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REVIEW

Finding the right thermal limit: a framework to reconcile ecological, physiological and methodological aspects of CT_{max} in ectotherms

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ABSTRACT

Upper thermal limits (CT_{max}) are frequently used to parameterize the fundamental niche of ectothermic animals and to infer biogeographical distribution limits under current and future climate scenarios. However, there is considerable debate associated with the methodological, ecological and physiological definitions of CT_{max}. The recent (re)introduction of the thermal death time (TDT) model has reconciled some of these issues and now offers a solid mathematical foundation to model CT_{max} by considering both intensity and duration of thermal stress. Nevertheless, the physiological origin and boundaries of this temperature–duration model remain unexplored. Supported by empirical data, we here outline a reconciling framework that integrates the TDT model, which operates at stressful temperatures, with the classic thermal performance curve (TPC) that typically describes biological functions at permissive temperatures. Further, we discuss how the TDT model is founded on a balance between disruptive and regenerative biological processes that ultimately defines a critical boundary temperature (T_c) separating the TDT and TPC models. Collectively, this framework allows inclusion of both repair and accumulation of heat stress, and therefore also offers a consistent conceptual approach to understand the impact of high temperature under fluctuating thermal conditions. Further, this reconciling framework allows improved experimental designs to understand the physiological underpinnings and ecological consequences of ectotherm heat tolerance.

KEY WORDS: Critical thermal maximum, Thermal tolerance limits, Heat stress, Thermal death time, Thermal performance curve, Homeostasis, Repair, T_c

Introduction

Thermal tolerance and performance are critical in defining the fundamental niche of ectothermic animals and, consequently, limits of thermal tolerance are strong predictors of the biogeographical distribution of ectotherms (Addo-Bediako et al., 2000; Bennett et al., 2021; Buckley and Huey, 2016; Deutsch et al., 2008; Kellermann et al., 2012; Pinsky et al., 2019; Sunday et al., 2012; Sunday et al., 2019). Projected increases in average temperature as well as increases in seasonal and daily temperature variation (Easterling et al., 2000; IPCC, 2021; Rahmstorf and Coumou, 2011; Seneviratne et al., 2014) underscore the importance of reaching a consensus on how to measure and analyse thermal tolerance estimates. In this process, it is important to understand the biological basis of thermal tolerance traits, e.g. critical thermal maximum (CT_{max}; see Glossary), and also to recognize how these acute tolerance traits relate to more chronic thermal performance traits, e.g. optimal temperature (T_{opt}; see Glossary). Such an understanding will ideally improve our ability to deliver relevant and consistent predictions of species' responses to climate change (Clusella-Trullas et al., 2021; Deutsch et al., 2008; Jørgensen et al., 2022; Kingsolver et al., 2013; Parmesan, 2006; Sinclair et al., 2016; Stoks et al., 2017).

There is currently little consensus on how to measure CT_{max} as this 'trait' can be determined with different endpoints, such as loss of coordination, onset of spasms, coma or death (Lutterschmidt and Hutchison, 1997a,b) and it is a source of confusion that CT_{max} is determined in assays with different time frames or heating rates, because the duration and intensity of heat exposure will invariably affect the CT_{max} estimate (Bates and Morley, 2020; Chown et al., 2009; Jørgensen et al., 2019; Kilgour and McCauley, 1986; Peck et al., 2009; Terblanche et al., 2007). An even bigger source of confusion comes from terminology, because CT_{max} is also often used to describe the upper endpoint of the thermal performance curve (TPC; see Glossary) for traits such as growth, metabolism or activity (in this Review, we define such upper limits as TPC_{max}) (Deutsch et al., 2008; Huey and Stevenson, 1979; Kellermann and van Heerwaarden, 2019). The somewhat indiscriminate use of the term CT_{max} results in ambiguity, which is problematic for comparison of CT_{max} across large comparative meta-analyses, and when CT_{max} is used in trait-based modelling to forecast the consequences of climate change (Cooper et al., 2008; Hoffmann et al., 2003; Jørgensen et al., 2022; Kellermann and van Heerwaarden, 2019; Sunday et al., 2012; Sunday et al., 2019; Woodin et al., 2013).

Some of the methodological challenges concerning CT_{max} have recently been addressed by (re)introducing the concept of the thermal death time (TDT) model (see Glossary), which combines information on the severity and duration of stressful temperature exposure (Fry et al., 1946; Jørgensen et al., 2019; Jørgensen et al., 2021b; Kilgour and McCauley, 1986; Maynard Smith, 1957; Rezende et al., 2014). However, these temperature–time models of critical tolerance have yet to be discussed extensively in relation to model boundaries and in relation to other measures of thermal performance, such as TPCs. Further, the physiological origin of TDT models is not well described (see discussion in Jørgensen et al., 2019 and Jørgensen et al., 2021b) nor have the models been properly integrated with the historical and ongoing debate on the mechanistic causes of CT_{max} (Bowler, 2018; Cossins and Bowler, 1987; González-Tokman et al., 2020; Jutfelt et al., 2018; Neven, 2000; Pörtner and Farrell, 2008; Schulte et al., 2011; Sørensen et al., 2003; Vornanen, 2020).

With this Review, we first discuss the current state of CT_{max} measurements and propose a common framework that can reconcile and differentiate the classic TPC model with the TDT model as models capturing how temperature affects rates of 'life' and 'death', respectively. We then establish an integrative model of

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Glossary

Critical temperature (T_c)
A species/population-specific temperature (or temperature zone) that defines the transition between the permissive and stressful temperature range. T_c is the temperature where homeostatic capacity rate and disruption rate are equal.

Critical thermal maximum (CT_{max})
Temperature at which locomotor function is lost during gradual heating (ramping assay). In static temperature assays, CT_{max} can be reported as the time to failure. CT_{max} therefore describes an acute heat failure that occurs at a temperature above the critical temperature (T_c) in static and dynamic assays.

Disruption rate
A theoretical rate describing the temperature sensitive increase in processes disrupting organismal homeostasis with increasing temperature.

Heat failure rate
The difference between the disruption rate and the homeostatic capacity rate determines the rate at which heat stress accumulates. This rate determines the TDT relationship and can be calculated as 1/time to failure at different stressful temperatures (above T_c).

Homeostatic capacity rate
A theoretical rate describing the capacity of biological processes that acts to support organismal homeostasis. This rate is temperature sensitive and the associated biological processes counter biological disruption caused by increased temperature.

Optimal temperature (T_{opt})
The temperature at which a trait/biological rate is maximized across a range of temperatures in a thermal performance curve (TPC).

Permissive temperature range
The temperature range that permits completion of the life cycle, i.e. development, maturation and reproduction, and where heat stress does not accumulate. This is comparable to the thermal niche breadth concept.

Stressful temperature range
The temperature range where homeostasis is disrupted such that heat stress accumulates until it causes heat failure or mortality.

Thermal death time (TDT) model
The TDT model describes the exponential relationship between temperature and time to heat failure. When time to heat failure is $\log_{10}$ transformed, the TDT becomes a linear regression that is easily described by the slope (or more often z=−1/slope) and a point on the line.

Thermal dose (Td)
A theoretical amount of thermal injury an organism can tolerate before heat failure. Once the thermal dose is reached, the organism will fail/die.

Thermal performance curve (TPC)
The TPC describes the relationship between temperature and biological rate of traits in ectotherms. These traits are often inferred to be fitness related and the TPC is often depicted as an asymmetric left-skewed curve.

Temperature quotient (Q_{10})
Quotient describing the fold-change in biological rate due to a 10°C increase in temperature.

thermal tolerance that is based on the balance between disruptive and homeostatic biological processes, providing a framework for understanding exposure to high temperature under naturally fluctuating thermal conditions, as it allows for the inclusion of repair in the assessment of cumulative heat stress. Together, this relatively simple and consistent view of critical thermal limits enables improved experimental design and a coherent understanding of animal heat tolerance in comparative, ecological and mechanistic studies.

Thermal performance or thermal death – defining the permissive and stressful temperature range

When discussing thermal limits of ectotherms, it is important to differentiate between the temperature range that permits completion of the life cycle, i.e. development, maturation and reproduction, and the temperatures that acutely limit the survival of the organism as a result of acute thermal stress (Fig. 1; Fry et al., 1946; Hollingsworth, 1969; Fry, 1971; Woodin et al., 2013; Kellermann and van Heerwaarden, 2019; Bates and Morley, 2020; Parratt et al., 2021; van Heerwaarden and Sgrò, 2021). Fry and colleagues (Fry, 1947; Fry, 1971; Fry et al., 1946) distinguished between these temperature domains as the ‘zone of tolerance’ and the ‘zone of resistance’, respectively. Here, we refer to these as the ‘permissive’ and ‘stressful’ temperature range, respectively (see Glossary), to avoid confusion with effects of acclimation on heat stress tolerance or resistance. An important aspect of the proposed framework is the introduction of the critical temperature (T_c; see Glossary), which separates the permissive temperature range from the upper stressful temperature range.

The permissive temperature range and the TPC

The effects of permissive temperatures on ectotherm performance/fitness have traditionally been described by the TPC (Fig. 1; Angilletta, 2009; Huey and Stevenson, 1979; Huey et al., 2012). As Darwinian fitness is difficult to assess directly, a proxy related to fitness is usually used instead, e.g. egg-laying capacity, developmental speed (Huey and Berrigan, 2001; Huey et al., 2012; MacLean et al., 2019; Overgaard et al., 2014) or rates of biochemical or physiological processes, such as growth rate or feeding rate (Angilletta, 2009; Cossins and Bowler, 1987; Hochachka and Somero, 2002; Schulte et al., 2011; Sinclair et al., 2016). The TPC is most often left-skewed with an initial positive effect of increasing temperature on performance (Buckley et al., 2022; Dell et al., 2011; Gilchrist, 1995). After the maximal rate of performance is reached at T_{opt}, the rate of performance quickly decreases when temperature increases further. The upper and lower endpoints of TPCs are frequently termed CT_{max} and CT_{min}, respectively, which can be confusing because the temperature limits of the TPC are not necessarily related to acute failure (Fig. 1). Thus, a complicating feature of TPCs is that the performance of some traits, e.g. speed of locomotion, oxygen consumption rate and heart rate, clearly span both the permissive and stressful temperature range, while TPCs for population growth and chronic behaviours are restricted to only the permissive temperature range. To illustrate this, we compiled estimates of thermal performance of various biological processes and acute thermal limits in three ectothermic species: vinegar fly, brook trout and zebra mussel (Fig. 1). Note that heat failure and heat knockdown are used interchangeably to describe either onset of heat coma or heat mortality (Lutterschmidt and Hutchison, 1997a,b). For all three species, the TPCs of fitness proxies and growth are restricted to the permissive temperature range and always decline to zero before the temperature reaches the stressful temperature range above T_c (Fig. 1). In contrast, TPCs for processes related to movement or metabolic rate span both the permissive and stressful temperature range such that the observed decline in these traits coincides more with the decrease in survival time conveyed by the TDT model (secondary axes in Fig. 1). The distinct nature of TPCs for different traits is partly a result of the time scales at which the performance is measured as fitness proxies of growth and reproduction inherently operate over long time frames, whereas locomotion and metabolic rate are instantaneous measures (Cossins and Bowler, 1987; Hoffmann and Todgham, 2010; Kellermann and van Heerwaarden, 2019; Kellermann et al., 2019; Kingsolver and Woods, 1997; Kingsolver and Woods, 2016; Schulte et al., 2011, 2020; Sinclair et al., 2016). Traits such as metabolic rate or movement are obviously indirectly related to Darwinian fitness and remain important and

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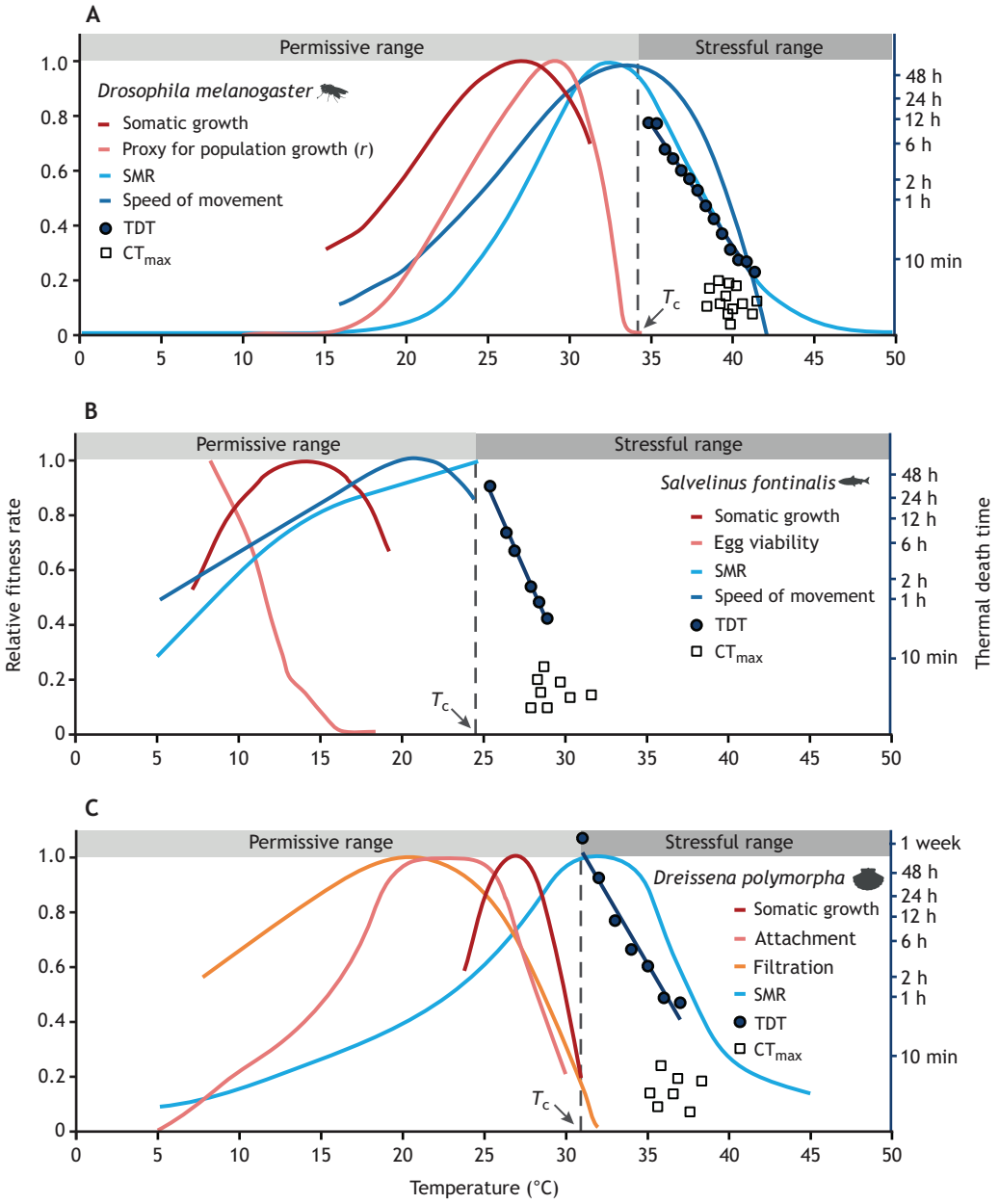

Fig. 1. Thermal performance cu rves (TPCs) differ markedly between traits. TPCs and measures of heat tolerance for (A) vinegar fly (*Drosophila melanogaster*), (B) brook trout (*Salvelinus fontinalis*) and (C) zebra mussel (*Dreissena polymorpha*). TPCs of chronic biological processes associated directly with Darwinian fitness [proxy of population growth rate (*r*) and somatic growth; red shades] generally have lower thermal optima (T_{opt}) and are restricted to the permissive temperature range, whereas those that are more indirectly associated with Darwinian fitness [speed of movement and standard metabolic rate (SMR); blue shades] span both the permissive and stressful temperature range and generally have a higher T_{opt}. Thermal death time (TDT) curves show the relationship between temperature and survival time in the stressful temperature range (dark blue circles and line refer to the secondary *y*-axis) along with acute measures of CT_{max} measured during dynamic (ramping) exposures (white squares separated vertically for increased visibility). A critical temperature (T_c; dashed line) separating the permissive and stressful temperature range is tentatively placed to indicate the transition between temperature ranges determining 'rate of life' and 'rate of death'. See Table S1 for references.

functional at temperatures above T_c, where they may be critical for escaping the heat stress. Even so, the optimal and critical temperature limits of these 'indirect' fitness traits may be confusing if their TPCs are interpreted as direct correlates of Darwinian fitness, e.g. the 'optimal' temperature for metabolic rate or running speed is clearly not aligned with the optimal temperature for population growth (Fig. 1).

The distinction between the permissive and stressful temperature range is also highlighted by the differential impact of temperature on lifespan (Fig. 2). In the permissive temperature range, lifespan is not limited directly by acute thermal injury but decreases with increasing temperature simply as a consequence of the thermal effect on metabolic rate (rate-of-living hypothesis; Brown et al., 2004; Munch and Salinas, 2009; Shaw and Bercaw, 1962) or through differences in the temperature-specific ageing threshold which precedes death (described in the threshold hypothesis; Maynard Smith, 1963). Irrespective of the mechanism, the rate of ageing/senescence/mortality (calculated as the inverse of lifetime)

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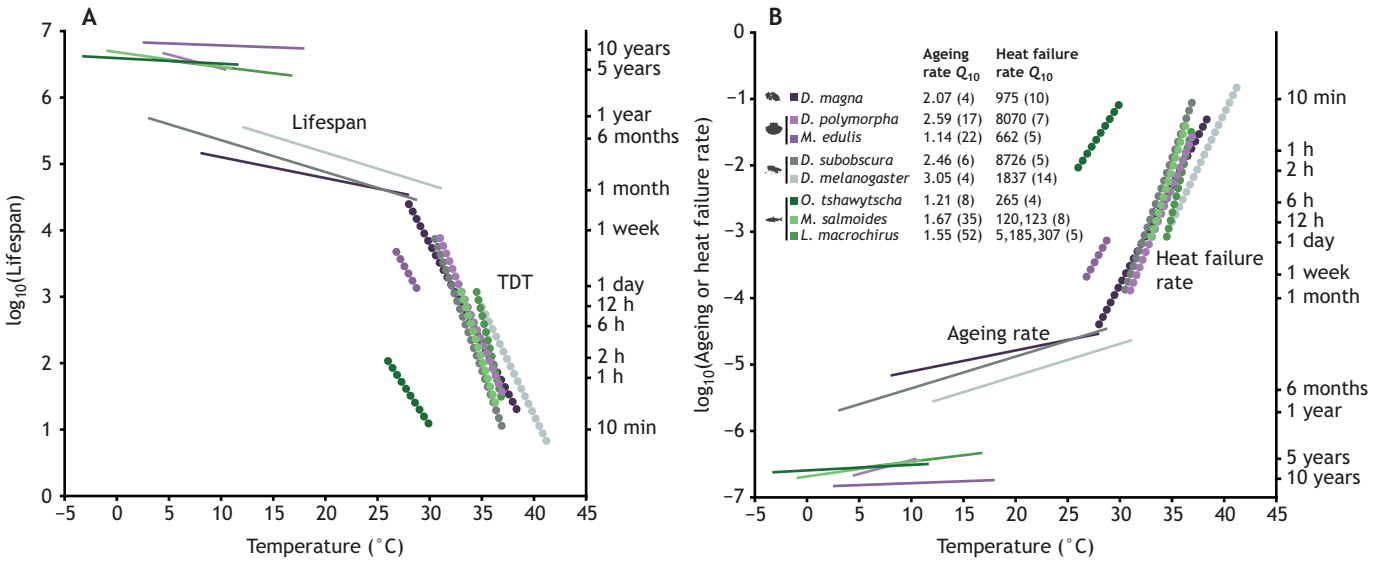

Fig. 2. Higher thermal sensitivity of survival in the stressful temperature range compared with lifespan in the permissive range. (A) Lifespans (min) and survival times for eight ectotherm species measured across temperatures in the permissive (solid lines) and stressful temperature range (dotted lines; TDT curves), respectively. (B) Lifespans and survival times are converted to ageing/mortality rate (permissive range) or heat failure rate (stressful range) in both cases calculated as 1/time to death (min^{-1}). Thermal sensitivity of heat failure rate in the stressful range (steepness of slope) is much more extreme than that for ageing rate in the permissive temperature range (note the logarithmic scale) as also shown in the species-specific temperature quotients, Q_{10}, reported separately for the permissive (ageing rate Q_{10}) and stressful (heat failure rate Q_{10}) temperature range. The number of data points used to calculate Q_{10} is indicated in parentheses. Lifespan data in the permissive temperature range were retrieved from Munch and Salinas (2009) except for *D. subobscura* (from Hollingsworth, 1969) and the references for the acute survival times (TDT) can be found in Table S2.

in the permissive temperature range has temperature quotients (Q_{10}; see Glossary) that are typical for 'normal' biological processes (Q_{10}=1.1–3.1; Fig. 2) (Dell et al., 2011; Doudoroff, 1945; Loeb and Northrop, 1916; Munch and Salinas, 2009; Seebacher et al., 2014). These Q_{10} estimates of ageing/senescence/mortality rates are in marked contrast to the very high Q_{10} values that characterize rates of heat failure conveyed in the TDT model (see below).

The stressful temperature range and the TDT curve

In the stressful range, temperature poses an acutely limiting factor on survival and does so in a dose-dependent manner where the severity of the thermal stress dictates survival time, i.e. faster heat death at higher temperature. As exemplified in Fig. 2, the slope between temperature and survival time is much steeper in the stressful temperature range than in the permissive range. The lethal domain of high temperatures therefore only spans a few degrees before survival time becomes extremely brief (Brown and Crozier, 1927; Cossins and Bowler, 1987; Fry et al., 1946; Jørgensen et al., 2019; Jørgensen et al., 2021b; Kilgour and McCauley, 1986; Rezende et al., 2014). Consequently, in all eight species examined here, Q_{10} values for heat failure rates (see Glossary) are much higher in the stressful temperature range (median Q_{10}=4953) compared with those for ageing rates in the permissive range (median Q_{10}=1.87; Fig. 2). These examples are in accordance with recent and historical evidence showing that the thermal sensitivity of processes related to heat failure are characterized by extreme Q_{10} values in the range 100–100,000 (Hollingsworth, 1969; Jørgensen et al., 2019; Jørgensen et al., 2022; Maynard Smith, 1957). To put this in perspective, a Q_{10} of 1500 (close to the median Q_{10} of heat failure rate in 112 ectotherm species; Jørgensen et al., 2022) causes a ∼100% increase in injury rate per 1°C in the stressful temperature range, causing the knockdown time to be halved for each 1°C increase. Contrastingly, in the permissive range, where Q_{10} is ∼2, lifespan is reduced by a modest 7% per 1°C increase as a result of accelerated biological activity. Importantly, the extreme thermal sensitivity in the stressful temperature range implies that the increase in intensity and frequency of heat waves associated with global warming could greatly amplify heat mortality for many ectotherms (Jørgensen et al., 2022).

The extreme thermal sensitivity of heat failure in the stressful temperature range is conveyed by the TDT model, which describes the exponential decline in survival time with increasing temperature (Fig. 3A,B; Fry et al., 1946; Kilgour and McCauley, 1986; Rezende et al., 2014; Jørgensen et al., 2019). Although the focus here is on heat tolerance, we emphasize that the TDT model can also be applied to cold stress (Chen and Walker, 1994; Nedvěd et al., 1998; Salt, 1966; Tarapacki et al., 2021).

A critical temperature, T_c, separates the permissive and stressful temperature zone

Here, we define a critical temperature (T_c) that separates the permissive and stressful temperature domains (Fig. 1). Philosophically, T_c can also be considered as a narrow temperature range, rather than a specific temperature, where thermal relationships of the organism transition from mainly temperature effects on 'processes supporting life' to those on 'processes causing injury and death'. Although it is difficult to empirically measure T_c (see 'Concluding remarks and future perspectives', below), this parameter is conceptually important for defining, parameterizing and reconciling measures of heat tolerance. For example, it is evident that any measure of acute heat failure will always be above T_c (Fig. 1). This definition helps separate measures of acute heat failure (often termed CT_{max}) from the upper thermal limits of TPCs (TPC_{max}) that must be below T_c for traits directly related to fitness and growth. The T_c concept presented in this review is largely analogous to 'the upper incipient lethal temperature' (UILT) defined by Fry and co-workers in their seminal studies on lethal temperatures in fish (Fry, 1947; Fry, 1971; Fry et al., 1946) or to the long-term survival temperature T_s, defined more recently by Richard et al. (2012) in

marine ectotherms. These transition temperatures (UILT, T_s or T_c) all represent the highest temperature that allows for chronic survival, and above these transition temperatures, heat stress accumulates with increasing intensity as temperature is raised further (as in Fig. 2). Importantly, modelling studies of heat tolerance in fish (Kilgour and McCauley, 1986; Kilgour et al., 1985) and insects (Kingsolver and Umbanhowar, 2018; Jørgensen et al., 2021a,b) have all concluded that inclusion of a critical thermal limit (T_c) is needed for models of heat stress to disregard the time spent below T_c during the gradual temperature increase in a ramping assay, as this time does not contribute to the accumulation of heat injury.

The TDT model – a tool to reconcile static and dynamic measures of acute thermal limits (CT_{max})

Heat tolerance is typically measured using either static assays, where time to heat failure is measured under exposure to a constant temperature, or dynamic/ramping assays, where the heat failure temperature under incrementally increased temperature exposure is assessed. Both static and dynamic protocols have been widely discussed (Overgaard et al., 2012; Rezende et al., 2011; Terblanche et al., 2007), and in particular it is debated whether static and dynamic measures of heat tolerance are comparable or even ecologically relevant (Bak et al., 2020; Cooper et al., 2008; Kingsolver and Umbanhowar, 2018; Rezende et al., 2014; Santos et al., 2011; Sgrò et al., 2010; Terblanche et al., 2011). Much of this methodological discussion can be settled by the TDT model (Fry et al., 1946; Jørgensen et al., 2019, 2021b; Kilgour and McCauley, 1986; Maynard Smith, 1957; Rezende et al., 2014; Willot et al., 2022) and here we elaborate on this discussion by reviewing the foundation and boundaries of the TDT model.

Foundations of the TDT model

The TDT model is based on the understanding that heat failure rate increases exponentially with temperature (Fry et al., 1946; Jacobs, 1919; Jørgensen et al., 2019, 2021b; Kilgour and McCauley, 1986; Kilgour et al., 1985) and that, at temperatures above T_c, the animal can only withstand a finite amount of accumulated heat injury before it succumbs to heat stress, here termed 'thermal dose' (see Glossary; Td in Fig. 3). This thermal dose is the same for all exposure temperatures above T_c and can graphically be represented as equal area rectangles in a plot of heat failure rate versus time to heat failure (Fig. 3A). As heat failure rate increases exponentially with temperature, it is clear that time to heat failure will decrease exponentially with temperature, as depicted in the classical TDT curve (Fig. 3B; Bigelow, 1921; Fry et al., 1946; Jørgensen et al., 2021b; Kilgour and McCauley, 1986; Maynard Smith, 1957). Time to heat failure is traditionally $\log_{10}$ transformed such that the TDT curve becomes a simple linear relationship described by the slope and a point on the line, e.g. the temperature causing heat coma after a 1 h exposure (Jørgensen et al., 2019, 2021b; Willot et al., 2022). In the TDT model, the slope represents the thermal sensitivity factor, z=−1/slope, describing the temperature change resulting in a one order of magnitude change in heat failure time (Rezende et al., 2014). TDT model parameters are typically derived from experiments where time to failure is measured under exposure to three or more static temperatures (Fig. 3B) and, once established, the TDT model can be used to estimate heat failure time at a given temperature or failure temperature for a given exposure duration.

High and low intensity heat stress are additive

If the thermal dose is truly independent of temperature, then the injury sustained at different static temperatures should be completely additive as is well supported empirically (Fry, 1947, 1971; Fry et al., 1946; Jørgensen et al., 2021b; Kashmeery and Bowler, 1977). This additivity suggests that the same physiological mechanisms are responsible for heat injury sustained under both intense and moderate heat stress at temperatures above T_c (see below). Consequently, it is possible to use TDT model parameters derived from static experiments to accurately predict heat failure time during (i) combinations of different static exposures, (ii) gradually increasing temperature where a linear temperature increase results in an exponentially increasing heat failure rate and/or (iii) fluctuating temperature exposure (Fig. 3C). These predictions of heat stress additivity above T_c have, to our knowledge, only been comprehensively tested experimentally in two species – the brook trout and vinegar fly – but here the predictions have been fully validated (Fry et al., 1946; Jørgensen et al., 2021b). First, using only static exposure temperatures, TDT models were parameterized. Subsequently, the TDT parameters were used to predict time to heat failure during several static, ramping or fluctuating conditions, and these predictions were then compared with actual experimental measures of time to heat failure (Fig. 3D). As is clear from these examples, the time to heat failure during variable temperature exposures can be accurately predicted from TDT model parameters by simply integrating heat failure accumulation over time (Fry et al., 1946; Jørgensen et al., 2021b). Importantly, in these experiments, additivity was not affected by the order of exposure (high or low intensity heat first), and the stressful temperatures were always above T_c, preventing the animals from repairing thermal injury, which could occur in the permissive zone (see below). Although these two examples provide strong support for the additivity of heat stress above T_c, it remains relevant to test this prediction broadly among ectotherms.

Static and dynamic assays measure the same type of heat stress

A consequence of additive heat stress accumulation above T_c is that static and dynamic assay types can be reconciled by considering that both assays report the heat failure endpoint once a given amount of heat stress (the thermal dose) has accumulated (Fry et al., 1946; Jørgensen et al., 2019, 2021b; Kilgour and McCauley, 1986). In other words, the model essentially claims that all static and dynamic measures of acute CT_{max} measure the same trait! It is simply the progression towards CT_{max} that differs when different static and dynamic protocols expose the animal to different failure rates (Fig. 3C). Consequently, it is mathematically possible to convert one assay type to another using the parameters from a TDT model (Fig. 3C,D; Fry et al., 1946; Jørgensen et al., 2019, 2021b; Kilgour and McCauley, 1986; Kilgour et al., 1985). Further, this reconciling model can explain the frequent observation that in dynamic assays, CT_{max} decreases with slower ramping rates, as the slower ramping invariably increases the duration of exposure to stressful temperatures above T_c (Chown et al., 2009; Jørgensen et al., 2019; Kingsolver and Umbanhowar, 2018; McMahon and Ussery, 1995; Mitchell and Hoffmann, 2010; Peck et al., 2009; Richard et al., 2012; Terblanche et al., 2007).

A TDT model can be parameterized from several static experiments (a classic TDT model) or using several dynamic assays with different temperature ramping rates (Jørgensen et al., 2021b; Kilgour and McCauley, 1986). In previous work (Jørgensen et al., 2021b), we introduced the mathematical framework and *R*-scripts allowing researchers to directly obtain TDT parameters enabling conversion and comparison of thermal tolerance measurements within and between static and dynamic experiments (see https://github.com/MOersted/Thermal-tolerances

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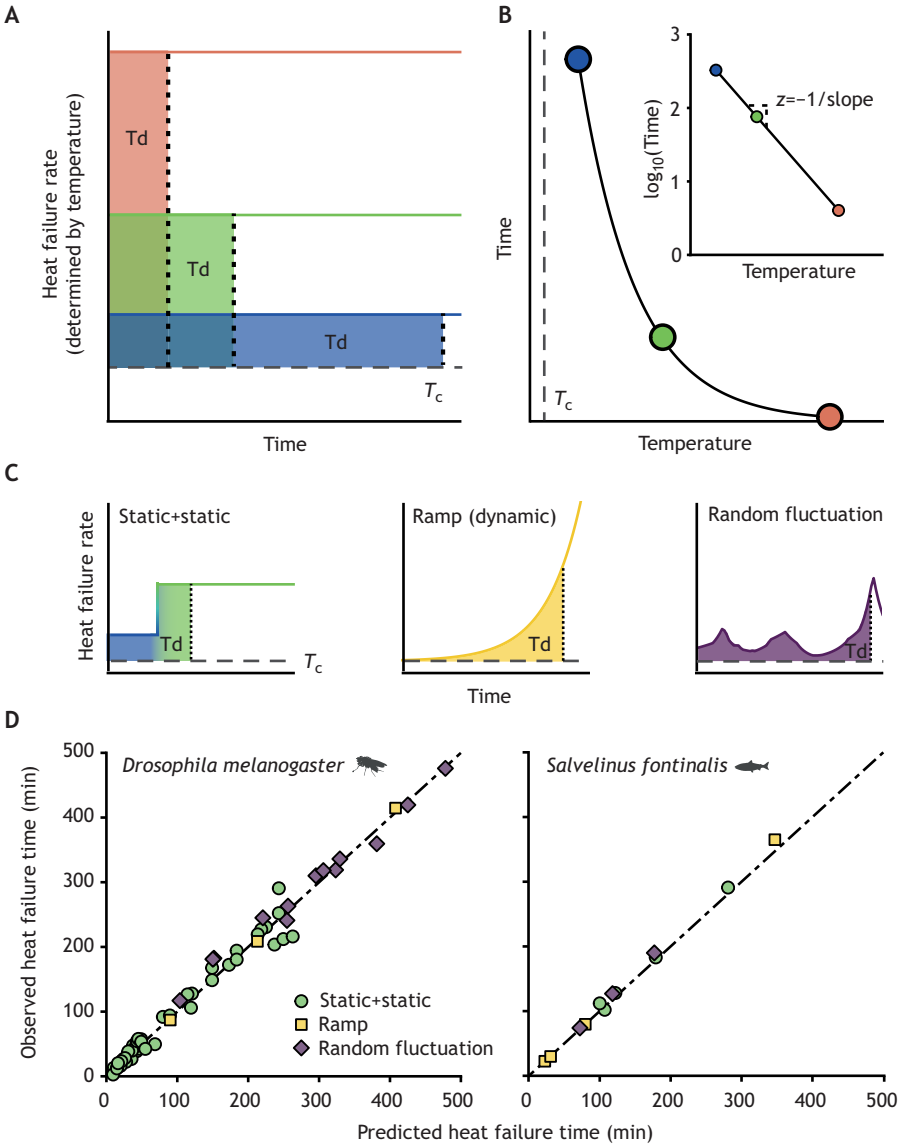

Fig. 3. Heat failure and tolerance can be explained using the TDT model. (A) Heat failure rate increases exponentially with temperature above the critical temperature T_c (dashed line) and heat failure (vertical dashed line) is assumed to occur once a finite amount of heat injury (indicated by the area of the rectangles) has accumulated (thermal dose, Td). Each of the equal-sized rectangles represents heat injury accrued at a specific constant temperature, i.e. with a specific rate calculated as 1/heat failure time. (B) The exponential relationship between temperature and heat failure rate in A leads to the negative exponential relationship between temperature and knockdown time (note that the axes are rotated). The exponential relationship is asymptotic towards the critical temperature T_c. A TDT curve can be produced by a linear regression of temperature on $\log_{10}$-transformed heat failure time. z=−1/slope of this line describes the thermal sensitivity of heat failure (see Figs 1 and 2 for species examples). (C) Examples of three temperature profiles that include exposure to more than a single temperature and result in different heat failure rates during the exposure. 'Static+static' refers to a sequential exposure to two constant temperatures where heat failure rate changes abruptly after the temperature change. 'Ramp' refers to a dynamic exposure where temperature increases linearly, resulting in an exponential increase in heat failure rate. 'Random fluctuation' refers to exposure to temperatures that fluctuate above T_c and can be regarded as a series of infinitesimal exposures to different constant temperatures, each with their specific heat failure rate. For each of the exposure types, Td eliciting heat failure is assumed to be the same, as conveyed by the TDT model. (D) TDT model parameters are derived from a series of static experiments. These parameters are then used to predict heat failure time of treatments (x-axis) plotted against the observed heat failure times (y-axis) in static+static, dynamic or fluctuating temperature experiments. Here, coma (*D. melanogaster*) and death (*S. fontinalis*) are used as the endpoint of heat failure. Irrespective of the type of temperature exposure profile (displayed in C), the predicted and experimentally observed heat failure times are highly correlated, attested by the closeness to the line of unity (dashed line). A–C are modified from Jørgensen et al. (2021b) and D uses data from Jørgensen et al. (2019, 2021b) (*D. melanogaster*) and Fry et al. (1946) (*S. fontinalis*).

for scripts and guidance). Furthermore, TDT parameters can be used to investigate the accumulation of heat stress under fluctuating temperatures (Fig. 3D). However, as with all models, considering limitations is crucial. Firstly, to establish a good TDT model, we recommend using several static experiments covering temperatures that result in a wide range of experimental durations as model extrapolation can be problematic (Jørgensen et al., 2019, 2021b). Secondly, it is important to consider the model boundaries as the TDT model is only valid at temperatures above T_c (Fig. 2; Jørgensen et al. (2021b)). Thus, TDT model parameters should never be used to assess temperature effects on thermal performance below T_c as tolerance and performance in the

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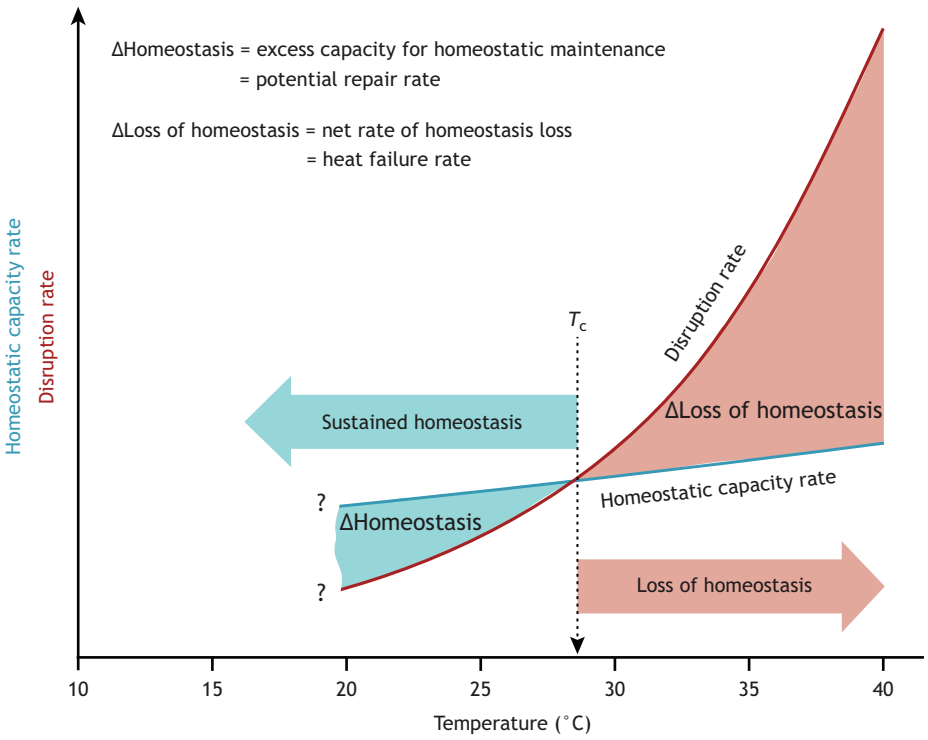

Fig. 4. Rate of disruption is balanced by a capacity for homeostatic maintenance. The red curve represents a theoretical rate of disruption while the blue curve represents a theoretical rate of homeostatic capacity. Below the critical temperature T_c, the homeostatic capacity surpasses the disruption rate, resulting in net sustained homeostasis, i.e. the animal is in a balanced state where disruption is mitigated and there is excess capacity for injury repair (ΔHomeostasis, blue area). Above T_c, the rate of disruption exceeds the capacity for homeostasis and accordingly a net loss of homeostasis occurs (ΔLoss of homeostasis, red area). The two antagonistic processes differ substantially in thermal sensitivity, which results in a very high thermal sensitivity of ΔLoss of homeostasis above T_c. The ΔLoss of homeostasis above T_c dictates the duration at a specific temperature required to lose homeostasis to an extent that results in organismal heat failure. A similar principle can be applied at low temperatures where ectotherms also suffer from thermal stress due to unbalanced rates of homeostatic disruption and repair (not shown).

permissive temperature range are related to biological rates of life with different thermal sensitivities (Figs 1 and 2; Jørgensen et al., 2022).

The physiological origin and boundaries of the TDT model

Considering the additive effects of heat stress outlined above, it is reasonable to assume that a single biological disruption rate accelerating above T_c is the cause of heat failure in ectotherms or that several processes leading to heat failure converge in a common disruptive process (Bowler, 2018; Schmidt-Nielsen, 1997). Further, the extreme and unconventionally high Q_{10} of the heat failure rate is indicative of a shared mechanistic cause of heat failure in ectotherms. However, at present there is little consensus regarding the physiological causes of heat failure. Below, we discuss how heat failure probably originates from a mismatch between rates of homeostatic and disruptive biological processes.

Balancing maintenance and loss of homeostasis

In the simplest model, the relationship between temperature and survival duration conveyed by the TDT model (Fig. 3) could be explained by a single temperature-dependent biological process that disrupts homeostasis with exponentially increasing intensity as temperature increases (‘disruption rate’ see Glossary; red curve in Fig. 4). However, heat injury only accumulates above a critical temperature threshold (T_c), necessitating the inclusion of an additional temperature-dependent biological rate representing the ‘capacity for homeostatic regulation’ (‘homeostatic capacity rate’ see Glossary; blue curve in Fig. 4). Introduction of this homeostatic capacity rate establishes T_c as the temperature where the rate of disruption exactly equals the capacity for restoration of homeostasis, i.e. with no net accumulation of heat injury or repair (for a comparable discussions of repair versus failure, see Colinet et al.; 2015; Kovacevic et al., 2019). According to this view, T_c defines the permissive temperature range as the range where the homeostatic capacity rate is greater than the disruption rate, and the stressful range as the range where the homeostatic capacity rate is less than the disruption rate (Figs 1–4). Accordingly, it is actually the net difference (ΔLoss of homeostasis) between the rate of disruption (‘injury’, red curve in Fig. 4) and the rate of homeostatic capacity (‘repair’, blue curve in Fig. 4) that determines the exponential increase in heat failure conveyed by the TDT model.

Repair of thermal injury at temperatures below T_c

A model with two antagonistic processes representing loss and re-establishment of homeostasis allows for modelling how net repair and net injury accumulation vary when ectotherms experience fluctuating temperatures (Colinet et al., 2015; Kovacevic et al., 2019; Nedvěd et al., 1998). Heat injury accumulates exponentially with temperature above T_c and even if an ectotherm survives a period above T_c and evades the stressful conditions, it will have accumulated a ‘load’ of heat stress (equivalent to a fraction of the thermal dose Td illustrated in Fig. 3A). This load of heat stress is additive (see above) in subsequent stressful exposures unless homeostasis is restored during exposure to temperatures below T_c. Accordingly, heat stress accumulated during a warm day can be partially or completely repaired during a cooler night (Colinet et al., 2015; Dillon et al., 2007; Fry et al., 1946; Kovacevic et al., 2019; Speights et al., 2017).

The potential for net repair at temperatures below T_c depends on the relative strength of the antagonistic processes determining repair and disruption of homeostasis, respectively (Fig. 4). The literature on repair rates is surprisingly scarce but studies indicate that net repair rate is temperature dependent with increasing repair rates found at higher temperatures (Bowler and Kashmeery, 1979; Dingley and Maynard Smith, 1968; Iandolo and Ordal, 1966). This observation is not fully compatible with the pattern in Fig. 4, where the rate of net repair should decrease at the highest permissive temperatures (because of an increasing disruption rate) and repair

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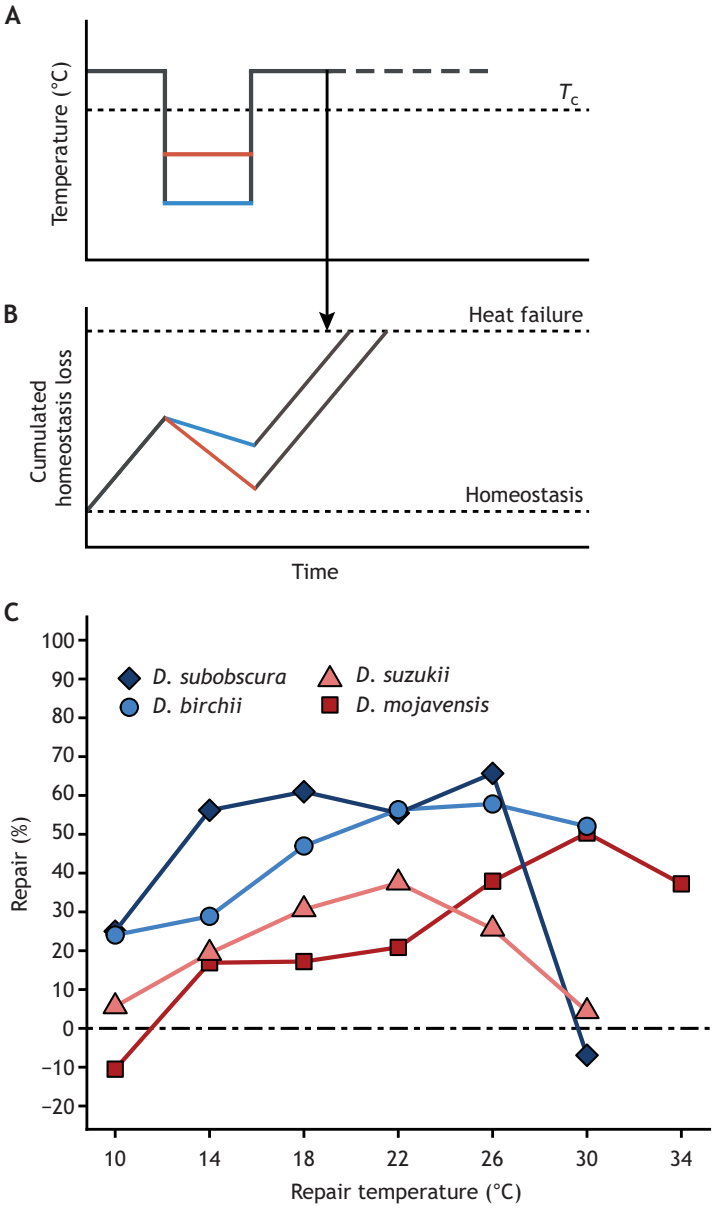

Fig. 5. Homeostatic capacity (repair) rates are temperature dependent. (A) Outline of a 'split-dose' temperature profile to quantify repair rates. Exposure to a constant temperature (above T_c) for the duration that corresponds to 50% of the predicted failure time is followed by a period at temperatures below T_c (blue and red lines) to allow re-establishment of homeostasis (repair). In a subsequent exposure to the same stressful temperature, time to heat failure is noted. If homeostasis has not been restored (repaired), failure is expected when the remaining 50% of the failure duration has passed (arrow), due to the additivity of heat stress. (B) A net loss of homeostasis accumulates during the initial heat stress dose. If repair processes restore homeostasis in a temperature-dependent manner, then re-exposure to heat stress following repair will result in different increases in heat failure time [compare the two treatments (arrow)]. (C) Preliminary data from *Drosophila*: *D. subobscura*, *D. suzukii*, *D. birchii* and *D. mojavensis* exposed to the species-specific stressful high temperature with a calculated heat knockdown time of about 3 h (based on initial TDT curves). In the first exposure, individuals were exposed to the stressful temperature for 50% of that time (ca. 1.5 h). Flies were subsequently transferred to temperatures between 10 and 34°C for 6 h to allow for repair, and then returned to the species-specific stressful temperature, and knockdown time was recorded. Here, the percentage of repair (percentage of initial thermal injury dose) is plotted against repair temperature. Thus, 0% indicates that knockdown time was unaffected by the time spent at benign temperature, i.e. no restoration of homeostasis, and 100% indicates that the homeostasis loss accumulated in the initial thermal dose was completely repaired, i.e. the treated individual had the same knockdown time as naive conspecifics. These pilot data indicate that repair is temperature sensitive, with the highest repair rate at intermediate temperatures and only the desert species (*D. mojavensis*) was able to repair at the highest temperature (34°C).

should converge to zero at T_c. It can also be argued that net repair capacity will not continue to increase indefinitely as temperatures decrease below T_c (Fig. 4). Ultimately, low temperatures will limit the capacity for repair and further it is assumed that at sufficiently low temperatures other processes will start to disrupt homeostasis as a result of cold stress (MacMillan and Sinclair, 2011; Nedvěd et al., 1998; Overgaard et al., 2021).

To briefly exemplify the thermal sensitivity of repair we used 'split-dose' experiments inspired by Kashmeery and Bowler (1977) where two stressful exposures are separated by a period at a permissive temperature allowing for repair. With this protocol, we examined the temperature dependence of repair in four species of *Drosophila* (Fig. 5A,B) by varying the temperature of the 'repair' period between the two stress exposures. The preliminary results support that net repair occurs when flies are allowed a period at permissive temperatures, and further that repair rate is temperature dependent (Fig. 5C) such that repair capacity declines at both low (probably due to slowed metabolism) and high temperature (due to the increased proximity to T_c and increased disruption rate).

The physiological cause of heat injury in ectotherms

There is currently no consensus on a single physiological dysfunction that is considered the proximal cause of acute heat failure in all ectotherms (Jutfelt et al., 2018; Pörtner, 2001; Somero et al., 2017; Vornanen, 2020). Several hypotheses have been proposed to explain ectothermic heat failure, and most of these can be linked to one or more of the five biological processes highlighted by Schmidt-Nielsen (1997): (i) protein denaturation, (ii) thermal inactivation of enzymes, (iii) inadequate oxygen supply, (iv) unbalanced temperature effects on interdependent metabolic reactions or (v) membrane dysfunction. These five 'heat failure hypotheses' are not mutually exclusive and several physiological dysfunctions may operate in unison to cause the high thermal sensitivity that characterizes heat failure (Fig. 2). For this review, we emphasize that these heat failure hypotheses easily integrate with a simple model balancing homeostatic capacity versus homeostatic disruption (Fig. 4). Specifically, we discuss this in relation to three examples: (i) oxygen limitation, (ii) protein denaturation and (iii) membrane effects on cellular excitability.

The much-debated 'oxygen- and capacity-limited thermal tolerance' hypothesis (OCLTT; Pörtner and Farrell, 2008; Clark et al., 2013; Verberk et al., 2016; Jutfelt et al., 2018) poses that ectotherms reach their critical thermal limit when the capacity for aerobic mitochondrial ATP production no longer matches the requirements of the standard metabolic rate (Pörtner, 2001). Limitations to aerobic ATP production at high temperature have often been linked to insufficient oxygen transport, including inadequate cardiac function (Pörtner, 2001; Pörtner and Farrell, 2008). However, compromised mitochondrial coupling and ATP production at high temperature are increasingly being discussed in relation to this hypothesis (Blier et al., 2014; Chung and Schulte, 2020; Hraoui et al., 2020; Iftikar and Hickey, 2013; Jørgensen et al., 2021a; Michaelsen et al., 2021). The OCLTT hypothesis has received mixed experimental support, particularly for terrestrial ectotherms (Fobian et al., 2014; Verberk et al., 2016). Nevertheless, the central tenets of the OCLTT hypothesis are easily integrated with a model of a temperature-sensitive (im)balance of homeostatic capacity (ATP synthesis rate) versus homeostatic disruption (ATP demand) (Fig. 4). The OCLTT hypothesis further aligns because it explains that reduced performance (declining TPC) at the highest permissive temperatures results from decreasing aerobic scope caused by the reduced difference between the antagonistic rates (ATP synthesis versus ATP demand) (Pörtner, 2001; Pörtner and Farrell, 2008; Ern, 2019).

A second hypothesis relates to increased expression or translation of heat shock proteins (hsps) which is a hallmark of heat stress in ectotherms (Feder and Hofmann, 1999; Parsell and Lindquist, 1993; Ritossa, 1962; Tomanek, 2010). The expression of inducible hsps during heat stress is somewhat proportional to net protein denaturation and heat stress can therefore be viewed as an (im)balance of homeostatic disruption (denaturation rate) versus homeostatic capacity (removal rate of denatured protein or rate of *de novo* protein synthesis). The imbalance between these antagonistic rates can be assessed from the magnitude of hsp expression, and several studies support that accumulated protein denaturation is tightly linked to heat failure. For example, (i) cumulative hsp expression is proportional to accumulated heat stress during fast and slow heat ramping in *Drosophila*, although the temperature of heat failure is different between ramping rates (Sørensen et al., 2013), (ii) heat hardening through a brief pre-exposure to heat stress increases hsp expression which subsequently augments the homeostatic capacity rate and therefore increases heat tolerance (Feder and Hofmann, 1999; Parsell and Lindquist, 1993; Sørensen et al., 2003; Tomanek, 2010), and (iii) protein degradation is extremely sensitive to temperature (Q_{10}=10–1000; Ushakov, 1964; Cossins and Bowler, 1987; Tattersall et al., 2012), which is similar to the very high Q_{10} found for organismal failure beyond T_c (Fig. 2; Jørgensen et al., 2022).

A third hypothesis compatible with the model outlined in Fig. 4 relates to the imbalance of membrane processes strengthening or weakening cellular excitability. In insects, it is well described that heat knockdown is associated with a shutdown of the central nervous system (CNS). This is caused by a surge in extracellular [K^+], resulting from an imbalance between active and passive ion transport, that ultimately depolarizes the neurons, causing neuronal silencing (Armstrong et al., 2009; Jørgensen et al., 2020; Robertson et al., 2020; Spong et al., 2016). Similar perturbations in CNS function are also found in other ectotherms at temperatures close to CT_{max} (Andreassen et al., 2022; Friedlander et al., 1976; Hamby, 1975; Jutfelt et al., 2019; Orr, 1955) and loss of ion balance in the extracellular fluid surrounding muscle has also been implicated in ectothermic heat failure (Gladwell et al., 1975; Grainger, 1975). Finally, Vornanen (2016, 2020) argues that heat tolerance is governed by the 'temperature-dependent deterioration of electrical excitability' (TDEE) hypothesis, which poses that unbalanced thermal sensitivity of excitatory (Na^+ current) and inhibitory currents (K^+ current) compromises the generation of cardiac action potentials, which eventually causes cardiac failure. From these examples, a temperature-sensitive antagonistic interaction between passive/active or excitatory/inhibitory ion transport across membranes could also explain why heat failure rates increase exponentially with increasing temperature.

Collectively, these three examples show that it is both straightforward and appropriate to integrate leading physiological hypotheses of heat failure with a model of two temperature-sensitive antagonistic rates. One rate determines the capacity for maintenance/restoration of homeostasis (e.g. ATP synthesis rate, protein synthesis rate or active ion transport) and the other rate, increasing faster with temperature, acts to disrupt homeostasis (e.g. ATP use, protein denaturation or passive ion leak). In the permissive temperature range, homeostatic capacity exceeds the disruption rate allowing for chronic survival, whereas the acutely stressful temperatures are dominated by disruption rates accumulating imbalance, which directly dictates survival duration (Figs 2, 3 and 4). The balance between these antagonistic rates will therefore also determine the critical transition temperature T_c and the potential for repair (Fig. 5).

Application of the TDT model in physiological, evolutionary and ecological studies

Comparing thermal tolerance limits between studies that use different CT_{max} protocols

Estimates of thermal tolerance are frequently compared within or between species to infer eco-physiological patterns (Addo-Bediako et al., 2000; Ghalambor et al., 2006; Hoffmann et al., 2013; Kovacevic et al., 2019; Pinsky et al., 2019; Sunday et al., 2011; Sunday et al., 2019). Such comparisons are complicated by the variable use of static and dynamic protocols to assess tolerance limits (Bak et al., 2020; Kovacevic et al., 2019; Rezende et al., 2014; Santos et al., 2011; Terblanche et al., 2007; Terblanche et al., 2011). However, as outlined above, tolerance measures can be reconciled and converted to a common estimate using the TDT parameters, e.g. the maximal static temperature tolerable for 1 h ($sCT_{max(1h)}$). To illustrate this, Jørgensen et al. (2021b) compiled and recalculated heat tolerance measures using data obtained from different sources of static or dynamic assays. Despite the original measures being based on very different protocols, populations and acclimation statuses, the recalculated $sCT_{max(1h)}$ values were remarkably similar within species (Fig. 6A; for more examples, see Jørgensen et al., 2021b), suggesting that the TDT model has promising applications in meta-analyses where a standardized measure of heat tolerance allows direct comparison.

Using TDT models in evolutionary/comparative studies of thermal physiology

The TDT model represents a powerful tool for designing experiments to test the heat tolerance of different species, populations, acclimation groups, etc. (Fig. 6B). Comparing groups with different heat tolerance limits and T_c can be problematic, as it may be difficult to find a treatment suitable for all groups. For example, if groups are tested at the same temperature and duration, this treatment may be above T_c for some groups, but below T_c for others (line *a* in Fig. 6B). Even above T_c, the level of stress inflicted on the groups may differ considerably for a given duration at the treatment temperature (line *b* in Fig. 6B).

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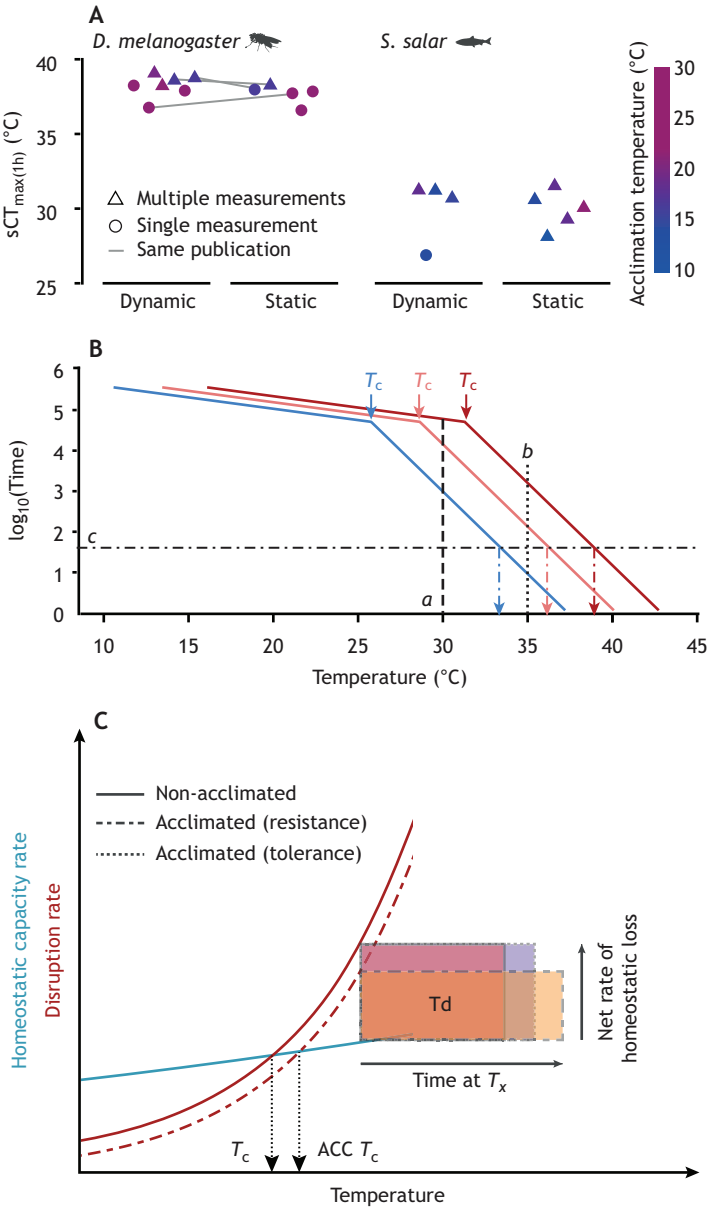

Fig. 6. Using the TDT model in comparative studies. (A) The TDT model can be used to compare thermal tolerance limits between studies that use different CT_{max} protocols. Heat tolerance measures from *Drosophila melanogaster* and *Salmo salar* were used to parameterize TDT models to calculate a single comparable metric – the static temperature causing heat failure after 1 h ($sCT_{max(1h)}$). Most estimates were derived from TDT models with multiple measurements (triangles), others with only a single measurement (circles; *z* approximated). A line between points indicates that the measurements are from the same study. Although the original measures are based on very different protocols, populations and acclimation statuses, the estimated $sCT_{max(1h)}$ values are remarkably similar within species. Adapted from Jørgensen et al. (2021b). (B) Three horizontally shifted TDT curves representing a comparison of hypothetical species/populations/acclimation groups. Exposure to the same treatment temperature for all treatments groups risks that some groups are tested above T_c and others below T_c (dashed line *a*). This design essentially compares acute effects of temperature on survival in some groups with chronic effects on life expectancy in others. Testing groups at the same temperature and duration (here, min) with all groups above T_c (dotted line *b*) will compare very different levels of heat stress. Finally, it is possible to test all groups to the same degree of thermal stress using group-specific temperatures, e.g. the group-specific $sCT_{max(1h)}$ (dash-dotted line *c* and coloured arrows). (C) Acclimation/adaptation to high temperatures can change the susceptibility to heat stress by changing resistance (change in T_c) or tolerance (change in Td). Solid coloured lines represent rates of disruption (red) and homeostatic capacity (blue), which intersect at the critical temperature T_c (see Fig. 4). Acclimation/adaptation-induced changes in any of the antagonistic rates (here exemplified by a decreased disruption rate; dashed line) result in an increased T_c (ACC T_c) and therefore an increased survival time (compare red and orange rectangles that have the same Td; see also Fig. 3). If acclimation/adaptation increases tolerance, then the amount of tolerable homeostatic loss increases (larger Td, compare increased area of purple versus red rectangle).

Accordingly, the associated behavioural or physiological responses of the groups may represent different information on chronic and acute temperature effects (see also Fig. 2). However, using information from the TDT model, it is possible to expose treatment groups to the same stress intensity (same level of homeostatic failure) using temperatures (or durations) specific to each group (line *c* in Fig. 6B). Such an approach will allow for more targeted experiments investigating the physiological mechanisms of thermal failure even if the experiments are performed on experimental groups that differ widely in thermal tolerance (Tarapacki et al., 2021).

Studying thermal adaptation and acclimation in the context of heat failure rates

Finally, the TDT model offers a framework to examine how temperature acclimation and adaptation (re)shape heat tolerance (Willot et al., 2022) or how nutritional status, water status and pathogens affect the heat stress response. In the context of the presented framework, we here discuss how acclimation or adaptation can affect heat susceptibility by changes in either resistance or tolerance to heat stress (Fig. 6C). Increased resistance can be achieved through modification of one (or both) of the two antagonistic rates determining net heat stress (rates of disruption and homeostatic maintenance, respectively; Fig. 4). Changing the rate of either disruption or homeostatic maintenance will increase T_c (compare solid and dashed red line in Fig. 6C). Heat stress susceptibility can also change by altering tolerance, which is equivalent to increasing Td (graphically an increased area of the thermal dose rectangle; Figs 3 and 6C). In this scenario, tolerance time increases even when homeostasis is lost at the same rate and above the same T_c (Fig. 6C). The difference between increased tolerance and resistance can be discerned by examining putative changes in T_c following acclimation/adaptation (Angilletta, 2009; Cossins and Bowler, 1987; Fry et al., 1946; Hoffmann and Todgham, 2010). Furthermore, increases in tolerance can be inferred from evaluating how markers of heat stress have accumulated at different levels of heat stress, e.g. indicated by higher lactate levels, hsp levels or the level of ionic imbalance (Sørensen et al., 2013; Tarapacki et al., 2021).

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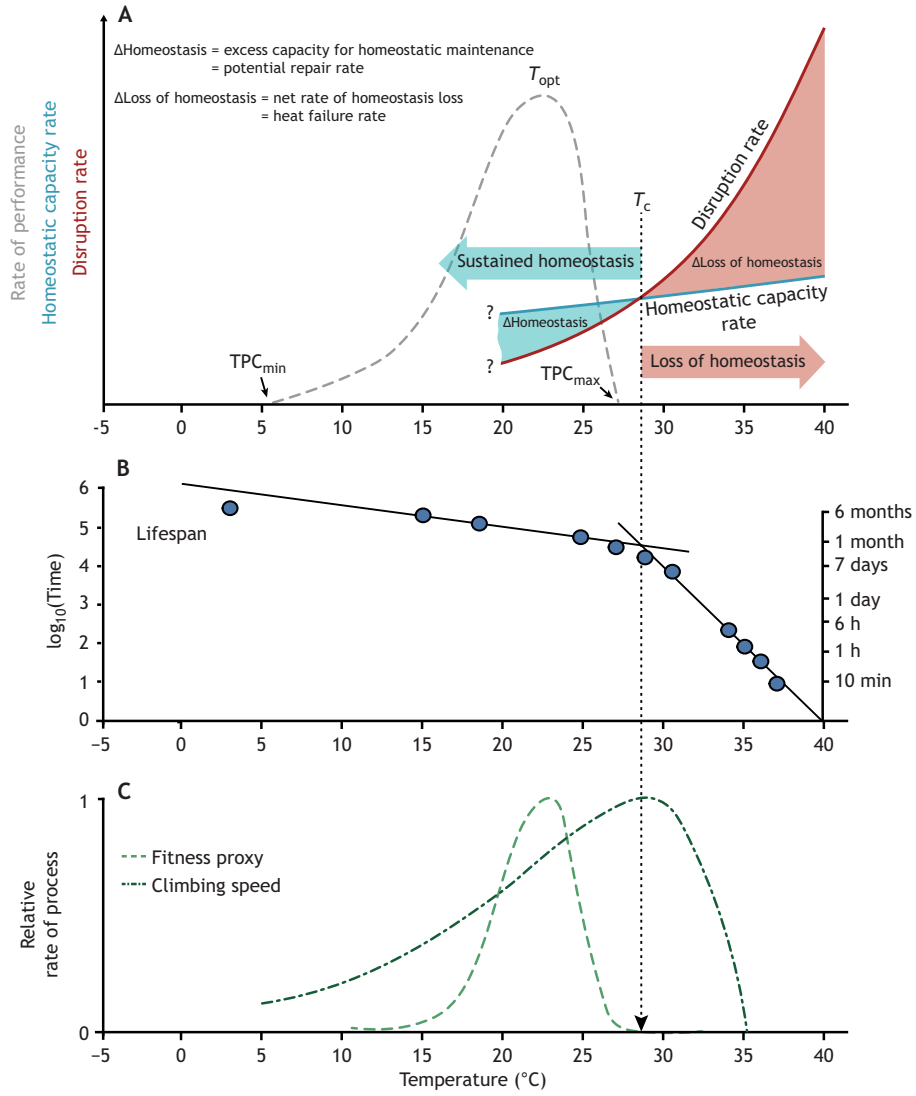

State	Suboptimal/lethal	Optimal/permissive	Lethal
Severity	Acute/chronic	Chronic	Acute
Seasonality	Winter	Growth season	Summer
Response	Survival	Growth and reproduction	Survival
Q_{10}	10–100	1–4	100–100,000

Fig. 7. Ectotherm temperature tolerance and performance across permissive and stressful temperature domains. Our proposed framework defines a critical temperature, T_c, separating the permissive temperature range allowing for population growth from the stressful temperature range causing acute thermal injury. The physiological origin of this separation stems from the balance between the two theoretical antagonistic biological processes responsible for maintaining or disrupting homeostasis ('injury' and 'repair', in red and blue curves, respectively). T_c is contrasted with a hypothetical TPC (dashed grey line) outlined by the lower and upper critical temperature where performance is zero (termed 'TPC_{min}' and 'TPC_{max}', respectively). Importantly, these TPC limits of population growth should not be related to any measure of acute heat/cold tolerance. (B) The net rate of homeostatic loss above T_c dictates the tolerable exposure duration as conveyed by the TDT model. Thus, T_c separates processes that are related to 'the rate of death' in the stressful temperature range from the processes determining 'the rate of life' in the permissive temperature range. This transition is illustrated with lifespan (in min) measured across a wide temperature range in *Drosophila subobscura* (Hollingsworth, 1969). Similar principles can be applied at low temperatures where ectotherms suffer from cold stress (not shown). (C) Comparing different TPCs to infer thermal limits can be problematic, particularly if the performance trait spans T_c. Here, we contrast two TPCs for *D. subobscura*; one trait is a proxy for population growth/fitness (product of egg-laying capacity, egg to adult viability and developmental speed; MacLean et al., 2019) and the other trait measures spontaneous activity (climbing speed; Mesas et al., 2021). The rate of the fitness proxy approaches zero at temperatures below T_c, while activity rate spans temperatures below and above T_c and in this example the apparent T_{opt} coincides with T_c. The activity trait is obviously important for many physiological and behavioural responses to temperature, but the T_{opt} and thermal limits of such 'indirect' fitness traits should be interpreted with caution. (D) Ageing rates and biological processes in the permissive temperature range are characterized by a 'normal' thermal sensitivity (Q_{10}≈1–4) which contrasts the extreme temperature sensitivity of acute heat failure rate in the stressful range (typically Q_{10}>1000). Collectively, these observations highlight why different analyses are relevant in the permissive and stressful temperature ranges, respectively.

Concluding remarks and future perspectives

The framework presented here (summarized in Fig. 7) separates permissive and stressful temperature ranges and allows for ecological and physiological assessment of heat stress based on the balance between two antagonistic biological processes (thermal injury and repair; Fig. 7A). This model therefore also provides an understanding of how temperature sensitivity of different biological processes underpins the TPC and the TDT model. Central to this framework is a clear boundary, T_c, which separates the permissive and stressful temperature ranges (Fig. 7). Acknowledging these temperature ranges with vastly different thermal sensitivities therefore allows for better integration of observations across studies and provides a conceptual model to study the temperature tolerance traits that are essential for predicting species' responses to climate change (Jørgensen et al., 2022).

Future efforts should investigate how T_c is best determined experimentally. Our suggestions for experiments that potentially may uncover this include; (i) creating TDT curves over sufficiently wide temperature ranges to uncover the actual breakpoints between permissive and stressful temperatures ranges (Fry, 1971; Fry et al., 1946; Hollingsworth, 1969) (Figs 2 and 7B), (ii) initiating dynamic assays at different temperatures, where a start temperature $>T_c$ would result in increased CT_{max}, whereas start temperatures $<T_c$ should have negligible effects on the CT_{max} estimate (Kingsolver and Umbanhowar, 2018), (iii) measuring CT_{max} at gradually slower ramping rates as the resulting CT_{max} estimates should then approach T_c asymptotically (Richard et al., 2012) and (iv) estimating T_c as the 'resting' temperature where thermal injury is equal to the repair capacity during experiments of alternating stressful and benign temperatures (compare species repair capacities in Fig. 5C).

Another perspective resulting from this synthesis concerns the temperature fluctuations that many ectotherms experience naturally. Fluctuations that occur only above or below T_c are easy to integrate into the TDT or TPC model, respectively (Colinet et al., 2015; Deutsch et al., 2008; Fry et al., 1946; Jørgensen et al., 2021b; Sinclair et al., 2016). However, the situation is much more complex when fluctuations occur between the permissive and stressful temperature ranges as they are dominated by different thermal sensitivities, and especially for TPCs, different traits can span the two temperature ranges (Fig. 7C,D). Studies on the thermal sensitivity of heat stress repair are still scarce (Bowler and Kashmeery, 1979; Dillon et al., 2007; Dingley and Maynard Smith, 1968; Kovacevic et al., 2019) and currently it is difficult to distinguish repair from rapid hardening processes which can increase heat resistance/tolerance during subsequent exposures to heat stress (Bowler, 2005; Krebs and Loeschcke, 1994; Sørensen et al., 2003). Although not the focus of this Review, many similar conceptual observations can also be applied to cold limits of ectotherms as these limits are also the result of unbalanced antagonistic processes (MacMillan and Sinclair, 2011; Overgaard et al., 2021). It may therefore be relevant to consult the cold tolerance literature, where the repeated transition from stressful to benign temperatures associated with 'fluctuating thermal regimes' and 'repeated cold exposures' has been investigated (Colinet et al., 2015; Colinet et al., 2018; Koštál et al., 2007; Marshall and Sinclair, 2012; Nedvěd et al., 1998).

Finally, the physiological rates underlying homeostatic capacity and disruption that ultimately determine CT_{max} are still intensely debated (Bowler, 2018; González-Tokman et al., 2020; Andreassen et al., 2022; Jutfelt et al., 2018; Neven, 2000; Pörtner and Farrell, 2008). We hope that appropriate integration of the TDT model in future experimental designs can help to identify the proximal physiological causes of heat stress susceptibility (Fig. 6B,C). The framework presented here will, for example, allow for a systematic approach to experimentally associate homeostatic capacity rate and/or perturbation rate with physiological mechanisms hypothesized to cause heat death in ectotherms (oxygen limitation, protein denaturation, membrane dysfunction, neurological failure, etc.).

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Competing interests

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