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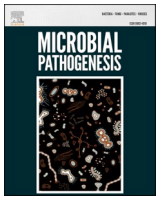

Impact of high temperature, CO_2 and parasitic infection on inflammation, immunodepression and programmed cell death in *Colossoma macropomum* at the transcriptional level

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ABSTRACT

The production of tambaqui *Colossoma macropomum* has recently reached a milestone, being considered the main native species produced in South American continental waters. Despite the importance of this fish, its immunity is poorly understood, and global warming could pose severe risks to its health as increasing water temperature leads to an increase in the incidence of parasitic diseases. In an experimental context based on the high-emission scenario of the 5th Intergovernmental Panel on Climate Change (IPCC) report, we evaluated the synergistic effect of exposure to the extreme climate change scenario (RCP8.5) during two exposure periods (7 and 30 days) and two levels of parasitism by monogeneans (low and high). The goal was to understand how the tambaqui immune system will react to this challenge. To achieve this goal, we analyzed the expression of nine immunity-related genes (*jak3, stat3, il-10, socs1, casp1, il-1β, tp53, bcl2,* and *hif-1α*) in the spleen. Our main findings showed downregulation in the *jak3/stat3* pathway, genes related to the control of inflammation and apoptosis, in addition to upregulation of proinflammatory genes and those related to pyroptosis during the first 7 days of exposure to the extreme climate scenario, also indicating a stage of immunodepression in these animals. After 30 days of exposure, all genes tended to return to similar levels in the current scenario, possibly due to the decrease in parasite load caused by chronic exposure to the extreme scenario. Our data strongly suggest that the increase in parasitism intensity caused by the extreme climate change scenario is responsible for disturbances in the host's immune system. However, more studies are needed to clarify this poorly understood cascade of events.

1. Introduction

The tambaqui *Colossoma macropomum*, a serrasalmid teleost is the most raised native fish in South America continental aquaculture [1,2]. Tambaqui has characteristics that stand out, favoring its success in aquaculture, such as easy adaptation to different farming systems, omnivory, high commercial value, in addition to its unique flavor [2,3]. However, as a result of an exponential increase in the world population, the demand for animal protein is rising and animal farming is using high density protocols, which makes animals more vulnerable to stress and several infectious diseases, being an obstacle to the development of tambaqui farming [1].

Infectious diseases are important drivers within ecosystems and fish farming, mostly because the infection caused by pathogens causes endemic diseases, impacting host-pathogen balance [4,5]. In this scenario, Monogeneans, highly diverse obligate ectoparasites, are a common cause of massive mortalities in fish. A simple direct lifecycle allows Monogeneans to rapidly multiply in fish farms environments, where they often harm their hosts' immune systems [6]. Due to the attachment and feeding, Monogeneans induce histopathological changes in the host epithelium, thus creating a gateway for secondary infections that pose a major risk. Under heavy infections, the host is unable to mount an effective defense against the parasites and thus damage becomes intense [7].

Furthermore, environmental disturbances, such as changing in climate are one of the main causes of imbalance in the host-pathogen interaction leading to severe infectious diseases and damage to the host's immune system [8]. According to the Intergovernmental Panel on Climate Change [9], the changes in climate are intensifying with at an astonishing speed and with potentially serious consequences for

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humans, animals and the planet. But why can climate change be so threatening for fish farming? As fish are poikilotherm animals, their body temperature and metabolism are regulated by the surrounding water which when above an upper threshold creates a stressful environment that can impact immunity and disease susceptibility and even host survival [10–12]. In our previous studies [10,13] we demonstrated that the monogenea-tambaqui interaction is impaired by exposure to the extreme climate change scenario (Representative Concentration Pathways 8.5, RCP8.5), causing disturbances to the immune system (such as activation of inflammatory mechanisms), ionic regulation, and increased oxidative stress in the host's gills.

Based on the promising results of these works [10,13], we carried out a third study, this time aiming to understand how the tambaqui immune system reacts to two periods (7 and 30 days) of exposure to extreme climate scenarios and two degrees of parasitism (low and high). To achieve this goal, we evaluated the expression of nine immunity-related genes (*jak3, stat3, il-10, socs1, casp1, il-1β, tp53, bcl2,* and *hif-1α*) in the spleen, that was chosen because it is one of the major peripheral lymphoid organs in fish, plays a key role in the immune and inflammatory response, besides being an important organ for the removal of invasive pathogens and is also a site for the production of immune substances, such as lymphocytes and immunoglobulin [14,15]. We speculated that a strong inflammatory response would be mounted in the first seven days of exposure to the extreme climate scenario in the group with greater parasitic intensity with a return to levels similar to the control (i.e. current climate scenario) after thirty days exposure, based on what we observed for oxidative stress and ionic regulation.

2. Materials and methods

2.1. Ethics statement

The experimental procedures were approved by the Ethics and Animal Welfare Committee (CEUA) of the Brazilian National Institute for Research of the Amazon (INPA), Manaus, AM, Brazil, under protocol number 053/2017 and was conducted in accordance with all relevant guidelines and applicable regulations.

2.2. Experimental design

Spleen samples used in this study were collected from the same fish used in the experiment described by Costa et al. [13]. All information discussed here is new, complementary to the initial work.

Sixty-four juvenile tambaqui (average weight: 45.25 ± 3.43 g; average length: 14.19 ± 1.15 cm; $N = 64$) were purchased from Fazenda Santo Antônio (02° 44′ 802'' S; 059° 28′ 836' 'W, Amazonas, Brazil) and were naturally parasitized. They were transported to the Laboratory of Ecophysiology and Molecular Evolution (LEEM) at INPA, where were randomly distributed in a 310 L tank, with forced aeration and continuous water flow, for 30 days. After acclimation, these fish were treated with 3 g L^{-1} of salt for 15 min during three consecutive days according to Schelkle et al. [16] to decrease the animals' parasitic burden and thus establish two degrees of parasitism, defined as Low and High group (LG and HG, respectively), according to the protocol used by Costa and Val [10]. The animals were then transferred to two real-time simulated controlled environmental rooms.

The rooms simulated the current (current temperature and CO_2 levels) and extreme (RCP8.5) scenarios according to the Fifth IPCC Assessment Report for the year 2100 [17], as described by Costa and Val [10]. The current conditions simulate the same conditions occurring in a forested area of the Amazon without human influence, collected every 2 min with Fieldlogger 512k (Novus Produtos eletrônicos LTDA). The extreme climate room was set to 4.5 °C and 900 pmm CO_2 above the current conditions (as described in Costa et al. [13]. The artificial light-dark cycle was 12L:12D, and humidity was set as a derived condition. Prior to the experiment, the gills, skin, and fins were carefully scraped with a coverslip, observed under an optical microscope and underlying subclinical infection and/or the presence of other parasites were assessed.

Tambaqui juveniles were transferred to the above climate scenarios two days prior to beginning the experiments. Eight 60 L PVC tanks in four replicates per treatment (Low and High levels of parasitism) containing four individuals in each tank were maintained in each climate room during seven and thirty days. Toxic ammonia accumulation was avoided by partial water renewal throughout the experiment. The pH, O_2 and CO_2 levels and temperature of the water were measured daily (data reported in Costa et al. [13]). All animals were fed commercial dry food pellets, with a 36% crude protein (Purina, BR), once a day. After each exposure period, two fish were removed from each tank, with a total of eight fish per scenario and treatment being collected (n = 8). The volume of water was adjusted after collecting the fish. Subsequently, fish were anaesthetized, weighed, measured, and euthanized by cervical sectioning with a scalpel. Spleen samples were collected using sterile tweezers and scissors and immediately immersed in liquid nitrogen and stored in an ultra-freezer at −80 °C until the isolation of ribonucleic acid (RNA). The mean intensity of parasitism data is available at Costa et al. [13].

2.3. Quantitative RT-qPCR

Total RNA was isolated from the spleen using TRIzol Reagent (Life Technologies, CA, USA). The extracted RNA integrity was verified by an electrophoretic run in agarose gel (2%), then DNA contamination was removed with use of the DNase I kit (Invitrogen, Life Technologies) and reverse transcribed to produce cDNA with the High-Capacity cDNA Reverse Transcription Kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Quantitative RT-PCR reactions were performed in triplicate using Fast SYBR® Green Master Mix (Applied Biosystems, Foster City, CA, USA) and Viia™7 Dx Real-Time PCR System (Applied Biosystem, Foster City, CA, USA). The following steps for the qPCR reaction were performed: heating for 2 min at 50 °C, plus 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C (annealing temperature of all primers). The data for each sample were expressed relative to the expression level of *β-actin* by using $2^{-\Delta\Delta Ct}$ method [18]. Other genes were assessed as housekeeping, such as *β-tubulin* and *gapdh*. *β-actin* was also chosen because it did not vary between treatments. The primers used for quantitative RT-qPCR are as listed in Table 1.

2.4. Statistical analysis

The statistical analyses were carried out using R software with Agricolae package [19]. Mean differences were evaluated by three factors analysis of variance (ANOVA) with exposure period, climate scenarios and parasitism as factors, and were discriminated using the Tukey post-hoc test and a significant difference was assumed when $p < 0.05$. We represented the similarity profile of each factor within each exposure period using a heatmap and were built using the heatmap function in R Software.

3. Results

The mRNA expression of immune-related genes is presented in Figs. 1–3. A heatmap was elaborated to help to better visualize the effect of climate scenario and parasitism on tambaqui immunity in seven and thirty days (Fig. 4). For the *jak3* gene (Fig. 1A), a decrease was observed in LG in the extreme scenario after 7 days of exposure ($p < 0.001$; F = 18.139); after 30 days, in contrast, it was increased in this group ($p = 0.034$; F = 4.747). There was an interaction between scenario, parasitism ($p = 0.018$; F = 5.978) and time; and scenario and parasitism ($p = 0.011$; F = 6.962). Decrease in *stat3* gene (Fig. 1B) was observed also in LG after 7 days of exposure ($p = 0.046$; F = 4.192), and after 30 days it

Table 1
Primers used in this study.

Primer	Sequence (5' - 3')	Eff (%)	R2	Reference/acession number
β-actin	F: GCTCCCCCTAGCGTAAATACT R: TTACAGGGAGGCCAAGAT	98.29	0.98	RNA-Seq by Prado-Lima and Val (2016)
jak3	F: CCCCAAACAGAAGCAGCAGT R: TTGAGGTGGCGGATGATGCT	96.94	0.99	RNA-Seq by Fé-Gonçalves et al. (2020)
stat3	F: CCTGTTTCATTTAGCGGCGG R: GCCATGTTGACCCTCTGTGT	96.88	0.99	
casp1	F: CAGGTGGTTACGATCAGCCG R: TGATCAGCAGAGCCAAACGC	99.27	0.99	
socs1	F: GCCAGCATCCGGATTACCT R: TGGGCGAGCTGGTGTAGTA	95.81	0.99	
tp53	F: GGAGTGGCTGATTCAGAG3 R: TTAAGGAGAGCGGTCATG	98.25	0.99	Souza et al. (2019)
bcl2	F: GCTGAAAACCGACTCCTTCT R: ACTATGGACGGCCAGGAACT	98.19	0.98	
il-1β	F: GCATGAACCTATCGACCTAC R: GAGGTTCCAGACTCCTTTGT	101.04	0.98	MN342243
il-10	F: AGCGTCCAACACACTGAC R: TTCTTCCATGGTCCACTG	104.42	0.97	MN342244
hif-1α	F: ATCAGCTACCTGCGCATG R: CTCCATCCTCAGAAAGCAC	97.48	0.99	Silva et al. (2019)

Eff = Primer Efficiency.

returned to levels similar to the current scenario ($p = 0.025$; F = 5.356). There was an interaction between time and scenario ($p = 0.029$; F = 5.068).

For the anti-inflammatory gene *il-10* (Fig. 1C), a drastic reduction was observed after 7 days of exposure to the extreme climate scenario in LG ($p < 0.001$; F = 16.413); however, after 30 days these levels were increased but the difference was about the same observed for the animals in the current scenario (p = 0.017; F = 5.065). There was an interaction of factors ($p = 0.036$; F = 4.701).

The expression of *socs1* (Fig. 2A) was influenced by time, scenario and parasitism ($p = 0.006$; F = 8.428). An increase in mRNA levels was observed in the LG group after 7 days of exposure to the extreme climate scenario ($p = 0.030$; F = 5.092) and a reduction occurred after 30 days (p = 0.048; F = 4.879). For the *caspase-1* gene (Fig. 2B), after 7 days the expression increased in fish from the LG maintained in the extreme scenario ($p = 0.047$; F = 4.202) when compared with the same group maintained in the current scenario; however, after 30 days, the same group returned to levels similar to that of the current scenario ($p = 0.040$; F = 5.044). There was no interaction of factors ($p = 0.099$; F = 2.858). A similar pattern to that described for *caspase-1* was observed for the *il-1β* gene (Fig. 2C) as expected, i.e., the expression of *il-1β* gene increased in the same group ($p = 0.032$; F = 5.547) in 7 days and after 30 days, a downregulation also occurred ($p = 0.049$; F = 4.686). There was an interaction of factors ($p = 0.0317$; F = 5.020).

For the *tp53* gene (Fig. 3A) no effects were observed in response to any of the factors analyzed. The mRNA levels were stable throughout the experimental period for all groups. For the anti-apoptotic gene *bcl2* (Fig. 3B), the expression was changed in the LG showing a reduction after 7 days ($p = 0.044$; F = 4.677) which remained after 30 days. The interaction between scenario and the parasitism ($p < 0.001$; F = 17.262) was observed. The *hif-1α* gene expression also did not change between exposed and not exposed groups to extreme climate scenario or over time, with levels of mRNA kept constant among the different groups

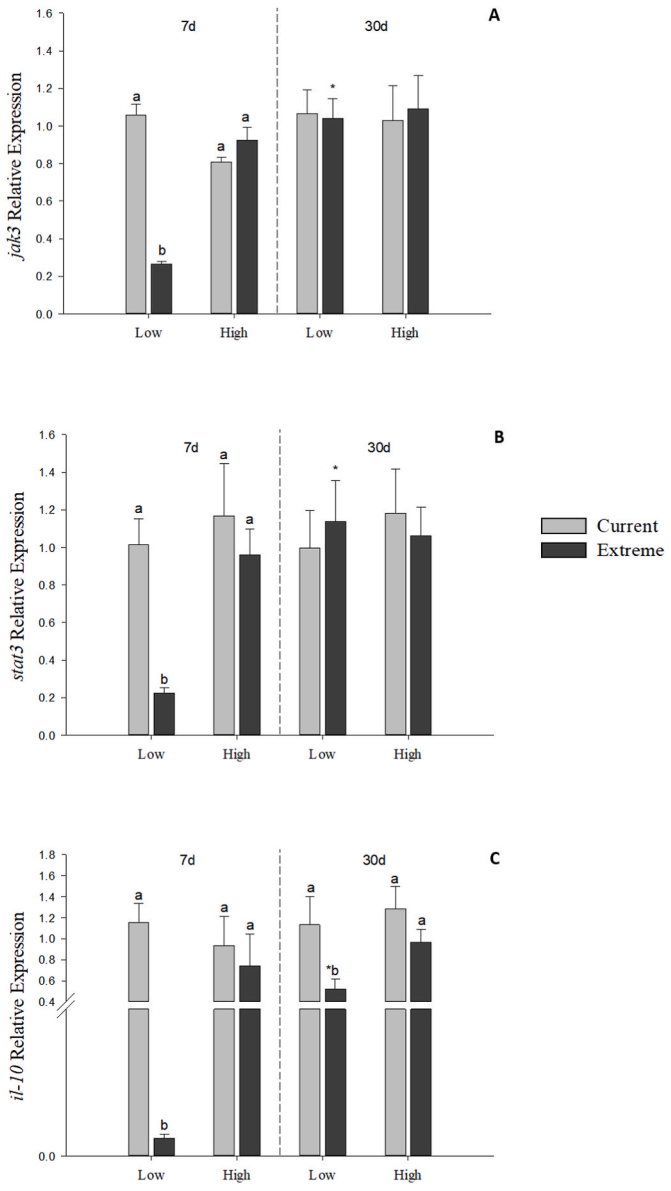

Fig. 1. Relative mRNA levels of *jak3* (A), *stat3* (B), and *il-10* (C) in spleen of tambaqui exposed to the extreme climate scenario as foreseen by IPCC (2014) compared to the current environmental conditions, and were analyzed by RT-qPCR. Bars with different letters indicate differences between environmental scenarios and asterisks indicate differences between exposure periods ($p < 0.05$). Low (LG) and High (HG) groups indicate the degree of parasitism after seven and thirty days of exposure to the experimental conditions. *β-actin* was used as housekeeping gene.

(Fig. 3C).

4. Discussion

We observed that exposure to the extreme climate scenario and parasitism is harmful to tambaqui immune system, based on the biomarkers presented here, especially in the first seven days in LG where most genes had their expression altered (see Fig. 4). Nonetheless, the group HG showed few changes, indicating that the pathways studied here remain at similar levels in both scenarios. As this group showed a lower increase in parasite load after seven days of exposure, when compared to LG, this suggests that these animals were already experiencing a chronic infection and consequently already had defenses against the infection, which explains the maintenance of similar

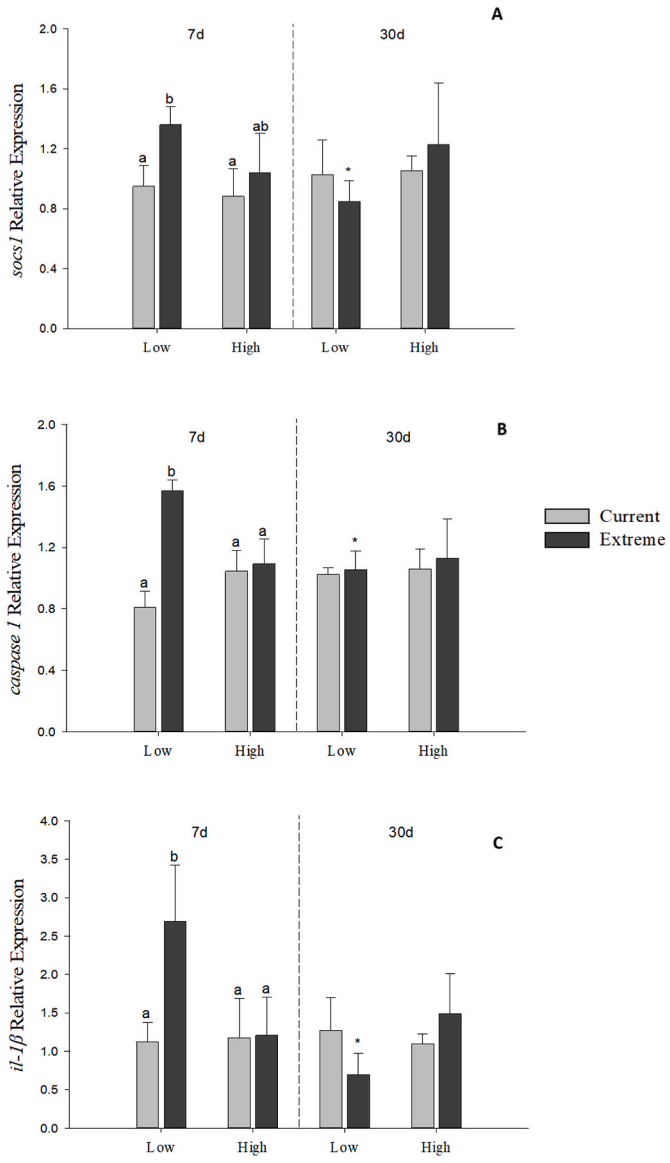

Fig. 2. Relative mRNA levels of *socs1* (A), *caspase1* (B), and *il-1β* (C) in spleen of tambaqui exposed to the extreme climate scenario as foreseen by IPCC (2014) compared to the current environmental conditions, and were analyzed by RT-qPCR. Bars with different letters indicate differences between environmental scenarios and asterisks indicate differences between exposure periods ($p < 0.05$). Low (LG) and High (HG) groups indicate the degree of parasitism after seven and thirty days of exposure to the experimental conditions. *β-actin* was used as housekeeping gene.

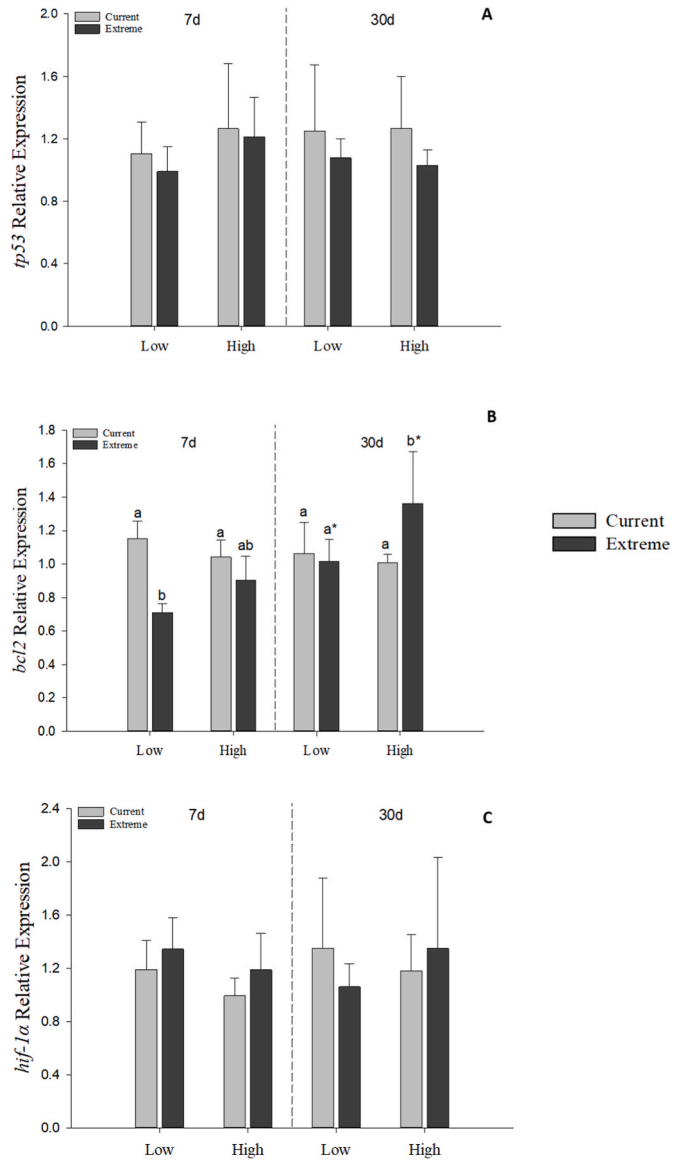

Fig. 3. Relative mRNA levels of *tp53* (A), *bcl2* (B), and *hif-1α* (C) in speen of tambaqui exposed to the extreme climate scenario as foreseen by IPCC (2014) compared to the current environmental conditions, and were analyzed by RT-qPCR. Bars with different letters indicate differences between environmental scenarios and asterisks indicate differences between exposure periods ($p < 0.05$). Low (LG) and High (HG) groups indicate the degree of parasitism after seven and thirty days of exposure to the experimental conditions. *β-actin* was used as housekeeping gene.

expression between the groups. This is corroborated by the data observed after 30 days of expression, where the levels of gene expression are similar between the groups, indicating already established defenses against the parasites. These data are in line with what was observed in our preliminary studies, where the increase in parasitism induced by exposure to the extreme scenario after seven days was harmful to tambaqui [10,13].

Our findings showed a downregulation of *jak3* and *stat3* genes in the LG after 7 days of exposure to the extreme climate scenario. The *jak3* gene is a member of the Janus family of tyrosine kinases and is crucial for the activation of the signal transducer and activator of transcription 3 (*stat3*) in response to cytokine stimulation [20]. In addition, it confers protection against apoptosis [21]. *Stat3* regulates cell-cycle progression and apoptosis, is involved in inflammation and is also known as an oncogene [22,23].

The reduction of *jak3* downregulates *stat3* and induces apoptosis [20] and inflammation, since *stat3*-mediates the expression of the anti-inflammatory gene *il-10* [24]. The *jak3/stat3* signaling pathway is still poorly understood even in mammals; however *jak3* knockout mice as well as patients with *jak3* mutations develop severe combined immunodeficiency [25]. On the other hand, different from what was observed in our study, in the grass carp (*Ctenopharyngodon idella*), the *stat3* gene was up-regulated significantly under the stimulation of LPS (lipopolysaccharide), suggesting that it is an anti-inflammatory mechanism [23]. Loss of *stat3* is associated with enhanced systemic cytokine production and tissue damage [26]. Our findings indicate that the *jak3/stat3* signaling pathway in tambaqui is affected by the challenge of the exposure to the extreme climate scenario plus parasitism for 7 days, which can result in the compromise of an inflammatory response, as well as the activation of apoptosis in this fish species.

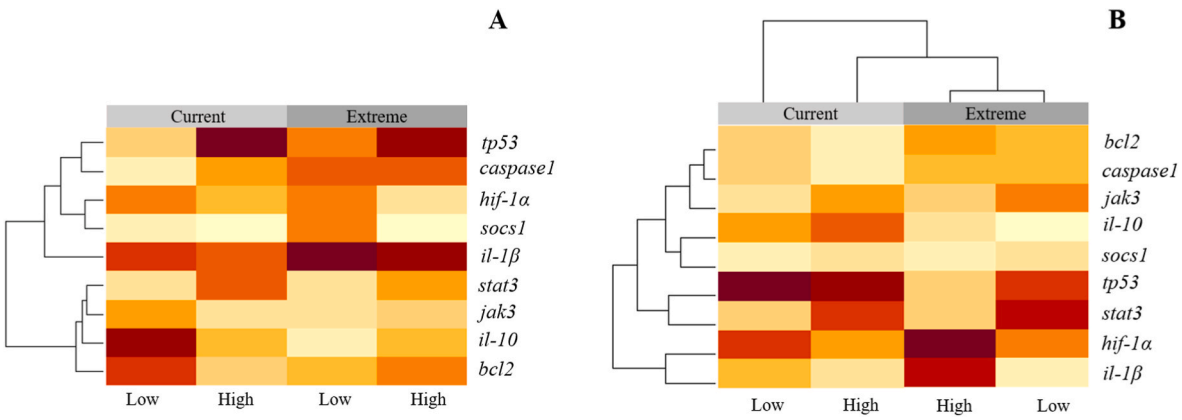

Fig. 4. Heatmap showing the similarity profile of the nine immunity-related genes within of each parasitism level and climate scenario during seven (A) and thirty days (B) in the spleen of tambaqui.

This is supported by the expression of *il-10*. It had transcripts downregulated in LG after 7 days of exposure, as was *stat3*, and increased expression after 30 days, but lower than that observed for the current scenario groups. Activation of *stat3* is essential for the regulation of *il-10* in immune cells and when this expression is promoted, they inhibit the expression of proinflammatory cytokines [27]. Downregulation of *il-10* shows that exposure to extreme climate scenario will initially promote an increase in parasitism, which will affect the *il-10/stat3* axis, which can be harmful to the tambaqui immune system. There are still few studies focusing on the effects of climate change on the immune system of fish. In our earlier study [10], we showed that mRNA levels of *il-10* in the gills of tambaqui was reduced in response to exposure to the extreme climate scenario and parasitism after 7 days. Unlike our results, Larsen et al. [28] experimentally exposed the Atlantic cod infected with the bacteria *Brucella pinnipedialis* to 15 °C and observed that the mRNA levels of *il-10* were up-regulated as a mechanism to reduce tissue damage.

Also in the jak/STAT pathway, we highlight the *Socs1* gene, a member of the suppressor of cytokine signaling family that are negative-feedback inhibitors of cytokine signal transduction [29,30]. *Socs1* is the most potent member of the SOCS family and is the primary regulator of several cytokines involved in the immune response, negatively controlling the inflammatory response in fish. In our study, the *socs1* was upregulated in the LG after 7 days of exposure to extreme climate scenario, but that expression was reduced after 30 days. These results suggest that there may have been an attempt to control the inflammatory process through *socs1*, since in our previous study [10], we observed that this same group had an overexpression of *il-1β* and *hsp70* in the gills, an indicative of inflammation. Tomalty et al. [31] observed that in *Oncorhynchus tshawytscha* exposed to acute thermal stress, 25 °C, *socs1* was upregulated, like that observed in our study. However, Saleh et al. [32] observed an increased in the mRNA levels of *socs1* in *Salmo trutta* following exposure to the parasite *Myxobolus cerebralis* as a strategy to inhibit the *il-1β*-induced inflammatory response and to modulate immune defense.

Here, the *il-1β* gene expression was also upregulated in spleen in the LG after 7 days of exposure to extreme climate scenario, returning to similar levels to the current scenario after 30 days. As described in our previous study [10], the expression of *il-1β* gene in the gills was similar to that observed in the spleen, suggesting that there is a systemic inflammatory response. The role of the *il-1β* in the host defense against monogenean is well described in the literature as having a vital importance in the early immune response in host defense [10,33,34]. Increased levels of *il-1β* mRNA have been observed in *Dicentrarchus labrax* exposed to high temperatures both in muscle and kidney, suggesting that heat stress causes inflammatory response [35].

The *il-1β* is a pro-inflammatory cytokine mainly proteolytically processed in the cytoplasm by the *caspase-1*, which also had its expression increased, suggesting the participation of this pathway in its production. The *caspase-1* gene is a member of a family of intracellular cysteine proteases that play an essential role in inflammation and apoptosis [36]. In addition to stimulating *il-1β* production, *caspase-1* contributes to the host's defense from invading pathogens through an inflammatory lytic cell death program known as pyroptosis, that is characterized by a rapid cell-membrane rupture and release of pro-inflammatory cytokines [37,38].

Pathogen infection is a common factor that causes pyroptosis that is thought to be more inflammatory and immunogenic than apoptosis [37]. In the present study, we observed an increase of mRNA levels of *caspase-1* in the LG after 7 days, as expected, as we also had an increase in *il-1β* in the same group [39]. also described the role of *caspase-1* in *Apostichopus japonicus* in the pyroptosis pathway regulating inflammatory responses. Our findings agree with the function described for *caspase-1*, including the pyroptosis effect, since it is essential for pathogen clearance and involves the release of intracellular signals to recruit immune system cells to the infection sites, corroborating the results of the expression of the other genes observed here. We suggest that the climate scenario and parasitism activate pyroptosis-related pathways.

As we observed the transcriptional activation of pro-inflammatory responses and cell death, via pyroptosis, we evaluated whether parasitism and climate change scenarios can promote apoptosis through the intrinsic mitochondrial pathway. Thus, we analyzed the expression of *tp53* and *bcl2* genes. Tumor suppressor gene *tp53* is a transcription factor that plays an important role in maintaining the integrity of the DNA, inhibits cell division or survival in response to various types of cellular stress and induces cell cycle arrest or apoptosis [40,41]. Contrary to our expectations, the exposure to extreme climate scenario and parasitism did not change the levels of *tp53* transcripts in tambaqui. For the conditions evaluated here, the maintenance of *tp53* mRNA levels is consistent with a pro-inflammatory response. In mammals it has been shown that *tp53* is an inhibitor of inflammation via NF-κB suppression [42]; therefore, an increase in the expression of *tp53* could compromise the inflammatory response.

Even though the *tp53* transcription remained stable throughout the experiment, the same was not observed for the anti-apoptotic *bcl2*, that was downregulated after 7 days of exposure to the extreme climate scenario in the LG, with a gradual return after 30 days. The *bcl2* gene (B cell lymphoma protein 2) is an important regulator of programmed cell death pathways. Its decline suggests the occurrence of mitochondrial pathway-mediated apoptosis [43]. The downregulation of *bcl2* in the LG fish exposed to the extreme scenario observed here may also be in response to decreased *jak3* mRNA levels, as this gene also regulates T lymphopoiesis, through its ability to selectively repress *bax* (pro apoptotic gene) expression and induce *bcl2* expression [44], suggesting a

failure of the normal function of this gene and possible apoptotic process. Reduced levels of *bcl2* also was observed in zebrafish exposed to 25 °C leading to an increase of apoptotic genes [45]. On the other hand, the transcriptome profile of the fat oyster (*Ostrea edulis)* infected with *Bonamia ostreae* showed an upregulation of *bcl2* as defense mechanism against the parasite [46].

We also evaluated the expression of the *hif-1α* gene and as observed for *tp53*, the expression of the *hif-1α* has not been changed in any group. The *hif-1α* gene is involved in hypoxia responses, but it has also been reported to regulate many innate immune functions in fish responses to water temperature, for example [47,48]. Although the *stat3* (that was downregulated) and *socs1* (upregulated) genes are involved in *hif-1α* expression by regulating the glycolytic pathway, which is essential for immune cell function [49], we did not observe changes in the expression of *hif-1α* in this study. In contrast, in our previous study [10] there was an upregulation of *hif-1α* in the gills in the LG after 7 days of exposure and this can be explained due to the gene tissue specificity, where the gills are the site of monogenean infection that may have created hypoxic microenvironment, and this distinct *hif-1α* expression patterns might be related to tissue-specific regulation [50–52].

5. Conclusion

The results obtained in the present work shed light on a poorly understood cascade of events involving synergistic exposure to climate change and parasitism in tambaqui. In addition, evidence of systemic inflammatory process, immunodepression, apoptosis and pyroptosis at the transcriptional level are also involved. Return of mRNA to control levels after 30 days represents a response to a reduced parasite infection, which did not necessarily decrease due to the inflammatory response mounted after 7 days. The reduction in parasite infection could also be due to the damage that the extreme climate change scenario caused to the parasite, which could influence the evolution of the tambaqui immune system if it is also accompanied by a loss of parasitic biodiversity, on a long-term scale. In this sense, we suggest studies that evaluate the effect of climate change on parasitic biodiversity in fish.

CRediT authorship contribution statement

Jaqueline Custódio da Costa: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Samara Silva de Souza:** Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Adalberto Luis Val:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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