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Take time to look at the fish: Behavioral response to acute thermal challenge in two Amazonian cichlids

Daiani Kochhann[1] | **Carolina G. Sarmento**[2] | **Jomara C. de Oliveira**[2,3] | **Helder L. Queiroz**[2] | **Adalberto L. Val**[4] | **Lauren J. Chapman**[5]

[1]Laboratory of Behavioural Ecophysiology, Center of Agrarian and Biological Sciences, Acaraú Valley State University, Sobral, Ceará, Brazil

[2]Laboratory of Ecology and Fish Biology, Mamirauá Institute for Sustainable Development–MISD, Tefé, Brazil

[3]Amazonas State Secretary for Education and Teaching Quality, SEDUC Amazonas, Manaus, Brazil

[4]Laboratory of Ecophysiology and Molecular Evolution, Brazilian National Institute for Research of the Amazon–INPA, Manaus, Brazil

[5]Department of Biology, McGill University, Montréal, Québec, Canada

Correspondence

Daiani Kochhann, Laboratory of Behavioural Ecophysiology, Center of Agrarian and Biological Sciences, Acaraú Valley State University, Av. da Universidade, 850 Campus da Betânia, Sobral, Ceará 62040370, Brazil.
Email: daia.kochhann@gmail.com

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Abstract

Critical thermal maximum (CT_{max}) is often used as an index of upper thermal tolerance in fishes; however, recent studies have shown that some fishes exhibit agitation or avoidance behavior well before the CT_{max} is reached. In this study, we quantified behavioral changes during CT_{max} trials in two Amazonian cichlids, *Apistogramma agassizii* and *Mesonauta insignis*. The thermal agitation temperature (T_{ag}) was recorded as the temperature at which fish left cover and began swimming in an agitated manner, and four behaviors (duration of sheltering, digging, activity, and aquatic surface respiration [ASR]) were compared before and after T_{ag}. Both *A. agassizii* and *M. insignis* exhibited high critical thermal maxima, 40.8°C and 41.3°C, respectively. Agitation temperature was higher in *M. insignis* (37.3°C) than in *A. agassizii* (35.4°C), indicating that *A. agassizii* has a lower temperature threshold at which avoidance behavior is initiated. Activity level increased and shelter use decreased with increased temperatures, and patterns were similar between the two species. Digging behavior increased after T_{ag} in both species, but was higher in *A. agassazii* and may reflect its substrate-oriented ecology. ASR (ventilating water at the surface film) was extremely rare before T_{ag}, but increased in both cichlid species after T_{ag} and was greater in *M. insignis* than in *A. agassizii*. This suggests that fish were experiencing physiological hypoxia at water temperatures approaching CT_{max}. These results demonstrate that acute thermal challenge can induce a suite of behavioral changes in fishes that may provide additional, ecologically relevant information on thermal tolerance.

KEYWORDS

agitation behavior, aquatic surface respiration, CT_{max}, thermal tolerance

1 | INTRODUCTION

Freshwater systems have suffered a number of anthropogenic impacts including habitat degradation and loss, dams and water diversions, influx of pollutants and contaminants, increase in frequency and extent of hypoxia, overfishing, and introductions of nonnative species, which has led to severe declines in the abundance of many freshwater fishes (Arthington et al., 2016; Dudgeon et al., 2006; Friedrich et al., 2014; Hermoso, 2017; Strayer & Dudgeon, 2010; Vörösmarty et al., 2010). In addition, a growing body of evidence from several continents suggests that freshwater systems are highly sensitive to climate change, which can lead to increased water temperature and changes in precipitation and hydrologic regimes and may interact with other stressors to increase the vulnerability of freshwater fishes (Ficke et al., 2007; Heino et al., 2009; Knouft & Ficklin, 2017; Myers et al., 2017; Reid et al., 2019). Climate warming may be especially significant for fishes that, as ectotherms, rely on environmental temperature to regulate metabolic functions. The ability of an ectotherm to carry out essential functions (e.g., growth and metabolic rate) over a range of water temperatures has been defined as its thermal performance window (Huey & Stevenson, 1979; Schulte et al., 2011). With increasing water temperature, ectotherms will reach a critical temperature above which they become reliant on anaerobic metabolism (Pörtner, 2010; Ern et al., 2016). Therefore, climate-induced warming may favor species with wider thermal windows and higher upper thermal limits over species with narrower windows (Angilletta et al., 2002; Magozzi & Calosi, 2015; Pörtner & Farrell, 2008). Tropical fishes may be particularly sensitive to climate warming because they experience small annual temperature fluctuations relative to temperate species and are therefore expected to be relatively stenothermal with narrow thermal safety margins (difference between the maximum environmental temperature and upper thermal tolerance) (Janzen, 1967; Magozzi & Calosi, 2015; Morley et al., 2019; Stillman, 2003; Tewksbury et al., 2008). Given the extraordinary diversity of tropical freshwater fishes and their potential vulnerabilty to climate warming, there is critical need to more fully understand and predict their thermal limits.

Extinction estimates for a wide range of taxas (birds, plants, invertebrates, fishes, mammals, reptiles, and amphibians) in South America are the highest of geographical regions with 23% of species predicted to be threatened by climate change (Urban, 2015). Applying this projection to the 5160 described species of freshwater fishes in the region (Reis et al., 2016), over 1100 species of South American fishes may be at risk of extinction due to climate change. A recent review of thermal tolerance and acclimation capacity for 106 species of South American freshwater fishes by Campos et al. (2021) supports a higher susceptibility of fish species living at the tropical areas of South America as they are living close to their thermal limits and have low acclimation capacity. These studies highlight the potential vulnerability of tropical South America fish species to climate change and the importance of increasing our understanding of thermal limits for fishes of this region.

To assess the upper thermal limits of fishes (and other ectotherms), many studies have estimated critical thermal maximum (CT_{max}), typically quantified as the temperature at which the fish loses equilibrium in response to acute linear temperature increases (Del Rio et al., 2019; McDonnell & Chapman, 2015; Zhou et al., 2019). Specifically, the fish is exposed to increasing water temperature at a standard rapid rate of increase (often 0.3°C per minute) until loss of equilibrium (LOE) (Becker & Genoway, 1979; Lutterschmidt & Hutchison, 1997). Although the mechanisms underlying LOE are still unclear (Brauner & Richards, 2020), CT_{max} has been demonstrated as a repeatable metric of tolerance (Morgan et al., 2018) that is sublethal and relatively simple, fast, and easy to measure even in field settings (e.g., Chrétien & Chapman, 2016; Turko et al., 2020), and in some cases, relates to thermal tolerance measured in response to slower warming. For example, Åsheim et al. (2020) found that upper thermal tolerance to rapid warming (CT_{max}) was correlated with upper thermal tolerance measured under slow warming conditions in zebrafish acclimated to 22°C and weakly correlated in fish acclimated to 34°C. Data on CT_{max} are available for many freshwater ectotherms providing a strong comparative framework for evaluating relative tolerance, plasticity in thermal tolerance, and for predicting shifts in geographic distributions (Campos et al., 2021; Morley et al., 2019; Sunday et al., 2012). However, recent studies have shown that some fish species exhibit avoidance behavior well before the CT_{max} is reached (McDonnell et al., 2019; McDonnell & Chapman, 2015; Turko et al., 2020; Wells et al., 2016), suggesting that behavioral metrics may provide additional, ecologically relevant information on thermal tolerance. McDonnell and Chapman (2015) observed consistent behavioral changes in the African cichlid *Pseudocrenilabrus multicolor* during CT_{max} tests, whereby fish would leave cover and begin swimming in a quick, agitated manner, which they interpreted as avoidance behavior. The temperature at which this behavior was initiated is referred to as the thermal agitation temperature (T_{ag}). The same shift in behavior during CT_{max} trials was observed in different populations of brook trout (*Salvelinus fontinalis*) (Wells et al., 2016). Wells et al. also proposed a new metric called thermal agitation window (the difference between CT_{max} and the agitation temperature). A small window implies a higher thermal tolerance as fish maintain routine behaviors over a wider range of increasing temperature, with clear behavioral changes occurring closer to CT_{max} (Wells et al., 2016). Agitation behavior was also noted in two temperate cyprinid fishes: redside dace (*Clinostomus elongates*, Turko et al., 2020) and pugnose shiner (*Notropis anogenus*, Potts, 2020). Barker et al. (2018) reported a CT_{max} of 37.1°C for lionfish (*Pterois* sp.) acclimated to 25°C, but they observed avoidance behavior at 30.9°C. These studies suggest that avoidance and agitation behavior may be a widespread response to acute thermal challenge and may provide additional insights into response of fishes to increasing water temperature.

Other behavioral responses to elevated water temperature have been reported in fishes including increases in swimming activity and aggression in the cichlids *Pseudocrenilabrus multicolor* (McDonnell et al., 2019) and *Cichlasoma paranaense* (Brandão et al., 2018), and the damselfishes *Pomacentrus moluccensis* and *P. bankanensis* (Biro et al., 2010). Kochhann et al. (2015) reported increased activity and a decrease in the stability of dominance hierarchies in a small Amazonian cichlid *Apistogramma agassizii* after a short-term exposure to a higher temperature. Increases in activity and risk taking behavior were also observed in the poeciliid *Pseudoxiphophorus jonesii* after a short-term increase in temperature (Culumber, 2020). Quigley and Hinch (2006) reported an increase in erratic swimming, abnormal posture, and aggregative behavior in the Chinook salmon *Oncorhynchus tshawytscha* in response to rapid increase of the water temperature in small streams. Together, these studies suggest that increasing water temperature can induce a suite of behavioral changes in fishes, in addition to avoidance behavior, that could potentially be used to identify sublethal thresholds of ecological significance. The overall objective of our study was to quantify a variety of behavioral changes during standardized acute thermal challenge (CT_{max}) in two Amazonian cichlid fish species *Apistogramma agassizii* and *Mesonauta insignis*. We predicted that fish would exhibit a suite of behaviors before reaching $\mathrm{CT}_{\mathrm{max}}$ that relate to avoidance of elevated temperature or compensation for elevated temperature, and that these behaviors may differ between species, reflecting differences in their ecological niche.

2 | METHODS

2.1 | Study species and collection site

Apistogramma agassizii (Steindachner, 1875) is a small (maximum standard length of 4.2 cm, [Kullander, 2003]) South American dwarf cichlid that inhabits streams and rivers of Central Amazonia. They can be found in clear, white and black water, near the substrate and leaves (Hercos et al., 2009). Campos et al. (2018) reported a $\mathrm{CT}_{\mathrm{max}}$ of 41.5°C for *A. agassizii*, but there is no information available on behavioral response of this species to elevated water temperature. The flag cichlid *Mesonauta insignis* (Heckel, 1840) is a larger (maximum standard length of 9.4 cm [Kullander, 2003]) widespread species in the Amazonian basin, inhabiting rivers and lakes of both white and black water. It is one of the most dominant species in lakes and swamps of the middle Solimões River floodplain (Hercos et al., 2013), widespread in Amazon River and the upper Negro River basins (Kullander, 2003), and often found in aquatic macrophytes (Hercos et al., 2009). There are no previous studies, to our knowledge, on the thermal tolerance of this widespread cichlid.

Apistogramma agassizii and *Mesonauta insignis* were live captured from black waters (lake Tefé and small adjacent streams 3°40'5254''S, 64°82'1485''W) using a combination of seining and dip netting. Fish were transferred back to the fish facility at Mamirauá Institute. *Mesonauta insignis* was transferred to 1000-L holding tanks (2 tanks, ~50 fish per tank), and *A. agassizii* (4 tanks, ~40 per tank) was held in 50-L tanks. Water temperature in the holding tanks averaged 26.9°C for *A. agassizii* and 28.1°C for *M. insignis*. The temperature for *M. insignis* is close to the average water temperature of 28.9°C for black waters in the region recorded in long-term monitoring carried out by the Mamirauá Institute [2012–2017, https://www.mamiraua.org.br/sistemas-de-monitoramento]. We held *A. agassizii* at a slightly lower water temperature as this species tends to inhabit cooler forest streams (Carvalho et al., 2014; Rodrigues et al., 2012). The averaged DO concentration, conductivity, and pH of the holding tanks was 4.6 mg L^{-1}, 88.2 µS, and 6.2, respectively.

Experimental trials were carried out over 26 days during which time estimates of thermal tolerance ($\mathrm{CT}_{\mathrm{max}}$) and suite of behavioral traits were collected for 10 *A. agassizii* and 11 *M. insignis* selected at random from the larger groups in which they were maintained. Acclimation time varied among individual fish but averaged 16.9 ± 1.8 days (SE) for *A. agassizii* (range = 9–23 days) and 18.5 ± 1.7 days for *M. insignis* (range = 8–26 days) (Table 1). The average mass and length for *A. agassizii* was 0.53 ± 0.11 g (SE) and 2.7 ± 0.2 cm, respectively. *Mesonauta insignis* was larger, averaging 9.8 ± 1.1 g and 6.3 ± 0.2 cm for mass and standard length, respectively (Table 1). Fish were fed tropical fish flakes (Oscar Gold, 38% of protein) twice a day to satiation. The experimental procedures were approved by the Animal Use Ethics Committee of the Brazilian National Institute for Research of the Amazon (CEUA-INPA). The permit for the collection of the biological material to carry out the research was authorized by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA/SISBio), number 29837-13.

TABLE 1 Mean values (± SE and range) for standard length (cm), mass (g), acclimation time for thermal tolerance trials, critical thermal maximum ($\mathrm{CT}_{\mathrm{max}}$), thermal agitation temperature (T_{ag}), digging initiation temperature, and ASR initiation temperature for two Amazonian cichlids exposed to an acute thermal challenge

Trait	Species	*N*	Mean	*SE*	Range
Length (cm)	*Apistogramma agassizii*	10	2.7	0.2	2.0–3.7
	Mesonauta insignis	11	6.3	0.3	4.6–7.5
Mass (g)	*A. agassizii*	10	0.5	0.1	0.2–1.3
	M. insignis	11	9.8	1.1	3.9–15.8
Acclimation time (days)	*A. agassizii*	10	16.9	1.8	9–23
	M. insignis	11	18.5	1.7	8–26
Critical thermal maximum (°C)	*A. agassizii*	10	40.9	0.2	39.9–42.1
	M. insignis	11	41.3	0.2	40.1–42.1
Agitation temperature (°C)	*A. agassizii*	10	35.4	0.3	33.9–37.5
	M. insignis	11	37.3	0.6	33.8–40.3
Dig initiation temperature (°C)	*A. agassizii*	10	31.5	1.0	28.4–37.2
	M. insignis	7	38.0	0.9	33.2–40.7
ASR initiation temperature (°C)	*A. agassizii*	7	37.7	1.0	33.8–41.5
	M. insignis	11	38.5	0.5	35.3–40.4

Abbreviation: ASR, aquatic surface respiration.

2.2 | Critical thermal maximum

Critical thermal maximum ($\mathrm{CT}_{\mathrm{max}}$) was quantified as LOE following methods described in several earlier studies (Becker & Genoway, 1979; Lutterschmidt & Hutchison, 1997; McDonnell & Chapman, 2015). Fish were starved for 24 h to ensure they were in a postabsorptive state and then transferred to the experimental tank (40 cm length × 20 cm width × 30 cm height, 24 L). Two fish were placed in small compartments with a piece of cover and situated within the larger buffer tank. The experimental (buffer) tank was covered, and fish were monitored using a webcam to minimize disturbance during both the acclimation period and the trial. After a 2-h acclimation period in the experimental compartments, water temperature was raised by 0.3°C min^{-1} using a temperature control system (TEMP-REG, Loligo Systems). This rate of temperature

TABLE 2 Behaviors measured in *Apistogramma agassizii* and *Mesonauta insignis* during the CT_{max} trials

Behavior	Description
Shelter use	Fish resting under or next the shelter
Swimming activity	Fish freely swimming
Digging	Fish touching the bottom of the aquarium with their lips
Aquatic surface respirationAbbrevi	Fish swimming near the surface with their mouth in contact with the surface layer of the water

Abbreviation: CT_{max}, critical thermal maximum.

increase, as described in previous studies, is constant but fast enough to impede acclimation (Lutterschmidt & Hutchison, 1997). Experimental tanks were supplied with constant aeration, and DO was monitored throughout the trials and maintained at >74% saturation.

During the trials, the cichlids exhibited a repeatable behavioral sequence. During the 2-h acclimation period and beginning of the trial, individuals of both species would rest next to or under the provided shelter. However, as the temperature increased, the fish would begin to swim around agitatedly, which was interpreted as an avoidance behavior, and likely reflects the point where fish begin searching for a cooler refuge (McDonnell et al., 2019; McDonnell & Chapman, 2015; Wells et al., 2016). The onset of this behavior was recorded as the "agitation temperature" (McDonnell & Chapman, 2015). CT_{max} was designated as the point when the fish lost equilibrium at which time the trial was ended, and the fish was placed in a recovery bath held at the original acclimation temperature to recover. The exact temperatures of the agitation temperature and the CT_{max} were confirmed by watching time-stamped video footage.

In addition to the agitation temperature, we recorded the duration of the following behaviors each minute of the trial: hiding in shelter, swimming, digging, and aquatic surface respiration (ASR). ASR involves a fish rising to the surface, skimming the surface film, and ventilating the layer of water in contact with the atmosphere, which is richer in dissolved oxygen (DO) than the underlying water (Chapman & Mckenzie, 2009; Kramer & McClure, 1982). A description of ASR and other behaviors can be found in Table 2. The behavior of experimental fish was quantified using the event recording software application The Observer XT 12.0 (Noldus, InfoTech.). We did not include the time when fish were resting outside of the shelter in presenting our results. And, in some cases, fish were doing two behaviors simultaneously (e.g., swimming and digging), so the duration for both behaviors would be recorded. For ASR and digging, we also recorded the temperature at which these behaviors were initiated, referred to as the ASR initiation and digging initiation temperature, respectively.

2.3 | Statistical analysis

We tested for effects of body mass and length on $CT_{max,}$ agitation temperature (T_{ag}), the thermal agitation window (T_{aw}), digging initiation temperature (T_{dig}), and ASR initiation temperature (T_{ASR}) for each species using linear regression. Since there were no significant effects of body size on all five traits for both species (range in p values: 0.162–0.855), body size was not included in subsequent analyses. A paired t test was used to test for differences in CT_{max} and $T_{ag,}$ within each species. Analysis of covariance was used to test for differences in $CT_{max,}$ $T_{ag,}$ $T_{aw,}$ T_{dig}, and T_{ASR} between *M. insignis* and *A. aggasizii*. Acclimation time was included as a covariate in the model, with species as the main effect. The interaction between species and acclimation time was not significant for CT_{max} ($p = 0.708$), T_{ag} ($p = 0.857$), T_{aw} ($p = 0.973$), T_{dig} ($p = 0.608$), or T_{ASR} ($p = 0.074$) and was therefore removed from the final models reported in the results. In Table 1 and Figure 1, we present mean values for $CT_{max,}$ $T_{ag,}$ $T_{aw,}$ T_{dig}, and T_{ASR} rather than estimated marginal means from the analysis of covariance (ANCOVA), as acclimation time was not significant for any traits. We used Pearson correlation to test for a relationship between CT_{max} and T_{ag} and relationships between T_{ASR} and both CT_{max} and T_{ag}. Correlations were run across individuals within each species.

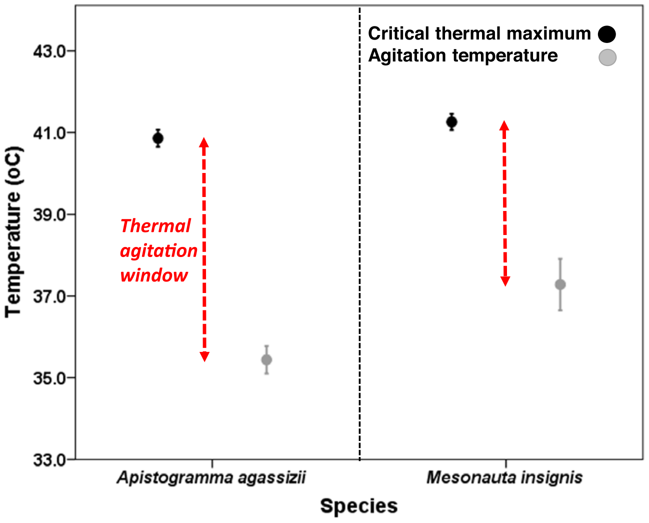

FIGURE 1 Mean critical thermal maximum (CT_{max}, °C, ± SE) and thermal agitation temperature (T_{ag}, °C) for two species of Amazonian cichlids *Apistogramma agassizii* and *Mesonauta insignis*. The difference between the CT_{max} and T_{ag} represents the thermal agitation window. CT_{max}, critical thermal maximum; T_{ag}, thermal agitation temperature [Color figure can be viewed at wileyonlinelibrary.com]

We calculated mean duration (sec/min) for each of the four behaviors (sheltering, activity, digging, and ASR) for two periods: before the water temperature at which T_{ag} was reached and subsequent to the T_{ag}. Because the behavioral data did not meet assumptions for a parametric approach, we tested for differences in the duration of the four behavioral metrics before and after T_{ag} using the nonparametric Wilcoxon signed rank test. This was done for each species separately. The nonparametric Mann–Whitney U test was used to test for differences between species in behavioral metrics before and after T_{ag}. All statistical analyses for were performed using SPSS version 24.

3 | RESULTS

$\mathrm{CT}_{\mathrm{max}}$ averaged 40.9 ± 0.2°C (SE) for *A. agassizii* and 41.3 ± 0.2°C for *M. insignis*. There was no difference between the two cichlid species in $\mathrm{CT}_{\mathrm{max}}$, and no significant effect of acclimation time (Tables 1 and 3). For both species, T_{ag} was significantly lower than $\mathrm{CT}_{\mathrm{max}}$ (paired *t* tests, *A. agassizii*, $t = 13.85$, $p < 0.001$; *M. insignis*, $t = 6.60$, $p < 0.001$, Figure 1). There was no effect of acclimation time on T_{ag}, but T_{ag} did differ between species averaging 35.4 ± 0.3°C (SE) for *A. agassizii* and 37.3 ± 0.6°C for *M. insignis* (Tables 1 and 3). Thus, *A. agassizii* started to exhibit avoidance behavior at a lower temperature than *M. insignis* (Figure 1). The thermal agitation window (difference between $\mathrm{CT}_{\mathrm{max}}$ and T_{ag}) for *A. agassizii* averaged 5.4 ± 0.3°C and 4.0 ± 0.6°C for *M. insignis* (Figure 1). There was no effect of acclimation time on the thermal agitation window, and a marginally higher window ($p = 0.070$) in *A. agassizii* than in *M. insignis* Figure 1, Tables 1 and 3) There was no effect of acclimation time on the initiation of digging behavior (T_{dig}) or ASR (T_{ASR}) (Table 3). Digging was initiated at a lower temperature in *A. agassizii* (31.5 ± 1.0°C) than *M. insignis* (38.0 ± 0.9°C), but there was no difference between species in the temperature at which they initiated ASR (Tables 1 and 3). There was no significant relationship between $\mathrm{CT}_{\mathrm{max}}$ and T_{ag} for either *A. agassizii* ($r = 0.028$, $p = 0.938$) or *M. insignis* ($r = 0.293$, $p = 0.383$). The temperature at which ASR was initiated was not related to $\mathrm{CT}_{\mathrm{max}}$ ($r = 0.045$, $p = 0.924$) in *A. agassizii*, but T_{ASR} was strongly related to T_{ag} ($r = 0.868$, $p = 0.011$). There was no relationship between T_{ASR} and either $\mathrm{CT}_{\mathrm{max}}$ ($r = -0.020$, $p = 0.954$) or T_{ag} ($r = 0.035$, $p = 0.918$) in *M. insignis*.

TABLE 3 Results of ANCOVA testing of effects of species and acclimation time (covariate) on thermal tolerance metrics for two Amazonian cichlids, *Apistogramma agassizii* and *Mesonauta insignis*

Thermal tolerance metric	Factor	*F*	*df*	*p* value
Critical thermal maximum	Species	2.266	1,18	0.150
	Acclimation time	0.892	1,18	0.357
Agitation temperature	Species	6.361	1,18	0.021
	Acclimation time	0.348	1,18	0.562
Thermal agitation window	Species	3.697	1,18	0.070
	Acclimation time	0.049	1,18	0.827
Digging initation temperature	Species	19.944	1,14	0.001
	Acclimation time	0.247	1,14	0.627
ASR initiation temperature	Species	0.476	1,15	0.501
	Acclimation time	0.061	1,15	0.808

Note: The interaction between species and acclimation time was not significant for all metrics and was not included in the final models.

Abbreviation: ANCOVA, analysis of covariance.

In both *M. insignis* and *A. agassizii*, shelter use decreased, and both swimming activity and digging behavior increased after T_{ag} (Figure 2a,b). ASR behavior was extremely rare before fish reached T_{ag}, but increased significantly after T_{ag} as fish approached $\mathrm{CT}_{\mathrm{max}}$ (Figure 2a,b). Digging was higher in *A. agassizii* than in *M. insignis* before T_{ag} ($p = 0.001$), but there was no difference between species in the duration of sheltering ($p = 0.085$), activity ($p = 0.426$), or ASR ($p = 0.468$). After T_{ag}, there was no difference between species in the duration of sheltering behavior ($p = 0.197$). *Apistogramma agassizii* showed a higher duration of digging ($p < 0.001$) and activity ($p = 0.001$) than *M. insignus*, but a lower duration of ASR ($p < 0.001$,

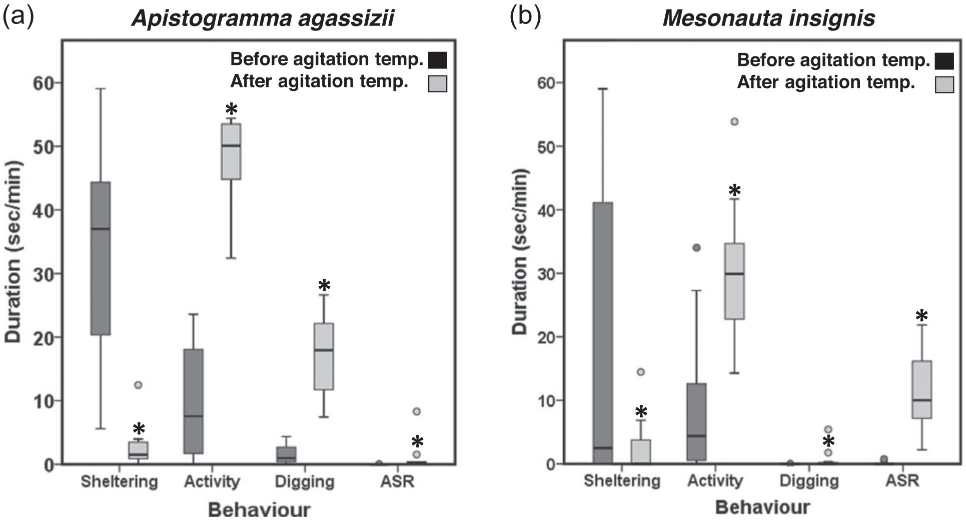

FIGURE 2 Box and whisker plots (median, quartiles, range, and outliers) for four behaviors of (a) *Apistogramma agassizii* and (b) *Mesonauta insignis* quantified during $\mathrm{CT}_{\mathrm{max}}$ trials. Fish can perform more than one behavioral category at the same time (e.g., swimming and digging); and in this case, both behaviors were recorded. *a significant difference ($p < 0.05$) between the behavioral metrics before and after the estimated thermal agitation temperature.$\mathrm{CT}_{\mathrm{max}}$, critical thermal maximum

Figure 2a,b). Fish sometimes performed more than one type of behavior at the same time, in particular digging and swimming, and ASR and swimming.

4 | DISCUSSION

Both *A. agassizii* and *M. insignis* exhibited high critical thermal maxima, 40.8°C and 41.3°C, respectively. There was no significant difference between the two species, and the CT_{max} of *A. agassizii* was very close to the CT_{max} of 40°C measured for *A. agassizii* in a previous study (Campos et al., 2019). In our study, fish were held at a temperature close to the average temperature of their natural environment, and their CT_{max} falls within the range reported for other equatorial fishes acclimated to temperatures reflecting their natural environment. For example, *Apistogramma hyppolitae* acclimated to 25°C exhibited a CT_{max} of 38.6°C (Campos et al., 2018). Lapointe et al. (2018) measured CT_{max} for six species of equatorial fishes from three locations (the characids *Brycon amazonicus* and *Colossoma macropomum* from Brazil; the cichlid *Oreochromis niloticus* and the cyprinid *Labeo victorianus* from Uganda, and the cyprinids *Barbonymus gonionotus* and *Labeo pierrei* from Cambodia). CT_{max} measured at the average maximum temperature encountered in the natural habitat of the six species ranged from ~39.2°C for *L. victorianus* to ~42.2°C for *C. macropomum*. In a recent review of thermal tolerance in South American fish Campos et al. (2021) summarized data for CT_{max} in 106 species; for those species from the Amazon acclimated to their average habitat temperature, CT_{max} averaged 38.6°C and ranged from 30.9 to 42.8°C (data extracted from aggregated data set). Nyboer and Chapman quantified the CT_{max} of Nile perch (*Lates niloticus*) held for 3 weeks to the average maximum water temperature of the home environment (Lake Victoria) and reported a CT_{max} of ~39.1°C. In the current study, the acclimation temperature was 1.2°C cooler for *A. agassizii* than for *M. insignis*. It is possible that CT_{max} and the agitation temperature would have been even more similar if we had acclimated the two species to the same temperature; however, we wanted to consider the natural conditions of these species in this study. We found no effects of acclimation time on CT_{max}, T_{ag}, or the thermal agitation window. However, a next step would be to calculate the T_{max} and T_{ag} for the two species acclimated to a range of temperatures to see if their acclimation capacity differs.

Species that have a CT_{max} well above environmental temperatures (a high thermal safety margin) may be relatively well buffered from the negative effects of warming water (Catullo et al., 2015). However, in recent review, Payne et al. (2021) showed that estimates of physiological heating tolerance estimated as the difference between CT_{max} and acclimation temperature in fishes are two to three times higher than population heating tolerance estimated as the difference between the temperature at which the abundance of a fish species is greatest and the temperature at the warm limit of its distribution. This is consistent with the idea that fish do not live in habitats with temperatures close to their CT_{max}. Ectotherms may have strategies for avoiding the physiological damage that occurs close to their maxima thermal tolerance, including searching for local thermal refugia, or on a larger scale, shifting their distribution to cooler regions. Avoidance behavior is also a common response of ectotherms to high temperature, which may represent an effort to seek thermal refugia (e.g., Barker et al., 2018; Christensen et al. 2021; Enders et al. 2019). We interpret agitation temperature (T_{ag}) as the temperature at which fish start to seek alternative habitat, and it is a threshold that is often several degrees lower than the CT_{max}. Agitation temperature was significantly higher in *M. insignis* (37.3°C) than in *A. agassizii* (35.4°C), indicating that *A. agassizii* has a lower temperature threshold at which avoidance behavior is initiated. In addition, there was a trend toward a greater thermal agitation window, estimated as the difference between CT_{max} and T_{ag}, in *A. agassizii* (5.4°C) than in *M. insignis* (4.0°C), suggesting that *A. agassizii* may be agitated for a longer period before LOE occurs. Together, these findings suggest that *A. agassizii* may be more sensitive than *M. insignis* to elevated water temperature, which may have important ecological consequences such as exposure to predation if avoidance behavior takes fish away from shelter. One might expect a positive relationship between CT_{max} and T_{ag} if both reflect thermal tolerance. We did not detect a significant relationship in either species, suggesting that T_{ag} may not reflect the same underlying physiological mechanisms as CT_{max}. Interestingly, CT_{max} in both species showed lower variation (range = 39.9°C–41.8°C for *A. agassizii*; 40.1°C–42.1°C for *M. insignis*) than T_{ag} (range = 33.9–37.5°C for *A. agasszii*; 33.8°C–40.3°C for *M. insignis*). A similar trend was observed in the lionfish (*Pterois* sp), where variation in thermal tolerance estimates was smaller than metrics of avoidance and preference behaviors (Barker et al., 2018).

After species reached $T_{ag,}$ we observed a clear change in a suite of behaviors, some of which differed between species. Activity level increased and use of shelter decreased with increased temperatures, and patterns were similar between the two species. The agitation temperature that we estimated as a threshold point reflects changes in activity and shelter use, both of which likely reflect a search for cooler temperatures. An increase in activity can affect social interaction of fish, especially aggressive interactions. Although not measured in the context of CT_{max} trials, other studies of cichlids have noted increases in both activity and aggression in response to elevated water temperatures. For the cichlid fish *Cichlasoma paranaense*, an increase in activity was observed at a temperature 6°C lower than CT_{max}, but the increase in activity did not increase aggressive behavior (Brandão et al., 2018). However, aggressive behavior increased in higher temperature and higher availability of shelters in the cichlid *A. agassizii* (Kochhann et al., 2015; Kochhann & Val, 2017). In the African cichlid *Pseudocrenilabrus multicolor*, fish reared under hot conditions showed increased activity and aggression relative to full sibs reared under cool conditions (McDonnell et al., 2019). Increased activity and decreased use of shelter may also have important ecological implications, potentially increasing the risk of predation or taking fish away from suitable foraging habitat. It should be pointed out that the increase in activity and the decrease in shelter use were observed in temperatures at least ~4°C below CT_{max}, so exposures to temperatures that clearly evoke an avoidance behavior can affect social interactions much before fish reach a temperature critical to survival.

Digging behavior increased after T_{ag} in both species, but was significantly higher in *A. agassazii* before and after T_{ag}. This behavior is routinely observed in the *A. agassizii* during feeding, as *Apistogramma* species tend to feed on debris that accumulate in dead leaves and other materials on the substrate (Oliveira et al., 2017; Rodrigues et al., 2012). *Apistogramma agassizii* also uses dead leaves as shelter (personal observation, DK). The lower duration of digging behavior in *M. insignis* may relate to its use of floating vegetation and weaker relationship with the bottom substrate in the field (Hercos et al., 2009; Santos et al., 2009). The increase in digging behavior after T_{ag}, which was particularly notable in *A. agassizii*, may reflect an attempt to seek lower temperature that might be expected under leaves on the bottom of streams and littoral areas. Digging behavior as a thermoregulatory strategy has also been reported in the flatfish (*Pseudopleuronectes americanus*) (Ziegler & Frisk, 2019) and highlights the importance of exploring behaviors that may facilitate access to thermal refugia in nature.

ASR (ventilating water at the surface film) was extremely rare before T_{ag}, but increased in both cichlid species after T_{ag} and was higher in *M. insignis* than in *A. agassizii*. ASR is a common and widespread adaptation of fishes in response to hypoxia (Chapman & Mckenzie, 2009; Kramer & McClure, 1982). During our CT_{max} trials, DO levels were maintained at relatively high levels, averaging 87%, with the lowest DO level measured at 74% air saturation. However, it is possible that at temperatures approaching CT_{max}, fish were unable to maintain sufficient oxygen supply to their tissues. This may have been exacerbated by metabolic costs associated with increased activity and digging behavior. Potential links between upper thermal limits and hypoxia in ectotherms have often been explored in the context of the oxygen- and capacity-limited thermal tolerance (OCLTT) framework (Pörtner, 2010; Pörtner & Farrell, 2008). OCLTT predicts that, in response to increasing water temperature, the maximum rate of oxygen consumption increases and exceeds the capacity of the cardiorespiratory system to supply oxygen to tissues, which results in a decline in aerobic scope (AS - difference between maximum and standard metabolic rate) and determines CT_{max} (Ern et al., 2016). The predicted relationship between oxygen supply capacity and upper thermal limits in fishes has received mixed support in the literature. Some studies have found little or no effect of DO on CT_{max} until fish are exposed to severe hypoxia (near the critical oxygen tension, P_{crit}) (Brijs et al., 2015; Ern et al., 2016), while other studies have reported a positive relationship between hypoxia tolerance and upper thermal tolerance (Chen et al., 2013). A recent study with 37 Amazonian fish species found a positive relationship between hypoxia and temperature tolerance, supporting the hypothesis that upper thermal limits are associated with the ability to supply oxygen to tissues (Jung et al., 2020). In our study, both species initiated ASR after the T_{ag}, a sign of thermal stress. Our results suggest that fish were experiencing physiological hypoxia at water temperatures approaching CT_{max} that led to the initiation ASR to increase oxygen delivery to the tissues. King and Sardella (2017) observed ASR during CT_{max} trials in the Mozambique tilapia (*Oreochromis mossambicus*), and they also reported ventilation cessation behavior, which we did not observe in *M. insignis* or *A. agassizii*. King & Sardella attributed the use of ASR in the Mozambique tilapia to increased metabolic demands on the fish during acute thermal increase and hypoxemia that would occur with ventilation cessation behavior. Jung et al. (2020) did not find a difference between CT_{max} of *Apistogramma borelli* in normoxia and hyperoxia; however, future experiments that measure CT_{max} across a range of DO in *M. insignis* and *A. agassizii* will be important in determining the link between environmental hypoxia and thermal tolerance in these species. Although ASR has been reported in many tropical fishes (Chapman et al., 2002; Chapman & Mckenzie, 2009; Kramer & McClure, 1982; Reid et al., 2013), this is the first report of ASR in *A. agassizii* and *M. insignis* and the second report of ASR during CT_{max} trials under normoxic conditions. The greater use of ASR in *M. insignis* than in *A. agassizii* may reflect differences in their ecology. The substrate-oriented behavior of *A. agassizii* may require increase travel time to the surface for ASR and potentially greater risk associated with leaving the cover of bottom structure (e.g., leaves).

The results of this study highlight the importance of measuring behavioral metrics during CT_{max} trials to improve our understanding of thresholds that may have important ecological consequences. Based on agitation temperature and the thermal agitation window, *A. agassizii* can be considered more sensitive than *M. insignis* to increasing temperature. An important next step is to examine effects of acclimation temperature and exposure time (acute, acclimation, developmental, multigenerational) on behavioral traits, particularly those that may have high metabolic costs and may influence ecological interactions (e.g., predation risk).

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

Data are fully available upon request (contact: Daiani Kochhann, daia.kochhann@gmail.com)

ORCID

Daiani Kochhann http://orcid.org/0000-0003-1665-9988
Adalberto L. Val http://orcid.org/0000-0002-3823-3868
Lauren J. Chapman http://orcid.org/0000-0002-2802-5889

REFERENCES

Angilletta, M. J., Cooper, B. S., Schuler, M. S., & Boyles, J. G. (2002). The evolution of thermal physiology in endotherms. *Frontiers in Bioscience-Elite*, *2 E*(3), 861–881. https://doi.org/10.2741/e148

Arthington, A. H., Dulvy, N. K., Gladstone, W., & Winfield, I. J. (2016). Fish conservation in freshwater and marine realms: Status, threats and management. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *26*(5), 838–857. https://doi.org/10.1002/aqc.2712

Åsheim, E. R., Andreassen, A. H., Morgan, R., & Jutfelt, F. (2020). Rapid-warming tolerance correlates with tolerance to slow warming but not growth at non-optimal temperatures in zebrafish. *The Journal of Experimental Biology*, *223*, 1–23. https://doi.org/10.1242/jeb.229195

Barker, B. D., Horodysky, A. Z., & Kerstetter, D. W. (2018). Hot or not? Comparative behavioral thermoregulation, critical temperature regimes, and thermal tolerances of the invasive lionfish *Pterois* sp. versus native western North Atlantic reef fishes. *Biological Invasions*, *20*(1), 45–58. https://doi.org/10.1007/s10530-017-1511-4

Becker, C. D., & Genoway, R. G. (1979). Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Environmental Biology of Fishes*, *4*(3), 245–256. https://doi.org/10.1007/BF00005481

Biro, P. A., Beckmann, C., & Stamps, J. A. (2010). Small within-day increases in temperature affects boldness and alters personality in coral reef fish. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1678), 71–77. https://doi.org/10.1098/rspb.2009.1346

Brandão, M. L., Colognesi, G., Bolognesi, M. C., Costa-Ferreira, R. S., Carvalho, T. B., & Gonçalves-de-Freitas, E. (2018). Water temperature affects aggressive interactions in a Neotropical cichlid fish. *Neotropical Ichthyology*, *16*(1), 1–8. https://doi.org/10.1590/1982-0224-20170081

Brauner, C., & Richards, J. G. (2020). Physiological performance in aquaculture: Using physiology to help define optimal conditions for growth and environmental tolerance. In T. J. Benfey, A. P. Farrell, & C. J. Brauner (Eds.), Aquaculture *(Fish Physi)*, (pp. 83–120). Elsevier Inc.

Brijs, J., Jutfelt, F., Clark, T. D., Gräns, A., Ekström, A., & Sandblom, E. (2015). Experimental manipulations of tissue oxygen supply do not affect warming tolerance of European perch. *Journal of Experimental Biology*, *218*(15), 2448–2454. https://doi.org/10.1242/jeb.121889

Campos, D. F., Val, A. L., & Almeida-Val, V. M. F. (2018). The influence of lifestyle and swimming behavior on metabolic rate and thermal tolerance of twelve Amazon forest stream fish species. *Journal of Thermal Biology*, *72*(June 2017), 148–154. https://doi.org/10.1016/j.jtherbio.2018.02.002

Campos, D. F., Braz-Mota, S., Val, A. L., & Almeida-Val, V. M. F. (2019). Predicting thermal sensitivity of three Amazon fishes exposed to climate change scenarios. *Ecological Indicators*, *101*, 533–540. https://doi.org/10.1016/j.ecolind.2019.01.051

Campos, D. F., Val, A. L., & Val, V. M. F. A. (2021). Climate vulnerability of South American freshwater fish: Thermal tolerance and acclimation. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology. Published online March 10, 2021*. 1–12. https://doi.org/10.1002/jez.2452

Carvalho, M. S., Zuanon, J., & Ferreira, E. J. G. (2014). Diving in the sand: The natural history of *Pygidianops amphioxus* (Siluriformes: Trichomycteridae), a miniature catfish of Central Amazonian streams in Brazil. *Environmental Biology of Fishes*, *97*(1), 59–68. https://doi.org/10.1007/s10641-013-0123-9

Catullo, R. A., Ferrier, S., & Hoffmann, A. A. (2015). Extending spatial modelling of climate change responses beyond the realized niche: Estimating, and accommodating, physiological limits and adaptive evolution. *Global Ecology and Biogeography*, *24*(10), 1192–1202. https://doi.org/10.1111/geb.12344

Chapman, L. J., Chapman, C. A., Nordlie, F. G., & Rosenberger, A. E. (2002). Physiological refugia: Swamps, hypoxia tolerance and maintenance of fish diversity in the Lake Victoria region. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, *133*(3), 421–437. https://doi.org/10.1016/S1095-6433(02)00195-2

Chapman, L. J., & Mckenzie, D. J. (2009). Behavioral responses and ecological consequences. In J. G. Richards, A. P. Farrell, & C. J. Brauner (Eds.), *Hypoxia - Fish Physiology Series* (27, Issue C, pp. 25–77). Academic Press. https://doi.org/10.1016/S1546-5098(08)00002-2

Chen, Z., Anttila, K., Wu, J., Whitney, C. K., Hinch, S. G., & Farrell, A. P. (2013). Optimum and maximum temperatures of sockeye salmon (*Oncorhynchus nerka*) populations hatched at different temperatures. *Canadian Journal of Zoology*, *91*(5), 265–274. https://doi.org/10.1139/cjz-2012-0300

Chrétien, E., & Chapman, L. J. (2016). Tropical fish in a warming world: Thermal tolerance of Nile perch *Lates niloticus* (L.) in Lake Nabugabo, Uganda. *Conservation Physiology*, *4*(1), 1–14. https://doi.org/10.1093/conphys/cow062

Christensen, E. A. F., Norin, T., Tabak, I., Van Deurs, M. & Behrens, J. W. (2021). Effects of temperature on physiological performance and behavioral thermoregulation in an invasive fish, the round goby. *The Journal of Experimental Biology*, *224*(1). https://doi.org/10.1242/jeb.237669

Culumber, Z. W. (2020). Thermal stress increases activity and risk-taking behavior but not anxiety in a livebearing fish. *Environmental Biology of Fishes*, *103*(4), 313–317. https://doi.org/10.1007/s10641-020-00966-9

Del Rio, A. M., Davis, B. E., Fangue, N. A., & Todgham, A. E. (2019). Combined effects of warming and hypoxia on early life stage Chinook salmon physiology and development. *Conservation Physiology*, *7*(1), 1–14. https://doi.org/10.1093/conphys/coy078

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., Naiman, R. J., Prieur-Richard, A. H., Soto, D., Stiassny, M. L. J., & Sullivan, C. A. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews of the Cambridge Philosophical Society*, *81*(2), 163–182. https://doi.org/10.1017/S1464793105006950

Enders, E. C., Wall, A. J., & Svendsen, J. C. (2019). Hypoxia but not shy-bold phenotype mediates thermal preferences in a threatened freshwater fish, Notropis percobromus. *Journal of Thermal Biology*, *84*, 479–487. https://doi.org/10.1016/j.jtherbio.2019.08.001

Ern, R., Norin, T., Gamperl, A. K., & Esbaugh, A. J. (2016). Oxygen dependence of upper thermal limits in fishes. *Journal of Experimental Biology*, *219*(21), 3376–3383. https://doi.org/10.1242/jeb.143495

Ficke, A. D., Myrick, C. A., & Hansen, L. J. (2007). Potential impacts of global climate change on freshwater fisheries. *Reviews in Fish Biology and Fisheries*, *17*, 581–613.

Friedrich, J., Janssen, F., Aleynik, D., Bange, H. W., Boltacheva, N., Çagatay, M. N., Dale, A. W., Etiope, G., Erdem, Z., Geraga, M., Gilli, A., Gomoiu, M. T., Hall, P. O. J., Hansson, D., He, Y., Holtappels, M., Kirf, M. K., Kononets, M., Konovalov, S., … Wenzhöfer F. (2014). Investigating hypoxia in aquatic environments: Diverse approaches to addressing a complex phenomenon. *Biogeosciences*, *11*(4), 1215–1259. https://doi.org/10.5194/bg-11-1215-2014

Heino, J., Virkkala, R., & Toivonen, H. (2009). Climate change and freshwater biodiversity: Detected patterns, future trends and adaptations in northern regions. *Biological Reviews*, *84*(1), 39–54. https://doi.org/10.1111/j.1469-185X.2008.00060.x

Hercos, A. P., Queiroz, H. L. de & Almeida, H. L. de (2009). Peixes Ornamentais do Amanã. Instituto de Desenvolvimento Sustentável Mamirauá.

Hercos, A. P., Sobansky, M., Queiroz, H. L., & Magurran, A. E. (2013). Local and regional rarity in a diverse tropical fish assemblage. *Proceedings of the Royal Society B: Biological Sciences*, *280* (1751). https://doi.org/10.1098/rspb.2012.2076

Hermoso, V. (2017). Freshwater ecosystems could become the biggest losers of the Paris Agreement. *Global Change Biology*, *23*(9), 3433–3436. https://doi.org/10.1111/gcb.13655

Huey, R. B., & Stevenson, R. D. (1979). Integrating thermal physiology and ecology of ectotherms: A discussion of approaches. *Integrative and Comparative Biology*, *19*(1), 357–366. https://doi.org/10.1093/icb/19.1.357

Janzen, D. H. (1967). Why mountain passes are higher in the tropics. *The American Naturalist*, *101*(919), 233–249. https://doi.org/10.1086/282487

Jung, E. H., Brix, K. V., Richards, J. G., Val, A. L., & Brauner, C. J. (2020). Reduced hypoxia tolerance and survival at elevated temperatures may limit the ability of Amazonian fishes to survive in a warming world. *Science of the Total Environment*, *748*, 141349. https://doi.org/10.1016/j.scitotenv.2020.141349

King, M., & Sardella, B. (2017). The effects of acclimation temperature, salinity, and behavior on the thermal tolerance of Mozambique tilapia (*Oreochromis mossambicus*). *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *327*(7), 417–422. https://doi.org/10.1002/jez.2113

Knouft, J. H., & Ficklin, D. L. (2017). The potential impacts of climate change on biodiversity in flowing freshwater systems. *Annual Review of Ecology, Evolution, and Systematics*, *48*(July), 111–133. https://doi.org/10.1146/annurev-ecolsys-110316-022803

Kochhann, D., Campos, D. F., & Val, A. L. (2015). Experimentally increased temperature and hypoxia affect stability of social hierarchy and metabolism of the Amazonian cichlid *Apistogramma agassizii*. *Comparative Biochemistry and Physiology -Part A: Molecular and Integrative Physiology*, *190*, 54–60. https://doi.org/10.1016/j.cbpa.2015.09.006

Kochhann, D., & Val, A. L. (2017). Social hierarchy and resting metabolic rate in the dwarf cichlid *Apistogramma agassizii*: The role of habitat enrichment. *Hydrobiologia*, *789*, 123-131(1). https://doi.org/10.1007/s10750-016-2806-7

Kramer, D. L., & McClure, M. (1982). Aquatic surface respiration, a widespread adaptation to hypoxia in tropical freshwater fish. *Environmental Biology of Fishes*, *7*(1), 47–55.

Kullander, S. O. (2003). Family Cichlidae. In C. J. Ferraris, S. O. Kullander, & R. E. Reis (Eds.), *Check list of the freshwater fishes of South and Central America* (pp. 605–654). EdiPUCRS.

Lapointe, D., Cooperman, M. S., Chapman, L. J., Clark, T. D., Val, A. L., Ferreira, M. S., Balirwa, J. S., Mbabazi, D., Mwanja, M., Chhom, L., Hannah, L., Kaufman, L., Farrell, A. P., & Cooke, S. J. (2018). Predicted impacts of climate warming on aerobic performance and upper thermal tolerance of six tropical freshwater fishes spanning three continents. *Conservation Physiology*, *6*(1), 1–19. https://doi.org/10.1093/conphys/coy056

Lutterschmidt, W. I., & Hutchison, V. H. (1997). The critical thermal maximum: History and critique. *Canadian Journal of Zoology*, *75*(10), 1561–1574. https://doi.org/10.1139/z97-783

Magozzi, S., & Calosi, P. (2015). Integrating metabolic performance, thermal tolerance, and plasticity enables for more accurate predictions on species vulnerability to acute and chronic effects of global warming. *Global Change Biology*, *21*(1), 181–194. https://doi.org/10.1111/gcb.12695

McDonnell, L. H., & Chapman, L. J. (2015). At the edge of the thermal window: Effects of elevated temperature on the resting metabolism, hypoxia tolerance and upper critical thermal limit of a widespread African cichlid. *Conservation Physiology*, *3*(1), 1–13. https://doi.org/10.1093/conphys/cov050

McDonnell, L. H., Reemeyer, J. E., & Chapman, L. J. (2019). Independent and interactive effects of long-term exposure to hypoxia and elevated water temperature on behavior and thermal tolerance of an equatorial cichlid. *Physiological and Biochemical Zoology*, *92*(3), 253–265. https://doi.org/10.1086/702712

Morgan, R., Finnøen, M. H., & Jutfelt, F. (2018). CTmax is repeatable and doesn't reduce growth in zebrafish. *Scientific Reports*, *8*(1), 1–8. https://doi.org/10.1038/s41598-018-25593-4

Morley, S. A., Peck, L. S., Sunday, J. M., Heiser, S., & Bates, A. E. (2019). Physiological acclimation and persistence of ectothermic species under extreme heat events. *Global Ecology and Biogeography*, *28*(7), 1018–1037. https://doi.org/10.1111/geb.12911

Myers, B. J. E., Lynch, A. J., Bunnell, D. B., Chu, C., Falke, J. A., Kovach, R. P., Krabbenhoft, T. J., Kwak, T. J., & Paukert, C. P. (2017). Global synthesis of the documented and projected effects of climate change on inland fishes. *Reviews in Fish Biology and Fisheries*, *27*(2), 339–361. https://doi.org/10.1007/s11160-017-9476-z

Oliveira, J., Amadio, S., & Queiroz, H. (2017). Populacional structure of *Apistogramma agassizii* (Steindachner, 1875) (Perciformes: Cichlidae) in aquatic environments of the Amana Sustainable Development Reserve (Amazonas - Brazil). *Acta of Fisheries and Aquatic Resources*, *05*(03), 61–67. https://doi.org/10.46732/actafish.2017.5.3.61-67

Payne, N. L., Morley, S. A., Halsey, L. G., Smith, J. A., Stuart-Smith, R., Waldock, C., & Bates, A. E. (2021). Fish heating tolerance scales similarly across individual physiology and populations. *Communications Biology*, *4*(1), 1–5. https://doi.org/10.1038/s42003-021-01773-3

Pörtner, H. O. (2010). Oxygen- And capacity-limitation of thermal tolerance: A matrix for integrating climate-related stressor effects in marine ecosystems. *Journal of Experimental Biology*, *213*(6), 881–893. https://doi.org/10.1242/jeb.037523

Pörtner, H. O., & Farrell, A. P. (2008). Physiology and climate change. *Science*, *322*(5902), 690–692. https://doi.org/10.1038/050458a0

Potts, L. (2020). *Hot and already bothered: Exploring the effects of elevated water temperature on an imperiled freshwater fish, Pugnose Shiner (Notropis anogenus)*. McGill University.

Quigley, J. T., & Hinch, S. G. (2006). Effects of rapid experimental temperature increases on acute physiological stress and behaviour of stream dwelling juvenile chinook salmon. *Journal of Thermal Biology*, *31*(5), 429–441. https://doi.org/10.1016/j.jtherbio.2006.02.003

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., Kidd, K. A., MacCormack, T. J., Olden, J. D., Ormerod, S. J., Smol, J. P., Taylor, W. W., Tockner, K., Vermaire, J. C., Dudgeon, D., & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, *94*(3), 849–873. https://doi.org/10.1111/brv.12480

Reid, A. J., Farrell, M. J., Luke, M. N., & Chapman, L. J. (2013). Implications of hypoxia tolerance for wetland refugia use in Lake Nabugabo, Uganda. *Ecology of Freshwater Fish*, *22*(3), 421–429. https://doi.org/10.1111/eff.12036

Reis, R. E., Albert, J. S., Di Dario, F., Mincarone, M. M., Petry, P., & Rocha, L. A. (2016). Fish biodiversity and conservation in South America. *Journal of Fish Biology*, *89*(1), 12–47. https://doi.org/10.1111/jfb.13016

Rodrigues, R. R., Zuanon, J., Del-Claro, K., & Carvalho, L. N. (2012). Reproductive behavior of the Amazonian dwarf cichlid *Apistogramma hippolytae* Kullander, 1982: Offsetting costs and benefits. *Acta Ethologica*, *15*(1), 47–53. https://doi.org/10.1007/s10211-011-0107-8

Santos, G. M. dos, Ferreira, E. J. G., & Zuanon, J. A. S. (2009). *Peixes comerciais de Manaus*. Editora INPA.

Schulte, P. M., Healy, T. M., & Fangue, N. A. (2011). Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. *Integrative and Comparative Biology*, *51*(5), 691–702. https://doi.org/10.1093/icb/icr097

Stillman, J. H. (2003). Acclimation capacity underlies susceptibility to climate change. *Science*, *301*(5629), 65. https://doi.org/10.1126/science.1083073

Strayer, D. L., & Dudgeon, D. (2010). Freshwater biodiversity conservation: Recent progress and future challenges. *Journal of the North American Benthological Society*, *29*(1), 344–358. https://doi.org/10.1899/08-171.1

Sunday, J. M., Bates, A. E., & Dulvy, N. K. (2012). Thermal tolerance and the global redistribution of animals. *Nature Climate Change*, *2*(9), 686–690. https://doi.org/10.1038/nclimate1539

Tewksbury, J. J., Huey, R. B., & Deutsch, C. A. (2008). Ecology: Putting the heat on tropical animals. *Science*, *320*(5881), 1296–1297. https://doi.org/10.1126/science.1159328

Turko, A. J., Nolan, C. B., Balshine, S., Scott, G. R., & Pitcher, T. E. (2020). Thermal tolerance depends on season, age and body condition in imperilled redside dace *Clinostomus elongatus*. *Conservation Physiology*, *8*(1), 1–15. https://doi.org/10.1093/conphys/coaa062

Urban, M. C. (2015). Accelerating extinction risk from climate change. *Science*, *348*(6234), 571–573.

Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S. E., Sullivan, C. A., Liermann, C. R., & Davies, P. M. (2010). Global threats to human water security and river biodiversity. *Nature*, *467*(7315), 555–561. https://doi.org/10.1038/nature09440

Wells, Z. R. R., McDonnell, L. H., Chapman, L. J., & Fraser, D. J. (2016). Limited variability in upper thermal tolerance among pure and hybrid populations of a cold-water fish. *Conservation Physiology*, *4*(1), 1–13. https://doi.org/10.1093/conphys/cow063

Zhou, L. Y., Fu, S. J., Fu, C., Ling, H., & Li, X. M. (2019). Effects of acclimation temperature on the thermal tolerance, hypoxia tolerance and swimming performance of two endangered fish species in China. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, *189*(2), 237–247. https://doi.org/10.1007/s00360-018-01201-9

Ziegler, C. M., & Frisk, M. G. (2019). Flatfish utilize sediment blanket to facilitate thermoregulation. *Marine Ecology Progress Series*, *609*, 179–186. https://doi.org/10.3354/meps12817

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