Science of the Total Environment 748 (2020) 141349

Contents lists available at ScienceDirect

Science of the Total Environment

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

Reduced hypoxia tolerance and survival at elevated temperatures may limit the ability of Amazonian fishes to survive in a warming world

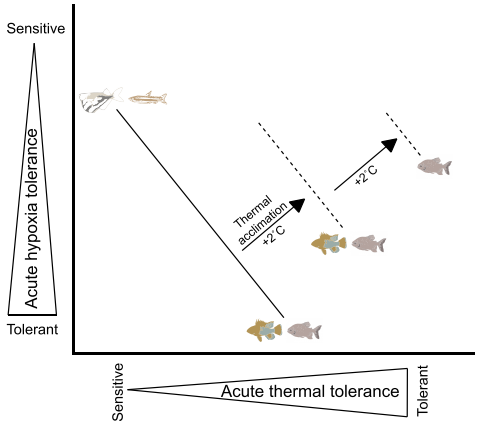
Ellen H. Jung [a,*], Kevin V. Brix [b], Jeffrey G. Richards [a], Adalberto L. Val [c], Colin J. Brauner [a,*]

[a] Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada
[b] EcoTox, Miami, FL, United States
[c] Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil

HIGHLIGHTS

- Correlation between thermal and hypoxia tolerance in 37 Amazonian fishes
- CTMax increases moderately with long-term exposure to higher temperatures.
- Hypoxia tolerance decreases with acute- and long-term thermal exposures.
- High morbidity following long-term exposure to temperatures above current levels

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 May 2020
Received in revised form 10 July 2020
Accepted 27 July 2020
Available online 2 August 2020

Editor: Daniel Wunderlin

Keywords:
Amazon
Thermal tolerance
Hypoxia tolerance
Heat waves
Climate change
Biodiversity

ABSTRACT

The Amazon basin contains more than 20% of the world's freshwater fishes, many of ecological and economical importance. An increase in temperature of 2.2 to 7 °C is predicted to occur within the next century in the worst-case scenario of climate change predictions, which will likely be associated with an increase in the prevalence and duration of reduced water oxygen levels (hypoxia). Furthermore, there is an increasing frequency of heat waves in the Amazon basin, which exacerbates issues related to temperature and hypoxia. Increases in temperature and hypoxia both constrain an organism's ability to supply oxygen to metabolizing tissues, thus the ability to cope with thermal and hypoxic stress may be correlated. Here, we reveal a positive correlation between acute thermal tolerance and acute hypoxia tolerance amongst 37 Amazonian fish species at the current river temperatures of 28–31 °C. The effects of long-term (10 days or 4 weeks) increases in temperature were investigated in a subset of 13 species and demonstrated that 2 species failed to acclimate and survive at 33 °C, 9 species failed at 35 °C, and only 2 species survived up to 35 °C. Of those that survived long-term exposure to 33 or 35 °C, the majority of the species demonstrated only an improvement in acute thermal tolerance. In contrast, hypoxia tolerance was reduced following acute- and long-term exposure to 33, 35 or 37 °C in all species investigated. The results of this study suggest that many of the fish species that inhabit the Amazon may be at risk during both short- and long-term temperature increases and these risks are exacerbated by the associated environmental hypoxia.

* Corresponding authors at: Department of Zoology, University of British Columbia, 6270 University Boulevard, Vancouver, British Columbia V6T 1Z4, Canada.
E-mail addresses: jung@zoology.ubc.ca (E.H. Jung), jrichard@zoology.ubc.ca (J.G. Richards), brauner@zoology.ubc.ca (C.J. Brauner).

https://doi.org/10.1016/j.scitotenv.2020.141349
0048-9697/

1. Introduction

The Amazon River system is inhabited by 20% of the world's freshwater fish species that live in a challenging environment characterized by a high stable temperature, but daily and seasonal periods of low oxygen (hypoxia; Val and Almeida-Val, 1995). Climate change is predicted to exacerbate the already extreme environmental conditions of the Amazon basin (IPCC, 2014). The predicted regional temperature increase will also be associated with a decrease in oxygen solubility (Benson and Krause, 1984) and an increase in biological oxygen demand leading to more frequent and severe bouts of hypoxia (Ficke et al., 2007). These disturbances may ultimately affect the distribution and abundance of freshwater fishes depending upon their capacity to acclimate and/or adapt to these changes. However, the effect of climate change induced increases in temperature have been studied much more extensively in marine tropical fishes (i.e. Eme and Bennett, 2009; Ospína and Mora, 2004; Shultz et al., 2016; Vinagre et al., 2016) than in tropical freshwater fishes.

Some tropical ectotherms are thought to be living close to their upper thermal limit with limited capacity to acclimate (Comte and Olden, 2017; Lapointe et al., 2018; Nyboer and Chapman, 2017; Rummer et al., 2014; Stillman, 2003), which may be the case for Amazonian fishes implying they may be vulnerable to future increases in temperature (Campos et al., 2019; Lapointe et al., 2018). Climate change-induced temperature increase is predicted to range from 2.2 to 7 °C in the worst-case scenario in the Amazon within the next century (IPCC, 2014). Furthermore, short-term heat waves, where temperatures are greatly elevated above normal, have profound effects on ecosystems (Frölicher and Laufkötter, 2018) and have already increased in frequency and duration in the Amazon basin, particularly in the last decade (Geirinhas et al., 2017). Within the last 50 years, the maximum temperature associated with heat waves in the Amazon increased by 3–3.5 °C (Geirinhas et al., 2017). Currently, the Amazon basin water temperature oscillates between 28 and 31 °C seasonally (Val and Almeida-Val, 1995). Thus, this study investigated the effects of temperatures at and above the current river thermal regime of 28–31 °C on species' thermal tolerance and hypoxia tolerance.

Some studies suggest that species-specific thermal limits may be, in part, dictated by limitations to aerobic metabolism, a concept embodied in the Oxygen- and Capacity- Limited Thermal Tolerance (OCLTT) hypothesis (Pörtner et al., 2017; Pörtner and Farrell, 2008) - but see Clark et al. (2013) and Jutfelt et al. (2018). According to this hypothesis, organismal upper thermal tolerance limits are reached at temperatures where the cardiorespiratory system fails to meet tissue metabolic demands, thus implying a functional association between tolerance to elevated temperature and low oxygen levels. To date, only two studies have been conducted to explore the direct relationship between thermal tolerance and hypoxia tolerance (Anttila et al., 2013; Smale and Rabeni, 1995), both on temperate fishes demonstrating a small but positive correlation. The correlation between these traits in tropical fishes, however, has not yet been investigated.

If the mechanisms underlying thermal tolerance and hypoxia tolerance are similar or related as suggested by the OCLTT, acclimation to one stressor may enhance tolerance to another (Burleson and Silva, 2011; McBryan et al., 2016). Such cross-tolerance will be beneficial in dealing with climate change and currently fluctuating environments. Whether the tropical fishes also have cross-tolerance to temperature and hypoxia is unknown. Furthermore, those fishes that currently live close to their upper thermal limits show a limited capacity to acclimate to higher temperatures relative to temperate species (Comte and Olden, 2017; Rummer et al., 2014; Stillman, 2003). Thus, the objective of this study was to investigate the interspecific relationship between thermal tolerance (Critical Thermal Maximum; CTMax and Chronic Lethal Maximum; CLMax) and hypoxia tolerance (time to loss of equilibrium (LOE) at $PO_2 = 3$ mm Hg or PO_2 at LOE) in a phylogenetically diverse group of Amazonian teleosts (37 species in total) to provide insight into their capacity to cope with projected short- and long-term temperature increases and associated environmental hypoxia.

2. Methodological approach

2.1. Temperature tolerance and hypoxia tolerance measurements following thermal trials at 3 research sites

Experiments were conducted in three locations: (i) in the field on board a research vessel (Ana Clara) in the Anavilhanas Archipelago of the Rio Negro, approximately 110 km upstream from Manaus (2°43′ 10.9″S, 60°45′18.8″ W); (ii) at the Instituto Nacional de Pesquisas da Amazônia (INPA) in Manaus, Brazil; and (iii) at the University of British Columbia (UBC), Canada. For clarity, these three locations will be referred to as (i) field, (ii) INPA and (iii) UBC throughout the remainder of this manuscript and in the figure legends.

A total of 37 species (Table S1) acclimated to current river temperature of 28–31 °C ('control') were investigated for acute thermal tolerance and hypoxia tolerance. Amongst the 37 species, there were 3 conspecifics investigated at different locations: *Carnegiella strigata* at (ii) INPA and (iii) UBC; *Colossoma macropomum* at (i) field and (ii) INPA; *Hemiodus gracilis at* (i) field and (ii) INPA (Table S2). In the (i) field, fish were caught and brought to the research vessel where they were kept at the river temperature of 30 °C for 1–5 days. At (ii) INPA, 15 species were held at their natural temperature of 28 °C (which involved a diurnal oscillation of 26 to 30 °C) for 10 days. At (iii) UBC, 6 species were held at 31 °C for 4 weeks. The control temperatures of three study sites varied but were within the current river temperature range of 28–31 °C. In the (i) field and (ii) INPA, we have used the average temperatures that the fish were naturally exposed to as the control temperatures (30 °C and 28 °C respectively). At (iii) UBC, we used the highest current river temperature of 31 °C as the control temperature.

Fish acclimated to these current control temperatures were also acutely exposed (<1.5 h) to higher temperatures of 33, 35 or 37 °C and then assessed for hypoxia tolerance. Studies involving long-term exposure to warmer temperatures were also conducted (ii) at INPA and (iii) at UBC: Fish were held at 31, 33 or 35 °C for 10 days or 4 weeks, respectively, then assessed for acute thermal tolerance and hypoxia tolerance. To achieve these temperatures, electric heaters plugged into temperature controllers (Fisherbrand™ Traceable™ Digital Temperature Controller, ON, Canada) were used to increase the temperature by (ii) 1 °C/12 h or (iii) 1 °C/day within the aquaria until the target temperature was reached at which point that temperature was maintained.

Acute thermal tolerance was assessed by measuring the CTMax with a rate of increase of 0.2 °C/min achieved using the same electrical heaters with temperature controllers as described above, and LOE as the endpoint. Fish ($n = 1$–10) were transferred to CTMax experimental tanks of (i) 50 L, (ii) 2 L, or (iii) 20 L glass aquaria and allowed an hour to acclimatize prior to initiating the CTMax test. During the test, water was constantly aerated to ensure adequate oxygenation and mixing of water, and also to avoid supersaturation, which can occur with warming. At LOE, fish were removed, euthanized and weighed. Additionally: (ii) at INPA, 7 of 15 species were acutely exposed to hyperoxic water and the CTMax was determined in hyperoxia to assess whether elevated environmental oxygen improved thermal tolerance. Fish were transferred and acclimatized in CTMax experimental tank for 1 h ($n = 8$ in 2 L). Then, O_2 was bubbled into the water until $PO_2 > 320$ mmHg and then the water was heated at 0.2 °C/min until LOE. Air was constantly bubbled to ensure continuous mixing of water, which did not lower PO_2 below 320 mm Hg throughout the experiment.

During long-term exposure to higher temperatures of 31, 33 or 35 °C at INPA (ii) and UBC (iii) some species exhibited high (>50%) mortality. If this was the case, the highest temperature that >50% fish were able to tolerate for 10 days or 4 weeks was noted as a chronic lethal maximum (CLMax).

In (i) the field, hypoxia tolerance was assessed by measuring time to LOE at a partial pressure of oxygen (PO_2) of 3 mm Hg. Fish (n = 6–8) were transferred to 50 L experimental tank with a netted top to restrict fish from coming up to the surface. After an hour of acclimatization, the PO_2 in the water was reduced within about 25 min using nitrogen gas bubbled into the water. The water was maintained at PO_2 = 3 mm Hg (monitored with YSI handheld DO meter, OH, USA) until LOE at which point the time was noted and fish was netted out carefully. The individuals that exhibited LOE prior to reaching 3 mm Hg were noted to last 0 min in hypoxia. At (ii) INPA and (iii) at UBC, a different hypoxia tolerance assay was performed. Individual fish were transferred to 200 or 300 mL mesh-sided chambers submerged in 50 or 40 L experimental tanks and allowed 1 h to acclimatize. Then, the water PO_2 was reduced from full saturation at a constant rate (11 mm Hg/min) using nitrogen gas (monitored with fibre optic oxygen probe; PreSens Precision Sensing, Regensburg, Germany) until LOE was observed (PO_2 at LOE).

In the (i) field, fish were not fed and were used within 1–5 days of capture. At (ii) INPA and (iii) UBC, fish were fed daily to satiation (TetraMin® Tropical Flakes, VG, USA). At all study sites, all fish were fasted at least 24 h prior to experimentation. All procedures were in compliance with Brazilian national and INPA animal care regulations and UBC animal protocol #A15-0266.

2.2. Statistical analysis

Statistical analyses were performed in R (version 0.98.1091), and graphs were created using Graphpad Prism (version 5.0a). Data are expressed as the means ± SEM (standard error, n = 1–10). Data were analyzed using a type III two-way ANOVA with temperature or hyperoxic treatment and species as factors. If the interaction terms were significant, the data were separated and analyzed independently using one-way ANOVA for each species and then post hoc tests were carried out using Tukey's HSD test. Correlation was determined using the Pearson's correlation test. A robust nonlinear regression outlier test (Motulsky and Brown, 2006) revealed an outlier in the 33 °C dataset (with false discovery rate < 1%), which we then removed and conducted the correlation (species #35: *N. trifasciatus*). Comparisons between the same species (*C. strigata*) or order (Characiformes, Siluriformes, Perciformes) measured in the different locations were conducted using Welch's *t*-test.

To test if thermal tolerance or hypoxia tolerance co-varied with body mass, we tested for a correlation between the phylogenetic independent contrast of thermal or hypoxia tolerance and absolute body mass (Felsenstein, 1985) and found no effect ($p > 0.05$). We used the most recent phylogeny published (Rabosky et al., 2018), where we imported 100 bayesian posterior probability trees, pruned them to only represent known species within our data set, and generated a maximum clade credibility tree, which was used in the analysis of phylogenetic signal and phylogenetic independent contrast. We used Pagel's lambda (Pagel, 1999) and found no significant phylogenetic signal for either variable (i. Thermal tolerance: 30 °C $\lambda = 0.024$, $p = 0.917$; Hypoxia tolerance: 30 °C $\lambda < 0.0001$, $p = 1$; 33 °C $\lambda < 0.0001$, $p = 1$; 35 °C $\lambda < 0.0001$, $p = 1$) (ii and iii. Thermal tolerance: 28 °C $\lambda = 0.303$, $p = 0.415$; 31 °C $\lambda < 0.001$, $p = 1$; 33 °C $\lambda = 0.110$, $p = 0.717$; Hypoxia tolerance: 28 °C $\lambda < 0.001$, $p = 1$; 31 °C $\lambda = 0.395$, $p = 0.334$; 33 °C $\lambda = 0.297$, $p = 0.514$). As a result, we did not correct for body mass or phylogenetic non-independence in the subsequent analysis. In all cases, α was set at 0.05.

3. Results

3.1. Current river temperatures

In this study, fish exposed to current river temperatures of 28–31 °C ('control') were characterized for acute thermal and hypoxia tolerance. The CTMax of fish acclimated to control temperatures measured in the (i) field (30 °C; N = 19), ranged from 36.1–42.8 °C; at (ii) INPA (28 °C; N = 15), ranged from 36.5–42.1 °C; and at (iii) UBC (31 °C; N = 6), ranged from 38.2–41.1 °C (Table S1). Hypoxia tolerance was assessed in two ways in this study: In the (i) field, time to LOE was measured at constant low PO_2; in the (ii and iii) laboratory settings, PO_2 at LOE was measured. The hypoxia tolerance of fish acclimated to control temperatures measured in the (i) field, ranged from 0 to 228.6 min at a PO_2 of 3 mm Hg; at (ii) INPA, all fish lost equilibrium at ≤17.2 mm Hg; and at (iii) UBC, all fish lost equilibrium ≤15.4 mm Hg (Table S1).

We found that interspecific thermal tolerance and hypoxia tolerance were positively correlated at the control temperatures in both the field and laboratory settings: (i) field (Fig. 1; 30 °C r = 0.6193, N = 19, $p = 0.0047$); (ii) INPA (Fig. 2A: 28 °C r = −0.6942; N = 15, $p = 0.0041$);

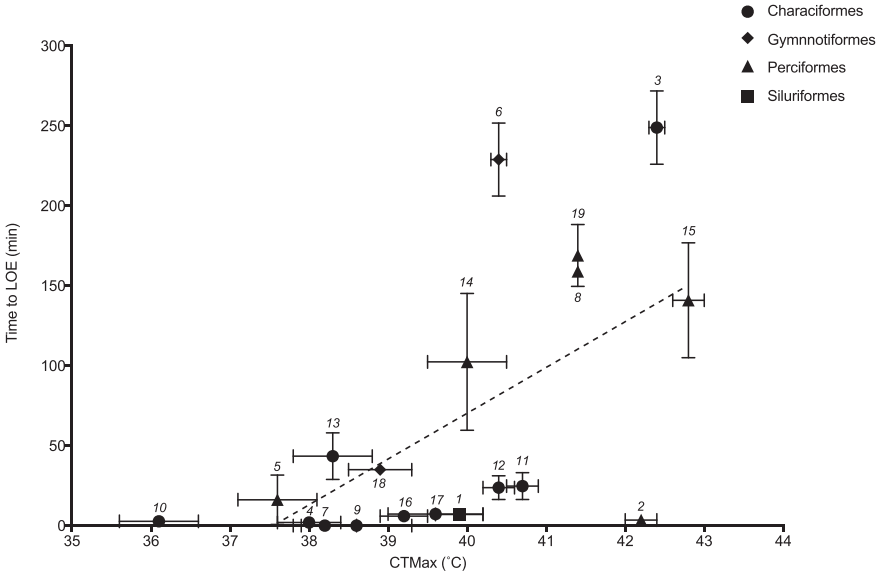


Fig. 1. The relationship between critical thermal maximum (CTMax, °C) and hypoxia tolerance (Time to LOE at 3 mm Hg) (r = 0.6193, N = 19, $p = 0.0047$) of 19 fish species collected (i) in the field (Rio Negro temperature = 30 °C). Values are means ± SEM (n = 1–10). The number above a symbol represents the species: (*1*) *Ancistrus dolichopterus*; (*2*) *Apistogramma diplotaenia*; (*3*) *Colossoma macropomum*; (*4*) *Exodon paradoxus*; (*5*) *Geophagus surinamensis*; (*6*) *Gymnotus anguillaris*; (*7*) *Hemiodus gracilis*; (*8*) *Heros severus*; (*9*) *Hyphessobrycon megalopterus*; (*10*) *Leporinus fasciatus*; (*11*) *Metynnis hysauchen*; (*12*) *Myloplus asterias*; (*13*) *Potamorhina pristigaster*; (*14*) *Satanoperca jurupari*; (*15*) *Satanoperca pappaterra;* (*16*) *Semaprochilodus insignis*; (*17*) *Serrasalmus rhombeus*; (*18*) *Sternopygus macrurus*; (*19*) *Uaru amphiacanthoides*. Symbols categorize species by order.

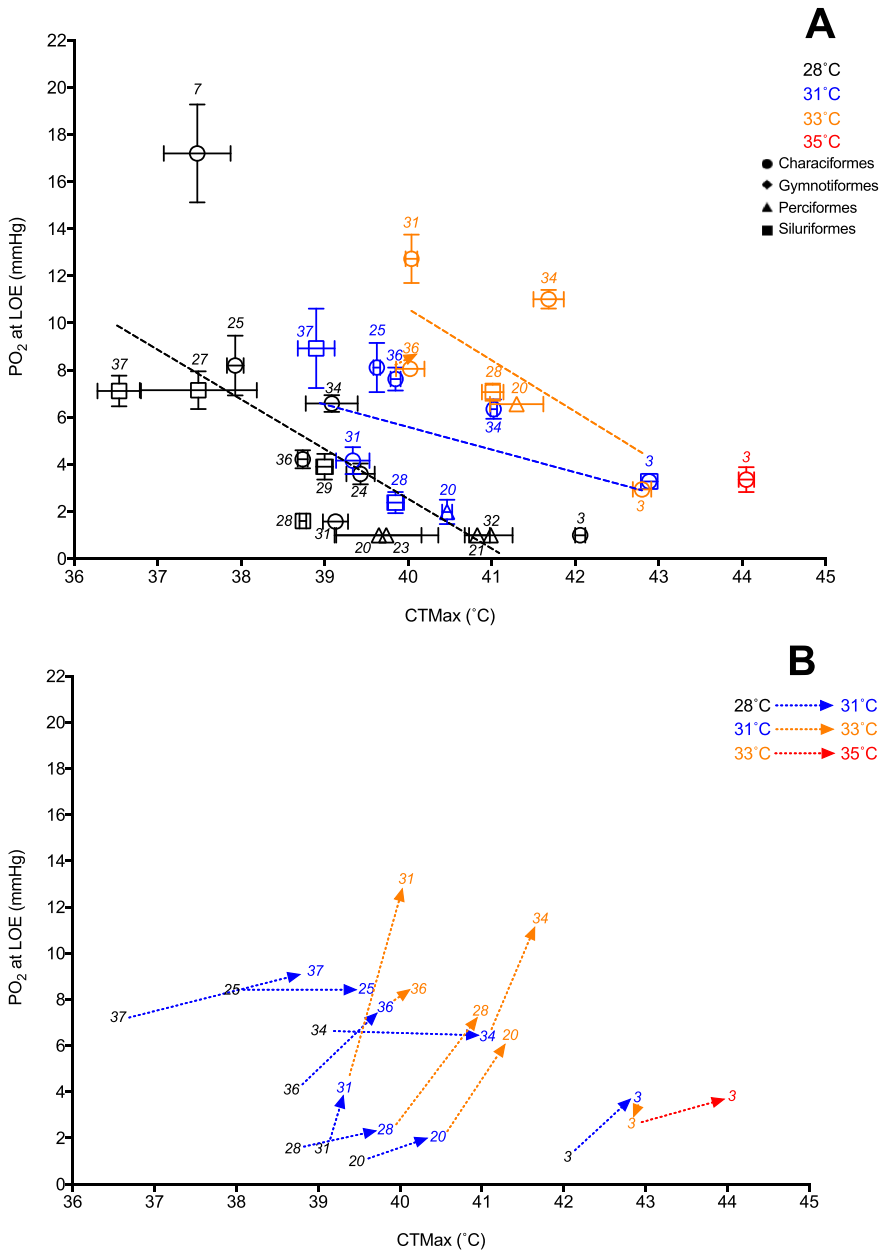


Fig. 2. A) The relationship between critical thermal maximum (CTMax, °C) and hypoxia tolerance (PO_2 at loss of equilibrium, mm Hg) of 15 fish species, prior to and following 10 days of thermal acclimation at (ii) INPA. Fish were housed under natural fluctuation of 26–30 °C and tested at 28 °C (black symbols, r = −0.6942; N = 15; $p = 0.0041$). Some fish were exposed to elevated temperatures of 31 °C (blue symbols, r = −0.4423; N = 8; $p = 0.2725$), 33 °C (orange symbols, r = −0.6655; N = 6; $p = 0.1491$) and 35 °C (red symbols; N = 1) for 10 days. Only 1 species survived 35 °C. Dashed lines represent linear regression at each temperature. B) Arrows connect a given species capable of surviving the next highest exposure temperature to simplify interpretation of how that affects CTMax and PO_2 at LOE. For example, a horizontal arrow indicates a change in CTMax with no change in PO_2 at LOE and a vertical arrow indicates no change in CTMax with a change in PO_2 at LOE. Blue arrow indicates a change from 28 °C to 31 °C for a given species, orange arrow from 31 °C to 33 °C, red arrow from 33 °C to 35 °C. Values are means ± SEM (n = 4–8). Symbols categorize species by order. The numbers represent the species: (*20*) *Apistogramma borellii*; (21) *Apistogramma gephyra*; (*23*) *Astronotus ocellatus*; (*24*) *Brycon amazonicus*; (*3*) *Colossoma macropomum*; (*27*) *Corydoras pulcher*; (*28*) *Corydoras schwartzi*; (*29*) *Corydroas splendens*; (*7*) *Hemiodus gracilis*; (*31*) *Hyphessobrycon erythrostigma*; (*32*) *Laetacara fulvipinnis*; (*34*) *Nannostomus eques*; (*36*) *Paracheirodon axelrodi*; (*37*) *Otocinclus* spp.

(iii) UBC (Fig. 3A; 31 °C r = −0.8448, N = 6, $p = 0.0343$). As a result of hypoxia tolerance assessed as PO_2 at LOE at (ii) INPA and (iii) UBC, the correlations are expressed as negative values but nonetheless still denote that species that are hypoxia tolerant are also thermally tolerant, and vice versa. We found no significant phylogenetic signal for either variable, thus the relationship does not appear to be influenced by phylogeny.

3.2. Warmer temperatures

Of the 37 species used in this study, some were further investigated for their capacity to respond to an increase in temperature both short-term (<1.5 h; (i) 7 species; (ii) 7 species; (iii) 6 species) and long-term ((ii) 8 species for 10 days; (iii) 6 species for 4 weeks). A short-term exposure to 33, 35 and 37 °C reduced hypoxia tolerance, resulting in reduced time to LOE at 3 mm Hg or LOE at a higher PO_2 than that at control temperatures in 16 of 18 species (Figs. 4 and 5; with 2 overlapping species).

Following long-term exposure to higher temperatures of 31, 33 and 35 °C, there was an increase in CTMax in species that survived (Figs. 2B and 3B). It did not, however, result in improved hypoxia tolerance. In fact, long-term exposure to elevated temperature relative to acute elevated temperature resulted in a reduced

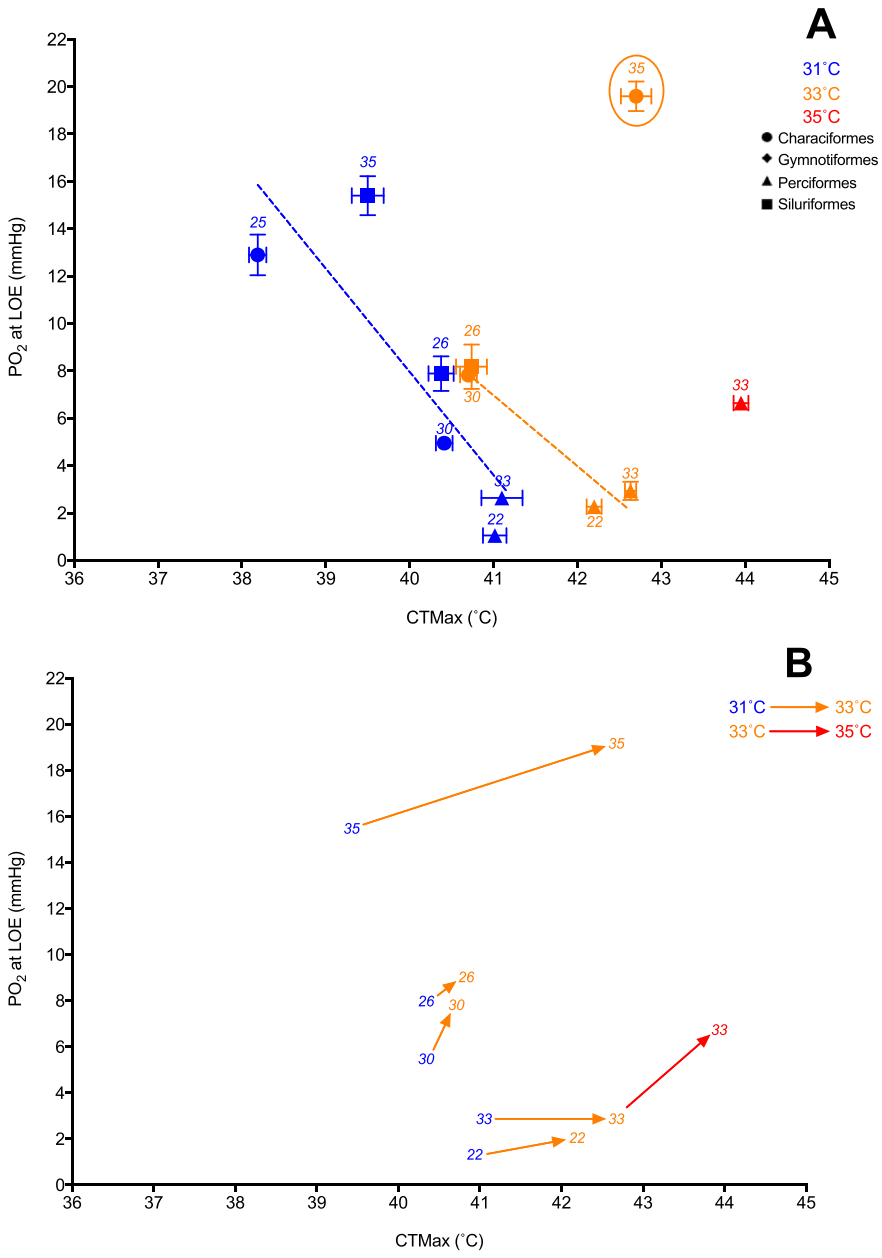


Fig. 3. A) The relationship between critical thermal maximum (CTMax, °C) and hypoxia tolerance (PO_2 at loss of equilibrium, mm Hg) of 6 fish species following 4 weeks of thermal acclimation to 31 °C (blue symbols), 33 °C (orange symbols) or 35 °C (red symbols) at (iii) UBC. Fish measured at 31 °C and 33 °C yield the following relationships: 31 °C ($r = -0.8448$, $N = 6$, $p = 0.0342$), 33 °C ($r = -0.9631$, $N = 4$, $p = 0.0369$, circled outlier species 35 is removed). Only 1 species survived 35 °C. Dashed lines represent linear regression at each temperature. B) Arrows connect a given species capable of surviving the next highest exposure temperature to simplify interpretation of how that affects CTMax and PO_2 at LOE. For example, a horizontal arrow indicates a change in CTMax with no change in PO_2 at LOE and a vertical arrow indicates no change in CTMax with a change in PO_2 at LOE. Orange arrow indicates a change from 31 °C to 33 °C for a given species, red arrow from 33 °C to 35 °C. Values are means ± SEM ($n = 8$). Symbols categorize species by order. The numbers represent the species: (*22*) *Apistogramma viejita*; (*25*) *Carnegiella strigata*; (*26*) *Corydoras julii*; (*30*) *Hemigrammus rhodostomus*; (*33*) *Mikrogeophagus ramirezi*; (*35*) *Nannostomus trifasciatus*.

hypoxia tolerance at the respective temperatures in 5 of 12 species (Fig. 5).

During long-term exposure to higher temperatures for (ii) 10 days and (iii) 4 weeks, high morbidity (>50%; defined as chronic lethal maximum; CLMax) was observed in many species: of the 13 species investigated, 2 species failed to survive above 31 °C, 9 failed to survive above 33 °C, and only 2 species survived above 35 °C. In addition, we found that the CTMax measured following 10-day acclimation to 28 °C or 31 °C was positively correlated with the CLMax at (ii) INPA (Table 1; 28 °C $r = 0.9366$; $N = 8$; $p = 0.0006$; 31 °C $r = 0.8442$, $N = 8$, $p = 0.0084$). Similarly at (iii) UBC, the CTMax measured following 4-week acclimation to 31 °C was positively correlated with CLMax (Table 1; $r = 0.83931$; $N = 6$; $p = 0.0367$).

Above the control temperature of 28 °C at (ii) INPA, the correlation between thermal tolerance and hypoxia tolerance was diminished (Fig. 2; 31 °C $r = -0.4423$; $N = 8$; $p = 0.2725$; 33 °C $r = -0.6655$; $N = 6$; $p = 0.1491$). Only 1 species survived 35 °C, thus we were unable to test for a relationship at this temperature. At (iii) UBC, initial analysis indicated no significant correlation between thermal tolerance and hypoxia tolerance in fish exposed to 33 °C for 4 weeks (Fig. 3; $r = 0.1414$; $N = 5$; $p = 0.8205$). However, a robust nonlinear regression outlier test revealed an outlier in the 33 °C dataset (supplementary information), and

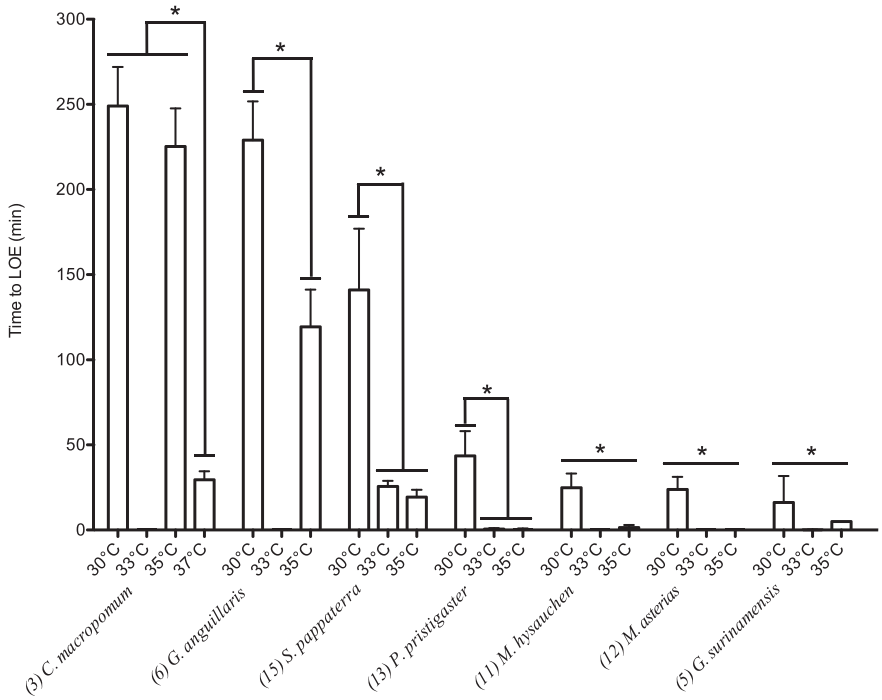


Fig. 4. Hypoxia tolerance (Time to LOE at $PO_2 = 3$ mm Hg) measured at 30 °C (i. Field; Rio Negro temperature) and following acute exposure to 33, 35, and 37 °C. Values are means ± SEM (n = 1–6). Asterisk represents a significant difference between temperatures within a species ($p < 0.05$).

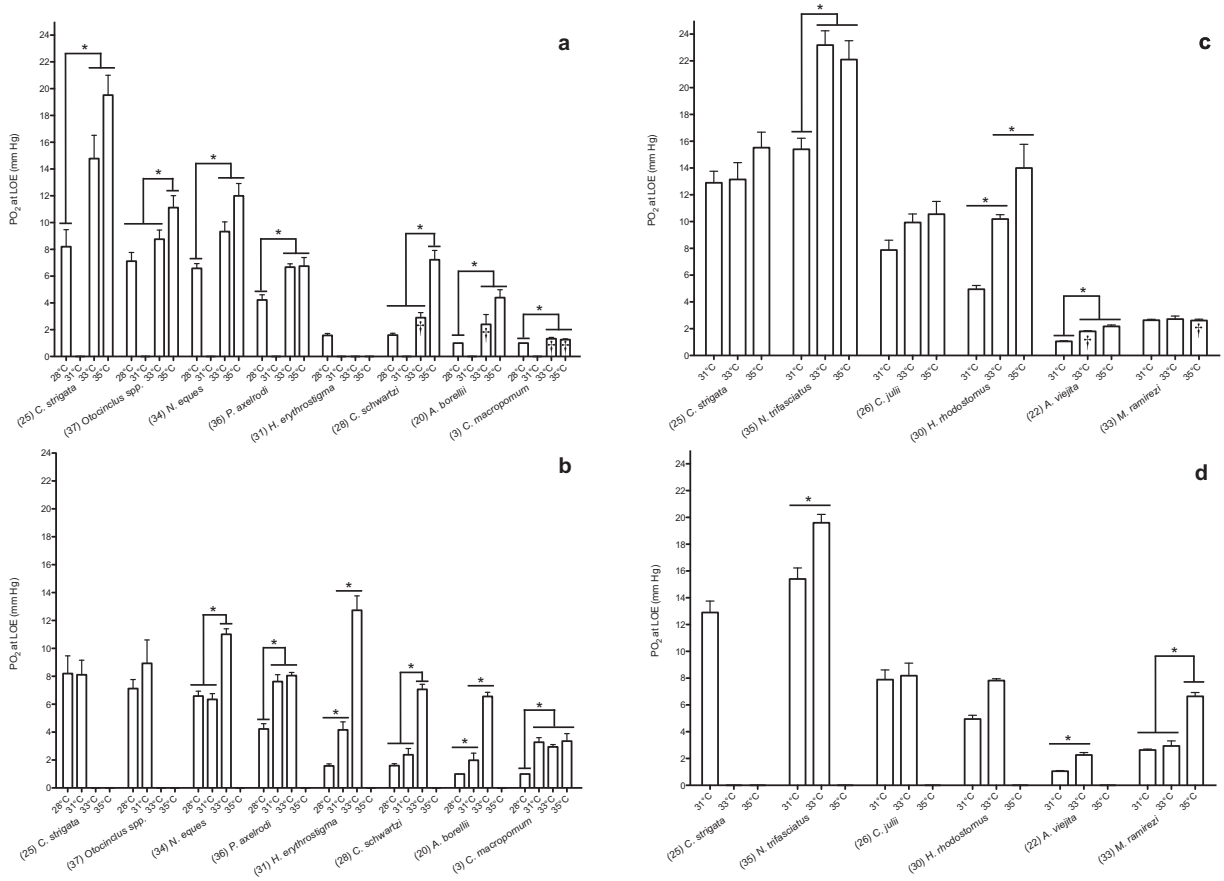


Fig. 5. Hypoxia tolerance (PO_2 at loss of equilibrium, mm Hg) of fish at 28 °C or 31 °C and following a) acute exposure and measurement at 33 and 35 °C at INPA; b) 10 days of exposure to 31, 33, and 35 °C at INPA; c) acute exposure and measurement at 33 and 35 °C at UBC; d) 4 weeks of exposure and measurement at 33 and 35 °C at UBC. Control 28 °C or 31 °C values in panel b and d are the same data as presented in a and c but included for comparative purposes. The absence of a bar in b and d) indicates fish were not able to survive in the respective temperature for the 10-day or 4-week duration. Values are means ± SEM (n = 4–8). Asterisk represents a significant difference between temperatures within a species ($p < 0.05$). † in bars of panel a and c indicate a significant difference in LOE between short- and long-term exposed fish at the same temperature within a species ($p < 0.05$). *A. borellii* and *C. macropomum* at 28 °C maintained equilibrium for some time in <1 mm Hg.

Table 1
Comparison of critical thermal maximum (CTMax) following (ii) 10 days or (iii) 4 weeks of exposure to 28 °C or 31 °C and chronic lethal maximum (CLMax). Species are separated by dotted line according to their CLMax.

Species (Study site: ii. INPA or iii. UBC)	CTMax (°C) at 28 °C	CTMax (°C) at 31 °C	CLMax (°C)
Carnegiella strigata (ii)	37.93 ± 0.10	39.63 ± 0.04	31
Carnegiella strigata (iii)	–	38.19 ± 0.10	31
Otocinclus spp. (ii)	36.54 ± 0.26	38.90 ± 0.22	31
Nannostomus eques (ii)	39.09 ± 0.31	39.50 ± 0.19	33
Nannostomus trifasciatus (iii)	–	41.03 ± 0.04	33
Paracheirodon axelrodi (ii)	38.74 ± 0.04	39.85 ± 0.06	33
Hyphessobrycon erythrostigma (ii)	39.13 ± 0.15	39.34 ± 0.20	33
Hemigrammus rhodostomus (iii)	–	40.41 ± 0.10	33
Corydoras julii (iii)	–	40.38 ± 0.15	33
Corydoras schwartzi (ii)	38.74 ± 0.04	39.85 ± 0.10	33
Apistogramma borellii (ii)	39.65 ± 0.51	40.47 ± 0.06	33
Apistogramma viejita (iii)	–	41.01 ± 0.14	33
Mikrogeophagus ramirezi (iii)	–	41.10 ± 0.24	>35
Colossoma macropomum (ii)	42.06 ± 0.06	42.89 ± 0.10	>35

we found a strong correlation after removal of this species ($r = -0.9631$; $N = 4$; $p = 0.0369$). Similarly to (ii) INPA, only 1 species survived 35 °C acclimation, thus we were unable to test for a relationship.

3.3. Thermal tolerance in hyperoxia

In an effort to determine whether limitations in oxygen supply contribute to defining a species' thermal tolerance, we determined CTMax for 7 species at (ii) INPA under normoxic (160 mm Hg) and hyperoxic conditions (>320 mm Hg) (Fig. 6). Overall, hyperoxia had a significant effect on CTMax ($p < 0.0001$). In 4 of the 7 species tested, CTMax was 0.5–1.7 °C higher in hyperoxic water compared with normoxic water ($p < 0.05$).

4. Discussion

4.1. Current river temperatures

As temperature ultimately imposes a limit on physiological and organismal function, thermal tolerance strongly governs biogeographic distributions of freshwater fishes (Carpenter et al., 1992). The CTMax of the fishes investigated in this study are within the typical range for tropical species in similarly warm habitats (i.e. Chrétien and Chapman, 2016; Eme and Bennett, 2009; Prodocimo and Freire, 2001). The mechanism(s) responsible for the LOE that occurs at CTMax is (are) still unknown, but some studies show that CTMax is not limited according to the OCLTT hypothesis (Lapointe et al., 2018; Nyboer and Chapman, 2017). The LOE at CTMax has also been associated with an impairment of enzyme function, increased membrane leakiness, and failure of the central nervous system (Friedlander et al., 1976; Jutfelt et al., 2019). A recent study on zebrafish (*Danio rerio*) (Åsheim et al., 2020) found that the CTMax with rapid (0.3 °C/min) and slow warming (0.025 °C/min) are correlated, suggesting that both may be due to similar physiological mechanisms.

In addition to high thermal tolerance, Amazonian fishes have also evolved diverse mechanisms to cope with diurnal hypoxia (Kramer et al., 1978; Val and Almeida-Val, 1995) that involve modification of the oxygen transport cascade and ultimately exploitation of anaerobic metabolism (Val et al., 1998; Val, 2000; Val et al., 2015). The interspecific variation in hypoxia tolerance of some fishes measured in this study seems to correlate well with previous findings of critical oxygen tension (P_{crit}) (Saint-Paul, 1984; Scott et al., 2008), haemoglobin-oxygen affinity (Hb-O_2 P_{50}) (Val et al., 2016), and habitat water flow rate (Powers et al., 1979) in other closely related species, clearly, a topic worthy of further investigation.

We found that Amazonian fishes that are thermally tolerant are also hypoxia tolerant at current river temperatures. This is the first study to reveal this correlation across multiple stenothermic tropical fishes. A recent study found a correlation between swimming lifestyle and acute thermal tolerance across families of Amazonian fish species, where those species with low factorial aerobic scope also had low CTMax (Campos et al., 2018). Taken together these findings are consistent with the thermal limit of these Amazonian fishes being associated with the ability to efficiently take up and use oxygen at the whole-organism level.

4.2. Warmer temperatures

The acute exposure to higher temperatures resulted in a reduction in hypoxia tolerance in most species investigated. An acute increase in water temperature decreases the amount of dissolved oxygen in the water for a given partial pressure (Benson and Krause, 1984), causes an increase in metabolic rate (Campos et al., 2019; Clark et al., 2011; Clarke and Johnston, 1999; Steinhausen et al., 2008) and often results in an increase in Hb-O_2 P_{50} (Val et al., 2016). Overall, the species in this study likely experienced difficulties in extracting and transporting sufficient oxygen to meet an increased energy demand in warmer water, resulting in a decrease in hypoxia tolerance.

In fish that survived long-term exposure to higher temperatures, most species exhibited an increase in CTMax to some degree, suggesting species-dependent plasticity (Anttila et al., 2013; Beitinger et al., 2000). Long-term exposure to higher temperatures, however, did not confer an improved hypoxia tolerance and in fact, it decreased hypoxia tolerance relative to acute exposure to the same temperatures in 5 of 12 species (Fig. 5). In the other 7 of 12 species, there were non-significant changes in hypoxia tolerance between acute- and long-term thermal exposures (Fig. 5). Closely related species acclimated to higher temperatures for 10 days (ii. INPA) and for 4 weeks (iii. UBC) resulted in some significant differences in CTMax and hypoxia tolerance (Table S2). However, the 4-week thermal acclimation did not always improve CTMax or hypoxia tolerance, indicating other factors than acclimation period that could have slightly influenced the results. Some species, mostly temperate, are found to re-establish homeostasis during thermal acclimation and undergo physiological adjustments such as an increase in gill surface area (Anttila et al., 2015; McBryan et al., 2016) that enhance oxygen uptake. The reduced hypoxia tolerance found in this study is consistent with others that have shown that tropical species lack metabolic compensation

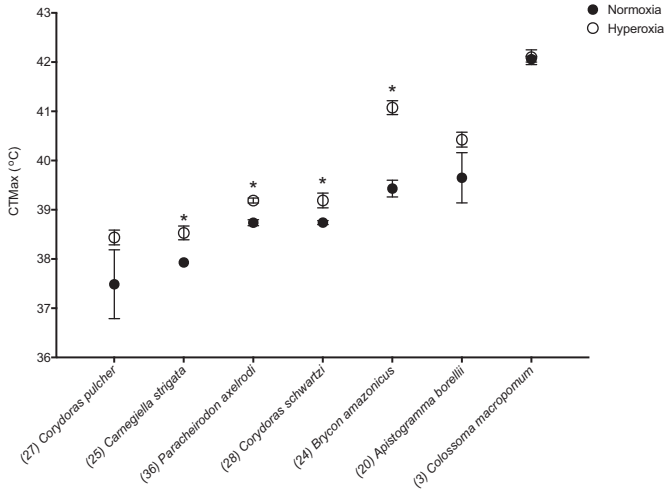


Fig. 6. Critical thermal maximum (CTMax, °C) of 7 fish species acclimated to 31 °C at (ii) INPA in normoxia (PO_2 = 160 mm Hg) and hyperoxia (PO_2 > 320 mm Hg). Normoxia CTMax values are reproduced from Fig. 2. Asterisk represents significant difference between two treatments within a species ($p < 0.05$). Mean ± SEM (n = 8).

with thermal acclimation, which consequently can hinder hypoxia tolerance (Corkum and Gamperl, 2009; Lapointe et al., 2018; Nilsson et al., 2010; Nyboer and Chapman, 2017; Rummer et al., 2014). Furthermore, it may indicate that although some species can survive the higher temperatures evaluated in this study, they are not fully acclimating.

We found a diminishing correlation between thermal tolerance and hypoxia tolerance following long-term exposure to temperatures above the control temperature at each study site. This was mainly a result of an increase in CTMax at higher acclimation temperatures that did not confer an improvement or even a recovery of hypoxia tolerance. There seems to be a limited capacity for acclimation of one trait – thermal or hypoxia tolerance – to act positively on another, at least in these Amazonian fishes. These findings are comparable to other studies that found no effect of hypoxia acclimation on CTMax (Ern et al., 2016; McDonnell et al., 2019) and consistent with a recent study that shows that the genetic bases for thermal and hypoxia tolerance are not associated (Healy et al., 2018). However, some studies have demonstrated cross-tolerance between these traits in other species (Anttila et al., 2015; Burleson and Silva, 2011; McBryan et al., 2016).

4.3. Chronic lethal maximum, CLMax

One of the most significant findings of this study is that many Amazonian species lack the capacity to acclimate and survive at 2–4 °C higher than current temperatures for a prolonged duration (Table 1). These data indicate that long-term increases in temperature only slightly above current river temperatures may impact a large number of Amazonian fishes and that acute thermal tolerance may be predictive of long-term thermal tolerance. Using this relationship between CTMax and CLMax, we would predict CLMax is <31 °C for ~15%, <33 °C for ~69% and > 35 °C for ~15% of the species tested. Even in the 2 species (3) *C. macropomum* and (33) *M. ramirezi* that could survive 35 °C, *M. ramirezi* exhibited reduced hypoxia tolerance after 4-weeks acclimation at that temperature relative to 33 °C. In (3) *C. macropomum*, we did not detect a significant change in survival rate or hypoxia tolerance following 10-day acclimation to 35 °C relative to that at 33 °C, implying that either the 10-day acclimation period may not be long enough to detect all physiological changes for this species or that they are not able to fully acclimate to 35 °C, consistent with Lapointe et al. (2018) that found a significant decrease in survival and specific growth rate of following 3-week acclimation to 35 °C of the same species.

There is a growing body of evidence that fishes already living in a high and narrow thermal range near their upper limit, as in equatorial regions, may be more prone to the effects of increases in temperature, but the duration and magnitude of thermal fluctuations that are lethal are species-specific (Campos et al., 2019; Comte and Olden, 2017; Neuheimer et al., 2011; Nilsson et al., 2010; Rummer et al., 2014; Tewksbury et al., 2008). Although it is difficult to predict population level effects and the possibility of adaptation or early life history acclimation from the results of this study, the observed high morbidity may imply a lack of capacity required to withstand future temperature increases in many Amazonian species. In particular, the temperature increases that occur in association with heat waves are directly relevant here. The duration of heat waves in Southern Brazil since 1980 range from 1 to 15 days and have been increasing since that time (Geirinhas et al., 2017). Heat waves that result in water temperature higher than the current maximum of 31 °C lasting for days to weeks could be lethal for the majority of the Amazonian fishes investigated in this study.

4.4. Thermal tolerance in hyperoxia

The increase in CTMax in hyperoxia indicates that greater oxygen availability alleviates some of the acute thermal stress. The effect of environmental oxygen availability on thermal tolerance in the literature is currently mixed (Brijs et al., 2015; Verberk et al., 2016), which implies that rather than oxygen being a sole determining factor, multiple limiting mechanisms contribute to thermal tolerance. However, an increase in CTMax in hyperoxia is directly relevant to these Amazonian fish species' life history considering that peak levels of hyperoxia (>400 mm Hg) occur mid-day when temperatures are the highest (Val and Almeida-Val, 1995).

5. Conclusion

In conclusion, we found an interspecific relationship between thermal tolerance and hypoxia tolerance in 37 Amazonian teleost species in both field and lab settings. In fish that could tolerate long-term exposure to temperatures above the current river temperature, an increased thermal tolerance but reduced hypoxia tolerance was observed. Acute exposure to hyperoxia, on the other hand, had a small but positive effect on thermal tolerance in some species. The absence of cross-tolerance in these Amazonian species suggests a limited capacity for acclimation of one trait to act positively on another. Thus, long-term exposure to higher temperatures may be helpful in dealing with the elevated temperature predicted by climate change and current heat waves but not in dealing with the concurrent hypoxia. Furthermore, hypoxia will occur in conjunction with an increase in water PCO_2 in nature, which may impose additional stress on the species; however, this was not investigated in this study. Clearly, more research is required to address these points but nonetheless, this is the first study to reveal a correlation in tolerance to both environmental stressors at the level of the whole organism in multiple stenothermic tropical fishes.

This study also demonstrates that climate change-induced disturbances may negatively impact the distribution and abundance of one of the world's hotspots of freshwater fish biodiversity. It appears that many species may have a limited capacity to survive long-term elevations of 2–4 °C over current river temperatures. This is of concern given that climate change models predict a regional 2.2–7 °C increase above the current river temperature over the next 100 years (IPCC, 2014), and more importantly short-term increases in temperature in this temperature range associated with heat-waves (Frölicher and Laufkötter, 2018; Geirinhas et al., 2017). In addition, the correlation between CTMax and CLMax imply that a species' potential risk level associated with fluctuations in temperature may be relatively easily predicted by measuring their acute thermal tolerance (CTMax). This may be used as a relatively simple protocol to identify species at risk associated with predicted regional changes in temperature (both short- and long-term) in the Amazon and the relevant species abundances in those regions; clearly an area worthy of further research.

CRediT authorship contribution statement

Ellen H. Jung: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Kevin V. Brix:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Jeffrey G. Richards:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Adalberto L. Val:** Resources, Writing - review & editing. **Colin J. Brauner:** Conceptualization, Methodology, Investigation, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper “Reduced hypoxia tolerance and survival at elevated temperatures may limit the ability of Amazonian fishes to survive in a warming world.”

Acknowledgements

We thank Dr. Vera M. F. Almeida-Val and Marcio S. Ferreira from INPA, and Dr. Chris M. Wood from UBC, for valuable assistance with this

study. We thank Dr. Christian Damsgaard for assistance with phylogenetic signal analysis.

Funding

Research was supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant to CJB (2018-04172), a UBC Go Global Award to EHJ, INCT ADAPTA to ALV (FAPEAM/CNPq/CAPES), and a NSF Post-Doctoral Fellowship to KVB (DBI-1306452).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.141349.

References

Anttila, K., Dhillon, R.S., Boulding, E.G., Farrell, A.P., Glebe, B.D., Elliott, J.A.K., Wolters, W.R., Schulte, P.M., 2013. Variation in temperature tolerance among families of Atlantic salmon (*Salmo salar*) is associated with hypoxia tolerance, ventricle size and myoglobin level. J. Exp. Biol. 216, 1183–1190. https://doi.org/10.1242/jeb.080556.

Anttila, K., Lewis, M., Prokkola, J.M., Kanerva, M., Seppanen, E., Kolari, I., Nikinmaa, M., 2015. Warm acclimation and oxygen depletion induce species-specific responses in salmonids. J. Exp. Biol. 218, 1471–1477. https://doi.org/10.1242/jeb.119115.

Åsheim, E., Andreassen, A., Morgan, R., Jutfelt, F., 2020. Rapid-warming tolerance correlates with tolerance to slow warming but not growth at non-optimal temperatures in zebrafish 1–23. Preprint at. https://doi.org/10.32942/osf.io/u6dpj.

Beitinger, T.L., Bennett, W.A., McCauley, R.W., 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. Environ. Biol. Fish 58, 237–275. https://doi.org/10.1023/A:1007676325825.

Benson, B.B., Krause, D., 1984. The concentration and isotopic fractionation of oxygen dissolved in freshwater and seawater in equilibrium with the atmosphere. Limnol. Oceanogr. 29, 620–632. https://doi.org/10.4319/lo.1984.29.3.0620.

Brijs, J., Jutfelt, F., Clark, T.D., Grans, A., Ekstrom, A., Sandblom, E., 2015. Experimental manipulations of tissue oxygen supply do not affect warming tolerance of European perch. J. Exp. Biol. 218, 2448–2454. https://doi.org/10.1242/jeb.121889.

Burleson, M.L., Silva, P.E., 2011. Cross tolerance to environmental stressors: effects of hypoxic acclimation on cardiovascular responses of channel catfish (*Ictalurus punctatus*) to a thermal challenge. J. Therm. Biol. 36, 250–254. https://doi.org/10.1016/j.jtherbio.2011.03.009.Cross.

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. J. Therm. Biol. 72, 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. Ecol. Indic. 101, 533–540. https://doi.org/10.1016/j.ecolind.2019.01.051.

Carpenter, S.R., Fisher, S.G., Grimm, N.B., Kitchell, J.F., 1992. Global change and freshwater ecosystems. Annu. Rev. Ecol. Syst. 23, 119–139. https://doi.org/10.1146/annurev.es.23.110192.001003.

Chrétien, E., Chapman, L.J., 2016. Tropical fish in a warming world: thermal tolerance of Nile perch *Lates niloticus* (L.) in Lake Nabugabo, Uganda. Conserv. Physiol. 4, cow062. https://doi.org/10.1093/conphys/cow062.

Clark, T.D., Jeffries, K.M., Hinch, S.G., Farrell, A.P., 2011. Exceptional aerobic scope and cardiovascular performance of pink salmon (*Oncorhynchus gorbuscha*) may underlie resilience in a warming climate. J. Exp. Biol. 214, 3074–3081. https://doi.org/10.1242/jeb.060517.

Clark, T.D., Sandblom, E., Jutfelt, F., 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. J. Exp. Biol. 216, 2771–2782. https://doi.org/10.1242/jeb.084251.

Clarke, A., Johnston, N.M., 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. J. Anim. Ecol. 68, 893–905. https://doi.org/10.1046/j.1365-2656.1999.00337.x.

Comte, L., Olden, J.D., 2017. Climatic vulnerability of the world's freshwater and marine fishes. Nat. Clim. Chang. 7, 718–722. https://doi.org/10.1038/nclimate3382.

Corkum, C.P., Gamperl, A.K., 2009. Does the ability to metabolically downregulate alter the hypoxia tolerance of fishes?: a comparative study using cunner (*T. adspersus*) and Greenland cod (*G. ogac*). J. Exp. Zool. Part A Ecol. Genet. Physiol. 311, 231–239. https://doi.org/10.1002/jez.520.

Eme, J., Bennett, W.A., 2009. Critical thermal tolerance polygons of tropical marine fishes from Sulawesi, Indonesia. J. Therm. Biol. 34, 220–225. https://doi.org/10.1016/j.jtherbio.2009.02.005.

Ern, R., Norin, T., Gamperl, A.K., Esbaugh, A.J., 2016. Oxygen dependence of upper thermal limits in fishes. J. Exp. Biol. 219, 3376–3383. https://doi.org/10.1242/jeb.143495.

Felsenstein, J., 1985. Phylogenies and the comparative method. Am. Nat. 125 (611-1–15).

Ficke, A.D., Myrick, C.A., Hansen, L.J., 2007. Potential impacts of global climate change on freshwater fisheries. Rev. Fish Biol. Fish. 17, 581–613. https://doi.org/10.1007/s11160-007-9059-5.

Friedlander, M.J., Kotchabhakdi, N., Prosser, C.L., 1976. Effects of cold and heat on behavior and cerebellar function in goldfish. J. Comp. Physiol. A. 112, 19–45. https://doi.org/10.1007/BF00612674.

Frölicher, T.L., Laufkötter, C., 2018. Emerging risks from marine heat waves. Nat. Commun. 9, 2015–2018. https://doi.org/10.1038/s41467-018-03163-6.

Geirinhas, J.L., Trigo, R.M., Libonati, R., Coelho, C.A.S., Palmeira, A.C., 2017. Climatic and synoptic characterization of heat waves in Brazil. Int. J. Climatol. 38, 1760–1776. https://doi.org/10.1002/joc.5294.

Healy, T.M., Brennan, R.S., Whitehead, A., Schulte, P.M., 2018. Tolerance traits related to climate change resilience are independent and polygenic. Glob. Chang. Biol. 1. https://doi.org/10.1111/gcb.14386.

IPCC, 2014. Climate Change 2014: Impacts, Adaptation, and Vulnerability Part. B: Regional Aspects, Climate Change 2014: Impacts, Adaptation, and Vulnerability. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA https://doi.org/10.1007/s13398-014-0173-7.2.

Jutfelt, F., Norin, T., Ern, R., Overgaard, J., Wang, T., McKenzie, D.J., Lefevre, S., Nilsson, G.E., Metcalfe, N.B., Hickey, A.J.R., Brijs, J., Speers-Roesch, B., Roche, D.G., Gamperl, A.K., Raby, G.D., Morgan, R., Esbaugh, A.J., Gräns, A., Axelsson, M., Ekström, A., Sandblom, E., Binning, S.A., Hicks, J.W., Seebacher, F., Jørgensen, C., Killen, S.S., Schulte, P.M., Clark, T.D., 2018. Oxygen- and capacity-limited thermal tolerance: blurring ecology and physiology. J. Exp. Biol. 221, 1–2. https://doi.org/10.1242/jeb.169615.

Jutfelt, F., Roche, D.G., Clark, T.D., Norin, T., Binning, S.A., Speers-Roesch, B., Amcoff, M., Morgan, R., Andreassen, A.H., Sundin, J., 2019. Brain cooling marginally increases acute upper thermal tolerance in Atlantic cod. J. Exp. Biol. 222, jeb208249. https://doi.org/10.1242/jeb.208249.

Kramer, D.L., Lindsey, C.C., Moodie, G.E.E., Stevens, E.D., 1978. The fishes and the aquatic environment of the Central Amazon basin, with particular reference to respiratory patterns. Can. J. Zool. 56, 717–729. https://doi.org/10.1139/z78-101.

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. Conserv. Physiol. 6, 1–19. https://doi.org/10.1093/conphys/coy056.

McBryan, T.L., Healy, T.M., Haakons, K.L., Schulte, P.M., 2016. Warm acclimation improves hypoxia tolerance in *Fundulus heteroclitus*. J. Exp. Biol. 219, 474–484. https://doi.org/10.1242/jeb.133413.

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. Physiol. Biochem. Zool. 92, 253–265. https://doi.org/10.1086/702712.

Motulsky, H.J., Brown, R.E., 2006. Detecting outliers when fitting data with nonlinear regression – a new method based on robust nonlinear regression and the false discovery rate. BMC Bioinforma. 7. https://doi.org/10.1186/1471-2105-7-123.

Neuheimer, A.B., Thresher, R.E., Lyle, J.M., Semmens, J.M., 2011. Tolerance limit for fish growth exceeded by warming waters. Nat. Clim. Chang. 1, 110–113. https://doi.org/10.1038/nclimate1084.

Nilsson, G.E., Östlund-Nilsson, S., Munday, P.L., 2010. Effects of elevated temperature on coral reef fishes: loss of hypoxia tolerance and inability to acclimate. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 156, 389–393. https://doi.org/10.1016/j.cbpa.2010.03.009.

Nyboer, E.A., Chapman, L.J., 2017. Elevated temperature and acclimation time affect metabolic performance in the heavily exploited Nile perch of Lake Victoria. J. Exp. Biol. 220, 3782–3793. https://doi.org/10.1242/jeb.163022.

Ospína, A.F., Mora, C., 2004. Effect of body size on reef fish tolerance to extreme low and high temperatures. Environ. Biol. Fish 70, 339–343.

Pagel, M., 1999. Inferring the historical patterns of biological evolution. Nature 401, 877–884. https://doi.org/10.1038/44766.

Pörtner, H.O., Farrell, A.P., 2008. Ecology: physiology and climate change. Science (80-.) 322, 690–692.

Pörtner, H.O., Bock, C., Mark, F.C., 2017. Oxygen- and capacity-limited thermal tolerance: bridging ecology and physiology. J. Exp. Biol. 220, 2685–2696. https://doi.org/10.1242/jeb.134585.

Powers, D.A., Fyhn, H.J., Fyhn, U.E.H., Martin, J.P., Garlick, R.L., Wood, S.C., 1979. A comparative study of the oxygen equilibria of blood from 40 genera of Amazonian fishes. Comp. Biochem. Physiol. A Physiol. 62, 67–85. https://doi.org/10.1016/0300-9629(79)90741-2.

Prodocimo, V., Freire, C.A., 2001. Critical thermal maxima and minima of the platyfish *Xiphophorus maculatus* Günther (Poecillidae, Cyprinodontiformes): a tropical species of ornamental freshwater fish. Rev. Bras. Zool. 18, 97–106. https://doi.org/10.1590/S0101-81752001000500007.

Rabosky, D.L., Chang, J., Title, P.O., Cowman, P.F., Sallan, L., Friedman, M., Kaschner, K., Garilao, C., Near, T.J., Coll, M., Alfaro, M.E., 2018. An inverse latitudinal gradient in speciation rate for marine fishes. Nature 559, 392–395. https://doi.org/10.1038/s41586-018-0273-1.

Rummer, J.L., Couturier, C.S., Stecyk, J.A.W., Gardiner, N.M., Kinch, J.P., Nilsson, G.E., Munday, P.L., 2014. Life on the edge: thermal optima for aerobic scope of equatorial reef fishes are close to current day temperatures. Glob. Chang. Biol. 20, 1055–1066. https://doi.org/10.1111/gcb.12455.

Saint-Paul, U., 1984. Physiological adaptation to hypoxia of a neotropical characoid fish *Colossoma macropomum*, Serrasalmidae. Environ. Biol. Fish 11, 53–62. https://doi.org/10.1007/BF00001845.

Scott, G.R., Wood, C.M., Sloman, K.A., Iftikar, F.I., De Boeck, G., Almeida-Val, V.M.F., Val, A.L., 2008. Respiratory responses to progressive hypoxia in the Amazonian oscar, *Astronotus ocellatus*. Respir. Physiol. Neurobiol. 162, 109–116. https://doi.org/10.1016/j.resp.2008.05.001.

Shultz, A.D., Zuckerman, Z.C., Suski, C.D., 2016. Thermal tolerance of nearshore fishes across seasons: implications for coastal fish communities in a changing climate. Mar. Biol. 163. https://doi.org/10.1007/s00227-016-2858-2.

Smale, M.a., Rabeni, C.F., 1995. Hypoxia and hyperthermia tolerances of headwater stream fishes. Trans. Am. Fish. Soc. 124, 698–710. https://doi.org/10.1577/1548-8659(1995)124<0698:HAHTOH>2.3.CO;2.

Steinhausen, M.F., Sandblom, E., Eliason, E.J., Verhille, C., Farrell, A.P., 2008. The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*). J. Exp. Biol. 211, 3915–3926. https://doi.org/10.1242/jeb.019281.

Stillman, J.H., 2003. Acclimation capacity underlies susceptibility to climate change. Science (80-.). 301, 65. https://doi.org/10.1126/science.1083073.

Tewksbury, J.J., Huey, R.B., Deutsch, C.A., 2008. Ecology: putting the heat on tropical animals. Science (80-.) 320, 1296–1297. https://doi.org/10.1126/science.1159328.

Val, A.L., 2000. Organic phosphates in the red blood cells of fish. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 125, 417–435. https://doi.org/10.1016/S1095-6433(00)00184-7.

Val, A.L., Almeida-Val, V.M.F., 1995. Fishes of the Amazon and Their Environment. Springer-Verlag, Berlin, Heidelberg https://doi.org/10.1007/978-3-642-79229-8.

Val, A.L., Silva, M.N.P., Almeida-Val, V.M.F., 1998. Hypoxia adaptation in fish of the Amazon: a never-ending task. South African J. Zool. 33, 107–114. https://doi.org/10.1080/02541858.1998.11448459.

Val, A.L., Gomes, K.R.M., de Almeida-Val, V.M.F., 2015. Rapid regulation of blood parameters under acute hypoxia in the Amazonian fish *Prochilodus nigricans*. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 184, 125–131. https://doi.org/10.1016/j.cbpa.2015.02.020.

Val, A.L., Paula-Silva, M.e.N., Almeida-Val, V.M.F., Wood, C.M., 2016. *In vitro* effects of increased temperature and decreased pH on blood oxygen affinity of 10 fish species of the Amazon. J. Fish Biol. 89, 264–279. https://doi.org/10.1111/jfb.13009.

Verberk, W.C.E.P., Overgaard, J., Ern, R., Bayley, M., Wang, T., Boardman, L., Terblanche, J.S., 2016. Does oxygen limit thermal tolerance in arthropods? A critical review of current evidence. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 192, 64–78. https://doi.org/10.1016/j.cbpa.2015.10.020.

Vinagre, C., Leal, I., Mendonça, V., Madeira, D., Narciso, L., Diniz, M.S., Flores, A.A.V., 2016. Vulnerability to climate warming and acclimation capacity of tropical and temperate coastal organisms. Ecol. Indic. 62, 317–327. https://doi.org/10.1016/j.ecolind.2015.11.010.