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

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

Abstract

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

Introduction

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

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

The critical thermal maximum (CT_{max}), a form of acute upper thermal tolerance, is one of the traits most commonly used to test the thermal biology of a species. CT_{max} is the temperature at which some specified endpoint occurs as the organism's body temperature is being steadily ramped upwards from its acclimation temperature (Becker and Genoway, 1979; Lutterschmidt and Hutchison, 1997; Morgan et al., 2018; Morgan et al., 2019). In fishes, two commonly used endpoints are the onset of muscle spasms and the loss of equilibrium (LOE), the latter being a state where the fish loses the ability to maintain an upright swimming position. The endpoint represents a state where the animal, while still alive, could be considered ecologically dead as in nature it would be unable to escape its condition. Measurements of CT_{max} has become a common measure of thermal tolerance, and it is increasingly being used to connect thermal physiology to the consequences of climate change (Comte and Olden, 2017; Deutsch et al., 2008; Sandblom et al., 2016; Sunday et al., 2012)

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

Syndrome theory is a theoretical framework for studying systems of correlated traits (Sih et al., 2004). Syndrome theory has mostly been applied to animal personality research, but based on the tight relationship between temperature, metabolism, and behaviour (Biro and Stamps, 2010), Goulet et al (2017a) suggested that thermal physiology could be included into the pace-of-life syndrome hypothesis (POLS). In this hypothesis, consistent differences in behavioural traits are suggested to co-vary with life-history and physiological traits like growth and metabolism, placing individuals along a fast-slow life-history axis (Réale et al., 2010). Goulet et al (2017a) proposed that the individual's thermal type would align with their behavioural and life-history types. According to this framework, thermal traits would be configured into a thermal syndrome with individuals distributed along a cold-hot axis, and their position in this continuum corresponding to different thermal types. The inclusion of thermal toleranc in this system has so far not been tested, and expanding this system to include this, cold-type individuals at one end of the axis would have left-shifted thermal performance curves, performing better at lower temperatures and having lower critical thermal limits; the opposite would be the case for hot-type individuals at the other end of the axis (Fig.1C, D). Recent studies on delicate skinks (*Lamphropolis delicata)* have revealed some interesting connections between thermal preference, thermal sprint performance, habitat selection, and traits related to boldness, exploration and social behaviour (Goulet et al., 2017a; Goulet et al., 2017b; Michelangeli et al., 2018). These findings support individuals existing on a cold-hot axis with corresponding behavioural traits, but the role of thermal tolerance in such a system is so far unexplored.

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

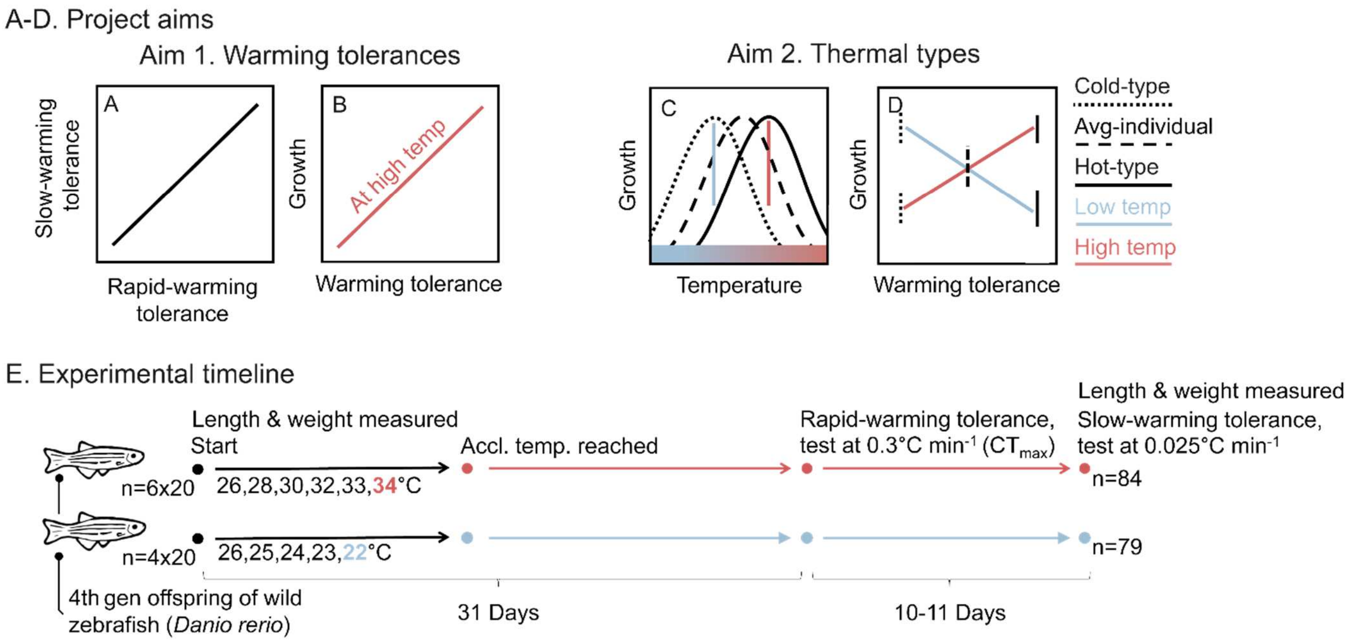

Fig. 1. Experimental aims and design. (A-D) Graphic representation of the predictions from the two experimental aims. Aim 1: testing the hypothesis that tolerance to rapid warming also confers tolerance to slow warming and increased growth at supra-optimal temperatures. This would lead to (A) a correlation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

Materials and methods

Experimental procedure

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

Holding conditions

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

Thermal tolerance measurements

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

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

The slow-warming tolerance test (0.025°C min^{-1}) was conducted in the holding tanks on the last day of the experiment. The water level was reduced to 10 cm (15 L), filters and ornamental plants were removed, and the titanium heater (TH-100, Aqua Medic, Bissendorf, Germany) was placed horizontally and close to the water surface on each tank's longest wall with the air stone placed underneath to provide circulation over the heater. The thermometer for recording water temperature at LOE was placed on the opposite side of the tank. A thermostat (ITC-310T, Inkbird, Shenzen, China) was used to control the titanium heater while gradually heating the water. The thermostat's thermal probe was placed close to the air stone to keep it close to the water flow but underneath the heater. Temperature was recorded as each individual reached LOE, and the individual was immediately euthanized, weighed, and measured.

Statistical analysis

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

Results

Weight and growth

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

Mortality and outliers

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

Correlations

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

Thermal tolerances

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

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

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

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

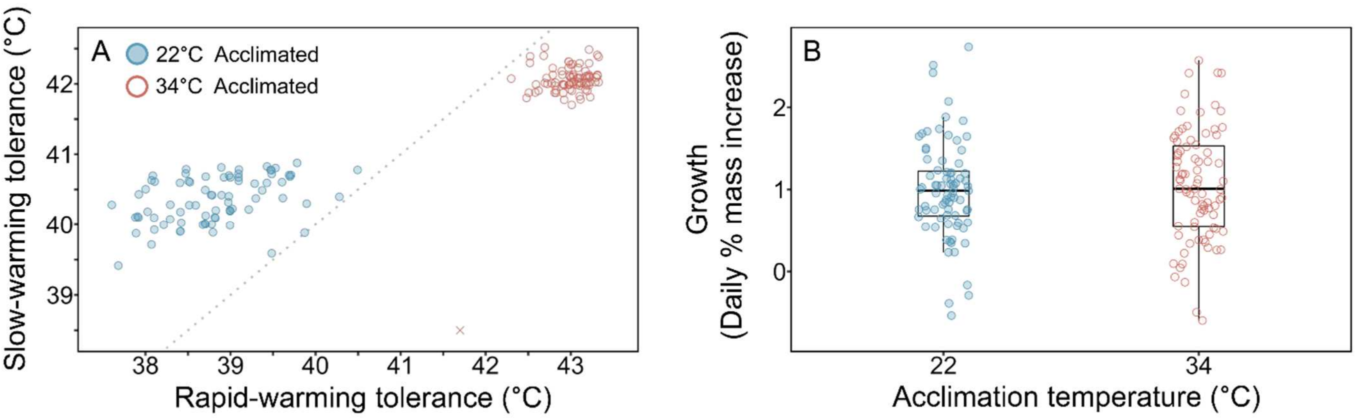

Fig. 2. Temperature; growth; and rapid- and slow warming tolerance. Results are for two acclimation treatments of juvenile zebrafish at 22°C (n = 79) and 34°C (n = 80). (A) Shows Individuals' rapid-warming tolerance, measured as the temperature where loss of equilibrium (LOE) occurs at a warming rate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

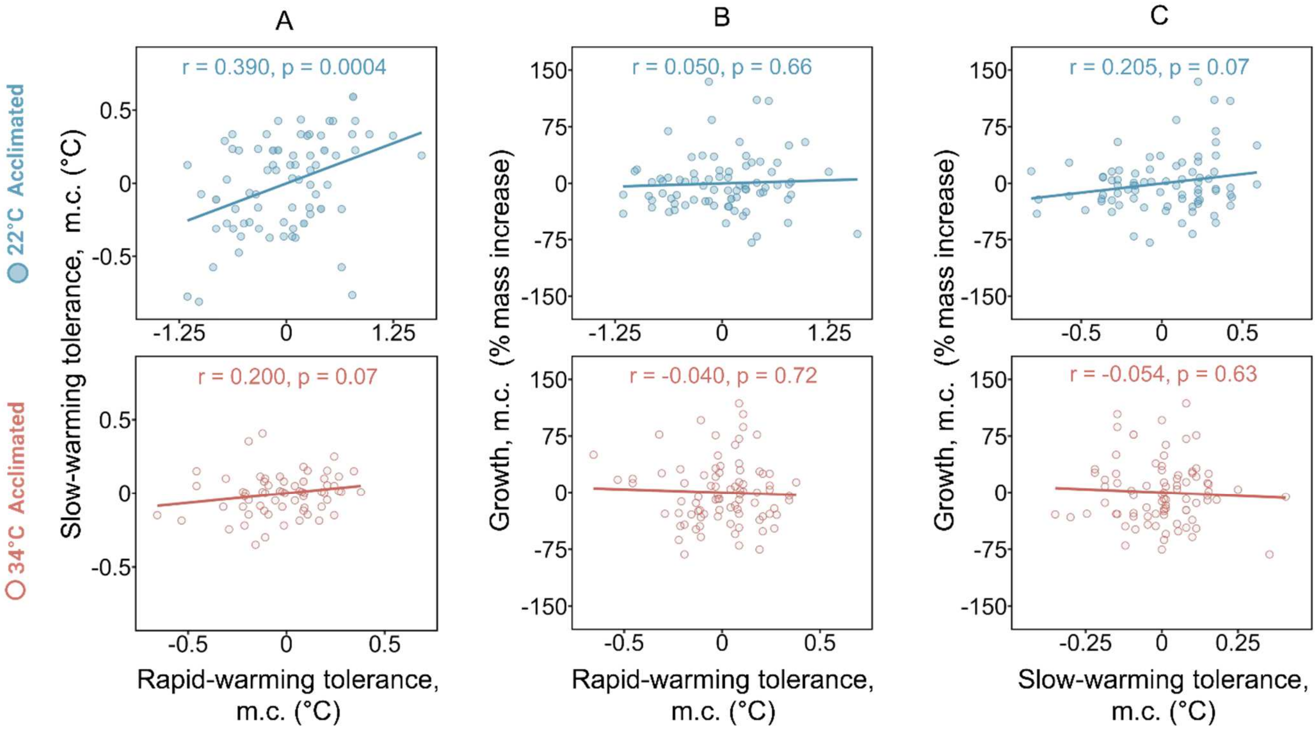

Fig. 3. Correlations between rapid-warming tolerance, slow-warming tolerance, and growth. The figure shows correlations including Person’s correlation coefficient (r) and respective p-values between all combinations (A, B, C) of rapid-warming tolerance, measured as the temperature where loss of equilibrium (LOE) occurs at a warming rate of 0.3°C min^{-1} (also known as CT$_{max}$; Critical thermal maximum); slow-warming tolerance (LOE at a warming rate of 0.025°C min^{-1}) and growth (% mass increase over 32 days) for two acclimation treatments of zebrafish at 22°C (n= 79) and 34°C (n = 80). Measurements are corrected for tank-effects by mean-centring (m.c.) all values on their respective tank-means. Lines are fitted using least-square regression for each plot’s values and are for illustrative purpose only.

Discussion

Upper thermal tolerance under rapid warming correlated significantly with upper thermal tolerance under slow warming, demonstrating that individuals with high tolerance to rapid warming (30-60 minutes until LOE) are also individuals tolerant to slow warming (5-12 hours until LOE). This means that rapid CT$_{max}$ measurements can be used as a quick and practical proxy for estimating more general thermal tolerance. The finding thus extends the importance of CT$_{max}$ measurements from a being a laboratory test of acute thermal tolerance to a potentially ecologically relevant metric for estimating tolerance to heat waves in nature.

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

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

A potentially important, although unquantified observation during our thermal challenges tests was that the nature of the LOE changed between the two warming rates. At the standard, rapid warming rate, zebrafish display a distinct disorganisation response, characterized by fast, erratic swimming coupled with an inability to remain upright. Under slow warming, however, it was more common for the fish to lose equilibrium from what appeared to be exhaustion. That is, instead of swimming without a righting response (as in the rapid-warming test), the fish simply stopped swimming, and thus also lost their ability to remain upright. These different responses leading to LOE suggest different underlying mechanisms ultimately causing the LOE at the two warming rates. The almost instant LOE and disorganization-reaction during the rapid warming suggest an immediate failure of some vital mechanism, like cardiac (Sidhu et al., 2014) or neurological malfunctioning (Jutfelt et al., 2019; Miller and Stillman, 2012; Robertson, 2004), while the slow exhaustion-like response during the slow warming may suggest a gradual build-up of some malfunction, metabolic waste products, or the exhaustion of some system. Still, the correlation found in this experiment suggest that important links between these two traits exist. One source of this correlation could be a more fundamental mechanism that ultimately governs both long-term and acute thermal tolerance, for example, membrane failure (Bowler, 2018), enzyme denaturation or a shared genetic or developmental component.

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

The growth rates observed in this experiment were close to what was observed previously at 22°C and 34°C (Morgan et al., 2020 in prep), and about half of the growth rate at optimal temperature (Morgan et al., 2020 in prep), showing that these temperatures had a strong negative effect on growth rates. We predicted a relationship between thermal tolerance and ability to grow in non-optimal temperatures. However, only a weak, near-significant positive correlation was found between these traits, and only in the 22°C treatment under slow warming. The lack of clear correlations between thermal growth performance and rapid-warming thermal tolerance suggests that acute thermal tolerance has little mechanistic connection with the ability to maintain growth-performance outside optimal temperatures. Whichever mechanism allows some individuals to have a higher CT_{max} does not give them a considerable advantage or disadvantage in growth when acclimated to temperatures outside their optimum. If, for example, oxygen limitation is reducing growth at high temperatures (Pörtner and Knust, 2007), it likely does not play a significant role during acute temperature increases such as during a CT_{max} trial. The results suggest that variation in thermal tolerance and growth-performance (under-supra optimal temperatures) are governed by disparate mechanisms.

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

Conclusions

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

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

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Competing interests

No competing interests declared.

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Abbreviations used

CT_{max}: Critical thermal maximum

LOE: Loss of equilibrium

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Appendices

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

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

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

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

Relationship	***Accl temp (°C)***	***SSq***	F_{df}	p
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

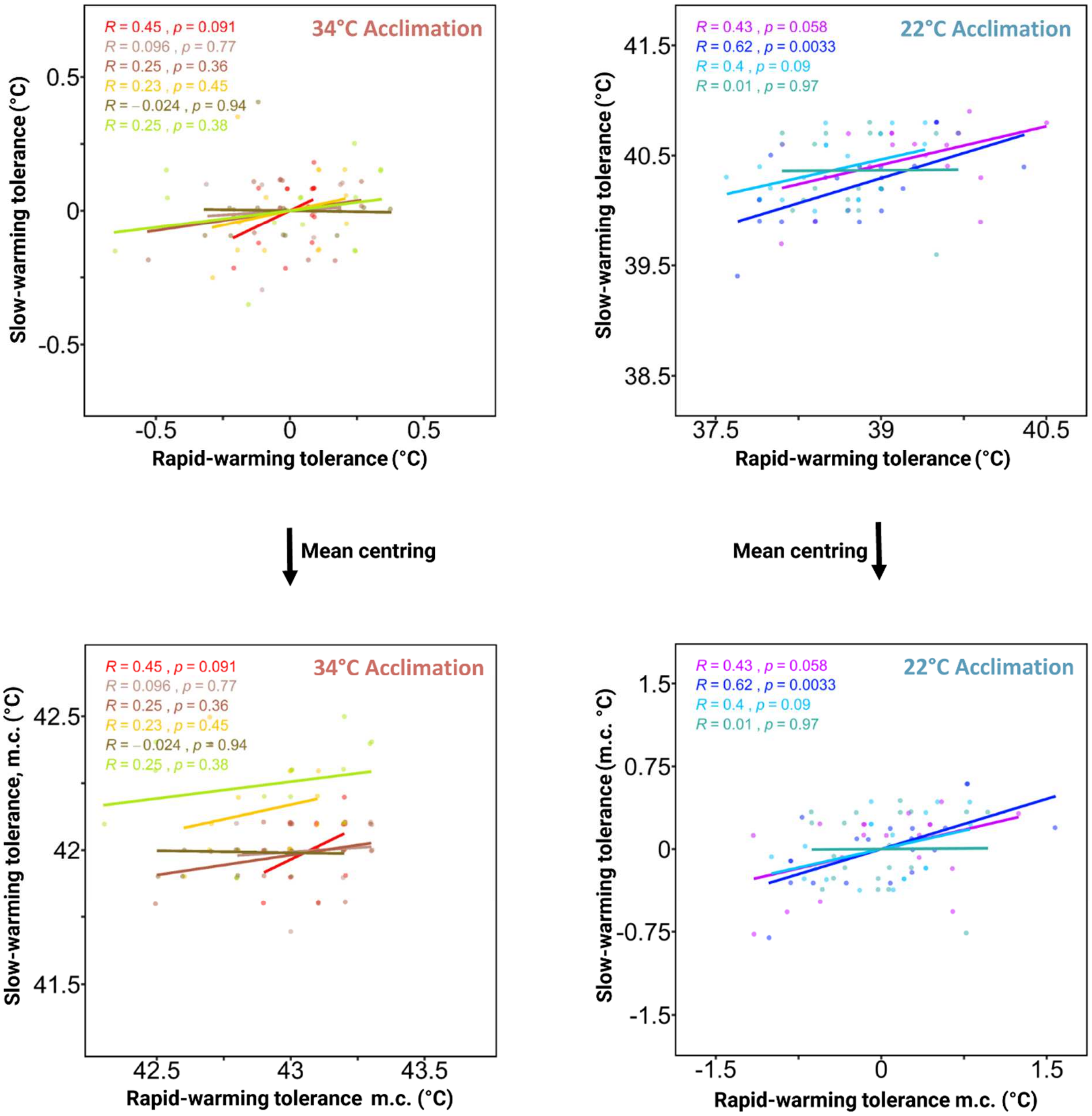

Fig. S2. Mean centring adjusting for tank effects. Shows of rapid-warming tolerance, measured as the temperature where loss of equilibrium (LOE) occurs at a warming rate of 0.3°C min^{-1} (also known as CT_{max}; Critical thermal maximum) and slow-warming tolerance (LOE at a warming rate of 0.025°C min^{-1}) for both acclimation treatments before and after mean centring (m.c.) of values. Mean centring redefines each value by subtracting the mean of its respective holding-tank from it, centring all tank-means on zero. Lines are fitted using least-square regression and are for illustrative purpose only.