Neotropical Ichthyology

Original article

https://doi.org/10.1590/1982-0224-2023-0130

Glycolytic modulations and antioxidant capacity in Amazonian fish, *Bryconops giacopinii* (Characiformes: Iguanodectidae), living at high temperature

Correspondence:
Derek Felipe de Campos
campos.derek@gmail.com

Derek Felipe de Campos[1,2], Susana Braz Mota[1], Vera Maria Fonseca de Almeida-Val[1] and Adalberto Luis Val[1]

Temperature is projected to continue increasing in the upcoming years. The effects of temperature warming in deforested stream populations have not been addressed yet and are a promising area to understand the consequences of increased temperature on fish physiology. Therefore, the aims of this study were to determine the manner in which *Bryconops giacopinii* from deforested habitat modulates the LDH kinetics in response to warming and whether the antioxidant system is able to withstand thermal stress. We collected individuals from two roadside streams (deforested) and one forested stream and measured the LDH kinetics parameters (Vmax and Km) for pyruvate and lactate, measured the total ROS production, and measured the activity of antioxidant enzymes and the oxidative stress biomarker. Our results showed lower affinity and higher LDH activity for lactate oxidation in road-side populations, suggesting that populations living in high temperatures use lactate as aerobic fuel. Besides, there was an increase in ROS production, and CAT and GSH levels in road-side populations, but not LPO levels, suggesting that *B. giacopinni* is able to neutralize the ROS production with the antioxidant systems. Our results bring important findings in the adaptation of this specie to a warm environment.

Keywords: Aclimatization, Antioxidant enzymes, Lactate dehydrogenase, Oxidative stress, Thermal adaptation.

Submitted December 18, 2023
Accepted April 5, 2024
by Bernardo Baldisserotto
Epub July 8, 2024

Online version ISSN 1982-0224
Print version ISSN 1679-6225

Neotrop. Ichthyol.
vol. 22, no. 2, Maringá 2024

1 Laboratório de Ecofisiologia e Evolução Molecular (LEEM), Instituto Nacional de Pesquisas da Amazônia (INPA), Av. André Araújo 2936, 69067-375 Manaus, AM, Brazil. (DFC) campos.derek@gmail.com (corresponding author), (SBM) susanabmota@gmail.com, (VMFAV) veraval30@gmail.com, (ALV) dalval.inpa@gmail.com.

2 Laboratório de Fisiologia Térmica Integrativa, Departamento de Morfologia e Fisiologia Animal, Universidade Estadual Paulista, Via de Acesso Prof. Paulo Donato Castelane, s/n, Vila Industrial, 14884-900 Jaboticabal, SP, Brazil.

A temperatura deverá continuar aumentando nos próximos anos. Os efeitos do aumento da temperatura nas populações de riachos desmatados ainda não foram abordados e são uma área promissora para compreender as consequências do aumento da temperatura na fisiologia dos peixes. Portanto, os objetivos deste estudo foram determinar a maneira pela qual *Bryconops giacopinii* de habitat desmatado modula a cinética do LDH em resposta ao aquecimento e se o sistema antioxidante é capaz de resistir ao estresse térmico. Coletamos indivíduos de dois riachos à beira de estradas (desmatados) e de um riacho florestado, e medimos os parâmetros cinéticos da LDH (Vmax e Km) para piruvato e lactato, medimos a produção total de EROs e medimos a atividade de enzimas antioxidantes e do biomarcador de estresse oxidativo. Nossos resultados mostraram menor afinidade e maior atividade de LDH para oxidação de lactato em populações à beira de estradas, sugerindo que populações que vivem em altas temperaturas utilizam lactato como combustível aeróbico. Além disso, houve um aumento na produção de EROs e nos níveis de CAT e GSH nas populações à beira da estrada, mas não nos níveis de LPO, sugerindo que *B. giacopinni* é capaz de neutralizar a produção de EROs com os sistemas antioxidantes. Nossos resultados trazem descobertas importantes na adaptação desta espécie a um ambiente quente.

Palavras-chave: Aclimatização, Adaptação térmica, Enzimas antioxidantes, Estresse Oxidativo, Lactato desidrogenase.

INTRODUCTION

Climate change stands as a principal concern for conservationists, posing a significant threat to biodiversity. Rising temperatures are reshaping environmental conditions and imposing novel selection pressures on organisms (Crozier, Hutchings, 2014). Consequently, understanding the capacity of species and populations to respond and adapt to climate change, particularly warming, is more pressing than ever. This understanding is crucial for predicting the consequences of climate change and enhancing our management and conservation efforts (Donelson *et al*., 2011; Sinclair *et al*., 2016; Campbell *et al*., 2017). Ectothermic animals are especially vulnerable to ambient temperature variations due to their reliance on external temperatures to regulate body temperature and metabolism (Schulte, 2015).

Rising temperatures exert a significant influence on ectothermic metabolism, with the ability to survive in warm environments being closely linked to an individual's capacity to meet its energetic requirements (Pörtner, Gutt, 2014; Pörtner *et al*., 2014). Lactate dehydrogenase (LDH) is a key metabolic enzyme involved in the final stages of glycolytic metabolism. It facilitates the interconversion of pyruvate and lactate while concurrently interconverting NADH and NAD+, thus playing a crucial role in energy metabolism (Driedzic, Almeida-Val, 1996).

While the effects of temperature on the kinetics of LDH in the forward reaction (pyruvate reduction) have been extensively studied, relatively few studies have focused on the lactate oxidation reaction (Fields, Houseman, 2004; Fields *et al*., 2015). The

utilization of lactate as an aerobic fuel has been associated with enhanced aerobic and swimming performance in fish (Omlin *et al*., 2014; Ferreira *et al*., 2018), suggesting its potential importance when fish are exposed to high temperatures. Therefore, a comprehensive understanding of LDH kinetics in both the forward and reverse reactions in fish acclimatized to different temperatures would provide valuable insights into the modulation of glycolytic metabolism to cope with thermal stress.

The ability of organisms to respond and survive increases in temperature hinges on their ability to supply energy and manage internal components and processes to maintain physiological homeostasis (Somero, 2010). As oxygen consumption rises to meet energy demands, there is a concomitant increase in the production of reactive oxygen species (ROS) due to warming. The excessive production of ROS can inflict severe damage to cells, leading to lipid peroxidation, protein carbonylation, and DNA damage – collectively known as oxidative stress.

Campos *et al*. (2019) reported a heightened vulnerability of the characid fish *Hyphessobrycon melazonatus* acclimated to a climate change scenario characterized by elevated temperatures (4°C) and atmospheric CO2 levels (900 ppm above current levels), with a notable link between increased mortality and oxidative damage. However, cells possess mechanisms to counteract the deleterious effects of ROS through the activation of ROS scavengers, including superoxide dismutase, catalase, glutathione-S-transferase, and others. This activation serves to reduce the flux of ROS generated during oxidative metabolism, thereby mitigating oxidative stress (Madeira *et al*., 2016a). Lopes *et al*. (2018) demonstrated that the activation of antioxidant enzymes provides an effective defense mechanism to cope with ROS formation in *Chiloscyllium plagiosum* exposed to a climate change scenario.

The vast majority of studies examining the effects of temperature on physiological and biochemical plasticity have focused on acclimating individuals to different temperature regimes (Campos *et al*., 2017, 2019). However, only a few studies have investigated the effects of natural populations inhabiting distinct thermal habitats. Phenotypic variation can arise from both environmental and genetic influences, underscoring the importance of understanding the relative contributions of local adaptation and phenotypic plasticity. This understanding is crucial for elucidating the impact of environmental variation on populations.

In this study, we investigate the physiological and biochemical adaptation of *Bryconops giacopinii* (Fernández-Yépez, 1950), a rheophilic species, residing in different thermal habitats. While *B. giacopinii* typically inhabits natural forested streams in the Amazon, the construction of roads through the Amazon Forest has altered its habitat. Deforestation of Amazonian streams has resulted in temperature increases of up to 6°C (Ilha *et al*., 2018) a rise warmer than projections for the year 2100 due to climate change. Consequently, our aim is to explore the physiology and biochemistry of *B. giacopinii* populations living in diverse thermal habitats. We hypothesize that: i) individuals from roadside streams will exhibit different LDH kinetics parameters compared to those from forested streams, and ii) individuals from roadside streams will display higher levels of antioxidants and oxidative stress. Therefore, we expect changes in LDH kinetics and redox balance related to the increase in energy demand to live in warm waters. Changes in glycolytic and redox balance will inform us about the physiological adjustment of this species to survive in a climate change world.

MATERIAL AND METHODS

Study area and fish collection. The individuals' collection was performed on February 2020 (Amazon wet season) in two road-side streams (BR#1 - 02°34'45"S 60°01'44"W; BR#2 - 02°44'51"S 60°01'35"W) and one forested stream (Ducke - 02°58'07"S 60°00'20"W). The two roadside streams are located along the Manaus-Boa Vista road, the BR-174 (Fig. 1). The highway BR-174 construction beginning in the 1970's years and its paving only finished in 1997. The construction and paving of the highways represent the main vectors of deforestation in the Amazon, and according to Rodrigues, Pinheiro (2012), there was a total of 2618, 58 km^2 of deforested areas near the BR-174. Deforestation has impacted the physical structure of the small aquatic ecosystems, what once was a forested lotic system, turned into a semi-lentic system without riparian forest, leading to the increase in the mean and fluctuation of the temperature. The forested stream is located inside the Reserva Adolpho Ducke (02°53'S 59°58'W), a protected area located in the central Brazilian Amazon near the confluence of the Negro and Solimões rivers and bordering Manaus. The Ducke Reserve encompasses an area of 100 km^2 and comprises two distinct watersheds: Puraquequara basin in the Tarumã basin. The animals were collected specifically from the Tarumã Basin (Acará streams). The distance between Ducke and the roadside streams was approximately 40 km in a straight line, with a distance of around 10 km between the roadside streams. Importantly, there is no connection between these streams.

In both sites, ten individuals were collected using a seine net. Captured fish were maintained in a container with stream water and aeration. The animals were collected over two consecutive days. On the first day, between 9 and 11 am, we gathered populations from the roadside. The following day, at the same time frame of 9 to 10 am, we collected animals from the Ducke reserve. After collection, fish were anesthetized and dissected, the muscle and gills were removed and immediately frozen in liquid N_2. The samples were stored in the ultra-freezer at -80°C until the enzymatic analyses were performed. Besides, temperature and oxygen were measured by YSI55 probe, and 5 mL of water was collected to measure pH in the laboratory with Ohaus ST2100-F Starter 2100 pHmeter. The species are included in the ichthyological collection of the Instituto Nacional de Pesquisas da Amazônia (INPA) under voucher number INPA-ICT 044364.

LDH Kinetics. Enzyme assays were realized according to protocols described in Driedzic, Almeida-Val (1996). The absolute activity of LDH was measured in the white muscle of fish that was homogenized according to Driedzic and Almeida-Val's method. Briefly, the portions were homogenized manually with a conic pistil and microtubes in a 1:10 (w:v) ice-cold buffer (150mM imidazole, 1mM EDTA, 1% triton X-100, pH 7.4). Homogenates were centrifuged during 15 min at 13.000g at 4°C to separate particulate material. Assays were performed in 300 µL microplate in 288 µL reaction buffer (NADH 0.15mM for pyruvate reductase or NAD^+ 0.15mM for lactate oxidase, KCN 1mM, and imidazole 50mM), pH 7.4 at 25°C, 2µL homogenate, and reaction was started adding 10µL pyruvate (0.0, 0.05, 0.10, 0.25, 0.50, 0.75, 1.00, 5.00 mM) or lactate (0.0, 0.5, 1.0, 2.0, 4.0, 8.0 and 16mM). In a spectrophotometer spectramax M5 plate reader the reaction was read at 340 nm. K_m and V_{max} for each substrate were calculated after assays and expressed as mM and µmol pyruvate/minute/g protein, respectively.

K_m and V_{max} were calculated with a nonlinear least square regression fit to the one site saturation ligand biding equation using SigmaPlot 3.0 (Systat Software Inc).

Antioxidant and reactive oxygen species analyses. Muscle and Gills were homogenized in buffer (pH 7.6) containing (in mM): Tris base 20, EDTA 1.0, dithiothreitol 1.0, sucrose 50, KCl 150. Homogenates were centrifuged at 15,000 g for 20 min at 4°C and used to determine GST, SOD and CAT activities, and LPO levels (1:10 w/v for GST and SOD and 1:4 w/v for CAT and LPO). The glutathione-S-transferase (GST) activity was determined using 1-chloro 2,4-dinitrobenzene (CDNB) as substrate, according to Keen *et al.* (1976). Changes in absorbance were recorded at 340 nm. The enzyme activity was calculated as nmol CDNB conjugate formed per min per mg protein. To determine the catalase (CAT) activity, the inhibition rate of H_2O_2 decomposition was monitored at 240 nm (Beutler, 1975), and expressed as μM H_2O_2 min^{-1} mg $protein^{-1}$. Superoxide dismutase (SOD) activity was quantified based on the inhibition of cytochrome c reduction rate by the superoxide radical at 550 nm and 25°C (Turrens, 1997). Enzyme activity is expressed as U SOD mg $protein^{-1}$, where 1 U of SOD corresponds to the quantity of enzyme that promoted the inhibition of 50% of cytochrome c. The lipid peroxidation (LPO) levels were quantified based on the oxidation of the Fe^{+2} to Fe^{+3} by hydroperoxides in the acid medium in the presence of ferrous oxidation-xylenol orange, at 560 nm, according to the method described by Jiang *et al.* (1991).

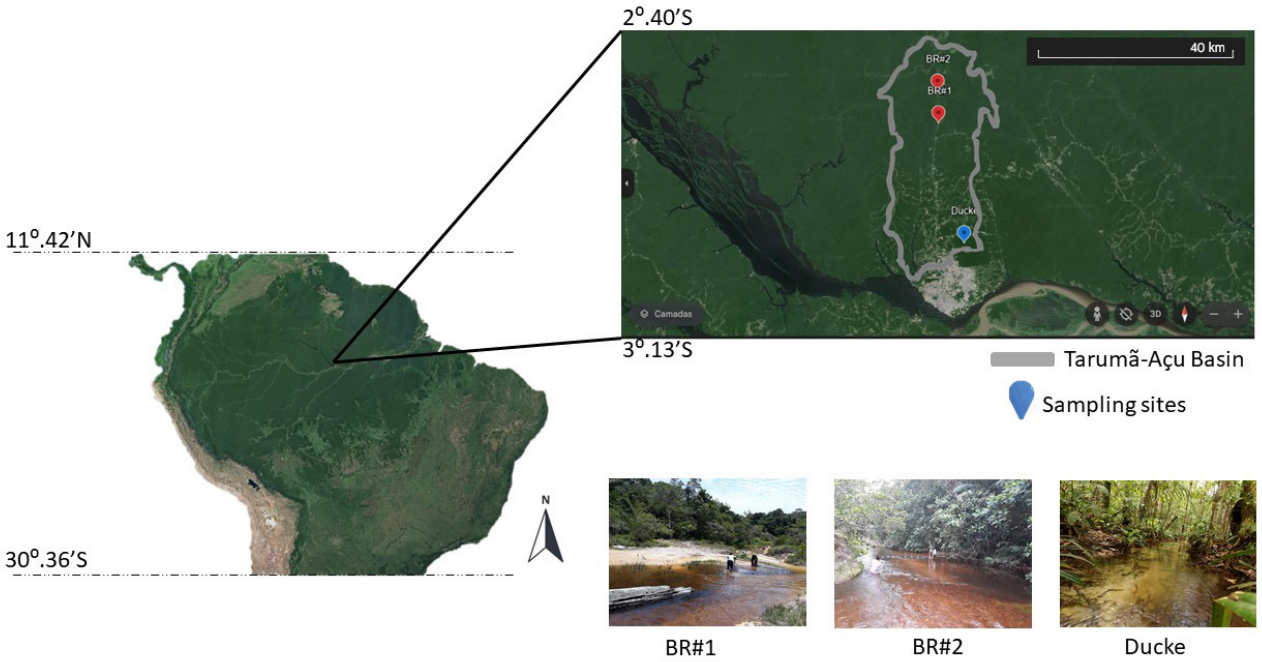

FIGURE 1 | Location map of the collection sites of *Bryconops giacopinii*. Blue spot indicates the forested streams (Reserve Ducke) and red spot indicates the roadside streams (BR#1 and BR#2). Both forested and roadside streams belong to the Taruma-Açu basin, highlighted in grey area. The collection sites are showed in the images below the map.

Reactive oxygen species (ROS) were measured according to the method of Amado *et al.* (2009). In the homogenates, as described above, were added the reaction buffer (30 mM HEPES, 200 mM KCl, and 1 mM MgCl2, pH 7.2) and the fluorescent dye 2′,7′ dichlorofluorescein diacetate (H2DCF-DA). The acetate groups of H2DCF-DA are cleaved by intracellular esterase present in sample supernatants. After that, the non-fluorescent compound H2DCF was oxidized by ROS to the fluorescent compound, DCF, which was detected in the wavelengths of 488 (excitation) and 525 (emission) nm. Total fluorescence production was calculated by integrating the fluorescence units (FU) over the measurement time and expressed as the area of fluorescence. The results were expressed as area difference of FU × min in the sample.

Statistics. The data are presented as mean ± SEM (n = 10). All statistical analyses were carried out in SigmaStat 3.1 using a significance level of 5%. Parametric Anova or ANOVA on ranks was performed to detect significant differences in the biomarker's response among different populations followed by the Tukey post hoc analysis.

RESULTS

The temperature and pH were higher, oxygen was lower in both roadside streams compared to the forested stream (see Tab. 1). The maximum activity (Vmax) of LDH for lactate formation surpasses that for pyruvate formation, indicating that this enzyme is more effective in catalyzing lactate formation. Significant differences were observed among collection sites for LDH kinetic parameters. Specifically, the maximum activity (Vmax) of LDH for lactate formation is higher in fish from the Ducke compared to those from BR#1. Conversely, the maximum activity (Vmax) of LDH for pyruvate formation is higher in fish from the BR#1 population than in those from the Ducke population (Fig. 2). This suggests that higher temperatures enhance the ability for pyruvate formation, which can be used to enter in citric acid cycle and improve oxidative phosphorilation. Furthermore, LDH Km^{pyr} does not exhibit significant differences among populations. However, LDH Km^{lac} is lower in the Ducke population compared to BR#1, indicating a higher affinity for lactate in the Ducke population (Fig. 3). Taken together, the kinetics of LDH suggest that populations inhabiting higher temperatures have adapted to increase lactate oxidation.

Regarding the antioxidant enzymes, there were significant differences among the collection sites in gills (Tab. 2). The SOD activity was lower in BR#1 compare to BR#2 and Ducke. The CAT was lower in BR#1 compare to BR#2, the GSH was higher in BR#1 and BR#2 compare to Ducke, and the GST activity was lower in BR#1 compare to Ducke. There were no differences in LPO and ROS among treatments, although BR#1 and BR#2 showed higher ROS mean values.

TABLE 1 | Temperature, oxygen and pH of the collection sites.

	Temperature (°C)	Oxygen (mgL^{-1})	pH
BR#1	29.3	5.4	5.3
BR#2	28.5	5.6	5.1
Ducke	25.3	6.4	4.5

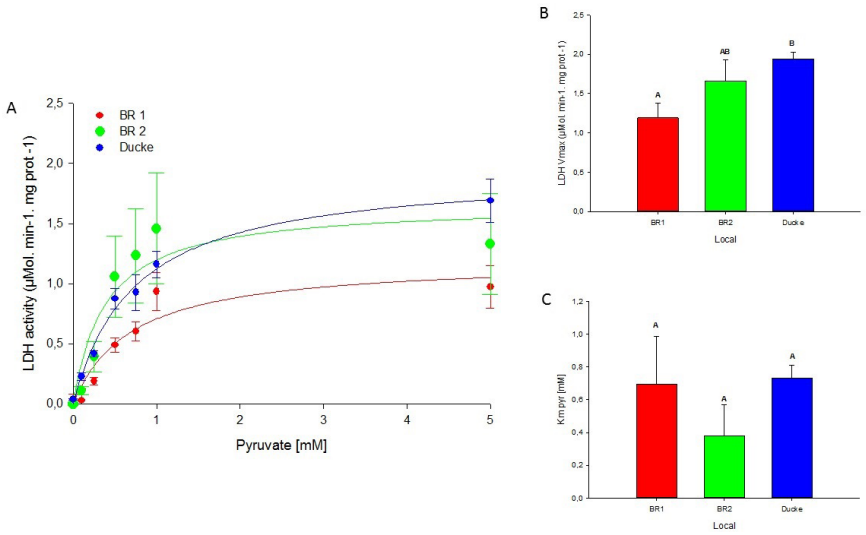

FIGURE 2 | **A.** LDH enzyme activity in different concentrations of substrate (pyruvate, mM); **B.** Maximum velocity of lactate dehydrogenase (LDH), and **C.** Enzyme affinity (Kmpyr) of lactate dehydrogenase (LDH) in muscle of different populations of *Bryconops giacopinii* that inhabit different thermal habitats (for details see Material and Methods).

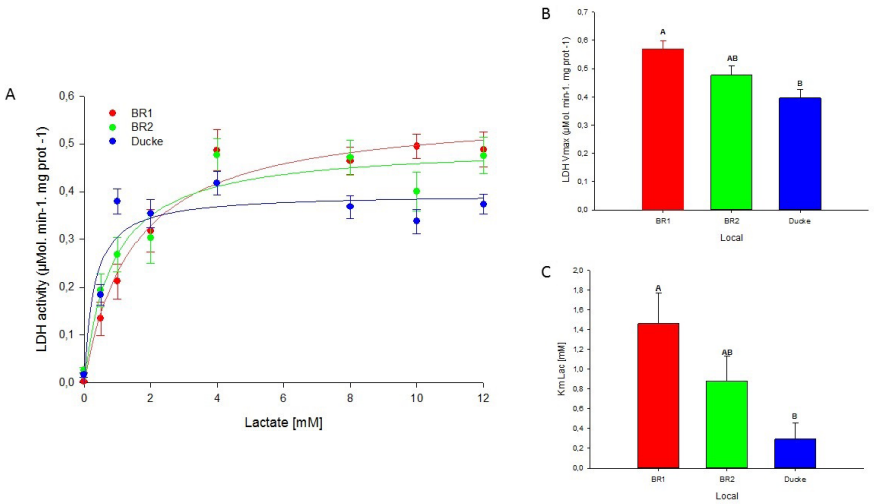

FIGURE 3 | **A.** LDH enzyme activity in different concentrations of substrate (lactate, mM); **B.** Maximum velocity of lactate dehydrogenase (LDH), and **C.** Enzyme affinity (Km^{lac}) of lactate dehydrogenase (LDH) in muscle of different populations of *Bryconops giacopinii* that inhabit different thermal habitats (for details see Material and Methods).

Also, there were significant differences in the muscle among the collection sites (Tab. 3). SOD activity was lower in BR#1 and BR#2 compare to Ducke, CAT activity was higher in BR#1 compare to Ducke, the GST activity was lower in BR#1 compare to Ducke and ROS was higher in BR#1 and BR#2 compare to Ducke. There were no differences in the GSH activity and LPO.

TABLE 2 | Superoxide dismutase (SOD) (µMol·min^{-1}·mg^{-1}protein), glutathione-S-transferase activity (GST) (µMol·min^{-1}·mg^{-1} protein), Catalase activity (CAT) (µMol H_2O_2·min^{-1}·mg^{-1} protein), Levels of lipoperoxidaton (LPO) (µMol cumeme hydroperoxide mg. protein), and reactive oxygen species (ROS) (area/mg protein) measured in gills of population of *Bryconops giacopinii* living at different temperatures. Different letters indicate statistical differences by one-way ANOVA $p < 0.05$.

	SOD	CAT	GSH	GST	LPO	ROS (10^5)
BR#1	$60.19^{a} \pm 7.03$	$2.22^{a} \pm 0.15$	$138.74^{a} \pm 20.58$	$8.76^{a} \pm 0.75$	12.75 ± 0.71	4.1 ± 0.44
BR#2	$121.74^{b} \pm 22.25$	$4.04^{b} \pm 0.46$	$104.87^{a} \pm 4.48$	$9.80^{ab} \pm 0.84$	13.98 ± 1.5	2.6 ± 0.15
Ducke	$136.07^{b} \pm 20.86$	$3.10^{ab} \pm 0.31$	$76.09^{b} \pm 6.26$	$13.21^{b} \pm 1.52$	12.42 ± 1.02	2.2 ± 0.36

TABLE 3 | Superoxide dismutase (SOD) (µMol·min–1·mg–1protein) glutathione-S-transferase activity (GST) (µMol·min–1·mg–1 protein), Catalase activity (CAT) (µMol H2O2·min–1·mg–1 protein), Levels of lipoperoxidaton (LPO) (µMol cumeme hydroperoxide mg. protein) and reactive oxygen species (ROS) (area/mg protein) measured in white muscle of population of *Bryconops giacopinii* living at different. Different letters indicate statistical differences by one-way ANOVA $p < 0.05$. temperatures.

	SOD	CAT	GSH	GST	LPO	ROS (10^5)
BR#1	$51.52^{a} \pm 10.41$	$0.54^{a} \pm 0.09$	121.12 ± 19.42	$5.02^{a} \pm 0.41$	11.18 ± 0.19	$0.2^{a} \pm 0.04$
BR#2	$78.43^{a} \pm 11.9$	$0.36^{ab} \pm 0.05$	156.26 ± 22.08	$7.06^{ab} \pm 0.40$	10.28 ± 0.62	$0.3^{a} \pm 0.04$
Ducke	$136.13^{b} \pm 15.21$	$0.21^{b} \pm 0.04$	98.65 ± 13.59	$7.68^{b} \pm 0.38$	12.68 ± 0.69	$0.1^{b} \pm 0.01$

DISCUSSION

In our study, we observed distinct environmental conditions among collection sites, particularly with roadside streams exhibiting higher temperatures and pH levels, along with lower oxygen concentrations. These changes are closely associated with the deforestation that has occurred along the BR-174 highway. While our data represents a single measurement and may not capture the entire thermal dynamics of the environments, other studies conducted in Amazonian streams have also documented warming trends linked to deforestation (see Ilha *et al*., 2018). Although data specific to the Tarumã-Açu basin are limited, a recent report indicated that temperatures in roadside streams ranged from 29.31°C to 36.07°C between September 2019 and July 2020 (Costa, 2020). Interestingly, this corresponds to the timeframe of our study, suggesting consistency with our observations and highlighting the impact of environmental changes on stream temperatures.

Our study revealed that *Bryconops giacopinii* inhabiting road-side streams exhibit alterations in LDH kinetics and antioxidant response systems. Through cellular analyses, we were able to identify physiological mechanisms in response to increased temperatures. Overall, individuals from roadside habitats, which experience higher temperatures, displayed elevated LDH activity for pyruvate formation in muscle, increased levels of

reactive oxygen species (ROS) production, and heightened glutathione (GSH) activity. Conversely, these populations exhibited decreased LDH activity for lactate formation in muscle, as well as reduced superoxide dismutase (SOD) and glutathione-S-transferase (GST) activity in both gills and muscle tissues.

The absence of oxidative stress, indicated by the lack of changes in lipid peroxidation (LPO), suggests that the antioxidant response exhibited by *Bryconops giacopinii* effectively detoxifies reactive oxygen species. These findings suggest that the species possesses physiological adaptations enabling it to thrive in warmer environments.

It has been established that the kinetic parameters of fish LDH enzymes are influenced by their environment (Almeida-Val *et al*., 2006). However, there is limited evidence regarding alterations in the kinetic properties of glycolytic enzymes in Amazonian natural populations inhabiting different temperature regimes. LDH, being a pivotal enzyme in glycolysis, has garnered attention for its kinetic parameters in the context of thermal adaptations (Fields *et al*., 2015).

Our current study revealed that LDH in the forward reaction from Ducke exhibits higher Vmax compared to BR#1, while the Km^{pyr} of LDH among sites collection did not differ significantly. The elevation of LDH Vmax in Ducke, despite a constant Km, could be attributed to increased anaerobic activity in populations inhabiting colder environments. Consequently, LDH activity for pyruvate formation is lower in Ducke compared to BR#1. These findings suggest that populations residing in high temperatures (BR#1; 29.3°C) enhance lactate oxidation, potentially reflecting their utilization of lactate as an aerobic fuel.

Indeed, during periods of high aerobic demand, such as elevated temperatures or exercise, fish tend to increase muscle lactate oxidation to sustain aerobic metabolism. Ferreira *et al*. (2018) demonstrated that the Amazon characid *Brycon amazonicus*, known for its high aerobic and swimming capabilities, enhances muscle lactate oxidation to meet the demands of exercise. Therefore, heightened metabolic demand may enable fishes to utilize lactate as an aerobic substrate, consequently improving swimming performance.

Consistent with this notion, the higher Km^{lac} (indicative of lower affinity) observed in the BR#1 population suggests that under high temperature conditions, this species may accumulate lactate in muscle to be utilized when needed. *Bryconops giacopinii*, being a rheophilic species inhabiting Amazonian streams, consistently swims against the flow, necessitating significant muscle work to sustain its lifestyle.

In vertebrates, the LDH reaction is widely recognized to be in equilibrium (Spriet *et al*., 2000; Quistorff, Grunnet, 2011), indicating that the enzyme's activity is regulated by concentrations of substrates and products. Consequently, alterations in equilibrium may be associated with changes in one of these compartments. Our finding shows that when there are changes that reduce the affinity for lactate, it can result in more lactate accumulating in tissues. As a result, there is an increase in LDH Vmax to speed up the rate at which lactate is utilized, especially in individuals living in high-temperature environments. This suggests that lactate plays a vital role as a metabolic fuel in such conditions. Indeed, a preference for lactate oxidation has been observed in skeletal muscle (Donovan, Pagliassotti, 2000) and cardiac muscle (Chatham *et al*., 2001) during exercised mammals.

Warming conditions elevate oxygen consumption in ectotherms to cope with

heightened physiological demands. However, as a by-product of respiration, increased oxygen consumption is also associated with the generation of potentially harmful reactive oxygen species (ROS) (Madeira *et al*., 2018). The production of ROS in mitochondria is directly linked to oxygen availability (Pelster *et al*., 2020). Therefore, the elevation of oxygen consumption to meet energy demands under warming conditions may incur cellular costs (Madeira *et al*., 2016a,b). Our findings revealed higher ROS production in the BR#1 population, which inhabits warmer streams. This suggests an upsurge in cellular respiration, supporting the hypothesis that these individuals enhance lactate oxidation to improve aerobic metabolism.

Warming-related oxidative stress occurs from the inability of the antioxidant defense system to avoid damage caused by ROS (Kültz, 2020; Somero, 2020). Herein, we showed that the population living in a high-temperature environment presents different levels of antioxidant enzymes to detoxify ROS production. In general, roadside populations showed lower levels of SOD and GST, while increased CAT and GSH in both muscle and gills. Therefore, we showed that to detoxify the increased levels of ROS at warming they increased the activity of specific enzymes, while decreased others. Theses physiological mechanism seems efficient to deal with oxidative stress since no difference was observed increase in LPO levels in both tissues.

The effects of warming on antioxidant and oxidative stress in Amazon species have attracted attention in recent years, and the physiological mechanism to detoxify ROS production seems specie, temperature, and time-specific (Campos *et al*., 2019; Baldissera *et al*., 2020). As we observed in the present work the decrease in some enzyme activity in fish exposed to warming has been reported by many authors. For instance, SOD activity and GPx activity were inhibited in *Brycon amazonicus* exposed to 34°C, compared to 28°C (Baldissera *et al*., 2020). Souza *et al*. (2018) reported significant decreases in muscle SOD activity of *Notothenia rossii* and *N. coriiceps* exposed 14 days to 8°C compared to 0°C, and Madeira *et al*. (2016b) showed a decrease in muscle SOD activity of *Amphiprion ocellaris* acclimated 28 days to 30°C compare to 26°C. In contrast, others antioxidant enzymes increased when fish are exposed to heat, especially CAT. Exposure to high temperatures can increase the formation of H_2O_2 as showed by Iftikar *et al*. (2015). CAT plays the primary role in the elimination of H_2O_2 (López-Cruz *et al*., 2010) converting it back to H_2O and O_2, decreasing the flow of ROS and preventing oxidative stress. Indeed, Campos *et al*., 2019 showed that acclimation for 30 days to the projected climate future (4°C and 900 ppm above current levels) increased CAT activity in the muscle of *Pyrrhulina brevis* and *Hyphessobrycon melazonatus*, while *Apistogramma agassizii* increased GST. In accordance, we found an increase in the CAT activity and GSH levels in roadside individuals, suggesting an activation of this antioxidant system to deal with the increase of H_2O_2 production in warm living individuals.

The ability to regulate ROS levels and prevent oxidative damage is a crucial mechanism for maintaining cellular homeostasis (Madeira *et al*., 2016a,b; Rosa *et al*., 2016). Lipids play a vital role in preserving the integrity of cellular membranes, regulating ion gradients, membrane fluidity, and permeability (Gaschler, Stockwell, 2017). The absence of elevated levels of lipid peroxidation (LPO) in populations inhabiting warm environments suggests that the antioxidant systems of this species effectively counteract ROS production, thereby preserving cellular homeostasis. This indicates that the species is capable of physiological adaptation to warming conditions.

The disparities observed between the roadside streams (BR#1 and BR#2) may stem from differences in intrapopulational responses. Factors such as the duration of exposure to high temperatures and the duration of species separation could contribute to these differences. Additionally, the degree of exposure to temperature variation can impact antioxidant enzyme levels. For instance, research by Madeira *et al*. (2016b) demonstrated that mild temperature exposure can enhance antioxidant enzyme activity, whereas extreme temperatures lead to a decrease in enzyme activities. The decline in SOD and CAT activity observed at BR#1 compared to BR#2 could be linked to enzyme denaturation or impaired synthesis within the cells. Enzymes are proteins held together by hydrogen bonds, which can weaken when temperatures exceed a certain threshold, resulting in denaturation and reduced catalytic activity (Rosa *et al*., 2016). Tropical species are particularly susceptible to temperature changes as they often live near their thermal limits. Therefore, even slight increases in temperature can significantly impact animal physiology (Campos *et al*., 2021).

Although our work presents important findings, it is not possible to identify if these responses are physiological plasticity or result of local adaptation among populations, since differences in phenotype are both genetic and environmental induced (Allendorf, Luikart, 2007). Phenotypic divergence can be caused by genetic differences or phenotypic plasticity (Via, Lande, 1985). Therefore, the differentiation between roadside and Ducke individuals does not necessarily indicate local adaptation (Conover, Schultz, 1995). Typically, the causes of phenotypic variation are assessed by removing the effect of environment via common garden and/or reciprocal transplant experiments (Hansen *et al*., 2012). To understand this evolutionary question, the next step is to acclimate these populations to different temperatures to identify possible mechanisms of local adaptation or physiological plasticity, which will be important to understand in light of climate change.

In summary, our findings indicate that roadside populations of *Bryconops giacopinii* inhabiting high-temperature environments exhibit modifications in muscle LDH kinetics parameters, enhancing lactate oxidation. This adaptation likely corresponds to increased energetic demands. Furthermore, these populations demonstrate elevated levels of CAT and GSH in both gills and muscle, aiding in the management of heightened ROS production. The robust antioxidant system effectively mitigates oxidative stress, suggesting that this population has adapted to survive in warmer environments. Therefore, our study reveals that *B. giacopinii* living in a warming world adjust their glycolytic metabolism and bolster antioxidant enzymes to meet energy demands without experiencing heightened oxidative stress.

ACKNOWLEGMENTS

This study was part of the INCT-ADAPTA supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, process 465540/2014–7), Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM, process 062.01187/2017), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Finance Code 001). ALV are the recipients of Research fellowships from CNPq. DFC is the recipient of a post-doctoral fellowship from FAPESP, and SBM was the recipient of a doctoral fellowship from CAPES.

REFERENCES

- **Allendorf FW, Luikart G.** Conservation and the genetics of populations. Malden, Massachusetts, Oxford and Carlton, Victoria: Blackwell Publishing; 2007.
- **Amado LL, Garcia ML, Ramos PB, Freitas RF, Zafalon B, Ferreira JLR *et al*.** A method to measure total antioxidant capacity against peroxyl radicals in aquatic organisms: application to evaluate microcystins toxicity. Sci Total Environ. 2009; 407(6):2115–23. https://doi.org/10.1016/j.scitotenv.2008.11.038
- **Almeida-Val VMF, Chipari Gomes AR, Lopes NP.** Metabolic and physiological adjustments to low oxygen and high temperature in fishes of the Amazon. In: Val AL, Almeida-Val VMF, Randall DJ, editors. The physiology of tropical fishes. Series fish physiology. Elsevier, London; 2006. p.443–500.
- **Baldissera MD, Souza CF, Barroso DC, Falk RB, Wagner R, Baldisserotto B *et al*.** Disturbance of oxidant/antioxidant status and impairment on fillet fatty acid profiles in *Brycon amazonicus* subjected to acute heat stress. Fish Physiol Biochem. 2020; 46(5):1857–66. https://doi.org/10.1007/s10695-020-00835-3
- **Beutler E.** Red Cell Metabolism: A manual of biochemical methods. New York: Grune & Straton; 1975.
- **Campbell BM, Beare DJ, Bennett EM, Hall-Spencer JM, Ingram JSI, Jaramillo F *et al*.** Agriculture production as a major driver of the Earth system exceeding planetary boundaries. Ecol Soc. 2017; 22(4):8. https://doi.org/10.5751/ES-09595-220408
- **Campos DF, Braz-Mota S, Val AL, Almeida-Val VMF.** Predicting thermal sensitivity of three Amazon fishes exposed to climate change scenarios. Ecol Indic. 2019; 101:533–40. https://doi.org/10.1016/j.ecolind.2019.01.051
- **Campos DF, Amanajás RD, Almeida-Val VMF, Val AL.** Climate vulnerability of South American freshwater fish: Thermal tolerance and acclimation. J Exp Zool A Ecol Integr Physiol. 2021; 335(9–10):723–34. https://doi.org/10.1002/jez.2452
- **Campos DF, Jesus TF, Kochhann D, Heinrichs-Caldas W, Coelho MM, Almeida-Val VMF.** Metabolic rate and thermal tolerance in two congeneric Amazon fishes: *Paracheirodon axelrodi* Schultz, 1956 and *Paracheirodon simulans* Géry, 1963 (Characidae). Hydrobiologia. 2017; 789:133–42. https://doi.org/10.1007/s10750-016-2649-2
- **Chatham JC, Des Rosiers C, Forder JR.** Evidence of separate pathways for lactate uptake and release by the perfused rat heart. Am J Physiol Endocrinol Metab. 2001; 281:794–802. https://doi.org/10.1152/ajpendo.2001.281.4.e794
- **Conover DO, Schultz ET.** Phenotypic similarity and the evolutionary significance of countergradient variation. Trends Ecol Evol. 1995; 10(6):248–52. https://doi.org/10.1016/S0169-5347(00)89081-3
- **Costa JS.** Relatório da situação hidrográfica do Tarumã-Açu: transformações antrópicas e qualidade da água. Universidade do Estado do Amazonas: Manaus-AM; 2020.
- **Crozier LG, Hutchings JA.** Plastic and evolutionary responses to climate change in fish. Evol Appl. 2014; 7(1):68–87. https://doi.org/10.1111/eva.12135
- **Donelson JM, Munday PL, McCormick MI, Nilsson GE.** Acclimation to predicted ocean warming by a tropical reef fish. Glob Chan Biol. 2011; 17(4):1712–19. https://doi.org/10.1111/j.1365-2486.2010.02339.x
- **Donovan CM, Pagliassotti MJ.** Quantitative assessment of pathways for lactate disposal in skeletal muscle fiber types. Med Sci Sporst Exerc. 2000; 32(4):772–77. https://doi.org/10.1097/00005768-200004000-00009
- **Driedzic WR, Almeida-Val VMF.** Enzymes of cardiac energy metabolism in Amazonian teleosts and fresh-water stingray (*Potamotrygon hystrix*). J Exp Zool. 1996; 274(6):327–33. https://doi.org/10.1002/(SICI)1097-010X(19960415)274:6%3C327::AID-JEZ1%3E3.0.CO;2-Q
- **Ferreira MS, Aride PHR, Val AL.** Could resistance to lactate accumulation contribute to the better swimming performance of *Brycon amazonicus* when compared to *Colossoma macropomum*? PeerJ. 2018; 6:e5719. https://doi.org/10.7717/peerj.5719

- **Fields PA, Dong Y, Meng X, Somero GN.** Adaptations of protein structure and function to temperature: there is more than one way to 'skin a cat'. J Exp Biol. 2015; 218(12):1801–11. https://doi.org/10.1242/jeb.114298
- **Fields PA, Houseman DE.** Decreases in activation energy and substrate affinity in cold-adapted A4-lactate dehydrogenase: evidence from the Antarctic notothenioid fish *Chaenocephalus aceratus*. Mol Biol Evol. 2004; 21(12):2246–55. https://doi.org/10.1093/molbev/msh237
- **Gaschler MM, Stockwell BR.** Lipid peroxidation in cell death. Biochem Biophys Res Commun. 2017; 482(3):419–25. https://doi.org/10.1016%2Fj.bbrc.2016.10.086
- **Hansen MM, Olivieri I, Waller DM, Nielsen EE.** Monitoring adaptive genetic responses to environmental change. Mol Ecol. 2012; 21(6):1311–29. https://doi.org/10.1111/j.1365-294x.2011.05463.x
- **Iftikar FI, MacDonald JR, Baker DW, Renshaw GMC, Hickey AJR.** Could thermal sensitivity of mitochondria determine species distribution in a changing climate? J Exp Biol. 2014; 217:2348–57. https://doi.org/10.1242/jeb.098798
- **Ilha P, Schiesari L, Yanagawa FI, Jankowski K, Navas CA.** Deforestation and stream warming affect body size of Amazonian fishes. PLoS ONE. 2018; 13(5):e0196560. https://doi.org/10.1371/journal.pone.0196560
- **Jiang Z-Y, Woollard ACS, Wolff SP.** Lipid hydroperoxide measurement by oxidation of Fe2+ in the presence of xylenol orange. Comparison with the TBA assay and an iodometric method. Lipids. 1991; 26(10):853–56. https://doi.org/10.1007/BF02536169
- **Keen JH, Habig WH, Jakoby WB.** Mechanism for several activities of the glutathione-S-transferase. J Biol Chem. 1976; 251(20):6183–88.
- **Kültz D.** Evolution of cellular stress response mechanisms. J Exp Zool. 2020; 333(6):359–78. https://doi.org/10.1002/jez.2347
- **Lopes AR, Sampaio E, Santos C, Couto A, Pegado MR, Diniz M *et al*.** Absence of cellular damage in tropical newly hatched sharks (*Chiloscyllium plagiosum*) under ocean acidification conditions. Cell Stress Chaperon. 2018; 23(5):837–46. https://doi.org/10.1007/s12192-018-0892-3
- **Madeira C, Madeira D, Diniz MS, Cabral HN, Vinagre C.** Thermal acclimation in clownfish: an integrated biomarker response and multi-tissue experimental approach. Ecol Indic. 2016b; 71:280–92. https://doi.org/10.1016/j.ecolind.2016.07.009
- **Madeira C, Mendonça V, Leal MC, Flores AAV, Cabral HN, Diniz M *et al*.** Environmental health assessment of warming coastal ecosystems in the tropics – application of integrative physiological indices. Sci Total Environ. 2018; 643:28–39. https://doi.org/10.1016/j.scitotenv.2018.06.152
- **Madeira D, Vinagre C, Diniz MS.** Are fish in hot water? Effects of warming on oxidative stress metabolism in the commercial species *Sparus aurata*. Ecol Indic. 2016a; 63:324–31. https://doi.org/10.1016/j.ecolind.2015.12.008
- **Omlin T, Langevin K, Weber J-M.** Exogenous lactate supply affects lactate kinetics of rainbow trout, not swimming performance. Am J Physiol. 2014; 307:1018–24. https://doi.org/10.1152/ajpregu.00200.2014
- **Pelster B, Wood CM, Campos DF, Val AL.** Cellular oxygen consumption, ROS production and ROS defense in two different size-classes of an Amazonian obligate air-breathing fish (*Arapaima gigas*). PLoS ONE. 2020; 15(7):e0236507. https://doi.org/10.1371/journal.pone.0236507
- **Pörtner HO, Gutt J.** Impacts of climate variability and change on (marine) animals: Physiological underpinnings and evolutionary consequences. Int Comp Biol. 2016; 56(1):31–44. https://doi.org/10.1093/icb/icw019
- **Pörtner HO, Karl DM, Boyd PW, Cheung WWL, Lluch-Cota SE, Nojiri Y *et al*.** 2014: Ocean systems. In: Field CB, Barros VR, Dokken DJ, Mach KJ, Mastrandrea MD, Bilir TE *et al*., editors. Climate change 2014: impacts, adaptation, and vulnerability. Part A: global and sectoral aspects. Contribution of working group II to the Fifth assessment report of the Intergovernmental panel on climate change. New York: Cambridge University Press; 2014. p.411–84.
- **Quistorff B, Grunnet N.** The isoenzyme pattern of LDH does not play a physiological role; except perhaps during fast transitions in energy metabolism. Aging (Albany, NY). 2011; 3(5):457–60. https://doi.org/10.18632%2Faging.100329

- **Rodrigues EP, Pinheiro ES.** O desflorestamento ao longo da rodovia BR-174 (Manaus/Am - Boa Vista/Rr). Soc Nat. 2012; 23(3).
- **Rosa R, Paula JR, Sampaio E, Pimentel M, Lopes AR, Baptista M *et al*.** Neuro-oxidative damage and aerobic potential loss of sharks under elevated CO_2 and warming. Mar Biol. 2016; 163(119). https://doi.org/10.1007/s00227-016-2898-7
- **Schulte PM.** The effects of temperature on aerobic metabolism: Towards a mechanistic understanding of the responses of ectotherms to a changing environment. J Exp Biol. 2015; 218(12):1856–66. https://doi.org/10.1242/jeb.118851
- **Sinclair BJ, Marshall KE, Sewell MA, Levesque DL, Willett CS, Slotsbo S *et al*.** Can we predict ectotherm responses to climate change using thermal performance curves and body temperatures? Ecol Lett. 2016; 19:1372–85. https://doi.org/10.1111/ele.12686
- **Somero GN.** The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. J Exp Biol. 2010; 213(6):912–20. https://doi.org/10.1242/jeb.037473
- **Somero GN.** The cellular stress response and temperature: Function, regulation, and evolution. J Exp Zool. 2020; 333(6):379–97. https://doi.org/10.1002/jez.2344
- **Souza MRDP, Herrerias T, Zaleski T, Forgati M, Kandalski PK, Machado C *et al*.** Heat stress in the heart and muscle of the Antarctic fishes *Notothenia rossii* and *Notothenia coriiceps*: Carbohydrate metabolism and antioxidant defence. Biochimie. 2018; 146:43–55. https://doi.org/10.1016/j.biochi.2017.11.010
- **Spriet LL, Howlett RA, Heigenhauser GJF.** An enzymatic approach to lactate production in human skeletal muscle during exercise. Med Sci Sports Exerc. 2000; 32(4):756–63. https://doi.org/10.1097/00005768-200004000-00007
- **Turrens JF.** Superoxide production by the mitochondrial respiratory chain. Biosci Rep. 1997; 17(1):3–08. https://doi.org/10.1023/a:1027374931887
- **Via S, Lande R.** Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution. 1985; 39(3):505–22. https://doi.org/10.2307/2408649

AUTHORS' CONTRIBUTION

Derek Felipe de Campos: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing-original draft.

Susana Braz Mota: Data curation, Investigation, Writing-review and editing.

Vera Maria Fonseca de Almeida-Val: Supervision, Validation, Writing-review and editing.

Adalberto Luis Val: Conceptualization, Supervision, Writing-review and editing.

ETHICAL STATEMENT

All procedures followed CONCEA, Brazilian Guide for Animal Use and Care approved by INPA's Council for Ethics in Animal Use (Protocol number 026/2015), collection license SISBio number 29837–24.

COMPETING INTERESTS

The author declares no competing interests.

HOW TO CITE THIS ARTICLE

- **Campos DF, Mota SB, Almeida-Val VMF, Val AL.** Glycolytic modulations and antioxidant capacity in Amazonian fish, *Bryconops giacopinii* (Characiformes: Iguanodectidae), living at high temperature. Neotrop Ichthyol. 2024; 22(2):e230130. https://doi.org/10.1590/1982-0224-2023-0130

Neotropical Ichthyology

OPEN ACCESS

SBI
Official Journal of the
Sociedade Brasileira de Ictiologia