

Sex differences in the thermoregulation and evaporative water loss of a heterothermic bat, *Lasiurus cinereus*, during its spring migration

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Summary

This study quantifies sex differences in thermoregulation and water loss of a small (20–35 g) insectivorous heterothermic mammal, the hoary bat *Lasiurus cinereus*, during its spring migration. We measured body temperature, metabolic rate and evaporative water loss, and calculated wet thermal conductance, for bats exposed to air temperatures ranging from 0 to 40°C for periods of 2–5 h. Pregnant females maintained normothermic body temperatures ($35.7 \pm 0.7^\circ\text{C}$; mean \pm S.E.M.) independent of air temperature. In contrast, males became torpid during the majority (68%) of exposures to air temperatures $<25^\circ\text{C}$. The thermal neutral zone (TNZ) ranged between approximately 30°C and 34°C in both sexes and, within the TNZ, females had lower mass-specific metabolic rates ($6.1 \pm 0.2 \text{ mW g}^{-1}$) than males ($9.0 \pm 0.9 \text{ mW g}^{-1}$). Wet thermal conductance values in torpid bats ($0.7 \pm 0.5 \text{ mW g}^{-1} \text{ deg}^{-1}$) were lower than those of

normothermic individuals ($1.1 \pm 0.3 \text{ mW g}^{-1} \text{ deg}^{-1}$). Mass-specific rates of evaporative water loss in males were consistently higher than in females at most air temperatures and rates of water loss in torpid bats were $63 \pm 6\%$ of normothermic values. These results suggest that male and pregnant female *L. cinereus* employ different thermoregulatory strategies during their spring migration. Females defend normothermic body temperatures, presumably to expedite embryonic growth, while males use torpor, presumably to minimize energy and water deficits. These variable thermoregulatory strategies may reflect continental differences in the summer distribution of the sexes.

Key words: Chiroptera, thermoregulation, energetics, body temperature, metabolic rate, conductance, evaporative water loss, torpor, sex difference, hoary bat, *Lasiurus cinereus*.

Introduction

Faced with the challenges of maintaining positive energy and water balance under variable environmental conditions, small endotherms often forgo the metabolic maintenance of high body temperatures by entering torpor (Lyman et al., 1982). Temperate insectivorous bats of the family Vespertilionidae are well known for such thermal lability (Lyman, 1977). The ubiquity of torpor among vespertilionid bats likely relates to the problems associated with the metabolic demands of living in temperate climates and the ecological limitations of feeding on aerial insects. Small body size, which confers a limited ability to store vital resources, when combined with high mass-specific rates of energy and water flux, as well as an energetically expensive mode of locomotion, can place significant demands on the ability of bats to balance energy and water budgets (Ewing et al., 1970; Henshaw, 1970; Licht and Leitner, 1967; Lyman, 1977; McNab, 1969). This can be especially true when bats are faced with unpredictable food resources or challenging thermal and hygric environments. When normothermic, bats must balance their water and energy budgets for periods of minutes to hours.

Torpor provides a temporary release from these short-term demands and allows bats to maintain energy balance over longer periods on limited endogenous resources.

Within a given species, however, torpor may not benefit each sex to the same degree. Differences are likely to be particularly pronounced during the periods when females are pregnant or lactating. Insectivorous bats produce offspring that exhibit some of the slowest rates of fetal development, and relatively longest periods of *postpartum* dependence known among mammals (Eisenberg, 1981; Hayssen, 1993; Kunz and Stern, 1995). Once weaned, juvenile bats must also learn to forage efficiently, and accumulate adequate energy stores before environmental conditions become unfavorable and prompt either migration or hibernation. For temperate species, these activities must occur within a relatively short period of time when climate and resource availability are amenable to successful reproduction. Fetal development and *postpartum* growth in bats are temperature-dependent processes that cooler temperatures generally retard (Kunz and Stern, 1995; Racey, 1973; Racey and Swift, 1981). Although torpor can ameliorate

both energy and water demands in reproductive females (Wilde et al., 1999), the associated low body temperatures would lengthen developmental periods and potentially lead to increased maternal and juvenile mortality (Kunz and Hood, 2000).

Male bats face different energy and time constraints compared to reproductive females (Barclay, 1991). Current evidence indicates that the greatest energy demand in males coincides with spermatogenesis (Racey and Entwistle, 2000) and, although considerable resources may be diverted toward sperm production, it is unlikely that reproductive costs for males exceed those of females (Gittleman and Thompson, 1988). In addition, the timing of reproductive demands differs between the sexes. Spermatogenesis in vespertilionids typically occurs in summer, followed by mating activity during late summer through winter (Racey and Entwistle, 2000). In contrast, for females, the ever-increasing energy demands of pregnancy and lactation begin in early spring and continue through autumn (Racey and Speakman, 1987). Given the phenological and quantitative differences in energy demands between male and reproductive female bats during spring, it seems plausible that differences in their use of torpor occur.

Field studies suggest that male insectivorous bats enter torpor more frequently than reproductive females (Kurta and Fujita, 1988). For instance, male big brown bats *Eptesicus fuscus* in Canada use torpor more frequently than reproductive females during summer (Grinevitch et al., 1995; Hamilton and Barclay, 1994). In light of such evidence, it has been proposed that males (and non-reproductive females) use torpor more frequently than reproductive females, are capable of withstanding more variable climatic conditions and may, in some cases, actually select roost microclimates that facilitate torpor (Barclay, 1991). Inherent in such hypotheses is the assumption that males and reproductive females are capable of different physiological responses when exposed to similar conditions, rather than simply preferentially selecting different microclimates. Unfortunately, it is impossible to control the conditions to which bats are exposed in the field, and laboratory studies aimed at quantifying sex differences in thermoregulation or use of torpor are lacking (Kurta and Fujita, 1988).

This study aimed to quantify sex differences in the short-term thermoregulatory strategies of hoary bats *Lasiurus cinereus* captured during their spring migration. In North America, *L. cinereus* is a long-distance migrant that winters in California and Mexico then moves into northern latitudes of the continent during spring and summer (Cryan, 2003). Pregnant female *L. cinereus* precede males north in early spring and move to more eastern summering grounds, while males occupy mountainous regions of western North America (Findley and Jones, 1964). *L. cinereus* is an ideal species for examining sexual differences in thermoregulatory behavior for several reasons: (1) females are pregnant during migration; (2) energetic demands on males during spring are presumably low and they are not yet sexually active; and (3) the microclimates of roosts used by *L. cinereus* (tree-foliage) are probably little

different from the general environment. Here we report laboratory work that quantifies variation in body temperature (T_b), metabolic rate (MR), wet thermal conductance (C_{wet}), and evaporative water loss (EWL) of both male and pregnant female *L. cinereus* over a range of air temperatures (T_a).

Materials and methods

Animals

Measurements were carried out on 122 adult *Lasiurus cinereus* Palisot de Beauvois 1796 (53 males and 69 females). Bats were captured in mist nets over streams in the Sandia and Manzano Mountains, Bernalillo County, New Mexico, USA, between 8 May and 22 July 2002 under permits issued by the New Mexico Department of Game and Fish with the approval of the University of New Mexico's animal care and use committee. One male included in this analysis was captured in late August 2002. Upon capture, data on the species, sex, age, reproductive status, mass and external measurements of each bat were recorded. Individuals were classified as adults based on the degree of epiphyseal ossification in the metacarpal-phalangeal joints of the wing (Anthony, 1988). Palpation of the abdomen was used to detect the presence of embryos in females. The majority of females captured for this study (>95%) were palpably pregnant, but we could not be sure that the others were not carrying embryos. Among specimens of female *L. cinereus* housed in the Museum of Southwestern Biology, University of New Mexico, 54 of 56 collected in New Mexico during spring carried one or more embryos. The range of variation in mass of the bats that were carrying embryos (24–41 g) was greater than that of the sample captured during the current study (range 25–40 g). Therefore, we are unable to conclude that pregnancy was always detected by palpation. Two of the males captured later in summer had descended testes, whereas all others did not. Individuals chosen for laboratory experiments were kept in cloth bags and maintained at room temperature ($26 \pm 2^\circ\text{C}$) and on a natural photoperiod until experimentation. Bats were only used for experiments during the day following capture, to eliminate bias associated with nutritional state (McNab, 1969). An elapsed time of >7 h between capture and experimentation ensured that bats were post-absorptive at the beginning of experimentation (Genoud, 1993; Morris et al., 1994). Respirometry experiments took place between 08:00 h and 18:00 h. Bats were given water prior to experiments and then hand fed meal worms (*Tenebrio* sp.) and more water after experimentation. Before release, a small tissue biopsy was taken from the flight membrane as part of another study; this tissue sampling guaranteed that we did not catch and experiment on the same bat twice.

Physiological measurements

We used flow-through respirometry to determine the MR, C_{wet} and EWL of *L. cinereus* over a range of T_a values. Metabolic rates were inferred from measurements of the rate of carbon dioxide production (\dot{V}_{CO_2}). Bats were placed in 2 l stainless steel metabolic chambers fitted with perches that

allowed them to hang in a normal roosting position. Excreta was trapped under 1 cm of mineral oil placed in the bottom of each chamber to prevent interference with EWL measurements. Metabolic chambers were situated within a temperature-controlled cabinet that maintained constant temperatures ($\pm 1^\circ\text{C}$) over a range of 0–40°C. Internal chamber temperatures were continuously measured with thermocouples and a digital thermometer (Sable Systems TC-1000, Las Vegas, NV, USA). Dry, CO₂-free air from a purge gas generator (Whatman FT-IR, Haverhill, MA, USA) was fed through rotameters (Scientific Model # FL-3402C, accurate to $\pm 2\%$ of full scale; Omega, Stamford, CA, USA) or mass flow controllers (FMA Model # FLA-A2409, accurate to $\pm 1\%$; Omega) before entering metabolic chambers. Flow meters were calibrated using a soap-bubble flow meter. Air from the purge gas generator was also directly sampled, and served as a baseline. The flow rate of the air into chambers ranged between 0.7 and 1.21 m³ min⁻¹, to maintain chamber humidities below 1.0 kPa; time to reach 99% chamber equilibrium ranged from 6.5 to 11.2 min (Lasiewski et al., 1966). Up to four metabolic chambers were used simultaneously and outlet air from the chambers was routed to a gas multiplexer (Sable Systems Respirometer Multiplexer V 2.0), which allowed for sequential sampling of individual chambers with the gas analyzer. During runs with more than one bat, each metabolic chamber was sampled for 7.7 min before the multiplexer switched to the next chamber. Chamber outlet air was monitored with a CO₂/H₂O analyzer (Li-Cor LI-7000, Lincoln, NE, USA) and the digital output from the gas analyzer was sampled 9 times per second by a computer using DATACAN V data-acquisition software (Sable Systems). The gas analyzer was calibrated daily using CO₂-free air and a reference gas of 1020 p.p.m. CO₂ for CO₂ calibration, while dry air and a dew point generator (LiCor Li-610) were used for water calibration. Accuracy of CO₂ and water channels of the LI-7000 were ± 1 p.p.m. and ± 0.01 kPa, respectively. All measurements were corrected to standard temperature and pressure (STP).

Most bats were run only once and at a single temperature, but 17 individuals were run twice at two temperatures with at least 2 h between runs; we did not detect differences in the measured parameters between individuals subjected to a second run and other bats. Experimental runs occurred at 5° increments from 0 to 40°C, and also at 32.5 and 37.5°C. With the exception of runs at 37.5 and 40°C (which lasted only 30–45 min), bats were acclimated to metabolic chambers for 1 h before measurements began. Bat activity was monitored visually and individuals that were not resting quietly after 30 min were excluded from measurements. Experimental runs lasted 1–3 h, during which time carbon dioxide and water readings typically reached stable levels for >10 min. All reported MR and EWL values are averages taken from 1 min of the lowest stable values.

In addition to CO₂ and evaporative water loss measurements, we recorded body temperature (T_b) to $\pm 0.1^\circ\text{C}$ within 15 s of removal from the chambers by inserting a lubricated Teflon coated copper-constantan thermocouple

(Physitemp # NJ07013, Clifton, NJ, USA) 1 cm into the rectum. Animals were classified as torpid when exit $T_b < 30^\circ\text{C}$; in three instances, \dot{V}_{CO_2} readings indicated that bats were using torpor, but T_b indicated a return to a normothermic state before the end of the run. Therefore, despite their normothermic exit T_b values, these bats were considered torpid for subsequent analyses of MR, EWL and C_{wet} , but not T_b . Respiratory rates of several bats were quantified by monitoring pressure fluctuations within the metabolic chambers using a pressure meter (Sable Systems PT-100B).

Calculation of metabolic rate and minimum wet thermal conductance

\dot{V}_{CO_2} was calculated as:

$$\dot{V}_{\text{CO}_2} = \frac{(\dot{V}_i + \dot{V}_{\text{H}_2\text{O}})F_{\text{ECO}_2}}{(1 + F_{\text{ECO}_2}[1/RQ - 1])}, \quad (1)$$

where \dot{V}_i is the STP-corrected flow rate of air entering the chamber, $\dot{V}_{\text{H}_2\text{O}}$ is the flow rate of water vapor into the chamber, F_{ECO_2} is the fractional concentration of CO₂ in air exiting the chamber, and RQ is the respiratory quotient. Bats were assumed to be post-absorptive and metabolizing lipids, thus we used an RQ value of 0.71. MR, in mW, was calculated from \dot{V}_{CO_2} assuming that 1 l of CO₂=27.8 kJ (Walsberg and Wolf, 1995).

Wet thermal conductance C_{wet} was calculated using the equation $C_{\text{wet}} = \text{MR}/(T_b - T_a)$, where MR is measured in mW g⁻¹ and T_b and T_a are in °C.

Statistical analysis

Statistical analyses were carried out using NCSS 2000 for Windows. Differences in mean values of variables showing no significant relationship to T_a were tested using *t*-tests. Analysis of covariance (ANCOVA; GLM ANOVA) was used to test for differences in regression slopes, intercepts and interaction terms between sexes and thermoregulatory groups (torpor versus normothermia), using sex (or group) as a fixed effect, and T_a as a covariate. EWL data were linearly transformed using a natural-log function prior to ANCOVA analysis. We estimated the thermoneutral zone (TNZ) from values within 1°C of the T_a values at which actual measurements were made. Reported values are expressed as means \pm standard errors (S.E.M.) and statistical significance was set at $P \leq 0.05$.

Results

Body temperature

Bats showed a dichotomous response in T_b to low chamber temperatures. At temperatures below 25°C, males either maintained a constant T_b (normothermic state), or dropped T_b below 30°C to values near T_a (herein defined as torpor; Fig. 1). In contrast, nearly all of the females ($N=67$) remained normothermic at low T_a values. At chamber temperatures below 25°C, the proportion of males using torpor (68%) considerably exceeded that of females (5%). There were no

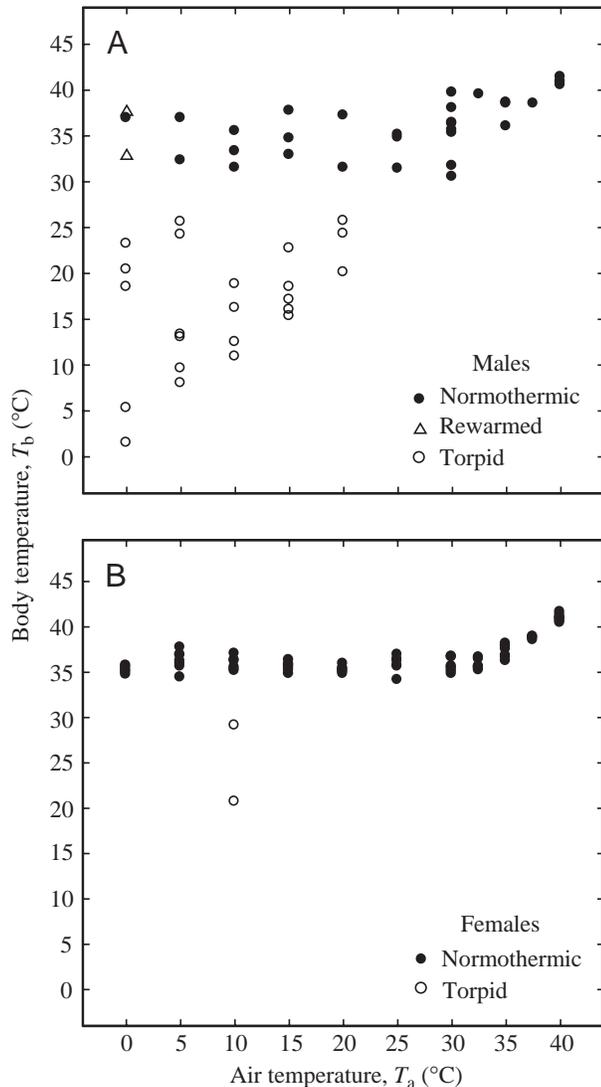


Fig. 1. Body temperature (T_b) of male and female *Lasiurus cinereus* as a function of air temperature (T_a). Closed symbols represent normothermic individuals, open symbols are torpid bats. Open triangles indicate bats that entered torpor but were normothermic upon exit from the metabolic chamber.

significant differences in T_b between normothermic males and females at $T_a < 35^\circ\text{C}$ ($t=1.5$, $\text{d.f.}=25.1$, $P=0.14$), although variation in T_b was greater in males (mean $T_b=34.9\pm 2.7^\circ\text{C}$) than in females (mean $T_b=35.7\pm 0.7^\circ\text{C}$). The T_b of males using torpor at $10^\circ < T_a < 20^\circ\text{C}$ were closer to T_a ($T_b - T_a = 3.6 \pm 3.0^\circ\text{C}$, range $0.1\text{--}8.8^\circ\text{C}$), than at $0^\circ < T_a < 5^\circ\text{C}$ ($T_b - T_a = 10.1 \pm 8.0^\circ\text{C}$, range $0.9\text{--}23.2^\circ\text{C}$).

Metabolic rate

As with T_b , metabolic measurements indicated a dichotomous thermoregulatory response (normothermia and torpor) by bats at $T_a < 25^\circ\text{C}$ (Fig. 2). The thermal neutral zone ranged between approximately 30°C (lower temperature T_{lc}) and 34°C (upper temperature T_{uc}) in both sexes. Within the

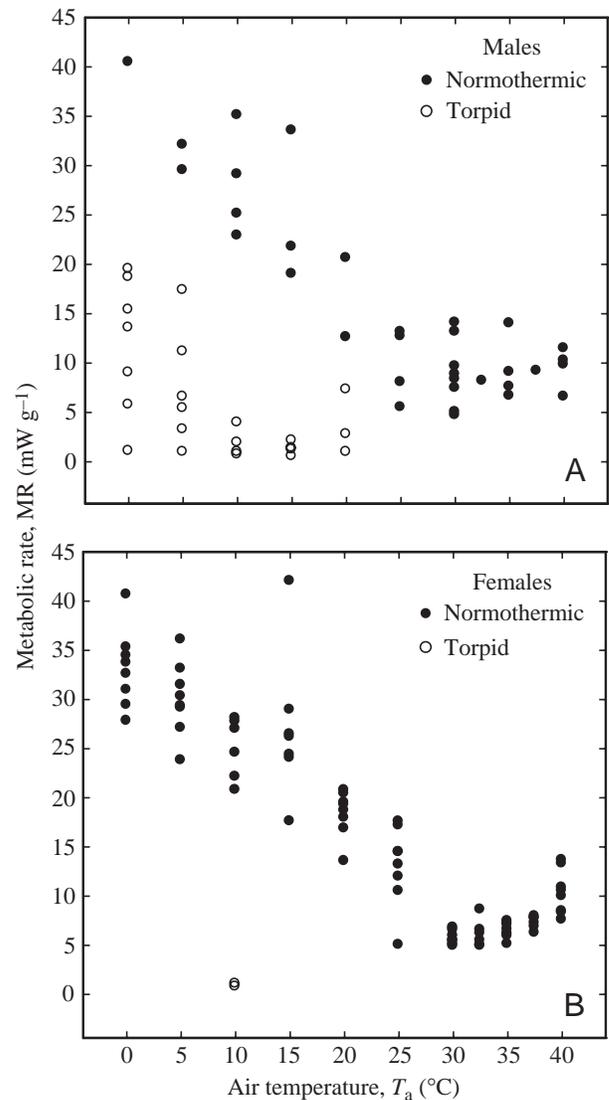


Fig. 2. Mass-specific metabolic rate of male and female *L. cinereus* as a function of air temperature (T_a). Closed symbols represent normothermic individuals, open symbols are torpid bats.

TNZ, females had significantly lower mass-specific MR values than males ($t=-3.3$, $\text{d.f.}=13.5$, $P<0.05$), but there were no differences in mass-specific MR between normothermic males and females below the T_{lc} (T_a , $F_{1,49}=27.2$, $P<0.0001$; sex, $F_{1,49}=0.2$, $P=0.69$; interaction, $F_{1,49}=1.1$, $P=0.38$). The metabolic rate of males in torpor was lower and less variable at chamber temperatures between 10 and 20°C ($2.1 \pm 0.5 \text{ mW g}^{-1}$) than at temperatures between 0 and 5°C ($9.8 \pm 1.8 \text{ mW g}^{-1}$). At $10^\circ < T_a < 15^\circ\text{C}$, torpor decreased metabolic expenditure to $4\text{--}7\%$ of normothermic values, but the savings of torpor were generally smaller ($22\text{--}29\%$ of normothermic values) outside of this temperature range. We detected no significant differences in the mass ($t=1.0$, $\text{d.f.}=57$, $P=0.34$), forearm length ($t=-0.7$, $\text{d.f.}=54$, $P=0.46$), or amount of guano produced ($t=-0.3$, $\text{d.f.}=55$, $P=0.75$) between heterothermic and normothermic bats.

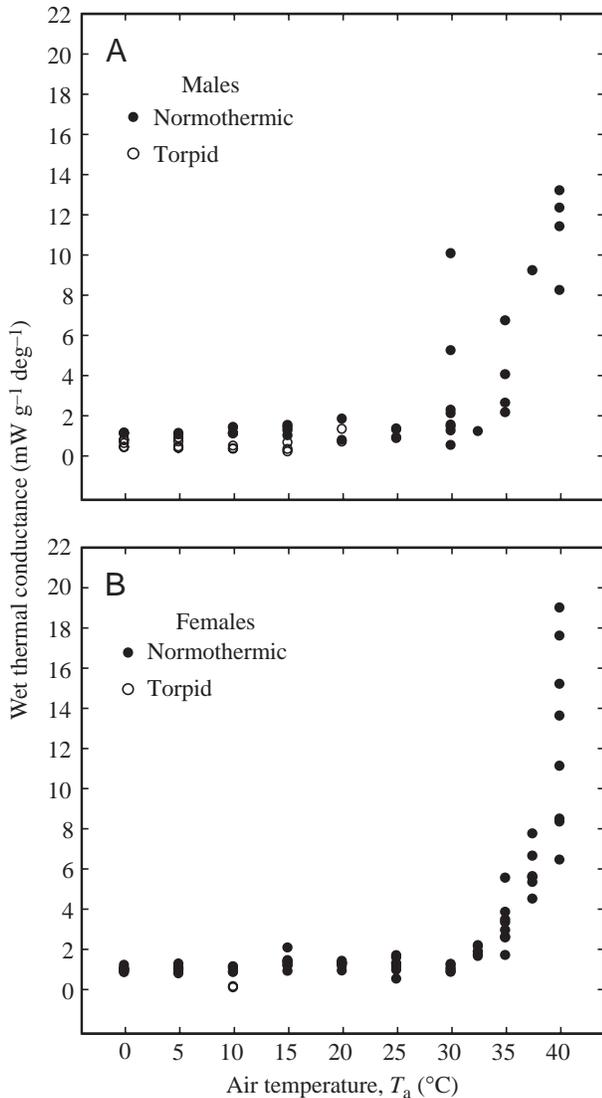


Fig. 3. Wet thermal conductance of male and female *L. cinereus* as a function of air temperature (T_a). Closed symbols represent normothermic individuals, open symbols are torpid bats.

Thermal conductance

Wet thermal conductance increased exponentially above minimum values at chamber temperatures $>30^\circ\text{C}$ in both sexes (Fig. 3). There was no significant relationship between T_a and C_{wet} below T_{lc} , nor were there differences in C_{wet} between sexes of normothermic bats (T_a , $F_{1,49}=1.1$, $P=0.36$; sex, $F_{1,49}=0.5$, $P=0.51$; interaction, $F_{1,49}=0.7$, $P=0.60$). However, C_{wet} values of bats in torpor were significantly lower than those of normothermic individuals ($t=4.2$, d.f.=29.6, $P<0.05$).

Evaporative water loss

Mass-specific rates of EWL were consistently higher in normothermic males than in females, and increased more rapidly with T_a in males (Fig. 4; T_a , $F_{1,99}=28.3$, $P<0.0001$; sex, $F_{1,99}=27.4$, $P<0.05$; interaction, $F_{1,99}=3.1$, $P<0.05$). EWL in torpid males was significantly less than in normothermic

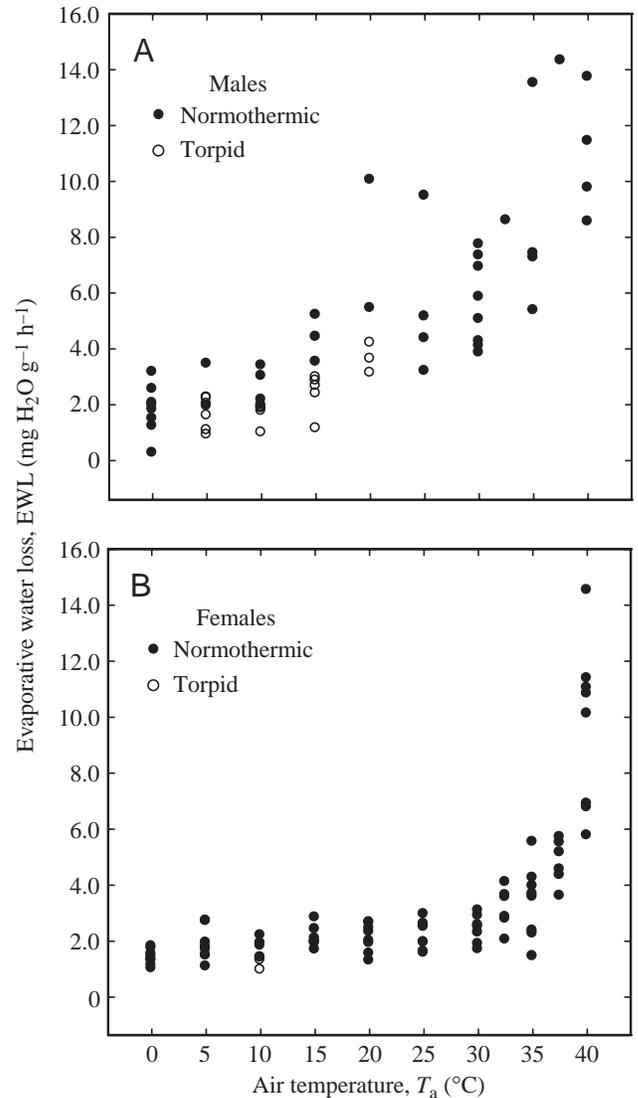


Fig. 4. Mass-specific evaporative water loss of male and female *L. cinereus* as a function of air temperature (T_a). Closed symbols represent normothermic individuals, open symbols are torpid bats.

individuals at similar T_a values, but the slopes of the regression lines did not differ significantly (T_a , $F_{1,27}=3.9$, $P<0.05$; group, $F_{1,27}=54.6$, $P<0.05$; interaction, $F_{1,27}=0.1$, $P=0.99$). EWL of bats in torpor was $63\pm 6\%$ of EWL of normothermic individuals at similar T_a values.

Discussion

Sex differences in thermoregulation

Thermoregulation in pregnant female *L. cinereus* differed from males during spring migration. Females rarely used torpor and maintained a high stable T_b over a broad range of air temperatures. In contrast, about two-thirds of males entered torpor when exposed to air temperatures below 25°C . Furthermore, males showed much wider variation in body temperature (Fig. 1) and metabolic rate (Fig. 2) than females

at most air temperatures. These differences in thermoregulation between males and females suggest that (1) torpor is not an obligate response to low air temperatures and (2) there may be sex-biased selective advantages to maintaining normothermy. Maintenance of normothermic T_b in females is likely to minimize the period of embryonic development and thus maximize the time available to both mother and young when favorable foraging conditions exist after weaning (Racey, 1982). The higher frequency of torpor use and more labile body temperatures observed in males may reflect the relative lack of selective pressure imposed on them by energetic demands associated with reproduction. If indeed the propensity for females to maintain normothermic temperatures during their spring migration is associated with expediting embryonic growth, we predict that males and females would use similar thermoregulatory strategies during the fall and winter.

Metabolic rates of normothermic males and females within the TNZ were $106 \pm 12\%$ and $74 \pm 3\%$, respectively, of values expected [basal metabolic rate (BMR) = $4.12 m^{-0.31}$, where m = body mass; White and Seymour, 2003]. The MR we observed was higher than that measured by Genoud (1993), who found that the BMR of a single *L. cinereus* captured during winter and maintained in captivity was 52% of the expected value (White and Seymour, 2003). Metabolic rates of lasiurines change with season and are generally lower in winter than during the warmer months (Genoud, 1990, 1993), potentially explaining some of these differences. However, comparisons of data collected from metabolic studies of bats captured and maintained under different conditions should be treated with caution (Kurta and Fujita, 1988). Unlike males, the mass-specific metabolic rates measured in females during this study were lower than expected. McLean and Speakman (2000) also observed lower metabolic rates in pregnant bats and suggested that lower metabolic needs of fetal tissues might contribute to this effect. Increases in the mass of ametabolic materials during pregnancy may also contribute to the low mass-specific metabolic rates that we observed in this study. In general, circulating fluid volume increases with pregnancy in mammals, and the water content of mammalian embryos is relatively high (Adolph and Heggeness, 1971). Sexual size dimorphism may also influence allometric differences in metabolic rate. Mass-specific metabolic rate decreases with increasing body size (White and Seymour, 2003; but see Gillooly et al., 2001) and *L. cinereus* is sexually dimorphic, with females averaging 3.9% larger than males in skeletal measurements (Williams and Findley, 1979) and about 40% larger in mass during pregnancy (this study). Comparative studies of male and non-reproductive female *L. cinereus* would help elucidate whether the lower mass-specific metabolic rates of pregnant females observed in this study were related to embryonic growth or body size alone.

Energetic savings associated with the use of torpor

In the hoary bat, as with small mammals in general, the metabolic cost of maintaining normothermic body temperatures increases rapidly as air temperatures decrease

below the lower critical temperature. Because maintenance costs in small endotherms comprise a large proportion of total daily energy expenditure (McNab, 2002), thermoregulation has a considerable influence on overall energy balance. Metabolic rates of *L. cinereus* increased sixfold as chamber air temperature decreased from the 30°C (T_{lc}) to 0°C (Fig. 2). Whereas most males used torpor to reduce thermoregulatory costs, the reluctance of pregnant females to do so suggests they must have much higher energetic demands when migrating through cold areas. Indeed, female *L. cinereus* routinely encounter sub-thermoneutral environmental temperatures ($<T_{lc}$) during their spring migration, as average air temperatures within the study area range from 3.4 to 23.4°C during the months when *L. cinereus* is found there (Climate Source, Corvallis, Oregon, USA). At the metabolic rates observed in this study, a roosting 30 g female *L. cinereus* would expend approximately 15.6 kJ of energy over a 24 h period at 30°C and a decrease in environmental temperature to 5°C for the same period would increase total energy expenditure more than fivefold to approximately 79.0 kJ. These values can be compared to estimates of field metabolic rate (FMR) in bats using equation 4 of Nagy et al. (1999), which predicts an FMR of 65.8 kJ day^{-1} (range 25.5 – $169.6 \text{ kJ day}^{-1}$) for a 30 g female *L. cinereus*. In general, these values are consistent with measured FMR values from other free-ranging insectivorous bats, e.g. pregnant 9 g *Myotis lucifugus*, 33.7 kJ day^{-1} (Kurta et al., 1989); pregnant 18 g *Eptesicus fuscus*, 48.6 kJ day^{-1} (Kurta et al., 1990). Given that the thermoregulatory costs incurred by a normothermic *L. cinereus* exposed to low air temperatures could exceed the total daily energy use of a free-ranging individual, pregnant female *L. cinereus* might not maintain a positive energy balance during spring migration through colder areas, if energy intake were limited (e.g. during inclement weather).

Males, in contrast, are not constrained physiologically or behaviorally by the needs of the young. As a consequence, males can use torpor to accrue energy or limit foraging. In the laboratory, torpor in males resulted in a reduction in metabolic rate of up to 97% ($T_a = 0^\circ\text{C}$, torpor MR = 1.1 mW g^{-1} , normothermic MR = 40.5 mW g^{-1}).

We can explore the relative benefits of torpor between the sexes by modeling the energy expenditure of males and females at a single T_a using several different torpor bout lengths for each sex and comparing these estimates to normothermic energy expenditure at the same T_a (Fig. 5). This model estimates energy use during a torpor bout by summing the torpor maintenance costs (MR duration) and the energetic cost of arousal. We calculated arousal costs using the following equation (A. McKechnie, personal communication):

$$E_{\text{arousal}} = s(T_{\text{norm}} - T_{\text{torpor}})M_b + \int_{t_1}^{t_2} Q, \quad (2)$$

where E_{arousal} = energetic cost of arousal, s = specific heat of tissues ($3.9 \text{ J g}^{-1} \text{ deg.}^{-1}$; Withers, 1992), T_{norm} and T_{torpor} are normothermic and torpor body temperatures, respectively, M_b

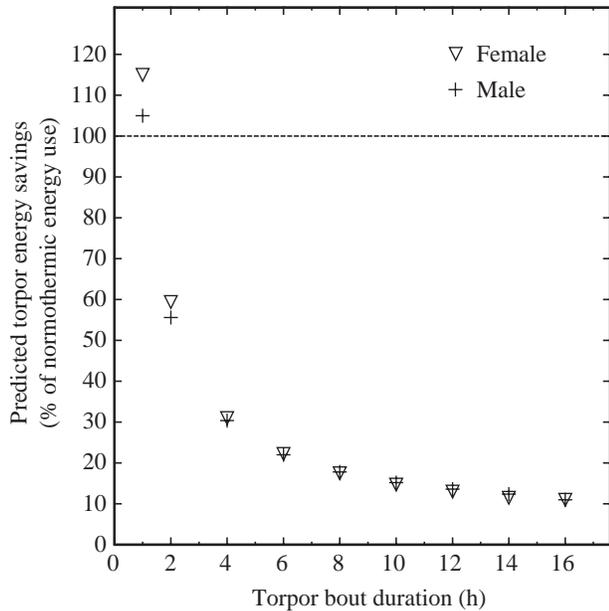


Fig. 5. Model-based predictions of torpor energy use, proportional to normothermic energy expenditure, as a function of torpor bout duration in male and female *L. cinereus*. See text for description of model. The broken line indicates the point at which torpor maintenance and arousal costs equal normothermic energy expenditure.

is body mass, Q is the rate of metabolic heat production required to balance heat loss to the environment, and t_1 and t_2 are the start and end times of rewarming, respectively. The calculation of the second term in Equation 2 was simplified by assuming that rewarming occurs linearly and by calculating Q using the simplified Newtonian equation $Q=C(T_b-T_a)$, where C is the thermal conductance and T_a is ambient temperature. Previous models for the energetic cost of arousal (Humphries et al., 2002; Prothero and Jürgens, 1986) assumed that rewarming occurred instantaneously and did not account for heat loss during the rewarming period. We assumed a rewarming rate of $1.0^\circ\text{C min}^{-1}$, a typical value for bats (Geiser and Baudinette, 1990). Using this model, arousal costs for male and female bats were calculated at $T_a=10^\circ\text{C}$ using our observed values for T_{norm} , M_b and normothermic C_{wet} . Because the two females that used torpor at 10°C were arousing by the time we removed them from the chambers, an average male value was used for T_{torpor} of both sexes.

The model suggested that the higher mass-specific metabolic rates of males lead to slightly greater energy saving for this sex during shorter bouts of torpor (1–4 h). However, it is evident that females potentially save substantial energy by using torpor as well. The propensity of females to avoid torpor during spring suggests that the short-term costs of defending a normothermic body temperature may be repaid later, perhaps through shorter embryonic developmental periods and earlier juvenile independence. Our model calculations are intended for comparison between sexes and probably overestimate the

actual energy expenditure, as they do not account for passive rewarming from torpor (Geiser and Drury, 2003). Selection of roosts that facilitate passive rewarming may be an important strategy for reducing energetic costs in bats (Chruszcz and Barclay, 2002; Vaughan and O'Shea, 1976; Willis, 2003). Regardless of the actual amount of energy saved during torpor under natural conditions, both sexes potentially benefit to the same degree.

Our data also show how environmental temperature potentially affects torpor T_b values and energetic savings. Torpid bats maintained the lowest T_b values at moderate environmental temperatures ($10\text{--}15^\circ\text{C}$), which resulted in the greatest energetic savings. At $T_a<10^\circ\text{C}$, T_b and MR were more variable and tended to be higher, resulting in smaller energy savings. Similar patterns of increasing metabolic rate and body temperature at increasingly low temperatures by bats in torpor have been observed among lasiurines (Genoud, 1993) and other insectivorous species (Hosken and Withers, 1997). It is unclear whether such variation in response at low air temperatures is associated with the ability of *L. cinereus* to efficiently thermoregulate at temperatures below 10°C or simply an artefact of our laboratory protocol. We did not choose to run individuals at low temperatures for >5 h, and the possibility exists that T_b and MR would have stabilized if we continued measurements for longer periods. In addition, bats may not reach steady state minima for extended periods (5–20 h) after torpor is induced (Riedesel and Williams, 1976), thus our measurements may overestimate minimum values. However, Genoud (1993) ran *L. cinereus* at chamber temperatures $<10^\circ\text{C}$ for relatively long time periods (up to 10 h) and observed similar patterns. Our data support Genoud's argument (Genoud, 1993) that sustained periods of torpor (i.e. hibernation) in *L. cinereus* at temperatures at or below 0°C are unlikely.

Thermal conductance

At $T_a<T_{lc}$, the conductance of normothermic *L. cinereus* was $78\pm 2.4\%$ of values expected for bats, based on body mass alone (Bradley and Deavers, 1980). Shump and Shump (1980) showed that the fur of *L. cinereus* provided relatively more insulation than the pelage of cave-roosting species and attributed the difference to an adaptation for roosting in foliage versus less exposed sites. Relatively low conductance values have also been observed in other insectivorous tree-roosting species (Hosken and Withers, 1997), but the ubiquity of this pattern in bats that roost in exposed sites is unclear.

Torpid *L. cinereus* exhibited lower conductance values than normothermic individuals. Lower conductance during torpor has also been observed in rodents (Snyder and Nestler, 1990) and other insectivorous bat species (Genoud, 1993; Hosken, 1997; Hosken and Withers, 1997, 1999; Morris et al., 1994). Conductance values for male *L. cinereus* during torpor were $38\pm 5.4\%$ of expected values based on body mass, whereas for the two females that used torpor values were 3% and 8% of those expected. It is unclear why conductance is sometimes lower during torpor (Snyder and Nestler, 1990), but possible

explanations include changes in breathing rate, posture or circulation (Hosken and Withers, 1997).

Sex differences in evaporative water loss rate

Normothermic male *L. cinereus* exhibited higher mass-specific rates of EWL than females. In addition, the rate at which males lost water increased more rapidly as a function of T_a than for females. There are several potential explanations for higher rates of water loss in males, including sex differences in breathing rate, body size and metabolism. Among similarly sized mammals, bats exhibit high rates of transepidermal and respiratory water loss because of their relatively large wing membranes and lungs (Bassett, 1980; Hattingh, 1972; Licht and Leitner, 1967; Studier, 1970). Among these routes of water loss, respiratory losses are probably the highest (Kurta, 1985). We measured the respiratory rates of 32 normothermic individuals and could detect no significant differences in ventilation frequency between males and females ($t=-0.1$, d.f.=30, $P=0.94$). Differences in metabolism could also potentially contribute to differences in water loss, with EWL increasing proportionally with metabolic rate (Studier, 1970). Although we detected metabolic differences between sexes within the thermal neutral zone, there were no significant differences in rates of mass-specific MR below the TNZ. However, differences in rates of water loss spanned all measured air temperatures. These consistent sex differences in EWL, but not MR, suggest that differences in metabolic rate are not the primary factor influencing sex differences in evaporative water loss rates. Smaller body size in male *L. cinereus* results in higher surface area to volume ratio and proportionally greater pulmonary and epidermal surface areas of males might explain the consistently higher rates of water loss over all air temperatures. Comparable to the findings of the current study, lower rates of water loss in pregnant females have been observed in other species as well (Proctor and Studier, 1970; Studier, 1970).

At air temperatures below 25°C, the use of torpor decreased water loss by as much as 29% in male *L. cinereus*. Similar reductions in water loss with torpor use have been noted in other species of temperate insectivorous bats (Carpenter, 1969; Dwyer, 1971; Hosken, 1997; Hosken and Withers, 1999; Maloney et al., 1999; Morris et al., 1994; Studier, 1970). Female *L. cinereus* migrate to areas of eastern North America (Cryan, 2003; Findley and Jones, 1964) where relative humidity (RH) generally exceeds 50% during summer (Baldwin, 1968). Unlike females, most males remain in arid (<50% RH) regions of western North America, where evaporative water loss may be problematic. For example, at rates of water loss measured during this study (1.6–12 mg g⁻¹ h⁻¹), males could potentially lose up to 6–18% of their mass in body water over the course of a typical 12 h roosting period under hot and dry conditions. Shump and Shump (1982) noted that *L. cinereus* could lose up to 28% of its body mass in water without noticeable effects, but other species suffer high (>50%) mortality after losing 23–32% of their body mass in water (Studier et al., 1970). Given the vulnerability of foliage-roosting bats to desiccation, torpor may

provide male *L. cinereus* with a means of saving water in arid regions of western North America. Although the maintenance of a positive energy budget is often cited as the principal factor governing summer torpor use in bats, water balance may also play an important role.

Sex differences in distribution

Selection of optimal microclimates by bats can minimize their thermoregulatory demands and, in many cases, behavioral thermoregulation may be just as important as physiological regulation (Studier and O'Farrell, 1972). Considering the potential selective advantages for bats that choose roosts which limit energy and water expenditure, accessibility to sites with adequate thermal and hygric properties probably plays an important role in determining species distribution (Baudinette et al., 2000; Bell et al., 1986; Humphrey, 1975; Morris et al., 1994; Webb et al., 1995). However, differences in distribution between sexes of vespertilionid bats are known to occur at both regional and continental scales during summer and many of these differences likely stem from differential energy needs (Barclay, 1991; Cryan, 2003; Cryan et al., 2000; Thomas, 1988). Are the different thermoregulatory strategies of male and female *L. cinereus* a reflection of their disparate distributions during the summer months? Why do females forgo so many western areas along their migration route, essentially flying hundreds of kilometers farther than males? The results of this study suggest that female *L. cinereus* may pass through the arid western regions of North America because climatic conditions there are unfavorable for raising young. The cooler night-time temperatures and more arid conditions of western regions during summer (Climate Source, Corvallis, Oregon) would be a liability to females that remain normothermic. In contrast, males are not faced with the same energy and water challenges as reproductive females, so torpor use may mitigate the occupancy of more challenging thermal and hygric environments found in western North America.

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