Thermal energetics of silver-haired bats (*Lasionycteris noctivigans*) and the implications for roost selection: Do all bats like it hot?

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A thesis submitted in partial fulfillment of the Honours Thesis Course (05.4111/6)

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2008

Abstract

Roost selection by temperate bats is variable. Some species exploit both natural and human-made structures, whereas others roost mainly in trees. Reasons for this variation are unknown and I wondered if inter-relationships between torpor use and roost selection during summer, and migration vs. hibernation during winter could play a role. Torpor results in large energy savings, but can be costly because it slows growth and development. This cost may be small for migratory bats because juveniles can feed over the winter but is likely larger for hibernators because juveniles will not survive if they grow slowly and fail to accumulate sufficient fat stores prior to hibernation. Therefore, hibernators likely face greater selection pressure than migrants to seek out warm summer roosts. I tested this hypothesis by comparing torpor and energy use of silver-haired bats (*Lasionycteris noctivigans*), a migratory tree-roosting species, when exposed to ambient temperature (T_a) profiles which simulated either cool tree-cavity roosts or a warm building roost. Bats relied more heavily on torpor in the tree roost when conditions simulated a cool day; however there was no difference in energy use when conditions simulated an average July day. My results suggest that migratory bats save energy by roosting in cooler trees only under certain conditions.

Acknowledgements

I would like to thank C. Willis for his admirable patience, and consistent help and expertise on the project in the field and lab. In addition, I thank D. Donald, J. Jameson, and A. Matheson for help capturing bats in the field, and for memorable pictures. I would also like to acknowledge R. Moodie for coordinating the thesis program, my committee members M. Wiegand and J. Huebner for help and support, A. Trachtenberg for the dollar sign, and the Natural Sciences and Engineering Research Council (NSERC) Undergraduate Student Research Award. Funding for this project was provided by the University of Winnipeg and a NSERC Discovery Grant to C. Willis. Lastly, but not least, I thank my family and friends who are solely responsible for keeping me sane.

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1.0 Introduction

In the wild, small endotherms often face energetic shortfalls because their small volume and large surface area result in rapid rates of heat loss. Therefore, they must often use large amounts of energy defending high, normothermic body temperatures (T_b). The energetic consequences of a small body size may be especially pronounced for species which rely on temperature-dependent or seasonally dependent food, such as flying insects, nectar or pollen (e.g., Law 1994; Coburn and Geiser 1998; Willis 2007; Wojciechowski *et al.* 2007). Behavioural strategies that reduce heat loss such as microhabitat selection may be critical for some species. For example, Phainopepla (*Phainopepla nitens*), a songbird, and the Verdin (*Auriparus flaviceps*) can substantially reduce their metabolic rate by selecting roosts which provide shelter from the wind (Walsberg 1986; Wolf and Walsberg 1996). In addition, other endotherms, such as the southern flying squirrel (*Glaucomys volans*), roost in nest-lined tree cavities to provide insulation and huddle in groups to benefit from increased heat production by a greater number of individuals over the winter (Stapp *et al.* 1990). Thus, selecting an appropriate roost may be essential to reduce heat loss.

In addition to, or in place of microhabitat selection, some endotherms may also avoid energetic shortfalls via periods of heterothermy or torpor, a state of reduced energy expenditure during which T_b is permitted to fall, often to within 1-2° C of ambient temperature (T_a), as a means to reduce metabolic rate (Geiser 2004; Turbill and Geiser 2005). Torpor may be essential for survival under unfavourable or unpredictable conditions because it saves the large amount of energy that would otherwise be needed to

maintain a high T_b (Turbill 2006a). Energy expenditure during torpor, or the torpid metabolic rate (TMR), is a function of T_b (and T_a in thermoconforming heterotherms) so lower T_a can result in larger energy savings for torpid animals. Therefore, under some circumstances heterothermic animals (those that can vary their T_b) may select relatively cool roosts to obtain the largest energetic benefits of torpor. In addition, torpor use appears especially common in species which live in variable climates and which are often exposed to periods of food shortage (Geiser 2004). However, actively warming up from torpor via shivering or non-shivering thermogenesis can be extremely costly at low T_a (McKechnie and Wolf 2004; Willis 2008). A number of mammals and birds have been shown to use passive warming to arouse from torpor to avoid this potential cost, relying on ambient heat energy to return to normothermic T_b at the end of a torpor bout (Lovegrove *et al.* 1999; Geiser and Drury 2003). For example, passive arousal results in an energy savings of 15% for the marsupial heterotherm, the stripe-faced dunnart (*Sminthopsis macroura*) compared to active arousals for the same change in T_b (Geiser and Drury 2003). Despite the potential benefits of passive arousal, the energetic demands of active arousal may be unavoidable under some circumstances, such as on cool, overcast days with little diurnal fluctuation in temperature, or for endotherms living in cool, stable microclimates (Geiser and Ruf 1995; Holloway and Geiser 1995; Lovegrove *et al.* 1999; Willis 2008).

Insect-eating bats are good models for studying the importance of roost selection and use of torpor for small endotherms because of their small size, high energy mode of locomotion, and insectivorous diet. As a result of these features, bats face frequent energy

bottlenecks and tend to rely on torpor throughout the year, especially in the temperate zones (e.g., Chruszcz and Barclay 2002; Lausen and Barclay 2003; Lausen and Barclay 2006) but also in subtropical and tropical habitats (e.g., Bartels *et al.* 1998; Geiser and Brigham 2000; Kelm and von Helversen 2007). They also exhibit great diversity in terms of their roosting habitat selection, making use of a wide variety of structures including natural roosts such as rocks, bamboo culm, tree cavities, underneath exfoliating bark, or underneath foliage, as well as different kinds of buildings (Cryan 2003; Kunz and Lumsden 2003). Despite this diversity, more than half of the roughly 1100 bat species worldwide depend on trees or other plants as roosts (Kunz and Lumsden 2003). Tree-living species may roost in foliage, under exfoliating bark, in cracks and crevices, or in large enclosed cavities (Cryan 2003; Kunz and Lumsden 2003) and suitable roost trees represent essential habitats for many species of temperate-zone bats (Kalcounis *et al.* 1999; Willis and Brigham 2004; 2005; Kofoky *et al.* 2007; Rancourt *et al.* 2007; Vonhof and Gwilliam 2007). Depending on the type of roost, tree roosts can help minimize predation risk, provide sites for rearing young, facilitate information sharing through social interactions and/or provide thermoregulatory benefits by offering a relatively stable microclimate (Kerth *et al.* 2001a; Kunz and Lumsden 2003). Microclimate is thought to be especially important to the roost requirements of bats. Many bats heavily rely on torpor, thus selecting roosts with a microclimate that enables them to frequently use torpor is often beneficial (Kunz and Lumsden 2003; Willis and Brigham 2007).

The implications of torpor use for offspring growth have been relatively well-studied in temperate-zone bats. It is clear that torpor slows gestation for pregnant bats and reduces

lactation output which, in turn, slows offspring growth (e.g., Racey 1973; Racey and Swift 1981; Lewis 1993; Wilde *et al.* 1999). This implies that, despite the immediate energetic benefits of torpor as a thermoregulatory mechanism, other strategies, such as selecting roosts with warmer microclimates, may be important for reproductive individuals and their developing young. A number of studies of free-ranging bats indicate that roost selection can help counteract potential negative fitness consequences of use of torpor for reproductive and lactating female bats (Chruszcz and Barclay 2002; Turbill and Geiser 2005; Willis and Brigham 2007). For example, reproductive females often form maternity colonies and roost with more individuals than their nonreproductive counterparts to counteract thermoregulatory costs (Parsons *et al.* 1986; Rancourt *et al.* 2007). Silver-haired bats (*Lasionycteris noctivigans*) have been observed to form small maternity colonies in tree cavities (Parsons *et al.* 1986), even though these bats are typically solitary for the rest of the year (Barclay *et al.* 1988). Female big brown bats select larger tree cavity roosts as opposed to other potential cavities, likely to accommodate a larger colony which increases T_{roost} and alleviates energy costs (Willis *et al.* 2006b, Willis and Brigham 2007). Chruszcz and Barclay (2002) showed that lactating female western long-eared bats (*Myotis evotis*) select roosts that remain warmer at night, so their pre-volant young (which remain in the roost while their mothers forage) can maintain a higher T_b, and thus avoid the delayed growth and development that results from heterothermy. In contrast to reproductive female bats, nonreproductive females and males (which do not invest in parental care) often select cooler roosts such that they can use torpor and significantly reduce their energy costs (Cryan and Wolf 2003; Solick and

Barclay 2006). Cryan and Wolf (2003) demonstrated that nonreproductive male hoary bats (*Lasiurus cinereus*) use torpor more than reproductive females, regardless of T_a.

While some bat species appear to roost only in trees, other species are more flexible in their roost selection behaviour and are able to take advantage of cavities in human-made structures, such as buildings, as well as natural sites such as trees or rock crevices (e.g., Lausen and Barclay 2006; Law and Chidel 2007). A persistent question with respect to habitat selection in temperate bats is why can some species exploit human-made structures as roosts (typically hibernators such as little brown bats, *Myotis lucifugus*) while others roost only in trees (typically migrants such as silver-haired bats)? Recent work suggests that roosts in buildings may be preferred in some situations because they provide warmer, more stable microclimates which are protected from predators, relative to natural roosts at the same study sites (Lausen and Barclay 2006). Although they did not study tree-roosting bats, Lausen and Barclay (2006) conducted the first study comparing thermoregulatory benefits, predation risk and a correlate of reproductive fitness (date of first juvenile flights) between building and natural roost sites. They demonstrated that reproductive female big brown bats (*Eptesicus fuscus*) roosting in buildings used torpor less frequently than those roosting in rock crevices in their study area in southern Alberta. Likely due to their less frequent use of torpor, juveniles in the building became volant at a younger age, suggesting a fitness benefit of the warmer building roost (Lausen and Barclay 2006). Predation risk was also substantially reduced in the building roost, whereas rattlesnakes (*Crotalus viridis*) and bull snakes (*Pituophis melanoleucus*) were potential predators of rock-roosting bats (Lausen and Barclay 2003;

Lausen and Barclay 2006). Thus, there is evidence of thermoregulatory, predator avoidance and reproductive fitness benefits for bats roosting in buildings as opposed to natural roosts at the same site.

Considering the abundance of human-made structures that could serve as roosts in many areas, their likely thermoregulatory and predator-avoidance benefits, and the decline of forest habitats due to harvesting (Jung *et al.* 1999), it is surprising that more forest-dependent bat species do not exploit buildings as roosts more frequently. One possibility is that, under some circumstances, energetic benefits of torpor in cooler and more variable microclimates of natural roost sites outweigh the potential costs associated with increased predation risk in natural roost sites. These cooler roosts could provide thermal benefits by allowing bats to use torpor and depress their T_b to a greater extent than if they were to choose a warmer roost, such as a building.

Although the influence of reproduction on use of torpor and roost selection has been relatively well-studied in bats (e.g., Cryan and Wolf 2003; Lausen and Barclay 2006; Solick and Barclay 2006), little is known about the relationship between over-wintering strategy, summer roost selection and use of torpor, despite evidence of its potential importance. Temperate bats can be loosely categorised into two groups: those which migrate to warmer climates for the winter (termed “migratory tree bats” by Cryan 2003) and those which over-winter in a state of prolonged hibernation in a protected hibernaculum (Nagorsen and Brigham 1995). Species which rely on prolonged hibernation must accumulate large fat stores prior to hibernation or they will not survive

until spring (Pagels 1975; Geiser 2004; Brack 2007). Therefore, during summer, reproductive females of these species are thought to face strong selection pressure to seek out the warmest possible roosts for rapid offspring growth. Available evidence supports this prediction for a range of bat species (Pagels 1975; Geiser 2004; Brack 2007). However, much less is known about summer roost selection and use of torpor in the migratory tree bats. Koehler and Barclay (2000) reported that hoary bats, one of these migratory species, have a very slow offspring growth rate which likely reflects heavy reliance on torpor by reproductive females (Willis 2006; Willis *et al.* 2006a). In contrast to resident hibernators, these migratory tree bats are likely able to successfully reproduce in the northern part of their range, despite a slow offspring growth rate, because migration provides them with year-round access to food supplies (Koehler and Barclay 2000). Koehler and Barclay (2000) predicted that, in general, reproductive female migratory tree bats might rely more heavily on torpor during summer, than reproductive individuals of hibernating species. This means they could benefit by selecting roosts with relatively cool microclimates to achieve greater energetic savings during torpor. One prediction of this hypothesis is that migratory tree bats exposed to cool T_{roost} will use torpor more frequently and gain greater energy savings than individuals exposed to warmer T_{roost}.

My objective was to test Koehler and Barclay's (2000) hypothesis that migratory tree bats can gain energetic benefits by roosting in trees rather than buildings. To do this, I compared use of torpor and daily metabolic rate (MR) of silver-haired bats, a common migratory tree bat in southern Manitoba, exposed to treatments based on measured

temperature profiles from two types of roosts: 1) potential tree cavity roosts of silver-haired bats characterised by relatively cool T_{roost}; and 2) a little brown bat roost in a building characterised by relatively warm T_{roost}.

Silver-haired bats are a good model species for testing this hypothesis because they are a relatively common migratory tree bat and exhibit morphological and behavioural similarity to the most common, sympatric hibernating bat species, the little brown bat. At 6-12 g, silver-haired bats are similar in size to 6-10 g little brown bats and they have comparable roosting strategies during summer in that they both form maternity colonies in enclosed structures (Nagorsen and Brigham 1995; Kalcounis-Rueppell *et al.* 2005).

Addressing this hypothesis is important for understanding microhabitat selection decisions of endotherms and for conservation of not only bats, but also forests. Populations of the migratory tree bats (i.e., silver-haired, hoary, and eastern red bats (*Lasiurus borealis*)), are thought to be declining throughout North America, possibly due to their reliance on shrinking forest habitats (Cryan 2008). Therefore, understanding factors influencing their dependence on forests is crucial for management efforts which aim to incorporate protection of forest-dwelling bats.

2.0 Material and Methods

2.1 Study area and species

I conducted this study near the town of Altamont in the Pembina Hills of Manitoba, 138 km southwest of Winnipeg (Figure 1). The area is best described as a forest-agriculture matrix composed of cultivated land and scattered woodlots dominated by bur oak (*Quercus macrocarpa*) and trembling aspen trees (*Populus tremuloides*). Bats were captured in four woodlots ca. 1.7 to 6.5 km north/northeast of Altamont (Figure 1). Capture and acoustic surveys indicate that all three of Manitoba's migratory tree bats occur in these woodlots although silver-haired bats are most abundant (Willis and Jameson 2008).

2.2 T_{roost} data collection

I measured T_{roost} in four potential silver-haired bat tree-cavity roosts using small (8.4 mm radius) temperature dataloggers, accurate to ± 0.5°C (iButton thermocron, Maxim Integrated Products, Sunnyvale CA). I selected these sites based on criteria defined by Betts (1998) as typical of silver-haired bat maternity roosts. All potential roosts were in trembling aspen trees which were tall relative to the surrounding forest and, despite the presence of cavity openings, were in relatively early decay stages with intact tops (Betts 1998). Dataloggers were placed in cracks or beneath exfoliating bark at least 3 m above the ground. All roost openings faced south or southeast and opened into clearings, features which are thought to be preferred by forest-living bats because they improve solar exposure while also allowing bats to enter and exit roost cavities more easily (Betts 1998; Lausen and Barclay 2002; Kalcounis-Rueppell *et al.* 2005). I found no evidence

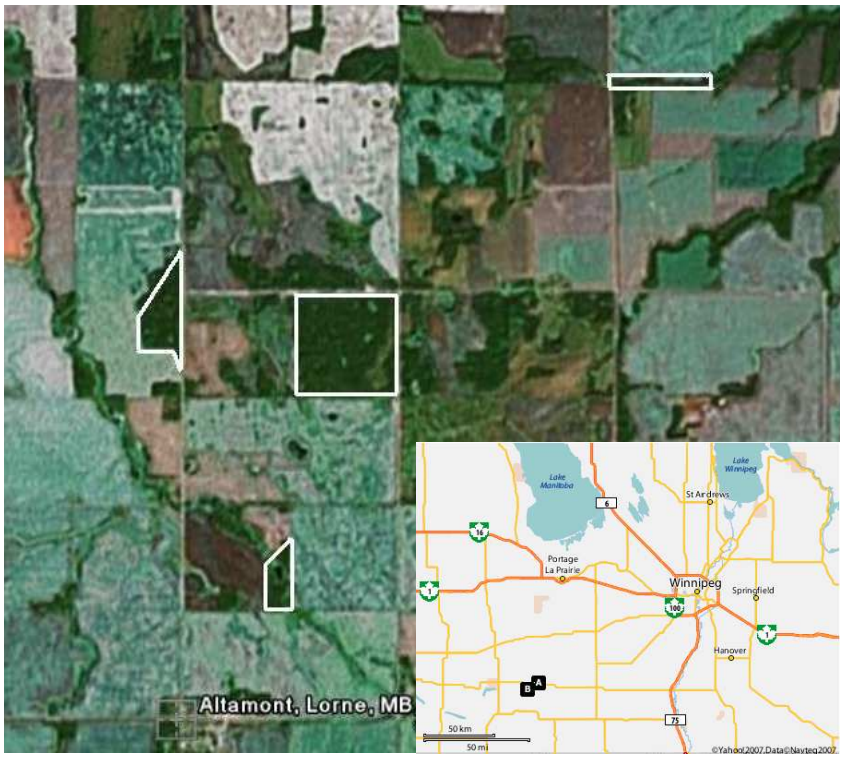

Figure 1. Map of the study area illustrating the location of the woodlots with inset map of Altamont (A) and St. Leon (B). Counterclockwise from the left, woodlots are 0.16 km^2, 0.07 km^2, 0.65 km^2, and 0.02 km^2. Image from Google earth and inset from Yahoo maps.

that bats used these cavities during the study period. However, evidence from other cavity roosting bats suggests that these bats use most if not all available cavities in a given patch of suitable forest habitat at some point during the summer (Willis and Brigham 2007). Thus, iButtons were attached to monofilament fishing line so they could be retrieved later and positioned in the roost openings so they were shaded from direct solar exposure.

I also used iButton dataloggers to record temperatures in a known little brown bat maternity roost in the Altamont Community Centre. This roost site housed about 120 individuals (based on emergence counts conducted at dusk). The bats roosted behind a brick wall and underneath the aluminium flashing at the junction of the roof and the wall, exclusively on the south and southeast side of the building. Dataloggers were attached to monofilament fishing line and lowered into the two openings from which most bats were observed emerging at dusk. I am confident that the temperatures I recorded accurately reflect the T_{roost} experienced by bats in this roost because, during the day, I could hear bats scurrying and making social calls behind the brick wall very close to where the iButtons were positioned. I also recorded outside T_a using a datalogger hanging from a nearby tree outside the Altamont Community Centre.

I programmed the iButtons in both roost types (tree and building) to record T_{roost} every 5 minutes and deployed them from 15-19 July 2007. I averaged hourly temperatures from the four different potential roost trees and also averaged hourly temperatures recorded by the two iButtons in the building roost (Figure 2). I then used these natural T_{roost} values to

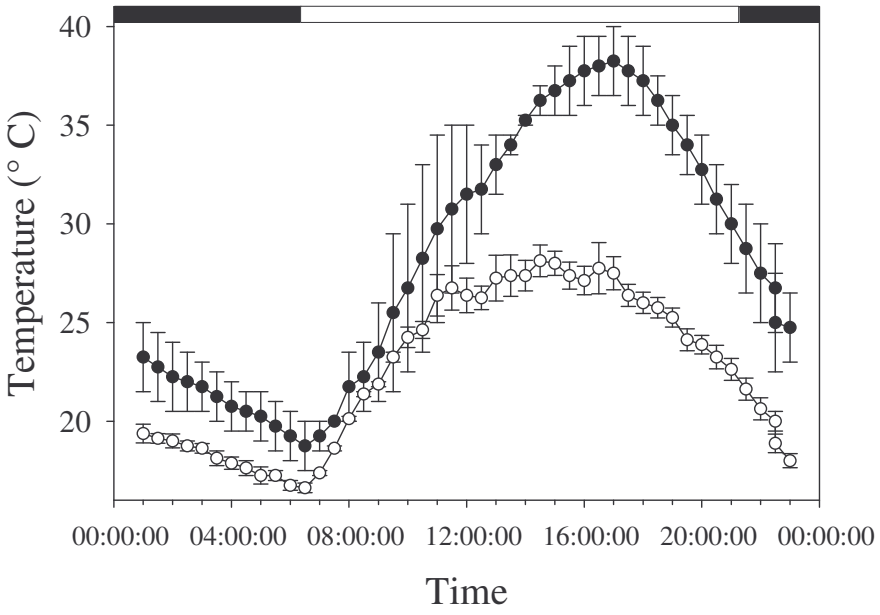

Figure 2. Values of T_{roost} measured in a building roost (●) and likely silver-haired bat tree roost (○) on 18 July 2008. These values were used as treatments during experimental trials. Dark bars represent the scotophase and the white bar represents the photophase. Values represent means ± SE.

design experimental treatments for use during two sets of metabolic trials in the laboratory (see below).

2.3 Capture and laboratory methods

I captured silver-haired bats for metabolic trials from the woodlots using mistnets set between 22:00 and 02:00, from 24 July to 20 August 2007. Within two hours of capture, I transported bats 11 km to a field laboratory in the town of St. Leon, MB, where all experiments were conducted. I measured standard body size parameters for bats (i.e., body mass, forearm length, tibia length), determined their sex, and individually marked each bat with a numbered, lipped, aluminium forearm band (Porzana, Ltd. East Sussex, U. K.). I distinguished adults (> 1 year old) from volant young-of-the-year based on the degree of fusion of the phalangeal, epiphyseal joint (Anthony 1988). Both adult and juvenile bats were captured in the study area but only juveniles were used for metabolic recordings for three reasons. First, juveniles could be captured more reliably, ensuring a larger sample size. Second, selection pressure for rapid growth in the summer and fall is likely to be strongest for juvenile bats, if this selection pressure exists. Third, the vast majority of field studies of thermoregulation by bats and other small mammals focus on adults and virtually no published data exist on thermoregulation by juveniles. Therefore, new data from juveniles are important. All bats were used for metabolic recording within three days of capture to avoid the influence of captivity on thermoregulation, use of torpor, and metabolism (e.g., Geiser *et al.* 2000). Bats held in captivity for more than one day were housed in cloth bags, hand-fed mealworms (*Tenebrio molitor*) once per day and were hand-watered with an eyedropper every four to six hours. Bats maintained body

mass each day during this brief captivity period and were released at the site of capture immediately after their metabolic trials.

I used open-circuit respirometry (Figure 3) to quantify oxygen consumption (VO_2) and carbon dioxide production (VCO_2) as proxies for MR, and temperature radio-telemetry to quantify skin temperature (T_{sk}) as a proxy for T_b. Carbon dioxide measurements were not used for analysis due to frequent problems with the CO_2 gas analyzer. Before each metabolic trial, I trimmed a small patch of fur in the inter-scapular region of each bat, and attached a 0.7 g temperature sensitive transmitter (BD-2T Holohil Systems Ltd., Carp ON) using a non-toxic, surgical adhesive (Torbot Bonding Cement, Torbot Group Inc., Cranston RI). These transmitters code temperature in their pulse rate and, prior to beginning the experiment, I calibrated them in a temperature-controlled cabinet against a digital thermometer traceable to a national standard. Once transmitters were affixed, I sealed each bat in a cylindrical, glass metabolic chamber. Chamber volume was 255 ml, and each chamber was 14.1 cm long and 4.8 cm in diameter. This was sufficiently large to allow bats to move freely during recording but small enough to ensure I could detect clear differentials between incurrent and excurrent O_2 and CO_2 concentrations (Withers 2001, Willis and Cooper 2008). I equipped each chamber with a perch made from plastic window screening to allow bats to hang comfortably. To mimic natural roost conditions and provide natural photoperiod cues, the cabinet was equipped with a shielded, compact fluorescent light connected to a light timer.

Metabolic chambers were air-tight except for inlet and outlet tubes, which were positioned at opposite ends of the chamber to improve gas mixing and minimise washout

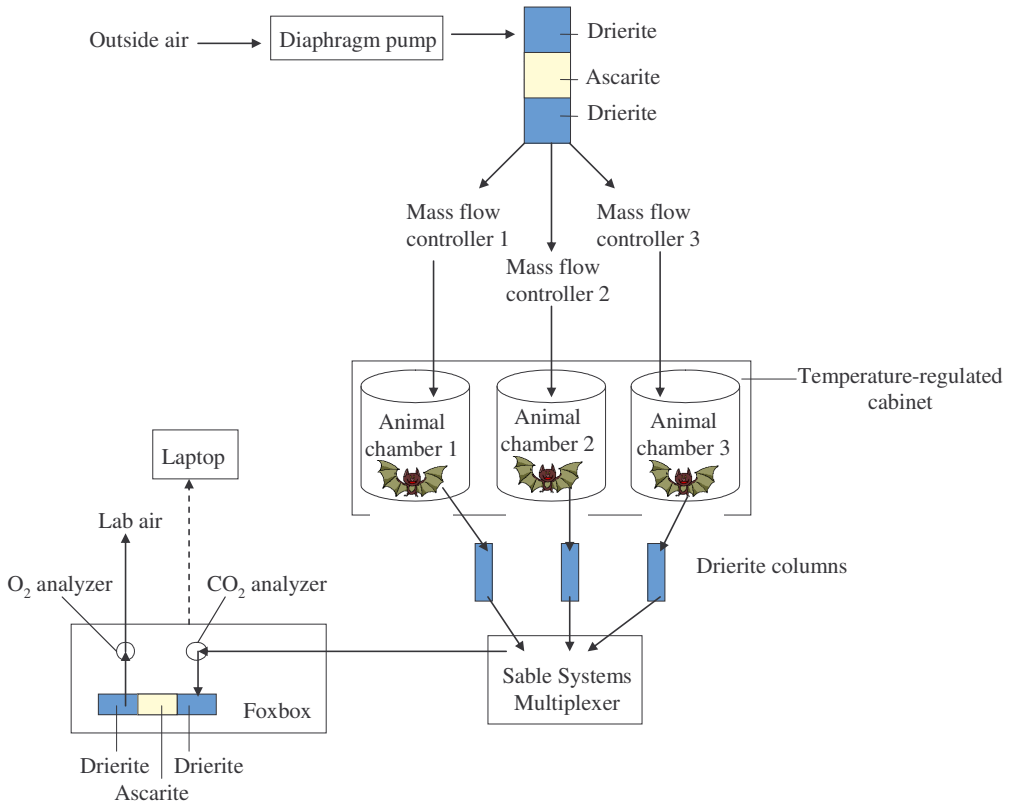

Figure 3. Open-circuit respirometry experimental setup. Solid arrows represent the direction of air flow through the system. O_2 and CO_2 measurements from the Foxbox were recorded on a laptop, indicated by the dashed arrow.

time (Withers 2001, Willis and Cooper 2008). Chambers were hung vertically in a temperature-regulated cabinet during metabolic recordings. During respirometry trials, outside air was scrubbed of CO_2, using Ascarite II (Sodium Hydroxide on Non-Fibrous Silicate Carrier; Thomas Scientific, Swedesboro, NJ), and water vapour, using Drierite Drying Agent (Anhydrous Calcium Sulfate; W.A. Hammond Drierite Co. Ltd., Ohio), and pumped, using a diaphragm pump (SG O 115-120 V/60 Hz, SCHEGO, Offenbach, Germany), through four separate channels (three animal channels plus an outside-air reference). Flow rate through each of the three animal chambers was regulated between 150 and 250 ml/min using mass flow controllers (GFC17, Aalborg Instruments Inc., Orangeburg NY). I adjusted flow rate depending on whether or not bats were torpid or normothermic to ensure it was high enough that O_2 concentration did not fall below 20.2%, and CO_2 concentration did not exceed 0.43%, but low enough that I could still detect small differences between incurrent and excurrent O_2 and CO_2 during torpor (Withers 2001, Willis and Cooper 2008).

The system maintained continuous airflow through all three chambers and I sub-sampled outlet air at 80 ml/min from the outside-air reference channel and then each animal chamber in sequence using the on-board pump and flow control system of the gas analyser (Foxbox, Sable Systems International) and a computer-controlled, respirometry multiplexer (Intelligent Multiplexer V3, Foxbox Sable Systems International). Excurrent air from all three animal channels was dried upstream of the multiplexer. I varied the recording time slightly for each animal chamber and the reference depending on how many animals I had captured and was able to run on a given night (i.e., between 1 and 3).

This ensured that VO_2 and VCO_2 were recorded for all animals at least every 30 minutes. If I ran three animals, I recorded from the reference channel for 90 seconds and from the chambers for 9.5 minutes each. If I ran two animals, I recorded from the reference channel for 4 minutes and from the chambers for 12 minutes. In these recordings, chamber recordings were interspersed with baseline measurements. If I ran one animal, I recorded from the reference channel for three minutes and from the chamber for 10 minutes. All recording times were more than adequate to accommodate the washout characteristics of the metabolic chambers and tubing (Withers 2001; Willis and Cooper 2008). Sub-sampled air flowed first through the CO_2 gas analyzer of the FoxBox and was subsequently scrubbed of CO_2 prior to O_2 measurement. Metabolic recordings were stored on a laptop using ExpeData (v. 1.0.24, Sable Systems International). At the end of each metabolic recording, bats were removed from metabolic chambers; their transmitters were removed using a non-toxic adhesive remover (Detachol Adhesive Remover, Ferndale Laboratories Ltd., Michigan) and their body mass was recorded to the nearest ± 0.1 g. I assumed a linear loss of body mass throughout metabolic trials for calculation of mass-specific MRs.

Respirometry trials began between 23:30 and 05:00. Trials could not be started at exactly the same time each night because bats were not necessarily captured at the same times. However I programmed the temperature-controlled cabinet to ensure that each bat would be exposed to the same temperature at the same time. For instance, during exposure to the simulated building T_{roost}, all bats were exposed to a T_a of 17.9 °C at 06:30. Metabolic trials lasted for 21-hour periods. I chose this recording length because bats forage for a

few hours each night, and thus generally do not spend a full 24-hours in their roost (Murray and Kurta 2004).

I performed weekly calibrations of gas analysers using 100% N_2 as a zero and 1% CO_2 in pure N_2 as a span gas for the CO_2 analyzer. I used outside air to span the O_2 analyzer because atmospheric air has a highly stable oxygen concentration of 20.95%. I also tested for leaks in each metabolic chamber weekly and in the system as a whole bi-weekly to ensure accurate measurements.

In addition to metabolic data, I recorded T_{sk} using a datalogging radiotelemetry receiver (SRX400, Lotek Wireless Inc., Newmarket ON). I entered pre-recorded calibration data for each transmitter into the receiver's memory and programmed the receiver to convert transmitter pulse rates to T_{sk} every five minutes during metabolic recordings. I also recorded T_a inside each metabolic chamber using iButton dataloggers synchronised with the receiver to record every five minutes.

2.4 Experiment 1 – T_{roost} profile experiment

For the first set of metabolic trials, I used the T_{roost} recorded in the tree and building roosts on 18 July 2007 as treatments (Figure 2). I selected this date because outside T_a on 18 July best represented conditions for an average day in July in the study area based on historical climate data from 2006 (weathernetwork.com). The maximum and minimum T_a recorded by a datalogger positioned outside the building roost on 18 July were 28° C and 16.6° C, respectively, and the averages of those recorded from the University of

Manitoba weather station at Carmen (51.7 km from Altamont) between 1 July and 31 July, 2006 were 28.1° C and 12.7° C, respectively (weathernetwork.com). Thus, the roost temperatures I recorded on 18 July likely reflected average conditions experienced by bats in their roosts at this time of year. I programmed each 24-hr T_{roost} profile into the memory of the temperature-regulated cabinet and, each day, randomly selected whether to apply the simulated building or simulated tree T_{roost} profile for that day's experimental run. Depending on how many bats I was able to capture on a given night, I obtained metabolic recordings from one, two, or three bats over a 21-hour period.

2.5 Experiment 2 – Constant T_{roost} experiment

For the second set of metabolic experiments I compared MRs of bats in the two roost types assuming it was a relatively cool, overcast day, as opposed to an average day because of the potential effect of cool weather on the energy budgets of bats. This also allowed for comparison to previous studies which have only measured O_2 consumption at constant temperatures as opposed to temperature profiles based on natural T_{roost} (e.g., Cryan and Wolf 2003; Dunbar 2007; Dunbar and Tomasi 2006). For these trials I used the overall daily average tree T_{roost} (21° C) to simulate conditions in the colder tree roost, and the overall daily average building T_{roost} (27.5° C) to mimic conditions in the warmer building roost. I programmed the temperature controlled cabinet to maintain these chamber temperatures, and temperatures did not deviate by more than ± 2° C throughout metabolic trials. The average T_{roost} for the tree was an accurate representation for an average day in July in the study area, based on historical climate data from 2006. The average calculated temperature from the iButtons was 21° C, and the average recorded

temperature from the Carmen University of Manitoba weather station was 20.4° C (weathernetwork.com). Again, I randomly selected whether to apply the tree or building constant T_{roost} for a given day's experimental run, and obtained metabolic recordings of one, two or three bats for 21-hour experimental trials.

2.6 Data processing and statistical analysis

I used ExpeData to record and process data from respirometry trials (i.e., to correct for gas analyser drift using data from the reference channel and to automate calculation of VO_2 and VCO_2). From each respirometry trace for each 9.5 – 12 minute recording, I selected the minimum, stable 30-second section from within the last 90 seconds of recorded measurements (Withers 2001). I calculated VO_2 and VCO_2 using standard respirometry equations from Withers (2001). I also quantified torpor duration (hours) and torpor frequency, and calculated daily whole animal (ml O_2/day) and mass-specific (ml O_2/g/day) MR for each bat. I defined the onset of torpor bouts based on obvious, abrupt declines in T_{sk} and whole animal MR and the end of torpor bouts based on a clear increase in MR coupled with a corresponding increase in T_{sk} (see results).

Inspection of histograms for data from both experiments clearly indicated that distributions of dependent variables violated equal variance assumptions of parametric analyses. Therefore, I used non-parametric ANCOVAs (with starting body mass as a covariate) or Kruskal-Wallis tests, as appropriate. For non-parametric ANCOVAs, I ranked values for all recorded variables and performed ANCOVA on the ranks (Edwards

1993). Significance for all tests was assessed at an alpha level of 0.05. Values are reported as averages ± standard deviation unless otherwise indicated.

3.0 Results

3.1 Experiment 1 – T_{roost} profile experiment

In total, for both experiments, I obtained metabolic recordings from 33 juvenile silver-haired bats (20 female, 13 male). Mean body mass prior to metabolic recording was 10.8 ± 0.21 g. Twelve bats (7 female, 5 male) were exposed to the simulated building T_{roost} profile (hereafter building roost), and 9 (6 female, 3 male) to the simulated tree T_{roost} profile (hereafter tree roost). Bats exposed to the building roost tended to use one deep torpor bout beginning in the early morning and ending with a passive arousal as T_{roost} increased in the late morning to early afternoon (e.g., Figure 4A). Some bats exposed to the building roost also used a second shallower torpor bout in the late afternoon/early evening. Bats exposed to the tree roost were much more variable in terms of torpor use. Some individuals used torpor rarely, or not at all (e.g., Figure 4B), while some individuals used several shallow torpor bouts and others used long, deep torpor bouts throughout most of the experimental run.

Despite qualitative difference in their responses to the two treatments, bats exposed to the tree roost did not spend significantly more time in torpor than those exposed to the building roost ($F_{1,19} = 0.014$, $p = 0.91$; Figure 5A). However, bats exposed to the tree roost did arouse from and re-enter torpor more often. That is, they used a larger number of discrete torpor bouts when exposed to the cooler tree roost (2.0 ± 0.78 compared to 1.0 ± 0.39 bouts in the building, $F_{1,19} = 7.56$, $p = 0.013$; Figure 5B).

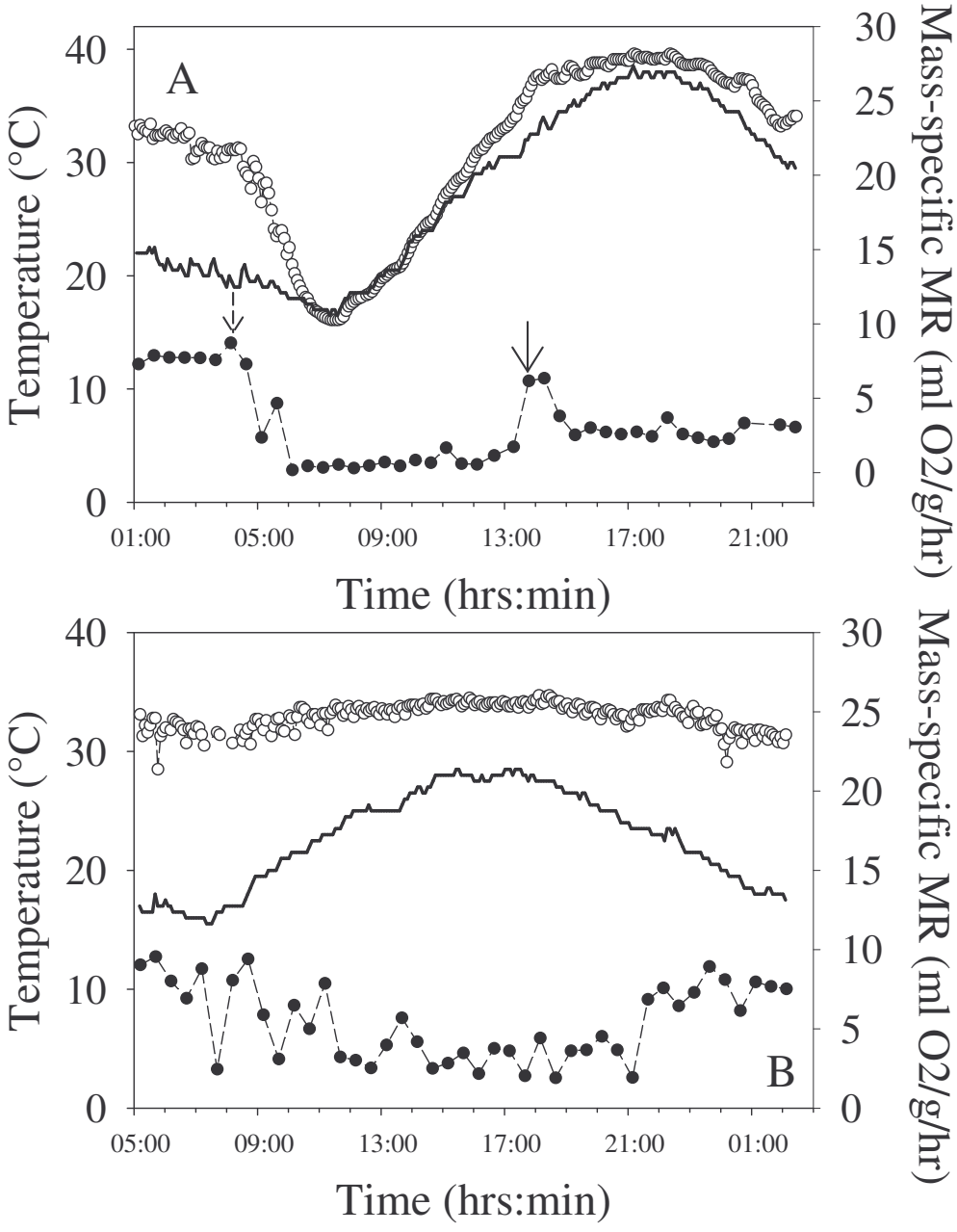

Figure 4. Representative traces of metabolic trials for individuals exposed to a building T_{roost} profile (A) and a tree T_{roost} profile (B). Empty circles represent T_{sk}, the solid line represents T_a in the chamber, and filled circles represent mass-specific MR (in half-hour intervals). The dashed arrow represents the beginning of a torpor bout, and the solid arrow represents the end of a torpor bout.

Roost type did not significantly affect daily mass-specific MR ($F_{1,19} = 0.65$, $p = 0.43$; Figure 5C) or daily whole-animal MR (nonparametric ANCOVA; $F_{1,19} = 0.11$, $p = 0.75$; Figure 5D). There was no significant difference between starting body masses of bats exposed to the tree (10.0 ± 0.49 g) or building roost (10.78 ± 0.21 g, Kruskal-Wallis; $U = 76$, $p = 0.12$) but, as a covariate in the ANCOVA models, body mass had a strong influence on both torpor duration ($F_{1,19} = 19.26$, $p < 0.001$; Figure 5A) and daily whole-animal MR ($F_{1,19} = 8.56$, $p = 0.01$; Figure 5D). Smaller bats spent more time in torpor and subsequently reduced their metabolic rate significantly more compared to larger bats (Figure 5). Starting body mass was not significantly related to torpor frequency ($F_{1,19} = 2.98$, $p = 0.101$), mass-specific MR ($F_{1,19} = 1.81$, $p = 0.20$; Figure 5C), minimum TMR (min TMR) ($F_{1,19} = 0.11$, $p = 0.75$) or T_a at min TMR ($F_{1, 19} = 1.09$, $p = 0.31$) so I removed it as a covariate from these analyses and used Kruskal-Wallis Tests to compare each variable between the two treatments. Roost type had a significant effect on torpor frequency ($U = 19$, $p = 0.006$), however there were no significant effects of roost type on mass-specific MR ($U = 49$, $p = 0.72$), min TMR ($U = 67$, $p = 0.14$), or T_a at min TMR ($U = 68$, $p = 0.12$).

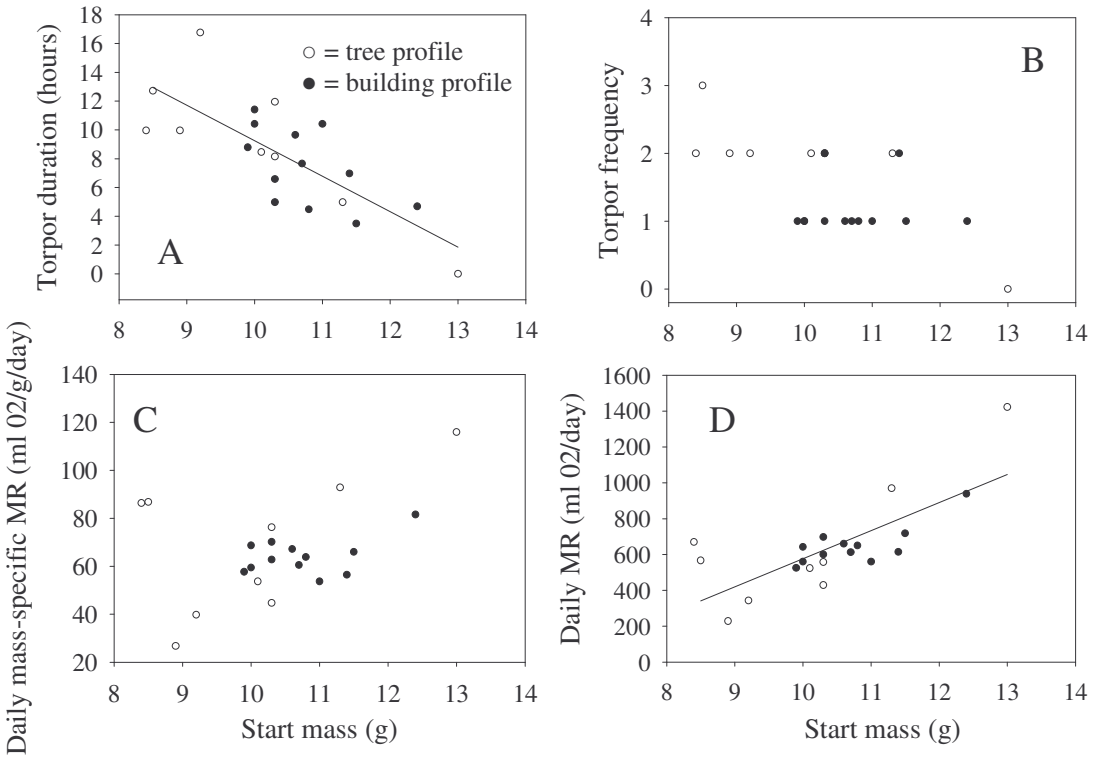

Figure 5. Relationship between start mass and (A) duration of torpor bouts, (B) torpor frequency, (C) daily mass-specific MR, and (D) daily MR in bats exposed to the building and tree T_{roost} profiles. Filled circles represent bats exposed to the building roost, and empty circles represent those in the tree roost. Regression line equations for A, and D are y = -2.466 + 33.914; and y = 156.8x – 991.89, respectively.

3.2 Experiment 2 - Constant T_{roost} experiment

Six bats (3 female, 3 male) were exposed to the constant building T_{roost} condition (hereafter constant building), and 6 (4 female, 2 male) to the constant tree T_{roost} condition (hereafter constant tree). Despite the fact that 27.5° C is likely below the lower critical temperature of the thermoneutral zone for silver-haired bats, four out of six bats exposed to the constant building did not make use of torpor at all (e.g., Figure 6A), and averaged 1.0 ± 1.33 torpor bouts over experimental trials. The two bats that did use torpor in the constant building generally entered short periods of shallower torpor. All bats in the constant tree used torpor at least twice during experimental trials, and averaged 3.0 ± 0.22 torpor bouts (Figure 6B). These bats used longer, deeper bouts of torpor than those in the constant building.

In contrast to results for the T_{roost} profile experiment, bats exposed to the constant tree spent significantly more time in torpor than those exposed to the constant building (Kruskal-Wallis; $U < 0.01$, $n = 6$, $p = 0.003$; Figure 7A) and used a greater number of discrete torpor bouts ($U = 6.0$, $p = 0.04$; Figure 7B). However, this effect did not translate into as pronounced a difference in daily MR. Although there was a strong trend for greater metabolic savings for bats exposed to the constant tree this effect did not quite reach significance for either daily mass-specific ($U = 31$, $p = 0.055$; Figure 7C) or whole animal MR ($U = 30$, $p = 0.055$; Figure 7D). Bats exposed to the constant tree had a lower min TMR than those exposed to the constant building (Kruskal-Wallis; $U = 18$, $p = 0.02$), but there was no significant effect of roost type on T_a at min TMR ($U = 15$, $p = 0.088$).

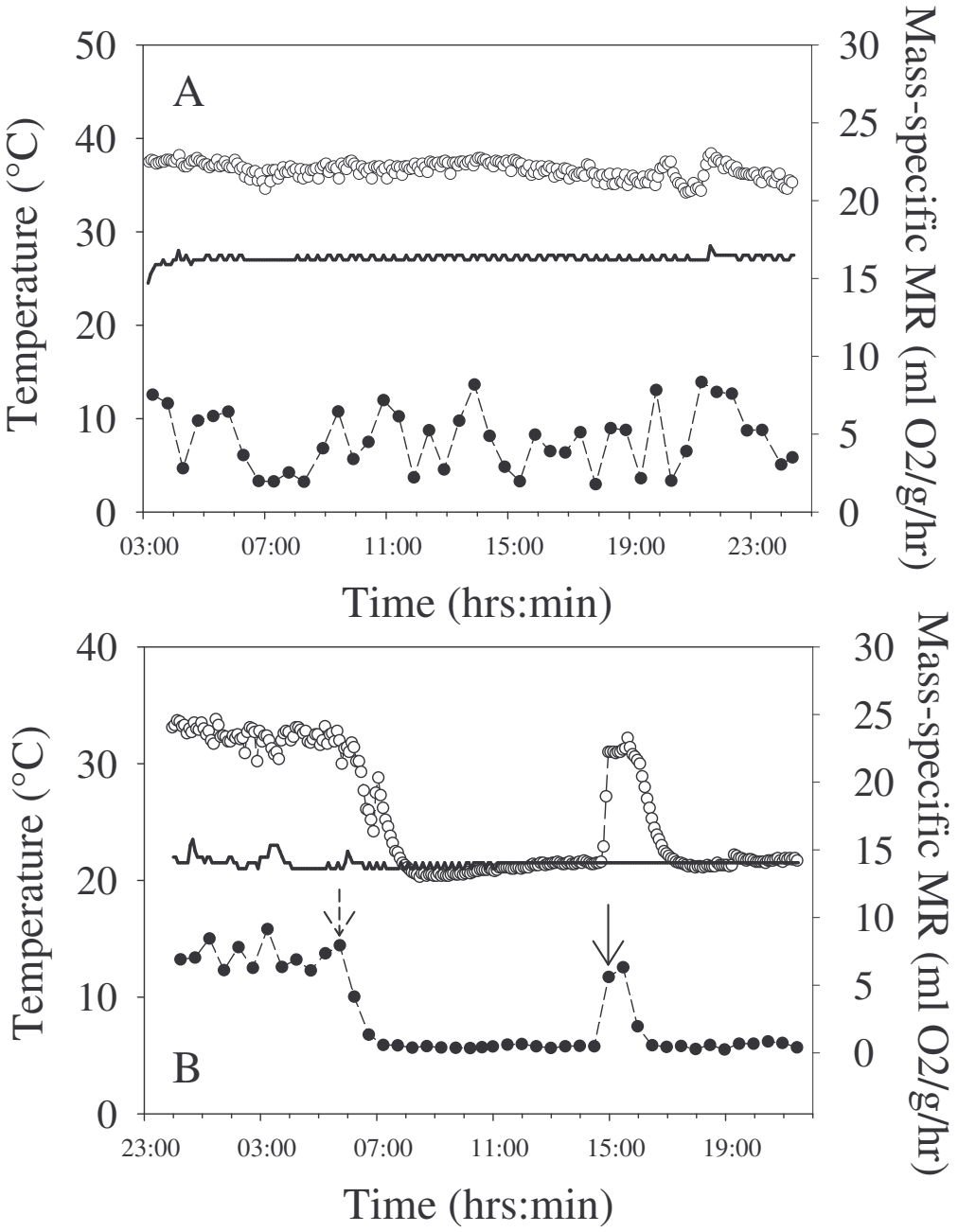

Figure 6. Representative traces of metabolic trials for individuals exposed to a constant building T_{roost} (A) and a constant tree T_{roost} (B). Empty circles represent T_{sk}, and the solid line represents T_a in the chamber. Filled circles represent mass-specific MR (in half-hour intervals). The dashed arrow represents the beginning of a torpor bout, and the solid arrow represents the end of a torpor bout.

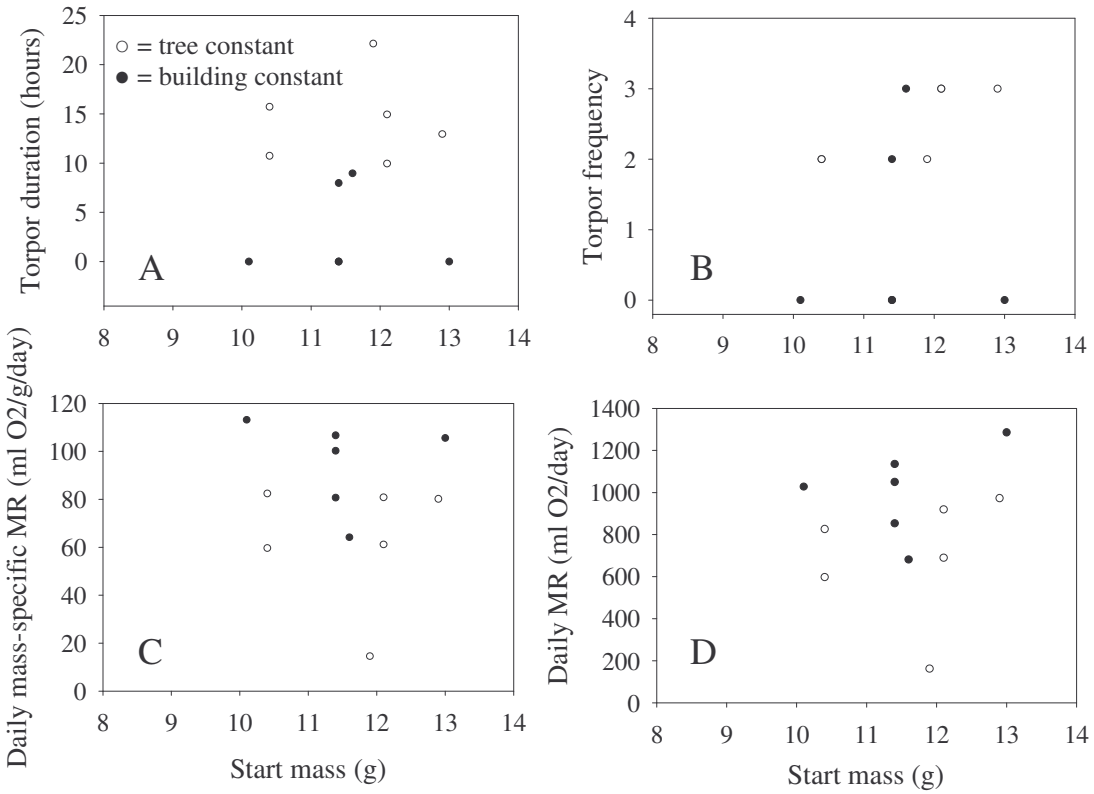

Figure 7. Relationship between start mass and (A) duration of torpor bouts, (B) torpor frequency, (C) daily mass-specific metabolic rate, and (D) daily metabolic rate in bats exposed to the constant building and tree T_{roost}. Filled circles represent bats exposed to the constant building temperature condition, and empty circles represent those exposed to the constant tree temperature condition.

The mean body masses of bats exposed to the constant building (11.5 ± 0.38 g) and constant tree (11.6 ± 0.41 g), were not significantly different (U = 14, p = 0.52) and in contrast to the T_{roost} profile experiment, surprisingly, did not have a significant effect as a covariate on any of the dependent variables (torpor duration: $F_{1,3} = 0.014$, p = 0.91, Figure 7A; torpor frequency: $F_{1,3} = 1.57$, p = 0.24, Figure 7B; daily mass-specific MR: $F_{1,3} = 0.26$, p = 0.62, Figure 7C; or daily whole animal MR: $F_{1,3} = 0.73$, p = 0.42, Figure 7D). Mass also had no effect on min TMR ($F_{1,3} = 1.21$, p = 0.31), or T_a at min TMR ($F_{1,3} = 1.39$, p = 0.28).

4.0 Discussion

This study provides some support for the hypothesis that migratory tree bats rely more heavily on torpor when exposed to conditions simulating a cooler tree-cavity roost compared to conditions simulating a warmer building roost, but only under certain circumstances. Juvenile silver-haired bats did not rely more heavily on torpor or benefit from a reduction in daily MR during exposure to a T_{roost} profile which simulated conditions in their tree roosts on an average July day, compared to a T_{roost} profile simulating conditions in a building roost of a different species. However, bats showed a greater proclivity for torpor use when exposed to conditions simulating their natural tree roosts on a relatively cool, overcast day. I used a conversion factor of 20.083 kJ per ml O_2 to quantify the energetic consequences of these different patterns (Withers 2001). Bats exposed to the constant building spent 20.2 ± 4.3 kJ per 21 hour trial compared to 14.0 ± 5.9 kJ per 21 hours in the constant tree, an energy savings of 31%. There are currently no data on field metabolic rates (FMR) of free-living silver-haired bats, but Kurta *et al.* (1989) used the doubly-labelled water method to demonstrate that free-ranging pregnant little brown bats required 33.7 kJ day^{-1}, and lactating females required 41.3 kJ day^{-1}. Foraging and flight will clearly account for a large portion of daily energy expenditure in bats and Kurta *et al.* (1989) reported flight costs of 61% of FMR in pregnant little brown bats, and 66% in lactating little brown bats. Assuming similar FMRs and a similar proportion of the daily energy budget spent on flight and foraging for juvenile silver-haired bats, my data suggest that individuals would expend 48.1 – 53.2 kJ d^{-1} in a building roost vs. 48.5 – 53.6 kJ d^{-1} in a tree roost on an average July day. However, on the cool, overcast day, my results predict a total daily energy expenditure of 72 – 79.6 kJ

d^{-1} in a building roost vs. 49.9 – 55.2 kJ d^{-1} in a tree roost, a savings of 31%. These values are clearly somewhat speculative for silver-haired bats as Kurta *et al.* (1989) calculated daily energy use in reproductive and lactating little brown bats, as opposed to juveniles. However, they support the hypothesis that migratory tree bats may save energy in their tree roosts compared to building roosts under certain conditions.

Despite the energetic benefits of roosting in trees on cool, overcast days, during exposure to conditions simulating an average day in July, there was no effect of roost type on torpor duration or energy use. This suggests that there may be selection pressures other than thermoregulatory benefits which influence silver-haired bats to roost in trees. Preference for tree-cavity roosts may partially be associated with the solitary-roosting habits or much smaller maternity colonies of silver-haired bats compared to the colonial roosting behaviour of many temperate hibernators (Barclay *et al.* 1988; Lausen and Barclay 2006; Willis and Brigham 2004). Colonial species require larger roosts to accommodate more individuals, whereas solitary species are not confined by this potential limitation. Some hibernators, such as little brown bats, often form large colonies (120 individuals at my study site), thus they may select larger roosts, such as buildings, to accommodate their large colony size. For example, Willis and Brigham (2007) demonstrated that forest-living big brown bats, a colonial species which hibernates, preferred roosting in relatively spacious tree cavities. This allowed for larger numbers of clustering bats, which elevated T_{roost} and resulted in significant energy savings (Willis and Brigham 2007). Although there are benefits to roosting in large aggregations, there are also some costs. For instance, roosting in colonies can deplete local food sources, in

which case bats would need to travel further to find food, increasing energetic costs of flight, while solitary roosting bats, or those in small groups, may benefit from reduced competition (Kunz and Lumsden 2003). Thus, roosting in an area with a greater abundance of smaller tree cavities, and where competition for food might be less, would likely facilitate a solitary-roosting bat's decision to select a forest habitat over a larger roost in a building.

My results provide some support for Koehler and Barclay's (2000) hypothesis that migrants face less selection pressure than hibernators to seek out the warmest possible summer roosts. Most studies of summer torpor use by free-ranging bats focus on species which hibernate for the winter, rather than migrate (Kurta *et al.* 1989; Kerth *et al.* 2001b; Lausen and Barclay 2006; Turbill and Geiser 2005; Turbill 2006a; Boyles *et al.* 2007). Hibernators are subjected to low T_a, often near 0°C, for periods lasting up to 6 months (Neubaum *et al.* 2006). Adequate energy reserves prior to hibernation and selection of an appropriate summer roost that minimizes energy expenditure while maximizing fat storage is essential to increase the likelihood of overwinter survival (Humphries *et al.* 2003). This is especially important for reproductive females and juveniles because juveniles are especially susceptible to starvation during their first winter. Migratory tree bats might enjoy more relaxed selection pressure for rapid offspring growth because they have access to food for most or all of the winter. Migration may be energetically costly, but likely represents a much less severe energetic bottleneck for migratory bats than long-term hibernation, allowing them to select cooler microhabitats and use torpor to obtain immediate benefits associated with reduced energy expenditure. The energy savings

gained by silver-haired bats in cool roosts would come with the cost of a reduced growth rate (Racey 1973) but for migratory species, the short term energetic gains of torpor could outweigh long-term reductions in growth rate (Koehler and Barclay 2000).

Even stronger than the influence of roost type on my results, there was a clear effect of starting body mass during the T_{roost} profile experiment, which suggests that smaller body mass may influence roost selection over the summer. Humphries *et al.* (2003) predicted that smaller mammals should use torpor more frequently to compensate for high rates of heat loss due to their large surface to volume ratio. Larger mammals with reduced heat loss and the potential for larger fat reserves could use torpor less often. Although this model was suggested for over-winter hibernators, Humphries *et al.*'s (2003) predictions about torpor expression based on body mass are consistent with patterns of torpor use exhibited by silver-haired bats in my study, as the larger bats used torpor less than the smaller bats. Larger bats likely faced reduced rates of heat loss and may have already accumulated larger fat reserves than smaller bats. Thus, smaller individuals may have been forced to use torpor because of higher rates of heat loss combined with smaller energy stores.

The significant effect of body mass on torpor duration and daily MR could also be related to relative ages of bats and the development of thermoregulation. My study took place over the course of just over a month during summer and the smallest bats were those captured earliest in the season. Thus it seems likely that smaller bats were younger and may not have developed the same thermoregulatory capability as older, larger

individuals. Geiser *et al.* (2006) described the development of thermoregulation in a small heterothermic marsupial, the stripe-faced dunnart, and reported that torpor bouts were longer and more frequent in smaller individuals early during the developmental period, vs. larger individuals which were older. Geiser *et al.* (2006) demonstrated that this pattern occurred shortly after dunnarts were capable of exploiting heterothermy. Although my study did not specifically evaluate development of thermoregulation from birth to adulthood, it is possible that the smaller bats, which used torpor for longer durations of the experimental trial, were younger and had most likely only recently developed the ability to maintain a high and constant T_b. Thus, developing the ability to remain normothermic could be related to increasing body mass over the developmental period. Smaller bats save more energy by exploiting deeper torpor bouts and use less energy arousing from torpor than larger bats due to their small volume (Turbill 2006a, b). As bats get larger they require more energy to actively arouse from torpor bouts (Turbill 2006a, b), thus, they might use shallower bouts of torpor and reduce the time spent in torpor to compensate for this increase in body mass. Another possibility is that the smaller bat's more frequent use of torpor could represent an inability to stay warm. These torpor patterns may reflect a shortage of food shortly after the development of endothermic thermoregulation (Geiser *et al.* 2006), or a critical period in development in which torpor is required to develop normal heterothermy. No published studies have explored the development of thermoregulation in bats so experiments are required to investigate these differences in energy use and their possible significance on developmental changes in torpor expression.

Laboratory studies may be limited in terms of what they tell us about behaviour and physiology of free-living animals due to the potential for captivity effects and the possible influence of stress on behavioural and physiological responses (Geiser and Ferguson 2001). I eliminated potential effects of long-term captivity because I only held bats for a maximum of three days. Stress may still have influenced my results because bats may not have adjusted to captivity in such a short period of time. However both treatment groups would have been equally stressed, thus it likely would not have affected the comparison. In addition, I took several steps to ensure semi-natural conditions in the lab. For instance, bats were not fed *ad libitum*, but rather were fed mealworms once a day, just prior to experimentation, typically during the time they might be expected to forage. Furthermore, the datalogger recordings from the building roost were an exact replica of natural conditions in a little brown bat building roost, and also accounted for the effects of clustering in elevating T_{roost}. Thus, temperature conditions were consistent between the natural and simulated roosts. The temperatures obtained from the tree cavities were taken from unoccupied cavities that may have served as roosts; however this likely did not have an effect on recordings obtained from the tree cavities, since silver-haired bat colonies tend to be small (e.g., 2-6 individuals; Nagorsen and Brigham 2005) and would likely not be large enough to elevate T_{roost} significantly.

5.0 Conclusions

[1] There was no difference in use of torpor or energy expenditure between bats in the tree or building roost conditions when temperatures simulated an average day in July.

[2] When conditions represented a cool, overcast day, bats used torpor significantly more in the simulated tree roost, thus suggesting that silver-haired bats may save energy by roosting in a cooler tree roost under these conditions.

[3] Torpor is associated with delayed growth and development; however with access to food year-round, forest-dwelling migratory bats may not face the same selection pressures as hibernators to select roosts with warmer microhabitats to reduce torpor use and increase growth and development rates over the summer.

[4] Body mass was a strong predictor of torpor duration and energy use, which may suggest a difference in heterothermy in younger newly volant vs. older juvenile bats, such that younger bats may be unable to defend a high T_b and thus use torpor more to counteract their high rates of heat loss due to their smaller volume and larger surface area.

[5] Further studies which investigate roost selection are important to maintain and preserve forest roosts to prevent the further decline of forest-dwelling bats, such as the migratory silver-haired bat.

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