

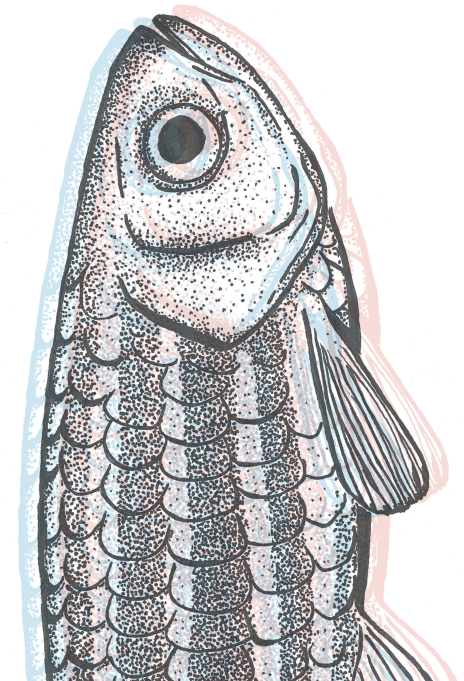
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Acute thermal tolerance correlates with longer-term warming tolerance but not growth during thermal stress in zebrafish

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Abstract

Global warming is causing an increase in both acute lethal and prolonged sub-lethal thermal challenges for aquatic ectotherms, but a relationship between individual performance and tolerance under these challenges remains unexplored. It has been suggested that thermal traits may be connected to behaviour and life-history syndromes, and that individuals in a population could be placed on a hot-type to cold-type axis. This implies that traits of thermal tolerance and thermal performance should be correlated. In this experiment, we tested whether different thermal traits correlate across individuals by acclimating 200 zebrafish (*Danio rerio*) to sub- and supra optimal temperatures for growth (22 and 34 °C) for 40 days, and measuring growth and critical thermal maximum under two different thermal ramping rates. We found that tolerance to rapid thermal ramping correlated across individuals with tolerance to slow thermal ramping, but a correlation with growth was only indicated with tolerance to slow thermal ramping in the 22 °C Treatment. The results suggest that tolerance to acute heating shares important underlying mechanisms with tolerance to slower heating, and indicate that the relevance of critical thermal limits extends beyond the rapid ramping rates used to measure them. We thus find some support for a syndrome-like organization of thermal traits, but the lack of connection between tolerance and growth-performance suggest a restricted generality of a thermal syndrome.

Sammendrag

For akvatiske ektoterme dyr medfører global oppvarming en økning i eksponering til både akutt-dødelige og langvarige ikke-dødelige temperaturutfordringer. Samtidig er sammenhengen mellom disse dyrenes yteevne og toleranse under disse utfordringene lite utforsket. Det har blitt foreslått at temperaturrekk, sammen med adferd og livshistorietrekk kan være organisert i et syndrom, og at individer i en populasjon kan sorteres på en akse fra kald-typer til varm-typer. Implisitt sier dette at temperaturløtoleranse og yteevne ved ulike temperaturer bør være korrelert. I dette eksperimentet testet vi om ulike temperaturrekk er korrelerte på tvers av individer ved å måle vekst og øvre kritiske temperatur ved to ulike temperaturøkningssrater for 200 sebrafisk (*Danio rerio*) akklimert til sub- og supraoptimale veksttemperaturer (22 og 34 °C) i 40 dager. Vi fant en korrelasjon mellom temperaturløtoleranse målt under hurtig og langsom temperaturøkning på tvers av individer, men en korrelasjon med vekst ble kun indikert med toleranse til langsom temperaturøkning hos individene akklimert til 22 °C. Disse resultatene tyder på at temperaturløtoleranse til akutt oppvarming deler viktige underliggende mekanismer med temperaturløtoleranse for langsom økning og indikerer at relevansen av øvre kritiske temperaturer strekker seg utover den akutte økningen som blir brukt for å måle dem. Resultatene gir noe støtte til en syndromlignende organisering av temperaturrekk, men mangelen på en kobling mellom temperaturløtoleranse og ytelseevne i form av vekst tilsier en begrenset utstrekning av et temperatursyndrom.

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Introduction

Climate change is bringing a range of different challenges upon life on earth. 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 heatwaves predicted to increase in both frequency and severity (Perkins *et al.*, 2012). In this context, ectothermic animals can be especially vulnerable as their body temperature directly follows that of their environment. These animals are thus forced to either migrate or face the consequences of higher and more variable body-temperatures.

When studying the consequences of climate change, an important question is to what extent generalisations can be made on the effects of temperature on ectotherm physiology. There are several methods that can be used to describe how temperature affects performance and survival. One way is to measure how different performances (Schulte *et al.*, 2011) like growth, reproduction, locomotion and oxygen consumption changes with temperature. Another way is to measure the critical thermal limits, which are the temperatures where whole-animal functioning collapses (MacMillan, 2019). However, knowledge is lacking on the causes of variation in these 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 of these traits but would also have major implications for how selection on these traits occur under climate change.

Syndrome theory is a theoretical framework for studying systems of correlated traits (Sih *et al.*, 2004). Historically, syndrome theory has mostly been applied to animal personality research, but physiology was eventually included with the proposal of the pace-of-life syndrome hypothesis (POLS). In this hypothesis, consistent differences in behavioural traits were suggested to co-vary with life-history and physiological traits like growth and metabolism, sorting individuals along a fast-slow life-history axis (Réale *et al.*, 2010). Recently, based on the tight relationship between temperature, metabolism, and behaviour (Biro and Stamps, 2010), Goulet *et al.* (2017b) suggested that thermal physiology could be included into the POLS framework, where 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 behaviour-syndrome where individuals are sorted along a cold-hot axis, and their position in this continuum corresponds to different thermal types: Cold-type individuals at one end of the axis would perform better at lower temperatures, have lower

critical thermal limits, and have a reduced performance at comparably high temperatures, with the converse being true for hot-type individuals. Configured into a syndrome, thermal traits like optimal performance temperatures, critical thermal limits, and temperature preference would be predicted to correlate, with consistent differences between individuals across situations. Thermal, behavioural, and life-history traits being organized like this in a single continuum would have major implications on both the evolution of thermal traits and the potential consequences of climate change. Selective pressure caused by changing thermal regimes could under this premise co-select for life-history and behavioural traits together with thermal physiology.

The critical thermal maximum is one of the thermal traits most commonly used to represent the thermal biology of a species, and hence a trait that should be considered in a thermal syndrome. By definition, 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). In fishes, two 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 it in nature would be unable to escape its condition. The CT_{max} measurement has become a common measure of thermal tolerance, and it is increasingly being used to connect thermal physiology to the consequences of climate change (Sunday *et al.*, 2012; Comte and Olden, 2017; Sandblom *et al.*, 2016; Deutsch *et al.*, 2008). A search in Google Scholar for “ CT_{max} ” and “Climate change” speaks for the prevalence of this method, returning over 700 results for the last two years. Despite its frequent use, knowledge is lacking on what the CT_{max} tells us about the overall thermal physiology of an ectotherm (MacMillan, 2019); Specifically, it is unclear whether inter-individual differences in CT_{max} can be expected to correlate and form a thermal syndrome together with other thermal traits that are relevant in a context of climate change. For example, in fishes, the standard CT_{max} test may use a ramping rate of $0.3^{\circ}\text{C}/\text{min}$ (Becker and Genoway, 1979), which limits its ecological relevance as it only measures thermal tolerance in a context of highly acute thermal stress. For fishes living in shallow, slow-flowing waters, the water temperature is likely to be affected by changes in weather. In these types of habitats, during a heatwave, it is possible that the temperature increase and thermal ramping rate could come close to or even surpass that of a standard CT_{max} trial. Yet, outside of this context, in other environments or during longer but less severe periods of temperature increase, the relevance of the $0.3^{\circ}/\text{min}$ CT_{max} for survival in fishes is less clear.

The rate of temperature increase during a thermal challenge is important, as ramping rates used for the CT_{max} assay has been shown to alter the final temperature reached (Mora and Maya, 2006). The relationship between ramping rate and the upper critical limit varies significantly between species and taxa (Kovacevic *et al.*, 2019), but the general pattern is for thermal tolerance to be reduced when the ramping rate is slower. One explanation for this is that slow heating rates are increasing the time spent at each successive temperature, exhausting the animal before a higher critical temperature is reached (Rezende *et al.*, 2014). Another possibility is that different ramping rates are stressing different physiological mechanisms, meaning that tolerance to different heating rates can be considered as disparate traits.

While critical thermal limits represent the temperature where an individual would be ecologically dead, these limits give little information on the state of the animal during less-than-lethal thermal challenges. One way of studying temperature-effects is to measure how performances are affected by exposure to different temperatures (Schulte, Healy and Fanguie, 2011). Together, thermal performance and thermal tolerance can give a detailed picture of the effects of temperature on an animal, but it is not yet known if there is a link between these two types of traits. Growth, for example, is an important measure of performance, but it has not been tested if thermal tolerance predicts growth performance outside optimal temperatures. If thermal traits are organized in a syndrome, it would be expected that consistent differences in growth performance and critical thermal limits correlate.

Studying how growth performance and tolerance covary, finding any correlations could hint at some shared mechanism, developmental process or genetic architecture. Alternatively, the ability to grow outside of optimal temperatures and to survive acute temperature changes may be conferred by different mechanisms, resulting in no correlation. A negative correlation would be expected if there's a trade-off between growth and CT_{max} . One scenario where such a trade-off could occur is if the variance in CT_{max} is given by the acclimated metabolic rate of the individual. Individuals with relatively high metabolic rates would then be sensitive to sudden increases in temperature but would also have a general advantage in for example growth (Álvarez and Nicieza, 2005). Additionally, the premise of thermal-traits being organized in a syndrome predicts that cold-type individuals would, compared to the rest of the population, have a lower CT_{max} as well as a lower optimal growth temperature. At temperatures below the species' optimal growth temperature, cold-type individuals would then be expected to grow better than hot-types, resulting in a negative correlation between CT_{max} and growth. At temperatures above the species optimal growth

temperature, this pattern would be reversed and there would be a positive correlation between CT_{max} and growth.

The underlying mechanism of the proposed linkages in the thermal behaviour syndrome has so far not been specified. Consistent intra-individual correlations in thermal traits could be expected to occur as long as there is some shared mechanism between them. Whether the linking mechanism is a shared physiological mechanism, a genetic component, or an environmental effect, any change happening in this link should affect all its connected traits and cause a consistent correlation between them. Differences in this mechanism between individuals should then be expected to cause consistent inter-individual differences in these traits as well, creating individuals of different thermal types.

The degree to which different thermal traits are linked by a shared physiological mechanism is a controversial question. The oxygen and capacity-limited thermal tolerance (OCLTT) hypothesis (Pörtner and Farrell, 2008) is based on the idea that temperature limits performance via reductions in aerobic scope, and that this form of oxygen limitation may be a unifying physiological principle for temperature tolerance and performance. From a thermal-trait syndrome perspective, individuals with different thermal types could occur as a consequence of between-individual variation in the ability to maintain aerobic scope at high and low temperatures. A central prediction of the OCLTT hypothesis is that the proposed relationship between performance and aerobic scope is causing thermal traits not only to correlate but to share the same optimum temperature (where the aerobic scope is highest). This hypothesis has for some time been dominating within the field of ectotherm thermal biology, and although this kind of temperature-dependent oxygen limitation seems to occur in some species and contexts (Pörtner and Knust, 2007), a growing amount of contraindicative evidence (Brijs *et al.*, 2015; Gräns *et al.*, 2014; Lefevre, 2016) has recently put the hypothesis in the centre of debate (Jutfelt *et al.*, 2018; Pörtner *et al.*, 2017). An alternative multiple performances – multiple optima hypothesis (MPMO) is based on the idea that disparate physiological functions have different thermal optima, instead of a single unifying mechanism centring on one single temperature (Clark *et al.*, 2013). Although this hypothesis rules out a shared optimum for different performances, it does not necessarily rule out the existence of a thermal syndrome, as thermal traits could still display consistent within-individual correlation and between-individual differences via the mechanisms mentioned above.

Aims and hypotheses. The aim of this experiment was to check if there's a correlation between a measure of thermal performance and two measures of thermal tolerance, a necessary prerequisite for these traits being organized in a syndrome. Using zebrafish (*Danio rerio*), We measured CT_{max} at ramping rates of 0.3 °C/min (rapid-rate) and 0.025°C/min (slow-rate) as well as growth-performance over 30 days at two temperature treatments, with one temperature on either side of the species' thermal optimum. Based on an earlier, unpublished acclimation experiment, we chose temperature treatments to be at 22 (low-temp) and 34 °C (high-temp), as these are the temperatures at which growth rate is equally reduced, being about half of what is observed at the optimal temperature for growth at around 28-30 °C.

We hypothesised that thermal tolerance at two ramping rates and growth performance would be organised in a syndrome, and predicted that rapid-rate CT_{max} , slow-rate CT_{max} , and growth above optimal temperatures would all correlate, so that hot-type individuals with a high CT_{max} would grow comparably better in the high-temp treatment, but not in the low-temp treatment, where we predicted cold-type individuals having a low CT_{max} but performing better in terms of growth than hot-type individuals.

Experimental methods

Experimental procedure. The fish used in this experiment were third generation offspring of wild-caught zebrafish (*Danio rerio*) from West Bengal, India, a strain brought into our lab in November 2016. We used a total of 200 zebrafish, about 40 days old, which were divided into two treatment groups to be acclimated at 22 ± 0.2 °C (Low-temp, N = 80) and 34 ± 0.2 °C (High-temp, N = 120). We used a higher sample size in the high-temp treatment to compensate for the predicted increase in mortality due to individuals reaching a higher CT_{max} in this treatment. Individuals were then divided into subgroups of 20 fish and sorted into 10 tanks, with six tanks for the high-temp (34 °C) treatment and four tanks for the low-temp (22 °C) treatment. Before being sorted into their tanks, fish were tagged and measured while under surgical anaesthesia using 110 mg/L buffered tricaine methane sulfonate (MS-222). Surgical anaesthesia was ensured when individuals no longer responded to tactile stimuli. 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 and combinations of yellow, blue, pink, orange and green tags. 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. Of the 200 fish tagged, one individual was found dead the next day and was replaced. Initial holding temperature was 26 ± 0.2 °C and temperature-acclimation started after two days of acclimation to the holding tanks. Temperature was reduced by 1 °C/day in the 22 °C treatment until 22 °C was reached. In the 34 °C treatment, temperature was increased by 2 °C/day until 32 °C, and 1 °C/day until 34 °C. Thus, final acclimation temperatures were reached after six days. Fish were tested for rapid-rate CT_{max} after 22 days at their respective acclimation temperature, and slow-rate CT_{max} 10 days after that (Figure 1).

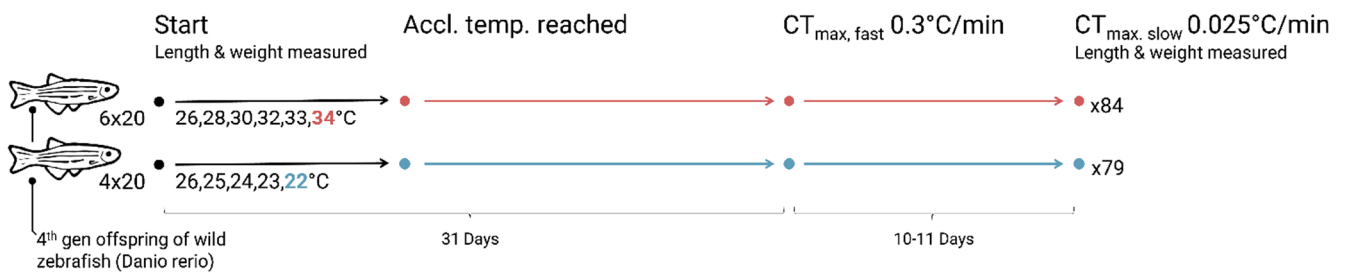


Figure 1 Schematic timeline of the experiment. A total of 200 zebrafish, raised at 26 °C, were divided into two treatments to be acclimated at either 22 or 34 °C. All fish were tested for CT_{max} at a ramping rate of 0.3 °C/min (rapid-rate) after 31 days in the experiment, and CT_{max} at a ramping rate of 0.025 °C/min (slow-rate) ten or eleven days after that. All individuals were measured for weight and length at the beginning and end of the experiment.

Acclimation temperatures. For this experiment, we wanted two temperatures at either side of the optimum temperature for growth, where we could expect an equal reduction in growth compared to this optimum. We also wanted the higher temperature close to the highest temperature tolerated by this species. In an earlier temperature-acclimation experiment we had performed on our population of zebrafish (unpublished), we found 28-30 °C to be the optimal temperature for growth, with a growth rate of 2.05 ± 0.19 % mass incr/day at 30 °C (mean \pm SEM), and we found 36 °C to be the highest temperature our fish could survive in the long term. Based on results from this earlier experiment, we chose to use 34 °C and 22 °C as treatment temperatures for this experiment. At these temperatures, we had had earlier observed a growth rate of 1.13 ± 0.15 % mass incr/day and 1.27 ± 0.17 % mass incr/day.

Holding conditions. Holding tanks of 45 L (50 x 30 x 30 cm), made of glass and filled to 35 L, were environmentally enriched with one red and green plastic ornamental plant, and had two sponge filters used for filtration, air bubbling, and water circulation. Temperatures were controlled using a thermostat-controlled (ITC-310T, Inkbird, Shenzhen, China) titanium heater (TH-100, Aqua Medic,

Bissendorf, Germany). 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 low- and high-temp tanks, respectively.

CT_{max} procedure. Two separate procedures were used to test CT_{max} at a 0.3 °C/min and a 0.025 °C/min ramping rate. In both procedures, loss of equilibrium (LOE) was used as the test's endpoint (Becker and Genoway, 1979). We defined the loss of equilibrium as the state where the fish has, 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 ±0.01 °C accuracy (Testo -112, Testo, Lenzkirch, Germany).

The 0.3 °C/min CT_{max} test was conducted using a heating tank (25 x 22 x 18 cm) filled with nine litres of 26 °C water, including a heating element and a pump for circulation. A detailed description of this CT_{max} setup can be found in (Morgan, Finnøen and Jutfelt, 2018). Ten individuals were tested simultaneously in the same heating tank, and temperature was recorded as each individual reached LOE. 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 0.025 °C/min CT_{max} test was conducted in the fish' 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, Shenzhen, 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 cold-euthanized, weighed, and measured.

Statistical analysis. All analysis was done using the R 3.5.1 software environment (R Core Team, 2018). Growth was defined as the percentage increase in body mass during the experiment, calculated using the initial and final weight, thus accounting for initial size differences. Growth rates, accounting for time in the experiment, and thus useful for comparing between experiments,

were calculated as percentage growth in mass per day (Assuming an equal growth rate each day)(Supplementary material, Equation S1). Only individuals that survived through the entire experiment were included in the analysis. All comparisons on growth and CT_{max} between the acclimation treatments were tested using two-tailed t-tests. Any tank-effect on CT_{max} or growth was tested using an ANOVA analysis on a linear model with holding tank as the independent variable against slow-rate CT_{max}, rapid-rate CT_{max} or growth as the dependent variable. Holding tank was found to significantly affect both types of CT_{max}, but not growth (Supplementary material, Table S2). Small variations in tank temperature are likely to play a part in this, causing differences in acclimated temperature. Testing for correlations on all individuals when there are subgroups (holding tanks) with significantly different mean values may lead to false positives or negatives, particularly if these differences are caused by directional tank-effects and not sampling error. Because we were testing for correlations on inter-individual differences, we corrected for the tank-effects by a way of mean-centring, where each measurement is re-defined as its difference from its respective tank mean. This resulted in a new set of mean centred (m.c.) values for growth and CT_{max} at both ramping rates, where the mean within each tank is centred on 0, but the variance remains the same (Supplementary material, Figure S3). We chose to use these corrections on the growth measurements as well for consistency. Correlations were tested using Pearson's product-moment correlation between all three measurements (rapid-rate CT_{max}, slow-rate CT_{max}, and growth). Two separate sets of correlation were tested, using either raw uncorrected values or mean-centred values (Table 1).

Results

Weight and growth. Mean weight of all individuals was 0.078 ± 0.02 g (Mean \pm SD) before acclimation and 0.118 ± 0.024 g at the end of the experiment, equivalent to a 56.9 ± 43.74 % increase, or a growth rate of 1.02 ± 0.05 % mass incr/day. There was no significant difference in growth between the acclimation groups ($t = 0.80$, $p = 0.42$)(Figure 1-a).

Mortality and outliers. The 22 °C group only had a mortality of 1 % through the entire experiment, while the 34 °C group experienced a mortality of 30 % after the rapid-rate CT_{max} test, leaving the final tally of individuals tested for both slow and rapid-rate CT_{max} 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 an outlier, as its Slow CT_{max} at 38.5 °C was over four times the interquartile range below the lower quartile.

CT_{max}. Acclimation temperature significantly affected CT_{max} at both the rapid ($t = -55.91$, $p < 0.0001$) and slow ($t = -41.00$, $p < 0.0001$) ramping rate (Figure 1-b). rapid-rate CT_{max} 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-rate CT_{max} was 40.36 ± 0.33 °C and 42.07 ± 0.16 °C. In the 22 °C treatment, rapid-rate CT_{max} was significantly lower than slow-rate CT_{max} ($t = -19.21$, $p < 0.0001$), while in the 34 °C treatment the rapid-rate CT_{max} was significantly higher than the slow-rate CT_{max} ($t = 29.96$, $p < 0.0001$). Average duration of the slow-rate CT_{max} test was 743 and 322 minutes in the 22 and 34 °C treatment, respectively. Average duration of the rapid-rate CT_{max} test was 56 and 30 minutes.

Correlations. A significant correlation was found between rapid-rate and slow-rate CT_{max} in the 22 °C treatment ($r = 0.390$, $p = 0.0004$). At 34 °C, this correlation was positive, but only near-significant ($r = 0.200$, $p = 0.071$). Growth and rapid-rate CT_{max} did not correlate in any of the treatments. Growth and slow-rate CT_{max} only correlated significantly in the 22 °C treatment when using uncorrected raw values ($r = 0.240$, $p = 0.070$). Correction using values controlled for tank-effects resulted in a near-significant, positive correlation ($r = 0.205$, $p = 0.070$) (Table 1).

Table 1 Pearson's correlation coefficient (r) and corresponding p -values for correlations between all combinations of Growth, rapid-rate CT_{max} (+0.3 °C /min) and slow-rate CT_{max} (+0.025 °C /min) at two acclimation temperature (22 & 34 °C) s. To correct for tank-effects, mean-centring (m.c.) was used 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.

Relationship	Accl. temp	Raw values		Controlled for tank-effects (M.C.)	
		Correlation I		Correlation II	
		r	p	r	p
<i>Growth — Slow-rate CT_{max}</i>	22 °C	0.240	0.033	0.205	<i>0.070</i>
	34 °C	-0.021	0.849	-0.054	0.629
<i>Growth — Rapid-rate CT_{max}</i>	22 °C	0.117	0.306	0.050	0.662
	34 °C	-0.019	0.863	-0.040	0.720
<i>Rapid-rate CT_{max} — Slow-rate CT_{max}</i>	22 °C	0.416	0.0001	0.390	0.0004
	34 °C	0.060	0.588	0.200	<i>0.071</i>

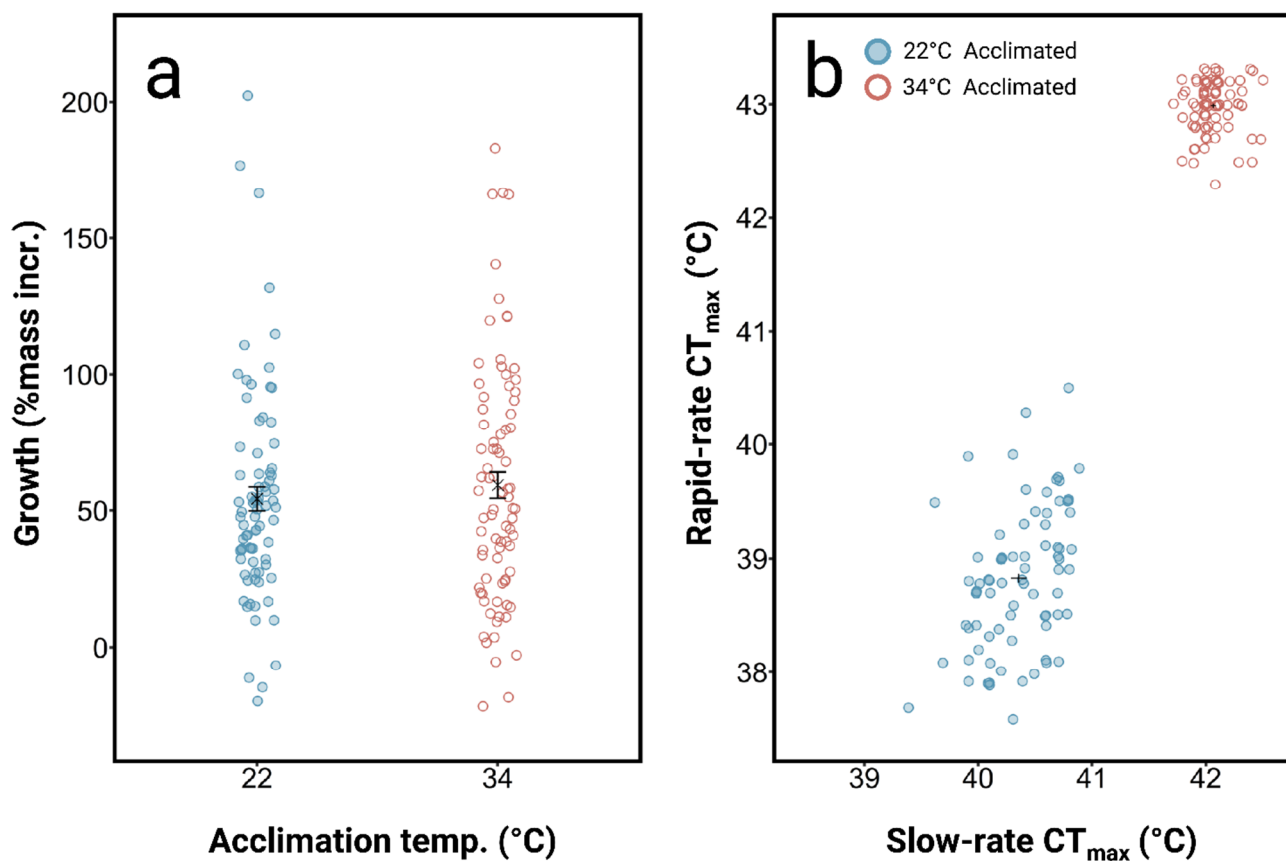


Figure 2 (a-b) Growth (Percent mass increase), rapid-rate CT_{max} (0.3 °C/min) and slow-rate CT_{max} (0.025 °C/min) For two acclimation treatments of fish at 22 °C (N = 79) and 34 °C (N = 80). Individuals were tested for rapid-rate CT_{max} after 22 days of acclimation and slow-rate CT_{max} after 32 days. **(a)** Displays percentage growth for all included individuals in the two treatment. Crosses and error bars display mean \pm SEM **(b)** Displays rapid-rate and slow-rate CT_{max} for all included individuals. Axes are on the same scale in (b). Points are jittered in both (a) and (b) to reveal overlapping points, but only horizontally in (a), and no more than 0.02 °C in (b).

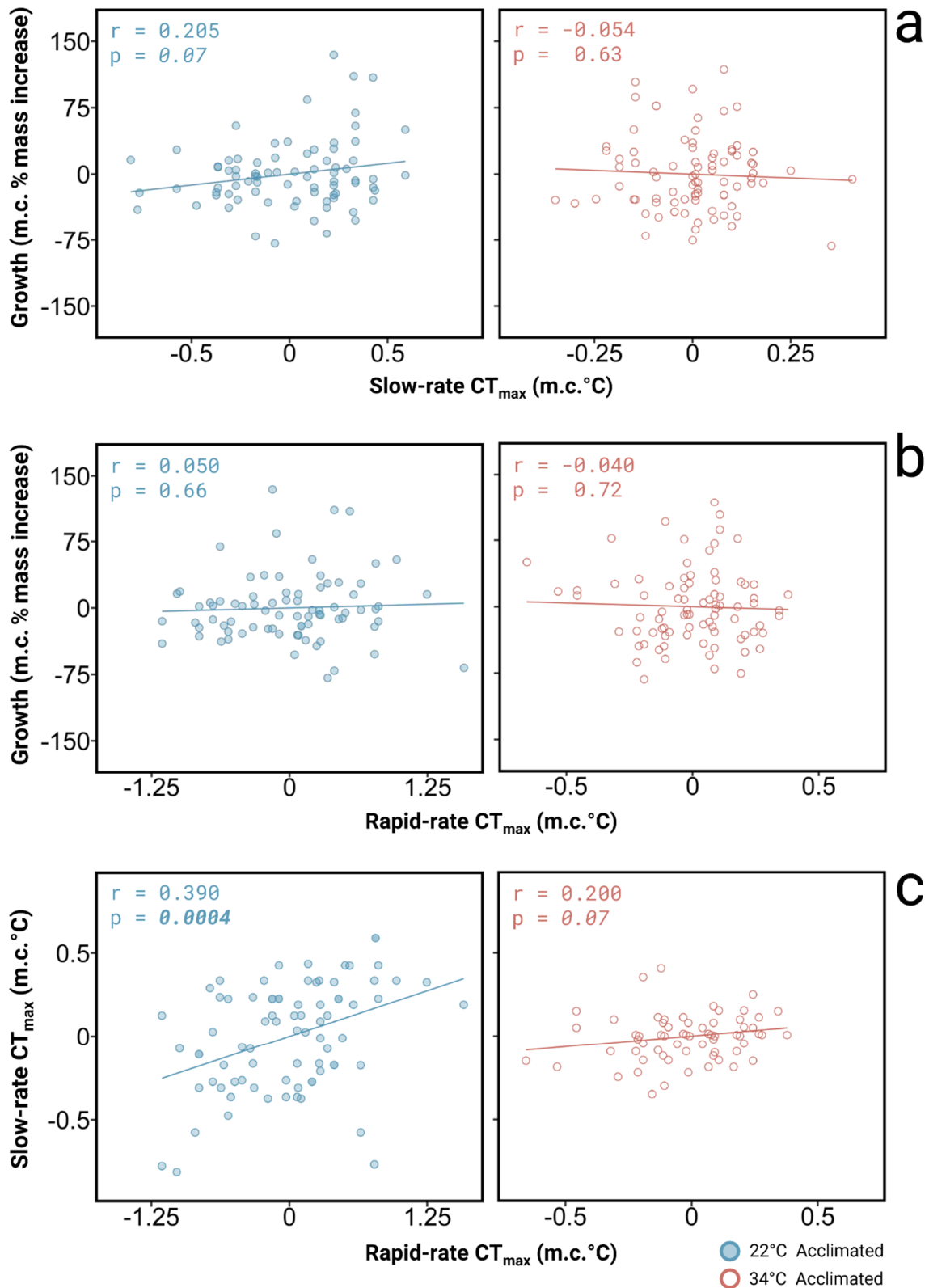


Figure 3 (a-c) Displays correlations, including Person's correlation coefficient (r) and respective p -values, between rapid-rate CT_{max} , slow-rate CT_{max} , and growth for both treatments. Measurements are corrected for tank-effects by mean-centring (m.c.) all values on their respective tank-means. Lines are fitted using least-square regression for each plot's values and are for illustrative purposes only.

Discussion

Critical thermal tolerance under rapid thermal ramping was significantly correlated with critical thermal tolerance under slow thermal ramping. This shows that individuals with a comparably high tolerance to rapid temperature increases are also comparably tolerant to slow rates of thermal ramping. At the 34 °C acclimation treatment, there was a near-significant, weak, but positive correlation. The reduced strength and significance 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 (Pintor *et al.*, 2016) and increasing the relative measurement error, masking the correlation and making it harder to detect. Although the result is less clear in this treatment, it still suggests of a link between these traits. It is a well-established pattern that ramping rate affects the outcome of the CT_{max} test (Kovacevic, Latombe and Chown, 2019), but this experiment is the first one to our knowledge that has, across individuals, tested for correlations between tolerance to different thermal ramping rates. Recently, CT_{max} at a 0.3°/min ramping rate was found to be a repeatable trait within zebrafish individuals, meaning that there are consistent inter-individual differences in acute thermal tolerance (Morgan, Finnøen and Jutfelt, 2018). The correlation we found between critical thermal tolerance at a rapid and slow ramping rate, together with the known consistent differences in rapid-rate CT_{max} , suggest that these two measures of thermal tolerance could be organized in a syndrome and are likely to coevolve as the climate changes.

In terms of the ecological relevance of the acute CT_{max} test, the results are promising as they show that the rapid-rate CT_{max} test, which ramps temperatures up to a critical limit in a matter of 30-60 minutes, is still relevant for high-temperature challenges happening at timespans eleven times longer. An individual with a high tolerance to rapid thermal ramping may very well also be an individual able to survive a slow increase in temperature happening over many hours or days during a heatwave. The correlation between the ability to tolerate short and longer thermal challenges demonstrates a broader relevance for the 0.3 °/min rapid-rate CT_{max} measurement.

A potentially important observation during rapid-rate and slow-rate CT_{max} tests was that the nature of the LOE changed between the two ramping rates. At the standard, rapid ramping rate, zebrafish display a distinct disorganisation response, characterized by fast, erratic swimming coupled with an inability to remain upright. At the slow ramping rate, however, it was more common for the fish to lose equilibrium from what seemed to be exhaustion. That is, instead of swimming without a

righting response (as in the rapid-rate test), the fish simply stopped swimming, and thus also lost their ability to remain upright. These different responses leading to LOE suggests different underlying mechanisms ultimately causing the LOE at the two heating rates. The almost instant LOE and disorganization reaction during the rapid-rate thermal ramping suggests an immediate failure of some vital mechanism, like cardiac (Sidhu *et al.*, 2014) or neurological malfunctioning (Miller and Stillman, 2011; Robertson, 2004), while the slow exhaustion-like response during the slow rate ramping suggests a gradual build-up of some malfunction, metabolic waste products, or the exhaustion of some organ. Still, the correlation found in this experiment suggests that some important link between these two traits exists. 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) or enzyme denaturation. Alternatively, the different endpoints observed at slow-rate and rapid-rate thermal ramping could suggest that a genetic or developmental component may be important for the observed correlation.

In the face of climate change, these findings could be particularly important for studies on the ecology of zebrafish, being a species commonly inhabiting shallow habitats with low flow (Arunachalam *et al.*, 2013). Historically, the peak temperature of heatwaves in the north-east of India (a central part of the zebrafish range) have 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 the most likely scenario of carbon emissions, the duration and frequency are likely to increase by two to five times in this region (Murari *et al.*, 2015). Survival of species in these areas is thus depending on the ability to survive very high increases in temperature over increasingly longer periods. Our results suggest that future studies as well as data already available on the critical thermal maximum of this species may very well be relevant in this context of thermal challenges.

Tolerance to rapid thermal ramping was higher than tolerance to slow thermal ramping 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 cumulative stress of a prolonged thermal challenge makes the fish loose equilibrium before reaching as high temperatures as during a shorter test using a more rapid ramping rate (Rezende, Castañeda and Santos, 2014). Interestingly, this pattern was reversed in the 22 °C acclimation treatment. One explanation for this is that the total time of the slow-rate CT_{max} test in the 22 °C acclimation treatment is over twice as long as in the 34°C treatment (742 vs 322 minutes), giving individuals in the 22 °C treatment more

time to acclimate during the trial. Alternatively, the exposure to a high temperature during the rapid-rate CT_{max} test, done ten days before the slow-rate test, might also have caused a slight upwards temperature-acclimation in the individuals of the 22 °C treatment, whereas in the 34 °C treatment, the individuals are already acclimated close to their upper limit.

The growth rates observed in this experiment were close to what we've observed at 22 °C and 34 °C acclimation in an earlier acclimation experiment (unpublished), being almost half of what we then observed in the 30 °C treatment, indicating that these temperatures had a strong negative effect on their growth rate. Given this, we had expected a strong relationship between their thermal tolerance and ability to grow in these temperatures. However, only a weak, near-significant positive correlation was found between these traits, and only in the 22 °C treatment when using a slow thermal ramping. Tolerance to rapid thermal ramping did not correlate significantly with growth in the 22 °C treatment, and neither measure of tolerance to thermal ramping correlated with growth in the 34 °C treatment. The lack of any strong correlation demonstrates that acute thermal tolerance has little 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 growth-performance and thermal tolerance are governed by disparate mechanisms.

The weak correlation found between tolerance to slow thermal ramping and growth at 22 °C still suggests some link between these traits to be present. The test of tolerance to slow thermal ramping from 22 °C was by far the longest one in terms of time and was also where the highest variation was observed. Based on this high variation, it is not surprising that a weak link between growth and thermal tolerance was only detectable in that combination of ramping rate and acclimation temperature. 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 CT_{max} and lower optimal temperature for growth, a negative correlation should have been observed. If both thermal tolerance and growth are influenced by metabolism, we would also predict a negative correlation, as higher metabolism would be assumed to increase growth but reduce thermal tolerance. Still, the correlation found in this specific context alludes to some link between thermal

tolerance and growth, but the lack of an overall strong correlation means this relationship is unlikely to be important in terms of climate change and evolution.

Under the premise of a thermal behaviour syndrome, we had predicted a correlation between all three measured traits. However, we only found a correlation between the two tests of thermal tolerance, leaving growth unlinked to these traits. The results show that the scope of a thermal-behaviour syndrome may be more limited than what was predicted. On the other hand, the lack of correlation between thermal tolerance and growth does not necessarily suggest that both these traits should be excluded from this suite of thermal traits, only that they are not strongly linked within it. Another important premise of the thermal behaviour syndrome and the extension of the POLS with thermal physiology is that consistent differences in thermal traits should co-vary with differences in life-history and behaviour traits. Recent studies on delicate skinks (*Lamprologis delicata*) have revealed some very 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). Although thermal tolerance has not been tested in this system, the findings have been otherwise supportive of individuals being ranked on a cold-hot axis with corresponding behavioural traits. The integration of thermal physiology into a framework of behaviour and life-history traits like POLS is an exciting prospect as it could help bridge studies of physiology and ecology, particularly in terms of evolution and consequences of climate change. In this experiment, we've focused on traits of thermal physiology in isolation from behaviour, but found important limits to the link between tolerance and performance, suggesting they're not strongly linked within a syndrome.

Conclusions. Tolerance to rapid warming correlates with tolerance to slow warming across individuals. This means that the measure of acute thermal tolerance also predicts tolerance to more prolonged thermal challenges, on the scale of what can be experienced during daytime under a heatwave. Thermal tolerance and growth under temperature-stress do not correlate significantly, but a weak correlation was found at the 22 °C treatment, suggesting some shared underlying mechanism between both traits.

Altogether, the results provide some support for the configuration of thermal traits into a syndrome but show that thermal tolerance and performance are not strongly linked within such a system.

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Supplementary material

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

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

Table S2 Results of ANOVA analysis on linear models modelling either slow-rate CT_{max}, fast-rate CT_{max}, or growth as the response variable against holding tank as the predictor variable.

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

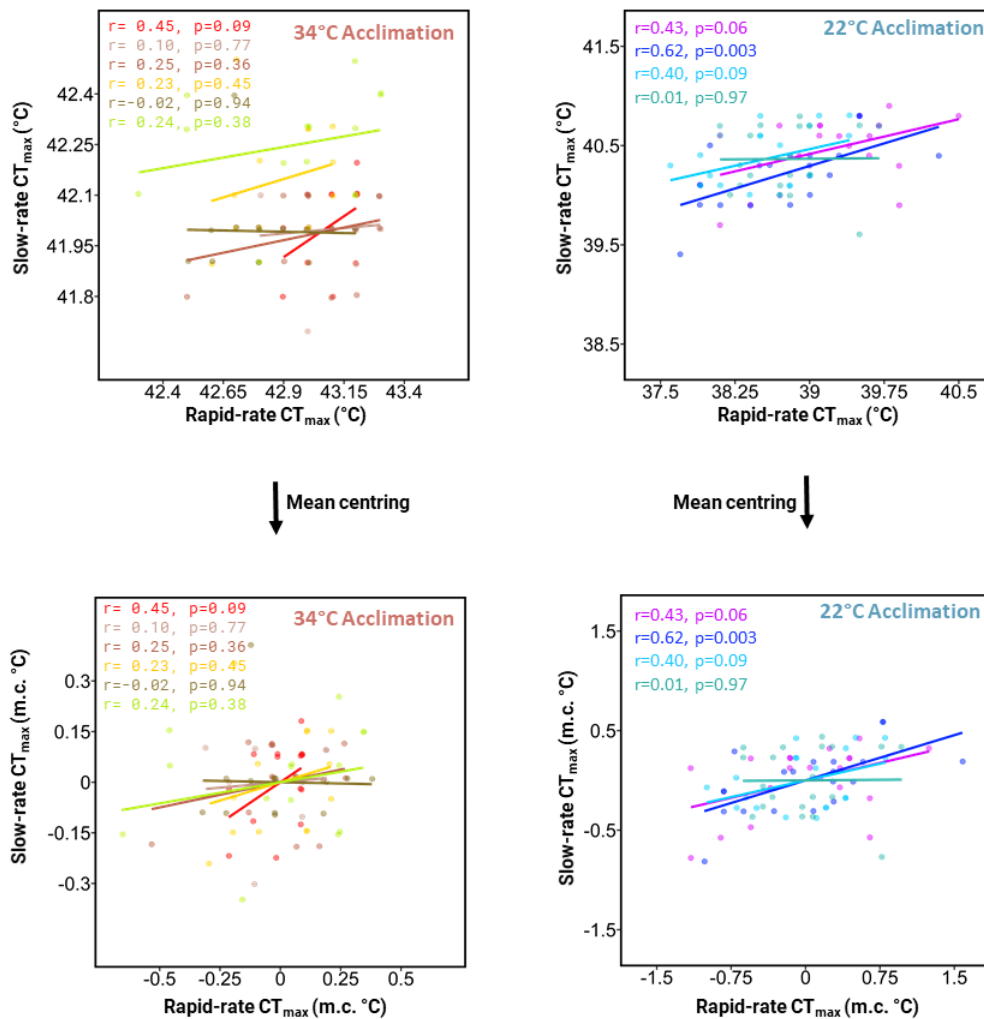


Figure S3 Shows slow-rate CT_{max} plotted against rapid-rate CT_{max} for both acclimation treatments before and after mean centring (m.c.) of values. Mean centring redefines each value by subtracting the mean of it's respective holding-tank from it, centring all tank-means on zero.

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