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Measuring behavioral thermal tolerance to address hot topics in ecology, evolution, and conservation

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ABSTRACT

Understanding the impacts of anthropogenic climate change requires knowing how animals avoid heat stress, and the consequences of failing to do so. Animals primarily use behavior to avoid overheating, but biologists' means for measuring and interpreting behavioral signs of stress require more development. Herein, we develop the measurement of behavioral thermal tolerance using four species of lizards. First, we adapt the voluntary thermal maximum concept (VTM) to facilitate its measurement, interpretation, and comparison across species. Second, we evaluate the sensitivity of the VTM to diverse measurement options (warming rate, time of day, etc) across four species with highly different life histories. Finally, we clarify the interpretation of VTM in two ways. First, we show the effects of exposure to the VTM on panting behavior, mass loss, and locomotor function loss of two species. Second, we compared the VTM with the preferred body temperatures (PBT) and critical thermal maximum (CTMAX) intraspecifically. We found that the VTM can be consistently estimated through different methods and methodological options, only very slow warming rates affected its estimates in one species. Exposure to the VTM caused panting between 5 and 50 min and induced exceptionally high mass loss rates. Loss of locomotion function started after 205 min. Further, the VTM did not show intraspecific correlations with the PBT and CTMAX. Our study suggests the VTM is a robust and flexible measure of thermal tolerance and highlights the need for multispecies evaluations of thermal indices. The lack of correlation between the VTM, the PBT and CTMAX suggests the VTM may evolve relatively free between the other parameters. We make reccommendations for understanding and using the VTM in studies of ecology, evolution, and conservation.

1. Introduction

Understanding how thermal constraints affect species' behavior and distributions has long been central to studies of ecology and evolution. More recently, the climatic crisis has increased the urgency of these pursuits (e.g. Huey and Stevenson, 1979; Williams et al., 2008; Huey et al., 2012; Sunday et al., 2014; Sinclair et al., 2016). Temperatures that lead to the suboptimal performance of individuals (also called, *pejus* temperatures, Pörtner and Farrell, 2008) can constrain the spatial distribution of individuals and species, leading to population decline or localized extinction. The failure to avoid *pejus* temperatures causes this constraint by decreasing physiological performance or by negative interactions with species capable of performing better at those temperatures (e.g. Buckley, 2008).

Popular indices of thermal constraints represent different parts of a thermal performance curve, which relates body temperature to an organism's performance levels (Huey, 1982; Angilletta et al., 2002). For example, some researchers use the temperature at which performance is optimal (i.e,. optimal temperature; e.g. Huey et al., 2009) because at higher temperatures, physiological function and survival lowers rapidly (Huey, 1982; Rezende et al., 2014). Others use the temperatures that cause loss of locomotion or muscle spasms (A.K.A. the critical thermal maximum (CTMAX); Lutterschmidt and Hutchison, 1997a, 1997b), to infer thermal constraints on species distributions (Kingsolver et al., 2013) and evolutionary drivers (Clusella-Trullas et al., 2011). Still, these two indices present specific disadvantages that may impair their wide applicability. For example, measures of optimal temperatures may take weeks to be measured (Hertz et al., 1993). Likewise, the CTMAX may be too insensitive for assessing thermal constraints because many animals thermoregulate behaviorally (e.g. shift their microhabitat use),

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keeping body temperatures within preferred ranges, often below optimal and critical temperatures (Cowles and Bogert, 1945; Heath, 1970; May, 1979; Martin and Huey, 2008).

In contrast, measuring behavioral thermal tolerance may only take the time needed to heat an animal until it shows first signs of thermal stress (e.g. Heatwole and Firth, 1982). Thus, measuring a species' voluntary thermal maximum (VTM) is not only the fastest way to infer thermal tolerance, but also safer than the CTMAX and accounts for animal's own perception of thermal stress. Despite these advantages, the VTM has been rarely used, compared with the wide application of PBT and the CTMAX to infer thermal constraints on the ecological and geographic distribution of species (Hillman, 1969; Cowles and Bogert, 1945; Curry-Lindahl, 1979; Sinervo et al., 2010; Sunday et al., 2014; Piantoni et al., 2016; but see Porter et al., 1973; Kearney and Predavec, 2000).

Several issues may explain the rare application of behavioral thermal tolerance in ecological and evolutionary studies. First, it requires a practical and consistent definition. Previous studies identified the voluntary thermal maximum (VTM) as the temperature that makes an animal look for shelter underground (Cowles and Bogert, 1945), startles the escape of a warming chamber (e.g. Hertz, 1979; Cadena and Tattersall, 2009), the maximum body temperature observed for an organism within a thermal gradient (e.g. Licht, 1965), the temperature at which panting is observed (Heatwole and Firth, 1982; Firth and Heatwole, 1976), or simply the highest body temperature observed in the field (Brattstrom, 1965). A second issue is that behavioral thermoregulation can be labile (e.g. Clusella-Trullas et al., 2007) and dependent on the costs for thermoregulation (Huey and Slatkin, 1976; Cadena and Tattersall, 2009). Finally, because exposure to the VTM does not induce immediate function loss, it becomes important to know how long it takes for the VTM to distress the behavior and function of animals exposed to it.

Thermal tolerance indices are often affected by a multitude of factors. However, comparative studies of thermal tolerance methods are rare (reviewed in Camacho and Rusch, 2017). For instance, the CTMAX can change depending on the starting temperature or heating rate (e.g. Lutterschmidt and Hutchison, 1997a, 1997b; Terblanche et al., 2007). Similarly, the PBT of lizards may change with time of day (e.g. Clusella-Trullas et al., 2007), photoperiod, or the position of the light source over a thermal gradient (Sievert and Hutchison, 1991). Furthermore, the CTMAX and the PBT seem to be more sensitive to acclimation than the VTM (at least in lizards). Therefore, the VTM might become an useful index of thermal tolerance, if its measurement and interpretation were better understood.

This study clarifies the measurement and application of the voluntary thermal maximum (VTM). We used lizards, a common model organism in thermal biology (e.g. Huey, 1982; Angilletta, 2009). First, we generalize the definition of VTM as the body temperature that induces a behavioral response seeking to cool down. This definition is wider from Cowles and Bogert (1945) "upper voluntary tolerance", defined for lizards, in which the animal specifically seeks shade or burrows. This change is in line with the use of the VTM in previous studies (e.g. Cadena and Tattersall, 2009). It has the advantage that it can be applied to any animal and to different measurement methods, as opposed to other measures of voluntary tolerance such as panting behavior, which is not even present in all lizard species (e.g. Taylor and Heatwole, 1987). Next, we tested whether the VTM can be consistently estimated with three different methods. In addition, we tested specific effects of both methodological and biological sources of variability over these methods: 1) time in captivity, 2) start temperature, 3) warming rate, 4) time of the day, 5) order of measure for repeated measures, 6) sex, and 7) body mass. Finally, we clarify the interpretation of VTM in two ways. We show how exposure to the VTM affects behavior and function of two species, and correlate the VTM with the PBT and CTMAX intraspecifically.

2. Methods

2.1. Supporting online materials

The link https://figshare.com/s/3cde18355ed47e79e7a5 leads to 3 files.

Supporting online file 1 includes details of instrumental calibration and extended results. The second file contains all the generated datasets for this study. The third file includes the R scripts necessary to repeat the analyses and graphs, using the tables in file 2.

2.2. Species accounts

All measures were done on four species of lizards; three phrynosomatid species and one earless and limb reduced skink. Thus, we sampled lizards from different geographic regions facing different thermal constraints. Specifically, we sampled *Urosaurus ornatus*, a microhabitat generalist distributed from low to high altitudes in United States, *Sceloporus tristichus*, another microhabitat generalist distributed across high altitude woodlands in Arizona, and *Sceloporus jarrovi*, a rock specialist distributed across highlands of Arizona, New Mexico, and northcentral Mexico. We collected *U. ornatus* in Tempe and Superior (AZ), *S. tristichus* from Show Low (AZ), and *S. jarrovi* from the western side of the Chiricahua Mountains in Cochise County (AZ). *Hemiergis peronii* was collected in Yorke peninsula, Australia. All lizards were captured by hand or noosing. During captivity, they were frequently weighed to ensure they were in good health.

2.3. Comparing methods of measuring the VTM

We used the species *U. ornatus* to compare VTM estimated by three different methods: a dark chamber, a light chamber, and a thermal gradient (Fig. 1). In the first two methods, we measured the VTM as the body temperature at which an individual exited a warming chamber. In the dark-chamber method, we placed each lizard inside a metal can and warmed the can with a fluorescent light bulb (20 W) until the animal decreased its body temperature by putting its head or tail outside the chamber. For the light-chamber method, each lizard was directly illuminated and heated by the same fluorescent light bulb, and VTM was the body temperature at which the animal moved into the connecting cool chamber. We observed this movement through a slit (aprox. 2 mm × 2 cm) in the box's upper side.

In the thermal gradient, lizards were placed individually in a rectangular acrylic box (e.g. Licht, 1965) that contained a linear gradient of operative temperatures ranging from 25° (room air temperature) to 48 °C. Operative temperatures were confirmed by measuring clay models mimicking the size and shape of an adult lizard along a whole activity period. Gradients were created by circulating hot water and attaching heat tapes under the hot extremes (Fig. S12). We placed the lizards in thermal gradients the day before measuring the VTM. The next day, we recorded body temperatures every 10 s between 0800 h and 1600 h. From each day, we extracted the four highest peaks in each individual temperature profile (e.g. Fig. S1 in the supporting online file 1).

For all methods, body temperature was continuously monitored by a type T thermocouple (1-mm in diameter, Omega Engineering) attached to the lizards' groin with medical tape. A thermocouple was also attached to the surface of the warming chamber contacting the animal. Thermocouples were connected to a factory-calibrated temperature recorder (picolog® TC H8), and downloaded to a computer during trials. We avoided measuring temperatures in the cloaca to reduce risk of injury and discomfort. Additionally, lizards have been found to use skin rather than internal temperature to thermoregulate (Barber and Crawford, 1979). In our study, differences between cloacal and groin temperatures during warming were small (< 1.01 °C; Supporting online file 1). The three methods were applied in random order among