



Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Unifying indices of heat tolerance in ectotherms

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ARTICLE INFO

Article history:

Received 10 December 2007

Accepted 12 April 2008

Keywords:

Critical thermal maximum

Heat tolerance

Knockdown temperature

Knockdown time

Temperature

Thermal performance curve

ABSTRACT

1. Researchers commonly rely on indices of heat tolerance to infer the limits of performance in nature. Unfortunately, many methods are used to estimate heat tolerance, creating difficulties when synthesizing or comparing results among studies. Here, we relate several measures of heat tolerance based on the concept of a performance curve.
2. To evaluate our ideas about the link between two measures of heat tolerance, we measured knockdown times and knockdown temperatures of terrestrial isopods (*Porcellio scaber*). We fit a linear model relating knockdown time to environmental temperature; this model was used to predict the knockdown temperature (i.e., the temperature at which the knockdown time equaled zero).
3. Our model predicted a knockdown temperature within 0.1 °C of the observed value. This result reinforces the possibility of conceptually unifying several measures of thermal physiology.

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1. Introduction

Survival and reproduction often depend on an organism's ability to deal with thermal extremes. Temperature determines rates of chemical reactions (Hochochka and Somero, 2002) inducing thermal sensitivities of function at every level of biological organization (Rome et al., 1992). These thermal sensitivities limit behavioral and physiological performances linked to development, growth and reproduction (Angilletta et al., 2002). Measures of heat tolerance identify the upper bounds under which these performances can occur (Huey and Stevenson, 1979). Consequently, heat tolerance directly limits the spread of a population by dictating the suitability of a particular range. Predictions about the biological responses to climate change depend strongly on thermal tolerances of organisms (e.g., critical thermal maxima; Buckley, 2008). Given the changes in regional and global temperatures (IPCC, 2001), a better understanding of heat resistance and its evolution should help to predict the future of biodiversity.

Unfortunately, various methods have been used to quantify heat resistance, making it onerous to compare results among studies (Hoffmann et al., 2003). Common measures include (i) the temperature that triggers the loss of motor function (critical thermal maximum or knockdown temperature; Huey et al., 1992; Lutterschmidt and Hutchison, 1997) and (ii) the temperature at

which 50% of a population succumbs to heat stress (LT_{50} ; Stillman and Somero, 2000). Both measures provide useful indices of heat tolerance, but the differences between them cause difficulties when seeking general patterns. To make matters worse, a parallel set of indices deal with the time that an organism can resist heat stress. For example, knockdown time measures the time required for an individual to lose its motor function during exposure to a high temperature (Huey et al., 1992; Jenkins and Hoffmann, 1994), whereas the lethal time measures the time required for 50% of a population to die from exposure to a temperature (also abbreviated as LT_{50} , but should not be confused with the lethal temperature; Berrigan, 2000). To achieve a general understanding of heat tolerance, we must develop a unified concept and identify the relationships among common indices (Berrigan, 2000).

The thermal performance curve (*sensu* Huey and Stevenson, 1979) provides a framework for unifying measures of heat tolerance. Based on this concept, survivorship improves as temperature approaches the thermal optimum and steadily declines as the organism approaches its lethal temperature (Fig. 1A). By multiplying the probability of surviving a finite exposure to a given temperature, we can calculate the probability that an organism survives any period of time (L_x):

$$L_x = \prod_{i=1}^x p_i(T),$$

where x equals the period of time, and p_i equals the probability of surviving during the interval i at temperature T . The LT_{50}

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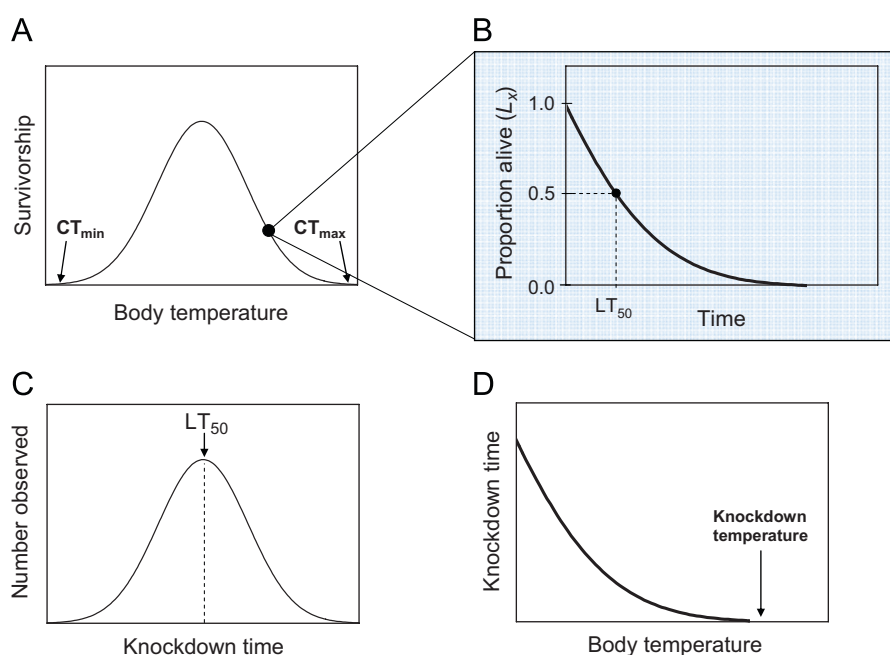


Fig. 1. Various measures of heat tolerance can be derived from the concept of a thermal performance curve. (A) A simple Gaussian function approximates the relationship between body temperature and the probability of survival (i.e., a thermal performance curve). The critical thermal minimum (CT_{min}) and maximum (CT_{max}) define the bounds of the curve. (B) At any given body temperature, the probability of survival determines the number of individuals in a population that survive to any time. Hence, the time until 50% of the population dies during exposure to an extreme temperature (LT_{50}) depends directly on the performance curve. (C) For a normal distribution of knockdown times, the mean knockdown time should equal the LT_{50} . (D) By tracing from plot C back to plot A, we see that the thermal sensitivity of knockdown time has the same form as the thermal performance curve for survivorship. Thus, we can use the thermal sensitivity of knockdown time to estimate the knockdown temperature (i.e., the temperature at which the knockdown time equals zero). In theory, a nonlinear model should best describe the thermal sensitivity of knockdown time, but a linear model might provide a sufficient approximation over a narrow range of temperatures.

corresponds to the time when $L_x = 0.5$ (Fig. 1B), which describes the median time until death (or immobility). When times until death are normally distributed, the LT_{50} equals the mean knockdown time (Fig. 1C). In this way, we can relate measures of knockdown time back to the concept of a performance curve (Fig. 1D).

Based on these considerations, we predicted a relationship exists between knockdown time and knockdown temperature. This relationship should enable researchers to design more comprehensive assays of heat tolerance and synthesize data from different experiments. Here, we empirically validate this relationship in a species of terrestrial isopod, *Porcellio scaber*. In doing so, we treat knockdown time as a function of temperature instead of a variable measured at a single temperature (see Fig. 1D). From this function, we accurately predict the knockdown temperature of *P. scaber*. This finding suggests integrated measures of heat tolerance will yield greater generality and facilitate comparative analysis.

2. Methods

We measured knockdown temperatures and knockdown times of terrestrial isopods. Because these isopods move their legs rapidly when thermally stressed, we scored knockdown as the time or temperature at which legs ceased to move. Isopods generally do not recover mobility when removed from thermal stress, suggesting these measures reveal a threshold for heat tolerance. We collected isopods from a suburban lot in Terre Haute, Indiana. Two collections were made: one for knockdown temperature and one for knockdown times. Between the times of collection and measurement, isopods were stored in plastic terraria with a substrate of small gravel and peat moss. These

terraria were stored in a dark incubator at 25 °C. A relatively high humidity was maintained by misting the containers daily.

Both knockdown temperatures and knockdown times were recorded in a water bath (Isotemp 228, Fisher Scientific). Isopods were placed individually in small vials (10 ml), affixed to a white sheet of plastic. These vials were then submerged in the water bath. First, we determined knockdown temperatures by steadily increasing the temperature of the water until isopods lost mobility. The water temperature initially equaled 38.0 °C and increased by approximately 0.2 °C min⁻¹. The temperature of knockdown was recorded when an isopod ceased to move its legs; at this time, we removed the vials from the bath for a few seconds to confirm the isopod could not respond to stimuli. We completed five trials, each including 4–10 isopods, to obtain 33 measurements of knockdown temperature.

We measured knockdown times at intervals of 0.5 °C between 38 ° and 42 °C. The procedure followed that used for knockdown temperature, except the water temperature remained constant and the time until knockdown was recorded. We used five isopods at each temperature for a total of 45 independent measurements. We fit a linear model relating knockdown time to environmental temperature. This model was used to predict the knockdown temperature (i.e., the temperature at which the knockdown time equaled zero). To determine whether a linear model provided a sufficient fit to the data, we compared this model to an exponential model using Akaike's information criterion. Both models were fit using Statistica 6.0 (Statsoft, Inc., 2003).

3. Results

Although we were primarily interested in predicting the knockdown temperature from a linear model, we also wanted to know whether this model provided a reasonable description of

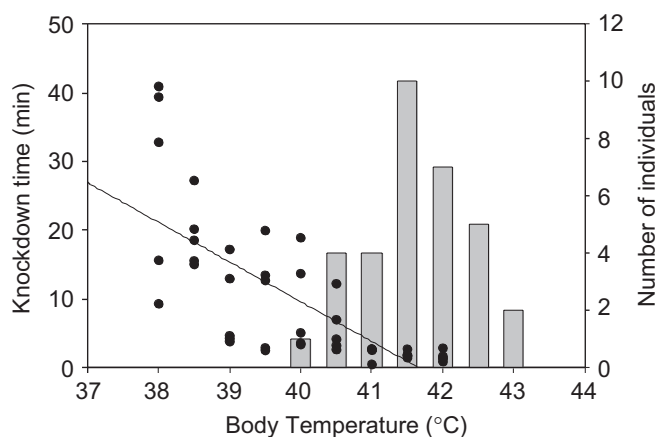


Fig. 2. A linear model approximates the thermal sensitivity of knockdown time over the range of 38–42 °C. The intercept of this model corresponds well to the mean of the distribution of knockdown temperatures (gray bars).

the data. In comparing linear and exponential models, we found substantial support for the linear model ($\Delta_{AIC} = 1.6$; see Burnham and Anderson, 2002).

Based on this model, knockdown time decreased nearly 6 min/1° increase in temperature ($F_{1,43} = 50.892$, $P < 0.00001$, $r^2 = 0.54$; Fig. 2). The predicted knockdown temperature equaled 41.7 °C (95% confidence interval = 41.2–42.4 °C).

The observed knockdown temperature corresponded nicely to the predicted knockdown temperature. Using a standard protocol, knockdown temperature averaged 41.8 °C (range = 40.4–43.3 °C). The distribution of knockdown temperatures appeared normal (Wilk's $W = 0.98$, $P = 0.69$; Fig. 2); consequently, the LT_{50} (41.9 °C) roughly equaled the mean knockdown temperature. Thus, by treating knockdown time as a function of environmental temperature, we accurately predicted the knockdown temperature and the LT_{50} of isopods.

4. Discussion

Among others, measurements of knockdown temperature, lethal temperature and knockdown time provide a means for understanding how organisms deal with thermal extremes. However, this diversity of indices inherently reduces the possibility of comparing results among populations. Many researchers use the critical thermal maximum (Hertz et al., 1979; Gaston and Chown, 1999; Addo-Bediako et al., 2002; Winne and Keck, 2005), knockdown time (Sorensen et al., 2001; Hoffmann et al., 2002), or lethal temperature (Stillman and Somero, 2000) to estimate heat tolerance. Few researchers use two or more indices in a single experiment (e.g., see Huey et al., 1992; Gaston and Spicer, 1998; Berrigan, 2000). Consequently, we can only compare heat tolerances among populations when the same indices were chosen. By recognizing the mathematical relationships among indices of heat tolerance, we can now design experiments that generate comparable data. Our ability to empirically relate knockdown times to the knockdown temperature support this conclusion.

Despite our success in relating indices of thermal tolerance, two artifacts might prevent us from extending this result to other species. First, cumulative heat shock during assays of knockdown temperature could reduce heat tolerance, resulting in a lower knockdown temperature. Second, if assays take too long, rapid acclimation of heat resistance could artificially inflate knockdown temperatures (Czajka and Lee, 1990). Presumably, neither artifact played a role in our study because our predicted and observed

temperatures were nearly identical. Nevertheless, future studies of this species and others could suffer from these artifacts.

To avoid artifacts caused by cumulative heat shock, we should measure the knockdown temperature in a timeframe shorter than the time known to initiate heat shock. Presently, we lack the data to evaluate the effect of cumulative heat shock on heat resistance, but anecdotal evidence leads us to assume cumulative heat shock might occur during measures of heat resistance. To quantify this phenomenon, we could design multiple treatments, each starting at a different temperature. Individuals starting at a lower temperature should have lower knockdown temperatures than those starting at a higher temperature. With this kind of experimental design, we could identify the protocol that provides the most accurate knockdown temperature for future comparative studies.

In contrast to our ignorance of cumulative heat shock, many researchers have shown that acclimation alters heat resistance, often in predictable ways. For example, populations of *Drosophila melanogaster* exposed to 29 °C for up to several days resisted heat better than control populations did, with the largest difference in heat tolerance occurring only 12 h after the onset of exposure (Levins, 1969). Likewise, heat resistance generally increases with increasing developmental temperature (e.g., see Worthen and Haney, 1999). In fact, brief exposure to a high temperature nearly always increases the heat tolerance of an organism (heat hardening; see Hoffmann et al., 2003 and references therein). Although rates of acclimation remain unknown for most species, we clearly need to consider the possibility of rapid acclimation when designing assays of heat tolerance. By considering this issue, researchers can increase the chances that one could accurately convert different indices of heat tolerances into a common measure.

Given the desire to ultimately compare thermal tolerances among populations and species, thermal biologists face the difficult task of choosing among many indices (Hoffmann et al., 2003). Berrigan (2000) showed the need to choose ecologically relevant measures of heat resistance when analyzing intraspecific variation in stress resistance. Still, researchers rarely consider ecological relevance when choosing among methods of estimating heat tolerance (Huey and Berrigan, 1996; Krebs and Feder, 1997). This problem increases the importance of unifying indices of thermal tolerance in ectotherms. Once we know the relationships among different indices, the particular index chosen for analysis becomes less important (Berrigan, 2000). The mathematical relationships among different indices enables us to precisely estimate the value of one index from a measurement of another.

This conceptual insight should guide the design of future experiments. Measuring knockdown time at a single temperature provides valuable information, but this index cannot easily be compared to other indices of heat resistance. In contrast, our approach (i) lessens the importance of choosing the appropriate index and (ii) makes comparisons among populations and species more feasible. With the appropriate experimental design, we can derive the knockdown temperature from knockdown times. Despite the benefit of unifying indices of heat tolerance, we are the first to attempt a formal integration of existing concepts. We expect a similar effort to unify indices of cold tolerance will further enhance our ability to uncover broad patterns of thermal physiology.

Acknowledgments

We would like to thank Ray Huey for constructive comments that improved our manuscript. This research was supported by grants from the Lilly Endowment, Indiana State University's Office

of the Provost and Vice President for Academic Affairs, the University Research Committee, the Stanley Foundation and the National Science Foundation (IOS 0616344).

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