

1 Rapid-warming tolerance correlates with tolerance to slow warming but
2 not growth at non-optimal temperatures in zebrafish

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11 Keywords: Thermal tolerance, CT_{max} , Warming rates, Growth, Thermal syndrome, Climate
12 change, Thermal biology, Teleost

13

14 Summary statement: We show that critical thermal maximum (CT_{max}), measured at a rapid
15 warming rate, is a relevant proxy for more prolonged thermal challenges, but cannot be used to
16 predict growth rate in zebrafish.

17 **Abstract**

18

19 Global warming is predicted to increase both acute and prolonged thermal challenges for aquatic
20 ectotherms. Severe short and medium-term thermal stress over hours to days may cause mortality,
21 while longer sub-lethal thermal challenges may cause performance declines. The interrelationship
22 between the responses to short, medium and longer thermal challenges is unresolved. We asked if
23 the same individuals are tolerant to both rapid and slow warming challenges, a question which has
24 so far received little attention. Additionally, we investigated the possibility of a thermal syndrome
25 where individuals in a population are distributed along a warm-type to cold-type axis. We tested
26 whether different thermal traits correlate across individuals by acclimating 200 juvenile zebrafish
27 (*Danio rerio*) to sub- or supra- optimal temperatures for growth (22 and 34°C) for 40 days and
28 measured growth and thermal tolerance at two different warming rates. We found that tolerance to
29 rapid warming correlated with tolerance to slow warming. However, individual tolerance to
30 neither rapid nor slow warming correlated with growth at the supra-optimal temperature. We thus
31 find some support for a syndrome-like organisation of thermal traits, but the lack of connection
32 between tolerance and growth-performance indicates a restricted generality of a thermal
33 syndrome. The results suggest that tolerance to rapid warming may share underlying physiological
34 mechanisms with tolerance to slower heating, and indicate that the relevance of acute critical
35 thermal tolerance extends beyond the rapid ramping rates used to measure them.

36 **Introduction**

37

38 Climate change is imposing a range of different thermal challenges on organisms. At the end of the
39 century, the mean global temperature is projected to increase by 1.5-5°C compared to pre-industrial
40 time (Pachauri et al., 2014). Additionally, weather is becoming more extreme and variable, with
41 heat waves predicted to increase in both frequency and severity (Perkins et al., 2012). In this context,
42 ectothermic animals may be especially vulnerable as their body temperature often directly follows
43 that of their environment (Angilletta Jr and Angilletta, 2009).

44

45 There are two main views on how aquatic ectotherms may be directly affected by a warming climate.
46 The acute upper thermal tolerance view proposes that survival during short-duration transient heat
47 waves (hours to days) is the dominant determinant of fitness during warming. This view has support
48 from observations that global distribution patterns of species match acute upper thermal tolerance
49 measurements (Sunday et al., 2012), from mass mortality in nature during warming (Wegner et al.,
50 2008) and from findings that populations can function and perform well up to very close to their
51 lethal temperature (Morgan et al., 2019; Sandblom et al., 2016). On the other hand, the upper thermal
52 performance view focuses on the level of functioning of important traits such as growth, fecundity,
53 and locomotion during longer time scales at temperatures above optimal but below lethal. The support
54 for this view comes from of medium- and long-term laboratory experiments, as well as field
55 observations (Pörtner and Knust, 2007), where sub-lethal fitness effects such as reduced growth and
56 fecundity occur at supra-optimal temperatures (Gräns et al., 2014; Morgan, 2020; Pörtner et al., 2001;
57 Rogers et al., 2011). It is unknown if these two views of thermal effects can be united by any common
58 principles of thermal physiology. That is, do traits for survival during acute warming correspond with
59 traits for higher thermal performance? Currently, knowledge is lacking on both the causes of variation
60 in thermal traits (Schulte, 2015; Somero, 2010) and whether different thermal traits are independent,
61 or linked by underlying mechanisms. A potential linkage between different thermal traits would not
62 only give clues to the underlying mechanisms but would also have major implications for how
63 selection on these traits occur under climate change.

64

65 The critical thermal maximum (CT_{max}), a form of acute upper thermal tolerance, is one of the traits
66 most commonly used to test the thermal biology of a species. CT_{max} is the temperature at which
67 some specified endpoint occurs as the organism's body temperature is being steadily ramped

68 upwards from its acclimation temperature (Becker and Genoway, 1979; Lutterschmidt and
69 Hutchison, 1997; Morgan et al., 2018; Morgan et al., 2019). In fishes, two commonly used endpoints
70 are the onset of muscle spasms and the loss of equilibrium (LOE), the latter being a state where the
71 fish loses the ability to maintain an upright swimming position. The endpoint represents a state
72 where the animal, while still alive, could be considered ecologically dead as in nature it would be
73 unable to escape its condition. Measurements of CT_{max} has become a common measure of thermal
74 tolerance, and it is increasingly being used to connect thermal physiology to the consequences of
75 climate change (Comte and Olden, 2017; Deutsch et al., 2008; Sandblom et al., 2016; Sunday et al.,
76 2012)

77

78 Despite its frequent use, knowledge is lacking on what the CT_{max} tells us about the overall thermal
79 physiology of an ectotherm (Kovacevic et al., 2019), and whether or not it can predict warming
80 tolerance in nature. While having been linked with geographical distributions of species, few
81 attempts have been made to link this trait with other thermal performance traits, like growth,
82 fecundity, or locomotion. Additionally, the recommended warming rate for CT_{max} assays of fish is
83 $0.3^{\circ}\text{C min}^{-1}$ (Becker and Genoway, 1979), but warming events in the wild may occur over longer
84 timeframes. To differentiate CT_{max} at $0.3^{\circ}\text{C min}^{-1}$ from tolerance to other warming rates we use the
85 terms rapid-warming tolerance and slow-warming tolerance. It is a well established pattern that the
86 rate of warming affects the temperature where LOE occurs (Mora and Maya, 2006) and that this
87 relationship varies between species and taxa (Kovacevic et al., 2019), but it remains unknown if
88 individuals with a high rapid-warming tolerance are also more tolerant to slow warming (Fig.1A).
89 Generally, thermal tolerance is reduced when the warming rate is slower, and it has been
90 hypothesised that this is because the slower warming rates increasing the time spent at each
91 successive temperature exhaust the animal before a higher critical temperature is reached (Morgan,
92 2020; Rezende et al., 2014). Another possibility is that different warming rates impact different
93 physiological mechanisms, meaning that slow- and rapid-warming tolerances should be considered
94 disparate traits.

95

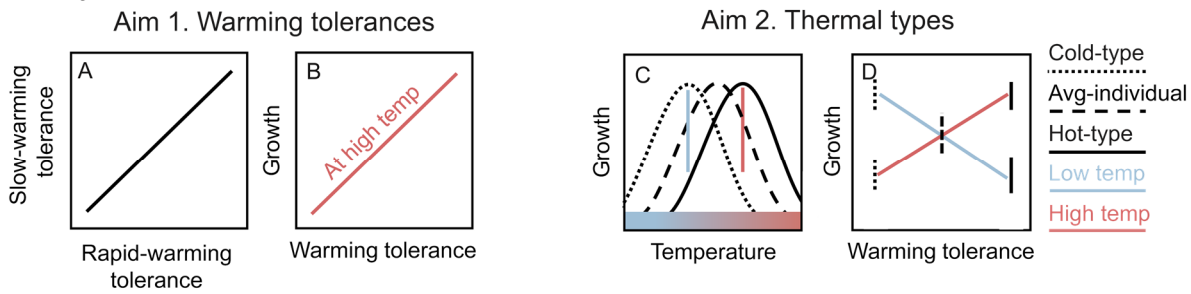
96 Syndrome theory is a theoretical framework for studying systems of correlated traits (Sih et al.,
97 2004). Syndrome theory has mostly been applied to animal personality research, but based on the
98 tight relationship between temperature, metabolism, and behaviour (Biro and Stamps, 2010), Goulet
99 et al (2017a) suggested that thermal physiology could be included into the pace-of-life syndrome

100 hypothesis (POLS). In this hypothesis, consistent differences in behavioural traits are suggested to
101 co-vary with life-history and physiological traits like growth and metabolism, placing individuals
102 along a fast-slow life-history axis (Réale et al., 2010). Goulet et al (2017a) proposed that the
103 individual's thermal type would align with their behavioural and life-history types. According to
104 this framework, thermal traits would be configured into a thermal syndrome with individuals
105 distributed along a cold-hot axis, and their position in this continuum corresponding to different
106 thermal types. The inclusion of thermal tolerance in this system has so far not been tested, and
107 expanding this system to include this, cold-type individuals at one end of the axis would have left-
108 shifted thermal performance curves, performing better at lower temperatures and having lower
109 critical thermal limits; the opposite would be the case for hot-type individuals at the other end of the
110 axis (Fig.1C, D). Recent studies on delicate skinks (*Lamprolophus delicata*) have revealed some
111 interesting connections between thermal preference, thermal sprint performance, habitat selection,
112 and traits related to boldness, exploration and social behaviour (Goulet et al., 2017a; Goulet et al.,
113 2017b; Michelangeli et al., 2018). These findings support individuals existing on a cold-hot axis
114 with corresponding behavioural traits, but the role of thermal tolerance in such a system is so far
115 unexplored.

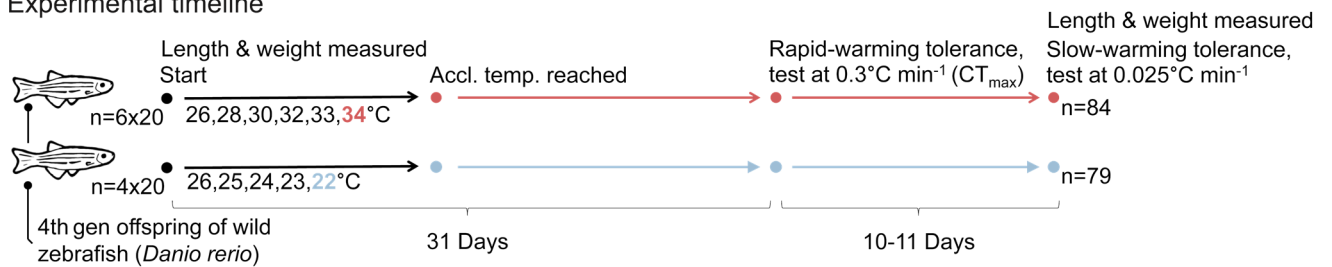
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117 This experiment had two aims. Aim 1 was to test the relevance of CT_{max} under rapid-warming as a
118 measure for predicting slow-warming tolerance as well as performance under supra-optimal
119 temperatures. We predicted correlations between rapid- and slow-warming tolerance, measured as
120 loss of equilibrium temperature at the warming rates $0.025^{\circ}\text{C min}^{-1}$ and $0.3^{\circ}\text{C min}^{-1}$ as well as
121 growth-performance at a supra-optimal holding temperature (Fig.1A, B). Aim 2 was to test the
122 hypothesis that thermal traits are linked in such a way that they form a thermal syndrome where
123 individuals are distributed along a cold-type to hot-type continuum. We predicted that thermal traits
124 are correlated so that cold-type individuals have both lower thermal tolerance and a left-shifted
125 thermal performance curve, giving them comparably higher growth at sub-optimal temperatures
126 than hot-type individuals. This would be seen as a correlation between thermal tolerance and growth
127 performance that becomes negative when temperature is below optimal, and positive when above
128 optimal (Fig.1C, D).

A-D. Project aims



E. Experimental timeline



129

130 **Fig. 1. Experimental aims and design.** (A-D) Graphic representation of the predictions from the
 131 two experimental aims. Aim 1: testing the hypothesis that tolerance to rapid warming also confers
 132 tolerance to slow warming and increased growth at supra-optimal temperatures. This would lead to
 133 (A) a correlation between rapid- and slow-warming tolerance, as well as (B) a correlation between
 134 warming tolerance and growth. Aim 2: testing the hypothesis that thermal traits like thermal
 135 tolerance and performance are linked within individuals, placing individuals on a continuum from
 136 cold-types to hot-types. Specifically, we hypothesised that thermal traits are correlated so that cold-
 137 type individuals have a lower thermal tolerance and a (C) left-shifted thermal performance curve for
 138 growth. Given this, we predicted that (D) individuals with low warming tolerance have higher
 139 growth at sub-optimal temperatures than individuals with a high thermal tolerance, while the
 140 opposite would be true for hot-type individuals. (E) Timeline of the experiment. A total of 200
 141 individually tagged zebrafish, raised at 26°C , were divided into two treatments to be exposed to
 142 either 22°C or 34°C . All fish were tested for thermal tolerance at a warming rate of $0.3^{\circ}\text{C min}^{-1}$
 143 (rapid-warming tolerance; CT_{max}) after 31 days of thermal exposure, and thermal tolerance at a
 144 warming rate of $0.025^{\circ}\text{C min}^{-1}$ (slow-warming tolerance) ten or eleven days after that. All
 145 individuals were measured for weight and length at the beginning and end of the experiment.

146 **Materials and methods**

147

148 *Experimental procedure*

149 The fish used in this experiment were third-generation offspring of wild-caught zebrafish (*Danio*
150 *rerio* Hamilton 1822) from West Bengal, India, a strain brought into the lab in November 2016. We
151 used a total of 200 juvenile zebrafish, about 40 days old, which were randomly divided into two
152 treatment groups to be acclimated at $22\pm 0.2^\circ\text{C}$ (sub-optimal temperature, $n = 80$) and $34\pm 0.2^\circ\text{C}$
153 (supra-optimal temperature, $n = 120$). We chose temperature treatments at 22°C and 34°C based on
154 an earlier unpublished acclimation experiment (Morgan et al., 2020 in prep), where we observed an
155 equally reduced growth at these two temperatures, being about 60% of what was observed at the
156 optimal temperature for growth ($28\text{--}30^\circ\text{C}$). We used a higher sample size in the 34°C treatment to
157 compensate for the possibility of increased mortality due to individuals reaching a higher CT_{max} in
158 this treatment group. Individuals were then divided into 10 tanks each containing 20 fish, with six
159 tanks for the 34°C treatment and four tanks for the 22°C treatment. Before being distributed into
160 their tanks, fish were tagged and measured while under anaesthesia (110 mg L^{-1} buffered tricaine
161 methanesulfonate (MS-222)). Visible implant elastomer tags (Northwest Marine Technologies,
162 Shaw Island, WA, USA) were subcutaneously injected at the left and right side of their dorsal fin
163 using a 0.5 mm syringe in different colour combinations (Hohn and Petrie-Hanson, 2013). Weight
164 was measured down to nearest microgram using a digital precision scale. Standard length, defined
165 as the distance from snout to base of tail, was measured down to nearest micrometre using a digital
166 calliper. Initial holding temperature was $26\pm 0.2^\circ\text{C}$ and temperature acclimation started after two
167 days of habituation to the holding tanks. The temperature was reduced by 1°C every day in the 22°C
168 treatment until 22°C was reached. In the 34°C treatment, the temperature was increased by 2°C
169 every day until 32°C , and 1°C every day until 34°C . Thus, final acclimation temperatures were
170 reached after six days. The fish were tested for rapid-warming tolerance (CT_{max} ; $0.3^\circ\text{C min}^{-1}$) after
171 22 days at their respective acclimation temperature, and slow-warming tolerance ($0.025^\circ\text{C min}^{-1}$)
172 10 days after that (Fig. 1B). Each fish was tested in both protocols. The experiment was approved
173 by the Norwegian Animal Research Authority (permit number: 8578). Experimental procedures and
174 care of animals were done following all relevant local guidelines and policies.

175 *Holding conditions*

176 Holding aquaria of 45 L (50 x 30 x 30 cm) were environmentally enriched with a red and green
177 plastic ornamental plant, had sponge biofilters used for filtration, air bubbling, and water circulation
178 (Fig. S1). Temperature was controlled using a thermostat (ITC-310T, Inkbird, Shenzhen, China) and
179 one titanium heater (TH-100, Aqua Medic, Bissendorf, Germany) in each tank. Tanks in the 34°C
180 treatment had an extra titanium heater installed, as well as an air-stone for improving water
181 circulation over the heaters. Lighting was set on a 12 h/12 h dark/light cycle. Salinity was kept at
182 0.3 ppt using natural sea salt. Fish were fed ground up TetraPro energy flakes ad libitum twice a day
183 (Tetra®, Blacksburg, VA, USA). Water was replaced after 13 and 15 days for the 22°C and 34°C
184 tanks, respectively.

185

186 *Thermal tolerance measurements*

187 Two separate procedures were used to test thermal tolerance, one with a 0.3°C min⁻¹ and one with a
188 0.025°C min⁻¹ warming rate. In both procedures, loss of equilibrium (LOE) was used as the test
189 endpoint (Becker and Genoway, 1979). We defined the loss of equilibrium as the state where the
190 fish had, for more than three seconds, been unable to right itself and maintain an upright swimming
191 position. Water temperature at LOE was recorded using a high precision digital thermometer with a
192 ±0.1°C precision (Testo -112, Testo, Lenzkirch, Germany).

193 The rapid-warming tolerance test (CT_{max}; 0.3°C min⁻¹) was conducted using a heating tank (25 x 22
194 x 18 cm) filled with nine litres of water at the acclimation temperature for each treatment. The tank
195 had a heating element and a pump for circulation and a detailed description of this CT_{max} setup can
196 be found in Morgan et al (2018). Ten individuals were tested simultaneously in the same heating
197 tank, and tolerance was defined as the temperature where LOE occurred for each individual. Each
198 individual was immediately removed from the heating tank after LOE and put in a small holding
199 tank at its respective acclimation temperature to recover before it was relocated to its holding tank.

200 The slow-warming tolerance test (0.025°C min⁻¹) was conducted in the holding tanks on the last day
201 of the experiment. The water level was reduced to 10 cm (15 L), filters and ornamental plants were
202 removed, and the titanium heater (TH-100, Aqua Medic, Bissendorf, Germany) was placed
203 horizontally and close to the water surface on each tank's longest wall with the air stone placed
204 underneath to provide circulation over the heater. The thermometer for recording water temperature
205 at LOE was placed on the opposite side of the tank. A thermostat (ITC-310T, Inkbird, Shenzhen,
206 China) was used to control the titanium heater while gradually heating the water. The thermostat's

207 thermal probe was placed close to the air stone to keep it close to the water flow but underneath the
208 heater. Temperature was recorded as each individual reached LOE, and the individual was
209 immediately euthanized, weighed, and measured.

210

211 *Statistical analysis*

212 All analyses were done using the R 3.5.1 software environment (R Core Team, 2019). Growth was
213 defined as the percentage increase in body mass during the experiment, calculated using the initial
214 and final weight. Growth rates accounting for time in the experiment (assuming an equal growth
215 rate each day) were calculated as percentage growth in mass per day and thus useful for comparing
216 between experiments (Eqn S1). Only individuals that survived through the entire experiment were
217 included in the analysis. All comparisons on growth and thermal tolerance between the acclimation
218 treatments were tested using two-tailed t-tests. Any tank-effect on thermal tolerance or growth was
219 tested using an ANOVA analysis on a linear model with holding tank as the independent variable
220 against slow-warming tolerance, rapid-warming tolerance or growth as the dependent variable.
221 Holding tank was found to significantly affect both types of thermal tolerance, but not growth (Table
222 S1). Small variation in tank temperature is a likely reason, causing differences in acclimated
223 temperature. To account for tank effects on the inter-individual differences these data were mean
224 centred (m.c.), which re-defined each measurement as its difference from its respective tank mean.
225 The mean centred values for growth and thermal tolerance at both warming rates have the same
226 variance as the raw values and the mean within each tank is centred on 0 (Fig. S2). For transparency,
227 results in Table 1 show correlations using both raw and mean centred values (Table 1). We chose to
228 use mean-centring on the growth measurements as well for consistency, even though tanks didn't
229 affect the growth results. Correlations were tested using Pearson's product-moment correlation
230 between all three measurements (rapid-warming tolerance, slow-warming tolerance, and growth).
231 Two separate sets of correlation were tested, using either raw uncorrected values or mean-centred
232 values (Table 1). Outliers were defined as values being over five times the interquartile range beyond
233 either the upper or lower quartile. After all data was collected, one individual from the 34°C with a
234 slow-warming tolerance at 38.5°C was removed, being a lower-range outlier and the cause of a
235 likely false correlation between rapid-warming and slow-warming tolerance.

236 **Results**

237

238 *Weight and growth*

239 Mean weight of all individuals was 0.078 ± 0.019 g (mean \pm SD) before acclimation and 0.118 ± 0.024
240 g at the end of the experiment, equivalent to a 56.85 ± 43.74 % increase, or a growth rate of 1.02 ± 0.05
241 % mass increase per day. There was no significant difference in growth between the acclimation
242 treatments ($t = 0.80$, $p = 0.42$)(Fig. 2B).

243

244 *Mortality and outliers*

245 In the 22°C treatment, there was a mortality of 1% through the entire experiment, while the 34°C
246 treatment had a mortality of 30% after the rapid-warming test, leaving the final number of
247 individuals tested under both slow and rapid-warming at 79 in the 22°C treatment and at 84 in the
248 34°C treatment. One individual from the 34°C treatment was removed as a lower-range outlier
249 having a slow-warming tolerance at 38.5°C, causing a likely false correlation between rapid-
250 warming and slow-warming tolerance (Fig. 2A). Given the high mortality, future experiments
251 should avoid CT_{max} testing on zebrafish acclimated to high temperatures.

252

253 *Correlations*

254 Rapid-warming tolerance and slow-warming tolerance correlated significantly in the 22°C
255 acclimation group ($r = 0.390$, $p = 0.0004$). In the 34°C group, this correlation was positive, but only
256 near-significant ($r = 0.200$, $p = 0.071$). Growth and rapid-warming tolerance (CT_{max}) did not
257 correlate in any of the treatments. Growth and slow-warming tolerance only correlated significantly
258 in the 22°C treatment when using uncorrected raw values ($r = 0.240$, $p = 0.070$). Correction using
259 mean centred values adjusting for tank-effects resulted in a near-significant, positive correlation (r
260 $= 0.205$, $p = 0.070$) (Table 1).

261

262 *Thermal tolerances*

263 Acclimation temperature significantly affected thermal tolerance at both rapid ($t = -55.91$, $p <$
264 0.0001) and slow warming ($t = -41.00$, $p < 0.0001$) (Fig 2A). Rapid-warming tolerance was

265 38.83±0.62°C and 42.99±0.23°C in the 22°C and 34°C treatment, respectively. In the same order,
 266 slow-warming tolerance was 40.36±0.33°C and 42.07±0.16°C. In the 22°C treatment, rapid-
 267 warming tolerance was significantly lower than slow-warming tolerance ($t = -19.21$, $p < 0.0001$),
 268 while in the 34°C treatment the rapid-warming tolerance was significantly higher than the slow-
 269 warming tolerance ($t=29.96$, $p<0.0001$) (identity line, Fig. 2A). Average duration of the slow-
 270 warming tolerance test was 743 and 322 minutes in the 22 and 34°C treatment, respectively. Average
 271 duration of the rapid-warming tolerance test was 56 and 30 minutes.

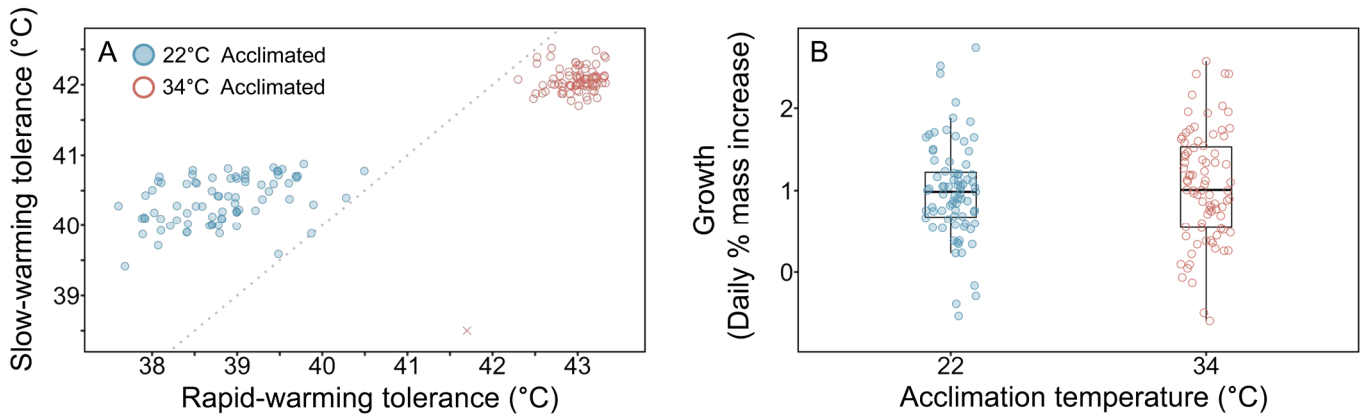
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273 **Table 1. Correlations between rapid-warming tolerance, slow-warming tolerance, and growth.**

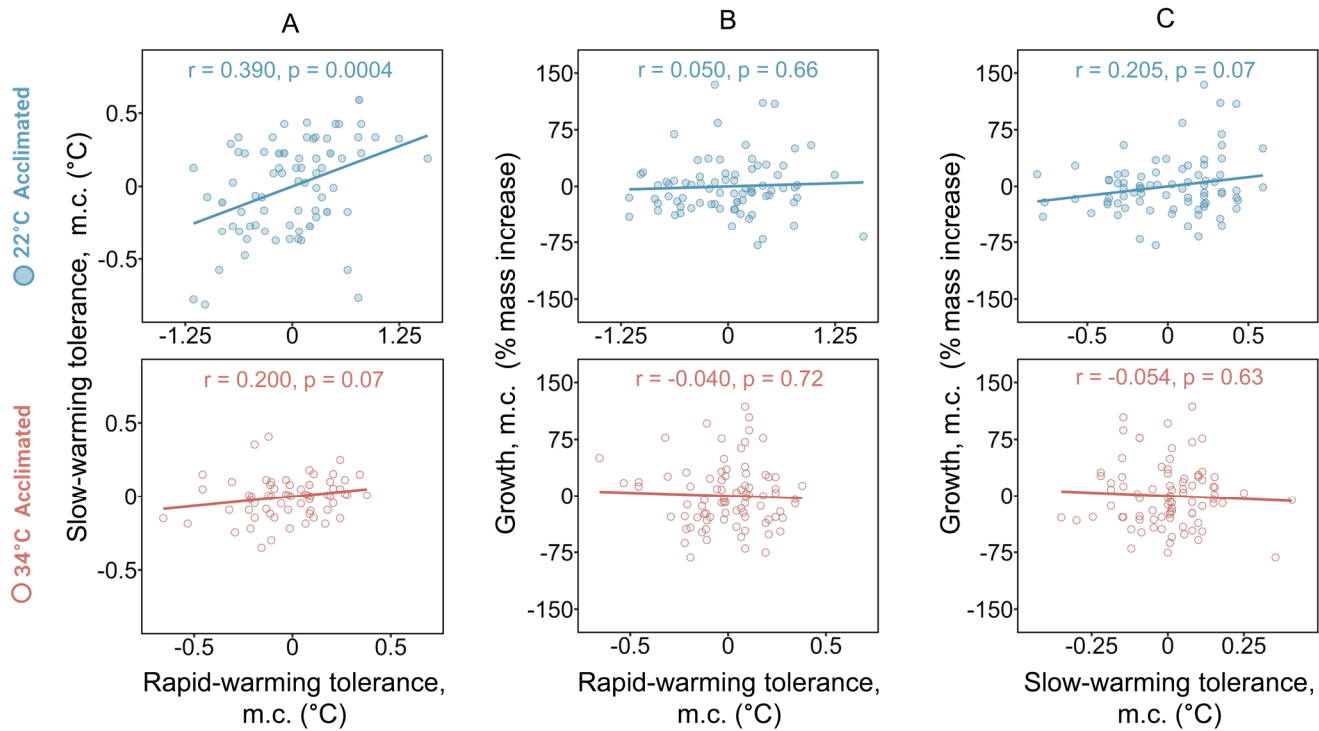
274 The table includes Pearson’s correlation coefficient (r) and corresponding p -values for correlations
 275 between all combinations of growth; rapid-warming tolerance, measured as the temperature where
 276 loss of equilibrium (LOE) occurs at a warming rate of $0.3^\circ\text{C min}^{-1}$ (also known as CT_{max} ; Critical
 277 thermal maximum) and slow-warming tolerance (LOE at a warming rate of $0.025^\circ\text{C min}^{-1}$) at two
 278 acclimation temperatures (22 and 34°C). To correct for tank-effects, mean-centring (m.c.) was done
 279 by redefining each value as its deviance from tank mean. Correlations were tested using both raw
 280 values and mean-centred values. Numbers in bold signify significant p -values below 0.05, and italics
 281 signify near-significant p -values below 0.1.

Relationship	Acclimated temp (°C)	Raw values		Mean centred (m.c.)	
		Correlation I		Correlation II	
		r	p	r	p
<i>Growth - slow-warming tolerance</i>	22	0.240	0.033	0.205	<i>0.070</i>
	34	-0.021	0.849	-0.054	0.629
<i>Growth - rapid-warming tolerance</i>	22	0.117	0.306	0.050	0.662
	34	-0.019	0.863	-0.040	0.720
<i>Rapid-warming tolerance - slow-warming tolerance</i>	22	0.416	0.0001	0.390	0.0004
	34	0.060	0.588	0.200	<i>0.071</i>

282



283
 284 **Fig. 2. Temperature; growth; and rapid- and slow warming tolerance.** Results are for two
 285 acclimation treatments of juvenile zebrafish at 22°C (n = 79) and 34°C (n = 80). (A) Shows
 286 Individuals' rapid-warming tolerance, measured as the temperature where loss of equilibrium (LOE)
 287 occurs at a warming rate of 0.3°C min⁻¹ (also known as CT_{max}; Critical thermal maximum) and slow-
 288 warming tolerance (LOE at a warming rate of 0.025°C min⁻¹) for both treatments. Fish were tested
 289 for rapid-warming tolerance after 22 days of acclimation and slow-warming tolerance after 32 days.
 290 The identity line is drawn with grey dots. The X indicates a removed outlier from the 34°C treatment.
 291 (B) Growth (displayed as daily per cent mass increase) for all included individuals in the two
 292 treatments. Points are jittered in both A and B to reveal overlapping points, but only horizontally in
 293 B, and no more than 0.02°C in A.
 294



295

296 **Fig. 3. Correlations between rapid-warming tolerance, slow-warming tolerance, and growth.**

297 The figure shows correlations including Person's correlation coefficient (r) and respective p-values
 298 between all combinations (A, B, C) of rapid-warming tolerance, measured as the temperature where
 299 loss of equilibrium (LOE) occurs at a warming rate of $0.3^{\circ}\text{C min}^{-1}$ (also known as CT_{max} ; Critical
 300 thermal maximum); slow-warming tolerance (LOE at a warming rate of $0.025^{\circ}\text{C min}^{-1}$) and growth
 301 (% mass increase over 32 days) for two acclimation treatments of zebrafish at 22°C ($n = 79$) and
 302 34°C ($n = 80$). Measurements are corrected for tank-effects by mean-centring (m.c.) all values on
 303 their respective tank-means. Lines are fitted using least-square regression for each plot's values and
 304 are for illustrative purpose only.

305

306 **Discussion**

307

308 Upper thermal tolerance under rapid warming correlated significantly with upper thermal tolerance
 309 under slow warming, demonstrating that individuals with high tolerance to rapid warming (30-60
 310 minutes until LOE) are also individuals tolerant to slow warming (5-12 hours until LOE). This
 311 means that rapid CT_{max} measurements can be used as a quick and practical proxy for estimating
 312 more general thermal tolerance. The finding thus extends the importance of CT_{max} measurements

313 from a being a laboratory test of acute thermal tolerance to a potentially ecologically relevant metric
314 for estimating tolerance to heat waves in nature.

315

316 At the 34 °C acclimation treatment, the correlation between slow- and rapid-warming tolerance was
317 weaker and only near-significant. The reduced strength of this correlation may have been caused by
318 individuals in this treatment being acclimated closer to their ceiling in terms of achievable thermal
319 tolerance, thus reducing variation (Morgan et al., 2019; Pintor et al., 2016) and increasing the
320 relative measurement error, masking the correlation and making it harder to detect. Although the
321 results were less clear in this treatment, they are still suggestive of a link between these traits.

322

323 Zebrafish is a species where acute upper thermal tolerance could be central to its populations'
324 survival under climate change. Historically, the peak temperature of heat waves in the north-east of
325 India (a central part of the zebrafish range) has been in the range of 40-45°C (air temperature), with
326 a duration around one to four days and a frequency of one to two occurrences per season (Murari et
327 al., 2015). With some scenarios of carbon emissions (Pachauri et al., 2014), the peak temperature,
328 duration, and frequency are likely to increase in this region (Murari et al., 2015). Zebrafish are often
329 found in shallow, low-flow freshwater habitats (Engeszer et al., 2007; Sundin et al., 2019), making
330 them naturally exposed to rapid thermal fluctuations. Survival of zebrafish in these areas is thus
331 depending on the ability to survive higher temperatures and longer heat waves. An increase in the
332 severity of thermal challenges may thus select for more thermally tolerant individuals. The results
333 of this experiment suggest that slow- and rapid-warming tolerance could be co-selected under these
334 circumstances as closely connected traits.

335

336 A potentially important, although unquantified observation during our thermal challenges tests was
337 that the nature of the LOE changed between the two warming rates. At the standard, rapid warming
338 rate, zebrafish display a distinct disorganisation response, characterized by fast, erratic swimming
339 coupled with an inability to remain upright. Under slow warming, however, it was more common
340 for the fish to lose equilibrium from what appeared to be exhaustion. That is, instead of swimming
341 without a righting response (as in the rapid-warming test), the fish simply stopped swimming, and
342 thus also lost their ability to remain upright. These different responses leading to LOE suggest
343 different underlying mechanisms ultimately causing the LOE at the two warming rates. The almost

344 instant LOE and disorganization-reaction during the rapid warming suggest an immediate failure of
345 some vital mechanism, like cardiac (Sidhu et al., 2014) or neurological malfunctioning (Jutfelt et
346 al., 2019; Miller and Stillman, 2012; Robertson, 2004), while the slow exhaustion-like response
347 during the slow warming may suggest a gradual build-up of some malfunction, metabolic waste
348 products, or the exhaustion of some system. Still, the correlation found in this experiment suggest
349 that important links between these two traits exist. One source of this correlation could be a more
350 fundamental mechanism that ultimately governs both long-term and acute thermal tolerance, for
351 example, membrane failure (Bowler, 2018), enzyme denaturation or a shared genetic or
352 developmental component.

353

354 Tolerance to rapid warming was higher than tolerance to slow warming in the 34°C acclimation
355 treatment, a pattern similar to what has been seen in other species (Kovacevic, Latombe and Chown,
356 2019). The current explanation for this difference is that the higher cumulative stress of a prolonged
357 thermal challenge makes the fish lose equilibrium before reaching as high temperatures as during a
358 shorter test using a more rapid warming rate (Rezende et al., 2014). Interestingly, this pattern was
359 reversed in the 22°C acclimation treatment, with fish reaching higher temperatures during slow
360 warming. One potential explanation for this could be that the total time of the slow-warming
361 tolerance test in the 22°C acclimation treatment was over twice as long as in the 34°C treatment
362 (742 vs 322 minutes), giving individuals in the 22°C treatment more time to rapidly acclimate during
363 the trial. It is, however, unclear which physiological or biochemical mechanisms would be amenable
364 for adjustment over such short timescales. One possibility could be production of heat shock
365 proteins. Alternatively, the exposure to a high temperature during the rapid-warming tolerance test,
366 done ten days before the slow-warming test, might also have caused a slight upwards temperature-
367 acclimation (heat hardening) in the individuals of the 22°C treatment (Morgan et al., 2018), whereas
368 in the 34°C treatment, the individuals were already acclimated closer to their upper limit.

369

370 The growth rates observed in this experiment were close to what was observed previously at 22°C
371 and 34°C (Morgan et al., 2020 in prep), and about half of the growth rate at optimal temperature
372 (Morgan et al., 2020 in prep), showing that these temperatures had a strong negative effect on growth
373 rates. We predicted a relationship between thermal tolerance and ability to grow in non-optimal
374 temperatures. However, only a weak, near-significant positive correlation was found between these
375 traits, and only in the 22°C treatment under slow warming. The lack of clear correlations between

376 thermal growth performance and rapid-warming thermal tolerance suggests that acute thermal
377 tolerance has little mechanistic connection with the ability to maintain growth-performance outside
378 optimal temperatures. Whichever mechanism allows some individuals to have a higher CT_{max} does
379 not give them a considerable advantage or disadvantage in growth when acclimated to temperatures
380 outside their optimum. If, for example, oxygen limitation is reducing growth at high temperatures
381 (Pörtner and Knust, 2007), it likely does not play a significant role during acute temperature
382 increases such as during a CT_{max} trial. The results suggest that variation in thermal tolerance and
383 growth-performance (under-supra optimal temperatures) are governed by disparate mechanisms.

384

385 The weak correlation found between tolerance to slow warming and growth at 22°C may suggest
386 some link between these traits to be present. However, the positive correlation between these traits
387 was not predicted under the premise of a thermal syndrome. If cold-type individuals are
388 characterized by both lower thermal tolerance and lower optimal temperature for growth, a negative
389 correlation should have been observed. This suggests that the scope of a thermal syndrome may be
390 more limited than we predicted. On the other hand, the lack of correlation between thermal tolerance
391 and growth does not necessarily mean that both these traits should be excluded from this suite of
392 thermal traits, only that they are not strongly linked within it.

393

394 **Conclusions**

395 Tolerance to rapid warming correlates with tolerance to slow warming across individuals. This
396 means that the measure of rapid-warming tolerance also predicts tolerance to slower warming
397 challenges. the scale of what can be experienced during daytime under a heat wave. This suggests
398 that CT_{max} tests may be useful for predicting impacts of climate change in a broader context than
399 what is given by the rapid warming rate usually used to measure it.

400 We did not find support for a thermal syndrome that links growth performance at non-optimal
401 temperature and thermal tolerance measures, suggesting these traits may be selected for
402 independently in thermally stressed populations.

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407

408 **Competing interests**

409 No competing interests declared.

410

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413

414 **Abbreviations used**

415 CT_{max}: Critical thermal maximum

416 LOE: Loss of equilibrium

417

418 **References**

419

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- 518

519 **Appendices**

520



521

522 **Fig S1. Experimental setup.** The picture shows the tanks used in this experiment. Red labels
523 indicates 34°C treatment and white label indicates 22°C treatment. The temperature in each
524 tank is controlled with a thermostat (seen on the shelves columns) connected to one or two
525 titanium heaters (seen on tank's left side). Each tank was equipped with one red and green
526 ornamental plastic plant and two sponge biofilters for filtration, aeration and circulation. The
527 34°C tanks had an extra air stone installed to increase circulation over their heaters, resulting in
528 a more even and stable temperature in their tanks.

529

530

$$Grow\ thrate = \left(\left(\frac{Initial\ weight}{Final\ weight} \right)^{\frac{1}{Number\ of\ days}} - 1 \right) * 100$$

531

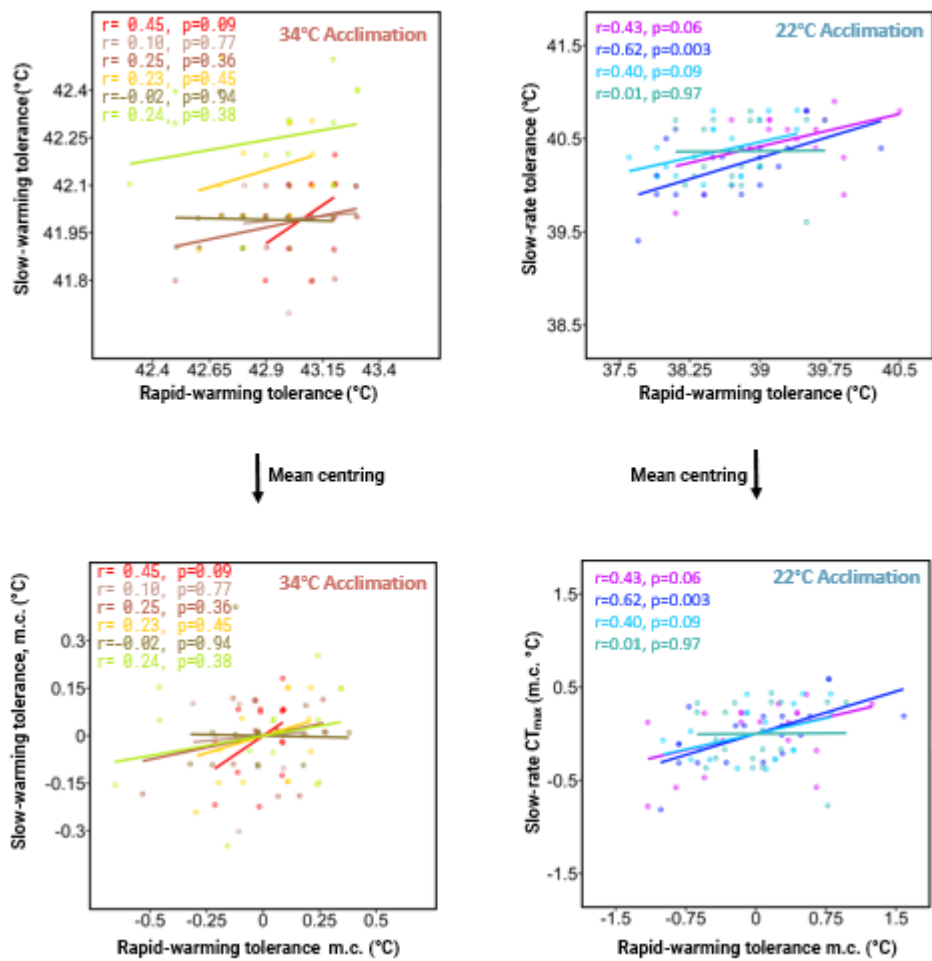
532 **Eqn S1. Equation for calculation growth rate expressed as percentage weight increase per**
533 **day.** This expression assumes equal growth rate every day throughout the period between the
534 measurement of initial and final weight.

535

536 **Table S1. Tank effects.** Results of analysis on linear models modelling either rapid-warming
 537 tolerance, measured as the temperature where loss of equilibrium (LOE) occurs at a warming
 538 rate of $0.3^{\circ}\text{C min}^{-1}$ (also known as CT_{max} ; Critical thermal maximum); slow-warming tolerance
 539 (LOE at a warming rate of $0.025^{\circ}\text{C min}^{-1}$) or growth as the response variable against holding
 540 tank as the predictor variable.
 541

<i>Relationship</i>	<i>Accl temp (°C)</i>	<i>SSq</i>	<i>F_{df}</i>	<i>p</i>
Slow-warming tolerance ~ Tank	22	0.717	$F_{3,75} = 2.247$	0.090
	34	0.774	$F_{5,76} = 8.915$	1.07e-06*
Fast-warming tolerance ~ Tank	22	5.105	$F_{3,75} = 5.071$	0.003*
	34	0.974	$F_{5,76} = 4.548$	0.001*
Growth ~ Tank	22	13060	$F_{3,75} = 1.313$	0.276
	34	6003	$F_{5,76} = 1.385$	0.239

542



543

544 **Fig. S2. Mean centring adjusting for tank effects.** Shows of rapid-warming tolerance,
 545 measured as the temperature where loss of equilibrium (LOE) occurs at a warming rate of 0.3°C
 546 min^{-1} (also known as CT_{max} ; Critical thermal maximum) and slow-warming tolerance (LOE at a
 547 warming rate of $0.025^{\circ}\text{C min}^{-1}$) for both acclimation treatments before and after mean centring
 548 (m.c.) of values. Mean centring redefines each value by subtracting the mean of its respective
 549 holding-tank from it, centring all tank-means on zero.