

Thermal energetics of female big brown bats (*Eptesicus fuscus*)

Craig K.R. Willis, Jeffrey E. Lane, Eric T. Liknes, David L. Swanson, and R. Mark Brigham

Abstract: We investigated thermoregulation and energetics in female big brown bats, *Eptesicus fuscus* (Beauvois, 1796). We exposed bats to a range of ambient temperatures (T_a) and used open-flow respirometry to record their metabolic responses. The bats were typically thermoconforming and almost always entered torpor at T_a s below the lower critical temperature T_{lc} of 26.7 °C. Basal metabolic rate (BMR, 16.98 ± 2.04 mL O₂·h⁻¹, mean body mass = 15.0 ± 1.4 g) and torpid metabolic rate (TMR, 0.460 ± 0.207 mL O₂·h⁻¹, mean body mass = 14.7 ± 1.3 g) were similar to values reported for other vespertilionid bats of similar size and similar to a value for *E. fuscus* BMR calculated from data in a previous paper. However, we found that big brown bats had a lower T_{lc} and lower thermal conductance at low T_a relative to those measured in the previous study. During torpor, the minimum individual body temperature (T_b) that we recorded was 1.1 °C and the bats began defending minimum T_b at T_a of approximately 0 °C. BMR of big brown bats was 76% of that predicted for bats based on the relationship between BMR and body mass. However, the Vespertilionidae have been under-represented in previous analyses of the relationship between BMR and body mass in bats. Our data, combined with data for other vespertilionids, suggest that the family may be characterized by a lower BMR than that predicted based on data from other groups of bats.

Résumé : Nous avons étudié la thermorégulation et les relations énergétiques chez des femelles de la grande chauve-souris brune, *Eptesicus fuscus* (Beauvois, 1796). Nous avons exposé les chauves-souris à une gamme de températures ambiantes (T_a) et enregistré leurs réponses métaboliques par respirométrie en circuit ouvert. En général, les chauves-souris ont un faible pouvoir de régulation thermique et elles entrent presque toujours en torpeur à une T_a s sous la température critique inférieure T_{lc} de 26,7 °C. Le taux de métabolisme de base (BMR, $16,98 \pm 2,04$ mL O₂·h⁻¹, masse moyenne du corps = 15,0 ± 1,4 g) et le taux de métabolisme durant la torpeur (TMR, $0,460 \pm 0,207$ mL O₂·h⁻¹, masse moyenne du corps = 14,7 ± 1,3 g) sont semblables à ceux signalés chez d'autres vespertilionidés de taille similaire et à une valeur de BMR calculée à partir de données sur *E. fuscus* dans une publication antérieure. Nos valeurs de T_{lc} et de conductance thermique à T_a basse chez les chauves-souris brunes sont plus basses que celles mesurées dans l'étude antérieure. Durant la torpeur, la valeur minimale de température corporelle (T_b) individuelle enregistrée est de 1,1 °C et les chauves-souris commencent à défendre la T_b minimale à T_a d'environ 0 °C. Le BMR des grandes chauves-souris équivaut à 76 % de la valeur prédite à partir de la relation entre BMR et la masse corporelle chez les chauves-souris. Cependant, les Vespertilionidae sont sous-représentés dans les analyses antérieures de la relation entre BMR et la masse corporelle chez les chauves-souris. Nos données, combinées à d'autres sur des vespertilionidés différents, indiquent que la famille est peut-être caractérisée par un BMR plus faible que celui prédit d'après les données sur les autres groupes de chauves-souris.

[Traduit par la Rédaction]

Introduction

Endothermic animals that regularly face cold ambient temperatures (T_a) have evolved a suite of physiological, morphological, and behavioural traits to enable them to withstand periods of cold weather and food shortage (Prosser 1991; Geiser 1996). Thermal conductance is negatively correlated with body size (Bradley and Deavers 1980), so the

cost of maintaining a stable warm body temperature (T_b) is particularly high for small endotherms. Therefore, selection pressure associated with the production and retention of metabolic heat likely exerts a strong influence on small endotherms that live in cold climates.

Many studies have quantified physiological responses of endotherms to temperature (e.g., Bartels et al. 1998; Liknes et al. 2002; Geiser and Drury 2003). For logistic reasons,

Received 11 January 2005. Accepted 26 May 2005. Published on the NRC Research Press Web site at <http://cjz.nrc.ca> on 5 August 2005.

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studies of energetics typically use laboratory-housed animals or animals captured at a single study site. However, captivity can influence patterns of thermoregulation (Geiser et al. 2000) and, for wide-ranging species, clearly not all populations experience the same conditions. For example, Geiser and Ferguson (2001) found evidence of intraspecific variation in some (though not all) physiological traits between two populations of feathertail gliders (*Acrobates pygmaeus* (Shaw, 1793)) from different parts of their range. The relative influence of different selection pressures will vary among sites for species that range widely, so it is reasonable to predict considerable intraspecific variation in physiology (Garland and Adolph 1991; Spicer and Gaston 1999). Despite this potential variation, subsequent analyses of physiological traits in literature reviews are based on the assumption that data points obtained for a species in one part of its range are representative of that species in general (e.g., Bradley and Deavers 1980; Hayssen and Lacy 1985; McNab 1988, 2002; Speakman and Thomas 2003).

Torpor is a thermoregulatory strategy employed by some heterothermic mammals and birds to offset the high energetic cost of endothermy during periods of inclement weather and reduced food availability (Wang 1989). During torpor, endotherms allow body temperature (T_b) and metabolic rate (MR) to fall substantially below normothermic levels (Wang 1989). Temperate-zone bats use torpor readily in both the laboratory and the field (Geiser and Brigham 2000; Lausen and Barclay 2003; Turbill et al. 2003a, 2003b). Female bats, however, are predicted to avoid torpor during gestation and lactation, and select relatively warm roost sites because low T_a delays the development of their offspring (Racey and Swift 1981; Tuttle and Stevenson 1982; Wilde et al. 1999; Willis 2005). Thermoregulation clearly plays a key role in the ecology and behaviour of bats. Therefore, quantifying the energetic costs of normothermia and benefits of torpor over a range of conditions is not only valuable from a physiological perspective but is relevant to understanding the habitat requirements and roosting ecology of bats, as well.

The North American big brown bat (*Eptesicus fuscus* Beauvois, 1796) is a common wide-ranging vespertilionid found from southern Canada to northwestern South America (Kurta and Baker 1990). In the northern part of its range it hibernates during winter (Kurta and Baker 1990) and regularly uses torpor during summer (e.g., Hamilton and Barclay 1994; Lausen and Barclay 2003). Big brown bats have been well studied in both the laboratory and the field (e.g., Kurta et al. 1990; Kalcounis and Brigham 1998; Lausen and Barclay 2003; Willis and Brigham 2003, 2004; Willis et al. 2004). However, only one previous study has quantified thermal energetics of big brown bats. Herreid and Schmidt-Nielsen (1966) captured male and female big brown bats at sites in North Carolina, housed them in the laboratory for up to several months, and measured O_2 consumption over a range of T_a . However, they did not quantify a number of physiological traits including thermal conductance, basal metabolic rate (BMR), the boundaries of the thermoneutral zone, the lower limit of the T_b set-point during torpor, and minimum body temperature and torpid metabolic rate (TMR). Herreid and Schmidt-Nielsen's (1966) study site was about halfway between the northern and southern extent

of the species' range (latitude = approximately 35°N). This raises the question of whether their data are applicable to populations living farther north where they will often face much colder T_a . It is difficult to replicate Herreid and Schmidt-Nielsen's (1966) study precisely and test the hypothesis that geographic variation in thermal energetics occurs among populations of big brown bats. If differences from the previous study are observed, it could reflect intraspecific geographic variability or the fact that slight differences in techniques and equipment have led to different results. Nevertheless, it is a question worthy of emphasis because the review literature relies heavily on the assumption that data from one study for a particular species are representative of that species in general, with the consequence that studies are rarely repeated within species (e.g., Bradley and Deavers 1980; Hayssen and Lacy 1985; McNab 1988, 2002; Speakman and Thomas 2003).

Our objectives were to (i) quantify metabolic rate during torpor and normothermia in big brown bats over a range of T_a from a study site in the northern part of their range; (ii) quantify physiological traits including thermal conductance, BMR, the boundaries of the thermoneutral zone, the lower limit of the T_b set-point during torpor, and minimum T_b and TMR; and (iii) determine if metabolic responses to temperature recorded for bats from southeastern South Dakota differ from those recorded for individuals by Herreid and Schmidt-Nielsen (1966).

Materials and methods

All procedures were approved by the University of Regina President's Committee on Animal Care and the University of South Dakota Animal Care Committee, and were in accordance with the principles and guidelines set by the Canadian Council on Animal Care. We captured bats using mist nets in riparian woodlands of southeastern South Dakota, near the town of Vermillion (42°47'N, 97°0'W). This site is about 1800 km west-northwest of Raleigh, North Carolina, near Herreid and Schmidt-Nielsen's (1966) field site, and 1000 km south of the northern extent of the known range of big brown bats. In Vermillion, daily average maximum and minimum temperatures are, respectively, about 27 and 16 °C in July and -7 and -18 °C during January. Daily average maximum and minimum temperatures in Raleigh are warmer: approximately 33 and 21 °C in July and 10 and -1 °C in January (NOAA 2003).

We performed all trials at the University of South Dakota on 10 individuals. Five nonreproductive/postlactating female bats were used from 2 to 13 September 2001, and five females, which were not palpably pregnant, were measured from 1 to 10 May 2002. All animals were adults. Postlactating bats were easily identified based on regrowth of hair around the nipples and by the fact that milk could not be expressed. Following capture, bats were held in cloth bags, exposed to outside photoperiod, and provided access to water every few hours. Within 1 day of capture, a temperature sensitive radio transmitter (0.75 g, model BD-2ATH, Holohil Systems Ltd., Carp, Ontario) was surgically implanted into the intraperitoneal cavity of each bat under inhalant anaesthesia (Isoflurane USP, Abbot Laboratories, Montréal, Quebec) to permit body temperature (T_b) record-

ing simultaneous to metabolic measurements. Bats were allowed 1–3 days to recover from surgery prior to metabolic measurements. We did not wait longer than 3 days to reduce potential effects of captivity on thermal physiology, which can be considerable (Geiser et al. 2000). Each day during captivity, bats were hand-fed mealworms and crickets and all bats were released after their metabolic trials within 5 days of capture. We used a hand-held telemetry receiver (R-1000, Communication Specialists Inc., Orange, California) and a 5-element yagi antenna (AF Antronics, Inc., Urbana, Illinois) to detect the transmitter signal during metabolic trials. The relationship between transmitter pulse rate and T_a was calibrated to ± 0.5 °C in a water bath by the manufacturer and verified prior to experiments. Every 2 min during metabolic trials, we recorded the time it took for a transmitter to emit 11 pulses (i.e., 10 interpulse intervals). We later calculated the average interpulse interval for each recording and then determined T_b from calibration curves provided by the manufacturer.

Food was withheld for at least 12 h prior to experiments to ensure that bats were postabsorptive during recording trials. We used open flow respirometry to determine BMR, resting metabolic rate (RMR), and TMR over a T_a range between -2.5 and 40 °C. We started recording at about 1430 to 1500 to ensure that BMR measurement took place during the inactive phase of the daily activity cycle (McNab 1997). Recording continued overnight so that bats would be more likely to remain normothermic at low T_a during the active phase. We measured each bat's mass to the nearest 0.01 g using an electronic balance (model C305-S, Ohaus, Pine Brook, New Jersey) immediately prior to and immediately after metabolic trials, and assumed a linear decrease in body mass for calculation of mass-specific metabolic rates. Bats were placed in a sealed metabolic chamber constructed from a 1.0-L glass jar and equipped with a small platform so that they could hang upside down. The chamber was lined with black paper to provide an emissivity near 1. We regulated flow rates of dry, CO₂-free air, between 97 and 290 mL · min⁻¹, depending on the bat's metabolic rate. The O₂ concentration in the excurrent airflow never fell below 20%. Flow rates were regulated with a precision rotameter (model FM082-03ST, Cole-Parmer, Vernon Hills, Illinois) previously calibrated to $\pm 1\%$ accuracy. Excurrent air was dried and fractional oxygen concentration (F_eO₂) was analyzed with an O₂ analyzer (model S-3A, Ametek, Paoli, Pennsylvania). For the five bats measured in September 2001, we also recorded fractional concentrations of CO₂ (F_eCO₂) in the excurrent gas stream with a CO₂ analyzer (model CD-3A, Ametek). We recorded both F_eO₂ and F_eCO₂ concurrent with T_b , every 2 min. We later calculated V_{O_2} and V_{CO_2} corrected to standard temperature and pressure. We calculated steady-state MR following Withers (1977) using the minimum 10-min average F_eO₂ concentration taken from the hour-long sampling session at each temperature. We ensured that V_{O_2} had stabilized before beginning the hour-long session. Respiratory exchange ratio (RER) was measured for five bats as V_{CO_2}/V_{O_2} .

To balance heat loss and heat gain, resting animals adjust thermal conductance (C) and metabolic heat production (i.e., MR) in direct proportion to the differential between T_b and T_a (Withers 1992; Schmidt-Nielsen 1997). The rate of heat transfer between an object and its surroundings depends on

the surface area of the object and is the sum of radiative heat loss and gain, convective heat transfer due to movement of the surrounding medium, conductive heat transfer due to direct contact with any surface, and heat loss due to the loss or phase change of water (Bakken and Kunz 1988; Schmidt-Nielsen 1997). To calculate heat balance for free-living animals, all of these factors must be considered, but for bats in a metabolic chamber at the low flow rates used in this study, the effects of forced convection owing to wind, radiative heat gain, and conductive transfer between the animal and solid surfaces are negligible. This is because the air is still, animals are shielded from solar radiation, and surfaces are essentially the same temperature as air. Furthermore, bats are hanging, so they have little contact with surfaces. Under these conditions, thermal conductance becomes mainly a function of free convection from the body surface, evaporative heat loss (EHL) across the respiratory surface, and radiative heat loss across the body surface. We calculated wet thermal conductance (C_{wet}), an estimate of the rate of heat loss that combines all these modes of heat transfer, following the standard equation of Schmidt-Nielsen (1997) and Withers (1992), as the rate of metabolic heat production divided by the differential between T_b and T_a (i.e., $MR/(T_b - T_a)$). Like many studies, we did not calculate dry thermal conductance (C_{dry}), which estimates conductance once effects of EHL are removed, because it is well established that EHL is negligible below thermoneutrality (Withers 1992; Schmidt-Nielsen 1997) and because we were unable to measure evaporative water loss. Thermal conductance depends on surface area, so ideally it should be expressed on a surface area specific basis. However, the surface area of any animal, especially a bat, is extremely difficult (if not impossible) to measure accurately and can vary widely within individuals depending on minor postural adjustments. In addition, body surface temperature varies over different portions of the body, which also complicates calculation of conductance per unit of surface. Because of these difficulties, we did not estimate conductance on a surface area basis but, instead, used the standard mass-specific units (Withers 1992; Schmidt-Nielsen 1997). This is entirely appropriate given the highly significant relationship between body mass and thermal conductance in endotherms and the well-established allometric relationship between body mass and surface area in mammals (Bradley and Deavers 1980; Withers 1992; Schmidt-Nielsen 1997).

Metabolic measurements were conducted at two to four different T_a for each bat. We maintained each test T_a for a minimum of 1 h after V_{O_2} stabilized. Ambient temperature in the metabolic chamber was regulated at ± 0.5 °C by submerging the chamber in a circulating bath (model 2095, Forma Scientific, Marietta, Ohio) filled with ethylene glycol and water. We measured chamber temperature concurrent with T_b , F_eO₂ and F_eCO₂ every 2 min using a copper constantan thermocouple attached to a thermocouple thermometer (model 8500-40, Cole Parmer, Vernon Hills, Illinois) calibrated against a thermometer traceable to the US Bureau of Standards.

We measured BMR first in all trials. Bats were allowed to equilibrate to the first test T_a in the metabolic chamber for a minimum of 1.5 h prior to metabolic recording. The first test T_a for each bat was a temperature that we predicted would fall within the thermoneutral zone (TNZ; 30.9 ± 2.4 , range

27.0–34 °C). We defined TNZ as the range of ambient temperatures over which MR did not vary. BMR was taken as the 10-min mean minimum MR recorded during the minimum 1-h period when T_a was within the TNZ after VO_2 had stabilized (Schmidt-Nielsen 1997).

Following BMR measurements, for four bats (one in fall 2001 and three in spring 2002) we increased T_a to between 35.0 and 40.0 °C, to determine the upper limit of TNZ. Two of these bats were used for TMR or RMR measurements, but the individuals exposed to the two highest temperatures (37.5 and 40 °C) were not. For six bats, immediately following BMR recording (and for the two bats recorded at 35 and 36.5 °C), we reduced the temperature of the circulating bath to the next highest test temperature (12.5–21.0 °C). We maintained this T_a until VO_2 stabilized and used the same procedure as for BMR to calculate RMR or TMR (depending on whether bats defended a normothermic T_b or entered torpor) at the new T_a . After at least 1 h, once VO_2 had stabilized at this temperature, we further reduced the temperature of the circulating bath to the next test T_a (2.0–11 °C) and RMR or TMR was recorded again. Four bats were exposed to a final test T_a (–2.5 to 1.5 °C). By using this approach we were able to measure the MR of bats at roughly 5 °C intervals between 0 and 25 °C, and BMR at approximately 2 °C intervals between 27 and 37 °C. In total, data for all 10 individuals were used to calculate mean BMR, four bats were exposed to a T_a of >35 °C, and two bats remained normothermic below a T_a of 27 °C. Six bats entered torpor immediately and the two bats that initially remained normothermic also eventually entered torpor. Torpor bouts were obvious in time course plots of metabolic trials because MR and T_b fell rapidly to a reduced steady-state level. We defined the onset of torpor bouts as the beginning of these rapid reductions of MR and T_b .

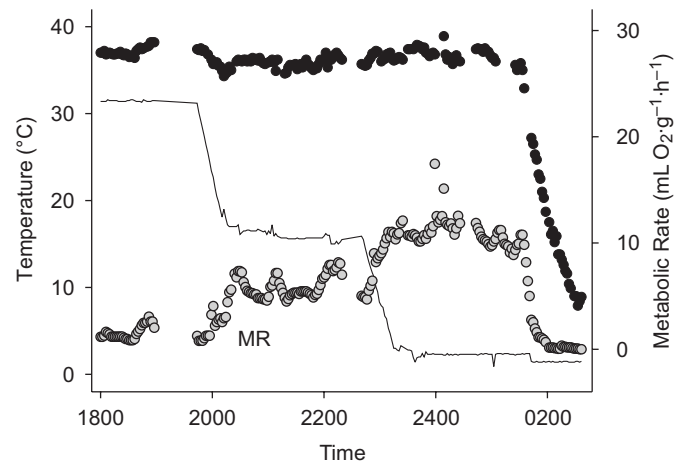
Statistical analysis

We used continuous two-phase regression (Nickerson et al. 1989) to determine the lower critical limit (T_{lc}) of the TNZ. We used ANCOVA, with body mass as a covariate, to compare whole animal BMR between bats captured in the spring and in the fall. Values presented are means \pm 1 SD. For null hypothesis testing, significance was assessed at the $\alpha = 0.05$ level. Analyses were conducted using SYSTAT® version 9 (SPSS Inc. 1998). Metabolic rates represent an animal's energy expenditure, so we report MR in energy units of milliwatts. However, to be consistent with previous studies we also report O_2 consumption values in millilitres O_2 per hour.

Results

A time course of the recording trial for one individual, which details the sampling protocol, is shown in Fig. 1. Bats either maintained a normothermic T_b for most of the recording session or went into torpor as soon as T_a was reduced below T_{lc} . Within the TNZ, T_b was 35.8 ± 2.1 °C and the minimum VO_2 was 16.98 ± 2.04 mL·h^{–1} at a mean body mass of 15.0 ± 1.4 g (1.13 ± 0.136 mL O_2 ·g^{–1}·h^{–1}, $n = 10$). Using a conversion factor of 0.179 mL O_2 ·h^{–1} per mW, this equates to a mass-specific BMR of 6.31 ± 0.76 mW·g^{–1}. There was no significant difference in BMR between bats captured in

Fig. 1. Time course of one metabolic recording trial for a nonreproductive big brown bat (*Eptesicus fuscus*). The solid line represents ambient temperature, solid circles represent T_b and shaded circles represent VO_2 .

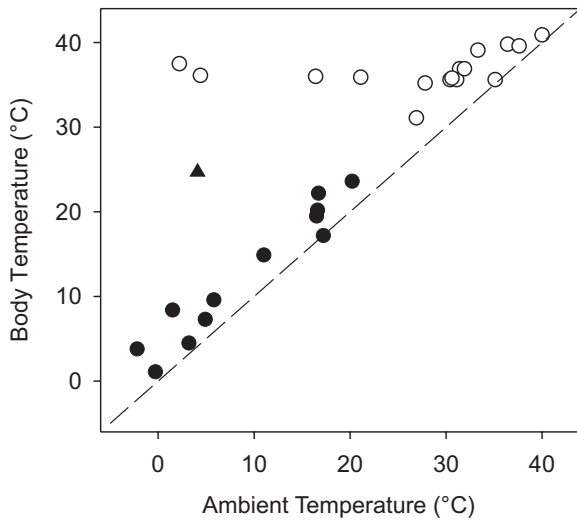


fall 2001 (6.63 ± 1.11 mW·g^{–1}) than in spring 2002 (6.16 ± 0.95 mW·g^{–1}; ANCOVA, $F_{[1,8]} = 0.6$, $p = 0.49$). There was no effect of T_a (ANCOVA, $F_{[1,6]} = 0.2$, $p = 0.72$) or body mass (ANCOVA, $F_{[1,6]} < 0.001$, $p = 0.93$) on BMR. Bats were also equally likely to enter torpor in both fall and spring (one bat remained normothermic at all temperatures in each season). Therefore, we pooled data from the two groups for the remaining analyses.

As is typical of heterothermic endotherms, below T_{lc} there were two distinct responses to reduced T_a (Fig. 2). Most bats were thermoconforming and entered torpor as soon as T_a fell below T_{lc} , allowing T_b to fall near T_a (Fig. 2). Two individuals initially remained normothermic as T_a was reduced and then entered torpor after several hours. Another individual aroused from torpor to near normothermic T_b when the temperature was lowered below zero. However, this bat defended T_b for only a few minutes and re-entered torpor almost immediately. Data for this individual were not used to calculate RMR. However, its stable TMR values, prior to arousal, were included in TMR calculations. The thermoneutral zone ranged from T_{lc} of 26.7 °C to upper critical temperature (T_{uc}) of about 36 °C. We did not calculate T_{uc} using two-phase regression because the regression line for points at high T_a was not statistically significant (Nickerson et al. 1989). However, above T_a of approximately 36 °C there was a clear increase in MR (Fig. 3A). Only two bats remained normothermic below 26 °C. Despite this small sample size, when we included the MR for the individual measured at T_a of 26.7 °C there was a strong linear relationship between T_a and mass-specific MR (in mW·g^{–1}) below T_{lc} (Fig. 3A, $RMR = 84.71 - 2.94T_a$, $r^2 = 0.76$, $F_{[1,3]} = 9.7$, $p = 0.05$). There was a sharp rise in C_{wet} above TNZ at approximately 33 °C (Fig. 3B). Wet thermal conductance of normothermic bats below 26 °C was 1.96 ± 0.78 mW·g^{–1}·°C^{–1} (0.35 ± 0.14 mL O_2 ·h^{–1}·g^{–1}·°C^{–1}) and was 0.20 ± 0.20 mW·g^{–1}·°C^{–1} (0.04 ± 0.04 mL O_2 ·h^{–1}·g^{–1}·°C^{–1}) for torpid bats.

One bat entered torpor but defended a T_b 15.3 °C above T_a (Fig. 2). Another did not reach a steady-state TMR at T_a of 1.5 °C in the time available for recording (Fig. 2). Data for these torpor bouts were excluded from torpid conductance

Fig. 2. Relationship between body temperature and ambient temperature for 10 bats during normothermia (open circles) and torpor (solid circles). The dashed line indicates a 1:1 relationship. The triangle represents data for one individual that defended a high T_b during torpor and which was excluded from later analyses. One data point for a torpid bat at $T_a = 1.5$ °C was also excluded from subsequent analyses because this individual likely did not reach steady-state TMR. Data for one individual at $T_a = 12.5$ °C is not included because the transmitter signal was lost during recording owing to temporary radio interference.



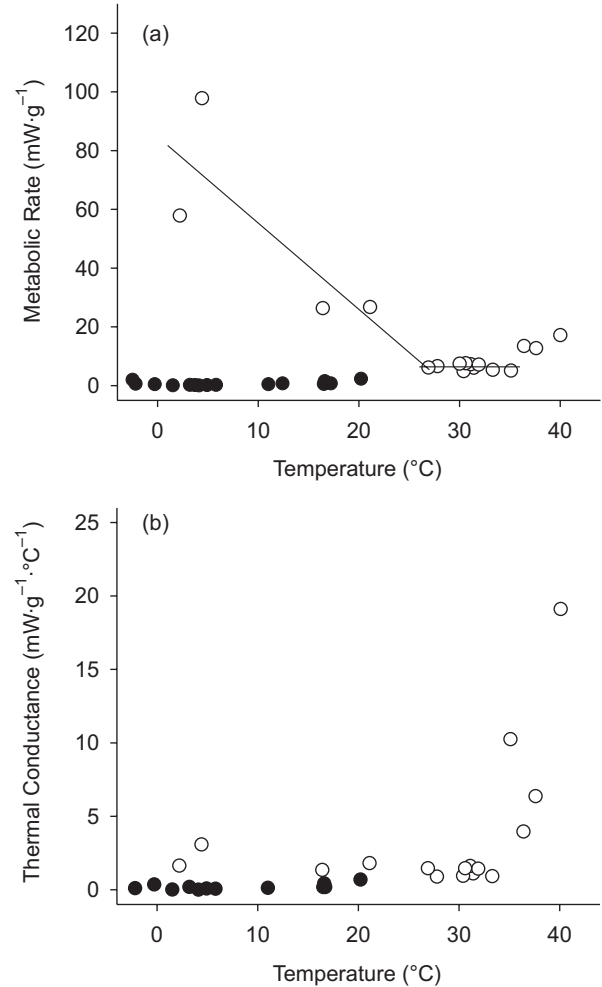
and all subsequent calculations. The differential between T_b and T_a during torpor at T_a below 10 °C was 2.6 ± 1.6 °C. The average minimum VO_2 recorded for four bats at T_a between 0 and 5 °C (mean = 3.5 ± 1.2 °C) was 0.408 ± 0.182 mL $O_2 \cdot h^{-1}$ at a mean body mass of 14.6 ± 1.5 g (0.028 ± 0.012 mL $O_2 \cdot g^{-1} \cdot h^{-1}$) and T_b of 4.3 ± 3.1 °C. This equates to a mass-specific TMR of 0.156 ± 0.070 mW \cdot g $^{-1}$. Below T_a of approximately 0 °C and at T_b of about 3.5 °C, bats increased TMR to defend a minimum threshold T_b . The minimum individual T_b we recorded was 1.1 °C at a T_a of -0.3 °C. Above T_a of approximately 0 °C and T_b of approximately 3.5 °C, there was a significant linear relationship between T_a and the logarithm of mass-specific TMR (mW \cdot g $^{-1}$, $\log(\text{TMR}) = 0.054T_a - 0.887$, $r^2 = 0.87$, $F_{[1,9]} = 61.7$, $p < 0.001$). There was also a significant relationship between T_b and \log mass-specific TMR (mW \cdot g $^{-1}$, Fig. 4, $\log(\text{TMR}) = 0.049T_b - 0.973$, $r^2 = 0.85$, $F_{[1,7]} = 40.7$, $p < 0.001$). We had a smaller sample size of T_b than T_a measurements for these analyses because implanted transmitter signals were lost occasionally owing to radio interference.

We did not calculate respiratory exchange ratios for torpid bats because the CO_2 analyser was not sensitive enough to detect a F_eCO_2 signal when CO_2 production dropped to very low levels during torpor. However, the respiratory exchange ratios of normothermic bats between approximately 26 and 40 °C ranged between 0.73 and 0.62, which indicates that all animals were post absorptive during metabolic trials and using fat as the metabolic substrate.

Discussion

Our objectives were to quantify energy expenditure during torpor and normothermia in big brown bats from the north-

Fig. 3. (a) Relationship between ambient temperature and metabolic rate for 10 big brown bats during normothermia (open circles) and torpor (solid circles). The solid horizontal line indicates the mean BMR. See text for RMR regression results. (b) Thermal conductance at different T_a for 10 big brown bats during normothermia (open circles) and torpor (solid circles).



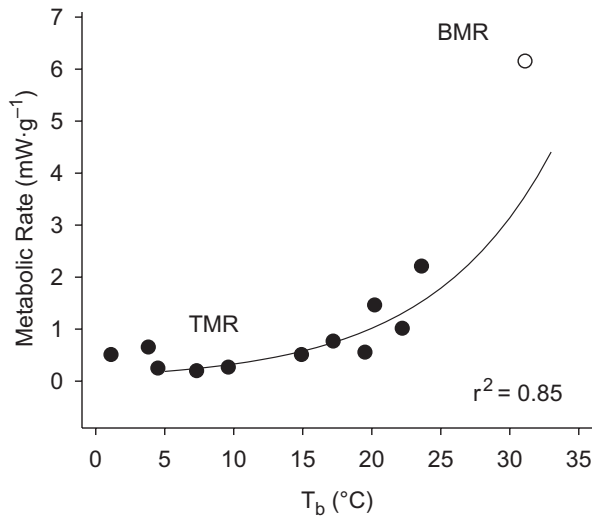
ern half of their range and to compare our results with previous data. This study is the first to rigorously quantify the thermal physiology of big brown bats and, to our knowledge, is the first to address intraspecific variation in thermal physiology data recorded for different populations of a temperate bat.

Basal metabolic rate

One important assumption associated with measurement of minimum maintenance metabolism is that animals are nonreproductive (McNab 1992). We ensured that bats met this assumption by restricting the study to the spring and fall. Some of the bats measured in the spring of 2002 may have been in the very early stages of pregnancy that we were unable to detect by palpation. However, any effect of this was so small as to be inconsequential because there was no difference in BMR values between bats captured in spring 2002 and those captured after lactation in September 2001.

Herreid and Schmidt-Nielsen (1966) did not explicitly report a value for BMR in their study. Kurta and Baker (1990)

Fig. 4. Relationship between metabolic rate and body temperature for 10 big brown bats during torpor bouts. Solid circles represent values for torpid bats. The solid line is the regression relationship between TMR and T_b (see text for regression results). The single open circle represents the BMR value for a bat at the lower boundary of the thermoneutral zone. This datum was not included in the regression analyses relating T_b with TMR.



calculated BMR from the regression equation relating T_a and RMR in Herreid and Schmidt-Nielsen's (1966) paper as $0.8 \text{ mL O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$, (A. Kurta, personal communication). However, Herreid and Schmidt-Nielsen (1966) did not define the TNZ (see below), so this value may be inaccurate. Hayssen and Lacy (1984) cited Herreid and Schmidt-Nielsen's (1966) BMR value of $1.20 \text{ mL O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$ for big brown bats, although no explanation is given on how they arrived at this value. Nevertheless, it more closely reflects the data presented in the former paper (Fig. 2 in Herreid and Schmidt-Nielsen 1966) and is consistent with our findings. Zoogeography and climate (Lovegrove 1996, 2000, 2003) or diet and feeding habits (McNab 1988, 1992; Speakman and Thomas 2003) may have implications for differences in BMR between mammal species, but there is no strong evidence that mass-specific BMR varies significantly among populations of the same species (Garland and Adolph 1991).

Geiser and Brigham (2000) found that mass-specific BMR in two species of Australian Vespertilionidae (*Nyctophilus geoffroyi* Leach, 1821 and *Nyctophilus gouldi* Tomes, 1858) was 65% of that predicted by Hayssen and Lacy's (1984) allometric equation for bats. Based on this, Geiser and Brigham (2000) and Geiser (2005) suggested that BMR may be lower in Australian bats than in microchiropterans generally, because of low primary productivity, variable climate, and unpredictable food availability in Australia. A low mass-specific BMR in mammals has been suggested as a consequence of living in zoogeographic regions with unpredictable climates, possibly to reduce energetic costs of maintenance metabolism during periods of food shortage and drought (Lovegrove 1996, 2000, 2003). However, residual BMR variation can also be explained by differences in diet (McNab 1988, 1992; Speakman and Thomas 2003) or could simply reflect phylogenetic affiliation (Elgar and Harvey 1987; Speakman and Thomas 2003). We found that the mass-

specific BMR of big brown bats was similar to that for *Nyctophilus* species at only 68% of that predicted by Hayssen and Lacy's (1984) allometric equation and 76% of that predicted by Speakman and Thomas's (2003) equation. Similar results have been found for the North American little brown bat (*Myotis lucifugus* (LeConte, 1831), 67% of predicted BMR; Hock 1951) and evening bat (*Nycticeius humeralis* (Rafinesque, 1818), 61% in spring and summer, 42% in fall and winter; Genoud 1993). Taken together, these data suggest that vespertilionids, in general, may be characterized by relatively low BMR. Further work is needed to address the allometry of BMR in bats, incorporating new data from the Vespertilionidae. This family is the most diverse among the Chiroptera (approximately 350 species), yet has been dramatically underrepresented in the physiological literature and in previous analyses of BMR (e.g., Hayssen and Lacy 1984; McNab 1988; Speakman and Thomas 2003). Even more critical is the need to resolve uncertainties surrounding phylogenetic relationships of bats to allow for phylogenetically independent analysis of residual mass-specific BMR variation.

Thermal neutral zone

Despite the similarity between the BMR values that we measured and those previously reported for big brown bats (Herreid and Schmidt-Nielsen 1966; Hayssen and Lacy 1984), the range of the thermal neutral zone was substantially different. Herreid and Schmidt-Nielsen (1966) found that O_2 consumption was lowest at $35 \text{ }^\circ\text{C}$ and increased immediately above and below this point. The upper critical temperature that we found was close to $35 \text{ }^\circ\text{C}$, but the TNZ was much broader with T_{lc} almost $10 \text{ }^\circ\text{C}$ lower. Close inspection of Herreid and Schmidt-Nielsen's (1966) Fig. 2 suggests that a thermal neutral point is not the best way to characterize the data, as VO_2 varied little between 33 and $38 \text{ }^\circ\text{C}$. Despite this interpretation, there remains a clear difference between T_{lc} for the previous study (approximately $33 \text{ }^\circ\text{C}$) and the value that we found ($26.7 \text{ }^\circ\text{C}$). Not surprisingly, thermal neutrality in mammals is a physiological trait that can vary within species, both seasonally and among populations that inhabit regions with different climates (Garland and Adolph 1991). Bats from the northern US Great Plains face much colder temperatures than those from a more southerly population in North Carolina. Bats from the colder site are likely to rely on physiological and morphological traits (e.g., fur thickness, pilomotor or vasomotor responses) that reduce T_{lc} and therefore save them energy. This hypothesis is supported by recent work on a population of adult female big brown bats from even farther north, in southern Alberta, which revealed a slightly broader TNZ with an even lower T_{lc} than the value that we found (L. Hollis, personal communication). Perhaps more convincing are differences in values for C_{wet} between bats from South Dakota and North Carolina. Herreid and Schmidt-Nielsen (1966) did not report C_{wet} , but based on the T_a and MR data presented in their Fig. 2 and assuming a constant T_b of $34 \text{ }^\circ\text{C}$ and T_a of $30 \text{ }^\circ\text{C}$, C_{wet} approximated $2.29 \pm 0.5 \text{ mW} \cdot \text{g}^{-1} \cdot \text{ }^\circ\text{C}^{-1}$ ($0.41 \pm 0.09 \text{ mL O}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1} \cdot \text{ }^\circ\text{C}^{-1}$, $n = 19$ data points). This value is significantly higher than the value that we found for the two South Dakota bats that remained normothermic at a T_a of below $30 \text{ }^\circ\text{C}$ ($1.96 \pm 0.78 \text{ mW} \cdot \text{g}^{-1} \cdot \text{ }^\circ\text{C}^{-1}$, Student's t test, $t = 2.2$, $p =$

0.04, $n = 6$ data points), although, clearly, our sample size of normothermic bats is small. Nonetheless, this is consistent with expectations based on geographic variation in climate between the two sites.

Variation in thermal traits within a species could be the manifestation of genetic differences between populations or a regional acclimatization effect. Both inheritance and regional acclimatization cause intraspecific physiological variation between populations of other taxa (Spicer and Gaston 1999). These two possibilities could be teased apart by a series of common garden experiments, exposing bats from each site, to ambient conditions at the other site to see if individuals are capable of acclimatizing to new conditions (Spicer and Gaston 1999). Whether genetically inherited or not, if they do exist, population differences in thermal tolerance will likely be especially pronounced for female bats, because they typically avoid deep torpor while rearing young to maximize offspring growth rate (Racey and Swift 1981; Tuttle and Stevenson 1982; Wilde et al. 1999; Willis 2005). Therefore, mechanisms that reduce T_{lc} and thermal conductance may be especially important for females in cold climates.

It is possible that differences from the previous study in T_{lc} and C_{wet} could reflect methodological differences between studies. For example, Herreid and Schmidt-Nielsen (1966) studied both male and female bats and kept animals in captivity for up to several months, whereas we studied only females, minimized time in captivity to no more than 5 days, and used a newer model of O_2 analyzer and different respirometry system. However, we did not observe a difference in BMR between studies, which we might have expected if methodological effects accounted for the differences in our results. Only variables predicted to vary under different climatic conditions (i.e., T_{lc} and C_{wet}) differed between studies. Another possibility is that some of the variance in mass-specific C_{wet} could reflect inter- and intra-individual variability in the body mass – surface area ratio. However, this variation will be small relative to that caused by changes in metabolic heat production, the $T_b - T_a$ differential, or body mass (Schmidt-Nielsen 1997), and more to the point, there is no reason to expect that this variation will differ between bats from the two studies. Thus our findings suggest, but do not confirm, the possibility that thermal physiology varies among populations of big brown bats. Our findings also reinforce the need to repeat metabolic physiology studies within species, to address intra-specific population variation in physiological traits, and to account for experimental variation between studies.

TMR and RMR

All but two bats entered torpor as soon as T_a dropped below about 27 °C, and once in torpor, bats rarely aroused. This was surprising given that much of each recording trial took place during the active phase when we predicted bats would remain normothermic, at least for a short time. In some free-ranging bats, torpor bouts are most common in the early morning when T_a is at a minimum (e.g., Turbill et al. 2003a, 2003b; Willis 2005), so we predicted that bats would remain normothermic for at least part of the night. It is possible that effects of captivity influenced the propensity for bats to use torpor (Geiser et al. 2000), but this seems un-

likely, as we took care to ensure bats were held captive for as short a period as possible before performing metabolic trials.

The energy savings associated with torpor in this study are consistent with previous results for microchiropteran bats (e.g., Studier 1981; Genoud 1993; Geiser and Brigham 2000). Minimum energy expenditure during torpor was as low as 2% of BMR and 0.2% of RMR at the same T_a . The propensity for big brown bats to use torpor in the laboratory, and the large energy savings associated with torpor, are consistent with results from field studies (e.g., Hamilton and Barclay 1994; Lausen and Barclay 2003) and highlight the important role of torpor for balancing the daily energy budget in this species.

Unfortunately, virtually all bats entered torpor as soon as T_a fell below T_{lc} , resulting in a very small sample size of normothermic bouts. However, despite this small sample size, there was a significant linear relationship between T_a and RMR, and the slope of this relationship agrees closely with data collected from a larger sample of normothermic female big brown bats from southern Alberta (L. Hollis, personal communication). Therefore, we argue that our RMR data provide a reasonable estimate of the relationship between RMR and T_a for the population of big brown bats in southeastern South Dakota.

Conclusions

Our results for big brown bats and data from other vespertilionids suggest that BMR in this family may be relatively low compared with that of other bats. Further work addressing this relationship is important. We found no difference in BMR between bats from a northern site and bats previously studied from North Carolina (Herreid and Schmidt-Nielsen 1966), but there were differences in the T_{lc} and thermal conductance of bats from the two study sites.

Meta-replication (i.e., repetition of studies addressing the same question) is an important emerging concept in field-based wildlife research. For example, replicating studies of habitat use by a certain species throughout its range is important to determining if patterns observed in one area are widely applicable to a broad-based management strategy (Johnson 2002). Our data illustrate the importance of replicating physiological studies within species, as well. A meta-replication approach is important because it can help address potential differences in physiological variables among populations and help authors of review papers account for variation between studies.

Acknowledgements

We are grateful to Azure-Dee Farago and Kristen Kolar for assistance in the laboratory and field. Fritz Geiser and Al Kurta provided excellent comments that dramatically improved an earlier draft of the manuscript, and we are also very grateful to Christine Cooper and Phil Withers for their helpful advice. Funding for this project was provided by a Natural Sciences and Engineering Research Council (NSERC) Canada Discovery grant to R.M.B. and NSERC postgraduate scholarships to J.E.L. and C.K.R.W.

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