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Physiological Ecology of Bat Migration

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A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy

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by

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THE UNIVERSITY OF WESTERN ONTARIO
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Abstract

Migration is perhaps the most poorly understood aspect of bat biology and the underlying physiological basis is virtually unstudied. Although distantly related, bats and birds are both endothermic flying vertebrates and bird migration physiology has been studied for decades. Therefore, I used migratory birds as a model system to make predictions regarding the physiological ecology of bat migration.

First, I compared brain size of migratory and sedentary bat species. Migratory species have smaller brains which suggests the costs of carrying and maintaining a large brain are incompatible with the demands of migration. Next, I studied silver-haired bats (*Lasionycteris noctivagans*) during migratory stopover. Bats arrived at the site with fat stores comparable to migratory birds, rarely foraged, and had short stopover durations. I proposed that bats use daily torpor to minimize energy expenditure during non-flight periods, thus sparing fuel stores for migratory flight. Finally, I compared body composition and flight muscle physiology in migrating and non-migrating hoary bats (*Lasiurus cinereus*). Changes in digestive and exercise organ sizes, the composition of adipose stores, and increased catabolic enzyme activities all reflected the increased energetic demands of migration. Sex-specific changes in muscle membrane fatty acid composition and the expression of fatty acid transport proteins suggest pregnant females are subject to different pressures than males.

The energetic demands of bat migration lead to many physiological changes as observed in migratory birds. However, several factors specific to bats (especially heterothermy and the timing of reproduction) result in bat migration as a distinct phenomenon compared to birds.

Keywords

Bat migration physiology, physiological ecology, birds, brain size, stopover, body composition, fatty acids, fatty acid transport protein, hoary bat, silver-haired bat

Co-Authorship Statement

A version of Chapter 2 was published in the *Journal of Mammalogy* with Christopher Guglielmo as a co-author. Dr. Guglielmo contributed substantially to discussion of the material included in the chapter and contributed editorial comments to the manuscript.

A version of Chapter 3 was published in *Biology Letters* with John Ratcliffe as a co-author. Dr. Ratcliffe conducted phylogenetic statistical analysis of the data, provided valuable insight into previous studies of brain size evolution in bats, and contributed editorial comments to the manuscript.

A version of Chapter 4 was published in *Journal of Animal Ecology* with Christopher Guglielmo, Stuart Mackenzie and Philip Taylor as co-authors. All co-authors contributed to the study design. Dr. Taylor and Dr. Guglielmo provided access to the equipment required, and Mr. Mackenzie provided logistic support in the field. All authors contributed to interpretation of the data and editorial comments on the manuscript.

Chapters 5 and 6 are co-authored by Brock Fenton and Christopher Guglielmo. Dr. Fenton and Dr. Guglielmo were involved in study design, provided equipment, helped interpret the data, and provided comments on the manuscripts.

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CHAPTER 1

1 INTRODUCTION TO THE THESIS

1.1 A brief history of bat migration research

Bats are the only mammals capable of flight, a trait they share with birds, pterosaurs, and insects (Rayner 1988). Other so-called 'flying' mammals (e.g. flying lemurs [Dermoptera], flying squirrels [Rodentia: Sciuridae: Petauristinae] and several other lineages), only glide short distances on extended membranes (Jackson 2002). Bats are the only mammals capable of sustained periods of true flapping flight. All bats fly, a character that evolved very early in the lineage. The oldest known fossil bat, *Onychonycteris finneyi*, dating from 52.5 Myr ago, had well developed wings capable of powered flight, as have all other known fossil bats (Simmons *et al.* 2008).

Flight was a key adaptation in the evolution of bats and plays an important role in nearly all aspects of bat life history (Barclay and Harder 2003). Of particular interest for my dissertation is the ability of flying animals to travel great distances in relatively short periods of time. Every spring and autumn billions of birds undertake long migrations between summer and winter habitats. Consequently, it is not difficult to imagine bats making similar movements on an annual basis. Yet while empirical evidence of bird migration was documented in the early part of the 19th century (in 1822 a white stork was found in Germany that was impaled with an African spear), the earliest suggestions of bats performing similar migrations to birds did not appear until the end of that century. At temperate latitutdes, many species of bats hibernate in caves and mines in spectacular aggregations of tens or hundreds of thousands of bats (Davis and Hitchcock 1965). Consequently, in the late 19th century the prevailing thought suggested all temperate bats overwintered locally in a state of hibernation. This sentiment was echoed by C. Hart Merriam (1887) when presenting some of the earliest evidence that some bat species, including hoary (*Lasiurus cinereus*) and silver-haired (*Lasionycteris noctivagans*) bats (the focal species for my thesis), migrate south for the winter:

> *"The belief that the bats of temperate and cold temperate regions pass winter in a state of hibernation, is so general and widespread, that an attempt to prove the contrary, even in the case of a single species, is likely to be received with surprise, if not with incredulity. Nevertheless, I shall be disappointed if the facts here brought together fail to demonstrate, that at least two species of Canadian bats regularly perform extended migrations to avoid the cold of our northern winters."* (Merriam 1887; p. 85)

In the years that followed, further evidence of annual bat migration began to emerge (e.g., Miller 1897; Grinnell 1918) including species from non-temperate regions (e.g., Ratcliffe 1932). By the middle of the 20th century, the concept of bat migration was sufficiently accepted that it began to regularly appear as a chapter in books about bats (e.g., Allen 1939) and there have now been several detailed reviews of bat migration (Griffin 1970; Fleming and Eby 2003; Hutterer *et al.* 2005).

Migration is not the ancestral condition among bats (Bisson *et al.* 2009), rather most species are sedentary, spending the entirety of their lives in the same region (Fleming and Eby 2003; Hutterer *et al.* 2005). However, migration has evolved repeatedly (Bisson *et al.* 2009) and members of at least nine bat families are known to migrate (McGuire and Ratcliffe 2011/ Chapter 3). Bat migration may be broadly classified into three general patterns: altitudinal migration, regional migration, and latitudinal migration.

1.2 Altitudinal migration

Altitudinal migration involves the seasonal return movement of all or part of a population along an elevational gradient. Although bat altitudinal migration has been frequently suggested, almost no empirical research has been done in this area (McGuire and Boyle *In Review*). In a thorough review of the topic, McGuire and Boyle (*In Review*) identified 60 species of bats from 5 families (20 countries, 4 continents) for which there is empirical or suggestive evidence of some form of altitudinal migration. Perhaps the best empirical evidence of altitudinal migration in bats comes from big brown bats (*Eptesicus fuscus*) in Colorado that have been radio-tracked between summer roosts in the lowlands to winter

hibernation sites in the nearby mountains (Neubaum *et al.* 2006). However, due to the lack of empirical research in this area, I do not treat it further here. Altitudinal migration deserves a much greater focus in future research, not least because bats may frequently mix elements of altitudinal migration with either regional or latitudinal migration (McGuire and Boyle *In Review*).

1.3 Regional migration

Regional migration involves bats migrating to and from a central hibernaculum where they spend the winter months in a state of hibernation. This type of movement is common among temperate species (Griffin 1970; Fleming and Eby 2003; Hutterer *et al.* 2005) and is the best documented form of bat migration. During the summer months, females typically form maternity colonies where pups are born and reared while males remain solitary. In late summer, bats return to hibernacula (typically caves and abandoned mines) where mating occurs in a behaviour known as swarming (Fenton 1969; Parsons *et al.* 2003; Piksa 2008; McGuire *et al.* 2009). The bats then hibernate through the winter, emerge in the spring when females become pregnant, and males and females migrate back to their separate summer quarters.

The aggregations of bats in hibernacula have enabled large-scale banding programs to document individual migratory movements. The United States Fish and Wildlife Service issued > 2 million bat bands during the course (1932 – 1972) of the US Bat Banding Program (Ellison 2008). Similar programs were undertaken by many European countries (Roer 1995; Hutterer *et al.* 2005). In the absence of large government sponsored banding operations, individuals have continued to band large numbers of regional migrants (e.g., Dubois and Monson 2007). Regional migration is characterized by a radiation type migration with no clear directional component. Bats migrate in to a central hibernaculum and radiate back out again in all directions (a "star-like pattern" as described by Hutterer *et al.* 2005). Based on the time and distance between recaptures, maximum migration rates have been estimated at ~ 50 – 100 km day^{-1} (Tuttle 1976; Fleming and Eby 2003; Hedenström 2009). Females generally depart earlier in spring (Fleming and Eby 2003), and limited evidence suggests females may also migrate more rapidly during autumn migration (Tuttle 1976). One-way migration distances are typically < 250 km, and

consequently, authors frequently refer to ‘short-distance migration’ (e.g., Bisson *et al.* 2009). However, some individuals may travel > 500 km between summer and winter quarters (Fenton 1969; Tuttle 1976). Therefore, the term ‘regional migration’ is preferable to ‘short-distance migration’ which invites classification based on arbitrary distance thresholds rather than behavioural patterns.

The relatively short distances involved in regional migrations have perhaps contributed to the lack of research regarding the migratory phase. What happens between the time the bats depart their summer quarters and reach the hibernacula (or vice versa) is poorly understood. However, there is evidence that migratory periods present a critical time in the annual cycle of the species. Using data from recoveries of banded bats, Tuttle and Stevenson (1977) demonstrated that migration was associated with elevated mortality rates, particularly for sub-adults. The energetic demands of regional migration are difficult to determine either through seasonal comparisons of body or fat mass (e.g., Ewing *et al.* 1970; Kunz *et al.* 1998) or mass changes of marked individuals (Tuttle 1976) because of the confounding effect of hibernation. For temperate insectivorous bats, the sub-zero temperatures of winter, unsuitable for bats and their insect prey, may last for more than half of the year (Humphries *et al.* 2002). Therefore it is impossible to determine whether bats deposit fat in anticipation of the demands of migration or the future demands of hibernation (likely a combination of both). Further complicating the matter is the fact that bats continue to deposit fat after arriving at hibernacula (Kunz *et al.* 1998; McGuire *et al.* 2009).

1.4 Latitudinal migration

The third general pattern of bat migration involves seasonal migration with a strong north-south component as bats move generally longer distances (> 500 km, but rarely > 2000 km) between summer and winter grounds. Compared to regional migrants, there is more variability in the migration patterns, largely arising from a greater diversity of species that migrate in this manner. Furthermore, latitudinal migration is not limited to temperate zones as generally observed in regional migration.

As for regional migrants, banding programs have been successful in elucidating the migration patterns of some latitudinal migrants. Some European species (e.g., *Nyctalus noctula, Pipistrellus nathusii*) migrate 1000 – 2000 km from northeastern to southwestern Europe where they hibernate over winter (Hutterer *et al.* 2005; Dietz *et al.* 2009). In the southwestern United States, the seasonal disappearance of millions of Brazilian free-tailed bats (*Tadarida brasiliensis*) was obvious to early observers. However, it was not until concerns regarding these bats as a rabies vector motivated large-scale banding efforts that it was revealed the bats migrated > 1000 km south to overwinter in Mexico (Constantine 1967; Cockrum 1969; Griffin 1970).

As in early observations of *T. brasiliensis*, seasonal appearance of large aggregations of bats in Africa and Australia identified these species as latitudinal migrants. In West Africa several species migrate from forested areas to savanna regions in the wet season, returning to the forest in the dry season (Thomas 1983). In central Africa, straw coloured fruit bats (*Eidolon helvum*) migrate in response to seasonally variable fruit abundance (Richter and Cumming 2006). Similar movements have long been known from several *Pteropus* species in Australia (Ratcliffe 1932). The large body size of the pteropodid migrants in Africa and Australia has made it possible to attach satellite transmitters to follow migratory movements in detail (Smith *et al.* 2011). Satellite tracked *E. helvum* migrated 1300 – 2500 km (one-way) from Zambia to the northwestern region of the Democratic Republic of the Congo (Richter and Cumming 2008). Migrating bats typically travelled ~ 90 km day^{-1}, but one bat travelled 370 km in a single night. Similarly, satellite tracked grey-headed flying foxes (*Pteropus poliocephalus*) in Australia migrated > 500 km in 2 – 3 week periods (Tidemann and Nelson 2004). Malayan flying foxes (*Pteropus vampyrus*) in southeast Asia migrate more nomadically, but provide further examples of long-distance movements in short time periods (Epstein *et al.* 2009). One individual equipped with a satellite transmitter travelled 363 km from mainland peninsular Malaysia to the island of Sumatra in 4 days (Epstein *et al.* 2009).

For many species of latitudinal migrants, the animals are too small to permit tracking and too rarely encountered for banding to be effective in determining movement patterns. For these species, the description of migration patterns has generally relied on observations of

seasonal occurrence and other indirect evidence. In the southwestern United States, lesser long-nosed bats (*Leptonycteris yerbabuenae*) arrive in late April and early May, where they give birth and raise their young before disappearing again in late September or early October (Cockrum 1991). Further observations on the seasonal occurrence of this species indicated a latitudinal migration as the bats moved north and south with the availability of nectar from blooming columnar cacti and agaves (Fleming and Eby 2003). This migratory pattern is further supported by stable carbon isotope evidence indicating the migration-related dietary shift from C3 photosynthetic plants to cacti and agaves which use the CAM photosynthetic pathway (Fleming *et al.* 1993). Genetic evidence further links summer populations in the northern part of the species range (Arizona, USA) to the overwintering range in southern Mexico (Wilkinson and Fleming 1996).

The above examples illustrate the variety of patterns associated with latitudinal migration in bats. In all cases, migration is associated with seasonal variability in resources (e.g., fruit abundance) or associated factors (e.g., cold winters and dry seasons limiting food availability). Large body size and/or colonial lifestyle have allowed reasonably effective documentation of the migration patterns in these species. Yet for smaller, more solitary species, the patterns remain somewhat unclear.

1.5 Migration in North American tree bats

Despite ample indirect evidence, direct empirical evidence of migration in North American tree bats is lacking (Cryan 2003). Three temperate species, hoary bat (*Lasiurus cinereus*), eastern red bat (*Lasiurus borealis*), and silver-haired bat (*Lasionycteris noctivagans*), spend their summers as far north as the boreal forest of Canada and migrate south in the winter months (Cryan 2003). Three chapters of my dissertation investigate the migration of either *L. cinereus* or *L. noctivagans* and hence I will describe these species in more detail (additional specific details are provided in the relevant chapters of my dissertation).

The three species of North American tree bat are too small for currently available telemetry devices that would enable long-distance tracking. Hoary bats weigh ~ 20 – 35 g (Shump and Shump 1982b; personal observation), whereas eastern red and silver-haired

bats weigh ~ 8 – 15 g (Kunz 1982; Shump and Shump 1982a; personal observation). Furthermore, hoary and eastern red bats are solitary foliage roosting bats that forage in open habitats and are thus rarely captured (Shump and Shump 1982a; Shump and Shump 1982b). Similarly, silver-haired bats form small colonies that roost in tree hollows or under shedding tree bark (Kunz and Lumsden 2003), and are not frequently encountered. Thus the probability of recapturing a banded bat is extremely low. Nonetheless, numerous studies provide convincing evidence of the latitudinal migratory habits of these species. The earliest observational evidence, based on seasonal absence from northern regions and appearance in southern regions, was presented by Merriam (1887). Since that time, numerous studies have reported seasonal observation patterns and particularly frequent encounters with migratory bats in specific locations during migratory periods (summarized in Cryan 2003). Museum records have also provided a valuable resource for demonstrating seasonal occurrence patterns (Cryan 2003) and as source material for studies of stable hydrogen isotope composition (Cryan *et al.* 2004). Based on differences between the stable hydrogen isotope composition of fur and local precipitation (hydrogen isotopic enrichment varies predictably with latitude in North America; Fraser 2011), Cryan *et al.* (2004) demonstrated that individual hoary bats may migrate > 2000 km.

Pups are born in the summer months when females are found into the northern regions of each species' respective distributions. Mating behaviour is poorly documented, but is believed to occur at least in part during fall migration (Kunz 1982; Shump and Shump 1982a; Shump and Shump 1982b; Cryan 2008). Sperm development peaks in late summer for silver-haired bats (Kunz 1982) and both silver-haired and hoary bats have viable sperm during autumn migration (Cryan *et al.* 2010). Females then store the sperm over winter. The overwinter ecology of these species is poorly understood, however eastern red bats and silver-haired bats apparently remain active as weather permits and enter short term hibernation/ extended torpor during colder periods (Perry *et al.* 2010; Dunbar and Tomasi 2006). In spring, females become pregnant and migrate back to their summer grounds several weeks earier than males (Valdez and Cryan 2009). Thus females carry developing foetuses during spring migration (Cryan and Wolf 2003) and arrive at their summer grounds prior to parturition (Willis *et al.* 2006). Hoary bats and silver-haired bats produce two offspring (Shump and Shump 1982b; Kunz 1982) while eastern

red bats may give birth to as many as five pups (Shump and Shump 1982a). Combined, newborn pups may account for 15 – 30% of the mother's body mass (Kunz 1982; Shump and Shump 1982a; Koehler and Barclay 2000). It is unclear whether the additional mass of near term foetuses affects the ability of females to migrate.

During the summer months, male and female hoary and silver-haired bats are geographically segregated (Cryan 2003). Male hoary bats are most commonly found in mountainous regions in the western part of the continent while females are found throughout the east. Female silver-haired bats are found in more northern regions than males. The sexes reunite during fall migration indicating that differential migration (varying migration pattern in a particular sex or age cohort) is common in these species, with female bats migrating greater distances than males. Furthermore, a population of silver-haired bats in the Pacific northwest are apparently non-migratory (Cryan 2003), and thus it is possible that silver-haired bats are partial migrants (not all populations migrate or only a subset of the population migrates while the remainder are sedentary) in some parts of their range. Eastern red bats may also undergo differential migration, although in the reverse pattern compared to hoary and silver-haired bats. Males and females typically occupy similar ranges during the summer months, but males overwinter further north than females (Cryan 2003) suggesting that females migrate farther than males.

The observation of high rates of bat mortality at wind energy facilities has led to increased interest in the migration of North American tree bats (Arnett *et al.* 2008). Bats are frequently killed by turbines, and may be killed either by direct turbine blade strikes, or indirectly due to barotrauma caused by low pressure zones around moving turbine blades (Baerwald *et al.* 2008). Latitudinal migrants account for the majority of bat mortality at wind turbines, and fatalities are concentrated during migratory seasons (Arnett *et al.* 2008; Cryan and Barclay 2009). Direct strikes and barotrauma provide plausible proximate mechanisms, yet the specific reasons for migratory bat mortality remain unknown. Several hypotheses have been proposed (Cryan and Barclay 2009) and research is ongoing. One of the greatest hindrances to developing effective mitigation and

management plans is lack of understanding regarding the basic biology of these species during migratory periods.

1.6 Partial and differential migration

As demonstrated for North American tree bats, partial and differential migration are common in bats. *Nyctalus noctula*, *Tadarida brasiliensis*, *Pteropus poliocephalus*, and *Leptonycteris yerbabuenae* are all frequently cited as examples of 'long-distance' migration in bats (e.g., Fleming and Eby 2003), however, none of these species can be said to be strictly migratory. Swedish and British populations of *N. noctula* are apparently sedentary (Dietz *et al.* 2009). In Australia, sedentary populations of *P. poliocephalus* are common (Fleming and Eby 2003). The migration of *T. brasiliensis* provides examples of both differential and partial migration. Populations in California do not migrate south in the autumn, and the migration of populations in Texas and New Mexico is strongly female biased (females more likely to migrate north than males; Cockrum 1969). Similarly, *L. yerbabuenae* from south-central Mexico do not undergo latitudinal migration (Rojas-Martínez *et al.* 1999) and may in fact migrate altitudinally (Herrera Montalvo 1997). For those populations that migrate latitudinally, females are more likely to migrate and move further north than males (Cockrum 1991).

A common pattern in each of these examples of differential migration is that migration is often female biased (females more likely to migrate and/or females migrate longer distances). The energetic demands of pregnancy and lactation (Kurta *et al.* 1989) likely place females under greater pressure to find optimal foraging habitat than males while males may remain more sedentary and tolerate sub-optimal conditions. However, in some cases the pattern may be reversed. In a recent study of migratory patterns in the tricoloured bat (*Perimyotis subflavus*), there was only limited evidence of female migration, and rather a male biased differential migration pattern (Fraser *et al.* 2012). Consequently, to interpret the results of migration studies it is important to consider sex effects and the life history of the particular species in question.

1.7 The mechanisms of migration- migratory birds as a model for studying bat migration

The sections above demonstrate that there is a considerable body of literature regarding bat migration. However, with few exceptions, previous research deals almost exclusively with documenting patterns of migration: questions of who, when, and where. For many species, little is known of their migration beyond drawing a straight line on a map to connect two capture locations of a banded individual. Virtually nothing is known about the mechanisms and processes involved in bat migration: the question of how. Ultimately, physiology limits animal performance, and combined with behaviour and extrinsic factors (e.g., environmental conditions) leads to observed migration patterns (Bowlin *et al.* 2010). Thus it is important to understand the underlying physiological processes involved to provide a comprehensive understanding of bat migration. The broad objective of my thesis was to examine the physiological ecology of bat migration. Specifically I was interested the physiological processes and associated behaviours that enable bats to travel hundreds or thousands of kilometers during their annual migration.

Although there is little published research about migration physiology in bats, the physiology of bird migration has been studied for decades and entire volumes have been published on the subject (e.g., Gwinner 1990). Bats and birds are both endothermic flying vertebrates but are distantly related phylogenetically. Therefore, I hypothesized that the common selective pressures of vertebrate flight would lead to evolutionary convergence. The continuing theme in my approach to addressing questions of bat migration was to make predictions about bats based on empirical evidence and theory developed for migratory birds.

1.8 Dissertation structure

Each of the chapters in my dissertation was conceived and prepared as a separate study for independent publication. Chapters 2 – 4 have been published, and chapters 5 and 6 are currently in preparation to be submitted for publication. The various chapters approach the question of bat migration physiology from different perspectives including evolutionary comparisons (multi-species phylogenetic comparisons), observation of free-

living bats and computer simulation based on these bats, body composition changes (whole animal, organs, cell membranes), and flight muscle physiology.

Chapter 2 ("What can birds tell us about the migration physiology of bats?") serves as a detailed introductory chapter, reviewing the migration physiology of bats and the use of migratory birds as a model for developing hypotheses and predictions in the absence of previous evidence from migratory bats. I conducted a detailed review of the bird migration physiology literature and compared the patterns to evidence (if any) from the bat literature. Specifically, I address questions about the energetic demands of migration, fuel selection, nutrient acquisition, storage and utilization. I conclude the chapter with a brief discussion of unique aspects of bat biology that may lead to substantial differences in migration physiology compared to birds

In Chapter 3 ("Light enough to travel: migratory bats have smaller brains, but not larger hippocampi, than sedentary species") I examine the energetic consequences of migration from an evolutionary perspective by comparing brain size of migratory and non-migratory bat species. Migratory bird species have smaller brains than non-migratory species. The energy trade-off hypothesis is one proposed explanation of this phenomenon. The hypothesis suggests that the energetic costs of carrying and maintaining a large brain are incompatible with the energetic demands of migration, although other non-mutually exclusive hypotheses have been proposed. Differences in the life-history of bats compared to birds enabled me to distinguish among these competing hypotheses. Therefore I tested the prediction that migratory bats would have smaller brains than non-migratory species if migration is indeed energetically demanding for bats.

In Chapter 4 ("Migratory stopover in the long-distance migrant silver-haired bat, *Lasionycteris noctivagans*") I address the stopover biology of migratory bats. Numerous studies have suggested that bats use or require sites to rest and refuel during migration, but there are no empirical studies documenting stopover biology in bats. I hypothesized that bats would use stopover sites in a manner similar to migrating passerines. I documented the behaviour of silver-haired bats (*L. noctivagans*) at a stopover site during

southward autumn migration. I used an automated digital radio-telemetry array to document landscape-scale movement patterns, roost sites, foraging behaviour, stopover duration, and departure direction. Furthermore, I measured body composition with quantitative magnetic resonance and used this data to conduct computer simulations of long-distance migration to make predictions about the migration range and rate of this species.

In Chapter 5 ("Phenotypic flexibility in migrating bats: seasonal variation in body composition, organ sizes, and fatty acid profiles") I investigated migration-related phenotypic flexibility in migratory bats. I hypothesized that bats, like birds, would alter their phenotype seasonally in response to the demands of migration. I compared aspects of nutrient acquisition and storage between summer non-migrating and spring migrating hoary bats (*L. cinereus*). Based on a large body of literature of migratory birds, I made predictions about migration-related variation in body composition (fat and lean mass), digestive and exercise organ sizes, and the fatty acid profiles of adipose lipid storage tissue and flight muscle membranes.

In Chapter 6 ("Seasonal upregulation of catabolic enzymes and fatty acid transport protein expression in the flight muscle of migrating hoary bats, *Lasiurus cinereus*") I examine factors related to fuel utilization in migratory flight. Migration is energetically demanding, and flying migrants should fuel flight with energy dense lipid stores. Birds do indeed fuel migratory flight primarily with stored fat, but previous studies of fuel selection at high intensity exercise in mammals indicated that carbohydrates provide the primary fuel source. I hypothesized that bats, like birds but unlike running mammals, fuel endurance high-intensity exercise (flight) with stored fat. I tested the predictions that the activities of mitochondrial catabolic enzymes and the expression of fatty acid transport proteins would both be upregulated during migration in response to the energetic demands of migratory flight.

Finally, I conclude my thesis (Chapter 7) with a summary of the emerging picture of bat migration physiology based on the combined results of my dissertation research.

Furthermore, I briefly discuss similarities and differences between bat and bird migration, and highlight future directions in the study of bat migration physiology.

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CHAPTER 2

2 WHAT CAN BIRDS TELL US ABOUT THE MIGRATION PHYSIOLOGY OF BATS?[1]

Many species of bats undergo annual migrations, in some cases covering distances of 1,000 km or more. However, very little is known about the physiological and biochemical mechanisms underlying bat migration. In contrast, the physiology of migrating birds has been studied for decades and many migration-related changes have been documented. Although bats and birds evolved flight and long-distance migration independently, they have likely experienced many similar selective pressures. We therefore suggest that knowledge of bird migration physiology can be used to generate predictions for emerging studies of bat migration physiology. In this review, we discuss major physiological and biochemical adaptations relating to fuel acquisition and fuel utilization. For each, we summarize knowledge gained from migration studies of birds and bats (if any) and make predictions of bat migration physiology. For many aspects, we predict that bats will have evolved similar physiological mechanisms to birds. However, there are some potentially major differences in the energetic models for bats and birds, including torpor, fuel selection at high-intensity exercise, and trade-offs between reproduction and migration.

2.1 Introduction

Our knowledge of bat migration was first reviewed nearly 40 years ago by Griffin (1970), and since that time, it is startling how few new data have been published. Recently, interest in all aspects of bat migration has surged, partly due to improvements in tracking techniques (Cryan *et al.* 2004; Wikelski *et al.* 2007), but also due to the high mortality rates of migrant bats at wind energy facilities (Baerwald and Barclay 2009; Barclay *et al.*

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2007; Cryan and Brown 2007; Cryan and Barclay 2009; Kunz *et al.* 2007). It is also important that we understand bat migration so that we may assess the impacts of climate change, as already observed in other migratory taxa (e.g., Both and Visser 2001; Cotton 2003).

Migration physiology examines the mechanisms that enable organisms to successfully complete migratory movements. The literature on migration physiology of bats is sparse, whereas birds have been studied intensively for many decades in this context (e.g., Berthold 1993; Gwinner 1990; McWilliams *et al.* 2004; and many others). Although bats and birds are taxonomically distant, both groups are endothermic vertebrates capable of flight and consequently should be subject to similar selective pressures on their physiology as it relates to flight and migration. For example, similar to birds, and unlike typical nonvolant mammals, bats have high rates of passive nutrient absorption in the intestine, which is thought to be a mass-reducing adaptation to minimize the cost of flight (Caviedes-Vidal *et al.* 2007, 2008). In this review, we focus on several key aspects of migration physiology of birds and address how they may apply to bat migration. We limit our discussion to fuel metabolism, and do not include other key areas such as orientation (see Griffin 1970; Holland 2007; Holland *et al.* 2006; Wang *et al.* 2007), endocrine control (Berthold 1996; Wingfield *et al.* 1990), or biological rhythms (Berthold 1996; Gwinner 1990). Specifically, we first discuss mechanisms of fuel acquisition and storage and then discuss fuel utilization during flight. We also discuss some potentially interesting features of bat migration (torpor and reproductive allocation) and summarize some key questions for future bat research.

2.2 Fuelling the Journey

Fuel selection by birds for migration has been reviewed previously (Jenni and Jenni-Eiermann 1998). Energy metabolism can be fuelled by any one or a mixture of the three macronutrients (carbohydrate, protein, or fat), but it is clear that fat should be the fuel of choice for migratory flight. The cost of flight increases with body mass and consequently fuel sources with a high ratio of energy to mass should be favoured. On a dry matter basis, lipids provide approximately twice the energy of either carbohydrates or protein, and when coupled with the fact that lipids are stored nearly anhydrously, the difference

increases to 8 – 10 times the amount of energy on a wet mass basis (Jenni and Jenni-Eiermann 1998; Ramenofsky 1990). Birds can fuel migratory flight almost exclusively with stored fat (Jenni and Jenni-Eiermann 1998; McWilliams *et al.* 2004), and often store large quantities of fat during migration seasons. Fat is stored in subcutaneous and abdominal fat depots and can account for > 50% of total body mass (Berthold 1993; Piersma and Gill 1998). Migratory birds may also increase protein stores by growing larger lean body components (muscles and organs) to increase the functional capacity of flight and digestive machinery, and to provide amino acids required for the maintenance of citric acid cycle intermediates (anaplerotic flux), gluconeogenesis, water production, or a combination of these, during flight (Jenni and Jenni-Eiermann 1998; Lindström and Piersma 1993).

There is limited evidence that bats use fat as a primary fuel source during flight, and their fuel storage patterns during migration are essentially unknown. A common method to determine fuel selection is the respiratory quotient, the ratio of CO_2 produced to O_2 consumed (Frayn 1983; Walsberg and Wolf 1995). Bats are often difficult to train to fly in controlled conditions (e.g., a wind tunnel) and echolocating bats are particularly reluctant to wear a mask required to measure gas exchange. Nevertheless, a few estimates of respiratory quotient have been obtained. The respiratory quotient of the frugivores *Eidolon helvum* and *Hypsignathus monstrosus* indicated that fat provided 77 – 92% of the fuel during flight, and a decline in respiratory quotient with flight time suggests that at stable conditions in longer flights, fat may prove to be the exclusive fuel source following initial carbohydrate use (Carpenter 1986). Similar patterns of declining respiratory quotient with flight time were found for *Pteropus poliocephalus*, another frugivore (Carpenter 1985). The respiratory quotient for the carnivore–frugivore *Phyllostomus hastatus* in short flights (~ 1 min) indicated that carbohydrates were the sole fuel source (Thomas and Suthers 1972), in agreement with the hypothesis that carbohydrates provide the initial fuel. When fasted, the hovering flight of the nectarivore *Glossophaga soricina* is fueled mainly by stored fat (Welch *et al.* 2008) suggesting that fat would be the primary fuel during migratory endurance flights.

If bats do indeed fuel migration with lipids, fat stores should increase during migration. In practice this has been difficult to establish. There is some evidence for migration-related increases in fat stores by the insectivore *Tadarida brasiliensis* (O'Shea 1976). However, most species for which fat stores have been quantified during times of migration spend the winter in hibernation and consequently it is impossible to determine if the fat was accumulated to fuel migration or hibernation (e.g., Ewing *et al.* 1970; Kunz *et al.* 1998).

The quantity of stored fat is important, but so is the quality. Differences in the chain length (number of carbon atoms) and the number of double bonds affect the energy density and mobilization rates of fatty acids (Price 2010). Some birds appear to adjust the quality of fat stores during migration by increasing the proportion of unsaturated fatty acids (McWilliams *et al.* 2004). Unsaturated fatty acids are mobilized more rapidly from adipocytes than are saturated fatty acids (Price *et al.* 2008), and may be preferentially oxidized by muscles (Leyton *et al.* 1987). Data about the variation in fatty acid composition in bats are limited. An evaluation of the prey items in fecal pellets suggested that bats prefer prey items high in unsaturated fatty acids (Schalk and Brigham 1995). Although no studies have investigated the fatty acid composition of adipose tissue in migrating bats, there are significant changes in the fatty acid profile of active and hibernating bats (Arévalo *et al.* 1990; Ewing *et al.* 1970).

There also are no data about variation in protein stores during bat migration. Studies of seasonal changes in body composition have been hindered by the elusive nature of many migratory bat species, and the fact that traditional methods of determining body composition require lethal sampling and lengthy chemical extraction processes. Recent advances in noninvasive measurement techniques for fat and lean mass, such as quantitative magnetic resonance analysis (McGuire and Guglielmo 2010) and dual-energy X-ray absorptiometry (Stevenson and van Tets 2008) may make it possible to determine fuel stores of migrant bats more easily.

2.3 Fuel Acquisition

To successfully complete a migratory movement, birds and bats must accumulate the energy and nutrients required to fuel the flight before departure. There are many physiological and behavioural adaptations that enable birds to rapidly deposit these nutrient stores and bats may potentially use similar mechanisms. In fact, the time and energy demands on bats may be even more severe. Many songbirds partition their time for different activities during migration by flying at night and resting and foraging during the day. In contrast, bats are not active during the day and must accomplish all foraging and migrating during the hours of darkness. This potential time limitation may enhance the selective pressure for efficiencies in any of the mechanisms described below, or perhaps bats have evolved an alternative mechanism to avoid the time constraint (see discussion of torpor).

2.3.1 Hyperphagia and diet selection

Migratory birds deposit fuel stores quickly by entering a state of hyperphagia, with substantial increases in daily food intake (Bairlein 1990; Bairlein and Gwinner 1994; Berthold 1993; Blem 1990; Lindström 1991). Although there is very little research that suggests hyperphagia by bats in preparation for or during migration, one example is *T. brasiliensis*, which becomes hyperphagic before fall and spring migrations (Widmaier *et al.* 1996). Current efforts to identify potential migration stopover sites for bats may provide study locations where fueling rate can be measured using plasma metabolite analysis (McGuire *et al.* 2009a,b), or perhaps relative frequency of feeding buzzes.

In addition to eating more food, many bird species shift their dietary preferences (e.g., seeds, fruits, or invertebrates) to obtain the nutrients they require or to take advantage of abundant resources (Bairlein and Gwinner 1994; McWilliams and Karasov 2005). Bats generally do not exhibit the same degree of dietary plasticity (e.g., an insectivorous bat will not eat berries). Therefore, the ability of bats to choose prey items based on physiologically relevant criteria (e.g., fat content) is probably limited. However, at least some species of insectivorous bats appear to prefer prey items high in unsaturated fatty

acids during the summer (Schalk and Brigham 1995), suggesting the possibility of diet selection during periods of migration.

Seasonal variability in available food resources also may affect diet selection. Bats may simply consume whatever resources are available at the time. In the case of *Leptonycteris yerbabuenae*, a nectarivorous migrant, the diet shifts from C3 plants in the winter to CAM plants during migration and the summer (Fleming *et al.* 1993). The change in nectar consumption appears to be driven by resource availability, rather than by active selection to enhance migration ability; *L. yerbabuenae* migration follows the timing of CAM flowering species, suggesting that the bats migrate because of nectar availability, and not selecting specific nectars to fuel the migration. Similarly, it has been suggested that spring migration of hoary bats (*Lasiurus cinereus*) is timed to coincide with seasonal irruptions of moths in New Mexico (Valdez and Cryan 2009).

In an interesting twist, some bat species may actually take advantage of migration by other species. *T. brasiliensis* feeds opportunistically on migrating moths (Lee and McCracken 2005) and in Europe, *Nyctalus lasiopterus* feeds on migrating songbirds (Ibáñez *et al.* 2001; Popa-Lisseanu *et al.* 2007).

2.3.2 Digestive system flexibility

The digestive system (alimentary tract, liver, and pancreas) exhibits remarkable variation in size and functional capacity in migratory birds (McWilliams and Karasov 2005). Hypertrophy is a generalized response of the digestive system to hyperphagia, which increases the demand to process food (Dykstra and Karasov 1992; McWilliams and Karasov 2001, 2005). Some birds that fly in short bouts and stop to refuel frequently along their migratory journey dramatically increase the size of digestive tract organs on a seasonal basis (Guglielmo and Williams 2003). On the other hand, species that make long-distance nonstop flights across inhospitable landscapes may reduce the size of the digestive system just before departure to minimize mass and metabolic cost during periods of flight; the so called ‘‘guts don’t fly’’ hypothesis (McWilliams and Karasov 2005; Piersma 1998; Piersma and Gill 1998; Piersma *et al.* 1999). Furthermore, the breakdown of protein for fuel in flight appears to be preferentially directed at the gut,

resulting in the ‘‘shrinking gut’’ phenomenon, which, according to the gut limitation hypothesis (McWilliams and Karasov 2001), can impair refueling capacity after arrival (Karasov and Pinshow 2000; Klaassen and Biebach 1994; Lee *et al.* 2002; McWilliams and Karasov 2001). All of these processes combined can mean that birds arrive after a long flight to a stopover with relatively small and impaired digestive systems, increase them dramatically during peak refueling, and then reduce them again just before departure (Piersma *et al.* 1999).

We predict that bats will generally maintain enlarged digestive systems during migration seasons and probably not decrease them before flight in most circumstances. With few diurnal records of migrating bats, we assume that bats migrate during overnight flights, stopping each morning. If bats refuel each night, then increasing the size of nutritional organs should be favored, minimizing the time required for refueling and increasing the time available for migrating. Preliminary evidence suggests that bats feed nightly. Analysis of stomach contents of *L. cinereus* and *Lasionycteris noctivagans* killed at wind turbines during migration revealed that 96% had fed that night (Reimer *et al.* 2010).

2.3.3 Hepatic lipid synthesis capacity

Elevated food intake and rapid fat deposition place elevated demands on the liver for postabsorptive processing of nutrients. Although some of the lipids in fat depots originate directly from the diet, fat also can be synthesized de novo in the liver from carbohydrate and protein precursors. Thus, it may be expected that the biochemical pathways of lipid synthesis may be up-regulated during the migration season. Fatty acid synthase is a hepatic enzyme that uses the substrates malonyl-coenzyme A (malonyl-CoA) and acetyl-CoA to produce the 16-carbon saturated fatty acid palmitic acid (Smith 1994), which can be further extended and desaturated by other enzymes known as fatty acid elongases and desaturases (Jakobsson *et al.* 2006). During the migration of western sandpipers (*Calidris mauri*) liver fatty acid synthase activity increases by about 2-fold (Egeler *et al.* 2000) and the mass of the liver doubles (Guglielmo and Williams 2003), resulting in greatly elevated lipid synthesis capacity. Similar changes occurred in Δ^9- desaturase activity, suggesting that increased desaturation capacity is also important during the migration season (Egeler *et al.* 2000).

The natural diets of insectivorous bats can be high in lipids, and therefore enhanced lipid biosynthetic capacity may not be required during migration. Stomach contents of *T. brasiliensis* during pregnancy and lactation averaged 60% lipids on a dry matter basis (Kunz *et al.* 1995). However, direct measurements of the diets and hepatic biosynthetic capacity of bats are required to assess the generality of this pattern.

2.3.4 Torpor and body temperature reduction

In birds, approximately two-thirds of the total energy cost of migration is incurred during stopover, where energy expenditure is strongly linked to thermoregulatory costs (Hedenström and Alerstam 1997; Wikelski *et al.* 2003). In cooler weather, a significant amount of the metabolic fuel acquired during feeding may be oxidized to maintain body temperature, and is therefore not available to be stored for later use during migratory flight. Hummingbirds appear to use torpor at night during stopover refueling, saving fat acquired during foraging to be used during migratory flight and not in overnight thermoregulation (Carpenter and Hixon 1988; Hiebert 1993). Many birds do not have the ability to use torpor (McKechnie and Lovegrove 2002), yet some appear to lower their body temperature during the migration period, potentially to enhance their ability to deposit and store fuel (Butler and Woakes 2001).

Temperate bats are well known for their ability to enter daily torpor, and for some, long periods of uninterrupted hibernation (Davis 1970; Lyman 1970; Speakman and Thomas 2003). Many bats need to deposit fuel stores before hibernation at a time when temperature and prey availability are both declining. One strategy used by these bats is bouts of daily torpor, which minimize daily energy expenditure and enable greater nutrient storage for less foraging effort (McGuire *et al.* 2009a; Speakman and Rowland 1999). Therefore, it is possible that migrating bats could minimize energetic costs during the inactive periods by entering torpor and saving their fuel stores for flight. During spring migration, male hoary bats (*L. cinereus*) readily entered torpor when placed in a cooled environment (Cryan and Wolf 2003).

The ability of bats to use torpor may prove to be a critical difference between birds and bats during migration. For birds, migration is very energetically costly and consequently

there are a large number of potential adaptations made to adjust to those energy demands. However, if bats are able to use torpor to substantially reduce the energetic costs of migration, many of the adaptations observed in birds relating to hyperphagia and digestive system function may not be needed by bats.

2.4 Fuel Utilization During Flight

In flight, birds and bats alike must maintain very high rates of energy expenditure (approximately 10 – 15 times basal metabolism—Speakman and Thomas 2003; Winter and von Helverson 1998), and thus they must be able to mobilize, transport, and oxidize metabolic fuels quickly enough to meet energy demand. Classic exercise models developed using data for running mammals predict that exercise as intense as flight should be fueled almost completely by carbohydrate oxidation with fat oxidation contributing < 20% of the energy (Roberts *et al.* 1996). Of the fat used by running mammals, most comes from intramuscular lipids, with only 25 – 50% delivered from adipose stores by the blood (Weber *et al.* 1996). Consequently, at high exercise intensities, only 5 – 10% of energetic requirement for a typical nonvolant mammal is supplied from stored fat. In stark contrast to this fuel selection pattern, birds fuel migratory flight almost exclusively with fat, yet the biochemical and physiological mechanisms that make this possible are very poorly understood (Guglielmo *et al.* 2002; McWilliams *et al.* 2004; Weber 1988).

2.4.1 Fatty acid transport proteins

The main factor apparently responsible for the limited use of extramuscular lipids in exercising nonvolant mammals is restricted transport across muscle membranes (Vock *et al.* 1996; Weber 1988). To achieve high rates of extramuscular fat oxidation, migratory birds substantially up-regulate fatty acid transport proteins in flight muscles (Guglielmo *et al.* 1998, 2002; McFarlan *et al*. 2009). Plasma membrane fatty acid binding protein (FABPpm) and fatty acid translocase (FAT/CD36) are muscle membrane proteins responsible for up to 80% of the transfer of fatty acids across muscle membranes (Luiken *et al.* 1997, 1999). They are strongly up-regulated during migration in white-throated sparrows (*Zonotrichia albicollis*—McFarlan *et al.* 2009). Heart-type fatty acid binding

protein (H-FABP) is a cytosolic protein required to receive incoming fatty acids and transport them to be oxidized in mitochondria. H-FABP is one of the most abundant cytosolic proteins in the flight muscles of migratory birds and is seasonally up-regulated during migration (Guglielmo *et al.* 2002; McFarlan *et al.* 2009).

Despite the phylogenetic constraints of their mammalian ancestry, bats may have converged on a similar solution to birds to overcome limitations on the use of extramuscular fat to fuel flight. We are currently testing this hypothesis by studying seasonal changes in the expression of FABPpm, FAT/CD36, and H-FABP in migrating and nonmigrating *L. cinereus*. Encouraging data come from *Myotis lucifugus*, which is known to up-regulate H-FABP expression during hibernation (Eddy and Storey 2004), a physiological challenge that also requires high dependence on extramuscular fat stores.

2.4.2 Muscle oxidative capacity

Bird flight muscles must have a high oxidative capacity to support the intense aerobic exercise required to fly, and this capacity may increase during migration to meet the high demands for fat oxidation. As a result, bird flight muscles have small-diameter muscle fibers, which increase surface area for oxygen and fuel transport, and a high proportion of fast oxidative glycolytic fibers (George and Berger 1966). Fiber diameter also is smaller in long-distance migrants compared to short-distance migrants and sedentary species (Lundgren and Kiessling 1988). Similarly for bats, comparisons of muscle in a migratory species (*T. brasiliensis*) and a nonmigratory species (*Artibeus jamaicensis*) suggest that migrants are better adapted for periods of sustained flight (Foehring and Hermanson 1984; Hermanson and Foehring 1988). To confirm that the differences are related to migration rather than due to foraging strategy or some other factor, more comparisons of migratory and nonmigratory species are necessary.

Mitochondrial volume density, as well as activities of key oxidative enzymes (e.g., citrate synthase, cytochrome oxidase, 3-hydroxy-acyl-CoA-dehydrogenase, and carnitine palmitoyl transferase) of bird flight muscles are typically high, and may become elevated during migration (Driedzic *et al.* 1993; Evans *et al.* 1992; Guglielmo *et al.* 2002;

Lundgren and Kiessling 1985; Marsh 1981). Migrant bats are predicted to exhibit similar characteristics.

The activities of oxidative enzymes in bat pectoralis muscle are among the highest reported for vertebrate muscle and the relative capacities of lipid- and carbohydrate-specific enzymes suggest that lipids are the primary fuel for flight (Armstrong *et al.* 1977; Yacoe *et al.* 1982). Furthermore, insectivorous bats depend more on fat than do frugivorous species, which use more glucose due to their increased dietary glucose (Yacoe *et al.* 1982). Although there are no data about migration-related changes in oxidative capacity in bats, oxidative capacity decreases during hibernation when energy demand is low (Armstrong *et al.* 1977). During migration, bats should increase the already high oxidative capacity of pectoralis muscle to compensate for the increased energy demands of migratory flight.

2.5 Trade-Offs Between Migration and Reproduction

For most of the factors we have described, we predict the selection pressures imposed by vertebrate flight will result in convergent evolutionary responses by bats and birds. In many ways, migration by bats and birds may be similar; however, the reproductive strategy of some bat species may make interpretations of physiological stress and energetic demands difficult. Temperate migratory birds travel from winter to summer grounds where they breed and rear young before returning to the wintering grounds. The demands of migration and reproduction are separated in time. For some bats, mating occurs (at least partially) during fall migration and females store the sperm over winter, becoming pregnant during spring migration (e.g., Cryan 2008; Kunz 1982; Shump and Shump 1982a, 1982b). The temporal overlap of migration and reproduction has physiological consequences resulting from competing time and energy demands. Adaptations or behaviors favoring migration may conflict with reproduction or vice versa.

An example of the conflict between migration and reproduction is the effect of sex on use of torpor during the spring migration of *L. cinereus*. At lower ambient temperatures, males readily enter torpor in response to cold challenge, whereas pregnant females

increase metabolic rate to maintain normothermic body temperature (Cryan and Wolf 2003). The use of torpor enables males to save energy and potentially to wait out unfavorable migration conditions with little energetic cost. At the same time, females incur greater energetic cost and are forced to remain active during conditions when they would otherwise enter torpor. Further studies of bat migration physiology are likely to identify other situations of conflict between the demands of migration and reproduction.

2.6 Conclusions

We have identified several areas where we predict that the selective pressures of vertebrate flight have resulted in convergence between birds and bats. The proposed common features of migration provide a starting point for generating testable predictions about the physiology, biochemistry, and energetics of bat migration. We propose a list of key research questions that should be addressed in the study of fuel acquisition and utilization by migratory bats (Table 2.1). Understanding the similarities and differences between bat and bird migration will lead to insights about the evolution of migration and flight.

Table 2.1 Research questions to be addressed in future studies of bat migration physiology.

Fuel Acquisition

- What is the size and composition of fuel stores?
- Do fat stores become more unsaturated?
- Do bats enter a state of hyperphagia during fuelling?
- Do bats store lean mass and how is it allocated between flight and digestive machinery?
- How flexible is the gut?
- How does liver size and metabolism change?
- What is the role of torpor in fuel acquisition?
- How do bats refuel during migration (stopover duration, refueling rate)?

Fuel utilization

- What is the fuel mixture used during flight?
- Do migrating bats use food ingested pre-flight or feed on the wing?
- Can bats maintain endurance flight using extra-muscular fat stores?
- How abundant are muscle fatty acid transporters and are they seasonally modulated?
- Does muscle aerobic capacity increase during migration (enzymes, capillarity, mitochondrial volume density)?

Reproductive allocation

- What are the tradeoffs in energetic demand for migration and reproduction?
- How do spring and fall migration differ due to reproductive activities?
- What sex-based variations in migration physiology result from reproductive behaviors?

To keep our review concise, we have only considered simplified, stereotypical models of migration. However, in many cases bat migration is anything but simple. Many species have differential migration, with females moving farther than males (Cryan 2003; Fleming and Eby 2003), potentially placing stronger pressure for physiological specialization on females. First-year bats may continue somatic growth during fall migration, and thus greater physiological stress on first-year bats could cause age-biased mortality, as suggested by Tuttle and Stevenson (1977). Some species also appear to be partial migrants, where not all individuals or populations migrate (e.g., *T. brasiliensis*—Fleming and Eby 2003), presenting valuable opportunities for comparative studies. These factors are important to consider when planning studies of migratory species.

Bat migration, and particularly migration physiology, is a field that is only beginning to receive the research attention that is needed. With much to learn, our objective was to present a summary of our knowledge about basic bird migration physiology and, where available, any data about bat migration. We hope this provides incentive for readers to address some of the questions that we have identified.

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CHAPTER 3

3 LIGHT ENOUGH TO TRAVEL: MIGRATORY BATS HAVE SMALLER BRAINS, BUT NOT LARGER HIPPOCAMPI, THAN SEDENTARY SPECIES[2]

Migratory bird species have smaller brains than non-migratory species. The behavioural flexibility/migratory precursor hypothesis suggests that sedentary birds have larger brains to allow the behavioural flexibility required in a seasonally variable habitat. The energy trade-off hypothesis proposes that brains are heavy, energetically expensive and therefore, incompatible with migration. Here, we compared relative brain, neocortex and hippocampus volume between migratory and sedentary bats at the species-level and using phylogenetically independent contrasts. We found that migratory bats had relatively smaller brains and neocortices than sedentary species. Our results support the energy trade-off hypothesis because bats do not exhibit the same degree of flexibility in diet selection as sedentary birds. Our results also suggest that bat brain size differences are subtler than those found in birds, perhaps owing to bats' shorter migration distances. In contrast, we found no difference in relative hippocampus volume between migratory and sedentary species, underscoring our limited understanding of the role of the hippocampus in bats.

3.1 Introduction

Several studies have examined avian brain size in relation to migratory behaviour at the species (Winkler *et al.* 2004; Sol *et al.* 2005; Sol *et al*. 2010) and sub-species level (Cristol *et al.* 2003; Pravasudov *et al*. 2007) and found that migratory taxa have smaller

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brains than non-migratory taxa. Two leading hypotheses to explain observed differences are the behavioural flexibility/migratory precursor hypothesis (Sol *et al.* 2005) and the energy trade-off hypothesis (Isler and van Schaik 2006a). The behavioural flexibility hypothesis suggests that sedentary species face changing environmental conditions over the course of the year and therefore, must be flexible in foraging behaviour and dietary breadth. It follows that, because they confer greater behavioural flexibility, larger brains are selected for in sedentary species, while species with relatively smaller brains are not capable of such flexibility and instead migrate to remain within favourable habitat.

Conversely, the energy trade-off hypothesis argues that because the brain is an energetically expensive organ to maintain (Isler and van Schaik 2006b), animals should partially offset its costs through minimizing others, including the costs of locomotion. Large brains may be particularly problematic for flying organisms because increased mass contributes substantially to the energetic costs of flight (Pennycuick 1972; Alerstam and Lidström 1990). The energy required for migration may limit that available for brains and, conversely, larger brains may increase migration costs. Migratory species are thus expected to have smaller brains than sedentary species (Winkler *et al.* 2004; Isler and van Schaik 2006a).

Studies have also considered variation in avian hippocampus size (e.g. Pravasudov *et al.* 2006) and mammalian neocortex size (e.g. Reader and Laland 2002). The hippocampus is involved in spatial memory in birds and mammals and hence may be important in bats for recalling landmarks and migratory routes. Although the role of the hippocampus is poorly understood in bats, we might expect to observe patterns similar to those in birds, where migratory species have relatively larger hippocampi than sedentary species (Pravasudov *et al*. 2006). In mammals, relative neocortex size correlates with enhanced cognition (Reader and Laland 2002; Dechmann and Safi 2009). In predatory bats, its relative size is reduced in bats that aerially hawk prey in open spaces, species that tend to have larger home ranges than other predatory bats and wings better suited to long-distance flight (Safi *et al*. 2005; Ratcliffe *et al.* 2006).

Addressing the issue of brain size in relation to migration in non-avian vertebrates could reveal general patterns of brain-size evolution (Sol *et al*. 2005). Bats and birds are the only extant vertebrate groups capable of powered flight and so may be subject to similar selective pressures (McGuire and Guglielmo 2009/Chapter 2). Like birds, some bats undertake seasonal migrations and thus experience similar environmental conditions throughout the year (Fleming and Eby 2003). However, few sedentary bats exhibit behavioural flexibility with respect to seasonal diet change on par with that which has been observed in many sedentary birds (McGuire and Guglielmo 2009/Chapter 2).

Furthermore, in temperate zones sedentary birds often experience dramatic environmental variation, while many species of bat (migratory and sedentary) simply hibernate. As a result, in many regions both sedentary and migratory bats that hibernate experience limited seasonal variation. Thus, neither sedentary nor migratory bat species should require as great a degree of overall behavioural flexibility as do most sedentary birds. We therefore posit that if migratory bats have relatively smaller brains than sedentary species this result would better support the energy trade-off hypothesis than the behavioural flexibility hypothesis in flying vertebrates.

3.2 Materials and Methods

3.2.1 Data Assembly

Baron *et al.* (1996) includes brain and body mass data for 342 bat species and specific brain region data for a subset of these species. We confined ourselves to these species and searched the literature for those that had been documented to have a migratory or a sedentary lifestyle (Figure 3.1 and Appendix A for supporting references). Because bats are mostly small and nocturnal, bat migration has been historically understudied; our list of species is almost certainly an underestimate. We also found sedentary species difficult to identify because staying put is the rule in bats and thus rarely explicitly described. To supplement our list of species explicitly described as sedentary, we included those for which there is no mention of migration and year-round, population-specific reproduction at the same location has been documented (Figure 3.1 and Appendix A).

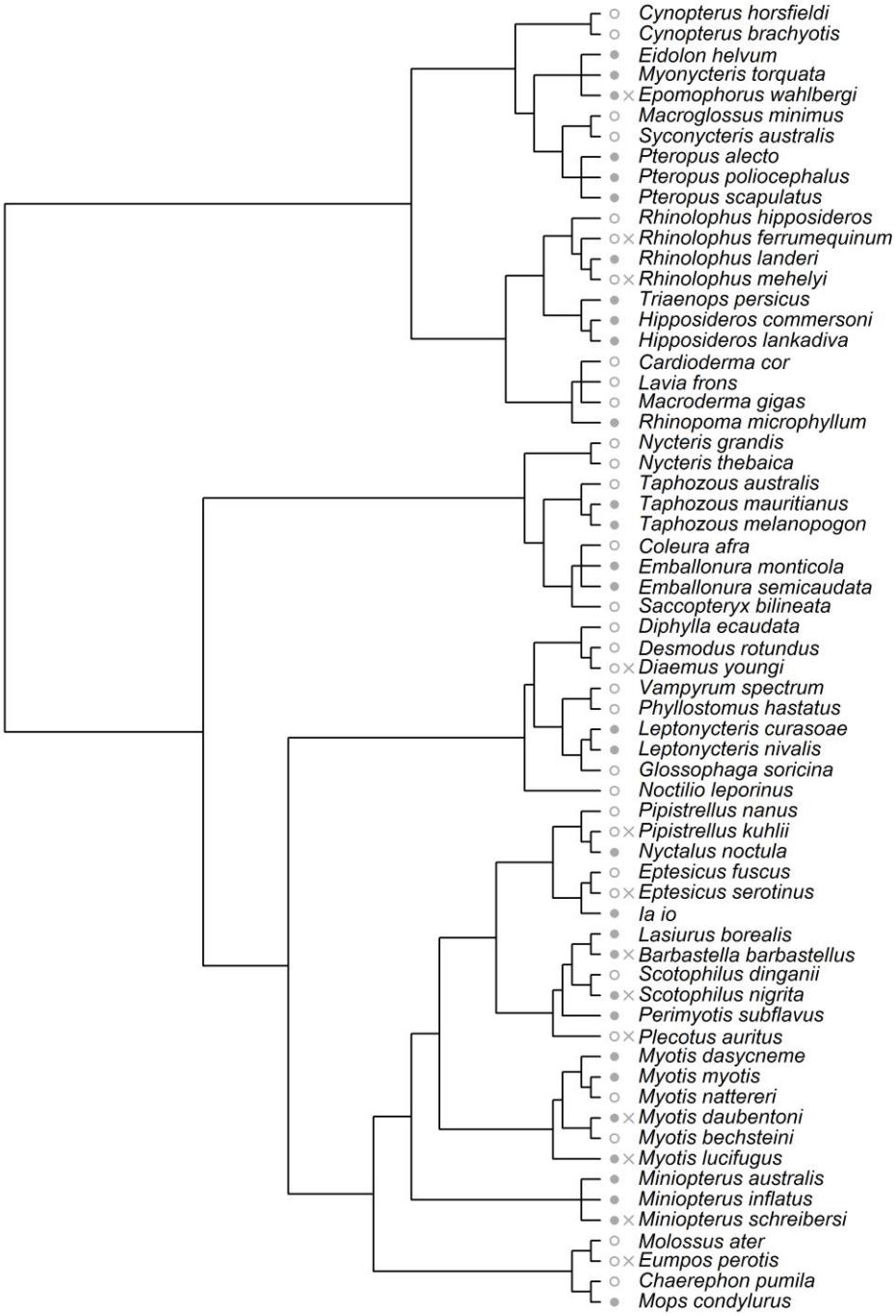

Figure 3.1 Composite phylogeny used to generate phylogenetically independent contrasts (PICs). Migratory species are indicated with filled circles, sedentary species with open circles. Species marked with (×) had only whole brain, not brain region. Data are available in Appendix A.

For character mapping, we constructed the composite phylogeny (Figure 3.1) based on Miller-Butterworth *et al.* (2007; phylogeny based on 16 nuclear genes) for interfamilial relationships. Where > 2 species from a family were present in our data set, we used Stoffberg *et al.* (2010; phylogeny based on 3 nuclear and 1 mitochondrial gene) for species relationships within Rhinolophidae, Hoofer and Van Den Bussche (2003; phylogeny based on 3 mitochondrial genes) and Stadelmann *et al.* (2007; phylogeny based on 2 nuclear and 1 mitochondrial gene) for Vespertilionidae, and Jones *et al*. (2002; supertree of 925 bat species) for all other families. We set branch lengths as equal because different methods were used to construct each of these phylogenies and because this is the preferred and most robust setting in CAIC (Comparative Analysis by Independent Contrasts phylogenetic software; Purvis and Rambaut 1995) when using incomplete phylogenies (see Purvis and Rambaut 1995 and references therein).

3.2.2 Comparative Analyses

We took the standardized residuals from log–log regressions of brain volume (converted from brain mass: brain volume = brain mass/1.036, Baron *et al.*, 1996) versus body mass, and neocortex and hippocampus volume versus brain volume remainder. Additionally, we used medulla oblongata and cerebellum volume versus brain volume remainder as controls. The cerebellum's primary function is motor control and calibration; the medulla oblongata (i.e. the lower portion of the brainstem) controls several autonomic functions (e.g. heart rate and respiration). Neither region was expected to differ with migratory status (Appendix A). For analyses at the species-level (SL) and to generate phylogenetically independent contrasts (PICs), we used these standardized residuals as data. We conducted two-sample t-tests at the SL. For those using PICs (generated by Brunch procedure in CAIC v. 2.6.9), we conducted one-sample t-tests (Purvis and Rambaut 1995). At both levels of analysis, all tests were two-tailed.

3.3 Results

Log brain volume was positively related to log body mass ($F_{1,62}$ = 774.2, r^2 = 0.93, p < 0.001), as were log brain region volumes to their respective log brain volume remainders (hippocampus: $F_{1,50}$ = 605, r^2 = 0.92, p < 0.001; neocortex: $F_{1,50}$ = 1835.2, r^2 = 0.97, p <

0.001; medulla oblongata: $F_{1,50} = 1085.2$, $r^2 = 0.96$, $p < 0.001$; cerebellum: $F_{1,50} = 770.5$, $r^2 = 0.94$, $p < 0.001$). Absolute and log-transformed body mass values did not differ significantly between migratory and sedentary species (two two-sample t-tests: $p > 0.05$ for both; Appendix A).

At the SL and for PICs, relative brain size was significantly greater in sedentary species than in migratory species (SL: $t = 2.26$, $p = 0.027$; PICs: $F_{1,16} = 7.27$, $p = 0.016$; Figure 3.2). Similarly, at both levels of analysis, relative neocortex size was significantly greater in sedentary species (SL: $t = 2.94$, $p = 0.006$; PICs: $F_{1,14} = 4.6$, $p = 0.049$; Figure 3.2). Relative hippocampus (SL: $t = 20.39$, $p = 0.7$; PICs: $F_{1,14} = 0.59$, $p = 0.453$), medulla oblongata (SL: $t = 21.42$, $p = 0.16$; PICs: $F_{1,14} = 3.48$, $p = 0.083$) and cerebellum (SL: $t = 20.99$, p = 0.325; PICs: $F_{1,14} = 2.83$, $p = 0.11$) volumes did not differ between categories (Figure 3.2).

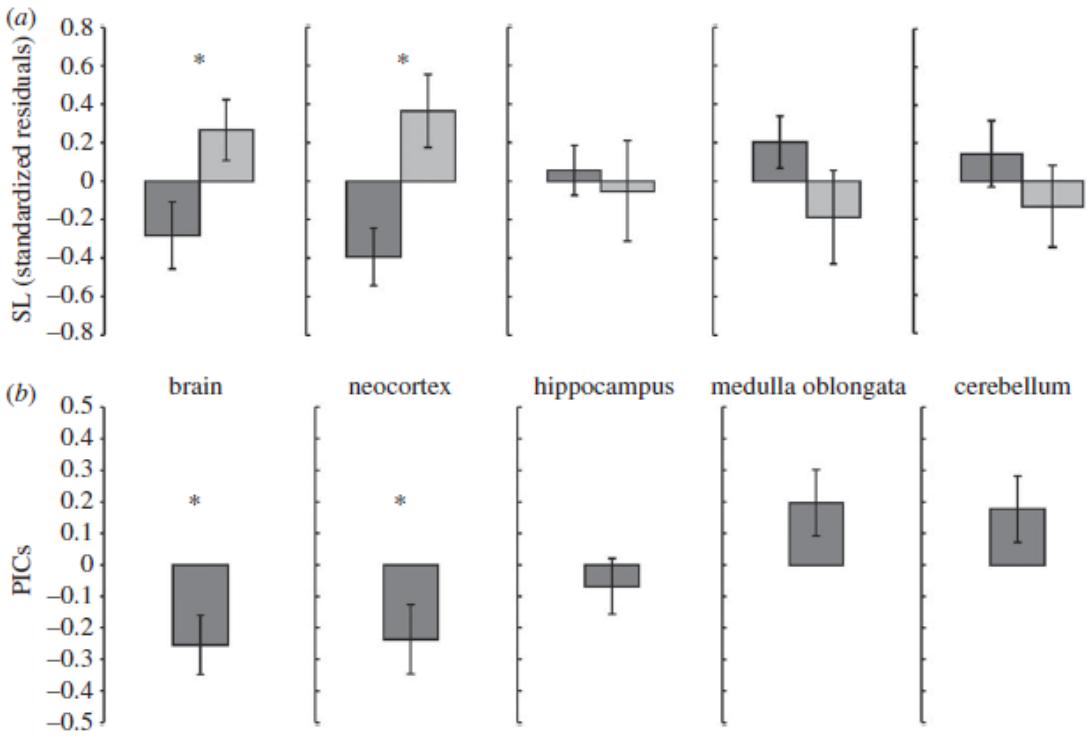

Figure 3.2(a) At the SL, five two-sample t-tests comparing relative brain and brain region volumes (transformed as described in § 3.2) in migratory bats to those of sedentary bats. (b) Based on PICs (generated as described in § 3.2), five one-sample t-tests comparing mean relative brain and region volumes in migratory bats to those for all species pooled (combined mean set to 0 for all). Data are presented as mean ± s.e. (asterisk indicates $p < 0.05$). Dark grey boxes, migratory; light grey boxes, sedentary.

3.4 Discussion

Our comparative analyses suggest that migrating bats have relatively smaller brains and neocortices than sedentary species (Figure 3.2) but do not differ significantly in body mass, supporting the energy trade-off hypothesis. In both bats and birds, two distantly related vertebrate groups with divergent life histories, migratory species have relatively smaller brains than do sedentary species, suggesting a general incompatibility between the high energy demands of migration and those of maintaining and carrying a large brain. In bats, however, the effect appears to be not as profound as in birds. In some bird species, brain size is negatively related to migration distance (Winkler *et al.* 2004). Few bat species are, relative to birds, long-distance migrants and this discrepancy may account, in part, for the apparently smaller effect size in bats.

By contrast, we found no difference in relative hippocampus size between migratory and sedentary bats (Figure 3.2). In birds, an enlarged hippocampus has been linked to migration (Pravasudov *et al.* 2006), but also to smaller scale spatial memory (Roth and Pravasudov 2009). While the role of the hippocampus in bats remains unclear, it may function in migratory navigation (Holland 2007) and, in frugivorous and nectarivorous species, for the relocation of food (Carter *et al.* 2010). Confoundingly, among phyllostomids, gleaners have relatively larger hippocampi than even frugivores and nectarivores (Ratcliffe 2009). Currently, limited data and the potential for multiple roles preclude making clear predictions about hippocampus size and bat migration.

Brain and brain region size variation in bats has been considered in relation to several behavioural, physiological and ecological factors (see Dechmann and Safi [2009] for review). While a variety of factors will influence observed phenotype, our results suggest that energetic limitations play a major role in determining brain size in migrating bats. Further support for the energy trade-off hypothesis comes from studies of brain size and foraging strategies in bats. Brain and neocortex size is smallest in obligate aerially hawking bats foraging primarily in open spaces (Ratcliffe *et al.* 2006; Safi *et al.* 2005), consistent with the idea that the energetic requirements of high powered fast flight (open space aerial-hawking and / or migratory flight) negatively impact brain size. Whether a

bat species' relative brain and brain region size reflects a migratory or sedentary evolutionary history, as our data suggest, or waxes and wanes as a result of individual experience, as in some migratory birds (e.g. Roth and Pravasudov 2009; Sol *et al.* 2010), are among a number of possibilities. Indeed, research into migration and brain development in birds has revealed many unexpected and puzzling factors (e.g. species-specific, latitudinal effects, basal metabolic rate, diet, developmental constraints) and we caution that ours is a preliminary study, demonstrating a correlative, not causal, relationship (see Dechmann and Safi [2009] for review). Furthermore, it should be noted that migration is a characteristic of individuals, not species. Differential and partial migration are both common among bats with many examples of sex-biased migration, and migratory and non-migratory populations within species. Whatever the underlying and interacting selective forces, future studies comparing bat and birds should yield further insight into the processes of vertebrate brain evolution.

3.5 Acknowledgements

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CHAPTER 4

4 MIGRATORY STOPOVER IN THE LONG-DISTANCE MIGRANT SILVER-HAIRED BAT, *LASIONYCTERIS NOCTIVAGANS*[3]

Some bat species make long-distance latitudinal migrations between summer and winter grounds, but because of their elusive nature, few aspects of their biology are well understood. The need for migratory stopover sites to rest and refuel, such as used by birds, has been repeatedly suggested, but not previously tested empirically in bats. We studied migrating silver-haired bats (*Lasionycteris noctivagans*) at Long Point, ON, Canada. We used digital radio-transmitters to track 30 bats using an array of five towers that effectively covered the entire region (*c.* 20 × 40 km). We measured stopover duration and departure direction, and documented movement patterns, foraging activity and roost sites. We measured body composition on arrival using quantitative magnetic resonance and simulated long-distance migration using observed body composition to predict migration range and rate. Migration occurred in two waves (late August and mid-September). Most bats stayed 1 – 2 days, although two remained > 2 weeks. One third of the bats foraged while at the site, many foraging opportunistically on nights when rain precluded continued migration. Bats roosted in a variety of tree species and manmade structures in natural and developed areas. Half of the bats departed across Lake Erie (minimum crossing distance *c.* 38 km) while half departed along the shoreline. Simulations predicted a migration rate of *c.* 250 – 275 km per day and suggest that all but one of the bats in our study carried sufficient fuel stores to reach the putative wintering area (estimated distance 1500 km) without further refuelling. Our results suggest that migrating bats stopover for sanctuary or short-term rest as opposed to extended rest and

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refuelling as in many songbirds. Daily torpor could reduce energy costs when not in flight, minimizing the need for extended stopovers and allowing bats to potentially complete their migration at a fraction of the time and energy cost of similar sized birds.

4.1 Introduction

Many species of bats make annual migrations between summer and winter grounds (Fleming and Eby 2003), yet migration is perhaps the most poorly understood aspect of the biology of bats. Many species are best described as regional migrants, travelling at most a few hundred kilometers from summer roosts to a central hibernaculum (Fleming and Eby 2003). Other species may move > 1000 km and are typically described as long-distance migrants. For regional migrants, migration may not be a particularly demanding period [but see Tuttle and Stevenson (1977) for migration-related mortality in a regional migrant]. However, identifying specific migration-related changes in body composition, physiology and other characteristics is difficult because regional migrants must hibernate for several months upon reaching their destination (McGuire and Guglielmo 2009/Chapter 2). To understand the energetic consequences of bat migration, studies should focus on long-distance migrants (longer migratory distances will magnify any effects) that remain active on the winter grounds, minimizing the confounding effects of preparing for hibernation.

While knowledge of bat migration is sparse (although interest is growing rapidly), there is an enormous literature on bird migration, which contains the accumulated knowledge gained from decades of study. For migratory landbirds (e.g. passerines), stopover to refuel between flights is a crucial component of migration (Hutto 2000). Birds expend more total energy during stopover than during flight, much of the cost arising from thermoregulatory expenses on the ground (Wikelski *et al.* 2003). However, many migratory bat species (and some birds- see § 4.4) are well known for their ability to regularly use torpor to minimize energetic costs (Cryan and Wolf 2003; Dunbar 2007) and could use torpor to minimize the costs associated with nonflight periods of migration (Barclay *et al.* 1988; McGuire and Guglielmo 2009/Chapter 2). Thus, while migratory bats may travel comparable distances to some migratory birds, torpor use during

nonflight periods may decrease the overall cost of migration and minimize the need for extended periods of stopover.

During migration, bats remain almost exclusively nocturnal and consequently it is not possible for a bat to complete a long-distance migration without making several diurnal stops *en route.* Furthermore, it is unclear whether long-distance migrating bats carry sufficient fuel stores to complete the journey without making multi-day stopovers to refuel. In a study of the Brazilian free-tailed bat (*Tadarida brasiliensis*, Geoffroy Saint-Hilaire 1824), O'Shea (1976) estimated flight range based on fat stores in spring and autumn migrating bats and concluded bats would not have been able to complete the autumn migration without refuelling. Similarly, Fleming and Eby (2003) suggested that lesser long-nosed bats (*Leptonycteris curasoae*, Miller 1900) would need to make several stopovers during migration. Serra-Cobo *et al.* (1998) identified temporary roosts used by migrating bent wing bats (*Miniopterus schreibersii*, Kuhl 1817) and it has been reported that migrating little red flying foxes (*Pteropus scapulatus*, Peters 1862) stop at temporary camps while *en route* (Ratcliffe 1932). However, despite frequent suggestions that bats use or would require stopover sites during migratory periods, there is little research describing these sites or the behaviour and ecology of bats during stopover (e.g. Barclay *et al.* 1988).

The silver-haired bat (*Lasionycteris noctivagans*, Le Conte 1831) is a long-distance migrant in North America, and several studies have suggested it uses migratory stopover sites (Barclay 1984; Barclay *et al.* 1988; Geluso 2006; Dzal *et al.* 2009). In spring, *L. noctivagans* migrate through Manitoba, Canada in large numbers (Barclay 1984) and some individuals stop at a site on the south shore of Lake Manitoba for several days (Barclay *et al.* 1988). In autumn, *L. noctivagans* appear to migrate south via the Long Point Peninsula on the north shore of Lake Erie (Dzal *et al.* 2009).

Studies of migratory landbirds have demonstrated that birds frequently stop in areas adjacent to geographic barriers (e.g. the Great Lakes of North America; Diehl *et al.* 2003; Bonter, Gathreaux. & Donovan 2009); it is not known if bats behave similarly or even if bats migrate across such barriers. At the south shore of Lake Manitoba (oriented north-

south) migratory bats are frequently encountered in forested patches along the shoreline and it is suggested that they migrate along the shoreline rather than crossing the lake (Barclay 1984; Barclay *et al.* 1988), although this may largely be due to the orientation of the lake. However, bats are known to fly across the open waters of the Mediterranean Sea (Amengual *et al.* 2007) and congregate at shoreline areas before crossing the North Sea (Ahlén *et al.* 2009). Although poorly documented, it appears that bats may follow geographic features during migration including riparian zones (Serra- Cobo *et al.* 1998; Furmankiewicz and Kucharska 2009), mountain ranges (Baerwald and Barclay 2009) and shorelines (Barclay 1984; Serra-Cobo *et al.* 1998; Ahlén *et al.* 2009).

The objective of our study was to document the behaviour of *L. noctivagans* at an autumn migration stopover site to better understand the occurrence and significance of stopover in bat migration. Specifically, we set out to document the timing of migration, roosting and foraging ecology during migration, and to determine stopover duration and departure direction (and any effects of sex, age or body composition). We followed the within-site movements and departures of individual bats using an automated digital radio-telemetry array. We also used measurements of body composition to model flight range and migration rate.

4.2 Materials and Methods

4.2.1 Study Species

Lasionycteris noctivagans is an 8 – 15 g bat from the family Vespertilionidae. Along with the hoary bat (*Lasiurus cinereus*, Palisot de Beauvois 1796), and eastern red bat (*Lasiurus borealis*, Müller 1776), *L. noctivagans* is one of three species of bat found in Canada that makes annual, long-distance latitudinal migrations. Although the exact wintering grounds are unknown, it has been suggested that populations winter in the south-eastern United States or perhaps southern California and Mexico (Izor 1979; Cryan 2003; Perry *et al.* 2010). There may be an eastern and western population of the species (or at least two general migration routes), with eastern populations migrating to and from the southeastern United States, while western populations migrate to and from the southwest (Cryan 2003). In the southeastern portion of the wintering range, *L. noctivagans* remain

active during winter, using torpor when temperatures drop below freezing (Perry *et al.* 2010). Autumn migration generally occurs August through September (Barclay 1984; Arnett *et al.* 2008). However, precise migration windows and interannual variation are not well understood. Behaviour during migration periods and the use of stopover sites have only briefly been addressed at a single site (Barclay 1984; Barclay *et al.* 1988). Migration rate and the total time required for an individual to complete migration are unknown.

Lasionycteris noctivagans is a tree-roosting species, typically found in crevices, cavities or under loose bark (Kunz and Lumsden 2003; Carter and Menzel 2007; Perry *et al.* 2010). While roost selection in summer is well studied for this species, roost selection during migration is poorly documented (Cryan and Veilleux 2007; but see Barclay *et al.* 1988) although it seems bats are less selective when choosing day roosts (e.g. roosting in man-made structures; Cryan and Veilleux 2007).

4.2.2 Study Site

Long Point, ON, Canada, is a 35 km peninsula extending from the north shore of Lake Erie (Figure 4.1). The lake is *c.* 70 km wide, although crossing distance is reduced to *c.* 38 km if departing from the tip of the point. The base of the point is dominated by wetlands, with land areas featuring small woodlots (Appendix B Figure B.2), residential (cottages) and commercial development. Development extends to Long Point Provincial Park (Figure 4.1), beyond which the point is uninhabited and characterized by a mixture of wetlands, sand dunes and mixed forests that are reduced to sparse stands of eastern cottonwood (*Populus deltoides*) and eastern red cedar (*Juniperus virginiana*) at the eastern tip.

We captured all bats at the Old Cut field station (Figure 4.1) of the Long Point Bird Observatory. The field station is located among a development of residential properties, adjacent to a small (9 ha) woodlot. The area is characterized by a mixed-forest dominated by scots pine (*Pinus sylvestris*), eastern white pine (*Pinus strobus*), eastern cottonwood (*P. deltoides*), dogwood (*Cornus spp.*) and willow (*Salix babylonica spp.*) with dense cover of riverside grape (*Vitis riparia*).

We captured bats in mist nets set around the Old Cut woodlot. Nets were opened nightly between 20 August and 17 September, 2009 (with the exception of 10 and 15 September). We recorded the species, sex, age, forearm length (± 0.05 mm) and mass (± 0.1 g) for each bat we captured. Age (adult or sub-adult) was determined by the degree of ossification of the metacarpal-phalanges joint (Anthony 1988). Bats were typically released within 30 min of capture.

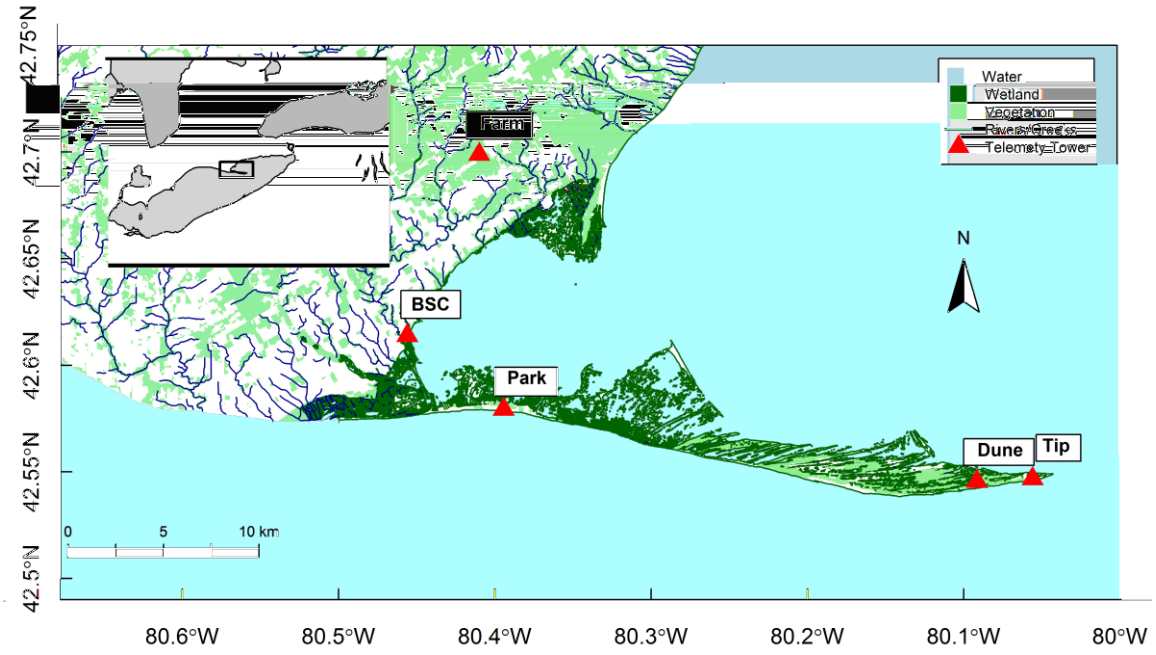

Figure 4.1 The study site indicating the location of the five telemetry towers (Farm, BSC, Park, Dune, Tip). Most towers were outfitted with nine-element antennas which have an approximate detection range of 12 km. The antennas at the Farm tower and the East antenna on the Tip tower were five-element and had an approximate detection range of 5 km. All bats were captured and released at the Long Point Bird Observatory, adjacent to the Park tower. The broader region is shown in the inset, with the study region in the box around Long Point.

4.2.3 Body Composition Measurement

We used quantitative magnetic resonance (QMR, EchoMRI-B; Echo Medical Systems, Houston, TX, USA) body composition analysis to determine fat mass and lean mass (McGuire and Guglielmo 2010). Unrestrained, non-anaesthetized bats were placed in a ventilated 3-cm diameter plastic holding tube that was inserted into a chamber between two fixed magnets. Body composition was measured using a *c.* 3 min scan. The system returned values in grams (0.001 g) for dry fat mass and wet lean mass (muscles and organs, but not skeletal components). Based on previous validation studies, the accuracy of the QMR measurement for fat mass was *c.* ±10% (McGuire and Guglielmo 2010; Guglielmo *et al.* 2011). Following the scan, the animal was released unharmed.

Bats are known to orient by magnetic compass (Holland *et al.* 2006; Holland 2007; Holland *et al.* 2010), and consequently, we sought to ensure that the magnetic fields of the QMR did not affect the behaviour of the bats following release. It is unlikely that the QMR procedure would affect the bats as they are only exposed to the altered magnetic field for a short period. In studies that manipulate magnetic orientation (Holland *et al.* 2006; Holland *et al.* 2010), bats are exposed to altered magnetic fields for *c.* 90 min (compare with *c.* 3 min for QMR). As a control, we performed QMR on only half the bats to which we applied radio-transmitters. The stopover behaviour and departure data did not indicate that the bats were affected by the QMR scans.

4.2.4 Radio-transmitters and Automated Digital Telemetry Array

We used an automated digital radio-telemetry array to monitor the movements and behaviours of the bats at the stopover site. Digitally encoded transmitters allow simultaneous monitoring of up to 200 transmitters on a single frequency; each transmitter is identified by a unique digital signature. The transmitters (Lotek NTQB-1 Nano Tags; Lotek Wireless Inc., Newmarket, ON, Canada) weighed 0.29 g, < 3.1 % of the body mass of the bats in this study. The transmitters had a pulse rate of 5 s resulting in a battery life of ≥ 23 days.

We trimmed a small section of fur from the upper dorsum and affixed the transmitter with ostomy bonding cement (Torbot, Cranston, RI, USA). Upon release, the movement of the transmitters was monitored by an array of five receiving towers (Figure 4.1), three on the point and two on the mainland. The Farm tower (furthest inland) was erected on 9 September 2009 while all other towers were active for the entire duration of the study. Each tower was equipped with a data logging receiver (SRX 600 or SRX DL; Lotek Wireless Inc., Newmarket, ON, Canada) and 1 – 4 Yagi antennas. The Farm, Park and Tip towers had four antennas oriented in the cardinal directions. The Dune tower had a single west-facing antenna. The BSC tower had two antennas facing NE and SW, respectively, ensuring detection of any animals moving between the peninsula and the mainland. Towers with > 1 antenna were equipped with a switching mechanism (ASP-8; Lotek Wireless Inc.) such that antennas were monitored on 8 s cycles (transmitters had 5 s pulse rate, ensuring detection of the transmitter if present and detectable). The BSC, Park, Dune and Tip towers (except Tip east antenna) were equipped with nine-element antennas (Lindsay Antennas, Lindsay, ON, Canada). The east antenna of the Tip tower and all antennas on the Farm tower were five-element Yagi antennas (AF Antronics Inc., Urbana, IL, USA). Calibration tests revealed a line-of-sight detection radius *c.* 5 km for the five-element antennas and *c.* 12 km for the nine-element antennas, although based on simultaneous detections on multiple towers, we can confirm that in some cases, detections occurred at ranges $\geq$ 14 km.

The receivers on each tower continuously recorded all signal detections. When a transmitter was detected, the receiver logged the transmitter ID, time (synchronized among receivers by GPS), antenna and signal strength. Signal strength is generally proportional to the distance from the tower (although a number of factors may decrease signal strength, most commonly physical interference from terrestrial objects that block the signal), and therefore, the direction of the transmitter relative to the tower can be deduced from the strength of the signal on multiple antennas. Over multiple detections, a flight path can be inferred (especially when detections span multiple towers).

To supplement the automated detections by towers, we conducted daytime searches for transmitters with a handheld antenna (3 or 5 – element Yagi) and a receiver (SRX 400 or

SRX 600; Lotek Wireless Inc). For manual telemetry searches, we drove along roads in the areas bound by Long Point Provincial Park to the east (*c.* 1.5 km east of the Park tower) and the base of the point, effectively the area between the Park and BSC towers in Figure 4.1. Where possible, we determined the exact location of the roosting bat and visually confirmed the location of the bat. Upon locating a roost, we noted the geographic coordinates, roost type (tree, building etc.), tree species and the condition of the tree (live, dead, live but partially decaying). We noted approximate roost locations when we could not obtain sufficient signal resolution or were unable to obtain landowner permission on private property.

4.2.5 Data Analysis

All analyses were conducted with the program R (version 2.9.2; R Development Core Team 2009). Before analysing the data, we error checked the detections for each bat. For cases of suspicious detections (typically a single detection well outside the time period of any other observations of the animal), we examined the fractional seconds (unique interval for each transmitter) of the detection time. Any suspicious observations that did not fall on the correct signal interval were discarded. An important consideration in any study involving animal handling is potential behavioural responses to the capture event itself. In our study, it is possible that capture stress may have affected the movement behaviour of the bats we monitored. By monitoring a large region (*c.* 20 × 40 km), we may have mitigated some of this concern, in that short relocation movements would be detected and only cases of longer flights outside the study region may have erroneously been considered departures.

When day roosting, the transmitter signal maintained a relatively constant strength as the bat remained in the same location. When the bat emerged from the roost, there was a sudden increase in signal strength and the strength became variable as the bat moved about within the detection area of the array. In some cases, the location of the roost blocked the signal from the transmitter and it was not detected until the bat emerged from the roost and was active again. In either case, it was possible to very accurately identify the timing of the emergence (see Appendix B Figure B.1 for an example).

We also noted any observations of presumed foraging activity based on transmitter detections. During periods of flight, we observed highly variable signal strength on multiple antennas (but not moving between towers) which we interpreted as foraging (see Figure 4.3). The pattern of the signal detections during presumed foraging was quite distinct from the pattern observed during departure flights. The pattern of signal detections on departure indicated a directed movement, typically fading in signal strength on a single antenna (i.e. a specific direction; Appendix B Figure B.1).

On the night of departure, we recorded the timing of emergence when possible (some cases were unclear), as well as the time when the bat began a directed flight presumed to be the commencement of departure (Appendix B Figure B.1). When possible we inferred a flight path (some cases were unclear) to document the departure direction and whether bats departed across Lake Erie or not (see Appendix B Figure B.3 and Figure B.4 for examples).

From the telemetry data, we calculated the minimum length of stay for each bat as the number of nights between initial capture and the final detection. A bat that departed on the night following capture would be recorded as a 1-day stopover duration.

4.2.6 Migration Simulation

We used the program FLIGHT (v1.21; Pennycuick 2008) to simulate long-distance migration. FLIGHT simulates migration in 6 min steps, calculating changes in fuel stores and flight speed at each step, based on a set of body composition, morphometric and metabolic rate parameters entered by the user. We used fixed values of wing span and area (Norberg and Rayner 1987), and basal metabolic rate (BMR) for all simulations. We calculated BMR from an allometric scaling equation (Speakman and Thomas 2003) using the mean body mass of the *L. noctivagans* we captured during our study. We converted BMR from units of mL O_2 h^{-1} to W using the oxyjoule equivalent described by Lighton (2008) assuming a respiratory quotient of 0.725 (*c.* 95% fat fuel mixture). Atmospheric conditions were calculated using the observed temperature and pressure (at 06:00 on the morning of capture for each individual bat) as described in Pennycuick (2008). We used

observed values for each of the bats in our study (body mass, proportion fat) to simulate long-distance migration for a range of realistic body compositions.

We considered two migration scenarios in our modelling. For the first scenario, we allowed migration to proceed until fuel stores were exhausted. Such an approach provides an estimate of the maximum range the bats may achieve without refuelling. To put the results in a more realistic context, we assumed a migration distance of 1500 km (approximately the distance from southern Ontario to the Gulf of Mexico) to determine whether the bats carried sufficient fuel stores to complete migration without refuelling stopover, and what the migration rate might be. We calculated migration rate by estimating the maximum possible flying time in each night as the difference in the observed departure time (mean of all radio-tracked bats) and the mean observed capture (assumed arrival) time. Dividing the total possible flight hours by the number of flight hours each night estimated the number of days required to complete migration.

4.3 Results

4.3.1 Arrival Timing

We captured 79 bats during the period of the study (20 August–17 September 2009) of which 60 were *L. noctivagans*. The majority (51 of 60) were sub-adults (young of the year), and the sex ratio was approximately equal for adults (4 : 5, M : F) and sub-adults (28 : 23, M : F).

Lasionycteris noctivagans migration occurred in two apparent waves (Figure 4.2). Approximately half of the bats were captured during a 10-day period centred around 27 August, followed by a period of *c.* 1 week with only a single capture. A second 10-day wave centred on 12 September accounted for the other half of the captures. All adults were captured in the second wave. There was no difference in the sex ratio in the two periods (two-sample test for equality of proportions; $\chi^2 = 0.60$, $p = 0.44$). Preliminary stable hydrogen isotope analysis does not suggest the two groups originated at different latitudes (Fraser 2011).

The timing of *L. noctivagans* migration at Long Point is consistent among years (Figure 4.2 inset). The two periods of arrivals that we observed are consistent with capture records from previous seasons (L. Hooton, A. Adams, E. Fraser, pers. comm.). Although capture efforts were not standardized among years, and there is surely some annual variation, migrants were predictably captured in two waves at the end of August and mid-September, respectively.

All *L. noctivagans* captures occurred in the latter part of the night, mostly in the hours preceding dawn. We captured one *L. noctivagans* at 12:05 am (386 min before sunrise) but this was an exception. Median capture time was 81 min before sunrise and 78% of captures were within 120 mins of sunrise. The pattern of early morning captures and the lack of evening captures suggests that bats were captured as they arrived at the site following a migratory flight. Although it is impossible to confirm the exact arrival time of unmarked animals, we assume for the purposes of our analysis that we captured bats as they arrived at the site.

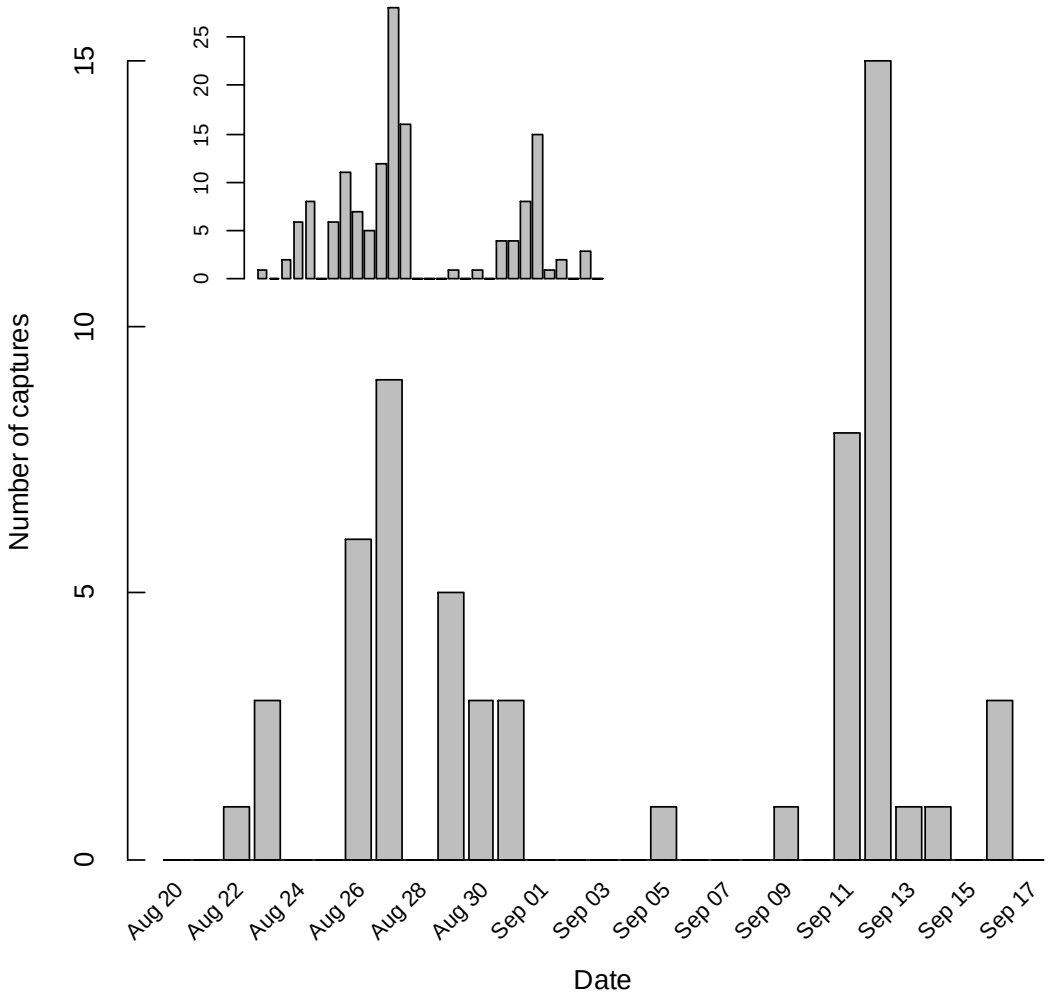

Figure 4.2 Silver-haired bats (*Lasionycteris noctivagans*) migrate through Long Point in two distinct waves. The number of bats captured by date in autumn 2009 (n = 60). Nets were not opened on Sept 10 or Sept 15. The inset combines the captures from this study with captures reported over the same time period in 2007 and 2008.

4.3.2 Stopover Behaviour and Departure

We applied radio-transmitters to 30 *L. noctivagans*, 17 males and 13 females including one adult of each sex. Twenty-one transmitters were applied to bats that arrived in the first wave (August), the remaining nine were applied to bats in the September group. The number of detections ranged from 89 to 14 911 (i.e. one transmitter was detected 14 911 times between the time the bat was released and when it departed the site).

We manually tracked 14 bats to 17 day roosts (Appendix B Figure B.2) including two consecutive day roosts for three bats. All detected roosts were within 3 km of the capture location, and all but one within 1.5 km. For consecutive roosts used by the same bat, roosts were within 700 m of each other. Bats used a variety of natural and man-made roosts. Roost trees included scots pine, eastern white pine, eastern cottonwood and willow. Some trees were healthy, others were dead or in varying stages of decay. Roost trees were located within natural woodlots or isolated trees in residential areas. Bats also roosted in a children's playhouse, a utility pole, and one roost appeared to be behind the window shutters of a cottage.

We observed presumed foraging bouts for 10 bats (excluding two bats that remained at the site for 13 and 22 days; Appendix B Figure B.5). Foraging occurred on arrival, prior to departure or throughout the night (on nights where bats remained at the site). Five bats appeared to forage (bouts 36 – 126 min long) before roosting for the day. Four bats appeared to forage prior to departure, either single bouts (59 – 72 min) or multiple bouts (18 – 46 min each). Three bats engaged in multiple foraging bouts on nights when they remained at the site (not the night of arrival or departure) (Figure 4.3).

Stopover duration (minimum length of stay) was generally quite short (Figure 4.4). Most bats (21 of 30) departed from the site on the night after capture. The pattern of detections for these bats is consistent with the bats arriving near dawn after completing a migratory flight, roosting for the day and continuing on migration the next evening. Two bats made extended stopovers (13 and 22 days) described in Appendix B Figure B.5.

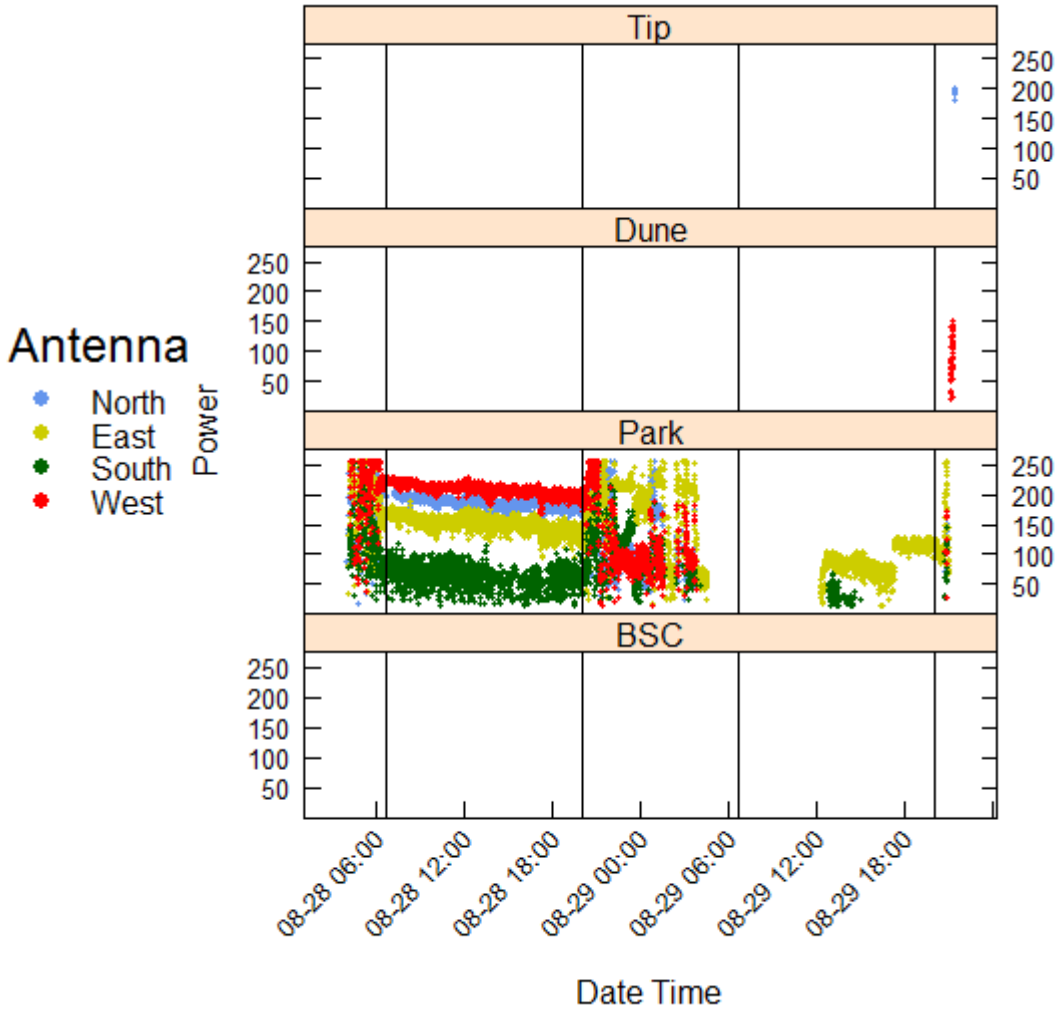

Figure 4.3 Telemetry observations for a sub-adult male *L. noctivagans*. Vertical lines indicate sunrise and sunset. The bat was captured and released on the morning of August 28. On release the signal strength is highly variable and the bat is detected on all four antennas of the Park tower, a period of suspected foraging. At dawn, the bat roosted very near to the Park tower. The transmitter was detected on all four antennas throughout the day, although signal strength is strongest to the west (the bat roosted in a children's playhouse). The following night the bat foraged on and off throughout the night (highly variable signal strength on multiple antennas) and roosted in the early hours of the morning. The bat spent the second day partially wedged beneath some shedding bark on a dead scots pine tree in Long Point Provincial Park, resulting in variable signal strength. Within minutes of emerging from the roost on the evening of August 29, the bat moved east of the Park, past the Dune and departed from the Tip of Long Point. See Appendix B Figure B.1 for an expanded view of the departure.

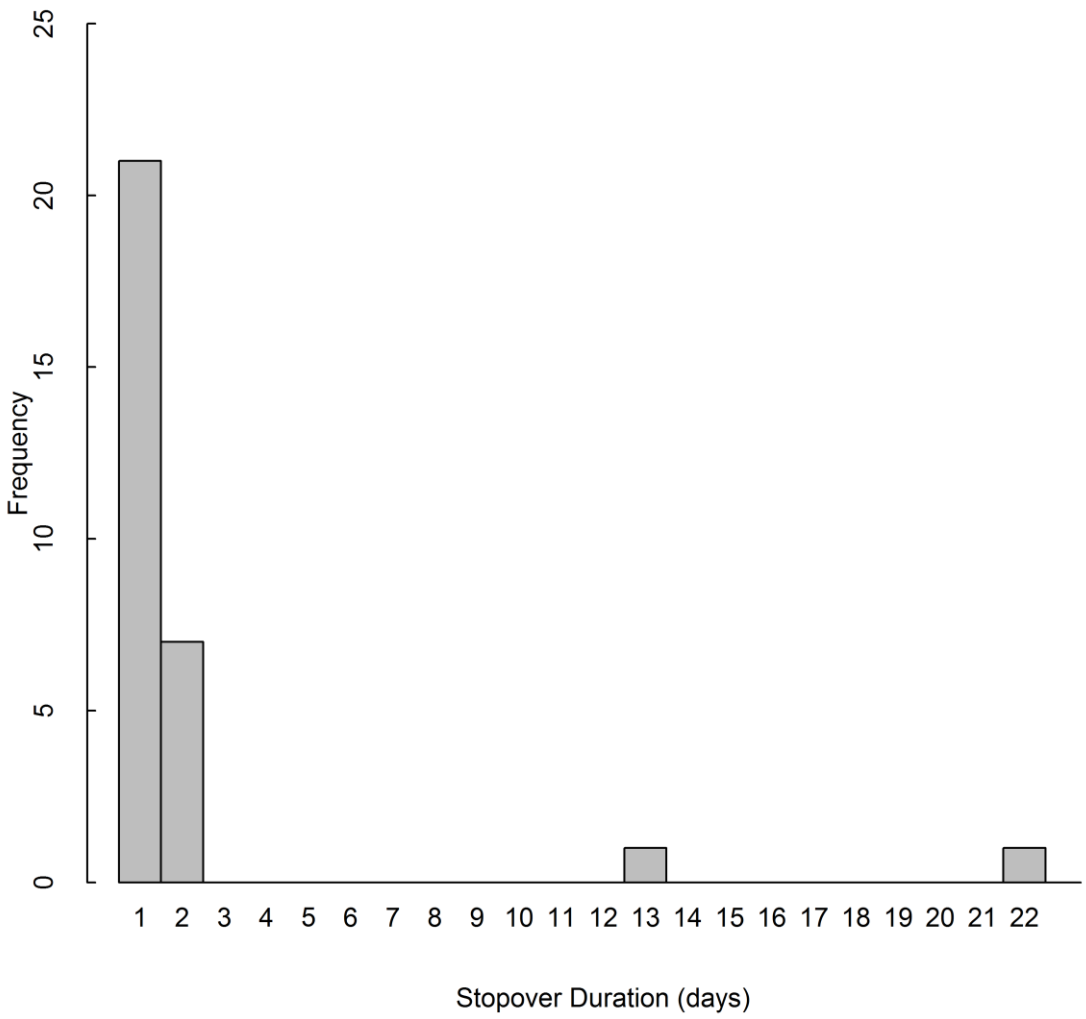

Figure 4.4 Stopover duration for *Lasionycteris noctivagans* at Long Point, Ontario, during autumn migration. A one-day stopover indicates the bat departed on the evening following capture the previous morning.

Seven bats made a 2-day stopover. For six of these, there was rain on the night after capture. On the night with rain, three of the bats remained close to the roost from the previous day (consecutive day roosts < 700 m apart; Appendix B Figure B.2), while the other three moved back to the mainland on the second night, a distance of at least 6 km. No bats departed on nights with rain.

On the night of departure, emergence timing was very consistent (29 ± 2.8 min after sunset, mean ± SEM, n = 24; Appendix B Figure B.1). Following emergence there was typically a brief period of erratic movement prior to departure (Appendix B Figure B.1). Three bats remained at the site for 72 – 104 min before departing, while the remainder began a departure flight shortly after emerging from the day roost (9 ± 1.4 mins mean ± SEM; n = 17).

Generally, departure was quite obvious and we assigned a departure direction to 24 bats. Departures typically followed one of three patterns. The most common route appeared to be for bats to depart across Lake Erie (n = 12). Some of the presumed cross-lake departures flew along the length of Long Point past the Tip tower (e.g. Appendix B Figure B.3). Others flew south from areas to the west of the Dune tower in the mid-regions of the point, some having crossed the bay from the Turkey Point area (point along the shoreline north of the Park tower and south-east of the Farm tower in Figure 4.1). Still others departed to the south while they were still within range of the Park tower.

Eight bats departed along the west shoreline of Lake Erie (e.g. Appendix B Figure B.4) and four others crossed the bay to the north east, presumably to follow the east shoreline. A further three bats did not have a clear departure but were last detected on the mainland. With no subsequent detections on the towers on the point, they likely continued along the shoreline. The final three bats did not have a clear departure event, rather the transmitter simply stopped being detected. The telemetry array remained active until 15 October for other research, and none of the bats were ever detected after the departure we recorded.

We recorded temperature, atmospheric pressure, wind speed and wind direction data at the time of departure. We then considered three departure patterns (cross-lake, west

shoreline and east shoreline) and assessed whether departure direction was related to the environmental variables using ANOVA. We found no evidence that departure direction was related to temperature at departure time (15.7 – 22.6 °C; $F_{2,21}$ = 1.46, p = 0.26), wind speed (3.61 – 9.17 m s^{-1}; $F_{2,21}$ = 1.92, p = 0.17) or atmospheric pressure ($F_{2,21}$ = 1.17, p = 0.33). Body composition (13.9 – 21.5 % body fat) also had no effect on departure ($F_{1,10}$ = 0.64, p = 0.44). The distribution of sexes was not different from the expected even distribution of males and females in each direction (χ^2 = 1.5, d.f. = 2, p = 0.47). Because of the circular nature of wind direction data, any statistical analysis with our small sample sizes would be inappropriate (Fisher 1993), but visual assessments of plots of wind direction and departure direction did not suggest an association.

4.3.3 Body Composition and Migration Simulations

We measured the body composition of 36 *L. noctivagans* (four adult male, five adult female, 14 sub-adult male, 15 sub-adult female). The bats carried 19.3 ± 0.7 % body fat on arrival at Long Point (range 11.9 – 28.7 %). When controlling for body mass, there were no sex or age differences in fat mass (all $p > 0.05$). However, despite the lack of a statistically significant age effect on fat mass, the mean and maximum fat values were greater for adults than subadults, suggesting that the small number of adults may limit the analysis.

Table 4.1 Results of simulated long-distance migration using observed body composition values for bats captured at Long Point. The top four rows present results for simulations where migration continued until fat stores were exhausted, while the bottom two rows present results for simulations where migration distance was fixed at 1500 km.

Parameter	Mean ± s.e.m	Range
Out of fuel time (days)	9.0 ± 0.3	(5.5 – 13.9)
Out of fuel distance (km)	2501 ± 110	(1393 – 4135)
Mean true air speed (m s^{-1})	9.04 ± 0.08	(8.18 – 9.84)
Migration rate (km day^{-1})	276.1 ± 2.4	(250.8 – 301.8)
Time to 1500 km (days)[a]	5.73 ± 0.03	(5.40 – 6.08)
Migration rate (km day^{-1})	262 ± 1.4	(246.6 – 277.8)

[a]one bat did not have sufficient fuel stores to complete a 1500km journey

The migration simulations predicted a mean true air speed of 9.04 ± 0.08 m s^{-1} (Table 4.1). The predicted flight speed closely matches a number of estimates of flight speed we made from the difference in detection times of an individual making a straight line flight between two towers of known distance (data not presented). The FLIGHT simulations suggest that the fuel stores on arrival at Long Point (presumably after having completed at least one migratory flight) were sufficient for all but one bat to complete a further 1500 km migration. At the predicted flight speeds, it would take 5 – 6 nights to complete migration (assuming a migratory flight takes place every night) resulting in a migration rate of *c.* 250 – 300 km day^{-1} (Table 4.1). Any periods of stopover will decrease the migration rate.

4.4 Discussion

Most of the *L. noctivagans* in our study appeared to stay only one day at Long Point before continuing migration. This is much shorter than most passerine birds monitored with the same telemetry system at this stopover site. Mean stopover duration was 3.4 and 8.9 days for Swainson's Thrushes (*Catharus ustulatus*, Nuttall 1840) and Hermit Thrushes (*Catharus guttatus*, Pallas 1811), respectively (Mills *et al.* 2011). The difference in stopover durations suggests that bats may be much more energy efficient during the stopover phase than birds, perhaps by using torpor to save their on-board fuel stores for subsequent flights. Some bats (7 of 30) stopped over for 2 days, but in almost every case, this was associated with rain which appeared to postpone migratory flight. During spring migration in Manitoba, a similar proportion of *L. noctivagans* stopped for at least 2 days (Barclay *et al.* 1988), although comparison is difficult as that study did not track individuals. Bats that remained at Long Point for more than one day in our study engaged in foraging, which suggests that refuelling may be a contingency behaviour for bats when continued movement is delayed. It may be that undistracted migration is a priority and multi-day stopovers are reserved for times when weather prevents bats from safely continuing on. Many studies of avian migration have documented lower departure likelihoods in rainy conditions (e.g. Schaub *et al.* 2004). Furthermore, rain increases energetic costs to flying bats (Voigt *et al.* 2011) potentially adding greater risk for bats compared with birds.

The bats in our study carried fat stores equivalent to about 19 % of their total body mass, similar to that observed in migratory passerines (mean 24.6 % body fat for 39 passerine species; Dunning 2008). Simulation of long-distance migration using the observed fat loads suggests that all but one of the bats in our study carried sufficient fuel stores to complete the migration without requiring an extended refuelling stopover. A limitation of the migration simulations is that they only consider energetic costs during periods of migratory flight (effectively the simulation models a nonstop flight and we have split that flight into multiple bouts). Daily torpor could provide dramatic energy savings during nonflight periods. The torpid metabolic rate of *L. noctivagans* at 21 °C was only 2.5 % of the metabolic rate of bats that remained euthermic (Parkinson 2008). The hourly daytime temperatures at our site ranged from 14.7 to 22.7 °C (18.9 ± 1.9 °C, mean ± SD; Environment Canada WMO ID 71464) suggesting bats at our study site could potentially realize similar energy savings. Arousal costs could be substantial, however, these costs may be mitigated through selection of roosts where passive rewarming is possible (Cryan and Wolf 2003). Furthermore, Cryan and Wolf (2003) have shown that when accounting for arousal costs, energetic benefits are realized even for short bouts of torpor lasting only a few hours.

Compared to estimates of energetic costs in bird migration where up to two-thirds of the total cost of migration is accounted for during periods of stopover (much of that because of thermoregulatory costs; Wikelski *et al.* 2003), bats that intersperse periods of migratory flight with roosting in torpor may accomplish similar migrations at a fraction of the costs for birds. We propose the term ‘torpor-assisted migration’ to describe this migratory strategy. Many temperate migratory bat species are likely to employ the ‘torpor-assisted migration’ strategy to minimize the energetic costs of migration. Although true torpor is uncommon among birds (McKechnie and Lovegrove 2002), it would be interesting to test for ‘torpor-assisted migration’ strategies among avian taxa which have been reported to use torpor (e.g. Trochilidae and Caprimulgidae). Carpenter and Hixon (1988) suggested that a Rufous Hummingbird (*Selasphorus rufus*, Gmelin 1788) they observed at a stopover site may have saved as much as 10% of its total fat store by using torpor during overnight roosting. Hiebert (1993) observed seasonal variation in *S. rufus* torpor use, suggesting that torpor is associated with migratory

refuelling. Hypothermia has been suggested to serve a similar function (Wojciechowski and Pinshow 2009; Carere *et al.* 2010). Blackcaps (*Sylvia atricapilla*, Linnaeus 1758) using overnight hypothermia at a stopover site may have achieved 30% energy savings (Wojciechowski and Pinshow 2009).

The small number of adult bats in our study was surprising. It may be that adults do not roost in the area where we captured bats, preferring to roost on the mainland or further along the point, or it may be that the adults follow a different migratory route. In a study that translocated White-crowned Sparrows (*Zonotrichia leucophrys gambelii,* Nuttall 1840) from the west coast to the east coast of the United States, hatch year birds instinctually continued to migrate due south while adults corrected for the displacement (Thorup *et al.* 2007), suggesting experienced adults use a map and compass orientation. The first year bats we captured in our study had never migrated before and thus encountered Lake Erie for the first time. Adults with experience from previous years may have selected an alternative route to avoid crossing the lake (neither of the two adults we tracked departed across the lake), although this seems unlikely. Lake Erie does not appear to present a significant barrier to bat migration. Of the bats we were able to assign a departure direction, half appeared to cross the lake as they continued on migration. Even if the bats did veer from this course before reaching the opposite shore, our results confirm that bats will readily fly >10 km over open water during migration.

The bats we observed were not selective in their roosting habits. Some roosted on the point, others moved back to the mainland. Bats roosted in a variety of trees (live or dead) and manmade structures, some in natural woodlots and others in residential areas. The only previous study of roost selection during migration (Barclay *et al.* 1988) found bats to be selective in choosing their roosts, contrary to our study. The opportunism we found may be a result of the characteristics of the site. Delta Marsh (site of the Barclay *et al.* 1988 study) is a small, relatively natural forest that runs along the lakeshore, compared with the relatively developed, heterogeneous landscape at our study site. Alternatively, climate may have played a role. Autumn temperatures during our study were relatively warm and consistent compared to the unpredictable and cool temperatures experienced during spring migration at Delta Marsh. Cooler, unpredictable temperatures combined

with energetic demands of pregnant females in spring may exert greater pressure on selecting favourable microclimate.

Our combined telemetry and simulation modelling results suggest that *L. noctivagans* are able to complete their migration without the need for extended stopovers. Some bats remained at the site for extended periods, but the majority stopped only during diurnal periods. Bats were opportunistic in roost selection, spending the day in a variety of natural and anthropogenic roosts. Some bats, although not all, foraged while at the site suggesting that refuelling may be accomplished in numerous opportunistic occasions during migration, particularly when inclement weather precludes further migration on a given night. Approximately equal numbers of bats departed across Lake Erie and along the shoreline, which suggests alternate strategies for dealing with an ecological barrier. Migration simulations based on body composition measured on arrival at the site indicate that the bats may reach the south-eastern United States in 5 – 6 nights and cover 250 – 300 km per night. The overall energetic cost of migration for bats may be only a fraction of the costs experienced by a similar sized bird because of the use of daily torpor, and this should be a high priority for future studies.

4.5 Acknowledgements

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Ontario Council on Animal Care (protocol no. 2008-003) and a Wildlife Scientific Collector's Authorization from the Ontario Ministry of Natural Resources (authorization no. 1050823).

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CHAPTER 5

5 PHENOTYPIC FLEXIBILITY IN MIGRATING BATS: SEASONAL VARIATION IN BODY COMPOSITION, ORGAN SIZES, AND FATTY ACID PROFILES

Aerial hawking insectivorous bats may fly continuously for long durations over great distances during regular foraging, and consequently it is unknown if, or to what degree, migratory flight poses additional physiological challenges. We tested the hypothesis that migration is physiologically demanding for bats by examining migration-related phenotypic flexibility. Based on previous studies of migratory birds, we made predictions about variation in body composition, organ sizes, and fatty acid profiles of adipose tissue and flight muscle membranes.

5.1 Introduction

The ability to fly allows many animal species to migrate great distances in relatively short periods of time, although the absolute cost of flight per unit time is extremely high (Schmidt-Nielsen 1972; Maina 2000). Thus, flying migrants experience high energy demands during their annual migrations, which are often reflected in dramatic changes in physiology (e.g., Guglielmo and Williams 2003; McWilliams *et al.* 2004; Guglielmo 2010). In birds, endurance flights required for migratory movements far exceed the typical amount of time spent flying at any other time of the year (e.g., Portugal *et al.* 2011) and this leads to seasonal changes in numerous characters at the molecular, biochemical, tissue, organ, and whole animal levels. Such reversible changes are referred to as phenotypic flexibility (Piersma and Drent 2003; Piersma and van Gils 2011).

In contrast to birds, very little is known about the migration of bats (McGuire and Guglielmo 2009/Chapter 2). Many species migrate long distances (> 1000 km; Fleming and Eby 2003) but the physiological adaptations and trade-offs involved have received little attention (reviewed by McGuire and Guglielmo 2009/ Chapter 2). Some species fly for long durations (Barclay 1989) and travel great distances (Best and Geluso 2003) when foraging in non-migratory periods and consequently the degree to which migration poses

physiological challenges to bats is unknown. A night of migratory flight may be no more demanding than a long night of foraging.

To investigate the physiological challenges of migration in bats, we examined migration-related phenotypic flexibility in migrating and non-migrating bats. We hypothesized that bats and birds, despite being distantly phylogenetically related, would face similar physiological challenges during migration (McGuire and Guglielmo 2009 / Chapter 2). Both groups are vertebrate endotherms that have evolved the capacity for true powered flight (Rayner 1988; Maina 2000) and thus may have converged on similar solutions to some of the physiological challenges of migration. Consequently, in the absence of previous studies of migratory bats, we made predictions regarding phenotypic flexibility in bats based on previous studies of migratory birds. Specifically, we examined seasonal variation in body composition (fat and lean stores), organ sizes (digestive and exercise organs), and the fatty acid composition of adipose tissue and flight muscle membranes. Generally, we predicted that migrating bats would have larger fat stores, enlarged exercise and digestive organs, a greater degree of unsaturation in adipose tissue fatty acids, and more polyunsaturated fatty acids in muscle membranes. Background, justification, and additional details for each of these predictions is given below.

5.1.1 Body Composition

Oxidation of fat provides 8 – 10 times more energy than either carbohydrates or protein (wet mass basis) (Jenni and Jenni-Eiermann 1998). Migratory birds deposit large fat stores prior to migration (McWilliams *et al.* 2004) and fuel migratory flight almost exclusively with stored fat (Jenni and Jenni-Eiermann 1998; McWilliams *et al.* 2004). In extreme cases fat may account for > 50% of pre-migration body mass (Piersma and Gill 1998). Several studies have assumed fat to be the primary fuel for migratory flight in bats (O'Shea 1976; Fleming and Eby 2003; McGuire *et al.* 2012/Chapter 4), but there have been no detailed studies of fuel metabolism in bat migration.

Seasonal fat deposition is common in many species of bats that migrate to and from hibernacula (e.g., Krulin and Sealander 1972; Ewing *et al.* 1970; Kunz *et al.* 1998) but it is difficult to distinguish between fat deposited to fuel migration or to survive months of

hibernation. Studies of non-hibernating migratory bat species are rare. Brazilian free-tailed bats (*Tadarida brasiliensis*) do not hibernate and deposit fat stores for spring and fall migration (O'Shea 1976). Fat deposition consistent with the timing of migration has also been reported in several West African species (O'Shea and Vaughan 1980) and lesser long-nosed bats (*Leptonycteris yerbabuenae*; Ceballos *et al.* 1997). Fall migrating silver-haired bats (*Lasionycteris noctivagans*) carried similar amounts of fat compared to migrating passerines (McGuire *et al.* 2012/Chapter 4) but without a non-migrating group for comparison it is unclear whether these bats increased the amount of fat stored seasonally. In the current study, we predicted that bats collected during spring migration would have larger fat stores than non-migrating bats.

5.1.2 Organ Size Variation

Migrants face two seemingly opposed activities, flight and refueling, each of which may require increased capacities of different physiological systems. During a migratory flight, animals maintain high intensity exercise for many hours or days while fasting and rapidly depleting nutrient stores (Wikelski *et al.* 2003; Gill *et al.* 2009). These flights are interspersed with relatively long periods of stopover where birds become hyperphagic and seek to rapidly replenish nutrient stores (Lindström 1991; Schaub and Jenni 2001; McWilliams *et al.* 2004). The contrasts between high intensity exercise and hyperphagia have led numerous studies to consider changes in 'exercise organs' (typically flight muscles, heart, lungs) and 'digestive' or 'nutritional organs' (stomach/gizzard, intestines, kidneys, liver) (Hume and Biebach 1996; Piersma 1998; Piersma and Gill 1998; Piersma *et al.* 1999; Battley *et al.* 2001; Guglielmo and Williams 2003; Landys-Ciannelli *et al.* 2003; Bauchinger *et al.* 2005).

5.1.2.1 Exercise organs

Several hypotheses may explain changes in exercise organ sizes during migration. The simplest explanation is that increased flight muscle mass is a response to increased body mass in migrants (Marsh 1984; Lindström *et al.* 2000; Guglielmo and Williams 2003). However, flight muscle mass may increase independent of body mass (Portugal *et al.* 2009). Heart mass is also frequently observed to increase during migration (Piersma *et al.*

1999; Guglielmo and Williams 2003; Bauchinger *et al.* 2005) which suggests increasing body mass alone can-not explain changes in exercise organ masses (Bauchinger *et al.* 2005). Exercise organs may be increased to enhance aerobic exercise performance (Pennycuick 1998; Guglielmo and Williams 2003), or may serve as protein stores which may be catabolised in flight to maintain blood glucose, replenish metabolite pools for the Krebs cycle, or to provide metabolic water (Jenni and Jenni-Eiermann 1998; Gerson and Gugliemo 2011). Regardless of the function, we predict that exercise organs will be larger during migration if migration results in a seasonal increase in body mass and time spent in flight.

5.1.2.2 Digestive organs

While exercise organs generally hypertrophy or vary in size with body mass change, digestive organ masses are far more variable (Piersma 1998). Digestive organ size is proportional to food intake or energy demand (McWilliams and Karasov 2005). During stopover, hypertrophy of digestive organs could increase fuelling rate and minimize time spent at stopover ('migration takes guts'; McWilliams and Karasov 2005). A time minimization strategy may be crucial to successful migration (Alerstam and Lindström 1990) as ~ 90 % of the total time taken by migration is at stopover (Hedenström and Alerstam 1997). Conversely, digestive organs represent additional mass that must be transported during migratory flights and there may be selective pressures to reduce digestive organ mass resulting in a more energy efficient flight ('guts don't fly'; Piersma and Gill 1998).

Changes in the size of digestive organs may be used as an indicator of migratory strategy. Birds that migrate long-distances without stopping to refuel (typically crossing ecological barriers such as oceans or deserts) reduce the size of digestive organs prior to or during flight, and rebuild these organs upon arrival at stopover sites (Hume and Biebach 1996; Piersma 1998; Piersma and Gill 1998; Piersma *et al.* 1999; Battley *et al.* 2000; Battley *et al.* 2001; Bauchinger *et al.* 2005). Alternatively, species that stop frequently along the migratory route may maintain enlarged digestive organs to minimize refuelling time at each stopover. Such is the case for Western Sandpipers (*Calidris mauri*) that migrate in 'short hops' (Guglielmo and Williams 2003).

Little information is available about the behaviour of bats at stopover sites. At a fall migration stopover site, silver-haired bats (*L. noctivagans*) did not forage extensively (McGuire *et al.* 2012/Chapter 4), but we lack information from other seasons and species. During migration, most bats remain nocturnal and therefore must complete all foraging and migratory flight within the hours of darkness, unlike nocturnally migrating songbirds that forage by day and fly at night. We predicted that the additional time pressure faced by migratory bats would select for enlarged digestive organs to facilitate rapid refueling.

5.1.3 Fatty Acid Profiles

5.1.3.1 Adipose stores

Migrants may increase the quantity of stored lipids (the size of fat stores), but qualitative changes in the fatty acid (FA) composition of these stores are equally important to consider. FAs serve both as energy substrates stored as triglycerides in adipose tissue, and as structural components (phospholipids; PL) of cell membranes in flight muscle. The dual role of FAs in migration is explicitly addressed by Price (2010) describing the 'fuel hypothesis' and the 'phospholipid hypothesis' in discussing optimal FA composition in migratory birds. There is an extensive body of literature describing whole animal performance effects of varying FA profiles. Several reviews have covered this issue in detail (e.g., Guglielmo 2010; Price 2010) and consequently we will only briefly review the pertinent issues here.

FAs vary both in chain length (number of carbon atoms) and the degree of unsaturation (number of double bonds). Furthermore, some FAs may be synthesized *de novo* by birds and mammals, while others must be derived from the diet (essential dietary FA). Poly-unsaturated fatty acids (FAs with multiple double bonds; PUFA) with the first double bond at the third or sixth carbon from the methyl end (n-3 / n-6 or omega-3 / omega-6) must be obtained from dietary sources. As an energy substrate (the fuel hypothesis), FA chain length and degree of unsaturation affect the potential energy that may be derived from fat stores (more ATP from longer FA with fewer double bonds) (Price 2010). However, these same factors affect the rate of mobilization; shorter FAs with more double bonds are preferentially mobilized (Price *et al.* 2008). Consequently adipose FA

profiles present a trade-off between rapid mobilization and energy density. Many studies of migratory birds predict an increase in the degree of unsaturation (favouring mobilization) in adipose stores during migration, yet only some studies actually find such a seasonal effect (Klaiman *et al.* 2009; Price 2010).

Adipose FA composition is largely determined by diet (although selective mobilization, *de novo* synthesis from non-lipid substrates, and post-absorptive modification may also affect composition), which may vary seasonally in many bird species (Price 2010). While bats generally do not present the same degree of dietary plasticity (an insectivore will not suddenly switch to a diet of seeds or berries) there is some limited evidence that bats select insect prey based on FA content (Schalk and Brigham 1995). If migrating bats are able to alter the FA composition of adipose stores through either dietary selection or preferentially retaining particular FAs, we predict a shift towards shorter chain length and more unsaturated FA.

5.1.3.2 Muscle membrane phospholipids

As components of cell membranes (the phospholipid hypothesis), variation in FA profiles has been suggested to affect whole animal exercise performance. A pervasive theme in both mammalian and avian literature is the importance of essential dietary PUFA (n-3 and n-6 FAs), though the pattern is not consistent. Some studies have suggested high n-6 PUFA increases exercise performance in birds (Pierce *et al.* 2005) and mammals (endurance- Ayre and Hulbert 1997; running speed- Ruf *et al.* 2006). Other studies have suggested that high n-3 PUFA is related to improved exercise performance (Maillet and Weber 2007). Still other studies consider the ratio of n-6:n-3, alternately finding increased (Klaiman *et al.* 2009) or decreased (Guglielmo *et al.* 2002) n-6:n-3 in migrants. Finally, it may be possible that the muscle PL composition does not affect exercise performance at all, rather differences may simply arise due to differences in adipose FAs (Price and Guglielmo 2009). Therefore the role of muscle PL composition in whole animal exercise performance remains unclear. Consequently we do not make a prediction for the muscle PL composition in regards to exercise performance, rather will consider any patterns observed in the context of previous studies.

Muscle PL composition has also been implicated in torpor and hibernation studies. In an extensive review Munro and Thomas (2004) describe tradeoffs between improved torpor performance with high membrane PUFA content, but caution that PUFA are particularly sensitive to autoxidation at low temperatures. For migrating insectivorous bats that likely have low dietary PUFA concentrations (compared to herbivorous rodents) and use torpor at warmer temperatures (if at all), the problems of autoxidation are likely negligible and thus these species should seek to maximize PUFA (Munro and Thomas 2004). However, another review has suggested that a high n-6:n-3 ratio is important for maintaining membrane function at low body temperatures, specifically affecting Ca^{2+}-Mg^{2+} ATPase pumps (Ruf and Arnold 2008). Given that male hoary bats are more likely to use torpor during spring migration than females (Cryan and Wolf 2003), we predict that sexes will differ in muscle PL composition during migration, with males having either greater total PUFA or n-6:n-3 if either the Munro and Thomas (2004) or Ruf and Arnold (2008) theories are supported.

5.2 Materials and Methods

5.2.1 Study Species

Hoary bats (*Lasiurus cinereus*) are the most widespread bat species in North America (Shump and Shump 1982), and are believed to migrate longer distances (in some cases > 2000 km), than other migratory species (Cryan *et al.* 2004). The hoary bat is the largest bat species in Canada (~ 20 – 35 g; Shump and Shump 1982) and females are larger than males (~3% larger; Williams and Findley 1979). Hoary bats are solitary and roost in exposed foliage (Willis and Brigham 2005; Carter and Menzel 2007; Cryan and Veilleux 2007). In summer the sexes are largely segregated with males more commonly found in mountainous regions in the western part of North America, whereas females are widespread throughout the eastern part of the continent (Cryan 2003). Mating occurs during autumn migration (Cryan 2008); females store sperm over winter and become pregnant in spring (females are pregnant with twins as they migrate north; Cryan and Wolf 2003). The winter distribution is not well understood, though it is thought that most individuals overwinter in southern California and Mexico (Cryan 2003).

5.2.2 Animal Collection

Migrating *L. cinereus* were captured May 5 – 17 2009 by setting mist nets across creeks in Bernalillo County, New Mexico, USA (35° 12’ N, 106° 18’ W) or around water pools in the Manzano Mountains, Cibola National Forest, New Mexico, USA (34° 59’ N, 106° 21’ W; see Cryan and Wolf 2003 for a description of the region). Assuming overwintering sites are in Mexico or southern California, migrants captured in New Mexico would be in the early stages of northward migration. Non-migrating bats were captured July 20 – August 1 2008 and 2009 in mist nets set across creeks in Cypress Hills Interprovincial Park, Saskatchewan, Canada (49° 34’ N, 109° 53’ W; see Willis and Brigham 2005 for description). For the non-migrants, we determined age (sub-adult or adult) by the degree of ossification of the metacarpal-phalanges joint (Anthony 1988). By autumn the metacarpal-phalanges joint is fully ossified, and thus it was not possible to determine age in spring migrating bats. Lactating females were identified by manually expressing milk from the mammary glands. Sub-adults and lactating females were released immediately upon capture. We collected 15 female and 15 male migrants (New Mexico), and 8 female and 7 male non-migrants (Saskatchewan). We euthanized the bats immediately following capture by cervical dislocation under isoflurane anesthesia. We recorded body mass (± 0.1 g) and forearm length (± 0.05 mm) and immediately excised samples of pectoral muscle, liver and adipose tissue, which we transferred to pre-weighed individual vials (2 mL Cryotube [Cryo.S, Grenier Bio-One] for liver and muscle, 600 μL o-ring-sealed screw cap microcentrifuge tubes [Fisherbrand; Thermo Fisher Scientific, Pittsburgh, Pennsylvania] for adipose) and stored frozen in a liquid nitrogen cooled dry-shipper (Taylor-Wharton CX-100). The remainder of the carcass was frozen in a sealed plastic bag at -20 °C. Samples were transported back to the lab either in liquid nitrogen cooled cryoshippers or packed in dry ice. Pectoralis, liver, and adipose tissue samples were stored at -80 °C, the remaining tissues at -20 °C. All animal collection and experimental protocols were approved by the University of Western Ontario Animal Use Sub-committee (protocol no. 2008-003-04) and conducted under permits from the New Mexico Department of Game and Fish (permit no. 3424), United States Department of Agriculture- Forest Service (permit no. SND502), Saskatchewan Ministry of Environment (permits no. 08FW080 & 09FW045), and Saskatchewan Ministry of

Tourism, Parks, Culture and Sport (permits no. SP-CHPP-02-08 & SP-CHPP-01-09). Samples from New Mexico were imported to Canada under the approval of the Canadian Food Inspection Agency (permit no. A-2009-01022-3).

5.2.3 Organ Size Measurement and Body Composition Analysis

Carcasses were thawed overnight at 4 °C prior to dissection. We removed the remaining pectoral muscle, intestines (large and small combined), stomach, remaining liver tissue, kidneys, heart and lungs. Intestines and stomach were opened to remove all contents, rinsed in 0.9 % NaCl and blotted dry. We recorded wet mass of each organ/tissue (± 0.0001 g), correcting for subsamples taken in the field. We dried organs to a constant mass at 70 °C, then placed them in pre-weighed filter paper envelopes (Whatman #1) and extracted them with petroleum ether (boiling point 30 – 60 °C) for 6 h in a Soxhlet apparatus. Similarly, we dried the remainder of the carcass at 70 °C, homogenized it with a heavy-duty blender (model CB15l Waring Commercial, Torrington, Connecticut). We divided the homogenate into 2 – 3 pre-weighed filter paper envelopes (Whatman #1) for Soxhlet extraction with petroleum ether.

5.2.4 Fatty Acid Analysis of Adipose and Muscle Tissue

Total lipids were extracted by adding the sample (75 – 120 mg muscle or 6 – 12 mg adipose) to 8 mL chloroform:methanol (1:1 v/v) containing butylated hydroxytoluene (25 mg L^{-1}), homogenizing 2 x 10 s (Polytron PT 10-35, Kinematica Inc., Bohemia, NY), adding 4 mL chloroform, and homogenizing an additional 1 x 10 s. The homogenizer was rinsed with an additional 6 mL chloroform methanol (2:1 v/v) to ensure complete transfer of the sample to the sample tube. The sample was then centrifuged for 15 min at 2056 *g*, and gravity filtered (Whatman #1) into a new tube. The previous sample tube was rinsed with 12 mL chloroform:methanol (2:1 v/v) which was also filtered into the new tube. To separate aqueous solutes, we added 7.5 mL 0.25 % KCl, incubated in a 70 °C water bath for 10 min, and discarded the aqueous layer. The remaining organic phase was transferred to a pear flask (25 mL) and evaporated under vacuum at 60 °C (Rotovapor, Buchi, Switzerland). Dried samples were either dissolved in 1 – 2 mL chloroform:methanol (1:1 v/v) under nitrogen for overnight storage at -20 °C, or immediately dissolved in 100 µL

chloroform for separation of the different lipid fractions. Phospholipid (PL), neutral lipid (NL; primarily triglycerides) and non-esterified FA (NEFA) fractions were separated with Supelclean solid phase extraction tubes (LC-NH_2, 100mg; Supelco, Sigma-Aldrich Canada, Oakville, ON, Canada). The columns were conditioned with 2 mL hexane prior to addition of the samples. After each elution the samples were centrifuged for 1 min at 1370 *g*. NL were eluted with 1.8 mL chloroform:isopropanol (2:1 v/v). Non-esterified FA were eluted with 1.6 mL isopropyl ether:acetic acid (98:2 v/v). PL were eluted with 3 mL methanol. We added heptadecanoic acid (17:0; 3 mg mL^{-1} in hexane) as an internal standard to the NL and PL fractions.

For transesterification, the NL and PL fractions were dried at 70 °C under a stream of N_2, redissolved in 2 mL of 1 M acetyl chloride in methanol, and incubated at 90 °C for 2 h. The samples were then dried under N_2, redissolved in 1 mL methanol and dried under N_2 again to remove any residual HCl and H_2O. Finally, the samples (now fatty acid methyl esters) were dissolved in dichloromethane for analysis by gas chromatography (Agilent Technologies 6890N, Hewlett Packard, Palo Alto, CA, USA). We used a J&W Scientific high resolution gas chromatography column (DB-23, Agilent Technologies), a flame ionization detector and He as a carrier gas (as described in Klaiman *et al.* 2009). The temperature program was 2 min at 80 °C, increase 5 °C min^{-1} for 20 min, hold 180 °C for 3 min, increase 1.5 °C min^{-1} for 13.3 min, hold 200 °C for 0 min, increase at 10 °C min^{-1} for 4 min, and hold 240 °C for 4 min. Fatty acids were identified by comparing relative retention time (retention time/retention time of internal standard) to known standards (Supelco C8 – C24 FAME mix, Supelco 37 component FAME mix, and Supelco PUFA no. 3 from Menhaden oil). For analysis, we did not consider short chain fatty acids (less than 16 carbons) or any fatty acids that comprised less than 0.5 % of the total FA content.

5.2.5 Statistical Analysis

All analyses were completed with the software R (version 2.9.2; R Development Core Team 2009). In all cases where we considered body mass, we used empty mass (capture mass – stomach content mass). We first used a linear model to test for sex and migration effects on forearm length to confirm there was no systematic size difference between the migrating and non-migrating bats. We then tested for sex and migration effects on body

mass, dry lean mass, and fat mass. For all linear models, we started with a full model including main effects and all 2-way interactions. We then removed non-significant terms and re-evaluated the model until only significant terms remained. All masses were $\log_e$ transformed prior to analysis.

For statistical analysis of organ sizes, we considered wet intestine mass, dry lung, kidney and stomach masses, and dry lean (extracted) liver, heart, and pectoralis masses as in Guglielmo and Williams (2003). When comparing organ sizes we originally considered forearm length as a measure of body size but found that organs sizes were more strongly correlated with body mass. Thus, we calculated corrected body mass as $\log_e$(body mass – organ mass) as a measure of body size accounting for part whole correlation, substituting total wet mass, total dry mass, or total dry lean mass as appropriate. For each organ, we used general linear models to test for the effects of sex and migration controlling for corrected body mass (including all 2- and 3-way interactions). We sequentially removed non-significant terms and re-evaluated the model until only significant terms remained. Whether considering models correcting for forearm length or body mass, the results were qualitatively the same.

We also used principal components analysis (PCA) to conduct a multivariate analysis of migration related changes in muscle and organ sizes. We entered all organ masses and total fat mass into a PCA and used MANOVA to test for effects of sex, migration, and body mass on the retained PC axes.

We compared the fatty acid profiles of adipose neutral lipids and muscle phospholipids as described in Klaiman *et al*. 2009. We arcsin square root transformed the proportions of each FA and conducted two-way analysis of variance to test for effects of sex, migration and sex*migration interaction. Furthermore we calculated the double bond index as DBI = Σ[(proportion FA_i)(number of double bonds per FA_i)]. We also calculated the n-6:n-3 ratio, the total proportion of mono-unsaturated FA (MUFA), total proportion of poly-unsaturated FA (PUFA), and total proportion of saturated FA.

5.3 Results

5.3.1 Body Composition

Body composition is summarized by sex and migration in Table 5.1. Female hoary bats were 3.4 % larger than males (forearm length; $F_{1,42} = 30.91$, $p < 0.0001$) but there was no size difference between migrants and non-migrants ($F_{1,42} = 0.081$, $p = 0.78$). Body mass was greater in females ($F_{1,42} = 88.55$, $p < 0.0001$) and migrating bats weighed approximately 15 % less than non-migrants ($F_{1,42} = 23.91$, $p < 0.0001$). As for body mass, dry lean mass was lower in migrants ($F_{1,42} = 5.73$, $p = 0.021$) and males ($F_{1,42} = 32.87$, $p < 0.0001$). The effect of migration on fat storage was sex dependent (Figure 5.1; sex*migration $F_{1,41} = 6.24$, $p = 0.017$). Absolute fat mass decreased during migration for males ($F_{1,20} = 4.92$, $p = 0.038$) but when controlling for fat-free mass (accounting for overall decrease in body mass) there was no difference in fat content of migrating and non-migrating males ($F_{1,19} = 1.31$, $p = 0.27$). For females, there was no difference in the absolute fat mass between migrating and non-migrating bats ($F_{1,21} = 2.19$, $p = 0.15$) but when controlling for fat-free mass the relative fat content increased during migration ($F_{1,20} = 5.53$, $p = 0.029$), accounting for as much as 24% of body mass. In summer, there was no difference in the relative fat content of males and females ($F_{1,12} = 0.0034$, $p = 0.95$).

Table 5.1 Summary statistics for body and organ size measurements of migrating and non-migrating hoary bats. Values presented are mean ± s.e.m. Significant sex and migration effects are indicated in the final column. See text for analysis details.

	Migrant		Non-migrant		Sig.
	Female	Male	Female	Male	
Forearm length (mm)	54.90 ± 0.26	52.98 ± 0.35	54.84 ± 0.20	53.25 ± 0.42	sex: $p < 0.0001$
Body mass (g)	29.39 ± 0.80	22.35 ± 0.49	33.01 ± 1.00	26.39 ± 0.87	sex: $p < 0.0001$, migration: $p < 0.0001$
Dry lean mass (g)	7.15 ± 0.15	6.09 ± 0.19	7.65 ± 0.15	6.54 ±0.24	sex: $p < 0.0001$, migration: $p = 0.02$
Fat mass (g)	4.67 ± 0.42	2.51 ± 0.15	3.78 ± 0.41	3.13 ± 0.22	sex*migration: $p = 0.017$
Dry lean pectoralis (g)	0.31 ± 0.009		0.29 ± 0.02		n.s.
Dry lungs (g)	0.12 ± 0.006		0.099 ± 0.006		migration: $p = 0.0057$
Dry lean heart (g)	0.078 ± 0.003		0.081 ± 0.005		n.s.
Wet intestine (g)	0.52 ± 0.03		0.92 ± 0.11		migration: $p < 0.0001$
Dry stomach (g)	0.036 ± 0.002		0.037 ± 0.003		n.s.
Dry kidneys (g)	0.075 ± 0.002	0.062 ± 0.001	0.082 ± 0.002	0.066 ± 0.002	sex: $p = 0.0045$, migration: $p = 0.026$
Dry lean liver (g)	0.22 ± 0.01	0.14 ± 0.008	0.25 ± 0.02	0.14 ± 0.01	sex: $p < 0.001$

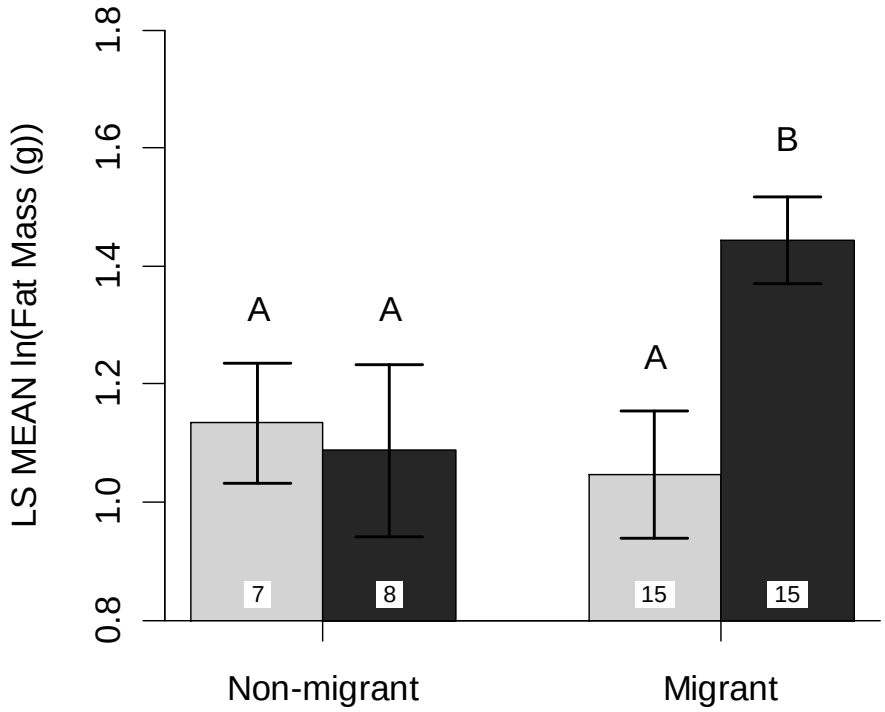

Figure 5.1 Fat storage in relation to migration was sex dependent. Male bats (light bars) decreased body and fat mass proportionally during migration and consequently relative fat mass was the same in summer and spring migration. Females (dark bars) maintained the same absolute fat mass in both samples, but a decrease in overall body mass during migration resulted in higher relative fat mass for migrants. Bars indicate least square means predicting fat mass controlling for fat-free mass. Error bars indicate ± s.e.m. Sample sizes are indicated inside the bars.

5.3.2 Organ Sizes

Organ masses are summarized by sex and migration in Table 5.1. Upon visual inspection of the untransformed organ masses, we noted obvious outliers for intestine and pectoralis mass (Figure 5.2). There were three female non-migrants with much larger intestines than any other bats. These were the first three females we captured in the summer. These same individuals and one other non-migrant bat (a male) had much smaller pectoralis muscles than would have been expected for their size. After applying the $\log_e$ transformation to intestine mass, the outliers were no longer problematic. Migrating bats had smaller intestines than non-migrating bats ($F_{1,42} = 14.97$, $p < 0.0001$). However the pectoralis mass outliers were still apparent even after data transformation and thus were excluded from the analysis. Excluding the outliers there was no effect of migration on pectoralis mass ($F_{1,38} = 1.09$, $p = 0.30$). However, if we simply consider the data it would suggest that at least some of the non-migrants have pectoralis muscles that are much smaller than would be expected for a bat of that size.

No other outliers were apparent in the dataset. Migrating bats had larger lungs than non-migrants ($F_{1,42} = 8.49$, $p = 0.0057$) but there was no difference in heart size ($F_{1,42} = 0.13$, $p = 0.72$). There was no difference between migrants and non-migrants in the size of stomach ($F_{1,42} = 0.15$, $p = 0.70$). Similarly, liver size was not affected by migration ($F_{1,41} = 0.36$, $p = 0.55$) though females had larger livers than males ($F_{1,41} = 33.01$, $p < 0.0001$). Kidney size was affected by both sex (females had larger kidneys; $F_{1,41} = 9.03$, $p = 0.0045$) and migration ($F_{1,41} = 5.33$, $p = 0.026$), with smaller kidneys in migrating bats.

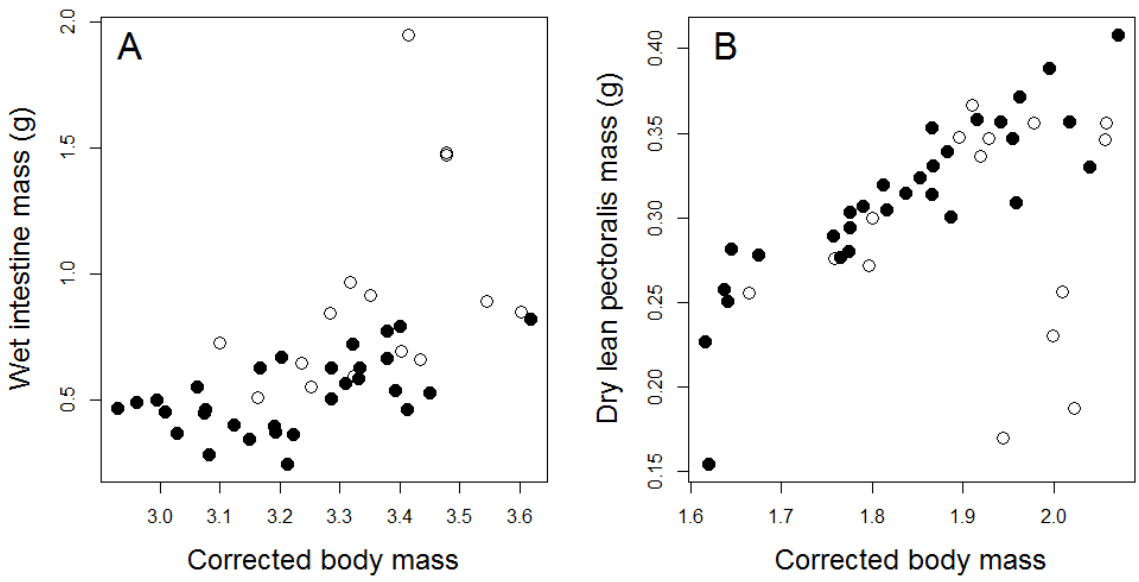

Figure 5.2 (A) Wet intestine mass relative to corrected body mass (see text for details) for spring migrating (●) and summer non-migrating (○) hoary bats. Note the three outliers (two overlaid) among the non-migrants. These three individuals are females and were captured earlier in the summer than the other females. (B) Dry lean pectoralis mass relative to corrected body mass for spring migrating (●) and summer non-migrating (○) hoary bats. Note the four outliers among the non-migrants, three of which are the same individuals as the outliers noted in (A).

Excluding the three outliers among the non-migrant females, the results of the principle components analysis largely reflected the conclusions of the individual organ analyses. The first two principle components (PC1 and PC2) were retained in the analysis, accounting for 43.1 and 13.4% of the total variance respectively (Table 5.2). All loadings in PC1 were positive and approximately equal suggesting that this PC reflects body size. This is further confirmed by the strong correlation between PC1 and body mass (Pearson correlation coefficient = 0.87). Lungs, heart and pectoralis loaded positively on PC2, while intestines, stomach, kidneys, and fat had negative loadings (Table 5.2) suggesting this PC represented an axis of exercise machinery and digestive machinery.

Including both PCs, MANOVA indicated significant effects of migration (Wilks λ = 0.764, $F_{2,37}$ = 5.73, p = 0.0068), sex (Wilks λ = 0.199, $F_{2,37}$ = 74.64, p < 0.0001) and body mass (Wilks λ = 0.408, $F_{2,37}$ = 26.84, p < 0.0001). PC1 was related to body mass ($F_{1,38}$ = 48.76, p < 0.0001) and sex ($F_{1,38}$ = 137.92, p < 0.0001), but not migration ($F_{1,38}$ = 0.098, p = 0.76), further confirming this PC as an indication of body size (the inclusion of sex reflects sexual size dimorphism). PC2 was related to migration ($F_{1,38}$ = 10.21, p = 0.0028), but not body mass ($F_{1,38}$ = 0.24, p = 0.63) or sex ($F_{1,38}$ = 0.27, p = 0.61). Therefore, migrating bats were shifted towards increased exercise machinery and reduced digestive machinery.

Table 5.2 Eigenvectors of the first two principal components of organ sizes in hoary bats. The first principal component (PC1) represents body size, while the second principal component (PC2) reflects a tradeoff between digestive organs and exercise organs.

	PC1 (Body Size)	PC2 (Digestive/Exercise organs)
Pectoralis	0.390	0.113
Lungs	0.332	0.415
Heart	0.253	0.688
Intestines	0.300	-0.465
Kidneys	0.451	-0.267
Liver	0.416	0.023
Stomach	0.237	-0.098
Adipose	0.389	-0.212
Proportion of Total Variance Explained	0.431	0.134

5.3.3 Fatty Acid Profiles

5.3.3.1 Adipose Neutral Lipids

We found migration-related differences in the proportions of all adipose neutral lipid FA except 18:0 (Figure 5.3). Although some sex*migration interactions indicated that the amounts of certain fatty acids changed for one sex but not the other, the general pattern was a decrease in 16:0 and increases in 18:2n-6 and 18:3n-3 during migration. This pattern is further supported by decreased saturated FA ($F_{1,42}$ = 35.53, p < 0.0001) and increased PUFA. A sex*migration interaction indicated a greater PUFA increase for males ($F_{1,20}$ = 15.12, p = 0.00091) than females ($F_{1,21}$ = 8.89, p = 0.0071). There was no overall change in MUFA ($F_{1,42}$ = 0.44, p = 0.51). DBI was greater in migrants ($F_{1,42}$ = 45.57, p < 0.0001) and also greater in males ($F_{1,42}$ = 5.91, p = 0.019). There was no difference in n-6:n-3 ratio of migrating and non-migrating bats ($F_{1,42}$ < 0.0001, p = 0.99).

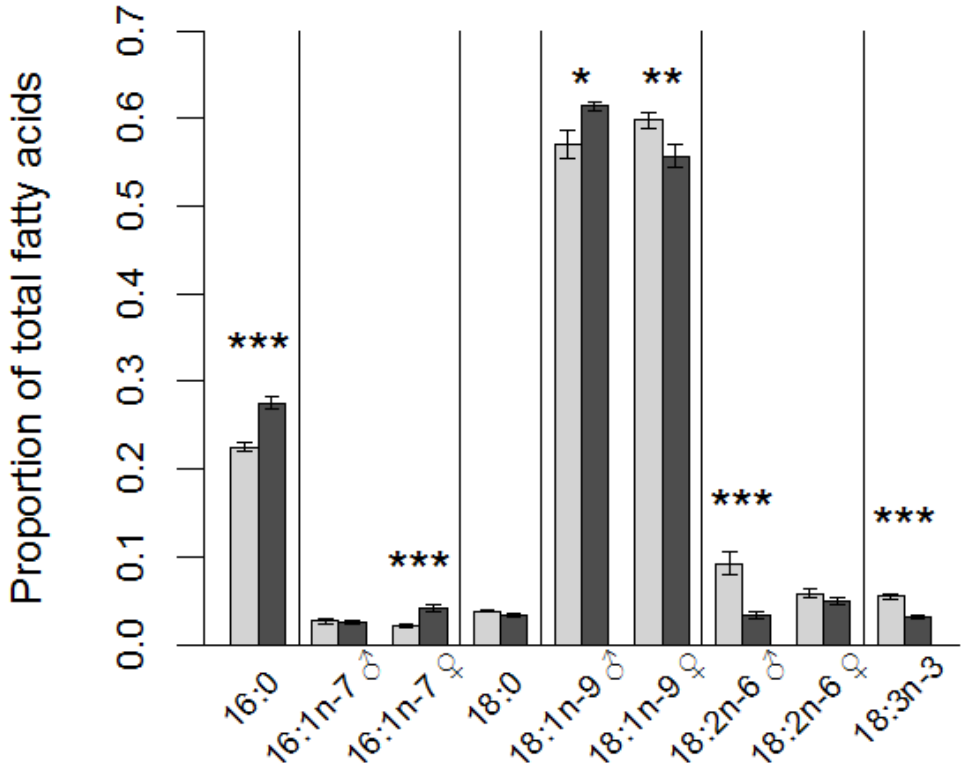

Figure 5.3 Fatty acid composition of adipose neutral lipids of migrating (light bars) and non-migrating (dark bars) hoary bats. In cases of sex*migration interaction, males and females are presented separately. Vertical lines separate individual fatty acids. Bars indicate mean ± s.e.m. * p < 0.1, ** p < 0.05, *** p < 0.01.

5.3.3.2 Muscle Phospholipids

There were differences in FA composition between migrants and non-migrants for 5 of the 9 FA comprising muscle phospholipids (Figure 5.4). Sex*migration interactions indicated sex specific responses for several fatty acids, notably 18:2n-6 and 22:6n-3. Males had more 18:2n-6 and less 22:6n-3 during migration while the pattern was reversed for females. This led to a significant change in n-6:n-3 ratio, higher during migration for males (migrants: 0.89 ± 0.06; non-migrants: 0.56 ± 0.02; $F_{1,20}$ = 12.4, p = 0.0022) and lower during migration for females (migrants: 0.59 ± 0.03; non-migrants: 0.74 ± 0.05; $F_{1,21}$ = 8.98, p = 0.0069). DBI index was similarly affected, decreasing during migration for males (migrants: 2.25 ± 0.04; non-migrants: 2.48 ± 0.05; $F_{1,20}$ =14.08, p = 0.0013) and increasing marginally for females (migrants: 2.43 ± 0.03; non-migrants: 2.31 ± 0.08; $F_{1,21}$ = 3.15, p = 0.091). The composition of MUFA, PUFA, and total saturated fatty acids did not differ between migrants and non-migrants (all $p > 0.05$).

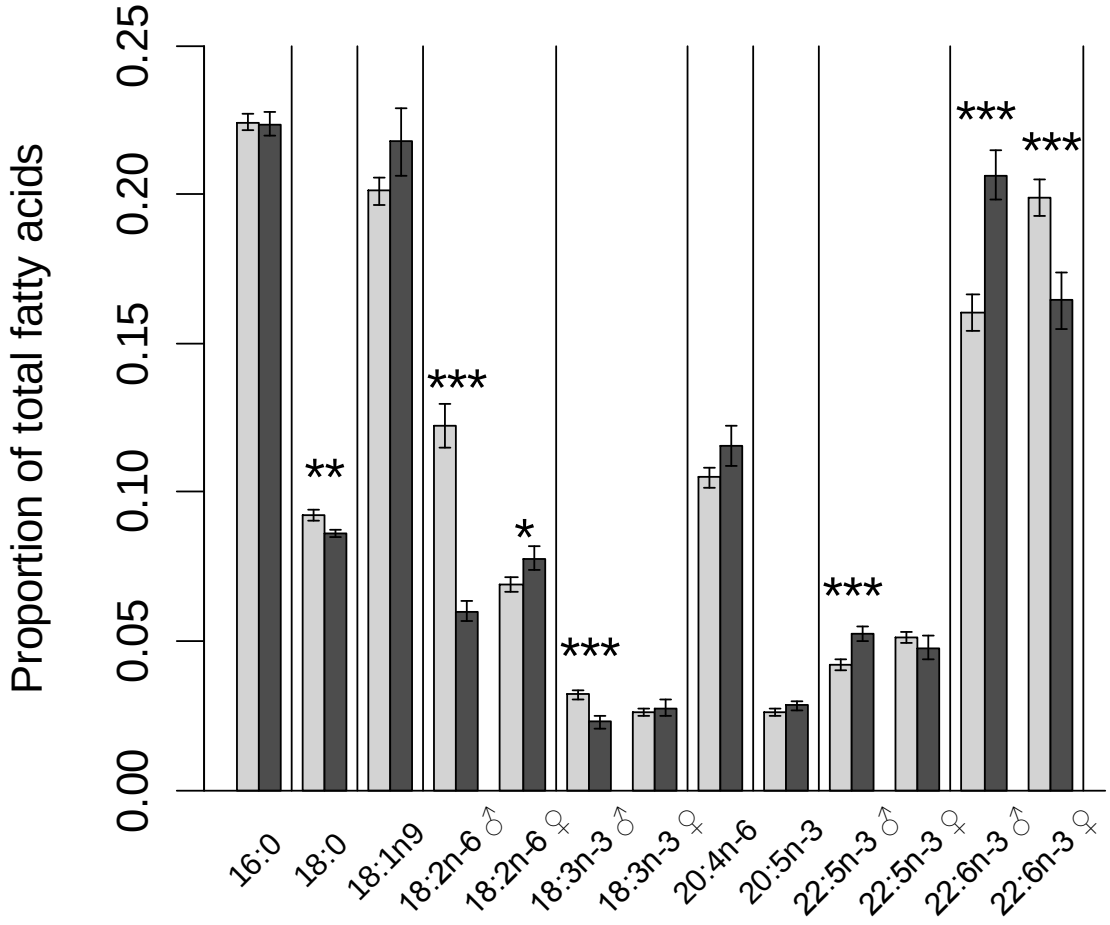

Figure 5.4 Fatty acid composition of muscle phospholipids of migrating (light bars) and non-migrating (dark bars) hoary bats. In cases of sex*migration interaction, males and females are presented separately. Vertical lines separate individual fatty acids. Bars indicate mean ± s.e.m. * p < 0.1, ** p < 0.05, *** p < 0.01.

5.4 Discussion

We found migration-related changes in all aspects of our study, suggesting that for these bats, as in birds, migration presents distinct physiological challenges compared to other times of the annual cycle. Sex was important for every aspect of physiology we examined, a pattern not typically observed in migratory birds. Migrating females carried relatively larger fat stores during migration, males did not. In summer, some females stood out as outliers with larger intestines and smaller pectoralis muscles. Separate male and female effects were evident in adipose FA composition, and males and females changed muscle PL composition in opposite directions. The simplest explanation for these sex differences is the reproductive demands imposed on females during spring migration and throughout the summer prior to the period when we made our collection.

5.4.1 Body Composition

We expected that migrating bats would weigh more than non-migrating bats due to the increased mass of fat stores and hypertrophy of muscles and digestive organs. Contrary to our expectations, body mass was reduced in migrating bats. Particularly surprising was the overall reduction of lean mass in migrants (see discussion of digestive organs below). A reduction in body mass reduces wing loading and consequently lowers the energetic cost of flight (Bowlin and Wikelski 2008). The wing loading effect is in addition to the efficient flight that hoary bats achieve due to a high aspect ratio. Of 81 bats measured in the family Vespertilionidae, hoary bats had the fourth highest aspect ratio (Norberg and Rayner 1987). High aspect ratio and seasonally lowered wing-loading suggest that the energetic costs of migration have played an important role in the evolution of this species (although wing morphology adaptations for an open-space aerial hawking foraging strategy have also surely contributed).

Relative increases in fat stores for migrating females, but not males, may reflect the cost of migration; female migration may be more costly and consequently females carry more fat. Differential migration among hoary bats results in females migrating greater distances than males, and therefore females may require larger fat stores. Alternatively, the discrepancy may arise due to differences in thermoregulatory strategy during

migration. McGuire *et al.* (2012/ Chapter 4) proposed the torpor-assisted migration hypothesis which states that bats could use daily torpor during migration and spare energy stores to fuel migratory flight. However, Cryan and Wolf (2003) demonstrated (at the same sites we collected our migrants) that during spring migration female hoary bats defend normothermic body temperature while males readily use torpor when ambient temperature is decreased. Females may be reluctant to lower body temperature due to the potential detrimental effects on the developing foetuses. If females do not use daily torpor, they would either need larger fat stores to support higher metabolic rates during diurnal roosting periods (compared to torpid males), or they would need to frequently replenish fuel stores that are depleted in both migratory flight and defense of body temperature. We argue (see below) that the former scenario is more consistent with our observations. Our observations provide a snapshot of the body composition at an early stage of spring migration. Future studies of body composition including overwintering, pre-migration, early and late migration, and summering are needed to determine whether the changes in body composition we observed indeed reflect a migration phenotype, or if body composition waxes and wanes in response to short term demands.

5.4.2 Organ Sizes

The general pattern of reduced digestive organs is similar to the pattern observed in numerous studies of migratory birds (e.g., Battley *et al.* 2000; Bauchinger *et al.* 2005), consistent with the 'guts don't fly' hypothesis (minimize gut mass to minimize the cost of transport; Piersma and Gill 1998). Individually, intestines and kidneys were smaller in migrating bats, and combined digestive organs (except liver) loaded together on the second PC axis indicating smaller organs in migrants. This pattern was unexpected given that most species for which reduced guts have been observed migrate vast distances over habitat which does not permit foraging. In the case of Bar-tailed Godwits (*Limosa lapponica baueri*), non-stop migratory flights may cover > 11 000 km and last 9 days as birds fly from Alaska to New Zealand (Gill *et al.* 2009; Battley *et al.* 2012). Garden Warblers (*Sylvia borin*) migrate > 2500 km across the Sahara desert without feeding (Bauchinger *et al.* 2005). However, digestive capacity (and digestive tract organ sizes) is typically linked to demand (McWilliams and Karasov 2005). For a hoary bat migrating

from southern California to Canada, there is ample suitable foraging habitat along the route, and therefore it is somewhat surprising that the changes in digestive organs are not similar to the increases observed in Western Sandpipers (*Calidris mauri*) that stop to refuel frequently along their migratory route (Guglielmo and Williams 2003). Instead, it would appear that the bats in our study were more likely to deposit fuel (larger fat stores for the females that could not save energy through the use of daily torpor) prior to migration and minimize time spent foraging along the migratory route. We did not observe sex effects in the reduction of digestive organs, suggesting that both sexes reduce foraging effort similarly. To compensate for reduced foraging during migration, females that do not use torpor could deposit larger fat stores prior to migration which is indeed what we observed. In the fall when females are not pregnant, and should be capable of using torpor, there should be no sex effects in either foraging effort or the size of fat stores. Both of these predictions are consistent with observations of fall migrating silver-haired bats (*L. noctivagans*) where few bats foraged and there was no difference in body composition with regards to sex (McGuire *et al.* 2012/ Chapter 4). However, only two of the migrants in our study had empty stomachs, therefore foraging does not cease completely during migration. Reimer *et al.* (2010) observed a similar ratio of hoary and silver-haired bats with stomach contents during autumn migration in Alberta, Canada. Therefore bats may forage briefly each night rather than alternating extended periods of refuelling and migratory flight.

Increased exercise components have been frequently observed in migratory birds, but the individual organ changes differ from the observations of the bats in our study. Birds may hypertrophy the heart and flight muscle (Marsh 1984; Piersma 1998; Piersma *et al.* 1999; Portugal 2009), but changes in lung mass are rarely recorded. In the hoary bat PCA all exercise organs (heart, lungs, pectoralis muscle) loaded together, indicating larger exercise organs in migrating bats. However the evidence for changes in flight muscle mass is weak (not significant in individual organ comparison, smaller factor loading in PCA) suggesting that flight muscle size varies with body mass as has been observed in many migratory birds (e.g. Marsh 1984). When comparing individual organs, migrating bats had larger lungs but there was no difference in heart or flight muscle size. Heart and flight muscle mass may change in parallel with body mass, but the fact that lungs are

larger in migrants independent of body mass indicates that these seasonal changes may be associated with increased capacity for aerobic exercise. Lung mass change appears to be a novel component of bat migration physiology that may result from the less rigid structure of the mammalian lung (Maina 2000) enabling phenotypic flexibility that is not possible given the design of the avian lung. An intriguing alternative explanation is that, in rodents, increased lung mass may be associated with exposure to low oxygen concentration (Burri and Weibel 1971), as may be naturally experienced at high altitudes (Hammond *et al.* 2001). If bats spend more time flying at high altitudes during migration, and over a long enough period, lung mass may be increased to compensate for lower oxygen concentration. This suggestion is highly speculative and requires further investigation.

The data we collected from some of the non-migrating female hoary bats suggests that all organ size changes we observed may be conservative estimates of seasonal migration-related phenotypic flexibility. Female bats are pregnant as they migrate north in the spring, arriving in Cypress Hills in late May or early June (Willis *et al.* 2006). Parturition occurs shortly after arrival at the summer habitat (mid-June), and the young become volant approximately 5 weeks later (Shump and Shump 1982). The earliest date we captured post-lactating females (in either year) was July 20; the latest date we captured lactating females was July 22. Therefore some of the earliest females we captured (outliers in Figure 5.2) may only have ceased lactating days earlier. The outliers in Figure 5.2 suggest that there may be a rapid and dramatic change in body composition at weaning. In these early-season females, intestines were approximately two times larger and pectoralis muscles were only ~ 60% of the size that would be expected for their body mass. If these organ size changes are typical then our late summer female samples may be better considered a pre-migratory sample. Comparison of migrating bats and lactating females may present a more extreme picture than the potentially conservative size changes we documented.

5.4.3 Fatty Acid Profiles

Adipose FA composition was shifted towards increased PUFA and decreased saturated FAs. Such changes would slightly reduce the net ATP production per gram of

triglycerides (Price 2010), but would greatly increase the potential mobilization of FA stores. In Ruffs (*Philomachus pugnax*), the relative mobilization of 18:2n-6 and 18:3n-3 (two fatty acids which increased in hoary bats; Figure 5.3) was approximately 50 % greater than 16:0 (decreased during migration in hoary bats; Figure 5.3) (Price *et al.* 2008) due to differences in unsaturation and chain length. A similar pattern (but less pronounced) is found in rats and humans (Raclot and Groscolas 1993; Raclot 2003). High intensity migratory flight demands a continuous supply of energy substrates. Consequently the trade-off between potential energy and mobilization rate may favour those fatty acids that permit a high rate of sustained energy substrate delivery to the flight muscles. The reduction in energy density may be compensated by deposition of larger fat stores, or refuelling more frequently.

Interpretation of migration-related changes in the FA composition of muscle PL is notoriously difficult (Price 2010). Most studies report correlations in the absence of plausible mechanisms, and it has proven difficult to experimentally isolate the effects of muscle PL from other possibly confounding effects. It has been suggested that many studies reporting effects of different muscle PL profiles may in fact simply reflect differences in adipose composition. This is not likely the case in our study. The most notable difference in muscle PL FA was changes in n-6:n-3 ratio, increasing for males and decreasing for females. The only adipose FA where males and females changed in opposite directions was 18:1n-9, which was not affected by either sex or migration in muscle PL. Sex-biased differences in muscle PL but not adipose NL suggest that any effects of muscle PL are not simply consequences of adipose NL composition.

It is difficult to interpret how muscle PL changes might affect exercise performance given that males and females changed in different manners. To interpret exercise performance effects we would need to understand sex differences in the energetic cost or energetic strategies associated with migratory flight. However, sex-based variation in muscle PL n-6:n-3 ratio is consistent with the Ruf and Arnold (2008) hypothesis that increased n-6:n-3 ratio is associated with torpor use. During spring migration, male hoary bats readily use torpor while females rarely lower their body temperature (Cryan and Wolf 2003), and accordingly we observe increased n-6:n-3 ratio in males and decreased ratio in females.

The prediction that migrating males should maximize total PUFA (Munro and Thomas 2004) is not supported by our data as we did not observe any differences in muscle PL total PUFA content.

5.4.4 Conclusion

Although bats and birds represent two phylogenetically distant lineages, they have converged on a number of similar physiological strategies associated with migratory behaviour. We observed patterns consistent with fat being used as the primary fuel for migration, as in previous studies of migratory birds. Bats are able to deposit large fat stores (as observed in the migrating females), and the FA profile of adipose stores indicates that mobilization of FA to maintain high delivery rates in flight is more important than the energy density of the fat stores. Furthermore, changes in digestive organ sizes indicate that bats may favour a time minimizing migration strategy, minimizing time spent foraging *en route*. Like birds, enlarged exercise organs reflect the increased aerobic exercise demands during migratory periods. However, the increase in lung size that we observed has not been previously documented in birds and highlights the fact that the migration physiology of bats is, in many ways, distinct from the migration of birds.

The coincidence of spring migration and pregnancy is perhaps the most important contrast between the migration of birds and bats. In birds, migration and breeding are temporally isolated, and thus physiological consequences of migration are more easily isolated than in bats. Clearly, reproductive physiology is an important consideration when interpreting bat migration-related phenotypic flexibility. Pregnancy and lactation may affect nearly all aspects of migration physiology that we considered. We collected bats during spring migration and summer non-migratory periods because these were the only times of year we could reliably capture hoary bats. Comparison of fall migrating and overwinter bats will help to isolate migration effects from reproductive effects. We were unaware of reliable sites for capturing fall migrants or overwintering bats. Future work should focus on finding such sites or studying species where such sites are known.

5.5 Acknowledgements

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CHAPTER 6

6 SEASONAL UPREGULATION OF CATABOLIC ENZYMES AND FATTY ACID TRANSPORT PROTEIN EXPRESSION IN THE FLIGHT MUSCLE OF MIGRATING HOARY BATS, *LASIURUS CINEREUS*

Flying migrants exercise at high metabolic rates for long periods of time. The high energy density of fat and limited capacity for carbohydrate storage suggest that migrating bats should fuel endurance flights with fat, similar to migrating birds. This is contrary to the typical mammalian exercise model where running mammals fuel high intensity exercise primarily with carbohydrates. We hypothesized that migratory flight is fuelled by stored fat as observed in migratory birds. We studied seasonal variation in mitochondrial catabolic enzyme activities and expression of fatty acid transport proteins in the flight muscle of migrating and non-migrating hoary bats (*Lasiurus cinereus*). We predicted that enzyme activities and transporter expression would be upregulated during migration to support aerobic metabolism from fatty acid substrates.

6.1 Introduction

Flying bats expend energy at about 15 times the basal metabolic rate (Speakman and Thomas 2003), and must maintain this high level of expenditure for several hours during migratory flights. While insectivorous bats fly for long, uninterrupted periods when foraging, there is no evidence that bats feed while in migratory flight. In this fasted, flying state, glycogen stores would be quickly depleted (Yacoe *et al.* 1982), so migrating bats, like birds, should use extra-muscular fat stores as their main source of fuel (Jenni and Jenni-Eiermann 1998; McGuire and Guglielmo 2009/ Chapter 2). This is unlike typical exercise in most mammals where high intensity exercise is fuelled primarily by carbohydrates with < 20 % of energy required coming from lipids at intensities near $\dot{V}O_2$ max (Roberts *et al.* 1996; Weber *et al.* 1996). In running mammals the contribution of lipids peaks at approximately 40 % of $\dot{V}O_2$ max (Roberts *et al.* 1996; Weber *et al.* 1996). In this study, we investigate mechanisms for migrating bats to support high intensity endurance exercise with stored fat. We hypothesize that bats represent an exception to the

mammalian exercise paradigm, and will have converged on similar physiological mechanisms as migratory birds due to the common selective pressures of vertebrate flight. Studies of migratory birds have focused on two key processes: lipid oxidation and aerobic capacity of flight muscles, and protein-mediated transport of fatty acids from the circulation to the mitochondria.

Many studies of muscle metabolism in migratory birds have focused on the oxidative capacity of flight muscles, documenting increases in catabolic enzyme activities during migratory periods (Marsh 1981; Lundgren and Kiessling 1985; Driedzic *et al.* 1993; Guglielmo *et al.* 2002; McFarlan *et al.* 2009). Three enzymes in particular are seasonally modulated: carnitine palmitoyl transferase (CPT), 3-hydroxyacyl-CoA dehydrogenase (HOAD), and citrate synthase (CS). CPT is involved in translocating fatty acids from the cytosol across the mitochondrial membrane. HOAD is an enzyme in the β-oxidation pathway which provides an index of fatty acid catabolism. CS is an enzyme in the Krebs cycle and is therefore indicative of muscle aerobic capacity. Studies of catabolic enzymes in bat flight muscle suggest a high aerobic capacity (Armstrong *et al.* 1977; Yacoe *et al.* 1982; Suarez *et al.* 2009) and fatty acid oxidation potential (Yacoe *et al.* 1982; Suarez *et al.* 2009). Comparisons of active and hibernating bats have demonstrated seasonal variation in enzyme activity (Armstrong *et al.* 1977; Brigham *et al.* 1990; Yacoe 1983; Kim *et al.* 2000), but seasonal variation associated with migration has not been considered in bats.

Recent studies of migratory birds have emphasized the capacity to transport fatty acids as a limiting factor in the chain of events required to maintain high rates of oxidative metabolism during migration (Guglielmo *et al.* 1998; Pelsers *et al.* 1999; Guglielmo *et al.* 2002; McFarlan *et al.* 2009; Price *et al.* 2010). Due to the low solubility of lipids, each step in the transport pathway from adipose stores to muscle mitochondria must be mediated by protein transporters (Guglielmo 2010). It is currently thought that migratory birds are able to maintain high metabolic rates for long periods of time due to high levels of fatty acid transport protein expression in flight muscles (McFarlan *et al.* 2009). Transport of fatty acids into muscle cells is achieved primarily by the action of two membrane-bound fatty acid transport proteins, plasma-membrane fatty acid binding

protein (FABPpm) and fatty acid translocase (FAT/CD36), although the exact mechanisms are unknown (Bonen *et al.* 2007). Inhibition studies have demonstrated that FAT/CD36 may be responsible for up to 50 % of protein mediated fatty acid transport in muscle cells (Luiken *et al.* 1999). Furthermore, FAT/CD36 may act synergistically with FABPpm to increase fatty acid transport rates (Bonen *et al.* 2007). FAT/CD36 and FABPpm are seasonally upregulated in migrating White-Throated Sparrows (*Zonotrichia albicollis*) with 70 – 150 % relative increases in mRNA expression (McFarlan *et al.* 2009). Within the cytosol, fatty acids are transported from the cell membrane to the mitochondria by heart-type fatty acid binding protein (H-FABP) (McWilliams *et al.* 2004). A large pool of H-FABP may act as a fatty acid sink, maintaining high rates of fatty acid flux across the cell membrane (Bonen *et al.* 2007). H-FABP is seasonally upregulated during migration, with increased protein concentration observed in Barnacle Geese (*Branta leucopsis*; Pelsers *et al.* 1999), Western Sandpipers (*Calidris mauri*; Guglielmo *et al.* 2002) and White-Throated Sparrows (McFarlan *et al.* 2009). In white-throated sparrows, relative mRNA expression in migrating birds was approximately 11 times greater than non-migrating birds (McFarlan *et al.* 2009). The role of fatty acid transporters in bat migration has not been studied. However, H-FABP is upregulated in hibernating bats (Eddy and Storey 2004), a period when the bats rely exclusively on stored fat, suggesting that fatty acid transporters may be upregulated in migration.

To test the hypothesis that bats, like birds but unlike running mammals, fuel high intensity exercise with stored fat, we studied seasonal changes in flight muscle fatty acid transport protein expression and catabolic enzymes in the long-distance latitudinal migrant hoary bat (*Lasiurus cinereus*). We predicted migrating bats would increase CPT, HOAD, and CS activities in flight muscles and mRNA expression of H-FABP, FAT/CD36, and FABPpm to enhance their capacity to use extra-muscular fat as fuel for endurance flights.

6.2 Materials and Methods

6.2.1 Animal Collection

The hoary bat (*Lasiurus cinereus*) is a ~ 20 – 35 g insectivore in the family Vespertilionidae. It is the most widely distributed bat species in North America, breeding as far north as the boreal forest of Canada (Shump and Shump 1982). The winter distribution is poorly documented, but it is thought that hoary bats overwinter in Mexico and southern California (Cryan 2003). Individuals may migrate > 2000 km between summer and winter grounds (Cryan *et al.* 2004).

Non-migrating bats were captured from 20 July to 1 August, 2008 and 2009 in mist nets set across creeks in Cypress Hills Interprovincial Park, Saskatchewan, Canada (49° 34' N, 109° 53' W). We collected migrating hoary bats May 5 – 17, 2009 by setting mist nets over water in Bernalillo County, New Mexico, USA (35° 12' N, 106° 18' W) and in the Manzano Mountains, Cibola National Forest, New Mexico, USA (34° 59' N, 106° 21' W). We identified sub-adult bats by the degree of ossification of the metacarpal-phalanges joint (Anthony 1988), and lactating females were identified by manually expressing milk from the mammary glands. All sub-adults and lactating females were released immediately.

In Saskatchewan we collected 8 female and 7 male non-migrants, and in New Mexico we collected 15 female and 15 male migrants. Immediately upon capture we euthanized the bats by cervical dislocation under isoflurane anaesthesia. We recorded body mass (± 0.1 g) and forearm length (± 0.05 mm) and quickly dissected a sample of pectoralis muscle. The pectoralis sample was transferred to a 2 mL cryotube (Cryo.S, Grenier Bio-one) and frozen in a liquid nitrogen cooled cryoshipper (Taylor-Wharton CX-100). The samples were transported back to the lab either in the liquid nitrogen cooled cryoshipper or packed in dry ice, and stored at - 80°C until analysis.

All animal collection and experimental protocols were approved by the University of Western Ontario Animal Use Sub-committee (protocol no. 2008-003-04) and conducted under permits from the New Mexico Department of Game and Fish (permit no. 3424), United States Department of Agriculture- Forest Service (permit no. SND502),

Saskatchewan Ministry of Environment (permits no. 08FW080 & 09FW045), and Saskatchewan Ministry of Tourism, Parks, Culture and Sport (permits no. SP-CHPP-02-08 & SP-CHPP-01-09). Samples from New Mexico were imported to Canada under the approval of the Canadian Food Inspection Agency (permit no. A-2009-01022-3).

6.2.2 Enzyme Assays

Approximately 100 mg of pectoralis muscle was combined with 9 volumes of homogenization buffer (20 mM Na_2HPO_4, 0.5 mM EDTA, 0.2 % defatted BSA, 50 % glycerol, 0.1 % Triton x-100, and 50 μg/mL aprotinin). Keeping the sample on ice, we homogenized 3 × 10 s (Polytron PT 10-35, Kinematica Inc., Bohemia, NY), allowing the sample to rest for 30 s between bouts. We set the speed of the homogenizer to the highest setting which did not result in foaming. Homogenates were sonicated 3 × 10 s, waiting 30 s on ice between bouts, then stored at -80 °C until analysis.

All enzyme assays were performed at 39 °C (approximate body temperature of flying bats, Carpenter 1985) with a 1 mL reaction volume on a Cary 100 Bio Spectrophotometer (Varian, Palo Alto, CA). CPT was assayed in 50 mM Tris buffer (pH 8.0) with 2.5 mM carnitine, 0.15 mM DTNB, 0.00875 mM palmitoyl CoA and 10 μL homogenate diluted 1:5 in homogenization buffer. HOAD was assayed in 50 mM imidazole buffer (pH 7.4) with 2 mM EDTA, 0.2 mM NADH, 0.2 mM acetoacetyl CoA, and 10 μL homogenate diluted 1:10 in homogenization buffer. CS was assayed in 50 mM Tris buffer (pH 8.0) with 0.6 mM acetyl CoA, 0.15 mM DTNB, 0.5 mM oxaloacetic acid, and 10 μL homogenate diluted 1:10 in homogenization buffer. CS and CPT activities were calculated from ΔA_{412} and HOAD activity was calculated from ΔA_{340}.

6.2.3 Transporter mRNA Expression

6.2.3.1 RNA Isolation and Reverse Transcription

RNA isolation and reverse transcription followed the methods of Price *et al.* (2010). We isolated total RNA by extracting 50 – 100 mg of frozen muscle tissue in 1 mL TRIzol reagent following the manufacturer's protocol (Invitrogen, Burlington, ON, Canada) with two additional chloroform extractions and ethanol washes. The RNA pellet was stored at

-20 °C until analysis. RNA samples were eluted in sterile water and dissolved by heating to 55 °C. To quantify the concentration and quality of the RNA, we measured absorbance (in Tris-EDTA buffer- 10 mmol L^{-1} Tris, pH 8, 1 mmol L^{-1} EDTA) at 260 nm and 280 nm. All samples were high quality as indicated by A_{260}:A_{280} > 1.8. DNA contamination was removed by incubating 5 µg RNA with 2 i.u. DNase I (New England Biolabs) at 37 °C for 15 min and inactivating at 75 °C for 10 min. We reverse transcribed RNA to complementary DNA (cDNA) with 0.5 µg RNA, 0.5 µg oligo-dT_{12-18} primer, 0.5 mM of each dNTP, 1 × FS buffer, 10 mM DTT, 1 µL RNase OUT, and 200 U SuperScript II reverse transcriptase in 20 µL total reaction volume (all reagents from Invitrogen). Samples were incubated for 90 min at 42 °C and then 10 min at 70 °C to stop the reaction. cDNA samples were stored at -80 °C until analysis.

6.2.3.2 Primer Design

For each target gene (H-FABP, FABPpm, FAT/CD36) and a housekeeping gene (glyceraldehyde 3-phosphate dehydrogenase; GAPDH) we developed degenerate primers designed from previously published sequences of other bat species, or if no bat species were available, a range of mammals. After using the degenerate primers to find the hoary bat sequence, we designed specific primers for use in real-time PCR. All primer design was performed with Primer3 software (Rozen and Skaletsky 2000). All sequencing (Applied Biosystems 3730 Analyzer) was performed by the Robarts Research Institute (London, ON, Canada), and all primers were commercially manufactured (Invitrogen, San Diego, CA, USA).

For H-FABP and GAPDH, previously published sequences were available from other bat species. We performed a BLAST search of *Tadarida brasiliensis* GAPDH (Genbank Acc. No.:ABD77189.1) against the *M. lucifugus* genome. We aligned the resulting sequence (Genbank Acc. No.: AAPE.02038885.1) with *T. brasiliensis* and used the consensus sequence to develop degenerate primers. H-FABP primers were designed from the *M. lucifugus* sequence (Genbank Acc. No.: AAO49500.1; Eddy and Storey 2004).

There were no sequences from bats available for either FAT/CD36 or FABPpm. For FAT/CD36 we used two alignments to find degenerate primers. In the first alignment we

performed a BLAST search of *Rattus norvegicus* FAT/CD36 (Genbank Acc. No.: AAC24876.1). We aligned the rat template with the sequences resulting from the search (*Mus musculus, Macaca mulatta, Homo sapiens, Canis lupus familiaris, Bos taurus,* and *Sus scrofa*) and designed degenerate primers based on conserved regions. A second set of FAT/CD36 degenerate primers was based on an alignment of only *R. rattus* (Genbank Acc. No.: NP_001153030) and *M. musculus* (Genbank Acc. No.: AF072411.1) sequences. For FABPpm we performed a BLAST search of *M. musculus* FABPpm (Genbank Acc. No.: NP_034455.1) and aligned the mouse template with the resulting sequences (*R. norvegicus*, *Macaca fascicularis*, *H. sapiens*, *Pongo abelii*, *S. scrofa*, and *B. taurus*). All degenerate and specific primer sequences are reported in Table C.1.

6.2.3.3 Real-time PCR

We conducted real-time PCR analysis for each gene separately with a Rotor-Gene 6000 Real-Time Rotary Thermocycler (Corbett Life Science, Concorde, New South Wales, Australia). The reaction conditions were 1 × reaction buffer, 3 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 μM primers, 0.75 U Platinum Taq polymerase, 0.7 × SYBR-Green I (all reagents from Invitrogen). Primers are listed in Table C.1. Cycling conditions were: 95 °C for 10 min, 40 cycles of 95 °C for 10 s, 56 °C for 15 s, 72 °C for 20 s, and 83 °C for 0 s, with a final melt curve analysis from 72 – 95 °C. Fluorescence, excitation at 470 nm and detection at 510 nm, was measured following the 72 °C elongation phase and at the 83 °C point of each cycle. All samples were analyzed in duplicate. An aliquot of cDNA from each bat was combined into a single pooled standard. We calculated the reaction efficiency for each gene with a serial dilution of the pooled standard and included one dilution as a calibrator on each run. Expression of the target genes was calculated relative to the expression of the housekeeping gene as described in McFarlan *et al.* (2009).

6.2.4 Data Analysis

All data analysis was performed with the software R (v. 2.9.2; R Core Development Team 2009). The distribution of target gene expressions was highly right skewed. Therefore, expression was $\log_e$ transformed prior to analysis. We used ANOVA to test

for sex and migration effects (including interaction) on gene expression, removing the interaction term when it was not significant.

For comparison of enzyme activities we used general linear models and a backwards stepwise selection process starting with sex, migration, forearm length, capture mass and all interactions as predictor variables and subsequently removing non-significant model terms until only significant terms remained in the model.

6.3 Results

Catabolic enzyme activities (Figure 6.1) increased 29 % during migration for CS ($F_{1,43}$ = 16.13, p = 0.0002), 53 % for HOAD ($F_{1,42}$ = 26.57, p < 0.0001), and 32 % for CPT ($F_{1,43}$ = 12.76, p = 0.0009). Sex did not affect either CPT or CS (both p > 0.05), but HOAD activity was greater for males ($F_{1,42}$ = 11.06, p = 0.0018).

We obtained partial sequences of reverse-transcribed mRNA for GAPDH (Genbank Acc. No.: JQ598170), H-FABP (Genbank Acc. No.: JQ598171), FABPpm (Genbank Acc. No.: JQ598173), and FAT/CD36 (Genbank Acc. No.: JQ598172). Hoary bat GAPDH was 97.8 and 98.3 % identical to the nucleotide sequences for *T. brasiliensis* and *M. lucifugus* respectively, and the predicted peptide sequences were both 100% identical. Our H-FABP sequence for hoary bats was 95 % identical to the nucleotide sequence for *M. lucifugus*, and the predicted peptide sequence was 92.8 % identical. Compared to the other mammalian nucleotide sequences we used (not including any bats), hoary bat FABPpm was 88.5 – 91.1 % identical (93.0 – 95.7 % identical predicted peptide) and FAT/CD36 was 80.5 – 87.3 % identical (75.8 – 82.0 % identical predicted peptide sequence).

We excluded two extreme outliers from H-FABP analysis (one non-migrating male and one non-migrating female) as well as one extreme outlier from FABPpm analysis, a non-migrating female. The three outliers clearly arose from some undetermined error as all three values were > 5 standard deviations from the mean expression excluding these values. Expression of FAT/CD36 did not vary seasonally (Figure 6.2; $F_{1,42}$ = 0.54, p = 0.47) or by sex ($F_{1,42}$ < 0.001, p = 0.99). A similar pattern was observed for FABPpm

with no season (Figure 6.2; $F_{1,41}$ = 0.95, p = 0.33) or sex effect ($F_{1,41}$ = 0.056, p = 0.81). A sex*migration interaction ($F_{1,39}$ = 3.41, p = 0.072) indicated that seasonal patterns in H-FABP mRNA expression varied by sex. H-FABP expression did not change during migration for male hoary bats ($F_{1,20}$ = 0.78, p = 0.39), whereas expression increased ~ 5-fold for migrating females ($F_{1,20}$ = 7.66, p = 0.012).

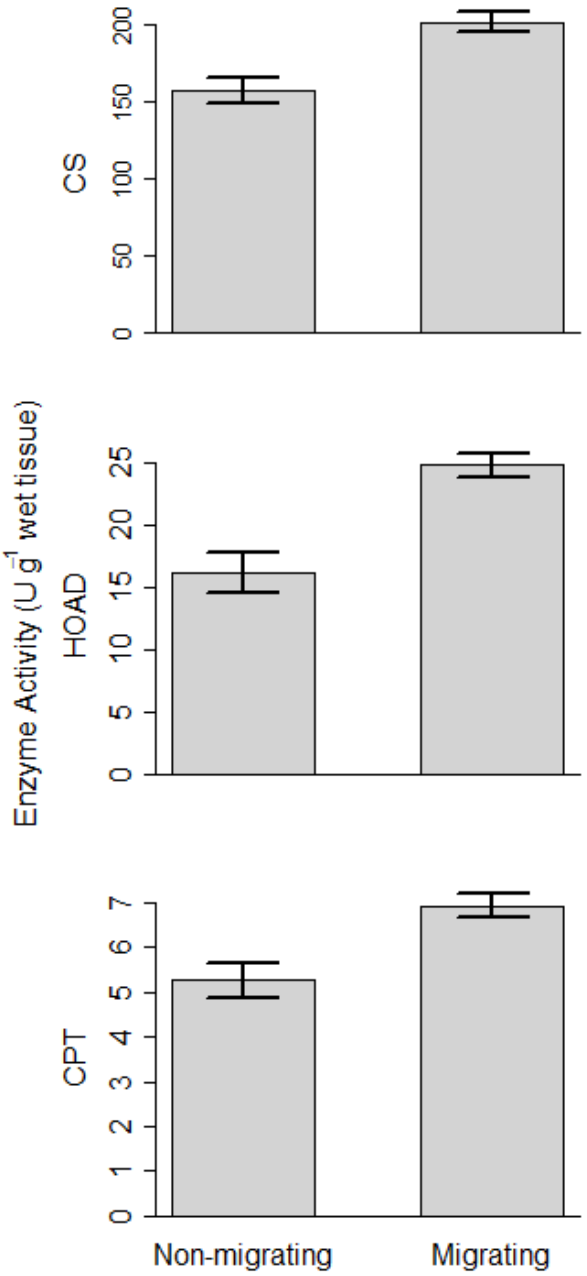

Figure 6.1 Seasonal increases in enzyme activities were observed for citrate synthase (CS; top), 3-hydroxyacyl-CoA dehydrogenase (HOAD; middle), and carnitine palmitoyl transferase (CPT; bottom). In addition to the migration effect indicated in these plots, HOAD activity was greater for males than females independent of season. Bars indicate mean ± s.e.m. Units are the same on all three plots.

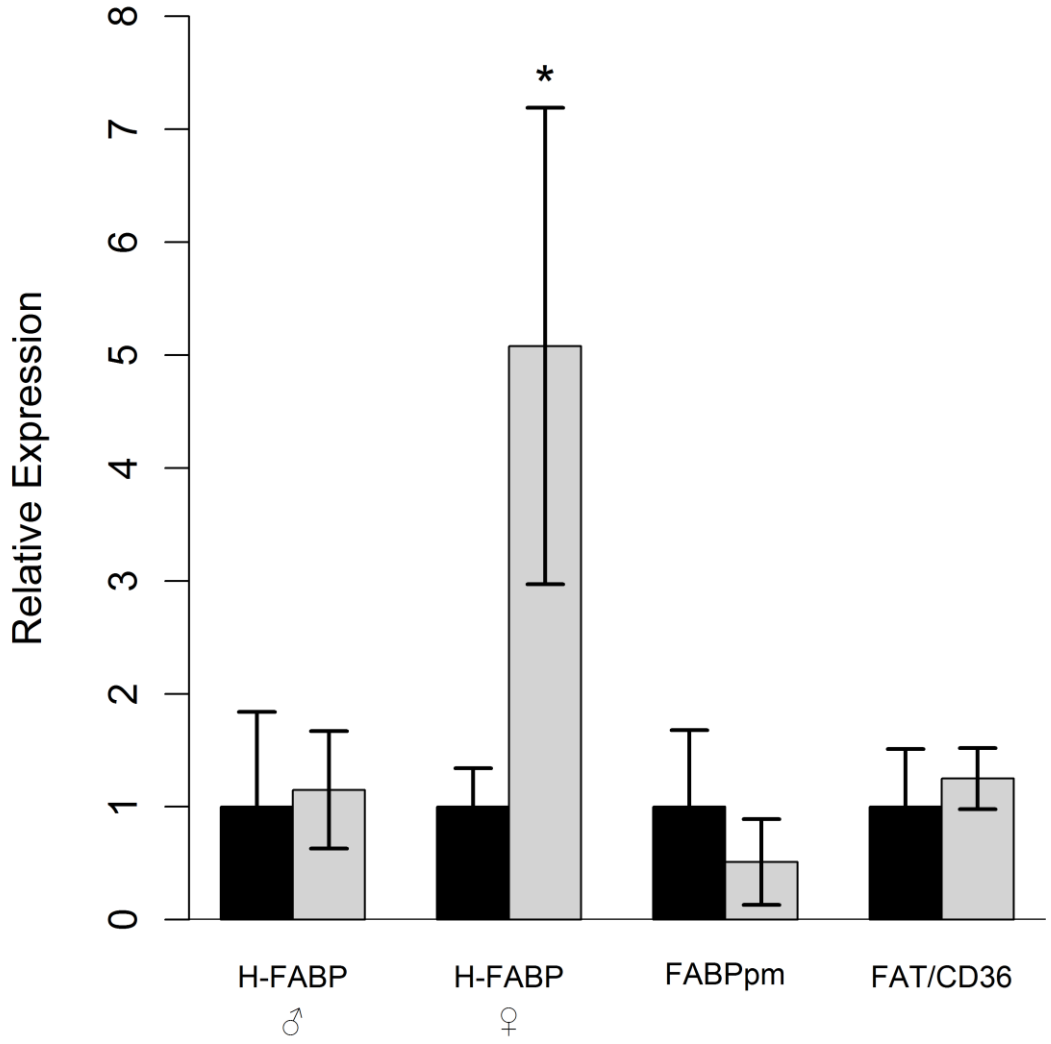

Figure 6.2 Seasonal variation in fatty acid transport protein mRNA expression. Bars indicate target gene expression for migrating bats (light bars) relative to non-migrating bats (dark bars). Males and females are presented separately for H-FABP because a sex*migration interaction indicated sex-specific migration effects. Female H-FABP expression was ~ 5-fold higher in migration compared to summer, but there were no other seasonal changes in expression. Expression of the three target genes was measured relative to the housekeeping gene GAPDH. Error bars indicate ± s.e.m.

6.4 Discussion

We observed an increased aerobic capacity and lipid oxidation potential and during migration. Citrate synthase activity increased by 29% during migration indicating that endurance migratory flights are more demanding than summer-time foraging flights. According to optimal flight speed theory (Hedenström and Alerstam 1995), migrating bats should fly near the maximum range speed which requires more energy than speeds near the minimum power speed predicted for foraging flight. Empirical evidence supports the idea of context dependent, energetically optimal flight speed in bats (Grodzinski *et al.* 2009). Furthermore, migratory flights may be more demanding than foraging flight due to the relatively shorter durations of foraging flights (Barclay 1989). The increases in CPT (+ 32%) and HOAD (+ 53%) activities highlight the importance of fat for fuelling migratory flight. Although it appears hoary bats forage regularly during migration (Valdez and Cryan 2009; Reimer *et al.* 2010; Chapter 5) we suggest these foraging periods are likely to be concentrated in the early night or pre-dawn hours, and bats will rely on stored fat during endurance flight through the middle hours of the night. CPT and HOAD are indicative of mitochondrial transport and oxidation of fatty acids, and thus the increased activity we observed during migration is consistent with the hypothesis that endurance flight is fuelled by stored fat and not recently ingested nutrients as is the case for foraging bats (Voigt *et al.* 2010). Studies of migratory birds have considered seasonal changes in both lipolytic and glycolytic enzymes to document seasonal changes in both fat and carbohydrate metabolism (e.g., Lundgren and Kiessling 1985; Driedzic *et al.* 1993). Similar comparisons should be a high priority for future studies of bat migration to determine the relative changes in both lipid and carbohydrate metabolism.

Unlike previous studies of migrating birds and contrary to our prediction, we did not observe increased fatty acid transport protein mRNA expression (with the exception of H-FABP in migrating females, see below). The lack of transporter upregulation may reflect differences in the ecology of hoary bats compared to previously studied birds. Seasonally increased fatty acid transport protein expression has been reported in migrating shorebirds (Guglielmo *et al.* 2002), geese (Pelsers *et al.* 1999), and songbirds (McFarlan *et al.* 2009). These types of birds show a dramatic difference between time

spent flying in migratory and non-migratory periods (e.g., Portugal *et al.* 2011). During non-migratory periods these birds spend relatively little time flying compared to migratory periods when they may fly hundreds or thousands of kilometres in a single flight. In contrast, hoary bats are aerial hawking insectivores and may fly continuously for several hours each night (Barclay 1989; Hickey and Fenton 1996). Therefore it is plausible that hoary bats maintain high fatty acid transport protein expression year-round. If so, we would not expect to observe a seasonal increase during migratory periods. Given the high energetic demands of continuous flight, it would be particularly interesting to examine seasonal fatty acid transport protein expression in aerial feeding migratory birds such as swifts or swallows. It may be that the difference we observed compared to previous studies may not be a taxonomic difference, but rather a consequence of foraging strategy.

Spring migrating females were an exception to the pattern of constant fatty acid transport protein expression. The ~ 5-fold increase in H-FABP expression is particularly interesting given that expression did not vary seasonally for males. Spring migrating females are pregnant which may have implications for their thermoregulatory strategy, precluding the use of torpor which males may use regularly (Cryan and Wolf 2003). Previously we found that females increase the relative size of their fat stores during spring migration, and we suggested that the additional fat may be required to defend body temperature during daytime roosting periods when males use torpor to save energy (Chapter 5). H-FABP upregulation may be associated with these elevated periods of fat metabolism as has been observed in hibernating bats (Eddy and Storey 2004). However, the difference in expression may also be a result of differential migration strategies in hoary bats. Females generally migrate farther than males (Cryan 2003) which may lead to greater energetic demands and further gene expression changes. Additionally, pregnant females must complete their migration in a timely manner to avoid giving birth prior to reaching the breeding grounds. Taken together, increased fat metabolism, longer migration distances, and possible faster pace of migration required by females than males may explain the sex-biased seasonal H-FABP expression pattern we observed.

Overall, our results are consistent with the hypothesis that migratory flight is an energetically demanding activity for bats, and that this flight is fuelled by stored fat. Therefore these bats are exceptional among mammals because they fuel high intensity exercise with fat, not carbohydrates. The migration physiology of bats presents an example of convergent evolution with migratory birds. In both groups, the selective pressures of maintaining high intensity exercise for long durations while minimizing mass have led to adaptations for maintaining high rates of aerobic metabolism supported by energy dense lipid substrates. Ultimately, upregulation of fatty acid metabolism and aerobic capacity to support migratory flight will involve up-regulation at multiple steps in the pathway which is consistent with our results (upregulation of fatty acid binding proteins, mitochondrial enzymes involved in fatty acid transport and oxidation, and aerobic capacity).

While migratory birds provide a valuable model system for making predictions about bat migration, it is clear from our results and previous studies (McGuire *et al.* 2012/Chapter 4; Chapter 5) that bat migration is different from bird migration. The mammalian affiliation of bats, different physiological strategies/capabilities, the capacity for heterothermy, and the coincidence of migration and reproductive periods all contribute to bat migration as a distinct phenomenon from bird migration. The immense body of literature regarding bird migration is an invaluable resource, but further study of bat migration specifically is required.

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CHAPTER 7

7 SUMMARY

The studies included in my dissertation have added substantially to our understanding of bat migration. The literature review in Chapter 2 highlights the paucity of previous research in this area. For many aspects of the review chapter, information on bats was minimal or lacking entirely. Combining the results of each of the studies presented in this dissertation, a general picture of bat migration physiology is beginning to emerge. It is now possible to provide at least partial answers to many of the questions posed in Table 2.1. Detailed discussion of the results of each chapter are presented in their respective discussion sections. Here I present a general summary of the cumulative findings of my dissertation and suggest directions for future research.

7.1 Bat migration physiology

Migration is an energetically demanding period for bats. In Chapter 3, I showed that migratory bat species have relatively smaller brains than sedentary bat species. This finding is consistent with the energy trade-off hypothesis (Isler and van Schaik 2006) which posits that for flying animals, the energetic cost of maintaining and carrying a large, heavy brain is incompatible with the energetic demands of migration. Thus migratory species would face selection for smaller brains, which is consistent with the patterns observed in extant bats. Enlarged brains confer numerous benefits including enhanced sensory performance, behavioural flexibility, and cognitive ability; large brains are associated with increased intelligence (Isler and van Schaik 2006; Ratcliffe *et al.* 2006; Dechmann and Safi 2009). Consequently the trade-off in reduced brain size associated with migration emphasizes the physiological demands placed on migratory bats. Although brain size is ultimately the product of numerous selective pressures (Dechmann and Safi 2009), taken with the evidence presented in my other chapters, the results of the brain size comparison point towards migration as a physiological challenge for bats. Changes in the quantity and quality of fat stores, patterns of nutrient acquisition and utilization, modulation of lean body components, and physiological changes in flight

muscle provide additional evidence of the heightened demands of migration, and the trade-offs bats make to meet them.

7.1.1 Quantity and quality of fat stores

Birds store large amounts of fat to fuel migratory flight. Pre-migratory fattening is a key feature of bird migration and there is a large body of literature devoted to the subject (e.g., Bairlein 2002). The results of my dissertation indicate that fat is also the primary fuel for bat migration. Yet seasonal patterns of migration-related fat accumulation have not been clearly documented in bats.

In fall migrating silver-haired bats (Chapter 4), fat mass was ~ 19 % of body mass (no sex differences), comparable to the fat stores carried by migrating passerines (Dunning 2008). The computer simulations I conducted suggest that the amount of fat was sufficient for the bats to complete their migration without the need for extended periods of stopover to refuel. However, without a non-migratory group for comparison, it is unclear whether these bats increased the quantity of stored fat for migration.

In spring migration, female hoary bats (Chapter 5) increased the relative size of their fat stores to as much as 24% of body mass. The seasonal increase in the relative quantity of stored fat is consistent with studies of bird migration, but the mechanism may be quite different. Birds increase the amount of stored fat by entering a state of hyperphagia and building a large fat store (McWilliams *et al.* 2004). While bats may become hyperphagic (Widmaier *et al.* 1996), female hoary bats I observed increased the relative size of their fat store by maintaining a consistent amount of fat and reducing the size of lean body components not needed in flight. Male hoary bats reduced the absolute size of their fat stores, but the decrease in fat was accompanied by a parallel decrease in lean mass and consequently the relative amount of fat was the same in both seasons (Chapter 5). Consequently, male hoary bats had smaller fat stores than females in spring migration, contrary to the observations of silver-haired bats in fall migration (Chapter 4). The energetic demands of migrating while pregnant may account for the sex difference in fat storage in spring; females may increase the size of fat stores in spring but not fall migration. Alternatively the absence of sex differences in fall migration may have arisen

due to the relatively small number of adult bats captured and analyzed in the fall. Age differences in fattening are well documented in regional migrants as they deposit fat stores for hibernation (Kunz *et al.* 1998; McGuire *et al.* 2009). It is noteworthy that Layne (1958) reported female silver-haired bats with larger fat stores than males in winter. Future studies including multi-season comparisons and finer temporal resolution in the fluctuation of both fat and lean body components are required to elucidate the seasonal pattern of fat storage in migratory bats.

In addition to quantity, it is also important to consider the quality of nutrient stores. The fatty acid composition of adipose stores affects performance in migratory birds (McWilliams *et al.* 2004; Price 2010). Due to increased mobilization and uptake rates, migrants should increase the proportion of unsaturated fatty acids (Price 2010). During migration, polyunsaturated fatty acids comprised a greater proportion of adipose triglyceride stores (Chapter 4), which may enable bats to maintain higher rates of fatty acid oxidation during migratory flight. Adipose fatty acid composition is largely determined by dietary composition (Price 2010). Some studies have suggested that insectivorous bats may select prey items based on fatty acid composition (Schalk and Brigham 1995). However, with few exceptions, moths accounted for > 90% of the identifiable prey items in the stomach contents of the bats collected in Chapter 5, regardless of sex or season (McGuire and Valdez, unpublished data). Therefore, future study is warranted to determine the mechanism by which bats alter the fatty acid profile of adipose stores.

7.1.2 Nutrient acquisition and use

Several observations from my dissertation suggest that bats primarily deposit fat prior to migration with minimal refueling while *en route*. Digestive organs were smaller in migrating hoary bats (Chapter 5) suggesting reduced foraging activity during migration. Consistent with this observation, most of the bats I radio-tracked at a stopover site did not forage (Chapter 4). However, bats do not completely refrain from foraging during migration. On nights where inclement weather precluded continued migration, several bats foraged extensively (Chapter 4). Only two of the migrating hoary bats I collected had empty digestive tracts (Chapter 5). In fall migration, a similar proportion of hoary and

silver-haired bats had insects in their digestive tracts indicating recent foraging (Reimer *et al.* 2010). Therefore, bats may supplement fuel stores deposited prior to migration by foraging briefly each night or extensively on nights when continued migration is not possible. The ability to use torpor to minimize energy expenditure during daytime roosting periods (the torpor-assisted migration hypothesis proposed in Chapter 4) is central to the ability of bats to forgo extensive refueling during migration. If energy expenditure is restricted primarily to active periods (migratory flight), the total amount of energy required and thus the size of fat depots could be substantially smaller than predicted. A variation on this strategy has been suggested for bats fattening in the pre-hibernation period (Krzanowski 1961; Speakman and Rowland 1999) whereby bats increase effective fattening rate by using torpor to minimize energy expenditure. In both cases, energetic balance is favourably altered by minimizing expenditure rather than maximizing intake as observed in hyperphagic fattening of migratory birds. Future studies should focus on the energetic implications of daily heterothermy during migration, and particularly how daily heterothermy affects nutrient acquisition.

7.1.3 Modulation of lean body components

Lean tissue components play a greater role in bat migration than has been previously considered. In early studies of body composition changes in migratory birds, it was assumed that lean body composition remained static and birds only modulated the amount of stored fat (Odum 1964). It was later realized that the size of lean body components also vary during migration (Piersma 1990). The few previous studies that have examined migration-related body composition changes in bats (O'Shea 1976; Fleming and Eby 2003) considered only fat. The results of my studies indicate that variation in both lean and fat components must be considered in migratory bats. If only considering the amount of fat in spring migrating hoary bats (Chapter 5), one would conclude that male bats decreased the size of fat stores, and female fat storage was the same in spring migration and summer. Taking lean mass into consideration, the conclusion is quite different and indicates that the relative size of fat stores was increased in migrating females while remaining unchanged in males.

Total dry lean mass was reduced in migrating hoary bats, but mass changes differed among exercise and digestive components. Multivariate analysis indicated a general increase in exercise organs of migrating hoary bats (Chapter 5) which may reflect increased machinery to support extended periods of high intensity aerobic exercise. The observation of enlarged lungs in migrants was particularly interesting as many studies of migratory birds find that lungs are one of the few organs that do not change in size during migration. This may represent a consequence of the structural differences between avian and mammalian lungs. In my dissertation, I have focused primarily on aspects of energy substrate (fat) acquisition, storage, and utilization. Studies of running mammals emphasize the importance of considering both energy and oxygen supply limitations in aerobic exercise (Weibel *et al.* 1996). Compared to running mammals, bat lungs are highly specialized to support the oxygen demands of high intensity aerobic exercise (Maina 2000) and seasonal hypertrophy of the lungs presents an intriguing avenue for future research.

Hypertrophy of exercise components was offset by reduced digestive organs, resulting in the overall decrease in lean mass during migration (Chapter 5). Digestive capacity is linked to demand (McWilliams and Karasov 2005), and therefore the reduction of digestive organs indicates a reduced demand. Reduced foraging activity during migratory periods would lower digestive capacity requirement allowing the bat to atrophy digestive organs. As an additional benefit, flight cost increases with body mass and therefore digestive organ atrophy leads to a reduction in the energetic cost of flight. The mean wet intestine mass of migrating hoary bats was only 56% of the mass in non-migrants, comparable to the 51% mass reduction observed in the small intestine of Garden Warblers (*Sylvia borin*) migrating across the Sahara desert (Bauchinger *et al.* 2005). Such reductions surely have functional consequences. Blackcap Warblers (*Sylvia atricapilla*) arriving in Israel after similarly crossing the Sahara desert had lower food intake on the first two days after arrival, presumably due to limitations of reduced digestive organs (Gannes 2002). Bats regularly eat during migratory periods as evidenced by the rarity of bats with empty digestive tracts (Chapter 5; Reimer *et al.* 2010), and hoary bats may in fact time their migration to coincide with seasonally abundant food resources (Valdez and Cryan 2010). I personally saw a mass emergence of geometrid moths while collecting

spring-migrating hoary bats in New Mexico. Such an abundance of prey is surely not an opportunity that a bat would want to pass up. Yet with reduced digestive organs, digestive capacity would limit the ability of the bat to take advantage of such punctuated pulses of prey availability. Further research is needed regarding the trade-offs between mass reductions for lowering flight cost and maintaining digestive capacity to take advantage of irruptive prey. Further study is also required to determine whether digestive organs are reduced prior to migration or decrease gradually during migration, and whether the digestive tract is rebuilt at stopover sites to facilitate hyperphagic refueling.

For seasonal comparisons of hoary bat body composition and physiology, I restricted the duration of collecting trips to < 2 weeks. The migrant vs. non-migrant comparisons are intended to present a 'snapshot' of the animals at a particular time in the annual cycle. However, even with the short duration of my collecting trips, there is evidence that seasonal organ-size plasticity may be more pronounced than what I generally observed. The earliest summer females I captured had greatly enlarged intestines and reduced pectoralis muscles, which suggests that examining late summer bats may underestimate the extent of atrophy/hypertrophy in migration. These females had presumably just recently completed lactation and therefore may indicate carryover effects of the physiological and nutritional challenges associated with milk production to support growing pups. More detailed studies of the timing and extent of organ size plasticity are required. Bats that remain fully active in winter months (don't enter regular multi-day torpor bouts or hibernate) would provide an ideal baseline for comparison with migrating and reproductive individuals.

7.1.4 Physiological changes in flight muscle

Increased activity of catabolic enzymes in flight muscle is consistent with the hypothesis that migration is more energetically demanding than non-migratory periods, and that bats fuel migratory flight with fat. Mitochondrial enzymes involved in transport (carnitine palmitoyl transferase) and oxidation (3-hydroxyacl-CoA dehydrogenase) of fatty acids were greater in migrating bats compared to non-migrating bats (Chapter 6). Citrate synthase activity was also increased during migration indicating an increased aerobic capacity (Chapter 6). Given the upregulation of lipid oxidation capacity I expected that

fatty acid transporters would also be upregulated. This prediction was not supported. One interpretation of these results is that limitations to supporting aerobic metabolism with extramuscular fat lie in mitochondrial transport and oxidation rather than sarcolemmal and cytosolic transport. However, before making such a conclusion, transporter upregulation must be considered at the protein level. In a study of White-Throated Sparrows (*Zonotrichia albicollis*) both mRNA expression and protein abundance of fatty acid transporters were upregulated, but the two measures were not correlated in individual birds (McFarlan *et al.* 2009). Thus mRNA provides a useful indicator, but to support these conclusions, future studies should use western blots to measure protein abundance.

Although most comparisons of fatty acid transport protein expression did not reveal seasonal variation, heart-type fatty acid binding protein (H-FABP) expression was ~ 5-times greater in migrating female (but not male) hoary bats compared to non-migrants (Chapter 6). Female hoary bats migrate greater distances than males and thus the increased H-FABP expression may reflect a more physiologically taxing migration. Furthermore, spring migrating females are pregnant which may lead to increased H-FABP expression through a faster pace of migration (pressure to arrive at summer habitat before giving birth), direct physiological costs of the developing young, increased flight costs due to the added mass of pregnancy, or increased costs due to forgoing torpor during daytime roosting periods. The fatty acid composition of flight muscle membranes supports the latter possibility. The ratio of n-6:n-3 poly-unsaturated fatty acids in the flight muscle membranes decreased in migrating females but increased in migrating males, consistent with increased torpor use in males and decreased torpor use in females (Ruf and Arnold 2008). In a recent study testing predictions of the torpor-assisted migration hypothesis (proposed in Chapter 4), I confirmed that both male and female silver-haired bats use torpor in fall migration (McGuire, Jonasson and Guglielmo, unpublished data). Consequently, I predict that comparisons of phospholipid profiles in the flight muscle of fall migrating bats should not be affected by sex. If sex differences in phospholipid profiles persist in fall migrants, alternative hypotheses related to membrane composition and exercise performance will warrant further investigation.

I suggested in Chapter 6 that the lack of seasonal variation in fatty acid transporters may be due to the aerial insectivore lifestyle of insectivorous bats. In summer, hoary bats may forage (fly) for up to 6 h per night (Barclay 1989) and consequently, the contrast to the energetic demands of migratory flight may be less pronounced. Conversely, the Barnacle Goose (*Branta leucopsis*) is a species for which H-FABP is increased during migration (Pelsers *et al.* 1999). During migration, individuals may fly continuously for up to 14 h or cumulatively for 49 out of 59 h (Butler *et al.* 1998). When compared to the typical winter activity of ~ 0.5 h of flight per day (Portugal *et al.* 2011), the contrast is obvious. Thus it is perhaps not surprising that fatty acid transporter expression did not vary seasonally in my study. The increased H-FABP expression in spring migrating female bats remains intriguing, emphasizing the importance of separating migration and reproduction. Comparison of non-reproductive spring migrant females would be informative, although it is probably not possible to obtain a sufficient sample size of females that are not pregnant. Comparisons of winter and fall migration would avoid the potential confounding effects of pregnancy, but would not be directly comparable to my results due to the potential for seasonally variable migration strategies as frequently observed in migratory birds (e.g., Karlsson *et al.* 2012; Tøttrup *et al.* 2012).

7.2 Migration Rate

One of the principal conclusions of my research is that migration poses physiological challenges for bats. However, this is somewhat contrary to what might be expected given previously published estimates of migration rate. Migration rates based on recaptured banded individuals (e.g., Tuttle 1976; Fleming and Eby 2003), satellite tracking (Tidemann and Nelson 2004), and theoretical models (Hedenström 2009) frequently indicate a migration rate of ~50 km per day or less. At a reasonable flight speed (7 m s^{-1}, typical for a 10 g bat [Norberg and Rayner 1987; McGuire *et al.* 2012/Chapter 4]), a bat would fly 50 km in approximately 2 h, assuming neutral winds. This distance and flight duration are well within the range observed during summer foraging where some species may spend up to 6 h per night flying (Barclay 1989) and travel 50 km from roost to foraging area (Best and Geluso 2003). If migrating bats do not fly longer durations and

greater distances than non-migratory periods, the degree of phenotypic flexibility would be minimal, contrary to the observations presented in my dissertation.

However, banding recaptures and some satellite telemetry techniques provide poor temporal resolution of departure and arrival from a migratory flight and thus true migration rate may be greater than previously estimated. Estimates from some studies of the movements of known individuals suggest migration rates of 70 – 90 km per day (Cockrum 1969; Richter and Cumming 2008). To my knowledge, the fastest individual migration rate reported comes from a straw-coloured fruit bat (*E. helvum*) that travelled 370 km in a single night (Richter and Cumming 2008). Based on presumed arrival and departure times (and hence nightly flight duration), I estimated silver-haired bats may migrate approximately 250 – 275 km per day (McGuire *et al.* 2012/Chapter 4). However, this may be an overestimate as my model did not account for non-flight energetic costs.

Current tracking technology (e.g., radio-tracking, satellite telemetry) is insufficient for tracking most bats over long-distances (Cyan and Diehl 2009). As newer technology becomes available it will be possible to determine migration rate more definitively. The ICARUS project presents one promising option for future long-distance tracking of individual migrating bats (Wikelski *et al.* 2007). When operational, the ICARUS project will track small radio-transmitters (< 1 g) from a low-orbit satellite (Wikelski *et al.* 20007), enabling researchers to document the migration of small bats over the entire globe.

The degree of phenotypic flexibility I observed suggests that the higher migration rate estimates are more appropriate than the lower rate estimates. Until such time as technology becomes available, the various estimates of migration rate serve as testable predictions.

7.3 Comparison of Bats and Birds

Migratory birds have served as an excellent model system for developing hypotheses and making predictions about bat migration. In Chapter 2, I predicted that bats and birds would have evolved similar physiological mechanisms to deal with the energetic

demands of migration. In many regards, the migration physiology of the two groups is similar. Both groups alter the quantity and quality of fat stores, modulate lean body mass components, and increase lipid oxidation and aerobic capacity in flight muscles.

However, despite the many similarities, bat migration is clearly distinct from bird migration. Two important factors to consider are the coincidence of migration and reproduction, and the ability of bats to use torpor. Reproduction and migration are temporally segregated in birds yet female bats are pregnant during spring migration. I did not observe sex effects when radio-tracking fall migrating silver-haired bats (Chapter 4), but sex*migration interactions were apparent in many aspects of spring migration in hoary bats (Chapters 5, 6). Female bats that are simultaneously subject to the energetic and physiological demands of pregnancy and migration present an unparalleled scenario in bird migration. I suggested above that female bats must arrive at the summer habitat before the pups are born, placing strict time constraints on spring migration. However, the time pressure may be even more severe. I captured hoary bats early in migration when females were in the early stages of pregnancy. As the pups continue to develop and grow, the energetic demands of late pregnancy and the increased flight costs incurred by carrying near-term foetuses may limit the energy available for migration, precluding females from migrating as they approach full term of their pregnancy. In autumn, the degree to which mating occurs during migration is not understood for many species, but trade-offs between optimal migration and mating opportunities are a possibility deserving further investigation.

For temperate bat species, the ability to use torpor has significant ramifications when comparing the energetics of bat and bird migration. Migratory birds spend twice as much energy at stopover compared to migratory flight (Wikelski *et al.* 2003). The thermoregulatory costs of defending euthermic body temperature reduce the effective refueling rate (energy is spent on warming rather than being stored) and result in prolonged stopover durations. Theoretical estimates suggest a 7:1 ratio of time spent refueling and time spent flying (Hedenström and Alerstam 1997). In Chapter 4, I observed very short stopovers in bats compared to similar sized birds at the same site. As a potential explanation, I proposed the torpor-assisted migration hypothesis, whereby

migrating bats could use daily torpor to minimize thermoregulatory costs during diurnal roosting periods. Since then, I have confirmed that fall migrating silver-haired bats do indeed use torpor during daytime roosting periods (regardless of sex or age), and that the time spent in torpor is inversely proportional to ambient temperature (McGuire, Jonasson and Guglielmo, unpublished data). Daytime torpor saves up to 94 % of the energetic cost of maintaining euthermia, but perhaps more importantly the bats achieve a predictable daytime roosting cost by increasing torpor bout duration on cold days when thermoregulatory costs would be elevated (McGuire, Jonasson and Guglielmo, unpublished data). By minimizing the costs associated with non-flight periods, bats are able to reserve their energy stores for migratory flight. Thus, bats may rely primarily on fat stores deposited prior to migration, minimizing time spent refueling at stopover sites and achieving a faster overall migration with a lower total energy cost compared to birds. However, even with the energy (and hence time) savings provided by daily torpor, bats still face substantial physiological challenges in migration as the numerous migration-related physiological changes identified in my dissertation demonstrate.

In future research I will continue to use migratory birds as a model system for studying bat migration. In addition, I will turn the tables and use the knowledge gained from migratory bats to ask questions about migratory birds. For example, contrary to previous studies of geese (Anseriformes), sandpipers (Charadriiformes), and sparrows (Passeriformes), I did not find seasonal upregulation of fatty acid transport protein expression and suggested this may be due to the aerial insectivore lifestyle of hoary bats (Chapter 6). Studies of seasonal variation in transporter expression in migratory insectivorous birds such as swifts (Apodiformes) or swallows (Passeriformes) could test this hypothesis. Similarly, the ability to use torpor appears to be one of the most important factors differentiating bat migration from birds. Although true torpor is uncommon among birds, hummingbirds (Apodiformes) have been observed to use torpor during migration, and goatsuckers (Caprimulgiformes) are known to use torpor during non-migratory periods. Shallow hypothermia may present a functionally similar strategy in birds that are not capable of using deep torpor. Further investigation of the prevalence and consequences of heterothermy among migratory birds will be informative.

7.4 Comparing North American tree bats to other migratory bat species

The study of bat migration physiology is difficult. Given the dearth of previous migration physiology research, I carefully chose a study system where seasonal physiological variation is maximized and not confounded with other life history factors (e.g., hibernation). I chose to study North American tree bats (*Lasiurus cinereus* and *Lasionycteris noctivagans*) because they are among the longest-distance bat migrants (Bisson *et al.* 2009), they do not hibernate over winter (Perry *et al.* 2010), and there is a growing body of literature regarding their migration biology (Cryan 2003; Cryan *et al.* 2004; Baerwald and Barclay 2009; Valdez and Cryan 2009; Reimer *et al.* 2010; Baerwald and Barclay 2011).

However, in many ways, these species are not ideal for migration studies. The basic natural history is poorly documented for most parts of the annual cycle. For hoary bats, it is not even entirely clear where they go in the winter months, but it is suspected they overwinter in southern California and/or Mexico (Cryan 2003). The situation is somewhat clearer for silver-haired bats, but suggestions of multiple migration routes and connections between summer and winter grounds remain purely speculative. These bats are also elusive. It took me two field seasons to collect seven adult male and eight adult female hoary bats. Ideally the sample sizes for seasonal comparisons would be larger, but it was simply not possible to catch enough bats. During migration it is easier to catch large numbers of bats, but only in particular locations. I spent one fall field season at a reported migration site in Manitoba and only collected three bats. At Long Point, ON I caught reasonable numbers of silver-haired bats, but these were dominated by sub-adults. I was unable to capture sufficient numbers of adults for meaningful age comparisons. The elusiveness of North American tree bats has also precluded any successful banding studies to determine their movement patterns. Furthermore, these bats are too small for many tracking technologies (Wikelski *et al.* 2007; Bridge *et al.* 2011). The radio-transmitters I used in Chapter 4 were the very first batch of that particular type of transmitter ever produced.

In an ideal world, bat migration studies would focus on species with all of the positive attributes of North American tree bats, but for which the basic biology was also well understood, were easy to capture, and large enough to permit tracking. Unfortunately, no such species exists. August Krogh (1929) would not have studied bat migration physiology. However, given the diversity of bats that undertake latitudinal migration, different combinations of these characteristics can be found in different species. Studies of North American tree bat migration provide a useful baseline for comparisons with other species, and combined, a unified picture of the migration physiology of bats may emerge.

Brazilian free-tailed bats (*T. brasiliensis*) are abundant (McCracken 2003; Betke *et al.* 2008) and may fly greater distances each night than hoary bats (Best and Geluso 2003) providing a valuable comparison as another species where the seasonal contrast between migratory and non-migratory periods may be muted. Furthermore, non-migratory populations in California provide the opportunity for intraspecific comparative studies. In Europe, *Nyctalus leisleri* migrate > 1000 km but are not ideal for migration physiology studies because they hibernate (Hutterer *et al.* 2005; Dietz *et al.* 2009). However, the particularly interesting opportunity with this species lies in their habit of returning to artificial bat boxes year after year (Schorcht *et al.* 2009). Schorcht *et al.* (2009) report a recapture rate of nearly 40% for females (compare with 0.5 % recapture rate in *T. brasiliensis*; Constantine 1967) presenting the opportunity for longitudinal studies. Furthermore, although their body size is too small for satellite or GPS tracking devices, they may be able to carry geolocator tags which log position based on sunrise and sunset times provided the tag can be recovered (Stutchbury *et al.* 2009). Documenting the actual movement patterns of a migratory bat over an entire annual cycle would be far more informative than a straight line connecting the dots of two banding recaptures. A similar opportunity exists with larger members of the family Pteropodidae that can carry satellite transmitters. Some tracking studies have been done (Tidemann and Nelson 2004; Richter and Cumming 2008; Epstein *et al.* 2009) and current research is investigating the use of GPS tracking to study migration in the straw-coloured fruit bat (*Eidolon helvum*) (D.K.N. Dechmann, personal communication). The migration of pteropodids is frequently associated with seasonally available fruits and flowers (e.g., Richter and Cumming 2006).

Lesser long-nosed bats (*Leptonycteris yerbabuenae*) similarly migrate in association with cactus flower phenology (Cockrum 1991; Fleming *et al.* 1993) and thus comparisons among these groups may be informative. The patterns of migration in these species are sufficiently described that it may soon be possible to conduct the types of mechanistic studies required to answer the 'big questions' about how and why bats migrate.

7.5 Conclusion

Bat migration is a topic that is finally beginning to receive the attention it deserves (Popa-Lisseanu and Voigt 2009) but further study is required in all aspects of bat migration. The degree of phenotypic flexibility and the number of physiological and behavioural changes associated with migration all indicate that migration is a non-trivial component of the annual cycle for bats. In my dissertation I have presented the first studies of the physiological ecology of bat migration. These studies provide a starting point for future work in this system as well as investigations of other systems including bats from different families (e.g., Pteropodidae, Molossidae), geographic regions (e.g., Europe, Africa, Australia), diets (e.g., frugivores, nectarivores), life histories (e.g., hibernating bats), and different migration systems (e.g., altitudinal and regional migration). Equally important is further study of the basic biology and natural history of migratory species. To fully understand the migration biology, it is crucial that we are able to place migration into the broader context of the annual cycle of the species. When large periods of the year remain a mysterious 'black box' it is difficult to understand how observations relate and even more difficult to develop conservation and management plans.

Migration is likely the single most important factor contributing to annual mortality rates in migratory bats. The inherent risks and energetic demands of migration lead to increased mortality (Tuttle and Stevenson 1977). Anthropogenic activities are further compounding the natural dangers faced by migratory bats. Wind turbines kill large numbers of migratory bats (Arnett *et al.* 2008; Baerwald and Barclay 2011). Because of the difficulty catching and tracking these solitary species, population size estimates are not available for most migratory species. Thus it is impossible to determine the long-term consequences for population viability and how current wind turbine mortality rates will affect migratory species. Furthermore, it is unclear how migratory species will respond to

changes in the North American landscape through climate change and human development. Understanding the physiological mechanisms that underlie migratory behaviours and strategies will aid in the development of conservation and management practices and the implications of both current and future threats to migratory bat populations.

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Appendix A: Chapter 3 Supplementary Material

Table A.1 Body mass (g) and brain, neocortex, hippocampus, cerebellum, and medulla oblongata volume data (mm^3) for the 64 species considered in our study (brain mass given converted to volume: brain volume = brain mass/1.036, Baron *et al.* 1996). We included the cerebellum and medulla oblongata as control / neutral regions for which we had no *a priori* reason to expect systematic differences in volume between migratory and sedentary species.

Family	Species	Body (g)	Brain (mm^3)	Neocortex (mm^3)	Hippocampus (mm^3)	Medulla oblongata (mm^3)	Cerebellum (mm^3)	Category	References
Pteropodidae	*Cynopterus brachyotis*	33.9	946.9	287.6	80.9	68.3	122.7	Sedentary	9,18
	Cynopterus horsfieldi	59.3	1325.3	432.3	110.3	92.7	180.5	Sedentary	18
	Eidolon helvum	262	4140.9	1406	258.1	273.7	463.3	Migratory	9,18,20,23,24,29
	Epomophorus wahlbergi	74.1	1751					Migratory	18,19
	Macroglossus minimus	14.6	541.5	155.7	47.0	41.3	79.2	Migratory	9
	Myonycteris torquata	36.2	1116.8	336.5	85.7	82.2	155.8	Migratory	9,18,20,29
	Pteropus alecto	595	6794.4	2716	357.4	392.1	843.7	Migratory	9,20
	Pteropus poliocephalus	695	6978.8	2710	358.5	431.6	880.3	Migratory	7,9,20,21,30
	Pteropus scapulatus	375	5173.7	2078	267.8	284.3	682	Migratory	8,9,20,21
	Syconycteris australis	14.7	550.2	170.0	48.4	37.9	64.9	Sedentary	9
Hipposideridae	*Hipposideros commersoni*	99.2	723	171.7	47.8	86.5	128.4	Migratory	6,19
	Hipposideros lankadiva	48.5	707.5	154.5	48.8	93	127.3	Migratory	11
	Triaenops persicus	13.7	261.6	48.1	17.4	38.7	66.1	Migratory	19
Rhinolophidae	*Rhinolophus ferrumequinum*	23.5	337.8					Sedentary	9,12,13,26,28
	Rhinolophus hipposideros	4.6	143.8	19.0	13.6	23	30.4	Sedentary	9,12,13,26,28
	Rhinolophus landeri	9.7	267.4	50.6	19.1	35.3	56.3	Migratory	19
	Rhinolophus mehelyi	9.7	299.2					Sedentary	9,13
Megadermatidae	*Cardioderma cor*	26.0	646.7	186.3	32.3	71.5	111.7	Sedentary	9
	Lavia frons	23.4	621.6	171.4	25.8	67.7	138.8	Sedentary	9
	Macroderma gigas	119.8	1644.8	510.5	68.9	186.7	301.4	Sedentary	9
Rhinopomatidae	*Rhinopoma microphyllum*	29.4	373.6	77.5	25.8	51.3	60.4	Migratory	1,11
Emballonuridae	*Coleura afra*	11.5	248.1	52.5	12.4	33.1	49.1	Sedentary	9
	Emballonura monticola	5.3	160.2	32.1	7.61	21	31.8	Migratory	17
	Emballonura semicaudata	7.0	177.6	36.0	10.1	25.7	36	Migratory	17
	Saccopteryx bilineata	8.4	223	43.9	12.2	27.2	37.8	Sedentary	9

	Taphozous australis	24.0	499	101.8	25.5	65.8	104.1	Sedentary	9
	Taphozous mauritianus	29.0	531.8	105.9	25.4	70.4	114	Migratory	19
	Taphozous melanopogon	22.7	517.4	113.2	23.7	67	112.7	Migratory	1,11
Nycteridae	*Nycteris grandis*	29.8	684.4	187.0	36.2	80.3	139	Sedentary	9
	Nycteris thebaica	8.9	311.8	85.7	17.4	39.6	58	Sedentary	19
Noctilionidae	*Noctilio leporinus*	58.0	1141.9	361.7	50.5	124.4	194.1	Sedentary	9
Phyllostomidae	*Desmodus rotundus*	36.3	964.3	312.5	42.4	80	166.4	Sedentary	9
	Diaemus youngi	34.6	944					Sedentary	9
	Diphylla ecaudata	30.9	770.3	231.1	41.0	63.1	132	Sedentary	9
	Glossophaga soricina	9.9	379.3	88.0	31.4	37.9	81.6	Sedentary	10,18
	Leptonycteris curasoae	24.5	588.8	140.3	47.7	62.2	119.4	Migratory	2,9,10,15,16,20,22
	Leptonycteris nivalis	22.8	565.6	144.3	42.2	56	104.4	Migratory	9,10,16,18,20
	Phyllostomus hastatus	91.8	1480.7	438.9	79.8	140.2	281.3	Sedentary	9
	Vampyrum spectrum	173	2497.1	757.7	110.4	233.5	532.7	Sedentary	9
Vespertilionidae	*Barbastella barbastellus*	5.8	176.6					Migratory	9,12,13
	Eptesicus fuscus	13.6	229.7	43.3	19.7	31.1	38.7	Sedentary	2,3,9,18
	Eptesicus serotinus	23.4	299.2	55.7	27.6	47.1	55.2	Sedentary	3,9,28
	Ia io	53.3	734.6	141.3	47.1	95.3	145.6	Migratory	18
	Lasiurus borealis	7.8	164.1	27.9	9.0	23.4	40.9	Migratory	2,3,4,9,12,18,20,22
	Myotis bechsteini	9.5	255.8	36.3	25.5	39.4	42.5	Sedentary	3,9,13
	Myotis dasycneme	15.0	299.2	46.5	25.2	49.3	49.8	Migratory	3,9,12,13,26,28
	Myotis daubentoni	7.0	222					Migratory	3,9,12,13
	Myotis lucifugus	7.9	163.1					Migratory	2,3,5,8,9,12,18,20,22
	Myotis myotis	25.6	468.1	78.3	35.6	70.3	87.1	Migratory	3,9,12,13,27
	Myotis nattereri	7.0	212.4	34.8	21.4	31.6	37.4	Sedentary	3,9,13,28
	Nyctalus noctula	27.0	351.4	54.9	19.2	57.6	80.5	Migratory	3,9,12,13,18,20,26,27,28
	Perimyotis subflavus	5.3	120.7	16.6	10.9	18.3	21.3	Migratory	3,9,22
	Pipistrellus kuhlii	6.3	160.2					Sedentary	3,9,13
	Pipistrellus nanus	3.9	96.5	15.7	6.27	15.2	18.5	Sedentary	3,19
	Plecotus auritus	6.6	229.7					Sedentary	3,9,13,28
	Scotophilus dinganii	24.3	421.8	87.2	22.7	58.2	81.5	Sedentary	3,6
	Scotophilus nigrita	101.3	1009.7					Migratory	19
Miniopteridae	*Miniopterus australis*	7.1	196.9	37.6	15.0	27.2	39.7	Migratory	17
	Miniopterus inflatus	14.9	313.7	55.9	19.0	43.8	79.5	Migratory	17

	Miniopterus schreibersi	11.4	245.2					Migratory	3,9,10,12,13,18,20,25,26,27,28
Molossidae	*Chaerephon pumila*	10.3	234.6	44.3	12.2	36.3	41.8	Sedentary	18
	Eumops perotis	47.5	774.1					Sedentary	18,22
	Molossus ater	33.6	507.7	113.8	24.4	66	93.8	Sedentary	18
	Mops condylurus	27.2	441.1	102.0	22.9	58.6	74.4	Migratory	19
Migratory (n = 31, mean ± s.e.)		86.0 ± 30.3	1152.8 ± 339.2	435.3 ± 166.2	73.9 ± 21.7	100.6 ± 22.9	184.0 ± 49.9		
Sedentary (n = 33, mean ± s.e.)		31.3 ± 6.2	625.0 ± 91.0	186.1 ± 34.9	38.7 ± 5.5	68.1 ± 9.8	119.1 ± 21.1		

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Appendix B: Chapter 4 Supplementary Material

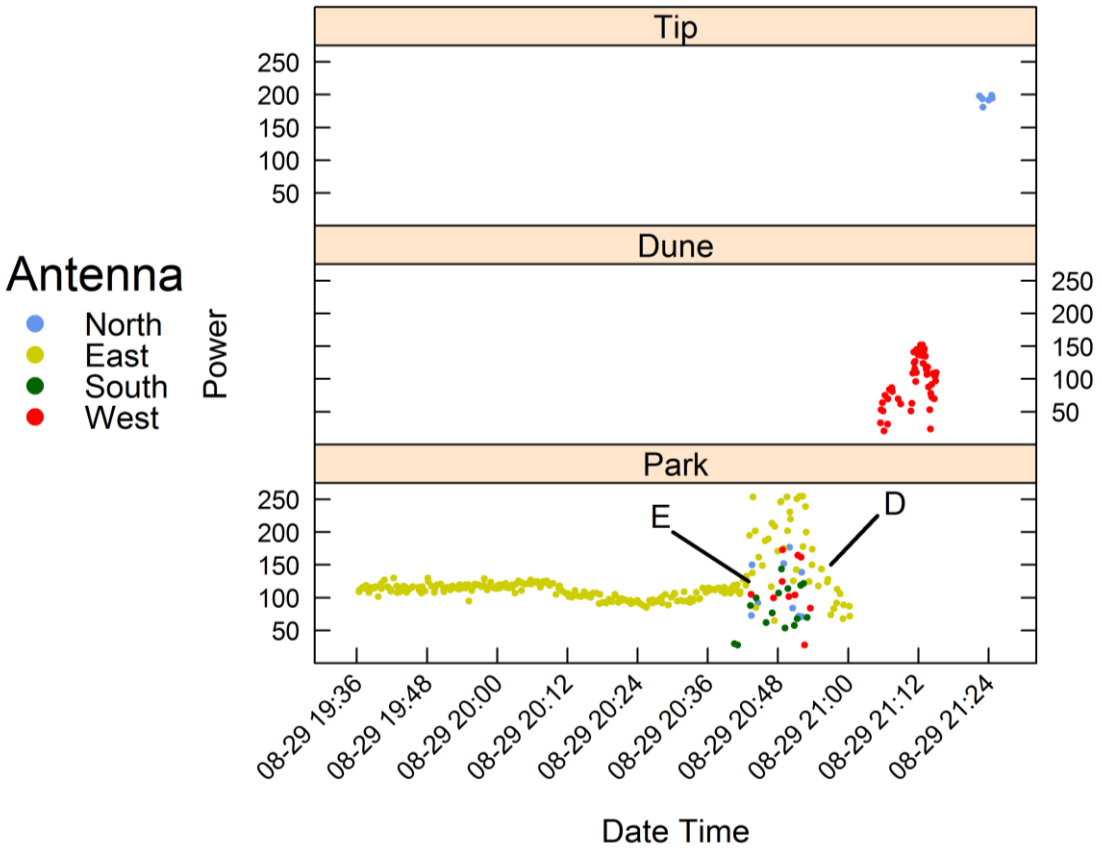

Figure B.1 Telemetry observations of a bat on the night of departure (August 29). The bat roosted to the east of the Park tower during the day and emerged from the roost (E) at 20:43. Fourteen minutes later, the bat began a directed departure flight (D) as it moved away to the east of the Park tower (signal fading on east antenna), past the west facing antenna of the Dune tower (signal strength increasing then decreasing) and past the tower at the Tip.

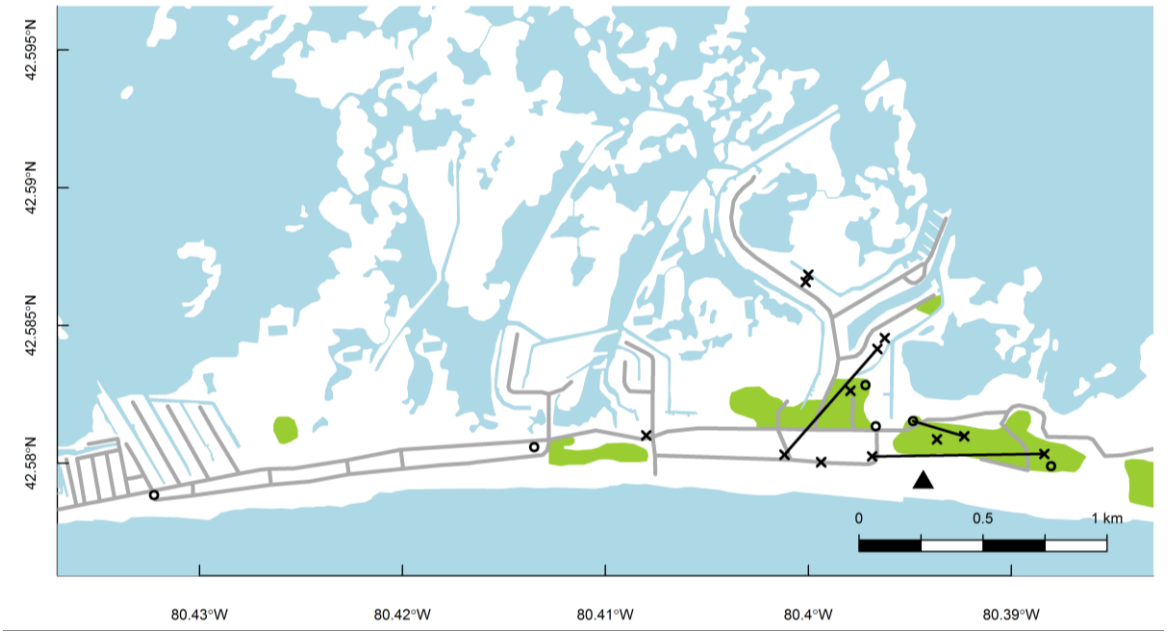

Figure B.2 Location of known day roosts. In some cases we determined the exact location of the day roost (×) and others we were only able to note an approximate roost location (○), either because the transmitter signal could not be sufficiently resolved or because we were unable to obtain landowner permission to access roosts on private property. We could only make direct visual confirmation of roosting bats on two occasions, one bat roosted under loose bark on a dead scots pine (roost height ~ 5 m), and another in a deep groove of the bark of an eastern cottonwood (roost height ~ 1.5 m). Solid black lines connect roosts used by the same bat on consecutive days. The majority of the area is marshland (white fill) with small isolated woodlots (green fill). Thick grey lines indicate roads, most of which are lined by houses. The park tower (▲) was located on the south beach of the peninsula.

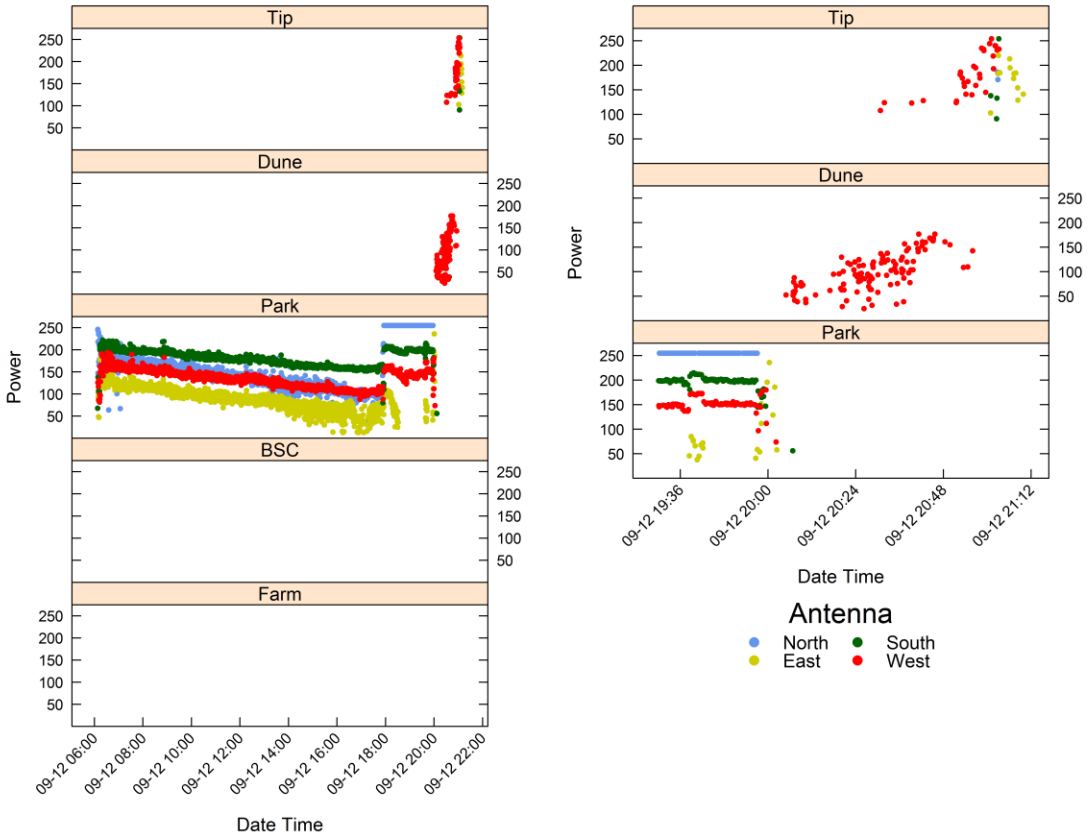

Figure B.3 An example of a Tip departure. The full contact period is shown on the left and on the right is an expanded view of the departure. The bat was captured the morning of September 12. It day roosted in a nearby scots pine in Long Point Provincial Park throughout the day (note the jump in signal strength as the bat changed roosting position). Just before 20:00, the bat emerged from the roost, moving east from the Park tower. Signal strength increased on the Dune tower as the bat approached from the west. There were simultaneous detections on the west facing Dune and Tip antennas before the bat flew almost directly over the Tip tower (brief north and south antenna hits) before departing from the tip as the signal faded on the east antenna.

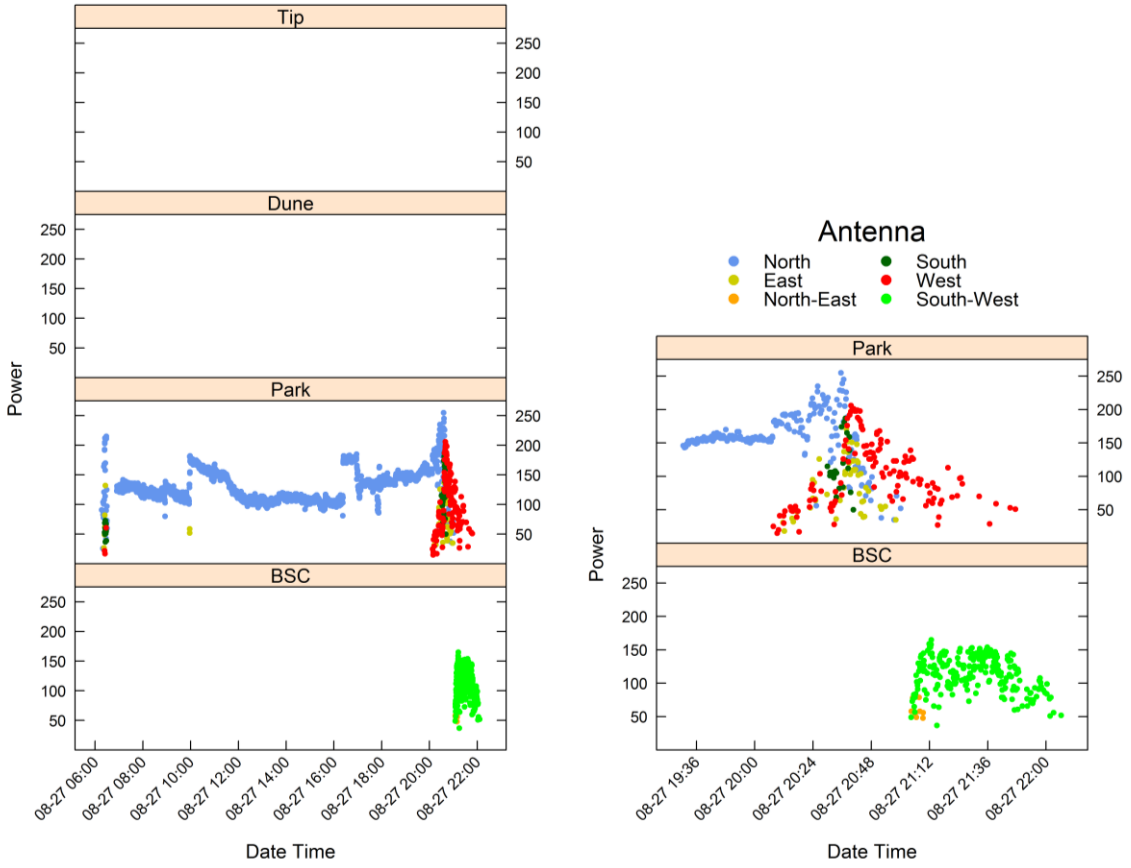

Figure B.4 An example of a west shoreline departure. The full contact period is shown on the left and on the right is an expanded view of the departure. The bat was captured the morning of August 27. The bat roosted in an unknown location north of the Park tower through the day. Shortly after 20:00, the bat emerged from the roost and flew west from the Park tower (signal fading on west facing antenna). The signal was simultaneously detected on the south-west facing antenna of the BSC tower. BSC signal strength increased and then faded as the bat passed perpendicularly through the beam, following the west shoreline.

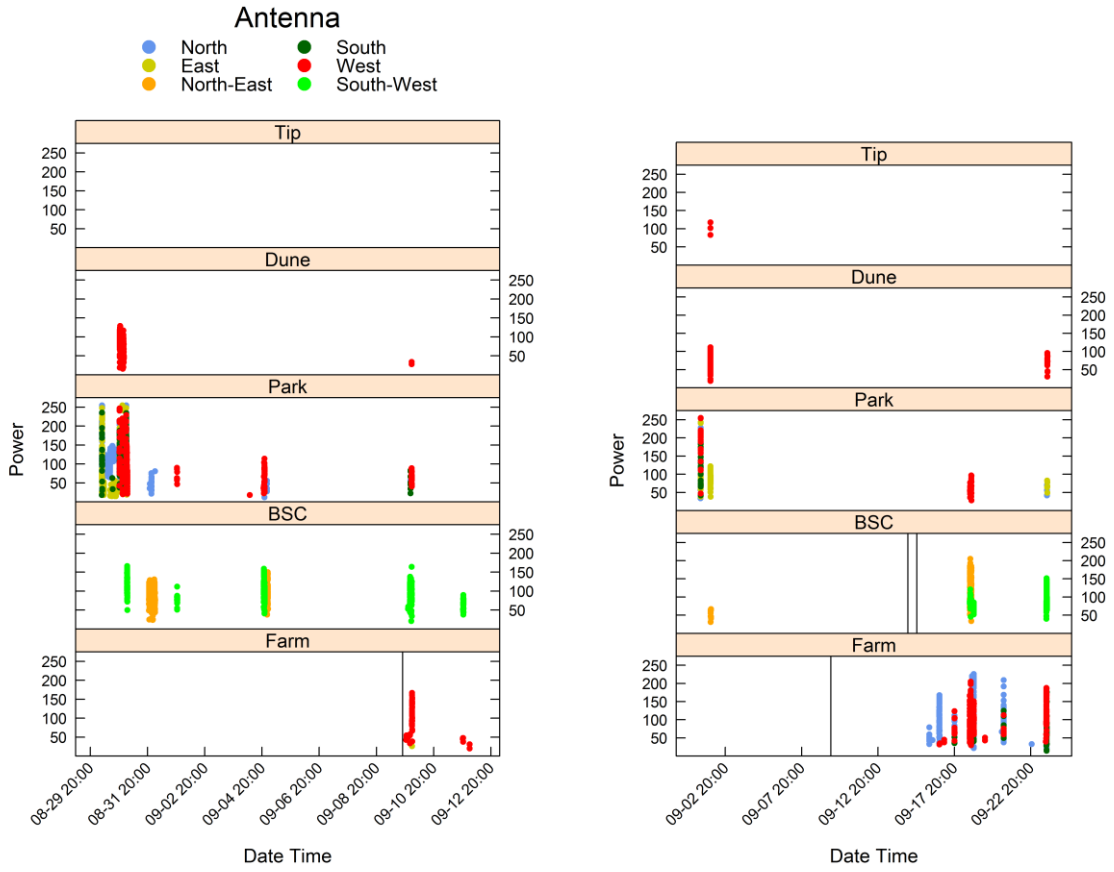

Figure B.5 Two bats made extended stopovers. We monitored one bat (left panel) for 13 days, as it spent time in the areas of the Park, BSC, and Farm towers. Note the vertical bar in the Farm panel indicates the date/time this receiver was established. The bat was immediately detected in the vicinity of the Farm, suggesting it may have been in the area prior to the establishment of the Farm tower. No clear departure was apparent for this bat. The other extended stopover was made by a bat that stayed 22 days (right panel). The bat originally appeared to depart on the night after capture, much as the majority of bats we observed. Fifteen days later the bat reappeared at the Farm tower and spent the remainder of the stopover in the area of the Farm and BSC towers. Presumably the bat was in the area for the full 23 nights and spent the initial 15 nights beyond the range of the telemetry array. Ultimately the bat departed to the south, heading across the lake. The vertical bar in the farm panel indicates the establishment of that receiver, and the area between the two vertical bars on the BSC panel indicates a brief period where that receiver was offline.

Appendix C: Chapter 6 Supplementary Material

Table C.1 Degenerate and specific primers developed for hoary bats. Primers pairs are presented as they were used in the study.

	Gene	Primer name	Sequence (5' – 3')	Product Size (bp)
Degenerate Primers	GAPDH	LMGAPDH5F	AGT CCA CCG GTG TCT TCA CT	
		LMGAPDH5R	GTC ATG AGT CCC TCC ACG AT	238
	H-FABP	HFABP10F	GGy ACC TGG AAG CTr GTG G	
		HFABP10R	TCA nGC yTC yTT yTC rTA AGT	385
	FAT/CD36	LMFAT2F	TGC AAA GAA GGA AAA CCT GTG	
		LMFAT2R	TTT TTG CTG GCT TGA CCA AT	342
		LMFAT7F	CAG CCT CCT TTC CAC CTT TT	
		LMFAT7R	CCC AGT CTC ATT TAG CCA CAG	462*
	FABPpm	LMFABPpm2F	TTG ACA TGG CCT ACC AAG G	
		LMFABPpm2R	TTG GTG ACC TGG TGA ATG G	567
Specific Primers	GAPDH	LMGAPDH7F	AAG GGT GGA GCC AAG AGG	
		LMGAPDH7R	TGC TGA CAA TCT TGA GGG AGT	110
	H-FABP	LMHFABP2F	TGG AGT TTG ACG AGA CAA CG	
		LMHFABP2R	CAT GGG TGA GTG TCA GGA TG	156
	FAT/CD36	LMFAT8F	AAA CGG CTG CAA GTC AAC AT	
		LMFAT8R	TCA GCC AAA GAA TAG GCA CA	97
	FABPpm	LMFABPpm4F	CTT GAT TCG CCC CAT GTA TT	
		LMFABPpm3R	CTT TCA CCT CCT GCA ACC AT	108

* Note: Combining overlapping regions of LMFAT2F/LMFAT2R and LMFAT7F/LMFAT7R sequences yields a 587 bp sequence.

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Appendix E: Ethics Approvals

Western

April 2, 2008

This is the Original Approval for this protocol
A Full Protocol submission will be required in 2012

Dear Dr. Fenton:

Your Animal Use Protocol form entitled:
Behavioural Ecology of Bats
Funding Agency NSERC - Grant #R3516A03

has been approved by the University Council on Animal Care. This approval is valid from **April 2, 2008 to April 30, 2009.** The protocol number for this project is **#2008-003-04 and replaces #2004-027-03** .

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

ANIMALS APPROVED FOR 1 YR.

Species	Strain	Other Detail	Pain Level	Animal # Total for 1 Year
Other, add to detail	Bats	various species M/F	C	~ 1500

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

1. Please ensure that the approximate number of bats used under this protocol is submitted to the AUS office by December each year for the annual CCAC report.

c.c. Approved Protocol - B. Fenton, L. McGuire, J. Weber, D. Cheshuk
Approval Letter - L. McGuire, J. Weber, D. Cheshuk

[redacted]

The **University *of* Western Ontario**
Animal Use Subcommittee / University Council on Animal Care

[redacted]

Western

05.01.09
***This is the 1st Renewal of this protocol**
*A Full Protocol submission will be required in **2012**

Dear Dr. **Fenton**

Your Animal Use Protocol form entitled:

Behavioural Ecology of Bats

has had its yearly renewal approved by the Animal Use Subcommittee.

This approval is valid from **05.01.09 to 04.30.10**

The protocol number for this project remains as **2008-003**

1. This number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this number.
3. If no number appears please contact this office when grant approval is received.
   If the application for funding is not successful and you wish to proceed with the project, request that an internal scientific peer review be performed by the Animal Use Subcommittee office.
4. Purchases of animals other than through this system must be cleared through the ACVS office. Health certificates will be required.

REQUIREMENTS/COMMENTS
Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.
The holder of this *Animal Use Protocol* is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

[redacted]

c.c. L McGuire, J Wasylenko

***The* University *of* Western Ontario**
Animal Use Subcommittee / University Council on Animal Care

[redacted]

Appendix F: Permits

Saskatchewan Ministry of Environment

Special Permit

Scientific Research

Permit Number: 08FW080

Under the authority of the *Wildlife Act and the Regulations* thereunder, permission is hereby granted

To:

LIAM	MCGUIRE	UNIVERSITY OF WESTERN ONTARIO
Given Name	**Surname**	**AKA**

of

[redacted]

Street/Box Number **City/Town** **Prov/State Postal Code**

To: capture, sample and ethanize Hoary bats to determine migration related variation in body composition and lipid metabolism related processess for migrating Hoary bats in the Cypress Hills Interprovincial Park in July and August of 2008.

- up to a maximum of 30 bats may be taken, blood and muscle samples will be collected
- no lactating females may be taken

Upon completion of your activities, you are required to submit a detailed report to Ministry of Environment, Fish & Wildlife Branch, 2nd Floor, 3211 Albert, REGINA SK S4S 5W6, attention Penny Lalonde outlining your research activities, numbers of species collected and locations (either land location or NAD 83 UTM), and any other relevant notes. This report must be received by December 31, 2008.

Every effort should be made to avoid unnecessarily disturbing wildlife and their habitats during your fieldwork activities. This permit does not allow you to access leased provincial Crown lands without prior permission of the landowner. Permittee must obtain permission from park supervisor within provincial parks and conservation officer outside provinical parks prior to any fieldwork commencing in the area. Failure to comply with any of the conditions of this permit will result in its immediate withdrawal, subsequent rejection of future permit applications, and possible prosecution under The Wildlife Act, 1998.

Ministry of Environment is also soliciting observations of rare species in the areas you may be in, it is a condition of this permit that you submit your observations of other rare species (including location, and date) directly to the Conservation Data Centre (http://www.biodiversity.sk.ca/docs/speciesreportform.pdf). See the Government of Canada's Species at Risk Public Registry (http://www.sararegistry.ca) for the most current list of endangered species. A Species Report Form must be completed for each rare species population found at a location as well as each reported occurrence (see the SKCDC Project Review Website), even if not found.

This permit expires on 2008-December-31
Issued at REGINA
This date 2008-May-28

Permittee Signature [redacted]

[redacted]
Signature - for Director, Resource Allocation
Fish and Wildlife Branch

[X] **- Permittee Copy**
- Fish and Wildlife Branch
- Field Office Copy
- Compliance and Field Services

630-01-01

Ministry of
Tourism, Parks,
Culture and Sport

Special Permit

Permit Number SP-CHPP-02-08

Under authority of The Parks Act and the Regulations thereunder, permission is hereby granted to

Liam McGuire, PhD student of University of Western Ontario

to: collect (i.e. euthanize and take back to University of Western Ontario), 30 hoary bats to serve as a baseline group for comparison of physiological changes during migration. Lauren Hooton (MSc student at UWO) to accompany Liam as a field assistant.

This permit is valid from July 16, 2008 to August 6, 2008

In order for this permit to be valid, it must be signed by the permittee in the space provided.

Issued Pursuant to Sec. 17(1), 17(2) and 17(3)(a) of The Saskatchewan Park Regulations, 1991, Ch.P-1.1 Reg. 6

Issued at Cypress Hills Interprovincial Park, Saskatchewan, this 9th day of May, 2008

[redacted]
Signature of Permittee

[redacted]
Signature of Park Supervisor

Permit Distribution:
Permittee
Park Supervisor

Saskatchewan Ministry of Environment

Special Permit

SCIENTIFIC RESEARCH

Permit Number: 09FW045

Under the authority of the *Wildlife Act and the Regulations* thereunder, permission is hereby granted

To:

LIAM	MCGUIRE	UNIVERSITY OF WESTERN ONTARIO
Given Name	**Surname**	**AKA**

of [redacted]

Street/Box Number	**City/Town**	**Prov/State**	**Postal Code**

To: capture, sample and ethanize Hoary bats to determine migration related variation in body composition and lipid metabolism related processess for migrating Hoary bats in the Cypress Hills Interprovincial Park in July and August of 2009.

- up to a maximum of 15 bats may be taken, blood and muscle samples will be collected
- no lactating females may be taken
- permit valid for permittee and designates

Upon completion of your activities, you are required to submit a detailed report to Ministry of Environment, Fish & Wildlife Branch, 2nd Floor, 3211 Albert, REGINA SK S4S 5W6, attention Penny Lalonde outlining your research activities, numbers of species collected and locations (either land location or NAD 83 UTM), and any other relevant notes. This report must be received by December 31, 2009.

Every effort should be made to avoid unnecessarily disturbing wildlife and their habitats during your fieldwork activities. This permit does not allow you to access leased provincial Crown lands without prior permission of the landowner. Permittee must obtain permission from park supervisor within provincial parks and conservation officer outside provinical parks prior to any fieldwork commencing in the area. Failure to comply with any of the conditions of this permit will result in its immediate withdrawal, subsequent rejection of future permit applications, and possible prosecution under The Wildlife Act, 1998.

Ministry of Environment is also soliciting observations of rare species in the areas you may be in, it is a condition of this permit that you submit your observations of other rare species (including location, and date) directly to the Conservation Data Centre (http://www.biodiversity.sk.ca/docs/speciesreportform.pdf). See the Government of Canada's Species at Risk Public Registry (http://www.sararegistry.ca) for the most current list of endangered species. A Species Report Form must be completed for each rare species population found at a location as well as each reported occurrence (see the SKCDC Project Review Website), even if not found.

If this permit is not used, it must be returned to the Fish and Wildlife Branch no later than 7 days following the expiry date.

This permit expires on 2009-December-31
Issued at REGINA
This date 2009-January-28

Permittee Signature _____ [redacted]

Signature - for Director, Resource Allocation
Fish and Wildlife Branch

[X] **- Permittee Copy**
- Fish and Wildlife Branch
- Field Office Copy
- Compliance and Field Services

Saskatchewan Ministry of Environment

Special Permit

SCIENTIFIC RESEARCH

Permit Number: 09FW045

630-01-01

Ministry of
Tourism, Parks,
Culture and Sport

Special Permit

Permit Number SP-CHPP-01-09

Under authority of The Parks Act and the Regulations thereunder, permission is hereby granted to

Liam McGuire, PhD student of University of Western Ontario

to: collect (i.e. euthanize and take back to University of Western Ontario), 15 hoary bats (Lasiurus cinereus) to serve as a baseline group for comparison of physiological changes during migration.

This permit is valid from July 1, 2009 to August 31, 2009

In order for this permit to be valid, it must be signed by the permittee in the space provided.

Issued Pursuant to Sec. 17(1), 17(2) and 17(3)(a) of The Saskatchewan Park Regulations, 1991, Ch.P-1.1 Reg. 6

Issued at Cypress Hills Interprovincial Park, Saskatchewan, this 20th day of April, 2009

[redacted]

Signature of Permittee

Signature of Park Supervisor

Permit Distribution:
Permittee
Park Supervisor

NEW MEXICO DEPARTMENT OF GAME AND FISH AUTHORIZATION FOR TAKING PROTECTED WILDLIFE FOR SCIENTIFIC AND/OR EDUCATION PURPOSES

**amendment request (4/3/2009); approved per authorization period below*

Name of Permittee: **Liam McGuire**

Address: [redacted]

Name(s) of Subpermittee(s): Quentin Hays, Chris Guglielmo

Authorization Number: 3424

Period of Authorization: *Period beginning with date of Director's signature (see below)* to Dec. 31, 2009

Means of Taking Wildlife: Mist nets

Disposition of Wildlife: University of Western Ontario

Conditions of Authorization:

Permittee and all subpermittees must have pre-exposure rabies prophylaxis (vaccination) previous to conducting any activities under this permit.

May capture and retain up to 15 female and 15 male specimens of *Lasiurus cinereus* (hoary bat), from the Sandia and Manzano Mountains (Sandoval, Bernalillo, Valencia and Torrance Counties) for laboratory analysis.

Within Sandia and Manzano Mountains, from above mentioned counties, Permittee may capture, collect (75-150 ml) blood samples from and release up to 100 *Lasiurus cinereus* (hoary bat) and up to 100 *Lasionycteris noctivagans* (silver-haired bat).

May possess and transport specimens within the state. The State of New Mexico cannot authorize permission to possess or transport specimens outside of New Mexico. Permission to possess specimens in other states or to transport specimens to other states must be obtained from the affected states.

This permit does not authorize activity on private land without consent of the landowner and does not apply to tribal or other lands where the New Mexico Department of Game and Fish does not have jurisdiction.

Any state protected species captured will be reported to the NMDGF Conservation Services Division (505-476-8101) within 24 hours of capture.

This permit does not authorize activity on private land without consent of the landowner and does not apply to tribal or other lands where the New Mexico Department of Game and Fish does not have jurisdiction.

Collection or possession of any federally protected species requires authorization from the U. S. Fish and Wildlife Service.

Permittee must obtain proper permits from the Canadian Food Inspection Agency for transport of specimens from the United States across the Canadian border.

An annual report summarizing the number of specimens of each species handled during the previous calendar year, geographic location (UTMs preferred) and date of collection, and the disposition of those animals, shall be submitted to the NMDGF's Conservation Services Division by January 31st following each calendar year for which the permit is in place.

[redacted]

Signature of Permittee
[Sign and retain in your possession]

signature: [redacted] *date:* 05-18-2007

Director, Department of Game and Fish
P.O. Box 25112, Santa Fe, NM 87504 USA

Note: Banding, collecting, and/or salvage of migratory birds require additional authorization from the U. S. Fish and Wildlife Service (USFWS). If your activities involve the taking of federally protected species, your state permit is not valid without the appropriate authorization from the USFWS. It is your responsibility to obtain the appropriate authorization from the USFWS.

United States Department of the Interior

United States Geological Survey

Fort Collins Science Center

[redacted]

MEMO

DATE: 30 April 2009

TO: Files, USDA Special Use Permit SND502 File

FROM: Paul Cryan, Research Biologist [redacted]

RE: Designation of Subpermittees

I designate the following individuals as subpermittees for activities involving bat research in New Mexico, as described in USDA Special Use Permit SND502 and amendments.

Ernest Valdez
Liam McGuire
Gabriel Reyes

Canadian Food Inspection Agency
Goverment of Canada

Agence canadienne d'inspection des aliments
Gouvernement du Canada

Permit No./N° de permis:
A-2009-01022-3
ORIGINAL
2009/03/18
year/mo/day
année/mois/jour

IMPORT PERMIT PERMIS D'IMPORTATION

Page 1 of/de 5

THIS PERMIT IS ISSUED PURSUANT TO:/CE PERMIS EST DÉLIVRÉ CONFORMÉMENT A:

THE HEALTH OF ANIMALS ACT AND REGULATIONS/LOI ET RÈGLEMENT SUR LA SANTÉ DES ANIMAUX

Importer/Importateur
UNIVERSITY OF WESTERN ONTARIO
[redacted]
Applicant Name: LIAM MCGUIRE
Phone: [redacted]

Exporter/Exportateur
LIAM MCGUIRE
[redacted]

Quarantine/Destination/Quarantaine
UNIVERSITY OF WESTERN ONTARIO
[redacted]

Producer/Producteur
SAMPLES COLLECTED IN THE FIELD

NEW MEXICO
UNITED STATES

Valid/Valide from/du 2009/03/18 year/month/day année/mois/jour **to/au** 2009/06/18 year/month/day année/mois/jour

Country of Origin/ Pays d'Origine UNITED STATES (NEW MEXICO)

For the entry of/ Pour l'entrée de: XX Single shipment/Chargement simple ____ Multiple shipments/Chargements multiples

Place of entry into Canada/Lieu d'entrée au Canada:
ALL REGULATED PORTS

FOR THE IMPORTATION OF:/POUR L'IMPORTATION DE:
(Description of things(s)/Description de la ou des choses)
1. Product Description: 230 FROZEN BAT SAMPLES FOR RESEARCH PURPOSES (30 HOARY BATS - WHOLE CARCASS, 100 HOARY BATS - BLOOD PLASMA SAMPLES, 100 SILVER-HAIRED BATS - BLOOD PLASMA SAMPLES) Treatment Description: ALL BATS WILL BE WILD CAUGHT IN NEW MEXICO

A PERSON WHO IMPORTS A THING UNDER THIS PERMIT SHALL COMPLY WITH ALL THE CONDITIONS SET OUT HEREIN/TOUTE PERSONNE QUI IMPORTE UNE CHOSE EN VERTU DE CE PERMIS DEVRA RESPECTER TOUTES LES CONDITIONS DÉCRITES CI-DESSOUS

Selected Conditions / Conditions Choisies

230 FROZEN BAT SAMPLES FOR RESEARCH PURPOSES (30 HOARY BATS - WHOLE CARCASS, 100 HOARY BATS - BLOOD PLASMA SAMPLES, 100 SILVER-HAIRED BATS - BLOOD PLASMA SAMPLES)

1. The original of this permit and any other necessary export documentation pertaining to the shipment must be provided for inspection at the first port of entry or to a Canadian Food Inspection Agency Import Service Center.

2. The conditions in this permit can only be changed or amended by a CFIA inspector. Any change to the permit by an unauthorized person will render the permit invalid.

3. Accompanying export documentation must be issued in either English or French.

4. The animal(s), germplasm or thing(s) described on this permit must be shipped by the most direct and appropriate route from the point of export to the

APR-30-2009 12:01 MNR PLANNING PTBO 705 755 3292 P.003

Ontario

Ministry of Natural Resources

Ministère des Richesses naturelles

Wildlife Scientific Collector's Authorization

Autorisation pour faire la collecte scientifique d'animaux sauvages

Authorization No. / N° d'autorisation	1050823
Local Reference No. / N° de référence local	
Issuer Account No. / N° de compte du délivreur de permis	10002364

This authorization is issued under Section 39 of the Fish and Wildlife Conservation Act, 1997 to:
Cette autorisation est délivrée en vertu de l'article 39 de la Loi sur la protection du poisson et de la faune de 1997 à:

	Last Name / Nom de famille	First Name / Prénom	Middle Name / Second Prénom
Name of Authorization holder / *Nom du titulaire de l'autorisation*	Mr. Fenton	Brock	M

Name of Business/Organization/Affiliation (if applicable) / Nom de l'entreprise/de l'organisme/de l'affiliation (le cas échéant)

University of Western Ontario, Dept. of Biology

	Street Name & No./PO Box/RR#/Gen. Del./N° rue/C.P./R.R./poste restante		
Mailing address of Authorization holder / *Adresse postale du titulaire de l'autorisation*	[redacted]		
	City/Town/Municipality / Ville/village/municipalité	Province/State / Province/Etat	Postal Code/Zip Code / Code Postal/Zip
	London	ON	N6A 5B7

This authorization permits the above-named person to:
Cette autorisation permet à la personne nommée ci-haut de:

☐ **Capture wildlife of the species and sex, in the numbers, and in the area set out be below.**
Capturer les espèces d'animaux sauvages selon le nombre et le sexe indiqués ci-dessous dans les lieux indiqués ci-dessous

and/or / et/ou

☐ Keep game wildlife or specially protected wildlife in captivity for the purposes of education or science.
Garder des animaux sauvages spécialement protégés et du gibier sauvage en captivité à des fins éducatives et scientifiques

☐ Release the captured wildlife in the area of capture, if the captured wildlife is not to be removed from that area
Remettre en liberté les animaux sauvages capturés dans la zone de capture si les animaux capturés ne doivent pas être enlevés de cette zone

OR / OU

☒ **Capture and kill wildlife of the species and sex, in the numbers, and in the area set out be below.**
Capturer et tuer les espèces d'animaux sauvages selon le nombre et le sexe indiqués ci-dessous dans les lieux indiqués ci-dessous

Species / Espocés	Sex / Sexe	Numbers / Nombre	Location / Endroit
See Attached			

Yes/Oui ☒ Additional list attached / Liste additionnelle ci-jointe

Authorization Dates / *Dates d'autorisation*	Effective Date / Date d'entrée en vigueur (YYYY-MM-DD)	Expiry Date / Date d'expiration (YYYY-MM-DD)
	2009-03-20	2010-03-20

Authorization conditions / **Conditions de l'autorisation**: This authorization is subject to the conditions contained in Schedule A if included. / Cette autorisation doit respecter les conditions de l'annexe A si celle-ci est jointe.

Yes/Oui ☒ No/Non ☐ Schedule A included. / Annexe A ci-jointe

Authorized by (please print) / Autorisé par (veuillez écrire en caractères d'imprimerie)	Signature of Authorizer / Signature de la personne chargée d'autoriser	Date of Issue/Date de délivrance (YYYY-MM-DD)
Monique Rolf von den Baumen-C[redacted]	[redacted]	2009-03-20
Signature of Authorization holder / Signature du ti[redacted]		Date (YYYY-MM-DD) 2009-03-20

Personal information contained on this form is collected under the authority of the Fish and Wildlife Conservation Act, 1997 and will be used for the purpose of licencing, identification, enforcement, resource management customer service surveys. Please direct further inquiries to the District Manager of the MNR issuing district.

Les renseignements personnels dans ce formulaire sont recueillis conformément à la Loi sur la protection du poisson de la faune, 1997 , et ils seront utilisés aux fins de délivrance du permis, d'identification, d'application règlements, de gestion des ressources et de sondage sur les services à la clientèle. Veuillez communiquer avec le chef du district du MRN qui délivre le permis si vous avez des questions.

FW2018 (04/00)

Curriculum Vitae

NAME: Liam P. McGuire

POST-SECONDARY EDUCATION AND DEGREES:

Carleton University
Ottawa, Ontario, Canada
2000-2005 B.Sc. in Biology, minor in Statistics

The University of Western Ontario
London, Ontario, Canada
2006-2012 Ph.D.

HONOURS AND AWARDS:

Natural Sciences and Engineering Research Council
Canada Graduate Scholarship. 2009-2012.

Ruth Horner Arnold Fellowship. 2011.

UWO Department of Biology Graduate Student Teaching Award. 2011.

Titley Electronics Award for outstanding student platform presentation, North American Society for Bat Research. 2010.

UWO Biology Graduate Travel Award. 2010.

Robert and Ruth Lumsden Graduate Award. 2009.

Ontario Ministry of Natural Resources research grant. 2009.

Dr. Irene Uchida Fellowship in Life Sciences. 2008.

Bat Conservation International
Student Research Scholarship. 2008.

Society for Integrative and Comparative Biology
Fellowship of Graduate Student Travel. 2008.

UWO Graduate Thesis Research Award. 2008.

UWO Faculty of Science Graduate Student Teaching Award. 2008.

RELATED WORK EXPERIENCE:

Teaching Assistant
The University of Western Ontario
2006-2012.

Research Associate
Iogen Corporation
2005-2006.

Laboratory Technician
Iogen Corporation
2003-2005.

Publications:

Fraser, E.E., **L.P. McGuire**, J.L. Eger, F.J. Longstaffe, and M.B. Fenton. 2012. Evidence of latitudinal migration in tri-colored bats, *Perimyotis subflavus*. *PLoS One*. 7: e31419.

McGuire, L.P., C.G. Guglielmo, S.A. Mackenzie, and P.D. Taylor. 2012. Migratory stopover in the long-distance migrant silver-haired bat, *Lasionycteris noctivagans*. *Journal of Animal Ecology*. 81: 377 – 385.

Taylor, P., S.A. Mackenzie, B.G. Thurber, A.M. Calvert, A.M. Mills, **L.P. McGuire**, and C.G. Guglielmo. 2011. Landscape movements of migratory birds and bats reveal an expanded scale of stopover. *PLoS One*. 6: e27054.

Guglielmo, C.G., **L.P. McGuire**, A.R. Gerson, and C.L. Seewagen. 2011. Simple, rapid, and non-invasive measurement of fat, lean, and total water masses of live birds using quantitative magnetic resonance. *Journal of Ornithology*. 152: 75 – 85.

Fenton, M.B., M.D. Skowronski, **L.P. McGuire**, and P.A. Faure. 2011. Variation in the use of harmonics in the calls of laryngeally echolocating bats. *Acta Chiropterologica*. 13: 169 – 178.

Dzal, Y., **L.P. McGuire**, N. Veselka, and M.B. Fenton. 2011. Going, going, gone: the impact of white-nose syndrome on the summer activity of the little brown myotis (*Myotis lucifugus*). *Biology Letters*. 7: 392-394.

McGuire, L.P. and J.M. Ratcliffe. 2011. Light enough to travel: migratory bats have smaller brains, but not larger hippocampi, than sedentary bats. *Biology Letters*. 7: 233 – 236.

McGuire, L.P. and C.G. Guglielmo. 2010. Quantitative magnetic resonance: a rapid, non-invasive body composition analysis technique for live and salvaged bats. *Journal of Mammalogy*. 91: 1375 – 1380.

McGuire, L.P. and M.B. Fenton. 2010. Hitting the wall: Light affects the obstacle avoidance ability of free-flying little brown bats (Myotis lucifugus). *Acta Chiropterologica*. 12: 247 – 250.

McGuire, L.P. and C.G. Guglielmo. 2009. What can birds tell us about the migration physiology of bats? *Journal of Mammalogy*. 90: 1290 – 1297.

McGuire, L.P., M.B. Fenton, P.A. Faure, and C.G. Guglielmo. 2009. Determining feeding state and rate of mass change in insectivorous bats using plasma metabolite analysis. *Physiological and Biochemical Zoology*. 82: 812 - 818.

McGuire, L.P., M.B. Fenton, and C.G. Guglielmo. 2009. The effect of age on energy storage during pre-hibernation swarming in little brown bats (*Myotis lucifugus*). *Canadian Journal of Zoology*. 87: 515 - 519.

McGuire, L., H. Van Gossum, K. Beirinckx, and T.N. Sherratt. 2006. An empirical test of signal detection theory as it applies to Batesian mimicry. *Behavioural Processes*. 73: 299 – 307.