



Tolerance of an acute warming challenge declines with body mass in Nile tilapia: evidence of a link to capacity for oxygen uptake

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1 Tolerance of an acute warming challenge declines with body mass in Nile tilapia:
2 evidence of a link to capacity for oxygen uptake

3

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12

13 **Abstract**

14 It has been proposed that larger individuals within fish species may be more sensitive to global
15 warming, due to limitations in their capacity to provide oxygen for aerobic metabolic activities.
16 This could affect size distributions of populations in a warmer world but evidence is lacking. In Nile
17 tilapia *Oreochromis niloticus* (n = 18, mass range 21 - 313g), capacity to provide oxygen for aerobic
18 activities (aerobic scope) was independent of mass at an acclimation temperature of 26 °C. Toler-
19 ance of acute warming, however, declined significantly with mass when evaluated as the critical
20 temperature for fatigue from aerobic swimming (CTS_{max}). The CTS_{max} protocol challenges a fish to
21 meet the oxygen demands of constant aerobic exercise while their demands for basal metabolism
22 are accelerated by incremental warming, culminating in fatigue. CTS_{max} elicited pronounced in-
23 creases in oxygen uptake in the tilapia but the maximum rates achieved prior to fatigue declined
24 very significantly with mass. Mass-related variation in CTS_{max} and maximum oxygen uptake rates
25 were positively correlated, which may indicate a causal relationship. When fish populations are
26 faced with acute thermal stress, larger individuals may become constrained in their ability to per-
27 form aerobic activities at lower temperatures than smaller conspecifics. This could affect survival
28 and fitness of larger fish in a future world with more frequent and extreme heatwaves, with con-
29 sequences for population productivity.

30 Introduction

31 Global change is having profound effects on aquatic ecosystems worldwide, with poorly known
32 consequences for fish populations. Global warming is causing a rise in average seasonal tempera-
33 tures and, more importantly, higher frequency and intensity of acute summer heatwaves (Smale
34 et al., 2019; Stillman, 2019). This has stimulated a wave of research into the mechanisms that un-
35 derly tolerance of warming in fishes, especially into the Fry paradigm and its mechanistic elabora-
36 tion, the Oxygen and Capacity Limited Thermal Tolerance hypothesis (here abbreviated for con-
37 venience as Fry-OCLTT, reviewed in Lefevre et al., 2021). The Fry-OCLTT posits that upper thermal
38 tolerance in fishes is defined by physiological capacity to provide oxygen for the demands of aero-
39 bic metabolism as these are increased by warming, a capacity that ultimately reaches a physiologi-
40 cal ceiling, leading to functional collapse (Eliason et al., 2011; Fry, 1971; Pörtner, 2010; Pörtner
41 and Farrell, 2008; Schulte, 2015). Although the Fry-OCLTT paradigm seems intuitive, empirical evi-
42 dence for it is mixed and therefore its universality remains open to question (Farrell, 2016; Jutfelt
43 et al., 2018; Lefevre, 2016; Lefevre et al., 2021; Pörtner, 2021).

44 A major biological phenomenon that has been correlated with ongoing global warming is a
45 progressive reduction in the adult size of many fish species (Audzijonyte et al., 2020; Baudron et
46 al., 2014; Daufresne et al., 2009). This may be a consequence of the temperature-size rule (TSR;
47 Atkinson, 1994), which derives from laboratory and field observations that adult body size declines
48 when aquatic ectotherms are grown at warmer temperatures (Audzijonyte et al., 2020; Hume,
49 2019; Loisel et al., 2019). The mechanisms underlying the TSR remain to be understood (Verberk
50 et al., 2021; Wootton et al., 2022) but it has been suggested that, at warmer temperatures, larger
51 individuals may be prone to limitations in their capacity for oxygen uptake, so that species may
52 have evolved to modify their growth trajectories and avoid constraints on aerobic activities in
53 adults (Atkinson et al., 2006; Hoefnagel and Verberk, 2015; Leiva et al., 2019; Rubalcaba et al.,
54 2020; Verberk et al., 2021). Modelling across species provides some support for potential oxygen
55 supply limitation of larger fishes at warm temperatures (Rubalcaba et al., 2020) but studies of how
56 body size and temperature interact to affect oxygen supply capacity within species are very rare
57 (Tirsgaard et al., 2015).

58 If we accept that oxygen supply limitation could define upper thermal tolerance in fishes
59 and that larger individuals might be more prone to suffer from oxygen limitation at warm tem-
60 peratures, a prediction would be that tolerance will decline with increasing body size. If true, this
61 would be important in projecting how global warming will affect fish populations. Leiva et al.

(2019) provided evidence that, in aquatic ectotherms generally, upper thermal tolerance declines with body and cell size of species. Within fish species, studies using the critical thermal maximum (CT_{max}) protocol have found that upper thermal tolerance drops with increasing body size in some species, although not in all (reviewed in McKenzie et al., 2020). The CT_{max} protocol is the standard method to evaluate acute thermal tolerance in fishes and ectotherms in general; animals are warmed incrementally until loss of equilibrium (LOE), the tolerance endpoint (Beitinger and Lutterschmidt, 2011). Although the CT_{max} is easy to perform and is a repeatable trait within fish individuals and populations (Claireaux et al., 2013; McKenzie et al., 2020; Morgan et al., 2018), it has the weakness that the mechanisms for LOE are not understood. They presumably reflect acute loss of function of critical organs such as the heart and central nervous system, but may differ among species and with warming rate (Ekstrom et al., 2018; Jutfelt et al., 2019; Lefevre et al., 2021; McKenzie et al., 2020; Morgenroth et al., 2021; Rezende and Bozinovic, 2019; Rezende et al., 2011). In particular, there is evidence that LOE may not always be attributable to problems with systemic oxygen supply, which has been a major source of contention in the debate surrounding the universality of the Fry-OCLTT paradigm (Blasco et al., 2020; Brijs et al., 2015; Ekström et al., 2016; Ern et al., 2016; Lefevre et al., 2021; Wang et al., 2014). Some studies have considered whether size-dependent warming tolerance might be linked to capacity for oxygen uptake within species (Christensen et al., 2020; Messmer et al., 2017) but this remains to be tested explicitly.

Blasco et al. (2020) proposed a measure of upper thermal tolerance whereby fish are warmed incrementally while they perform intense but steady aerobic exercise in a swimming respirometer (Steinhausen et al., 2008), with fatigue as the tolerance endpoint. In two tropical freshwater species, warming caused a progressive and profound increase in oxygen uptake up to a maximum rate, at which both species transitioned from steady aerobic swimming to an unsustainable anaerobic swimming gait, which rapidly led to fatigue. Fatigue in both species occurred at temperatures significantly lower than their CT_{max} based on LOE (Blasco et al., 2020). The protocol evaluates capacity for oxygen uptake because it challenges a fish to meet the oxygen demands of a constant metabolic load, aerobic exercise, plus an incremental metabolic load, progressive warming; fatigue presumably occurs when a fish can no longer meet these combined demands (Blasco et al., 2020; Steinhausen et al., 2008).

We used this swimming protocol (called CT_{swim} in Blasco et al. 2020 but see later) to investigate whether tolerance of acute warming declined with increasing body mass in Nile tilapia *Oreochromis niloticus*, and whether this could be related to a reduced capacity for oxygen uptake

94 with increasing mass. The Nile tilapia is a relatively eurythermal tropical freshwater teleost (natu-
95 ral thermal range 14-33°C) that provides important fisheries in tropical and sub-tropical countries
96 across the globe (De Silva et al., 2004; Schofield et al., 2011). We studied a group of fish that
97 ranged over one order of magnitude in body mass; we first measured key traits of respiratory me-
98 tabolism and performance at acclimation temperature (26°C) by swimming respirometry, in order
99 better to interpret any effects of mass on thermal tolerance. We then investigated effects of mass
100 on thermal tolerance using the CT_{max} and the critical thermal maximum for aerobic swimming
101 (CTS_{max} , previously called CT_{swim}) protocols. We correlated variation in CTS_{max} with variation in
102 maximum rates of oxygen uptake at fatigue, as evidence of a causal relationship.

103 **Material and Methods**

104 **Ethical approval**

105 Experiments were performed according to Brazilian National Council for Control of Animal
106 Experimentation (CONCEA) regulations, the protocol was approved by the Ethics Committee on
107 Animal Use of the Federal University of São Carlos (CEUA/UFSCAR), protocol number CEUA
108 3927151016.

109 **Animals**

110 Animals came from populations of Nile tilapia of different sizes and age classes, obtained from the
111 same supplier and reared in recirculating biofiltered water at $25 \pm 1^\circ\text{C}$ in multiple outdoor tanks
112 (vol. 1000 L) at the Department of Physiological Sciences, UFSCar, São Carlos (SP), Brazil.
113 Individuals ($n = 18$, mass range 20.6 g to 313.0 g) were selected in three sequential groups of six
114 relatively size-matched individuals, who each underwent the tagging and experimenting protocol
115 shown in Figure 1. That is, they were initially tagged for identification (PIT under benzocaine
116 anaesthesia) then recovered for at least 96 h in an indoor tank (vol. 100 L) in the same biofiltered
117 water system and with routine feeding. Animals were fasted 24 h prior to experimentation, and
118 weighed prior to overnight recovery in each experimental setting. They did not change in mass
119 between the sequential tests of performance and tolerance (see below).

120 **Metabolic and performance phenotype at acclimation temperature**

121 Following tagging, each individual's metabolic and performance phenotype was measured at
122 acclimation temperature (Fig 1), with a Steffensen-type swim-tunnel (volume 13.4 L) supplied with
123 vigorously aerated, biofiltered water at $26 \pm 0.1^\circ\text{C}$, as described in (Blasco et al., 2020). Briefly,
124 fish were placed in the tunnel and left overnight at a low swimming speed equivalent to 1
125 bodylength·s⁻¹ (BL·s⁻¹, corrected for solid blocking effect (Blasco et al., 2020)). The following

126 morning swimming speed was increased each 30 min in steps of either 1 BL (mass range 20.6 to
127 86.5 g, n=12) or 0.5 BL (204 to 313 g, n=6), up to either 5 or 2.5 BL·s⁻¹, respectively. Speeds differed
128 for the size groups because relative swimming speed declines with size (length) in fishes
129 (Bainbridge, 1958; Beamish, 1978), and the objective was to obtain a range of speeds at which all
130 individuals exclusively engaged steady aerobic body-caudal swimming (Webb, 1998).
131 Measurements of oxygen uptake ($\dot{M}O_2$) were made by stopped-flow respirometry at each speed
132 and calculated as both absolute (mmol·fish⁻¹·h⁻¹) and mass-corrected (mmol·kg⁻¹·h⁻¹) rates, to
133 subsequently derive standard metabolic rate (SMR) (Chabot et al., 2016) and active metabolic rate
134 AMR (Norin and Clark, 2016), and calculate AS as AMR-SMR.

135 Following this element of the swim test, the swimming speed was increased by 0.1 BL
136 every 10 s until fatigue, with this speed noted as maximum swimming speed (U_{max} ; Blasco et al.,
137 2020; Marras et al., 2013). When the fish fatigued and fell back against the rear screen of the swim
138 tunnel, speed was immediately reduced to 1 BL s⁻¹ and, after 30 min, it was returned to a second
139 holding tank for at least 96 h under normal rearing conditions, prior to testing thermal tolerance.
140 Blank measurements were made before and after each trial and, if they increased over a trial,
141 were presumed to do so linearly. Background respiration did not exceed 10% of fish $\dot{M}O_2$, all data
142 were corrected accordingly.

143 During the constant acceleration element of the protocol, gait transition speed (U_{GT}) was
144 identified as when an individual began to engage an unsteady anaerobic gait, which presaged
145 fatigue (Blasco et al., 2020; Marras et al., 2013). The aerobic gait comprised steady body-caudal
146 swimming (rhythmic beating of the tail) that relies upon slow twitch oxidative muscle (Hachim et
147 al., 2021; Webb, 1998). The unsteady anaerobic gait comprised intermittent powerful tailbeats
148 that propelled the fish forward in the swim tunnel, after which it coasted back and then repeated
149 the powerful tailbeat, so-called 'burst and coast' swimming that relies on recruitment of fast-
150 twitch glycolytic muscle (Hachim et al., 2021; Webb, 1998). The net difference between U_{max} and
151 U_{GT} ($U_{max}-U_{GT}$) was calculated as a potential indicator of relative anaerobic swimming capacity.

152 **Tolerance of warming**

153 After suitable recovery in their holding tank, fish were measured for thermal tolerance by CT_{max}
154 and CTS_{max} . The order of the two tests was reversed for 50% of animals (Fig 1) to avoid any
155 potential systematic effect of exposure history on responses to the second test. CT_{max} was
156 measured on groups of tilapia that had acclimated overnight in a 68 L tank containing vigorously
157 aerated water at 26 °C. The following morning, water was warmed 1 °C every 30 min until loss of

equilibrium (LOE). Individual CT_{max} was then recorded as the highest temperature step fully completed plus the proportion of the last step that the fish endured prior to LOE (Blasco et al., 2020). Immediately upon LOE, fish were recovered at 26 °C for at least 30 min, in a 68 L tank of aerated water. If this was the first test applied (Fig 1), the animal was then returned to a holding tank for at least 96 h prior to the CTS_{max} .

For CTS_{max} each individual recovered overnight in the swim-tunnel at 1 BL·s⁻¹ then, the next day, speed was increased over 30 min until 85% of its own U_{GT} (Blasco et al., 2020). That is, each fish was exercised at a fixed proportion of their own aerobic swimming capacity, which was expected to impose a metabolic load that represented a similar proportion of their AS. After 30 min at that speed, temperature was increased by 1°C every 30 min until fish fatigued, resting against the rear screen (Blasco et al., 2020). The CTS_{max} was calculated as for CT_{max} but using fatigue as endpoint (Blasco et al., 2020). The fish was immediately placed in a recovery tank at 26 °C for 30 min. If this was the first test applied (Fig 1), the animal was then returned to a holding tank for at least 96 h prior to the CT_{max} .

At each temperature step during the CTS_{max} , measurements of $\dot{M}O_2$ were made over the last 20 min and the highest rate achieved was denoted as $\dot{M}O_{2max}$ (Blasco et al., 2020). Fatigue in the CTS_{max} was not simply due to lack of endurance, Nile tilapia acclimated to 26 °C can swim steadily at 85% of U_{GT} for at least 9 h, exceeding the duration of the CTS_{max} (Blasco et al., 2020). Blank measurements were made before all trials and at the highest temperature achieved after all trials, increases in background were presumed to be linear over temperature. Background respiration did not exceed 10% of fish $\dot{M}O_2$ and all data were corrected proportionally.

Statistical analysis

Statistics were performed with SigmaPlot 11. Relationships with body mass, of metabolic, performance and tolerance variables, were assessed by least squares regression of log-transformed data. Correlations between variables (CT_{max} vs CTS_{max} ; CTS_{max} vs $\dot{M}O_{2max}$) were assessed by Pearson product-moment. Mean CT_{max} and CTS_{max} were compared by paired T-test, assumptions of normality and equal variances were tested by Shapiro-Wilks and F-test, respectively. $P < 0.05$ was the limit for statistical significance.

Results

Metabolic and performance phenotype at acclimation temperature

Mass-independent SMR, AMR and AS showed highly significant positive relationships with mass (Table 1, Fig. S1, Electronic Supplementary Material). Slopes for SMR and AMR were less than 1,

190 indicating an allometric decline in metabolic rates with increasing mass (Table 1). By contrast, the
191 slope for AS was very close to 1, indicating that this trait scaled isometrically with mass (Table 1).
192 In order better to evaluate how metabolic traits depended on body mass (especially the $\dot{M}O_{2max}$
193 during CTS_{max} , see below), data are presented here as plots of mass-specific $\dot{M}O_2$ (Table 1, Figures
194 2 and 4). At 26 °C, log mass-specific SMR and AMR both showed a clear decline with increasing log
195 body mass, with significant negative linear relationships, (Table 1, Figure 2). Log mass-specific AS
196 did not, however, show any significant relationship to mass, indicating that it was maintained
197 unchanged as mass increased (Table 1, Figure 2). Relative swimming performance declined
198 significantly with mass, log U_{GT} and U_{max} both fell significantly with log mass, but $U_{max}-U_{GT}$ was
199 independent of mass (Table 2).

200 Thermal tolerance

201 As fish were warmed during CT_{max} they first exhibited erratic behaviour, rolling sideways, followed
202 by complete LOE. During CTS_{max} , fish swam using a steady aerobic gait up to a certain
203 temperature, beyond which they progressively engaged unsteady burst and coast anaerobic
204 swimming that led to fatigue. No fish lost equilibrium during CTS_{max} . All fish from both protocols
205 recovered normal swimming behaviour within 10 min at 26 °C. The two thermal thresholds were
206 not correlated ($r=0.371$, $P=0.129$) but overall mean (\pm SEM) CT_{max} , at $40.7\pm0.23^\circ\text{C}$, was significantly
207 higher than CTS_{max} , at $38.1\pm0.29^\circ\text{C}$. Log CT_{max} had no relationship with log body mass whereas log
208 CTS_{max} declined significantly with mass (Figure 3, Table 2).

209 At the outset of the CTS_{max} , when individuals were swimming at 85% of their U_{GT} at
210 acclimation temperature, absolute and mass specific $\dot{M}O_2$ declined against body mass with slopes
211 that were similar to those for AMR (Table 1, Figure S2, Electronic Supplementary Material). When
212 each individual's mass specific SMR was subtracted from their mass-specific $\dot{M}O_2$ at 85% of their
213 U_{GT} , the net difference represented $71 \pm 3\%$ (mean \pm SEM) of mass specific AS across all 18
214 individuals, with no significant relationship to their mass (Figure S3, Electronic Supplementary
215 Material). During the CTS_{max} , the $\dot{M}O_{2max}$ occurred at the temperature step that was either the
216 penultimate or final step endured, in all individuals except for one (mass 204g, see data in (Blasco
217 et al., 2022)). There was a highly significant negative relationship between mass-specific $\dot{M}O_{2max}$
218 and mass (Figure 4A, Table 1, see Figure S1 for the reciprocal allometric scaling relationship).
219 There was a significant positive correlation between log $\dot{M}O_{2max}$ and log CTS_{max} (Figure 4B).

220 Discussion

221 The results demonstrate that tolerance of acute warming declines with increasing body mass in a

222 teleost fish, when measured as the temperature at which individuals fatigue from sustained
223 aerobic swimming (CTS_{max}). The decline in CTS_{max} with mass was significantly correlated with a
224 decline in $\dot{M}O_{2max}$, which may indicate a causal relationship.

225 **Metabolic and performance phenotype at acclimation temperature**

226 The tilapia exhibited the significant mass-specific declines in SMR and AMR at acclimation
227 temperature that reflect the allometric mass-scaling relationships of metabolic rate found in all
228 fish species. Their underlying mechanisms remain a matter of debate (Glazier, 2020; Killen et al.,
229 2010; Killen et al., 2016). An absence of any effect of mass on AS has also been observed in
230 multiple species, the implication being that, although SMR and AMR decline with mass the animal
231 maintains the same absolute scope to provide its tissues with oxygen (Killen et al., 2016; Lefevre et
232 al., 2017). At the outset of the CTS_{max} protocol, when individuals were swimming at 85% of their
233 U_{GT} at acclimation temperature, their associated $\dot{M}O_2$ declined with mass but nonetheless
234 represented a constant proportion of their AS ($\sim 70\%$). The fact that relative U_{GT} and U_{max} ,
235 measured in $BL\ s^{-1}$, declined with mass at acclimation temperature was of course a direct
236 reflection of a tight relationship between mass and length in the tilapia. It is well-established that
237 relative swimming performance declines with length in most fish species (Bainbridge, 1958;
238 Beamish, 1978).

239 **Effects of mass on thermal tolerance and potential underlying mechanisms**

240 The finding that CT_{max} did not decline with increasing body mass is consistent with a previous
241 study on Nile tilapia (Recsetar et al., 2012). It may indicate that LOE during CT_{max} in tilapia is not
242 due to limitations in oxygen supply but instead occurs due to another mechanism. Although LOE
243 presumably reflects loss of function of critical organs such as the heart and central nervous
244 system, further study is required to understand the exact mechanism in tilapia or, for that matter,
245 in any fish species (Jutfelt et al., 2019; Lefevre et al., 2021). Given the absence of any dependence
246 of CT_{max} upon mass, it was interesting to find a clear negative dependence of CTS_{max} . This
247 dependence of CTS_{max} on mass in Nile tilapia should be investigated in other fish species, to
248 establish the generality of the phenomenon.

249 The profound decline in $\dot{M}O_{2max}$ with body mass, and the significant correlation of $\dot{M}O_{2max}$
250 with CTS_{max} , are also worthy of further investigation to establish whether capacity for oxygen
251 uptake is indeed a mechanism underlying size-dependence of CTS_{max} . This could, for example,
252 include measurements of cardiac performance, blood flows and blood gases during a CTS_{max} in
253 individuals of different sizes, in species that are amenable to both blood vessel catheterisation and

254 placement of flow probes. These results in the tilapia lend support to the Fry-OCLTT paradigm
255 whereby thermal tolerance in ectotherms can be limited by capacity for oxygen uptake (Blasco et
256 al., 2020; Fry, 1971; Pörtner, 2010). The fact that size-related variation in $\dot{M}O_{2max}$ only accounted
257 for about 60% of the variation in CTS_{max} indicates, however, that other mechanisms must have
258 contributed to fatigue in the tilapia. All individuals engaged unsustainable anaerobic swimming
259 prior to fatiguing (Blasco et al., 2020) and capacity for anaerobic exercise varies among individuals
260 in fish species (Marras et al., 2010; Marras et al., 2013). If, however, the difference between U_{max}
261 and U_{GT} ($U_{max}-U_{GT}$) is considered an indicator of relative anaerobic swimming capacity (Marras et
262 al., 2013), this was independent of mass and length.

263 It is also important to understand why $\dot{M}O_{2max}$ declined so significantly with mass, and to
264 establish whether this is also a general phenomenon across fish species. A regression coefficient of
265 0.93 on 18 fish indicates that the relationship is unlikely to be spurious in the tilapia, which is
266 therefore a stimulus to investigate the phenomenon in other fish species. The slope for the decline
267 in log mass-specific $\dot{M}O_{2max}$ with log mass in the tilapia, of -0.33, would be expected if the capacity
268 for oxygen uptake were subject to surface to volume geometric constraints (Glazier, 2018; Glazier,
269 2020), notably if fishes were subject to the simple geometric constraints of spherical bodies (Pauly,
270 1981). Fishes are not, of course, spherical bodies; their gills are folded surfaces which, as an
271 individual grows, can perfectly compensate increases in body volume with increases in respiratory
272 surface area (Lefevre et al., 2017). This is why, at a given acclimation temperature, AS scales
273 isometrically with body mass across multiple species (Lefevre et al., 2017), as it did with the tilapia
274 in this study. It is known, however, that gill functional respiratory surface area declines with mass
275 in fishes, in tilapia it does so with a slope of -0.30 (Kisia and Hughes, 1992). This decline in gill
276 surface area with mass may be linked to the decline in mass-specific SMR, whereby basal
277 metabolic costs decline and the fish gills are then 'tailored' to maintain a constant scope for
278 aerobic performance at any given mass. Maintaining a greater gill respiratory surface area than is
279 needed for AS, at any given acclimation temperature, could have costs in terms of, for example,
280 regulating water and ion balance (Lefevre et al., 2017; Taylor, 1998). As a consequence, however,
281 the fish also show a decline in their maximum mass-specific oxygen uptake capacity at acclimation
282 temperature, estimated as AMR. This design characteristic, of reduced gill surface area with
283 increasing mass, would then become a constraint when large individuals face the profound
284 challenge to oxygen uptake that the CTS_{max} protocol imposed. Although the established mass
285 related decline in gill respiratory surface area in the Nile tilapia seems like a coherent

286 explanation for the decline in $\dot{M}O_{2\max}$ with mass, there may be multiple contributing factors,
287 including other elements of the oxygen transport cascade. This is a topic that deserves further
288 investigation, especially on species that are amenable to catheterisation and placement of flow
289 probes.

290 **Ecological implications**

291 Extreme warming events are predicted to increase in frequency and severity in aquatic habitats
292 worldwide due to accelerating global climate change (Field et al., 2012; Frölicher et al., 2018;
293 Stillman, 2019). There are clear ecological implications to the fact that, if tilapia populations
294 experience an acute warming event, the ability of larger fishes to perform a given level of aerobic
295 work would be constrained at lower temperatures than that of smaller conspecifics. Although we
296 concentrated on aerobic swimming, larger tilapia would also presumably be constrained in their
297 ability to perform other activities at warm temperatures, for example the specific dynamic action
298 response that follows ingestion of a meal and is believed to be in large part the cost of tissue
299 anabolism and growth (McCue, 2006). The law of averages would predict that larger fishes are
300 more likely to encounter temperatures sufficiently warm to limit their performance (Field et al.,
301 2012; Stillman, 2019). Therefore, stochastic extreme warming events could threaten ecological
302 performance, fitness and survival of fishes in a size-dependent manner (Habary et al., 2017;
303 Stillman, 2019). If fitness and survival of larger animals is compromised more frequently, this could
304 have important consequences for Nile tilapia fisheries, an important source of dietary protein in
305 many countries (De Silva et al., 2004).

306 Overall, the data indicate that capacity for oxygen uptake may be a factor limiting thermal
307 tolerance in fishes, and also that global warming may 'favour the small' (Daufresne et al., 2009).
308 Further work is required to understand if this size-dependence in temperature tolerance is true in
309 other fish species. The evidence we provide, that larger individuals are more prone to limitations
310 in their capacity for oxygen uptake at warmer temperatures, is coherent with a mechanism
311 suggested to underly the TSR (Verberk et al., 2021). That is, that species may have evolved to
312 modify their growth trajectories at warmer temperatures, to avoid constraints on aerobic
313 activities in adults (Atkinson et al., 2006; Hoefnagel and Verberk, 2015; Leiva et al., 2019;
314 Rubalcaba et al., 2020; Verberk et al., 2021). This, in turn, might be linked to ongoing declines in
315 maximum body size of fishes globally (Audzijonyte et al., 2019; Baudron et al., 2014; Daufresne et
316 al., 2009). The rate of global warming is too fast for adaptive evolutionary responses by most fish
317 species, so survival and resilience will depend upon the options for migration, the prevailing

318 plasticity in tolerance, and the existence of tolerant genotypes (Bell, 2013; Gunderson and
319 Stillman, 2015; Habary et al., 2017; Stillman, 2019). The current data indicate that capacity for
320 migration may be impaired at lower temperatures in larger fish, and that smaller animals are more
321 tolerant. This could also contribute to a decline in maximum body size in future populations.

322

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328

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515 Figure legends

516 **Figure 1.** Schematic of the treatment protocol. Individuals were PIT-tagged, recovered at least 96h
517 then submitted to a swim performance test (Swim test) in a swim tunnel with respirometry (R) to
518 measure the performance and metabolic phenotype at acclimation temperature (26°C). After at
519 least 96h recovery, 50 % of individuals were submitted to a critical thermal maximum (CT_{max}) test,
520 warming in small groups until loss of equilibrium, followed by at least 96h recovery. They were
521 then submitted individually to a critical thermal maximum for aerobic swimming (CTS_{max}) test in a
522 swim tunnel until fatigue with R, to measure the maximum rate of oxygen uptake achieved prior
523 to fatigue. The other 50% were submitted in the opposite order, CTS_{max} and then CT_{max}.

524 **Figure 2.** Least squares regression of the log:log relationship between body mass and mass-specific
525 rates of oxygen uptake equivalent to standard metabolic rate (SMR); active metabolic rate (AMR),
526 and aerobic scope (AS). Data are for n = 18 Nile tilapia, mass range 21 to 313g, acclimated to 26°C.
527 Regression lines are for variables where relationship is significant, lines denote best fit, see Table
528 1.

529 **Figure 3.** Least squares regression of the log:log relationship between body mass and two critical
530 temperatures (CT) for tolerance, critical thermal maximum (CT_{max}, red symbols) and critical ther-
531 mal maximum for aerobic swimming (CTS_{max} blue symbols, dotted regression line, see Table 2 for
532 best fit regression information). Data are for n = 18 Nile tilapia, mass range 21 to 313g, acclimated
533 to 26°C, the CTS_{max} showed a significant negative relationship with mass, see Table 1.

534 **Figure 4.** (A) Least squares regression of the log:log relationship between body mass and mass-
535 specific maximum rate of oxygen uptake achieved during CTS_{max} ($\dot{M}O_{2max}$, see Table 1 for best fit
536 regression information) and (B) Pearson correlation between critical thermal maximum for aerobic
537 swimming (CTS_{max}) and $\dot{M}O_{2max}$, dotted line is described by $\log CTS_{max} = (\log \dot{M}O_{2max}) \times 0.061 +$
538 1.491 ($r = 0.637$, $P = 0.004$). Data are for n = 18 Nile tilapia, mass range 21 to 313g, acclimated to
539 26°C.

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