. Ecotoxicol. Environ. Saf. 231, 113187.
Espinoza, C.V., Hernandez-Miranda, E., Gilabert, H., Ojeda, F.P., 2023. Temporal and spatial dissimilarities in an intertidal fish assemblage in the South Pacific Ocean: the role of the ENSO process and intrinsic habitat conditions in emerging patterns. Sci. Total Environ. 872, 162220.
Fuentes, E.N., Ruiz, P., Valdes, J.A., Molina, A., 2012. Catabolic signaling pathways, atrogenes, and ubiquitinated proteins are regulated by the nutritional status in the muscle of the fine flounder. PLoS One 7, e44256.
Glickman, M.H., Ciechanover, A., 2002. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol. Rev. 82, 373–428.
Gonzalez, P., Dettleff, P., Valenzuela, C., Estrada, J.M., Valdes, J.A., Meneses, C., et al., 2019. Evaluating the genetic structure of wild and commercial red cusk-eel (*Genypterus chilensis*) populations through the development of novel microsatellite markers from a reference transcriptome. Mol. Biol. Rep.
Hori, T.S., Gamperl, A.K., Afonso, L.O., Johnson, S.C., Hubert, S., Kimball, J., et al., 2010. Heat-shock responsive genes identified and validated in Atlantic cod (*Gadus morhua*) liver, head kidney and skeletal muscle using genomic techniques. BMC Genom. 11, 72.
Hori, T.S., Gamperl, A.K., Nash, G., Booman, M., Barat, A., Rise, M.L., 2013. The impact of a moderate chronic temperature increase on spleen immune-relevant gene transcription depends on whether Atlantic cod (*Gadus morhua*) are stimulated with bacterial versus viral antigens. Genome 56, 567–576.
Hsiung, K.M., Kimura, S., Han, Y.S., Takeshige, A., Iizuka, Y., 2018. Effect of ENSO events on larval and juvenile duration and transport of Japanese eel (*Anguilla japonica*). PLoS One 13, e0195544.
Johnston, I.A., Bower, N.I., Macqueen, D.J., 2011. Growth and the regulation of myotomal muscle mass in teleost fish. J. Exp. Biol. 214, 1617–1628.

Joy, S., Alikunju, A.P., Jose, J., Sudha, H.S.H., Parambath, P.M., Puthiyedathu, S.T., et al., 2017. Oxidative stress and antioxidant defense responses of *Etroplus suratensis* to acute temperature fluctuations. J. Therm. Biol. 70, 20–26.
Khansari, A.R., Balasch, J.C., Vallejos-Vidal, E., Parra, D., Reyes-Lopez, F.E., Tort, L., 2018. Comparative immune- and stress-related transcript response induced by air exposure and Vibrio anguillarum bacterin in rainbow trout (*Oncorhynchus mykiss*) and gilthead seabream (*Sparus aurata*) mucosal surfaces. Front. Immunol. 9, 856.
Klak, K., Maciuszek, M., Marcinkowska, M., Verburg-van Kemenade, B.M.L., Chadzinska, M., 2022. The importance of CXC-receptors CXCR1-2 and CXCR4 for adaptive regulation of the stress axis in teleost fish. Fish Shellfish Immunol. 127, 647–658.
Kumar, N., Kumar, S., Singh, A.K., Gite, A., Patole, P.B., Thorat, S.T., 2022. Exploring mitigating role of zinc nanoparticles on arsenic, ammonia and temperature stress using molecular signature in fish. J. Trace Elem. Med. Biol. 74.
Lesser, M.P., 2006. Oxidative stress in marine environments: biochemistry and physiological ecology. Annu. Rev. Physiol. 68, 253–278.
Li, J., Xue, L., Cao, M., Zhang, Y., Wang, Y., Xu, S., et al., 2020. Gill transcriptomes reveal expression changes of genes related with immune and ion transport under salinity stress in silvery pomfret (*Pampus argenteus*). Fish Physiol. Biochem. 46, 1255–1277.
Li, P., Liu, Q., Li, J., Wang, F., Wen, S., Li, N., 2021. Transcriptomic responses to heat stress in gill and liver of endangered *Brachymystax lenok tsinlingensis*. Comparative biochemistry and physiology Part D. Genom. Proteonomics 38, 100791.
Li, R., Liu, J.Q., Leung, C.T., Lin, X., Chan, T.F., Tse, W.K.F., et al., 2022. Transcriptomic analysis in marine medaka gill reveals that the hypo-osmotic stress could alter the immune response via the IL17 signaling pathway. Int. J. Mol. Sci. 23.
Liu, M., Hu, R., Li, W., Yang, W., Xu, Q., Chen, L., 2022. Identification of antibacterial activity of hepcidin from antarctic notothenioid fish. Front. Microbiol. 13, 834477.
Logan, C.A., Somero, G.N., 2011. Effects of thermal acclimation on transcriptional responses to acute heat stress in the eurythermal fish *Gillichthys mirabilis* (Cooper). Am J Physiol Regul Integr Comp Physiol, 300. Am J Physiol Regul Integr Comp Physiol, pp. R1373–R1383.
Ma, J.L., Xu, D.P., Tao, Y.F., Zheng, T., Xu, P., Qiang, J., 2023. Integrated transcriptome and miRNA sequencing analyses reveal that hypoxia stress induces immune and metabolic disorders in gill of genetically improved farmed tilapia (GIFT, *Oreochromis niloticus*). Fish Shellfish Immunol. 139.
Madeira, D., Narciso, L., Cabral, H.N., Vinagre, C., Diniz, M.S., 2013. Influence of temperature in thermal and oxidative stress responses in estuarine fish. Comparative Biochemistry and Physiology Part A. Molecular & Integrative Physiology 166, 237–243.
Madeira, C., Madeira, D., Diniz, M.S., Cabral, H.N., Vinagre, C., 2017. Comparing biomarker responses during thermal acclimation: a lethal vs non-lethal approach in a tropical reef clownfish. Comparative Biochemistry and Physiology Part A. Molecular & Integrative Physiology 204, 104–112.
Mahapatra, S., Ganguly, B., Pani, S., Saha, A., Samanta, M., 2023. A comprehensive review on the dynamic role of toll-like receptors (TLRs) in frontier aquaculture research and as a promising avenue for fish disease management. Int. J. Biol. Macromol. 253.
Mokhtar, D.M., Zaccone, G., Alesci, A., Kuciel, M., Hussein, M.T., Sayed, R.K.A., 2023. Main components of fish immunity: an overview of the fish immune system. Fishes-Basel 8.
Molina, A., Dettleff, P., Valenzuela-Muñoz, V., Gallardo-Escarate, C., Valdés, J.A., 2023a. High-temperature stress induces autophagy in rainbow trout skeletal muscle. Fishes-Basel 8, 303.
Molina, A., Dettleff, P., Valenzuela-Muñoz, V., Gallardo-Escarate, C., Valdés, J.A., 2023b. High-temperature stress induces autophagy in rainbow trout skeletal muscle. Fishes-Basel 8.
Montie, S., Thomsen, M.S., Rack, W., Broady, P.A., 2020. Extreme summer marine heatwaves increase chlorophyll a in the Southern Ocean. Antarct. Sci. 32, 508–509.
Nitz, L.F., Pellegrin, L., Maltez, L.C., Pinto, D., Sampaio, L.A., Monserrat, J.M., et al., 2020. Temperature and hypoxia on oxidative stress responses in pacu *Piaractus mesopotamicus*. J. Therm. Biol. 92, 102682.
Ong, J.J., Rountrey, A.N., Meeuwig, J.J., Newman, S.J., Zinke, J., Meekan, M.G., 2015. Contrasting environmental drivers of adult and juvenile growth in a marine fish: implications for the effects of climate change. Sci. Rep. 5, 10859.
Palti, Y., 2011. Toll-like receptors in bony fish: from genomics to function. Dev. Comp. Immunol. 35, 1263–1272.
Pietretti, D., Wiegertjes, G.F., 2014. Ligand specificities of Toll-like receptors in fish: indications from infection studies. Dev. Comp. Immunol. 43, 205–222.
Resseguier, J., Dalum, A.S., Pasquier, L.D., Zhang, Y., Koppang, E.O., Boudinot, P., et al., 2020. Lymphoid tissue in teleost gills: variations on a theme. Biology 9.
Santaclara, F.J., Perez-Martin, R.I., Sotelo, C.G., 2014. Developed of a method for the genetic identification of ling species (*Genypterus* spp.) in seafood products by FINS methodology. Food Chem. 143, 22–26.
SERNAPESCA, 2023. In: SNdPy, Acuicultura (Ed.), Anuario estadístico de pesca y acuicultura 2022.
Shi, H.N., Liu, Z., Zhang, J.P., Kang, Y.J., Wang, J.F., Huang, J.Q., et al., 2015. Effect of heat stress on heat-shock protein (Hsp60) mRNA expression in rainbow trout *Oncorhynchus mykiss*. Genet. Mol. Res. 14, 5280–5286.
Soto, E., Fast, M.D., Purcell, S.L., Denver Coleman, D., Yazdi, Z., Kenelty, K., et al., 2022. Expression of immune markers of white sturgeon (*Acipenser transmontanus*) during Veronaea botryosa infection at different temperatures. Comparative biochemistry and physiology Part D. Genom. Proteonomics 41, 100950.
Sun, J.L., Zhao, L.L., Liao, L., Tang, X.H., Cui, C., Liu, Q., et al., 2020. Interactive effect of thermal and hypoxia on largemouth bass (*Micropterus salmoides*) gill and liver: aggravation of oxidative stress, inhibition of immunity and promotion of cell apoptosis. Fish Shellfish Immunol. 98, 923–936.
Tort, L., 2011. Stress and immune modulation in fish. Dev. Comp. Immunol. 35, 1366–1375.
Umasuthan, N., Bathige, S., Thulasitha, W.S., Oh, M., Lee, J., 2020. Comparative characterization of two cxcl8 homologs in *Oplegnathus fasciatus*: genomic, transcriptional and functional analyses. Biomolecules 10.
van der Aa, L.M., Chadzinska, M., Tijhaar, E., Boudinot, P., Verburg-van Kemenade, B. M., 2010. CXCL8 chemokines in teleost fish: two lineages with distinct expression profiles during early phases of inflammation. PLoS One 5, e12384.
Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., et al., 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3, RESEARCH0034.
Vargas-Chacoff, L., Regish, A.M., Weinstock, A., McCormick, S.D., 2018. Effects of elevated temperature on osmoregulation and stress responses in Atlantic salmon Salmo salar smolts in fresh water and seawater. J. Fish. Biol. 93, 550–559.
Vinagre, C., Madeira, D., Narciso, L., Cabral, H.N., Diniz, M.S., 2012. Effect of temperature on oxidative stress in fish: lipid peroxidation and catalase activity in the muscle of juvenile seabass, *Dicentrarchus labrax*. Ecol. Indicat. 23, 274–279.
Vinagre, C., Madeira, D., Mendonça, V., Dias, M., Roma, J., Diniz, M.S., 2014. Effect of increasing temperature in the differential activity of oxidative stress biomarkers in various tissues of the Rock goby, *Gobius paganellus*. Mar. Environ. Res. 97, 10–14.
Wang, B., Luo, X., Yang, Y.M., Sun, W., Cane, M.A., Cai, W., et al., 2019. Historical change of El Nino properties sheds light on future changes of extreme El Nino. Proc. Natl. Acad. Sci. U.S.A. 116, 22512–22517.
Wentworth, S.A., Thede, K., Aravindabose, V., Monroe, I., Thompson, A.W., Molyneaux, N., et al., 2018. Transcriptomic analysis of changes in gene expression of immune proteins of gill tissue in response to low environmental temperature in fathead minnows (*Pimephales promelas*). Comparative Biochemistry and Physiology Part D. Genom. Proteonomics 25, 109–117.
Yao, T., Wang, R., Han, P., Liu, X., Wang, X., 2023. Identification of olive flounder (*Paralichthys olivaceus*) toll-like receptor genes: involvement in immune response to temperature stress and Edwardsiella tarda infection. Fish Shellfish Immunol. 138, 108841.
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.
Zhao, Y., Li, S., Tang, S., Wang, Y., Yao, X., Xie, J., et al., 2023. Effects of chloride, sulfate, and bicarbonate stress on mortality rate, gill tissue morphology, and gene expression in Mandarin fish (*Siniperca chuatsi*). Environ. Sci. Pollut. Res. Int. 30, 99440–99453.
Zhong, Y., Duan, Z.Y., Su, M.L., Lin, Y.Q., Zhang, J.B., 2021. Inflammatory responses associated with hyposaline stress in gill epithelial cells of the spotted scat *Scatophagus argus*. Fish Shellfish Immunol. 114, 142–151.