Tolerance landscapes in thermal ecology

Enrico L. Rezende*[,1], Luis E. Castañeda[2,3] and Mauro Santos[4]

[1]*Department of Life Sciences, University of Roehampton, Holybourne Avenue, London SW15 4JD, UK;* [2]*Instituto de Ecología y Biodiversidad (IEB), Universidad de Chile, Casilla 653, Santiago, Chile;* [3]*Instituto de Ciencias Ambientales y Evolutivas, Universidad Austral de Chile, PO 5090000, Valdivia, Chile; and* [4]*Departament de Genètica i de Microbiologia, Grup de Biologia Evolutiva (GBE), Universitat Autònoma de Barcelona, 08193 Bellaterra (Barcelona), Spain*

Summary

1. How thermal tolerance estimated in the laboratory can be extrapolated to natural settings remains a contentious subject. Here, we argue that the general premise that a single temperature can accurately describe upper or lower tolerance limits is incorrect.

2. Survival probability is determined by both the intensity and the duration of a thermal stress, and the association between these variables can be adequately conveyed by a thermal tolerance landscape. Employing this framework, we demonstrate that the temperature range that an organism can tolerate is expected to narrow down with the duration of the thermal challenge.

3. Analyses suggest that a trade-off exists between tolerances to acute and chronic exposition to thermal stress, and that changes in temperature means or extremes may result in drastically different selective pressures and subsequent evolutionary responses.

4. After controlling for the duration of the thermal challenge, we also uncover latitudinal effects on upper lethal temperatures in insects that remained unnoticed in previous broad-scale comparative analyses.

5. Ultimately, critical thermal limits have been adopted in the ecological literature for logistic reasons and are inadequate descriptors of thermal tolerance on conceptual grounds. We consider that tolerance landscapes provide a more suitable framework to study temperature tolerance and its potential impact in ecological settings.

Key-words: climate change, cold tolerance, critical thermal limits, heat tolerance, thermal adaptation

Introduction

Studies of thermal tolerance have recently gained considerable attention given their relevance to forecast the impact of climate change on species persistence and distribution (Deutsch *et al.* 2008; Angilletta 2009; Huey *et al.* 2009; Terblanche *et al.* 2011; Araújo *et al.* 2013; Hoffmann, Chown & Clusella-Trullas 2013). The possibility to predict from first principles when environmental temperatures may become restrictive is tantalizing, as is the capability to quantify how and how fast species may respond to potential thermal challenges (Mitchell & Hoffmann 2010; Hoffmann & Sgrò 2011; Santos, Castañeda & Rezende 2012). However, empirical observations and theoretical analyses show that critical thermal limits are highly dependent on

the methodological context, raising doubts regarding the adequacy and validity of different estimates of thermal tolerance for comparative purposes and extrapolations to natural settings (Lutterschmidt & Hutchison 1997; Terblanche *et al.* 2007; Chown *et al.* 2009; Santos, Castañeda & Rezende 2011).

This issue reflects to a large degree differences in methodology and in the end points employed to estimate thermal tolerance (Lutterschmidt & Hutchison 1997; Cooper, Williams & Angilletta 2008). Tolerance has been quantified either as the time to collapse at a constant stressful temperature (static method) or the end point temperature to collapse as temperature is increased or decreased at a controlled rate (dynamic method), and a wide variety of temperatures, starting points and ramping rates have been employed in different studies across multiple organisms. Additionally, 'collapse' has been operationally defined in many different ways, such as loss of motor coordination,

*Correspondence author. E-mail: enrico.rezende@roehampton.ac. uk

the onset of muscle spasms, coma or death. This diversity of measures has jeopardized efforts to compare results across studies and lineages (Hoffmann, Sørensen & Loeschcke 2003; Cooper, Williams & Angilletta 2008; Rezende & Santos 2012) and, consequently, the quest for a more general understanding of temperature tolerance. Recently, Cooper, Williams & Angilletta (2008) demonstrated that static and dynamic methods provide comparable estimates of temperature tolerance, opening the venue for a unified framework to study thermal limits. Here, we go one step further and address a more fundamental aspect: whether the premise that there is such a thing as a critical thermal limit (i.e. a temperature that establishes a frontier between two physiological states, such as alive vs. dead) is adequate or realistic.

From a theoretical perspective, this idea is intrinsically flawed because the impact of any stress depends both on its intensity and duration; hence, a single parameter is insufficient to describe a response that is embedded in two dimensions (this explains why thermal tolerance can be quantified in either temperature or time units, see Lutterschmidt & Hutchison 1997; Cooper, Williams & Angilletta 2008; Mitchell & Hoffmann 2010; Santos, Castañeda & Rezende 2011). Different thermal tolerance estimates likely represent snapshots of a single underlying relationship between temperature, exposure time and mortality, and here, we explore some of the implications of the formal framework that emerges from this relationship. Because this study is primarily conceptual, we deal with thermal tolerance in a generic sense and are not concerned with the multitude of confounding factors (age, sex, acclimatory effects, desiccation or starvation, measurement error, etc.) that have already been covered in previous studies (e.g. Lutterschmidt & Hutchison 1997; Terblanche *et al.* 2007; Chown *et al.* 2009; Rezende, Tejedo & Santos 2011; Santos, Castañeda & Rezende 2011; Castañeda *et al.* 2012; Rezende & Santos 2012).

Background

The association between thermal tolerance and time is straightforward and should follow a typical dose–response relationship: the higher the thermal stress, the shorter the time an organism can tolerate it. Accordingly, when organisms are exposed to high constant stressful temperatures, thermal tolerance decreases nearly linearly with the logarithm of elapsed time (Fig. 1). This relation is known for almost a century (Bigelow 1921) and has been repeatedly obtained empirically (e.g. Maynard Smith 1957; Cerdá, Retana & Cros 1998; Cerdá & Retana 2000; Tang *et al.* 2000; Armstrong, Tang & Wang 2009) and also on theoretical grounds (Santos, Castañeda & Rezende 2011). This so-called thermal death time (TDT) curve has been extensively studied during the development of quarantine heat treatments for food processing and rests on a solid basis borrowed from thermobacteriology (see Tang *et al.* 2007). Succinctly, TDT curves can be described as
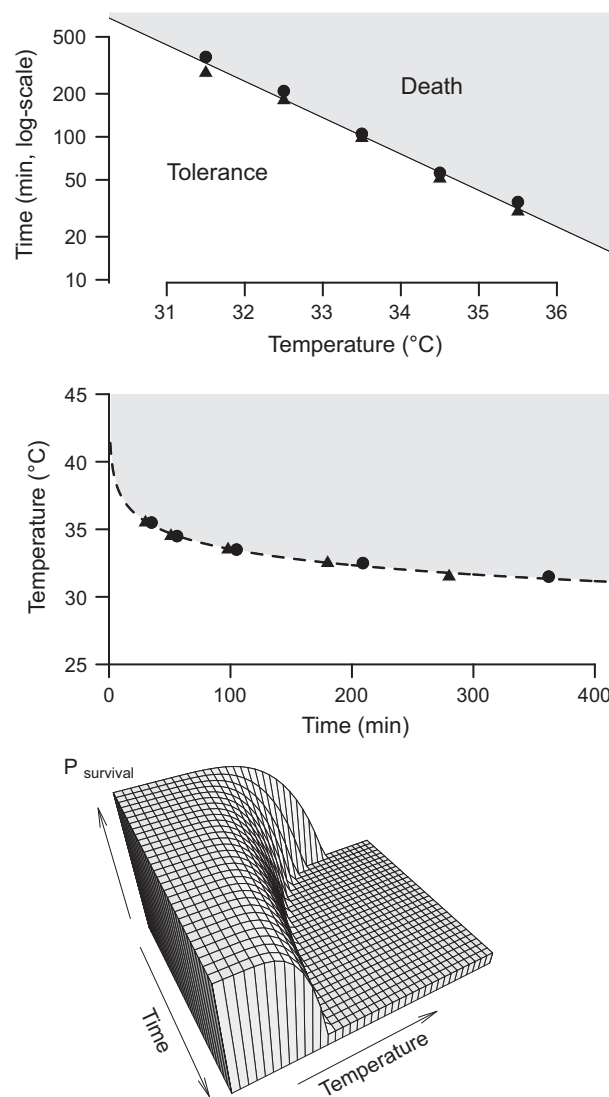
Fig. 1. *Top.* Average survival times of *Drosophila subobscura* in dry air, males in triangles and females in circles (data from Maynard Smith 1957). *Middle.* The same dataset represented as a thermal death time (TDT) curve, showing how thermal tolerance decreases with time. The general association between knockdown temperatures and times simulated with our theoretical model is also well described by a semilogarithmic association between temperature tolerated and total time of exposure (see fig. 3b in Santos, Castañeda & Rezende 2011). *Low.* The thermal tolerance landscape results from the superimposition of TDT curves describing different survival probability isoclines.

$$\log_{10} t = \frac{(CT_{max} - T)}{z}, \qquad \text{eqn 1}$$

where T corresponds to the assay temperature (°C), CT_{max} is the upper critical thermal limit (°C), t the knockdown time (min), and z a constant that characterizes the sensitivity to temperature change.

Rearranging this equation, one obtains the more tractable linear model, which can be generalized for ramping assays (in which T increases linearly with t):

$$T_{ko} = \text{CT}_{\max} - z \log_{10} t, \qquad \text{eqn 2}$$

where T_{ko} corresponds to the knockdown (or death) temperature (°C). Consequently, the capacity to withstand heat at any given temperature is characterized by two parameters: $\text{CT}_{\max}$ (i.e. the temperature that would result in knockdown or death at $\log_{10} t = 0$ that corresponds, by definition, to 1 min) and the constant of thermal susceptibility z, which describes how thermal tolerance decays with the duration of the heat challenge (i.e. the net reduction in T_{ko} resulting from a tenfold increase in exposure time). These parameters can be easily estimated with an ordinary linear regression of $\log_{10} t$ on T or vice versa, depending on the experimental design and which sums of squares must be minimized. Because most studies reviewed here controlled for T and measured the elapsed time to attain a given relative mortality, we included T as the independent variable and then calculated parameters from back-transformation as $\text{CT}_{\max} = -$ intercept/slope and $z = 1/$slope (eqn 1).

The impact of temperature on mortality rates can also be expressed as a kinetic model describing mortality as a function of time (Tang *et al.* 2000, 2007; Wang *et al.* 2002; Armstrong, Tang & Wang 2009). Mathematically, logarithmic TDT curves correspond to a first order of reaction, in which mortality rate at any given temperature remains constant through time (Tang *et al.* 2007). Given stressful temperature, population size changes in time as:

$$\frac{d(N/N_0)}{dt} = -k(N/N_0), \qquad \text{eqn 3}$$

where k is the thermal death rate constant (min^{-1}), and N_0 and N are the initial and surviving numbers of individuals, respectively. The death rate constant k can be calculated regressing survival (N/N_0) against exposure time t (min) because the integration of eqn 3 corresponds to:

$$\ln(N/N_0) = -kt + c. \qquad \text{eqn 4}$$

Although in reality, death rate can often change in time, which reflects other reaction orders and curvilinear mortality rates with time, the fundamental kinetic model is simple to obtain and employ with acceptable precision to estimate lethal times and compare different organisms (see Tang *et al.* 2007). Note that relative survival (N/N_0) corresponds to survival probability (given a large sample size); hence, these models can also be expressed in probabilistic terms. This is particularly important because thermal tolerance limits under more complex scenarios, in which temperature varies with time as in ramping assays or in the field, may eventually be calculated from first principles if survival probability curves as a function of exposure time at different temperatures are known (see Santos, Castañeda & Rezende 2012).

Thermal tolerance landscapes

Ultimately, TDT curves and kinetic models offer complementary approaches to describing the interacting effects of temperature and exposure time in mortality. Whereas eqns 1 and 2 describe how estimates of thermal tolerance change in time, kinetic models address how mortality rates are affected by temperature (the activation energy E_a required for thermal death to occur indicates the sensitivity to temperature change, and can be readily converted to parameter z of TDT curves; see Tang *et al.* 2000, 2007). This information can be succinctly conveyed as a thermal tolerance landscape, which provides a detailed description of how survival probabilities change as a function of temperature and exposure time (Fig. 1).

Time of exposure will have an impact on thermal tolerance, and yet the magnitude of this effect has remained unexplored in the ecological literature. How does $\text{CT}_{\max}$ and z vary across organisms? For a preliminary assessment of this question, we compiled lethal times measured at different temperatures from the literature (Appendix S1, Supporting information). We selected studies that reported lethal times (LT_{50} and LT_{100}) from static assays employing at least three different temperatures, and $\text{CT}_{\max}$ and z were estimated by regressing $\log_{10}$-transformed lethal times on assay temperature (eqn 1). This resulted in 56 TDT curves for 49 species of insects, bivalves and fishes (Fig. 2). Curves exhibited consistently high goodness-of-fit (median $r^2 = 0.985$, 95% CI between 0.876 and 0.999).

Parameters $\text{CT}_{\max}$ and z varied considerably across curves, with $\text{CT}_{\max}$ ranging between 17·0 °C and 61·6 °C and z exhibiting values between 0·82 °C and 9·3 °C. Analyses show that parameters $\text{CT}_{\max}$ and z are highly positively correlated (Fig. 2). In the pooled dataset, variation in z accounts for 85% of the variation in $\text{CT}_{\max}$ as estimated by the coefficient of determination (slope = 3·20, $P < 0.0001$, $r^2 = 0.851$), after excluding three arctic fish species and controlling for relative survival as a categorical factor (LT_{50} vs. LT_{100}, $P < 0.001$). There are no significant differences between insects, bivalves and fishes in this model ($P = 0.426$) and no interaction effects ($P > 0.344$), suggesting that the association between $\text{CT}_{\max}$ and z is general across groups. Within insects and bivalves, the variance explained by the model corresponds to 70% and 76%, respectively (slopes = 3·35 and 2·59, $P < 0.0001$ in both cases). Although such a tight correlation is expected to hold with phylogenetic analyses (Carvalho, Diniz-Filho & Bini 2006), this result is preliminary and should be studied more thoroughly in future analyses.

Under the constraints imposed by the dose–response relationship, the general shape of the thermal tolerance landscape including cold stress can be readily established on theoretical grounds (Fig. 3). Then, it becomes clear that the thermal tolerance landscape constitutes an expansion of the standard thermal performance curve (Huey & Bennett 1987; Huey & Kingsolver 1989; Angilletta 2009) to accommodate different time exposures to a thermal stress. However, freezing temperatures involve an additional level of complexity that might apparently disrupt the association between mortality, cold stress and exposure times expected from a dose–response relation. For instance, a
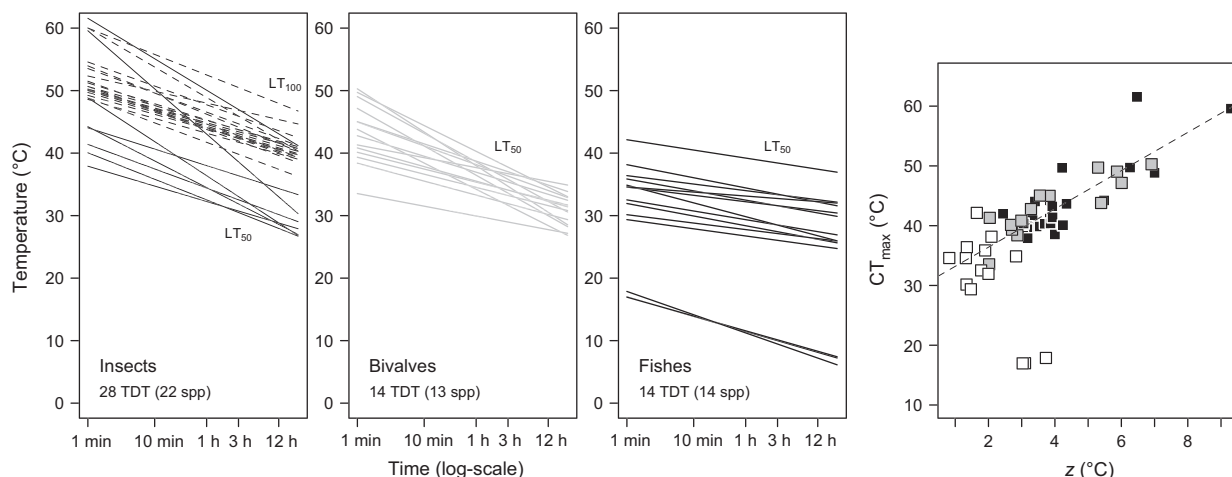
Fig. 2. Thermal death time (TDT) curves obtained under heat stress from the literature for insects, bivalves and fishes (Appendix S1), reported for 50% or 100% mortality (LT_{50} and LT_{100}). Parameters CT_{max} and z correspond to the intercept and slope of these curves, respectively (eqn 2). The right panel depicts the association between these parameters after controlling for mortality differences in CT_{max} (black = insects, grey = bivalves, white = fishes).

freezing tolerant organism that tolerates 5 °C for 120 min might cope with temperatures below zero for longer periods because freezing results in a different physiological state (see Lee 1989; Delinger & Lee 1998; Sinclair 1999; Sinclair *et al*. 2003).

Nonetheless, a linear relationship between cold tolerance and the logarithm of exposure time has been described many instances in the literature (e.g. Fields 1992; Renault *et al*. 2004; Imai & Harada 2006; Abdelghany *et al*. 2010; Loganathan *et al*. 2011). As with upper thermal limits, we compiled data on survival from static assays at different temperatures to analyse TDT curves under cold stress (Appendix S2, Supporting information). Analyses are fundamentally identical except that, for lower limits, the temperature resulting in death following 1 min of exposure is CT_{min}, and the association between lethal temperatures and the logarithm of exposure times is positive with a slope z' (whereas for heat stress, it was negative with a slope z, eqn 2). The range of variation of these parameters observed across 22 TDT curves encompassing 13 species of insects (Fig. 4) was substantially higher than for upper thermal limits, with CT_{min} ranging between -107.2 °C and -22.2 °C and z' between 6·0 °C and 23·0 °C (median $r^2 = 0.955$, 95% CI between 0·876 and 0·993), which is in agreement with recent variance analyses comparing upper and lower thermal limits (Araújo *et al*. 2013). Additionally, parameters were highly correlated (Fig. 4), with variation in z' explaining 96·5% of the variability in CT_{min} (slope = -4.99, $P < 0.0001$, $r^2 = 0.965$) after controlling for relative survival (LT_{50} vs. LT_{100}, $P = 0.002$).

To summarize, the available data suggest that, in spite of the inherent complications associated with freezing temperatures, the proposed framework is applicable for both upper and lower tolerance limits. In practice, physiological responses to cope with freezing conditions may give rise to more than one TDT curve (see also David *et al*. 2003),

each one described by a single CT_{min} and z' relevant to the physiological state and temperature range in which tolerance was quantified (i.e. for descriptive and comparative purposes, parameters are calculated under the assumption that the relation between tolerance and time observed in the experimental setup holds across a larger range of temperatures). Nonetheless, for any given physiological state, restrictions imposed by the dose–response relationship imply that the range of temperatures tolerated by an organism is expected to narrow down as exposure times increase. In spite of the fragmented nature of the available data, empirical measurements of lethal temperatures and times across multiple studies support this proposition (Fig. 5).

Acute vs. chronic stress

The tight association between CT_{max} and z (or CT_{min} and z') suggests that these variables cannot evolve independently. For heat tolerance, for instance, species with a high CT_{max} also exhibit a high z, which suggests that the ability to withstand extreme temperatures, encapsulated in CT_{max}, cannot be sustained during long periods of time as evidenced by higher values of z. A heat tolerant jack-of-all-trades would combine the best of both worlds and exhibit both a high CT_{max} and a low z, which seems to be prevented by a general trade-off between these variables. Species with high CT_{max} and low z are less sensitive to temperature changes (i.e. large changes in temperature have only a minor impact in survival times) at the expense of tolerating high temperatures for a brief amount of time. One might argue, for instance, that plastic responses to a heat shock, in which molecular chaperones are recruited to actively maintain proteins folded employing ATP (Hochachka & Somero 2002), have evolved to partly circumvent this constraint. In our dataset, only arctic fishes
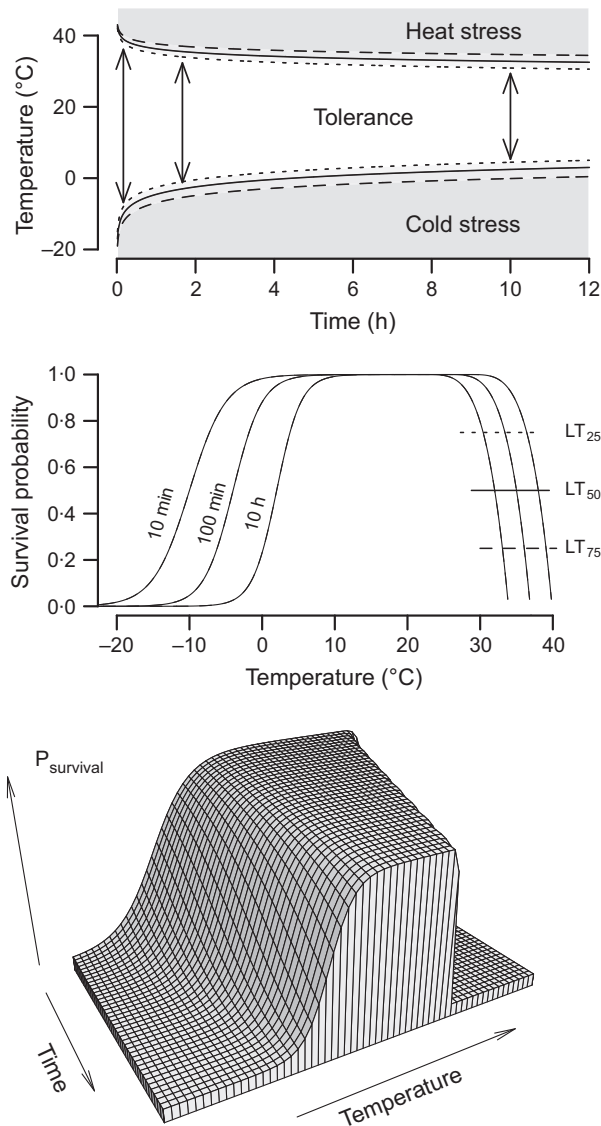
Fig. 3. *Top.* Upper and lower TDT curves resulting in a mortality of 25% (LT_{25}), 50% (LT_{50}) and 75% (LT_{75}) of the population. *Middle.* Thermal tolerance curves describing how survival probability changes as a function of temperature and time of exposure, which have been described empirically in *D. melanogaster* (figs 1a, b in Overgaard *et al.* 2008), *Cydia pomonella* (figs 2a and 3a in Chidawanyika & Terblanche 2011) and *Glossina pallidipes* (figs 2a, b in Terblanche *et al.* 2008). *Low.* The thermal tolerance landscape, which describes the probability to survive a thermal stress as a function of the intensity and duration of the thermal stress.

deviate from this general relation (Fig. 2), which, we speculate, might be indicative of relaxed selection for heat tolerance.

Therefore, short-term measurements may not adequately reflect thermal tolerance at longer temporal scales, which is more of an issue in organisms with higher z (eqn 2). Within the range of z of our dataset, 100-fold increase in exposure time results in a drop in tolerated temperatures from 1·64 °C ($z = 0·82$ °C) to 18·6 °C ($z = 9·8$ °C). The immediate consequence is that, in some cases, the effects of exposure time can override differences in CT_{max} (Fig. 6). It

can be readily shown (eqn 2) that a species a with high CT_{max} and z collapses at the same temperature as a species b with a low CT_{max} and z at:

$$\log_{10} t' = \frac{CT_{max_a} - CT_{max_b}}{z_a - z_b}. \qquad \text{eqn 5}$$

Consequently, species a will exhibit a higher heat tolerance than species b at exposure times below t' and a lower tolerance at times above t', which can disrupt or invert the correlation between tolerance estimates obtained from assays with different durations (Fig. 6). Even in absence of stochasticity and measurement error (Santos, Castañeda & Rezende 2011), the correlation between T_{ko} and CT_{max} is expected to decrease with assay duration. For example, in the bivalve dataset, the initial correlation between T_{ko} following 1 min of exposure and CT_{max} ($r = 1$ by definition, eqn 2) decreases and eventually becomes non-significant ($r_{12} = 0·457$, 1-tailed $P > 0·05$) in assays lasting more than 7·4 h (Fig. 2). In these species, assays lasted up to 96 h, and the correlation between T_{ko} at this time and CT_{max} is expected to become negative ($r_{12} = -0·162$, $P = 0·29$). In summary, the ability to withstand a thermal challenge depends both on its intensity and duration; hence, adaptive responses in thermal tolerance across different organisms have likely been shaped by the interaction between these two factors.

Several counterintuitive results in the literature, such as a higher heat tolerance to an acute challenge in *Drosophila melanogaster* from cold regions (Sgrò *et al.* 2010), can be understood having this framework in mind (Fig. 6). These populations exhibit an increased tolerance to an acute 5 min challenge but collapsed sooner in longer static assays at 39 °C (Sgrò *et al.* 2010), supporting a higher sensitivity to prolonged exposure than their warm regions counterparts (see also Santos, Castañeda & Rezende 2011, 2012). We obtained qualitatively similar results in *D. subobscura* populations from Chile exposed to 35 °C, 36 °C, 37 °C and 38 °C (Castañeda, unpublished results). Because most heat tolerance measurements in reptiles involve ramping assays that generally last a few minutes, the positive association reported with latitude in these organisms (Huey *et al.* 2009), or negative with environmental temperature (Araújo *et al.* 2013), might have a similar basis. Overall, the two parameters of TDT curves emphasize that thermal tolerance estimates obtained in brief assays may not be representative of tolerance to prolonged exposure to less-extreme – albeit still stressful – temperatures.

A trade-off between tolerances to acute vs. chronic thermal stress has enormous implications. Adaptive strategies are expected to differ depending on the nature of the thermal regime, and selective pressures due to changes in temperature means or extremes may result in different evolutionary responses. For upper thermal limits, for instance, our results suggest that low values of z should generally be favoured in relatively constant environments at the expense of a high CT_{max}, whereas high CT_{max} should be favoured in highly variable thermal
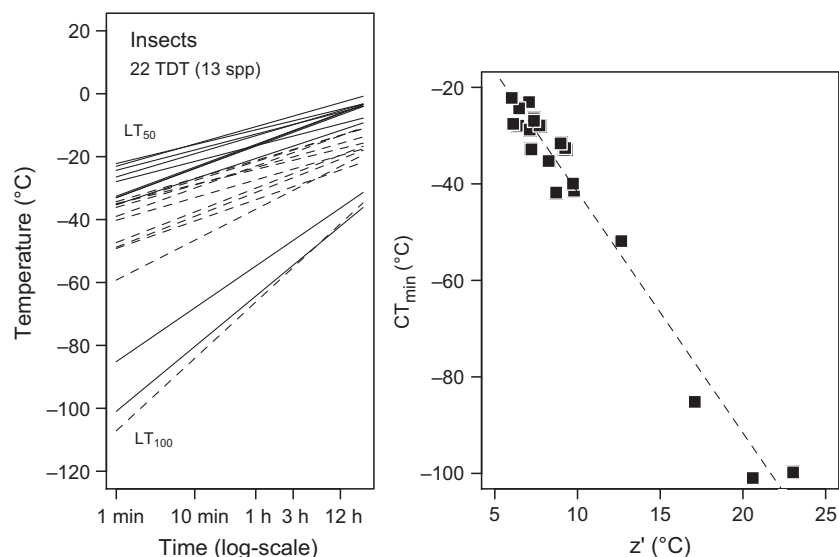
Fig. 4. Thermal death time (TDT) curves obtained under cold stress for insects (Appendix S2), reported for 50% or 100% mortality (LT_{50} and LT_{100}). Parameters CT_{min} and z' correspond to the intercept and slope of these curves, respectively. The association between these parameters, after controlling for mortality differences in CT_{max}, is shown in the right panel.
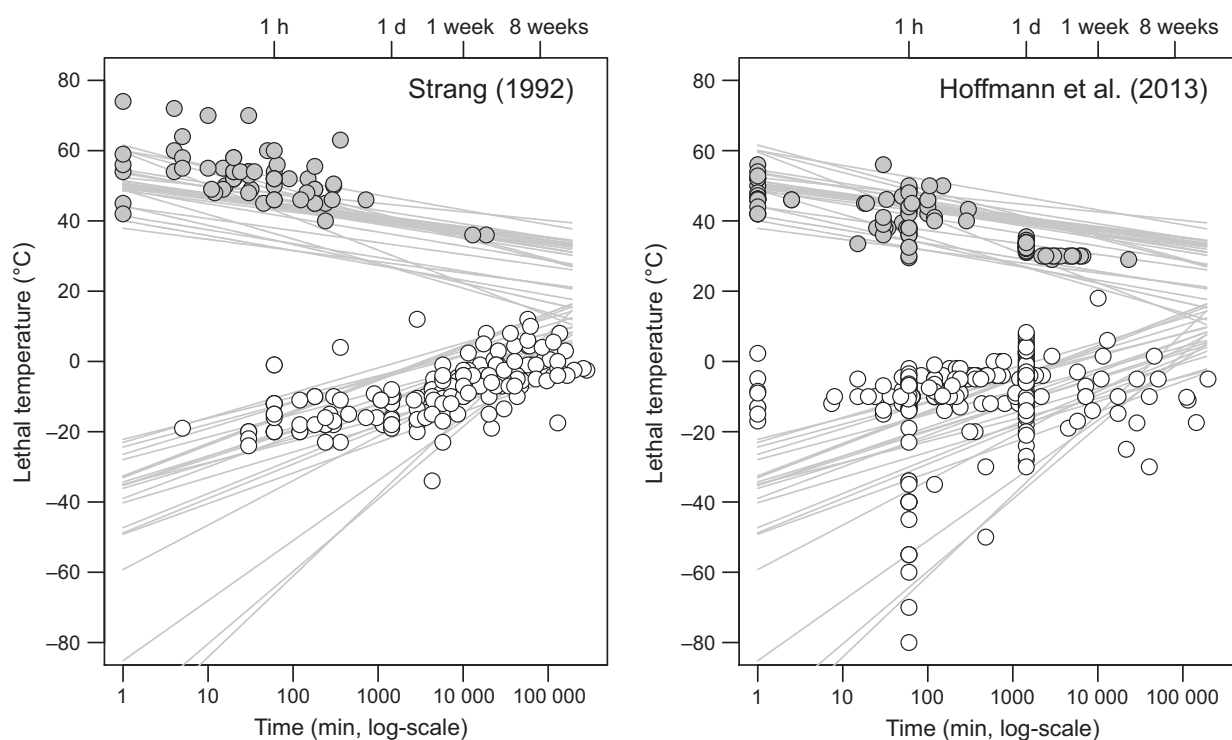
Fig. 5. Upper and lower lethal temperatures across insect species and life stages. Published temperatures for the control of different pest insects in museums (Strang 1992) and lethal temperatures compiled from thermal tolerance studies (Hoffmann, Chown & Clusella-Trullas 2013) decay linearly with log-transformed time and overlap with the TDT curves gathered for insects (Figs 2 and 4).

environments at the expense of low z values. Although this dichotomy represents extremes along a continuum and an overly simplistic view of reality (a multitude of factors such as the duration of exposure to thermal extremes, behavioural thermoregulation or past history are also expected to affect CT_{max} and z), it raises interesting predic-

tions that are worth testing. For example, tropical and temperate environments are expected to exert very different selective regimes on thermal tolerance, and the same is true for marine vs. terrestrial environments. In this context, it is noteworthy that aquatic fishes exhibit low CT_{max} and z in comparison with bivalves inhabiting intertidal
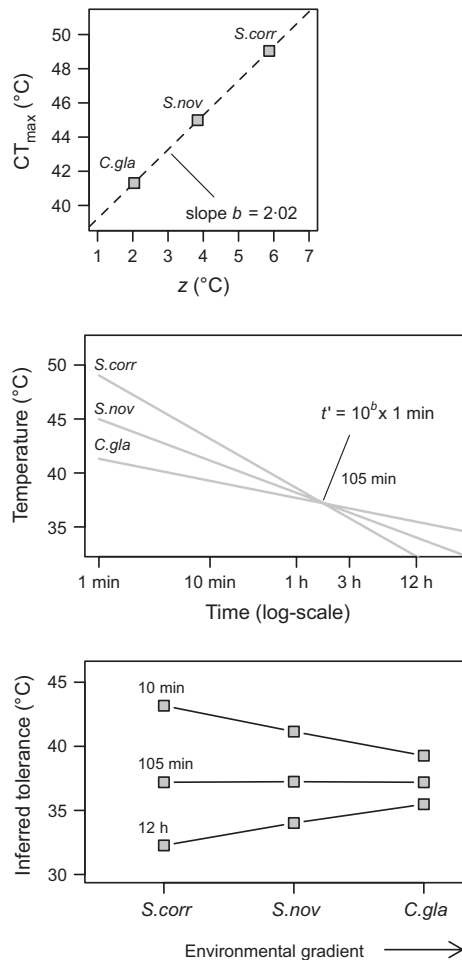
Fig. 6. A negative association between CT_{max} and z across species (or populations) implies that TDT curves intersect at a given time t', which can be calculated from the slope b of this relation (eqn 5). Species ranking based on observed tolerated temperatures is expected to change with exposure times and, if they are distributed along an environmental gradient, so is the sign of thermal tolerance clines. TDT curves were plotted for bivalve species *Semele corrugata*, *Sphaerium novaezelandiae* and *Cardium glaucum* (Appendix S1).

habitats and terrestrial insects, which provides circumstantial support to this suggestion (Fig. 2).

Alternatively, taxonomic and developmental effects aside, this pattern might partly reflect differences in body size. Variation in thermal tolerance with size may result, for example, from differences in organismal complexity and aerobic metabolism (see Pörtner 2002, 2010), in cumulative effects of thermal stress (larger organisms exhibit a lower metabolic rate per gram of tissue and, consequently, lower rates of resources depletion; see Rezende, Tejedo & Santos 2011; Santos, Castañeda & Rezende 2011) and in thermal inertia. Small and large organisms may exhibit very different strategies to cope with temperature stress, and size-mediated effects in thermal tolerance might partly account for the general trend of shrinking sizes with warmer temperatures observed in a variety of organisms (Sheridan & Bickford 2011). Ultimately, these alternative

explanations to account for the general association between CT_{max} and z are not mutually exclusive and, as with many other physiological traits, the shape of tolerance landscapes likely varies with body size and the thermal environment.

Broad-scale comparative analyses

Analyses suggest that differences in exposure times may account for an important fraction of the variation in thermal tolerance. For instance, heat tolerance estimates quantified in assays lasting 5 min vs. 2 h are expected to differ 5·5 °C for an organism with $z = 4$ °C and 8·3 °C for $z = 6$ °C; hence, differences in assay duration might impact broad-scale comparative studies (Sunday, Bates & Dulvy 2011; Diamond *et al.* 2012; Hoffmann, Chown & Clusella-Trullas 2013; Araújo *et al.* 2013). To address this potential problem, we reanalysed the dataset on upper and lower lethal temperatures compiled by Hoffmann, Chown & Clusella-Trullas (2013). Because this exercise is primarily illustrative, we employed ordinary regressions to compare the relative effects of assay duration vs. absolute latitude and did not account for other variables (taxonomic affiliation, acclimation temperature, etc.). We pooled estimates reported for 50% and 100% mortality and assay duration (their Table S3, Supporting information) to obtain upper and lower lethal temperatures for 127 and 242 insect species, respectively. These estimates overlap with the expectations of TDT curves (Fig. 5), supporting the general association between stress intensity and duration proposed here.

For upper lethal temperatures, latitude was not statistically significant when included as a single predictor in the regression, whereas assay duration was highly significant (Table 1). Importantly, latitudinal effects were significant in the model including both variables; hence, controlling for assay duration seems to have uncovered latitudinal differences in heat tolerance. Consequently, from an initial model in which latitude is not significantly different than zero and only accounts for 2·1% of the variation in heat tolerance, we end up with a model explaining roughly 68% of the variation and significant latitudinal effects that are, comparing slope estimates, five times larger than in previous analyses (−0·15 in Table 1 vs. −0·03 reported for terrestrial organisms by Sunday, Bates & Dulvy 2011). For lower lethal temperatures, both latitude and assay duration were statistically significant in the regression as single predictors (Table 1). Assay duration accounted only for a small fraction of the variation in cold tolerance in this dataset and its inclusion in the model did not affect latitudinal trends. Nonetheless, for both heat and cold tolerance, the best model according to Akaike Information Criterion (AIC) included both latitude and exposure times (Table 1).

Several authors have raised concerns about comparing thermal tolerance estimates obtained with different protocols (Lutterschmidt & Hutchison 1997; Chown *et al.*

Table 1. Regressions of upper (UTL) and lower thermal limits (LLT) against absolute latitude and/or $\log_{10}$-transformed exposure time (t)

Model estimates ($\pm$ SE)	Significance	r^2	AIC	AIC_w
UTL = 42·34 ($\pm$ 2·41) − 0·11 ($\pm$ 0·07) lat	$P_{lat} = 0.102$	0·021	865·4	0·000
UTL = 48·75 ($\pm$ 0·79) − 4·83 ($\pm$ 0·32) $\log_{10} t$	$P_{\log t} < 0.001$	0·638	739·2	0·002
UTL = 54·16 ($\pm$ 1·57) − 0·15 ($\pm$ 0·04) lat − 4·91 ($\pm$ 0·31) $\log_{10} t$	$P_{lat} < 0.001$, $P_{\log t} < 0.001$	0·677	726·4	0·998
LTL = 16·07 ($\pm$ 2·81) − 0·63 ($\pm$ 0·07) lat	$P_{lat} < 0.001$	0·278	1838·0	0·007
LTL = −17·15 ($\pm$ 2·24) + 2·74 ($\pm$ 0·82) $\log_{10} t$	$P_{\log t} < 0.001$	0·044	1905·0	0·000
LTL = 9·52 ($\pm$ 3·36) − 0·62 ($\pm$ 0·06) lat + 2·38 ($\pm$ 0·70) $\log_{10} t$	$P_{lat} < 0.001$, $P_{\log t} < 0.001$	0·311	1828·0	0·993

2009; Santos, Castañeda & Rezende 2011). Our analysis constitutes the first quantitative assessment of the magnitude of methodological effect on comparative analyses, and results show that assay duration can be a major confounding factor even in broad-scale geographic studies. Previous analyses suggest that upper thermal limits are highly conserved across species and generally uncorrelated with environmental temperatures (Addo-Bediako, Chown & Gaston 2000; Sunday, Bates & Dulvy 2011; Araújo et al. 2013; Hoffmann, Chown & Clusella-Trullas 2013), and these results have been interpreted as evidence of limited evolutionary potential in heat tolerance (Araújo et al. 2013; Hoffmann, Chown & Clusella-Trullas 2013). It is possible, however, that existing geographical trends may have been partly overshadowed by differences in exposure times. This is particularly true for upper thermal limits, which exhibit a lower variability than lower thermal limits (see Araújo et al. 2013), and therefore, assay duration seems to have a disproportionally larger impact on this trait (Table 1). Nonetheless, these alternatives are by no means mutually exclusive and highlight the necessity to account for methodological differences between studies and for the adoption of standardized methods. Although controlling statistically for assay duration and/or selecting tolerance estimates obtained within a narrower temporal range can partly circumvent this issue, these approaches neglect differences in z across populations or species that might be potentially relevant from a comparative and/or ecological perspective (Fig. 6).

Conclusions

Here, we develop a framework to study thermal tolerance based on the premise that thermal tolerance decreases with exposure time to a thermal challenge, which has been long recognized in the thermobacteriology and food processing literature. This perspective departs from the general approach view held in the ecological literature, that a single estimate can adequately describe upper or lower thermal tolerance, which was adopted primarily for logistic convenience (to estimate thermal tolerance fast and with fewer animals than in static methods; Lutterschmidt & Hutchison 1997) without any support on theoretical grounds. The absence of a formal theoretical framework surrounds much of the debate on the genetic and physiological basis of thermal tolerance, and its ecological and

evolutionary repercussions (see Santos, Castañeda & Rezende 2011). Given the high fit of TDT curves to the data currently available, the proposed framework seems to provide a very good compromise between biological realism and mathematical tractability, opening the venue for combined theoretical and empirical work (see Angilletta 2009; Rezende, Tejedo & Santos 2011; Santos, Castañeda & Rezende 2011).

Extrapolations must be carried out with caution, however, because the semilogarithmic association between the intensity and duration of a thermal stress remains fundamentally empirical, and deviations from this relationship will have an impact on the outcome of predictive models. Stressful temperatures affect many levels of organization simultaneously and, because their cumulative effects may disrupt different biochemical and physiological processes, break points are expected to occur along the TDT curve (Fig. S2, Supporting information; see also Santos, Castañeda & Rezende 2011; David et al. 2003). For instance, whereas enzyme denaturation or oxygen limitation may account for mortality during an acute heat stress, metabolic imbalance and resource depletion may be more important during chronic stress. Similarly, non-lethal thermal end points in ramping experiments, such as the onset muscle spasms and loss of motor coordination (see Lutterschmidt & Hutchison 1997; Sunday, Bates & Dulvy 2011; Hoffmann, Chown & Clusella-Trullas 2013 and references therein), could be caused by neural dysfunction during an acute heat shock and might not be suitable for chronic assays. Because the dose–response relationship is expected to hold at lower levels of organization and multiple factors probably contribute to thermal limits to performance, it is not surprising that different measures of thermal tolerance are often uncorrelated (e.g. Hoffmann et al. 1997; Berrigan 2000; for issues associated with measurement error and statistical power, see Santos, Castañeda & Rezende 2011; Castañeda et al. 2012). However, consistent patterns across species support a common denominator underlying these different indices (e.g. Berrigan & Hoffmann 1998; Berrigan 2000; Mitchell & Hoffmann 2010; see also Cooper, Williams & Angilletta 2008) that, nuances aside, seem to be adequately described by a thermal tolerance landscape.

Two parameters (i.e. CT_{max} and z or CT_{min} and z') are necessary to fully describe the TDT curve for any given relative mortality (around 50% when dealing with average

estimates). Even though, in principle, both the intensity and duration of a thermal stress must be taken into account to have a complete description of what thermal tolerance entails, the use of a single parameter could provide a reliable approximation as has been intrinsically assumed in the ecological literature. Nonetheless, our results suggest otherwise. First, estimated values of z (and z') conclusively show that temporal effects are important and can have a major impact on thermal tolerance. This is crucial for comparative purposes and if we ought to estimate species thermal niche in the field based on laboratory measurements (see Chown *et al.* 2009; Overgaard, Hoffmann & Kristensen 2011; Terblanche *et al.* 2011). Secondly, there is enough interspecific variation in z (and z') to cast doubts on the adequacy of estimates obtained in short-term assays as relevant indicators of tolerance during longer periods of time (Figs 2 and 4). Although in some systems variation in z (or z') may be less of an issue – for example, among the fish species studied here, a trade-off between CT_{max} and z is not immediately apparent and TDT curves are nearly parallel (Fig. 2) –, susceptibility to prolonged thermal stress remains a potential confounding factor that ought to be appropriately quantified. Thirdly, inclusion of a temporal component suggests that species might respond differently to changes in temperature averages or extremes. Even though a potential trade-off between tolerances to acute vs. chronic thermal stress remains contentious, thermal biologists should start considering which temporal window may be selectively relevant in the field.

During the development of this work, some colleagues have argued that lethal assays are not feasible or desirable for many organisms. We certainly agree. Inferring the overall shape of the thermal tolerance landscape from a limited number of measurements remains a challenge and only a handful of model organisms may be employed for experimentation with the proposed framework (but see Fig. 2). Nonetheless, ignoring the potential limitations of current approaches can be substantially more problematic not only because many studies might be reporting spurious patterns (see also Santos, Castañeda & Rezende 2011), but also because some lethal assays currently used may ultimately be uninformative. For instance, fast ramping assays may still provide useful estimates of tolerance to acute – but not chronic – thermal stress, whereas it becomes eventually impossible to discern whether a given end point in a slow heat tolerance assay reflects variation in CT_{max} or z (see also Rezende, Tejedo & Santos 2011; Santos, Castañeda & Rezende 2011). Consequently, static assays at different temperatures should be preferred whenever possible because they are easier to control, replicate, compare and analyse (see Supplementary Information), even in the absence of less invasive methods to study physiological tolerance limits.

Since the seminal work by Cowles & Bogert (1944), critical thermal limits have provided a useful approach to quantify thermal tolerance. Their prevalence in the

ecological literature can be explained solely on practical grounds (Lutterschmidt & Hutchison 1997), whereas from a conceptual perspective, the very notion of a unique critical thermal limit constitutes a gross oversimplification of what thermal tolerance limits actually entail. In this context, thermal tolerance landscapes provide a general, intuitive and mathematically tractable framework to circumvent this limitation. In spite of the inherent logistic complications, the incorporation of a temporal dimension is mechanistically realistic and conceptually necessary. Thermal stress comes in different flavours, and there seems to be more than a way to cope with it. Only by studying the combined effects of the intensity and duration of a thermal stress on survival or other fitness components, we might predict how temperature changes may impact biological systems across the globe.

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Statement of authorship

ELR designed the study; ELR, LEC and MS compiled and analysed the data; all authors contributed to the final draft.

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Appendix S1. CT_{max} and z from heat tolerance TDT assays.
Appendix S2. CT_{min} and z' from cold tolerance TDT assays.

Fig. S1. Building a thermal tolerance landscape from experimental data.
Fig. S2. Thermal tolerance landscapes and subordinate traits.