

The upper limit of thermoneutrality is not indicative of thermotolerance in bats

Shaun Welman^{a,b,*}, Ana M. Breit^{c,d}, Danielle L. Levesque^c, Julia Nowack^e

^a Department of Biological Sciences, University of Cape Town, Cape Town, 7701, South Africa

^b Department of Zoology, Nelson Mandela University, Gqeberha, 6031, South Africa

^c School of Biology and Ecology, University of Maine, 04469 Orono, ME, USA

^d School of Life Sciences, University of Nevada Las Vegas, 89154, Las Vegas, NV, USA

^e School of Biological and Environmental Sciences, Liverpool John Moores University, L3 3AF, Liverpool, UK

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ABSTRACT

To assess the vulnerability of birds and mammals to climate change recent studies have used the upper critical limit of thermoneutrality (T_{UC}) as an indicator of thermal tolerance. But, the association between T_{UC} and thermal tolerance is not straightforward and most studies describe T_{UC} based solely on a deviation in metabolism from basal levels, without also considering the onset of evaporative cooling. It was argued recently that certain torpor-using bat species who survived prolonged exposure to high ambient temperatures (i.e. high thermal tolerance) experienced during extreme heat events did so by entering torpor and using facultative heterothermy to thermoconform and save on body water. Assuming that T_{UC} is indicative of thermal tolerance, we expect T_{UC} in torpor-using species to be higher than that of species which are obligate homeotherms, albeit that this distinction is based on confirmation of torpor use at low temperatures. To test this prediction, we performed a phylogenetically informed comparison of bat species known to use torpor ($n = 48$) and homeothermic ($n = 16$) bat species using published thermoregulatory datasets to compare the lower critical limit of thermoneutrality (T_{LC}) and T_{UC} in relation to body temperature. The influence of diet, biogeographical region, body mass and basal metabolic rate (BMR) was also considered. Body mass had a positive relationship with BMR, an inverse relationship with T_{LC} and no relationship with T_{UC} . Normothermic body temperature scaled positively with BMR, T_{LC} and T_{UC} . There was no relationship between diet or region and BMR, but both influenced thermal limits. Torpor-using bats had lower body mass and body temperatures than homeothermic bats, but there was no difference in BMR, T_{LC} and T_{UC} between them. Exceptional examples of physiological flexibility were observed in 34 torpor-using species and eight homeothermic species, which included 15 species of bats maintaining BMR-level metabolism at ambient temperatures as high as 40 °C (and corresponding body temperatures ~ 39.2 °C). However, we argue that T_{UC} based on metabolism alone is not an appropriate indicator of thermal tolerance as it disregards differences in the ability of animals to tolerate higher levels of hyperthermia, importance of hydration status and capacity for evaporative cooling. Also, the variability in T_{UC} based on diet challenges the idea of evolutionary conservatism and warrants further consideration.

1. Introduction

As climate change progresses there is a need to know how species will respond to future increases in ambient temperature (T_a) (Sherwood and Huber 2010; Willett and Sherwood 2012). However, our understanding of the thermoregulatory responses of endotherms at high T_a s, where animals face hyperthermia, is limited – especially for mammals (reviewed by McKechnie and Wolf 2019). To gain a broader perspective

of the vulnerability of endotherms to the effects of climate change, recent studies have equated the upper critical limit of thermoneutrality (T_{UC}) in birds and mammals to their potential vulnerability to heat stress (Araújo et al. 2013, 2016; Khaliq et al. 2014, 2015). This has stimulated debate on the topic with some expressing concern surrounding the quality of the dataset used in those studies (McKechnie et al., 2017; Wolf et al., 2017) (but see also Hof et al., 2017a; Hof et al., 2017b), while others expressed concern regarding the nature of the physiological

* Corresponding author. Department of Biological Sciences, University of Cape Town, Cape Town, 7701, South Africa.

E-mail address: shaun.welman@uct.ac.za (S. Welman).

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relationship between T_{UC} and heat tolerance (Mitchell et al., 2018). We agree with the view that T_{UC} should not be used as an indicator of heat tolerance in mammals and, here, we aim to address the misconception that it can.

By the International Union of Physiological Sciences (IUPS) Thermal Commission (2003)'s definition, T_{UC} marks the highest temperature of the thermoneutral zone (TNZ) which is "the range of ambient temperature at which temperature regulation is achieved only by control of sensible heat loss, i.e., without regulatory changes in metabolic heat production (H) or evaporative heat loss". Therefore, the temperature marking T_{UC} should include consideration of both increases in metabolic rate and rates of evaporative cooling. However, most studies only consider metabolism when determining T_{UC} . Irrespective of this bias, Khaliq et al. (2014), for example, reported that many species reside in areas where the *in situ* T_a s are below T_{UC} and thus should be able to persist across much of their current distribution well into the future. Indeed, many mammals living in warm climates spend the majority of their time at T_a s nearing or surpassing T_{UC} without ill effect (Mitchell et al., 2018). It is thus unclear how T_{UC} relates to heat tolerance. Furthermore, as the parameters of the TNZ are measured under restrictive conditions (in captivity, at rest and often in post-absorptive animals) the ecological relevance of these parameters for many endotherms is questionable (reviewed in Levesque and Marshall 2021). One potential exception to this, however, is that roost conditions in nocturnal, particularly arboreal, endotherms which rest in the heat of the day does somewhat resemble the conditions under which thermoregulation is measured in the laboratory (Lovegrove et al., 2014).

In the strictest sense of the IUPS Thermal Commission (2003)'s definition of TNZ, researchers should report T_{UC} as the upper temperature at which metabolism deviates from basal levels or the temperature coincident with the onset of evaporative cooling, whichever comes first (Withers et al., 2016). Presumably, due to the complexity of accurate evaporative water loss measurements, the majority of thermoregulatory studies do not include simultaneous measurements of metabolism (oxygen consumption and/or carbon dioxide production) and water vapour (see Muñoz-García et al., 2022). Arguably, T_{UC} informed by or based on evaporative heat loss measurements can be informative regarding thermal tolerance as one can determine the risk of lethal dehydration based on the animal's body mass (M_b) and length of exposure to heat (Riddell et al., 2021). But, this would remain a crude estimate without also considering how hydration status influences thermoregulatory behaviour (Sawka et al., 2001; Fuller et al., 2007; Ben-Hamo et al., 2013; Ramirez et al., 2022). For example, seasonal variation in water availability influences how animals respond to heat, with some enduring facultative hyperthermia (i.e. allowing an increase in body temperature above normothermic levels) during periods when water availability is low but otherwise defending a lower body temperature (Dawson et al., 2007; Hetem et al., 2014b).

In general, M_b does offer some predictability as to how a mammal is likely to respond to heat. Large-bodied mammals benefit from high thermal inertia and many opt to endure periods of moderate-to-serve hyperthermia to save body water (Hetem et al. 2014a, 2014b). In contrast, small mammals, with larger surface area to volume ratios, typically heat faster and invest in evaporative cooling much sooner to avoid lethal hyperthermia (Tattersall et al., 2012). However, evidence is mounting in support of the argument that small mammal species which are capable of using torpor at low T_a (characterised by a controlled decrease in body temperature and metabolic rate associated with abandoning the defence of normothermia (Geiser 2004)), can also use torpor at warm-to-high T_a s, albeit that a reduction in body temperature would be impeded by the high T_a (Song et al., 1997; Bronner et al., 1999; Grimpo et al., 2013; Bondarenko et al., 2014; Busse et al., 2014; Bondarenko et al., 2016; O'Mara et al., 2017; Reher et al., 2018; Reher and Dausmann 2021; Sørås et al., 2023). In some cases, torpid animals display a facultative increase in body temperature to levels typically considered severely hyperthermic (body temperature, $T_b \geq 40$ °C),

enhancing heat tolerance and reducing the risk of dehydration without necessarily showing a pronounced increase in metabolism (Reher et al., 2018; Reher and Dausmann 2021). This adds another layer of uncertainty regarding the use of T_{UC} as an indicator of thermal tolerance.

Although torpor at high temperature has now been reported in several species of small mammals, the most compelling support for the argument that heterotherms may have improved heat tolerance is observed in bats (for example Reher et al., 2018; Reher and Dausmann 2021). Yet, bats also seem particularly vulnerable to heat stress because there have been several reports of catastrophic mass die-offs during extreme heat events observed around the world including India, the Pacific Islands (O'Shea et al., 2016), South Africa (McKechnie et al., 2021b) and especially amongst fruit bats in Australia (Welbergen et al., 2008; Ratnayake et al. 2019, 2020; Mo et al., 2022). Their vulnerability is likely due to their sun exposed diurnal roosts without access to water and limited means by which to cool evaporatively (Bondarenko et al., 2014; Lovegrove et al., 2014; Czenze et al., 2022). Worryingly, extreme heat events are predicted to increase in frequency and intensity due to climate change, placing many species at risk of local extinctions and range contractions (Festa et al., 2023). To the best of our knowledge, the majority of the bats that have succumbed during extreme heat events have thus far been members of the Pteropodidae (Festa et al., 2023). But, this is not to say that other bats are not at risk (Pruvot et al., 2019). To date, however, none of the species experiencing mass die-offs are known to use torpor. Given the contrast in response to heat observed among bats and the possibility of torpor-users to display a form of hypometabolism at high temperatures (O'Mara et al., 2017), bats may be a good model to test for a potential association between T_{UC} - determined using metabolism only - and thermal tolerance.

Here, we compiled data from published thermal profiles to test the hypothesis that species known to use torpor, due to their ability to use torpor under heat stress, would exhibit higher T_{UC} s than species which are obligated to defend their normothermic body temperatures (i.e. homeothermic species). The hypothesis is based on the premise that torpor improves thermal tolerance, as argued earlier. Further, the hypothesis hinges on the assumption that hypometabolism due to torpor would require higher T_a to force an increase in metabolism to a level above that of basal metabolic rate (BMR), whereas homeothermic species should display heat related tachymetabolism at comparative lower T_a marking the end of TNZ. A consideration, based on the classic Scholander-Irving model (Scholander et al., 1950) (which should be noted did not include upper limits), is that for any given value of conductance, species with higher BMR should display broader TNZs relative to those with lower BMRs. Thus, it may be possible that T_{UC} would be higher in species with higher BMR or high normothermic body temperatures. Firstly, because body temperature is correlated to BMR even though the relationship may not be straight-forward. Although it is known that a high body temperature requires a high BMR (Clarke and Pörtner 2010), the interdependence is confounded by a variety of factors including phylogeny (Lovegrove 2012), M_b (Clarke et al., 2010), diet (Clarke and O'Connor 2014) and climate (Lovegrove 2003, 2005), amongst others. Secondly, the larger temperature differential between body temperature and ambient temperature (i.e. $T_b - T_a$) would allow passive heat loss at higher T_a s and delay the metabolic investment related to active cooling or Arrhenius effects on metabolic rate due to increased heat storage (Schmidt-Nielsen 1997; Tattersall et al., 2012; McKechnie et al., 2021a). As such, we included the species-specific normothermic body temperature (i.e. the body temperature associated with the lower end of the TNZ from animals not in torpor) and BMR as part of the study. Furthermore, because BMR may influence the breadth of TNZ, and M_b , biogeographic region and diet are known to influence BMR in bats (Lovegrove 2000; Marroquin et al., 2023), we also considered these factors to provide better context for the limits of thermoneutrality. Including the lower critical limit (T_{LC}) provides the opportunity to determine if there is any difference in the breadth of TNZ between bat groups and to test the association between BMR, diet and

TNZ breadth. Ultimately, for T_{UC} to serve as a measure of thermal tolerance we expect that after accounting for the effect of phylogeny, M_b , biogeography and diet, torpor-using species would have a higher heat tolerance, measured as a higher T_{UC} compared to homeothermic species. Further, if T_{UC} is higher in torpor-using species we then expect that the temperature differential between T_{UC} and normothermic body temperature would be larger compared to that of homeothermic species, necessary to show that the higher T_{UC} in torpor-using species was not due to any benefit from passive heat loss as explained earlier.

2. Methods and materials

2.1. Dataset

We assembled an independent dataset of physiological and ecological data for 64 species using information from original sources only (Supplementary Table 1). We initially compiled a list of candidate species from citations listed in datasets such as Clarke et al. (2010), Riek and Geiser (2013) and Khaliq et al. (2014) and supplemented this list using an independent literature search in Web of Science. We conducted the literature search in Web of Science using keywords such as “metabolic rate”, “thermoneutral zone” and “thermoregulation” as search criteria. This yielded over 48 000 article matches which we subsequently refined to 500 articles and then 262 articles through an iterative process by searching within the list of articles firstly for the word “bats”, and then searching within the resultant set of articles for the words “body temperature”. We reviewed these 262 studies to determine their eligibility. To be considered eligible, studies needed to provide thermal profiles including concurrent measures of metabolism and body temperature. This yielded 114 candidate species. As an attempt to assemble a good-quality dataset (*sensu* Wolf et al., 2017), based on the general scientific principle of three independent replicates, all candidate species whose thermal profiles were based on fewer than three individuals (i.e. $n < 3$) were excluded. We used PlotDigitizer 2.6.8 (Huwaldt and Stenhorst 2015) to digitise the metabolic rate and body temperature thermal profiles of each bat and then performed a broken-stick regression analysis on the metabolic rate profile of the remaining candidate species using the R package “segmented” (Muggeo 2008) to determine if any inflection points could be detected. Based on this, we excluded species whose thermal profiles consisted of fewer than 10 data points as the analysis was unable to detect any inflection point along the species metabolic profile. Furthermore, recent studies of thermoregulation in bats seem to prefer a sliding scale (or stepped) temperature profile to study heat tolerance. Given the fundamental difference in the approach between these and classic studies (sliding scale vs static temperature) and the fact that animals in sliding scale temperature experiments would only be exposed to any one temperature for a short period of time, data of six species from sliding scale studies were also excluded from our analyses. For species where eligible thermoregulatory data were available from multiple studies, the study that reported the higher number of samples was preferred. From the remaining 42 studies we extracted information for 68 species about capture location, body temperature, BMR, T_{LC} , T_{UC} and M_b and assigned a subjective score to the reported T_{UC} value based on a visual inspection of the thermal profile. If there was a clear inflection in metabolism after the reported T_{UC} it was scored as “reliable” ($n = 45$). If the T_{UC} reported represented the highest experimental temperature of the study it was either classed as “special inclusion” if the T_{UC} was a higher temperature compared to the species’ normal body temperature ($n = 19$), or “unreliable” if it was lower ($n = 4$). In the latter instances it is likely that the experimental temperatures used were simply too low to elicit a heat related metabolic response characteristic of T_{UC} whereas in the former instances the experimental temperatures used should have been sufficiently high to elicit a metabolic response because heat storage ensues as T_a approaches T_b . Thus, although an inflection in metabolism was not evident, those bats would certainly have been heat challenged. All species whose T_{UC} value was

deemed unreliable were excluded. The accuracy of the digitisation process was validated using a paired *t*-test to compare the normothermic body temperature reported within the original studies (mean = 34.9 ± 0.3 °C, $n = 64$) and the body temperature of bats at T_{LC} (mean = 35.1 ± 0.3 °C, $n = 64$) determined from the digitised data.

Bats were assigned to one of six biogeographical regions (*sensu* Lovegrove 2000), based on the location of capture. Bats were classified as torpor-users or homeothermic based on all available evidence from literature, following the classification of bat species listed in Lazzaroni et al. (2018) and Geiser and Stawski (2011) and irrespective of whether or not the original source provided evidence of torpor. Any species without evidence of torpor use was classed as a homeotherm. Notably, we disagreed with Lazzaroni et al. (2018)’s classification of *Pteronotus quadridens* as being a homeothermic species because there was clear evidence to the contrary ($T_b < 30$ °C accompanying metabolic rates \approx BMR) reported by Rodríguez-Durán (1995).

Diet was classified as either 1) frugivore (consumes fruit, pollen, nectar or plants), 2) omnivore (consumes both plant and animal parts), 3) carnivore (consumes vertebrates), 4) insectivore (consumes mainly invertebrates) or 5) sanguinivore (consumes blood). We based this on the criteria and classification of bat diets listed in Marroquin et al. (2023). When dietary information was not provided in the original source, we considered the International Union for the Conservation of Nature (IUCN) RedList entry to be a reliable source of information.

2.2. Statistical analyses

To test for evidence of a phylogenetic effect in the dataset, the R package “picante” (Kembel et al., 2010) was used to determine Blomberg et al.’s (Blomberg et al., 2003) *K*-statistic and *P*-values for each physiological variable. *K*-values < 1 were taken to indicate that closely related species resemble each other less than expected under a Brownian motion (BM) evolutionary model, whereas *K*-values > 1 were taken to indicate that closely related species resemble each other more than expected under a BM evolutionary model. We obtained the most recent time-calibrated species-level mammal phylogeny from <https://data.vertlife.org/> (Upham et al., 2019). The phylogeny containing approximately 6000 species was pruned to include only the 64 bat species used in this study using the R package “ape” (Paradis et al., 2004). For any conflicts in taxonomic classification we resolved to the classification used in the VertLife phylogeny (Fig. 1). The test for phylogenetic signal showed that there was a significant phylogenetic signal for M_b (*K*-statistic = 0.33, $p = 0.00.05$) and BMR (*K*-statistic = 0.32, $p = 0.022$). Thus, because a phylogenetic signal was detected in the dataset and the analysis showed a low *K*-statistic it was decided to include the potential influence of phylogeny during comparisons using Pagel’s evolutionary model. The Pagel evolutionary model performs comparisons that includes an estimation of the phylogenetic correlation between traits (Pagel’s λ). The estimated correlation varies between 0 (where traits are phylogenetically independent of each other i.e. ordinary least squares model) and 1 (where traits are directly proportional to shared ancestry i.e. Brownian motion model) (Freckleton et al., 2002). This allows for a better fit compared to a *prior* selection of a pure Brownian motion model or ordinary least squares model.

All variables were \log_{10} -transformed to normalize the data before analyses. The R package “nlme” (Pinheiro et al., 2019) was used to perform phylogenetic generalized least squares (PGLS) regression analyses and phylogenetic comparisons using a phylogenetic generalized ANCOVA (Revell and Harmon 2022). To account for the effect of M_b on physiological variables it was included as a covariate in all models. We then tested for an effect of torpor, region and diet on physiological variables. As an attempt to account for the effect of body temperature in comparisons of thermal limits, we compared T_{LC} and T_{UC} relative to normothermic body temperature in addition to comparisons of absolute values. To determine the values relative to body temperature, for the lower critical limit we calculated the difference between normothermic

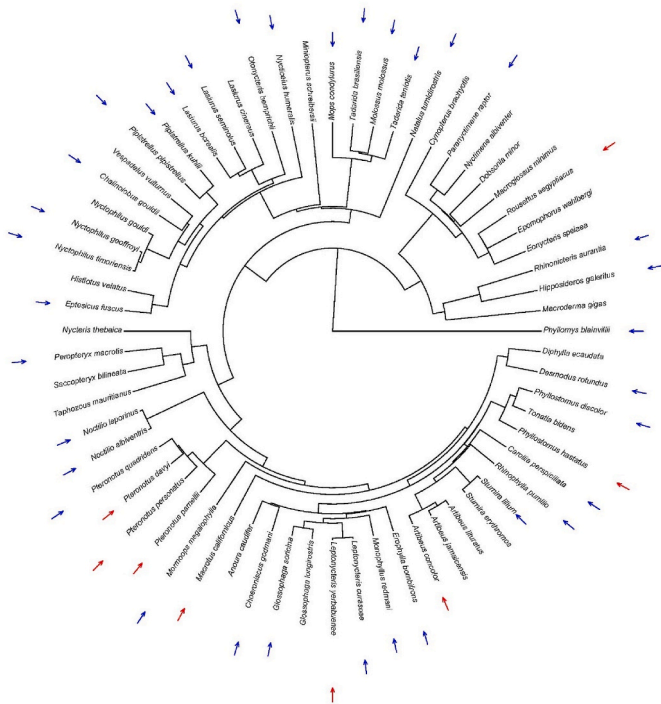


Fig. 1. Phylogenetic tree of the 64 species of bats used in the study. The arrows indicate bats whose thermoneutral zone extends above their normothermic body temperature. *Blue arrows* indicate species known to use torpor and *red arrows* indicate homeothermic species. The phylogeny was pruned from the mammalian phylogeny obtained from <https://data.vertlife.org/>. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

body temperature and T_{LC} (i.e. $T_b - T_{LC}$) and for the upper critical limit we subtracted normothermic body temperature from T_{UC} (i.e. $T_{UC} - T_b$). In both cases, the smaller the value the nearer the critical limit is to normothermic body temperature.

Statistical analyses of T_{UC} and its associated body temperature presented below were performed using the full dataset (i.e. including data from species deemed as “special inclusions” where $T_{UC} \geq$ normothermic body temperature). Results from statistical analyses based on the restricted dataset (i.e. excluding data from species deemed as “special inclusions”) are provided as supplementary material. We opted to present results based on the full dataset because that is generally what has been used in previous work arguing in favour of T_{UC} serving as an indicator of thermal tolerance. While there is no fundamental difference in the comparison of T_{UC} between bat groups, the outcome of some comparisons based on diet and biogeographical region differed. We clearly identify such cases in the supplementary. All statistical procedures were performed using R version 4.2.2 (R Core Team 2022) and tests were conducted using $\alpha = 0.05$. Where applicable a Tukey post hoc test was used to determine specific differences for comparisons involving diet and region. The data are presented as means \pm standard error and the relevant sample sizes are provided alongside.

3. Results

3.1. Phylogenetically informed relationship between metabolism, body mass, body temperature and thermoneutrality

Basal metabolic rate scaled positively with M_b (Fig. 2; $t = 18.32$, $p < 0.001$, $df = 62$). There was also a significant and positive relationship between normothermic body temperature and BMR (Fig. 3; $t = 3.31$, $p = 0.002$, $df = 62$). Absolute T_{LC} scaled negatively with BMR (Fig. 4a; $t = -2.59$, $p = 0.012$, $df = 62$) and M_b (Fig. 4b; $t = -2.46$, $p = 0.017$), and

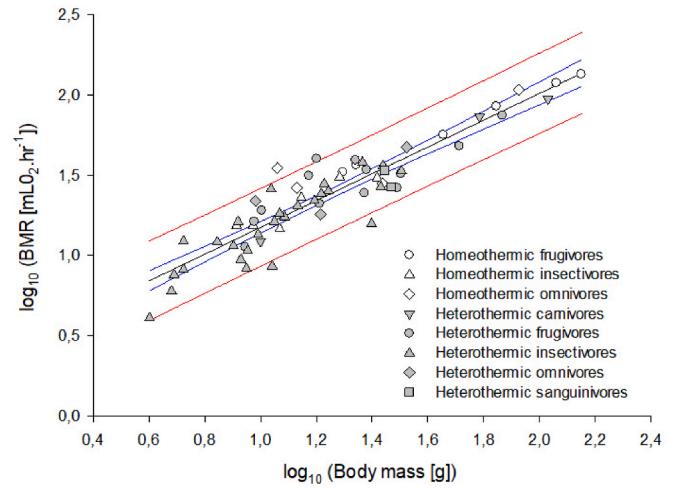


Fig. 2. Basal metabolic rate relative to body mass in bats. *Grey filled* symbols represent heterothermic species, *open* symbols represent homeothermic species, *hexagons* represent carnivorous species, *circles* represent frugivorous species, *triangles* represent insectivorous species, *diamonds* represent omnivorous species and *squares* represent sanguinivorous species. The blue and red lines are the 95% upper and lower confidence intervals and the prediction intervals, respectively, determined using ordinary least squares. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

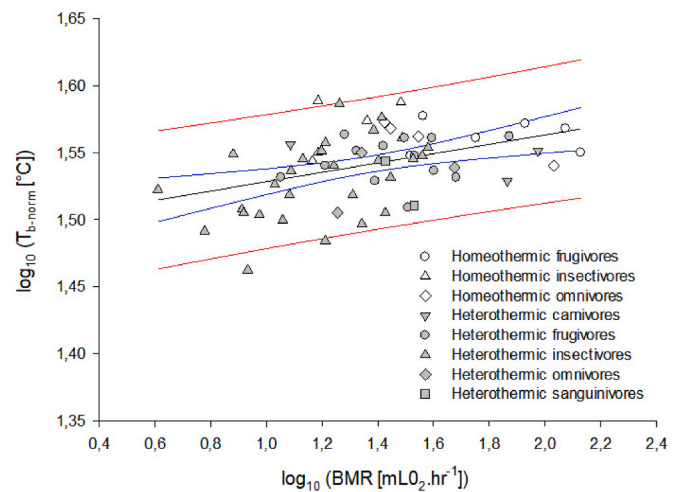


Fig. 3. Normothermic body temperature relative to basal metabolic rate in bats. *Grey filled* symbols represent heterothermic species, *open* symbols represent homeothermic species, *hexagons* represent carnivorous species, *circles* represent frugivorous species, *triangles* represent insectivorous species, *diamonds* represent omnivorous species and *squares* represent sanguinivorous species. The blue and red lines are the 95% upper and lower confidence intervals and the prediction intervals, respectively, determined using ordinary least squares. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

positively with normothermic body temperature (Fig. 5a; $t = 2.12$, $p = 0.038$, $df = 62$). Absolute T_{UC} scaled positively with normothermic body temperature (Fig. 5b; $t = 2.13$, $p = 0.038$, $df = 62$) but did not scale with M_b or BMR. In contrast, the breadth of TNZ in bats was unrelated to BMR, M_b and body temperature.

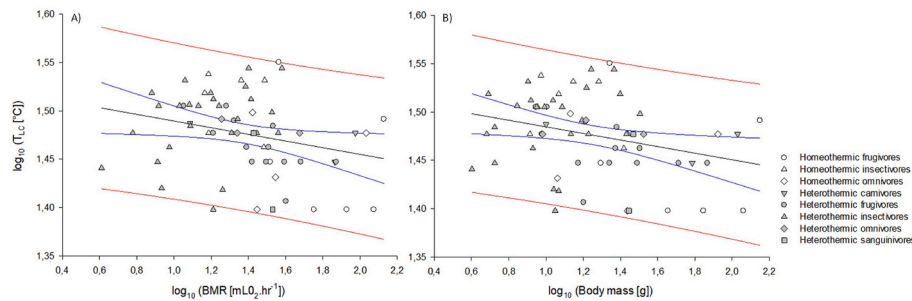


Fig. 4. Lower critical limit of thermoneutrality relative to a) basal metabolic rate and b) body mass in bats. *Grey filled* symbols represent heterothermic species, *open* symbols represent homeothermic species, *hexagons* represent carnivorous species, *circles* represent frugivorous species, *triangles* represent insectivorous species, *diamonds* represent omnivorous species and *squares* represent sanguivorous species. The blue and red lines are the 95% upper and lower confidence intervals and the prediction intervals, respectively determined using ordinary least squares. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

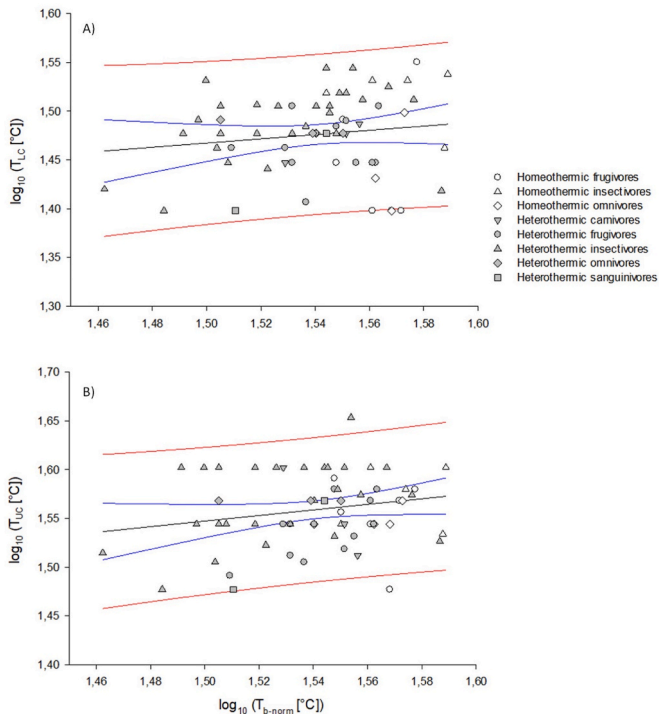


Fig. 5. a) Lower and b) upper critical limits of thermoneutrality relative body temperature in bats. *Grey filled* symbols represent heterothermic species, *open* symbols represent homeothermic species, *hexagons* represent carnivorous species, *circles* represent frugivorous species, *triangles* represent insectivorous species, *diamonds* represent omnivorous species and *squares* represent sanguivorous species. The blue and red lines are the 95% upper and lower confidence intervals and the prediction intervals determined using ordinary least squares, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.2. Interspecific comparison of metabolism, body mass, body temperature and thermoneutrality

The phylogenetically informed comparison of M_b showed that homeothermic species ($M_b = 39.91 \pm 9.93$ g, $n = 16$) were significantly heavier than torpor-using species ($M_b = 20.49 \pm 2.73$ g, $n = 48$) ($t = -2.65$, $p = 0.010$, $df = 62$), whereas the analysis failed to detect an effect of region or diet on M_b . Accounting for M_b , region had no effect on BMR but did influence normothermic body temperature ($F_{5,57} = 4.36$, $p = 0.002$). The post-hoc test showed that normothermic body temperature was significantly higher in Afrotropical species ($T_b = 37.5 \pm 0.7$ °C, $n =$

4) compared to both Neotropical species ($T_b = 34.6 \pm 0.4$ °C, $n = 33$) and Indomalayan species ($T_b = 32.7 \pm 0.6$ °C, $n = 3$), but there were no other differences (Australasia species: $T_b = 35.0 \pm 0.5$ °C, $n = 12$; Nearctic species: $T_b = 35.1 \pm 0.5$ °C, $n = 7$; Palearctic species: $T_b = 34.9 \pm 0.5$ °C, $n = 5$). Diet had no effect on BMR or normothermic body temperature. Similarly, accounting for M_b , there was no difference in BMR between torpor-using species (1.38 ± 0.06 mL O_2 ·g $^{-1}$ ·hr $^{-1}$, $n = 48$) and homeothermic (1.54 ± 0.12 mL O_2 ·g $^{-1}$ ·hr $^{-1}$, $n = 16$) species, but torpor-using species were found to have significantly lower normothermic body temperatures ($F_{1,61} = 16.00$, $p < 0.001$). Mean body temperature for torpor-using species was 34.3 ± 0.3 °C while in homeothermic species it was 36.7 ± 0.3 °C.

Mean absolute T_{UC} did not differ between torpor-using species (36.3 ± 0.5 °C, $n = 48$) and homeothermic species (36.5 ± 0.6 °C, $n = 16$), but there was a significant difference when comparing T_{UC} relative to normothermic body temperature ($F_{1,61} = 4.40$, $p = 0.040$). Whereas T_{UC} in homeothermic bats was, on average, lower than normothermic body temperature ($T_{UC} - T_b$: 0.16 ± 0.71 °C, $n = 16$), in torpor-using bats T_{UC} was higher than normothermic body temperature ($T_{UC} - T_b$: 2.05 ± 0.48 °C, $n = 48$). The body temperature of bats observed at T_{UC} was significantly lower in torpor-using species (37.6 ± 0.4 °C, $n = 48$ vs 39.1 ± 0.3 °C, $n = 16$) ($F_{1,61} = 4.39$, $p = 0.040$). However, both groups showed a similar increase in body temperature from T_{LC} to T_{UC} ($\sim 3.1 \pm 0.3$ °C).

Further exploration of the data showed that apart from the 19 species considered as “special inclusions” to the dataset, there were 23 other species (19 torpor-users and four homeotherms) for which information about T_{UC} was considered reliable that also displayed $T_{UC} \geq$ normothermic body temperature. Combined, this list comprised 34 (of 48) torpor-using species and eight (of 16) homeothermic species (Figs. 1 and 6). Mean absolute T_{UC} did not differ between torpor-using species (37.7 ± 0.5 °C, $n = 34$) and homeothermic species (38.3 ± 0.7 °C, $n = 8$) in this subset of species. There was also no difference between the temperature differential between normothermic body temperature and T_{UC} of bats in this subset (torpor-using species = -3.6 ± 0.4 °C vs homeothermic species = -1.9 ± 0.7 °C). Similarly, the body temperature observed at T_{UC} in this subset of bats did not differ (torpor-using species = 38.3 ± 0.4 °C vs homeothermic species = 39.1 ± 0.7 °C). All diets were represented within these 42 species, with one sanguivore, one carnivore, four omnivores, 10 frugivores and 26 insectivores. Similarly, all regions were represented with one Afrotropical species, one Indomalayan species, five Australasian species, five Palearctic species, six Nearctic species and 24 Neotropical species.

Diet had a significant influence on mean absolute T_{UC} ($F_{4,58} = 3.00$, $p = 0.024$) but not when comparing T_{UC} relative to normothermic body temperature. The post hoc test showed that mean absolute T_{UC} differed between insectivores (37.4 ± 0.6 °C, $n = 33$) and sanguivores (33.5 ± 2.5 °C, $n = 2$), but no other differences were detected (carnivores: 35.8

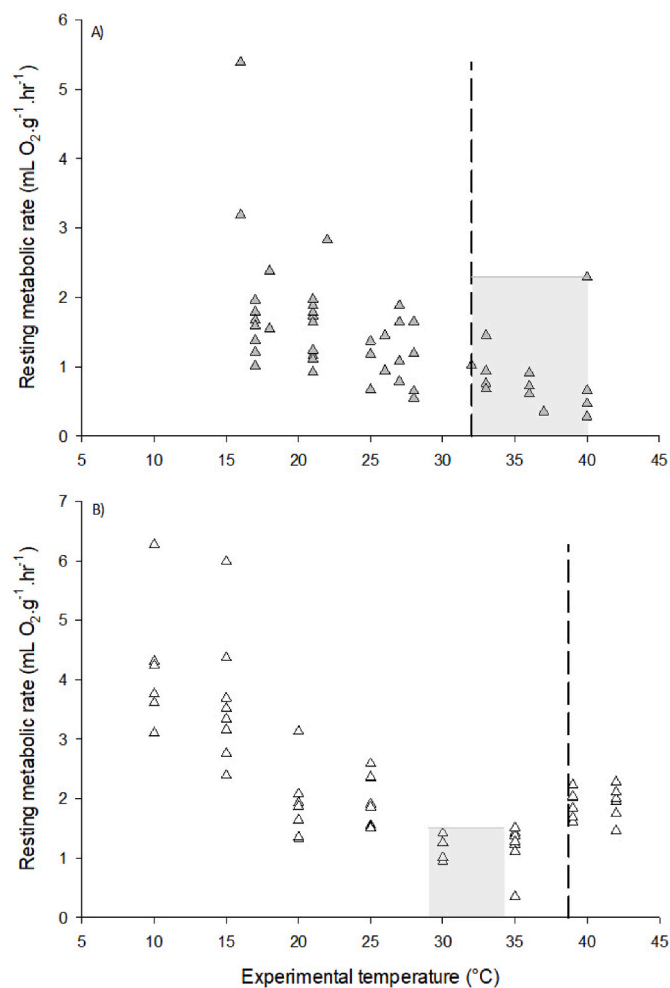


Fig. 6. Thermal profile illustrating resting metabolic rate in a) a heterothermic and insectivorous 9-g bat *Phyllostomus blainvillii* and b) a homeothermic and insectivorous 26-g bat *Taphozous mauritanicus*. Data for *P. blainvillii* were extracted from Rodríguez-Durán (1995), whereas raw data for *T. mauritanicus* were supplied by Toussaint and McKechnie (2012). The symbols used are consistent with those in other figures. Black vertical lines represent the species normothermic body temperature and the grey shade represents the reported range of thermoneutral temperatures.

± 1.8 °C, $n = 3$; frugivores: 35.1 ± 0.6 °C, $n = 19$; omnivores: 36.1 ± 0.4 , $n = 7$). Diet did not influence the body temperature of bats at T_{UC} . Region had no effect on absolute T_{UC} , but there was a significant effect when considering T_{UC} relative to normothermic body temperature ($F_{5,57} = 4.09$, $p = 0.003$). On average, T_{UC} was lower than normothermic body temperature in Afrotropical species ($T_{UC} - T_b$: -1.8 ± 3.7 °C, $n = 4$) and Indomalayan species ($T_{UC} - T_b$: -0.9 ± 0.5 °C, $n = 3$) but higher than normothermic body temperature in Australasian species ($T_{UC} - T_b$: 1.1 ± 1.1 °C $n = 12$), Nearctic species ($T_{UC} - T_b$: 2.6 ± 1.1 °C $n = 7$), Neotropical species ($T_{UC} - T_b$: 1.8 ± 0.5 °C $n = 33$) and Palearctic species ($T_{UC} - T_b$: 2.9 ± 0.8 °C $n = 5$). The post hoc test showed that Afrotropical species differed from Australasian species, Nearctic species, Neotropical species and Palearctic species, but no other differences were detected. Region had no effect on the body temperature observed at T_{UC} .

Mean absolute T_{LC} did not differ between torpor-using species (30.1 ± 0.3 °C, $n = 48$) and homeothermic species (29.8 ± 0.9 °C, $n = 16$). There was also no difference when comparing T_{LC} relative to normothermic body temperature ($T_b - T_{LC}$: torpor-using species = 4.1 ± 0.4 °C, $n = 48$ vs homeothermic species = 6.8 ± 0.9 °C, $n = 16$). Diet had no influence on T_{LC} or TNZ breadth irrespective of whether normothermic

body temperature was considered or not. Biogeographical region had a significant influence on absolute T_{LC} ($F_{5,57} = 3.16$, $p = 0.014$) but not T_{LC} relative to body temperature. The post hoc test showed that mean absolute T_{LC} differed between Neotropical species (29.4 ± 0.5 °C, $n = 33$) and species from the Palearctic (32.1 ± 0.4 °C, $n = 5$) and Nearctic (32.1 ± 0.7 °C, $n = 7$) respectively. No other differences were detected (Afrotropical species: $T_{LC} = 28.8 \pm 2.2$ °C, $n = 4$; Australasian species: $T_{LC} = 30.7 \pm 0.7$ °C, $n = 12$; Indomalayan species: $T_{LC} = 28.7 \pm 0.3$ °C, $n = 3$).

There was no difference in TNZ breadth between torpor-using species and homeothermic species irrespective of whether BMR or body temperature was included as a covariate in the model or not. However, TNZ breadth differed significantly between biogeographical regions ($F_{5,57} = 3.97$, $p = 0.004$). The post hoc test showed that TNZ breadth was significantly broader in Neotropical species (7.1 ± 0.4 °C, $n = 33$) compared to Indomalayan species (3.2 ± 0.7 °C, $n = 3$), but no other differences were detected (Afrotropical species: TNZ breadth = 6.9 ± 1.2 °C, $n = 4$; Australasian species: TNZ breadth = 5.4 ± 0.6 °C, $n = 12$; Nearctic species: TNZ breadth = 5.6 ± 0.8 °C, $n = 7$; Palearctic species: TNZ breadth = 5.7 ± 0.9 °C, $n = 5$).

4. Discussion

Given the argument that torpor improves thermal tolerance in bats (Reher et al., 2018; Reher and Dausmann 2021), if T_{UC} was indicative of thermal tolerance, it is reasonable to expect that T_{UC} would differ between bats capable of torpor and bats incapable of torpor. Although lower normothermic body temperatures in torpor-using species resulted in them having a larger temperature differential between body temperature and T_{UC} , our results did not support the hypothesis that bat species which are known to use torpor exhibit higher T_{UC} s compared to homeothermic species. The results do, however, show that the metabolic response of bats at high T_a is flexible. Using a few key species as examples, below we make clear that the association between T_{UC} and thermal tolerance in mammals is a misconception. Furthermore, we also challenge the argument that T_{UC} may be an evolutionarily constrained variable (Araújo et al., 2013).

On average, bats showed an increase of ≈ 3 °C in body temperature from the start to the end of TNZ irrespective of grouping. If we consider that the standard deviation (SD) for normothermic body temperature calculated across all bats in our dataset is ± 2.1 °C, then based on the IUPS Thermal Commission (2003)'s criteria of normothermy as normothermic body temperature ± 1 SD, the results show wide-spread use of low-to-moderate heterothermy in bats at ambient temperatures typically considered to be thermoneutral. Furthermore, 42 (of 64) species of bats achieved BMR-level metabolism even at T_a exceeding normothermic body temperature, despite increases in body temperature of up to ≈ 8 °C (a high level of heterothermy) in some species. By contrast, a few bats maintained metabolic rate at basal levels with little change in body temperature even at T_a s of 39–40 °C. This high degree of physiological flexibility is best showcased when we contrast torpor-using species such as the Antillean ghost-faced bat (*Phyllostomus blainvillii*, normothermic $T_b = 32.0$ °C, $M_b = 8.9$ g; Rodríguez-Durán 1995) and the sooty mustached bat (*Pteronotus quadridens*, normothermic $T_b = 31.2$ °C, $M_b = 4.8$ g; Rodríguez-Durán 1995) to the lesser long-eared bat (*Nyctophilus geoffroyi*, normothermic $T_b = 31.6$ °C, $M_b = 8.0$ g; Hosken and Withers 1999). In all three species T_{UC} was reported as 40 °C but *N. geoffroyi* showed an increase in body temperature of ≈ 7.6 °C within TNZ, whereas the other two species showed an increase in body temperature of ≈ 1 –3 °C. High physiological flexibility was also observed in a few species that were classed as homeotherms (based on the current lack of evidence of torpor use). This is best showcased by the brown fruit-eating bat (*Artibeus concolor*, normothermic $T_b = 35.3$ °C, $M_b = 19.7$ g; McNab 1969), Parnell's mustached bat (*Pteronotus parnellii*, normothermic $T_b = 36.4$ °C, $M_b = 19.2$ g; Bonaccorso et al., 1992) and Davy's naked backed Bat (*Pteronotus davyi*, normothermic $T_b = 38.8$ °C,

$M_b = 9.4g$; Bonaccorso et al., 1992). All three of these species showed heterothermy at high T_a as they maintained BMR-level metabolism at $T_{a,s}$ of 39–40 °C and showed increases in body temperature of ≈ 2.7 –4.6 °C within their TNZ. Despite any potential thermoregulatory benefit that a higher normothermic body temperature may have afforded these species (*sensu* Levesque et al., 2018), heat stress would surely have become severe as their body temperatures approached 39 °C. However, corresponding information about evaporative cooling effort is needed to confirm that this response was adaptive and to provide insight to the benefit thereof for survival.

It is interesting that in bats lethal body temperature and T_{UC} both seem to vary with diet but, even so, our results do not support T_{UC} as a reliable measure of thermal tolerance. We found no difference in T_{UC} between frugivores and insectivores even though these groups appear to have vastly different lethal body temperatures. For example, lethal body temperatures in fruit bats are reported to be ≈ 37 °C, whereas in insectivorous bats it is ≈ 45 °C (McKechnie and Wolf 2019). In addition, variation in lethal body temperature exists within dietary guilds. For example, McNab (1969) reported lethal experiments involving several species of bats including five frugivores, two omnivores and one carnivore. The experimental temperatures at which bats succumbed varied greatly within each dietary guild ranging in frugivores from $T_{a,s}$ of ≈ 37 °C in Seba's short-tailed bats (*Corollia perspicillata*) to ≈ 42 °C in Jamaican fruit bats (*Artibeus jamaicensis*), and in omnivores from $T_{a,s}$ of ≈ 35 °C in tailed tailless bats (*Anoura caudifer*) to ≈ 42 °C in greater spear-nosed bats (*Phyllostomus hastatus*). The carnivorous greater bulldog bat (*Noctilio leporinus*) succumbed at $T_a \approx 42$ °C. While the exact duration of exposure until death is uncertain, collectively, these observations reinforce the argument against T_{UC} as an indicator of thermal tolerance. In addition, the variability of T_{UC} with diet contradicts the argument that T_{UC} may be an evolutionarily constrained variable (Araújo et al., 2013).

Avoiding lethal hyperthermia is intrinsically linked to dehydration tolerance, which is dependent on M_b , to the duration of exposure and the intensity of heat (Tattersall et al., 2012). Although bat-specific dehydration tolerance limits are likely to vary between many species, earlier work by Studier et al. (1970) showed that dehydration limits in *Myotis* ranged from $\approx 23\%$ to 33% loss of body weight. This range is higher than the common textbook threshold of dehydration tolerance in mammals reported as 15% loss of body weight (Hill et al., 2012; Sherwood et al., 2012) (but see also Adolph 1947). Given the small body size of many bats, which limits the total volume of body water available to use for evaporative cooling, one would be hard-pressed to conclude that the aforementioned bats with high T_{UC} s have a high thermal tolerance without also having information about their risk of dehydration due to heat stress (Czenze et al., 2020). Thus, for example, data for the previously mentioned insectivore *N. geoffroyi* (Hosken and Withers 1999) extracted using PlotDigitizer 2.6.8 shows a dramatic increase in evaporative water loss associated with cooling effort from ≈ 3.7 mg $H_2O \cdot g^{-1} \cdot hr^{-1}$ at $T_{LC} = 35$ °C to ≈ 10.6 mg $H_2O \cdot g^{-1} \cdot hr^{-1}$ at 40 °C, which is approximately a 3-fold increase. Assuming a linear response, it seems as though this 8g bat would lose approximately 25% of its weight (i.e. 2g of body water) through evaporation per 24hrs under continuous exposure to 40 °C. By comparison, we may consider another insectivore the homeothermic 26g Mauritian tomb bat (*Taphozous mauritanus*) for which evaporative water loss was also measured at 40 °C (Toussaint and McKechnie 2012). In this species, TNZ ranges from 29 to 35 °C, with evaporative water loss ≈ 1.5 mg $H_2O \cdot g^{-1} \cdot hr^{-1}$ at T_{LC} increasing to ≈ 8.1 mg $H_2O \cdot g^{-1} \cdot hr^{-1}$ at a T_a of 40 °C (5-fold increase). As before, assuming a linear response to a continuous exposure to 40 °C this bat would lose approximately 19% of its weight per 24hrs. Based on these estimations, the higher T_{UC} in *N. geoffroyi* does not support the argument of higher thermal tolerance as this species would reach the typical mammalian lethal dehydration threshold *ca.* 4hrs sooner than *T. mauritanus*, and this time difference increases to *ca.* 6.5hrs if basing it on a 23% threshold.

As expected, M_b had a positive scaling effect on BMR, but contrary to

broad scale mammalian patterns (Lovegrove 2000; McNab 2008) BMR of bats in our study did not vary between biogeographical regions. Further, while diet is known to influence BMR in mammals in general (McNab 1992, 2008), the results support a previous observation that once phylogeny is considered the effect of diet on BMR in bats is negated (Cruz-Neto et al., 2001). However, the results conformed to the expectation that a higher body temperature necessitates a higher BMR (Clarke and Pörtner 2010), and the results showed that a higher body temperature was associated with a higher T_{UC} in bats. However, while we observed variations in normothermic body temperature between regions, this was not the case for BMR or T_{UC} . Whereas Indomalayan bats had the lowest body temperatures, Afrotropical bats had the highest. Similarly, we found that torpor-using species had lower body temperatures but not lower BMR or T_{UC} . This speaks to the complicated nature of the relationship between BMR and body temperature, showing that while they may be interdependent, M_b is a dominant factor influencing BMR but not body temperature (Clarke et al., 2010; Clarke and O'Connor 2014) or T_{UC} .

The final argument that we present against the use of T_{UC} as an indicator of thermal tolerance is related to the assigning of T_{UC} itself. We found that the reported T_{UC} for 20 of the 64 species included in our study was simply the highest experimental temperature measured in the original study, without any obvious change in the metabolic rate of bats. As discussed earlier, this may or may not be associated with large increases in body temperature. Nevertheless, this creates an artificial T_{UC} value. Without any further consideration of the physiology of heat stress, on its own, artificial T_{UC} s would undermine the use of T_{UC} as an indicator of thermal tolerance. Alternatively, by disregarding those species one may likely be disregarding those with high thermal tolerance. Consequently, this renders useless any attempt at determining estimates of an average T_{UC} or comparisons of TNZ breadth without a careful scrutiny of the dataset (Wolf et al., 2017). It is unfortunate that only a few thermoregulatory studies of bats include measurements of evaporative water loss (Czenze et al., 2020; Muñoz-García et al., 2022). The inclusion of evaporative water loss measurements in thermal studies is pertinent for understanding patterns of thermoregulation, needed to more accurately determine the upper limit of TNZ and the onset of heat stress. Available reports show a high variability among bat species regarding the onset of evaporative cooling effort. For example, evaporative cooling begins in some bats at T_a that, if thermoneutrality was based purely on metabolism, would typically be considered to be within the TNZ (Maloney et al., 1999; Baudinette et al., 2000; Cryan and Wolf 2003), in other bats evaporative cooling begins at T_a well below TNZ (Hosken and Withers 1997; Marom et al., 2006) or coincident with the start of TNZ (Hosken and Withers 1999; Cooper and Withers 2012). Thus, we stress the point that thermal tolerance based on metabolism alone is inadequate as it disregards the impact of hydration on survival.

We also acknowledge the need for accurate measures of body temperature. Techniques used to measure body temperature in bats, as with many other study models, varied widely between bats in our dataset, caused by advances in technology and equipment becoming more accessible. For example, body temperatures were based on measures of rectal temperature using probes (Bonaccorso et al., 1992), subcutaneous (Toussaint and McKechnie 2012) and core temperature (Welman et al., 2017) using temperature sensitive passive integrated transponder (PIT) tags, and core temperature using surgically implanted iButtons (Downs et al., 2012), to mention a few. Thus, we urge future studies to include concurrent measures of evaporative water loss alongside measures of metabolism and core body temperature to allow a more holistic assessment of the risks associated with higher environmental temperatures in future. Lastly, while the focus of the present study was a comparison between species capable of torpor and homeotherms, the inclusion of diet as a factor revealed an interesting avenue for further exploration of why diet influences lethal body temperatures and T_{UC} in bats, and whether this holds true for other mammals as well. While the results seem to support the idea that tropical species are more vulnerable

to heat stress (Lovegrove et al., 2014), appropriate studies of thermal tolerance are urgently required to develop a more realistic metric to assess the risk of lethality due to warmer temperatures and extreme heat events in future.

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CRedit authorship contribution statement

Shaun Welman: Writing – review & editing, Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Ana M. Breit:** Writing – review & editing. **Danielle L. Levesque:** Writing – review & editing, Conceptualization. **Julia Nowack:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The dataset used in the study is provided as supplementary data to this article and can be found online at <https://doi.org/10.1016/j.jtherbio.2024.103933>.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jtherbio.2024.103933>.

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