

2017

# Roosting Ecology and Behaviour of Small-footed Bats (*Myotis ciliolabrum*) and Presence of Bats in Winter in Dinosaur Provincial Park, Alberta

Findlay, Stephanie

---

Findlay, S. (2017). Roosting Ecology and Behaviour of Small-footed Bats (*Myotis ciliolabrum*) and Presence of Bats in Winter in Dinosaur Provincial Park, Alberta (Master's thesis, University of Calgary, Calgary, Canada). Retrieved from <https://prism.ucalgary.ca>. doi:10.11575/PRISM/27693  
<http://hdl.handle.net/11023/3618>

*Downloaded from PRISM Repository, University of Calgary*

UNIVERSITY OF CALGARY

Roosting Ecology and Behaviour of Small-footed Bats (*Myotis ciliolabrum*) and Presence of  
Bats in Winter in Dinosaur Provincial Park, Alberta

by

Stephanie Viola Findlay

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE

GRADUATE PROGRAM IN BIOLOGICAL SCIENCES

CALGARY, ALBERTA

JANUARY, 2017

© Stephanie Viola Findlay 2017

## Abstract

I studied roosting behaviours of western small-footed bats (*Myotis ciliolabrum*) in Dinosaur Provincial Park, a semi-arid badland region in Alberta. Individuals roosted solely in the prevalent erosion-holes within the park. Regardless of sex or reproductive condition, *M. ciliolabrum* exhibited low roost fidelity but high fidelity to coulees. Individuals selected roosts which were high off the ground with an internal microclimate significantly buffered from external conditions, with depth not affecting roost buffering capacity.

I also measured the effect of habitat on the echolocation calls for three *Myotis* species within the coulees and along tree edges. *Myotis lucifugus* and *Myotis ciliolabrum* significantly alter their calls between the two habitat types. Using local calls to construct a call library allowed me to determine that both *M. ciliolabrum* and *M. evotis* were present over winter. The higher proportion of *M. evotis* activity may be explained by gleaning insects during warmer periods.

## Acknowledgements

I would like to thank my amazing supervisor Robert Barclay for giving me the opportunity to take this leap into a new field of study. Your guidance and support has been beyond anything I have come to experience from a mentor. I would also like to thank Ralph Cartar for pushing me to draw all those graphs, they really did help! Thanks also to Jessica Theodor and Katherine Ruckstuhl for their support.

I would also like to thank my two superhero field assistants, Jill Sick and Nicole Besler. You two kept me sane over the months of field work, listened to my crazy ideas, and are amazing hard working people. I would never have accomplished as much as I did without your help.

I would like to thank the staff at Dinosaur Provincial Park for allowing me access to the restricted areas and offering us a wonderful place to stay during the many, many months of field work. Also to the fellow researchers in the park, for giving me the opportunity to see into the other scientific wonders Dino has to offer.

I would like to thank Cindy Kam and Sandie Black. For helping me find my passion and the strength to follow it.

Last but absolutely not least I would like to thank my wonderful, supportive, patient husband Duncan Findlay. You have always had faith in me, you have always known I was smart enough and brave enough to take up this challenge. You are always on my side, helping me, guiding me, telling me when I am being ridiculous, and picking me up every time I fell. I love you.

*For Duncan*

## Table of Contents

Abstract .....	ii
Acknowledgements.....	iii
Dedication .....	iv
Table of Contents.....	v
List of Tables .....	vii
List of Figures.....	viii
CHAPTER 1: GENERAL INTRODUCTION .....	1
CHAPTER 2: SUMMER ROOSTING ECOLOGY OF <i>MYOTIS CILIOLABRUM</i> IN DINOSAUR PROVINCIAL PARK.....	7
Introduction.....	7
Methods.....	13
<i>Study Species</i> .....	13
<i>Field Methods</i> .....	13
<i>Roost Identification</i> .....	14
<i>Statistical Analysis</i> .....	15
Results.....	17
<i>Physical Roost Characteristics</i> .....	18
<i>Internal Microclimate</i> .....	20
Discussion.....	21
CHAPTER 3: ECHOLOCATION CALL CHARACTERISTICS AND SPECIES IDENTIFICATION OF <i>MYOTIS</i> DURING WINTER IN DINOSAUR PROVINCIAL PARK.....	45

Introduction.....	45
Methods.....	50
<i>Study Species</i> .....	50
<i>Field Methods</i> .....	51
<i>Acoustic Call Processing</i> .....	52
<i>Statistical Analysis</i> .....	53
Results.....	54
Discussion .....	57
<i>Model Performance</i> .....	57
<i>Winter Activity</i> .....	60
Conclusions.....	62
CHAPTER 4: GENERAL CONCLUSIONS.....	72
LITERATURE CITED .....	77

## LIST OF TABLES

Table 2.1. Comparison of physical characteristics of erosion-holes selected by <i>Myotis ciliolabrum</i> and random suitable roosts in Dinosaur Provincial Park .....	40
Table 2.2. Physical characteristics measured at erosion-hole roosts selected by various sex groups of <i>M. ciliolabrum</i> .....	41
Table 2.3. The 15 logistic-regression models generated to compare the physical characteristics of erosion-holes selected by <i>M. ciliolabrum</i> to those of randomly available holes in Dinosaur Provincial Park .....	42
Table 2.4. Mean ( $\pm$ SEM) for daily, maximum and minimum ambient temperatures ( $T_a$ ; °C) near Dinosaur Provincial Park for July 2014, July 2015, and August 2015 .....	43
Table 2.5. Mean ( $\pm$ SEM) depth (cm), roost temperature ( $T_r$ ; °C) and corresponding ambient temperature ( $T_a$ ; °C) for selected and suitable roosts for <i>Myotis ciliolabrum</i> in Dinosaur Provincial Park .....	44
Table 3.1. Mean ( $\pm$ SEM) values for all call parameters for <i>Myotis ciliolabrum</i> (MYCI) and <i>Myotis lucifugus</i> (MYLU).....	68
Table 3.2. Discriminant function analysis performance for species-species level accuracy for both coulee and tree-edge models.....	69
Table 3.3. Model classification results using known bat calls in two habitat types from Dinosaur Provincial Park.....	70
Table 3.4. Mean ( $\pm$ SEM) values for echolocation call parameters for three <i>Myotis</i> species in Dinosaur Provincial Park .....	71

## LIST OF FIGURES

Figure 2.1.	<i>Myotis ciliolabrum</i> roost locations within Dinosaur Provincial Park.....	29
Figure 2.2.	Photo of an erosion-hole used as a roost by a male <i>Myotis ciliolabrum</i> in Dinosaur Provincial Park .....	30
Figure 2.3.	Mean distance to nearest horizontal surface below (cm) for selected versus suitable erosion-holes for <i>Myotis ciliolabrum</i> .....	31
Figure 2.4.	Mean slope (°) of the coulee for the selected (53.74 ±2.98cm) versus suitable (46.99 ± 2.99cm) erosion holes of <i>Myotis ciliolabrum</i> in Dinosaur Provincial Park .....	32
Figure 2.5.	Mean values for the largest dimension (cm) of openings in selected (17.00 ± 3.19cm) versus suitable (13.12 ± 2.027cm) holes for <i>Myotis ciliolabrum</i> in Dinosaur Provincial Park.....	33
Figure 2.6.	Mean values for the largest dimension (cm) of roost opening for lactating female (9.6 ± 1.5cm), non-reproductive female (37.1 ± 12.0 cm) and male (12.1 ± 2.5cm) <i>Myotis ciliolabrum</i> in Dinosaur Provincial Park .....	34
Figure 2.7.	Roost aspect represented as compass direction (°) for selected and suitable erosion holes for <i>Myotis ciliolabrum</i> .....	35
Figure 2.8.	Buffering effect of erosion-hole roosts in Dinosaur Provincial Park .....	36
Figure 2.9.	Buffering effect for relative humidity of erosion-hole roosts in Dinosaur Provincial Park.....	37
Figure 2.10.	The mean temperature (°C) at various depths within selected <i>M. ciliolabrum</i> erosion-hole roosts (n=37).....	38
Figure 2.11.	Roost temperatures (°C) in selected erosion-holes at various depths (cm) .....	39
Figure 3.1.	Measurements for each parameter per bat call using AnaLookW software.....	63
Figure 3.2.	Mean call duration (ms) for <i>Myotis ciliolabrum</i> recorded in the coulees or at the tree edge in Dinosaur Provincial Park .....	64
Figure 3.3.	Mean minimum frequency (kHz) for <i>Myotis ciliolabrum</i> recorded in the coulees or at the tree edge in Dinosaur Provincial Park .....	65
Figure 3.4.	Linear discriminant analysis results for three bat species, <i>Myotis ciliolabrum</i> (MYCI), <i>Myotis evotis</i> (MYEV), and <i>Myotis lucifugus</i> (MYLU).....	66

Figure 3.5. Bat passes per night with respective temperatures in Dinosaur Provincial Park during the winter of 2014-2015 ..... 67

## Chapter 1

### General Introduction

Endothermic organisms are able to maintain an elevated body temperature ( $T_b$ ) by using the energy released as heat from a high metabolic rate (MR) (Willis and Cooper 2009). As a result, these organisms are able to tolerate ambient temperatures ( $T_a$ ) below their thermoneutral zone (TNZ) (Studier 1981). When  $T_a$  is below the TNZ, individuals must increase their MR to ensure their  $T_b$  remains stable, which is energetically costly (Kleiber 1947). The cost of endothermy is not equal among all individuals; as body size decreases, the rate of energy lost to the surrounding environment increases (McNab 1970, Willis and Cooper 2009). The rate at which energy is lost is dependent on the surface area to volume ratio of an individual (Kleiber 1947). Small individuals, such as bats, must therefore maintain higher MR to maintain  $T_b$  than larger individuals do under the same environmental conditions (Kleiber 1947).

Individuals may compensate for this high energy demand via the use of torpor (Wang and Wolowyk 1988, Geiser 2004). Torpor is the periodic decrease in  $T_b$  and MR which results in a substantial energy savings (10-88%; Wang and Wolowyk 1988, Webb et al. 1993, Bogan et al. 2003). The physiological state of torpor is most advantageous when  $T_a$  is low, when food is unavailable, or during periods of inactivity (Willis and Cooper 2009). Torpor bouts can range from a few hours to several weeks. Prolonged periods of torpor are also known as hibernation (Speakman and Racey 1989, Willis and Cooper 2009).

Homeothermic animals can also reduce the costs of a high MR by selecting nests, burrows or roosts with microclimates within their TNZ. In bats, roost selection can provide insight into the overall fitness of individuals as they spend over half of their lives in roosts (Kunz

1982, Holloway 1998, Bogan et al. 2003). Roosts can be used for a variety of different purposes; protection from elements/predation, social interactions, mating, rearing young, and hibernation (Kunz 1982, Vonhof and Barclay 1996, Bogan et al. 2003). Roost microclimate (temperature and humidity) must be suitable and will depend on the purpose of the roost (Chruszcz and Barclay 2002). For example, in maternity roosts, the internal temperatures must maintain conditions close to the TNZ thereby minimizing the need for torpor and promoting juvenile development (Solick and Barclay 2006a). To increase temperature within roosts, bats can take part in social thermoregulation (Willis and Brigham 2007) or use shallow roosts (Vaughan and O'Shea 1976) that take advantage of warm  $T_a$ .

By selecting roosts with higher relative humidity, bats can decrease evaporative water loss (EWL). When compared with other similar sized mammals, bats lose more water via EWL (Studier 1970). In many bats species, the wing membrane is hairless and increases the surface area to volume ratio resulting in a greater rate of water loss (Webb et al. 1995). Water is also lost through respiration and the rate of respiration is related to  $T_a$ , where high temperatures can increase EWL (Studier and O'Farrell 1976, Webb et al. 1995). Furthermore, there is a significant effect of reproductive condition on EWL in females; lactating females lose more water compared to non-reproductive females (Studier and O'Farrell 1976). The rate of EWL is also affected by ambient relative humidity; more arid conditions lead to a greater loss of water when compared to higher humidity (Webb et al. 1995). Bats which roost and reproduce in arid regions with low ambient relative humidity need to effectively balance their water budget, requiring them to alter their behaviour when compared with populations which inhabit areas with higher humidity.

Bats which roost in trees and rocks tend to exhibit low roost fidelity, switching roosts frequently (Lewis 1995, Hayes et al. 2009). Roost switching is common among bats which roost

in non-permanent structures, and individuals may switch to avoid predation/parasites, select more favourable microclimates, move closer to resources, and increase social interactions (Lewis 1995, Nixon et al. 2009). However, the energy budget of a bat also may influence roost switching; the cost of flight (which can vary depending on reproductive status or  $T_a$ ) must be lower than the energy gained from acquiring a new roost (Vonhof and Barclay 1996). As a result, roost switching and roost selection can have a large effect on the overall fitness of an individual, and the study of these behaviours can provide valuable insight regarding these species.

The roosting behaviours of non-migratory bat species inhabiting the prairie regions of Canada are poorly understood. Yet the need to understand these behaviours has increased in North America since the introduction of *Pseudogymnoascus destructans*, which causes white-nose syndrome (WNS). This fungus arrived in New York state in 2006 and has since spread to 29 states and 5 provinces with a drastic jump into Washington state in the winter of 2015-2016 (Blehert et al. 2009, U.S. Fish and Wildlife Service 2016). *P. destructans* is a psychrophilic fungus, which takes advantage of a bat's suppressed immune system while hibernating and grows on any exposed dermis, disrupting torpor bouts and pre-maturely depleting fat-stores leading to starvation (Reeder et al. 2012, Raudabaugh and Miller 2013). The fungus is easily transmitted through physical contact with an infected individual or roosting site, with transmission occurring in both summer and winter as the fungus can survive on various substrates (Lorch et al. 2011). Research is needed to understand year-round habitat use by bats to obtain a clear picture of hibernating ecology and behaviour to better manage unique bat populations across North America.

I conducted my research in Dinosaur Provincial Park (DPP) (50.7537, -111.528, 621–727 m) which is located in southeastern Alberta, Canada. The park primarily consists of badland

terrain (sandstone cliffs and eroded coulees) and is sparsely vegetated (Chruszcz and Barclay 2002). The climate is arid, receiving <350mm of precipitation annually, with temperatures ranging from -45°C to 40°C (Lausen and Barclay 2006). The Red Deer River Valley runs through the northern portion of the park, with a public campground within the riparian region along Little Sandhill Creek which drains into the Red Deer River. Summer roosting habitat for bats in this region is limited due to the lack of complex vertical structures, buildings and caves (Holloway 1998). Trees are present in the riparian zones, but consist solely of cottonwood (*Populus deltoides*) which provides few large trees with a suitable degree of decay favourable for roosting (Vonhof and Barclay 1996, Holloway 1998). As a result, bats in this region trend to roost primarily in rock crevices on ridges, erosion valley walls and boulders (Chruszcz and Barclay 2002, Lausen 2007).

Several bat species hibernate in Dinosaur Provincial Park through the winter, including the big brown bat (*Eptesicus fuscus*) and with one or more *Myotis* species (the small-footed bat, *Myotis ciliolabrum*; the long-eared bat, *Myotis evotis*; the little brown bat, *Myotis lucifugus*; Lausen and Barclay 2006). Although they have been observed roosting in cave systems in western Montana (Schwab and Mabee 2014), winter hibernation sites for these *Myotis* species in southeastern Alberta are unknown and difficult to ascertain. Capture rates of *Myotis* bats over winter are low due to a decrease in abundance and activity. As a result, acoustic surveying (recording echolocation calls) has been conducted in the area but the acoustic similarities of these three *Myotis* species makes differentiation problematic (Lausen and Barclay 2006).

Acoustic surveys can be used to determine overall bat activity, habitat use over time and species identification (Hayes et al. 2009, Parsons and Szewczak 2009). This method has increased in popularity recently with creation of automated software packages designed for

species identification, with some boasting real-time species classification in the field (Russo and Voigt 2016). However, the accuracy of such software is subject to debate; when these packages (Bat Call ID, EchoClass, Kaleidoscope Pro and SonoBat) were tested against large acoustic samples of unknown bat species, the packages were only in agreement in terms of species identification 40% of the time (Lemen et al. 2015). As multiple variables can be measured from a single bat call, multivariate discriminant function analysis is the best approach when modeling echolocation calls, however, this requires more human input when compared to automated classification software (Russo and Voigt 2016).

Echolocation calls are difficult to differentiate because they are primarily used as a sensory aid (Barclay 1999). Echolocation evolved for a different function, prey identification and spatial orientation, when compared with bird song; bird song evolved to communicate with individuals from the same species (Barclay 1999). Echolocation calls are less variable when compared with bird song, but that does not mean they are unchanging; they can vary individually, geographically, ecologically or environmentally (Barclay 1999, Hayes et al. 2009, Parsons and Szewczak 2009, White et al. 2014). Some species of bat have very distinct echolocation call characteristics, making them easy to identify, but some variables of bat calls overlap significantly with other species making it difficult to discern species with any degree of accuracy (Barclay 1999, Gannon et al. 2001, Broders et al. 2004, Agranat 2013, Russo and Voigt 2016). Building a model with a call library from known individuals local to your study site, in the habitat type they are being passively monitored in, and using recording techniques which will least alter natural behaviour, is crucial when attempting to discriminate acoustically similar species (Barclay 1999, Mukhida et al. 2004, Parsons and Szewczak 2009, Clement et al. 2014).

In this thesis, I investigated the roosting ecology of *Myotis ciliolabrum* in Dinosaur Provincial Park. I also monitored the acoustic activity of *Myotis evotis*, *Myotis lucifugus*, and *Myotis ciliolabrum* over winter in my study site. In Chapter 2, I report on the physical roost characteristics and the internal microclimatic conditions for roosts used by males, non-reproductive females and lactating females during the summer in this semi-arid region of Alberta. My goals were to determine a) which physical characteristics *M. ciliolabrum* select by comparing their roosts to other randomly available holes in the vicinity, b) what internal conditions bats are selecting based on their reproductive condition, and c) how well their roosts are buffered from the extreme ambient conditions in the area. In Chapter 3, I determined which *Myotis* species (*M. ciliolabrum*, *M. evotis*, *M. lucifugus*) are present in Dinosaur Provincial Park over the winter by creating a call library from local known bats within their natural habitat. I predicted habitat would have an effect on echolocation call characteristics and therefore would alter model performance. Using discriminant function analysis, I modeled the known calls from the call library and applied them to unknown *Myotis* bat passes recorded over winter to determine which species are using the study site to hibernate in. Chapter 4 is a summary of my results with overall conclusions and future research possibilities.

## Chapter 2

### Summer roosting ecology of *Myotis ciliolabrum* in Dinosaur Provincial Park

Bats are the only mammals capable of true flight and it requires a large amount of energy per minute travelled when compared with swimming or running (Schmidt-Nielsen 1972). Bats must maintain a high metabolic rate to generate enough energy to fly and as a result bats reduce their energetic costs in numerous ways; limiting flight during periods of poor ambient conditions or lack of food, and reducing their metabolic rate when roosting. Bats spend the majority of their lives in roosts, and roost selection can have a significant effect on an individual's fitness (Kunz 1982). An individual will select a roost based on its current energetic needs (Hayes et al. 2009, Altringham 2011), and as different roost characteristics can alter internal conditions (Kunz 1982) bats can reduce their energy expenditure through roost selection.

Bats use several types of roosts (day, night or hibernacula), which act as protection from the environment and predators (Kunz 1982, Vonhof and Barclay 1996, Bogan et al. 2003). The type of roost depends on the individual needs of the bat. Continuous flight can be costly and often individuals do not need to fly all night to obtain enough food they require on a nightly basis. As a result, bats may roost periodically throughout the night to digest food while remaining protected from environmental elements or predators. Males, reproductive and non-reproductive females can use these temporary night roosts to maximise the net energy gain per foraging bout (Anthony et al. 1981, Barclay 1982). Reproductive females have greater energy demands due to embryo development and lactation. As such, these females tend to congregate prior to parturition in maternity roosts during the day (Williams and Brittingham 1997, Altringham 2011). Maternity colonies, with warmer microclimates (compared to non-maternity roosts), are only used by reproductive females and their offspring, and allow them to minimise

the costs of maintaining higher body temperatures for lactation and pup growth (Hamilton and Barclay 1994). Non-reproductive females and males have lower energetic constraints when roosting during the day, and these individuals can afford to use day roosts where microclimate temperatures are lower to better facilitate the use of torpor (Hamilton and Barclay 1994, Altringham 2011).

Bats may not reuse a roost over the entirety of their lives and in some cases individuals switch roosts every night (Lewis 1995). Roost switching is poorly understood and there are several postulated underlying causes, such as avoiding detection by predators, limiting parasite transmission, selecting more favourable roost microclimates, moving closer to resources, or increasing social interactions (Lewis 1995, Nixon et al. 2009). Roost fidelity is used to describe this switching behaviour and is a measure of consecutive roost use in bats. The benefits of switching need to outweigh the costs if it is to have a positive effect on an individual's overall fitness. As the energetic requirements of the individual vary, so does the cost of switching, with the cost being higher in reproductive females carrying a pup compared to males or non-reproductive females (Vanhof and Barclay 1996).

The type of roost an individual selects will also have an effect on roost fidelity. Bats use a variety of different physical structures as roosts; caves, buildings, trees/tree cavities, rock crevices or manmade bat houses (Altringham 2011). The degree of permanence of a roost is correlated with roost fidelity. Bats roosting in/on non-permanent structures (trees, boulders and rock crevices) exhibit low roost fidelity, and bats roosting in more permanent features (caves or buildings) exhibit high roost fidelity (Lewis 1995, Hayes et al. 2009). Structures such as caves provide year-round stability and often bats will frequent these types of roosts over several years, whereas bats which roost in trees and rock crevices tend to exhibit low roost fidelity and switch

often (Lewis 1995, Hayes et al. 2009). In more ephemeral roosts, which are less stable both climatically and physically, bats often exhibit fidelity to a given area rather than to a specific roost (Altringham 2011). Studying the frequency of roost switching can provide valuable insight into a species' success in a given area (Kunz 1982). Determining the underlying cause of roost switching can indicate which ecological pressures are acting on bat roosting behaviour.

The physical characteristics (aspect, opening dimensions and depth) of a roost have an effect on its internal microclimate (Vonhof and Barclay 1996, Lausen and Barclay 2003, Garroway and Broders 2008). Shallow roosts which are nearer the surface are less buffered from ambient environmental conditions and therefore internal microclimatic conditions are less stable (Lausen and Barclay 2002, 2003, Solick 2004), but shallow roosts also allow individuals to take advantage of warmer external temperatures through passive warming (Vaughan and O'Shea 1976). As depth of a roost increases, the buffering ability of that roost also increases. As a result, there exists an internal temperature gradient with conditions similar to ambient near the opening and buffered conditions at the deepest point. This gradient allows bats to alter their position within a roost as their thermoregulatory requirements vary throughout the day (Vaughan and O'Shea 1976).

To further increase individual fitness, bats can also use torpor, which involves reducing body temperature and heart rates to below normothermic levels (Wang and Wolowyk 1988, Geiser 2004). Torpor can vary in duration from a few hours to several weeks and bats employing torpor can experience substantial energetic savings (10-88%; Wang and Wolowyk 1988, Webb et al. 1993, Cryan and Wolf 2003). Once in torpor, a bat must return body temperature and metabolic rates to normothermic levels before they are able to fly. This can be achieved by

internal heat generation or by taking advantage of increasing ambient temperatures during the later portion of the day, known as passive warming (Geiser 2004, Solick and Barclay 2006b).

Torpor-use patterns employed by individuals depend on their energetic requirements, as the costs of its use are unequal across reproductive states. Males and non-reproductive females select cooler roost microclimates which facilitates the use of torpor during the day or during periods when foraging is unfavourable (heavy rainfall, high winds or low ambient temperatures; Solick and Barclay 2007, Willis and Cooper 2009). Reproductively-active females must limit torpor use as it slows fetal development and reduces milk production during lactation (Racey and Swift 1981, Racey 2009). Pregnant and lactating females should therefore remain homeothermic and employ other strategies to reduce the costs of maintaining a high body temperature. Microclimates near the thermal neutral zone (TNZ) for reproductive females allow for increased energy savings as minimal metabolic activity is required to ensure stable body temperatures (Kleiber 1947). Therefore, the selection of a roost within the TNZ will allow reproductive females to avoid using torpor and remain warm (Geiser 2004, Solick and Barclay 2006a). Reproductive females may also roost together in large numbers to increase roost temperatures as well as take advantage of social thermoregulation (Willis and Brigham 2007). They may also select for south facing slopes (in the Northern hemisphere) as they tend to receive more solar radiation, and therefore are often warmer than north facing slopes.

By examining roosting behaviour in habitats which experience extreme ambient climatic conditions, as roosts must act as a buffer from harsh external elements, you can assess the effect of habitat on an individual's fitness (Nixon et al. 2009). Temperate arid regions can experience extreme variability in both temperature and relative humidity. During summer months, temperatures can range from high 30's to just above freezing (°C), with low relative humidity

punctuated by infrequent precipitation events (Holloway 1998, Alberta Climate Information Service. 2016). As a result, roost selection can have a large effect on fitness as ambient environmental extremes will need to be mitigated by selecting for an optimal roost (Chruszcz and Barclay 2002, Lausen and Barclay 2003).

The goal of my study was to test hypotheses regarding the ecology and roosting behaviour of bats in arid regions which provide atypical roosting options for bats. Specifically, I studied the roosting behaviour and ecology of *Myotis ciliolabrum*, the western small-footed bat, during the summers of 2014 and 2015 in Dinosaur Provincial Park (50.7537, -111.528, 621–727 m), a semi-arid region in southwestern Alberta, Canada. During the summer months, daily maximum and minimum temperatures reached 37.7(°C) and 4.3(°C), respectively, with little precipitation and low relative humidity (Alberta Climate Information Service 2016). The landscape is sparsely vegetated, and primarily consists of badland terrain (sandstone cliffs and eroded bentonitic coulees; Chruszcz and Barclay 2002). The Red Deer River runs through the northern portion of the park with the surrounding riparian zones containing the only trees (cottonwood, *Populus deltoides*; Holloway and Barclay 2000). Small ephemeral creeks also run through the park, with Little Sandhill Creek being the largest, draining into the Red Deer River. Summer roosting habitat in this region is limited due to the lack of caves, buildings and suitable large decaying trees (Holloway 1998). Large boulders, rock cracks/crevices, and erosion holes are the most common roosting opportunities present in my study site (Schowalter and Allen 1981, Holloway and Barclay 2001, Chruszcz and Barclay 2002, Lausen 2007)

*Myotis ciliolabrum* is common in this region and exhibits crevice-roosting behaviour in the area, as well as in other arid environments (Schowalter and Allen 1981, Holloway and Barclay 2001, Lausen 2007). I hypothesized that sex and reproductive condition influence the

roosting behaviour of bats in terms of the physical and microclimate characteristics of sites they select. I predicted that reproductive female *M. ciliolabrum* switch roosts less often and use roosts that are closer together than males and non-reproductive females do. I also hypothesized that, as reproductive females have greater energy demands compared to non-reproductive females or males, roost selection by reproductive females would differ from that of non-reproductive females and males. I predicted that reproductive females select warmer and more humid roosts than non-reproductive females and males do.

I measured external physical roost characteristics, internal microclimatic conditions, roost switching and distance between roosts in males, non-reproductive females and lactating females. I compared selected roosts with random suitable crevices (referred to as suitable roosts) to determine which physical characteristics are important when bats are selecting roosts. Within selected roosts, I also compared physical and microclimatic characteristics of roosts used by males, non-reproductive females and lactating females, to assess the effect of sex and reproductive condition on roost selection. I hypothesized that bats select roosts that buffer them from ambient conditions. I predicted that roosts selected by *M. ciliolabrum* differ significantly from other holes, with selected roosts being warmer when ambient temperatures are cool, and cooler when ambient conditions are warmer. I also predicted that selected roosts are more humid than ambient conditions thereby limiting evaporative water loss. Finally, I predicted that deeper roosts are cooler than roosts which are closer to the surface.

## Methods

### *Study Species*

The western small-footed bat (*Myotis ciliolabrum*) is an aerial insectivorous species prevalent in the prairies of southeastern Alberta (Schowalter and Allen 1981). *Myotis ciliolabrum* is the smallest species of bat in this region, weighing on average 4.5g (Holloway and Barclay 2001). This species is common in arid regions but uses the riparian zones in these areas, roosting in small colonies (1-6 individuals) in adjacent outcrop cracks/crevices composed mostly of bentonite (Holloway and Barclay 2000, 2001). Individual *M. ciliolabrum* exhibit low roost fidelity, switching every one to two days but remaining within a small home range, with a mean of  $45 \pm 6$  m between roosts (Lausen 2007). Females give birth to a single pup, with parturition ranging from early to late June (Holloway and Barclay 2001). In day roosts, reproductive females may roost in small groups (2-6 individuals) or solitarily. Males are absent from maternity colonies and may roost alone but both sexes use the same foraging spaces (Schowalter and Allen 1981, Holloway and Barclay 2001).

### *Field Methods*

I captured bats in July 2014, and July to mid-August 2015 in Dinosaur Provincial Park, Alberta. Bats were captured using mist nets placed across various locations within the park; along the edge of Little Sandhill Creek, in the cottonwoods within the riparian zone, and in the coulees (away from water sources or canopy cover). Nets were placed 1-3 meters off the ground to target *M. ciliolabrum* (Vonhof 2006). For each captured individual I determined species, age, sex and reproductive condition. I determined age by assessing the cartilaginous epiphyseal growth plates in the finger joints; if fused, bats were classified as adult (Vonhof 2006, Brunet-

Rossinni and Wilkinson 2009). Reproductive condition of females was divided into four categories (a) non-reproductive (b) pregnant, determined by palpating lower abdomen (c) lactating, hair surrounding nipples worn, can express milk or nipples appear enlarged (d) post-lactation, fur worn around nipples but no milk expressed and nipples reduced (Vonhof 2006, Racey 2009). All bats were banded with small metal split-rings (forearm) for future identification (2.4mm, Porzana, UK). I attached radio transmitters to males, non-reproductive females and lactating females (LB-2X and LB-2XT, 0.23g and 0.25g; Holohil Systems, Carp, Ontario). Fur was trimmed between the scapulae, and nontoxic surgical adhesive (Osto-Bond, Montreal Ostomy) was used to attach the transmitter. All tags were less than 5% of the bat's body mass to minimize the transmitter altering natural behaviour (Aldridge and Brigham 1988).

### *Roost Identification*

I tracked bats daily to their roost location within the coulees, and recorded GPS location to assess movements between roost sites. I used receivers (R-1000 Telemetry Receiver, Communication Specialists, Inc., Orange, CA, USA) with three and five element Yagi antenna to track individuals. I classified roosts as an erosion hole (naturally occurring hole, mostly circular, within a sandy/bentonitic mudstone), a boulder (solidified independent structure with vertical or horizontal fissures), or a tree. I measured the length and width of the roost opening, slope of the coulee surface and aspect using compass direction. Distance from the nearest horizontal surface above (DHA) and below (DHB) the roost were also measured as an indicator of predation risk (Neubaum et al. 2006).

I recorded internal roost microclimate (temperature and relative humidity) using HOBO Pro v2 Loggers® (Onset Computer Corp). Detectors were only inserted into a roost once the bat had switched to a new roost to avoid trapping or disturbing it. Ambient conditions were recorded

in a solar and wind shade approximately 1m off the ground in the coulee using a HOBO® logger (Onset Computer Corp). Temperatures at certain depths in roosts were measured using a Traceable® Big-Digit Memory Thermometer (Control Company). Once daily (between 0900 and 1300h), temperature was measured at three depths, Max (maximum depth reached by temperature probe), Mid (half of the maximum depth) and Min (roost aperture). To maintain consistency, ambient temperatures were measured concurrently with depth temperatures with the same unit under similar physical conditions (under direct sunlight, shaded by vegetation or overcast).

For each roost measured, a “suitable” roost was also measured. I defined a suitable roost as any hole in the ground or a boulder or tree located near the selected bat roost, and with an opening large enough to fit an *M. ciliolabrum*, with no upper limit for opening size. A random direction from the bat roost was selected and no maximum or minimum distance between selected and suitable roosts was imposed. All suitable roosts were measured using the same parameters as selected roosts for both physical and microclimatic characteristics.

### *Statistical Analysis*

I compared consecutive roost use among roosts used by males, non-reproductive females and lactating females. GPS coordinates of roost locations were used to measure the distance between roosts measured with Google Earth’s measuring tool and these were compared between sex groupings using either analysis of variance (ANOVA) or Kruskal-Wallis rank sum test for non-parametric data. Physical roost characteristics were compared using t-tests or Mann–Whitney U (the latter for data that were not normally distributed).

To test whether bats selected roosts based on physical characteristics, I analysed the physical roost characteristics using a generalised linear model (GLM) and selected the best model using the Akaike information criterion (AIC). This method allowed me to determine and remove measured variables that were not biologically important (Whitlock and Schluter 2009, Zuur et al. 2009). I used six different variables, opening length and width, DHB, DHA, slope and aspect. Due to the circular nature of the aspect data, aspect was removed from analysis and analyzed separately using a Rayleigh test to compare aspect of selected roosts to random direction (Pewsey et al. 2013). Prior to the GLM, I checked for correlated variables ( $R^2 \geq 0.70$ ), and removed one of the correlated variables. I ranked the results using  $AIC_c$  (corrected for small sample size) values and calculated the  $AIC_c$  weights ( $w_i$ ), with the best model resulting in the lowest  $AIC_c$  values and highest  $w_i$  (Burnham and Anderson 1998, Wagenmakers and Farrell 2004). I also calculated the McFadden Pseudo  $R^2$  value for the top model to determine what proportion of the variability was explained by the model. I used a multivariate analysis of variance (MANOVA) on physical roost characteristics to test my prediction that sex and reproductive status influence roost selection in *M. ciliolabrum* (Tabachnick and Fidell 2013). Significant results were then ranked using Tukey multiple comparisons of means.

I compared roost and random hole temperature and relative humidity using analysis of covariance (ANCOVA) to test whether or not bats were selecting roosts based on microclimatic conditions. Selected roosts were compared with suitable holes and I also compared among the three sex groupings. ANCOVAs were also used to test temperature-at-depth data (ambient temperature as a covariate), comparing temperatures at various depths in selected versus suitable roosts, as well as among sex groups. An ANCOVA was also used to compare the effect of depth on internal temperatures at the three depths (Max, Mid, and Min). Lastly, I used a Kruskal-

Wallis rank sum test to compare Max, Mid and Min roost depths in selected versus suitable roosts. All statistical analysis was completed using RStudio (version 0.99.489; R. RStudio, Inc) with  $\pm$ SEM reported unless otherwise specified.

## Results

Over the entire period of my field research I caught a total of 168 *M. ciliolabrum* in Dinosaur Provincial Park, including 34 reproductive females (pregnant, lactating or post lactating), 44 non-reproductive females, and 90 males, in a total of 67 netting days. Other species captured included *Eptesicus fuscus* (8 males), *Myotis evotis* (8 males and 14 females), and *Myotis lucifugus* (39 males and 7 females).

I attached radio transmitters to 8 lactating female (3 in 2014 and 5 in 2015), 8 non-reproductive female (3 in 2014 and 5 in 2015), and 12 male (4 in 2014 and 8 in 2015) *M. ciliolabrum*. I successfully tracked 27 (one non-reproductive female was never located) bats to their roosts and recorded physical roost characteristics, internal temperature and relative humidity. Bats were tracked for from 1 to 11 days with a mean radio transmitter life of 4.7 days. In total I collected 126 days of roosting data, 45 days for lactating females, 31 days for non-reproductive females and 50 days for male bats. Bats remained in the same roost for from 1 to 6 consecutive days, changing every 1.7 ( $\pm$  0.23) days on average. With the exception of one lactating female, bats did not reuse any previous roost occupied by either themselves or other monitored bats. There was a significant difference in switching frequency among sex groups (Kruskal-Wallis chi-squared= 7.27, df= 2, P= 0.026), with males switching more frequently than non-reproductive females or lactating females, switching on average every 1.2 ( $\pm$  0.10), 2.1 ( $\pm$  0.66), and 2.2 ( $\pm$  0.40) days, respectively.

Although bats switched roosts frequently, individuals did exhibit high site fidelity by returning to the same coulee. The distance between roosts varied between 14.89 and 2004.07m (mean =  $211.63 \pm 87.36$ m). On average males traveled  $354.08(\pm 200.83$ m), non-reproductive females  $77.70 (\pm 23.25$ m) and lactating females  $134.02 (\pm 60.87$ m) between consecutive roosts. There was no significant difference in distance between roosts among sex groups (Kruskal-Wallis chi-squared = 0.58, df = 2, P = 0.75). There was no observed sexual segregation of roost locations, with males, non-reproductive females and lactating females roosting in the same or adjacent coulees (Figure 2.1).

### *Physical Roost Characteristics*

All bats, with the exception of one male roosting in a rock fissure, roosted in the sides of coulees in erosion-holes which were abundant features within my study site (Figure 2.2). I recorded physical roost characteristics from 174 erosion-holes (87 selected and 87 suitable; Table 2.1). Among all selected roosts, the distance from nearest horizontal ground below (DHB) (mean =  $241.4 \pm 20.1$ cm) was significantly greater than that for random suitable holes (mean =  $136.32 \pm 15.6$ cm; Mann-Whitney U = 4775.5, P = 0.003), with bats selecting for erosion holes that were higher off the ground (Figure 2.3). Distance from nearest horizontal ground above (DHA) was not significantly different (t = 0.98, P = 0.33) between selected and suitable holes and neither was the slope (t = 1.60, P = 0.11; Figure 2.4) of the coulee or the largest dimension of the hole (Ldim; Figure 2.5) (Mann-Whitney U = 3664.5, P = 0.72).

I measured roost characteristics for erosion holes used by individuals in each reproductive state and sex group. Of the 87 selected roosts that I measured, 38 were used by males, 29 by lactating females, and 20 by non-reproductive females. Comparing among these groups, there was a significant difference in Ldim (F = 6.79, P = 0.002), with non-reproductive

females selecting larger erosion holes ( $37.1 \pm 12.0$  cm) than males ( $12.1 \pm 2.5$ cm) or lactating females ( $9.6 \pm 1.5$ cm; Figure 2.6), with no significant difference between males and lactating females. For DHB, DHA and slope of erosion holes, there were no significant differences among the groups (Table 2.2). I also measured slope aspect ( $^{\circ}$ ) for all erosion holes for selected ( $188.8 \pm 8.77^{\circ}$ ) and suitable ( $191.8 \pm 10.70^{\circ}$ ) roosts. Within selected roosts, I also measured holes chosen by males ( $235.9 \pm 15.35^{\circ}$ ), non-reproductive females ( $199.8 \pm 15.85^{\circ}$ ) and lactating females ( $173.0 \pm 13.31^{\circ}$ ). Bats did not preferentially select roosts with a specific aspect as there was no significant difference between the aspect of selected holes and a random direction. This was also the case among sex groups, with no significant difference from random in any group (see Figure 2.7 for respective p-values).

Of the five physical roost characteristics, opening length and width were highly correlated ( $R^2=0.8$ ; Zuur et al. 2009). Instead of arbitrarily removing length or width, I used the largest dimension (Ldim) from these measurements and ran these in the generalised linear model, as erosion holes with larger openings have more potential fitness consequences (e.g. predation) than the smaller dimension, which is limited only by body size. I created 15 candidate models using one, two and three-way comparisons with the global model comparing all four parameters (Table 2.3). The highest ranked model, with the lowest  $AIC_c$  value (231.148) and highest AIC weight ( $w_i = 0.189$ ), contained two variables, distance to nearest horizontal ground below (DHB) and slope. However, the McFadden Pseudo  $R^2$  value for this model was 0.045 suggesting that the model only explains a small portion of the difference between selected and random holes. Also the  $\Delta w_i$  ( $w_i/w_j$ ) values for the next three models were low ( $w_i/w_j = 1.047, 1.071, 1.161$ , respectively) further suggesting that all variables in the top four models could be predictors for roost selection (Burnham and Anderson 1998). The variables present in the top four models were

DHB, slope and Ldim suggesting that all these are important features during roost selection by *M. ciliolabrum*.

### *Internal Microclimate*

I measured the internal microclimate conditions (temperature and relative humidity) for selected erosion-holes (n=53) and those deemed suitable (n=39) every 30 minutes for 24 hours. As field-site ambient temperature ( $T_a$ ) and ambient relative humidity (RH) did not vary significantly ( $F = 2.12$ ,  $P = 0.13$ ;  $F = 0.91$ ,  $P = 0.41$  respectively) between the two field seasons (Table 2.4), all erosion-hole microclimate data were pooled for analysis. There was no significant difference between temperatures in selected versus suitable holes ( $F = 0.25$ ,  $P = 0.62$ ), with mean temperatures of  $23.8 (\pm 0.43^\circ\text{C})$  and  $23.4 (\pm 0.57^\circ\text{C})$ , respectively. Relative humidity also was not significantly different ( $F = 0.74$ ,  $P = 0.40$ ) between selected and suitable holes, with random suitable holes at  $58.7 (\pm 3.31\%)$  and selected holes at  $55.07 (\pm 2.48\%)$ . Temperatures in all erosion holes, those selected by *M. ciliolabrum* or deemed suitable, were warmer than ambient temperature when ambient conditions were below  $\sim 26^\circ\text{C}$ , and cooler than ambient during high temperatures (Figure 2.8). Internal relative humidity was also generally higher than ambient conditions for both selected and suitable erosional-holes (Figure 2.9).

Within selected roosts, I measured microclimate conditions for 24 roosts used by males, 12 used by non-reproductive females, and 17 used by lactating females. Internal temperature for roosts used by males ( $23.5 \pm 0.64^\circ\text{C}$ ), lactating females ( $24.0 \pm 0.65^\circ\text{C}$ ), and non-reproductive females ( $24.2 \pm 1.09^\circ\text{C}$ ), was not significantly different ( $F = 0.09$ ,  $P = 0.92$ ). Relative humidity in the roosts was also not significantly different among sex groups ( $F = 0.72$ ,  $P = 0.49$ ) with mean relative humidity in erosion holes selected by males at  $51.8 (\pm 3.29\%)$ , lactating females  $57.2 (\pm 4.03\%)$ , and non-reproductive females  $58.2 (\pm 6.74\%)$ .

I also measured internal temperature at various depths for 37 roosts (19 used by males, 12 by lactating females, 6 by non-reproductive females), and 29 suitable holes (Table 2.5). The maximum recorded depth averaged 39.31 ( $\pm 5.91$  cm) for selected and 29.77 ( $\pm 4.54$  cm) for suitable erosion holes and were not significantly different (Kruskal-Wallis chi-squared = 0.50,  $P = 0.48$ ). There were no significant differences (Kruskal-Wallis chi-squared = 1.34,  $P = 0.51$ ) in depth of erosion holes selected by males (48.40  $\pm 8.86$  cm), non-reproductive females (37.25  $\pm 19.95$  cm) and reproductive females (24.72  $\pm 3.54$  cm). There was also no significant difference in erosion-hole temperature between Max, Mid, or Min depths (Kruskal-Wallis chi-squared = 67.196,  $P = 0.436$ ) in selected roosts (Figure 2.10). However, internal temperatures within selected erosion holes at all depths were significantly different from ambient temperatures (Max:  $t = -5.73$ ,  $df = 61.70$ ,  $P = 3.2^{-7}$ ; Mid:  $t = -0.28$ ,  $df = 47.52$ ,  $P = 3.2^{-6}$ ; Min:  $t = -4.26$ ,  $df = 63.51$ ,  $P = 6.9^{-5}$ ).

## Discussion

Each individual *M. ciliolabrum* monitored within Dinosaur Provincial Park selected roosts in erosional-holes within the coulees. Other types of roosts available within my study site included rock cracks/crevices, tree crevices or buildings. Random erosion-holes did not differ significantly from those selected by *M. ciliolabrum* in terms of coulee slope, aspect, opening dimensions, and distance from the top of the coulee. Erosion-holes selected by bats were significantly higher off the ground when compared with random holes. These erosional features also act as a significant buffer from ambient conditions for both temperature and relative humidity. Depth did not have a significant effect as internal temperatures were significantly buffered from ambient conditions even in very shallow erosion-holes. Erosion-holes are

abundant in my study site and *M. ciliolabrum* switched roosts frequently but exhibited preference to a specific coulee.

I expected that as all roosts selected by *M. ciliolabrum* were erosion-holes that experience a low degree of roost stability, bats in my study site would exhibit low roost fidelity with lactating females switching least often (Lewis 1995, Kunz and Lumsden 2003). Males did switch roosts more frequently than both reproductive and non-reproductive females. This supports my hypothesis that lactating females would experience a higher cost of switching as they must carry non-volant pups between roosts (Vonhof and Barclay 1996). However, lactating females switched roosts every two days on average, suggesting that the costs of frequent roost switching are less than the benefits gained by relocating (Kunz and Lumsden 2003). Similar findings of daily roost switching in lactating females were observed for New-Zealand long-tailed bats (*Chalinolobus tuberculatus*) in areas where roosting opportunities were abundant (Sedgeley and O'Donnell 1999). My observations indicate there is an abundance of suitable bat roosts in Dinosaur Provincial Park and as a result, on average, bats relocate to a roost with similar qualities compared to their previous roost (Kunz 1982, Lewis 1995).

In areas which experience low roost fidelity and frequent roost switching, individual bats often exhibit high fidelity towards a given area (Kunz and Lumsden 2003). This type of fidelity is known as site fidelity; bats select roosts  $\leq 400\text{m}$  from their previous roost (Kunz and Lumsden 2003). Based on measurements for distance between bat roosts, I confirmed my prediction of high site fidelity. Males, non-reproductive females and lactating females selected roosts an average of  $211.63\text{m} (\pm 87.36\text{m})$  from their previous roosts. Individuals often exhibited high site fidelity to a given coulee, rather than to any specific roost location. With the exception of one lactating female, no roost was re-used by the same individual or by another bat being monitored.

As no individual bat ventured beyond their preferred coulee, roost switching is likely not motivated by the need to reduce proximity to resources.

There was no significant difference between selected and suitable roosts with regards to slope of the coulee, roost aspect, distance to horizontal above or largest dimension. Distance from nearest horizontal surface below was the only physical roost characteristic which was significantly different, with selected erosion-holes higher off the ground than random suitable holes. The top four models supported by the AIC analysis included DHB, Ldim and slope as potentially important roost characteristics when comparing selected to suitable roosts. Erosion-holes higher off the ground with narrow entrances and on a steeper slope may make it more difficult for terrestrial predators to access bats during the day. There are two species of snake in my study site, *Crotalus viridis* (prairie rattlesnake) and *Pituophis catenifer* (bullsnake) which may prey on roosting bats. The model suggests that these three variables could be important in selection of erosion-holes. The lack of significant difference in selected and suitable erosion-holes for slope and Ldim could be the result of all roosts being located in the same rock formation and these physical roost characteristics are limited by the nature of this rock type.

Comparing among selected erosion-holes, males, non-reproductive females and lactating females used roosts which did not differ significantly in aspect, slope, DHB or DHA. This contradicts my prediction. I expected that lactating females would exhibit different roost selection as they experience more energetic demands when compared to non-reproductive females or males. I expected lactating females would select more south-facing erosion-holes to reduce their need to use daily torpor with males and non-reproductive females selecting cooler roosts to facilitate torpor use (Hamilton and Barclay 1994, Solick and Barclay 2006a). This was not supported by my data. As there was no significant difference between roost aspect and a

random direction, this could suggest that the internal microclimate of the roosts is not significantly affected by the amount of direct sunlight received (Kunz and Lumsden 2003). The only significant difference between physical roost characteristics among sex groups was the largest dimension. Non-reproductive females roosted in erosion-holes with a larger opening when compared to males and lactating females. Non-reproductive females are not likely transient in my study site as I was able to track them for a similar number of days compared to reproductive females or males. Therefore, it is unlikely that these females are selecting larger erosion-holes due to lack of experience in my study site. My results do not offer any supporting data to explain why non-reproductive females selected these types of erosion-holes.

The internal microclimate within a bat roost should match the energetic demands of the bat which selected it. I predicted that reproductive females would have higher energetic demands compared to males and non-reproductive females and that this would be reflected in higher, more stable roost temperatures. However, roost temperatures in selected and suitable erosion-holes were statistically similar. In addition, roosts used by males, non-reproductive females and reproductive female *M. ciliolabrum* did not vary significantly from one another with regards to temperature. All roosts were equally buffered from ambient conditions, with holes cooler during warm ambient conditions (day) and warmer when ambient conditions were cooler (night). The similar internal conditions within erosion-holes suggest that bats experience similar roosting conditions regardless of roost selection. If, on average, erosion-holes have similar microclimates, it would also lower the risk of daily roost switching as an individual will most likely end up in a roost with similar environmental properties as their previous roost.

Internal relative humidity within selected erosion-holes was also statistically similar to random holes. All roosts experienced significantly higher humidity compared with ambient

conditions. This supported my prediction as these bats live in an arid region with low ambient relative humidity and as a result they may experience high rates of evaporative water loss (Thomas and Geiser 1997, Bogan et al. 2003). *M. ciliolabrum* were found in erosion-holes with mean relative humidity of 55% while average ambient conditions for my study site during this period was 44%. These erosional features are able to maintain higher levels of moisture, limiting EWL for those individuals roosting within these features. The fact that bats use this type of feature over more exposed roosts such as trees or under rocks, could suggest that limiting EWL is important in my study site.

As *M. ciliolabrum* uses roosts with relative humidity levels higher than ambient conditions, they may be at risk for increased ectoparasite loads (Moyer et al. 2002). Although the semi-arid conditions in Dinosaur Provincial Park may imply low parasite loads in resident bat species, humidity within bat roosts may provide a more suitable microclimate for some insect species. Individuals infected with parasites may experience a decrease in overall fitness as hosts may be required to divert energy away from other tasks to remove or avoid parasites (Richner et al. 1993, Moyer et al. 2002). Parasitism in bats has also been linked with the frequency of roost switching; infested roosts cause resident bats to switch regardless of reproductive condition (Lewis 1996, Bartonička and Gaisler 2007). Studies have shown that relative humidity levels lower than 60% can decrease survivorship of the early instar stages of bat bug (*Cimex pipistrelle*); bat bugs are a common ectoparasite found on bats in my study site (Bartonička 2010). If parasite levels in the park are high, it could be more beneficial for a bat to select a roost humid enough to reduce EWL but low enough to limit ectoparasite load. This trade-off may be observed by comparing relative humidity between sex groupings. Lactating females leave non-volant young within roosts while foraging, and therefore may need to select for more humid

roosts to offset risk of EWL in pups. There was no significant difference in relative-humidity levels among roosts of males, non-reproductive females or lactating females, which could imply that this trade-off is not significant for this species in my study site. The observed high frequency of roost switching may be related to limiting parasite load as bat roosts may provide a favourable microclimate for ectoparasites.

All selected erosion holes were located within the Dinosaur Park Formation (DPFm). This formation is approximately 75m thick and is situated on top of the Oldman Formation (40m) and below the Bearpaw Formation (prairie surface level; Eberth and Evans 2011). The DPFm consists of three layers; basal sandstone, angled layers of sand and mud, and bedded sandstones (Eberth and Evans 2011, Hathway et al. 2011). These units were deposited in a fluvial environment which is characterised by a fining upwards grain size trend from medium grained sandstone at the base to very fine grained sandstone or siltstone at the upper portion of the DPFm (Eberth and Evans 2011, Hathway et al. 2011). The DPFm is the primary formation exposed within the coulees in Dinosaur Provincial Park. This exposed unit is often overlaid by “popcorn” muddy-siltstone which erodes from the upper layers of the coulees and is transported down via runoff (Eberth and Evans 2011). This unit is repeatedly exposed through every successive coulee within the park indicating that bats are selecting roosts with relatively uniform geologic characteristics. As a result, the ability of each roost to retain heat and moisture should be relatively consistent across individual coulees. Finer grained substrate can retain moisture for longer periods of time which would allow for roosts to remain more humid for longer periods. As *M. ciliolabrum* are primarily roosting within these features it could explain why there is so little variation between selected erosion-hole roosts and random erosion-holes within my study.

The depth of the erosion-holes did not have a significant effect on how effective these features were as buffers from ambient conditions. There was no significant difference among erosion-hole temperatures at Max, Mid or Min distances. Temperatures at each of these depths were equally effective in limiting a bat's exposure to harsh ambient temperatures. As these data were recorded during the day, average internal conditions were significantly cooler than ambient temperatures. When looking at the actual distance measured for roost depth and its effect on temperatures, erosion-holes need not be deeper than 20cm to achieve the maximum buffering capacity for that roost (Figure 2.11). Erosion-hole temperatures at the aperture were also significantly different from ambient conditions suggesting there is little influence of air flow exchanging cooler erosion-hole temperatures with warmer ambient air.

Although there may not appear to be much selection exhibited by *M. ciliolabrum* when choosing erosion-holes, the act of selecting this type of roost may indicate a different level of selection within this species. Other potential roosting possibilities in my study site included manmade structures, rock crevices or tree crevices. Other bat species within this area, such as *Eptesicus fuscus* (Big Brown Bat) and *Myotis evotis* (Long-eared Bat), roost in rock crevices of fractured, more cemented boulders or rock layers (Holloway and Barclay 2000, Solick and Barclay 2006a, Nixon et al. 2009). *M. ciliolabrum* has also been observed roosting in these rock crevices in other similar environments (Rodhouse and Hyde 2014) but has also been observed roosting within caves or under tree bark (Holloway and Barclay 2001). Lausen (2001) measured the temperature variation in rock-crevices vs erosion-holes in a similar environment as my study site and found rock-crevices can reach exceedingly high daily temperatures (50°C). The erosion-holes were significantly cooler (20-25°C) with less variability than these thin walled crevices. Crevices with thicker walls also experienced more variability than the erosion-holes, as the

opening dimensions were larger and may have better facilitated airflow within these roosts (Lausen 2001). I thus suggest that *M. ciliolabrum* are selecting for roosts which can provide the most stable microclimate available within their habitat as erosion-holes are more adequately buffered from ambient conditions with limited air exchange ensuring microclimatic stability.

*Myotis ciliolabrum* in my study site roosts in shallow erosion-holes which do not differ significantly in temperature, humidity, slope, aspect, distance to horizontal above or largest dimension from randomly measured holes. Selected roosts are significantly higher off the ground, which could indicate a risk of terrestrial predation from snakes. This is supported by the GLM, as distance from the ground appears in the top four models along with other possible predation deterrents such as slope and largest opening dimensions. This species also switched roosts frequently which can be related to the abundance of suitable roosting sites, reducing predation risk, and limiting parasite load. Additionally, this species selects for structures that provide stable microclimate conditions that are buffered from the extreme ambient temperatures and reduce EWL. My results thus suggest that protection from environmental conditions, limiting predation risk and reducing parasite loads are important for *M. ciliolabrum* within Dinosaur Provincial Park.

## Figures

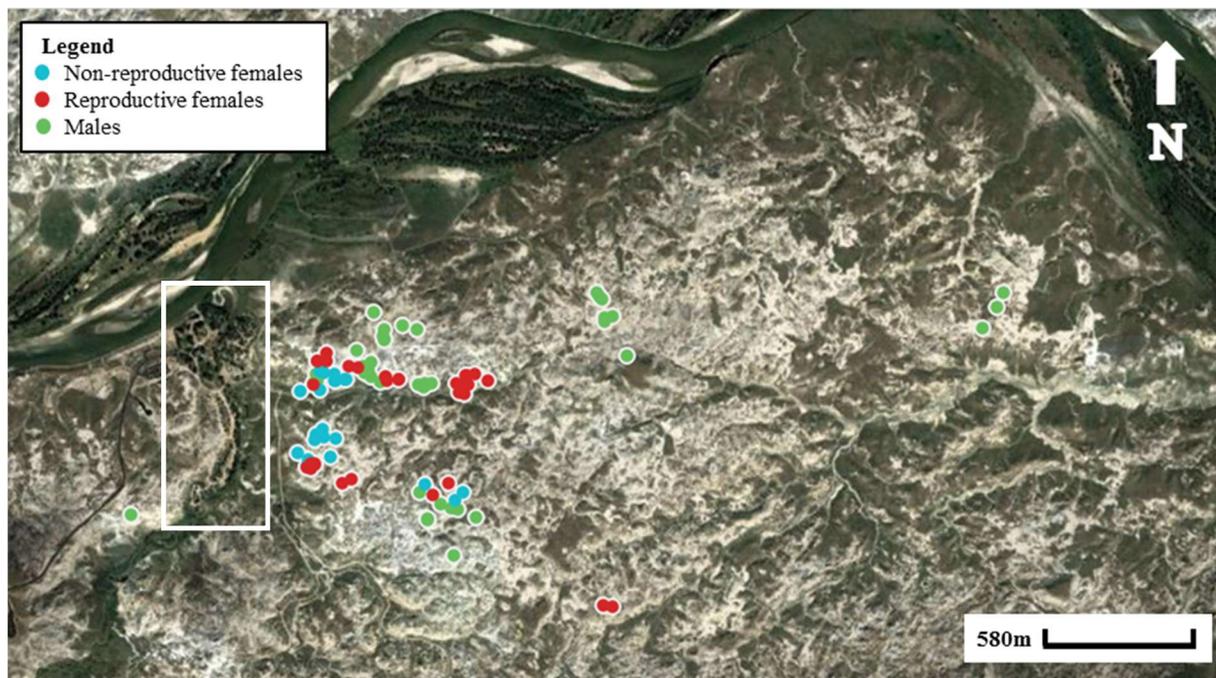


Figure 2.1: *Myotis ciliolabrum* roost locations within Dinosaur Provincial Park. The area within the white square is the public campground. Little Sandhill Creek runs through this site and drains into the Red Deer River.



Figure 2.2: Photo of an erosion-hole used as a roost by a male *Myotis ciliolabrum* in Dinosaur Provincial Park. These holes were the only roost type selected by *M. ciliolabrum* in this area regardless of sex or reproductive condition. Pen pointing towards opening for scale.

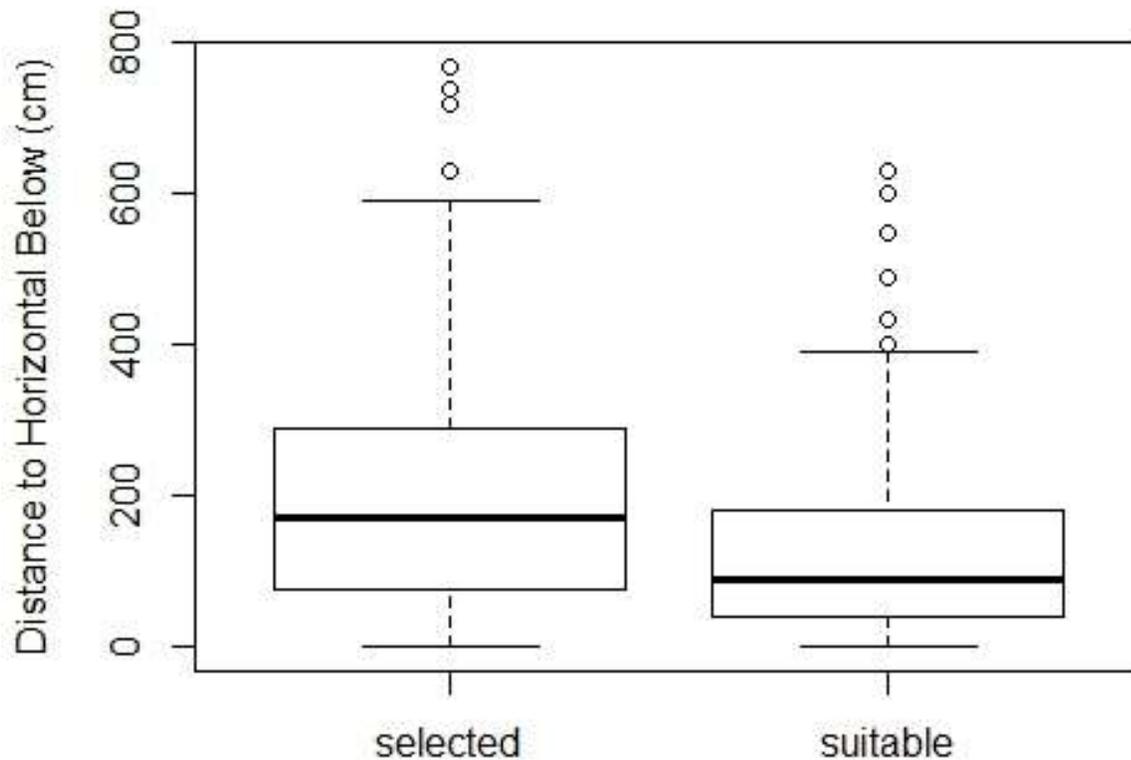


Figure 2.3: Mean distance to nearest horizontal surface below (cm) for selected versus suitable erosion-holes for *Myotis ciliolabrum*. Distance in selected roosts was significantly greater compared to suitable roosts (Mann–Whitney  $U = 4775.5$ ,  $P = 0.0029$ ). In this and subsequent Figures of this type, the median is represented by bold lines within the box, and the 75<sup>th</sup> quartile lies above while the 25<sup>th</sup> quartile is below the median line. Whiskers represent the maximum and minimum values with the circles indicating possible outliers.

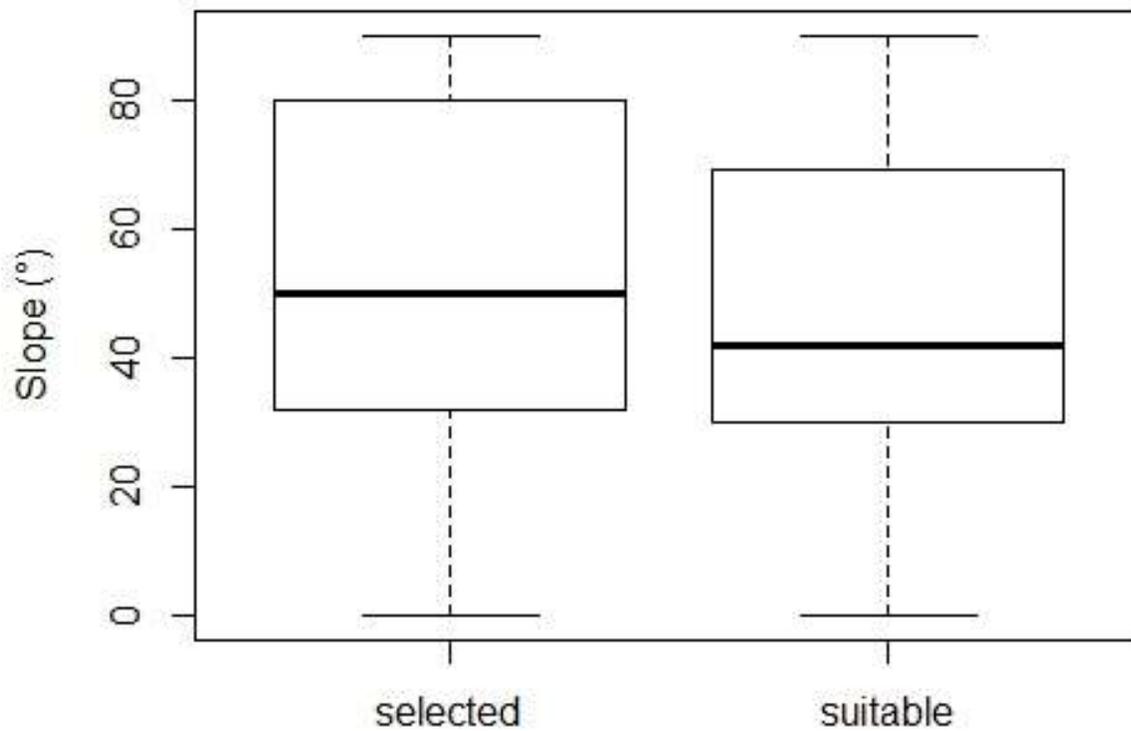


Figure 2.4: Mean slope (°) of the coulee for the selected ( $53.74 \pm 2.98$ cm) versus suitable ( $46.99 \pm 2.99$ cm) erosion holes of *Myotis ciliolabrum* in Dinosaur Provincial Park. The slope was not significantly different between selected and suitable roosts ( $t = 1.6015$ ,  $P = 0.1111$ ).

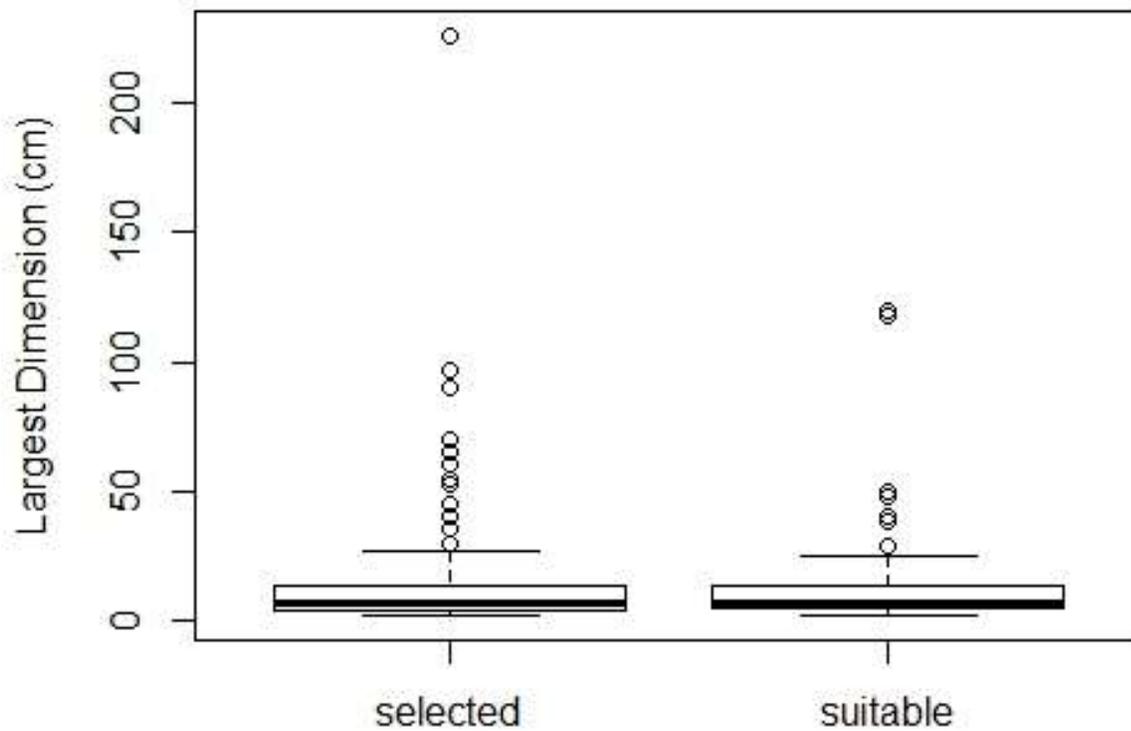


Figure 2.5: Mean values for the largest dimension (cm) of openings in selected ( $17.00 \pm 3.19\text{cm}$ ) versus suitable ( $13.12 \pm 2.027\text{cm}$ ) holes for *Myotis ciliolabrum* in Dinosaur Provincial Park.

There was no significant difference between the roosts selected by bats and randomly chosen holes deemed suitable (Mann–Whitney  $U = 3664.5$ ,  $P = 0.7185$ ).

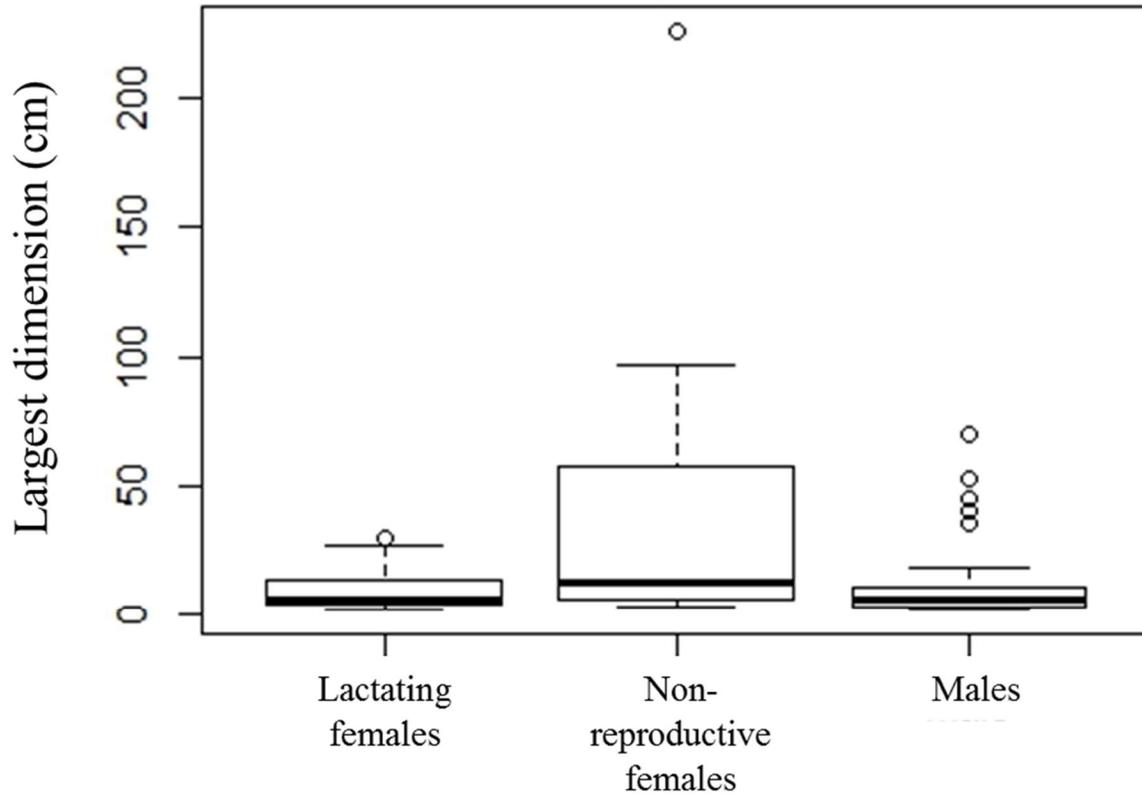


Figure 2.6: Mean values for the largest dimension (cm) of roost opening for lactating female ( $9.6 \pm 1.5$ cm), non-reproductive female ( $37.1 \pm 12.0$  cm) and male ( $12.1 \pm 2.5$ cm) *Myotis ciliolabrum* in Dinosaur Provincial Park. There was a significant difference among sex groups ( $F = 6.786$ ,  $P = 0.00185$ ); non-reproductive females selected crevices with larger opening dimensions.

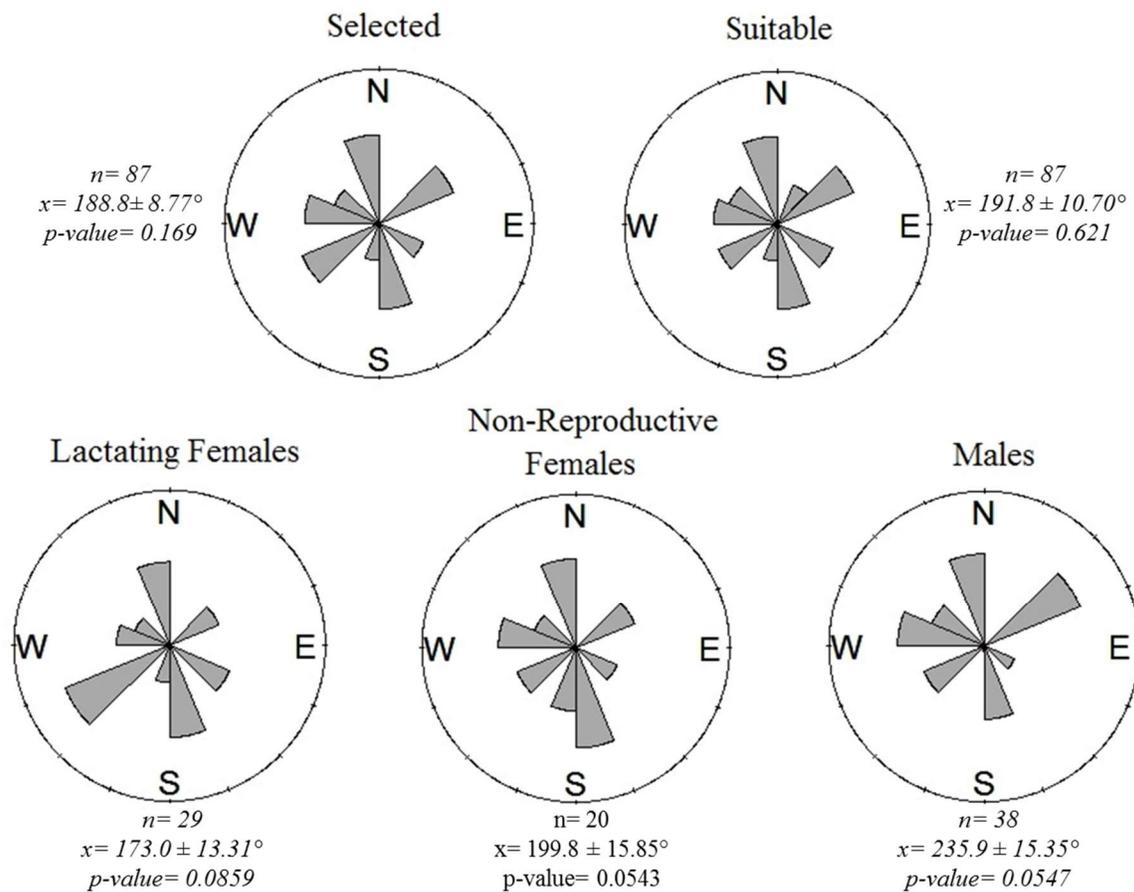


Figure 2.7: Roost aspect represented as compass direction ( $^\circ$ ) for selected and suitable erosion-holes for *Myotis ciliolabrum*. Among selected roosts are those selected by lactating females, non-reproductive females and male bats. There is no significant difference between aspect of roosts versus a random direction (Rayleigh's test  $p > 0.05$ ) for any roost type.

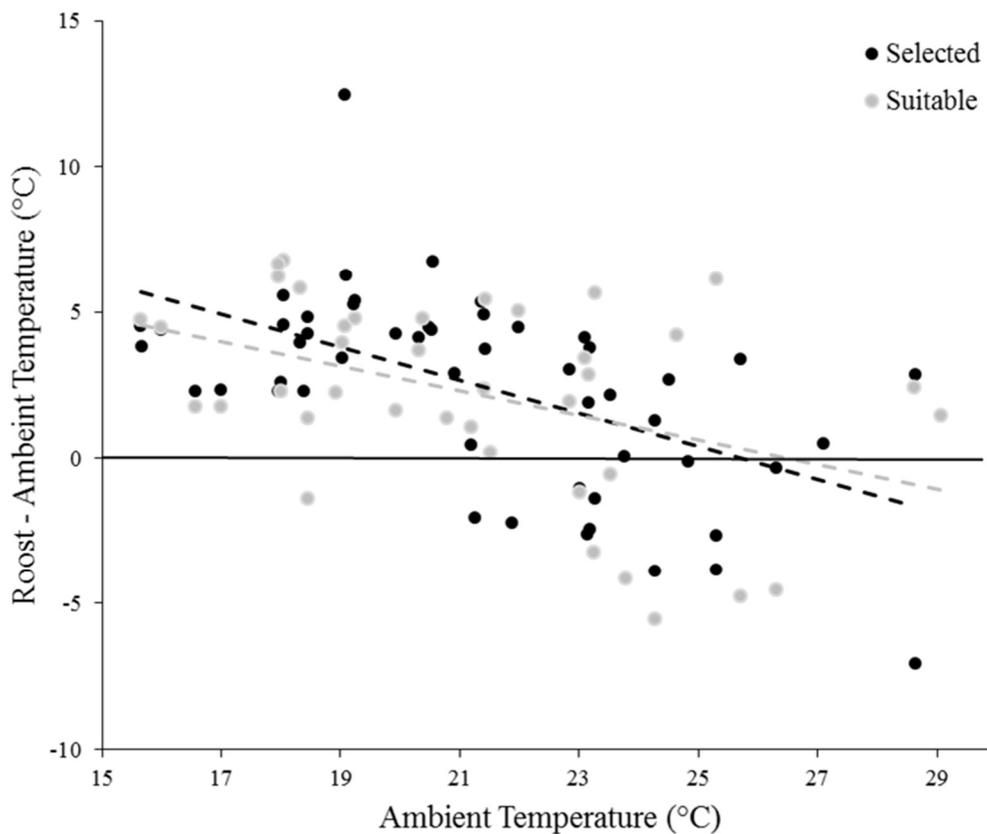


Figure 2.8: Buffering effect of erosion-hole roosts in Dinosaur Provincial Park. All holes, either selected by *Myotis ciliolabrum* or those deemed suitable for use, were warmer than ambient during low ambient temperatures and cooler than ambient during high ambient temperatures. Solid line represents no difference between ambient and erosion hole roost temperatures.

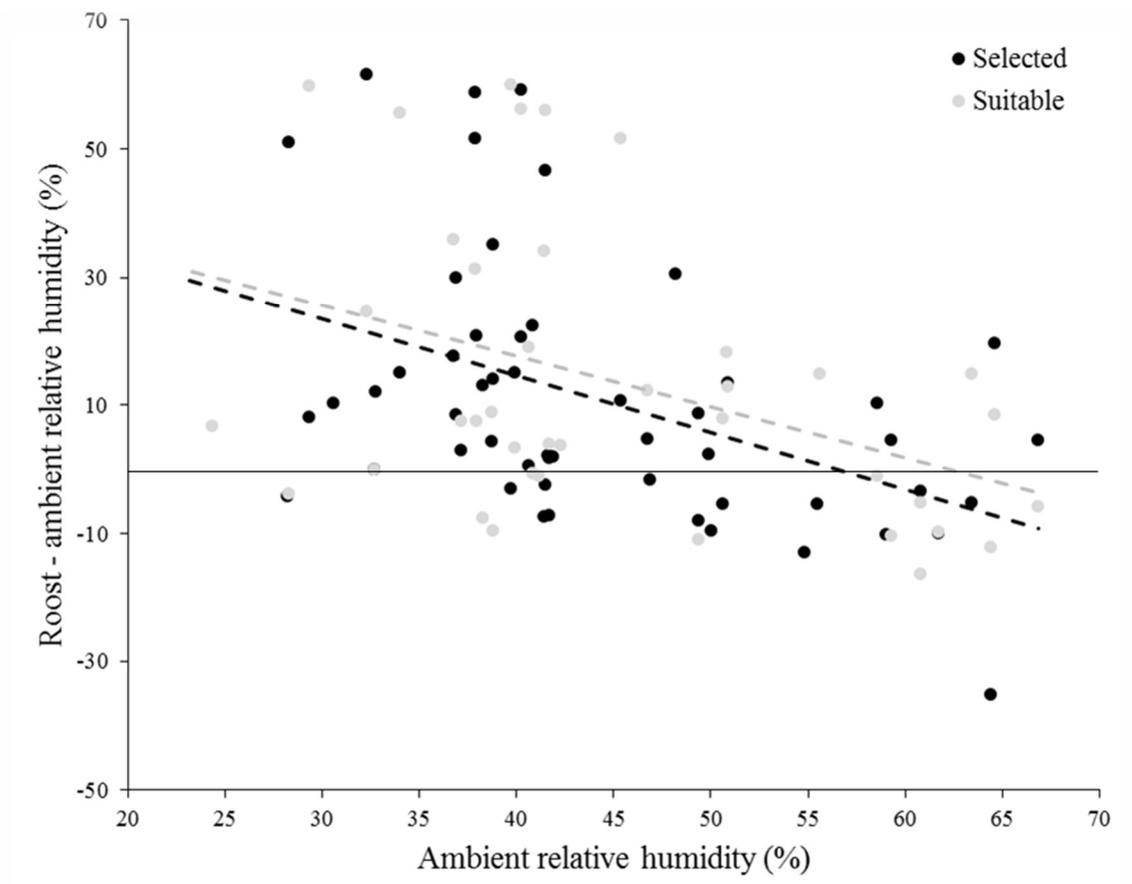


Figure 2.9: Buffering effect for relative humidity of erosion-hole roosts in Dinosaur Provincial Park. On average, erosion-holes, either selected by *Myotis ciliolabrum* or those deemed suitable for use, were more humid than ambient conditions. The solid line represents no difference between ambient and erosion-hole relative humidity.

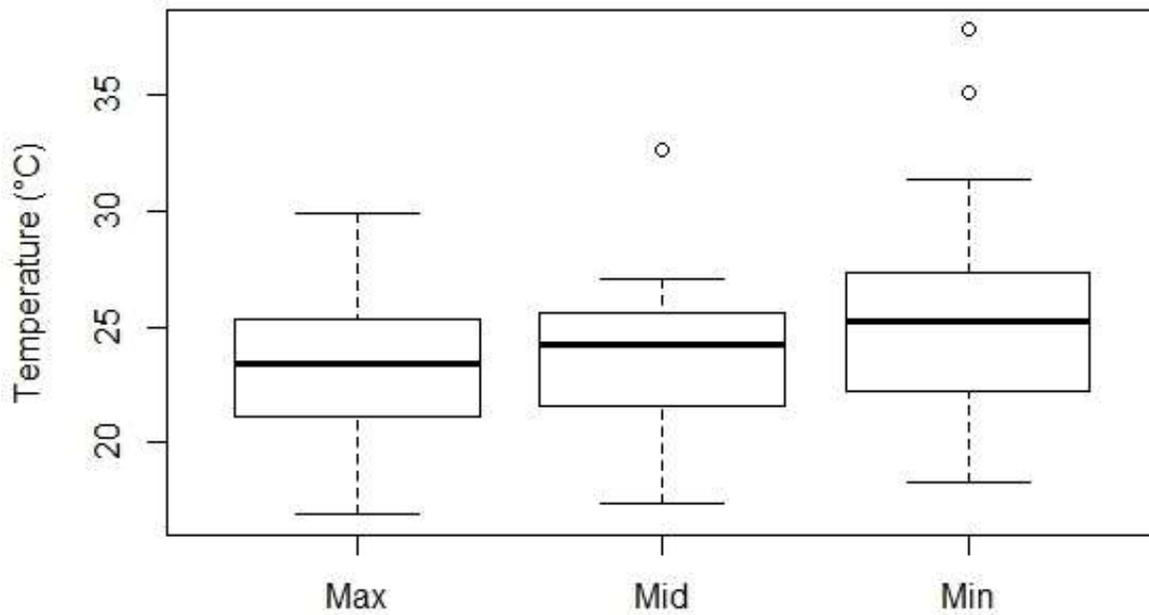


Figure 2.10: The mean temperature (°C) at various depths within selected *M. ciliolabrum* erosion-hole roosts (n=37). There was no significant effect of depth on roost temperature (Kruskal-Wallis chi-squared = 67.20, P = 0.44). Mean values for Max, Mid and Min depths were  $23.5 \pm 0.37^{\circ}\text{C}$ ,  $24.0 \pm 0.40^{\circ}\text{C}$  and  $25.2 \pm 0.50^{\circ}\text{C}$  respectively.

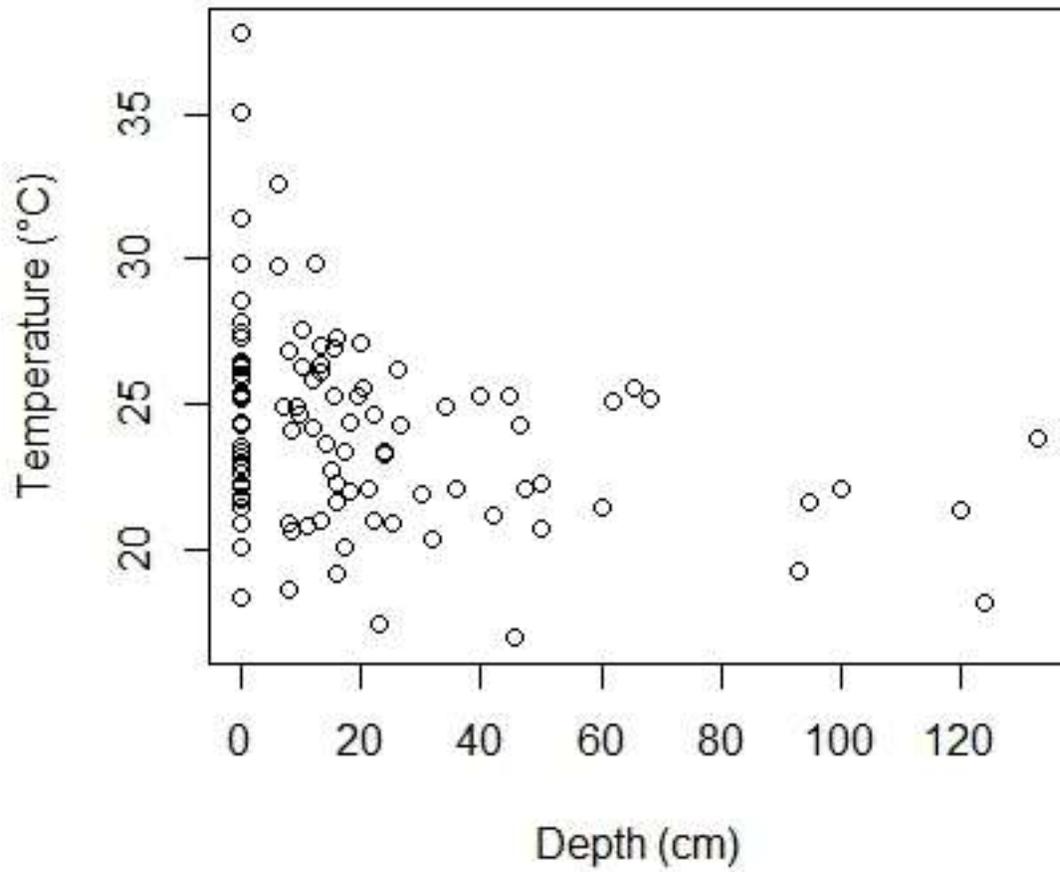


Figure 2.11: Roost temperatures (°C) in selected erosion-holes at various depths (cm). Roosts were selected by males, non-reproductive females and lactating female bats in Dinosaur Provincial Park.

## Tables

Table 2.1: Comparison of physical characteristics of erosion-holes selected by *Myotis ciliolabrum* and random suitable roosts in Dinosaur Provincial Park. The largest dimension was taken from the largest length (cm) or width (cm) measurements. The following variables were used in the generalised linear model.

Variable	Selected Roost ( <i>n</i> =87)		Suitable Roost ( <i>n</i> =87)		P
	x	SD	x	SD	
Distance horizontal below (cm)	211.2	187.4	136.3	145.2	0.0029*
Distance horizontal above (cm)	241.4	201.0	212.4	194.6	0.3308
Slope (°)	53.8	27.8	47.0	27.8	0.1111
Largest dimension (cm)	17.0	29.7	13.1	18.9	0.7185

\* indicates significant result

Table 2.2: Physical characteristics measured at erosion-hole roosts selected by various sex groups of *M. ciliolabrum*. The largest dimension was taken from the largest length (cm) or width (cm) measurements. An ANOVA was used to compare variables among sex groups.

Variable	Males ( <i>n</i> = 38)		Lactating females ( <i>n</i> = 29)		Non-reproductive females ( <i>n</i> = 20)		P
	x	SD	x	SD	x	SD	
Distance to horizontal below (cm)	187.1	172.0	222.3	171.6	240.9	235.8	0.5456
Distance horizontal above (cm)	234.4	30.6	268.3	199.4	216.8	230.7	0.6539
Slope (°)	55.8	29.0	48.1	25.4	58.0	28.6	0.3974
Largest dimension (cm)	12.1	15.6	9.6	7.9	37.1	53.6	0.0019*

\* indicates significant result

Table 2.3: The 15 logistic-regression models generated to compare the physical characteristics of erosion-holes selected by *M. ciliolabrum* to those of randomly available holes in Dinosaur Provincial Park. Models are ranked by the  $AIC_c$  results; lower scores indicate the higher ranked models. AIC weight ( $w_i$ ) indicates the probability of each being the best model given the subset of models run. If the evidence ratio ( $w_i/w_j$ ) is low it indicates support for both of the models being compared. For a complete description of each variable, see the Methods.

Model	$AIC_c$	$\Delta AIC_c$	$w_i$	$w_i/w_j$
DHB+SLOPE	231.148	0.000	0.189	
DHB	231.240	0.092	0.181	1.047
DHB+LDIM	231.285	0.137	0.177	1.071
DHB+SLOPE+LDIM	231.446	0.298	0.163	1.161
DHB+DHA+SLOPE	233.085	1.937	0.072	2.634
DHB+DHA	233.207	2.059	0.068	2.800
DHB+DHA+LDIM	233.336	2.096	0.063	2.986
Global Model (DHB+DHA+SLOPE+ LDIM)	233.482	2.242	0.059	3.212
SLOPE	237.577	6.337	0.008	24.891
LDIM	238.700	7.460	0.004	43.641
SLOPE+LDIM	238.827	7.587	0.004	46.502
DHA+SLOPE	238.848	7.608	0.004	46.993
DHA	239.063	7.823	0.004	52.326
DHA+LDIM	240.196	8.956	0.002	92.204
DHA+SLOPE+LDIM	240.200	8.960	0.002	92.388

Table 2.4: Mean ( $\pm$  SEM) for daily, maximum and minimum ambient temperatures ( $T_a$ ; °C) near Dinosaur Provincial Park for July 2014, July 2015, and August 2015. Daily mean ( $\pm$  SEM) for relative humidity (%) is also provided along with total precipitation (mm) with total days of precipitation events in parentheses (Alberta Climate Information Service 2016). There is no significant difference in daily mean  $T_a$  ( $F = 2.118$ ,  $P = 0.126$ ) or daily mean relative humidity ( $F = 0.908$ ,  $P = 0.407$ ) across field seasons.

Period	Mean $T_a$	Max $T_a$	Min $T_a$	Daily Mean Relative Humidity	Total Precipitation
July 2014	$20.0 \pm 0.47$	$28.2 \pm 0.75$	$11.3 \pm 0.39$	$61.3 \pm 1.61$	32.2 (13)
July 2015	$19.5 \pm 0.61$	$27.8 \pm 0.84$	$10.8 \pm 0.54$	$57.2 \pm 2.11$	22.7 (9)
August 2015	$18.3 \pm 0.71$	$26.6 \pm 0.99$	$10.0 \pm 0.46$	$59.4 \pm 2.60$	79.5 (9)

Table 2.5: Mean ( $\pm$  SEM) depth (cm), roost temperature ( $T_r$  °C) and corresponding ambient temperature ( $T_a$  °C) for selected and suitable holes for *Myotis ciliolabrum* in Dinosaur Provincial Park. Within selected roosts are mean ( $\pm$  SEM) values for lactating females, non-reproductive females and males.

Roost Type	Max			Mid			Min		
	Depth	$T_r$	$T_a$	Depth	$T_r$	$T_a$	Depth	$T_r$	$T_a$
Selected ( $n=37$ )	39.3 $\pm 5.91$	23.4 $\pm 0.52$	28.7 $\pm 0.53$	22.3 $\pm 3.32$	23.7 $\pm 0.54$	29.3 $\pm 0.90$	0.0 $\pm 0.00$	24.9 $\pm 0.66$	29.5 $\pm 0.92$
Suitable ( $n=29$ )	29.8 $\pm 4.54$	23.7 $\pm 0.53$	27.7 $\pm 0.79$	17.3 $\pm 2.53$	24.4 $\pm 0.59$	28.5 $\pm 1.08$	0.0 $\pm 0.04$	25.1 $\pm 0.77$	28.4 $\pm 0.99$
Lactating Females ( $n=12$ )	24.7 $\pm 3.54$	24.5 $\pm 0.66$	28.7 $\pm 1.25$	13.5 $\pm 1.96$	25.0 $\pm 0.69$	28.3 $\pm 1.44$	0.0 $\pm 0.00$	26.5 $\pm 1.06$	29.0 $\pm 1.33$
Non- Reproductive Females ( $n=6$ )	37.3 $\pm 19.95$	24.6 $\pm 1.53$	29.6 $\pm 1.66$	32.1 $\pm 17.17$	23.6 $\pm 1.53$	31.2 $\pm 1.32$	0.0 $\pm 0.00$	26.5 $\pm 1.42$	33.1 $\pm 2.73$
Males ( $n=19$ )	48.4 $\pm 8.86$	22.3 $\pm 0.72$	28.4 $\pm 1.17$	25.1 $\pm 4.58$	23.1 $\pm 0.78$	29.5 $\pm 1.32$	0.0 $\pm 0.00$	24.1 $\pm 0.97$	29.8 $\pm 1.31$

### Chapter 3

#### Echolocation call characteristics and species identification of *Myotis* during winter in Dinosaur Provincial Park

Chiroptera is the largest order of mammals capable of using echolocation to navigate and forage without light. By emitting sounds that are generally high in frequency (ultrasonic), bats are able to interpret the echoes and respond to the environment around them. This ability is present in both suborders of bats, with Microchiroptera as the primary implementers of echolocation. When an individual echolocates, the power, or amplitude, of the call decreases as it passes through the air and as a result the returning echoes are relatively weak (Altringham 2011). As nocturnal flying mammals, bats can be cryptic or difficult to capture via netting. As a result, determining habitat use, activity patterns or foraging habits of bats can be difficult. Acoustic surveys can be conducted to gather this type of information (Hayes et al. 2009, Parsons and Szewczak 2009). This type of research can be beneficial as it requires less manpower, is non-invasive and gives good insight regarding the natural behaviours of the species (Flaquer et al. 2007). However, not all species can be accurately differentiated acoustically and these species are either lumped together or misidentified. Creating a quality call library of calls from known individuals can aid in species identification (Barclay 1999, Hayes et al. 2009, Parsons and Szewczak 2009, White et al. 2014). However, other factors need to be considered when creating a call library as echolocation characteristics vary individually, geographically, ecologically or environmentally (Barclay 1999, Hayes et al. 2009, Parsons and Szewczak 2009, White et al. 2014).

Frequency modulated (FM) bat calls can be classified into three different stages of calls (1) search calls, (2) approach calls and (3) terminal calls. Typically, search calls sweep from high

to low frequencies in the bat's echolocation range. Once prey is located with a search call, bats switch to approach calls in which the time between pulses and call duration get shorter but the same frequency sweep is maintained (Griffin et al. 1960). The terminal set of calls is like a buzz; the time between calls is a fraction of a second and calls generally exclude the highest frequencies of a bats' range (Griffin et al. 1960). Search calls have characteristics which can be used to identify species; duration, maximum and minimum frequencies, time between calls and the overall shape of a search call are typically useful for species identification (Betts 1998, Barclay 1999, O'Farrell et al. 1999, Britzke et al. 2011). As a result, many studies passively monitor free-flying bats using acoustic recording devices and use these recordings to identify species' presence in a given area (e.g. White et al. 2014, Heim et al. 2015, Kaiser and O'Keefe 2015). This process is often automated using various software packages and can lead to erroneous results (Lemen et al. 2015). There are a number of different factors (bat ecology, environmental conditions, habitat type or population variation) which can effect species identification which need to be considered when acoustically identifying species. It is unclear if any of these factors were considered when these automated packages were constructed and often these models do not report validated model performance values which can affect software reliability (Russo and Voigt 2016).

The size and ecology of a bat influences the physical characteristics of its echolocation call characteristics. The size of an individual can affect the frequency a bat uses while echolocating. Smaller bat species feed on smaller insects which requires them to use higher frequencies. Low frequencies have long wavelengths which may not reflect off smaller insects. Larger species of bats can feed on larger prey items allowing for the use of lower frequencies (Griffin et al. 1960). For example, *Eptesicus fuscus* (big brown bat; 11-23g), has echolocation

calls ranging from 48 to 27 kHz and feeds on relatively large insects including moths and beetles (Kurta and Baker 1990). *Myotis lucifugus* (little brown bat; 8g), has calls sweeping from 80 to 40 kHz (Fenton and Barclay 1980). Both species are generalists, feeding on a variety of different insects in various habitats, but *M. lucifugus* is approximately 45% smaller than *E. fuscus* and requires the use of higher frequencies to detect smaller prey. Bat species with specialized feeding strategies have unique echolocation frequencies when compared to more generalist species. Some bat species are able to capture prey via aerial hawking as well as by gleaning insects off surfaces (Altringham 2011). Gleaning species use a number of different techniques to differentiate between insects and the surface they are on. They increase pulse rates and lower amplitude to gain enough detail to distinguish prey from surface (Altringham 2011).

Flying in clutter, requires additional alterations to frequencies and call duration used while maneuvering in complex spaces. Echoes from obstacles return more rapidly in highly cluttered environments and bats experience overlapping echolocation calls and the returning echoes (Fenton 1990). For bats to safely fly through complex cluttered environments, echolocation calls must be short in duration to limit call/echo overlap and avoid colliding with vegetation (Schumm and Neuweiler 1991, Schnitzler and Kalko 2001, Jung et al. 2007). Species which feed within clutter or off vegetation surfaces must also be able to discriminate prey from a variety of different surface types (Schnitzler and Kalko 2001, Jung et al. 2007). Echolocation calls with wide frequency sweeps allow for individuals to analyse textural patterns to locate prey on solid surfaces (Schmidt 1988). *Myotis evotis* is a small (5-8g) gleaning species with a broad range of echolocation frequencies, ranging from well above 100 kHz to 37 kHz, with short pulse intervals (Manning and Jones 1989). In contrast, species which fly in open spaces only experience call/echo overlap from potential prey items or other bats. They, however, require calls

that allow for detection of prey at greater distances. To increase the likelihood of hearing a returning echo, calls are lower in frequency, have narrower frequency sweeps and increased call duration (Fenton 1990).

Echolocation variation among populations in different geographic regions has also been observed (Parsons 1997, Barclay 1999, Parsons and Szewczak 2009). Call characteristics such as duration, minimum and maximum frequencies, and time between calls change as bats alter their calls to suit local conditions. Therefore, studies which aim to determine species via acoustic identification should include a call library which contains echolocation calls from known species of bats recorded within the environment they are being passively monitored (Barclay 1999, Parsons and Szewczak 2009). Collecting search calls from known individuals without altering a bats' natural search call characteristics is difficult. To ensure quality natural search phase calls you need to ensure bats are flying in an area/environment that mimics the conditions they are being passively monitored in (Parsons and Szewczak 2009). Recording bats in unnatural areas, such as enclosed spaces or within buildings, could have an effect on the type of echolocation call being made (Mukhida et al. 2004). Also, distance from the recording device and interference from passing unknown bats will have an effect on the quality of echolocation calls compiled for the library. Detectors placed far from a known bat may not record the higher search call frequencies with a bias towards the lower frequencies because as frequency increases, the rate at which the amplitude is absorbed by the atmosphere also increases (Lawrence and Simmons 1982). However, if the detector is too close, strong harmonics from a call will be recorded and will need to be removed prior to addition to the library (Parsons and Szewczak 2009).

Discriminating echolocation calls using a call library is often done via discriminant function analysis (DFA). DFA is a statistical tool used to evaluate multidimensional data such as

bat call characteristics (Parsons and Szewczak 2009, Tabachnick and Fidell 2013). The use of DFA to identify unknown bat species has become a common tool in bat research and is widely used (e.g. Vaughan et al. 1997, Gannon et al. 2004, Mukhida et al. 2004, Britzke et al. 2011). To increase model accuracy, echolocation calls should be analysed as sequences, not individually, to account for call variation (Britzke 2003). Furthermore, calls recorded in cluttered environments may result in different echolocation characteristics, therefore call-library sequences should be recorded in similar environments in which unknown species are being classified. This type of model is limited, however, as it is only able to identify species which were used to construct the model. As a result, if there are any unknown species in a study site, the model will fail to classify them (Britzke et al. 2011).

I tested the hypothesis that, within a habitat type, echolocation call characteristics vary among species. I predicted that the different foraging behaviours of three species of *Myotis* in Dinosaur Provincial Park allows for species' discrimination using echolocation calls, especially when call libraries are created from calls recorded in the appropriate habitat type. I also hypothesized that bats use echolocation calls with different characteristics in different habitat types. I predicted that bats alter their echolocation calls significantly in cluttered spaces when compared with open spaces. I predicted that echolocation calls within open spaces have longer duration and lower minimum frequencies when compared with calls from more cluttered habitats.

One of the goals of my research was to identify *Myotis* species active in my field site over the winter. Netting these species in the winter is inefficient as it is labour and time intensive due to the low numbers of bats present. I therefore conducted an acoustic survey throughout the winter. Previous research in Dinosaur Provincial Park over winter indicated that bats are

potentially hibernating in the park (Lausen and Barclay 2006). I predicted that creating an appropriate call library is necessary to accurately identify bat species. Therefore, I recorded calls from known individuals, flying in the same type of habitats I was passively monitoring in. The call library was used to construct a model which could then be used to identify unknown bat passes over winter.

## **Methods**

### *Study Species*

The western small-footed bat (*Myotis ciliolabrum*) is prevalent in the prairies of southeastern Alberta but ranges from western North America into northern Mexico (Holloway and Barclay 2001, Lausen 2007). *M. ciliolabrum* is the smallest species of bat in my study site weighing on average 4.5g (Holloway and Barclay 2001). This species is common in arid regions but uses the riparian zones in these areas for foraging, and roosts in adjacent outcrop cracks/crevices (Holloway and Barclay 2000, 2001). This is an insectivorous species, feeding on a variety of flying insects (Holloway and Barclay 2001). These bats are slow and maneuverable fliers, foraging near the ground and along rock bluffs (Schowalter and Allen 1981, Holloway and Barclay 2001). Echolocation calls vary geographically with average frequencies sweeping from >60 kHz to 40 kHz with call duration ranging from 1 to 5ms (Holloway and Barclay 2001).

The little brown bat (*Myotis lucifugus*) is a common species with a wide distribution across North America. It weighs on average 8g (Davis and Hitchcock 1965, Fenton and Barclay 1980). This species roosts in a variety of different habitats and uses various roost types (in tree hollows, under rocks, and in caves, buildings, or bat houses). *M. lucifugus* feeds primarily on insects with an aquatic larval stage and forages over slow moving creeks, streams, and lakes

(Fenton and Bell 1979). Echolocation calls last between 1-5ms and their sweep can range from 80 to 40 kHz (Fenton and Bell 1979, Fenton and Barclay 1980).

Western long-eared bats (*Myotis evotis*) inhabit temperate regions of western North American. These bats use a variety of habitats but are primarily found in forested areas, roosting in trees (crevices or under bark), in rock slabs, crevices in rock walls, caves or man-made structures (Manning and Jones 1989, Chruszcz and Barclay 2002). *M. evotis* is a gleaning species but can also hawk insects from the air (Faure and Barclay 1994). Echolocation calls vary depending on whether an individual is gleaning or hawking insects, but on average search calls range from 70 kHz to 30 kHz (Faure and Barclay 1994). Call duration varies from 1 to 2ms depending on an individuals' foraging strategy, whether hawking or gleaning (Faure and Barclay 1994).

### *Field Methods*

I captured bats in July to mid-August 2015 in Dinosaur Provincial Park, Alberta (50.7537, -111.528, 621–727 m). Bats were captured using mist nets in various locations within the park; along the edge of Little Sandhill Creek, in the cottonwoods (*Populus deltoides*) within the riparian zone, and in the coulees (away from water sources or canopy cover). Adult male and non-reproductive females were “kited” to collect calls from known individuals; reproductive females were excluded from the kiting process to reduce stress. Kiting is similar to ziplining bats, in which an elastic sewing thread is tethered to the bat via a loop around its neck (Parsons and Szewczak 2009). Once the thread was attached, the bat was released and it was given enough line to maintain flight while I held onto the other end of the thread. Once a bat attained steady flight, a field assistant recorded the echolocation calls using an Anabat SD2 (Titley Scientific, Ballina, NSW, Australia) as the bat flew in circles for several rotations. These calls from known

individuals were used to compile a call library specific to the local bat species within their natural environment. Environments were classified into two habitat types, the coulees and along the tree edge. The coulees are considered an open space lacking complex vertical structures whereas the tree edge are more cluttered due to the close proximity of trees.

I passively monitored the echolocation calls of bats in three different coulees within Dinosaur Provincial Park from late September 2014 until early March 2015. I used AnaBat SD2 (Titley Scientific, Ballina, NSW, Australia) units housed in water proof boxes running on an external 9V battery charged by two solar panels. All detectors were elevated approximately 1m above ground level with detectors pointing parallel to the ground.

#### *Acoustic call processing*

All bat calls (from known individuals in the call library and from passive monitoring) were analysed using AnaLookW (version 4.1t, Titley Electronics) with calls filtered to remove excess environmental noise such as wind or sounds from non-bats. *Myotis* calls from unknown individuals were selected for comparison with the call library by removing calls with frequencies <30 kHz. Any sequence of calls that had fewer than five calls, was not considered a bat pass and was removed. Lastly, echoes, overlapping bat calls and harmonics were manually removed from each sequence. I recorded 11 different call parameters (Figure 3.1). The knee divides the call into two halves. The first half is the more vertical portion of the call, with the second, or the body, the more horizontal portion of the call. I measured all call parameters including the location of the knee, using AnaLookW software to ensure repeatability of these measurements (Britzke et al. 2011).

### *Statistical Analysis*

I performed a correlation analysis on all call parameters to remove correlated variables; if the  $R^2$  value was equal to or greater than 0.70, I considered the variables to be highly correlated and one or both were removed (Zuur et al. 2009). Variable removal was dependant on which other parameter it was correlated with. For example, the maximum frequency (Fmax) and the slope before the body ( $S_1$ ) were correlated and as higher frequencies do not travel far, it is unlikely that passive monitoring stations capture the true maximum frequency of a call. Therefore, the  $S_1$  is more useful for call identification when compared with Fmax. A Multivariate Analysis of Variance (MANOVA) was used to compare call parameters for each species between the two habitats. Next I used a Discriminant Function Analysis (DFA) to determine which call parameter(s) was most important when discriminating between the *Myotis* species (Tabachnick and Fidell 2013). After correlation analysis, the model was constructed using six of the 11 parameters present, call duration (Dur), minimum frequency (Fmin), time between calls (TBC), frequency of the body (Fc), slope before the body ( $S_1$ ) and slope of the body ( $S_c$ ). Slope is expressed as octaves per second (OPS). Q-Q plots suggested, however, that some variables deviated slightly from normal. According to Tabachnick and Fidell (2013), a DFA is a robust analysis and can tolerate minor deviations from normality. They caution against arbitrarily transforming data to fit normality at the expense of altering the relationship of the parameters that could in turn skew the results of the DFA. Therefore, I ran the analysis on non-transformed data.

Cross-validation was performed on the models by removing values from the data set to independently test model accuracy (Tabachnick and Fidell 2013). To further assess the accuracy and sensitivity of the models, I also calculated the True Positive Rate (TPR), False Positive Rate

(FPR), Positive Performance Value (PPV) and Correct Classification Rate (CCR) for each species (Agranat 2013). The TPR was calculated by dividing the total number of times the model correctly classified species by the actual number of that species included in the model. FPR divides the number of times the model falsely identifies a species by the number of times that species was identified by the model in total. The PPV takes into consideration both the TRP and the FPR; the TPR was divided by the TPR and the FPR. Lastly, the CCR is the number of times the model predicts a species correctly divided by the total number of times the model predicted that species.

To test my hypothesis that habitat type influences call characteristics and the ability to correctly identify individuals, I used models from one habitat type to identify calls from known individuals recorded in the other habitat type. I then used a 3x3 contingency table to determine whether any of the models performed significantly differently in terms of TRP, PPV or CCR per species. Lastly, both models were used to identify unknown species collected from passive monitoring stations. A 3x3 contingency table was used to compare the proportions of each species identified by each model to test whether or not there was a significant effect of different habitat models and the number of each species identified from unknown bat passes. All statistical testing was performed using RStudio (version 0.99.489; R. RStudio, Inc) with  $\pm$ SEM provided unless otherwise specified.

## Results

I recorded a total of 1559 calls via kiting from known species of *Myotis*, 796 within the coulees and 730 next to trees. Within the coulees, 653 calls were collected from *Myotis ciliolabrum* (n=11 individuals), 121 from *Myotis evotis* (n=3) and 280 from *Myotis lucifugus* (n=9). Along the tree edge, 370 calls were from *Myotis ciliolabrum* (n=13), 158 from *Myotis*

*evotis* (n=4) and 202 from *Myotis lucifugus* (n=6). Of the 11 call parameters measured, five were highly correlated ( $R^2 > 0.70$ ) and were removed from further analysis leaving call duration (Dur), minimum frequency (Fmin), time between calls (TBC), frequency of the body (Fc), slope prior to the body ( $S_1$ ) and slope of the body ( $Sc$ ) in the MANOVAs and the DFA.

Within species, there was a significant difference between call parameters recorded in the two habitats (Table 3.1). *M. ciliolabrum* echolocation calls in the coulees were significantly different compared to tree-edge recordings ( $F_{6,19} = 4.1$ , Wilks  $\lambda = 0.436$ ,  $P = 0.008$ ). Call Dur and Fmin were significantly different between environments, with longer Dur (Figure 3.2) in the coulees ( $3.1 \pm 0.10$ ) compared to tree edges ( $2.65 \pm 0.10$ ), and Fmin (Figure 3.3) lower ( $39.8 \pm 0.36$ ) in the coulees versus tree edge ( $41.44 \pm 0.32$ ). *M. lucifugus*' echolocation calls were also significantly different ( $F_{6,4} = 27.98$ , Wilks  $\lambda = 0.023$ ,  $P = 0.003$ ) between coulees and tree edge. Call Dur, Fmin, TBC,  $S_1$  and  $Sc$  were significantly different between the two habitats (Table 3.1). I was unable to compare the effect of habitat type on echolocation calls of *M. evotis* due to small sample sizes.

Two models to identify calls were created using DFA, the first with known *M. ciliolabrum* and *M. lucifugus* calls within the coulees, and the second using calls collected along the side of a forested area (tree edge; Table 3.2). As I was unable to determine any significant effect of habitat conditions on call characteristics for *M. evotis*, all calls were pooled in both models for this species. Both models were then used to classify bat calls on known individuals recorded in various habitats (Table 3.3). Using TPR, there was a significant effect of habitat recording condition on model accuracy (chi-square test,  $X^2 = 29.51$ ,  $df = 6$ ,  $P = 4.9^{-5}$ ) for *M. ciliolabrum* and *M. lucifugus*. Models which classified known calls from the same environmental condition resulted in high TPRs when compared to those classifying calls from a different

environment, with the exception of *M. evotis*. For identifying *M. ciliolabrum*, the TPR was highest for the coulee model (95%), whereas the tree edge model more accurately identified both *M. evotis* (86%) and *M. lucifugus* (83%).

I also used both models to identify unknown species within the coulees. There was a significant difference between the two models in the total number of each bat species identified ( $X^2= 171.05$ ,  $df= 2$ ,  $P= 2.2^{-16}$ ). The coulee model identified 299 *M. ciliolabrum*, 232 *M. evotis*, and 115 *M. lucifugus*, whereas the tree-edge model identified 292, 64 and 290, respectively. The coulee model predicted significantly more *M. evotis* and significantly fewer *M. lucifugus* with the reverse when using the tree-edge model. Model had no effect on the number of times *M. ciliolabrum* was identified.

Echolocation calls passively monitored during the winter of 2014-2015 were in the coulees, therefore the calls used to build the model were library calls recorded in the coulees to ensure more accurate classification for unknown bats. The DFA was compiled using calls from 21 individual *M. ciliolabrum*, 9 *M. lucifugus*, and 7 *M. evotis* (Figure 3.4). The most important call parameters used to define species within the model were call duration, minimum frequency and the slope prior to the body (Table 3.4). The model was used to identify 646 bat passes, with 300 classified as *M. ciliolabrum*, 114 *M. lucifugus* and 232 *M. evotis* (Figure 3.5). There were high levels of activity in late September to early November, with a maximum of 140 bat passes per night, and a total of 619 bat passes over 46 days between September 25<sup>th</sup> and November 9<sup>th</sup>. Over this period, the model classified 294 as *M. ciliolabrum*, 111 as *M. lucifugus*, and 214 as *M. evotis*. Bat activity gradually decreased with the cooling of ambient temperature until November 10<sup>th</sup> 2014, when the first substantial cold period occurred (high of -10.8 and low of -20.5 °C) for the region. After this, bat activity rapidly decreased, with only 27 bat passes over the course of

the following 136 days. Of those, six were identified as *M. ciliolabrum*, three as *M. lucifugus* and 18 as *M. evotis*.

## **Discussion**

### *Model Performance*

My results supported my hypothesis that there is a significant environmental effect, with regards to the degree of clutter, on echolocation call characteristics for *M. ciliolabrum* and *M. lucifugus*. Within the coulees, *M. ciliolabrum* increased call duration and decreased minimum frequency when compared with tree-edge calls. The decrease in minimum frequency can increase the likelihood that a bat would perceive returning echolocation calls from a greater distance (Lawrence and Simmons 1982, Fenton 1990). Increasing proximity to more cluttered environments (i.e. in the tree-edge), resulted in *M. ciliolabrum* decreasing call duration and increasing minimum frequencies. This suggests that higher frequencies and short call durations are better features of echolocation calls to navigate complex spaces (Schnitzler and Kalko 2001).

*Myotis lucifugus* also altered echolocation calls when flying in cluttered compared to open spaces. Near tree edges, *M. lucifugus* increased call duration and time between calls, while decreasing minimum frequency, frequency of the body and the slope of the body. In open spaces, *M. lucifugus* echolocated using short calls with higher minimum frequencies and longer time between calls. These results do not support my predictions for bats echolocating near cluttered vs open environments. I predicted an increase in call duration, with lower minimum frequencies within open spaces. Previous research regarding this species flying in cluttered environments were only able to observe significant effects of clutter on echolocation call characteristics when the degree of clutter was high (Broders et al. 2004). As I recorded *M. lucifugus* echolocation

calls only next to, and not within, cluttered spaces, this suggests that this species might further adjust its echolocation calls in my study site if the density of the clutter was greater. The variation in echolocation call characteristics was significant for both *Myotis* species, indicating that these species are able to alter their echolocation calls depending on the nature of the habitat (Fenton 1990).

My results support my hypothesis that it is possible to determine species using acoustic call characteristics for three *Myotis* species within Dinosaur Provincial Park. Both models successfully identified all three species, with the tree edge model providing the best overall model performance for all three. My results supported my prediction that differences in behavioural ecology effect echolocation characteristics. *M. evotis* and *M. ciliolabrum* are clutter and open space specialists, respectively. As a result, there was little overlap between these species within the model. *M. lucifugus* is a generalist, overlapping both *M. evotis* and *M. ciliolabrum* in the model resulting in a higher error rate when identifying *M. lucifugus*.

Models performed significantly more poorly when using known individuals from the other habitat, with optimal model performance occurring when models were predicting species in the same type of environment as the model library was built on. This effect was most apparent when identifying *M. lucifugus*; there was a 40-50% decrease in model accuracy when identifying them in the different habitat. When using the tree-edge model on known coulee bat calls, the TPR was 22% and the CCR was 40%. In other words, the model was only capable of recognizing approximately two out of ten bat passes as *M. lucifugus*, and of those two, the model only correctly identified the species 40% of the time. However, when evaluating the tree-edge model using tree-edge recordings, the TPR was 75% with a CCR of 100%; i.e. three out of four *M. lucifugus* passes were recognized and they were correctly classified 100% of the time.

When evaluating model performance and species identification, my data suggest that species identification accuracy is highest in the bats' primary foraging habitat. *M. evotis* often forage in cluttered environments and are gleaning specialists with the ability to detect and capture prey from surfaces (Faure and Barclay 1994). The model with the best performance for identifying this species was the tree-edge model, with a TPR of 86% compared to 71% for the coulee model. *M. ciliolabrum* forage in open spaces with the coulee model achieving a higher TPR (95%) when compared to the tree-edge model (92%). Although typically generalists, *M. lucifugus* were captured more frequently within the forested areas of my field site, suggesting that this species may be spending more time within the trees compared to over the coulees or along the creek. This habitat preference is reflected in model performance for this species, with the TPR along the tree edge at 83% compared to 77% for the coulees. As bats alter their echolocation calls depending on habitat type, habitat-specific models can translate into better overall model performance.

The distinctiveness of the echolocation calls produced in more cluttered habitats has also been observed in other research, in which the degree of clutter affected model performance (Broders et al. 2004). *M. lucifugus* and *M. septentrionalis* (Northern long-eared bat; a gleaning species similar to *M. evotis*) were monitored acoustically in various clutter categories. Using a neural network model, researchers were able to correctly classify species with 82% accuracy within low (10m to horizontal clutter) to medium (3-10m to horizontal clutter) clutter. However, individuals in high clutter, where bats were within 3m of overhanging clutter, were correctly classified >94% of the time (Broders et al. 2004). This effect was observed in my model performance as the tree edge model resulted in a higher TPR for both *Myotis evotis* and *Myotis*

*lucifugus*. However, this may not apply for species such as *M. ciliolabrum*, which do not make use of these habitat types as the coulee model was more accurate when identifying this species.

Previous research into species identification using echolocation with my three study species is limited and often different modelling approaches have been applied, making direct comparisons with regards to model performance difficult. In my study, collecting echolocation calls from local individuals and identifying those within similar habitat types delivered more accurate results compared with more complicated modelling approaches. In one previous study, a researcher modelled various search calls from several species across several environmental types using the Hidden Markov Model (HMM), a commonly used method in speech recognition programs (Agranat 2012). Their models' TPR for *M. ciliolabrum*, *M. evotis*, and *M. lucifugus* were 63.2%, 36.4% and 56.3% respectively, when tested against their training data. The CCR for these species was considerably larger than their TPR (97.3%, 98.5%, and 94.5%), however, the nature of their model allows for calls to remain unclassified if they are deemed too poor in quality, whereas DFA will always identify calls regardless of call quality. As a result, comparing TPR is a more appropriate approach to compare model performance in this case, as the CCR in an HMM model can be skewed towards higher CCR values. The TPR in my DFA for both coulee and tree-edge models were higher for all three species when compared to this study, further emphasizing the importance of building models specific to local habitat type; this can optimise model performance and increase the TPR of more simplistic modelling approaches.

### *Winter Activity*

I applied the coulee DFA to echolocation calls from over 600 unknown bat passes over the winter in Dinosaur Provincial Park. All three *Myotis* species were identified from late September to the end of March. The first period of several successive days where maximum

daily temperatures were less than 0°C began on 10 November 2014, and bat activity fell sharply after this date. I therefore considered activity after this date to be those of local hibernating individuals. During periods of warming, when temperatures rose above 0°C, bat activity increased. These periodic arousals are regular occurrences during the hibernation cycle (Thomas et al. 1990) and winter flight during these periods has been previously observed in my study site (Lausen and Barclay 2006).

Prior to 10 November, bat activity was characterised as 47% *M. ciliolabrum*, 18% *M. lucifugus*, and 35% *M. evotis*. Lower detection rates for *M. lucifugus* and *M. evotis* coincides with low capture rates in this region during the fall (Unpublished data 2015). Activity after 10 November was characterised as 67% *M. evotis*, 22% *M. ciliolabrum* and 11% *M. lucifugus*. Lower actual numbers of bat passes may be the result of low populations, as Dinosaur Provincial Park lacks large cave structures preventing clustering of large numbers of hibernating bats (Lausen and Barclay 2006). Larger proportions of *M. evotis* activity during the winter months' conflict with previous research on cave-roosting populations, where *M. evotis* activity is absent over winter with emergence only occurring in April (Schwab and Mabee 2014). *M. evotis* was acoustically monitored during hibernation within several cave systems using acoustic detectors placed outside the hibernacula (Schwab and Mabee 2014). The data suggested that *M. evotis* did not fly out of the cave system over winter, but the study was unable to measure activity levels within the hibernacula where perhaps *M. evotis* may be more active than the data suggest. Winter flights by *M. evotis* in my study area may be profitable from a foraging perspective due to this species' gleaning specialisation, as terrestrial insects may be present, while aerial insects are rare/absent (Taylor 1963). This behaviour has been observed during summer periods, when *M. evotis* are able to extend foraging bouts later into the night when ambient temperatures drop and

aerial insect activity decreases but insects are still present on surfaces (Chruszcz and Barclay 2003).

## **Conclusions**

Conclusions drawn using echolocation identification using discriminant function analysis need to be taken with a degree of caution as such models may have several limitations. The model can only classify bat species according to the groupings used to construct the model. If other bat species are in the area but not included in the call library, the model will misidentify bat passes. This will affect the accuracy for the species within the model as they will now include data from the undocumented species. Further automation through complex software packages allow for confidence values for each species classification, but even this approach is not infallible (Russo and Voigt 2016). The quality of the call library (Barclay 1999), increased numbers of bat passes and manual processing of bat call sequences, allow for greater accuracy when identifying bat species (Russo and Voigt 2016).

Although my model suggests that all three *Myotis* species were present in Dinosaur Provincial Park over the winter, there were low numbers of bat passes after 10 November with only 3 bat passes classified as *M. lucifugus*. To determine whether or not this species is present in the area over winter, further analysis in areas with increased clutter should be monitored, as model performance for this species is more accurate. By monitoring over all habitat types, constructing call libraries from known individuals within similar environmental conditions can result in a more accurate assessment of species' presence within my study site.

## Figures

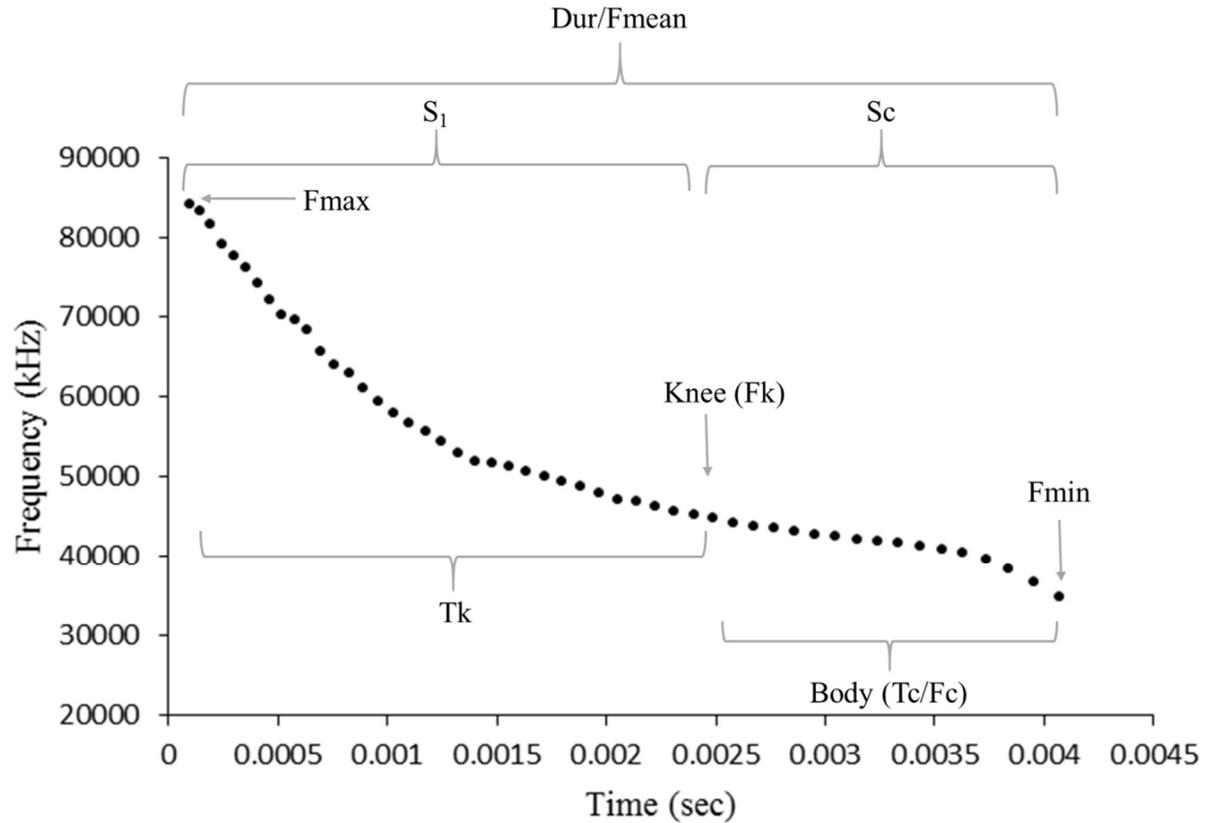


Figure 3.1: Measurements for each parameter per bat call using AnaLookW software. The knee is the point where a bat call changes from more vertical to more horizontal. The horizontal portion of a bat call is known as the body. A total of 11 parameters were measured (1) Dur: duration (ms) (2) F<sub>max</sub>: maximum frequency (kHz) (3) F<sub>min</sub>: minimum frequency (kHz) (4) F<sub>mean</sub>: mean frequency (kHz) (5) T<sub>k</sub>: duration to the knee (ms) (6) F<sub>k</sub>: frequency of the knee (kHz) (7) S<sub>1</sub>: initial slope (8) S<sub>c</sub>: slope of the body (9) T<sub>c</sub>: duration of the body (ms) (10) F<sub>c</sub>: frequency of the body (kHz) (11) TBC: time between calls (ms) (*not shown above*).

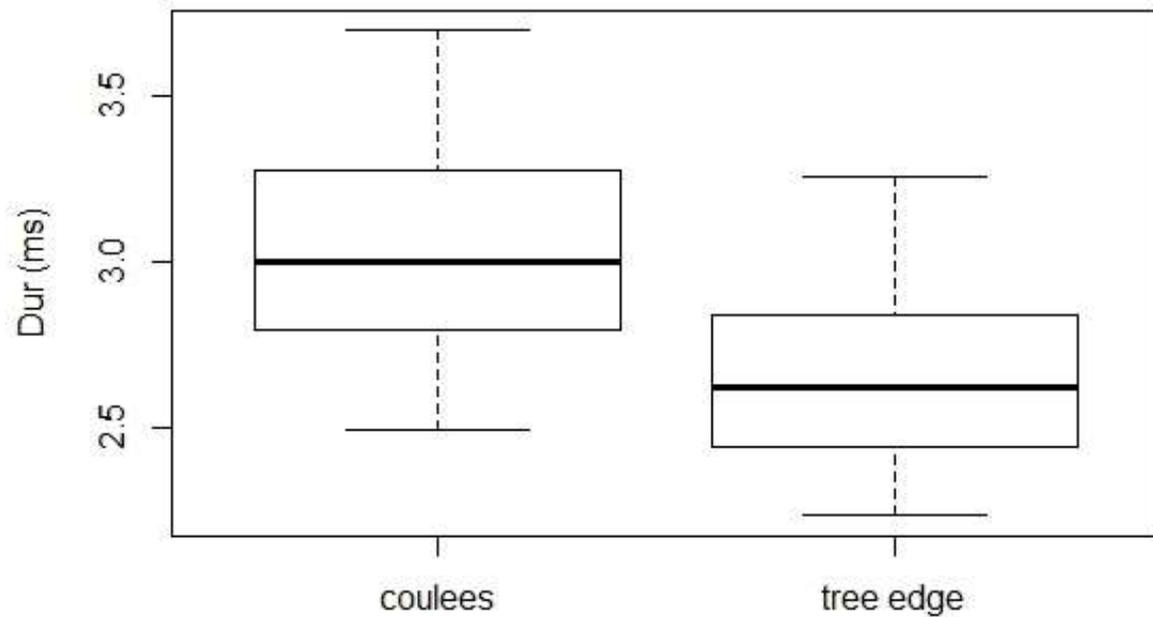


Figure 3.2: Mean call duration (ms) for *Myotis ciliolabrum* recorded in the coulees or at the tree edge in Dinosaur Provincial Park. *M. ciliolabrum* significantly decreased the duration of calls in more cluttered habitat compared to the coulees ( $F= 8.16$ ,  $P= 0.009$ ). For this and all subsequent boxplots, the median is represented by bold lines within the box and the 75<sup>th</sup> quartile lies above while the 25<sup>th</sup> quartile is below the median line. Whiskers represent the maximum and minimum values.

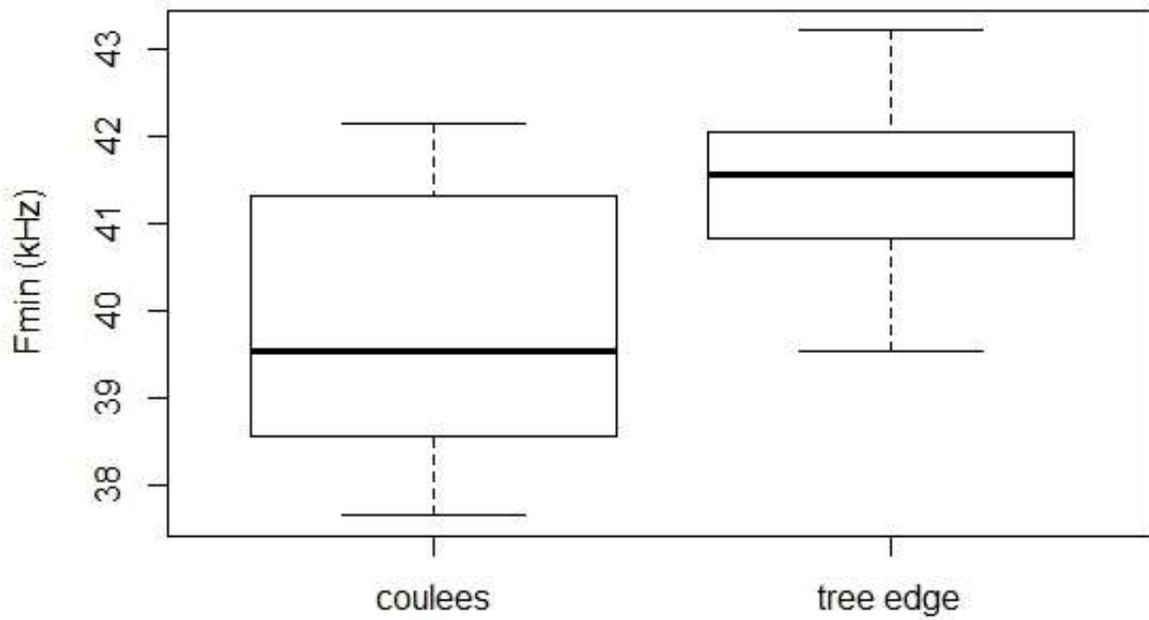


Figure 3.3: Mean minimum frequency (kHz) for *Myotis ciliolabrum* recorded in the coulees or at the tree edge in Dinosaur Provincial Park. *M. ciliolabrum* significantly increased minimum frequency of calls in more cluttered habitat compared to the coulees ( $F= 9.66$ ,  $P= 0.005$ ).

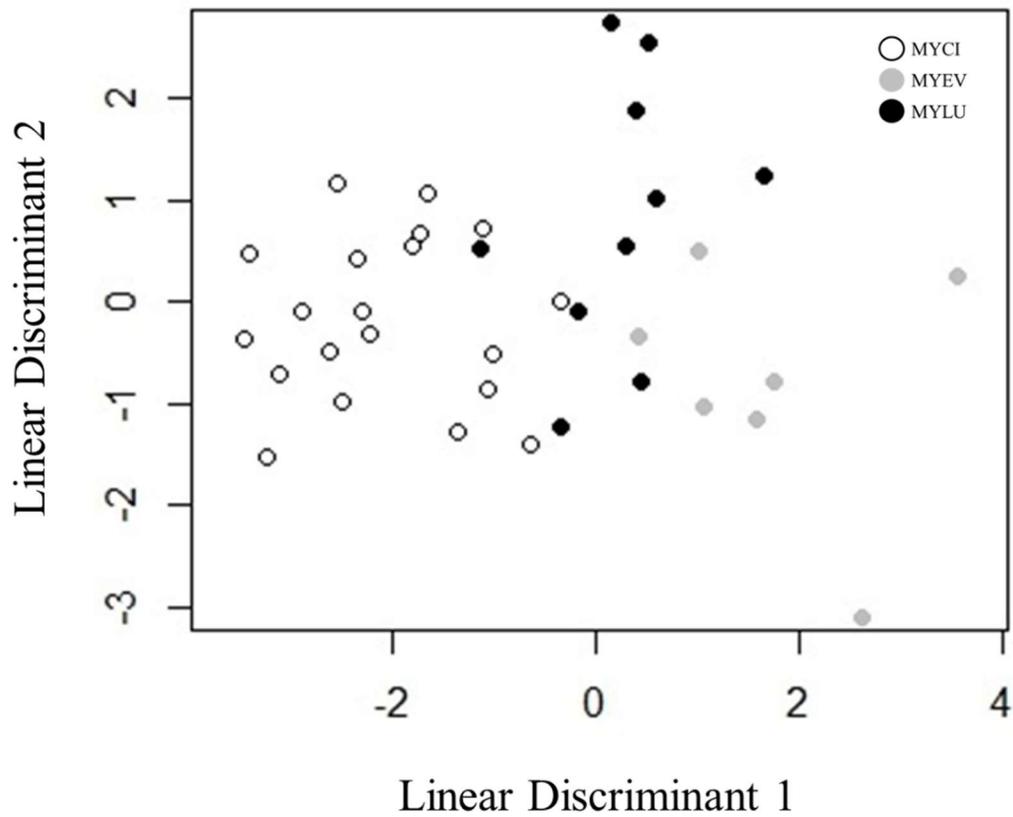


Figure 3.4: Linear discriminant analysis results for three bat species, *Myotis ciliolabrum* (MYCI), *Myotis evotis* (MYEV), and *Myotis lucifugus* (MYLU). Acoustic call characteristics, duration, minimum frequency, time between calls, frequency of the body and slope were used to calculate the two linear discriminants in the coulees.

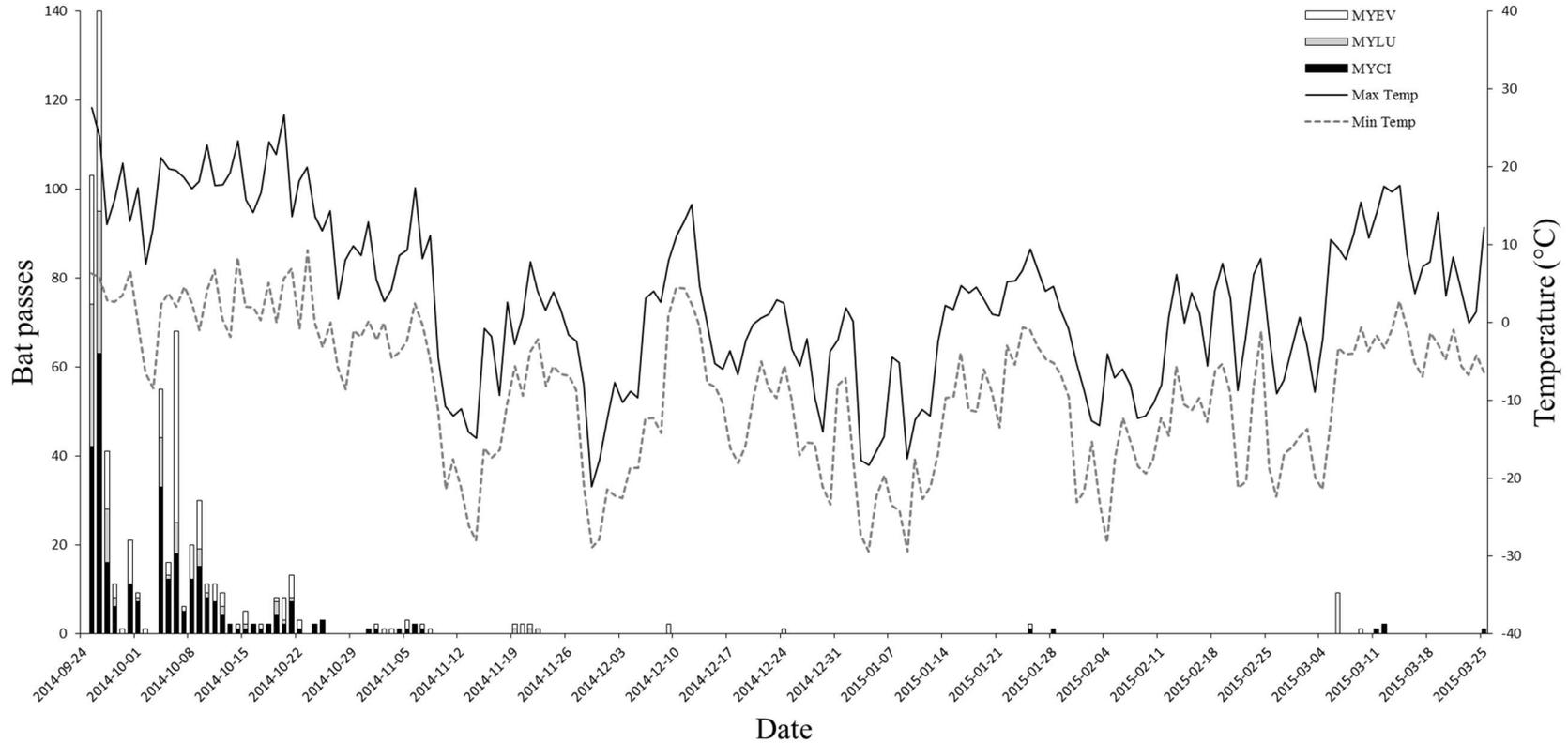


Figure 3.5: Bat passes per night with respective temperatures in Dinosaur Provincial Park during the winter of 2014-2015. The solid line represents average daily maximum temperature and the dashed line is daily average minimum temperature. *Myotis* species were classified using the DFA, black bars as *Myotis ciliolabrum* (MYCI), grey bars as *Myotis lucifugus* (MYLU) and white bars as *Myotis evotis* (MYEV).

## Tables

Table 3.1: Mean ( $\pm$ SEM) values for all call parameters for *Myotis ciliolabrum* (MYCI) and *Myotis lucifugus* (MYLU). There was a significant difference in some echolocation call parameters with the environment in which they were recorded. Call parameters outlined in the Methods section.

Parameter	MYCI			MYLU		
	Coulees ( <i>n</i> =21)	Tree Edge ( <i>n</i> =13)	p-value	Coulees ( <i>n</i> =9)	Tree Edge ( <i>n</i> =6)	p-value
Dur	3.1 $\pm$ 0.09	2.65 $\pm$ 0.10	0.009*	3.05 $\pm$ 0.15	4.0 $\pm$ 0.44	0.0004*
Fmin	39.8 $\pm$ 0.36	41.44 $\pm$ 0.32	0.005*	38.3 $\pm$ 0.25	36.5 $\pm$ 0.58	0.032*
TBC	69.7 $\pm$ 3.35	79.00 $\pm$ 2.57	0.06	82.9 $\pm$ 5.53	105.5 $\pm$ 6.00	0.009*
Fc	42.9 $\pm$ 0.30	43.65 $\pm$ 0.40	0.15	41.5 $\pm$ 0.43	40.0 $\pm$ 0.64	0.0005*
S <sub>1</sub>	729.6 $\pm$ 32.83	692.46 $\pm$ 27.90	0.44	573.5 $\pm$ 34.92	604.9 $\pm$ 38.69	0.48
Sc	189.0 $\pm$ 9.82	213.11 $\pm$ 13.62	0.15	225.7 $\pm$ 25.34	179.9 $\pm$ 40.85	0.027*

\* Indicates significant difference

Table 3.2: Discriminant function analysis performance for species-species level accuracy for both the coulee and tree-edge models. Numbers in bold represent correct species identification by the model. The coulee model is able to identify *M. ciliolabrum* with the highest accuracy while the tree-edge model most accurately identified *M. evotis* and *M. lucifugus*.

Predicted Species	True Species					
	Coulee Model			Tree Model		
	<i>Myotis ciliolabrum</i>	<i>Myotis evotis</i>	<i>Myotis lucifugus</i>	<i>Myotis ciliolabrum</i>	<i>Myotis evotis</i>	<i>Myotis lucifugus</i>
<i>Myotis ciliolabrum</i>	<b>20</b>	0	2	<b>12</b>	1	0
<i>Myotis evotis</i>	1	<b>5</b>	0	1	<b>6</b>	1
<i>Myotis lucifugus</i>	0	2	<b>7</b>	0	0	<b>5</b>
<b>Cross-validation</b>						
<i>Myotis ciliolabrum</i>	<b>18</b>	0	3	<b>11</b>	2	0
<i>Myotis evotis</i>	1	<b>5</b>	1	2	<b>4</b>	1
<i>Myotis lucifugus</i>	2	2	<b>5</b>	0	1	<b>5</b>

Table 3.3: Model classification results using known bat calls in two habitat types from Dinosaur Provincial Park. Model accuracy is measured using True Positive Rates (TPR), Positive Predictive Values (PPV) and Correct Classification Rate (CCR). See Methods section for specific calculations.

<b>Model : Calls</b>	<i>Myotis ciliolabrum</i>			<i>Myotis evotis</i>			<i>Myotis lucifugus</i>		
	<b>TPR</b>	<b>PPV</b>	<b>CCR</b>	<b>TPR</b>	<b>PPV</b>	<b>CCR</b>	<b>TPR</b>	<b>PPV</b>	<b>CCR</b>
Coulee : Coulee	95%	91%	90%	71%	81%	83%	77%	78%	77%
Coulee : Tree Edge	77%	77%	76%	71%	72%	71%	33%	33%	33%
Tree Edge: Tree Edge	92%	92%	92%	86%	77%	75%	83%	83%	100%
Tree Edge : Coulee	81%	84%	85%	86%	63%	50%	22%	26%	40%

Table 3.4: Mean ( $\pm$ SEM) values for echolocation call parameters for three *Myotis* species in Dinosaur Provincial Park. The following call characteristics were used to create the coulee model to predict species from unknown bat calls recorded in the region. See Methods for parameter definition.

Parameter	Dur (ms)	Fmin (kHz)	TBC (ms)	Fc (kHz)	S <sub>1</sub>	Sc
<i>Myotis ciliolabrum</i> (n=21)	3.1 $\pm$ 0.09	39.2 $\pm$ 0.38	74.3 $\pm$ 3.91	42.4 $\pm$ 0.40	711.4 $\pm$ 30.97	192.0 $\pm$ 13.38
<i>Myotis lucifugus</i> (n=9)	3.1 $\pm$ 0.15	38.3 $\pm$ 0.25	82.9 $\pm$ 5.53	41.5 $\pm$ 0.43	573.5 $\pm$ 34.92	225.7 $\pm$ 25.34
<i>Myotis evotis</i> (n=7)	2.6 $\pm$ 0.07	36.1 $\pm$ 1.06	83.2 $\pm$ 5.23	42.2 $\pm$ 0.99	601.5 $\pm$ 53.08	308.5 $\pm$ 53.84
P-value	0.026*	0.002*	0.31	0.55	0.026*	0.013*

\* Indicates significant difference

## Chapter 4

### General Conclusions

Bats living in temperate regions face a number of energetic challenges as ambient conditions are not always suitable, and as small, flying, homoeothermic mammals, they need to use various strategies to limit energy lost during these unfavourable periods. Investigating roost selection ecology and behaviour can provide valuable insight into the success of bat populations and roost microclimates can act as a buffer from ambient conditions. Bats inhabiting unique landscapes, such as the badland areas of Alberta, including Dinosaur Provincial Park, will have adapted to local conditions, and may behave differently when compared with individuals in other populations in less harsh landscapes. Therefore, understanding these behaviours and identifying habitat use over time is necessary when trying to successfully assess the effect of white-nose syndrome in these populations prior to its arrival in Alberta.

In Chapter 2, I showed that *Myotis ciliolabrum* in Dinosaur Provincial Park roost exclusively in erosion-holes over the summer. As I predicted, these impermanent roost types lead to low roost fidelity, with individuals switching every 1 to 2 days, depending on sex. Although *M. ciliolabrum* switched roosts frequently, individuals all exhibited high fidelity to small home regions with less than 400m between previous roosting sites. Physical roost characteristics within selected roosts did not differ from other randomly available holes, with the exception of distance from nearest horizontal surface below, with selected roosts being higher off the ground. This suggests that terrestrial predator avoidance may be an important factor for bats when selecting roosts within the park. When comparing selected to random suitable roosts using a general linear model, the top four models included distance from horizontal below, slope and largest dimension as important features for selected roosts. However, the low  $R^2$  value (0.045) for the highest

ranked model suggests these features alone do not account for all the variation between selected and suitable roosts. These results could indicate this species is exhibiting a different level of selection in terms of the type of roost *M. ciliolabrum* are using. Other suitable roosting features in my study site included manmade structures, under tree bark, or in cracks/crevices in rocks. However, these other features are not as prevalent as erosion-holes and exhibit different internal microclimates (Holloway 1998, Lausen 2001).

Internal roost conditions within selected erosion-holes were not significantly different from those of other randomly available holes, or when comparing roosts used by individuals of different sex and reproductive status. This contradicted my prediction that reproductive status would have an effect on roost selection, specifically that lactating females with their pups would select warmer, more humid roosts to better facilitate lactation and pup development. All selected roosts were significantly buffered from ambient temperature and relative humidity. Roosts were warmer at night and cooler during the day, with higher relative humidity within the roost when compared to ambient conditions. Depth also had no effect on the buffering capacity of these roosts during the day, with temperatures at the aperture of the roost significantly lower than ambient conditions. My results indicated roosts need not be deeper than 20cm to gain the maximum buffering capacity of any erosion-hole within the park.

My research leads to follow-up questions which can be addressed in future research regarding the roosting ecology of *M. ciliolabrum* and other *Myotis* species within Dinosaur Provincial Park. As I only measured the effect of depth on internal roost temperature during the day, measuring temperature at various depths overnight would give clearer results with regards to the effect of depth on the roost microclimate. Previous research has shown that depth had little effect on daily maximum temperatures within erosion-holes, but more variation was observed at

different depths when roost temperatures were recorded overnight (Lausen 2001). Also, accurately measuring depth in these types of roosts proved difficult, as these features are erosional in nature, resulting in convoluted channels within the coulees. By using ultrasound or other geophysical methods it may be possible to map the internal structure of erosion-holes. Currently however, this technology lacks an affordable method with a suitable resolution to effectively measure these small features. Lastly, measuring the buffering capacity of these roost types over winter could provide insight on whether or not erosion-holes are suitable structures in which *M. ciliolabrum* can hibernate over winter in Dinosaur Provincial Park. My results in Chapter 3 indicate two *Myotis* (*M. ciliolabrum* and *Myotis evotis*) species are present in my study site over winter and their winter roost selection is currently unknown. Gathering accurate data regarding species presence in a given habitat is crucial if management strategies are going to be effective.

In Chapter 3, I used echolocation calls from known individuals to construct a model which was used to identify unknown bat passes over winter in Dinosaur Provincial Park. My results supported my predication that habitat type would have an effect on the echolocation call characteristics in *Myotis* species. In both *Myotis lucifugus* and *M. ciliolabrum*, echolocation calls recorded within the coulees significantly differed when compared with those recorded along the tree edges. This translated into a significant effect on the accuracy of species identification. Models constructed using calls recorded in one habitat type had a higher True Positive Rate (TPR) when identifying bat calls within the same habitat. My research failed to identify the effect of habitat on the echolocation characteristics for *M. evotis* due to a small sample size. Future research is needed to provide more accurate results regarding the effect of habitat on this species.

Habitat specific models also had a significant effect on the proportions of *Myotis* species identified from unknown bat passes over winter. The coulee model predicted more *M. evotis* and fewer *M. lucifugus* with the inverse of this relationship when using the tree edge model. However, there was no effect of model type and the proportion of *M. ciliolabrum* identified. My results indicate that habitat type needs to be taken into consideration when constructing a model to increase model performance when identifying acoustically similar bat species. My results also indicate that optimal model performance on a species level occurred when using a model constructed within the bats' primary habitat type. The coulee model exhibited a higher TPR when identifying *M. ciliolabrum*, an open space specialist, whereas the tree edge model performed better at identifying both *M. lucifugus* and *M. evotis*, both of which were primarily captured in more cluttered environments.

I passively monitored *Myotis* in the coulees of Dinosaur Provincial Park over the winter of 2014-2015. Bat activity dropped as ambient conditions decreased with only 27 *Myotis* passes recorded over winter. Any activity occurring over winter in my field site was correlated with periods of warming during chinooks. This correlation has also been observed in previous research (Lausen and Barclay 2006). Over winter, *M. evotis* was observed more frequently in my study site followed by *M. ciliolabrum* and *M. lucifugus*. My data suggest that *M. ciliolabrum* and *M. evotis* hibernate in Dinosaur Provincial Park over winter. Although the model did identify three *M. lucifugus* over this period, the coulee model is not optimal for identifying this species. Given the low number of bat passes for this species, it is unclear whether these results are the product of model error. Further research is needed to determine whether or not *M. lucifugus* is present in the park over winter.

*Myotis lucifugus* was primarily captured within the trees along the riparian zones of the park, whereas other populations of this species typically forage over water. A model built using calls collected over water may provide more accurate results for this species in the future. Passively monitoring in every habitat type over winter will give a clearer picture with regard to habitat use in winter as well as determine whether or not *M. lucifugus* is indeed hibernating in the area. I only monitored in the coulees, which could result in species bias when determining presence or absence in my study site. Future research could also test which modelling approach would be most accurate when identifying these three *Myotis* species. There are other approaches to modelling multiple variables; neural networks, geometric morphometry, or custom built algorithms for pattern recognition have also been used to identify bats acoustically (Parsons and Szewczak 2009, Russo and Voigt 2016). Comparing modelling approaches could increase modelling accuracy for species identification giving a clearer picture of species presence in Dinosaur Provincial Park.

### Literature Cited

- Agranat, I. 2012. Bat species identification from zero crossing and full spectrum recordings with new techniques using HMMs and Fisher scores, unsupervised clustering and balanced Winnow pairwise classifiers. Pages 1–27 *in* Wildlife Acoustics, Inc. Concord, Massachusetts, USA.
- Agranat, I. 2013. Bat species identification from zero crossing and full spectrum echolocation calls using Hidden Markov Models, Fisher scores, unsupervised clustering and balanced winnow pairwise classifiers. Pages 1–9 *in* Proceedings of Meetings on Acoustics. Montreal, Canada.
- Alberta Climate Information Service. 2016. Interpolated Weather Data Since 1961 for Alberta Townships. <http://agriculture.alberta.ca/acis/township-data-viewer.jsp>.
- Aldridge, H. D. J. N., and R. M. Brigham. 1988. Load carrying and maneuverability in an insectivorous bat: A test of the 5% “rule” of radio-telemetry. *Journal of Mammalogy* **69**:379–382.
- Altringham, J. D. 2011. *Bats: From evolution to conservation*. Second Edition. Oxford University Press, Oxford.
- Anthony, E. L.P., M. H. Stack, and T. H. Kunz. 1981. Night roosting and the nocturnal time budget of the little brown bat, *Myotis lucifugus*: Effects of reproductive status, prey density, and environmental conditions. *Oecologia* **51**:151–156.
- Barclay, R. M. R. 1982. Night roosting behavior of the little brown bat, *Myotis lucifugus*. *Journal of Mammalogy* **63**:464–474.
- Barclay, R. M. R. 1999. Bats are not birds: A cautionary note on using echolocation calls to identify bats: A comment. *Journal of Mammalogy* **80**:290–296.
- Bartonička, T. 2010. Survival rate of bat bugs (*Cimex pipistrelli*, Heteroptera) under different microclimatic conditions. *Parasitology Research* **107**:827–833.
- Bartonička, T., and J. Gaisler. 2007. Seasonal dynamics in the numbers of parasitic bugs (Heteroptera, Cimicidae): A possible cause of roost switching in bats (Chiroptera,

- Vespertilionidae). *Parasitology Research* **100**:1323–1330.
- Betts, B. J. 1998. Effects of interindividual variation in echolocation calls on identification of big brown and silver haired bats. *Journal of Wildlife Management* **62**:1003–1010.
- Blehert, D. S., A. C. Hicks, M. Behr, C. U. Meteyer, B. M. Berlowski-Zier, E. L. Buckles, J. T. H. Coleman, S. R. Darling, A. Gargas, R. Niver, J. C. Okoniewski, R. J. Rudd, and W. B. Stone. 2009. Bat white-nose syndrome : An emerging fungal pathogen? *Science* **323**:227.
- Bogan, M. A., P. M. Cryan, E. W. Valdez, L. E. Ellison, and O. T.J. 2003. Western crevice and cavity-roosting bats. Pages 69–77 in T. O’Shea and M. Bogan, editors. *Monitoring Trends in Bat Populations of the United States and Territories: Problems and Prospects*. US Geological Survey, Biological Resources Discipline, Information and Technology.
- Britzke, E. 2003. Use of ultrasonic detectors for acoustic identification and study of bat ecology in the Eastern United States [PhD thesis]. Cookeville (TN): Tennessee Technological University.
- Britzke, E. R., J. E. Duchamp, K. L. Murray, R. K. Swihart, and L. W. Robbins. 2011. Acoustic identification of bats in the eastern United States: A comparison of parametric and nonparametric methods. *Journal of Wildlife Management* **75**:660–667.
- Broders, H., C. S. Findlay, and L. Zheng. 2004. Effects of clutter on echolocation call structure of *Myotis septentrionalis* and *M. lucifugus*. *Journal of Mammalogy* **85**:273–281.
- Brunet-Rossinni, A. K., and G. S. Wilkinson. 2009. Methods for age estimation and the study of senescence in bats. Pages 315–325 in T. Kunz and S. Parsons, editors. *Ecological and Behavioral Methods for the Study of Bats*. The John Hopkins University Press, Baltimore, MD.
- Burnham, K. P., and D. R. Anderson. 1998. *Model selection and multimodel inference: A practical information-theoretic approach*. Second Edition. Springer, New York.
- Chruszcz, B. J., and R. M. R. Barclay. 2002. Thermoregulatory ecology of a solitary bat, *Myotis evotis*, roosting in rock crevices. *Functional Ecology* **16**:18–26.
- Chruszcz, B. J., and R. M. R. Barclay. 2003. Prolonged foraging bouts of a solitary

- gleaning/hawking bat, *Myotis evotis*. Canadian Journal of Zoology **81**:823–826.
- Clement, M. J., K. L. Murray, D. I. Solick, and J. C. Gruver. 2014. The effect of call libraries and acoustic filters on the identification of bat echolocation. Ecology and Evolution **4**:3482–3493.
- Cryan, P. M., and B. O. Wolf. 2003. Sex differences in the thermoregulation and evaporative water loss of a heterothermic bat, *Lasiurus cinereus*, during its spring migration. The Journal of Experimental Biology **206**:3381–3390.
- Davis, W. H., and H. B. Hitchcock. 1965. Biology and migration of the bat, *Myotis lucifugus*, in New England. Journal of Mammalogy **46**:296–313.
- Eberth, D. A., and D. C. Evans. 2011. International Hadrosaur Symposium. in Geology and Palaeontology of Dinosaur Provincial Park, Alberta. Royal Tyrell Museum.
- Faure, P. A., and R. M. R. Barclay. 1994. Substrate-gleaning versus aerial-hawking: plasticity in the foraging and echolocation behaviour of the long-eared bat, *Myotis evotis*. Journal of Comparative Physiology A **174**:651–660.
- Fenton, M. B. 1990. The foraging behaviour and ecology of animal-eating bats. Canadian Journal of Zoology **68**:411–422.
- Fenton, M. B., and R. M. R. Barclay. 1980. *Myotis lucifugus*. Mammalian Species 142:1-8.
- Fenton, M. B., and G. P. Bell. 1979. Echolocation and feeding behaviour in four species of *Myotis* (Chiroptera). Canadian Journal of Zoology **57**:1271–1277.
- Flaquer, C., I. Torre, and A. Arrizabalaga. 2007. Comparison of sampling methods for inventory of bat communities. Journal of Mammalogy **88**:526–533.
- Gannon, W. L., M. J. O'Farrell, C. Corben, and E. J. Bedrick. 2004. Call character lexicon and analysis of field recorded bat echolocation calls. Pages 478–486 in J. Thomas, C. Moss, and M. Vater, editors. Echolocation in Bats and Dolphins. University of Chicago Press, IL, USA.
- Gannon, W. L., R. E. Sherwin, T. N. DeCarvalho, and M. J. O'Farrell. 2001. Pinnae and echolocation call differences between *Myotis californicus* and *M. ciliolabrum* (Chiroptera:

- Vespertilionidae). *Acta Chiropterologica* **3**:77–91.
- Garroway, C. J., and H. G. Broders. 2008. Day roost characteristics of northern long-eared bats (*Myotis septentrionalis*) in relation to female reproductive status. *Ecoscience* **15**:89–93.
- Geiser, F. 2004. Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annual Review of Physiology* **66**:239–274.
- Griffin, D. R., F. A. Webster, and C. R. Michael. 1960. The echolocation of flying insects by bats. *Animal Behaviour* **8**:141–154.
- Hamilton, I. M., and R. M. R. Barclay. 1994. Patterns of daily torpor and day-roost selection by male and female big brown bats (*Eptesicus fuscus*). *Canadian Journal of Zoology* **72**:744–749.
- Hathway, B., C. J. Banks, and D. C. Hay. 2011. Measured outcrop section T17-R3W4-01 of the Foremost, Oldman and Dinosaur Park Formations (Belly River Group), White Rock Coulee, South Saskatchewan River Valley, Southeastern Alberta (NTS 72L/08). Edmonton, AB Report No.:2011-04 Available from: Energy Resources Conservation Board, Alberta Geological Society.
- Hayes, J. P., H. K. Ober, and R. E. Sherwin. 2009. Survey and monitoring of mats. Pages 112–129 in *Ecological and Behavioral Methods for the Study of Bats*. The John Hopkins University Press, Baltimore, MD.
- Heim, O., J. T. Treitler, M. Tschapka, M. Knörnschild, and K. Jung. 2015. The importance of landscape elements for bat activity and species richness in agricultural areas. *PLoS ONE* **10**.
- Holloway, B. G. L., and R. M. R. Barclay. 2001. *Myotis ciliolabrum*. *Mammalian Species* **4**:1–5.
- Holloway, G. L. 1998. Ecology of prairie bats [master's thesis]. Calgary (AB): University of Calgary.
- Holloway, G. L., and R. M. R. Barclay. 2000. Importance of prairie riparian zones to bats in southeastern Alberta. *Ecoscience* **7**:115–122.
- Jung, K., E. K. V Kalko, and O. Von Helversen. 2007. Echolocation calls in Central American emballonurid bats: Signal design and call frequency alternation. *Journal of Zoology*

**272**:125–137.

- Kaiser, Z. D. E., and J. M. O’Keefe. 2015. Data acquisition varies by bat phonic group for 2 types of bat detectors when weatherproofed and paired in field settings. *Wildlife Society Bulletin* **39**:635–644.
- Kleiber, M. 1947. Body size and metabolic rate. *Physiological Reviews* **27**:511–541.
- Kunz, T. H., and L. F. Lumsden. 2003. Ecology of cavity and foliage roosting bats. Page 1-81 *in* T.H. Kunz and M.B. Fenton, editors. *Bat Ecology*. The University of Chicago Press, Chicago and London.
- Kunz, T. H. 1982. Roosting ecology. Pages 1–55 *in* *Ecology of Bats*. Plenum Press, New York.
- Kurta, A., and R. H. Baker. 1990. *Eptesicus fuscus*. *Mammalian Species* **356**:1–10.
- Lausen, C. L. 2001. Bat thermoregulation and roost selection [master's thesis]. Calgary (AB): University of Calgary.
- Lausen, C. L. 2007. Roosting ecology and landscape genetics of prairie bats [PhD thesis]. Calgary (AB): University of Calgary.
- Lausen, C. L., and R. M. R. Barclay. 2002. Roosting behaviour and roost selection of female big brown bats (*Eptesicus fuscus*) roosting in rock crevices in southeastern Alberta. *Canadian Journal of Zoology* **80**:1069–1076.
- Lausen, C. L., and R. M. R. Barclay. 2003. Thermoregulation and roost selection by reproductive female big brown bats (*Eptesicus fuscus*) roosting in rock crevices. *Journal of Zoology* **260**:235–244.
- Lausen, C. L., and R. M. R. Barclay. 2006. Winter bat activity in the Canadian prairies. *Canadian Journal of Zoology* **84**:1079–1086.
- Lawrence, B. D., and J. A. Simmons. 1982. Measurements of atmospheric attenuation at ultrasonic frequencies and the significance for echolocation by bats. *Journal of the Acoustical Society of America* **71**:585–590.
- Lemen, C., P. Freeman, J. A. White, and B. R. Andersen. 2015. The problem of low agreement among automated identification programs for acoustical surveys of bats. *Western North*

- American Naturalist **75**:218–225.
- Lewis, S. E. 1995. Roost fidelity of bats: A review. *Journal of Mammalogy* **76**:481–496.
- Lewis, S. E. 1996. Low roost-site fidelity in pallid bats: Associated factors on group stability. *Behavioral Ecology and Sociobiology* **39**:335–344.
- Lorch, J. M., C. U. Meteyer, M. J. Behr, J. G. Boyles, P. M. Cryan, A. C. Hicks, A. E. Ballmann, J. T. H. Coleman, D. N. Redell, D. M. Reeder, and D. S. Blehert. 2011. Experimental infection of bats with *Geomyces destructans* causes white-nose syndrome. *Nature* **480**:376–378.
- Manning, R., and J. Jones. 1989. *Myotis evotis*. *Mammalian Species* **329**:1–5.
- McNab, B. K. 1970. Body weight and the energetics of temperature regulation. *Journal of Experimental Biology* **53**:329–348.
- Moyer, B. R., D. M. Drown, and D. H. Clayton. 2002. Low humidity reduces ectoparasite pressure: implications for host life history evolution. *Oikos* **97**:223–228.
- Mukhida, M., J. Orprecio, and M. B. Fenton. 2004. Echolocation calls of *Myotis lucifugus* and *M. leibii* (Vespertilionidae) flying inside a room and outside. *Acta Chiropterologica* **6**:91–97.
- Neubaum, D. J., T. J. O’Shea, and K. R. Wilson. 2006. Autumn migration and selection of rock crevices as hibernacula by big brown bats in Colorado. *Journal of Mammalogy* **87**:470–479.
- Nixon, A. E., J. C. Gruver, and R. M. R. Barclay. 2009. Spatial and temporal patterns of roost use by western long-eared bats (*Myotis evotis*). *The American Midland Naturalist* **162**:139–147.
- O’Farrell, M. J. O., B. W. Miller, and W. L. Gannon. 1999. Qualitative identification of free-flying bats using the Anabat detector. *Journal of Mammalogy* **80**:11–23.
- Parsons, P. A. 1997. Search-phase echolocation calls of the New Zealand lesser short-tailed bat (*Mystacina tuberculata*) and long-tailed bat (*Chalinolobus tuberculatus*). *Canadian Journal of Zoology* **75**:1487–1494.
- Parsons, S., and J. M. Szewczak. 2009. Detecting, recording and analyzing the vocalizations of

- bats. Pages 91–111 in T.H. Kunz and S. Parsons, editors. *Ecological and Behavioral Methods for the Study of Bats*. The John Hopkins University Press, Baltimore, MD.
- Pewsey, A., M. Neuhäuser, and G. D. Ruxton. 2013. *Circular statistics in R*. Oxford University Press, Oxford.
- Racey, P. 2009. Reproductive assessment of bats. Pages 249–264 in T.H. Kunz and S. Parsons, editors. *Ecological and Behavioral Methods for the Study of Bats*. The John Hopkins University Press, Baltimore, MD.
- Racey, P. A., and S. M. Swift. 1981. Variations in gestation length in a colony of pipistrelle bats (*Pipistrellus pipistrellus*) from year to year. *Journal of Reproduction and Fertility* **61**:123–129.
- Raudabaugh, D. B., and A. N. Miller. 2013. Nutritional capability of and substrate suitability for *Pseudogymnoascus destructans*, the causal agent of bat white-nose syndrome. *PLoS ONE* **8**:1–9.
- Reeder, D. M., C. L. Frank, G. G. Turner, C. U. Meteyer, A. Kurta, E. R. Britzke, M. E. Vodzak, S. R. Darling, C. W. Stihler, A. C. Hicks, R. Jacob, L. E. Grieneisen, S. A. Brownlee, L. K. Muller, and D. S. Blehert. 2012. Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. *PLoS ONE* **7**:1–10.
- Richner, H., A. Oppliger, and P. Christe. 1993. Effect of an ectoparasite on reproduction in great tits **62**:703–710.
- Rodhouse, T. J., and K. J. Hyde. 2014. Roost and forage site fidelity of western small-footed Myotis (*Myotis ciliolabrum*) in an Oregon Desert Canyon. *Western North American Naturalist* **74**:241–248.
- Russo, D., and C. C. Voigt. 2016. The use of automated identification of bat echolocation calls in acoustic monitoring: A cautionary note for a sound analysis. *Ecological Indicators* **66**:598–602.
- Schmidt, S. 1988. Evidence for a spectral basis of texture perception in bat sonar. *Nature* **331**:617–619.

- Schmidt-Nielsen, K. 1972. Locomotion : Energy cost of swimming , flying , and running. *Science* **177**:222–228.
- Schnitzler, H. U., and E. K. V. Kalko. 2001. Echolocation by insect-eating bats. *Bioscience* **51**:557–569.
- Schowalter, D. B., and A. Allen. 1981. Late summer activity of small-footed, long-eared and big brown bats in Dinosaur Park, Alberta. *Blue Jay* **39**:50–53.
- Schumm, D. K., and G. Neuweiler. 1991. Echolocation in the notch-eared bat, *Myotis emarginatus*. *Behavioral Ecology and Sociobiology* **28**:255–261.
- Schwab, N. A, and T. J. Mabee. 2014. Winter acoustic activity of bats in Montana. *Northwestern Naturalist* **95**:13–27.
- Sedgeley, J. A., and C. F. J. O'Donnell. 1999. Roost selection by the long-tailed bat, *Chalinolobus tuberculatus*, in temperate New Zealand rainforest and its implications for the conservation of bats in managed forests. *Biological Conservation* **88**:261–276.
- Solick, D. 2004. Differences in the morphology and behaviour of western long-eared bats (*Myotis evotis*) within and between environments [master's thesis]. Calgary (AB): Univeristy of Calgary.
- Solick, D. I., and R. M. R. Barclay. 2006a. Thermoregulation and roosting behaviour of reproductive and nonreproductive female western long-eared bats (*Myotis evotis*) in the Rocky Mountains of Alberta. *Canadian Journal of Zoology* **84**:589–599.
- Solick, D. I., and R. M. R. Barclay. 2006b. Morphological differences among western long-eared myotis (*Myotis Evotis*) populations in different environments. *Journal of Mammalogy* **87**:1020–1026.
- Solick, D. I., and R. M. R. Barclay. 2007. Geographic variation in the use of torpor and roosting behaviour of female western long-eared bats. *Journal of Zoology* **272**:358–366.
- Speakman, J. R., and P. A. Racey. 1989. Hibernation ecology of the pipistrelle bat: Energy expenditure, water requirements and mass loss, implications for survival and the function of winter emergence flights. *Journal of Animal Ecology* **58**:797–813.

- Studier, E. H. 1970. Evaporative water loss in bats. *Comparative Biochemistry and Physiology* **35**:935–943.
- Studier, E. H., and M. J. O’Farrell. 1976. Biology of *Myotis thysanodes* and *Myotis lucifugus* (Chiroptera: Vespertilionidae) III. metabolism, heart-rate, breathing rate, evaporative water-loss and general energetics. *Comparative Biochemistry and Physiology A* **54**:423–432.
- Tabachnick, B. G., and L. Fidell. 2013. Using multivariate statistics. Sixth Edition. Pearson, Boston.
- Taylor, L. R. 1963. Analysis of the effect of temperature on insects in flight. *Journal of Animal Ecology* **32**:99–117.
- Thomas, D. W., M. Dorais, and J.-M. Bergeron. 1990. Winter energy budgets and cost of arousals for hibernating little brown bats, *Myotis lucifugus*. *Journal of Mammalogy* **71**:475–479.
- Thomas, D. W., and F. Geiser. 1997. Periodic arousals in hibernating mammals: Is evaporative water loss involved? *Functional Ecology* **11**:585–591.
- U.S. Fish and Wildlife Service. 2016. White-nose syndrome map. <https://www.whitenosesyndrome.org/resources/map>.
- Vaughan, N., G. Jones, and S. Harris. 1997. Identification of British bat species by multivariate analysis of echolocation call parameters. *Bioacoustics* **7**:189–207.
- Vaughan, T. A., and T. J. O’Shea. 1976. Roosting ecology of the pallid bat, *Antrozous pallidus*. *Journal of Mammalogy* **57**:19–42.
- Vonhof, M. 2006. Handbook of inventory methods and standard protocols for surveying bats in Alberta. Edmonton, AB. Available from: Alberta Fish and Wildlife Division.
- Vonhof, M. J., and R. M. R. Barclay. 1996. Roost-site selection and roosting ecology of forest-dwelling bats in southern British Columbia. *Canadian Journal of Zoology* **74**:1797–1805.
- Wagenmakers, E. J., and S. Farrell. 2004. AIC model selection using Akaike weights. *Psychonomic Bulletin & Review* **11**:192–196.
- Wang, L. C. H., and M. W. Wolowyk. 1988. Torpor in mammals and birds. *Canadian Journal of*

Zoology **66**:133–137.

- Webb, P. I., J. R. Speakman, and P. A. Racey. 1993. The implication of small reductions in body temperature for radiant and convective heat loss in resting endothermic brown long-eared bats (*Plecotus auritus*). *Journal of Thermal Biology* **18**:131–135.
- Webb, P. I., J. R. Speakman, and P. A. Racey. 1995. Evaporative water-loss in two sympatric species of vespertilionid bat, *Plecotus auritus* and *Myotis daubentoni*: relation to foraging mode and implications for roost site selection. *Journal of Zoology* **235**:269–278.
- White, J. A., B. R. Andersen, H. W. Otto, C. A. Lemen, and P. W. Freeman. 2014. Winter activity of bats in Southeastern Nebraska :An acoustic study. *Transactions of the Nebraska Academy of Sciences* **34**:80–83.
- Whitlock, M. C., and D. Schluter. 2009. *The analysis of biological data*. Roberts and Company Publishers, Colorado (USA).
- Williams, L. M., and M. C. Brittingham. 1997. Selection of maternity roosts by big brown bats. *Journal of Wildlife Management* **61**:359–368.
- Willis, C. K. R., and R. M. Brigham. 2007. Social thermoregulation exerts more influence than microclimate on forest roost preferences by a cavity-dwelling bat. *Behavioral Ecology and Sociobiology* **62**:97–108.
- Willis, C. K. R., and C. E. Cooper. 2009. Techniques for studying thermoregulation and thermal biology in bats. Pages 647–658 *in* T H.. Kunz and S. Parsons, editors. *Ecological and Behavioral Methods for the Study of Bats*. The Johns Hopkins University Press, Baltimore, MD.
- Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. *Mixed Effects Models and Extensions in Ecology in R*. Springer, New York.