

Torpor energetics are related to the interaction between body mass and climate in bats of the family Vespertilionidae

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Keywords: Body mass, climate, energy-saving strategies, mammals, torpor, Vespertilionidae.

Summary statement

The interaction between body mass and climate influences torpor energetics in bats of the family Vespertilionidae. As a result, torpid traits change based on body mass and climate.

Abstract

Torpor is an adaptive strategy that allows animals to cope with energy limitations under adverse environmental conditions. In birds and mammals, intrinsic and extrinsic factors such as body mass (M_b) and ambient temperature (T_a) are well established triggers of torpor. Interestingly, the interplay between M_b and climate with different T_a on torpor traits in bats remains unexplored. Using open flow respirometry, we calculated T_a upon entering torpor (T_{at}), the reduction in torpid metabolic rate relative to the basal metabolic rate (TMR_{red}), the T_a at which torpor metabolic rate reached its minimum ($T_{a\,adjust}$), and minimum torpid metabolic rate (TMR_{min}) in 11 bat species of the family Vespertilionidae that differ in M_b from warm and cold climates. We also included TMR_{min} data retrieved through a bibliography review. We tested the effects of M_b and climate on torpor traits using mixed-effect phylogenetic models. All models showed a significant interaction

between M_b and climate. This interaction was inversely related to T_{at} , TMR_{red} , $T_{a\ adjust}$, and positively related to TMR_{min} . These results are likely explained by the differences in M_b and the metabolic rate of bats from different climates, which may allow individuals to express torpor in places with different T_a . Further studies to assess torpor use in bats of different climates are proposed.

Introduction

Torpor is an adaptive strategy that enables animals to cope with energy limitations under adverse environmental conditions (Geiser, 2004; Geiser and Baudinette, 1988; Geiser and Brigham, 2012; Heldmaier et al., 2004; Körtner and Geiser, 2000). This physiological response is characterized by a controlled and reversible depression of animals' metabolic rate, accompanied by a decrease in their body temperature (T_b) (Fig. 1) (Geiser, 1988; Geiser, 2004; Geiser and Ruf, 1995). By using torpor, individuals can reduce their energy expenditures by 50–90 % while passively rewarming to return to normothermia (i.e., when utilizing external heat sources such as basking in the sun) (Geiser, 2008; Warnecke et al., 2008; Körtner and Geiser, 2009; Pretzlaff et al., 2010). It is well documented that intrinsic factors such as body mass (M_b), as well as extrinsic factors such as ambient temperature (T_a) and food availability are the primary selection pressures that trigger torpor in birds and mammals (McNab, 1989; Geiser and Brigham, 2000; Willis et al., 2005; Machado and Soriano, 2007; Wojciechowski et al., 2007; Doucette et al., 2012). Nevertheless, torpor can also facilitate energy balance during high energy-demand periods such as migration (Carpenter and Hixon, 1988; McGuire et al., 2014), development (Geiser, 2008), pregnancy (Willis et al., 2006), lactation (Chruszcz and Barclay, 2002), environmental disasters (Stawski et al., 2015) or situations involving high predation risk (Stawski and Geiser, 2010).

Torpor can be measured under controlled conditions (Geiser, 2021). Laboratory experiments have shown that in post-absorptive euthermic adult non-reproductive animals, a typical response to a decrease in T_a below the lower critical temperature (T_{LC}) is to increase resting metabolic rate to prevent hypothermia (Young et al., 1989). However, when environmental conditions are severe, animals may use torpor, thus reducing their metabolism below the basal metabolic rate (BMR) (Song et al., 1995; Geiser, 2004). Basal

metabolic rate represents the minimal metabolic rate to sustain thermoregulation during normothermia and is limited by the T_{LC} and the upper critical temperature (T_{UC}) (representing the T_a where animals begin expending energy to avoid hyperthermia) (Fig. 1) (Terrien et al., 2011). Speakman and Thomas (2003) proposed that the relationship between the metabolic rate and T_a can be similar during torpor and normothermia. For instance, when individuals experience T_a below their T_{LC} , they seem to abandon normothermia by regulating T_b at a very low rate, as if they were thermoconforming. In this scenario, the first inflection point of the torpor metabolic rate that falls below the BMR indicates the T_a at which animals enter torpor (T_{at}). As T_a continues to drop, the metabolic rate reaches a second inflection point ($T_{a\text{ adjust}}$), where torpid metabolic rate approaches its minimum values (TMR_{min}). Speakman and Thomas (2003) found a positive relationship between residual TMR_{min} (the metabolic rate that was not explained by T_b) and body mass (M_b) in 18 species from five families of bats. The authors also demonstrated that the minimum T_b of animals during torpor (T_{bmin}) was higher in species inhabiting warmer climates compared to colder ones. Interestingly, T_{bmin} has also been positively related to M_b in other species of birds and mammals (Ruf and Geiser, 2015; Geiser, 2021). Given that T_b is closely related to the metabolic rate of animals, this strongly suggests that TMR_{min} and other torpor traits, such as those described above, might vary among bats with different M_b inhabiting warm and cold climates. Nevertheless, to the best of our knowledge, no empirical attempts have been made to assess the effect of the relationship between M_b and climate on torpor traits in any mammal. This is surprising, as torpor energetics significantly influence many other aspects of species' biology, ecology, and geographical distribution (Law, 1994).

Bats are an especially well-suited group in which to explore the effects of M_b and climate on torpor energetics. They exhibit an exceptionally wide range of M_b among species—from the ~1.5 g bumblebee bat (*Craseonycteris thonglongyai*) to the ~1,500 g giant golden-crowned flying fox (*Acerodon jubatus*) (Stawski et al., 2014). Furthermore, bats are the mammal group with the highest proportion of species that employ torpor as an energy-saving strategy across many environmental conditions (Stawski et al., 2014; Czenze and Dunbar, 2020; Geiser, 2021;). Within this group, bats from the family Vespertilionidae: i) differ in M_b by approximately one order of magnitude (Moratelli et al., 2019), ii) have distributions spanning both cold and warm environments (Nowak and Walker, 1994), and

iii) most species, regularly use torpor in a variety of conditions (Audet and Fenton, 1988; McWilliam, 1988; Geiser and Brigham, 2000; Genoud and Christe, 2011). In vespertilionid bats, torpor traits could be influenced by M_b , climate, and/or the interaction among these explanatory variables. To test this hypothesis, we evaluated the relationship among torpor traits, M_b , and climate in bats of the family Vespertilionidae that differ in M_b from warm and cold climates using comparative phylogenetic models. We predicted a positive relationship between M_b and torpor traits (T_{at} , $T_{a\text{ adjust}}$ and TMR_{min}). Nevertheless, bats from the warmer sites should present higher values of torpor traits compared to bats from the colder ones.

Material and methods

Study area

Bats were captured in four locations with either cold or warm climates in central Mexico (Fig. 1): La Malinche National Park, in Tlaxcala state, and Santa Cruz Moxolahuac, in Puebla state (colder sites); and Sierra de Huautla Biosphere Reserve, in Morelos state, and Autlán, in Jalisco state (warmer sites). Climate classification followed Köppen's categorization, considering both the T_a and precipitation of the capture sites. In this classification, warm climates exhibit a mean annual T_a between ~ 24 to 40 °C, with the minimum annual T_a above 10 °C, and annual precipitation ranging between 600- and 2,000-mm. Cold climates have an average annual T_a between ~ 10 and 22 °C, an annual minimum T_a that tends to fall below ~ 0 °C, and annual precipitation that ranges between ~ 400 and 1,400 mm (Beck et al., 2005; Oliver, 2008) (Table 1).

Bat care and housing

Bats were captured between September and February 2021 and 2022 using mist nets (2 x 3 m, 2 x 6 m and 2 x 9 m) deployed for two consecutive nights each month at each locality during the new moon period near water bodies used by bats to drink and feed. Nets were opened at dusk and closed at ~ 01:00 am. Adult non-reproductive males of 11 bat species of the family Vespertilionidae that vary in M_b , were selected (Table 2). Some species were caught in limited numbers due to their low population size at the study localities (LaVal, 2004; Perry and Carter, 2010). Species identification followed Medellín et al. (2008). Taxonomic names were assigned following Ramírez-Pulido et al. (2014). We recorded the M_b of each bat to the nearest 0.2 g using an electronic balance (Ohaus, Newark, NJ, USA).

Age was determined by observing the epiphyseal space of the fourth metacarpal bone of the third and fifth fingers, since juvenile individuals have some visible space. Reproductive condition of males was determined by observing the testes, which increase in size during spermatogenesis (Wilkinson and Brunet-Rossinni, 2009). Bats were captured under permission of the Wildlife Department granted to our institution (SEMARNAT: SGPA/DGVS/06795/21) and the ethics committee of the University of Tlaxcala.

Once captured, bats were placed in cloth bags and transferred to captive conditions near the study sites where they were placed in individual flight cages (75 cm x 75 cm x 75 cm) with 12/12 light/dark cycles, a T_a of ~28 °C (near the *TNZ* of bats), and a relative humidity above 50 %.

Determination of thermal energetics in euthermic bats

Torpor is defined as a reduction of metabolic rate below *BMR* (Geiser and Ruf, 1995; Geiser, 2004), and *BMR* is measured between the T_{LC} and T_{UC} displayed by individuals (Withers et al., 2016). So, we initially measured *BMR*, T_{LC} , and T_{UC} of bats during normothermia by quantifying their resting metabolic rate (in mL O₂ h⁻¹) over a T_a range of 8 to 43 °C. Measurements were taken ~ 12 hours post-capture in a postabsorptive state during the resting phase (i.e., from ~ 10:00 to 19:30 hours) (Genoud et al., 2018). Thermal traits were measured by indirect calorimetry, estimating O₂ consumption and CO₂ production using an open-flow respirometer (FoxBox®; Sable Systems International, Las Vegas). Measurements were done following Medina-Bello et al. (2023). To do this, each bat was placed in a 410 mL metabolic chamber inside a temperature-controlled cabinet (PELT5®; Sable Systems International, Las Vegas, USA). The chamber had plastic mesh on the walls and the ceiling to allow bats to hang upside down as in natural conditions, but it was too small for them to fly. This allowed us to minimize variation in the measurements due to the individuals' movements. The temperature inside the cabinet was controlled to within ± 0.5 °C. Measurements were recorded when the readings reached an asymptote after the T_a stabilized at ± 0.5 °C of each experimental T_a . Flow rates (FR) of dry CO₂-free air scrubbed with Drierite (calcium sulfate for humidity) and Ascarite (sodium hydroxide for CO₂) passed upstream with the air pushed through the respirometry chamber at a rate between 180- and 350-mL min⁻¹ depending on the bats' metabolic rate. Flow rates were estimated following Lighton and Halsey (2011) using the formula:

$$FR = \dot{V}O_2 / \Delta O_2$$

Where $\dot{V}O_2$ corresponds to the predicted oxygen consumption of the experimental animal and ΔO_2 is the difference in fractional concentration between the incurrent and excurrent O_2 . $\dot{V}O_2$ was estimated assuming that bats' metabolic rates increase with M_b with a slope of 0.744 ($\log_e BMR \text{ (mL } O_2 \text{ h}^{-1}) = 1.0895 + 0.744 \log_e M_b \text{ (g)}$) (Speakman and Thomas 2003). ΔO_2 was taken from our FoxBox analyzer (0.0005 of ΔO_2 expressed as fractional concentration). Excurrent air was dried with Drierite, and fractional concentrations (%/100) of both oxygen ($F_e O_2$) and CO_2 ($F_e CO_2$) were measured every second. We placed an empty hermetic chamber of the same size (410 mL) inside the T_a cabinet to obtain reference measurements, since we did not have a system that allowed us to shift the airflow between the empty chamber and the animal chamber with the same respirometer. The empty chamber was connected to a second respirometer of the same brand (FoxBox®, Sable Systems International, Las Vegas, Nevada, USA) with the same type of sensors (O_2 fuel cell and CO_2 infrared sensors), and the same flow rate as that of the experimental chamber. Both respirometers were calibrated simultaneously and showed the same pattern of oxygen readings. Data from respirometers, including O_2 and CO_2 readings and data from the flowmeter and thermocouple, were sent to computers through Sable Systems-UI2 ports running Expedata software.

We placed each bat inside the metabolic chamber for 60 min at a T_a of 28 °C before taking any measurement to allow each bat to recover from the stress of handling. The first T_a tested was 28 °C, which we have previously determined falls within the *TNZ* of all of the bat species studied here (Medina-Bello et al., unpublished data). We measured the metabolic rate of individuals for 30 min at 28 °C, then decreased the T_a to 8 °C, taking measurements for 5 min for each drop in 1 °C and 30 min for each 5 °C drop (i.e., at 23, 18, 13, and 8 °C). Subsequently, we increased the chamber's T_a to 28 °C for 30 min before raising it to 43 °C. Measurements were recorded for 5 min for each 1 °C increase and 30 min for each 5 °C increase (i.e., at 33, 38, and 43 °C). It took 5 to 10 min for the T_a to stabilize for each change in T_a within the metabolic chamber. The metabolic measurements we obtained from the animals at each 1 °C increment allowed us to obtain a more precise estimation (1 °C rather than a 5 °C accuracy) of the *BMR*, T_{LC} , and T_{UC} . One individual was measured in the set of T_a 's at each time. We increased the T_a inside the metabolic chamber

and repeated the measurements if the bats reduced their metabolic rate below their *BMR* and intended to use torpor. With this methodology, bats from all the sites increased their resting metabolic rate when the T_a fell below their *TNZ*, indicating that they defended normothermia. At the end of these procedures, bats were provided with mealworms (*Tenebrio molitor*) and water *ad libitum* and kept in captivity for the torpor experiments.

Determination of thermal energetics during torpor

To induce bats to use torpor, we deprived the individuals of food for 36 h before conducting any experiment (Ruf et al., 1993). This period of food deprivation mirrors the short-term food restrictions that bats may encounter in the field (Geiser, 2021). With this methodology, all bats used torpor during our trials. During captivity, bats were kept in the previously described flight cages. After the 36 hrs., we measured the metabolic rate of individuals during their resting phase (from 8:00 to 17:00 hours). We placed each bat in the metabolic chamber for 60 min at a T_a of 28 °C to allow them to recover from handling. As we were particularly interested in identifying the inflection points below the *BMR* that defined torpor traits, the first T_a we tested was the mean T_{UC} we calculated for each species. This allowed us to maintain the bats in their thermoneutral zone before they reached their T_{LC} and used torpor. One individual was measured at a time. We measured the metabolic rate of individual for 75 min, then we decreased the T_a to 8 °C, with measurements taken for 7 min for each 1 °C drop and 75 min for each 5 °C drop. Using this protocol, we were able to measure torpid metabolic rate for five to six hours continuously, which allowed us to obtain precise measurements of torpor traits in our study species (Geiser, 2021). Since individuals exhibited a metabolic rate below the *BMR* during torpor, we adjusted the FR between 110 mL min⁻¹ and 180 mL min⁻¹ to achieve accurate measurements of T_{at} , $T_{a\text{ adjust}}$, and TMR_{min} . In both euthermic and torpor measurements, we obtained the M_b by weighing the individuals at the beginning of the experiments by using the electronic balance previously described. These measurements were used for data analyses. No bats died during the experiments. The individuals were fed, provided with water, and released at their captured sites the night following the conclusion of the experiments.

Data analyses

Metabolic rate

All data analyses were conducted using RStudio software (version 1.2.5042). We measured the metabolic rate of bats through oxygen consumption (VO_2) (in $\text{mL O}_2 \text{ h}^{-1}$) for each bat tested at each T_a . The metabolic rate was calculated using the formula proposed by Lighton (2018):

$$VO_2 = \frac{FR [(FiO_2) - (FeO_2) - FeO_2 * (FeCO_2 - FiCO_2)]}{(1 - FeO_2)}$$

Where FR is the flow rate (in mL min^{-1}), FiO_2 is the fractional concentration of O_2 in the incurrent (baseline) air, FeO_2 is the fractional concentration of O_2 in the excurrent air, $FiCO_2$ is the fractional concentration of CO_2 in the incurrent air, and $FeCO_2$ is the fractional concentration of CO_2 in the excurrent air. To conduct our analyses, we used the mean values of the metabolic rate obtained from the measurements of the experimental T_a 's and the mean values obtained from the animals at each $^{\circ}\text{C}$. We defined BMR (in $\text{mL O}_2 \text{ h}^{-1}$) as the mean metabolic rate of the bats between T_{LC} ($^{\circ}\text{C}$) and T_{UC} ($^{\circ}\text{C}$) (Genoud et al., 2018, Geiser, 2021). Critical temperatures were obtained using two-phase regression (TPR) with the 'chngtm' function ('chngtm' package). TPR identifies thresholds of abrupt changes in the relationship between the dependent and independent variables (Nickerson et al., 1989). In bats, TPR has been used to estimate thermal traits including BMR , T_{LC} , and T_{UC} (see Willis et al., 2005a and 2005b and Machado and Soriano, 2007; among others). In our models, the metabolic rate of the bats was the dependent variable, and T_a the independent one.

To determine the torpor traits of bats, we first fitted a simple linear regression model using the 'lm' function of the 'stats' package. In this model, the response variable was the mean value of the metabolic rate of bats at different T_a below the T_{UC} , and T_a was the explanatory variable. We calculated the inflection points of the regression model by using the 'davies.test' function of the 'segmented' package (Muggeo, 2008). In this calculation, the first inflection point indicated T_{at} of bats, signaling that they went into torpor. The second inflection point indicated $T_{a \text{ adjust}}$. Finally, we obtained the minimum torpid metabolic rate (TMR_{min}) by calculating the mean of the torpid metabolic rate for ~ 2 hrs. below $T_{a \text{ adjust}}$. Because some authors have measured TMR_{min} for certain bat species of the family Vespertilionidae, we conducted a systematic search in Google scholar and the Web of Knowledge to look for those values to include them in our data. The search was done

using the keywords: “torpor Vespertilionidae”, “vespertilionid minimum metabolic rate”, “torpor bats Vespertilionidae”. We found calculations of TMR_{min} for 14 species of bats. However, only data from *Nyctophilus geoffroyi*, and *Chalinolobus gouldii* (Hosken and Whitters, 1999), *Myotis myotis* (Hanus, 1959), *Myotis keaysi*, and *Myotis oxyotus* (Manchado and Soriano, 2007) were useful for our analysis, since those were the only studies in which TMR_{min} was measured in short-term experiments that were comparable to our study design.

Effect of M_b and climate on the torpid metabolic rate of bats

We evaluated the relationship of M_b and climate with 1) BMR , 2) percent reduction of the metabolic rate below BMR (TMR_{red}), and 3) torpor traits of bats. To conduct our analyses, we used phylogenetic mixed-effect models, using the ‘gls’ function of the ‘nlme’ package’. We employed a Brownian correlation structure based on the phylogenetic tree published by Amador et al. (2018) for the order Chiroptera. This correlation accounts for the shared evolutionary history among the bat species (Paradis and Schliep, 2019). We used the ‘drop.tip’ function of the ‘ape’ package to trim the tree to the 590 species of bats of the family Vespertilionidae and two species of the family Molossidae (*Eumops floridanus* and *Eumops glaucinus*) that were used as outgroups. To meet model assumptions of normality, we transformed the BMR , T_{at} and $T_{a\ adjust}$ data using the ‘bestNormalize’ function of the ‘bestNormalize’ package. Given that phylogenetic models have been reported to offer a better fit than conventional linear regressions (Riek and Geiser, 2013), we created simple linear regressions and compared them with our phylogenetic models using the ‘ltest’ function from the ‘ltest’ package. In the models, BMR , TMR_{red} , and torpor energetics (i.e., T_{at} , $T_{a\ adjust}$ and TMR_{min}) were the dependent variables, while M_b and climate (classified as categorical—either cold or warm) were the independent ones. To conduct our analyses, we utilized the M_b , TMR_{red} , T_{at} , $T_{a\ adjust}$ and TMR_{min} measurements we obtained from our experiments. For the TMR_{min} , we also integrated the data we gathered from our bibliographic search. The slopes of the relationships between explanatory and response variables (β_0) along with their respective standard error were extracted from model summaries. As our primary interest was to assess the effect of the combination of M_b and climate on the torpor energetics of bats, we tested the interaction between these explanatory variables. Notably, we found significant interactions between M_b and climate in all our

models. Given that these interactions provided a more comprehensive representation of the relationship we intended to describe, we present the results in terms of the interactions.

For the models, we constructed a vector containing the position of each measurement of the corresponding bat species in the phylogenetic tree using the ‘match’ function from the ‘base’ package. We employed this vector as a grouping factor and to account for the heteroscedasticity of the repeated measures obtained from our measurements using the ‘weight’ and ‘varIdent’ functions from the ‘sjstats’ and the ‘nlme’ packages, respectively. As *P* values for parameter estimates within mixed models are not as straightforward as those obtained from linear regressions, we obtained the *P* values from our phylogenetic models by comparing the likelihood ratio against null models using the ‘lrttest’ function from the ‘lme4’ package. Tests were considered significant at an α value ≤ 0.05 .

Results

In this section, data are presented as the mean \pm standard error unless otherwise noted. We obtained euthermic and torpor traits from 33 individuals belonging to 11 bat species from the family Vespertilionidae and TMR_{min} for five species from the bibliography search (Table 2). Bats from the warm climates were in general smaller (7.05 ± 1.03 g) and presented higher *BMR* (21.01 ± 1.63 mL O₂ h⁻¹), TMR_{red} (76.78 ± 2.82 %), T_{at} (27.89 ± 1.19 °C) and $T_{a\ adjust}$ (22.50 ± 1.19 °C) than bats from the cold climates (11.87 ± 1.42 g, 16.58 ± 0.86 mL O₂ h⁻¹, 67.98 ± 2.01 %, 27.89 ± 1.19 °C, 22.50 ± 1.19 °C, for the M_b , *BMR*, TMR_{red} , T_{at} , and $T_{a\ adjust}$, respectively). Nevertheless, bats from the warm climates presented lower values of TMR_{min} (3.72 ± 0.48 mL O₂ h⁻¹) than bats from the cold climates (5.02 ± 0.41 mL O₂ h⁻¹).

All phylogenetic models fit the data better than conventional ones (Table 3). Therefore, we utilized the phylogenetic models to interpret our results (Freckleton 2009). Our results are conceptually summarized in Fig. 3. In our models we found a positive relationship between the interaction of M_b and climate with the *BMR* of bats ($\beta_0 = 0.10 \pm 0.01$ and $\beta_0 = 0.09 \pm 0.00$ for bats from the warm and cold climate, respectively) ($X^2 = 6.05$, $df = 0$, $p < 0.0001$), such that *BMR* increased more strongly with increasing M_b in bats from warm climates than those from cold climates (Fig. 4A). We also found a remarkably strong relationship between T_{LC} and T_{at} of bats ($r^2 = 0.91$, $t = 27.1$, $df = 31$, $p < 0.0001$) (Fig. 4B).

When individuals entered torpor, they experienced TMR_{red} between 50 % and 90 % below BMR . This reduction was negatively affected by the interaction of M_b with climate ($\beta_0 = -0.52 \pm 0.04$ and $\beta_0 = -0.45 \pm 0.15$ for bats from the warm and cold climate, respectively) ($X^2 = 8.89$, $df = 0$, $p < 0.0001$) (Fig. 4C), such that TMR_{red} decreased with increasing M_b more strongly in bats from warm climates than in those from cold climates. We also found a negative relationship between the interaction of M_b with climate with T_{at} ($\beta_0 = -0.09 \pm 0.00$ and $\beta_0 = -0.19 \pm 0.01$ for the warm and the cold climate, respectively) ($X^2 = 4.58$, $df = 0$, $p < 0.0001$) (Fig. 4D), as well as the $T_{a\ adjust}$ ($\beta_0 = -0.03 \pm 0.01$ and $\beta_0 = -0.10 \pm 0.01$ for the warm and the cold climate, respectively) ($X^2 = 29.8$, $df = 0$, $p < 0.0001$) (Fig. 4E). Thus, both T_{at} and $T_{a\ adjust}$ decreased more strongly with increasing M_b in bats from cold climates than in those from warm climates. Finally, we found a positive relationship between the interaction of M_b with climate with the TMR_{min} of bats ($\beta_0 = 0.19 \pm 0.02$ and $\beta_0 = 0.17 \pm 0.02$, for bats from the warm and cold climate, respectively) ($X^2 = 3.34$, $df = 0$, $p < 0.0001$) (Fig. 4F), such that TMR_{min} increased with increasing M_b more strongly in bats from warm climates than in those from cold climates.

Discussion

In this study, we found that phylogenetic models were a better fit for our data than conventional linear regressions. This finding is consistent with previous research on thermal energetics during euthermia and torpor for some species of birds and mammals, including bats (e.g., Rezende et al., 2004; Cory Toussaint and McKechnie, 2012; Geiser, 2013; Riek and Wolf, 2020). Because phylogenetic models account for shared evolutionary relationships among taxa (Paradis and Schliep, 2019), this makes them a more realistic tool for interpreting the relationships among morphological, ecological, and physiological traits (Garland et al., 2005; Freckleton, 2009). Therefore, their use in comparative studies should be promoted.

In our models, we found a positive relationship between the interaction of M_b and climate with the BMR of bats. This can be explained by the combination of several factors associated with the morphology and physiology of individuals. First, we found smaller bats in warm climates compared to the colder ones. A similar trend in M_b variation has been observed among bats inhabiting sites with a variety of T_a 's, suggesting adaptations in M_b linked to thermoregulation. For example, Alston et al. (2023) compiled data from ~ 31,000

individuals across 20 bat species captured over a decade in North America. The authors found that bats from colder climates typically exhibited larger M_b than those from warmer environments. In colder climates, larger M_b can be advantageous because the surface-area-to-volume ratio decreases with increasing M_b , which may help individuals to lose less metabolic heat to the environment compared to their smaller counterparts (Austad and Fischer, 1991; Speakman, 2005). Second, smaller individuals exhibited lower BMR values than larger ones. This result aligns with findings in other mammals and birds worldwide (Hayssen and Lacy, 1985; Symonds and Elgar, 2002; White and Seymour, 2003; White et al., 2007, 2009; Packard and Birchard, 2008; among others) and can be explained simply because larger animals possess more metabolizable tissue than the smaller ones (Williams and Tieleman, 2000). However, bats from the warm climates had higher BMR values than those from the cold climates. Because smaller animals require greater metabolic heat production to maintain a high T_b due to their higher surface-area-to-volume ratio, this could lead to elevated BMR 's in bats from the warmer sites (Speakman and Thomas, 2003; Clarke et al., 2010). Third, individuals from the warm climates increased more strongly their BMR with M_b than those from the cold climates. In our trials, we found exceptionally high BMR values in very small M_b species like *Rhogeessa parvula*, *R. alleni* and *E. furinalis* from the warmer sites, which presented BMR values four to six times higher than what would be expected for their M_b and as much as double the BMR of *Myotis californicus* and *M. volans*, two bat species of similar M_b from the colder sites. This could be the reason because we found a higher slope in the relationship between M_b and climate with the BMR of bats for the warm climates. Differences in BMR have been associated to organ size in birds and mammals (Kersten and Piersma, 1987; Konarzewski and Diamond, 1995; Nespolo et al., 2002). In bats, there is scant information about this topic, yet larger brains have been correlated to higher BMR 's (McNab and Köhle, 2017). It has been also demonstrated that bats living in more complex environments tend to present larger brains than those living in simpler environments (Safi et al., 2005). Because warmer sites tend to present more vegetation compared to colder sites (and hence more complexity) (Gaston, 2000; Kraft and Ackerly, 2014), bats from warmer sites should have presented larger brains, leading to higher metabolic rates. Although differences in brain mass can explain the differences we observed here, this theme calls for further exploration. Higher BMR 's would be more

sustainable in warmer climates where prey abundance tends to be higher, and T_a is typically more stable than in colder environments (Wolda, 1978). Nonetheless, the use of torpor might help individuals in both warm and cold climates achieve energy balance when facing energy constraints due to both T_a and food availability.

In our measurements, we found a strong correlation between T_{LC} 's and T_{at} experienced by bats. This suggests that our study species efficiently conserved energy when faced with energy constraints due to the 36-hour food deprivation before experiments, combined with exposure to T_a 's below their T_{LC} . Lower critical temperature represents the T_a at which individuals start expending energy to generate heat and maintain a stable T_b that is in most cases higher than T_a (Fristoe et al., 2015). However, under energy-restricted conditions, minimizing metabolic expenditures becomes crucial (Vuarin et al., 2014, 2015). To achieve energy balance, our study species employed torpor, significantly reducing their metabolic rate to 50 % to 90 % below their BMR (i.e., TMR_{red}). Similarly, other daily heterotherms from the orders Rodentia, Insectivora, Chiroptera and Carnivora have been observed displaying a TMR_{red} ranging from 43 % to 96 % when using torpor in both laboratory and natural conditions (Ellison, 1992; Holloway and Geiser, 1995; Geiser, 2004 and the references cited there; Geiser et al., 2019). Nevertheless, in our experiments we found that TMR_{red} was negatively affected by the interaction of M_b and climate. In our trials, we found high TMR_{red} values in smaller M_b bats compared with their larger counterparts. Geiser (2021) has proposed that higher TMR_{red} in small animals has likely evolved to maximize energy-savings. Differences in TMR_{red} can be attributed to the evaporative cooling capacity experienced by animals when entering torpor, which could be influenced by their M_b . Smaller animals tend to exhibit elevated resting metabolic rates (RMR) at low T_a . When these individuals enter torpor, their RMR undergoes substantial reduction towards torpid values. This reduction, combined with the large surface-area-to-volume ratio, leads to a rapid decrease in the T_b of individuals, promoting a steep and deep decline in metabolism. This could be the reason because *R. parvula*, *R. allenii* and *E. furinalis*, which presented the highest BMR values and smallest M_b among our study species exhibited the highest TMR_{red} values (averaging from 79 to 85 %) in our experimental trials. This can also explain why the slope of the relationship between TMR_{red} and the interaction of M_b and climate was higher for bats from the warmer climates

compared to the colder ones. However, in our experimental trials, all bats were confronted with T_a 's below their T_{LC} when animals were within their TNZ (see methods section). Under these circumstances, physiological inhibition coupled with the abandonment of T_b regulation could have contributed to significant reductions in the metabolic rate of bats, especially of those of the warm climates (Reher and Dausmann, 2021). While physiological inhibition has been reported more for hibernating animals rather than daily heterotherms (Geiser, 1988; Lyman, 1982), it is plausible that a similar phenomenon has occurred in our study species. Nevertheless, this topic needs further exploration. Larger bats, which are adapted to colder climates, exhibit lower T_{LC} values (Speakman and Thomas, 2003; Geiser, 2021), and the disparity between RMR and BMR tends to be less pronounced. In our study sites, bats from colder climates exhibited a mean T_{LC} of 23.7 ± 0.89 °C compared to the 29.3 ± 0.90 °C observed in bats from warmer climates. As larger bats enter torpor, their metabolic rate drops may be less pronounced. Due to this condition and their lower surface-to-volume ratio, this leads to slower cooling rates that could result in higher torpid metabolic rates compared to bats from warmer sites (Geiser, 2004). This also can explain the lower slope of bats from the colder climates we found for the relationship between TMR_{red} and the interaction of M_b and climate compared to the warmer ones. Cooling rates have important co-evolutionary implications in roost selection by bats. Czenze et al. (2021) demonstrated that bat species occupying poorly buffered roosting sites exhibited higher evaporative cooling capacity compared to those using well-isolated ones. This can explain why small M_b bats such as *R. parvula*, *R. allenii*, and *E. furinalis* roost in poor isolated roosts, such as underneath exfoliating bark and inside shallow tree cavities in our study sites (Mies et al., 1996; Roots and Baker, 2007). Because roosting places are closely related to torpor use in bats (Alston et al., 2022), this topic needs more investigation.

In this study we identified a negative relationship between the interaction of M_b and climate with the T_{at} and $T_{a\,adjust}$ of bats, along with a positive relationship with TMR_{min} . In our experiments, smaller M_b bats presented higher values of T_{at} and $T_{a\,adjust}$ and lower values of TMR_{min} compared to their larger counterparts. This can explain why bats from warm climates in nature are able to use torpor at high T_a 's in the tropics (e.g., Turbill et al., 2003; Geiser et al., 2011; Liu and Karasov, 2011). These results can be explained by the strong correlation we found between T_{LC} and T_{at} of bats, as well as differences in TMR_{red}

observed among bats of the different climates. As T_{LC} inversely relates to M_b , smaller M_b bats display higher T_{LC} values leading to elevated T_{at} compared to larger ones.

Furthermore, due to the sequence of torpor events, $T_{a\text{ adjust}}$ occurred as the second inflection point after T_{at} , resulting in warmer $T_{a\text{ adjust}}$ values in smaller bats as well. Interestingly, we observed that smaller bats presented higher TMR_{red} values, which ultimately contributed to lower TMR_{min} than their larger counterparts. Given that bats from warmer sites had lower M_b , this may account for the differences we found in bats from cold and warm climates in our study sites.

Conclusion

This investigation underscores the significant role of the interaction between M_b and climate on the torpor traits of bats. However, more comprehensive research on this subject needs to be conducted. While numerous avenues for investigation exist, we propose several ideas related to the results we found. For instance, investigations should attempt to understand why extremely small bats endure significantly cold climates over extended periods of time. For example, *Myotis californicus* and *M. melanorhinus* (~ 4 g), and *M. volans* (~ 6 g) are found in La Malinche National Park for a significant portion of the year (Ayala-Berdon et al., 2017). Despite limited reports of hibernation (Aguilar-Rodríguez et al., 2021) the utilization of torpor by these species remains largely unexplored. Further investigation should also explore regional disparities in torpor usage among bats with extensive geographic ranges. In this regard, *Eptesicus fuscus* and *Lasiurus borealis* from North America presented lower T_{at} 's in higher latitudes where T_a tends to be colder than lower latitudes where T_a is typically warmer (Dunbar and Brigham, 2010). Although these differences have not been assessed in different climates, *M. velifer* presents higher BMR , thermal conductance, and T_{LC} and T_{UC} during euthermia when captured from warm sites compared to a cold site in central Mexico (Medina-Bello et al., 2023). Nevertheless, no information from torpor traits from this species has been collected. Therefore, more work in this area needs to be done.

Acknowledgments

This work supported by the program CONACYT FOSEC CB2017-2018 (A1-S-39572) granted to JAB. We are grateful to L. Orozco-Lugo for her help capturing bats in Quilamula, to R. Vázquez-Fuerte for her comments on the manuscript and the ejido of Moxolahuac and the Quilamula Biological Station for logistical support.

Competing interests

The authors declare there are no competing interests

Funding

This investigation was supported by the program CONACYT FOSEC CB2017-2018 (A1-S-39572) granted to JAB. Supporters had no participation in study design, data collection, analysis, or the writing of the manuscript.

Data availability

All data obtained in this work is fully presented in the manuscript.

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Figures and Tables

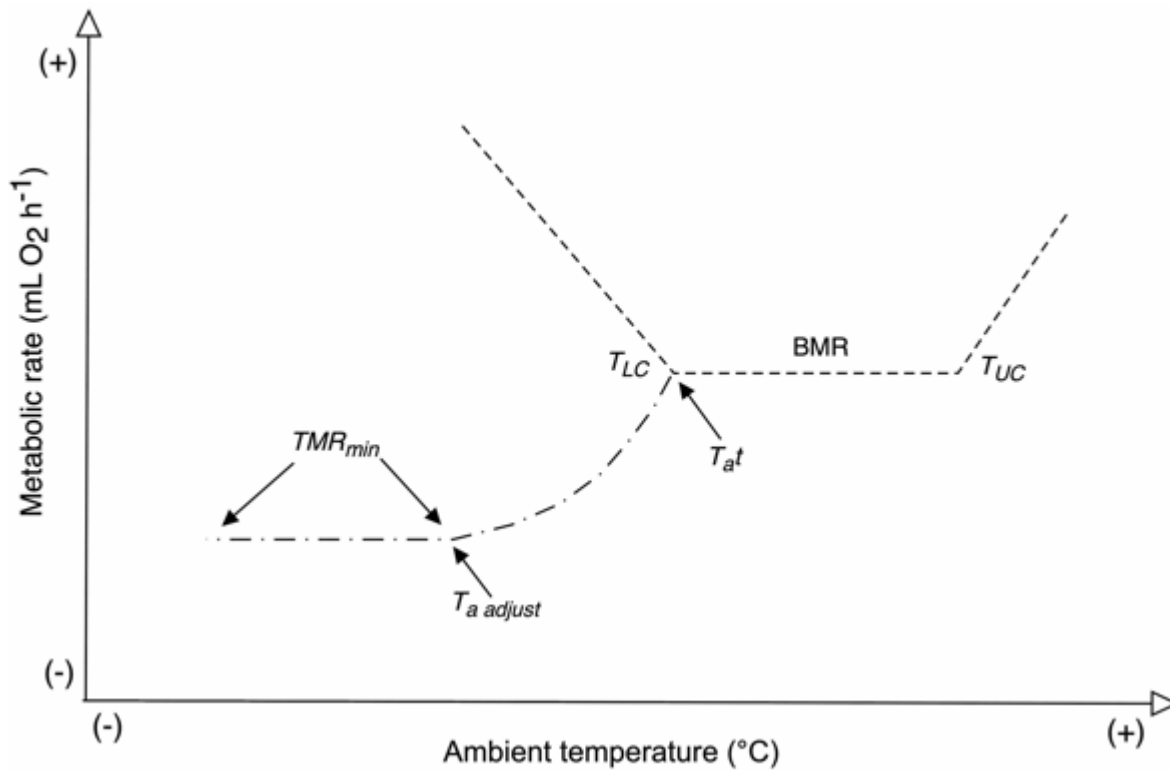


Figure 1. Conceptual model proposed by Speakman and Thomas (2003) of torpor energetics in heterothermic animals. In this representation the first inflection point of the metabolic rate below lower critical temperature (T_{LC}) indicates the T_a at which animals enter torpor (T_{at}). As T_a continues to drop, the metabolic rate reaches a second inflection point ($T_{a\text{ adjust}}$), where the torpid metabolic rate starts reaching its minimum values (TMR_{min}).



Figure 2. Bats were captured in four locations with either cold or warm climates in central Mexico; La Malinche National Park (LMNP) and Santa Cruz Moxolahuac (SCM) were the cold sites and Sierra de Huautla Biosphere Reserve (SHBR), and Autlán were the warm sites.

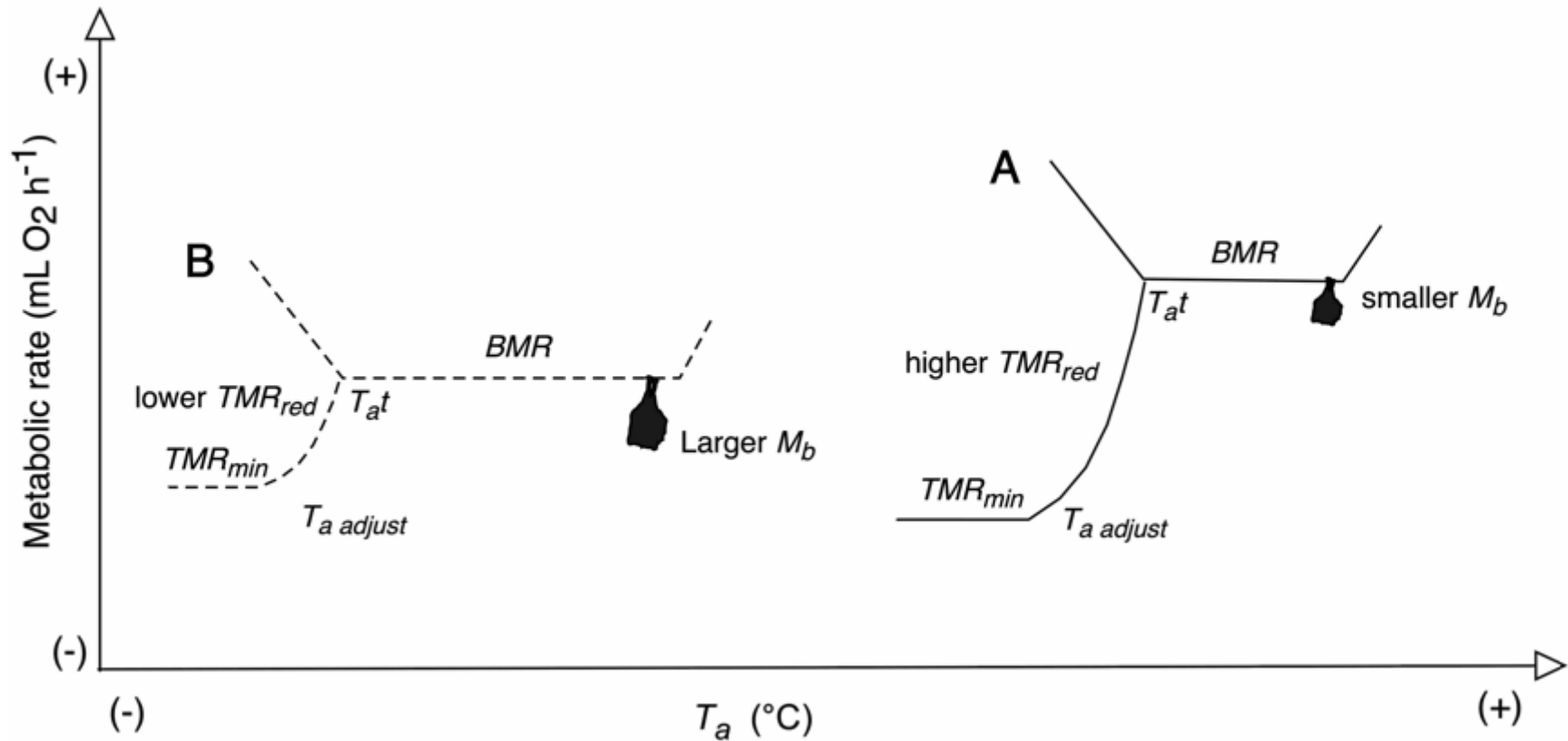


Figure 3. Summary of the thermal energetics of bats from warm (A) and cold (B) climates of central Mexico obtained in this study. Here we show how bats from the warm climates were smaller and showed higher basal metabolic rate (*BMR*) and torpid metabolic rate reductions (*TMR_{red}*), warmer temperatures at which bats entered torpor (*T_{at}*) and reached its lower values (*T_a adjust*) compared to bats from the cold climates. Nevertheless, because bats from the warm climates presented higher *TMR_{red}*, minimum torpid metabolic rates (*TMR_{min}*) were lower than bats from the colder ones.

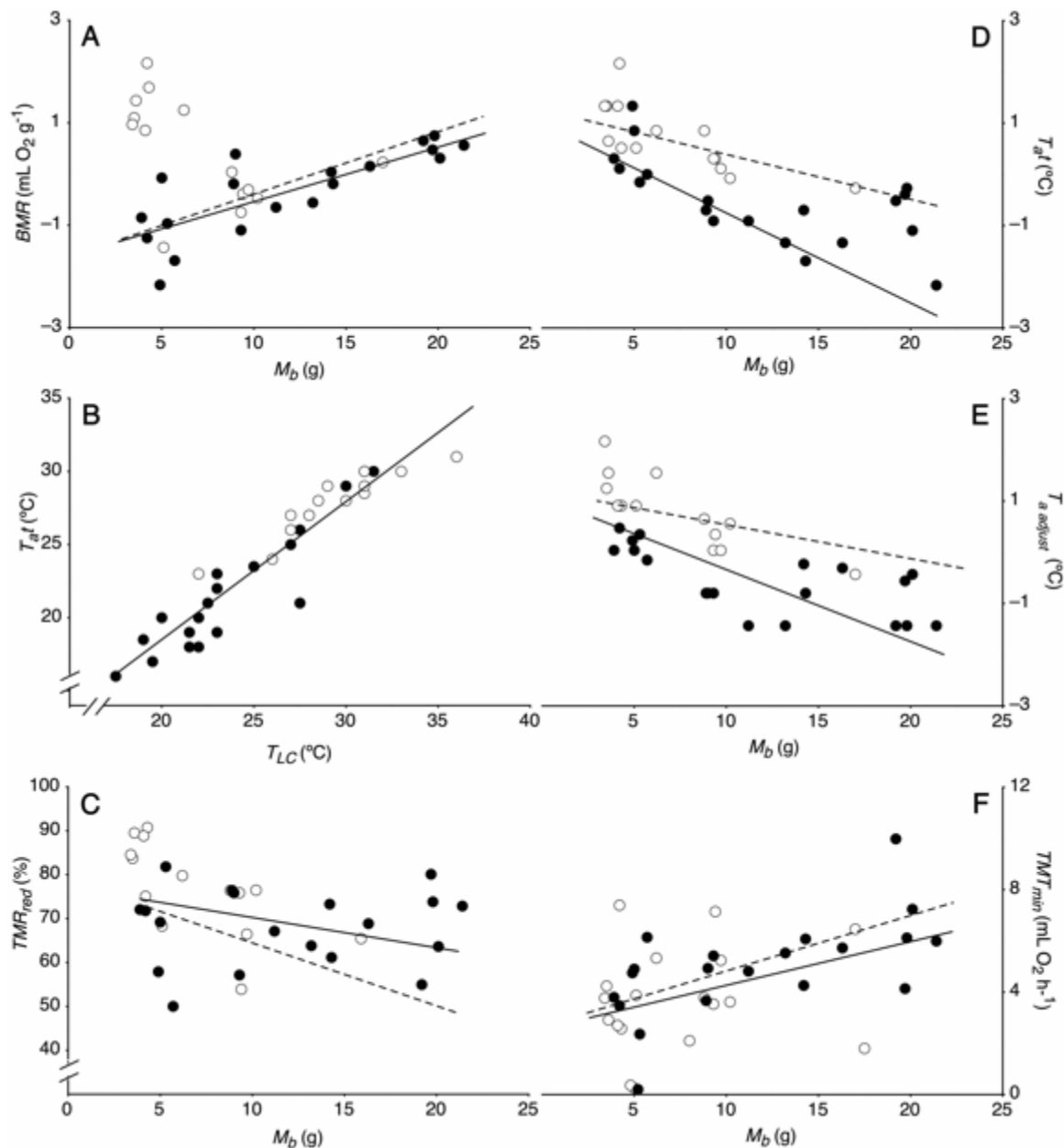


Figure 4. Effect of body mass (M_b) and climate (either warm-open symbols- or cold-closed symbols-) on basal metabolic rate (BMR), lower critical temperature (T_{LC}) during euthermia, and torpor energetics (T_a at which animals entered torpor (T_{at}), and the inflection point ($T_{a\text{ adjust}}$) where torpid metabolic rate started reaching its lower values (TMR_{min})) from vespertilionid bats living in central Mexico. Regression lines (dashed lines -warm climates-, and continuous lines -cold climates-) were fitted by using the estimated parameters from the phylogenetic models.

17

18 Table 1. Environmental variables of the capture sites for the measurements of torpor energetics of bats of the family Vespertilionidae
19 from central Mexico. Climate classification followed Köppen's categorization, considering both T_a and precipitation.

State	Locality	Köppen's climate clasification	Mean annual T_a (°C)	Mean annual minimum T_a (°C)	Mean annual rainfall (mm)	Dominant Vegetation	References
Tlaxcala	La Malinche Nacional Park (19° 14' 30.93" N 98° 8' 32.21" W)	Cold	13.0	5.0	1,150	Coniferous forest	Villers et al. 2006; INEGI 2005-2017
Puebla	Santa Cruz Moxolahuac (19° 27' 2.18" N 98° 34' 10.13" W) Quilamula	Cold	16.0	8.0	1,400	Coniferous forest	INEGI 2014, 2017

Morelos	(18° 30′ 41.98" N 98° 58' 16.55" W)	Warm	24.3	15.5	665	Tropical dry forest	INEGI 2014, 2017
Jalisco	Autlán (19° 41' 22.9" N, 104° 22' 03.7" W)	Warm	25.8	13.6	861	Tropical dry forest	INEGI 2014, 2017; Chávez 2019

20

21

22 Table 2. Thermal energetics of bats from the family Vespertilionidae living in cold and warm climates of central Mexico.
 23 Measurements were taken by using open flow respirometry of postabsorptive adult non-reproductive males during their resting phase
 24 from ~ 10:00 to 19:30 hours.

Bat species (climate)	M_b (g)	<i>Euthermic</i>			<i>Torpid</i>		
		BMR (mL O ₂ h ⁻¹)	T_{LC} (°C)	T_{UC} (°C)	T_{at} (°C)	$T_{a\ adjust}$ (°C)	TMT_{min} (mL O ₂ h ⁻¹)
<i>Eptesicus fuscus</i> (cold)	13.2	15.2	21.5	35.5	18	8	5.5
	11.2	14.6	23	32	19	8	4.8
	14.3	15.7	19.5	35	17	10	6.1
	16.3	18.3	22	36	18	13	5.7
	14.2	16.1	20	32.5	20	14	4.3
<i>Lasiurus cinereus</i> (cold)	21.4	22.1	17.5	33	16	8	6.0
	19.8	23.3	23	33	23	8	6.1
	19.2	22.2	22.5	32	21	8	10.0
	20.1	19.8	19	34	18.5	11	7.2
	19.7	20.6	23	34	22	10.5	4.1

<i>Lasiurus blossevillii</i>	9.0	20.3	27.5	36	21	10	4.9
(cold)	8.9	15.7	22	33	20	10	3.7
	9.3	12.6	21.5	31.5	19	10	5.4
<i>Myotis volans</i>	4.9	11.4	31.5	40.5	30	18.5	4.8
(cold)	5.3	13.2	25	36	23.5	20	2.4
	5.7	12.2	27	35	25	16.5	6.1
	5.0	15.9	30	39	29	18	4.9
<i>Myotis californicus</i>	4.2	12.4	27.5	38	26	20.5	3.5
(cold)	3.9	13.6	28	38	27	18	3.8
<i>Myotis myotis</i>	30.0						6.0*
(cold)							
<i>Myotis fortidens</i>	5.1	12.3	30	38	28	25	3.9
(warm)							
<i>Rhogeessa tumida</i>	3.5	25.8	31	42	30	25.5	4.2
(warm)	3.6	27.7	31	41	28.5	26	2.9
	3.4	24.6	31	43	30	27	3.8

<i>Rhogeessa parvula</i>	4.2	29.7	36	43	31	25	7.4
(warm)	4.3	28.1	28.5	36.5	28	25	2.6
	4.1	24.2	33	42	30	25	2.7
<i>Eptesicus furinalis</i>	6.2	26.2	31	38	29	26	5.3
(warm)							
<i>Myotis velifer</i>	9.4	15.4	27	34	27	20	7.1
(warm)	9.3	14.5	28	36	27	18	3.5
	9.7	15.5	27	35	26	18	5.2
	10.2	15.3	26	35	24	21.5	3.6
	8.8	16.1	29	36	29	22	3.8
<i>Lasiurus intermedius</i>	15.9	18.8	22	33	23	11	6.5
(warm)							
<i>Nyctophilus geoffroyi</i>	8.0						2.1*
(warm)							
<i>Chalinolobus gouldii</i>	17.5						1.8*
(warm)							
<i>Myotis keaysi</i>	5.0						0.25*

(warm)

Myotis oxyotus

4.8

0.36*

(warm)

-
- 25 M_b = body mass, BMR = basal metabolic rate, T_{LC} = lower critical temperature, T_{UC} = upper critical temperature, TMR_{red} = percentage of reduction of the
 26 metabolic rate relative to BMR , $T_{at} = T_a$ at which the metabolic rate reached its first inflection point below T_{LC} , signaling animals entered torpor, $T_{a\ adjust} = T_a$ at
 27 which the second inflection point occurred, indicating that torpid metabolic rate started reaching its lower values, TMR_{min} = minimum torpid metabolic rate.
 28 Measures marked with * were taken from the literature search.

29 Table 3. Statistical comparison between models used to evaluate the relationship between
30 M_b and climate in torpor traits of bats of the family Vespertilionidae. Comparisons were
31 made by using the 'lrtest' function from the 'lmodel2' package in RStudio (version 1.2.5042).
32

Model structure	Model type	AIC	Statistics
$BMR \sim M_b : \text{climate}$	phylogenetic	- 426.5	$X^2 = 658.4$, $df = 10$, $p < 0.0001$
	conventional	211.8	
$TMR_{\text{red}} \sim M_b : \text{climate}$	phylogenetic	- 268.1	$X^2 = 2243.4$, $df = 10$, $p < 0.0001$
	conventional	248.7	
$T_{at} \sim M_b : \text{climate}$	phylogenetic	- 2152.9	$X^2 = 2250.0$, $df = 10$, $p < 0.0001$
	conventional	77.0	
$T_{a \text{ adjust}} \sim M_b : \text{climate}$	phylogenetic	-2142.7	$X^2 = 2241.2$, $df = 10$, $p < 0.0001$
	conventional	78.4	
$TMR_{\text{min}} \sim M_b : \text{climate}$	phylogenetic	-2053.1	$X^2 = 2255.8$, $df = 16$, $p < 0.0001$
	conventional	170.7	

33 M_b = body mass (g), BMR = basal metabolic rate ($\text{mL O}_2 \text{ h}^{-1}$); TMR_{red} = percentage of reduction of the
34 metabolic rate relative to BMR (%), $T_{at} = T_a$ at which the metabolic rate reached its first inflection point below
35 T_{LC} , signaling animals entered torpor ($^{\circ}\text{C}$), $T_{a \text{ adjust}} = T_a$ at which the second inflection point occurred,
36 indicating that torpid metabolic rate started reaching its lower values ($^{\circ}\text{C}$), TMR_{min} = minimum torpid
37 metabolic rate ($\text{mL O}_2 \text{ h}^{-1}$)