

WINTER ECOLOGY AND ECOPHYSIOLOGY OF PRAIRIE-LIVING BIG
BROWN BATS (*EPTESICUS FUSCUS*)

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Brandon Jeremiah Baerwald, candidate for the degree of Doctor of Philosophy in Biology, has presented a thesis titled, ***Winter ecology and ecophysiology of prairie-living big brown bats (ptesicus fuscus)***, in an oral examination held on March 24, 2017. The following committee members have found the thesis acceptable in form and content, and that the candidate demonstrated satisfactory knowledge of the subject material.

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ABSTRACT

Hibernation allows animals to survive lengthy periods of energetic deficit, but is not without costs. Hypometabolism, low body-temperature, and inactivity are associated with a variety of costs such as immuno-incompetence, dehydration, and build up of harmful metabolites. Additionally, conditions within hibernacula have a profound influence on hibernation patterns and survival. Periodic arousals and site selection are thought to mitigate these costs, and often involve timing arousals to foraging opportunities and overwintering in locations with stable temperatures and high humidity. I studied prairie-living big brown bats (*Eptesicus fuscus*) that overwinter in rock crevices and take flight outside of the hibernacula despite a lack of foraging opportunity. My goal was to describe their winter ecology and behaviour, and investigate reasons for winter flight. I found that *E. fuscus* in my study area use relatively dry hibernacula compared to known cavernous sites and show fidelity to sites between and within years. I found that temperature and wind are important predictors of winter flight, and that arousals remain under diurnal influence. My data suggest that individuals from this particular population spend the majority of their winter energy-stores during steady-state torpor and have mechanisms to decrease evaporative water loss during hibernation. I found typical levels of dehydration as winter progressed and my data indicate no use by bats of a supplemental water source. My research elucidates novel behaviours and traits of this population of *E. fuscus*, and reduces the paucity of knowledge about winter bat-ecology in the prairies.

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DEDICATION

I dedicate this chunk of work to my lovely, brilliant, beautiful wife, Dr. Erin Baerwald. She started me down this academic path and gave me all the support and inspiration I needed to see it through this last step. From being super flexible with my field schedule and holding down the fort while I was away (which was a lot!) to numerous manuscript reviews and putting up with being ditched in a new city with few friends and a car you could not drive... you are truly the best! I am proud to be your partner and I love you (even more than beer!).

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CHAPTER ONE—GENERAL INTRODUCTION

1.1 BACKGROUND

Torpor is a physiological state during which metabolic rate (MR) is depressed and as a consequence body temperature (T_b) is lowered to substantially conserve energy (Geiser 1988; Heldmaier 1992; Wang and Wolowyk 2011). Hibernation (seasonal or long-term torpor) is mainly used by species living in temperate regions that experience extended periods of food shortage during winter (Wang and Wolowyk 2011; Ruf and Geiser 2015). By suppressing metabolic activity and reducing T_b , animals can survive for months on energy reserves stored in the form of fat (Humphries et al. 2003). A little brown bat (*Myotis lucifugus*) entering hibernation with an energy reserve of fat that is 38% of its body mass can survive up to 585 days without consuming additional energy (Humphries et al. 2003). Aside from considerable energy savings, there are other proposed benefits to torpor expression. The rate of evaporative water loss is reduced as an inherent benefit of suppressed metabolic rate and respiration (Hosken and Withers 1997; 1999), beneficial in periods of drought (Geiser and Brigham 2012).

Despite the many benefits of hibernation (Geiser and Brigham 2012), reductions in T_b and metabolic rate also have consequences for a number of biological processes. Suppression of metabolism reduces maintenance and growth, and possibly decreases fitness by inhibiting reproductive activity (Barnes et al.

1986; Lillegraven et al. 1987). Muscle catabolism and degeneration of muscle tissue is associated with hibernation (Wickler et al. 1987; Tinker et al. 1998), and immunosuppression leaves individuals susceptible to parasites or infectious diseases (Bouma et al. 2010). Given these physiological costs and the diminishing energetic returns of reducing body temperature (Studier 1981), small mammals can be expected to minimize time spent in torpor (Humphries et al. 2003). Indeed, hibernation is not a continuous state of quiescence and nearly all small mammals periodically arouse, for reasons poorly understood and extensively debated (Willis 1982; French 1985). Restoration of neural function (Popov et al. 1992), removal of metabolic waste (Malan 1986), mounting of an immune response (Bouma et al. 2010), mating (Barnes et al. 1986), maintenance of muscle tissue (Yacoe 1983), and access to food and water (Humphries et al. 2003) have all been suggested.

Arousals are necessary and have an essential role in hibernation biology, using a disproportionately large amount of energy (e.g., Armitage et al. 2003; Dunbar and Tomasi 2006). Over 80% of an individual's energy reserves can be spent warming up and maintaining high T_b during arousals and euthermia (Thomas et al. 1990; Geiser 2004). Balancing the costs and benefits of torpor bouts and arousals is crucial for small hibernators with finite energy reserves, and the hibernation patterns (i.e., torpid skin temperature, duration of torpor bouts, and frequency of arousals) of most animals are profoundly influenced by the microclimate (e.g., temperature and humidity) within their hibernacula (French 1985). Ambient temperatures above an animal's body-temperature setpoint during

hibernation (T_{set} ; Heller 1979) restrict the depth of T_b reduction and result in higher metabolic rates and more frequent arousals (e.g., Geiser and Kenagy 1988), whereas T_a below T_{set} stimulates increased metabolic activity and arousal frequency to avoid freezing (e.g., Geiser and Broome 1993; Buck and Barnes 2000; Humphries et al. 2002; Dunbar and Tomasi 2006). Humidity is also important because evaporative water loss persists during hibernation (Thomas and Cloutier 1992). Torpor bouts shorten (Thomas and Geiser 1997) and arousals become more frequent (Ben-Hamo et al. 2013) with increasingly dry conditions. Even small changes in temperature or humidity increase energy use and reduce overwinter survival (Boyles and Brack 2009).

Many hibernators seek out shelters with particular characteristics, often subterranean cavities (e.g., dens, burrows, caves, or mines) with stable microclimates (i.e., temperature and humidity). For example, burrows of hibernating arctic ground squirrels (*Urocitellus parryii*) occur in areas with higher soil temperatures than surrounding sites (Buck and Barnes 1999), and bats in eastern North America often overwinter in large, mixed-sex clusters within caves or mines with high levels of humidity and stable temperatures (Webb et al. 1996; Perry 2012). Cavernous hibernacula allow for flight and possibly mating (Thomas et al. 1979) within open chambers during arousals, and may contain water and hibernating insects that can be consumed (Swanson and Evans 1936; Rysgaard 1942).

Winter activity outside of these hibernacula appears to be associated with foraging opportunities in areas with mild winters. Warm calm nights are associated with increased insect abundance (Taylor 1963) and activity of many insectivorous hibernators. Tri-colored bats (*Perimyotis subflavus*), little brown myotis (*Myotis lucifugus*), northern long-eared myotis (*M. septentrionalis*), and brown long-eared bats (*Plecotus auritus*) are more active during warmer, calmer winter nights (Hays et al. 1992; Whitaker and Gummer 1992; Whitaker and Rissler 1993). Hibernators buffered from outside conditions likely use falling barometric pressure to cue activity on warm, Austral-winters nights (Turbill 2008), and to cue emergence from hibernation in some populations of bats (e.g., *M. lucifugus*; Czenze and Willis 2015). Diurnal rhythms in winter activity ostensibly facilitate foraging in a number of small mammals, such as the pocket mouse (*Perognathus longimembris*; French 1977) and mountain pygmy possum (*Burramys parvus*; Körtner et al. 1998), and in building- and crevice-hibernating bat populations when flight outside of the hibernaculum occurs (Twente and Twente 1987; Halsall et al. 2012; Hope and Jones 2013).

Much research on hibernation biology has focused on rodents (e.g., ground squirrels, marmots, and dormice), and our limited knowledge of bats largely comes from studies on species that overwinter in caves or mines. Little is known about the behavior of bats using noncavernous sites. Use of rock-crevices and persistent mid-winter flights despite the lack of insect prey (Lausen and Barclay 2006) by bats overwintering in the prairies presents a case of interesting

hibernation biology. Some available hibernacula in the prairies (e.g., rock crevices) are likely smaller, drier, and less thermally stable than most known cave hibernacula (Lausen and Barclay 2006). Fluctuating temperatures experienced by crevice-hibernating bats may increase energy consumption and cause bats to move to other sites with more suitable microclimates (Boyles et al. 2006). Increased evaporative water loss and limited opportunities to fly within hibernacula may also drive bats to fly mid-winter. Chronic negative water balance may be a principal factor in arousal and cause bats to exit the hibernacula in search of open water sources throughout the winter (Speakman and Racey 1989; Thomas et al. 1990; Thomas and Cloutier 1992; Thomas et al. 2003). Flight muscles are critical for locomotion in bats and, although limited muscle loss occurs during hibernation in most species (Lee et al. 2008; Cotton and Harlow 2010), including bats (Yacoe 1983; Brigham et al. 1990), the role of movement and flight in maintenance of muscle tone is unknown. Without space to fly within hibernacula, bats overwintering in rock-crevices may need to exit the roost to exercise muscles.

Bats that overwinter in the prairies may experience unique physiological and energetic challenges, arouse more frequently, and fly outside of the hibernacula more often compared to conspecifics that hibernate in caves. The results of my research will reduce the paucity of knowledge about winter bat-ecology in the prairies and provide comparative hibernation biology data for a group that is relatively understudied compared to rodents.

1.2 OBJECTIVES

I investigated the winter ecology and behaviour of crevice-roosting big brown bats (*Eptesicus fuscus*; Order: Chiroptera) hibernating at a site in the arid Canadian prairies. My study area in Dinosaur Provincial Park, Alberta is an ideal location to investigate how water balance influences arousals given the semiarid climate and physical and microclimate conditions in which bats hibernate.

Eptesicus fuscus is an ideal species in which to study hibernation biology, particularly to test hypotheses related to the importance of water balance and muscle retention. This species is widespread across heterogeneous landscapes, and also relies on flight for locomotion. Large wing membranes exacerbate evaporative water loss and place importance on maintaining flight muscle mass.

The specific objectives of my research were to:

1. Determine physical and microclimate characteristics of winter roosts (i.e., hibernacula) used by *E. fuscus* in the arid, non-mountainous landscape of Dinosaur Provincial Park, Alberta, and to compare these characteristics with those of known cave hibernacula.
2. Determine under what environmental conditions bats make mid-winter flights, and model the energy expenditure of these flights.
3. Describe thermoregulatory patterns of *E. fuscus* during hibernation, investigate predictors of arousals and activity, and model energy expenditure given the conditions in which bats in this area hibernate.

4. Test the hypothesis that bats hibernating in arid conditions have evidence of physiological adaptations to those environments (i.e., those that decrease evaporative water loss).
5. Investigate potential causes for mid-winter flight in free-ranging *E. fuscus*), specifically those related to dehydration, by examining bats captured in mid-winter flight for physiological signs of dehydration, such as increased serum ion concentrations and elevated hematocrit.
6. Test if bats in this area during winter would make use of a water feature, which would suggest that they fly mid-winter to drink and that artificial water developments may be a useful management tool to mitigate dehydration in bats that overwinter in arid regions.

Each of the following chapters addresses one or more of the above objectives. I wrote the thesis as a series of manuscripts with the intent to publish each of them. Thus, there is some overlap between chapters in introductory material, methodology, and reference material. Chapters 3 and 5 have been published in the *Canadian Journal of Zoology* (Klüg-Baerwald et al. 2016) and the journal *Oecologia* (Klüg-Baerwald and Brigham 2017), respectively. Chapter 2 has been accepted with minor revisions by the *Journal of Mammalogy*, and Chapter 4 is in review for the *Journal of Experimental Biology*. I have yet to submit Chapter 6.

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CHAPTER TWO—HOME IS WHERE YOU HANG YOUR BAT: WINTER
ROOSTING BEHAVIOUR OF PRAIRIE-LIVING BIG BROWN BATS (*EPTESICUS*
FUSCUS)

2.1 INTRODUCTION

Dens, burrows, and roosts play a crucial role in the biology of animals. These shelters buffer ambient conditions (Buck and Barnes 1999; Solick and Barclay 2006), provide a place to raise young and interact with conspecifics (East et al. 1989; Willis and Brigham 2004), and offer refuge from predators and catastrophic events (Fenton et al. 1994; Stawski et al. 2015). Animals spend a considerable proportion of their time within such structures. Habitat selection and the roosting, nesting, or denning behaviours of temperate-zone animals are generally well-studied during summer (Kalcounis Rüppell et al. 2005; Lesmeister et al. 2008; Gardiner et al. 2015), but less is known about the winter ecology of many species. This represents a considerable knowledge gap about the annual cycle and resource requirements of many animals.

Small mammals can spend up to 8 months hibernating (Davis 1967; Humphrey 1982; Murie and Boag 1984). During this time, metabolic rate (MR) is reduced and body temperature (T_b) regulated to just above ambient (T_a) for an extended period (Ruf and Geiser 2015), thus conserving a considerable amount of energy (Studier 1981). The energy budgets of hibernators are constrained by

available energy reserves (i.e., food or fat stores) and heavily influenced by ambient conditions (Humphries et al. 2003). Temperatures above or below an animal's torpid, T_b set-point lead to increased MR and more frequent arousals (Geiser 2004). Thus, even small changes in temperature or humidity tend to increase energy use and reduce overwinter survival (Thomas and Geiser 1997; Boyles and Brack 2009). For these reasons and others, many hibernators seek out shelter with particular characteristics, often subterranean cavities (e.g., burrows, caves, or mines) with a stable microclimate (i.e., temperature and humidity) to maximize energy savings (Geiser 2004). For example, bats in the eastern United States and Canada are often found roosting in large clusters in hibernacula with stable temperatures a few degrees above freezing and near-saturated humidity (Webb et al. 1996; Perry 2012). Such environments provide some protection from predators, minimize energy use, and mitigate water loss during hibernation.

Variation in geological conditions across North America means that access to caves and mines that could be used as hibernacula is often limited. To compensate, seasonal movements of up to 100s of km between summering and wintering grounds are common by many species (Fleming and Eby 2003; Norquay et al. 2013). However, some species appear relatively sedentary. For example, the big brown bat (*Eptesicus fuscus*) is thought to move only tens of kilometres between summer and winter habitat (Beer 1955; Goehring 1972; Mills et al. 1975). Although winter habitat requirements likely constrain the range of most temperate-zone bat species (Humphries et al. 2002), *E. fuscus* is one of the most

widespread in North America. Roost selection by crevice-roosting *E. fuscus* has been described during both summer (Lausen and Barclay 2002; 2003; 2006a) and autumn (Neubaum et al. 2006), but overwintering behaviour is known mainly for bats using buildings (Whitaker and Gummer 1992; Halsall et al. 2012).

Documented use of rock crevice roosts in winter (Lausen & Barclay, 2006b) suggests this species may use natural crevice roosts year-round in areas where extensive cavernous systems are absent.

Summer roosts of *E. fuscus* occur mostly in trees, buildings, and sometimes in rock crevices (Kurta and Baker 1990). Although trees may potentially be used as hibernacula in locations with milder climates (Burles et al. 2014), they are unlikely to buffer bats from ambient conditions in areas with harsh winters. *Eptesicus fuscus* is one of few bat species known to use building hibernacula (Whitaker and Gummer 1992; Halsall et al. 2012) and reliance on heated anthropogenic structures would suggest a limited historical range for this species. Few studies have investigated use of rock crevices by hibernating *E. fuscus* (Neubaum et al. 2006; Lausen and Barclay 2006b). If rock crevices meet the requirements of this species, their importance as naturally-occurring, non-cavernous hibernacula may have been underestimated. Rock-crevice hibernacula may also be used by other temperate-zone bat species with similar roosting ecology and limited evidence of seasonal movements, such as the western small-footed myotis (*Myotis ciliolabrum*) and long-eared myotis (*M. evotis*). Mid-winter acoustic monitoring in prairie river valleys has revealed winter activity by these two hibernating species

(Lausen and Barclay 2006b), and limited gene-flow in *M. ciliolabrum* suggests limited movement for mating (Lausen 2007). The prairies have extensive networks of river valleys that likely house a considerable number of bats during the winter, yet almost nothing is known about the use of these types of roosts. Microclimate and landscape characteristics of locations where these bats hibernate need to be determined to characterize overwintering habitats of bats in non-cavernous regions. This is especially important for predicting the spread and impact of white-nose syndrome (WNS)—an infection caused by the fungus *Pseudogymnoascus destructans* (*Pd*; Frick et al. 2015), which has been shown to grow optimally in cool moist cave conditions (Verant et al. 2012).

I studied male *E. fuscus* in southeastern Alberta, Canada to investigate the physical and microclimate characteristics of hibernacula used by this species in a prairie river valley. I hypothesized that microclimate would influence the hibernaculum selection decisions of *E. fuscus* in my study area. Specifically, I predicted that temperature and humidity of rock crevices used as hibernacula would be higher and less variable than that of all available (i.e., randomly selected) crevices. I also hypothesized that microclimates of rock-crevice hibernacula would differ from that of known cavernous hibernacula elsewhere, specifically that rock-crevice hibernacula would be drier, colder, and less thermally stable than known cave hibernacula in central and northern Canada. Lastly, I hypothesized that the thermal variability, small size, and potentially large number of available rock-crevice hibernacula at my study site would affect mid-

winter movements of bats. Specifically, I predicted that bats overwintering in the prairies would hibernate alone or in small groups and occasionally switch roosts to sites with more suitable microclimates as the weather varied.

2.2 METHODS

2.2.1 Study site and captures

I monitored the roosting behaviour and movement by individuals from a population of *E. fuscus* in Dinosaur Provincial Park (DPP), Alberta, Canada (50°45'09.2"N, 111°31'03.6"W) during the winters (November–March) of 2012–2015. The park is comprised of prairie and riparian areas with an extensive network of creeks and drainages. It has a semi-arid climate with winters characterized by low temperatures and little precipitation (Bailey 1979). At least three species—*E. fuscus*, *M. ciliolabrum*, and *M. evotis*—overwinter in deep (ca. 1–2 m) rock-crevices within the park and are active during the winter when the weather is favourable (Lausen and Barclay 2006b; Klüg-Baerwald et al. 2016; see also Chapter 3). Dinosaur Provincial Park is more than 300 km east of known cavernous sites in the Rocky Mountains and the only known hibernation area in the grasslands region of Alberta. The landscape contains no large subterranean features (i.e., caves or mines) but crevices provide access to underground habitat below the frost line (Lausen & Barclay, 2006b).

I captured bats in mist nets set across the Little Sandhill creek, a tributary to the Red Deer River. Although typically frozen during winter, these are the only

non-ephemeral water features within the study area. I took morphometric measurements (e.g., forearm length and mass) of all captured *E. fuscus* and permanently marked individuals with a 0.1 g passive integrated transponder (PIT) tag (Trovan ID100 nanotransponders; EIDAP Inc., Sherwood Park, Alberta) injected under the skin of the lower back. After tagging, I used tissue adhesive (Vetbond™; 3M Canada, London, Ontario) to close the injection site and then monitored each bat during the next hour for signs of distress or injury and to ensure proper insertion of the PIT tag. For a subset of individuals, I also affixed radio-transmitters (Lotek Ag392 1.2 g temperature-sensitive radiotransmitters; Lotek Wireless Inc., Newmarket, Ontario) to the interscapular region using latex adhesive (Perma-Type™; The Perma-Type Company Inc., Plainville, Connecticut). The combined mass of a transmitter and PIT tag represented less than 5% of body mass (Aldridge and Brigham 1988), and both have been used in *E. fuscus* without adverse effects on behaviour or survival (Neubaum et al. 2005; Wimsatt et al. 2005). I released all bats at the site of capture within 1.5 h of processing.

The University of Regina President's Committee on Animal Care approved all methods and procedures (Animal Use Protocol #12-12), which conformed to the guidelines for animal care and use outlined by the American Society of Mammalogists (Sikes 2016). I performed fieldwork under research and collection permits issued by Alberta Sustainable Resource Development and Alberta Tourism, Parks and Recreation Division.

2.2.2 Microhabitat and landscape measurement

I tracked bats to hibernacula and measured microclimate (temperature, humidity), microhabitat (area of opening, aspect and orientation of opening, crevice depth, material) and landscape variables (distance to level ground below, distance to water) at each. I inserted the probes of microclimate dataloggers (HOBO U23 Pro v2; Onset Computer Corp., Bourne, Massachusetts) as far into the hibernacula as possible (up to 1.8 m) to record hibernacula temperature (T_{hib} ; accuracy: $\pm 0.21^{\circ}\text{C}$, resolution: 0.02°C) and relative humidity (RH_{hib} ; accuracy: $\pm 2.5\%$, resolution: 0.03%) every 3 hours. Bats continued to occupy the roosts despite presence of the probes. I concurrently measured ambient temperature (T_a) and relative humidity (RH_a). Given the limitation of using RH as a means of assessing the rate at which evaporative water loss would occur (Kurta 2014), I converted all RH measurements to absolute water vapour pressure (WVP_{abs} ; kPa; Brice and Hall, 2016) for analyses.

In the winters of 2013–2015, I paired each hibernaculum with a randomly selected rock- and tree-crevice, and used the same type of datalogger (HOBO U23 Pro v2; Onset Computer Corp.) to record microclimate inside (T_{rock} , WVP_{rock} , T_{tree} , and WVP_{tree}). To locate random crevices, I first moved to a point on the map within park boundaries at a random distance (500–2500 m) and direction ($0-359^{\circ}$) from each hibernaculum, then found the nearest rock- or tree-crevice with an opening of sufficient size to allow big brown bats to enter (entrance at least 2 cm x

2 cm; (Greenhall 1982). In selecting tree-crevices, I focused on cavity volume rather than tree height or diameter (Willis et al. 2006).

In the winter of 2013–14, I also recorded microclimate at four known cave hibernacula across central and northern Canada (Figure 2.1). Cadomin cave (CA), a large sandstone cave located in the Rocky Mountains near Cadomin, Alberta (53.0325° N, 117.3265° W); Walk-In cave (WI), a limestone cave located in the boreal forest of northern Alberta near Fort Smith, Northwest Territories (60.0055° N, 111.8849° W); Abyss cave (AB), part of a series of limestone caves located in the boreal forest near Grand Rapids, Manitoba (53.1842° N, 99.2679° W); and Richard Lake mine (RL), an abandoned mine located in the boreal forest near Kenora, Ontario (49.7670° N, 94.4894° W). I deployed microclimate dataloggers (HOBO U23 Pro v2 in CA, WI, and AB; HOBO H21-002 micro station in RL; Onset Computer Corp.) to measure temperature (T_{hib}) and relative humidity (RH_{hib}) in a chamber where bats are known to hibernate. All sites are known to have overwintering populations of little brown myotis (*M. lucifugus*) and northern myotis (*M. septentrionalis*; Olson et al. 2011; Reimer et al. 2014; C. Willis pers. comm.), and CA also houses hibernating long-legged myotis (*M. volans*; Olson et al. 2011). However, *E. fuscus* occurs only in WI, RL, and DPP during winter (Reimer et al. 2014; C. Willis pers. comm.).

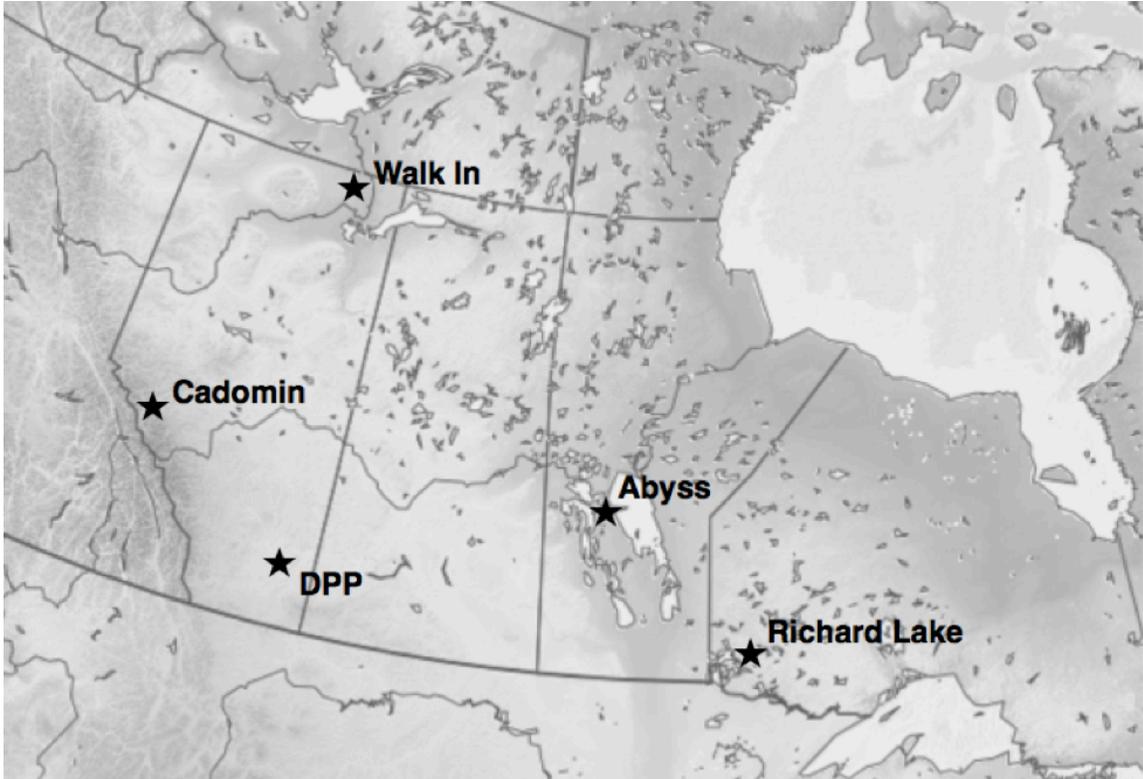


Figure 2.1. Map of central Canada showing sites where hibernacula microclimates were recorded during the winter of 2013–14.

2.2.3 Movement and group size

To estimate group size and determine frequency of roost switching by bats overwintering in DPP, I continuously monitored locations and movements of marked (with a radiotransmitter, PIT-tag, or both) individuals. In all three winters, I used receiver dataloggers (SRX400A; Lotek Wireless Inc.) with five-element yagi antennas placed at the base of each hibernaculum to monitor the location of radiotagged bats. Each receiver was housed in a waterproof box and powered by a 12 V deep-cycle marine battery connected to a 10 W solar panel. I programmed receivers to scan for the presence of radiotagged individuals every 15 minutes. In 2013–2015, I also mounted PIT-tag readers (Trovan LID650; EIDAP Inc.) with custom flexible antennas around the entrance of each of the three rock-crevice hibernacula to log activity of PIT-tagged bats. Each decoder was housed in a waterproof box and powered by two 12 V deep-cycle marine batteries connected to a 30 W solar panel; this array ensured continuous, instantaneous data collection. I defined an “emergence” as an event when: 1) the signal for a radiotagged bat was lost for 2 or more scans following arousal; or 2) when paired bouts of activity (i.e., exit and return) of PIT-tagged bats were recorded at the entrance of the hibernacula. I assumed a bat did not leave the hibernaculum if only a single PIT-triggered event was recorded (i.e., no clear exit and return) and the bat was recorded in the same hibernaculum at a later date.

2.2.4 Statistical Analyses

I used an information-theoretic approach (i.e., Akaike Information Criterion, AIC) to compare logistic regression models differentiating hibernacula microhabitat and landscape characteristics. Before constructing models for AIC analysis, I eliminated highly correlated variables ($r > 0.70$) and performed an initial logistic regression with an arbitrarily high α -value ($P = 0.35$) to reduce the number of variables but retain those that may be biologically important. I constructed four candidate binomial models containing the remaining variables—crevice depth (Depth) and distance to level ground below (DistGB)—and ranked them according to their AIC_c values (AIC value corrected for small sample size) and weights (w_i ; Burnham and Anderson 2002).

I confirmed all microclimate data met assumptions of normality with Kolmogorov–Smirnov goodness-of-fit tests and used quantile–quantile (Q-Q) plots to visually compare data against normal distributions. To compare microclimate data between roost types in DPP during the winters of 2013–2015, I used linear mixed models (LMMs) with crevice temperature and humidity as response variables, roost type (hibernaculum, random rock-crevice, and random tree-crevice) and T_a as predictors, and month and year as crossed random effects for the temperature model only; I removed these random effects from the humidity model because estimates of the variance explained by these effects were indistinguishable from zero. I also used a LMM to compare temperature of DPP hibernacula to that of known cavernous hibernacula (i.e., CA, WI, AB, and RL)

across Canada, with T_h as a response variable, site as a predictor, and month as a random effect. The random effect of month was indistinguishable from zero in the mixed model for humidity data, so I used a linear model (LM) to compare humidity data between sites, with $WVP_{\text{abs-h}}$ as a response variable and site as a predictor. I used Analysis of Deviance (Type II Wald chi-square tests) to derive P -values for predictors in each mixed model, and set statistical significance at $\alpha < 0.05$. I conducted analyses using R (R Development Core Team 2016) and present all data as means with standard deviation ($\bar{X} \pm S.D.$).

2.3 RESULTS

2.3.1 *Hibernacula microhabitat*

I PIT-tagged 75 *E. fuscus* (23 females and 52 males) and attached radiotransmitters to a subset of 36 individuals (3 females and 33 males). I did not re-locate any of the females after initial capture, and typically did not re-locate males caught earlier in the season (before 1 November). I successfully tracked 25 males to three hibernacula in DPP (Figure 2.2). Hibernacula were situated high ($\bar{X} = 10 \pm 2.5$ m) above the ground in south-facing ($\bar{X} = 160 \pm 32.8^\circ$), vertically oriented sandstone crevices (Table 2.1). Despite the small sample size of roosts, binomial models of physical characteristics were able to detect effects crevice characteristics. The highest ranked model with the lowest AIC_c value (7.800) and highest w_i (0.4127) suggested crevice depth (Depth) was a key variable differentiating hibernacula from randomly selected crevices (Table 2.2).

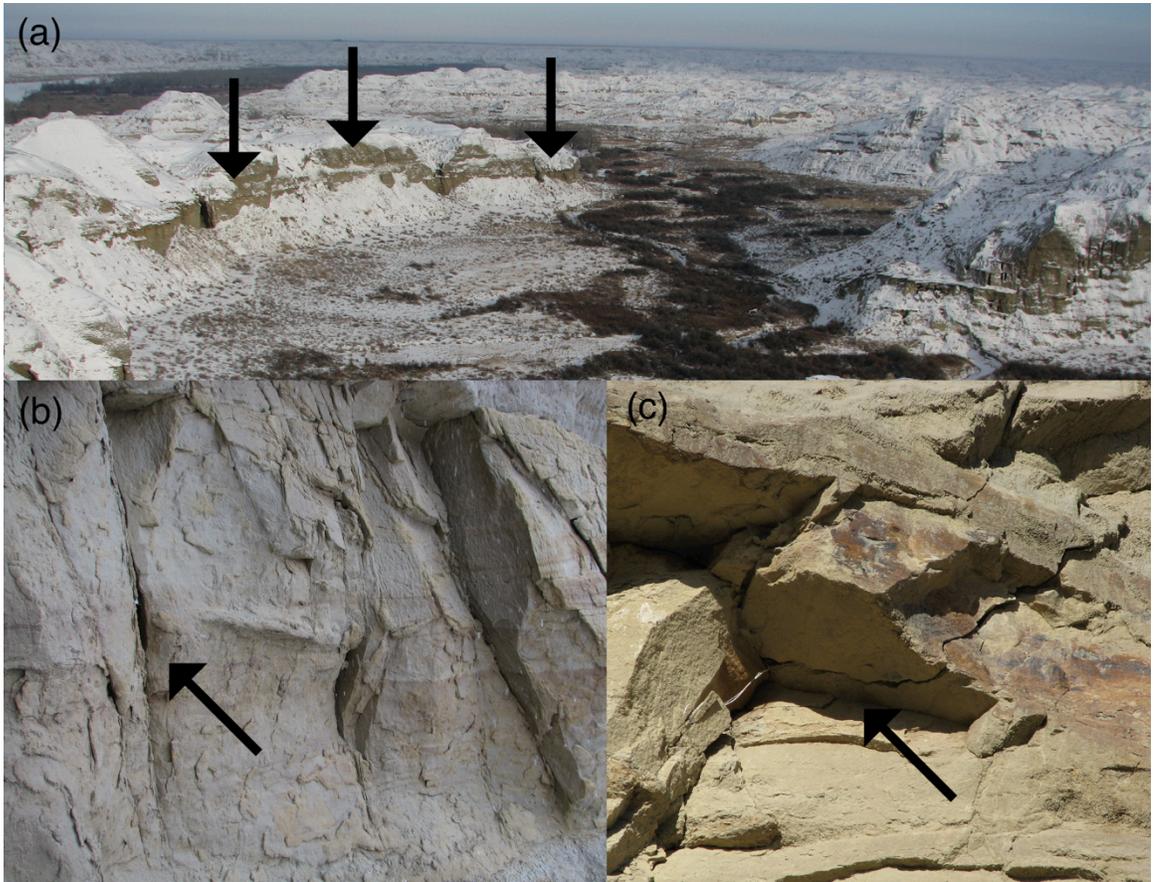


Figure 2.2. Photograph of (a) the cliff face containing the three rock-crevice hibernacula (approximate locations indicated by arrows) used by *Eptesicus fuscus* in Dinosaur Provincial Park, Alberta, Canada, and close-up photographs of (b) the largest and furthest east hibernaculum and the (c) middle hibernaculum (indicated by arrows).

Table 2.1. Mean Microhabitat and landscape characteristics ($\bar{X} \pm S.D.$) of 3 rock-crevice hibernacula used by *Eptesicus fuscus*, 3 random rock-crevices, and 3 random tree-crevices in Dinosaur Provincial Park, Alberta during the winters of 2013–2015.

Variable	Hibernacula	Random rock-crevice	Random tree-crevice
Microclimate			
Temperature (°C)	1.1 ± 0.87	-4.9 ± 5.86	-7.6 ± 8.70
Humidity (WVP _{abs} ; kPa)	0.35 ± 0.10	0.38 ± 0.18	0.36 ± 0.22
Microhabitat			
Area of opening (cm ²)	235 ± 281.1	302 ± 157.3	377 ± 242.7
Aspect of opening (deg.)	160 ± 32.8	165 ± 44.5	305 ± 39.8
Depth of crevice (cm)	150 ± 26.5	112 ± 27.7	34 ± 9.1
Material	Sandstone	Sandstone	Wood
Landscape			
Distance to ground above (m)	19 ± 3.1	2.6 ± 3.38	N/A
Distance to ground below (m)	10 ± 2.5	5.5 ± 5.01	2.2 ± 1.02
Distance to water (m)	78 ± 35.7	1 876 ± 664.2	153 ± 210.1

Table 2.2. Logistic regression models comparing microhabitat and landscape characteristics used in hibernacula selection by *Eptesicus fuscus* in Dinosaur Provincial Park, Alberta. Models are ranked by Akaike's Information Criterion corrected for small sample size (AIC_c) score and are presented with AIC weight (w_i) and evidence ratio (w_1/w_i).

Model	AIC_c	ΔAIC_c	w_i	w_1/w_i
Depth	7.800		0.6837	
DistGB	9.869	2.069	0.2430	2.814
Global (both variables)	12.743	4.943	0.0577	11.840
Null (no covariates)	15.365	7.565	0.0156	43.827

Hibernacula were deeper ($\bar{X} = 150 \pm 26.5$ cm) than random rock- ($\bar{X} = 112 \pm 27.7$ cm) and tree-crevices ($\bar{X} = 34 \pm 9.1$ cm). The high (> 2) ΔAIC_c values of subsequently ranked models suggests empirical support for the top ranked model (Burnham and Anderson 2002).

In the winters of 2013–2015, T_a in DPP ranged from -40.8°C to 19.4°C ($\bar{X} = -8.2 \pm 9.97^\circ\text{C}$), but mean temperature of rock-crevice hibernacula was considerably less variable (Figure 2.3). Mean temperature within DPP crevices differed with T_a ($\chi^2_1 = 6265.2, P < 0.001$) and crevice type ($\chi^2_2 = 4839.1, P < 0.001$; Figure 2.4). Rock-crevice hibernacula were warmer and more thermally stable than other available crevices in DPP (random rock- and tree crevices), but less humid. Mean temperature of hibernacula ($\bar{X} = 1.1 \pm 0.87^\circ\text{C}; N = 3$) ranged from -1.6°C to 2.7°C , and was higher and more stable than mean temperatures of random rock- ($\bar{X} = -4.9 \pm 5.86^\circ\text{C}$) and tree-crevices ($\bar{X} = -7.6 \pm 8.70^\circ\text{C}$), which ranged from -18.0°C to 9.0°C and -36.7°C to 16.9°C , respectively. Humidity also varied with $WVP_{\text{abs-a}}$ ($\chi^2_1 = 11\,447.2, P < 0.001$) and crevice type ($\chi^2_2 = 99.6, P < 0.001$; Figure 2.4). Mean WVP_{abs} of hibernacula ranged from 0.12 to 0.58 kPa ($\bar{X} = 0.35 \pm 0.10$ kPa). Hibernacula had less variable but lower humidity than random rock-crevices ($\bar{X} = 0.38 \pm 0.18$ kPa), and less variable but similar humidity compared to random tree-crevices ($\bar{X} = 0.36 \pm 0.22$ kPa). Mean WVP_{abs} ranged from 0.11 to 1.02 kPa in random rock-crevices and 0.02 to 1.41 kPa in random tree-crevices. Ambient WVP_{abs} ranged from 0.01 to 1.03 kPa ($\bar{X} = 0.32 \pm 0.20$ kPa).

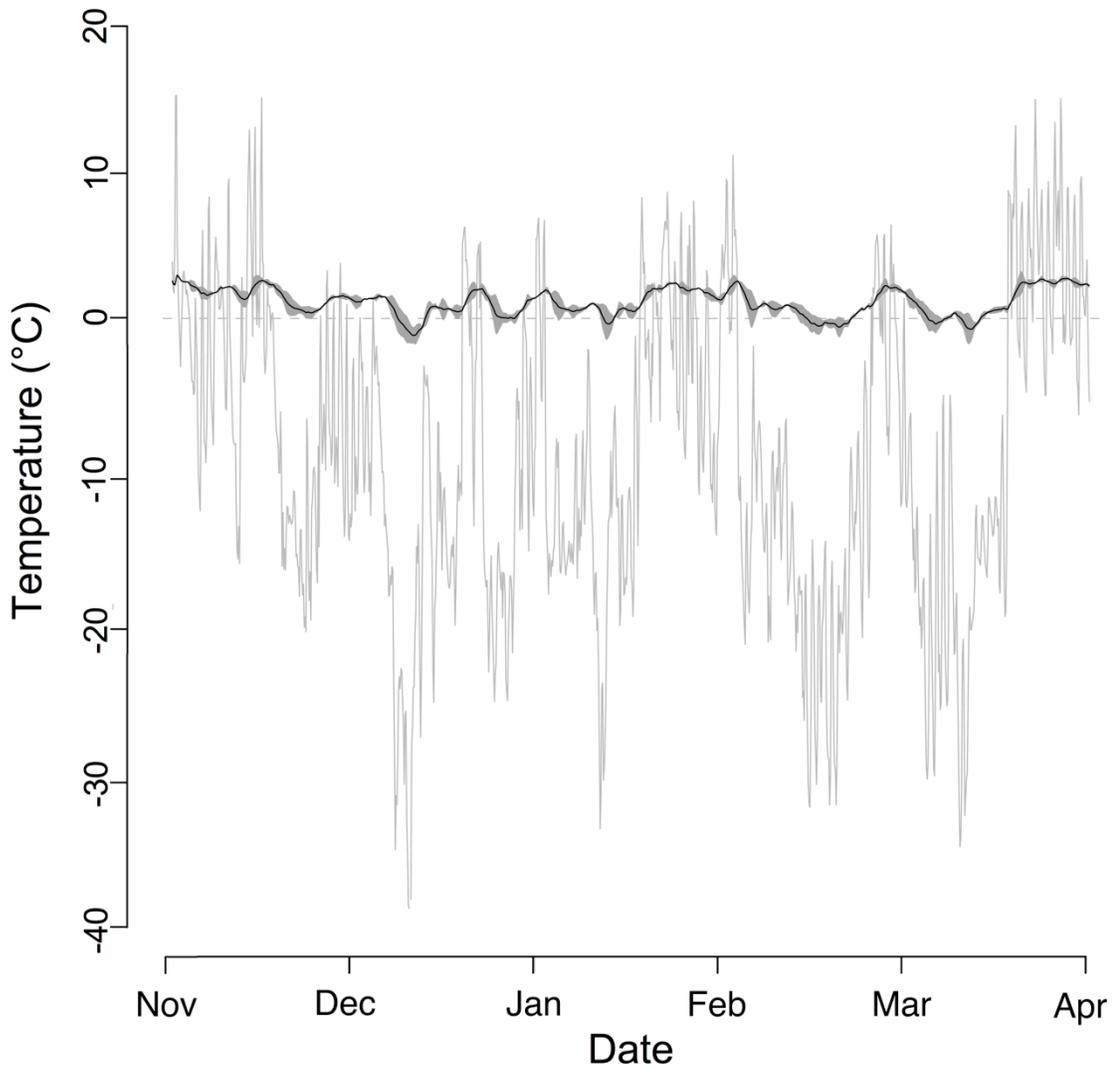


Figure 2.3. Ambient temperature (light grey line) and mean temperature inside 3 rock-crevice hibernacula (black line) with 95% CI (dark grey shade) in Dinosaur Provincial Park, Alberta during the winter (November–March) of 2013–14. Light grey dashed line represents 0°C.

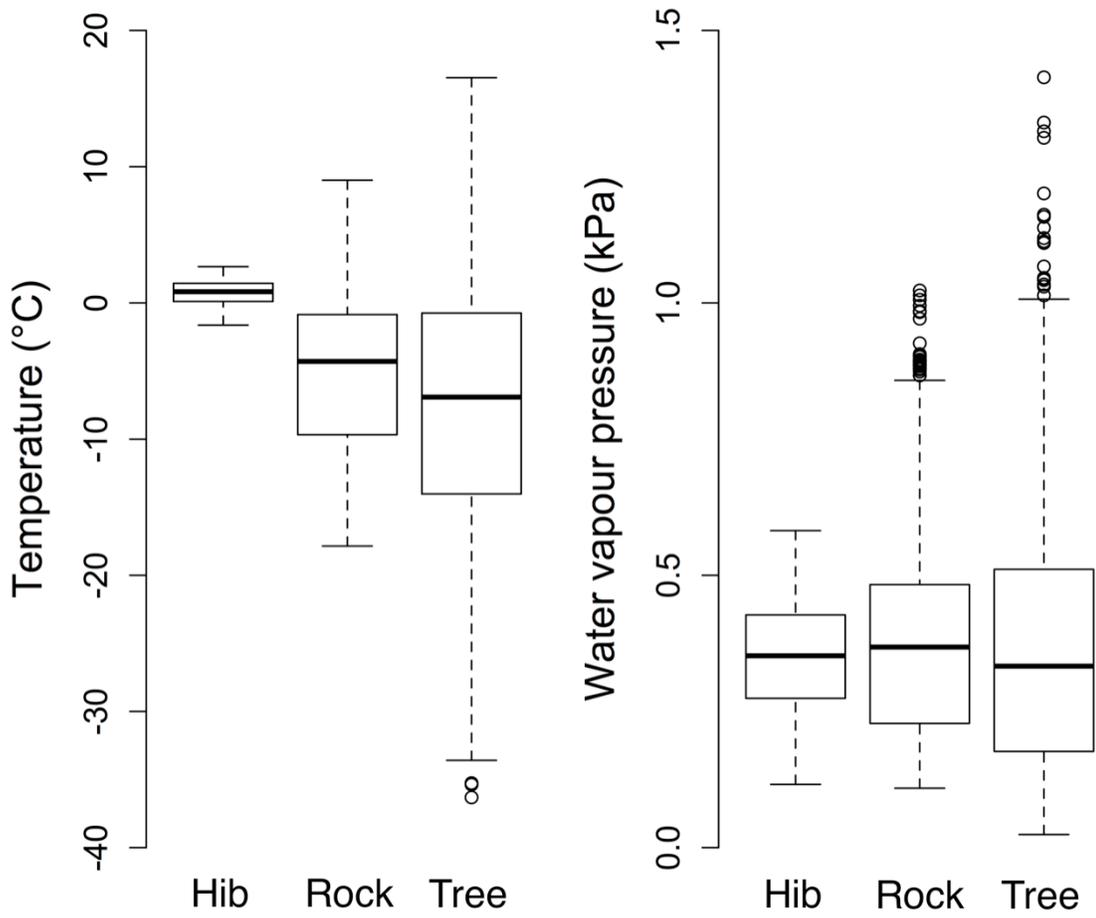


Figure 2.4. Boxplot of mean temperature and absolute water vapour pressure recorded inside 3 rock-crevice hibernacula (Hib), 3 random rock-crevices (Rock), and 3 random tree-crevices (Tree) in Dinosaur Provincial Park, Alberta during the winters (November–March) of 2013–2015.

During the winter of 2013–14, rock-crevice hibernacula in DPP were drier but not necessarily colder compared to known cave hibernacula in central and northern Canada. Temperatures varied with site ($\chi^2_4 = 28\ 969$, $P < 0.001$; Figure 2.5), and temperatures of DPP hibernacula ($\bar{X} = 0.8 \pm 0.91^\circ\text{C}$) were higher than those in WI cave ($\bar{X} = 0.0 \pm 0.63^\circ\text{C}$) but lower than those of all other sites. Temperatures were similar between CA ($\bar{X} = 2.0 \pm 0.01^\circ\text{C}$) and RL mine ($\bar{X} = 2.1 \pm 1.88^\circ\text{C}$). Humidity also varied with site ($\chi^2_4 = 19\ 308$, $P < 0.001$; Figure 2.6). In particular, mean WVP_{abs} of DPP hibernacula ($\bar{X} = 0.33 \pm 0.09$ kPa) was lower than that of all other sites.

2.3.2 Movement and group size

I recorded 622 days of radiotelemetry data from 13 male *E. fuscus* (48 ± 28.5 days bat⁻¹) during the three winters of my study. I also continuously recorded PIT-tag activity at the hibernacula entrances for 112 days in 2013–14 and 146 days in 2014–15. I detected 48% of the captured and uniquely identified (i.e., 52 PIT-tagged, 33 of which were also radiotagged) male *E. fuscus* in at least one of the three rock-crevice hibernacula during at least one winter. Bats roosted in small groups (maximum of 7 radiotagged and/or PIT-tagged individuals detected at one time) and 5 of 7 returning individuals used the same hibernaculum each year. Despite 107 recorded emergences (46 in 2013–14 and 61 in 2014–15), bats rarely moved between hibernacula mid-winter. The majority of monitored individuals (22/25) returned to the same hibernaculum after mid-winter flights. Bats that

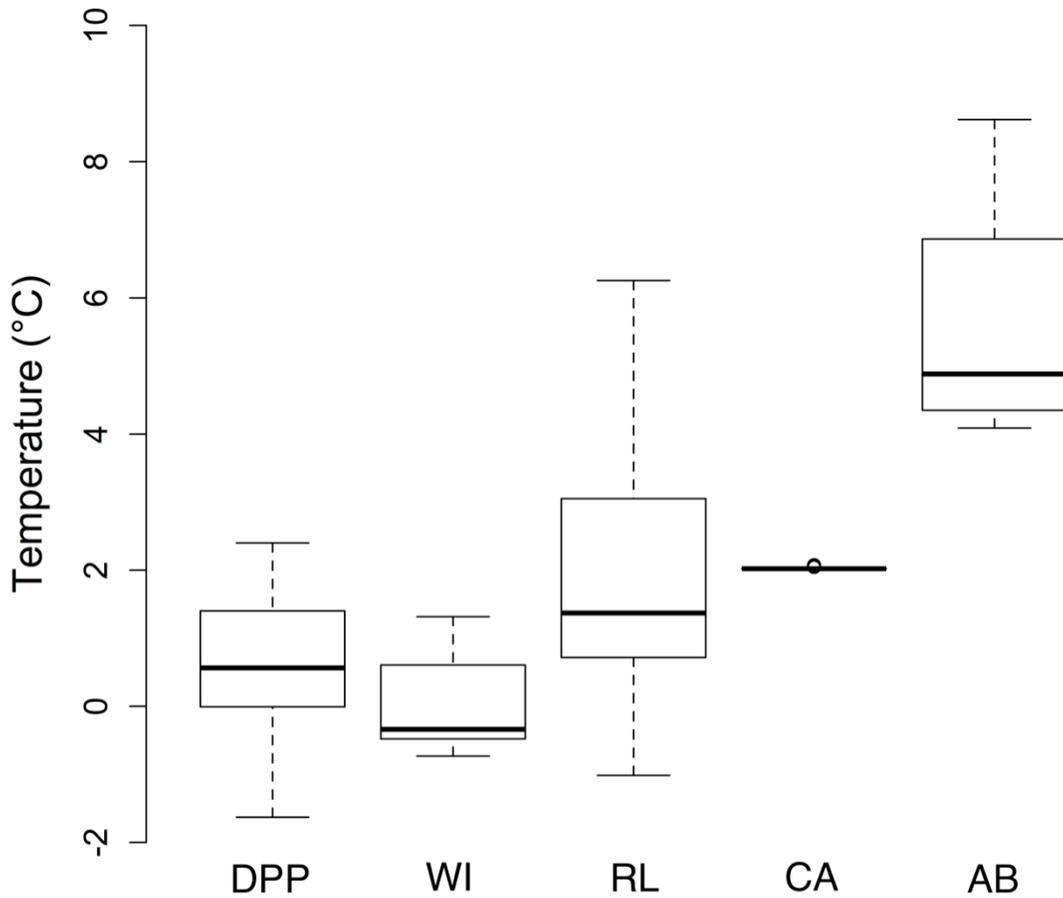


Figure 2.5. Boxplot of mean temperature recorded inside rock-crevice hibernacula in Dinosaur Provincial Park (DPP) and known cave hibernacula across northern and central Canada—Walk In (WI), Richard Lake (RL), Cadomin (CA), and Abyss (AB)—during the winter (November–March) of 2013–14.

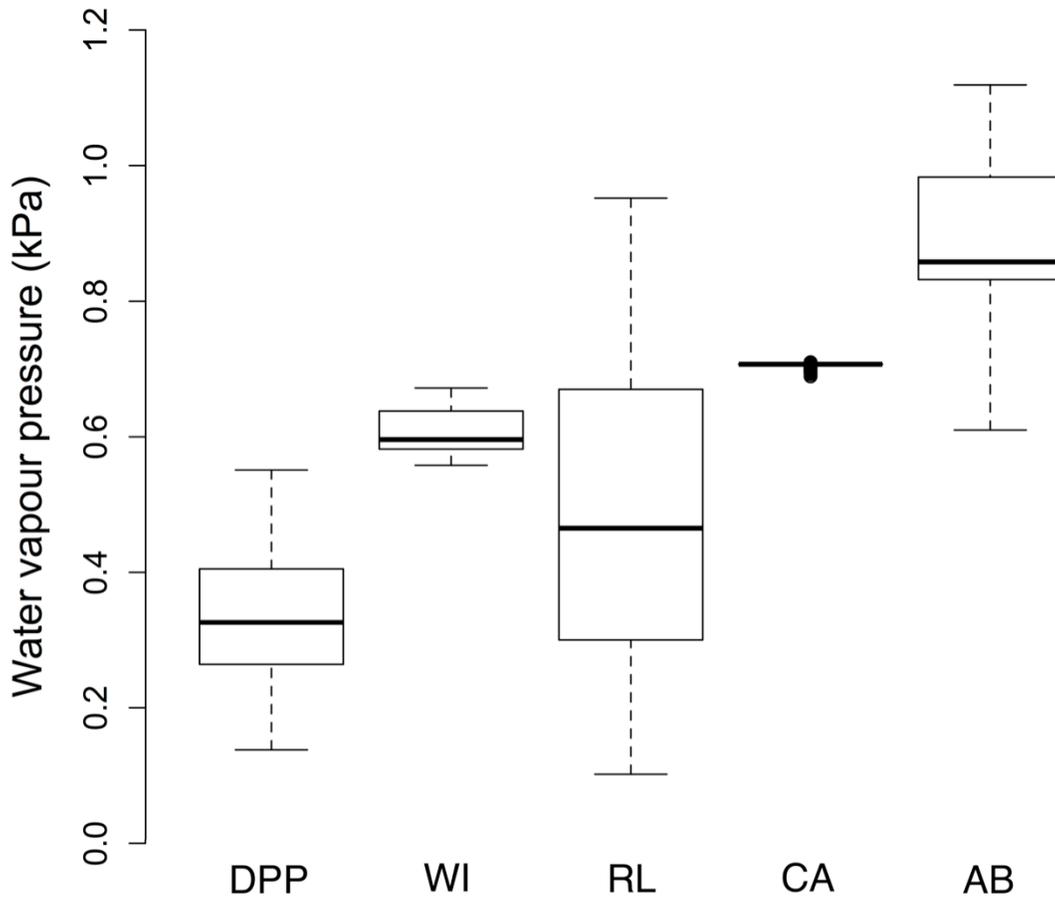


Figure 2.6. Boxplot of mean absolute water vapour pressure recorded inside rock-crevice hibernacula in Dinosaur Provincial Park (DPP) and known cave hibernacula across central Canada—Walk In (WI), Richard Lake (RL), Cadomin (CA), and Abyss (AB)—during the winter (November - March) of 2013-14.

moved between hibernacula mid-winter ($N = 3$) did so multiple (3–7) times, and two of those individuals also changed roosts between winters.

2.4 DISCUSSION

Documented use of rock crevices by hibernating bats is rare (Neubaum et al. 2006; Lausen and Barclay 2006b) and my study is the first to examine crevice roost selection during winter. My results show that male *E. fuscus* in DPP use specific rock-crevice hibernacula with particular microclimate and microhabitat characteristics, with evidence of winter roost fidelity between and within years. Bats I captured used only three rock crevices during hibernation, despite numerous crevices being potentially available. Furthermore, my data also show that mid-winter flight is common in my study area, yet there is little movement by *E. fuscus* between hibernacula. I also show that crevice hibernacula in my study area were dramatically less humid than cave hibernacula in other parts of Canada.

As predicted, despite a small sample size, I readily discriminated that rock-crevice hibernacula in DPP were deeper, warmer, and less thermally variable than random rock- or tree-crevices. Deep hibernacula buffer hibernating animals from ambient conditions and freezing temperatures can be avoided at depths beyond the limit of frost penetration in my study area (~2 m; Crawford 1955). Temperature is crucial to overwinter survival in hibernators and most species avoid hibernacula with sub-freezing temperatures if possible (Geiser 2004). Therefore, I was surprised to record occasional subzero temperatures within hibernacula. In DPP,

bats may roost deeper than I could monitor, although I did observe bats roosting close to the probes, which reached to depths of 1.8 m. The temperature inside WI also dropped below freezing at times (as low as -0.7°C). *Eptesicus fuscus* is commonly observed using cooler, drier areas of hibernation sites than other species (Beer and Richards 1956; Davis 1970; Fenton 1970), and has been recorded hibernating in freezing temperatures elsewhere (Fenton 1970) and moving to cooler rock-crevices despite the availability of warmer buildings during autumn in some areas (Neubaum et al. 2006). The three sites used by overwintering *E. fuscus* in this study were the driest and coldest of the hibernacula I examined, and temperatures were much lower than the average reported for bat hibernacula in North America (6°C ; Webb et al. 1996), further suggesting the relative cold-hardiness of this species.

Although humidity was more stable inside crevice hibernacula, contrary to my predictions, randomly selected rock- and tree-crevices had higher mean humidities than hibernacula. Water vapour pressure is a function of temperature, with higher levels of saturation in warmer air (Campbell and Norman 1998) and the higher levels of humidity I recorded in random crevices occurred mainly during periods with higher temperatures. For bats to benefit from higher humidity in hibernacula, they would also need to tolerate higher, more variable temperatures. Instead, *E. fuscus* appeared to favour thermal stability over high humidity during hibernation, highlighting the importance of hibernaculum temperature for this species (see also Beer and Richards 1956). Adaptation or

acclimatization of this species to arid conditions (Klüg-Baerwald and Brigham 2017; see also Chapter 5) may be especially important in its ability to hibernate in the conditions found in DPP.

Measurements of hibernacula microclimates have been compiled in published literature reviews (Webb et al. 1996; Perry 2012), but mine is the first study to directly compare sites across a large spatial scale. Rock-crevice hibernacula in DPP were drier and less thermally stable than known cave hibernacula, which likely influences hibernation energetics. Nearly all small hibernators arouse periodically during hibernation (Willis 1982) and winter roosting ecology of bats likely influences the motivation for, and frequency of, these arousals. Cavernicolous bats may have more opportunities to mate, drink, and forage during arousals than crevice-roosting bats. Use of drier, less thermally stable hibernacula may increase evaporative water loss and arousal frequency, and require that bats overwintering in the prairies exit the hibernacula to drink (Thomas and Cloutier 1992; Thomas and Geiser 1997). Bats in some areas may forage during arousals (Brigham 1987) but insect prey are not available during winter in my study area (Lausen and Barclay 2006b), which adds to the energetic constraint and importance of fat stores for bats in the prairies. In support of this, mean mass of *E. fuscus* entering hibernation in DPP (23.6 g; B.J. Klüg-Baerwald, unpublished data) is higher than that reported for a population of cave-hibernating *E. fuscus* in Ontario (21.6 g; Fenton 1972) where winter foraging may

be possible (Brigham 1987), but spring emergence masses are similar (16.8 g and 16.4 g, respectively).

Roost fidelity is thought to be low among bats that roost in ubiquitous, ephemeral structures (e.g., tree foliage and crevices; but see Willis et al. 2003; Klüg et al. 2012) and high when rare, permanent roosts are used (e.g., caves and buildings; Lewis 1995). I predicted the large number of rock-crevices across the landscape and variable temperatures within crevices would prompt *E. fuscus* in DPP to use multiple roosts and switch between them often. However, my data suggest that *E. fuscus* rarely move during winter and that winter roosts with suitable microclimate are not ubiquitous in my study area. I also documented between-year fidelity to rock crevice hibernacula, supporting the hypothesis that suitable roosts in the area are limiting, much like that documented for cavernicolous populations (Glover and Altringham 2008). The need to exit the hibernaculum to switch roosts, rather than move locations within a cave as cavernicolous hibernators could, may contribute to the rarity of roost switching during winter flights in my study area. Outdoor winter flights are energetically costly (Klüg-Baerwald et al. 2016; see also Chapter 3) and could increase predation risk (Thomas and Jacobs 2013). That bats still occasionally fly outside of the hibernacula despite such costs suggests these flights are necessary and beneficial for reasons not related to roost switching.

Limited space within rock-crevice hibernacula undoubtedly constrains the number of bats able to roost inside a given crevice and limits potential for social

thermoregulation. Bats may occasionally arouse together to save energy during arousals (Boyles et al. 2008) and may even cluster to reduce evaporative water loss (Boratyński et al. 2015). Although group size and thus associated benefits are likely reduced in narrow crevice hibernacula, roosting in tight quarters may still be beneficial from a heat loss perspective if the surrounding substrate insulates bats during arousals or reduces surface area exposed to cold, open air in the hibernaculum, thereby reducing convective heat loss. Previous studies have investigated heat conductance of summer roosts (Lausen and Barclay 2002) but none have evaluated the influence of substrate conditions on energetics and water balance during hibernation. Data on this are clearly needed.

The use of ubiquitous features such as rock-crevices for hibernacula could increase available winter habitat and allow a relatively sedentary species to occupy a larger range than otherwise available. However, rock-crevice hibernacula may be particularly vulnerable to climate change because temperatures inside hibernacula generally reflect trends in external temperatures (Figure 2.3). Rising winter temperatures may expand suitable overwintering habitat at the northern edge of a species range (Humphries et al. 2002; 2004), but the consequences for sites within existing overwintering areas is uncertain. Temperature strongly affects hibernation patterns and energy expenditure, and changes in climate are likely to have profound effects on overwinter survival of bats. Warming temperatures are associated with decreased survival in many species of hibernator (Geiser and Kenagy 1988; Geiser and Broome 1993; Turbill and Prior 2016). If global surface

temperatures in the area continue to rise, existing hibernacula may become too warm for some species, particularly those like *E. fuscus* that appear cold-adapted.

Benefits of smaller, drier, colder hibernacula may become apparent as WNS continues to impact North American hibernating bats. The fungus grows best at temperatures between 12.5 and 15.8°C and in sites with high humidity (Verant et al. 2012). The disease is associated with dehydration (Willis et al. 2011; Cryan et al. 2013; Ehlman et al. 2013; Warnecke et al. 2013), increased arousal frequencies, and starvation (Storm and Boyles 2011; Reeder et al. 2012; Warnecke et al. 2012). If conditions and behaviours of bats in the prairies are not conducive to the growth and spread of the fungus, the prairies may act as a barrier to the spread of WNS (Hallam and Federico 2012). Given the importance of bat-to-bat contact to transmission of the fungus (Lorch et al. 2011; Langwig et al. 2012) and the tendency for the fungus to grow best in damp conditions (Verant et al. 2012), small clusters of bats hibernating in dry hibernacula may be less impacted by the disease. My data on male *E. fuscus* support the prairie barrier hypothesis, but study of hibernacula conditions of other prairie bat species is needed. Susceptibility of *Eptesicus fuscus* to WNS mortality appears low (Frank et al. 2014), partly due to its winter roosting ecology (Langwig et al. 2012), and mortality may also be low for other prairie-living bat species detected in this area (e.g., *M. ciliolabrum* and *M. evotis*; Lausen and Barclay 2006b) if their overwintering behaviours are similar.

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CHAPTER THREE—OUT IN THE COLD: ENVIRONMENTAL CORRELATES
AND ENERGETIC ESTIMATES OF WINTER FLIGHT BY BATS IN SOUTHERN
ALBERTA, CANADA

3.1 INTRODUCTION

During hibernation, metabolic rate (MR) and body temperature (T_b), are decreased for days to weeks (Ruf and Geiser 2015). Hibernating animals save considerable energy and can survive on limited resources (i.e., body fat or cached food) for extended periods, up to months at a time (Humphries et al. 2003). Benefits of hibernation are obvious (Geiser 2004; Geiser and Brigham 2012) but there are associated costs, such as suppressed molecular synthesis (Lillegraven et al. 1987), ceased or delayed reproduction (Racey 1969; Barnes et al. 1986), and immunosuppression (Bouma et al. 2010). For these reasons and likely others, nearly all hibernating mammals arouse periodically (Willis 1982; French 1985), ostensibly to excrete metabolic wastes (Baumber et al. 1971), mount immune responses (Burton and Reichman 1999), mate (Thomas et al. 1979), eat (Humphries et al. 2001), and possibly drink (Thomas and Cloutier 1992; Thomas and Geiser 1997; Ben-Hamo et al. 2013).

Much of what is known about arousals and winter activity of temperate-zone bats comes from research on cavernicolous species. In eastern North America, bats often roost in large groups in sizeable, humid, thermally-stable hibernacula

(Webb et al. 1996; Perry 2012). Most activities typical of arousals occur within the hibernacula; caves and mines allow flight within hibernacula and may contain open water sources and hibernating insects that can be consumed (Swanson and Evans 1936; Rysgaard 1942). Opportunistic mating is also possible with mixed-sex groups typically found in cave hibernacula (Thomas et al. 1979).

Bats are also active outside of hibernacula for reasons poorly understood. Early evidence suggested these events were occasional and likely in response to starvation or dehydration (Speakman and Racey 1989; Boratyński et al. 2015). That some bats emerge in winter with low body mass supports the idea that bats emerge to forage (Brigham 1987). More recently, increased activity outside hibernacula has been associated with white-nose syndrome (WNS)—an invasive fungal disease responsible for the deaths of at least 6 million bats in eastern North America since 2006 (Frick et al. 2015). Interest in winter bat-activity due to WNS has made it apparent that mid-winter flight by bats is common (e.g., Falxa 2007; Schwab and Mabee 2014), even in northern areas with harsh winter climates (Lausen and Barclay 2006).

Winter bat-activity in areas with mild winters is often associated with favourable foraging conditions. In summer, warm calm nights are associated with increased insect abundance (Taylor 1963) and foraging behaviour by bats (e.g., Racey and Swift 1985; Fukui et al. 2006). Likewise, tri-coloured bats (*Perimyotis subflavus*), little brown myotis (*Myotis lucifugus*), northern long-eared myotis (*M. septentrionalis*), and brown long-eared bats (*Plecotus auritus*) are more active

during warmer, calmer winter nights (Hays et al. 1992; Whitaker and Rissler 1992). Bats also use barometric pressure to determine when to forage or move in summer. High activity levels of flying insects and *P. subflavus* occur during periods of low or falling barometric pressure (Paige 1995), and falling barometric pressure cues movement of migratory bat-species (Cryan and Brown 2007; Baerwald and Barclay 2011). Likewise, bat activity increases on warm nights with falling barometric pressure during Austral winters (Turbill 2008), and *M. lucifugus* uses barometric pressure to cue emergence from hibernation (Czenze and Willis 2015).

Although mid-winter foraging is likely in milder winter climates (e.g., Avery 1985; Brigham 1987; Dunbar et al. 2007), bats are active despite a lack of insects in northerly areas with harsher winters (Lausen and Barclay 2006). Without opportunity to supplement fat stores, the energetics of mid-winter flights become particularly important. Cold-weather activity may be especially energetically costly given the small body size of bats; a high body surface area to volume ratio and large surface area of the wings increase the potential heat loss (Phillips and Heath 2001). Conditions that increase thermal exchange during flight, such as high winds and precipitation (Voigt et al. 2011), may negate benefits of leaving the hibernacula.

Mechanisms to mitigate heat loss in the cold may enable activity through a low range of T_a . Spatial heterothermy in Brazilian free-tailed bats (*Acarida brasiliensis*) reduces heat loss from their wings while in flight (Reichard et al.

2010). In addition, heat produced through activity is used for thermoregulation in a wide range of animals (Humphries and Careau 2011). Metabolic heat production in cold temperatures is equivalent in perching and foraging black-capped chickadees (*Poecile atricapillus*; Cooper and Sonsthagen 2015), and flight metabolic rate is independent of ambient temperature in pigeons (*Columba livia domestica*; Rothe et al. 1987). Humans (*Homo sapiens*) also maintain steady rates of heat production despite drops in ambient temperature while active (Nielsen and Nielsen 1962). These data suggest that heat produced through movement substitutes for shivering and metabolic thermogenesis typically required to compensate for heat loss in cold conditions. In fact, one reason for nocturnality in bats might be the inability to dissipate heat produced during flight while exposed to solar radiation (Speakman 1991; 1995; Voigt and Lewanzik 2011). Activity-thermoregulatory heat substitution has not been investigated in bats but may mitigate the cost of winter flight.

I investigated correlates of winter bat-activity and estimated the energetic costs of flight by a population of hibernating bats in southern Alberta, Canada. Winter foraging is not possible at this site, yet bats continue to fly. Thus, I hypothesized factors influencing heat loss and the energetic cost of flight (e.g., wind, temperature, and precipitation) predict winter activity outside the hibernacula. Specifically, I predicted that winter bat-activity decreases during conditions of high wind, low T_a , and precipitation, and increases during warmer, calmer nights. I also proposed that interspecific differences in activity relative to

ambient temperature can be explained by body size and associated rates of heat loss. Thus, I predicted that larger-bodied big brown bats (*Eptesicus fuscus*) fly in colder temperatures than smaller-bodied *Myotis* bat species (genus *Myotis*). Finally, I accounted for activity-thermoregulation heat substitution (heat produced through flight) and estimated species-specific energy expenditure during winter flight. I expected my energetic models to corroborate inter- and intraspecific patterns in activity of bats in relation to ambient temperatures observed at my study site.

3.2 METHODS

3.2.1 Study area

I monitored bat activity in Dinosaur Provincial Park (DPP), Alberta, Canada from October 2012 through April 2015. The park is located along the Red Deer River in southern Alberta (50°45'09.2"N, 111°31'03.6"W) and represents a mixed landscape of prairie and riparian habitat with an extensive network of creeks and drainages. The semi-arid climate is characterized by hot, dry summers, cold winters, and low overall precipitation (Bailey 1979). At least three species—*E. fuscus*, western small-footed myotis (*M. ciliolabrum* (Merriam, 1886)), and western long-eared myotis (*M. evotis* (Allen, 1864))—over-winter in deep rock crevices in the area (Lausen and Barclay 2006). Dinosaur Provincial Park is the location of the first natural bat hibernation area discovered in Alberta's grasslands natural region.

The park provides numerous access points to underground habitat below the frost line, making it a potentially strategically important hibernation site for bats.

3.2.2 Acoustic monitoring

I acoustically monitored bat activity in DPP for three winters, October through April, 2012–2015. I used Anabat (Titley Electronics, Ballina, New South Wales, Australia) and SM2Bat+ (Wildlife Acoustics, Maynard, Massachusetts, USA) detectors set to record every night from an hour before sunset to an hour after sunrise. I calibrated Anabat units (see Larson and Hayes 2000) and deployed one facing out over the Red Deer River and one in an open field at the edge of a stand of cottonwood trees (*Populus deltoids*). I deployed one SM2Bat+ detector on the bank of the Little Sandhill Creek, the only permanent creek in the park. The creek is the only location where bats can be reliably captured in mist nets during winter despite being frozen. I housed Anabat detectors in custom waterproof boxes mounted on wooden stands approximately 1.5 m above ground, with microphones shielded and pointed parallel to the ground. I housed the SM2Bat+ in a plastic bin and mounted the microphone on a 1.5 m pole with a small plastic hood located 10 cm above to shield it from precipitation. I powered each detector with a 12 V, 12 Ah sealed lead acid battery coupled with a 10 W solar panel.

I analyzed echolocation calls in zero-crossing format using AnalookW software (version 3.9c; C. Corben, Columbia, Missouri, USA). I used a custom filter to separate background noise (e.g., insects and wind) from identifiable calls (see

Lausen et al. 2014 for details). I then manually identified bat calls to species using a combination of call characteristics, such as minimum frequency (F-min) and call duration, slope, and shape (Corben 2002; Lausen et al. 2014). Overlap occurs in call characteristics between sympatric silver-haired bats (*Lasionycteris noctivagans*) and *E. fuscus* (Barclay 1999), but I attributed all low-frequency (i.e., F-min between 20 and 23 kHz) calls recorded between October and April to *E. fuscus* given that *L. noctivagans* is migratory and does not occur in the area during winter, I attributed all high-frequency calls (i.e., F-min > 35 kHz) to *Myotis* but did not differentiate between *M. ciliolabrum*, *M. evotis*, or *M. lucifugus*. I used the presence of “feeding buzzes”, segments of quickly repeating pulses characteristic of an individual locating and closing in on prey to identify foraging by bats (Fenton 2003).

I recorded hourly ambient temperature (T_a ; °C) with a HOBO data logger (U23 Pro v2; Onset Computer Corporation, Bourne, Massachusetts, USA) shielded by a solar radiation shield (RS3; Onset Computer Corporation, Bourne, Massachusetts, USA) and located next to the SM2Bat+ detector on the bank of the Little Sandhill Creek. I gathered all other hourly environmental data (e.g., wind speed; $m\ s^{-1}$) 2 m above ground, precipitation (mm), and barometric pressure (kPa)) from the Alberta Agriculture and Forestry weather station (ACIS 2015) located in Patricia, Alberta, 15 km from the study site.

3.2.3 Statistical analyses

I used bat passes per hour (passes hour⁻¹) across all detectors to quantify bat-activity. I defined a bat pass as a sequence of ≥ 2 echolocation calls (Vonhof 2006). Hourly bat-activity data was not normally distributed due to overdispersion, which precluded the use of parametric tests for analyses. Instead, I used negative-binomial regression analyses to model hourly bat-activity as a function of temperature (Temp), wind speed (Wind), precipitation (Precip), and 24-hour change in barometric pressure (BPDiff). I used an information theoretic approach (i.e., Akaike information criterion (AIC)) to construct and compare *a priori* models (Hosmer and Lemeshow 1989; Anderson et al. 2000).

I analyzed data separately for *E. fuscus* and *Myotis* activity. Before model construction, I ran correlation analyses to eliminate highly correlated ($r > 0.70$) predictor variables. I constructed 14 candidate models containing the variables of interest for each group and ranked them according to their quasi-likelihood modified AIC_c values (QAIC_c) and weights (w_i). The AIC_c value considers sample size, the QAIC_c value accounts for overdispersion of the data, and w_i is a normalized value reflecting the probability the given model is correct given the entire subset of candidate models (Burnham and Anderson 2002). The best model has the lowest QAIC_c and highest w_i . However, I considered all models with a QAIC_c value within 2 of the best model ($\Delta\text{QAIC}_c < 2$) to have empirical support (Burnham and Anderson 2002). To determine the probability of detecting bats in

relation to ambient temperature based on my data, I ran binomial logistic regressions of hourly activity of *E. fuscus* and *Myotis* as functions of temperature.

To exclude late-stage summer and swarming activity and ensure I examined winter activity only, I defined the start of hibernation as the first day of the season with a daytime high $T_a \leq 0^\circ\text{C}$ (23 October 2012, 28 October 2013, 9 November 2014). I also excluded data beyond the last day of the season with a daytime high $T_a < 0^\circ\text{C}$ (24 March 2013, 1 April 2014, 4 March 2015). I used analyses of variance (ANOVA) with Tukey's post-hoc adjustments to test for interannual differences in weather patterns. I conducted all statistical analyses using R (R Development Core Team 2016) and present all data as means with standard deviation ($\bar{X} \pm S.D.$).

3.2.4 Energetic modelling

To estimate energy expenditure by resting euthermic bats (E_{rest}) in temperatures below the thermoneutral zone, I used the equation of Humphries et al. (2006):

$$E_{\text{rest}} = \text{BMR} + (T_{\text{lc}} - T_a)C_{\text{eu}},$$

which accounts for basal metabolic rate (BMR) and metabolic heat needed to compensate for convective heat loss (H_c , represented by $(T_{\text{lc}} - T_a)C_{\text{eu}}$). In this equation, T_{lc} is the lower critical temperature, T_a is the mean ambient temperature of known hibernacula in Dinosaur Provincial Park during my study (1.1°C ; see Chapter 2), and C_{eu} is wet thermal conductance ($\text{mW g}^{-1} \text{ }^\circ\text{C}^{-1}$) at euthermic T_b . I

used published values of BMR (6.31 mW g^{-1}) and T_{lc} (26.7°C) reported for *E. fuscus* by (Willis et al. 2005). However, Willis et al. reported C_{eu} for *E. fuscus* measured in summer when conductance of fur is higher than in winter (Shump and Shump 1980). Thus, I estimated C_{eu} for *E. fuscus* during winter ($1.41 \text{ mW g}^{-1} \text{ }^\circ\text{C}^{-1}$) as that of *M. lucifugus* measured in winter ($1.47 \text{ mW g}^{-1} \text{ }^\circ\text{C}^{-1}$; Stones and Wiebers 1967) and corrected for the 4.6% higher insulative value of *E. fuscus* fur (Shump and Shump 1980). I used published values of BMR (14.53 mW g^{-1}) and T_{lc} (33°C) reported for *M. lucifugus* by (Stones and Wiebers 1967). For whole animal estimates, I used the mean masses of *E. fuscus* captured during winter in DPP (21.7 g; B.J. Klüg-Baerwald, unpublished data) and *M. lucifugus* captured during early hibernation at a site located in Manitoba, Canada (10.1 g; Jonasson and Willis 2012).

To estimate net energy expenditure by active bats flying in cold conditions (E_{flight}), I modified the heat balance equation of Schmidt-Nielsen (1997) to include the energetic costs of flight (P_{flight}) and temperature-dependent rates of heat loss via forced convection (H_c) and long-wave radiation (H_r), as well as the energetic offset of flight thermogenesis:

$$E_{flight} = 0.2P_{flight} + H_c + H_r.$$

I assumed 20% muscle efficiency during flight (Speakman and Thomas 2003), with the remaining 80% of P_{flight} contributing to activity-thermoregulation heat substitution (Humphries and Careau 2011). If exercise thermogenesis exceeds total heat loss, then $E_{flight} < P_{flight}$; in such cases, I considered P_{flight} as the total cost of activity.

To calculate P_{flight} , I used the equation of Speakman and Racey (1991):

$$\log_{10}P_{\text{flight}} = -0.638 + 0.808 \log_{10}m,$$

where m is the mass reported above for each species. Given that this equation was extrapolated from measurements of bats flying in mild thermal conditions (room-temperature air; Speakman and Racey 1991), I assumed it does not include added thermoregulatory costs (i.e., heat produced during flight compensated completely for heat lost) and could thus be used to estimate the energetic cost of flight only.

I calculated H_c of body and wings using the equation of Schmidt-Nielsen (1997):

$$H_c = (T_{\text{sk}} - T_a)C_f.$$

For wings, I assumed skin temperature (T_{sk}) to approximate T_a but not drop below 1°C to avoid freezing, and used the convective coefficient of wing tissue during flight (C_f ; $\text{W m}^{-2} \text{ }^\circ\text{C}^{-1}$) reported for *T. brasiliensis* (Reichard et al. 2010). For body calculations, I used euthermic T_{sk} for *E. fuscus* (35.8°C ; Willis et al. 2005) and *M. lucifugus* (35°C ; Stones and Wiebers 1965) to calculate temperature-dependent rates of heat loss via forced convection from skin surface through fur. I accounted for forced convection experienced in flight by using the equation for wind-dependent thermal conductance of fur (K_{fur} ; $\text{W m}^{-2} \text{ }^\circ\text{C}^{-1}$) of Bakken (1991):

$$K_{\text{fur}} = a + bu^c.$$

In this non-linear equation, a is the thermal conductance of fur in the absence of wind ($3.969 \text{ W m}^{-2} \text{ }^\circ\text{C}^{-1}$ and $4.154 \text{ W m}^{-2} \text{ }^\circ\text{C}^{-1}$ for *E. fuscus* and *M. lucifugus*, respectively; Shump and Shump 1980), u is wind speed (m s^{-1}), which I set as the

reported minimum-power flight-speed of *E. fuscus* (3.8 m s^{-1} ; Brigham and Fenton 1991) and *M. lucifugus* (3.7 m s^{-1} ; Patterson and Hardin 1969), and b and c are constants. Given that no such data exist for bats, I set b at 1.3, a value reported for a small rodent in winter (Boyles and Bakken 2007), and used a common exponent of 1 (Campbell et al. 1980).

To estimate thermal radiation exchange between bat and night sky, I used the equation of Schmidt-Nielsen (1997):

$$H_r = \sigma \epsilon_{\text{bat}} \epsilon_{\text{sky}} (T_s^4 - T_{\text{sky}}^4) A,$$

where σ is Stefan-Boltzmann's constant ($5 \times 10^{-8} \text{ W m}^{-2} \text{ K}^{-4}$), ϵ_{bat} is emissivity of the bat (0.98; Monteith and Unsworth 1990), and ϵ_{sky} is emissivity of the sky calculated using the equation of Swinbank (1963):

$$\epsilon_{\text{sky}} = 0.398 \times 10^{-5} \times (T_a + 273.15)^{2.148}.$$

To calculate the radiative temperature of the night sky (T_{sky}), I used the equation of Gates (1980):

$$T_{\text{sky}} = T_a - 20.4 + 0.22 T_a.$$

Rates of heat loss depend on the temperature differential between environment and the surface of an object (T_s ; Schmidt-Nielsen 1997). Little data exist on flight temperatures of bats while in flight, so I assumed T_s of the body to approximate the lowest surface temperature measured during flight in *T. brasiliensis* ($\sim 18^\circ\text{C}$; Reichard et al. 2010) and calculated T_s of the wings as described above for H_c . I calculated surface area (A) of the body to be that of a cylinder (Chappell 1980) with dimensions based on the morphometrics of each species (van Zyll de Jong

1985; Nagorsen and Brigham 1993) and used surface areas reported in the literature for the wings (Farney and Fleharty 1969).

For my estimates, I assumed that bats fly at minimum-power speed (i.e., they maximize flight time with a given amount of energy; Thomas 1975), and that there is no influence of wind beyond that experienced via flight speed. I also assumed heat loss as a consequence of convection and radiation only. Heat is lost from a warm body to a colder environment through conduction, evaporation, radiation, and convection (Schmidt-Nielsen 1997). However, I excluded conduction given that bats are not in contact with any solid surface during flight, and evaporative heat loss is negligible in temperatures below thermoneutral. Finally, I assumed peripheral cooling minimizes heat loss from the wings during flight (Reichard et al. 2010) and did not consider effects of piloerection and vascular adjustments.

3.3 RESULTS

3.3.1 *General patterns in weather and bat activity*

I recorded 1 310 bat passes over 405 nights ($\bar{X} = 3.2 \pm 7.41$ passes night⁻¹) of monitoring during the winters of 2012-13, 2013-14, and 2014-15 (Table 3.1). Activity occurred during every month (October through April) in each year of the study. I detected bat-activity on 34.1% (138 of 405) of the nights sampled, when temperatures were as low as -10.4°C. Over the three winters of this study, mean temperature at sunset was -7.3 ± 8.20 °C and mean nightly low was -14.0 ± 13.34 °C.

Table 3.1. Summary of nightly low ambient temperatures and acoustic bat-activity recorded in three locations (see Methods for details) at Dinosaur Provincial Park over three hibernation seasons: 23 October 2012 through 24 March 2013, 10 November 2013 through 1 April 2014, and 9 November 2014 through 4 March 2015. Means are presented with standard deviation.

Mean Night					
Winter Season	Mean Nightly Low T_a (°C)	Wind Speed (m s ⁻¹)	Detector Nights	Number of Passes	Mean Passes Night ⁻¹
2012-13	-11.3 ± 6.944	2.5 ± 1.42	146	574	3.9 ± 7.76
2013-14	-16.9 ± 19.666	2.5 ± 1.46	143	470	3.3 ± 8.56
2014-15	-13.2 ± 8.522	3.5 ± 1.72	116	266	2.3 ± 4.97

Nightly low ambient temperature varied among winters ($F_{2,402} = 5.85, P = 0.003$); in particular 2013-14 was colder ($\bar{X} = -16.9 \pm 19.66^{\circ}\text{C}$) than 2012-13 ($\bar{X} = -11.3 \pm 6.94^{\circ}\text{C}; P = 0.003$). Mean nightly wind speed also varied among winters ($F_{2,402} = 18.58, P < 0.001$); 2014-15 ($\bar{X} = 3.5 \pm 1.72 \text{ m s}^{-1}$) was windier than 2012-13 ($\bar{X} = 2.5 \pm 1.42 \text{ m s}^{-1}; F_{2,402} = 5.85, P = 0.003$) and 2013-14 ($\bar{X} = 2.5 \pm 1.46 \text{ m s}^{-1}; F_{2,402} = 5.85, P = 0.003$). Total nighttime precipitation during the winter hibernation periods was 32.6 mm in 2012-13, 24.7 mm in 2013-14, and 27.9 mm in 2014-15. Nearly all calls (97.9%, $N = 1283$) occurred creekside, with only 17 (1.3%) passes at the riverbank detector and 10 (0.8%) at the Cottonwood flats detector. I identified 91.3% ($N = 1196$) of calls as belonging to *E. fuscus* and 8.7% ($N = 114$) to *Myotis* bat species. I did not detect any other phonic group of bats during the hibernation period. For both groups, the majority of activity occurred within 4–5 hours of sunset, but activity continued during all hours of the night with a notable peak in *Myotis* activity in the final few hours of the night (Figures 3.1a, 3.1b). I did not observe any feeding buzzes in recordings made within the hibernation period in any of the years.

3.3.2 Influence of weather and environment on bat activity

After initial correlation, I included four variables in candidate models: ambient temperature (Temp), wind speed (Wind), precipitation (Precip), and 24-hr change in barometric pressure (BPDiff). For both species groups, the highest-ranked model with the lowest QAIC_c value and highest AIC weight (w_i) contained

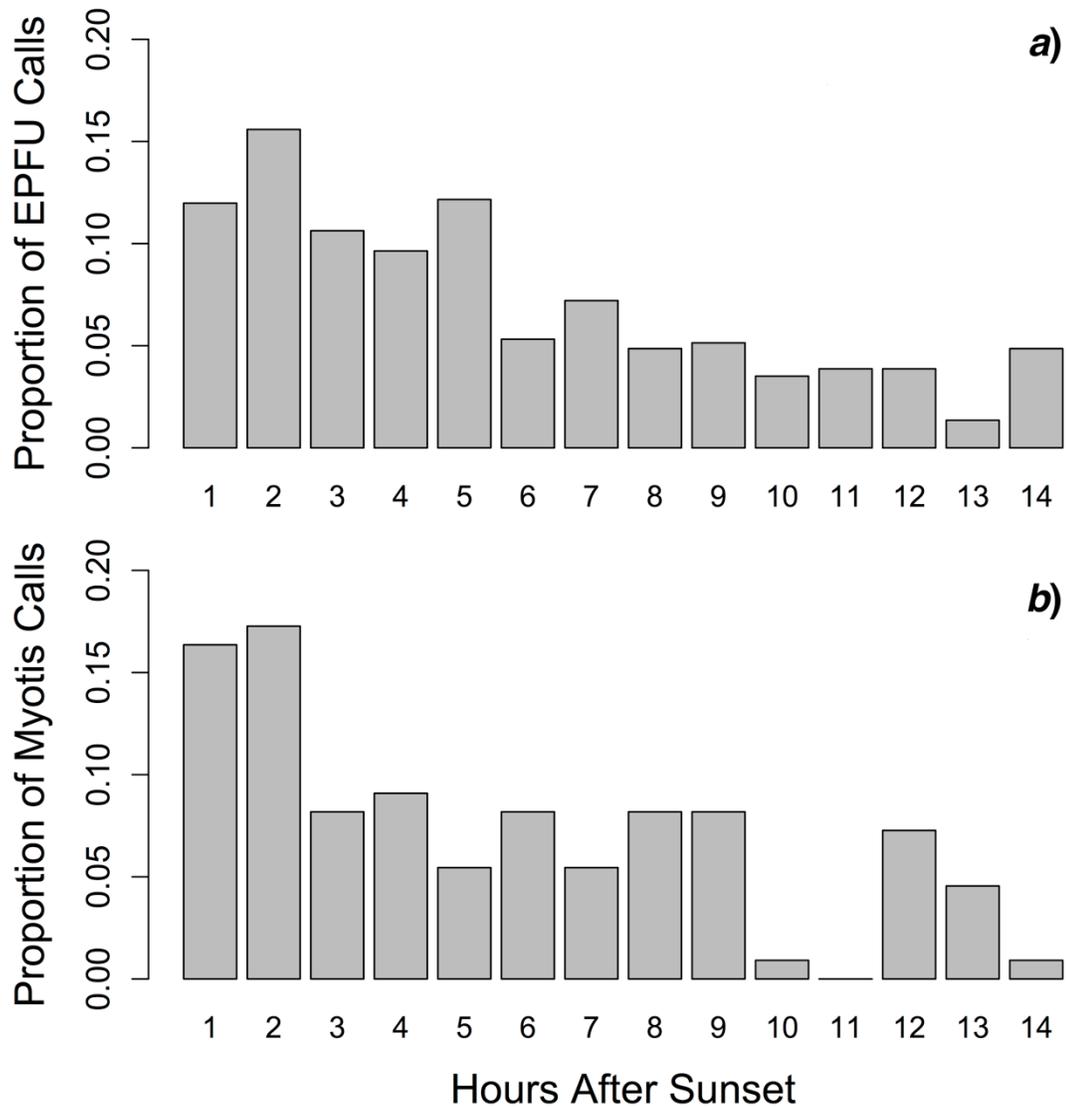


Figure 3.1. Hourly distribution relative to sunset of a) 1 196 *Eptesicus fuscus* calls and b) 114 *Myotis* calls recorded by three acoustic detectors over 405 winter nights in Dinosaur Provincial Park, Alberta from 23 October 2012 to 4 March 2015.

an interaction between the variables Temp and Wind (Tables 3.2, 3.3). For *E. fuscus*, there was no support for any other candidate model (Table 3.2). The second best model for *Myotis* had $\Delta\text{QAIC}_c < 2$ and a relatively low evidence ratio (w_1/w_2 ; Table 3.3), which suggests it could also explain considerable variation in the model (Burnham and Anderson 2002).

The two variables present in the top-ranked models for both species are temperature and wind, which suggests these variables are key predictors of hourly bat-activity. Temperature was positively correlated with bat activity (Tables 3.4a, 3.4b) with an estimated increase of 1.75 *E. fuscus* calls and 1.72 *Myotis* calls detected for every 1°C increase. Wind was negatively correlated with bat activity (Tables 3.4a, 3.4b), with an estimated decrease of 0.78 *E. fuscus* calls and 0.73 *Myotis* calls detected for every 1 m s⁻¹ increase. However, the intercepts of each model (1.54 for *E. fuscus* and 0.17 for *Myotis*) and probability of detection in relation to ambient temperature (Figure 3.2) suggest a lower temperature threshold for activity by *E. fuscus*. Most importantly, the inclusion of the interaction between temperature and wind produced a better model fit for both species, suggesting the effects of temperature and wind speed on bat activity are interdependent. Bats were more active when temperatures were high, but this was influenced by wind such that even in warmer conditions, bats were not active if it was windy.

Table 3.2. The top 5 models used to test the influence of weather and environmental variables on hourly activity (calls hour⁻¹) of *Eptesicus fuscus* activity during winter in Dinosaur Provincial Park. Models are ranked according to QAIC_c scores, with the lowest score indicating the top ranked model. ΔQAIC_c is the difference in QAIC_c scores between model *i* and the top ranked model. The probability of each being the best model, given the entire subset of models, is indicated by AIC weight (w_i).

Model	QAIC _c	ΔQAIC _c	w_i	w_1/w_i
Temp + Wind + Temp*Wind	3760.239		1.0000	
Temp + BPDiff + Temp*BPDiff	3815.315	55.076	0.0000	>1000
Temp + Wind + Precip	3816.247	56.008	0.0000	>1000
Global model (all variables)	3817.704	57.465	0.0000	>1000
Temp + Wind + BPDiff	3819.297	59.058	0.0000	>1000

Note: A complete description of each variable is given in materials and methods.

Table 3.3. The top 5 models used to test the influence of weather and environmental variables on hourly activity (calls hour⁻¹) of *Myotis* bats during winter in Dinosaur Provincial Park. Models are ranked according to QAIC_c scores, with the lowest score indicating the top ranked model. ΔQAIC_c is the difference in QAIC_c scores between model *i* and the top ranked model. The probability of each being the best model, given the entire subset of models, is indicated by AIC weight (*w_i*).

Model	QAIC _c	ΔQAIC _c	<i>w_i</i>	<i>w_i</i> / <i>w₁</i>
Temp + Wind + Temp*Wind	731.998		0.4876	
Temp + BPDiff	732.894	0.896	0.3116	1.57
Temp + Wind + BPDiff	735.119	3.121	0.1024	4.76
Temp + Precip + BPDiff	736.643	4.645	0.0478	10.20
Temp	739.052	7.054	0.0143	34.01

Note: A complete description of each variable is given in materials and methods.

Table 3.4. Top-ranked model based on negative binomial regression used to explain the influence of environmental conditions on hourly activity (passes hour⁻¹) of a) *Eptesicus fuscus* and b) *Myotis* bats in Dinosaur Provincial Park.

a) *E. fuscus*

Model term	Coefficient	S.E.	z-value	P-value
Intercept	0.4335	0.0143	3.03	0.003
Temp	0.5607	0.0288	19.46	<0.001
Wind	-0.2438	0.0323	-7.55	<0.001
Temp*Wind	-0.0567	0.0053	-10.69	<0.001

b) *Myotis* bats

Model term	Coefficient	S.E.	z-value	P-value
Intercept	-1.7578	0.3580	-4.91	<0.001
Temp	0.5393	0.0735	7.34	<0.001
Wind	-0.3173	0.0863	-3.68	<0.001
Temp*Wind	-0.0611	0.0137	-4.46	<0.001

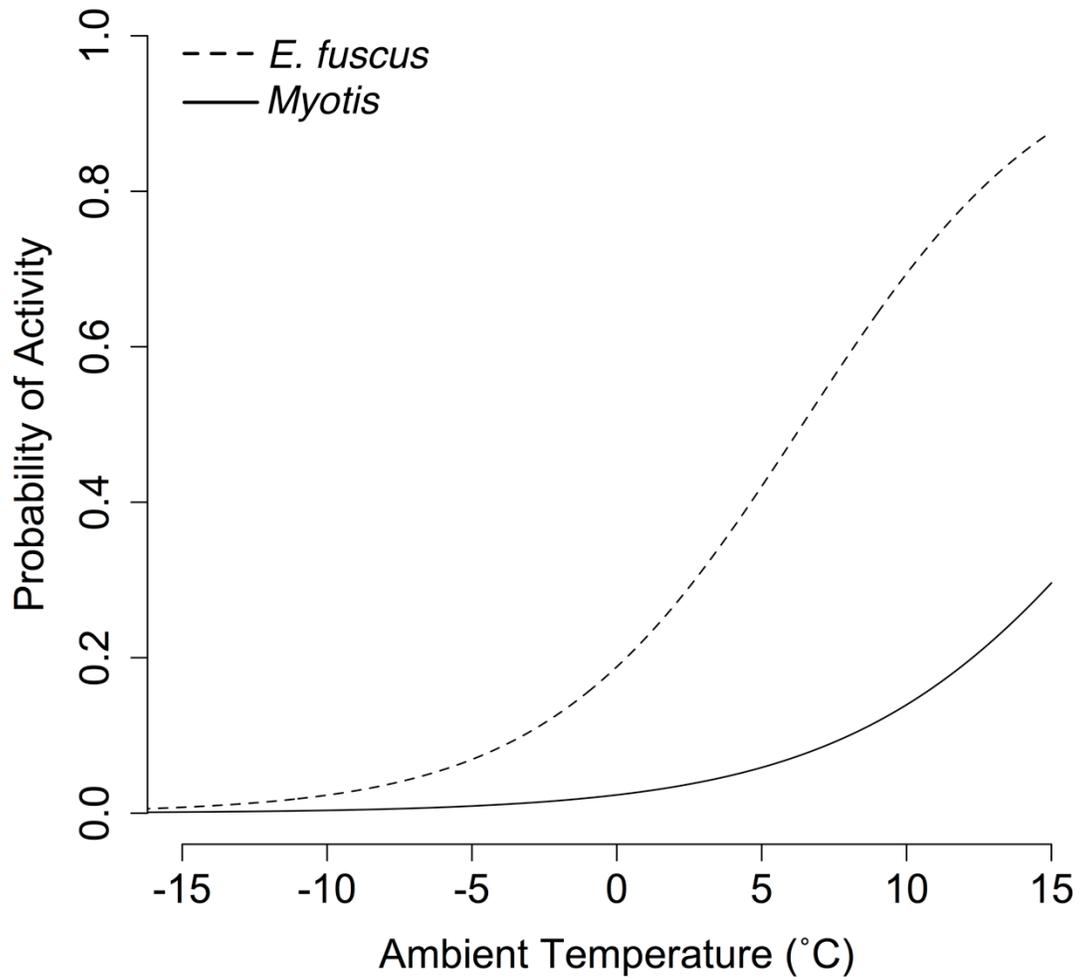


Figure 3.2. Cumulative probability plot showing the probability of detecting winter activity by *Eptesicus fuscus* (dashed line) and *Myotis* (solid line) in relation to ambient temperature in Dinosaur Provincial Park.

3.3.3 Energetic estimates of flight

To maintain euthermic T_b in a crevice hibernaculum with a T_a of 1.1°C , I estimated energy expenditure of 0.92 W for a 21.7 g *E. fuscus* and 0.61 W for a 10.1 g *M. lucifugus*. Based on these body masses, I estimated the cost of flight for bats to be 2.77 W for *E. fuscus* and 1.49 W for *M. lucifugus*. Estimated rates of heat loss via convection and radiation during flight differed with species, body region, and T_a (Table 3.5). When I included convective and radiative heat loss, the temperature below which heat loss exceeded metabolic heat production during flight was 5°C in *M. lucifugus* and 1°C in *E. fuscus* (Figure 3.3). Flight activity was more energetically costly per gram body-mass for *M. lucifugus* than *E. fuscus* at all T_a (Figure 3.3), and the rate of increase in the energetic cost of flight as T_a drops (i.e., slope) was higher for *M. lucifugus*. When converted to fat usage, I estimate *E. fuscus* and *M. lucifugus* spend an extra $0.19\text{--}0.87\text{ g}$ and $0.13\text{--}0.51\text{ g}$ of fat, respectively, per hour of flight in T_a ranging from 0°C through -10°C compared to that used per hour during euthermic rest within the hibernacula (0.08 g and 0.06 g , respectively). At 20% muscle efficiency (Speakman and Thomas 2003), I calculated 2.22 W and 1.19 W is available for activity-thermoregulatory heat substitution for *E. fuscus* and *M. lucifugus*, respectively. By my estimates, in T_a ranging from -10°C – 0°C , flight thermogenesis could offset heat loss by $23\text{--}89\%$ in *E. fuscus* and $20\text{--}70\%$ in *M. lucifugus*.

Table 3.5. Surfaces areas and estimated rates of heat transfer via convection (H_c) and radiation (H_r) from big brown bats (*Eptesicus fuscus*) and little brown bats (*Myotis lucifugus*). Rates of thermal radiation depend on ambient temperature (T_a) and are presented as ranges calculated for T_a 15°C through -10°C.

Species and body region	Surface area (m ²)	Heat transfer (W m ⁻² °C ⁻¹)	
		H_c	H_r
<i>Eptesicus fuscus</i>			
Body	0.00491	8.91	66.8–118.0
Wings	0.01517*	44.27 [†]	50.0–70.8
<i>Myotis lucifugus</i>			
Body	0.00314	8.96	66.8–118.0
Wings	0.00860*	44.27	51.1–70.8

*Farney and Fleharty 1969

†Reichard et al. 2010

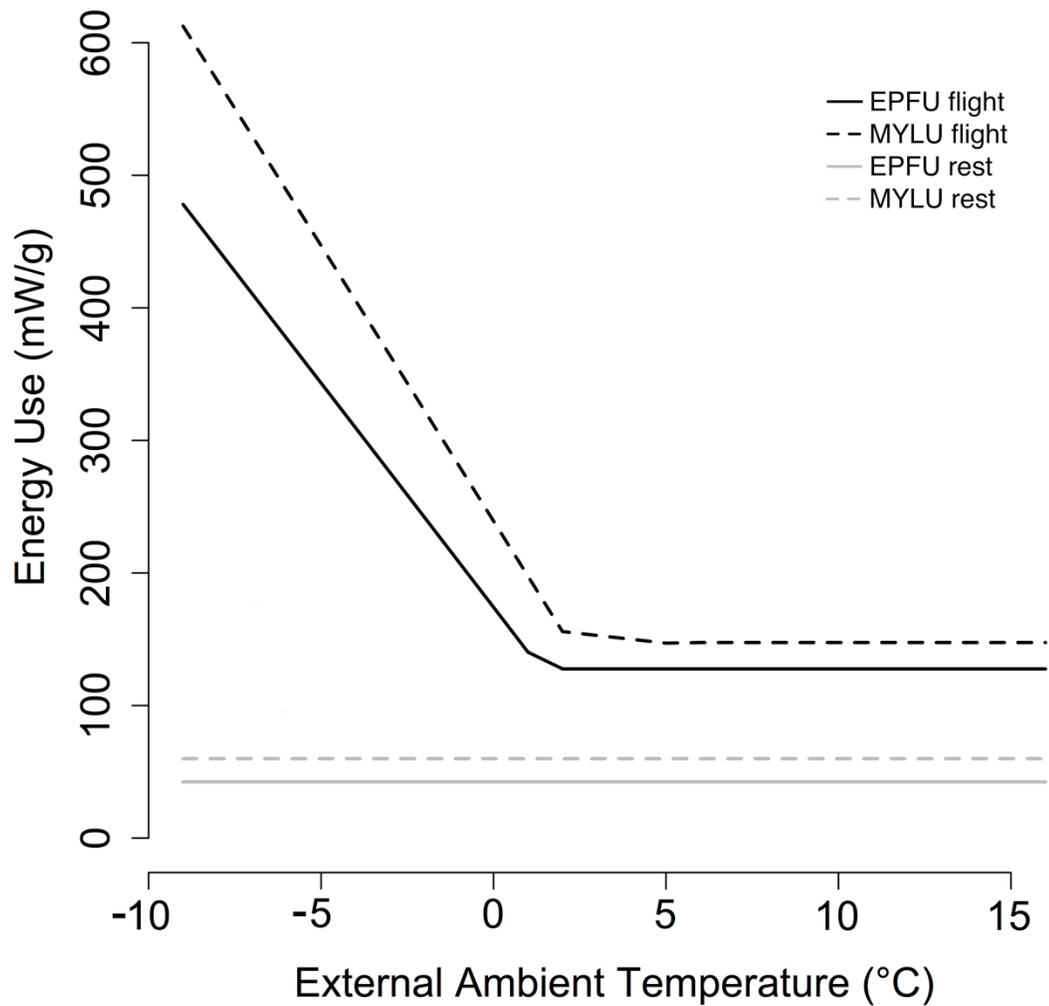


Figure 3.3. Estimated cost of flight (black lines) in relation to external ambient temperature for *Eptesicus fuscus* (solid lines) and *Myotis lucifugus* (dashed lines). Estimates for energy use during rest (grey lines) in a crevice hibernaculum with an internal temperature of 1.1°C are given for reference.

3.4 DISCUSSION

I recorded bat activity in Dinosaur Provincial Park throughout the entire winter each year of my study, which is consistent with other studies on winter bat-activity. Bat activity appears tied to foraging opportunity in areas with mild winter climates temperatures. For example, *L. noctivagans* and California myotis (*M. californicus*) foraged all winter in Washington State (mean sunset temperature = 9.1°C; Falxa 2007). Likewise, eastern red bats (*Lasiurus borealis*), *L. noctivagans*, and *E. fuscus* captured during winter in Missouri at sunset temperatures of 4–12°C showed evidence of feeding (Dunbar et al. 2007). In stark contrast, winters at my site in the Canadian prairies are colder (mean temperature at sunset = -7.3°C). No insects occur at my site during winter (Lausen and Barclay 2006) and no feeding buzzes have been detected (this study). Few other studies have reported mid-winter flight without evidence of insects or feeding (Whitaker and Rissler 1992; 1993; Schwab and Mabee 2014). These data suggest winter foraging is opportunistic and not the impetus for flight. Bats may instead be to find water (Speakman and Racey 1989; Hays et al. 1992; Thomas and Geiser 1997), switch roosts for more favourable microclimates (Whitaker and Gummer 1992; Whitaker and Rissler 1992; Boyles et al. 2006), or excrete built-up waste and metabolites outside hibernacula (Baumber et al. 1971).

Regardless of the reasons for mid-winter flight, my data support the hypothesis that weather conditions, particularly temperature and wind, influence bat-activity. Warmer temperatures and calmer winds led to higher activity in both

E. fuscus and *Myotis*. my results are consistent with other studies, but given the lack of insects at my site, such conditions likely reflect the energetic costs of flight rather than the possibility to forage. The rate of heat transfer is proportional to the temperature gradient between bat and environment (Bakken and Kunz 1988). Wind exacerbates convective heat loss by disturbing the insulative layer of air within fur (Bakken 1991) and also increases the power needed for flight. Although tailwinds can benefit airborne animals, power required for flight typically increase under conditions of headwinds or crosswinds (Tucker and Schmidt-Koenig 1971; Liechti et al. 1994). Avoidance of colder, windier conditions minimizes energetic costs of mid-winter flights and conserves fat stores, which likely improves body condition and chance of survival upon emergence in the spring (Humphries et al. 2003).

The interaction between temperature and wind may be particularly important in describing trends in bat activity recorded in my study area. Similarly to other studies (e.g., O'Farrell et al. 1967; Schwab and Mabee 2014), I recorded most calls during the warmer, early-evening period 4–5 hours after sunset. However, some activity persisted through all hours of the night with a slight increase in *Myotis* activity just prior to sunrise. Mild winter temperatures in southern Alberta are sometimes the consequence of “chinooks”—warm, westerly winds that can quickly and drastically increase ambient temperatures. Chinook winds often arrive during the night but die down by morning, leaving warm temperatures to persist through morning. Based on my data, it appears as though

bats remain in the hibernacula despite warm temperatures due to high winds during chinooks and emerge later in the night, after winds have subsided.

My data also suggest interspecific differences in the variables that influence activity. *Eptesicus fuscus* activity is predominantly influenced by temperature and wind, while *Myotis* activity is also influenced by changes in barometric pressure. *Eptesicus fuscus* roost close to hibernacula entrances (Rysgaard 1942), which may allow them to detect changes in ambient temperature. Conversely, *Myotis* roost deep in caves or crevices where temperature is relatively stable; detecting changes in barometric pressure to track approaching storm fronts (Paige 1995; Turbill 2008) reduces the risk of emerging during inclement weather. In addition, larger-bodied *E. fuscus* were more active at all temperatures and had a lower thermal threshold for activity than small-bodied *Myotis*. I recorded *E. fuscus* at temperatures as low as -10.4°C and *Myotis* as low as -5.1°C and there was a distinct difference between groups in the probability of activity relative to temperature. *Eptesicus fuscus* likely tolerate lower temperatures than smaller-bodied *Myotis* given that *Myotis* have higher surface area to volume ratios and lose more heat per unit of metabolically active mass (Kleiber 1947), and that *Myotis* fur has a lower insulation value than *E. fuscus* (Shump and Shump 1980).

I suggested that differences in net energetic costs of flight underlie disparities observed between species in winter activity. Based on the metrics of body size, fur insulation, and metabolic rates during flight and rest that I included in my calculations, my energetic estimates suggest that smaller-bodied *M.*

lucifugus spend more net energy per gram body mass in flight than larger-bodied *E. fuscus*. I estimated the temperature below which convective heat loss exceeds metabolic heat production via activity to be 5°C for *M. lucifugus* and 1°C for *E. fuscus*, which corroborates that larger-bodied *E. fuscus* energetically tolerate flight in colder temperatures than *Myotis*. Although mid-winter flight is energetically expensive, I estimated that activity-thermoregulatory heat substitution could mitigate costs by at least 20% and as much as 89% in milder temperatures. This phenomenon is especially important in areas devoid of prey where non-energetic benefits of flight may be attainable only with such reductions in net energetic cost. Interestingly, the mean mass of *E. fuscus* entering hibernation in October at my study site (23.6 g; B.J. Klüg-Baerwald, unpublished data) appears higher than that reported in Ontario (21.6 g; Fenton 1972) where winter foraging is possible (Brigham 1987), but masses observed in March-April post-hibernation are similar (16.8 g and 16.4 g, respectively); extra fat reserves may go toward fuelling mid-winter flight.

My study presents data on the winter activity and energetics of mid-winter flight of bats in an environment where foraging is unlikely. I show that temperature and wind are important predictors of *E. fuscus* and *Myotis* winter activity, and that *Myotis* may also use changes in barometric pressure to cue activity. I suggest these environmental factors relate to minimizing the energetic cost of flight. That bats take part in the behaviour of mid-winter flight without the opportunity to forage suggests other causal reasons and emphasizes the

importance of winter energy budgets in hibernating animals. My energetic estimates suggest that exercise thermogenesis partly mitigates energetic cost of flights in the cold, and that differences in winter activity between species likely stem from differences in rates of heat loss and potential for activity-thermoregulatory heat substitution. Winter activity of bats has been poorly studied to date, but as interest in studying the winter behaviours of bats increases, it is becoming apparent that activity following arousal is not an abnormal behaviour (Boyles et al. 2006). Given that bats can spend over half their lives in hibernation and that mid-winter flight ostensibly consumes a substantial amount of energy during this time of energetic constraint, it is important that I increase my understanding of this phenomenon.

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CHAPTER FOUR—FLIGHT CLUB: BEHAVIOURAL ADAPTATION AND
PERSISTENT FLIGHT IN HIBERNATING, PRAIRIE-LIVING BIG BROWN BATS
(*EPTESICUS FUSCUS*)

4.1 INTRODUCTION

Hibernation is an adaptive strategy used by many small mammals and one bird to save energy, mainly during long periods of food shortage (i.e., winter; Wang and Wolowyk 2011; Ruf and Geiser 2015). By suppressing metabolic activity and reducing T_b , animals can survive for months on stored energy reserves (e.g., fat; Humphries et al. 2003). However, winter energy stores are finite and hibernation is not a period of constant quiescence; nearly all small mammals periodically arouse for reasons that remain unclear (Willis 1982). Removal of metabolic waste (Malan 1986), mounting of immune responses (Bouma et al. 2010), mating (Barnes et al. 1986), maintenance of muscle tissue (Yacoe 1983), restoration of neural function (Popov et al. 1992), and access to food and water (Humphries et al. 2003) have all been suggested reasons for activity. Arousals use a disproportionately large amount of energy given the relatively short duration hibernators spend euthermic; over 80% of energy reserves can be spent warming up and maintaining high T_b during these events (e.g., Thomas et al. 1990; Armitage et al. 2003). Arousals are however an essential part in hibernation

biology and thus factors influencing these patterns are crucial for overwintering energetics and survival.

Activity patterns (e.g., torpor bout length, torpid T_b , arousal frequency) of hibernating animals are highly dependent on the microclimates within the hibernacula. Metabolic heat production is reduced until T_b reaches a newly established setpoint (T_{set} ; Heller 1979). Ambient temperatures above T_{set} restrict the depth T_b falls, and result in higher metabolic rates and more frequent arousals (e.g., Geiser and Kenagy 1988). The metabolic response of hibernating animals when T_a drops below T_{set} is equally important; metabolic activity and arousal frequency are increased to avoid freezing (e.g., Geiser and Broome 1993; Dunbar and Tomasi 2006). Evaporative water loss persists during hibernation and humidity is also an important factor influencing patterns of small-mammal hibernation (Thomas and Cloutier 1992). Duration of torpor bouts shorten (Thomas and Geiser 1997) and arousal frequency increases (Ben-Hamo et al. 2013) with increasingly dry conditions. The metabolic response of hibernators to changes in their environment means that even small changes in temperature or humidity tend to increase energy use and thus reduce the probability of overwinter survival (Boyles and Brack 2009).

The hibernaculum microclimate is important in determining how hibernators use finite energy stores. For this reason, many hibernators seek out sites with particular microclimates—high humidity and stable temperatures that remain above freezing—to maximize energy savings (Geiser 2004). Hibernating in

groups likely confers additional benefits due to social thermoregulation, including buffered temperature changes (e.g., Arnold et al. 1991) and reduced water loss (Boratyński et al. 2015). Our knowledge of hibernating bats largely comes from studies on cavernicolous species, which appear to fit this archetype. In eastern North America, bats typically overwinter in large clusters within caves or mines with high levels of humidity and stable temperatures (Webb et al. 1996; Perry 2012). Cavernous hibernacula also allow for flight or mating (Thomas et al. 1979) within open chambers during arousals, and often contain standing water sources, and possibly hibernating insects, that can be consumed (Swanson and Evans 1936; Rysgaard 1942).

Roosting ecology strongly influences the activity patterns and energy budgets of most small hibernators, yet little is known about the behaviour of bats using noncavernous sites. Some available hibernacula in the prairies (e.g., rock crevices) are smaller, drier, and less thermally stable than most known cave hibernacula (see Chapter 3). Such conditions may increase evaporative water loss, and limit opportunities to mate and fly within hibernacula. Fluctuating temperatures experienced by crevice-hibernating bats may also increase energy consumption and cause bats to search for sites with more suitable microclimates (Boyles et al. 2006). Thus, I predict that bats that use rock-crevices to overwinter in the prairies will arouse more frequently and fly outside of the hibernacula more often than bats that hibernate in caves. Although arousals in some populations of cave-hibernating bats show no diurnal pattern (Czenze et al. 2013), patterns of

activity may remain under some diurnal influence if flight is a priority (Hope and Jones 2013). Factors associated with favourable weather, such as falling barometric pressure (Paige 1995) may also cue arousal. Thus, hibernation patterns of prairie-living, crevice-hibernating bat populations are likely to be influenced by a unique suite of factors, and their energy budgets may be different than those of more oft studied, cave-roosting conspecifics.

I monitored hibernation patterns of free-ranging big brown bats (*Eptesicus fuscus*) overwintering in rock-crevices in the Canadian prairies. Given that changes in environmental conditions influence the hibernation patterns of most hibernators, I hypothesized that hibernation patterns of this population would be influenced by changes in microclimate detectable within hibernacula—therefore hibernacula temperature and humidity as well as barometric pressure. Specifically, I predicted that bats in my study area would arouse frequently in response to rising or falling crevice temperature, decreased crevice humidity, and falling barometric pressure. I further hypothesized that bats adapt behaviourally to offset the energetic cost of frequent arousals, and predicted that they would maintain T_b close to T_a to lower the energy spent during steady-state torpor. Given the prevalence of mid-winter flight in this population of *E. fuscus* (Klüg-Baerwald et al. 2016; see also Chapter 3), I also hypothesized that activity would follow a diurnal pattern, specifically that arousals would coincide with dusk to ensure the opportunity for flight. Related to this, I predicted that ambient temperature would be positively correlated with arousal duration, and thus flight. Finally, I estimated

the winter energy budget for *E. fuscus* in my study area and compared it to that of a population of building- hibernating conspecifics. I expected the overall energy requirements of both populations to be similar, but with divergent emphases on differing hibernation activities (e.g., flight, arousal, euthermia, etc.).

4.2 METHODS

4.2.1 Study site, captures, and radiotelemetry

My study took place at Dinosaur Provincial Park (DPP), Alberta, Canada (50°45'09"N, 111°31'03"W) during the winters (November through March) of 2012–13 through 2014–15. The park has a semi-arid climate (Bailey 1979) and is comprised of riparian and prairie habitat with an extensive network of creeks and drainages. *Eptesicus fuscus* hibernates in deep (1–2 m) rock-crevices within the park (see Chapter 2) and are active during the winter when the weather is favourable (Lausen and Barclay 2006; Klüg-Baerwald et al. 2016; see also Chapter 3).

I captured bats in mist nets set across a creek and recorded sex, age, and morphometrics (e.g., mass and forearm length). For a subset of 29 individuals, I affixed radio-transmitters (Lotek Ag392 1.2 g temperature-sensitive radiotransmitters; Lotek Wireless Inc., Newmarket, ON) to the interscapular region using latex adhesive (Perma-Type™; The Perma-Type Company Inc., Plainville, CT). Before attachment, I calibrated ($\pm 1^\circ\text{C}$) radiotransmitters and associated temperature curves every 5°C from 0°C to 40°C in a water bath using one mercury thermometer and one digital thermometer (Model 8402-00; Cole-

Parmer Canada Inc., Montreal, QC) as references. Radiotransmitters weighed less than 5% of body mass, thus minimized effects of the load on flight maneuverability and behaviour (Aldridge and Brigham 1988; Neubaum et al. 2005). I released all bats at the site of capture within 1 h of capture. The University of Regina President's Committee on Animal Care (PCAC) approved all methods and procedures (Animal Use Protocol #12-12), which conformed to the guidelines for animal care and use outlined by the American Society of Mammalogists (Sikes 2016). I carried out fieldwork under research and collection permits issued by Alberta Sustainable Resource Development and Alberta Tourism, Parks and Recreation Division.

I tracked radiotagged bats to three rock-crevice hibernacula located in DPP. I positioned datalogger receivers (SRX400A; Lotek Wireless, New Market, Ontario, Canada) at each hibernaculum to record skin temperature (T_{sk}) of radiotagged individuals within range every 15 min. Skin temperature recorded by external radiotransmitters is an accurate and relatively noninvasive method of measuring core T_b in small mammals (Barclay et al. 1996; Willis and Brigham 2003). I powered each datalogger with 12 V batteries charged by 10 W solar panels to ensure continuous, non-interrupted data logging. I used microclimate dataloggers (HOBO U23 loggers; Onset Computer Corp., Bourne, MA) with probes placed as far into the hibernacula as possible (up to 1.8 m) to record hibernacula temperature (T_h ; °C) and relative humidity (RH_h ; % RH) every 20 min. Given the limitation of RH as an ecological variable (Kurta 2014), I converted RH

measurements to absolute water vapour pressure ($WVP_{\text{abs-h}}$; kPa; Brice and Hall 2016). I gathered barometric pressure (kPa) from the Alberta Agriculture and Forestry weather station (ACIS 2015) located 15 km from the study site in Patricia, Alberta.

4.2.2 Definitions and statistical analyses

I did not set a T_{sk} threshold to distinguish between torpor and euthermia given the unambiguous difference between the two states. Constant, low T_{sk} maintained $< 10^{\circ}\text{C}$ characterized steady-state torpor, and sudden increases in T_{sk} typically $> 25^{\circ}\text{C}$ characterized arousals (Figure 4.1). Arousals can be broken down into periods of warming, euthermia, and cooling. I defined the start of the warming phase as the first of ≥ 2 consecutively rising T_{sk} readings, the start of the cooling phase as the first of ≥ 2 consecutively decreasing T_{sk} readings, and euthermia as the period of relatively stable T_{sk} readings in between. I recorded the time of each arousal relative to sunset and converted these data to radians. I excluded data recorded before the first arousal to avoid the influence of capture and radiotagging on my results. I also excluded data for any period between arousals that was missing 2 or more consecutive T_{sk} readings to avoid missing an arousal cycle (Willis and Brigham 2003).

To analyze hibernation pattern data, I used generalized linear mixed models (GLMMs) with individual bat as a random effect to account for

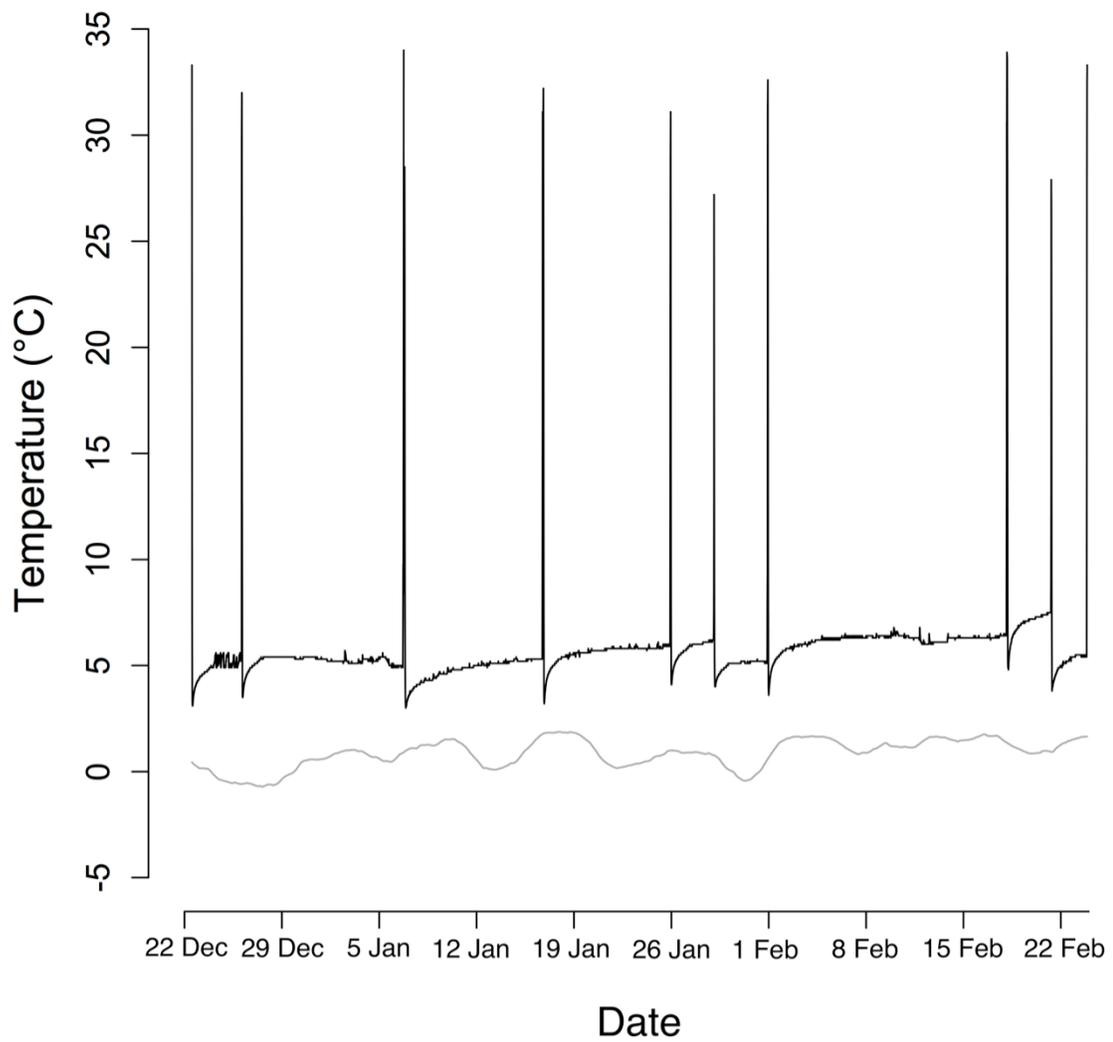


Figure 4.1. Thermoregulatory pattern of a big brown bat (*Eptesicus fuscus*) using a rock-crevice hibernaculum in Dinosaur Provincial Park, Alberta during winter 2012–13. The grey line represents temperature within the hibernaculum (T_h) and the black line represents recorded skin temperature (T_{sk}).

autocorrelation in my measurements (Zuur et al. 2009). For torpor bouts, I assessed the influence of hibernaculum microclimate (mean T_h and WVP_{abs-h}) and mean torpid T_{sk} (T_{sk-tor}) during each bout on torpor bout length, and the influence of hibernaculum microclimate on mean torpid T_{sk} . For arousals, I assessed the influence of hibernaculum microclimate, ambient temperature (T_a), and mean euthermic T_{sk} (T_{sk-eu}) on arousal length. I also used binomial logistic regression to assess the probability of arousal as a function of current and changing (over 24 hours) hibernaculum microclimate, as well as 24-hour change in barometric pressure (ΔBP); I did this by comparing conditions recorded at the start of each arousal with conditions recorded during an equal number of randomly sampled time periods from my entire dataset. I recorded the date and time of each arousal and used Rayleigh's test for circular distributions to determine if there was a temporal pattern in these events relative to sunset. I used R (R Development Core Team 2016) to conduct statistical analyses with a significance level of $\alpha = 0.05$ and present data as means with standard deviation ($\bar{X} \pm S.D.$).

4.2.3 Energetic Estimates

To calculate a single estimate of energy expenditure representative of bats in my study area, I used the mean values reported in this study (e.g., torpid T_{sk} , torpor bout duration, arousal frequency and duration, euthermic T_{sk} , flights per winter, flight duration, and T_h ; Table 4.1). I then used reported means for the same variables (except those associated with winter flight) reported for a population of

Table 4.1. Mean values ($\pm S.D.$) used to estimate and compare winter energy expenditure of *Eptesicus fuscus* hibernating in a building in western Indiana and rock crevices in Dinosaur Provincial Park, Alberta.

Variable	Building (Halsall et al. 2012)	Rock-crevices (this study)
Hibernaculum temperature (T_h ; °C)	12.0 \pm 1.6	1.1 \pm 0.87*
Torpid skin-temperature (T_{sk-tor} ; °C)	10.3 \pm 2.5	5.6 \pm 0.95
Torpor bout duration (d)	3.3 \pm 3.1 days	9.0 \pm 6.01
Euthermic skin-temperature (T_{sk-eu} ; °C)	31.9 \pm 1.7	31.7 \pm 2.15
Arousals per winter	28	17
Arousal duration (h)	5.1 \pm 2.7	1.0 \pm 0.73
Number of flights per winter	-	6
Duration of flight (h)	-	0.7 \pm 0.46
Approximate length of hibernation (d)	90	150

*see Chapter 2

E. fuscus hibernating in a building to compare energy budgets (Table 4.1). For all other parameters used in my calculations, I used values reported in the literature for this species (Table 4.2).

I based my estimates of energy expenditure during winter (E_{win}) on a model used by others (Humphries et al. 2002; 2006; Boyles and Brack 2009):

$$E_{win} = E_{tor} + nE_{bout},$$

where E_{tor} is the energetic cost of torpor bouts, and n and E_{bout} are the number and cost of arousal bouts, respectively. In this model, E_{tor} is calculated depending on hibernaculum temperature (T_h) as:

$$TMR_{min} Q_{10}^{(T_{sk-tor} - T_{tor-min})/10}; \text{ if } T_h > T_{tor-min}, \text{ OR}$$

$$TMR_{min} + (T_{sk-tor} - T_h)C_{tor}; \text{ if } T_h \leq T_{tor-min},$$

where TMR_{min} is minimum torpid metabolic rate, Q_{10} is the temperature-dependent change in TMR, $T_{tor-min}$ is lower ambient set-point temperature, and C_{tor} is wet thermal conductance of torpid *E. fuscus*. I altered the second equation to use torpid skin-temperature (T_{sk-tor}) instead of $T_{tor-min}$ given that *E. fuscus* in this study maintained T_{sk} above $T_{tor-min}$. I calculated E_{bout} as three separate phases (see Boyles and Brack 2009): arousal (E_{ar}), euthermia (E_{eu}), and cooling (E_{cool}). For E_{ar} , I use the equation:

$$E_{ar} = (T_{sk-eu} - T_{sk-tor})S,$$

where T_{sk-eu} is euthermic T_{sk} and S is specific heat capacity of tissue. For E_{eu} , I used the equation:

$$E_{eu} = BMR + (T_{lc} - T_h)C_{eu},$$

Table 4.2. Values reported in the literature used in estimating winter energy budgets of hibernating *Eptesicus fuscus*.

Parameter	Value	Source
Mean winter capture mass	21.7 g	1
Minimum torpid metabolic rate (TMR _{min})		2
Alberta population	0.404 ml O ₂ h ⁻¹	
Indiana population	0.473 ml O ₂ h ⁻¹	
Lower critical temperature limit (T _{lc})	26.7°C	3
Minimum body-temperature set-point (T _{tor-min})		2
Alberta population	2.0°C	
Indiana population	10.0°C	
Temperature-dependent change in TMR (Q ₁₀)	$1.6 + 0.26T_{sk} - 0.006T_{sk}^2$	4
Torpid wet thermal conductance (C _{tor})	0.04 ml O ₂ h ⁻¹ g ⁻¹ °C ⁻¹	3
Specific heat capacity of tissue (S)	0.131 ml O ₂ g ⁻¹ °C ⁻¹	5
Basal metabolic rate (BMR)	1.13 ml O ₂ h ⁻¹ g ⁻¹	3
Euthermic wet thermal conductance (C _{eu})	0.25 ml O ₂ h ⁻¹ g ⁻¹ °C ⁻¹	1
Cost of mid-winter flight (at T _a of 1°C)	15.5 ml O ₂ h ⁻¹ g ⁻¹	1

1 - Klüg-Baerwald et al. 2016, see also Chapter 3; 2 - Dunbar and Brigham 2010

3 - Willis et al. 2005; 4 - Hock 1951; 5 - Thomas et al. 1990

where BMR is basal metabolic rate, T_{lc} is the lower critical temperature, and C_{eu} is wet thermal conductance at euthermic T_b in winter. I set energetic cost of cooling (E_{cool}) at 67% of E_{ar} (Thomas et al. 1990). Lastly, I added the cost of mid-winter flight (E_{flight}) to the energy budget of bats in DPP (Klüg-Baerwald et al. 2016; see also Chapter 3) and subtracted the equivalent number of E_{eu} bouts as flights ($N = 6$) from the overall estimated energy budget given the likelihood of activity-thermoregulatory heat substitution (Humphries and Careau 2011).

To convert estimates of energy expenditure to g fat used, I assumed that use of 1 ml O_2 equates to 20.1 Joules from fat and that fat contains 39.3 kJ g^{-1} . To calculate whole animal estimates, I used mean winter capture mass (21.7 g) of *E. fuscus* in my study area (Klüg-Baerwald et al. 2016; see also Chapter 3).

4.3 RESULTS

I collected 585 days of individual T_{sk} data from 13 male *E. fuscus* (45 ± 31.1 days bat^{-1}) over three winters. I recorded 63 complete torpor bouts (5 ± 2.9 bouts bat^{-1}). Mean length of torpor bouts was 9.0 ± 6.01 days and mean T_{sk} of bats in steady state torpor was $5.6 \pm 0.95^\circ C$. I recorded 75 arousals (6 ± 2.8 arousals bat^{-1}). Mean length of arousals was 1.0 ± 0.73 h (59 ± 44.1 min) and mean T_{sk} during arousals was $31.7 \pm 2.15^\circ C$. Bats emerged and took flight outside of hibernacula 36% ($N = 27$) of the times they aroused. Mean length of mid-winter flights was 0.7 ± 0.46 h (44 ± 27.9 min) and mean T_a during these flights was $0.4 \pm 4.97^\circ C$ (range = $9.7-9.3^\circ C$).

I found no influence of hibernaculum microclimate during a torpor bout on the length of the bout ($P > 0.322$) or torpid T_{sk} ($P = 0.106$) during the bout, and no correlation between torpor bout length and torpid T_{sk} ($P > 0.689$). Probability of arousal increased when hibernaculum temperature warmed over 24 hours ($d.f. = 143$, $z = 2.16$, $P = 0.031$) and length of an arousal bout was positively correlated with T_a ($t_{53,13} = 4.78$, $P < 0.001$; Figure 4.2). No other variables I recorded influenced the length of arousal bouts ($P > 0.510$) or the probability of an arousal ($P > 0.468$). Although arousal bouts occurred during all hours of the day (Figure 4.3), timing of arousals relative to sunset was not random ($\bar{R} = 0.46$, $P < 0.001$) and the majority (73%, $N = 55$) occurred between sunset and sunrise; the mean time of arousals was 1.8 ± 4.94 h (108 ± 296.1 min) after sunset.

I estimate that 9.4 g of fat is required for *E. fuscus* to overwinter in my study area given the conditions and activities typical during hibernation by this population (Table 4.3). The majority (76%) of their fat stores are spent maintaining T_b on average 5°C above T_h during steady-state torpor, with considerably less (24%) spent on activities associated with arousal. Conversely, I estimate *E. fuscus* hibernating in a building in Indiana expend the majority (86%) of their total winter fat stores (8.2 g) during arousals, and less during steady state torpor (14%).

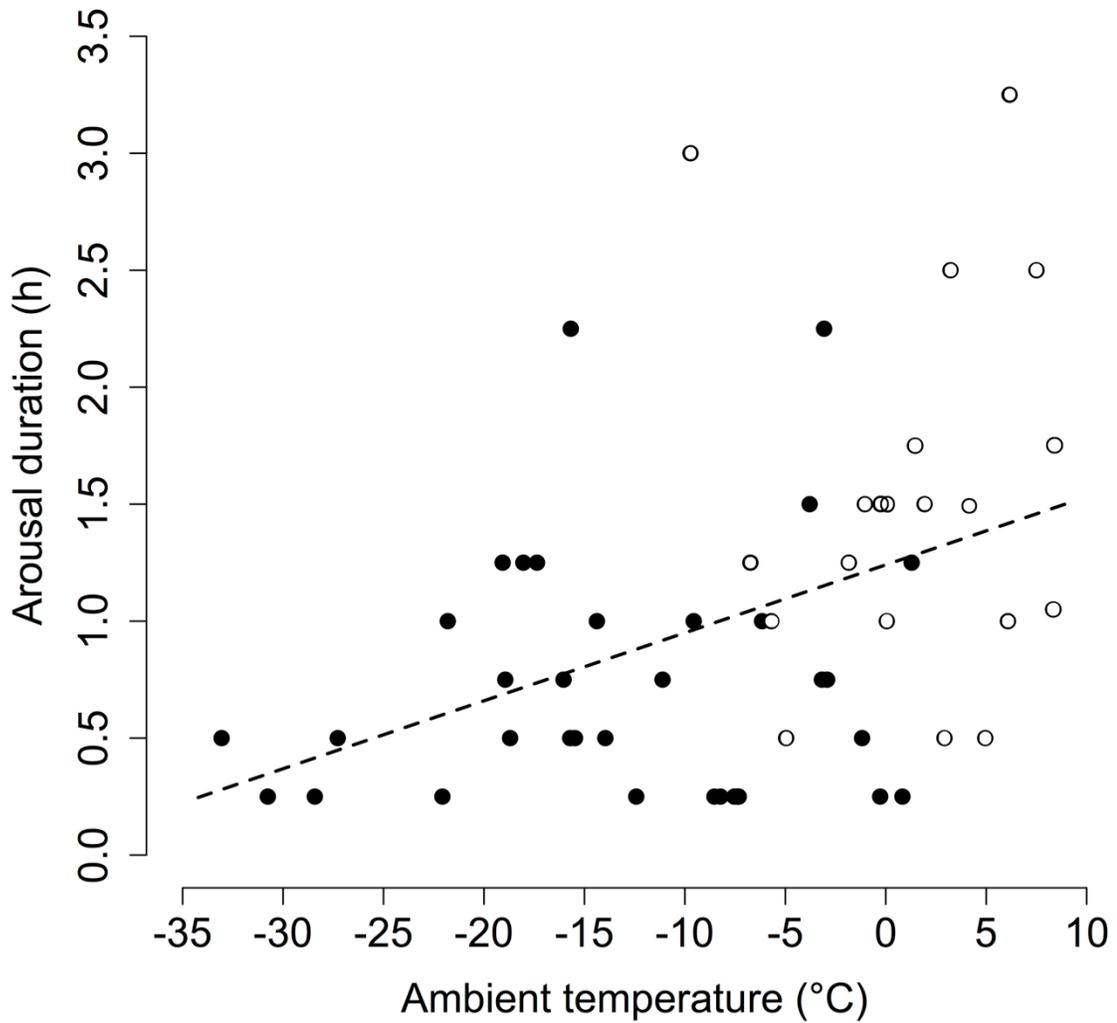


Figure 4.2. Relationship between ambient temperature (T_a) and duration of arousal bouts of big brown bats (*Eptesicus fuscus*) in Dinosaur Provincial Park, Alberta during the winters of 2012–15. Closed circles represent arousals during which bats did not leave the hibernacula and open circles represent arousals accompanied by flight. Dashed lines denote the least-squares fit for all data.

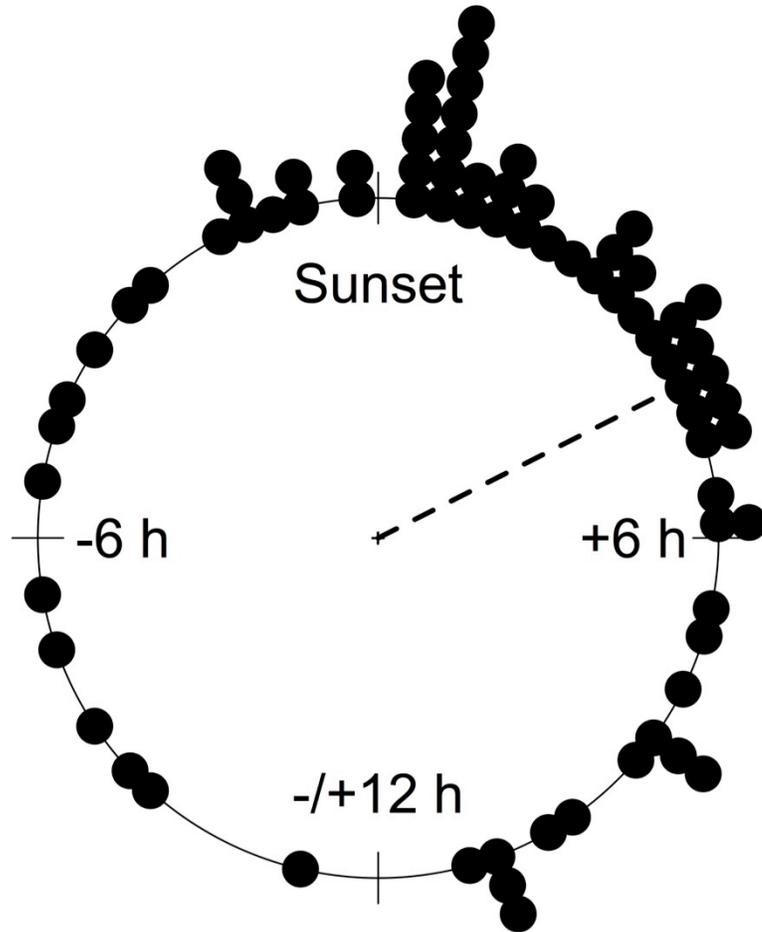


Figure 4.3. Circle plot of arousal timing relative to sunset. Each solid circle represents a recorded arousal and the dashed line is the mean arousal time.

Table 4.3. Total winter energy budgets estimated for populations of *Eptesicus fuscus* hibernating in a building in Indiana (Halsall et al. 2012) and rock-crevices in southern Alberta (this study). Estimates are given in grams of fat used broken down into individual activities following the equation of (Humphries et al. 2002).

Activity	Energetic Cost for Building Population	Energetic Cost for Crevice Population
Torpor bouts	0.6 g fat (14%)	7.1 g fat (76%)
Arousal bouts	7.6 g fat (86%)	2.3 g fat (24%)
Warming	0.7 g fat	0.6 g fat
Euthermy	6.4 g fat	0.7 g fat
Cooling	0.5 g fat	0.4 g fat
Flight	-	0.6 g fat
Total winter energy budget	8.2 g fat	9.4 g fat

4.4 DISCUSSION

Few studies have described patterns in body temperature of free-ranging, temperature-zone bat species while hibernating (Park et al. 2000; Hope and Jones 2012; Jonasson and Willis 2012; Czenze et al. 2013; Czenze and Willis 2015). Even fewer have done so for noncavernicolous populations (Halsall et al. 2012). Ours is the first to focus on a population of bats hibernating in natural rock-crevices. I found differences in the hibernation patterns of these bats compared to those typical of small hibernating mammals, and even compared to those of building-roosting conspecifics. Contrary to my hypotheses, conditions within hibernacula had little influence of over hibernation patterns (i.e., torpor length and depth). However, I did find an increased probability of arousal with warming hibernaculum temperature, as well as longer arousal bouts during warmer weather, often associated with flight. Further suggesting the importance of mid-winter flight to this population, I found a trend toward a nocturnal timing of arousals. Lastly, this population of bats maintained T_b higher than I expected, and appear to use behavioural adaptations to balance the energetic cost by arousing less frequently and for shorter durations than expected.

In many hibernators, length of torpor bouts shorten when hibernaculum temperature moves away from a particular T_b setpoint (e.g., Geiser and Kenagy 1988; Dunbar and Tomasi 2006; Jonasson and Willis 2012). Likewise, humidity within hibernacula influences arousal patterns, with drier conditions resulting in shorter torpor bouts (Thomas and Geiser 1997; Ben-Hamo et al. 2013). Despite

these widespread trends and contrary to my predictions, I found no influence of hibernaculum microclimate on torpor bout length or torpid T_{sk} . Consistent observations have been made of conspecifics hibernating elsewhere (Brack and Twente 1985; Halsall et al. 2012). Overwintering behaviour of *E. fuscus* in the wild is known mainly from populations using buildings (Whitaker and Gummer 1992; Halsall et al. 2012), with limited observations on movement by cave-hibernators (Brack and Twente 1985). Documented use of rock-crevice roosts in winter by *E. fuscus* (Lausen and Barclay 2006) further demonstrates their proclivity for using natural crevice roosts year-round. Thermal variability of these types of hibernacula likely favoured a muted response to environmental variables; being highly sensitive to external cues would likely result in overly frequent arousals and excessive energy expenditure.

Given the prevalence of mid-winter flight by individuals in this population (Klüg-Baerwald et al. 2016; see also Chapter 3), I hypothesized that ambient conditions would influence the probability and length of arousal. I also hypothesized that activity would follow a diurnal rhythm, specifically that arousals would coincide with dusk to enhance the opportunity for flight. My data support these hypotheses; probability of arousal increased with rising hibernaculum temperature, arousal bout length was positively correlated with ambient temperature, and the majority of arousals occurred at night. Energetic costs of winter flights increase as temperatures drop (Klüg-Baerwald et al. 2016; see also Chapter 3) and predation risk ostensibly precludes leaving the

hibernacula if arousal occurs during daylight hours (Thomas and Jacobs 2013). My results also add to a growing body of evidence that suggests a pronounced influence of ecology on the diurnal rhythms of small mammal hibernators. Whereas cave-hibernating bats synchronize arousals to sunset only with the potential for spring emergence (e.g., Czenze and Willis 2015) or not at all (Thomas 1993), a diurnal influence on winter activity persists in building- and crevice-hibernating populations where flight outside of the hibernaculum is important (Twente and Twente 1987; Halsall et al. 2012; Hope and Jones 2013). Diurnal rhythms facilitate foraging throughout hibernation in a number of other small mammals, such as the pocket mouse (*Perognathus longimembris*; French 1977) and the mountain pygmy possum (*Burramys parvus*; Körtner et al. 1998). Thus, a diurnal influence on the timing of arousals appears largely dependent on the ecology of a population and potential for extra-hibernacular activity.

That timing and length of arousals are heavily influenced by conditions that facilitate flight suggests that this is a crucial winter activity for bats in my study area. Caverniculous bats have opportunity to mate, drink, and potentially forage during arousals regardless of timing. Use of drier, less thermally stable crevices or buildings may increase evaporative water loss and arousal frequency, and require well-timed exits to drink or mate outside of these hibernacula. Although the exact reasons are unknown, bats in my study area do not appear to forage (Lausen and Barclay 2006) or drink (see Chapter 6) during arousals, but flight may confer neuromuscular and immunological benefits. Muscle is crucial to

hibernators for shivering thermogenesis and locomotion. Most hibernators have adaptations that inhibit muscle protein loss (reviewed in Klüg and Brigham 2015) and muscle atrophy is minimal in most species (e.g., Lee et al. 2008; Cotton and Harlow 2010; Gao et al. 2012). Indeed, the muscle fiber properties of flight muscles in *E. fuscus* under natural conditions are the same pre- and post-hibernation (Hermanson 2009). The role of active movement (i.e., contraction) in supplementing muscle tissue maintenance during an arousal bout or reinforcing neuromuscular function to maintain coordination remains largely unexplored, as does possible immunological purposes of winter flight.

Immune function declines during bouts of torpor (Burton and Reichman 1999; Bouma et al. 2010) but is restored during arousals, which are often accompanied by fever (Prendergast et al. 2002). Increases of 1–2°C above normothermic T_b can enhance host immune response and increase chances of clearing infection (Kluger et al. 1975; Kluger 1986). The febrile response is conserved across many animal taxa, including ectothermic arthropods (Casterlin and Reynolds 1980), fishes (Reynolds and Casterlin 1976; Reynolds 1977; Boltaña et al. 2013), amphibians (Casterlin and Reynolds 1977; Myhre et al. 1977), reptiles (Vaughn et al. 1974), and neonate mammals (Satinoff et al. 1976) that seek out warmth when challenged by pathogens—a phenomenon called behavioural fever. Although behavioural fever has not been described in bats, elevated flight temperatures are implicated in promoting the large diversity viruses that bats asymptotically carry (O'Shea et al. 2014). The T_b of bats in flight can be as high

as 41°C (reviewed in O'Shea et al. 2014), considerably higher than typical euthermic mammalian T_b (<37°C; Hock 1951). The short (< 1 h) flights made by *E. fuscus* at my study site might be as effective at mounting immune responses as maintaining euthermic T_b while resting for considerably longer periods of time (e.g., ~5 h; Halsall et al. 2012). Thus, flight may not only complete the final stages of warming during arousal (Willis and Brigham 2003), but also serve as a behavioural adaptation to clear infection and promote survival.

Winter energy budgets of hibernating animals are commonly overestimated when compared to actual body mass data collected in the field (e.g., Jonasson and Willis 2012). My estimates of the amount of fat required for hibernation by the Indiana (8.2 g) and Alberta (9.4 g) populations equate to a 46% and 52% gain from pre-hibernation mass (18 g; van Zyll de Jong 1985), respectively. This is higher than that estimated for the little brown myotis (29.6–32.9%; Kunz et al. 1998) and also that expected based on decreases in winter mass reported for conspecifics in DPP and elsewhere (5.2–6.8 g; Fenton 1972; Brigham 1987; Klüg-Baerwald et al. 2016; see also Chapters 2 and 3). However, I did not consider in my calculations the potential for passive rewarming to reduce energy consumption by as much as 47% during the warming phase (Halsall et al. 2012). Foraging may also compensate for this deficit; bats overwintering in areas with mild winters, such as Indiana, have the opportunity to forage and supplement fat stores during arousals (Whitaker and Rissler 1993; Dunbar et al. 2007; Falxa 2007). With no ability to forage, my estimates suggest that bats overwintering in DPP are under greater

energetic constraints than more southerly conspecifics. However, intraspecific differences in physiology occur in this species (Dunbar and Brigham 2010; Klüg-Baerwald and Brigham 2017; see also Chapter 5) and such metabolic adaptation of northern populations may ease energetic constraints. Discrepancies between measured and actual hibernaculum microclimate and T_{sk} will also negatively influence the accuracy of energy budget estimations; insulative properties of small roosts and social thermoregulation may lower energetic costs of warming, and euthermia (Boyles et al. 2008; Boyles and Brack 2009), and even slight changes in the values used for T_h (see Chapter 2) and T_{sk-tor} in my estimates have a large effect on E_{tor} calculations.

Despite the uncertainties inherent in my estimates of winter energy budgets, my data allow us to elucidate remarkable trends in the hibernation patterns of crevice-hibernating *E. fuscus* and to confirm disparate behavioural strategies between populations. Like most other hibernators, *E. fuscus* overwintering in buildings in Indiana spend a large proportion of energy on arousal events (Thomas et al. 1990; Heldmaier et al. 1993; Armitage et al. 2003; Dunbar and Tomasi 2006). Conversely, I found that crevice-hibernating bats in DPP atypically use most of their fat stores during steady-state torpor to maintain T_b a few degrees above T_h and even above that predicted based on measures of $T_{tor-min}$ in northern populations of this species (2°C; Dunbar and Brigham 2010). Although unexpected, this could be a behavioural response by this population to their particular hibernation environment. Hibernators should reduce torpor depth

and duration to mitigate the non-energetic costs associated with prolonged periods of low metabolic activity and T_b (Humphries et al. 2003; Boyles et al. 2007; Angilletta et al. 2010), which may be particularly important given the long (~150 d) hibernation season experienced by the bats in my study area. The trade-off in this population appears to be a decrease in the amount of energy spent during arousals. This appears to contradict the importance of arousals suggested by the hibernation patterns of other small hibernators but, as I argue above, it may be that whatever metabolic or physiological benefit there is to arousal and euthermia is being concentrated into a short period of time in this populations, and I suggest this is made possible by the high T_b and activity associated with flight.

In summary, a reduced sensitivity to environmental cues appears to be a general characteristic of *E. fuscus* (Brack and Twente 1985; Halsall et al. 2012), and maintenance of a diurnal rhythm to arousals, ostensibly due to the importance of winter activity outside of hibernacula, is also common to this species and many other small hibernators (e.g., French 1977; Körtner et al. 1998; Hope and Jones 2013). However, despite estimating similar overall energy requirements for hibernation by building- and crevice-hibernating populations of *E. fuscus*, I show that there are stark differences in their behavioural strategies. Whereas building-hibernating populations show a typical pattern of placing energetic emphasis on frequent, prolonged arousal bouts, bats in DPP spend the majority of their energy stores on maintaining higher than expected T_{sk} while torpid and arouse less often and for much shorter periods of time to compensate. This strategy likely mitigates

the non-energetic consequences of prolonged periods of low T_b that could be experienced inside cold, thermally variable rock-crevice hibernacula (Humphries et al. 2003). I suggest that flight expedites the benefits of arousals and is a critical component in the hibernation biology of this population of *E. fuscus*, which is supported by the fact that arousal timing and length are largely influenced by factors that facilitate flight. My data suggest the hibernation environment (i.e., roost, den, or burrow) is an important predictor of hibernation patterns and highlights the importance of future research on the behaviours of populations overwintering in noncavernous structures, such as trees, rock crevices, and buildings. Hibernators may be more flexible in their behaviour than previously thought, and display a spectrum of environment-dependent strategies across heterogeneous conditions.

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CHAPTER FIVE—HUNG OUT TO DRY: INTRASPECIFIC VARIATION IN WATER LOSS IN A HIBERNATING BAT

5.1 INTRODUCTION

Torpor allows individuals to lower their energetic demands during times of resource scarcity (Ruf and Geiser 2015). Metabolic rate (MR), and consequently body temperature (T_b), are reduced, resulting in suppression of biological processes and considerable energy savings (Ruf and Geiser 2015). Water loss is also lower during torpor relative to euthermia (e.g., Studier 1970; Muñoz-García et al. 2012a), and short-term torpor can be used as an adaptive strategy to avoid dehydration (Carpenter 1969). Rates of evaporative water loss (EWL) during torpor are reduced by over 90% from euthermic rates in some species (Morris et al. 1994; Webb et al. 1995). However, EWL persists and metabolically produced water often does not compensate for water lost through evaporation over the long term (Thomas and Cloutier 1992). Thus, torpor bouts are a period of negative water balance, and humidity is an important factor influencing patterns of small-mammal hibernation.

As humidity within or between hibernacula drops, the duration of torpor bouts shorten (Thomas and Geiser 1997) and arousal frequency increases (Ben-Hamo et al. 2013). In a similar vein, arousal rates also increase when hibernating animals are experimentally administered a diuretic (Németh et al. 2010). The

implications of the effects of EWL are increased energy use throughout hibernation and ultimately decreased survival (Thomas and Cloutier 1992; Thomas and Geiser 1997; Willis et al. 2011; Ehlman et al. 2013). All small mammals periodically arouse during hibernation; although the exact causes are unclear (Willis 1982), dehydration may stimulate the need to drink (Fisher and Manery 1967; Speakman and Racey 1989; Thomas and Geiser 1997). Given sufficient energy stores (i.e., fat deposits), dehydration may be more likely to be a cause of death during hibernation than starvation (Speakman and Racey 1989).

To mitigate the effects of low humidity and avoid dehydration, many small mammals hibernate in humid roosts, dens, or burrows (e.g., Webb et al. 1996; Speakman and Thomas 2003). The rate of evaporation is determined by difference in absolute water vapour pressure (WVP_{abs}) between a surface and the surrounding air (Δe_v ; Schmidt-Nielsen 1997). Convection and the temperature gradient between surface and air affect water vapour pressure and the rate of evaporation (Phillips 1966), but are likely negligible for animals thermoconforming in cool, still air typical of hibernacula (Webb et al. 1996). Thus, Δe_v and the rate of EWL are minimized in near saturated conditions (~100% RH). Hibernators that experience lower rates of EWL arouse less often and use less energy (i.e., fat) throughout winter, which increases their chance of survival (Thomas and Cloutier 1992; Thomas and Geiser 1997; Ben-Hamo et al. 2013).

Bats present a particularly interesting model for water balance during hibernation. Although ventilation is reduced and completely arrested for periods

of time (e.g., Thomas et al. 1990; Hays et al. 1991), bats have large lungs compared to birds and terrestrial mammals of similar size (Maina 2000) and likely experience higher rates of respiratory evaporative water loss (REWL). Additionally, the large surface area of the wings is a major avenue of cutaneous evaporative water loss (CEWL) in resting bats (Hosken and Withers 1997; Hosken and Withers 1999). On a wet mass basis, lipid oxidation produces more water than other fuel sources (Schmidt-Nielsen 1964), enough to keep larger, fully-furred hibernators, such as bears, hydrated throughout the winter (Nelson 1980). However, metabolically produced water does not compensate for water lost through evaporation in bats (Thomas and Cloutier 1992). Thus, bats likely face challenges in maintaining water balance not incurred by other hibernators. Even at a humidity slightly below that typical of cave hibernacula used by vespertilionid bats in North America ($\sim 100\%$ RH, $WVP_{\text{abs}} = 0.9$ kPa at 6°C ; Webb et al. 1996), energy savings are diminished and survival is impacted (Thomas and Cloutier 1992; Geiser and Broome 1993).

Despite the profound influence of humidity on hibernating bats, North American big brown bats (*Eptesicus fuscus*) hibernate in a wider variety of conditions and tolerate lower humidity during hibernation than most other bat hibernators (e.g., Beer and Richards 1956; Kurta and Baker 1990). Populations of *E. fuscus* in the Canadian prairies hibernate in rock-crevices that are drier and less thermally stable than most cave hibernacula (see Chapter 2). Water balance and the energy budgets of bats hibernating in the arid prairies appear different than

those in other habitats and the risk of dehydration may be elevated. Factors that increase arousal frequency ultimately decrease overwintering survival (Ehlman et al. 2013). Given that water balance is critical during hibernation, some bat populations that overwinter in arid, “suboptimal” conditions may have mechanisms to mitigate water loss that are not evident in populations that hibernate in more typical cavernous hibernacula with high humidity. My aim was to determine if *E. fuscus* that hibernate in prairie habitats show evidence of acclimatization or adaptation to dry conditions.

I compared torpid metabolic rate (TMR) and total evaporative water loss (TEWL) between two populations of bats with differing winter ecologies: one that overwinters in humid caves, and another that hibernates in arid rock-crevices. Given that both populations hibernate in similar thermal conditions, I hypothesized that there would be no difference in TMR between populations under similar conditions. However, given the differences in humidity in which each population typically hibernates, I hypothesized that crevice-hibernating bats would be particularly well adapted to an arid environment, and thus have lower rates of TEWL in dry conditions than those of cave-hibernating bats.

5.2 METHODS

5.2.1 Study Species

Eptesicus fuscus is a medium sized (mean mass = 18 g; van Zyll de Jong 1985), insectivorous bat (Family: Vespertilionidae) found throughout most of

Canada and the U.S., through Central America and parts of the Caribbean, and into northern South America (Kurta and Baker 1990). Its distribution includes a variety of habitats, including urban, desert, forest, and prairie. Its roosting ecology is highly variable, using buildings (Barbour and Davis 1969), trees (Kalcounis and Brigham 1998), and rock crevices (Lausen and Barclay 2002) during summer, and buildings (Whitaker and Gummer 1992; Halsall et al. 2012), tree cavities (Rainey et al. 1992), rock crevices (Lausen and Barclay 2006), and caves (Mills et al. 1975; Reimer et al. 2014) as hibernacula in the winter. The wide range and varied roosting ecology of *E. fuscus* make it an ideal species to assess differences in physiology among multiple populations across heterogeneous environments.

5.2.2 Study Sites

I sampled bats in Wood Buffalo National Park (WBNP) and Dinosaur Provincial Park (DPP), Alberta, Canada. Wood Buffalo National Park is located in northern Alberta and is comprised primarily of boreal forest with scattered wetlands and streams and some extensive karst formations (e.g., sinkholes and caves). Dinosaur Provincial Park is located along the Red Deer River in southern Alberta in a mixed landscape of prairie, badlands, and riparian habitat. It has a semiarid climate and contains an extensive network of creeks and drainages where *E. fuscus* overwinter in deep rock crevices (Lausen and Barclay 2006). I captured bats at Walk In Cave in WBNP (exact location withheld for confidentiality), a cavernous limestone hibernaculum used by *E. fuscus* and other species (Reimer et

al. 2014). Conditions within this cave during winter are more humid (ca. 100% RH, mean $WVP_{abs} (\pm S.D.) = 0.61 \pm 0.03$ kPa at a mean $T_a (\pm S.D.)$ of $0.0 \pm 0.63^\circ\text{C}$; Klüg-Baerwald, unpublished data) than those of rock-crevice hibernacula in DPP (ca. 52% RH, mean $WVP_{abs} (\pm S.D.) = 0.33 \pm 0.09$ kPa at a mean $T_a (\pm S.D.)$ of $0.6 \pm 0.91^\circ\text{C}$; see Chapter 2).

5.2.3 *Respirometry*

My study took place in mid- to late-September 2015, just prior to the hibernation season of *E. fuscus*, which typically begins mid-October in the WBNP region (Reimer et al. 2014) and late-October in DPP (Klüg-Baerwald et al. 2016; see also Chapter 3). I captured bats within 3 h of sunset in mist nets set across the entrance to Walk In Cave in WBNP, and over the Little Sandhill Creek in DPP. I held captured bats in cloth bags and took morphometric measurements (e.g., forearm length, mass). I recorded body mass (m_b) before and after metabolic trials and assumed a linear decrease in m_b during metabolic measurements for use in mass-specific calculations. I then placed bats in closed metabolic chambers and allowed them to acclimate to experimental conditions for at least 8 h to ensure they were torpid and post-absorptive before measurements. During this time, I set chamber temperature to 6°C and provided them with humidified air at a rate of 100 ml min^{-1} . This prevented disturbance of bats prior to trials, and acclimated them in a microclimate resembling that typically experienced by hibernating bats in North America (Webb et al. 1996), thus increasing the likelihood of inducing

torpor. I trimmed a small patch of hair from the interscapular region to within 1 mm of the skin and attached temperature sensitive dataloggers (iButton® model DS1921G; Maxim Semiconductors, Dallas, TX) to each bat using surgical adhesive (Skinbond®, Smith and Nephew United Inc., Largo, FL) to record skin temperature (T_{sk}) at 1-min intervals. Skin temperature provides a reliable estimate of T_b in small bodied insectivorous bats (Willis and Brigham 2003). I considered an individual to be in steady-state torpor if T_{sk} was within 3°C of the chamber temperature and whole animal oxygen consumption (V_{O_2}) was stable for ≥ 1 h prior to metabolic measurements (Willis and Brigham 2003).

For metabolic chambers, I used 250 ml glass jars lined with metal mesh to allow bats to hang comfortably during trials, and covered the bottom of each chamber with a layer of mineral oil to prevent evaporation of water from feces and urine. I used thermocouple probes (TC-2000; Sable Systems International, Las Vegas, Nevada, USA) in each metabolic chamber to monitor temperature and placed chambers in a temperature-controlled cabinet (12 V portable cooler-warmer; Koolatron Refrigeration, Brantford, ON) set to 6°C. I used Tygon tubing (Cole-Parmer, Montreal, QC) on all incurrent lines and Bev-A-Line tubing (Cole-Parmer) on all excurrent lines (Lighton 2008).

I used flow-through respirometry to measure oxygen consumption and determined total evaporative water loss (TEWL; cutaneous and evaporative) based on the difference in water vapour density between the incurrent and excurrent airstreams. I first measured MR and TEWL of bats exposed to relatively high

humidity. To humidify incurrent air, I passed air at room temperature ($\sim 20^{\circ}\text{C}$) through a bubbler made from a 500 ml glass jar filled with distilled water with an aquarium stone mounted on the inlet tubing. I then used a dew point generator and RH controller (DG-3; Sable Systems International) set to provide a saturated incurrent airstream at a dew point of 2°C (~ 0.7 kPa). This level of humidity was not high enough for water vapour to condense inside the tubing and chambers. For low-humidity trials, I bypassed the bubbler and dew point generator, and instead used desiccant (Drierite; W. A. Hammond Drierite Co. Ltd., Xenia, OH) to remove water vapour from incurrent air. From there, I used a subsampler (SS-4; Sable Systems International) and factory calibrated flow controllers (MFC-2; Sable Systems International) to push air at a precisely controlled rate (100 ml min^{-1}) into each of four metabolic chambers.

I used a water vapour analyzer (RH-300; Sable Systems International) to measure water vapour density (WVD; mg ml^{-1}) of baseline air and excurrent air from the animal chambers. I then passed air through soda lime (to remove carbon dioxide) and Drierite (W. A. Hammond Drierite Co. Ltd.) before measuring oxygen concentration (%) with a factory calibrated oxygen analyzer (FC-10A; Sable Systems International). I sampled each animal for 15 min, with 5-min baselines before and after each chamber, and ran three consecutive sets of high-humidity trials. Following high-humidity trials, I allowed animals to acclimate to dry-air conditions for 3 h before beginning measurements for low-humidity trials. Again, I sampled each animal for 15 min, with 5-min baselines before and after each

chamber, and ran three consecutive sets of low-humidity trials. I recorded data at a rate of 1 Hz, discarded the first 2.5 min of data of each trial to account for system washout between samples, and later used ExpeData (ver. 1.8.5; Sable Systems International) to correct for drift and lag. I gave bats water *ad libitum* immediately after completion of the experiment and before they were released at the site of capture.

To calculate torpid metabolic rates (TMR; mW) of bats in steady-state torpor, I first calculated whole animal oxygen consumption (V_{O_2} ; ml O_2 min⁻¹) of each trial for each individual using the equation of Withers (2001):

$$V_{O_2} = V_I(F_I O_2 - F_E O_2)(1 - F_E O_2)^{-1},$$

where V_I is incurrent airflow (ml min⁻¹) and $F_I O_2$ and $F_E O_2$ are fractional O_2 composition for incurrent and excurrent air, respectively. I then multiplied these values by 60 to calculate hourly metabolic rates and used a conversion factor of 0.179 ml O_2 h⁻¹ per mW (Willis et al. 2005). I used the trial with the lowest mean TMR for each individual at each humidity treatment in my analyses.

I calculated whole animal total evaporative water loss (TEWL; mg H_2O min⁻¹), which includes respiratory and cutaneous EWL, as the difference in water vapour density between incurrent and excurrent air streams following equation 10.9 of Lighton (2008):

$$TEWL = FR_i(F_e H_2O - F_i H_2O)(1 - F_e H_2O)^{-1},$$

where FR_i is incurrent airflow (ml min⁻¹ STP), and $F_e H_2O$ and $F_i H_2O$ are fractional water vapour concentration (mg ml⁻¹) in excurrent and incurrent air, respectively.

I then multiplied these values by 60 to derive hourly rates of TEWL. For analyses, I used rate of TEWL recorded during the trial that corresponded with the lowest mean TMR trial for each individual at each humidity treatment, as described above.

5.2.4 Statistical Analyses

I tested all data for normality using Shapiro-Wilk tests, as well as for homogeneity of variance between statistically compared groups using Bartlett tests. I used a Wilcoxon rank sum test to compare m_b between populations because these data did not fit a normal distribution. I used Welch's two sample t-tests to compare excurrent WVP_{abs} between populations within humidity treatments. The rate of EWL is negatively correlated with humidity of the microclimate surrounding the animal (i.e., WVP within the respirometry chambers; Thomas and Cloutier 1992). I found no difference in excurrent WVP between populations during high- and low-humidity treatments so I treated it as a categorical variable in further analyses. An analysis of variance (ANOVA) showed no significant variation in T_{sk} or chamber temperature across trials, so I excluded this variable from further analyses. To compare TMR and TEWL between populations (WBNP and DPP) in humid and dry air, I used generalized linear mixed models (GLMM) with m_b as a covariate, population and humidity as fixed effects, and individual as a random effect to account for the repeated measures design of my experiment. I did not remove any terms from my models given the

importance of all independent variables and interactions to the hypotheses being tested. I conducted all statistical analyses using R (R Development Core Team 2016) and used an α -value of 0.05 to assess significance. I present all data as means \pm S.D.

5.3 RESULTS

I captured 15 male *E. fuscus* in WBNP and 12 males in DPP. Of these, 20 individuals ($N_{\text{WBNP}} = 10$, $N_{\text{DPP}} = 10$) entered steady-state torpor within 6 h of being placed in the metabolic chambers and remained torpid during all metabolic measurements; I included only these individuals in my analyses. Body masses differed between populations ($W = 78$, $P = 0.035$); bats from DPP had higher m_b ($\bar{X} = 20.2 \pm 0.85$ g) than those from WBNP ($\bar{X} = 19.1 \pm 1.25$ g). Excurrent WVP did not vary between populations during high humidity ($t_{17.7} = 0.22$, $P = 0.828$) or low humidity ($t_{17.8} = 0.04$, $P = 0.9705$) treatments. Mean excurrent WVP was 0.78 ± 0.043 kPa for the high humidity trials and 0.07 ± 0.012 kPa for low humidity trials. Mean temperature inside metabolic chambers did not vary between treatments or populations ($F_{3,36} = 0.23$, $P = 0.854$) and was $6.3 \pm 0.15^\circ\text{C}$ during trials used in TMR and TEWL analyses. Mean T_{sk} of bats recorded during analyzed trials was $7.4 \pm 0.92^\circ\text{C}$ and did not vary with treatment and population ($F_{3,36} = 2.11$, $P = 0.105$).

Torpid metabolic rate (TMR) did not differ between bats measured in humid or dry air ($t_{18} = 1.08$, $P = 0.297$) or between populations ($t_{17} = 1.03$, $P = 0.319$; Table 5.1; Figure 5.1). Mean whole animal TMR of DPP bats was 4.2 ± 1.70 mW in

Table 5.1. Results of the generalized linear mixed model assessing the variation in whole animal torpid metabolic rate (TMR) of *Eptesicus fuscus* from Walk In hibernacula (Wood Buffalo region) and Dinosaur Provincial Park, Alberta measured in dry or humid air.

Model Term	Estimate	S.E.	d.f.	t-value	P-value
(Intercept)	-1.20	6.98	18	-0.17	0.865
Humidity	1.05	0.97	18	1.08	0.297
Population	1.08	1.06	17	1.03	0.319
Mass	0.26	0.34	17	0.77	0.451
Humidity*Population	-0.38	1.38	18	-0.27	0.787

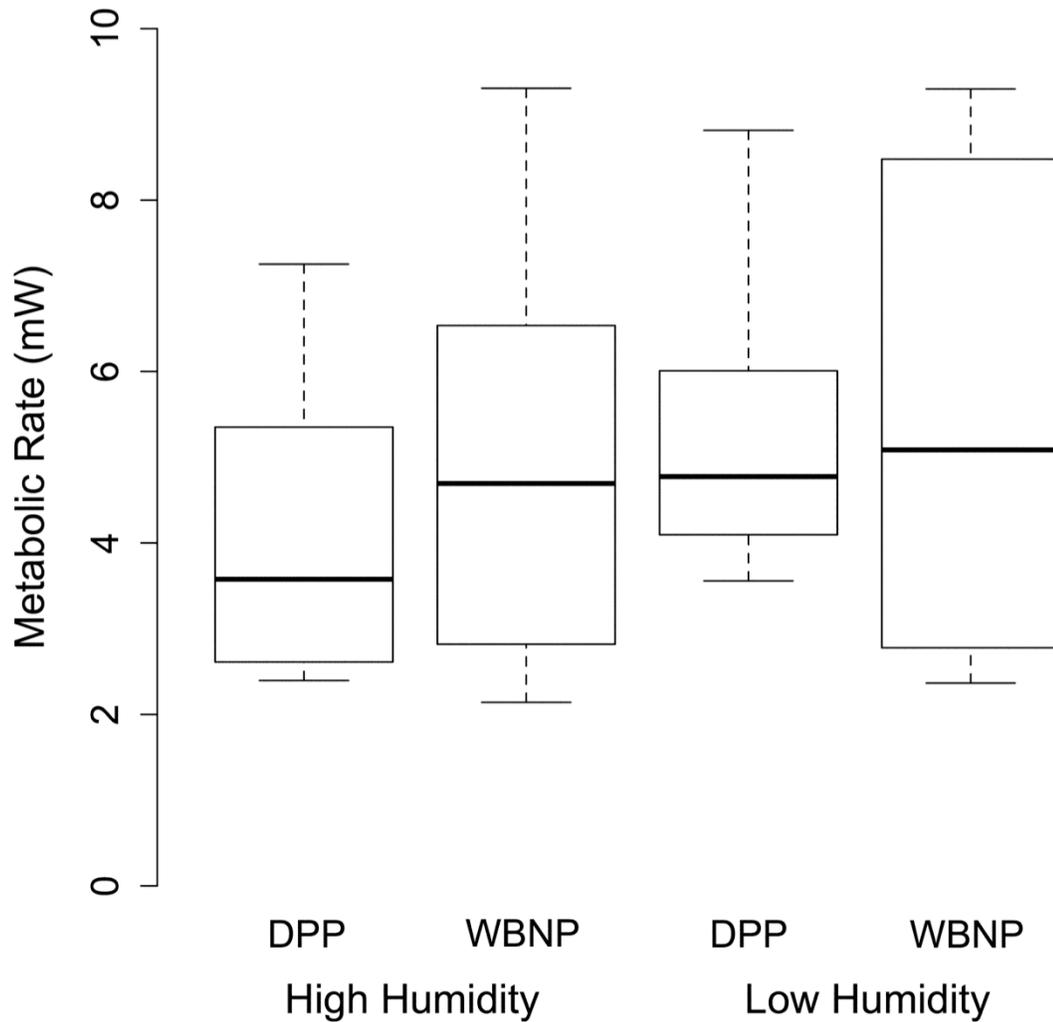


Figure 5.1. Boxplot of whole animal metabolic rates of torpid *Eptesicus fuscus* from Dinosaur Provincial Park ($N_{\text{DPP}} = 10$) and Wood Buffalo National Park ($N_{\text{WBNP}} = 10$) exposed to high humidity and low humidity conditions. Bold bars represent the median, upper and lower box limits are the 75th and 25th quartiles, respectively, and whiskers extend to the minimum and maximum values of each group.

humid air and 5.2 ± 1.58 mW in dry air. Mean whole animal TMR of WBNP bats was 4.9 ± 2.22 mW in humid air and 5.6 ± 2.91 mW in dry air. Conversely, rates of total evaporative water loss (TEWL) differed with humidity ($t_{18} = 3.33$, $P = 0.004$), and the interaction between humidity and population was significant ($t_{18} = 6.55$, $P < 0.001$; Table 5.2; Figure 5.2). For both WBNP and DPP populations, whole animal TEWL was higher in dry air ($\bar{X}_{\text{WBNP}} = 15.9 \pm 4.74$ mg H₂O h⁻¹, $\bar{X}_{\text{DPP}} = 4.8 \pm 1.27$ mg H₂O h⁻¹) than in humid air ($\bar{X}_{\text{WBNP}} = 1.4 \pm 0.72$ mg H₂O h⁻¹, $\bar{X}_{\text{DPP}} = 1.0 \pm 1.03$ mg H₂O h⁻¹). Mean whole animal TEWL of WBNP bats was approximately 3.3-fold higher than that of DPP bats in low humidity.

5.4 DISCUSSION

My data support the hypothesis that bats hibernating in the prairies are particularly well adapted to an arid environment, and thus have lower rates of evaporative water loss in dry conditions than bats from a more humid habitat. As expected, I found that the rate of total evaporative water loss (TEWL) of bats measured in dry air was higher than that of bats measured in humid conditions and, more importantly, the rate of TEWL did not differ between populations in humid air, but was approximately 3.3-fold higher in bats from the more mesic environment of Wood Buffalo National Park than in bats from the arid habitat of Dinosaur Provincial Park. Furthermore, I did not find differences in torpid metabolic rate between populations or between humidity treatments. This suggests that cutaneous evaporative water loss (CEWL) is important for the

Table 5.2. Results of the generalized linear mixed model assessing the variation in the rate of whole animal total evaporative water loss (TEWL) of *Eptesicus fuscus* from Walk In hibernacula (Wood Buffalo region) and Dinosaur Provincial Park, Alberta measured in dry or humid air.

Model Term	Estimate	S.E.	d.f.	t-value	P-value
(Intercept)	3.46	8.22	18	0.42	0.679
Humidity	3.81	1.15	18	3.33	0.004
Population	0.32	1.24	17	0.26	0.802
Mass	-0.12	0.40	17	-0.30	0.765
Humidity*Population	10.61	1.62	18	6.55	<0.001

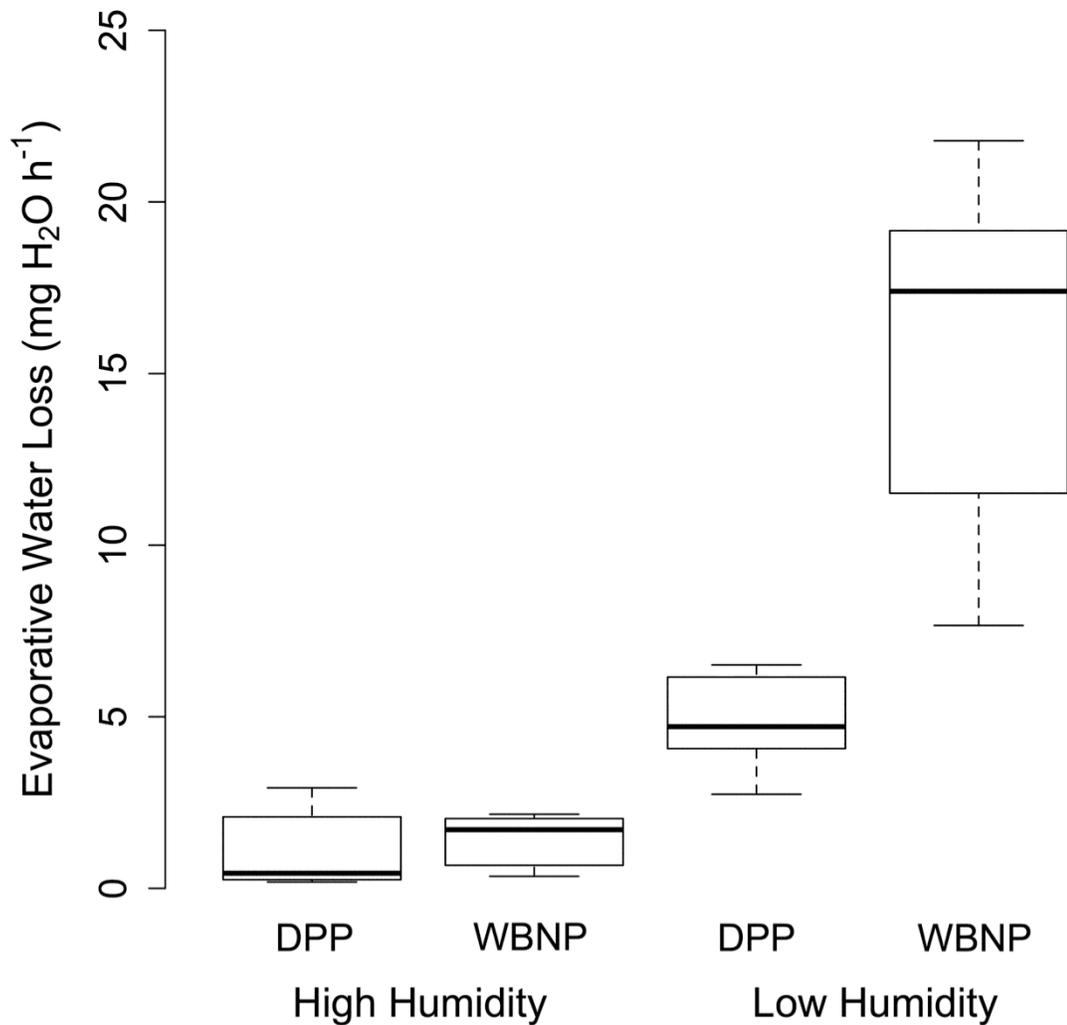


Figure 5.2. Boxplot of whole animal total evaporative water loss (TEWL; respiratory and cutaneous) of torpid *Eptesicus fuscus* from Dinosaur Provincial Park ($N_{DPP} = 10$) and Wood Buffalo National Park ($N_{WBNP} = 10$) exposed to high humidity and low humidity conditions. Bold bars represent the median, upper and lower box limits are the 75th and 25th quartiles, respectively, and whiskers extend to the minimum and maximum values of each group.

relationship between humidity and TEWL, with a lesser influence of variation in metabolism and respiratory evaporative water loss (REWL).

Small mammals use behavioral strategies to decrease EWL during hibernation. Clustering decreases the energetic cost of arousals (Roverud and Chappell 1991; Boyles et al. 2008) and reduces EWL (e.g., Boratyński et al. 2015; but see Proctor and Studier 1970; Studier 1970), presumably because it reduces exposed surface area (McNab 1969). Selection of humid hibernacula also mitigates water loss by minimizing the difference in water vapour pressure between air and skin (Schmidt-Nielsen 1997). Although many species of bats huddle in large groups during hibernation, *E. fuscus* is more commonly found solitarily or in small groups of less than 20 individuals (e.g., Phillips 1966), which likely limits the importance of huddling for water conservation. Furthermore, as evident in the population from DPP, *E. fuscus* commonly chooses hibernacula with variable and relatively low humidity compared to many other species of hibernating bat (Webb et al. 1996).

Small mammals also have physiological mechanisms to decrease EWL during hibernation. Although determining the specific mechanism of water retention is beyond the scope of this study, my data suggest a reduction in the rate of CEWL has a primary role. The rate of CEWL is largely determined by the composition of the *stratum corneum* (SC), the outermost layer of the dermis (Bouwstra et al. 2003). Higher proportions of waxy lipids (cerebrosides and ceramides), and fewer free fatty acids and triacylglycerols, result in lower rates of

CEWL (Haugen et al. 2003b). Indeed, greater amounts of cerebroside and ceramides are found in birds (Muñoz-Garcia and Williams 2005; Champagne et al. 2012; Clement et al. 2012) and bats (Muñoz-Garcia et al. 2012a) from arid environments than are found in conspecifics from more mesic habitats. The role of SC composition in reducing water loss during hibernation remains largely unexplored.

The lipid profile of bat integument is not well described, but recent evidence suggests that the SC of epidermal wing tissue in some species of bat is comprised mostly of sphingomyelin (SM; Pannkuk et al. 2015). Breakdown of SM produces ceramide (van Smeden et al. 2014), thus regulation of this process may be involved in altering ceramide concentration of the SC and water permeability of the skin. There may also be a link between polyunsaturated fatty acids (PUFA) and CEWL. Increased consumption of dietary PUFA and a shift to higher proportions of n-6 fatty acids, particularly linoleic acid, facilitates entrance into torpor and lengthens torpor bouts during hibernation (Geiser and Kenagy 1987; Geiser 1991; Frank 1992). Although maintenance of cell membrane function at low temperature and reduction of metabolism are the ostensible benefits of high n-6 to n-3 PUFA ratios (Ruf and Arnold 2008), linoleic acid is also associated with ceramides and skin barrier function (Bowser et al. 1985). Deficiencies in ceramides or fatty acids within the SC result in increased transepidermal water loss (Menon et al. 2012). Determining the specific roles and interactions of lipids and fatty acids in permeability of wing membranes is an important area of future research.

Ventilation patterns during hibernation may also contribute to water conservation. Evaporative water loss from pulmonary structures (e.g., tracheal and alveolar surfaces) is reduced with decreased ventilation rates (Milsom and Jackson 2011). Additionally, episodic breathing allows some small hibernators to completely close the epiglottis or glottis during apneic periods, further reducing REWL while passively allowing sufficient gas exchange (e.g., Wilz et al. 2000). In some species, the respiratory tract remains open during hibernation only in high humidity (Thomas et al. 1990; Hays et al. 1991; Szewczak and Jackson 1992), which suggests an influence of REWL on the closing of the epiglottis or glottis to conserve water. Apneic periods are common in small hibernators (Milsom and Jackson 2011) and have been observed in hibernating *E. fuscus* (Szewczak and Jackson 1992).

Given that torpid big brown bats breathe approximately every 6.5 min at 5°C (Szewczak and Jackson 1992), I expected to observe some sign of episodic breathing. However, I did not observe any evidence of this phenomenon (excurrent O₂ and water vapour pressure were constant within trials, as well as between the 3 trails taken for each individual). Further, sampling of individuals during apneic periods, thus measuring passive diffusion of O₂ into the lungs, would underestimate metabolic rates by ~35-54% (Szewczak and Jackson 1992). My values for TMR recorded at ~6°C (4.9 mW) are realistic given those recorded for this species at a temperature of 0-5°C (2.3 mW; Willis et al. 2005). Other studies on hibernating *E. fuscus* also do not report apneic periods during sampling

(Willis et al. 2005; Dunbar and Brigham 2010). Given the conflicting evidence for episodic breathing of hibernating *E. fuscus* despite the obvious benefit of apnea in reducing REWL, the potential for intraspecific variation in ventilation patterns and physiology to contribute to drought tolerance warrants further study.

Efficient mechanisms for water conservation are not unique to the population of *E. fuscus* from DPP. In fact, my measurements of TEWL are similar to those measured in desert conspecifics from populations in Arizona and California (Carpenter 1969). Thus, drought tolerance may be widespread in this species and may facilitate the sedentary nature of *E. fuscus*. Limited availability of appropriate overwintering habitat likely imposes constraints on the range of many temperate-zone species of bat. Seasonal, long-distance movements from summer breeding grounds to humid, thermally-stable subterranean (i.e., cave or mine) hibernacula (e.g., Norquay et al. 2013) or locations where winters are milder (e.g., Bisson et al. 2009; Cryan et al. 2014) are common. However, *E. fuscus* is generally thought to move only tens of kilometres between summering and wintering grounds (e.g., Beer 1955; Goehring 1972; Mills et al. 1975). Much of the habitat within the range of *E. fuscus* is non-mountainous, thus extensive cave systems are unavailable and roosts occur mostly in rock crevices, trees, and buildings (Kurta and Baker 1990). That *E. fuscus* is able to use such ubiquitous features as hibernacula, despite drier conditions within, may explain how it persists as one of the most common, widely distributed species of bat in North America without the need for seasonal, long distance movements.

Resilience of a population to changes in its environment depends on the physiological responses of individuals (Canale and Henry 2010). Acclimatization to dry conditions occurs within weeks of exposure in some species of bird, such as hoopoe larks (*Alaemon alaudipes*; (Haugen et al. 2003a) and house sparrows (*Passer domesticus*; (Muñoz-Garcia et al. 2008). Thus, individuals in these populations are likely to tolerate more variable conditions of humidity than those with less plastic physiological responses. Although I show *E. fuscus* can adapt to hibernate in arid environments, whether this is a result of acclimatization (i.e., phenotypic plasticity) or evolutionary adaptation (i.e., natural selection) remains unanswered. If the low rates of EWL I observed in *E. fuscus* are associated with phenotypic plasticity, individuals of this species may be less susceptible to novel threats or challenges that disrupt water balance during hibernation, such as increasingly arid conditions associated with climate change, or pathophysiologies associated with disease.

Dehydration may play a significant role in mortality from white-nose syndrome (WNS), an invasive fungal disease that has caused the deaths of over 6 million bats in North America (US Fish and Wildlife Service 2016). Bats die during winter while hibernating in cold, damp caves and mines, conditions under which the causative agent of WNS, *Pseudogymnoascus destructans* (*Pd*), grows best (Verant et al. 2012). Disrupted wing physiology caused by *Pd* infection leads to electrolyte imbalance and possible hypotonic dehydration (Cryan et al. 2013; Warnecke et al. 2013). Any mechanism to mitigate water loss during hibernation is

likely to be advantageous given the pathology of WNS. Recent research even suggests the unique fatty acid composition of *E. fuscus* wing epidermis may even inhibit fungal growth (Frank et al. 2016). In addition, microclimates of hibernacula in the prairies are drier and colder than those of known cave hibernacula (Lausen and Barclay 2006; see also Chapter 2) and outside the optimal growing conditions of *Pd* (Langwig et al. 2012). Bat populations that are able to hibernate in these colder, drier conditions may experience decreased WNS-related mortality.

In summary, my data support the hypothesis that bats overwintering in the prairies are well adapted to survive dry conditions, and experience lower rates of evaporative water loss than conspecific from more mesic environments. Drought tolerance is likely key in determining the range of conditions and habitats a species can inhabit successfully and may help predict the ability of a species to adapt to changes in climate or pathological threats that may alter their hibernation physiology. I also provide clear evidence of intraspecific differences in physiology between populations. Most energetic models based on physiological parameters sample individuals from a single population and do not account for the physiological differences associated with habitat or latitude (e.g., Humphries et al. 2002; but see Dunbar and Brigham 2010). Given that latitude, habitat, and possibly even microclimate can influence physiology, conclusions based on geographically restricted samples may not accurately represent the entire species. In general, more research is needed on intraspecific differences in physiology

across heterogeneous habitats, and attempts to model energetics or survivorship based on physiological parameters should consider plasticity in these metrics.

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CHAPTER SIX—MEET YOU AT THE LOCAL WATERING HOLE?
DEHYDRATION AND DRINKING DURING WINTER FLIGHT BY HIBERNATING
BATS IN THE PRAIRIES

6.1 INTRODUCTION

Hibernation allows animals to survive prolonged periods of food shortage by greatly reducing energy expenditure (Ruf and Geiser 2015). Despite the obvious energetic benefits, there are nonenergetic costs to hibernation that lead to periodic arousals (Humphries et al. 2003). Although the exact reasons remain poorly understood (Willis 1982; French 1985). Access to food or water has been suggested. Arousal patterns facilitate foraging for some small mammals overwintering in areas with mild winters (e.g., French 1977; Körtner et al. 1998). Some hibernators may exit their den, burrow, or roost to find water (Thomas and Geiser 1997) because metabolically produced water does not fully compensate for evaporative water loss during hibernation (Thomas and Cloutier 1992).

The need to periodically drink during hibernation may be exacerbated by the microclimate of the hibernacula (temperature and humidity). In bats, the combination of relatively large lungs compared to birds and terrestrial mammals of similar size (Maina 2000) and large surface area of the wings results in high rates of respiratory evaporative water loss (e.g., Kurta et al. 1989; Hosken and Withers 1997; 1999). Potential hibernacula for bats in the prairies (e.g., rock

crevices) are small, dry, and thermally variable (see Chapter 2) compared to most known cavernous hibernacula (Webb et al. 1996; Perry 2012), and these hibernation conditions may increase the risk of dehydration. Common winter flight by prairie bat-populations despite the unavailability of insect prey (Lausen and Barclay 2006) leads to the hypothesis that these individuals may fly in search of water.

Arid-adapted species rely on dietary water intake and physiological or behavioural adaptations, but often still experience water deficits (McKechnie and Wolf 2010). Artificial provision of water is often used as a management tool for wildlife and livestock in many arid regions (Rosenstock et al. 1999). A common technique to track the use of these structures involves the analysis of stable isotope ratios. Consumption of water with experimentally enriched concentrations of deuterium (^2H)—a stable isotope of hydrogen—have been used to track water use by wildlife and through trophic levels (e.g. McKechnie et al. 2004; McCluney and Sabo 2010). Comparison of the $\delta^2\text{H}$ in an individual's tissues relative to that of their isoscape (all potential foods or drinking sources) allows for the approximation of the relative contribution of each source (Ben-David and Flaherty 2012).

I proposed that prairie bats experience increased evaporative water loss and make frequent mid-winter flights to find water. I thus predicted that bats captured in mid-winter flight would exhibit physiological signs of dehydration, such as increased serum ion concentrations and elevated hematocrit. I provided a

heated water tank as an open source of water all winter and hypothesized that bats would preferentially drink from this structure relative to other sources of water, such as the creek, river, or melt water. I enriched the tank water with ^2H and used stable isotope analysis to look for evidence of elevated $\delta^2\text{H}$ in the blood of bats captured in winter. To supplement the isotope analysis, I also used passive acoustic monitoring, video surveillance, and passive integrated transponder (PIT) tags to determine if bats visited the heated water tank.

6.2 METHODS

6.2.1 Study site and sampling

My study took place in Dinosaur Provincial Park (DPP), Alberta, Canada ($50^{\circ}45'09''\text{N}$, $111^{\circ}31'03''\text{W}$) during October through March of 2012–15. The park is comprised of riparian and prairie habitat with an extensive network of creeks and drainages, and has a semi-arid climate (Bailey 1979). Three species—the big brown bat (*Eptesicus fuscus*), Western small-footed myotis (*Myotis ciliolabrum*), and long-eared myotis (*M. evotis*)—are known to overwinter within the park and are active during the winter when the weather is favourable (Lausen and Barclay 2006; Klüg-Baerwald et al. 2016; see also Chapter 3). In the summer of 2008, I installed a modified hot tub (~1700 l, 2 m in diameter) as a heated, open water source specifically designed for use by bats (Figure 6.1). The tank was located ~500 m from the three known rock-crevice hibernacula in the park (see Chapter 2) and ~25 m from the Little Sandhill Creek, which is the only permanent creek in the



Figure 6.1. Photograph of the heated water tank installed in Dinosaur Provincial Park from 2008–15 as a continuously accessible source of water all winter. Tank water was treated with deuterium (^2H) and maintained at $\delta^2\text{H}$ levels of +400–925‰.

park and the only location where bats can be reliably captured in mist nets during winter. I captured bats in mist nets set across this creek and recorded sex, age, and morphometric measurements (e.g., mass and forearm length).

I collected 50–75 μl of blood from the interfemoral vein of each *E. fuscus*. I used ~ 25 μl in a handheld blood gas analyzer (iStat[®]; Abaxis North America, Union City, California) to analyze Na^+ , K^+ , and Cl^- concentrations (mmol/l) and hematocrit (Hct; % packed cell volume [PCV]). I transferred the remaining portion (~ 50 μl) to a 200 μl o-ring microcentrifuge tube filled with 95% ethanol (K. Hobson, personal communications) and kept it frozen until analysis at the University of Regina. Before release, I permanently marked all *E. fuscus* with a 0.1 g passive integrated transponder (PIT) tag (Trovan ID100 nanotransponders; EIDAP Inc., Sherwood Park, Alberta) injected under the skin of the lower back. After tagging, I used tissue adhesive (Vetbond[™]; 3M Canada, London, Ontario) to close the injection site and then monitored each bat during the next hour for signs of distress or injury and to ensure proper insertion of the PIT tag.

The University of Regina President's Committee on Animal Care approved all methods and procedures (Animal Use Protocol #12-12), which conformed to the guidelines for animal care and use outlined by the American Society of Mammalogists (Sikes 2016). I performed fieldwork under research and collection permits issued by Alberta Sustainable Resource Development and Alberta Tourism, Parks and Recreation Division.

6.2.2 Stable isotope analysis

At my study site, ^2H occurs naturally in precipitation at an annual mean $\delta^2\text{H}$ of -84‰ Vienna Standard Mean Ocean Water (VSMOW; Bowen and Revenaugh 2003). To trace the use by bats of the supplied water source (i.e., heated water tank) by bats, I treated the tank water with small amounts (100–200 ml) of enriched (99.8%) deuterium oxide ($^2\text{H}_2\text{O}$). I took a 100 ml sample of water from the tank every month from October through April, as well as other potential sources of drinking water (i.e., snow and river water). I stored water samples in airtight collection vials at cool (but above freezing) temperatures and blood samples at subzero temperatures to minimize ^2H exchange between water and the storage solution (i.e., kinetic and equilibrium fractionation, respectively; Ben-David and Flaherty 2012; K. Hobson pers. comm.).

I transported samples on ice to the Institute of Environmental Change and Society (ICES) at the University of Regina for analysis. Prior to analysis, I freeze-dried blood samples. I performed stable isotope analyses on a Thermo Finnigan Delta plus XL isotope ratio mass spectrometer (precision: $\pm 0.5\text{‰}$; Thermo Electron Corporation Canada, Gormley, Ontario) that was coupled to a conversion/elemental analyzer (TC/EA). I standardized isotopic values of blood to Kudu Horn Standard (KHS) and Caribou Hoof Standard (CBS), and those of water to Picarro standards Zero, Mid, High, and DI water. I report all isotope results in delta notation in permil (‰) representing:

$$\delta^{j/i}\text{X} = [(j\text{X}/i\text{X})_{\text{sample}} / (j\text{X}/i\text{X})_{\text{standard}}] - 1,$$

where R is the ratio of heavy to light isotopes in the sample and standard, respectively (Bond and Hobson 2012).

6.2.3 Acoustic, video, and PIT-tag monitoring

I used an Anabat (Titley Electronics, Ballina, NSW, Australia) detector to record acoustic bat activity at the water tank every night from an hour before sunset to an hour after sunrise. I housed the detector in a custom built waterproof box mounted on the side of the tank and powered with a 12 V, 12 Ah sealed lead-acid battery coupled to a 10 W solar panel. I calibrated the detector (see Larson and Hayes 2000) to recorded activity only in a small volume of space directly above the water. I used AnalookW software (version 3.9c; C. Corben, Columbia, MS, USA) with a custom filter to separate background noise (e.g., insects and wind) from bat calls (for details see Lausen et al. 2014). I used the presence of “feeding buzzes” (segments of rapidly produced short duration pulses; (Fenton 2003) to indicate bats possibly drinking from the tank. On nights when I anticipated high bat activity (Klüg-Baerwald et al. 2016; see also Chapter 3), I also used a handheld camcorder with high definition night vision capability (HDR-CX730; Sony Corp., Tokyo, Japan) to record activity at the tank during the first 4–5 h after sunset. I also used a PIT-tag decoder (Trovan 650, EIDAP Inc.) connected to a waterproof plate antenna (IP68; EIDAP Inc.) placed just below (~2 cm) the surface of the water to record the time and date when any PIT-tagged *E. fuscus* came within range of the antennae (~5cm).

6.2.4 Statistical analyses

I used linear models to assess the influence of hibernation date (i.e., days since onset of hibernation; see Klüg-Baerwald et al. 2016 and also Chapter 3 for specific dates) on serum electrolyte concentrations (Na^+ , K^+ , Cl^-) and hematocrit (Hct). I also used linear models to produce baseline regressions with 95% confidence intervals (CI) of $\delta^2\text{H}$ of surface and tank water over the course of the sampling period (October – March). I compared $\delta^2\text{H}$ of blood sampled from bats during winter to the values from surface and tank water, and considered any points above the 95% CI of surface water as indicative that those bats had consumed at least some water from the tank. I conducted all statistical analyses using R (R Development Core Team 2016) and present all data as means \pm *S.D.*

6.3 RESULTS

I captured 102 *E. fuscus* during the 3 years of my study and PIT-tagged each one. I collected 31 blood samples for serum electrolyte and hematocrit analysis, and 54 blood samples for stable isotope analysis. Hibernation date was positively correlated with Hct ($t = 3.12$, $P = 0.004$; Figure 6.2) and the serum electrolyte concentration of Na^+ ($t = 4.52$, $P < 0.001$) and Cl^- ($t = 5.60$, $P < 0.001$), but not K^+ ($t = -0.109$, $P = 0.914$). From pre-hibernation (i.e., October) to late hibernation (i.e., February and March), mean Hct rose from 0.53 ± 0.05 to $0.58 \pm 0.03\%$, mean Na^+ concentration rose from 147 ± 4.3 to 158 ± 7.0 mmol/ml, and mean Cl^- concentration rose from 121 ± 3.9 to 134 ± 6.8 mmol/ml. Mean $\delta^2\text{H}$ of blood samples taken from bats ($\delta^2\text{H}_{\text{bat}}$) was -157 ± 7.7 ‰ VSMOW. All $\delta^2\text{H}_{\text{bat}}$ data

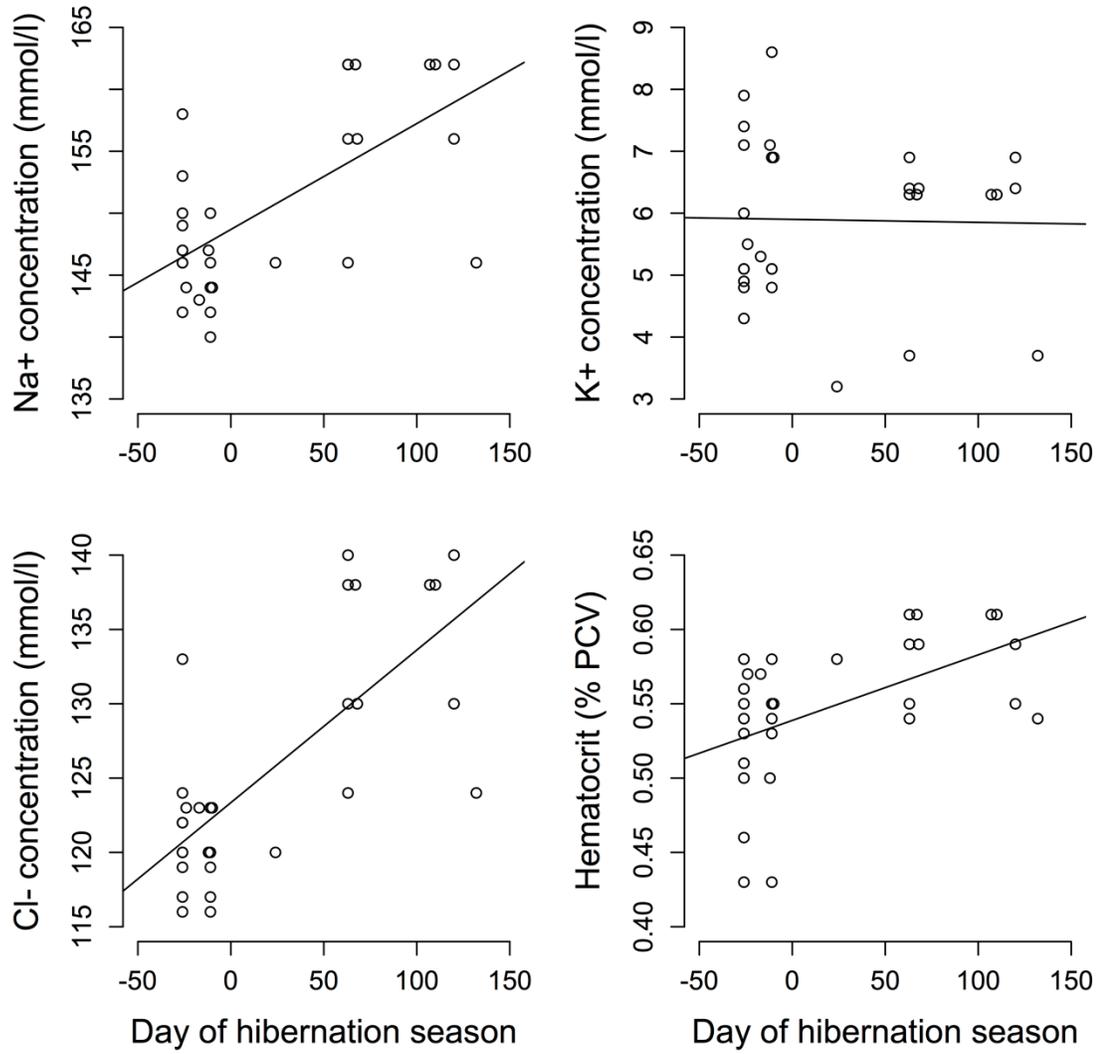


Figure 6.2. Serum electrolyte concentrations and hematocrit of *Eptesicus fuscus* captured mid-flight in Dinosaur Provincial Park, Alberta during the winters of 2012–15.

points fell within below the 95% CI of surface water $\delta^2\text{H}$ (mean $\delta^2\text{H}_{\text{surf}} = -143 \pm 33.6$ ‰ VSMOW; Figure 6.3), considerably lower than that of the water tank (mean $\delta^2\text{H}_{\text{tank}} = 719 \pm 180.2$ ‰ VSMOW).

The acoustic detector recorded 33 bat echolocation calls over the surface of the water tank, but I did not identify any as a “feeding buzz”. In 41 h of video footage over 9 nights (4.5 ± 1.08 h night⁻¹), I did not see bats visit the tank. The PIT-tag reader did not record the presence of any tagged *E. fuscus* over the surface of the water tank.

6.4 DISCUSSION

Winter bat activity is of considerable interest given the emergence of white-nose syndrome as a threat to hibernating bats (Frick et al. 2015). Initially thought of as atypical, we now know that bats in many areas exhibit some activity throughout hibernation and mid-winter flights are not uncommon (Boyles et al. 2006). Although the exact reasons for winter activity remain unclear (Willis 1982), in arid areas such as the prairies, evaporative water loss and negative water balance may drive bats to arouse and seek water often. In such cases, the provision of water may become an important management tool for bat conservation. Other studies have documented the use of artificial water developments for a range of animals, including bats in arid environments (Adams and Hayes 2008), but ours is the first to investigate this in winter.

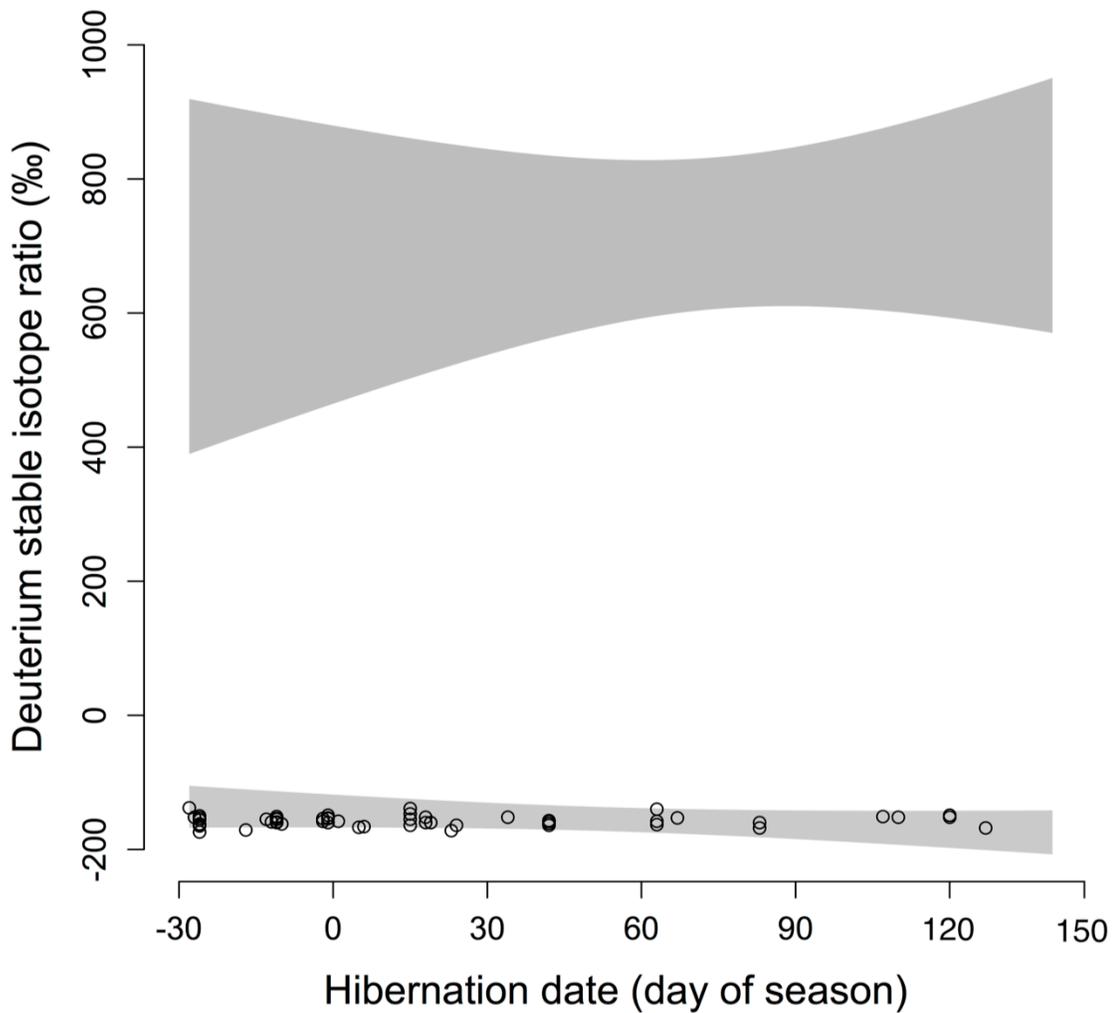


Figure 6.3. Plot of deuterium stable isotope ratios detected in the blood of *Eptesicus fuscus* ($\delta^2\text{H}_{\text{bat}}$; open circles) over time in Dinosaur Provincial Park, Alberta. Shaded areas represent 95% confidence intervals of $\delta^2\text{H}$ detected in surface water (snow and melt; light grey) and a heated water tank treated with 99.8% ^2H (dark grey). Data points lying above the 95% CI limit for surface water would suggest at least some use of the water tank by bats.

My data show hypertonic dehydration occurs in *E. fuscus* as hibernation progresses. Hematocrit and all serum electrolyte concentrations except K^+ increased from pre-hibernation (i.e., October) to late hibernation (i.e., February – March). Other studies have investigated hematological changes during hibernation with mixed results depending on species and depth of hibernation (Riedesel and Folk 1958). Both serum K^+ concentrations and Hct decrease within days of *E. fuscus* entering hibernation (Riedesel and Folk 1958) but no study has evaluated changes in blood composition of this species over the course of an entire hibernation period. Despite hibernating for <24 h and not likely being dehydrated, hematocrit and K^+ concentration were higher for post-hibernating little brown bats (*Myotis lucifugus*) than active pre-hibernating controls (Riedesel and Folk 1958). Thus observations of increased Hct and serum electrolyte concentrations may reflect a normal post-hibernation state of dehydration. It would be difficult to parse out the added effect of aridity on the hydration state of bats in my study area given the inconsistent results of other serum electrolyte studies and my limited dataset. The homeostasis of serum K^+ in the blood samples of bats is not entirely unexpected. Even bats experimentally infected with the fungus that causes white-nose syndrome (*Pseudogymnoascus destructans*) show little change in serum K^+ concentrations from healthy control groups, despite hypotonic dehydration and fluctuations in most other blood parameters (Warnecke et al. 2013).

Contrary to my prediction, I found no evidence for the use of the water tank by bats in DPP. Although I detected bats in the vicinity of the water tank, I did not identify any acoustic signs (i.e., feeding buzzes) indicating bats actually drank from tank. Further, I did not detect any PIT-tagged bats within range of the submersed antenna, nor did I collect any video evidence of visitation by bats to the water tank. Most conclusively, I did not find evidence of elevated $\delta^2\text{H}_{\text{bat}}$, which would be indisputable, direct evidence of water consumption. Even slight irregular drinking from the water tank should have been easily detected in blood samples given the large differences in $\delta^2\text{H}$ between the water tank (400–900‰ VSMOW) and ground water (annual mean level of -84‰ VSMOW). The lack of foraging in winter means δH of body tissues is likely not influenced by the contribution of dietary lipids and should reflect that of environmental water (Soto et al. 2013). Conversely, the low metabolic rate of hibernating bats (Geiser 2004) could result in the isotopic signature of consumed prey persisting in the blood for months after ingestion (Storm-Suke et al. 2012). The $\delta^2\text{H}$ values I detected likely reflect consumption of surface water or water released from fat stores amassed in the fall.

In summary, I found no evidence that bats in DPP used the water provided for them. Dehydration could still be the motivation behind bats arousing and leaving the hibernacula. Although the influence of arid conditions on dehydration is unclear, I did find increased levels of hypertonic dehydration in late winter. There are natural sources of water in the area that bats may drink from; the

weather occasionally warms enough to melt snow and produce puddles, and the creek and river occasionally experience superficial melts. Artificial sources can also be found across the landscape, such as heated cattle troughs, and there are reports of bats drinking from (and often falling into) water developments associated with agricultural use. However, bats have been reported to quickly find and exploit artificial water provisions (Adams and Hayes 2008). The water tank at my study site was functional for several years as a reliably open resource located close to the hibernacula during a time in which access to water is constrained. If bats are flying to find water in my study area, it seems unlikely that none would make use of this resource. Further research into the causes of winter bat-activity and the possible use of artificial water developments to mitigate dehydration is needed, especially as climate change and WNS add to the challenge of maintaining water balance faced by hibernating bats in some areas.

6.5 REFERENCES

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CHAPTER SEVEN—GENERAL CONCLUSION; OR BECAUSE THEY ARE BATS

7.1 SUMMARY AND SYNTHESIS

Hibernation is an effective strategy for energy conservation (Geiser 2004) and has other benefits (Geiser and Brigham 2012), but it is not without its costs and limitations. Nonenergetic costs associated with hypometabolism, low body-temperature, and inactivity include reduced molecular synthesis (Lillegraven et al. 1987), immuno-incompetence (Burton and Reichman 1999), dehydration (Thomas and Geiser 1997), neurological degeneration (Popov et al. 1992), and build up of harmful metabolites (Buzadžić et al. 1990). Furthermore, conditions within hibernacula have a profound influence on hibernation patterns (torpid skin temperature, torpor bout length, and arousal frequency; (French 1985) and survival (Boyles and Brack 2009). Periodic arousals and site selection are thought to mitigate these costs. For many hibernators, this involves overwintering in locations with stable temperatures and high humidity (Webb et al. 1996; Perry 2012), and timing arousals and activity to ensure efficient use of finite energy reserves to maximize the likelihood of survival (Boyles and Brack 2009).

I studied the hibernation biology of a population of bats that superficially do not appear to fit the archetype of well-studied rodents or cave-hibernating bats. Big brown bats (*Eptesicus fuscus*) in Dinosaur Provincial Park, Alberta hibernate in relatively small, dry, thermally-labile rock-crevices and make regular

flights outside of the hibernacula in subzero temperatures despite the lack of foraging opportunities. This behaviour is likely shared by other populations of *E. fuscus* throughout the prairies—and perhaps other species known to overwinter in the area (Lausen and Barclay 2006)—and seems counterintuitive given the precarious energy budgets (Boyles and Brack 2009) and vulnerability to temperature fluctuations (e.g., Geiser 2004) and low humidity (e.g., Thomas and Cloutier 1992; Willis et al. 2011) documented for other hibernators. The obvious questions that arise from this situation are: why do the bats fly if not to forage? and how do they handle the dry conditions? The focus of my research was an attempt to answer these questions.

In Chapter Two, I report data collected while monitoring the roosting behaviour and movement of *E. fuscus* hibernating in rock crevices in Dinosaur Provincial Park (DPP), Alberta, Canada. I also compared microclimate conditions (temperature and humidity) within these crevice hibernacula to those of random crevices within the study area, and to conditions inside four known cave hibernacula in central and northern Canada. My results show that male *E. fuscus* in DPP use rock-crevice hibernacula with particular microclimate and landscape characteristics. I also found evidence of winter roost fidelity between and within years. Bats used only three hibernacula and, although mid-winter flight is common in my study area, there was little movement by bats between hibernacula. Rock-crevice hibernacula were warmer and more thermally stable than other available crevices that I measured in DPP, and drier but not necessarily

colder than known cave hibernacula elsewhere. My study is the first to examine crevice roost selection by bats during winter, and suggests that specific hibernacula are important for individual bats, despite the fact that numerous crevices are available.

In Chapter Three, I report acoustic data where I used echolocation calls as a proxy for activity to determine correlates of hourly bat-activity in Dinosaur Provincial Park, Alberta, Canada (Klög-Baerwald et al. 2016). I documented bat activity in temperatures as low as -10.4°C . I observed *E. fuscus* flying at colder temperatures than species of *Myotis* bats (genus *Myotis*). I show that temperature and wind are important predictors of winter activity by *E. fuscus* and *Myotis*, and that *Myotis* may also use changes in barometric pressure to cue activity. In the absence of foraging opportunity, I suggest these environmental factors relate to heat loss and thus the energetic cost of flight. To understand the energetic consequences of bat flight in cold temperatures, I estimated energy expenditure during winter flights made by *E. fuscus* and *Myotis lucifugus* using species-specific parameters. I estimated that winter flight uses considerable fat stores but that flight thermogenesis could mitigate energetic costs by 20% or more. I also show that temperature-dependent interspecific differences in winter activity likely stem from differences between species in heat loss and potential for activity-thermoregulatory heat substitution.

In Chapter Four, I report data on the hibernation patterns of free-ranging *E. fuscus* overwintering in rock-crevices in the Canadian prairies. I found the bats

to exhibit reduced sensitivity to environmental cues, and that the probability of arousal remains under diurnal influence, ostensibly due to the importance of extra-hibernacular activity. Based on the hibernation patterns I observed, I also estimate that this population of bats spend the majority of their winter energy reserves on maintaining a slightly elevated body temperature, contrary to the archetype of small mammal hibernators. I propose that flight is an important winter activity in this population that expedites the physiological benefits of euthermic periods and allows for short but physiologically effective arousals.

In Chapter Five, I report comparative data on torpid metabolic rate (TMR) and total evaporative water loss (TEWL) from two populations of *E. fuscus* with differing winter ecologies: one that hibernates in humid karst caves and one that hibernates in relatively dry rock-crevices (Klüg-Baerwald and Brigham 2017). I used flow-through respirometry to measure TMR and TEWL of bats in dry and humid conditions. Neither population nor humidity influenced TMR. However, population and humidity influenced rates of TEWL, which were lower for bats that hibernate in dry conditions of DPP compared to those from WBNP. My results suggest that *E. fuscus* hibernating in arid environments have evolved mechanisms to decrease evaporative water loss that are not evident at more humid sites. Drought tolerance may facilitate the sedentary nature of the species, allowing them to tolerate more variable microclimates during hibernation and thus increasing the availability of overwintering habitat. The ability to survive arid

conditions may also lessen the susceptibility of *E. fuscus* to diseases that affect water balance.

In Chapter Six, I report data on the hydration status and possible drinking behaviour of *E. fuscus*. I hypothesized that bats experience increased evaporative water loss and make frequent mid-winter flights to find water. I measured serum ion concentrations and hematocrit to assess level of dehydration in bats captured during winter. I also provided a heated water tank treated with deuterium (^2H) and used stable isotope analysis to look for elevated ^2H ratios (δH) in the blood of bats as evidence of consumption of tank water. In addition, I used passive acoustic monitoring, video surveillance, and passive integrated transponder (PIT) tags to determine if bats visited the heated water tank. I found evidence of hypertonic dehydration (elevated hematocrit and concentrations of some serum ions) in bats as winter progressed. Blood δH of bats was similar to that of water on the landscape, which coupled with acoustic and video surveillance data indicated no visits by bats to the water tank. Post-arousal dehydration is not uncommon in hibernators, and thus I conclude that bats in my study area did not drink from the water tank. They may exploit other sources of water, but further study of the use of artificial water developments by bats is needed.

The results of my research reduce the paucity of knowledge about winter bat-ecology in the prairies and provide comparative hibernation biology data for a group that is relatively understudied compared to rodents. I conclude that adaptive behaviours (e.g., decreasing arousal frequency and duration) and

physiologies (i.e., mechanisms to reduce evaporative water loss) allow this species to hibernate in arid conditions, such as those found in Dinosaur Provincial Park. My findings add to the body of knowledge that suggests a relative cold-tolerance and overwinter hardiness of *E. fuscus* compared to other species of bat (Beer and Richards 1956; Davis 1970; Fenton 1970; Neubaum et al. 2006). As interest in the winter ecology of bats increases due to white-nose syndrome (Frick et al. 2015) and data continue to be collected on the site selection, behaviour, and physiology of populations across a wide geographic range, we may see similar flexibility and variation in hibernation biology in other species or populations.

A goal of my project was to evaluate the causes of winter flight in this population of bats. My data did elucidate some novel behaviours and traits of individuals in the population, but they did not lend support to the hypotheses that bats in my study are at greater risk of dehydration than conspecifics elsewhere or that they take flight to search for water. The reason for winter flight in this particular population of bats remains unclear. Dehydration could still be the motivation behind bats arousing and leaving the hibernacula from natural (e.g., surface melt) or artificial (e.g., cattle troughs) sources of water in the area. But if not to forage or drink, then why do *E. fuscus* in the prairies fly in the winter? Why is it important that these bats physically move during hibernation? What are they accomplishing that cannot be done during arousals while stationary within the hibernacula? Who the h-e-double-hockey-sticks knows? Bats are not known to read the literature and act accordingly. There are other hypotheses for arousals

in small hibernators that remain to be tested, such as those associated with muscle maintenance. Future work aimed at investigating the causes of winter flight in this bat population—and others where foraging is not obvious—is clearly warranted.

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APPENDIX 1—CITATIONS FOR THESIS CHAPTERS PUBLISHED, ACCEPTED,
OR SUBMITTED

Chapter Two:

Klüg-Baerwald, B.J., C.L. Lausen, C.K.R. Willis, and R.M. Brigham. In press. Home is where you hang your bat: winter roost selection by prairie-living big brown bats. *Journal of Mammalogy*.

Chapter Three:

Klüg-Baerwald, B.J., L.E. Gower, C.L. Lausen, and R.M. Brigham. 2016. Environmental correlates and energetics of winter flight by bats in Southern Alberta, Canada. *Canadian Journal of Zoology* 94: 829–836. DOI: 10.1139/cjz-2016-0055.

Chapter Four:

Klüg-Baerwald, B.J., C.L. Lausen, and R.M. Brigham. Submitted. Saturday flight fever; behavioural adaptation in hibernating, prairie-living bats. *Journal of Experimental Biology*.

Chapter Five:

Klüg-Baerwald, B.J. and R.M. Brigham. 2017. Hung out to dry? Intraspecific variation in water loss in a hibernating bat. *Oecologia* 183:977–985. DOI: 10.1007/s00442-017-3837-0

APPENDIX 2—ANIMAL CARE CERTIFICATE

The following is the clearance certificate from the University of Regina President's Committee on Animal Care (PCAC) associated with research described in this dissertation:

University
of Regina

Office for Research, Innovation and Partnership

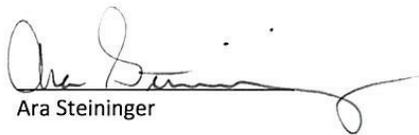
DATE: November 26, 2012
TO: Mark Brigham, Biology Department
FROM: Ara Steininger
RE: **Animal Use Protocol # 12-12**
Winter ecology of bats hibernating in the Canadian prairies

Following the meeting of the President's Committee on Animal Care on November 26, 2012, the above protocol has been granted approval.

Your AUP approval date is November 1, 2012 and your first renewal date is November 1, 2013.

Your protocol will expire on November 1, 2016.

Best wishes for success with your project.


Ara Steininger

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