A Technique for Modelling Thermoregulatory Energy Expenditure in Free-ranging Endotherms

Craig K. R. Willis,[1] Jeffrey E. Lane,[1] Eric T. Liknes,[2] David L. Swanson,[2]
and R. Mark Brigham[1]
[1] University of Regina, Department of Biology, Regina, Saskatchewan, Canada
[2] University of South Dakota, Vermillion, South Dakota, USA

Abstract. The development of small temperature-sensitive radiotransmitters has allowed researchers to measure skin temperature (T_{sk}) or body temperature (T_b) and quantify temporal patterns of torpor use by free-ranging animals (e.g., number of bouts, length of bouts). However, simply addressing temporal patterns of heterothermy limits the scope of potential research questions because temporal patterns may not correlate with energy savings. Our objective was to devise a predictive model for thermoregulatory energy expenditure, using T_{sk} and ambient temperature (T_a) as independent variables. We used open-flow respirometry and temperature telemetry to quantify the metabolic rate (MR) and T_{sk} of big brown bats (*Eptesicus fuscus*) over a range of T_a from 0° C to 40° C. We calculated regression equations relating T_a, T_{sk}, and MR for each of four different thermoregulatory states: steady-state normothermia, cooling, steady-state torpor, and warming. Our approach may prove useful for quantifying thermal energetics in other free-ranging heterothermic endotherms.

Introduction

Much of our understanding of the energetics of torpor in heterothermic endotherms is based on laboratory research (e.g., Geiser and Brigham, 1999; Hosken, 1997). However, within species, torpor patterns can differ markedly between free-ranging vs. captive (Geiser et al., 2000) or captive-bred (Geiser and Ferguson, 2001) individuals. Field research quantifying torpor patterns in

---

Barnes BM, Carey HV, eds (2004) *Life in the Cold: Evolution, Mechanisms, Adaptation, and Application. Twelfth International Hibernation Symposium.* Biological Papers of the University of Alaska, number 27. Fairbanks, Alaska, USA: Institute of Arctic Biology, University of Alaska.

free-ranging animals is essential if we are to address questions about the adaptive value of torpor in the wild (Willis and Brigham, 2003). Despite this, comparatively few field studies have addressed the energetics of torpor in free-living endotherms, largely because obtaining real-time measurements of metabolic rate (MR) is logistically difficult for most species in the field (but see Dausmann et al., 2000).

The development of temperature-sensitive radiotransmitters has contributed a great deal to our understanding of temporal patterns of torpor in free-ranging animals. For studies of relatively large or small sedentary animals, transmitters can be surgically implanted to measure body temperature (T_b). For smaller animals, external transmitters must be used to record skin temperature (T_{sk}) because the size and reception range limitations of implanted tags make them impractical (e.g., Hamilton and Barclay, 1994; Barclay et al., 1996; Brigham et al., 2000). Both types of transmitters can provide valuable information about temporal patterns of torpor in free-living animals (Chruszcz and Barclay, 2002; Lausen and Barclay, 2003). For example: What ambient conditions are associated with frequent torpor use? What is the frequency of torpor use at different times of year? However, quantifying temporal patterns of torpor tells us little about the energetic savings associated with heterothermy because, clearly, not all torpor bouts are energetically equivalent. For example, at a given ambient temperature (T_a), a short deep bout of torpor will save a small endotherm much less energy than a shallow bout of longer duration (Willis and Brigham, 2003). In this circumstance, simply quantifying the frequency of torpor use may limit research questions about animals' physiological decisions. What is the level of energy savings associated with torpor use in the wild? How does this level of savings balance against potential costs of torpor use? How do reproductive status and life history traits influence these trade-offs? Without some way to estimate MR in free-ranging animals, we cannot evaluate the real costs and energetic benefits of torpor use under different circumstances.

One approach to addressing this issue is to use laboratory data to model thermoregulatory and basal energy expenditure (hereafter thermal energy expenditure) based on independent variables that can be readily measured in the field (i.e., T_a and T_b or T_{sk}). Such models would need to address several features of normothermy and torpor. Within the thermal neutral zone (TNZ), predicting thermal energy expenditure is simple because model-predicted costs should equal basal metabolic rate (BMR; for a resting, post-absorptive, nongrowing animal). Below the lower critical temperature (T_{lc}) of the TNZ, thermal energy expenditure, or resting metabolic rate (RMR), scales linearly with T_a and T_b for normothermic animals. During torpor, the logarithm of torpid metabolic rate (TMR) scales linearly with both T_a and T_b. Therefore, for both torpid and normothermic animals, linear regression models could describe these relationships. Any predictive model for the energy savings associated with torpor must also address the fluctuations in energy expenditure that occur during cooling and active and passive warming at the beginning and end of a torpor bout. Recent evidence indicates that free-ranging mammals and birds may rely on ambient heat to arouse from torpor passively (Brigham et al., 2000; Geiser et al., this volume; Körtner and Geiser, 2000; Lovegrove et al., 1999; Willis, 2003). For example, tree cavity-roosting big brown bats, (*Eptesicus fuscus*) and foliage-roosting hoary bats (*Lasiurus cinereus*) are exposed to dramatic diurnal fluctuations in temperature within or at their roost sites and may rely on rising roost temperatures to arouse from morning torpor bouts (Willis, 2003). Geiser and Drury (2003) provided a heat source to torpid dunnarts (*Sminthopsis macroura*) and demonstrated that average energy expenditure during passive arousal was only about 70% of BMR. They also showed that the relationship between T_b and MR during passive warming in thermoconforming animals is nearly identical to the relationship between T_b and MR for thermoconforming animals in steady-state torpor (Geiser and Drury, 2003). Thus, predicting MR associated with arousal from torpor in many free-ranging endotherms may simply be a matter of relying on the relationship between T_{sk} and steady-state TMR.

Our objective was to use open-flow respirometry and temperature telemetry data to devise a predictive model of thermoregulatory energy expenditure for a small heterothermic endotherm, *E. fuscus*. We used T_{sk} and T_a as independent variables because they can be readily measured for free-ranging individuals. We selected T_{sk}, as opposed to T_b, because T_{sk} is much more easily measured in free-ranging bats due to the very small reception ranges of surgically implanted radiotransmitters.

Methods

We captured bats using mistnets in riparian woodlands of southeastern South Dakota, near the town of Vermillion (42° 47' N, 97° 0' W). We performed all trials at the University of South Dakota on five nonreproductive/post-lactating female bats from 2–13 September 2001, and five females that were not palpably pregnant from 1–10 May 2002. All animals were adults. Mean mass was 15.0 ± 1.4 g. Following capture, bats were held in cloth bags, exposed to natural photoperiod, and provided access to water every few hours. Within one day of capture, a temperature-sensitive radiotransmitter (0.75 g BD-2ATH, Holohil Systems Ltd., Carp ON) was glued to the skin between the scapulae, after trimming a small (0.5–1 cm^2) patch of fur. We used a hand-held telemetry receiver (R-1000, Communication Specialists Inc., Orange, CA) and five-element yagi antenna (AF Antronics, Inc. Urbana, IL) to detect transmitter signals 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 two minutes we recorded the time required for a transmitter to emit 11 pulses (i.e., 10 inter-pulse intervals). We later calculated the average inter-pulse interval for each recording and determined T_{sk} from calibration curves provided by the manufacturer.

Food was withheld for 12 hours prior to experiments to ensure bats were post-absorptive during recording. We measured each bat's mass to the nearest 0.01 g using an electronic balance (C305-S, Ohaus, Pine Brook, NJ) immediately before and after metabolic trials and assumed a linear decrease in body mass to calculate mass-specific metabolic rates. We used open flow respirometry to determine MR over a range of T_a between 0° and 40° C. For details of the equipment and protocols used to record BMR, RMR and TMR see Willis (2003). In brief, we recorded oxygen consumption at two to four different test T_a for each of the 10 bats using an oxygen analyser (S-3A, Ametek, Paoli, PA). Bats were exposed to each test T_a for one hour, and the minimum 10-minute average MR recorded during that hour was calculated. We recorded BMR first for each individual at a T_a within the TNZ (30.9 ± 2.4, range 27.0–34° C). For two of the bats we then increased temperatures above 35° C to determine the upper boundary of the TNZ. These individuals were not used to record MR at temperatures below T_{lc}. For the remaining eight bats, we decreased the temperature following BMR recording to obtain RMR and TMR values. Overall, we recorded steady-state MR of bats at roughly 5° C intervals between 0 and 25° C, and BMR at ca. 2° C intervals between 27 and 34° C. Data for all 10 bats were used to calculate mean BMR; two bats remained normothermic below $T_a = 27°$ C and six bats entered torpor. We also recorded instantaneous metabolic rates concurrent with T_{sk} and T_a during periods of cooling during entry into torpor as well as active and passive warming from torpor. In total we recorded four bouts of passive warming from four bats, four bouts of active warming from three bats, and 23 bouts of cooling from eight bats. Twelve cooling bouts were recorded when bats entered torpor at low T_a, and 11 were recorded from thermoconforming bats that allowed T_{sk} and MR to decline as soon as T_a was reduced.

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, OH) filled with ethylene glycol and water. Concurrent with T_{sk} and fractional oxygen concentration (FeO_2) of excurrent air, we measured T_a in the metabolic chamber every two minutes using a copper constantan thermocouple and thermocouple thermometer (Model 8500-40, Cole Parmer).

Model Equations

Values are presented as means ± 1 S.D. All model regression analyses were conducted using Systat (Version 9, SPSS Inc.). Non-normal data were log-transformed and significance was assessed at $p < 0.05$. For our predictive model, when ambient temperature was greater than T_{lc} we assumed MR = BMR. Below T_{lc} bats were either torpid or normothermic. For normothermic bats we used a General Linear Model (GLM) to calculate the relationship between the independent variables T_a and T_{sk} and the dependant variable RMR. During steady-state torpor, T_a and T_{sk} were highly correlated (Pearson r = 0.99), so we eliminated T_{sk} from the analysis. We used linear regression to quantify the relationship between T_a and the logarithm of TMR because T_{sk} was a linear function of T_a (see below). We identified individual bouts of cooling and warming by inspecting each bat's time course of MR and T_{sk} for each recording trial. Cooling bouts were divided into two categories: (1) those during which T_a was constant (i.e., cold) when bats entered torpor; and (2) those during which Ta was declining and T_{sk} and MR of thermoconforming bats decreased simultaneously with T_a. Similarly, warming bouts were divided into (1) those in which warming was spontaneous with T_a remaining constant during the warm-up period (i.e., active warming); and (2) those in which T_a was increasing during the warm-up period. The latter category of warming bouts occurred because for some trials we increased the temperature in the metabolic chamber at the end of a recording session while still recording MR and T_{sk}. For entry into torpor, we calculated total energy expenditure for each cooling bout. We used GLM to quantify the relationship between energy expenditure for the bout and five independent variables: Bout duration (Dur, minutes), starting T_a (T_a), change in T_a during the bout ($^{\Delta}T_a$), starting T_{sk} (T_{sk}), and change in T_{sk} during the bout ($^{\Delta}T_{sk}$). For the analysis of cooling bouts at a constant T_a, $^{\Delta}T_a$ was not included. We were not able to use GLM to model energy expenditure associated with warming bouts because of a small sample size.

For passive warming bouts we relied on our model equation for TMR (Geiser and Drury, 2003; see above). To predict the cost of active warming we calculated the average metabolic rate for all the warming bouts and used this value in our calculation of daily energy expenditure.

To demonstrate the applicability of the model to field data, we used it to predict daily energy expenditure values based on roost T_a and T_{sk} for a hypothetical free-ranging bat. Roost T_a was based on a typical temperature time course recorded from an *E. fuscus* roost tree in the Cypress Hills of Saskatchewan, Canada. Similarly, T_{sk} was based on a typical time course recorded from bats in the Cypress Hills (Fig. 1). The hypothetical T_{sk} time course included a deep bout of early morning torpor and an early evening shallow torpor bout. For comparison we also calculated the predicted energetic expenditure for a hypothetical bat that remained normothermic at T_{sk} = 33° C for the entire 24-hour period. We used a conversion factor of 20.083 J per mL O_2.

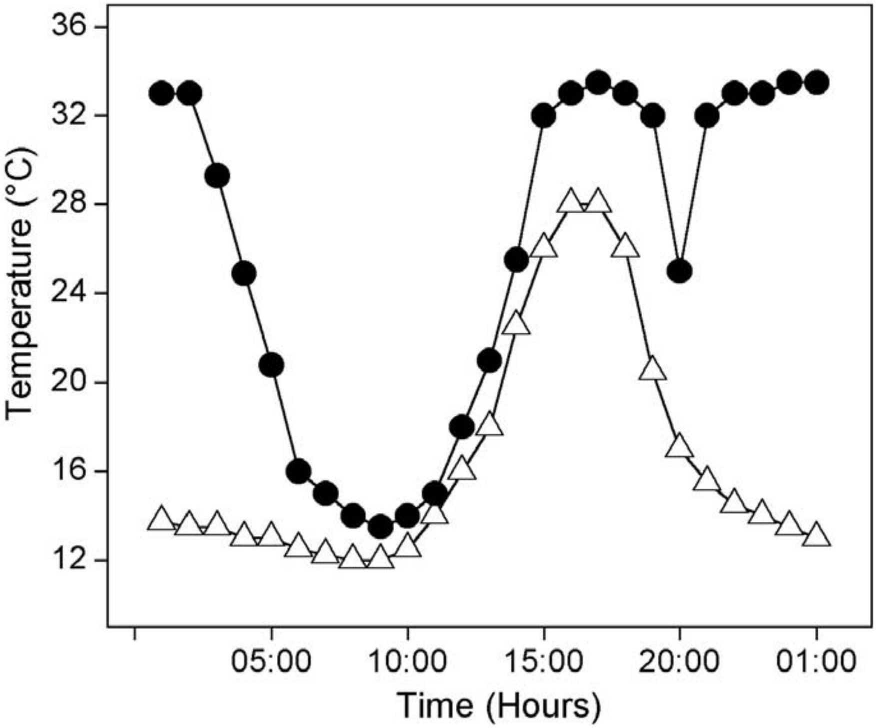

Fig. 1. Time course of T_a (open triangles) and T_{sk} (closed circles) used to calculate daily energy expenditure for a hypothetical free-ranging bat.

Results

As reported elsewhere, mass-specific BMR for *E. fuscus* is 1.14 ml $O_2\ g^{-1}\ hr^{-1}$ (Willis, 2003). Despite a small sample size (n = 6 datapoints from four bats), the general linear model predicting the RMR of normothermic bats below the T_{lc} of 27° C was significant ($r^2 = 0.98$, $F_{2,2} = 52.1$, $p = 0.019$), and the model equation for these bats was:

$$\text{Mass-Specific RMR} = (0.105 \pm 0.05)T_{sk} - (0.038 \pm 0.005)T_a - (2.22 \pm 1.72) \quad (1)$$

During steady-state torpor, above $T_a = 0°$ C, there was a significant linear relationship between T_{sk} and T_a ($r^2 = 0.98$, $F_{1,9} = 225.9$, $p < 0.001$), so we only used T_a as an independent variable to describe TMR. There was a significant linear relationship between the logarithm of TMR and T_a ($r^2 = 0.92$, $F_{1,9} = 96.5$, $p < 0.001$). The TMR model equation was:

$$\text{Log Mass-specific TMR} = (0.062 \pm 0.006)T_a - (1.73 \pm 0.096) \quad (2)$$

During constant T_a cooling during entry into torpor, the general linear model including Dur, T_a, T_{sk}, and $^{\Delta}T_{sk}$ as independent variables was significant ($r^2 = 0.92$, $F_{4,7} = 18.8$, $p = 0.001$). The constant T_a cooling model equation was:

$$\text{Mass-specific Energy Expenditure} = (0.010 \pm 0.004)\text{Dur} - (0.063 \pm 0.022)T_a + (0.075 \pm 0.020)T_{sk} - (0.053 \pm 0.027)^{\Delta}T_{sk} - (0.648 \pm 0.145) \quad (3)$$

For bouts of cooling when the T_{sk} of thermoconforming bats decreased concurrently with T_a, the general linear model was also significant ($r^2 = 0.91$, $F_{5,5} = 9.6$, $p = 0.01$). In this case the model included the variables above as well as $^{\Delta}T_a$ and the equation was:

$$\text{Mass-specific Energy Expenditure} = (-0.008 \pm 0.003)\text{Dur} + (0.086 \pm 0.019)T_a - (0.028 \pm 0.010)^{\Delta}T_a - (0.022 \pm 0.014)T_{sk} - (0.043 \pm 0.021)^{\Delta}T_{sk} - (0.465 \pm 0.160) \quad (4)$$

Due to a small sample size of warming bouts, we were unable to devise a predictive model for energetic costs of arousal from torpor. However, we did calculate metabolic rates and estimate energetic costs during passive and active rewarming (Table 1). The average MR of bats was 1.04 times BMR during passive

Table 1. Average metabolic rates (MR) recorded during bouts of active (n = 4) and passive (n = 4) rewarming from torpor, as well as the warming bout duration (Dur), starting T_a (T_a), change in T_a ($^{\Delta}T_a$), starting T_{sk} (T_{sk}), and change in T_{sk} ($^{\Delta}T_{sk}$).

	MR ($mL0_2g^{-1}hr^{-1}$)	**Dur (min)**	**T_a (° C)**	**$^{\Delta}T_a$ (° C)**	**T_{sk} (° C)**	**$^{\Delta}T_{sk}$ (° C)**
Passive	1.86 ± 1.91	34 ± 20	14.3 ± 8.6	17.1 ± 9.1	13.3 ± 7.5	14.1 ± 5.8
Active	9.43 ± 2.68	22 ± 10	4.4 ± 2.1	0	7.5 ± 4.8	19.5 ± 5.8

warming and 8.27 times BMR during active warming. We used the average active warming MR to predict energy expenditure during active warming for our hypothetical bat example (see below). For passive warming we used our equation relating TMR and T_a.

Combining these equations and based on values from Fig. 1, we calculated a time course of energy expenditure for each hour of a 24-hour period (Fig. 2) and used these values to calculate daily energy expenditure. The predicted daily energy expenditure was 22.8 kJ/day for a 21 g bat that employed two bouts of torpor, as shown in Fig. 1. The predicted daily energy expenditure for a 21 g bat defending T_{sk} at 33° C over the same 24 hour T_a profile was much greater at 47 kJ/day (Fig. 2).

Discussion

Our predictive model provides a reasonable estimate of the costs of thermoregulation and basal metabolism based on T_a and T_{sk}, independent variables that can be readily measured for free-ranging animals. Using doubly labelled water (DLW) to quantify field metabolic rate, Kurta et al. (1990) reported daily energy expenditures of up to 70 kJ/day for free-ranging big brown bats, much higher than the values we calculated. This makes sense in light of energetic costs we did not consider (e.g., foraging and flight, digestion, reproduction) but indicates that T_{sk} and T_a can be used to calculate accurate estimates of thermal energy expenditure for free-ranging animals. Future studies employing DLW both in the lab and the field are important to validating the accuracy of this type of model.

Predicted energetic expenditure was nearly twice as high for our hypothetical bat defending T_{sk} at 33° C compared to energy expenditure if the bat used torpor. This difference illustrates one potential application of our modeling approach. Previous studies have addressed temporal patterns of torpor use in free-ranging animals (e.g., Brigham et al., 2000; Chruszcz and Barclay, 2002; Lausen and Barclay, 2003) but none have quantified the energetic implications of torpor use or avoidance on a given day. Addressing detailed questions about costs and benefits of torpor under different circumstance requires that we estimate energetic benefits associated with different depths and durations of torpor. For example, defending a high T_{sk} was obviously costly for our hypothetical bat. However, if the bat was pregnant or lactating, this energetic cost could be balanced by the potential selective benefit of an increased offspring growth rate. Testing this hypothesis also requires specific information about selective benefits of rapid offspring growth, but quantifying energy expenditure is a necessary first step.

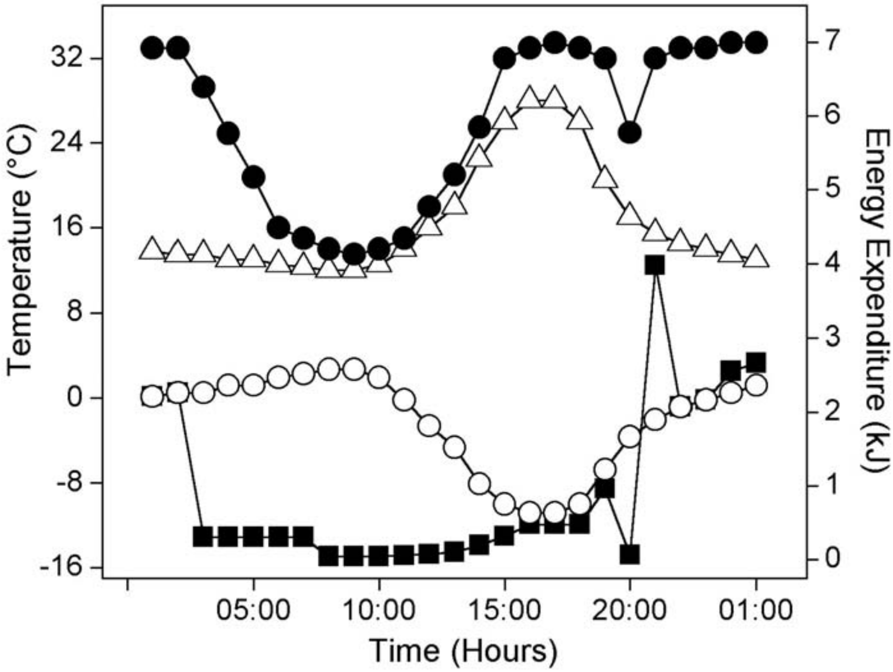

Fig. 2. Time course of T_a and T_{sk}, as shown in Fig. 1, along with two time courses of predicted energy expenditure. Open circles represent model-predicted values for energy expenditure calculated for a hypothetical bat that remained normothermic for the entire 24-hour period at the T_a shown. Closed squares represent model-predicted values calculated for the same bat but based on both the T_a and T_{sk} time courses shown. For the purpose of this illustration the energetic cost of the entire cooling bout between 02:00 and 05:00 was divided evenly between each hour.

The most obvious flaw in our model is the prediction of the energetic costs associated with active warming. We have included a crude estimate of warming costs in our calculation of daily energy expenditure, but it is an overestimate because it does not incorporate any variation in T_a or T_{sk} and is an average of active arousals, which included long warming bouts from deep torpor. It would make little sense for the bat in our hypothetical example to employ the shallow evening torpor bout because the costs of active warming from this bout far outweigh the energetic benefits (Fig. 2). A much larger sample size of spontaneous rewarming bouts over a wide range of T_a and T_{sk} is required to model this relationship.

Our approach is clearly not a replacement for techniques such as DLW, which is well-suited to integrating field metabolic rates averaged over a day or several days. However, this type of model may prove useful in quantifying real-time temporal patterns of energy expenditure and savings associated with torpor and other physiological states in heterothermic endotherms.

Acknowledgements

We are grateful to Azure-Dee Farago and Kristen Kolar for help in the lab and field. Fritz Geiser and an anonymous reviewer provided very helpful comments. Financial support was provided by NSERC (Canada) Discovery Grants to RMB and a scholarship to CKRW.

References

Barclay RMR, Kalcounis MC, Crampton LH, Stefan C, Vonhof MJ, Wilkinson L, Brigham RM (1996) Can external radiotransmitters be used to assess body temperature and torpor in bats. *J Mammal* 77:1102–1106.

Brigham RM, Körtner G, Geiser F, Maddocks TA (2000) Seasonal use of torpor by free-ranging Australian owlet nightjars. *Physiol Biochem Zool* 73:613–620.

Chruszcz BJ, Barclay, RMR (2002) Thermoregulatory ecology of a solitary bat, *Myotis evotis* roosting in rock crevices. *Funct Ecol* 16:18–26.

Dausmann KH, Ganzhorn JU, Heldmaier G (2000) Body temperature and metabolic rate of a hibernating primate in Madagascar: Preliminary results from a field study. In Heldmaier KM (ed), *Life in the Cold: Proceedings of the 11th International Hibernation Symposium, 13–18 August 2000, Jungolz Austria.* Berlin: Springer Verlag. Pp. 41–47.

Geiser F, Brigham RM (1999) Torpor, thermal biology, and energetics in Australian long-eared bats (*Nyctophilus*). *J Comp Physiol B* 153–162.

Geiser F, Drury RL (2003) Radiant heat affects thermoregulation and energy expenditure during rewarming from torpor. *J Comp Physiol B* 173:55–60.

Geiser F, Ferguson C (2001) Intraspecific differences in behaviour and physiology: Effects of captive breeding on patterns of torpor in feathertail gliders. *J Comp Physiol* 171:569–576.

Geiser F, Holloway JC, Körtner G, Maddocks TA, Turbill C, Brigham RM (2000) Do patterns of torpor differ between captive and free-ranging mammals and birds? In Heldmaier KM (ed), *Life in the Cold: Proceedings of the 11th International Hibernation Symposium, 13–18 August 2000, Jungolz Austria.* Berlin: Springer Verlag. Pp. 95–102.

Hamilton IM, Barclay RMR (1994) Patterns of daily torpor and day-roost selection by male and female big brown bats (*Eptesicus fuscus*). *Can J Zool* 72: 744–749.

Hosken DJ (1997) Thermal biology and metabolism of the greater long-eared bat, *Nyctophilus major* (Chiroptera: Vespertilionidae). *Austral J Zool* 45: 145–156.

Körtner G, Geiser F (2000) Torpor and activity patterns in free-ranging sugar gliders, *Petaurus breciveps* (Marsupialia). *Oecologia* 123:350–357.

Kurta A, Kunz TH, Nagy KA (1990) Energetics and water flux of free-ranging big brown bats (*Eptesicus fuscus*) during pregnancy and lactation. *J Mammal* 71:59–65.

Lausen CL, Barclay RMR (2003). Thermoregulation and roost selection by reproductive female big brown bats (*Eptesicus fuscus*) roosting in rock crevices. *J Zool (Lond)* 260:235–244.

Lovegrove BG, Körtner G, Geiser F (1999) The energetic cost of arousal from torpor in the marsupial *Sminthopsis macroura:* benefits of summer ambient temperature cycles. *J Comp Physiol B* 169:11–18.

Willis CKR (2003) Physiological Ecology of Roost Selection in Female, Forest-living Big Brown Bats (*Eptesicus fuscus*) and Hoary Bats (*Lasiurus cinereus*). PhD Thesis, University of Regina. Regina, SK, Canada.

Willis CKR, Brigham RM (2003) Defining torpor in free-ranging bats: Experimental evaluation of external temperature-sensitive radiotransmitters and the concept of active temperature. *J Comp Physiol B* 173:379–389.