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Stable isotope analyses of bat fur: Applications for investigating North American bat migration

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Biology
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STABLE ISOTOPE ANALYSES OF BAT FUR: APPLICATIONS FOR INVESTIGATING NORTH
AMERICAN BAT MIGRATION

(Spine title: Stable isotopes in bat fur)

(Thesis format: Integrated Article)

by

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Graduate Program in Biology

A thesis submitted in partial fulfillment
of the requirements for the degree of
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The School of Graduate and Postdoctoral Studies
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THE UNIVERSITY OF WESTERN ONTARIO
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Erin Elizabeth Fraser

entitled:

**Stable isotope analyses of bat fur: Applications for investigating North
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is accepted in partial fulfillment of the
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Abstract

Most North American bats undertake seasonal migrations and many aspects of bat migration are not well documented. Stable isotope analyses of animal tissues can elucidate migratory origin, but this technique has not been widely applied to bats. The objectives of this dissertation were i) to investigate North American bat migration using stable isotope analyses of fur and ii) to highlight some of the strengths and weaknesses of this analytical technique when applied to bat systems. I conducted stable hydrogen (δD), carbon (δ^{13}C), and nitrogen (δ^{15}N) isotope analyses on fur samples from five bat species.

First, I documented δD$_{fur}$ heterogeneity in summer resident populations of little brown bats (*Myotis lucifugus*), eastern red bats (*Lasiurus borealis*), hoary bats (*Lasiurus cinereus*) and silver-haired bats (*Lasionycteris noctivagans*). In some species, δD$_{fur}$ composition varied systematically within and among individual bats, age groups, species, sites, and over time. Future investigations should standardize fur collection according to the above variables and data from multiple species should not be pooled. Bats from proximate colonies can be discriminated using multiple stable isotopes and stable isotope correlations existed in some species. Multi-isotope studies may be used to detect population mixing.

Second, I investigated the origins of fall migrant *L. noctivagans* and *L. borealis* passing through an Ontario migration monitoring station. There was no stable isotope

evidence that migrant *L. noctivagans* originated from a wide range of latitudes, or that latitudinal origin varied with time or migrant "wave". *Lasiurus borealis* stable isotope results were variable between years and further work is required before stable isotope results can be used to draw ecological conclusions about this species.

Third, I investigated the annual migratory movements of tri-colored bats (*Perimyotis subflavus*), a presumed regional migrant. Stable hydrogen isotope results indicated that >30% of males migrated south in the fall. Only 16% of females migrated south and their movements were shorter than the male movements. Most of the migrants were captured at the northern and southern extremes of the species' range. I concluded that these bats engage in a pattern of partial and differential latitudinal migration that has previously not been described for this species.

Keywords

Bats, Carbon, Fur, Hydrogen, *Lasionycteris noctivagans, Lasiurus borealis, Lasiurus cinereus,* Migration, *Myotis lucifugus,* Nitrogen, North America, *Perimyotis subflavus,* Stable isotope analysis, Variation

Co-Authorship Statement

1) Dr. M. Brock Fenton (Professor emeritus, Department of Biology, The University of Western Ontario) – Acted as primary supervisor and provided support in project design, execution, and funding.

2) Dr. Fred J. Longstaffe (Distinguished Research Professor, Department of Earth Sciences, The University of Western Ontario) – Acted as joint supervisor and provided support in project design, execution, and funding.

3) Liam McGuire (PhD student, Department of Biology) – Collected fur samples for Chapters 2 and 3 and provided project planning and statistical support for Chapter 4.

4) Dr. Judith Eger (Mammals curator, Royal Ontario Museum) – Provided support and advice in planning and obtaining fur samples for Chapter 4.

5) Johnston Miller (University of Western Ontario) – Provided field and logistical support for Chapter 2.

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Chapter 1

1   Introduction

Stable isotope analysis has become a widely used and effective tool for researching animal migration (see Hobson and Wassenaar [2008] for a complete review), although to date it has been used infrequently for this purpose on bats (but see Britzke *et al.* 2009; Cryan *et al.* 2004; Fleming *et al*. 1993; Fraser *et al.* 2010). Bat migration is not as well understood as bird migration (Holland and Wikelski 2009; McGuire and Guglielmo 2009), but has received increased attention in recent years (Popa-Lisseanu and Voigt 2009). High bat mortality at some wind energy facilities during late summer and fall migration (hereafter fall migration) has resulted in an urgent need to learn more about the ecology of North American bat migration to inform management decisions.   Given the challenges inherent in successfully using established migration monitoring techniques on bats (Holland *et al.* 2007; Holland and Wikelski 2009), stable isotope analysis has great promise for answering some fundamental questions about bat migration (Cryan and Diehl 2009).

The over-arching goal of my dissertation is to use stable isotope analysis to investigate North American bat migration. In particular, I have studied fur samples from five species of North American insectivorous bats from multiple locations across North America. These include three species of latitudinal migrants (hoary bats [*Lasiurus cinereus*]; eastern red bats [*Lasiurus borealis*]; and silver-haired bats [*Lasionycteris noctivagans*]) and two species of presumed regional migrants (little brown bats [*Myotis*

lucifugus] and tri-colored bats [*Perimyotis subflavus*]). The work includes four main contributions: (i) a comprehensive study of stable hydrogen isotope heterogeneity in the fur of summer resident bat populations to supplement the existing body of knowledge about stable hydrogen isotope variation in other animal groups (chapter 2); (ii) an examination of the origins of migratory silver-haired bats (*Lasionycteris noctivagans*) and eastern red bats (*Lasiurus borealis*) passing through a migration monitoring station (Long Point, Ontario) over the period of fall migration (chapter 3); (iii) a continental-scale examination of the annual movements of tri-colored bats *(Perimyotis subflavus*) (chapter 4); and (iv) the development of fur standards with known non-exchangable stable hydrogen isotope compositions for use in conducting stable hydrogen isotope analyses of fur samples (appendix A).

In this chapter (chapter 1), I provide the rationale for my work by briefly describing the state of knowledge about North American bat migration and the current threats to bats that make migration studies increasingly important. I also present a summary of the uses of stable isotope analysis for studying migration. I discuss the necessary considerations when choosing a tissue type for stable isotope analysis and justify my use of bat fur. Finally, I describe the objectives of the three data chapters of this dissertation.

1.1   Bats and migration

Bat migration in North America is widespread among species and in many ways, only partially documented. All of the bats investigated in this dissertation reproduce during

the summer months and during this time, are believed to be sedentary. Throughout this work, I will refer to these bats as "summer residents". There is evidence to suggest that the majority of North American bat species are migratory to some extent, and several distinct migratory life history strategies have been reported (e.g., Fleming and Eby 2003). However, migratory timing, routes, and connectivity are not well understood for most North American bat species. There is evidence that some species migrate across latitudes, travelling south in the autumn and north in the spring (Cryan 2003; Cryan *et al.* 2004; Findley and Jones 1964). Although there are no direct measurements of migratory distance, stable isotope results suggest that some latitudinal migrants make continental scale flights that can exceed 2000 km and span much of North America (Cryan *et al.* 2004). Other species are considered regional migrants and have been documented moving from summer residency sites to autumnal swarming sites and winter hibernacula over distances exceeding 500 km (Dubois and Monson 2007; Fenton 1969). Bats engaging in regional migration have been documented migrating in all directions, not just north and south (Davis and Hitchcock 1965). Some species are considered sedentary, showing little evidence of substantial annual movement between summer resident colonies and winter hibernacula (Fleming and Eby 2003).

In many cases, variation in bat density both temporally (e.g., Dzal *et al.* 2009; Furmankiewicz and Kucharska 2009; Ibáñez *et al.* 2009; Reynolds 2006) and spatially (e.g., Baerwald and Barclay 2009; Hooton 2010) provide the sole evidence of bat migration and migratory routes across the landscape. Variation in bat density can be

detected based on changes in capture success and/or bat acoustic activity and more recently, seasonal peaks in bat mortality at some wind energy facilities (Arnett *et al*. 2008). Cryan (2003) identified broad trends in the latitudinal migrations of several species of bats based on the locality of museum specimens collected at various times throughout the year. Comparisons of bat activity across time and space can provide valuable information about migratory routes (Baerwald and Barclay 2009; Furmankiewicz and Kucharska 2009; Dzal *et al.* 2009; Hooton 2010; McGuire *et al.* 2011), but unless coordinated across a range of locations (e.g. Cryan 2003), they do not provide information about the geographical extent of migration. To develop a fuller picture of bat migration, it is necessary to track the movements of individual bats.

Methods for monitoring individual movement of animals include tracking with transmitters, mark-recapture studies, and the use of intrinsic markers such as stable isotopes and trace elements to collect proxy evidence about earlier locations. Current limitations on transmitter size make satellite tracking unfeasible for North American insectivorous bat species (Wikelski *et al.* 2007). Mark-recapture methods have provided valuable information about the movements of many species of bats (e.g., Ellison 2008; Hutterer *et al.* 2005; Thomas 1983) and band recoveries have been particularly effective in allowing links to be made among summer colonies, autumn swarming sites, and winter hibernacula of regional migrants (Davis and Hitchcock 1965; Dubois and Monson 2007; Fenton 1969; Rivers *et al.* 2006; Rodrigues and Palmeirim 2007). However, it is difficult to capture many of the North American latitudinal migrants once (never mind

twice) and there is an enormous area over which they are likely migrating. The lack of a

coordinated, centralized banding organization in North America has made mark-

recapture studies of many of these species unrealistic (Cryan and Veilleux 2007; Holland

and Wikelski 2009). To date, stable isotope analysis has been used infrequently, but

effectively, as a tool to address targeted questions about migration in several species of

bats (Cryan *et al.* 2004; Fleming *et al.* 1993).

1.2   Threats to bats

1.2.1   Wind energy facility associated mortalities

In recent years, wind energy facility associated bat mortalities have emerged as a

threat to bat populations. In 2004 Kerns and Kerlinger reported substantial bat mortality

around some wind turbines at the Mountaineer Wind Energy Centre (West Virginia,

USA), where the results of mortality surveys indicated that more than 2000 bats were

killed during a short period in August and September around 44 turbines. Since then,

substantial research effort has gone into documenting and studying the incidence of bat

mortality around wind turbines (much of which is summarized by Arnett *et al.* (2008)

and Cryan and Barclay [2009]) as these installations become increasingly common across

the North American landscape.

The majority of bat mortality occurs during the presumed fall migratory period (late

August and September), and while the composition of affected species varies among

locations, latitudinal migrants are the most affected, including hoary bats (*Lasiurus

cinereus*), silver-haired bats (*Lasionycteris noctivagans*) and eastern red bats (*Lasiurus

borealis) (Arnett *et al.* 2008). Bats are killed both by physical collision with turbine blades as well as by pulmonary barotrauma as a result of rapid air pressure change near moving turbine blades (Baerwald *et al.* 2008). Further, there is evidence that taller turbines pose a greater threat (Barclay *et al.* 2007).

Across North America, management decisions are required to mitigate bat mortality around wind energy facilities. Experimental results indicate that increasing the windspeed at which turbines are operational can decrease bat mortality by as much as 60% (Arnett *et al.* 2010; Baerwald *et al.* 2009). Further, there is some evidence that geographical features, such as the edge of the Canadian Rocky mountain range (Baerwald and Barclay 2009) are higher risk areas, perhaps because they are being used as flight corridors for migrating bats. Results such as these provide a basis for siting wind power installations, and these excellent studies represent huge strides in the collective knowledge about bats and wind energy. However, there are still many gaps in the literature about the basic bat life history, including migration, which make bat management challenging. A more thorough knowledge of bat migratory phenology and routes would be very useful in making informed decisions for bat management.

1.2.2   *White nose syndrome*

White nose syndrome (WNS), a contagious fungal infection (Blehert *et al.* 2009) experienced by bats during hibernation, was first discovered in upper New York State in 2006 and has since spread across eastern North America. It is not unusual for hibernating colonies to experience 99% mortality within three years of being exposed,

and there are serious concerns about the long-term viability of some previously common species, such as little brown bats (*Myotis lucifugus*) (Frick *et al.* 2010). A coordinated action plan exists in the United States to deal with this epizootic, and the top research priorities address the mechanics of White Nose Syndrome, including diagnostics, etiology and epidemiology, and disease management and surveillance; as well as a focus on the development of a conservation strategy for affected North American bats (US Fish and Wildlife Service 2011). Bats travelling among swarming sites and hibernacula may be a main cause of fungal dispersal. A better understanding of bat movement among swarming sites and hibernacula may be helpful in addressing this threat.

1.3   Intrinsic markers – stable isotope analysis

1.3.1   *Stable isotopes and animal ecology*

The fundamental theory of using stable isotope analyses to study animal ecology is that the ratio of heavy:light stable isotopes (usually one or several of the elements carbon, nitrogen, oxygen, hydrogen, and sulphur, reported as δ values in per mille [‰]) vary within the environment in a predictable way, often according to biogeochemical processes. Animals incorporate these stable isotope compositions into their own tissues (usually offset by a discrimination factor) as a result of consuming local food and water. Subsequent stable isotope analyses of these tissues can provide information about what the animal has been eating or where it has been in the past. Tissue stable isotope

compositions provide intrinsic markers of animal life history, allowing researchers to learn about an individual animal's past while only catching it once.

Wassenaar (2008) lists the two prerequisites for using stable isotope analysis as a tool to learn about animal movement: (i) there is a measurable amount of the element of interest in the tissue sampled, and (ii) the animal moves among areas that are isotopically distinct. In order to interpret tissue stable isotope results, it is further necessary to know when the tissue of interest is replaced or at what rate it turns over in order to establish the time period you are learning about. There can also be inter- and intra-sample isotopic heterogeneity (Wassenaar 2008), both within and among samples taken from one individual (e.g., Smith *et al.* 2008) as well as among samples taken from multiple individuals at the same location (e.g., Langin *et al.* 2007).

Animals incorporate the stable isotopes in their environment into their tissues through diet and drinking water. Insectivorous bats consume a tremendous diversity of insects (e.g., Clare *et al.* 2009), which occupy a wide range of habitats, dietary niches and trophic levels. Bat diets vary among species (*e.g.,* Reimer *et al.* 2010), between adults and volant subadults (*e.g.,* Adams 1996; Belwood and Fenton 1976), and may change over the course of the summer as different insects emerge (Clare *et al.* 2011). There are fewer data on bat drinking habits, although much anecdotal evidence and some published research report that most species consume water by dipping over nearby water bodies (Adams and Simmons 2002; Kurta and Teramino 1992). Bats also

get water from their food and some species, when provided with food, can survive for

extended periods of time without drinking water (Geluso 1978).

Stable hydrogen and oxygen isotope analyses are the most common stable isotope

approaches for investigating global scale animal movements (Wassenaar 2008),

although strontium isotope analysis also has great promise in this area (e.g., Sellick *et al.*

2009). Stable carbon, nitrogen, and sulphur isotope analyses can be used as indicators of

local spatial-scale movement (Wassenaar 2008).

1.3.2   *Stable hydrogen isotope analysis*

Stable hydrogen isotope compositions are reported as the ratio of the heavy

isotope (deuterium; D) to the light isotope (hydrogen) and are written using the notation

δD. The effectiveness of stable hydrogen isotope analysis as an indicator of continental

scale movement is linked to the presence of predictable patterns in the stable hydrogen

isotope compositions of meteoric water (δD_{precip}) across large distances. This is

particularly effective in North America, where generally south to north patterns in the

movement of atmospheric moisture masses result in an approximately latitudinal

gradient in δD_{precip} values, with declining sample enrichment in D with increasing

latitude. The δD_{precip} values also vary predictably with other topographical features, such

as mountain ranges and distance from the coast (Bowen and Revenaugh 2003).

Animals consuming food and water at a given location should, in theory, acquire

δD values indicative of that location into their own tissues (δD_{tissue}), making them an

isotopic reflection of their local environment (Hobson [2008] provides a thorough summary of animal δD_{tissue} values across latitudes varying predictably with latitude and or δD_{precip}). However, the relationship between δD_{tissue} and mean growing season δD_{precip} is stronger for some species than others, and variation does exist among bat species (e.g. Britzke *et al.* 2009). A number of factors may lead to deviation of δD_{tissue} values from the predicted values based on δD_{precip}. Hydrogen is incorporated into animal tissue not only from drinking water but also from food (Ehleringer *et al.* 2008; Hobson 1999). Consuming food with δD values that differ substantially from those of local meteoric water could result in a consumer having δD_{tissue} values that differ from what is expected.

To understand the role of diet in determining the δD_{tissue} values of insectivorous bats, we must consider what is known about stable hydrogen isotope compositions in insects. While insect δD values have been shown to vary predictably at a continental scale with local δD_{precip} values (Gröcke *et al.* 2006; Hobson *et al.* 1999), insect δD variability has not been well-documented at the community level. There appears to be substantial stable hydrogen isotope variation among local insects: Langin *et al.* (2007) found that the δD values of ten whole insects (orders Diptera and Lepidoptera) captured at one location in southeastern Ontario varied by more than 70‰. Britzke *et al.* (2009) explain that the weak relationship found between the δD_{fur} values of *Myotis lucifugus* and mean growing season δD_{precip} values was due to this species' tendency to forage over standing water, which may differ isotopically from local meteoric water. Physiological factors may also impact how dietary hydrogen is incorporated into animal

tissues. Several studies have shown that tissue δD values increase when moving up the food chain because of a trophic enrichment effect (Birchall *et al.* 2005; Bowen *et al.* 2009; Reynard and Hedges 2008). Further, McKechnie *et al.* (2004) showed that the D enrichment of Rock dove (*Columba livia*) body water compared to drinking water was positively related to evaporative water loss, suggesting that water stress may impact δD_{tissue} values in other species.

In summary, there is strong evidence that δD_{tissue} values are broadly indicative of local growing season δD_{precip} values. However, a number of factors may blur this relationship, and the extent to which δD_{tissue} heterogeneity exists within a resident population may vary among species, age and sex classes, and even among samples from a single individual, depending on its life history during the time of tissue growth. There is a wealth of ecological knowledge about animal movements to be gained using stable hydrogen isotope analysis, and quantifying δD_{tissue} heterogeneity within a resident population is an important step for accurate interpretation of stable isotope data.

1.3.3 *Stable carbon and nitrogen isotope analyses*

Stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope analyses of animal tissues can be used to derive information about community food webs, specifically dietary source and trophic level (Kelly 2000). Generally, animal tissues are enriched in ^{15}N by 3-5‰ compared to average diet (DeNiro and Epstein 1981), resulting in a trophic effect, whereby animals at higher trophic levels have tissues with higher $\delta^{15}N$ values than those at lower trophic levels (Minagawa and Wada 1984, *cf*. Hobson *et al.* 1993). Studies have

repeatedly demonstrated that a variety of insects are enriched in ^{15}N compared to their diet (Menke *et al.* 2010; Oelbermann and Scheu 2010; Ostrom *et al.* 1997). Further, there is evidence that a stable nitrogen isotope trophic effect exists in natural insect food webs (Bennett and Hobson 2009), with carnivorous groups, such as odonates and some coleopterans having higher δ^{15}N values than completely herbivorous groups, such as orthopterans. Previous studies have shown that the δ^{15}N values of bat tissues reflect diet (offset by a fractionation factor) (Mirón *et al.* 2006; Voigt and Matt 2004), and this technique has been used to investigate the contribution of insects to the diets of several mainly phytophagous bat species (Herrera *et al.* 2001a,b).

While stable carbon isotope compositions often increase slightly with trophic level, δ^{13}C values are typically used to distinguish tissues formed from sources with distinct δ^{13}C signatures (for instance, C$_3$ or C$_4$ plant dominated diets; marine, aquatic, or terrestrial dominated diets) (Kelly 2000; Tieszen *et al.* 1983). Gratton and Forbes (2006) found that beetle δ^{13}C values changed markedly when the beetles were switched from a C$_3$ to C$_4$ diet and Bennett and Hobson (2009) found that insect δ^{13}C values were lower in aquatic insects than terrestrial ones. Stable carbon isotope analysis has also been used to draw conclusions about dietary sources in several species of bats, including common vampire bats (*Desmodus rotundus*) (Voigt and Kelm 2006) and several species of nectar-feeding bats (Fleming *et al.* 1993). Stable nitrogen and carbon isotope analyses of animal tissues can be used in combination to learn about both dietary trophic level and source (Urton and Hobson 2005). A combination approach has been used to study feeding

ecology and habitat use in a number of neotropical bats (Herrera *et al.* 2001a,b; York

and Billings 2009).

1.3.4   Stable isotope analysis and animal migration

Stable isotope analysis can be used to investigate animal migration. Hobson

(2008) describes two broad categories of research to date: studies that have used local-

scale differences in environmental stable isotope compositions to detect animal

movements among biomes (usually using stable carbon, nitrogen, and sulphur isotope

analyses) and those that have employed stable hydrogen isotope analysis to detect

continental scale movement. The main focus of this dissertation is on the use of stable

hydrogen isotope analysis to study bat migration across large distances, so for the most

part, I will focus on previous examples of this second application.

Perhaps the most common and informative application of stable hydrogen

isotope analysis for migratory studies is through the species-specific quantification of

the relationship between the stable hydrogen isotope compositions of animal tissues

and those of precipitation at the time of tissue growth (bat-specific examples include

Britzke *et al.* 2009; Cryan *et al.* 2004). This approach, in combination with an

understanding of natural isotopic and analytical variation (Wunder and Norris 2008), can

be used to assign unknown migrants to a putative area of origin. Using analyses of

multiple elements can increase assignment precision (e.g. Sellick *et al.* 2009), and while

stable isotope studies of migration frequently span large geographic areas, it can also be

possible to use multiple element stable isotope analyses and multivariate statistics to distinguish among relatively nearby populations (e.g. Torres-Dowdall *et al.* 2009).

In the absence of a well-defined relationship between δD_{precip} and δD_{tissue}, it is still possible to use relative measures to investigate some aspects of animal migration, including phenology, philopatry, and connectivity, using stable isotope analysis. For instance, Kelly *et al.* (2002) and Kelly (2006) measured feather stable hydrogen isotope compositions of wood warblers migrating through a site in the southern US and detected that feather stable isotope ratios changed systematically as the migratory season progressed. Based on these results, they concluded that migratory timing varied among populations originating from different latitudes. Møller and Hobson (2004) used a multi-element approach (hydrogen, carbon and nitrogen) and found a bimodal distribution in the stable carbon and nitrogen isotope compositions of winter-grown feathers in a summer population of Danish Barn Swallows. They used these results as evidence that summer individuals originated from two geographically separate African wintering populations.

A reference resident population can be helpful in study design: Hobson *et al.* (2004) found that feather stable hydrogen isotope variation among after-second-year warblers was less than that of second-year birds and concluded that older birds had relatively high breeding philopatry, compared to second-year birds, who dispersed widely from their nesting locations. Similarly, Møller *et al.* (2006) found a strong correlation between feather stable carbon and nitrogen isotope compositions in Barn

Swallow feathers at a summer breeding population and used the lack of this correlation at a nearby population to suggest that individuals at the second site were not returning to their site of birth (i.e. the second site was a population sink).

To date, only a few published studies have used stable isotope analysis to investigate bat migration. Cryan *et al.* (2004) used fur samples from hoary bat (*Lasiurus cinereus*) museum specimens to conclude that this species engages in annual long distance latitudinal migratory movements and Fleming *et al.* (1993) used stable carbon isotope analyses to detect dietary changes and thus migratory corridors for the nectarivorous species, *Leptonycteris curasoe.* Fraser *et al.* (2010) compared neotropical bat fur and claw stable hydrogen isotope compositions to detect altitudinal migration, but uncertainty about the incorporation of environmental stable isotopes into hair and claw tissues precluded any conclusions about bat behaviour. Britzke *et al.* (2009) analyzed summer fur samples from four species of North American bats collected across most of Eastern North America and demonstrated a relationship between fur stable hydrogen isotope compositions and both latitude and mean growing season δD values for all of these species.

1.4   Tissue choice for stable isotope analysis

Stable isotope analyses of animal tissues provides information about an animal's life during tissue growth. Some tissues, such as teeth, hair/fur and nails are metabolically inactive; that is, once the tissue is formed, its composition is fixed and stable isotope analysis will provide information about the period of time when it was

grown (Voigt *et al.* 2003). Other tissues, such as blood, muscle, organs, etc., are metabolically active and are constantly being replaced. The time period represented by stable isotope analysis of metabolically active tissues varies depending on the rate of tissue replacement (turnover) and the species being studied. Among birds, stable carbon isotope turnover rates in various tissues range from several days for blood plasma (Hobson and Clark 1993) to several months for bone collagen (Hobson and Clark 1992).

The timing of tissue growth combines with a host of other important considerations, such as the logistics of tissue collection, storage and analysis, to make the choice of tissue an important decision. Other researchers have conducted stable isotope analyses on a wide variety of tissues, including whole blood (Mirón *et al.* 2006), blood plasma (Jenkins *et al.* 2001), the cellular fraction of blood (Mazerolle and Hobson 2005), muscle (Lohuis *et al.* 2007), liver (Wolf and Martinez del Rio 2000), wing membrane (Voigt and Kelm 2006), breath (Voigt *et al.* 2008), milk (Miller *et al.* 2011), bone collagen (Cormie *et al.* 1994), teeth (Metcalfe *et al.* 2010), feathers (Langin *et al.* 2007), hair/fur (Cryan *et al.* 2004), nails (Fraser *et al.* 2010), stomach contents (Hwang *et al.* 2007) and fecal matter (Des Marais *et al.* 1980). Migration studies using stable isotope techniques are frequently conducted using keratinous tissues, such as feathers, fur or claws (*e.g.* Clark *et al.* 2006), although some researchers have used metabolically active tissues (*e.g.* Marra *et al.* 1998).

All of the analyses reported in this dissertation were conducted on bat fur samples. I selected fur as the tissue for analysis for four reasons. First, it is consistent

with the work that has already been done in this field. The majority of stable hydrogen

isotope studies investigating bird migration analyze feathers, a keratinous tissue to

which fur is the best mammalian analogue. All of the current published literature using

stable hydrogen isotope analysis to investigate bat migration has used fur (Cryan *et al.*

2004; Britzke *et al.* 2009; Fraser *et al.* 2010), and several other bat researchers are

currently using fur for this purpose. A key contribution of my dissertation is to describe

natural variation in wild populations of resident bats to provide baseline data for future

research, so it is logical to use the most frequently analyzed tissue type. Previous

research indicates that temperate bats moult once annually during the summer (Cryan

et al. 2004; Quay 1970; Tiunov and Makarikova 2007), and analyses of bat fur should

provide information about the bat's life during the previous summer.

A second reason to use fur for stable isotope analyses is that turnover rates in

metabolically active bat tissues are not well understood. Currently, no studies exist

examining stable hydrogen isotope turnover in bat tissues. However, several lab studies

investigated blood carbon turnover rates in nectarivorous bats and found widely varying

results. Mirón *et al.* (2006) estimated a carbon half life in *Glossophaga soricina* whole

blood of approximately 19 to 44 days and a nitrogen half life of approximately 20 to 34

days; Voigt *et al.* (2003) reported blood carbon turnover values of 120 to 126 days in

both *G. soricina* and *Leptonycteris curasoe*; and Voigt and Matt (2004) estimated blood

nitrogen turnover values of 274 days in *G. soricina* and 514 days in *L. curasoe*. Mirón *et*

al. (2006) cited differences in diet as the main causal factor in these widely varying

estimates. They showed that bats fed a diet with higher nitrogen content had shorter

blood carbon turnover times than those fed a diet with lower nitrogen content. Voigt *et al.* (2003) fed their bats a diet containing low amounts of nitrogen and protein and

reported much longer carbon turnover times. A further conclusion by Mirón *et al.* (2006)

was that turnover rates of individual elements within tissues (in this case, carbon and

nitrogen) are not necessarily synchronous. Voigt *et al.* (2003) also suggested torpor use

and nutritional stress as potential factors in determining tissue half life.

Although turnover rate estimates for bat tissue hydrogen do not exist, the

aforementioned results for carbon and nitrogen suggest that turnover rates in

metabolically active bat tissues are plastic and highly related to individual-specific

variation in life history. Uncertainty about the timing of tissue turnover poses difficulties

to research projects aiming to use stable isotope analysis to learn about migratory origin

within a specific timeframe and is an argument for selecting non-metabolically active

tissues for analysis. Inert, non-lethal tissues that can be sampled from bats are limited to

claws and fur. Bats use their hind claws during roosting and sampling enough of this

tissue to conduct stable isotope analyses could have a negative effect on the bat's

roosting abilities. Further, recent evidence suggests that claw keratin is not formed in a

linear fashion from the base of the claw to the tip in some mammal species, but rather

that the claw tip has both old and new tissue, while the lateral walls may present linear

time-series data (Ethier *et al.* 2010). These results indicate that sampling from the claw

tip may in fact present an integrated sample of the bat's life over an extended period of

time. In contrast, enough fur for several isotope analyses can easily be sampled from a live bat with little potential for adverse effects on the bat's health.

The third and fourth reasons for selecting fur as the tissue to analyze are logistical, both from the perspectives of sample collection and analysis. In terms of sample acquisition, fur is much easier to collect, store and ship than metabolically active tissues, which must be frozen for storage and dried prior to analysis. Many migration studies employing stable hydrogen isotope analysis take place across a large geographic scale, requiring cooperation and sample collection from a large and varied team of researchers (e.g. Britzke *et al.* 2009; Cryan *et al.* 2004). It is sensible to concentrate efforts on a tissue that can be easily and non-lethally sampled, and that can be stored at ambient temperature.

In terms of laboratory analysis, tissue choice for stable hydrogen isotope analysis is further complicated by analytical considerations. In order to account for the presence of exchangeable hydrogen in complex organic tissues (for more detail, see appendix A), it is necessary to use tissue-specific standards to calibrate raw sample results to an internationally accepted standard value (Vienna Standard Mean Ocean Water, VSMOW) (Wassenaar and Hobson 2003). Tissue standards are not widely available and must be experimentally developed on a case by case basis in individual laboratories. The experimental procedure for developing these standards requires some assumptions about the chemical and physical properties of the samples and these assumptions are better understood for some materials (such as keratin) than others (Schimmelmann

1991; Schimmelmann *et al.* 1999). The use of non-tissue specific standards increases the potential for error in determining final stable hydrogen isotope results (Chesson *et al.* 2009).

1.5   Dissertation structure

The three data chapters of this thesis are separate research projects, each intended for publication and united by the common theme of investigating bat migration using stable isotope analysis. In addition to these three data chapters, I have included an appendix (A), which details fur standard development and the determination of the non-exchangeble stable hydrogen isotope compositions of these standards. Below, I provide a brief outline of the components of my dissertation.

First, stable isotope results from animal tissue provide a proxy for aspects of individual life history. To most accurately interpret these results, it is important to have a thorough understanding of how environmental stable isotopes are reflected in animal tissue and the amount of natural isotopic variation that exists. I have contributed to this body of knowledge by documenting stable isotope variation in resident populations of four species of insectivorous bats (Chapter 2). Here I have addressed the effects of the location of fur sampling, bat age, bat species, sampling time, and geographic location on stable hydrogen isotope heterogeneity in resident bat fur. Further, I investigated the relationships among the stable hydrogen, nitrogen, and carbon isotope compositions of fur samples taken from individual bats within a resident population, as well as the

efficacy of a combined stable isotope approach for separating bats of the same species from relatively nearby roost locations.

Second, I investigated the origins of fall migrant silver-haired bats (*Lasionycteris noctivagans*) and eastern red bats (*Lasiurus borealis*) passing through Long Point, Ontario, a well-documented stopover site for a variety of migratory animals (Chapter 3). Using the stable hydrogen, carbon, and nitrogen isotope compositions of migratory bat fur samples, and stable isotope results from resident populations of the same species as comparison points, I investigated whether the migrants passing through the study site originated from a wide variety of roost locations. Further, I tested whether migratory origin varied over the length of the migratory season and investigated whether bats coming through the site in temporal "waves" originated from different locations.

Third, I analyzed fur samples taken from museum specimens collected from across eastern North America to examine the scale of annual movements of tri-colored bats (*Perimyotis subflavus*) (Chapter 4). This species roosts in colonies in the summer, hibernates during the winter, and had previously been assumed to be a regional migrant. I determined the relationship between fur stable hydrogen isotope compositions and both latitude and mean growing season precipitation stable hydrogen isotope compositions at the location of capture during the summer. Using this relationship as a measure of natural stable hydrogen isotope heterogeneity at the location of annual fur growth, I examined the offset between the fur-

precipitation/latitude relationship during the fall, winter, and spring seasons to look for evidence of latitudinal migration in this species.

Finally, there are several analytical complications in conducting stable hydrogen isotope analysis on organic samples. The most worrisome is that a consistent fraction of sample hydrogen exchanges freely with atmospheric water vapour hydrogen at room temperature. As a result, standards must be developed for each tissue type being analyzed, and their non-exchangeable stable hydrogen isotope compositions must be determined using comparative equilibration techniques. The final contribution of my dissertation has been to develop fur-specific standards for stable hydrogen isotope analysis and to report on the effectiveness of conducting comparative equilibration at room temperature (Appendix A).

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Chapter 2

2 Stable isotope variation in the fur of four species of summer resident bats

2.1 Introduction

There is a critical need to better understand all aspects of the migratory behavior and ecology of North American bats. Wind energy associated mortalities (Arnett *et al.* 2008) and white nose syndrome (Frick *et al.* 2010) increasingly expose bats to threats during the autumn and winter periods of their life cycle. It is important to have a better understanding of the movements of bats across the landscape in order to best inform conservation decisions. The challenges inherent in using traditional mark-recapture and radio-tracking techniques (Holland & Wikelski 2009) to learn about long distance movement of bats make the alternate use of intrinsic markers very attractive. The hydrogen isotope ratio of animal tissues is one marker that has been commonly used to investigate animal migration (Hobson and Wassenaar 2008), particularly in birds and less frequently in other migratory groups, such as insects (*e.g.* Brattström *et al.* 2010; Dockx *et al.* 2004; Hobson *et al.* 1999a) and bats (Britzke *et al.* 2009; Cryan *et al.* 2004; Fraser *et al.* 2010).

The stable hydrogen isotope compositions of precipitation (δD_{precip}) vary predictably with several large-scale geographical features in North America, including latitude, altitude, and distance from the coast (Bowen and Revenaugh 2003). Many studies of animal migratory behaviour are based on these relationships and the

accompanying assumption that the δD values of animal tissues (δD_{tissue}) are reflective of

the δD_{precip} values at the location where the tissues were grown (Cormie *et al.* 1994;

Hobson 1999). Stable hydrogen isotope data from animal tissues can serve as a broad

proxy of the location where the animal was living when that tissue was grown and can

provide evidence of migration. A strong correlation between δD_{tissue} and latitude and/or

local δD_{precip} at the location of tissue formation has been demonstrated many times in

birds (*e.g.* Chamberlain *et al.* 1997; Clark *et al.* 2006; Kelly *et al.* 2002; Lott *et al.* 2003;

Meehan *et al.* 2003; Sellick *et al.* 2009; Wassenaar and Hobson 2000) and less frequently

in summer resident bats (Cryan *et al.* 2004; Britzke *et al.* 2009; Chapter 4).

2.1.1   Stable hydrogen isotope variation

Numerous statistical techniques have been used to estimate migratory origin of

animals based on the stable hydrogen isotope compositions of their tissues. These vary

in complexity from "map lookup" and regression analyses (Wunder and Norris 2008a)

that estimate migrant origin based on an observed correlation between δD_{tissue} and

latitude or local δD_{precip} (*e.g.* Cryan *et al.* 2004) to more complicated probabilistic

assignment methods (Wunder and Norris 2008b). Fundamental to the accurate

application of these techniques for migrant assignment is quantification of how δD_{tissue}

values reflect δD_{precip} at the location of tissue formation across the species' range and

the variation inherent in this relationship. Some variation is unavoidably created during

sample and data analysis, as a result of analytical and modelling imprecision (Wunder

and Norris 2008b), while other variation exists naturally. Natural stable isotope variation

occurs both in the environment (such as temporal changes in δD_{precip} [e.g. Farmer *et al.* 2008]), and among individual animals living in the same area. Studies of δD_{tissue} variation among resident birds have shown that variation can exist within and among individuals of the same and different species (Smith *et al.* 2008; Torres-Dowdall *et al.* 2009) and between age classes within species (Meehan *et al.* 2003).

One objective of the present study was to document stable hydrogen isotope variation in resident populations of North American bats (who should, in theory, be isotopically identical). There is a body of literature that documents natural levels of stable isotope variability in resident bird populations (*e.g.* Clark *et al.* 2006; Farmer *et al.* 2003; Langin *et al.* 2007; Hobson *et al.* 2004; Powell & Hobson 2006; Torres-Dowdall *et al.* 2009). These studies have reported $\delta D_{feather}$ values for many bird populations and have identified differences in resident $\delta D_{feather}$ variation among species (Torres-Dowdall *et al.* 2009), ages (Meehan *et al.* 2003; Langin *et al.* 2007) and samples taken from different locations on the same individual (Smith *et al.* 2008). There is comparatively little known about species-specific natural variation in resident bat δD_{fur} values or the factors that contribute to this variation (*cf.* Fraser *et al.* 2009). A stronger understanding of species-specific δD_{fur} variation in resident bats will be helpful in the development and execution of bat migration studies using stable isotope techniques. Specifically, knowledge of δD_{fur} variation at all levels can serve to inform sampling protocol and data analysis in order to quantify and in some cases, maximize, precision when conducting migrant origin assignment.

2.1.2   Stable carbon and nitrogen isotope analyses

The predictable continental scale of δD_{precip} variation makes stable hydrogen

isotope analysis a good choice for studying long distance movements in animals.

However, the use of only stable hydrogen isotope analysis limits the precision of origin

assignment to a latitudinal band and is not necessarily conducive to the study of smaller

scale animal movements. Other researchers have demonstrated that when stable

isotope analyses of additional elements are included, populations can have unique

stable isotope profiles that differ significantly among proximate populations (e.g. Torres-

Dowdall *et al.* 2009) and that occasionally include predictable and systematic

relationships among stable isotopes within the population. Accordingly, a further

objective of the present study was to construct such stable isotope profiles for

populations of several species of bats using stable hydrogen, nitrogen, and carbon

isotope analyses.

Stable isotope compositions of animal tissues are largely driven by individual diet

(DeNiro and Epstein 1978; DeNiro and Epstein 1981; Ehleringer *et al.* 2008; Hobson *et al.*

1999b). Stable nitrogen isotope ($\delta^{15}N$) values typically increase by approximately 3-5 ‰

with each increasing trophic level (Minagawa and Wada 1984; Kelly 2000), resulting in

consumers with tissues that are predictably enriched in ^{15}N compared to their diet.

Stable carbon isotope ($\delta^{13}C$) values also increase slightly with trophic level (Kelly 2000),

but are more strongly affected by dietary source at the base of the food web. Plants

using the C_4 photosynthetic pathway are enriched in ^{13}C relative to plants using the C_3

photosynthetic pathway and these compositions are reflected in consumer tissue (Kelly

2000). The stable hydrogen isotope compositions of animal tissues are determined by a

combination of the animal's diet and drinking water. Some evidence also exists to

suggest that the δD values of animal tissue may reflect diet similarly to both $\delta^{15}N$ and

$\delta^{13}C$. Several studies have shown that tissue δD values increase upwards through the

food chain as the result of a trophic enrichment effect (Bowen *et al.* 2009; Birchall *et al.*

2005; Reynard and Hedges 2008).

Habitat variation at the landscape level and subsequent variation in diet can

result in individuals from relatively close locations having different stable isotope

profiles (e.g. Urton and Hobson 2005; York and Billings 2009; Torres-Dowdall *et al.*

2009). For instance, Torres-Dowdall *et al.* (2009) used stable isotope analyses of multiple

elements (carbon, nitrogen, hydrogen, oxygen, and sulphur) of shorebird feathers to

differentiate populations from several sites within northern Argentina and to

successfully assign a large number of individuals to the correct origin site.

Some authors have found intra-specific correlations among stable isotopes (e.g.

Kelly 2000; Møller *et al.* 2006; Torres-Dowdall *et al.* 2009). Identification of predictable

relationships within stable isotope profiles of resident populations can allow for future

differentiation of single vs. mixed origin populations. For example, Møller *et al.* (2006)

identified a correlation between feather stable carbon and nitrogen isotope

compositions in breeding populations of Ukrainian Barn Swallows in non-contaminated

sites. They then used the absence of this correlation among breeding swallows from

post-1986 Chernobyl to infer that this site is a sink population, populated mainly by

immigrants (a mixed population).

2.1.3   Nursing young

A complicating factor for defining fur stable isotope variation in resident bats is

that bat pups subsist wholly on milk for the first several weeks of their life, during which

time they grow fur. Subadult bats are consuming a very different diet from conspecific

adults during fur growth, and it is likely that their tissues will be isotopically distinct. The

stable isotope compositions of the tissues of dependent young should be directly related

to the stable isotope composition of their mother's milk (Jenkins *et al.* 2001). Several

researchers have found that cow milk water (approximately 85% of milk – Vaughan *et al.*

2000) is enriched in both D and/or ^{18}O compared to cow drinking water (Camin *et al.*

2008; Chesson *et al.* 2010; Lin *et al.* 2003; Renou *et al.* 2004), and the magnitude of this

enrichment increased with the water content of the animal's plant food (Renou *et al.*

2004). Assuming this is also true in bats, we would expect that milk-fed young will be

consuming a diet enriched in D compared to the drinking water of their mother and

would predict that subadult tissues will be enriched in D compared to adult tissues. We

know of no other studies that have examined stable hydrogen isotope differences

between the tissues of mammalian mothers and their dependent offspring, but other

studies have found that the structural carbonate of dependant humans (Williams *et al.*

2005; Wright and Schwarcz 1998) and other mammals (woolly mammoths [*Mammuthus

primigenius*] - Metcalfe *et al.* 2010) is enriched in ^{18}O compared to maternal tissues.

Mother-young differences in δ^{13}C and δ^{15}N tissue values have been extensively studied. Results have varied, but it is common for adult tissues to be depleted of ^{15}N compared to subadult tissues (Hobson *et al.* 2000; Metcalfe *et al.* 2010; Polischuk *et al.* 2001; Sare *et al.* 2005). However, Jenkins *et al.* (2001) found substantial variation among species, including some (moose, *Alces alces*) where no age difference existed. Milk tends to be depleted of ^{13}C relative to other maternal tissues (Jenkins *et al.* 2001; Miller *et al.* 2008; Polischuk *et al.* 2001), and offspring tissues that were grown during the period of milk dependency are often depleted of ^{13}C relative to maternal tissues (Polischuk *et al.* 2001; Sare *et al.* 2005), although in some cases they may be the same (Jenkins *et al.* 2001), or in one case, enriched (Hobson *et al.* 2000).

2.1.4   Hypotheses and predictions

We captured and sampled fur from four bat species during the summer residency period. Three of these species (eastern red bats [*Lasiurus borealis*], hoary bats [*Lasiurus cinereus*], and silver-haired bats [*Lasionycteris noctivagans*]) are considered latitudinal migrants and are believed to migrate south in the fall and north in the spring (Cryan 2003; Cryan *et al.* 2004). The little brown bat (*Myotis lucifugus*) is a regional migrant that travels between winter hibernacula and summer maternity colonies (females) or bachelor roosts (males) (Davis and Hitchcock 1965; Fenton 1969). Temperate bats species are presumed to moult once annually during the summer (Constantine 1957; Tiunov and Makarikova 2007; Quay 1970; Cryan *et al.* 2004) and thus fur samples taken from bats represent the conditions experienced by the bat during the

summer. The pattern of fur replacement varies among species, but in general the lower

dorsal area is one of the first areas of new fur growth (Constantine 1957; Dwyer 1963;

Tiunov and Makarikova 2007), and so was our chosen sampling location.

In this paper, we have addressed three hypotheses and seven associated

predictions regarding stable isotope variability in bat fur.

1)   We hypothesized that δD_{fur} sampled from resident populations of bats would

vary both within and among individual bats, age classes, species, and over the period of

summer residency. Based on the results of similar avian research, we made the following

predictions:

   i)   *Intra-individual variation* – Fur samples taken from multiple loci on a bat's

         body will vary isotopically (Smith et al. 2008);

   ii)   *Age class variation* – When analyzed at the level of whole age classes,

         subadult fur will be enriched in D compared to adult fur;

   iii)   *Intra- and inter-species variation* – Total intra-species variation will vary

         among species (e.g. Clark et al. 2006; Langin et al. 2007; Powell &

         Hobson 2006);

   iv)   *Variation over time* – Average δD_{fur} values will vary over the course of the

         summer season as the annual moult occurs and fur from the previous

         year is replaced with fur from the current year.

2)   We hypothesized that analyses of stable carbon and nitrogen isotope

compositions for a subset of our samples would create population-specific stable

isotope profiles that would be unique for populations and would contain predictable

characteristics. We predicted that:

> *v)   Relationships among stable isotopes* – Within-population correlations will
>
>   exist among the three stable isotopes investigated (δD_{fur}, $\delta^{15}N_{fur}$, and
>
> $\delta^{13}C_{fur}$);
>
> *vi)   Discrimination among populations* – Using stable carbon, hydrogen, and
>
>   nitrogen isotope analyses, it will be possible to distinguish among
>
>   populations of the same species - *M. lucifugus* - even those captured
>
>   within relatively close proximity to each other.

 3)   We hypothesized that dependent young, having grown their fur while consuming

a milk-based diet, would be isotopically distinct from their mothers. We anticipated that

if average δD_{fur} values differed between adult and subadult bats, this difference could be

relatively small compared to overall resident population variation and so might not be

statistically detectable in a within-species comparison of all adults and all subadults (as

addressed in hypothesis 1). We sampled fur from known mother-pup pairs to test the

prediction that

> *vii)   Nursing young* – fur from dependent pups will be enriched in both
>
>   deuterium and ^{15}N and depleted of ^{13}C compared to adult fur.

2.2   Methods

2.2.1   Field methods

Bats were captured during the period of summer residency from 2007-2009 at

four locations in Canada. Unless otherwise noted, all bats were captured using mist nets.

Lasiurus borealis and *M. lucifugus* were sampled during summer 2007 in Pinery

Provincial Park, Ontario (43° 15' N 81° 48' W). *Lasiurus borealis* were captured nightly

while foraging around streetlights from June 1 until August 15. *Myotis lucifugus* were

captured on a weekly basis as they emerged from a building roost at dusk from June 1

until August 29, when the colony dispersed. Additional *M. lucifugus* were trapped on

two occasions in July 2008 at a site in the Kananaskis Valley, Alberta (50° 42' N 115° 7'

W); and in July 2009 at a building roost at Benmiller, Ontario (43° 46' N 81° 43' W). The

Pinery and Benmiller sites are quite close to each other: the Benmiller site is

approximately 60 km northeast of the Pinery site. *Lasionycteris noctivagans* and *L.

cinereus* were captured nightly in late July and early August 2008 (16 days) and 2009 (20

days) in Cypress Hills Inter-Provincial Park, Saskatchewan (49° 33' N 109° 52' W).

To make a mother-pup comparison, we captured and sampled fur from nine

female *M. lucifugus* and their associated pups within the Benmiller roost. Pups were

considered to be with their mother if they were nursing at the time of capture.

A small fur sample was taken using surgical scissors from the lower back of each

bat (putative early moult area) to maximize the likelihood that the fur we sampled

would have been grown before the bat began autumn migration. Fur samples were also

taken from the lower venter for all bats captured at Pinery Park. Weight (±0.1g), forearm length (±1mm), sex and age class (adult/subadult - determined based on fusion of the metacarpal-phalangeal joint [Buchler 1980]) were recorded from each bat before it was released at the site of capture. Fur samples were stored at room temperature until the time of analysis.

In 2007, water samples were taken on a weekly basis in 30 mL Nalgene^TM containers from the Old Ausable River Channel (OARC) in Pinery Provincial Park, the most probable source of drinking water for the bats in the area. All water samples were sealed with screw tops and parafilm^TM and stored in a refrigerator at 4˚C until the time of analysis.

2.2.2   Laboratory analyses

All stable isotope analyses were conducted at the Laboratory for Stable Isotope Science at the University of Western Ontario. Fur samples were soaked overnight in a solution of 2:1 chloroform:methanol. Samples were drained, rinsed with more 2:1 chloroform:methanol and left to dry in a fume hood for >48 h.

For stable hydrogen isotope analyses of fur, samples were weighed into silver capsules. Initially 350 µg of fur was weighed into 3.5x5 mm capsules (all Pinery samples), but as the method was further refined, sample weight was reduced to 175 µg and 3.2x4 mm capsules were used (all remaining samples). Samples were run alongside five fur standards specifically developed for this study (Appendix A) and standardized to

VSMOW using comparative equilibration techniques (Wassenaar and Hobson 2003).

Through external verification and internal experimentation, non-exchangeable δD and

percent exchangeable hydrogen values were determined for all fur standards and used

to normalize results to VSMOW. All Pinery samples were left to equilibrate with

laboratory air along with standards before being weighed and were stored under

vacuum in a desiccator until the night before analysis, when they were transferred to a

vacuum oven at 70˚C. All other samples and standards were weighed and left to

equilibrate with laboratory air for a minimum of four days (Bowen *et al.* 2005) and not

dried before analysis. I conducted several laboratory trials to confirm that final δD_{fur}

values were consistent (relative to VSMOW) regardless of drying protocol. Samples were

combusted in a Thermo Finnigan high temperature conversion elemental analyzer at

1450˚C interfaced with a Thermo Finnigan Delta[plus] XL mass spectrometer in continuous

flow mode. Each run included one internationally accepted standard (NBS-22; δD

(VSMOW) = -120‰), 10% sample duplication, and five *Lasiurus borealis* samples taken

from the same bat as a drift check. The average (± standard deviation) difference

between duplicates of the same sample was 2±2‰ and this precision did not differ

among species (Appendix B).

For stable carbon and nitrogen isotope analyses of fur, 0.500 ± 10 µg of sample

was weighed into 3.5x5.0 mm tin capsules and combusted in a Costech Elemental

Analyzer. The stable carbon and nitrogen isotope ratios of the resultant N_2 and CO_2

gases were determined using a Thermo Finnigan Delta[plus] XL Mass Spectrometer that

was coupled to the Elemental Analyzer in continuous flow (He) mode. Samples were run

alongside four standards (IAEA-CH-6; NBS-22; IAEA N1 and IAEA N2) and calibrated to

VPDB (carbon) and AIR (nitrogen) with 10% duplication. The average (± standard

deviation) difference between duplicates of the same sample was 0.11±0.16‰ (carbon)

and 0.14±0.12‰ (nitrogen).

For stable hydrogen isotope analyses of water, 2 µL of each sample was injected

into an evacuated pyrex tube containing 80 mg of zinc. The tubes were sealed under

vacuum and heated at 480°C for 20 minutes, causing reduction of water into H_2 gas.

Samples were analyzed on a PRISM dual inlet mass spectrometer and normalized to

VSMOW using two water standards (δD=-151‰, +89‰).

All stable isotope results are presented as δ values in parts per thousand (‰) and

are calculated using the standard equation:

$$\delta = \left(\left(\frac{R_{sample}}{R_{standard}} \right) - 1 \right) \times 1000$$

where *R* is the ratio of the heavy:light stable isotope of the sample and standard,

respectively (i.e. D:H, ^{15}N:^{14}N, ^{13}C:^{12}C).

2.2.3   *Statistical Analyses*

All statistical analyses were conducted using IBM SPSS version 19 (SPSS Inc.

2011). For tests where normal distribution and homogeneity of variance were

underlying assumptions, the data were visually examined for both characteristics (Zuur

2007).

2.2.4 *Stable hydrogen isotope variation*

2.2.4.1 Intra-individual variation

Dorsal and ventral δD_{fur} values from *L. borealis* and *M. lucifugus* captured at

Pinery were compared within-species using paired t-tests. Total variance in the

difference between dorsal and ventral δD_{fur} values ($\Delta D_{dorsal-ventral}$) was compared

between species using a Levene's test for homogeneity of variance. Simple linear

regression analyses were used to test for within-species changes in $\Delta D_{dorsal-ventral}$ values

over time.

2.2.4.2 Age class variation

Where sufficient numbers of both ages classes were captured, mean δD_{fur} values

of adults and subadults were compared using the appropriate test (Pinery *M. lucifugus*

and *L. borealis* – ANCOVA, with date as a covariate; *L. cinereus*, *L. noctivagans,* and

Benmiller *M. lucifugus* – Mann-Whitney U tests) and adult and subadult δD_{fur} variance

was compared using Levene's tests for homogeneity of variance. For species with age

classes that had different mean stable hydrogen isotope compositions, or different

stable hydrogen isotope population variances, adult and subadult age classes were

considered separately in the remaining analyses. If there was no age difference in mean

stable hydrogen isotope compositions, then age classes were combined for the

remaining analyses.

2.2.4.3 Intra- and Inter-specific variation

Stable hydrogen isotope variance was compared among the three populations of

M. lucifugus using a Levene's test for homogeneity of variance and when there was no

difference among these groups, their stable hydrogen isotope compositions were

transformed and pooled. Transformation was achieved by determining the offset of the

Kananaskis and Benmiller population medians from the Pinery median and adding the

appropriate offset value to all individuals from Kananaskis and Benmiller.

Variance in fur stable hydrogen isotope composition was compared among all

four study species (adults only if adult/subadult populations differed in mean δD_{fur}

values or δD_{fur} variance and pooled age class data if not) using a Levene's test for

homogeneity of variance. When this test indicated that there were significant

differences among species, pairwise comparisons were made using Bonferroni-corrected

Levene's tests. The mean δD_{fur} values of species captured at the same location were

compared using Mann-Whitney U tests (*L. borealis* and *M. lucifugus* from Pinery and *L.

cinereus* and *L. noctivagans* from Cypress Hills).

2.2.4.4 Variation over time

Simple linear regression was used to test for variation in δD_{fur} values over time in the

M. lucifugus and *L. borealis* fur samples from Pinery.

2.2.5   Stable carbon and nitrogen isotope analyses

2.2.5.1 Correlations among stable isotopes

Not all data were normally distributed, so Spearman's rank correlations were conducted on all pairwise combinations of δD_{fur}, $\delta^{15}N_{fur,}$ and $\delta^{13}C_{fur}$ for adults and subadults of all species where sample size was sufficient.

2.2.5.2 Distinguishing among populations

Discriminant function analysis followed by leave-one-out classification was used to determine if the three populations of *M. lucifugus* could be discriminated based on their stable hydrogen, carbon, and nitrogen isotope compositions.

2.2.6   Nursing young

2.2.6.1 Nursing young

Fur stable isotope compositions of mother-pup pairs from the Benmiller *M. lucifugus* population were compared using paired t-tests.

2.3   Results

2.3.1   Sampling

Dorsal fur samples were collected from 28 *L. noctivagans* and 43 *L. cinereus* in Cypress Hills, SK; 30 *L. borealis* and 67 *M. lucifugus* from Pinery, ON; 33 *M. lucifugus* from Benmiller, ON and 24 *M. lucifugus* from Kananaskis, AB (Appendix C). Ventral fur samples were also collected from all of the *L. borealis* and 32 of the *M. lucifugus* captured at Pinery. Stable hydrogen isotope analyses were conducted on all fur samples and stable carbon and nitrogen isotope analyses were conducted on a subset of the

dorsal fur samples (40 *L. cinereus*, 26 *L. noctivagans,* 14 *L. borealis,* 22 *M. lucifugus* [Pinery], 24 *M. lucifugus* [Benmiller], and 15 *M. lucifugus* [Kananaskis]).

2.3.2 Stable hydrogen isotope variation

2.3.2.1 Intra-individual variation

Individual dorsal and ventral δD_{fur} differences ($\Delta D_{dorsal-ventral}$) had an absolute difference of 6±5‰ (mean ± standard deviation) in *L. borealis* and 3±2‰ in *M. lucifugus* from Pinery, with dorsal values being both more enriched in and depleted of D relative to ventral values (Figure 2.1)*.* The most positive and negative $\Delta D_{dorsal-ventral}$ values for *L. borealis* were +17‰ and -16‰, respectively, and 6‰ and -9‰ for *M. lucifugus.* Overall, mean dorsal values were significantly depleted of D compared to ventral values in both species (paired t-test: *Lasiurus borealis* – *t*=-2.233, *p*=0.034, *df*=27; *Myotis lucifugus* – *t*=-3.172, *p*=0.003, *df*=30) and there was significantly greater $\Delta D_{dorsal-ventral}$ variance among the *L. borealis* samples than the *M. lucifugus* samples (Levene's test: *F*=6.041, *p*=0.017, *df*=57) (Figure 2.1). There was no significant change in $\Delta D_{dorsal-ventral}$ values over time for either *L. borealis* (SLR –r^2=0.018, *F*=0.470, *p*=0.499, *df*=27) or *M. lucifugus* (r^2=0.071, *F*=0.149, *p*=0.703, *df*=30).

2.3.2.2 Age class variation

Adult *L. cinereus* and *L. noctivagans* fur was significantly enriched in D compared to subadult fur (mean ± standard deviation: *L. cinereus* (adults) = -94±20‰, (subadults) = -121±8‰; *L. noctivagans* (adults) = -97±11‰, (subadults) = -110±14‰; Mann-Whitney U tests: *L. cinereus* – *U* = 23, *p* < 0.01; *L. noctivagans* – *U* = 28, *p* = 0.016) (Figure 2.2).
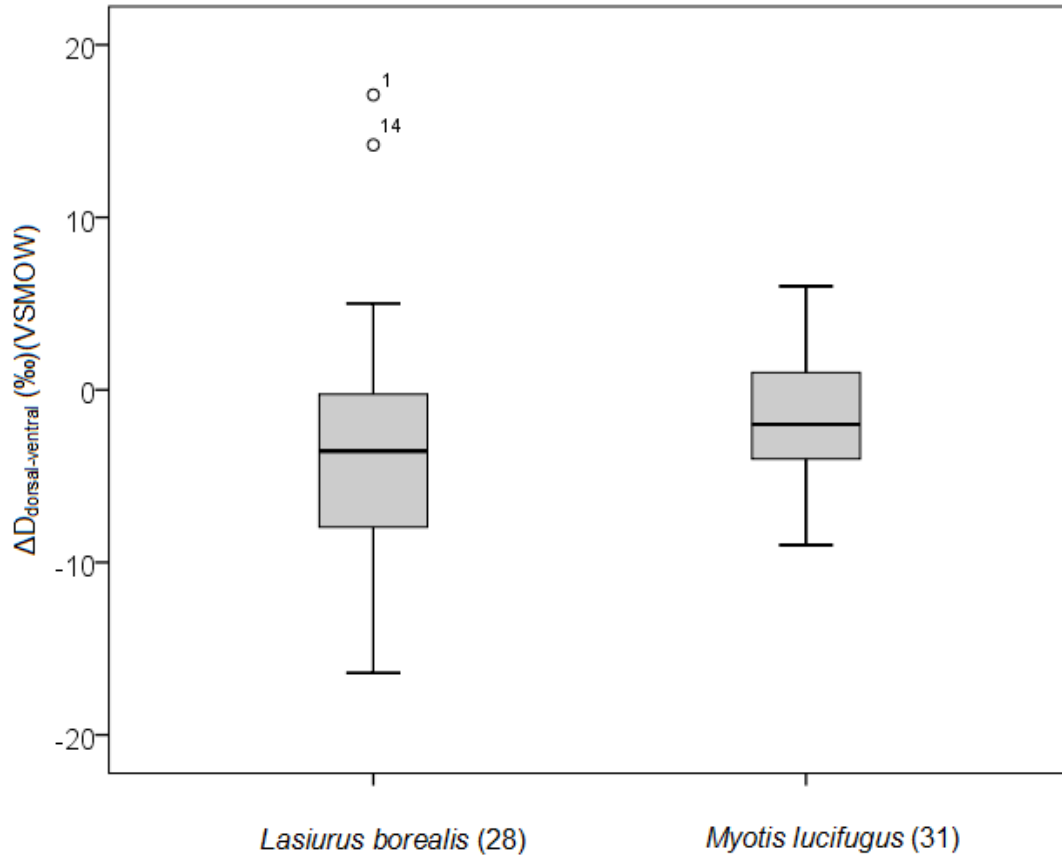
Figure 2.1. Mean differences between dorsal and ventral δD$_{fur}$ values (ΔD$_{dorsal-ventral}$) for little brown bats (*Myotis lucifugus*) and eastern red bats (*Lasiurus borealis*) captured at Pinery Provincial Park.

For both species captured at Pinery, mean dorsal δD$_{fur}$ values were significantly depleted of D compared to ventral values (as indicated by the difference between dorsal and ventral δD$_{fur}$ values [ΔD$_{dorsal-ventral}$]). There was significantly greater variance in ΔD$_{dorsal-ventral}$ values among the *L. borealis* sampled than among the *M. lucifugus*. Sample sizes are in parentheses.
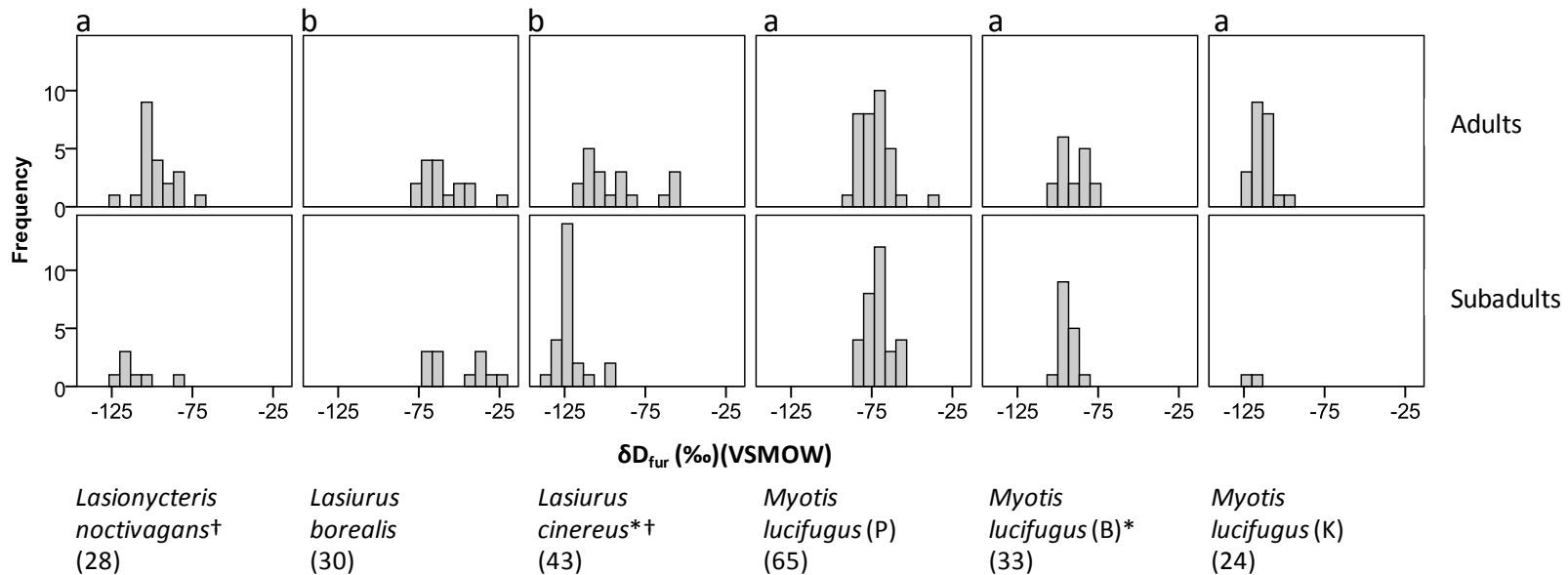
Figure 2.2. Distribution of δD$_{fur}$ values from adults and subadults of four bat species.

Adult *Lasiurus cinereus* and Benmiller *Myotis lucifugus* had greater δD$_{fur}$ variance than subadults from the same populations (significant age-related differences in variance indicated by *), while no age differences in variance existed for the other species. Adult *L. cinereus* and *Lasionycteris noctivagans* fur was enriched in D compared to subadult fur (significant age-related difference in mean δD$_{fur}$ values indicated by †). Populations of *Lasiurus borealis* and *L. cinereus* had significantly greater δD$_{fur}$ variances than populations of *M. lucifugus* and *L. noctivagans* (significant differences indicated by lower case letters). For *M. lucifugus* populations, (P)=Pinery; (B)=Benmiller; (K)=Kananaskis. Sample sizes are in parentheses.

There was no difference between mean adult and subadult δD_{fur} values for either *M. lucifugus* or *L. borealis* captured at Pinery (mean ± standard deviation: *M. lucifugus* (adults) = -73±10‰, (subadults) = -71±7‰; *L. borealis* (adults) = -59±14‰, (subadults) = -51±18‰; ANCOVA: *M. lucifugus* – F=2.260, p=0.138, df=64; *L. borealis* - F=0.235, p=0.631, df=37) or *M. lucifugus* captured at Benmiller (mean ± standard deviation: (adults) = -89±8‰, (subadults) = -94±4‰; Mann-Whitney U test: *U* = 85, p=0.066). Subadult sample size was not sufficient to make age comparisons for the Kananaskis population of *M. lucifugus*. Subadult δD_{fur} variance was significantly less than that of adults in the *L. cinereus* population and the Benmiller population of *M. lucifugus* (Levene's test: *L. cinereus* – F=17.870, df=1,41, p=0.000; Benmiller *M. lucifugus* - F=12.610, df=1,31, p=0.001) and there was no difference in variance between adults and subadults in the populations of *L. borealis* and *M. lucifugus* from Pinery and the population of *L. noctivagans* (Levene's test: *L. borealis* – F=3.797, df=1,26, p=0.062; Pinery *M. lucifugus* - F=1.253, df=1,63, p=0.267; *L. noctivagans* - F=0.428, df=1,26, p=0.519).

2.3.2.3 Intra- and inter-specific variation

   Intra- and inter-specific comparisons were made among adults only in populations where there were significant age-related differences in δD_{fur} mean and variance and among pooled adults and subadults where no age differences existed. The resident *L. cinereus, L. noctivagans* and *L. borealis* populations had the greatest overall inter-individual range in δD_{fur} values (59‰, 58‰, and 54‰, respectively). All three

populations of *M. lucifugus* had smaller ranges (Pinery [excluding one early season outlier]=34‰; Benmiller=25‰; Kananaskis=29‰) (Figure 2.2).

There was no difference in δD_{fur} variance among any of the *M. lucifugus* populations, (Levene's test: $F=1.858$, $df=2, 103$, $p=0.161$) so all data from *M. lucifugus* were pooled. There were no differences in δD_{fur} variance between the populations of *Lasiurus cinereus* and *L. borealis* (Levene's test: $F=1.177$, $df=1, 46$, $p=0.284$) or the populations of *M. lucifugus* and *L. noctivagans* (Levene's test- $F=1.909$, $df=1, 125$, $p=0.170$). Both *L. cinereus* and *L. borealis* had greater δD_{fur} variance than either *M. lucifugus* or *L. noctivagans* (pairwise Levene's tests: *L. cinereus/M. lucifugus* - $F=45.484$, $df=1, 123$, $p<0.001$; *L. cinereus/L. noctivagans* - $F=8.563$, $df=1, 38$, $p=0.006$; *L. borealis/M.lucifugus* - $F=42.830$, $df=1, 133$, $p<0.01$; *L. borealis/L.noctivagans* - $F=7.424$, $df=1, 48$, $p=0.009$).

Lasiurus borealis from Pinery had significantly higher mean δD_{fur} values than did sympatric *M. lucifugus* (mean ± standard deviation: *L. borealis* = -55±16‰, *M. lucifugus* = -72±9‰; Mann Whitney U test: $U=323.5$; $p<0.01$). Sympatric populations of *L. cinereus* and *L. noctivagans* did not differ (mean ± standard deviation: *L. cinereus* = -94±20‰; *L. noctivagans* = -97±11‰; Mann Whitney U test: $U=184.5$; $p=0.685$).

2.3.2.4 Variation over time

Lasiurus borealis δD_{fur} values did not change significantly over time (SLR: $r^2=0.111$, $F=3.388$, $p=0.077$, $df=28$). *Myotis lucifugus* δD_{fur} values from Pinery decreased

significantly with time (SLR: r^2=0.178, *F*=13.650, *p*<0.001, *df*=64) (Figure 2.3). Throughout

the summer, almost all δD_{fur} values from Pinery *M. lucifugus* were more negative than

δD_{water} values from the Old Ausable River Channel. Pinery *L. borealis* fur was both

enriched in and depleted of D relative to the Old Ausable Channel samples.

2.3.3 *Stable carbon and nitrogen isotope analyses*

2.3.3.1 Correlations among stable hydrogen, carbon and nitrogen isotope compositions

There was a positive correlation between δD_{fur} and $\delta^{13}C_{fur}$ values in the adult *L.*

noctivagans, and *L. borealis* populations, and a negative correlation between $\delta^{13}C_{fur}$ and

$\delta^{15}N_{fur}$ values in adult *L. noctivagans* and subadult Benmiller *M. lucifugus* populations

(Table 2.1). There were no correlations between δD_{fur} and $\delta^{15}N_{fur}$ values in any of the

populations (Table 2.1). There were insufficient data to make these comparisons for the

L. borealis, L. noctivagans, and the Kananaskis *M. lucifugus* subadults.

2.3.3.2 Distinguishing among populations

Using a discriminant function analysis, the three populations of *M. lucifugus*

could be distinguished based on their stable hydrogen, carbon, and nitrogen isotope

compositions with varying success (Figure 2.4). Discriminant functions 1 and 2 (DF1 and

DF2) were both significant (DF1: Eigenvalue=6.601, Wilks' λ=0.073, *p*<0.001; DF2:

Eigenvalue=0.803, Wilks λ=0.555, *p*<0.001). DF1 explained 89.2% of the variance among

the three populations and DF2 explained the remaining 10.8%. DF1 completely

separated the Kananaskis (Alberta) population from the Benmiller and Pinery (Ontario)

populations and partially separated the Pinery and Benmiller populations. Discrimination
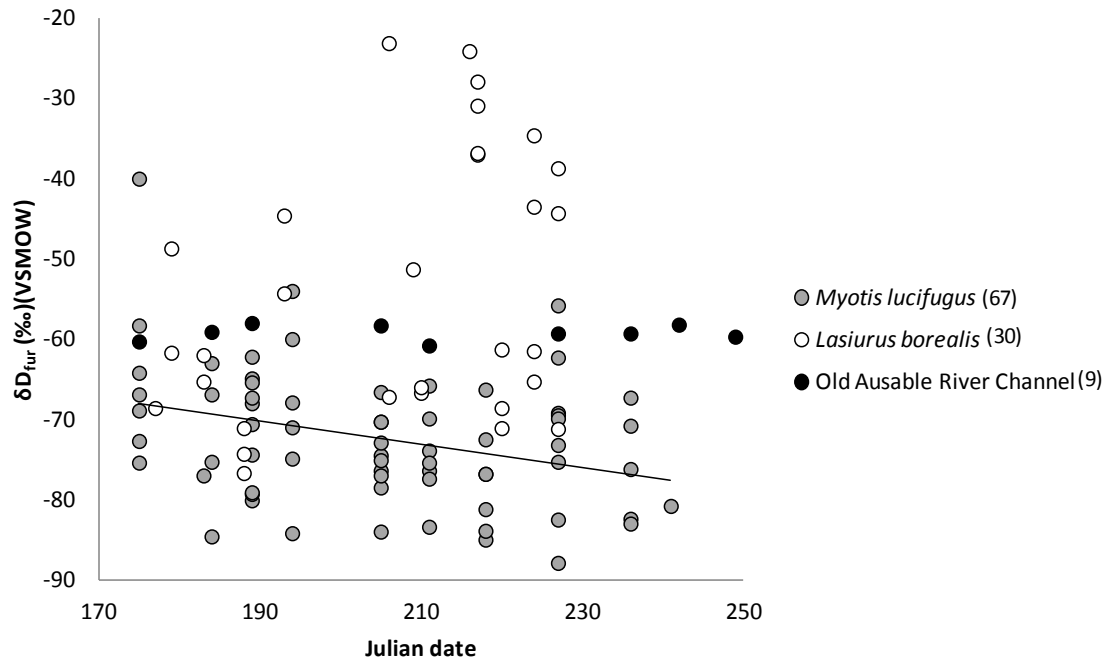
Figure 2.3. Stable hydrogen isotope composition of little brown bat (*Myotis lucifugus*) and eastern red bat (*Lasiurus borealis*) fur and Old Ausable River Channel water at Pinery Provincial Park over the 2007 summer season.

Myotis lucifugus δD_{fur} values decreased significantly over time (indicated by a solid line) while δD values of *L. borealis* fur and Old Ausable River Channel (OARC) water did not. Almost all fur samples from *M. lucifugus* were depleted of D compared to OARC water, while fur samples from *L. borealis* were both depleted of and enriched in D compared to water samples. Sample sizes are in parentheses.

Species	Age	δD vs. δ¹³C			δD vs. δ¹⁵N			δ¹³C vs. δ¹⁵N		
		n	*R*	*p*-value	*N*	*R*	*p*-value	*n*	*R*	*p*-value
Lasionycteris noctivagans	Adult	20	0.649	**0.002***	21	0.168	0.466	20	0.465	**0.039***
Lasiurus borealis	Adult	15	0.704	**0.003***	14	-0.310	0.281	14	0.385	0.175
Lasiurus cinereus	Adult	18	0.395	0.104	18	-0.390	0.109	18	0.269	0.280
	Subadult	21	0.184	0.425	23	-0.027	0.902	21	0.316	0.152
Myotis lucifugus (Pinery)	Adult	9	0.477	0.194	9	-0.555	0.121	10	-0.511	0.132
	Subadult	12	0.190	0.555	12	-0.127	0.709	12	-0.105	0.746
Myotis lucifugus (Benmiller)	Adult	13	-0.027	0.929	13	-0.096	0.754	13	-0.525	0.065
	Subadult	11	-0.009	0.979	11	-0.127	0.709	11	-0.845	**0.001***
Myotis lucifugus (Kananaskis)	Adult	13	0.270	0.372	13	0.255	0.400	13	-0.080	0.795

Table 2.1 Correlation statistics for pairwise comparisons of fur stable isotope compositions from four bat species.

Significant correlations are in bold and indicated by *.
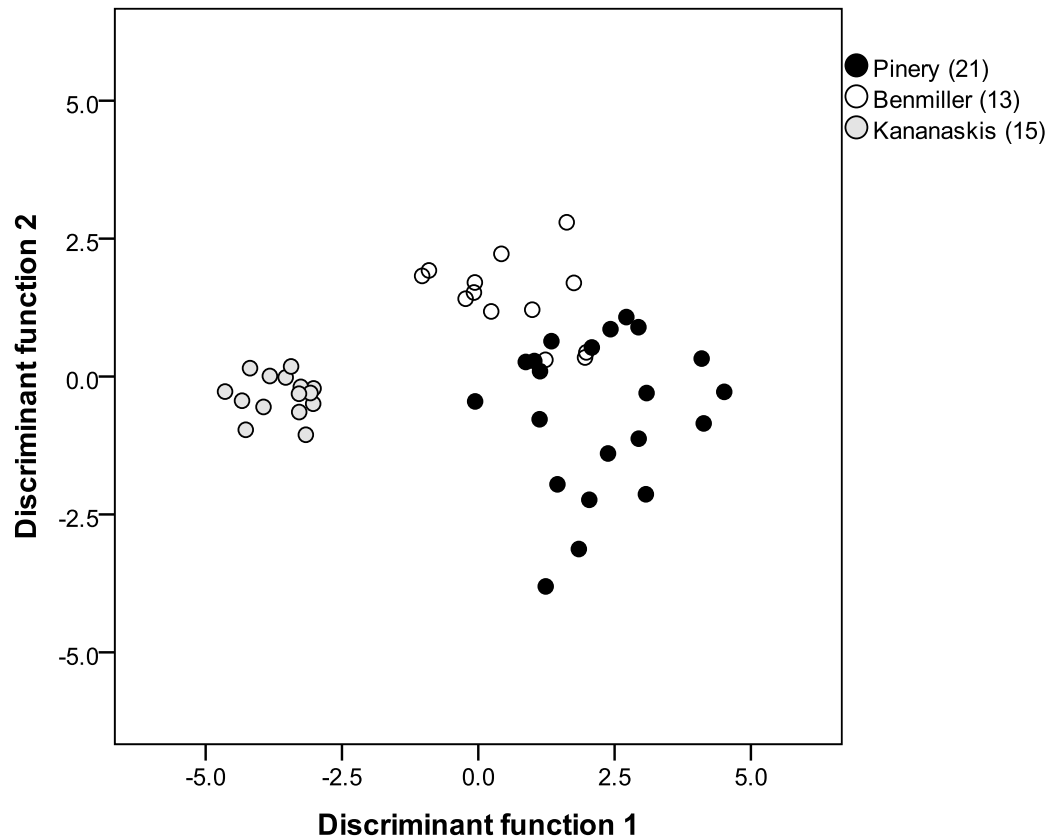
Figure 2.4. Discrimination of little brown bats (*Myotis lucifugus*) from three distinct colonies based on the stable hydrogen, carbon, and nitrogen compositions of their fur.

A discriminant function analysis incorporating the δD_{fur}, $\delta^{13}C_{fur}$, $\delta^{15}N_{fur}$ values of the *M. lucifugus* captured at three locations in Canada (one in southern Alberta [Kananaskis] and two in southern Ontario [Pinery and Benmiller]) separated the three populations with varying success. Leave-one-out classification correctly assigned 100% of the Kananaskis individuals, 76.2% of the Pinery individuals and 76.9% of the Benmiller individuals. Sample sizes are shown in parentheses.

was predominantly driven by δD_{fur} values (standardized canonical discriminant function coefficients: δD=0.917; $\delta^{13}C$=0.257; $\delta^{15}N$=0.190). DF2 further separated the Pinery and Benmiller populations and was more evenly contributed to by the three stable isotopes, although it was mainly driven by the $\delta^{15}N_{fur}$ values (standardized canonical discriminant function coefficients: δD=-0.376; $\delta^{13}C$=0.833; $\delta^{15}N$=1.109). Leave-one-out classification correctly identified 100% of *M. lucifugus* from Kananaskis, 76.9% of *M. lucifugus* from Benmiller and 76.2% of *M. lucifugus* from Pinery.

2.3.4 Nursing young

2.3.4.1 Nursing young

Nursing *Myotis lucifugus* pups had fur that was depleted of D, enriched in ^{15}N, and similar in ^{13}C compared to their mothers (Figure 2.5; mean ± standard deviation: δD (mothers) = -85±9‰, δD (pups) = -93±2‰; $\delta^{15}N$ (mothers) = 10.5±0.7‰, $\delta^{15}N$ (pups) = 11.2±0.8‰; $\delta^{13}C$ (mothers) = -22.4±1.4‰, $\delta^{13}C$ (pups) = -22.6±1.7‰; paired t-test: δD - t=2.852, df=8, p=0.021; $\delta^{15}N$ - t=-3.667, df=8, p=0.006; $\delta^{13}C$ - t=0.630, df=8, p=0.546).

2.4 Discussion

Overall, we found substantial stable hydrogen isotope variation among fur samples at each level that we investigated, including samples taken from the same individual, between age classes, among populations and species, and over time. There were strong correlations between δD_{fur} and $\delta^{13}C_{fur}$ and between $\delta^{15}N_{fur}$ and $\delta^{13}C_{fur}$ for some but not all species, a relationship that could be used in the future to
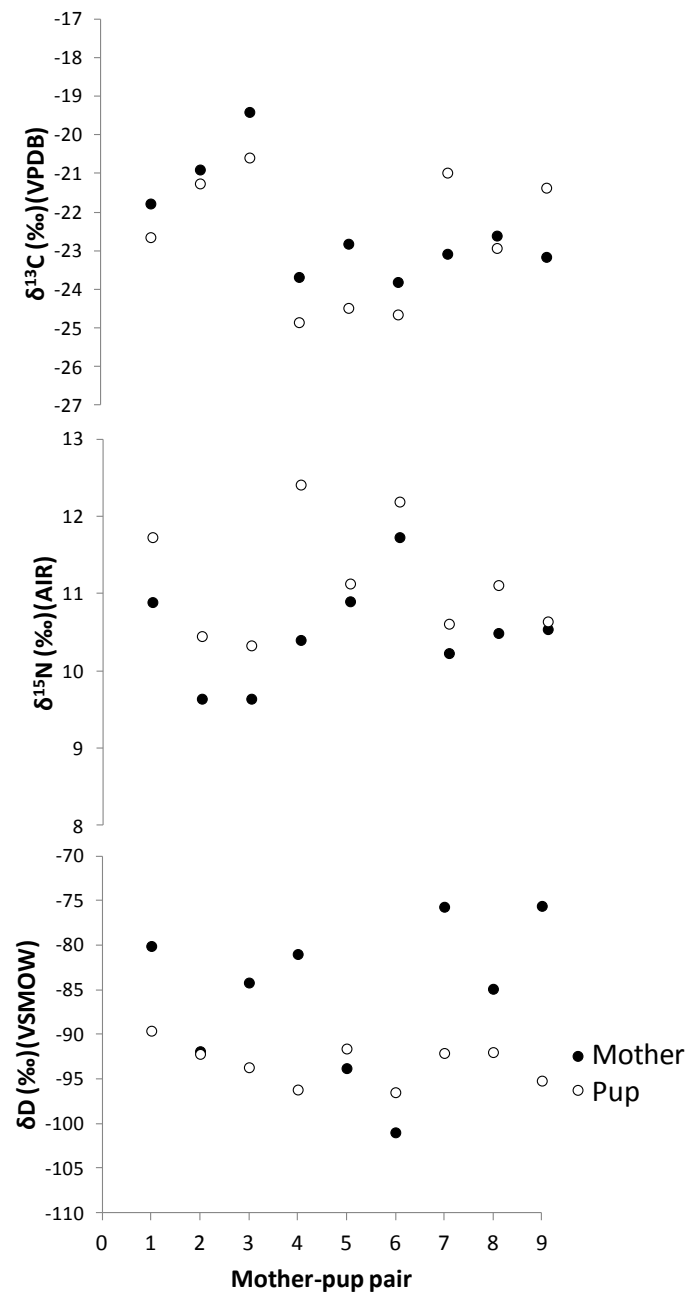
Figure 2.5. Comparison of *Myotis lucifugus* adult and pup stable isotope compositions.

A comparison of fur samples from nine matched mother-pup pairs indicated that subadult fur was significantly depleted of deuterium and enriched in ^{15}N compared to adult fur and that there was no significant age-related difference in fur δ^{13}C values.

discriminate groups of bats from single and multiple origins. Further, we found evidence that multi-element stable isotope analysis in origin assignment may make it possible to distinguish bats originating from relatively proximate roosts.

2.4.1 Stable hydrogen isotope variation

2.4.1.1 Intra-individual variation

Individual δD_{fur} variation between dorsal and ventral samples ranged from within experimental error to as much as 17‰ (*L. borealis*) and was greater among the *L. borealis* sampled than the *M. lucifugus*. This variation between dorsal and ventral samples may be the result of moulting or may reflect variation in rates of fur growth. Other researchers (Constantine 1957; Dwyer 1963) have shown that dorsal and ventral fur replacement can occur asynchronously, which could result in isotopic variation across an individual pelage if temporal dietary shifts are recorded across the pelage. Additionally, Smith *et al.* (2008) postulated that raptor $\delta D_{feather}$ values varied among feather types as a result of variable rates of feather growth; a similar phenomenon may occur during bat fur replacement. In either case, it is clear that fur samples should always be taken from a standardized location on the bat's body if those samples are to be used as comparable proxies of the location of fur growth.

2.4.1.2 Age class variation

In all cases where the adult age class had mean δD_{fur} values different than the subadult age class, subadult fur was depleted of D compared to adult fur. Relative

depletion of D in subadult fur values was unexpected, as published records of milk being enriched in D compared to drinking water led us to predict that subadult fur would be enriched in D compared to adult fur. It is challenging to explain our results without a more thorough understanding of the routing of maternal hydrogen into milk and then the metabolism of the milk into juvenile tissue. While the relationship between drinking water D and milk water D has received attention, a clear model does not exist for how δD values of diet relate to those of milk water or the other components of milk (*cf.* Camin *et al.* 2008).

When we found differences in δD_{fur} variance between adults and subadults, subadults had lower variance than adults. This may be the result of subadults growing all of their fur over a shorter time frame than the adult moulting period and thus having less of an opportunity to incorporate temporal shifts in bat diet into their fur. At the extreme of this scenario, it is possible that adult bats are still completing their moult at the end of the summer and may engage in some fur replacement while they are migrating. If this is the case, then adult δD_{fur} values would represent an average of diet and drinking water at the bat's summer residency site as well as during some of its migration. Such a scenario would almost certainly result in a higher δD_{fur} variance than for fur all grown at the same site.

The presence of an age effect in δD_{fur} values is an important consideration when samples are taken for stable isotope studies of bat migration. The suitability of grouping adult and subadult fur samples will depend on the scale of the project, as well as the

species being studied. In studies examining individuals that have travelled across a large latitudinal range, pooling age class data may increase sample size and only slightly add to assignment error. In a study attempting to detect finer isotopic differences, it may be helpful to separate age classes during analysis.

2.4.1.3 Intra- and inter-specific variation

Variance in the δD_{fur} values of resident bat populations varied among species: it was smallest in the populations of *Myotis lucifugus* and *L. noctivagans* and greatest in the populations of *L. borealis* and *L. cinereus*. While different bat populations had distinct stable hydrogen isotope profiles, all of the intra-specific δD_{fur} ranges that we recorded were similar to values that have been reported for various bird species (Clark *et al.* 2006; Langin *et al.* 2007; Powell & Hobson 2006). Of particular relevance for origin assignment of migrant bats, we found significant differences in both δD_{fur} variances of species caught at the same site (*L. borealis* and *M. lucifugus* from Pinery Provincial Park and *L. cinereus* and *L. noctivagans* from Cypress Hills Interprovincial Park) and mean δD_{fur} values (*L. borealis* and *M. lucifugus* from Pinery Provincial Park). These clear inter-specific differences in the δD_{fur} profiles of resident bat populations supports the findings of other authors (Britzke *et al.* 2009; Torres-Dowdall *et al.* 2009) that pooling stable hydrogen isotope data from multiple species should be avoided.

Britzke *et al.* (2009) attributed species-specific differences in δD_{fur} values to differences in foraging ecology. They suggested that the δD_{fur} values of *M. lucifugus* across eastern North America did not correlate with local δD_{precip} as well as some other

species because *M. lucifugus* forage primarily on aquatic emergent insect species (*e.g.* Clare *et al.* 2011) and therefore will have δD_{tissue} values driven by the water in local ponds and streams as opposed to precipitation. Our results from Pinery Provincial Park support this assertion: with few exceptions, all *M. lucifugus* fur was depleted of deuterium relative to water of the OARC, over which the bats forage. In contrast, *L. borealis* typically have much more variable diets than *M. lucifugus*, consuming both terrestrial and aquatic insects (Clare *et al.* 2009). We found that δD_{fur} values from *L. borealis* captured at Pinery were both depleted of and enriched in D compared to OARC water, suggesting isotopic decoupling from the river eco-system.

The δD variability among insect prey could result in individual variation in bat δD_{fur} values through several mechanisms. First, individual bats may prefer specific insects; there is little evidence to support individual food preferences, although intra-specific cohorts (such as age, sex, or reproductive groups) have been recorded having significantly different diets (e.g. Anthony and Kunz 1977). Second, slight temporal variation in the time of moult among bats may lead to fur growth occurring when bats are consuming different diets. Insect availability varies considerably over the summer (e.g. Anthony and Kunz 1977) and bats are known to take advantage of mass insect emergences (e.g. Valdez and Cryan 2009).

A further potential source of variation may result from differential thermoregulatory strategies. Many species of temperate bats are well-documented using torpor on a daily basis, and the extent of torpor use may vary among individuals

and sex classes (Cryan and Wolf 2003). The effects of frequent torpor bouts on fur

growth and the stable hydrogen isotope composition of growing fur are currently

unknown and are topics worthy of further consideration.

2.4.1.4 Variation over time

The slight decline over the summer in the δD_{fur} values of adult *M. lucifugus* from

Pinery likely coincides with the annual moult of this species. Most accounts of bat fur

replacement suggest that moulting mainly occurs once a year, during the summer

(Constantine 1957, 1958; Dwyer 1963; Tiunov & Makarikova 2007; Quay 1970). As such,

females arriving at summer maternity colonies will have fur that was grown the previous

summer. That fur is then gradually replaced over the summer season (though the time

required for a complete moult is not well understood). *Myotis lucifugus* females are

philopatric (Dubois and Monson 2007) and so fur samples taken at the beginning of the

summer (grown in the previous year) should still be isotopically reflective of the location

of capture. In our data set, we detected one *M. lucifugus* outlier (adult female) at the

beginning of the season with a very high δD_{fur} value. We suggest that this bat moulted

the previous year at a location different from Pinery Provincial Park.

Stable isotope variation in tissues across years has been recorded among birds

captured at the same site in different years (Langin *et al.* 2007). If substantial inter-

annual variation exists in the mean δD_{fur} values of *M. lucifugus* colonies, then this

variation would be detected as a change in δD_{fur} values over the course of the summer,

as fur from the previous year was replaced by fur from the current year. Based on our

results, we feel that stable isotope studies requiring bat fur from summer locations

should attempt to standardize fur sample collection time and if the research questions

are specific to a given year, then sampling should occur near the end of the summer,

after all or most of the moult has occurred.

2.4.2 *Stable carbon and nitrogen isotope analyses*

2.4.2.1 Correlations among stable isotopes

We did not find consistent and widespread correlations between any of the

stable isotopes included in this study; however, some relationships existed within

individual populations/species. The strong correlations between δD_{fur} and $\delta^{13}C_{fur}$ values

in the populations of *L. borealis* and *L. noctivagans* may have resulted from variation in

the original source of dietary carbon and hydrogen in the plants at the base of the food

web. The correlations that we detected between $\delta^{15}N_{fur}$ and $\delta^{13}C_{fur}$ in the *L. noctivagans*

and the subadult *M. lucifugus* may have resulted from differences in dietary trophic level

(among insects) that were detected by both stable isotopes.

The lack of correlation between δD_{fur} and $\delta^{15}N_{fur}$ values in any of the populations was

unexpected, as Fraser *et al.* (2010) found preliminary evidence to suggest that trophic

differences in average bat diet may have led to species-specific differences in average

δD_{fur} values among neotropical bats. Fraser *et al.* (2010) studied bats occupying highly

divergent dietary niches (frugivores, nectarivores and sanguinivores) and it is possible

that the trophic differences among these dietary sources was greater than would be

found among insectivorous species. It is also possible that the $\delta^{15}N_{tissue}$ values of the bats

varied not only as a result of diet, but also as a result of individual-specific physiological factors. Specifically, individuals that are nutritionally stressed (Hobson *et al.* 1993), or water stressed (Kelly 2000) may have tissues enriched in [15]N (*cf.* Kempster *et al.* 2007; Smith *et al.* 2010). Voigt and Matt (2004) show that dietary nitrogen stress can lead to increased ^{15}N enrichment in two species of nectar-feeding bats. If the bats included in this study were experiencing any of the stressors listed above, then their $\delta^{15}N_{fur}$ values may not have been an accurate indicator of dietary trophic level at the time of fur growth. In either case, the presence of correlations between the stable isotope ratios of different elements within resident populations of some species of bats may serve as a useful indicator of population mixing, as long as comparisons are made within species.

2.4.2.2 Distinguishing among populations

We had good success using δD_{fur}, $\delta^{13}C_{fur}$, and $\delta^{15}N_{fur}$ values to assign individual *M. lucifugus* to their original colonies. Given how geographically distinct the Kananaskis (Alberta) population is from the Ontario populations, it was not surprising that these individuals were perfectly assigned. The Kananaskis site was both farther north and at a higher elevation than the Ontario sites. Increasing latitude and elevation both correlate to increasingly negative δD_{precip} values (Poage and Chamberlain 2001), which should lead to more negative δD_{fur} values. Similarly, plants and subsequently animals (Bowen and Revenaugh 2003; Körner *et al.* 1988; Hobson *et al.* 2003) at higher elevations are more depleted of ^{13}C than plants and animals at lower elevations.

The high assignment success between the populations at Pinery and Benmiller is more surprising, particularly given the importance of the stable hydrogen isotope results in distinguishing among the populations. The Benmiller roost is slightly north of the Pinery roost and the predicted mean growing season δD_{precip} values at each site are very similar (Pinery=-50‰; Benmiller=-52‰ [Bowen 2010; Bowen and Revenaugh 2003]), yet the fur of the *M. lucifugus* from Pinery is more enriched in D than the fur of the bats from Benmiller. The main underlying assumption of using stable hydrogen isotope analysis to answer questions about migratory origin is that the δD values of the tissues sampled are representative of the mean growing season δD_{precip} values at the location of capture. Here, we see two populations from locations with very similar δD_{precip} values and quite different δD_{fur} profiles. As described earlier, Britzke *et al.* (2009) found that fur samples taken from *M. lucifugus* during the moult period were not as isotopically representative of the δD_{precip} values at their sampling location or of sampling latitude, as was the case with some other species. They suggested that the δD_{fur} values of *M. lucifugus* were primarily driven by the δD values of the body of water over which they forage, as both the bats' drinking water and insect prey would primarily originate from that body of water. Our results further support this, as a late summer water sample taken from the Benmiller River (BR; the water body nearest to the Benmiller colony) was substantially lower than any of the samples taken from the Old Ausable River Channel (OARC) at Pinery (δD_{BR} = -80‰; mean δD_{OARC}=-59±1‰).

We observed within-population δD_{fur} variance in *M. lucifugus* that was relatively constrained (as compared to other species). Significant δD_{fur} differences among nearby populations suggests that the high intra-latitudinal variation in δD_{fur} values from *M. lucifugus* detected by Britzke *et al.* (2009) is the result of differences among populations at the same latitude, as opposed to high variance within populations.

2.4.3   *Nursing young*

2.4.3.1 Nursing young

Depletion of D in the fur of dependent young compared to their mothers is consistent with our findings from the whole group age comparisons, where the subadult age groups had fur depleted of D relative to the adult age groups. However, we detected no difference in δD_{fur} values between age groups in the *M. lucifugus* population at Benmiller when we conducted an unpaired analysis. It was only when we compared matched mothers and pups that we detected a difference. The fact that we did not detect an age difference at the population level, but did detect one when comparing matched pairs suggests that small but systematic age differences in δD_{fur} values may exist between adults and subadults in other species that we studied using a whole group comparison, but that in some cases, that difference was too small to be detected within the context of the overall population variation.

Our observation of ^{15}N enrichment in fur from adult *M. lucifugus* compared to the fur of dependent pups supports a growing body of literature showing ^{15}N enrichment in dependent young tissues compared to their mothers (Jenkins *et al.* 2001;

Polischuk *et al.* 2001; Sare *et al.* 2005). The mean difference that we reported of

0.68±0.57‰ between pup and mother $\delta^{15}N_{fur}$ values was very similar to the mean

interspecific difference between mother and offspring plasma values reported by

Jenkins *et al.* (2001) and less than that reported for fur from mother and offspring voles

(Sare *et al.* 2005). Despite the fact that we did not detect a significant difference

between mother and pup $\delta^{13}C_{fur}$ values, seven of nine pups had fur that was depleted of

^{13}C compared to their mothers, which supports previous findings.

In interpreting our findings about stable isotope differences between adult and

subadult bat fur, it is important to consider the temporal offset in the time of fur growth

between mothers and pups (Hobson *et al.* 2000). Some studies of bat moulting have

shown that reproductive females may arrest moulting during lactation (Dwyer 1963) or

not start until late in the summer (Constantine 1957, 1958), while others have records of

some females engaging in moulting throughout the entire summer (Tiunov and

Makarikova 2007). Previous evidence suggests that *M. lucifugus* are highly loyal to their

summer colony sites (Dubois and Monson 2007), so adult females likely spent previous

summers at the site of capture. However, the adult maternal fur that we sampled may

have been grown in the summer preceding sampling or may be a mixture of previous

and current fur growth, while the pup fur was likely grown only a few weeks before the

sampling date. In our comparison of the two, we are assuming no inter-annual variation

among average δD_{fur} values, which is unlikely. However, in the populations where we

detected isotopic differences between adults and subadults/pups, our results were

consistent across three populations at two locations (deuterium) as well as with previous literature (nitrogen) (Jenkins *et al.* 2001; Polischuk *et al.* 2001; Sare *et al.* 2005), supporting that the effect we observed is a real age-related difference and not simply an artefact of inter-annual variation and time of fur growth.

2.4.4   Summary & recommendations for sampling

Significant differences in δD_{fur} composition existed within and among individuals, age classes, populations, species, and over time. These differences included variation in δD_{fur} mean values and variance, were present in some but not all groups that we studied, and were likely the result of differences in diet, moulting, and individual physiology. The δD_{fur} values correlated positively with $\delta^{13}C_{fur}$ values in the populations of *L. noctivagans* and *L. borealis*, and $\delta^{15}N_{fur}$ correlated negatively with $\delta^{13}C_{fur}$ in the *L. noctivagans* and subadult Benmiller *M. lucifugus* populations. There was no relationship between δD_{fur} and $\delta^{15}N_{fur}$ in any of the populations. Using a combination of stable carbon, nitrogen and hydrogen isotope analyses, it was possible to distinguish among individuals of the same species that were sampled from nearby roosts.

Understanding natural isotopic variation in the fur of resident bats is crucial when researching bat migration. This information can inform sampling design to reduce unnecessary error and increase precision of migrant assignment. Based on our findings, we recommend that researchers conducting bat research using stable isotope analyses of fur standardize their sampling and analysis procedures in the following four ways: (i) sample fur from a consistent location on each bat's body; (ii) if sampling to obtain

representative δD_{fur} values of summer residency sites, conduct sampling near the end of the summer in order to sample growth from the current moult; (iii) avoid pooling stable isotope data from multiple species or using δD_{fur} variation from one species as reference for another; and (iv) analyze data from adult and subadult age classes separately. Further, we suggest that smaller scale assignment of bats in individual colonies may be possible if multiple stable isotope tracers are used, and that the intraspecific correlations that we detected among the stable isotope compositions in the resident populations of some species could be used to test for population mixing.

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Chapter 3

3 A stable isotope investigation of the origins of fall migrant bats captured at a migration monitoring station in Ontario, Canada

3.1 Introduction

Many species of North American bats migrate (Fleming and Eby 2003) and some are latitudinal migrants, travelling south in the fall and north again in the spring (Cryan 2003; Cryan *et al.* 2004). Most evidence for latitudinal migration in North American bats consists of seasonal changes in observed abundance (Barclay 1984; Dzal *et al.* 2009; Geluso *et al.* 2004; Geluso 2006; Hooton 2010; but see Cryan 2003; Cryan *et al.* 2004), and more recently, peaks in mortality around wind energy facilities during the fall migration period (*e.g.,* Arnett *et al.* 2008). There is still much to be learned about the migratory origins, routes, connectivity and phenology of North American bats. The purpose of this study was to use stable isotope analysis to investigate the migratory origins of silver-haired bats (*Lasionycteris noctivagans*) and eastern red bats (*Lasiurus borealis*), two species of North American latitudinal migrants, captured during the fall migratory period at Long Point, Ontario, a site of high migrant activity (Dzal *et al.* 2009; Hooton 2010).

The spring and fall migration periods for migratory birds in North America are monitored at a series of Migration Monitoring Stations (MMSs) (Bird Studies Canada – Canadian Migration Monitoring Network website 2011). Migration monitoring data has been shown to reliably reflect bird population trends at more northern breeding sites

(Dunn *et al.* 1997), which can inform conservation decisions. It is valuable to understand the catchment areas of MMSs (the geographic breeding or wintering areas from which the captured migrants originate) in order to interpret migratory population data. Wassenaar and Hobson (2001) used stable isotope techniques to investigate the catchment area of two MMSs – Delta Marsh, Manitoba and Long Point, Ontario – for Swainson's Thrushes (*Catharus ustulatus*). They investigated whether Swainson's Thrushes captured at the MMSs during the migratory periods ranged from a variety of latitudes or were local, and they found evidence that birds captured during fall at both MMSs originated from broad boreal catchment areas. Similarly, Mazerolle *et al.* (2005) used stable isotope analysis of feathers to determine that White-throated Sparrows (*Zonotrichia albicollis*) captured at Delta Marsh, MB during the fall migration originated from latitudes spanning the entire western boreal forest.

MMSs are typically located in habitats that attract a broad diversity of migratory species at high seasonal concentrations and are commonly situated on land features that "funnel" migrants, such as coastal or peninsular areas (Hussell and Ralph 1998). There is substantial evidence that *L. noctivagans* and *L. borealis* bat activity increases substantially during the fall migration period at some MMSs (Delta Marsh - Barclay 1984; Barclay *et al.* 1988; Long Point - Dzal *et al.* 2009; Hooton 2010; McGuire *et al.* 2011, personal observation), suggesting that, like the birds captured at these sites, the bats are also migrants originating from a broad catchment area. Many latitudinal migratory bat species are very difficult to capture in parts of their range and bat populations in

northern Canada have received very little study. High concentrations of south-travelling

latitudinal migrants at Canadian MMSs could provide a unique opportunity to monitor

northern bat populations. However, little is known about the origins of migratory bat

species captured at these sites, limiting the biological information that can be gained

from long-term monitoring.

3.1.1   *Stable isotope techniques*

Stable isotope analysis of migratory animal tissues is a frequently used technique

for determining migrant origin (Hobson and Wassenaar 2008). Predictable

biogeographical patterns in stable isotope ratios are incorporated through food and

drink into animal tissues and can provide information about animal ecology. Predictable

latitudinal patterns in the stable hydrogen isotope composition of meteoric water have

made stable hydrogen isotope analysis of animal tissues, which reflect meteoric water

stable isotope composition, a useful approach for investigating long distance latitudinal

migration (Hobson and Wassenaar 2008). Stable carbon and nitrogen isotope analyses

are frequently used to investigate animal diet (both trophic level and dietary source)

(Kelly 2000), but can also be used to track local-scale movement among habitats (e.g.,

Torres-Dowdall 2009). Many studies have applied these techniques to investigate the

origins of birds captured during migration (*e.g.,* Atkinson *et al.* 2005; Kelly *et al.* 2002;

Kelly 2006; Langin *et al.* 2009; Mazerolle 2005; Mazerolle and Hobson 2007; Meehan *et

al.* 2001; Smith *et al.* 2003; Wassenaar and Hobson 2001).

Stable isotope compositions can also be used as an indicator of population mixing, that is, whether individuals captured at one location originated from the same or different (mixed) locations, as well as to investigate migratory timing. Hobson *et al.* (2004) interpreted a larger $\delta D_{feather}$ variance among second-year wood warblers (as compared to after-second-year wood warblers captured at the same breeding grounds) to conclude that second-year birds originated from more geographically diverse (natal) breeding grounds than after-second-year birds. Similarly, Møller *et al.* (2006) identified a strong correlation between feather $\delta^{13}C$ and $\delta^{15}N$ values in breeding populations of Barn Swallows at one site in the Ukraine. They did not find this correlation among breeding swallows from post-1986 Chernobyl, implying that this site is a sink population, populated mainly by immigrants (a mixed population). Several studies have suggested, based on stable hydrogen isotope analysis of feathers, that migratory timing of North American birds of the same species can vary across latitudes (Kelly *et al.* 2002; Kelly 2006; Langin *et al.* 2009; Mazerolle and Hobson 2007; Smith *et al.* 2003, but see Meehan *et al.* 2001).

Fewer researchers have used stable isotope techniques to investigate bat migration (but see Britzke *et al.* 2009; Cryan *et al.* 2004; Fleming *et al.* 1993; Fraser *et al.* 2010). However, there is consistent evidence that the stable hydrogen isotope composition of fur (δD_{fur}) from temperate North American bat species captured during the summer (the presumed time of fur growth) is representative of the stable hydrogen isotope composition of the local meteoric water (δD_{precip}). As a result, bat fur samples

collected during the summer typically correlate well with both local δD_{precip} values (Britzke *et al.* 2009; Cryan *et al.* 2004; Chapter 4 of this dissertation) and latitude (as δD_{precip} values vary predictably with latitude) (Britzke *et al.* 2009; Chapter 4). We know of no work that has documented this relationship for *L. noctivagans* specifically, but we assume that one exists. Britzke *et al.* (2009) reported the predicted relationship between latitude/meteoric water δD values and δD_{fur} values for female *L. borealis*, but there was an unexpected opposite relationship for males (at higher latitudes, males had lower average δD_{fur} values than at lower latitudes).

In Chapter 2 of this dissertation, I presented stable isotope data for summer resident populations of both *L. borealis* and *L. noctivagans.* These data quantified the extent of stable hydrogen isotope heterogeneity expected within resident populations and therefore can be used as comparison points for other populations consisting of individuals of unknown origin (*e.g.*, Møller *et al.* 2006). In Chapter 2, I found that the resident population of *L. borealis* had significantly higher δD_{fur} variation than the population of resident *L. noctivagans*. There was a strong correlation between $\delta^{13}C_{fur}$ and δD_{fur} values for both species. These samples were collected in late July and early August in an attempt to maximize that likelihood that the bats sampled were residents and not migrants (i.e. before the start of fall migration), and that the annual moult had occurred and the fur was representative of the location of capture.

3.1.2   Study site and species

Lasionycteris noctivagans ranges across North America, from the southern US

north to the Canadian boreal forest (Kunz 1982). *Lasiurus borealis* is found across part of

South America and through Eastern North America, north to the boreal forests of

Canada (Shump and Shump 1982; Dobbyn 1994). The northern extent of the Ontario

ranges of both species has not been well defined; although both species can be found at

the southern tip of James Bay (Dobbyn 1994), and at similar latitude sites to the east and

west (Kunz 1982; van Zyll de Jong 1985). Recent evidence may indicate summer activity

at mid-James Bay latitudes (D. Phoenix, pers. comm.). In eastern North America, both

species are found in Canada during the summer and at mid-latitudes and south during

the winter (Cryan 2003).

Long Point is a 35 km long sand spit that projects into Lake Erie and is a globally

important wildlife refuge and stopover site for migratory songbirds. Dzal *et al.* (2009)

reported that *L. noctivagans* activity, as indicated by capture records and acoustic

recordings, was low in June and very high in August and September, indicating fall

migratory activity. Hooton (2010) showed that *L. noctivagans* activity peaked during

autumn on Long Point, but not in the surrounding mainland, suggesting that migratory

bats funnel through Long Point as they cross Lake Erie. McGuire *et al.* (2011) provided

radio telemetry evidence for such behaviour by describing two waves of migratory *L.*

noctivagans during the fall migration period. They presented multi-year data for two

peaks in capture rates: the first centering on August 27 and the second on September

12. These observations support those made by Barclay (1984) that this species may migrate in waves.

There is less evidence that *L. borealis* migrate through Long Point. While it is possible to catch this species in low numbers during the late summer migration period (Dzal *et al.* 2009; Hooton 2010; personal observation), Dzal *et al.* (2009) did not find significant changes in acoustic activity of this species at Long Point between June and August. Hooton (2010) also did not find higher levels of *L.borealis* activity on Long Point compared to the surrounding area during the late summer but reported two late summer peaks in acoustic activity of this species in some mainland sites compared to Long Point.

3.1.3   *Hypothesis and predictions*

In the present study, we used a multi-element stable isotope approach to investigate the catchment area and effects of latitudinal origin on migration timing of two latitudinal migrants – *L. borealis* and *L.noctivagans* – captured at Long Point, Ontario during the fall migratory period. We hypothesized that these bats would have multiple origins, ranging from near the location of capture to the northern extent of the species' range. We investigated this hypothesis by testing the predictions that (i) total δD_{fur} variability (range and variance) would be greater among migrants than resident bats of the same species; and (ii) that there would be no correlation between δD_{fur} and $\delta^{13}C_{fur}$ values within the migratory group, unlike the resident populations, where a correlation existed for both species (Chapter 2). Further, we hypothesized that latitudinal origin

would influence the timing of bat migration and tested the predictions that (iii) mean

δD_{fur} composition would vary systematically with time during the fall migration period

and (iv) the first wave of migrant *L. noctivagans* would originate from a different

location than the second group and would have stable isotope compositions distinct

from that of the second wave.

3.2   Methods

3.2.1   Sample Collection and Analysis

We used mist nets to capture *L. noctivagans* and *L. borealis* from August 24 to

September 17, 2008 and from August 20 to September 16, 2009 at Long Point, Ontario

(42.58˚N, -80.03˚W). Fur samples were cut from the lower dorsal portion of each bat and

stored at room temperature in glass vials and plastic bags until analysis. All bats were

released at their location of capture.

Fur samples were soaked overnight in a solution of 2:1 chloroform:methanol and

then rinsed with the same solution. They were left to dry in a fume hood for more than

48 hours. Samples were weighed and analyzed for stable hydrogen, carbon and nitrogen

isotope compositions using the protocol outlined in Chapter 2. For silver-haired bats,

stable hydrogen isotope analyses were conducted on both 2008 and 2009 samples,

while stable carbon and nitrogen isotope analyses were conducted only on 2009

samples. Stable hydrogen, carbon and nitrogen isotope analyses were conducted on all

L. borealis samples. The average difference between the stable isotope compositions of

fur samples from the same individual (mean ± standard deviation) was 2±2‰

(hydrogen), 0.09±0.10‰ (carbon), and 0.15±0.12‰ (nitrogen).

3.2.2   Statistical methods

All statistical analyses were conducted using IBM SPSS (SPSS Inc. 2011). Data

from summer populations of *L. noctivagans* (Cypress Hills, SK) and *L. borealis (*Pinery

Provincial Park, ON) (both included in Chapter 2) were used as species-specific resident

comparisons to the data collected from the migrants in the present study. All means are

reported ± one standard deviation. Hereafter, bats captured at Cypress Hills and Pinery

will be referred to as resident bats and the bats captured at Long Point as migrant bats.

We used independent sample t-tests or Mann-Whitney U tests in combination

with Levene's tests for homogeneity of variance to determine if there were differences

in stable isotope compositions between sampling years and age classes for both species.

When no differences existed, we pooled the data and then compared the fur δ^{13}C, δ^{15}N,

and δD variances between the migrant and resident populations using Levene's tests for

homogeneity of variance. We were able to pool the stable isotope compositions of the

migrant silver-haired bats from both years and age classes as well as the data from

resident eastern red bats of both age classes.

Data from Chapter 2 indicated that fur from resident subadult silver-haired bats

was depleted of D relative to adult fur and that there was no difference in variance

between the age classes. We calculated the difference between the median values of

the adult and subadult δD_{fur} values, which was then added to each of the subadult δD_{fur}

values. Having normalized the δD_{fur} values from resident adults and subadults, we also

pooled those age classes for the site and compared those data to the migrant

population. There were differences in the distribution and mean δD_{fur} values of the

migrant eastern red bats between 2008 and 2009, so we considered the years

separately.

Results from Chapter 2 indicated that there was a significant correlation between

δD_{fur} and $\delta^{13}C_{fur}$ values of adult resident silver-haired bats and eastern red bats. We

conducted Pearson's correlations on the δD_{fur} and $\delta^{13}C_{fur}$ values for the migrant silver-

haired and eastern red bat populations and then tested for a difference between

resident and migrant population Pearson's correlation coefficients for each species using

the methods described in Sokal and Rohlf (1981) (following the methods of Møller *et al.*

2006).

3.2.3   Statistical testing for latitudinal variation in migration timing

To assess latitudinal variation in migration timing of migrant *L. noctivagans*, we

used a two-way ANCOVA with date as a covariate to test for age and sex-specific

differences in δD_{fur} values in 2009. After detecting no differences between either age or

sex groups, we pooled all 2009 migrant *L. noctivagans* data and used a simple linear

regression to test for a systematic change in δD_{fur} values over the migratory period. We

also conducted a simple linear regression on the δD_{fur} values of the 2008 migrant *L.

noctivagans* and all of the migrant *L. borealis* over the migratory period, but sample size

precluded an investigation of age and sex effects. We used a canonical discriminant

function analysis (including δD_{fur}, $\delta^{13}C_{fur}$, and $\delta^{15}N_{fur}$ values as variables) to test for

isotopic differences between the first and second wave of 2009 migrant *L. noctivagans*.

3.3   Results

3.3.1   Sampling

Fur samples were collected from 14 *L. noctivagans* and 8 *L. borealis* in 2008 and

58 *L. noctivagans* and 7 *L. borealis* in 2009 (Appendix C). Stable hydrogen isotope

analyses were conducted on all samples and stable carbon and nitrogen isotope analyses

were conducted on all *L. borealis* samples and 28 of the 2009 *L. noctivagans* samples.

3.3.2   Comparison of δD, $\delta^{13}C$ and $\delta^{15}N$ variance

The migrant *L. noctivagans* population had a smaller range of δD values and a

greater range of $\delta^{13}C$ and $\delta^{15}N$ values than the resident population but only the

variance of $\delta^{13}C$ values was significantly different (the range of δ values are denoted as

$\Delta\delta$) (migrant ranges: $\Delta\delta D=33‰$, $\Delta\delta^{13}C=6.9‰$, $\Delta\delta^{15}N=5.2‰$; resident ranges: $\Delta\delta D=57‰$,

$\Delta\delta^{13}C=3.1‰$, $\Delta\delta^{15}N=2.0‰$; Levene's test: δD – $F=3.543$, $df=1,99$, $p=0.063$; $\delta^{13}C$ –

$F=14.174$, $df=1,53$, $p<0.001$; $\delta^{15}N$ – $F=3.373$, $df=1,53$, $p=0.072$) (Figure 3.1a).

Among the migrant *L. borealis*, the distribution of the δD_{fur} values was unimodal

in 2008 and bimodal in 2009 (Figure 3.1b). The 2008 migrant *L. borealis* population had a

much smaller range of δD_{fur} values than did the 2009 migrant or resident populations.

All three populations had similar ranges of $\delta^{15}N_{fur}$ values, and the 2009 migrant
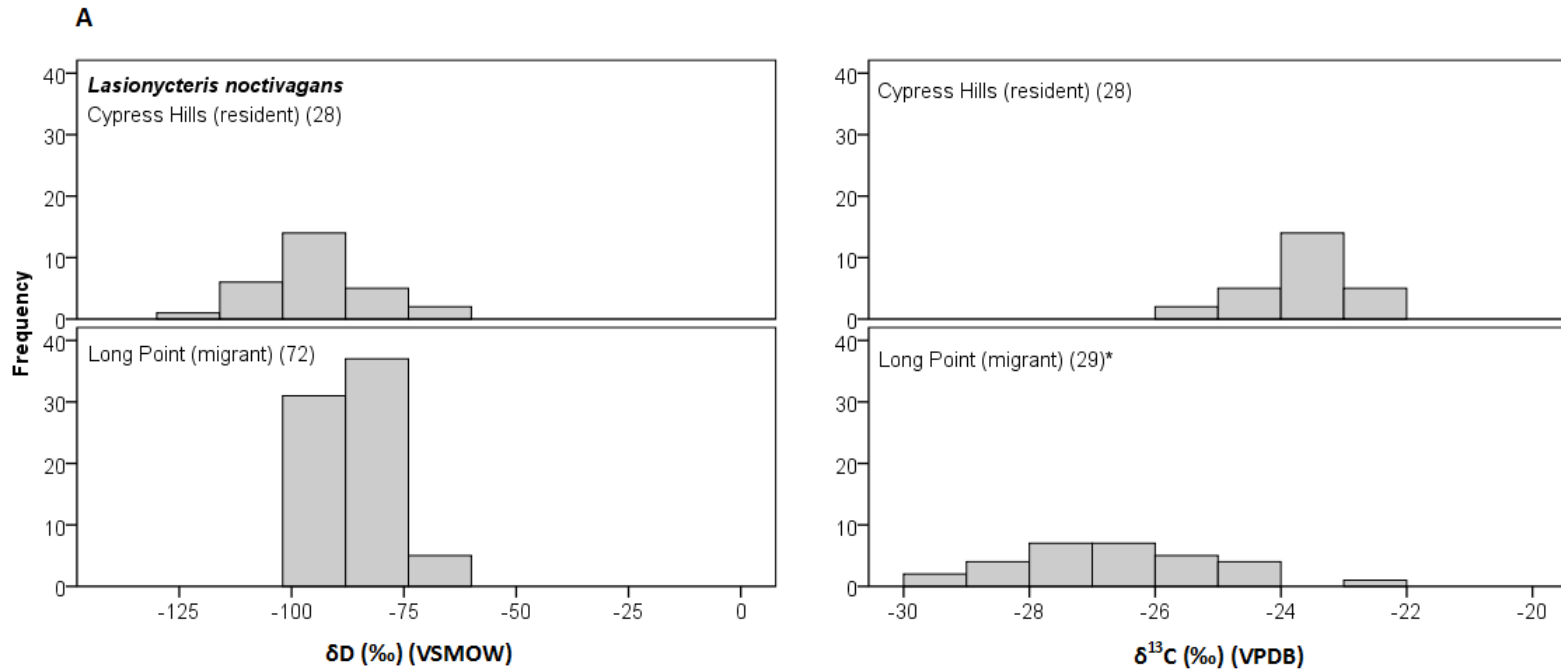
Figure 3.1. Frequency histograms of δD$_{fur}$ and δ^{13}C$_{fur}$ variation for migrant and summer resident populations of (a) silver-haired bats (*Lasionycteris noctivagans*) and (b) eastern red bats (*Lasiurus borealis*) (following page).

Significant differences in variance between the migratory and resident populations are indicated with an asterisk (*) next to the migrant population. Sample sizes are indicated in parentheses.
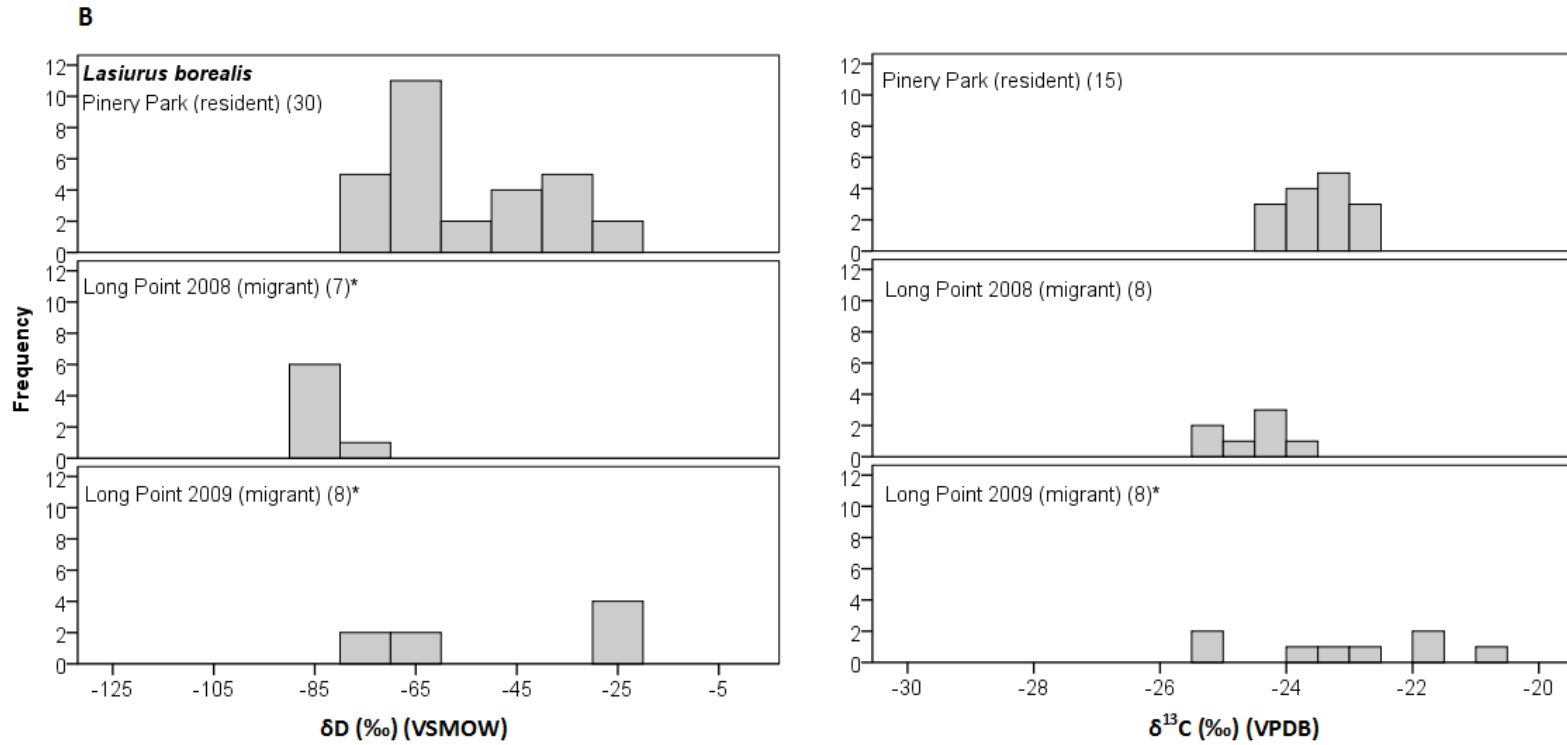
Figure 3.1

population had a larger range of $\delta^{13}C_{fur}$ values than did the other two populations (resident ranges: $\Delta\delta D=54‰$, $\Delta\delta^{13}C=1.8‰$, $\Delta\delta^{15}N=3.8‰$; migrant (2008) ranges: $\Delta\delta D=7.1‰$, $\Delta\delta^{13}C=1.8‰$, $\Delta\delta^{15}N=4.8‰$; migrant (2009) ranges: $\Delta\delta D=57‰$, $\Delta\delta^{13}C=4.5‰$, $\Delta\delta^{15}N=4.0‰$).

Migrant *L. borealis* captured during 2008 had smaller δD_{fur} variance than those captured from the resident population, while those captured in 2009 had greater variance and a bimodal distribution (Levene's test: 2008 – *F*=18.477*, df*=1,34*, p*<0.001; 2009 – *F*=12.930*, df*=1,35*, p*=0.001). There was no difference in $\delta^{15}N_{fur}$ variance between resident and migrant populations in either 2008 or 2009, and $\delta^{13}C_{fur}$ variation was not different between residents and migrants in 2008 and greater among migrant bats than residents in 2009 (Levene's test for homogeneity of variance: in 2008, $\delta^{13}C$ – *F*=0.312*, df*=1,19*, p*=0.583; $\delta^{15}N$ – *F*=0.387, *df*=1,19, *p*=0.541; in 2009, $\delta^{13}C$ – *F*=10.306*, df*=1,20*, p*=0.004; $\delta^{15}N$ – *F*=0.363, *df*=1,20, *p*=0.554) (Figure 3.1b).

3.3.3   *Comparison of $\delta D/\delta^{13}C$ correlations*

In Chapter 2, we reported a significant correlation between the δD_{fur} and $\delta^{13}C_{fur}$ values of the adult resident *L. noctivagans* and all *L. borealis* (Pearson's correlation coefficients: resident *L. noctivagans* – *F*=0.509, *df*= 20 *p*=0.022; resident *L. borealis* – *F*=0.767, *df*=18, *p*<0.001). There was no correlation between the δD_{fur} and $\delta^{13}C_{fur}$ values of the total migrant *L. noctivagans* (Pearson's correlation: *F*=0.315, *df*=30, *p*=0.090). However, we identified two individuals that appeared to be outliers. When these

individuals were removed from the data set, there was a significant correlation between the δD_{fur} and $\delta^{13}C_{fur}$ values (Pearson's correlation: $F=0.585$, $df=28$, $p=0.001$). After removing the two migrant outliers, there was no difference between the correlation coefficients of the migrant and resident *L. noctivagans* populations ($t=0.24$, $p=0.810$) (Sokal and Rohlf 1981) (Figure 3.2).

There was also a $\delta D_{fur}/\delta^{13}C_{fur}$ correlation among the 2009 migrant *L. borealis*, but not the 2008 bats (Pearson's Correlation Coefficient: 2008 – $F=0.534$, $df=7$, $p=0.217$; 2009 – $F=0.844$, $df=8$, $p=0.005$). There was no difference in correlation coefficients between the resident and 2009 migrant *L. borealis* populations (2009 - $t=-0.260$, $p=0.8026$) (Figure 3.3).

3.3.4   Latitudinal variation in migration timing

There were no age- or sex-related differences in the mean δD_{fur} values of migrant *L. noctivagans* (two-way ANCOVA: Age – $F=0.006$, $p=0.939$; Sex - $F=0.739$, $p=0.395$; Julian date - $F=0.882$, $p=0.577$) and no interactions among groups. There was no change in δD_{fur} values over the length of the fall migratory period for either species in either year (Simple linear regression: *L. noctivagans* (2008) - $r^2=0.212$, $F=3.237$, $p=0.097$, $df=13$; (2009) – $r^2=0.003$, $F=0.146$, $p=0.704$, $df=58$; *L. borealis* (2008) - $r^2=0.482$, $F=1.513$, $df=6$, $p=0.273$; (2009) $r^2=0.000$, $F=0.000$, $df=7$, $p=0.999$) (Figure 3.4).

Lasionycteris noctivagans
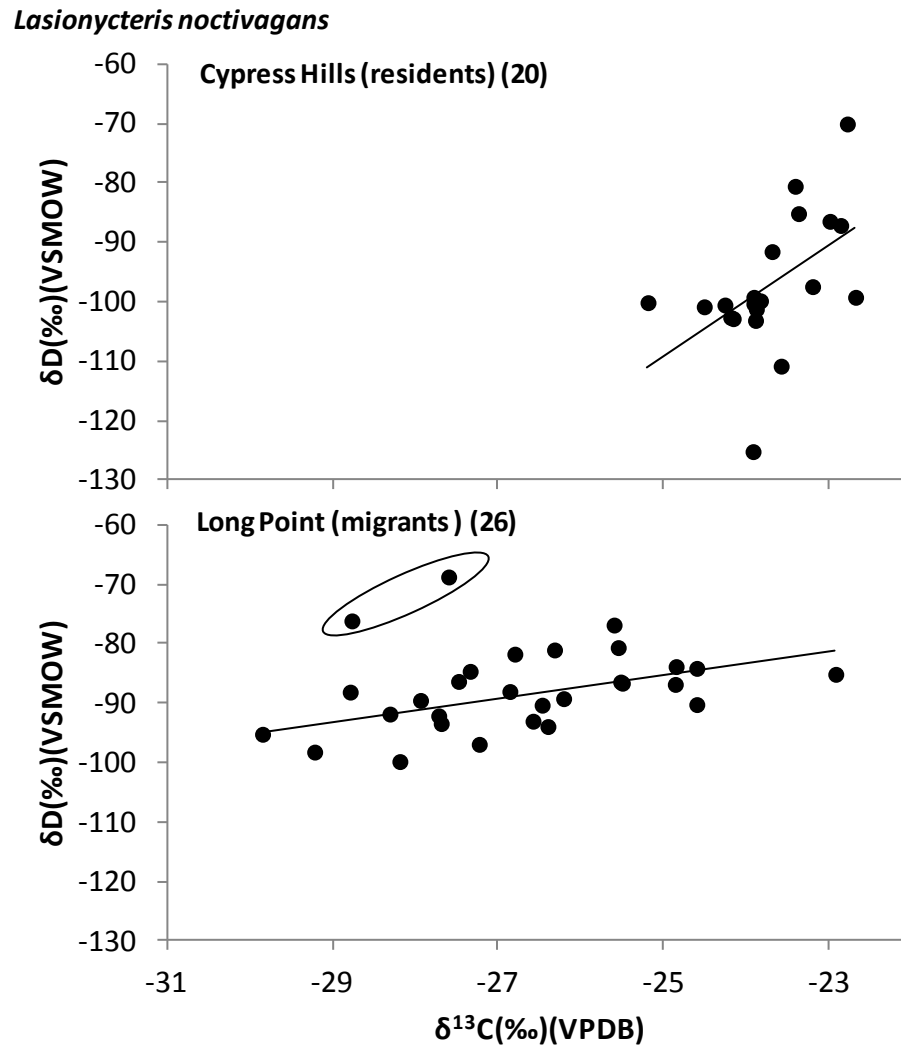
Figure 3.2. Relationship between the stable hydrogen and carbon isotope compositions of fur from migrant and resident silver-haired bats (*Lasionycteris noctivagans*).

There was a positive correlation between δD_{fur} and $\delta^{13}C_{fur}$ values for both resident and migrant *L. noctivagans.* Two outliers from the migrant silver-haired bat population are indicated by the ellipse and were not included in the correlation. Correlation coefficients did not differ between resident and migrant populations. Sample sizes indicated in parentheses.
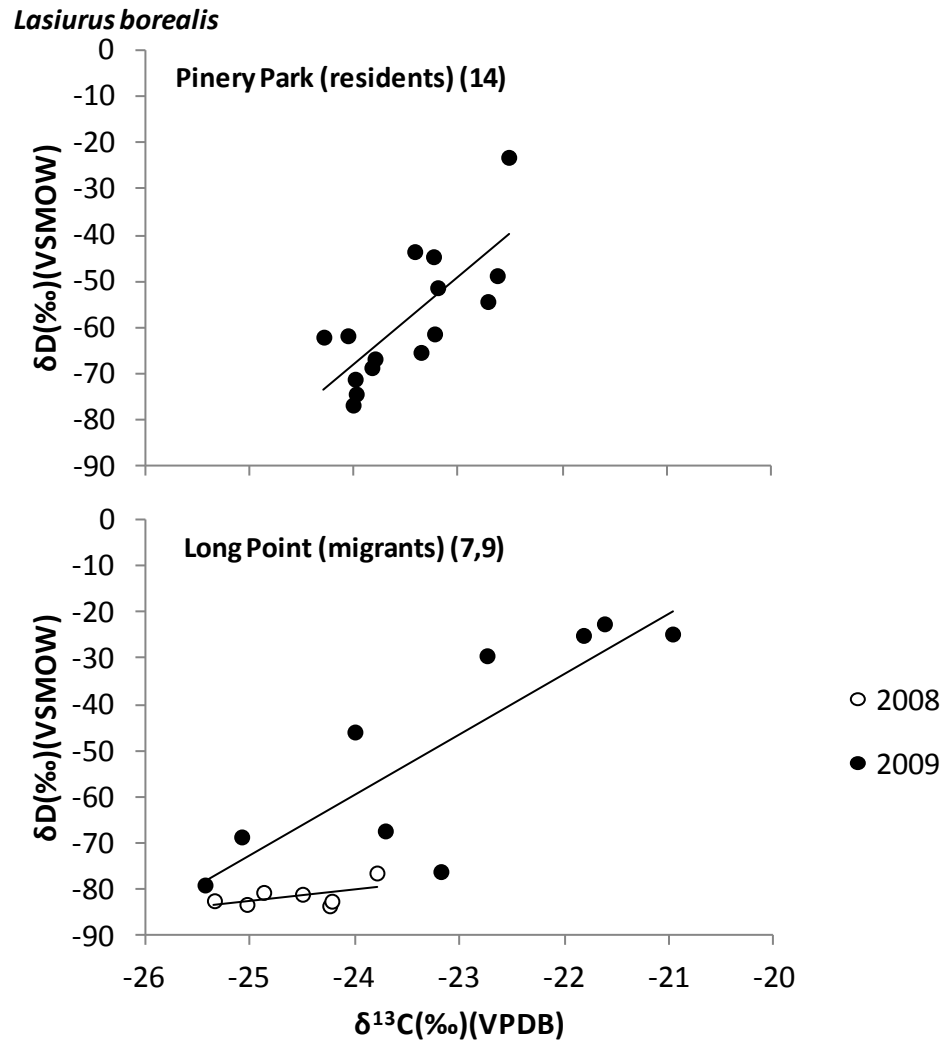
Figure 3.3. Relationship between the stable hydrogen and carbon isotope compositions of fur from migrant and resident eastern red bats (*Lasiurus borealis*).

There was a positive correlation between δD_{fur} and $\delta^{13}C_{fur}$ values for the resident and 2009 migrant *L. borealis,* but not for the 2008 migrants. Correlation coefficients did not differ between the resident and migrant populations in 2009. Sample sizes indicated in parentheses.

(A) *Lasionycteris noctivagans*

(B) *Lasiurus borealis*
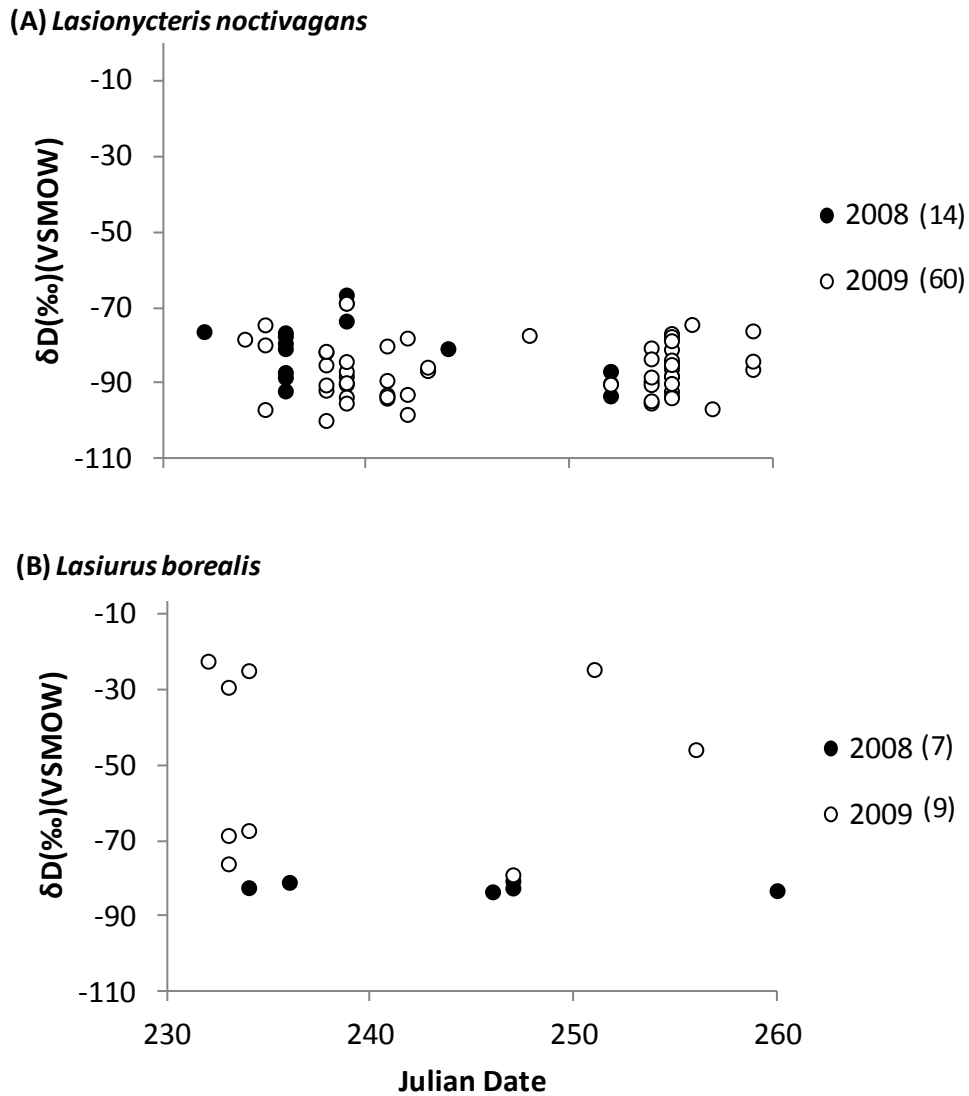
Figure 3.4. Stable hydrogen isotope composition of fur from migrant silver-haired bats (*Lasionycteris noctivagans*) and eastern red bats (*Lasiurus borealis*) over the length of the fall migratory period.

There was no change in δD_{fur} values over the course of the fall migratory period in either study year for either (a) *L. noctivagans* or (b) *L. borealis* captured at Long Point, Ontario. Sample sizes indicated in parentheses.

3.3.5   Origin of migratory waves

The discriminant function analysis was unable to discriminate between *L. noctivagans* from the two migratory waves based on their fur stable carbon, hydrogen and nitrogen isotope compositions (where individuals were assigned to either the first or second wave by date, as defined in McGuire *et al.* 2011) (Eigenvalue = 0.226; Chi-square=4.983; Wilks' λ = 0.816, *df*= 3, *p*=0.173).

3.4   Discussion

To our knowledge, this study is the first investigation into the catchment area of migratory bats passing though a Migration Monitoring Station. The stable isotope profile of the migrant *L. noctivagans* bat population was very similar to that of the reference resident population, providing no evidence of migrants from a wide range of latitudes. There was no isotopic evidence of systematic variation in migrant origin with any measure of time (over the migration season or between waves of migrants). There were substantial differences in the stable isotope profiles of the migrant *L. borealis* between 2008 and 2009, making it difficult to draw conclusions about the origin of the migratory bats and how this population compares to the resident population.

3.4.1   Silver-haired bats (Lasionycteris noctivagans)

The stable hydrogen isotope composition of the migrant *L. noctivagans* did not indicate a mixed population consisting of bats from a wide variety of latitudes. This conclusion is based on the assumption that the resident population *of L. noctivagans* is

isotopically representative of a typical resident population of the species. In Chapter 2, we showed homogeneity of variance in δD_{fur} values for three independent populations of little brown bats (*Myotis lucifugus)* from Ontario and Alberta in three different habitats and at different elevations, suggesting that there is some consistency among sites for at least this species. However, this has not been demonstrated specifically for *L. noctivagans*. Even if the resident bats were not isotopically representative of an average population for this species, the range of δD_{fur} values obtained among the migrant *L. noctivagans* ($\Delta\delta D_{fur}$=33‰) is smaller than the ranges that we reported for almost all of the populations of summer resident bats at single sites in Chapter 2 and is smaller than or similar to the ranges of δD_{fur} values for other species of bats sampled at the same latitude or from areas with the same mean growing season $\delta D_{precipitation}$ values (Britzke *et al.* 2009; Cryan *et al.* 2004; Chapter 4 of this dissertation). As a further comparison, Wassenaar and Hobson (2001) used the stable hydrogen isotope compositions of Swainson's thrushes migrating south through Long Point to indicate their origin from breeding sites throughout the boreal forest. The Swainson's thrushes had $\delta D_{feather}$ values ranging from -160‰ to -74‰, a range almost three times that which we observed among the migrant *L. noctivagans* (and compared to a range of 26‰ in a wild population of Swainson's thrushes that moulted in southern Quebec [Wassenaar and Hobson 2006]).

The $\delta^{13}C$ variance in *L. noctivagans* fur was much greater in the migrant population than the resident population, and the total range of migrant $\delta^{13}C_{fur}$ values

(>7‰) was greater than the ranges from several other studies reporting δ^{13}C values

from resident bats (Painter *et al.* 2009; York and Billings 2009, but see Voigt and Kelm

2006). The stable carbon isotope compositions of animal tissue vary largely as the result

of the dietary source carbon within a food web. Carbon from C_3, C_4, aquatic, or marine

eco-systems all have distinct δ^{13}C values (Tieszen *et al.* 1983), and as a result, tissue δ^{13}C

values may be proxies of variable habitat use or niche breadth. For instance, York and

Billings (2009) used variation in the $\delta^{13}C_{fur}$ values of neotropical bats in the genus

Carollia to indicate variation in foraging habitat. The stable carbon isotope compositions

of the Long Point *L. noctivagans* may suggest that they grew their fur while foraging in a

variety of habitats and while the range of δD_{fur} values does not indicate origins from a

wide range of latitudes, the high $\delta^{13}C_{fur}$ variation may indicate that the migrant

population consisted of bats from a mixture of locations.

The similarity in $\delta D_{fur} - \delta^{13}C_{fur}$ correlations that we detected between the

resident and migrant populations is further evidence that the Long Point individuals did

not originate from a wide range of latitudes. If the covariation in stable hydrogen and

carbon isotope compositions in the resident population were caused by dietary factors

such as dietary trophic level or source, then individuals from a range of latitudes would

obscure the correlation (*e.g.,* Møller *et al.* 2006).

A further step in investigating the origins of the migrant *L. noctivagans* would be

to create a calibration curve of moult time δD_{fur} values at all latitudes within the species'

northeastern range. Such a model would allow us to quantify the range of stable isotope

compositions that we could expect at sites ranging from Long Point to the northern

extent of the *L. noctivagans* range and to make a better estimate concerning the origins

of the bats captured at Long Point during the fall migration period. In the absence of

such a tool, we conclude that the stable hydrogen and carbon isotope compositions of *L.*

noctivagans from Long Point suggest a mixed population drawn from a variety of

habitats at similar latitudes.

Given the relative homogeneity of the stable hydrogen isotope composition of

the migrant *L. noctivagans*, it is not surprising that we did not find any temporal trend in

latitude of origin or variation among the migratory waves. Previous avian studies that

detected latitudinal trends in migration (*e.g.,* Kelly 2006; Kelly *et al.* 2002; Langin *et al.*

2009; Paxton *et al.* 2007) were conducted at more southern banding stations than Long

Point (New Mexico, California, Arizona and Louisiana). Replicating the methods of the

present study at a more southern location with high migratory bat activity would

provide a better continental-scale picture of the timing of bat migration across latitudes.

3.4.2   Eastern red bats (Lasiurus borealis)

The difference between the stable isotope compositions of the 2008 and 2009

migrant eastern red bat populations is striking. It is unclear why the bats would differ so

substantially between years. The narrow range of δD_{fur} and $\delta^{13}C_{fur}$ values for the 2008

bats certainly suggest their origin from one site or several nearby sites, while the greater

range in δD_{fur} and $\delta^{13}C_{fur}$ values in 2009 could represent bats originating from a wider

range of habitats and latitudes. Møller and Hobson (2004) observed similar bimodal data

in the stable carbon and nitrogen isotope compositions of winter-grown Barn Swallow feathers collected at a summer resident site and concluded that the summer population consisted of individuals migrating from two separate wintering grounds. In the present study, the range in migrant *L. borealis* δD_{fur} values in each of the two 2009 stable hydrogen isotope groups was far less than that of the resident population ($\Delta\delta D_{fur}$ values were 13‰ and 23‰, compared to 55‰, respectively). In fact, the total range of δD_{fur} values for the 2009 bats, including both stable hydrogen isotope groups, was fairly similar to that of the resident population ($\Delta\delta D_{fur}$ was 60‰ compared to 55‰), although the variance was significantly different. The bimodal distribution that we observed within the 2009 migrant *L. borealis* could be the result of a small sample size.

It is possible that the Pinery population was not isotopically representative of a typical population of resident *L. borealis.* One factor that could cause unusually high stable hydrogen isotope variation would be the presence of individuals that were not actually resident (for instance, highly mobile males). However, the majority of the adult bats included in the Pinery population were reproductive females, suggesting that these individuals truly were residents. Without a larger sample size of sites*,* it is not possible to know how isotopically representative the Pinery population was for the species.

The stable hydrogen isotope composition of *L. borealis* fur may not be as effective a proxy of the latitude of fur growth as it is for other species. Britzke *et al.* (2009) found that while female *L. borealis* δD_{fur} values decreased with increasing latitude (as expected), the opposite relationship existed among males of the same species. They

suggested that males may engage in non-typical migratory flights. While this may be the

case, the total range in *L. borealis* δD_{fur} values reported by Britzke *et al.* (2009) across 14

degrees of latitude was smaller than the range of δD_{fur} values that we detected within

the resident population at Pinery Provincial Park ($\Delta\delta D_{fur}$ values were approximately 45‰

compared to 55‰, respectively). Given the potential for extremely high *L. borealis* δD_{fur}

variation at one site, it is possible that the unexpected pattern in male *L. borealis* δD_{fur}

composition across latitudes (Britzke *et al.* 2009) could be an artefact of a relatively

small sample size. At this time, based on the findings of the present study and those of

Britzke *et al.* (2009) I feel that not enough is known about how environmental stable

isotopes are reflected in the fur of *L. borealis* to draw ecological conclusions about bat

behaviour.

3.4.3   Summary

Overall, we did not find stable isotope evidence to suggest that either the *L.*

noctivagans or *L. borealis* captured at Long Point during the fall migratory period were

originating from a wide range of latitudes or that latitude of origin varied over the

course of the fall migratory period or among temporal waves of migrants. The migrant

L. noctivagans population and the 2009 *L. borealis* population had greater $\delta^{13}C_{fur}$

variance than did their respective reference resident populations, suggesting that the

migrant bats were a mixed population originating from a variety of habitats. The stable

isotope compositions of the migratory *L. borealis* populations varied substantially

between years. In 2008, the δD_{fur} variance was apparently much lower than that of the

resident population, and points to the need for analysis of much larger populations before stable isotope data can be confidently used to interpret *L. borealis* ecology.

3.5   References

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Chapter 4

4   Evidence of latitudinal migration in tri-colored bats, *Perimyotis subflavus*[1]

4.1   Introduction

Many species of North American bats migrate and employ several strategies to do so. Some species are regional migrants, radiating annually from winter hibernation sites to summer sites, then travelling among swarming sites in the autumn (Davis and Hitchcock 1965; Fenton 1969; Rivers *et al.* 2006; Rodrigues and Palmeirim 2007). Bats engaging in this type of migration have been recorded travelling up to 500 km (Dubois and Monson 2007; Fenton 1969) and may move in any direction to hibernacula. Other species are latitudinal migrants, travelling south in the autumn and north in the spring (Cryan 2003; Cryan *et al.* 2004; Findley and Jones 1964). There is evidence that these migrants may travel > 2000 km one way during their annual movements (Cryan *et al.* 2004).

Tri-colored bats (*Perimyotis subflavus*; formerly included in genus *Pipistrellus* [Menu 1984; Hoofer *et al*. 2006]) are common in eastern North America ranging from Central America in the south to southern Canada in the north (Barbour and Davis 1969; Fujita and Kunz 1984). During summer they roost both in buildings (Allen 1921) and in

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[1] A version of this chapter has been submitted for publication (Appendix D). Citation: Fraser, E.E., McGuire, L.P., Eger, J.L., Longstaffe, F.J. and Fenton, M.B. Evidence of latitudinal migration in Tri-colored bats, *Perimyotis subflavus.* Submitted October 11, 2011 to *PLoS ONE* (submission #: PONE-D-11-20148v).

foliage (Poissant *et al.* 2010; Perry and Thill 2007; Veilleux *et al.* 2003). Females may

roost alone or in colonies, while males roost singly (Fujita and Kunz 1984; Perry and Thill

2007). In autumn, *P. subflavus* engage in swarming behavior, after which, they hibernate

in caves, abandoned mines and occasionally human-made structures (Davis 1964;

Goehring 1954; Griffin 1940; Kurta *et al*. 2007; Kurta and Teramino 1994; Sandel *et al*.

2001; Trombulak *et al*. 2001). There is little information about their movements among

summering grounds, swarming sites, and hibernacula (Cryan and Barclay 2009), but they

are currently believed to be a short-distance regional migrant (Bisson *et al.* 2009;

Fleming and Eby 2003; Fujita and Kunz 1984). Seasonal variation in abundance and sex

ratios *of P. subflavus* has led some authors to speculate that individuals migrate farther

distances than previously suspected (Cryan and Barclay 2009; LaVal and LaVal 1980) and

that this migration may be sex-specific (Davis 1959; Ferrara *et al.* 2008). Further, recent

studies have found an increase in *P. subflavus* activity during the autumn migration time

period (Dzal *et al.* 2009; Reynolds 2006). At some wind energy sites within the species'

range, *P. subflavus* are among the most frequently killed species and may account for up

to 25% of total bat mortality (Arnett *et al.* 2008), a much higher proportion than known

regional migrants. The bat species most frequently killed at wind turbines tend to roost

in trees and engage in long-distance latitudinal migration (Arnett *et al.* 2008; Cryan and

Barclay 2009).

Stable hydrogen isotope analysis is now a common tool used to learn about the

origin of migratory animals (Hobson 1999; West *et al.* 2006). There is a latitudinal

pattern in the stable hydrogen ratios of precipitation that is recorded in the tissues of

local animals, providing information about the latitudinal origin of a migrant. Several

authors have investigated tissue stable hydrogen isotope variability in bats across

latitudes (Britzke *et al.* 2009), and used this tool to investigate altitudinal migration

(Fraser *et al.* 2010) and to describe annual migration in hoary bats (*Lasiurus cinereus*)

(Cryan *et al.* 2004).

The purpose of the current study was to document the annual movements of *P.*

subflavus at a continental scale using stable hydrogen isotope analysis. We predicted

that the stable hydrogen isotope values of fur (δD_{fur}) from June-August, when the annual

moult is believed to occur (Cryan *et al.* 2004), would correlate closely with the latitude

of capture (Britzke *et al.* 2009), as well as the predicted growing season stable hydrogen

isotope values of the precipitation (δD_{precip}) at that location (Bowen and Revenaugh

2003; Cryan *et al.* 2004). Further, we predicted that the difference between δD_{fur} and

δD_{precip} at the site of capture would be smallest during summer months (when fur is

grown) and greater during winter (when the bats have migrated from the site of fur

growth).

4.2   Methods:

4.2.1   Sample collection and analysis:

We obtained 184 fur samples taken from the lower dorsal region of *P. subflavus* study

skins from four North American museum collections: the Royal Ontario Museum

(Toronto, ON), the Louisiana University Museum of Zoology (Baton Rouge, LA), the

Harvard Museum of Comparative Zoology (Cambridge, MA) and the Cornell Museum of

Vertebrates (Ithaca, NY). Museum specimens were collected between 1878 and 1986

during all seasons and across most of the species' range (Figure 4.1; Appendix D).

Fur samples were soaked overnight in 2:1 solution of chloroform:methanol

(Paritte and Kelly 2009), rinsed the next day in the same solution and then dried in a

fume hood for ≥ 48 hrs. Standards and samples were weighed into silver capsules (0.175

mg +/- 10 µg) with a 10% rate of duplication. Complex organic materials contain a

fraction of hydrogen that is readily exchangeable with ambient vapour at room

temperature. To correct for this uncontrolled exchange, we analyzed all samples

alongside five in-house fur standards with known non-exchangeable δD_n values using a

comparative equilibration approach (Wassenaar and Hobson 2003). After being

weighed, samples and standards were left to equilibrate with laboratory air for a

minimum of four days (Bowen *et al.* 2005). Samples were pyrolysed using a high

Temperature Conversion Elemental Analyzer (TC/EA) and analyzed using online

continuous-flow isotope ratio mass spectrometry (IRMS). Results are expressed as parts

per thousand (‰) relative to Vienna Standard Mean Ocean Water (VSMOW). Analytical

precision, based on repeated analyses of fur from the same individual bat during each

analysis, was less than 2‰ (1σ). The average (± standard deviation) difference between

duplicates of the same sample was 2±2‰.
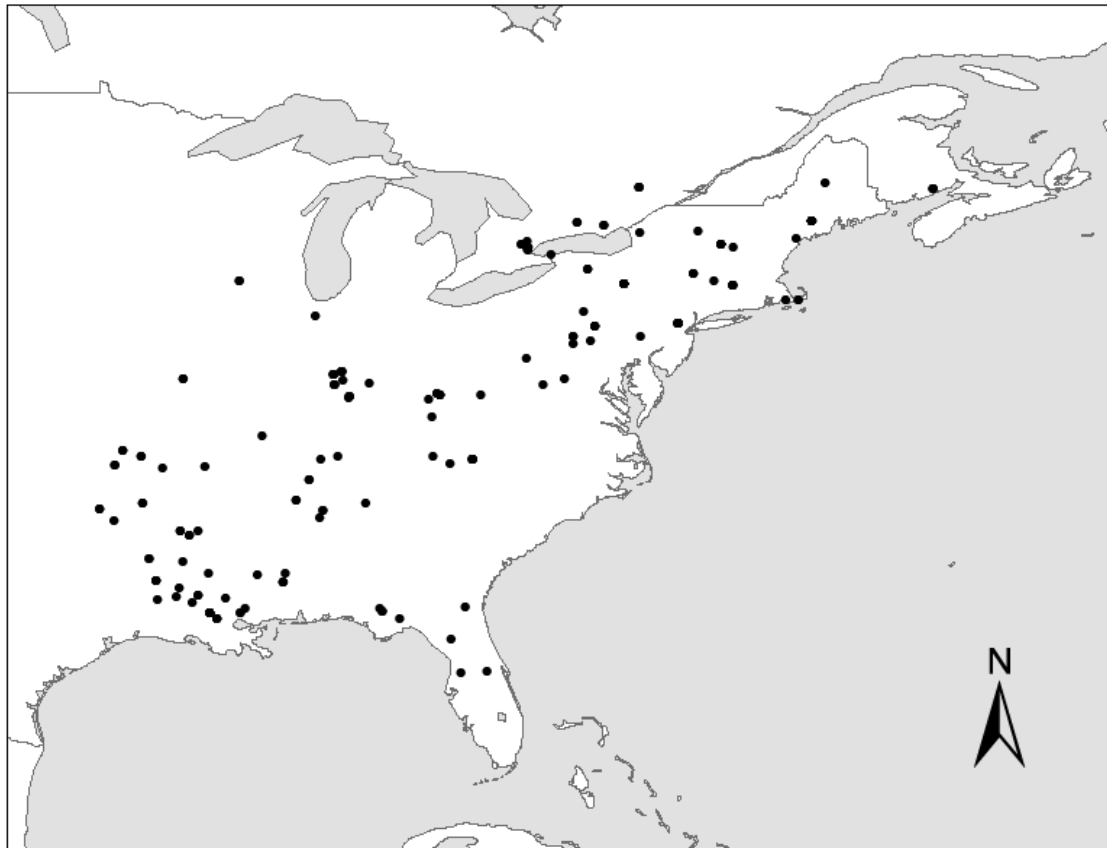
Figure 4.1. Tri-colored bat (*Perimyotis subflavus*) collection sites.

Dorsal fur samples from 184 *Perimyotis subflavus* museum study skins were sampled from individuals collected across most of the species' range. Each black dot represents a collection location; multiple individuals were collected from some locations.

4.2.2   Data analysis:

In some instances, GPS coordinates were available for specimen collection

locations from individual museum databases. When this was not the case, coordinates

were determined for the centroid of the county of collection using the USGS Geographic

Names Information System (http://geonames.usgs.gov/domestic/). Predicted growing

season δD_{precip} values were determined for the collection locality of each specimen using

the geospatial data available from waterisotopes.org (Bowen 2010; Bowen and

Revenaugh 2003).

We conducted analyses of males and females separately and first plotted the

difference between δD_{fur} and predicted growing season δD_{precip} at the location of

capture ($\Delta D_{fur\text{-}precip}$) for individual bats of both sexes against Julian date. Based on these

results, we visually determined the time when bats are at their location of moult as the

time period when $\Delta D_{fur\text{-}precip}$ was most constrained (similar to the approach taken by

Cryan *et al.* 2004). We then correlated both latitude (Britzke *et al.* 2009) and predicted

growing season δD_{precip} values (Bowen 2010; Bowen and Revenaugh 2003) with the

mean moult-time δD_{fur} values of bats from each sampling location (number of bats from

each location ranged from one to seven), using linear and quadratic regressions for both

predictors (latitude and precipitation). We used the equations of the male and female

δD_{fur} /δD_{precip} regression lines to calculate predicted δD_{fur} values for each individual

(based on the predicted growing season δD_{precip} value). In order to determine if there was isotopic evidence of latitudinal movement, and if so, if this behaviour was more prevalent in some parts of the species' range than others, we plotted the difference between δD_{fur} and predicted δD_{fur} ($\Delta D_{furactual-predict}$) of all bats against Julian date and of all non-moulting bats against latitude.

We calculated the approximate origin of the individual bat that was farthest from its location of fur growth by using the equation of the $\delta D_{fur}/\delta D_{precip}$ quadratic equation to calculate the predicted δD_{precip} value at the location of fur growth. Substantial variation exists in the δD_{fur} values of resident bats from the same location (which theoretically should be isotopically identical) (e.g. Langin *et al.* 2007; Torres-Dowdall *et al.* 2009). The expectation that one can estimate the δD_{precip} value at the location of fur growth based on the stable hydrogen isotope composition of one tissue sample is overly simplistic. Accordingly, to account for resident variation in δD_{fur} values, we referred to the sex-specific range of $\Delta D_{furactual-predict}$ values that we recorded during the moult period and used the equation of the $\delta D_{fur}/\delta D_{precip}$ correlation line to calculate the δD_{precip} value associated with the individual bat's δD_{fur} value ± 50% of the resident δD_{fur} variation. The results provide conservative estimates of the maximum and minimum δD_{precip} values at the location of fur growth according to the available data on resident variation in $\Delta D_{furactual-predict}$ values.

4.3   Results:

Male $\Delta D_{fur\text{-}precip}$ values were least variable during the summer months, indicating

that male *P. subflavus* moulted between June 23 and October 16 (though a period for

which we had no samples means that this end date may have been as early as

September 9). For females, there was no clear period of low variability that would

indicate a moult and then movement away from the moult location (Figure 4.2).

Hereafter, we refer to the moult period as the time when the bat is at its location of fur

replacement and define that time as being between June 23 and the period between

September 9 and October 16. This time period was indicated only by male bats and we

make the explicit assumption that the female moult timing is identical to that of males.

Both male and female moult period δD_{fur} values correlated significantly with

latitude and δD_{precip} (Figure 4.3). For each sex-predictor combination, the quadratic

regression fit better than a linear regression, as defined by the highest adjusted r^2 value,

and these are the relationships that we report. Male δD_{fur} values correlated significantly

with latitude (r^2=0.85; p<0.01) and δD_{precip} (r^2=0.86; p<0.01). Female δD_{fur} values

correlated significantly with latitude (r^2=0.73; p<0.01) and δD_{precip} (r^2=0.75; p<0.01).

Because latitude and δD_{precip} were almost equally effective at predicting δD_{fur}, we

followed the advice of Wunder and Norris (2008) and used the relationship between

known moult period δD_{fur} values and δD_{precip} to generate predicted δD_{fur} values for all

non-moult period bats.
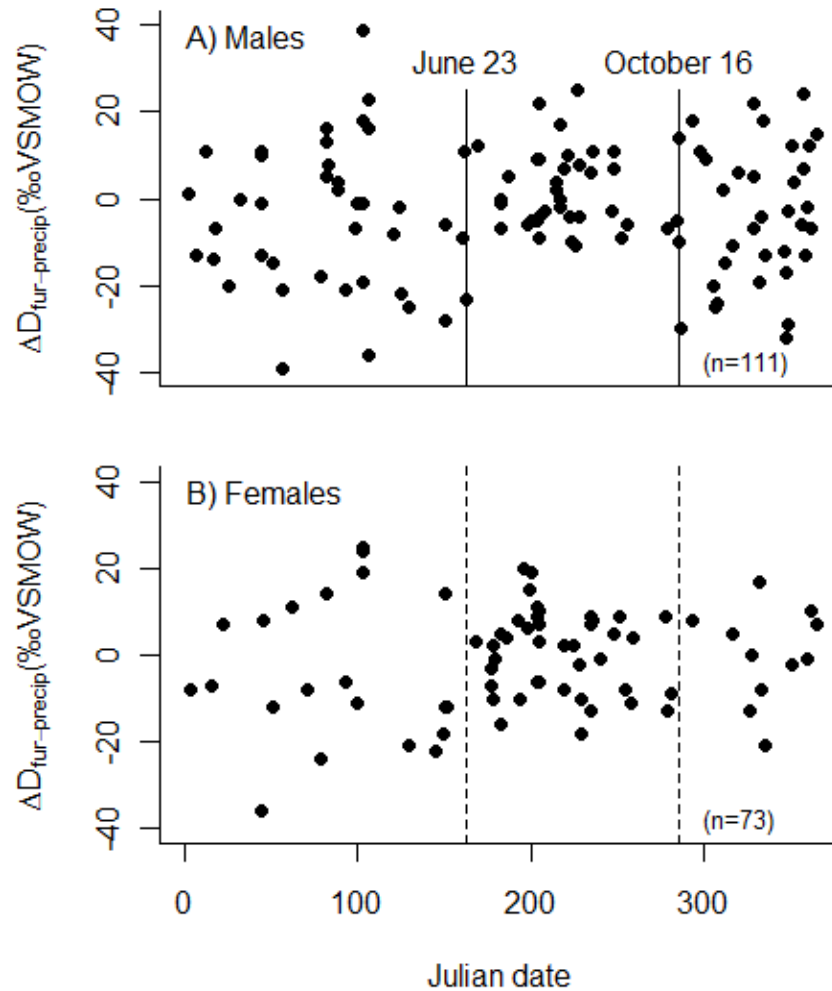
Figure 4.2. Differences between fur and local meteoric water stable hydrogen isotope compositions ($\Delta D_{fur\text{-}precip}$) for males and females across seasons.

The difference between δD_{fur} and δD_{precip} ($\Delta D_{fur\text{-}precip}$) for (a) males was smallest between June 23 and October 16, as indicated by two vertical lines representing the presumed moult period for males. (b) There was no clear trend in female $\Delta D_{fur\text{-}precip}$ values across seasons, so the male presumed moult period was applied to females (dashed lines). Sample sizes included in parentheses.
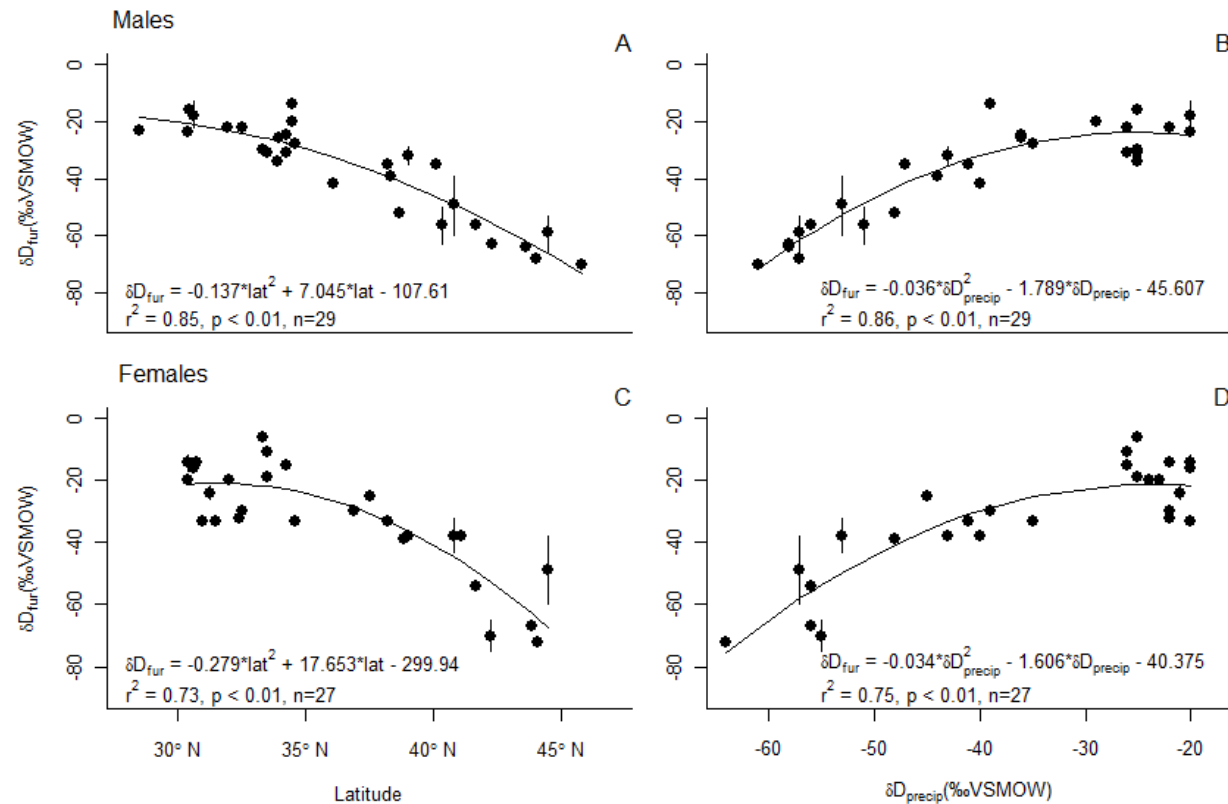
Figure 4.3. Sex-specific correlations between moult period δD$_{fur}$ and growing season δD$_{precip}$ or latitude at the collection site.

Male moult period δD$_{fur}$ values (site mean ± SD) correlated with latitude of capture (panel A) and local estimated growing season δD$_{precip}$ (panel B). The same was true for females (panels C and D). Number of individuals captured per site ranged from one to seven.

Our initial plot of $\Delta D_{fur\text{-}precip}$ against Julian date implicity assumed a linear relationship between δD_{fur} values and δD_{precip} values at all locations. Our data suggest that a quadratic curve better describes the relationship between δD_{fur} values and δD_{precip} values for both male and female bats. To correct for this, we used the sex-specific quadratic regression equations to calculate the predicted δD_{fur} value for each bat based on the estimated growing season δD_{precip} value at its location of capture (Bowen 2010; Bowen and Revenaugh 2003). We re-did the initial plot to show $\Delta D_{furactual\text{-}predict}$ against Julian date for both male and female bats (Figure 4.4).

During the male-defined moult period, the difference between maximum and minimum $\Delta D_{furacutal\text{-}predict}$ values was 35‰ for both males and females. During the non-moult period, the difference was 76‰ for males and 53‰ for females. Twenty-four of 73 males sampled during the presumed non-moult period had $\Delta D_{furactual\text{-}predict}$ values that were more negative than any observed during the moult period, indicating that these individuals were captured south of their location of fur growth. One individual showed evidence of northward movement. Five of 32 females sampled during the non-moult period had $\Delta D_{furactual\text{-}predict}$ values indicative of a more northern location of fur growth, although in general, these values were not as negative as those observed among the male migrants. Two females had a $\Delta D_{furactual\text{-}predict}$ value that may have indicated a slight northward movement.
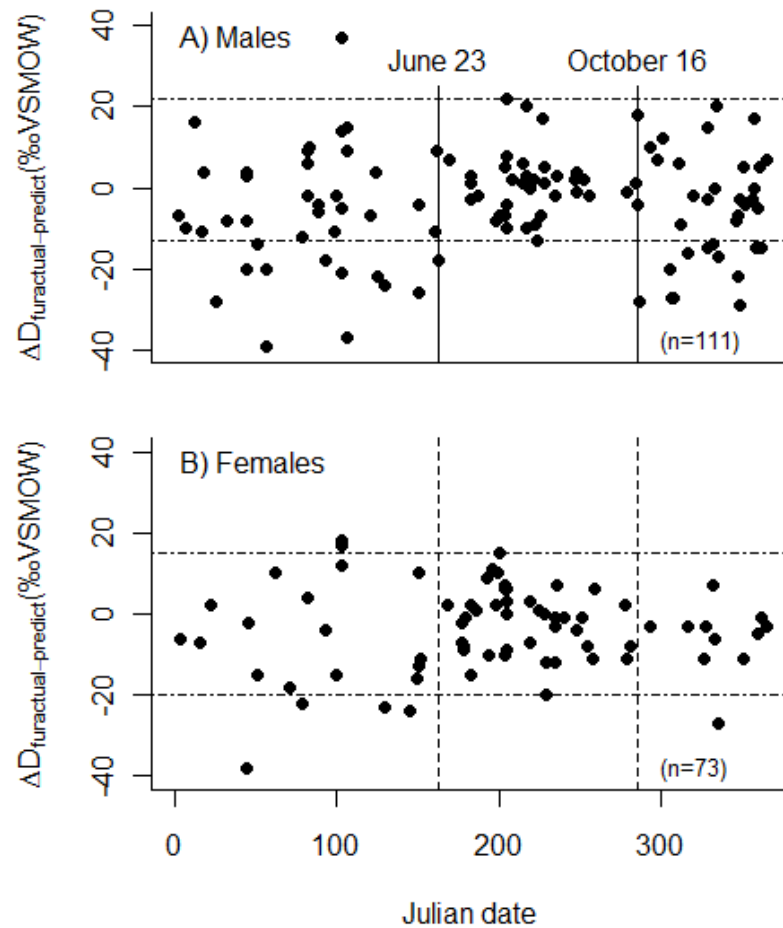
Figure 4.4. Differences between male and female actual and predicted fur stable hydrogen isotope compositions ($\Delta D_{\text{furactual-predict}}$) across seasons.

The range of variation in $\Delta D_{\text{furactual-predict}}$ values within the presumed moult period is indicated with horizontal dashed lines. Non-moult period individuals represented by dots below the horizontal band are assumed to have migrated from a more northern location. (a) 33% of non-moult period males and (b) 14% of non-moult period females appeared to be south of their location of fur growth. Sample sizes are indicated in parentheses.

For male bats, $\Delta D_{furactual-predict}$ values were most negative at high (>40˚) and low (<35˚) latitudes and closest to the range of moult period values at mid-latitudes (Figure 4.5), suggesting that high and low latitude individuals migrate south while mid-latitude males are more sedentary. The majority of the females with $\Delta D_{furactual-predict}$ values indicating they were south of their location of fur growth were captured at the northern end of the species' range.

The bat that was most distinct in isotopic composition from the precipitation at its location of capture was a male collected from southwestern Ontario (43.62 decimal degrees N, -80.13 decimal degrees W). This bat also was one of two that had the most negative δD_{fur} composition (-93‰) of all bats sampled, indicating the most northern point of fur growth/origin. Based on an extrapolation of the relationship that we established between δD_{precip} at the location of fur growth and δD_{fur}, this individual bat grew its fur at a location with a mean growing season δD_{precip} composition between -63‰ and -74‰ (Figure 4.6).

4.4   Discussion:

Our results indicate a fundamentally different picture of the annual migratory ecology of *P. subflavus* than has previously been assumed. *Perimyotis subflavus* visit swarming sites in autumn and hibernate during the winter (Fujita and Kunz 1984), sharing many characteristics with other species that have been documented to engage in a regional radiation pattern of migration from central hibernacula to summer
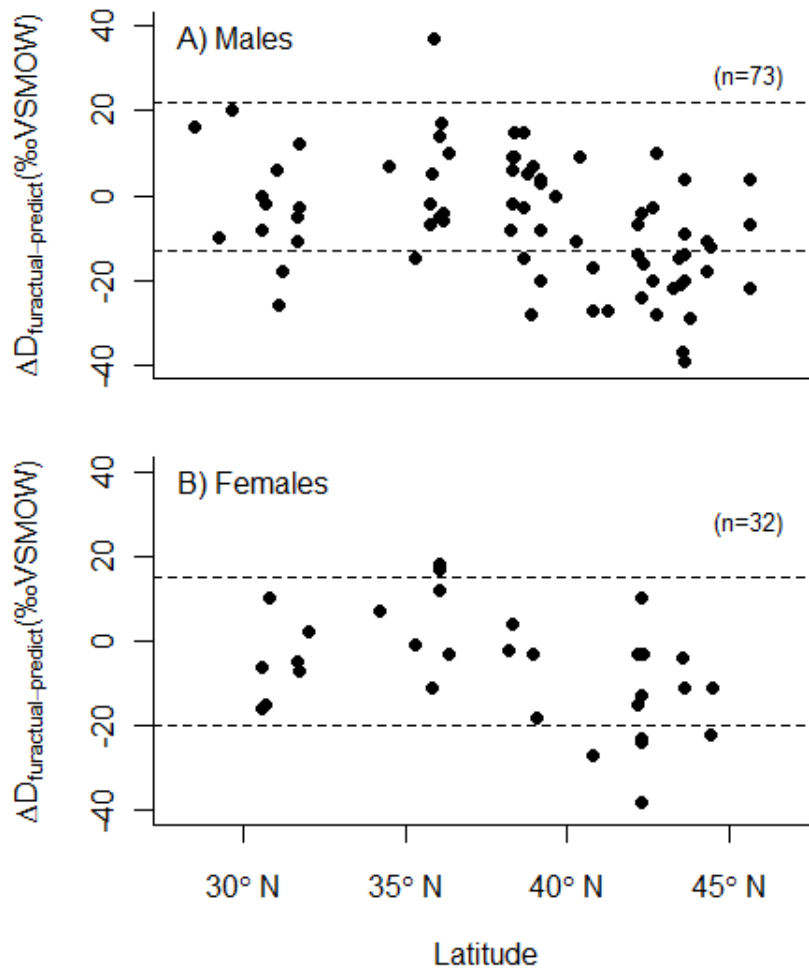
Figure 4.5. Differences between male and female non-moult period actual and predicted fur stable hydrogen isotope compositions ($\Delta D_{furactual-predict}$) across latitudes.

Non-moult period $\Delta D_{furactual-predict}$ values were more negative than moult period values at both northern and southern latitudes for (a) males (n=73) and only at northern latitudes for (b) females (n=32), indicating southern migration from the latitude of fur growth by these individuals. The range of moult period $\Delta D_{furactual-predict}$ values is indicated by horizontal dashed lines. Sample sizes indicated in parentheses.
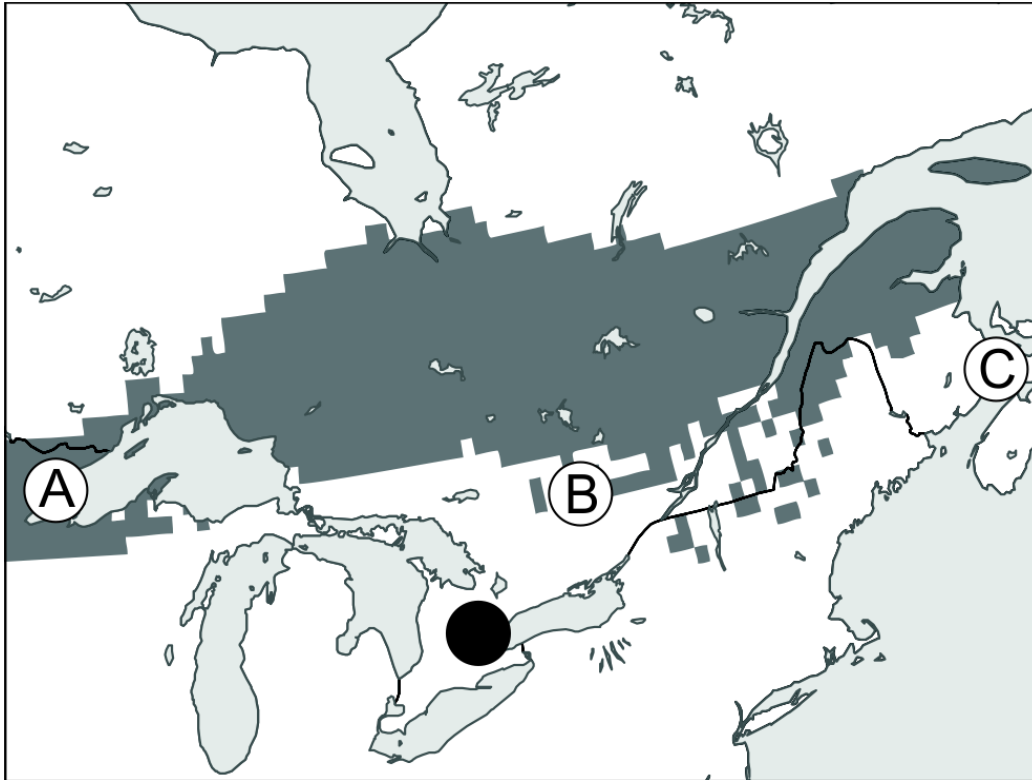
Figure 4.6. Range of potential origins of the individual with the most northern δD_{fur} signature.

The bat with the δD_{fur} signature indicating that it was from the most northern location (collection site indicated by black circle) was also the bat with the $\delta D_{furactual-predict}$ value suggesting that it had migrated the farthest. It likely originated at a location where the mean growing season δD_{precip} composition was between -63 and -74‰ (the area shaded in grey [Bowen 2010; Bowen and Revenaugh 2003]). The lettered points indicate the existing most northern records of *Perimyotis subflavus* (A=Knowles 1992; B=Fenton 1969; C=Broders *et al.* 2001).

maternity colonies (e.g., little brown bats (*Myotis lucifugus*); Davis and Hitchcock 1965;

Fenton 1969). However, 33% of males captured during the non-moult period (24

individuals) had δD_{fur} values indicating that they were south of their location of fur

growth and only 1 individual appeared to be north of the location of fur growth. Sixteen

percent of non-moult period females (5 individuals) appeared south of their location of

fur growth and only 6% (2 individuals) appeared to have been north of the location of

fur growth. Consequently we conclude that at least some *P. subflavus* of both sexes

engage in the type of latitudinal migration that is more typically associated with hoary

bats (*Lasiurus cinereus)*, eastern red bats *(Lasiurus borealis),* and silver-haired bats

(*Lasionycteris noctivagans)* (Cryan 2003), and that this behaviour is more common for

males than for females.

4.4.1   *Sex-biased migration*

Sex-biased migratory behaviour is common among bats (Fleming and Eby 2003).

Typically, when sex-biased differences in migration exist, females move farther between

winter and summer grounds than do males (e.g. Cryan 2003; Ibáñez *et al.* 2009), likely as

a result of more stringent habitat requirements for reproduction by females. Our data

are in contrast to this trend, suggesting that males have a greater tendency to make

long-distance north-south migrations than do females. Thomas (1983) found evidence of

male-biased migration in the West African species *Myonycteris torquata* and suggested

that males may be following rain and subsequent fruiting events, while pregnant

females were limited in their movement as they prepared to give birth and care for their

young. It is possible that temporal differences in reproductive-based energetic

limitations may be a factor in causing the sex-biased migratory tendencies that we have

observed in *P. subflavus.*

Male and female bats experience reproductive stress at different times of the

year (Speakman and Thomas 2003). Females ovulate and become pregnant following

emergence from hibernation and have increased energetic requirements throughout

pregnancy and lactation in the early to mid-summer (e.g. Anthony and Kunz 1977; Kurta

et al. 1989). Conversely, males experience reproductive costs in late summer during

spermatogenesis (e.g. Encarnação *et al.* 2004, 2006). Variation in reproductive status has

been linked to behavioural changes, such as increased food consumption (e.g. Kunz *et al.*

1995), differential torpor use (e.g. Dietz and Kalko 2006) and changes in the length of

foraging bouts (e.g. Barclay 1989) and home range sizes (Henry *et al.* 2002). It is possible

that during the female reproductive time period, immediately following emergence from

hibernation, it is not energetically feasible for females to undertake a large-scale

migration, whereas males are not subject to such constraints.

4.4.2 *Effects of latitude on migratory tendency*

All of the female and many of the male bats with fur stable isotope compositions

indicating that they were south of their location of fur growth were collected from the

northern part of the *P. subflavus* range. In some cases, δD_{fur} values indicated that the fur

had been grown at least at the northern extent of the previously known range for the

species, and perhaps even farther north than these locations (Fujita and Kunz 1984;

Knowles 1992). We propose that *P. subflavus* summering at the extreme northern edge of the range may migrate south (although still remaining in the northern portion of the known species range) to hibernate at sites where winters are shorter and their probability of survival is higher (Humphries *et al.* 2002).

Previous research (Boyles and Brack 2009) suggests that while winter length is a factor in survivorship of hibernating little brown bat (*Myotis lucifugus*), a more important factor is the extent to which the bats hibernate in clusters. Hibernating in clusters decreases the amount of energy lost during normothermic bouts within the hibernation period (Boyles *et al.* 2008) and as long as bats are hibernating in clusters, the impact of varying winter length on survivorship is negligible (Boyles and Brack 2009). However, the hibernation ecology of *P. subflavus* differs from that of many other species: *P. subflavus* is well documented hibernating singly or occasionally in small clusters, but not frequently in large clusters (Briggler and Prather 2003; Broders *et al.* 2001; Fujita and Kunz 1984; Jones and Suttkus 1973; Sandel *et al.* 2001). McNab (1974) cites solitary hibernation as a primary reason that *P. subflavus* are able to hibernate in southern hibernacula with warmer ambient temperatures while other clustering species are not. We suggest that hibernating singly makes *P. subflavus* more susceptible to the longer winter lengths at northern hibernacula than clustering species, driving them to migrate south during the winter.

4.4.3   Bat residency and the moult period

Interpretation of our results relies heavily on the timing and location of bat fur

growth, so it is appropriate to consider moulting patterns of *P. subflavus.* Previous

studies of moulting patterns of temperate bats suggest that moult usually occurs once

annually between mid-June and late-August (Constantine 1957; Cryan *et al.* 2004;

Tiunov and Makarikova 2007), similar to the period that we have defined for male *P.*

subflavus. However, it is important to remember that our isotopically identified moult

period does not describe the period of actual hair replacement, but only the period

when the bat remains a resident at its location of fur growth; *i.e.*, fur replacement

happens at some point during the isotopically defined "moulting" period. Currently no

data exist on inter-annual consistency in resident bat δD_{fur} values, and *P. subflavus*

philopatry is poorly understood, although there is evidence that females return to the

same summer roosting sites each year (Allen 1921; Veilleux and Veilleux 2004). It is

unclear if the initial moult date (June 23) is actually indicative of the beginning of fur

replacement or just the date when all sampled bats had returned to regular summering

grounds and hence had fur grown the previous year that was still isotopically indicative

of their location of capture.

September 9, our most conservative estimate for when bats may have begun to

leave their location of fur growth, is a later date for the beginning of autumn migratory

movement than has previously been recorded for *P. subflavus* specifically, and for other

North American bat species in general. Laval and Laval (1980) recorded subadult *P.*

subflavus arriving at swarming sites in Missouri on August 5 and studies of other

swarming and hibernating species indicate that male bats begin to arrive at swarming

sites as early as August 1 (Fenton 1969). Our stable isotope results may indicate a

tendency for bats to only engage in long distance southern migration in mid to late fall,

but we think that the late migration date that we detected is more likely an artefact of

sampling bias. Logistically, the easiest places to capture and collect a colonial species

such as *P. subflavus* are summer colonies and winter hibernacula. Little is known about

the mobility and roosting habits of these bats during the late summer and fall when they

engage in swarming behavior, making them challenging to reliably locate. Minimal

collection data exists for the specimens used in this study, so we do not know the

circumstance in which most were collected. If the late summer bats included in this

study were collected from known summer colonies, then they may represent a minority

of bats that remained at their summer location into the late summer and early fall and

may not be representative of the majority of *P. subflavus*. A follow-up study using

samples collected from bats captured at swarming sites during the fall season may

clarify this point.

Variation in female fur isotope compositions was similar throughout the year, so

we could not identify a time period for female moult. Throughout this study, we have

applied the male isotopically defined moult period to both sexes, which is a necessary

but potentially problematic approach. Other authors (Constantine 1957; Cryan *et al.*

2004; Dwyer 1963) reported that male and female moult patterns may vary in timing,

likely as a function of the increased energetic demands faced by females during reproduction. Constantine (1957) found that male *Tadarida brasiliensis* and *Myotis velifer* moulted before female conspecifics. Dwyer (1963) suggested that female *Miniopterus schreibersii* ceased moulting during lactation. Delayed moult timing in females could translate to decreased isotopic migration detectability. If females are still moulting as they begin to migrate, then the stable isotope signature of the food and water that they drink at a range of locations will be integrated into their fur. The δD_{fur} values would not be indicative of one location of fur growth, but the average of a range of locations.

4.4.4   Summary - Perimyotis subflavus as a partial and differential latitudinal migrant

We found that *P. subflavus* δD_{fur} values are a good predictor of both the latitude and the estimated local growing season δD_{precip} of the location of bat fur growth and so can be used as an indicator of bat movement away from that location. Stable isotope evidence suggested that some bats of both sexes underwent a southern fall migration during the non-moult period and that this behaviour was more prevalent in males than in females. The majority of individuals for which isotope values suggested a latitudinal migration (both sexes) were captured in the northern portion of the species' range and male bats at mid-latitudes appeared sedentary. Some non-moult period males from the southern portion of the range also showed evidence of southern migration.

It is important to note that migration is a characteristic of individuals and not of populations or species (Dingle 1996; Fleming and Eby 2003). Migratory behaviour varies greatly among individuals based on a variety of intrinsic and extrinsic factors. Both partial migration (movement of some members of a species, but not all) and differential migration (sex or age cohorts exhibiting different migratory patterns) (Hobson and Norris 2008) are common in bat species (e.g. *Nyctalus* sp. – Estok 2007, Ibáñez *et al.* 2009; *Lasiurus* sp. – Cryan 2003; *Tadarida brasiliensis* – Villa and Cockrum 1962). Our evidence for migration in *P. subflavus* strongly suggests that this species can be described as both a partial migrant and differential migrant, as we found differences in migratory behaviour between sexes and among latitudes. Though some individuals undertake regional radiation migrations as previously suggested, our evidence suggests that latitudinal migration is a strategy that is also common for some members of this species.

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Chapter 5

5   Summary

5.1   Introduction

The main goal of this dissertation was to use stable isotope analysis to contribute to the body of knowledge about North American bat migration. I addressed this goal in two ways: first, an investigation of the methodology of using bat fur stable isotope analyses to assign origin of fur growth; and second, the application of this knowledge in the design, implementation and analysis of bat migration studies. Included in the methodology section is a quantification of natural stable isotope heterogeneity within and among fur samples taken from four species of bats captured at their summer resident grounds (the presumed location of fur replacement); identification of multi-element stable isotope covariation among samples taken from individual bat populations; an investigation into the utility of using multi-element analyses to differentiate among local populations; and the quantification of the relationship between fur stable hydrogen isotope compositions and latitude/local meteoric water stable hydrogen isotope composition for one species of bat (the tri-coloured bat, *Perimyotis subflavus*). My contribution to bat migration research includes an investigation into the migratory bat catchment area of a Canadian Migration Monitoring Station (Long Point, ON) and the description of the annual latitudinal migratory movements of *P. subflavus* at a continental scale.

Here, I summarize my findings about (i) isotopic studies using bat fur and (ii) ecology of migrating bats. Finally, I conclude with a description of the remaining main challenges and knowledge gaps, and the future potential, of using stable isotope techniques to study bat migration.

5.2   Contribution to isotopic studies of bat tissues

An integral component of using stable isotope analyses to study animal migration is an understanding of natural heterogeneity in tissue stable isotope compositions, as well as the factors that contribute to this variation. Such information is necessary for effective sampling design, data analysis, and interpretation of results. I have contributed to this field in the following four ways:

5.2.1   *Stable isotope heterogeneity in resident populations of bats during the summer*

Many factors, such as sampling location, bat age, sampling time, and bat species can have a systematic effect on δD_{fur} values. In general, samples taken from the venter were more enriched in D compared to those from the dorsum and when age classes differed isotopically, fur of subadults was depleted of D compared to adult fur. In some, but not all species, there was a slight effect of time on δD_{fur} values that appears to reflect moulting and interannual stable hydrogen isotope variation, with new fur growth having a slightly different mean δD_{fur} values than the previous year's growth. I found substantial species-specific differences in δD_{fur} variance, with some species (hoary bats [*Lasiurus cinereus*] and eastern red bats [*Lasiurus borealis*]) having much greater

variance than other species (silver-haired bats [*Lasionycteris noctivagans*] and little brown bats [*Myotis lucifugus*]). These results indicate that fur sampling for stable isotope studies should be as controlled as possible, using samples from the same location on the bats' bodies, that age should be noted, and that if possible, sampling should occur within a constrained time frame that is consistent across sites. Finally, I echo the recommendations of other authors (Britzke *et al.* 2009; Torres-Dowdall *et al.* 2009) that stable isotope results of multiple species should not be pooled in analyses.

5.2.2   Covariation among stable isotopes in resident populations

Throughout the stable isotope literature, there are many instances of covariation among stable isotope compositions within populations, frequently between $\delta^{13}C$ and $\delta^{15}N$ values (*e.g.* Møller *et al.* 2006; Torres-Dowdall 2009; Kelly 2000). I detected a strong correlation between the δD_{fur} and $\delta^{13}C_{fur}$ values of bats in both of the summer resident *L. borealis* and *L. noctivagans* populations, and a correlation between $\delta^{13}C_{fur}$ and $\delta^{15}N_{fur}$ values in the *L. noctivagans* population. I speculate that these covariations resulted from individual-specific differences in diet, but feel that further investigation is warranted. I suggest that these correlations provide an indicator of population mixing and have used them as such in the Chapter 3 examination of fall-caught *L. noctivagans* and *L. borealis* at Long Point, Ontario.

5.2.3   Among-colony differentiation

Stable hydrogen isotope analysis has been used to detect long-range movement across latitudes, but multi-element analyses have recently been shown to effectively

differentiate populations at a finer geographic scale (*e.g.,* Torres-Dowdall *et al.* 2009).

Using stable isotope analyses of three elements, I had high success differentiating *M.*

lucifugus from three separate colonies, two of which were within 60 km of one another.

This technique could have useful applications at swarming sites and hibernacula, where

bats from many discrete colonies congregate.

5.2.4   Tri-colored bat (Perimyotis subflavus) stable isotopes across latitudes

To conduct migrant assignment at a continental scale using stable hydrogen

isotope analysis, it is necessary to demonstrate that the δD_{fur} values of the study species

are representative of the mean growing season δD_{precip} values at the site of fur growth.

Other authors have shown this to be true for several species of North American bats

(Cryan *et al.* 2004, Britzke *et al.* 2009), and I have added to this body of work in Chapter

4 by quantifying the δD_{fur}- δD_{precip}/latitude relationship for *P. subflavus*, a species that, to

my knowledge, has not previously been investigated using stable isotope techniques.

While this relationship was necessary for making an ecological interpretation of the

results in Chapter 4, it can also be used by other researchers to assign *P. subflavus* of

unknown origin to an approximate latitude of fur growth.

5.3   Contribution to bat biology

In this dissertation, I investigated migratory behaviour in three species of North

American bats: *L. noctivagans, L. borealis,* and *P. subflavus*. *Lasionycteris noctivagans*

and *L. borealis* are latitudinal migrants (Cryan 2003) and *Perimyotis subflavus* has

traditionally been considered a regional migrant, moving between summer resident

colonies and swarming sites and hibernacula (Fujita and Kunz 1984). In Chapter 3 I used

multi-element stable isotope analyses to investigate migratory origin and timing of *L.*

noctivagans and *L. borealis* at a migration monitoring station in Ontario, Canada. In

Chapter 4 I conducted a continental-scale investigation of *P. subflavus* migratory

movements across most of the species' range in eastern North America.

5.3.1   Origins of migrant bats at a Migration Monitoring Station

I compared the stable isotope profiles (including stable hydrogen, carbon, and

nitrogen values) of presumed migrant *L. notivagans* and *L. borealis* captured at Long

Point, Ontario, during the fall migration period with those of bats of the same species

captured during the summer at resident sites (Cypress Hills, SK for *L. noctivagans* and

Pinery Park, ON for *L. borealis*). I predicted that if the bats captured at the migration

monitoring station originated from a diversity of sites across the species' northern

range, then the migratory populations would have greater stable isotope variance than

the resident populations and that the $\delta D_{fur}/\delta^{13}C_{fur}$ correlation that I detected in resident

populations of both species would not be present among the migrants. I further

predicted that bats at more northern latitudes would migrate south through Long Point

before more southern bats, resulting in a positive trend in δD_{fur} values over the course of

the migratory period. Finally, several authors (Barclay 1984, McGuire *et al.* 2011) have

reported *L. noctivagans* migrating in temporal waves and I predicted that the bats in

these waves originated from discrete locations and would be isotopically distinct.

I found that migrant *L. noctivagans* were isotopically very similar to the summer resident population, having similar population-level stable hydrogen and nitrogen isotope variance and similar $\delta D_{fur}/\delta^{13}C_{fur}$ correlations. These results indicated that the migrant bats were not originating from a wide range of latitudes. However, the migrant *L. noctivagans* did have greater stable carbon isotope variance than the resident population, which suggests that the migrant population is comprised of bats from a variety of habitats. There was no temporal trend in δD_{fur} values and it was not possible to distinguish the migratory waves isotopically. In summary, these data suggest that the migrant *L. noctivagans* passing through Long Point are a mixed population originating from a variety of proximate sites. The population structure of the migratory waves remains unknown.

The stable isotope profiles of the migrant *L. borealis* were widely divergent between the two sampling years, making interpretation difficult. In 2008, the stable hydrogen isotope variance of the migrant population was far smaller than the variance of the known resident population, suggesting that the bats were local. In 2009, the migrant stable hydrogen isotope composition was bimodal, with each sub-population also having extremely low variance. These results augment previous results by Britzke *et al.* (2009) to suggest that a better understanding of natural heterogeneity in *L. borealis* stable hydrogen isotope compositions is required before these techniques can effectively and reliably be used to study bat movement.

5.3.2 *Evidence of latitudinal migration in tri-coloured bats, Perimyotis subflavus*

I obtained *P. subflavus* fur samples from museum collections in Canada and the US. Bats were collected from across most of the species' range (Ontario and Quebec south to Florida and Lousiana) during all seasons. I compared the difference between bat δD_{fur} values and mean growing season δD_{precip} values at the location of bat collection across seasons, following the methods of Cryan *et al.* (2004). Almost one third of the male bats had greater offset between fur and precipitation compositions during the fall, winter, and spring, indicating latitudinal movement away from the location of fur growth. Sixteen percent of non-moult period females were south of their location of fur growth, and most did not appear to be as far from their location of fur growth as the migratory males. The majority of bats that had engaged in latitudinal migration were from the northern and southern extents (males) or the northern extent (females) of the species' known range. Some of the bats that appeared south of their location of capture had δD_{fur} values indicating that they grew their fur at latitudes at the farthest northern extreme or even farther north than previously recorded for this species. I hypothesize that bats at high northern latitudes must migrate south to hibernate in order to take advantage of shorter winters at lower latitudes and to reduce the amount of time that they spend in hibernation. The results of this study suggest a fundamentally different migratory life history for this species than had previously been assumed, including both partial and differential latitudinal migration.

5.4   Future research

5.4.1   Isotopic studies of bat tissues

5.4.1.1 Discrimination and tissue turnover studies

To use stable isotope analysis to address ecological questions, it is important to know

how environmental stable isotopes are incorporated into animal tissues. Some of the

most important variables include the magnitude of discrimination, tissue turnover time

and the effect of individual physiology on both of these variables. Gannes *et al.* (1997)

put out a call for more laboratory experiments investigating the integration of

environmental stable isotopes into animal tissues and the role of individual physiology in

discrimination and tissue turnover rates. Since that time a large volume of work has

been done in this area (*e.g.,* Miller *et al.* 2008; Kempster *et al.* 2007; Podlesak *et al.*

2008; Sare *et al.* 2005; Tuross *et al.* 2008). A number of researchers have investigated

stable nitrogen and carbon isotope compositions in nectarivorous bat tissues (Mirón *et

al.* 2006; Voigt and Matt 2004; Voigt *et al.* 2003; Voigt *et al.* 2008), but I know of no

laboratory work that has investigated stable hydrogen isotope discrimination or tissues

turnover in captive bat tissues.

   It would be useful to have a more complete understanding of how the stable

hydrogen isotope compositions of bat food and drinking water are reflected in different

bat tissues, as well as the turnover rates of those tissues. Previous research on Rock

Doves (McKechnie *et al.* 2004) has shown that water stress in these birds leads to tissues

that are enriched in D compared to the tissues of non water-stressed birds. It would be helpful to know if this is the case for bats.

A further complication in understanding how the stable isotope compositions of bat tissues reflect diet is the frequency with which bats use torpor. Many insectivorous bats engage in torpor use on a daily basis and during times of topor, experience a reduced metabolic rate. Within species, torpor use varies among sexes (*e.g.,* Cryan and Wolf 2003; Dietz and Kalko 2006) and with ambient temperature and food availability (*e.g.,* Hickey and Fenton 1996). With the exception of some work examining the stable nitrogen isotope compositions of hibernating bear muscle (Lohuis *et al.* 2007), I know of no efforts to study the effect of hibernation and torpor use on stable isotope discrimination and tissue turnover rates.

5.4.1.2 Moulting studies

Stable isotope analyses of animal tissues provide information only about the time period in the animal's life when that tissue was being grown and so data interpretation requires a strong understanding of tissue growth patterns. The moulting patterns of a handful of bat species have been studied in detail and the overwhelming consensus among them is that bats moult once annually during the summer season (Constantine 1957, 1958; Cryan *et al.* 2004; Jones and Genoways 1967; Quay 1970; Tiunov and Makarikova 2007, Chapter 4 of this dissertation, but see Dwyer 1963). These data allow us to know that stable isotope analyses of bat fur provide information about the bat during the previous summer. A more detailed understanding of moulting patterns on a

species by species basis, however, would allow finer scale interpretation of stable isotope results. For instance, for each species studied, it would be helpful to know the exact timescale of moulting, how it varies among males and females, the timing of the subadult first moult, the order in which the bats' pelage is replaced and perhaps most importantly, the extent to which moulting may continue to occur while the bat is engaging in fall migratory movement.

In-depth studies of bat moulting are challenging, as it is often not possible to visually discern newly grown fur from old in most species (but see Constantine 1957, 1958; Dwyer 1963). A common methodology for determining mammalian moulting patterns is lethal, and involves collecting specimens, skinning them, and analyzing the pigmentation patterns on the back of the hide (*e.g.,* Constantine 1957; Tiunov and Makarikova 2007). Given the current conservation issues faced by North American bats, I do not advocate collecting them for this purpose. However, a detailed fall-time moulting analysis would be a good use of the carcasses of some of the many migrant bats that are killed at wind turbines each year and would provide valuable information about the extent to which bats are growing fur while migrating. A more sustainable approach to studying moulting involves dying the fur of captive bats and observing replacement patterns in the ensuing months (*e.g.,* Miller 2006). Dying bat fur is very challenging (personal observation), and as with any captive study, there is uncertainty about how representative the captive bats are of their wild counterparts. However,

there may be a role for this type of study in captive colonies of insectivorous North American bat species.

5.4.2   *Using stable isotopes to investigate bat migration*

While there are certainly limits to the precision with which stable isotope techniques can inform our knowledge about the migratory movements of animals (Farmer *et al.* 2008; Wunder and Norris 2008), I think that there is a tremendous potential for the use of these techniques in future bat migration studies. We know so little about the large scale movements of most bat species, that even very broad generalizations about migratory behaviour are novel, for example, our findings of latitudinal migratory movement in *P. subflavus*.

Successful application of stable isotope techniques in migratory studies requires the formulation of appropriate research questions that incorporate the unknowns of the study system into the study design. In order to interpret the stable isotope results of migrants, it is necessary to have a comparative measure of natural isotopic heterogeneity and it is my hope that future researchers will find the results of Chapter 2 of this thesis helpful in this respect. In studies where the objective extends to include migrant assignment to a specific area of origin, it is absolutely necessary to know the relationship between the stable isotope compositions of the tissues being studied and the environmental stable isotope compositions at the location of tissue growth.

Cryan *et al.* (2004) presents an example of a study design that effectively investigates

the migratory patterns of hoary bats (*L. cinereus*), while simultaneously incorporating

measures of natural stable isotope heterogeneity among summer resident bats, and

identifying a putative moulting period. I replicated these methods in Chapter 4 of this

dissertation and I think that there is great potential to conduct similar continental-scale

studies of other migratory bat species. Museum collections are a tremendous resource

and can allow researchers access to a breadth of samples that would otherwise take

years to collect. The addition of strontium isotope analysis to these studies has the

potential to further increase the precision with migrant assignment can occur. The

recent results of Sellick *et al.* (2009) indicate that while stable hydrogen isotope analysis

is most effective as an indicator of latitudinal movement, strontium isotope analysis at

the continental scale can indicate longitudinal movement in some parts of North

America.

Target species for future studies include *L. noctivagans* and *L. borealis*, both of which

are long-distance latitudinal migrants. Also, brazilian free-tailed bats *(Tadarida

brasiliensis*) are a partial migrant, and some sub-groups of this species engage in

substantial migratory movements that are currently not well understood (reviewed in

Russell *et al.* 2005) and could likely be better elucidated using stable isotope techniques.

5.5   References

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Appendices

Appendix A: Non-exchangeable δD_{fur} values of fur standards

Introduction

Most organic substances contain a fraction of hydrogen that exchanges readily with hydrogen in the surrounding atmospheric water vapour. Typically, exchangeable hydrogen is bonded to nitrogen or oxygen, while non-exchangeable hydrogen is bonded to carbon. The percentage exchangeable hydrogen varies among organic tissue types (Wassenaar and Hobson 2000; Chesson *et al.* 2009). The δD values of atmospheric water vapour vary both geographically and temporally, resulting in variability of the total δD values of otherwise identical organic samples among laboratories and across seasons. In order to standardize organic sample δD analyses, it is necessary to develop standards that are made of the same material as the samples of interest, but that have known non-exchangeable δD values. Based on the Principle of Identical Treatment (PIT) (Werner and Brand 2001), these standards can be prepared alongside samples and left to equilibrate with laboratory air (Wassenaar & Hobson 2003). Assuming that standards and samples have the same exchange and water absorption properties, the known non-exchangeable δD values (δD_n) of standards can be used to calibrate sample values. There currently are no internationally accepted standards for stable hydrogen isotope analysis of most organic tissue types. Therefore, to conduct stable hydrogen isotope analysis on organic samples, it is necessary to create tissue-specific standards and identify their % exchangeable hydrogen (f_e) and δD_n, either experimentally or through external

validation from a laboratory that already has known standards of that tissue type. Experimental determination of δD_n values involves analyzing tissues standards that have been equilibrated with several (minimum three) isotopically distinct water vapours and conducting a series of calculations (e.g. Cormie *et al.* 1994; Schimmelmann 1991, 1999; Wassenaar and Hobson 2000; Wassenaar and Hobson 2003).

Accordingly, I designed an experiment to identify and isotopically characterize five fur types for use as standards in the present study. The three objectives of this experiment were: (i) to create isotopically distinct fur standards for eventual use in stable hydrogen isotope analyses of North American bat fur samples; (ii) to obtain three independent estimates of the δD_n (VSMOW) values for each standard (two estimates from external laboratories calibrated using pre-existing keratin standards and one from an in-house comparative equilibration experiment); and (iii) to create and compare the three calibration curves resulting from the fur standard δD_n estimates and to conduct an experiment to evaluate which curve most accurately corrects sample δD_{total} values to δD_n (VSMOW).

Methods

Fur standards

Fur samples for use as standards were obtained from a variety of sources, including a local fur harvester's organization, the University of Western Ontario Zooarcheology Laboratory, and other researchers. Fur standards came from mammals

originating from a range of latitudes in North America. These include samples from: one

Northern Bog Lemming (*Synaptomys borealis*) from Nunavut, Canada; one Wolf (*Canis

lupus*) from Quebec, Canada; one domestic dog (*Canis familiaris*) from Ontario, Canada;

one Black-tailed Jackrabbit (*Lepus californicus*) from Arizona, USA; and one Spotted

skunk (*Spilogale putorius*) from West Virginia, USA. Fur was removed from the animal's

pelt using scissors and soaked overnight in 2:1 chloroform:methanol, then rinsed with

2:1 chloroform:methanol and left to dry for >48 hours in a fume hood (Paritte and Kelly

2009). Samples were chopped to a powder-like consistency using dissection scissors.

External validation of fur standard $\delta D_{non\text{-}exchangeable}$ values

Five samples of 350±10 μg of each standard were weighed into 3.5x5 mm silver

capsules and sent for stable hydrogen isotope analysis to the Environment Canada

Stable Isotope Hydrology and Ecology Research Laboratory in Saskatoon, Saskatchewan,

Canada (hereafter referred to as EC) and to the Stable Isotope Core Laboratory at

Washington State University in Pullman, Washington, USA (hereafter referred to as

WSU). At the Environment Canada laboratory, the samples were analyzed alongside

three homogenized keratin standards (made of cow hoof, chicken feathers and whale

baleen, respectively) whose non-exchangeable δD values had been experimentally

determined using a high temperature steam equilibration method (Wassenaar & Hobson

2003). At the Washington State University laboratory, the samples were analyzed

alongside three keratin standards normalized based on keratin standards at Northern

Arizona State University and which can originally be traced back to the three standards

from the Environment Canada laboratory.

Experimental determination of fur standard δD $_{non-exchangeable}$ ***values through***

comparative equilibration

The internal experimentation portion of this project was conducted at the

Laboratory for Stable Isotope Science at the University of Western Ontario in London,

Ontario (hereafter referred to as UWO). Dissecting pins were used to punch 10-20 small

holes in the bottom of 15 3.5x5 mm silver capsules to ensure that complete exchange

occurred between standard fur samples and the surrounding atmospheric water vapour

(Sauer *et al.* 2009). Three 175±10 μg samples of each standard were weighed, resulting

in three complete sets of all five standards. Each set was then sealed in a chamber at

room temperature with one of three isotopically distinct waters and left to fully

equilibrate. The three waters will be referred to as light, medium and heavy (light=-

151‰, medium=-53‰, heavy=+89‰).

Traditional comparative equilibration experiments are typically conducted at

temperatures greater than 100˚C, which allow the samples to exchange fully with steam

over a short time period (usually several hours) (Wassenaar and Hobson 2000, 2003;

Sauer *et al.* 2009; Schimmelmann 1991, 1999). However, Chesson *et al. (*2009) have

shown that f_e varies with temperature and that higher temperatures make more

hydrogen available for exchange. It is not ideal to calculate standard f_e and $δD_n$ values at

high temperatures, as the standards will eventually be used alongside samples that are left to equilibrate with laboratory air at room temperature. High temperature equilibration experiments may yield higher f_e values than typically encountered at room temperature, resulting in δD_n values that may be either too high or too low for samples equilibrated at room temperature.

Bowen *et al.* (2005) determined that it is possible to equilibrate hair samples with isotopically distinct waters at room temperature by sealing the samples in desiccators alongside small reservoirs of water. Within four days, exchangeable hydrogen in the samples fully exchanges with that in the ambient water vapour. Following these methods, we placed each set of three weighed standards in one of three sealed desiccators (without desiccant), each containing approximately 200 mL of isotopically distinct waters. Samples were left to equilibrate with the water vapour at room temperature in the desiccators for > 4 days (Bowen *et al.* 2005). Samples were quickly removed from the desiccators and placed under vacuum in a freeze dryer for six days (Bowen *et al.* 2005). Dried samples were transferred quickly from the freeze dryer into an empty glass desiccator and placed under vacuum. They were then immediately transferred into an autosampler, placed under vacuum, and flushed with helium. In total, the equilibrated samples were open to the laboratory atmosphere for less than five minutes before being sealed in the autosampler.

Samples were combusted in a Thermo Finnigan high Temperature Conversion Elemental Analyzer interfaced with a Thermo Finnigan Delta[plus] XL mass spectrometer in

continuous flow mode. Equilibrated fur samples were corrected to VSMOW using a

calibration curve based on two internationally accepted standards, NBS-22 (δD value -

120‰) and KGa-1 (δD value -57‰). These two standards were dried in a vacuum oven

overnight at 70˚C and analyzed alongside the fur samples.

Calculations

   The percent exchangeable hydrogen in each standard was calculated using

equation 1 (Wassenaar & Hobson 2000):

   (1)

$$f_e = \frac{\delta D_{ta} - \delta D_{tb}}{\left((\delta D_{wa} - \delta D_{wb}) \left(1 + \frac{\epsilon_{ex\text{-}liquid}}{1000} \right) \right)}$$

where f_e is the % exchangeable hydrogen, δD_{ta} and δD_{tb} are the total stable hydrogen

isotope values of samples exchanged with isotopically distinct waters, δD_{wa} and δD_{wb} are

the stable hydrogen isotope values of the isotopically distinct waters, and $\epsilon_{ex\text{-}lquid}$ is the

per mille fractionation (‰) between the δD value of the standard exchangeable

hydrogen (δD_{ex}) and δD_w. The δD_n values were calculated using equation 2 (adapted

from Schimmelmann 1991):

(2)

$$\delta D_n = \frac{\delta D_t - f_e\left(\delta D_w + \epsilon_{ex\text{-}liquid}\right)}{1 - f_e}$$

For both equations 1 and 2, there are two unknowns: the % exchangeable hydrogen (f_e) and the per mille fractionation ($\epsilon_{ex\text{-}liquid}$). Difficulties in experimentally determining fractionation factors for some complex organics make it necessary to use an estimate of $\epsilon_{ex\text{-}liquid}$ (Bowen *et al.* 2005; Chesson *et al.* 2009; Sauer *et al.* 2009; Schimmelmann 1999; Wassenaar and Hobson ([2000; 2003]). The magnitude of the fractionation is temperature dependent and may vary among organic compounds (Bigleisen 1965), with greater fractionation occurring at lower temperatures. In this experiment, there are two steps where fractionation can occur: first, as some (but not all) of the equilibration waters vapourize within the chambers and the hydrogen in the water moves between the liquid and vapour phases ($\epsilon_{liquid\text{-}vapour}$) and second, as the hydrogen in the water vapour exchanges with the exchangeable hydrogen in the samples ($\epsilon_{ex\text{-}vapour}$). For the purpose of this experiment, we are concerned with the net fractionation of these two steps ($\epsilon_{ex\text{-}liquid}$); however, it is necessary to consider them separately. Majoube (1971) shows that stable hydrogen isotope fractionation between the liquid and vapour phases of water varies with temperature (in Kelvin) according to equation 3, which can be combined with equation 4 (Fry 2006; Sharp 2007) to determine $\epsilon_{liquid\text{-}vapour}$ at varying temperatures:

(3)

$$1000\ln\alpha=24.844\left(10^6T^{-2}\right)-76.248\left(10^3T^{-1}\right)+52.612$$

(4)

$$\epsilon = 1000(\alpha - 1)$$

According to these equations, $\epsilon_{liquid-vapour}$ at 298K (25° C - the temperature at which the standards were equilibrated with water vapours of varying isotopic compositions) is 79.5‰. Therefore, when the standards were sealed in chambers with waters of -151, -53 and +89‰, they were equilibrated with water vapours of -230.5, -132.5 and 9.5 ‰.

In high temperature (>100°C) comparative equilibration experiments where 100% of the experimental waters are converted to steam (e.g. Wassenaar and Hobson 2000, 2003; Sauer *et al.* 2009; Schimmelmann 1991, 1999) $\epsilon_{ex-liquid}$ is not the result of a two-step process. Because all of the water is vaporized, $\delta D_{liquid}= \delta D_{vapour}$ and no fractionation occurs ($\epsilon_{liquid-vapour}=0$). Therefore, $\epsilon_{ex-liquid}= \epsilon_{ex-vapour}$ and in the remainder of this paper, when high temperature equilibration experiments are discussed, will be referred to as $\epsilon_{ex-vapour}$.

Schimmelmann (1991) experimentally determined that $\epsilon_{ex-vapour}$ for cellulose is +80‰ at 114°C and Schimmelmann (1999) suggested that values for $\epsilon_{ex-vapour}$ ranging from +60

to +100‰ are sufficient for high temperature equilibration experiments on complex

organics. Values of $\epsilon_{\text{ex-vapour}}$ within this range have been assumed in subsequent high

temperature equilibration experiments involving other complex organics, including

keratinous tissues such as feathers and butterfly wing (Wassenaar and Hobson 2000)

and cow hoof and whale baleen (Wassenaar and Hobson 2003).

Because we conducted our equilibration experiment at room temperature, the

values of $\epsilon_{\text{ex-vapour}}$ values assumed above are likely too low. Following the assumption

that the $\epsilon_{\text{ex-vapour}}$ value of cellulose is representative of other complex organics (in our

case, keratin) (Schimmelmann 1999; Wassenaar and Hobson 2000, 2003), we have

estimated the value of $\epsilon_{\text{ex-vapour}}$ at 25˚C using fractionations that have been

experimentally measured by other authors for cellulose at various temperatures. We

refer to these values as $\epsilon_{\text{ex-water}}$, as samples were equilibrated with steam (vapour) at

high temperatures (Filot *et al.* 2006; Schimmelmann 1991), and with liquid at low

temperatures (Feng *et al.* 1993). Some papers (Feng *et al.* 1993; Filot *et al.* 2006)

reported fractionation as a factor, $\alpha_{\text{ex-water}}$. These $\alpha_{\text{ex-water}}$ values were converted to $\epsilon_{\text{ex-water}}$ values using equation 4.

Figure A.1 compares results for $\epsilon_{\text{ex-water}}$ obtained by Schimmelmann (1991) at 114˚C,

Filot *et al.* (2006) at 105˚C and Feng *et al.* (1993) at 0˚C for cellulose. Assuming a linear
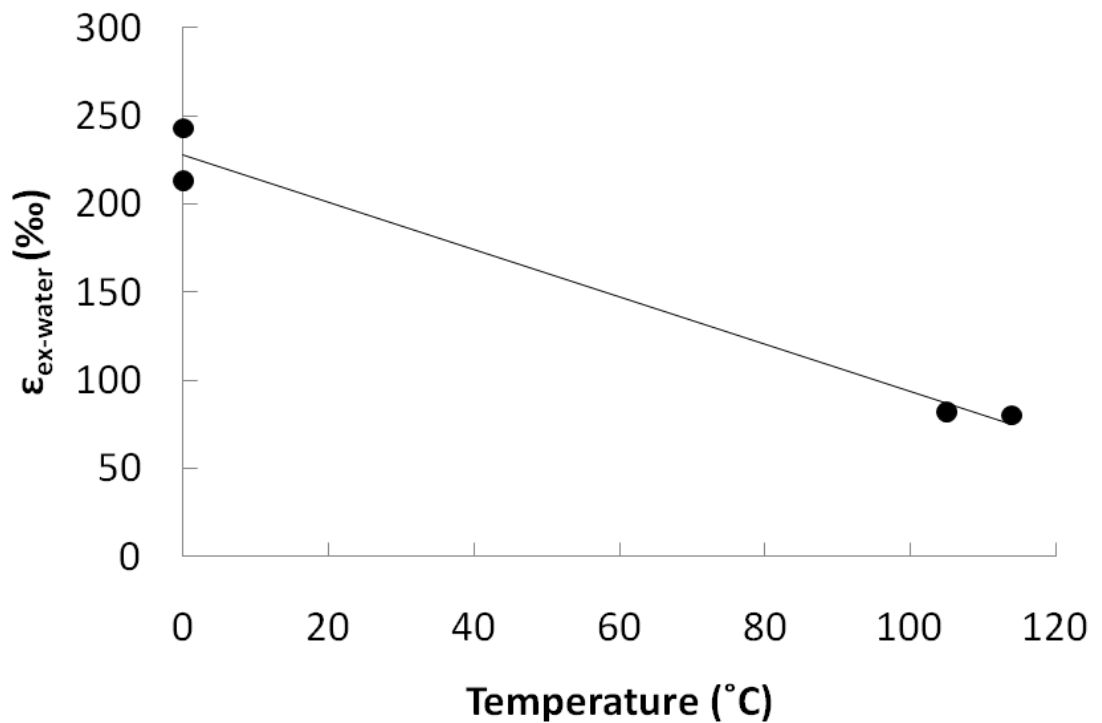
Figure A.1. Relationship between equilibration temperature and fractionation factor.

Assumed linear relationship between equilibration temperature and $\epsilon_{\text{ex-water}}$ (the per mille fractionation factor between the sample exchangeable hydrogen and the equilibration waters). At temperatures >100˚C, samples equilibrated with steam and at temperatures <100˚C, they equilibrated with liquid. Data taken from Schimmelmann (1991), Feng *et al.* (1993) and Filot *et al.* (2006).

relationship between $\epsilon_{ex\text{-}water}$ and temperature in ˚C, the two variables are related by equation 5:

(5)

$$\epsilon_{ex\text{-}water} = -1.3387(temperature) + 227.79$$

Using equation 5, $\epsilon_{ex\text{-}water}$ is 194‰ at 25˚C and we use this value as our estimate for $\epsilon_{ex\text{-}vapour}$.

Assuming that at 25˚C $\epsilon_{liquid\text{-}vapour} = 79.5‰$ and $\epsilon_{ex\text{-}vapour} = 194‰$, then the net fractionation between the experimental water δD values and the sample exchangeable hydrogen δD values ($\epsilon_{ex\text{-}liquid}$) is 114.5‰. Using this value to describe the per mille fractionation between the water and the standard exchangeable hydrogen during the experimental equilibration period, we can then use equations 1 and 2 to calculate % exchangeable hydrogen (f_e) and $\delta D_{non\text{-}exchangeable}$ of the five fur standards.

Comparison and evaluation of fur standard δD_n calibration curves

In anticipation of obtaining slightly different results from the two external laboratories and the internal experiment, I implemented an external check to evaluate the efficacy of the three data sets as calibration curves for normalizing sample δD values to VSMOW. Three sets of fur standards were weighed into silver capsules and left to equilibrate with laboratory air for >4 days. They were then transferred to a freeze drier

under vacuum for six days to ensure complete dryness (Bowen *et al.* 2005). Three sets of

two non-exchangeable standards with known δD values (NBS-22 and KGa-1) were also

weighed into silver capsules and dried overnight in a vacuum oven under vacuum at

70˚C. On the same day that the fur standards were transferred to the freeze drier, a

small sample of laboratory water vapour was collected and analyzed for its stable

hydrogen isotope composition. Stable hydrogen isotope analysis was conducted on all

standards using the methods described above.

Calculations

The calibration curve created by the fur standards is used to correct for multiple

factors: (i) the offset between the mass spectrometer raw δD values and δD values

normalized relative to VSMOW; (ii) variation in fur total δD values as a result of

exchange between sample exchangeable hydrogen and laboratory water vapour

hydrogen, and; (iii) variation in fur δD values as a result of water that has been absorbed

by the sample. Assuming that sample and standard tissues have identical exchange and

absorption properties, the standard calibration curve should produce sample δD

(VSMOW) values that account for all sources of error provided that samples and

standards are processed together according to the PIT (Werner and Brand 2001).

However, NBS-22 and KGa-1 do not have exchange and absorption properties that are

similar to the fur standards. Neither contains hydrogen that is readily exchangeable at

room temperature, and in this case, absorbed water was removed from all fur standards

and NBS-22 and KGa-1 prior to analysis. Using the fur calibration curve to calculate δD_n

(VSMOW) values for the non-exchangeable standards (e.g., NBS-22, KGa-1) should yield

δD values that are offset from their actual δD (VSMOW) values by a factor determined

by the exchange differences between the exchangeable and non-exchangeable

standards. If the actual δD (VSMOW) values are known for the non-exchangeable

standards, as is the δD value of the atmospheric water vapour and the % exchangeable

hydrogen in the fur standards, then it is possible to back-calculate the δD values

expected for the non-exchangeable samples using a calibration curve based on the

exchanged fur standards. It is then possible to use the three available calibration curves

to adjust the non-exchangeable standard δD values to VSMOW and thus evaluate which

produces the most accurate offset values for NBS-22 and KGa-1. The adjusted standard

δD values for NBS-22 and KGa-1 can be calculated by replacing the δD_{ex} variable in

equation 6 with the $\epsilon_{ex\text{-}vapour}$ and δD_{vapour} variables in equation 7 and rearranging to form

equation 8, which solves for δD_n:

(6)

$$\delta D_t = \left(\%_{non\text{-}exchangeable\ hydrogen}\right)\left(\delta D_n\right) + \left(\%_{exchangeable\ hydrogen}\right)\left(\delta D_{ex}\right)$$

(7)

$$\epsilon_{ex\text{-}vapour} = \delta D_{ex} - \delta D_{vapour}$$

(8)

$$\delta D_n = \delta D_t - \frac{\left(\%_{\text{exchangeable hydrogen}}\right)\left(\epsilon_{\text{ex-vapour}} + \delta D_{\text{vapour}}\right)}{\%_{\text{non-exchangeable hydrogen}}}$$

where δD_t is the total δD value of the sample, δD_{ex} is the δD value of the exchangeable

hydrogen in the sample, δD_{vapour} is the δD value of the water vapour in the laboratory,

and $\epsilon_{ex-vapour}$ is the difference between δD_{ex} and δD_{vapour}, estimated at +194‰.

Results

External validation and experimental results

There was a systematic difference in the total δD values of each of the fur

standards following total equilibration with isotopically distinct water vapour at 25˚C

(Figure A.2).

External laboratory analyses and the experimental determination of fur δD_n

values yielded similar, but not identical results (Table A.1). Overall, the WSU δD_n values

were systematically the most negative and the UWO values were the least negative.

Calibration curve comparison

The EC and WSU calibration curves produced offset VSMOW values for NBS-22

and KGa-1 that were more negative than or equal to the predicted values, while the
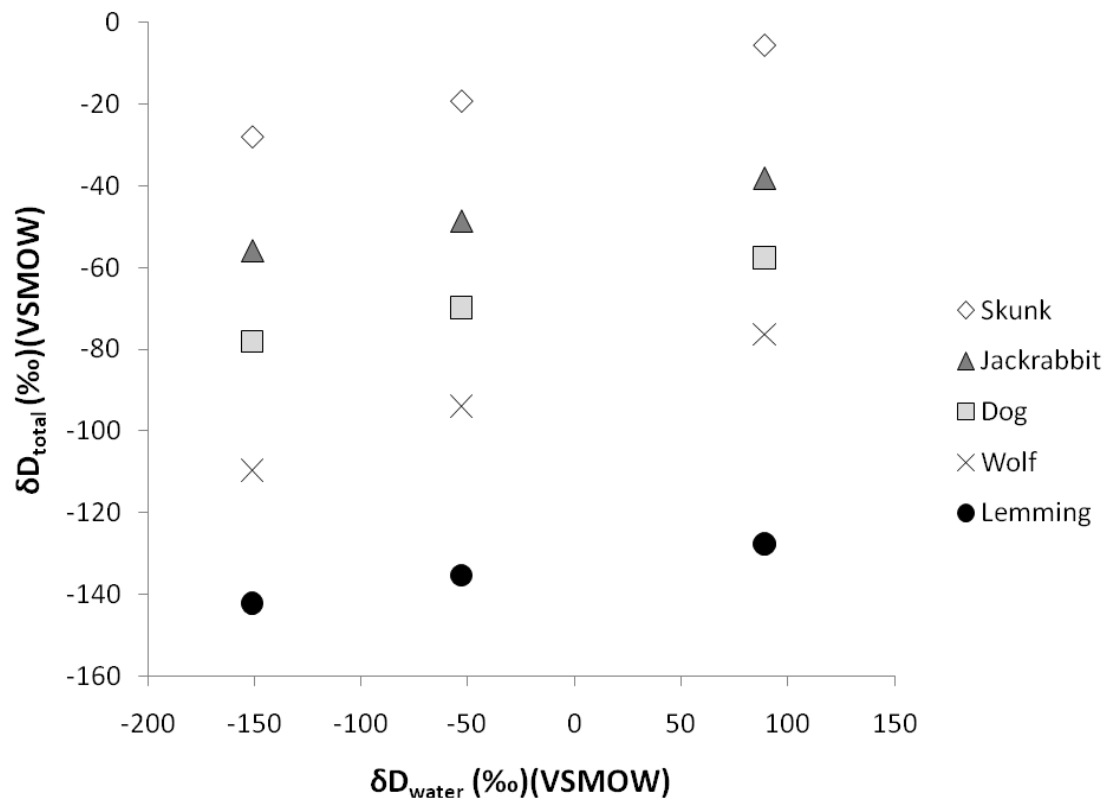
Figure A.2. Fur total stable hydrogen isotope compositions after equilibration with isotopically distinct waters.

Mean δD_{total} values (‰) (VSMOW) of five fur standards equilibrated with three isotopically distinct waters (light =-151‰; medium=-53‰; heavy=89‰) at room temperature for four days. Each data point is the average of three replicates. Standard deviation values (in ‰): Lemming – light=1, medium=0, heavy=1; Wolf – light=2, medium=0, heavy=1; Dog – light=1, medium=3, heavy=1; Jackrabbit – light=2, medium=1, heavy=1; Skunk – light=2, medium=1, heavy=2.

	δD_n (‰) (VSMOW)			F_e (%)
STANDARD	**EC**	**WSU**	**UWO**	**UWO**
LEMMING	-155 ± 1	-161 ± 1	-147±2	5.5±0.01
WOLF	-118 ± 2	-129 ± 3	-118±5	12.7±0.02
DOG	-88 ± 1	-97 ± 2	-81±0	7.6±0.00
JACKRABBIT	-59 ± 1	-65 ± 1	-57±0	6.6±0.00
SKUNK	-34 ± 0	-36 ± 3	-27±0	8.3±0.00
MEAN	-	-	-	**8.1**

Table A.1. Fur standard non-exchangeable stable hydrogen isotope compositions and % exchangeable hydrogen.

Mean non-exchangeable stable hydrogen isotope values (δD_n) of fur standards as analyzed and reported by the Environment Canada Stable Isotope Hydrology and Ecology Research Laboratory (EC), and the Stable Isotope Core Laboratory at Washington State University (WSU), and as experimentally determined in the Laboratory for Stable Isotope Science at the University of Western Ontario (UWO) (estimating $\epsilon_{ex\text{-}liquid}$=114.5‰ at 25˚C). The % exchangeable hydrogen (f_e) for experimentally determined UWO samples is also presented. Five of each standard were analyzed by EC and WSU; three of each standard were analyzed at UWO.

UWO curve produced less negative values (Table A.2). The EC values were the closest to, and the WSU values furthest from, the predicted values.

Discussion

We produced five fur standards for stable hydrogen isotope analysis that produce replicable results (standard deviation ≤±5‰) and span a range of >120‰. Through external laboratory validation as well as an internal laboratory experiment, we obtained three sets of δD_n values for the standards, which we used to create three independent calibration curves. We experimentally tested the efficacy of each calibration curve for producing accurate fur δD_n (VSMOW) values and determined that the curve created from the EC δD_n values produced the most accurate results. The UWO experimental results were second and the WSU results, third. Based on the calibration curve comparison, we have chosen to use the EC calibration curve when reporting our results for fur samples.

Differences in % exchangeable hydrogen (f_e) among the external laboratory standards and the fur standards may have caused some of the variation among the three fur standard calibration curves. Such differences could results from variation in standard tissue types as well as a mismatch between the temperature at which standard δD_n values were experimentally determined and the temperature at which they were used for analysis. While both external laboratories used keratinous standards, evidence suggests that f_e may vary among keratinous materials (Chesson *et al.* 2009; Wassenaar

	ACTUAL (non-corrected)	PREDICTED (corrected)	Calibrated values		
			EC	WSU	UWO
NBS-22	-120	-136	-136	-145	-132
KGa-1	-57	-68	-69	-75	-65

Table A.2. Non-exchangeable standard calibrations.

The δD (VSMOW) values of non-exchangeable standards analyzed at the Laboratory for Stable Isotope Science at the University of Western Ontario (UWO), as calibrated using fur standard (VSMOW) values provided by the Environment Canada Stable Isotope Hydrology and Ecology Research Laboratory (EC), the Stable Isotope Core Laboratory at Washington State University (WSU) and an experimentally determined curve at UWO. Predicted δD values for non-exchangeable standards are based on a mass balance equation using the average % exchangeable hydrogen of the fur standards (8.1%), a known laboratory water vapour δD value (-127‰) and an estimation of $\epsilon_{ex\text{-}vapour}$=194‰.

and Hobson 2000) as well as within materials that have been prepared differently (e.g. cut hair vs. ground hair) (Bowen *et al.* 2005; Chesson *et al.* 2009). The average fur standard f_e value was 8.1%, while the EC standards (ground cow hoof, chicken feathers and whale baleen) had an average f_e value of 15±3% (Wassenaar and Hobson 2003). The WSU standards were originally calibrated from the EC standards using comparative equilibration (Wassenaar and Hobson 2003), and were made from chitin, human hair and powdered commercial keratin. Ground human hair and commercial keratin have f_e values of 9.4% and 8.4%, respectively [Chesson *et al.* 2009]).

The use of high temperatures in exchange experiments may also have resulted in a slight difference between calculated standard f_e values (based on high temperature equilibration) and the actual standard f_e values at room temperature. The EC laboratory experimentally determined f_e and δD_n values for their standards using a high temperature equilibration experiment (Wassenaar and Hobson 2003) and the WSU standard curve was created by running WSU standards alongside EC standards after equilibrating with laboratory air at room temperature. Chesson *et al.* (2009) suggested that equilibration at high temperatures may make a higher fraction of hydrogen available for free exchange than would be at room temperature. A mismatch among the f_e values of the external laboratory standards combined with δD_{vapour} differences between the Saskatchewan and Washington laboratories may have led to the externally derived fur calibration curves being offset.

Assumptions in both the experimental design and calculations of the comparative equilibration experiment may have led to the experimental results being systematically less negative than both the EC and WSU results. Assumptions of all comparative equilibration experiments are that (i) equilibration occurs completely between samples and equilibration water or water vapour; (ii) all absorbed water is removed from the samples after equilibration and before analysis; and (iii) no back-equilibration with ambient water vapour occurs as samples are being dried or during transfer through laboratory air. We relied on the results of Bowen *et al.* (2005) to determine sufficient equilibration and drying times. However, Chamberlain *et al.* (1997) found that feather samples did not reach equilibrium with water vapour as quickly as was reported by Bowen *et al.* (2005). We feel that the results by Bowen *et al.* (2005) are more representative of our work, as they describe horse hair, a tissue type that is fairly similar to fur, and were collected using similar laboratory techniques to our own. The work by Chamberlain *et al.* (1997) was conducted on feathers that were cleaned, prepared and analyzed using different laboratory methods and so are likely less comparable. However, we cannot completely exclude the possibility that equilibrium had not been reached between the exchangeable hydrogen in the fur standards and the surrounding water vapour.

A necessary assumption in the experimental calculations for f_e and δD_n is the estimation of an $\epsilon_{ex\text{-}liquid}$ value. As our assigned value for $\epsilon_{ex\text{-}liquid}$ is a combination of estimates for $\epsilon_{liquid\text{-}vapour}$ and $\epsilon_{ex\text{-}vapour}$, we must consider the validity of both estimates

separately. Hydrogen per mille fractionation between the liquid and vapour phases of

water is well documented and our estimate of $\epsilon_{liquid\text{-}vapour}$=76.5 at 25˚C is supported

approximately by other sources (e.g. Kakiuchi and Matsuo 1979).

Our estimate for $\epsilon_{ex\text{-}vapour}$ at room temperature is subject to two potential errors.

First, the experimental values for $\epsilon_{ex\text{-}vapour}$ that other authors have used in comparative

equilibration experiments of complex organics (Schimmelmann 1999; Wassenaar &

Hobson 2000; 2003) and that we have used to interpolate a room temperature value for

$\epsilon_{ex\text{-}vapour}$, are based on cellulose. Cellulose is an ideal material for studying fractionation,

as the nature of its structure allows $\epsilon_{ex\text{-}vapour}$ to be determined precisely using nitration

techniques. It is not possible to conduct nitration techniques on many other complex

organics, keratin included, making cellulose a convenient and necessary proxy for

estimating $\epsilon_{ex\text{-}vapour}$.

Second, our interpolation of an $\epsilon_{ex\text{-}vapour}$ value at 25˚C was based on two end

point values and an assumed linear relationship between the two. Wang *et al.* 2009

showed slight non-linear relationships between $\epsilon_{ex\text{-}vapour}$ and temperature for carbon

bound hydrogens in seven ketones, suggesting that our assumption of linearity may be

inadequate. The use of an inaccurate $\epsilon_{ex\text{-}vapour}$ value would certainly have resulted in

offset δD_n values for each of the fur standards. To investigate the potential effect of an

inaccurate $\epsilon_{ex\text{-}vapour}$ value, we repeated the experimental calculations using $\epsilon_{ex\text{-}vapour}$

values that were 50‰ less than and greater than the value obtained through linear

interpolation. The range in final δD_n values based on $\epsilon_{ex\text{-}vapour}+50$ and $\epsilon_{ex\text{-}vapour}-50$ was 5‰ (lemming) to 12‰ (wolf).

Summary

All three sets of fur standard δD_n values obtained through this work indicate that we have created five reproducible standards for stable hydrogen isotope analysis and that these standards span a wide range of δD_n values. However, the differences among the three calibration curves highlight the sensitivity inherent in this type of analysis and the accompanying need to match standard type and preparation with samples as closely as possible. We propose that conducting exchange experiments at room temperature should theoretically produce standard f_e and δD_n values that will more closely match those of standards being equilibrated at room temperature than do high temperature exchange experiments, but that the uncertainty in predicting $\epsilon_{ex\text{-}vapour}$ at low temperatures could potentially overshadow any benefit to this approach. Future research on the relationship between temperature and $\epsilon_{ex\text{-}vapour}$ in keratinous substances would be highly beneficial in guiding low temperature exchange experiments. Based on our evaluation of the three fur standard curves created, we have accepted the δD_n values produced by the EC external validation as the basis of the calibration curve for the hydrogen isotopic analyses of bat fur conducted in this study.

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Appendix B: Fur stable hydrogen isotope precision by species for all individuals sampled.

Table B.1 There were no differences in stable hydrogen isotope precision among groups of fur samples taken from the five species studied (Kruskal Wallis test – F=2.79, df=4, p=0.594). Precision was measured as the mean absolute difference between the stable hydrogen isotope compositions of fur sample duplicates ($\Delta D_{first\text{-}second}$).

Species	Mean absolute $\Delta D_{first\text{-}second}$ ± standard deviation (‰ VSMOW)	n
Lasionycteris noctivagans	1±1	17
Lasiurus borealis	2±3	14
Lasiurus cinereus	2±2	6
Myotis lucifugus	2±2	23
Perimyotis subflavus	2±2	23

Appendix C: Stable isotope compositions for all fur samples reported in Chapters 2 and 3.

Table C.1. All stable isotope compositions are reported in ‰ and are relative to VSMOW, VPDB, and AIR for hydrogen, carbon, and nitrogen, respectively. F=female, M=male, A=adult, S=subadult, U=unknown age. Unless otherwise indicated, all samples were taken dorsally.

Species	Location	Date	Bat	Sex	Age	δD (dorsal)	δD (ventral)	δ^{15}N	δ^{13}C
Lasionycteris noctivagans	Cypress Hills	20-Jul-08	c1-08	F	A	-99		10.2	-22.7
L. noctivagans	Cypress Hills	26-Jul-08	c38-08	F	A	-101		9.4	-24.5
L. noctivagans	Cypress Hills	1-Aug-08	c11-08	F	A	-100		8.8	-23.9
L. noctivagans	Cypress Hills	1-Aug-08	c23-08	F	A	-70		10.5	-22.8
L. noctivagans	Cypress Hills	1-Aug-08	c31-08	F	A	-125		10.1	-23.9
L. noctivagans	Cypress Hills	20-Jul-09	CL1-09	F	A	-103		9.5	-24.2
L. noctivagans	Cypress Hills	20-Jul-09	CL2-09	F	A	-103		9.4	-24.2
L. noctivagans	Cypress Hills	22-Jul-09	CL4-09	F	A	-101		9.6	
L. noctivagans	Cypress Hills	28-Jul-09	CL10-09	F	A	-100		9.9	-23.8
L. noctivagans	Cypress Hills	28-Jul-09	CL9-09	F	A	-103		8.9	-23.9
L. noctivagans	Cypress Hills	29-Jul-09	CL11-09	F	A	-101		9.7	-24.3
L. noctivagans	Cypress Hills	29-Jul-09	CL12-09	F	A	-101		10.0	-23.9
L. noctivagans	Cypress Hills	1-Aug-09	CL14-09	F	A	-97		9.6	-23.2
L. noctivagans	Cypress Hills	31-Jul-08	c37-08	M	A	-99		10.2	-23.9
L. noctivagans	Cypress Hills	1-Aug-08	c10-08	M	A	-111		10.3	-23.6
L. noctivagans	Cypress Hills	21-Jul-09	CL3-09	M	A	-81		9.9	-23.4
L. noctivagans	Cypress Hills	22-Jul-09	CL5-09	M	A	-92		9.9	-23.7
L. noctivagans	Cypress Hills	22-Jul-09	CL6-09	M	A	-100		8.5	-25.2

Species	Location	Date	Bat	Sex	Age	δD (dorsal)	δD (ventral)	δ¹⁵N	δ¹³C
L. noctivagans	Cypress Hills	22-Jul-09	CL7-09	M	A	-86		10.1	-23.0
L. noctivagans	Cypress Hills	1-Aug-09	CL13-09	M	A	-87		8.8	-22.9
L. noctivagans	Cypress Hills	2-Aug-09	CL15-09	M	A	-85		9.5	-23.4
L. noctivagans	Cypress Hills	21-Jul-08	c4-08	F	S	-82		8.7	-23.5
L. noctivagans	Cypress Hills	1-Aug-08	c30-08	F	S	-118		10.3	-23.8
L. noctivagans	Cypress Hills	27-Jul-09	CL8-09	F	S	-113		10.0	-25.8
L. noctivagans	Cypress Hills	5-Aug-09	CL16-09	F	S	-115		10.0	-24.2
L. noctivagans	Cypress Hills	21-Jul-08	c3-08	M	S	-102		10.4	-22.9
L. noctivagans	Cypress Hills	27-Jul-08	c39-08	M	S	-115			
L. noctivagans	Cypress Hills	1-Aug-08	c32-08	M	S	-126		10.0	-23.3
L. noctivagans	Long Point	5-Sep-09	S31-09	F	A	-77			
L. noctivagans	Long Point	12-Sep-09	S47-09	F	A	-81		6.7	-26.3
L. noctivagans	Long Point	12-Sep-09	S50-09	F	A	-92		7.5	-27.7
L. noctivagans	Long Point	12-Sep-09	S54-09	F	A	-88		7.1	-26.9
L. noctivagans	Long Point	13-Sep-09	S56-09	F	A	-74			
L. noctivagans	Long Point	24-Aug-08	106-08	M	A	-78			
L. noctivagans	Long Point	9-Sep-08	119-08	M	A	-90			
L. noctivagans	Long Point	9-Sep-09	S32-09	M	A	-90			
L. noctivagans	Long Point	12-Sep-09	S41-09	M	A	-85		7.5	-27.3
L. noctivagans	Long Point	12-Sep-09	S53-09	M	A	-74			
L. noctivagans	Long Point	16-Sep-09	S58-09	M	A	-86		7.5	-27.5
L. noctivagans	Long Point	20-Aug-08	92-08	F	S	-76			
L. noctivagans	Long Point	24-Aug-08	103-08	F	S	-87			
L. noctivagans	Long Point	24-Aug-08	104-08	F	S	-92			
L. noctivagans	Long Point	24-Aug-08	107-08	F	S	-88			
L. noctivagans	Long Point	27-Aug-08	110-08	F	S	-67			

Species	Location	Date	Bat	Sex	Age	δD (dorsal)	δD (ventral)	δ¹⁵N	δ¹³C
L. noctivagans	Long Point	9-Sep-08	120-08	F	S	-87			
L. noctivagans	Long Point	9-Sep-08	121-08	F	S	-93			
L. noctivagans	Long Point	23-Aug-09	S3-09	F	S	-97		7.8	-27.2
L. noctivagans	Long Point	23-Aug-09	S4-09	F	S	-75			
L. noctivagans	Long Point	26-Aug-09	S6-09	F	S	-85		7.0	-22.9
L. noctivagans	Long Point	26-Aug-09	S9-09	F	S	-82		9.1	-26.8
L. noctivagans	Long Point	27-Aug-09	S16-09	F	S	-94			
L. noctivagans	Long Point	27-Aug-09	S17-09	F	S	-88		7.4	-28.8
L. noctivagans	Long Point	27-Aug-09	S18-09	F	S	-90		6.9	-24.6
L. noctivagans	Long Point	27-Aug-09	S19-09	F	S	-84			
L. noctivagans	Long Point	29-Aug-09	S21-09	F	S	-93		7.0	-26.6
L. noctivagans	Long Point	29-Aug-09	S22-09	F	S	-89		7.4	-26.2
L. noctivagans	Long Point	30-Aug-09	S26-09	F	S	-93			
L. noctivagans	Long Point	31-Aug-09	S28-09	F	S	-87		6.0	-25.5
L. noctivagans	Long Point	11-Sep-09	S38-09	F	S	-95		7.0	-29.9
L. noctivagans	Long Point	12-Sep-09	S42-09	F	S	-78			
L. noctivagans	Long Point	12-Sep-09	S43-09	F	S	-86			
L. noctivagans	Long Point	12-Sep-09	S44-09	F	S	-84		7.0	-24.9
L. noctivagans	Long Point	12-Sep-09	S46-09	F	S	-77		6.0	-25.6
L. noctivagans	Long Point	12-Sep-09	S48-09	F	S	-88			
L. noctivagans	Long Point	12-Sep-09	S49-09	F	S	-93		7.6	-27.7
L. noctivagans	Long Point	12-Sep-09	S52-09	F	S	-79			
L. noctivagans	Long Point	12-Sep-09	S55-09	F	S	-85			
L. noctivagans	Long Point	14-Sep-09	S57-09	F	S	-97			
L. noctivagans	Long Point	16-Sep-09	S60-09	F	S	-84		7.4	-24.6
L. noctivagans	Long Point	24-Aug-08	102-08	M	S	-81			

Species	Location	Date	Bat	Sex	Age	δD (dorsal)	δD (ventral)	δ¹⁵N	δ¹³C
L. noctivagans	Long Point	24-Aug-08	105-08	M	S	-77			
L. noctivagans	Long Point	24-Aug-08	108-08	M	S	-79			
L. noctivagans	Long Point	27-Aug-08	109-08	M	S	-74			
L. noctivagans	Long Point	1-Sep-08	114-08	M	S	-81		10.3	-25.3
L. noctivagans	Long Point	22-Aug-09	S1-09	M	S	-78			
L. noctivagans	Long Point	23-Aug-09	S2-09	M	S	-80			
L. noctivagans	Long Point	26-Aug-09	S10-09	M	S	-100		7.6	-28.2
L. noctivagans	Long Point	26-Aug-09	S5-09	M	S	-82			
L. noctivagans	Long Point	26-Aug-09	S7-09	M	S	-92		8.4	-28.3
L. noctivagans	Long Point	26-Aug-09	S8-09	M	S	-90			
L. noctivagans	Long Point	27-Aug-09	S11-09	M	S	-95			
L. noctivagans	Long Point	27-Aug-09	S12-09	M	S	-90			
L. noctivagans	Long Point	27-Aug-09	S13-09	M	S	-69		9.3	-27.6
L. noctivagans	Long Point	27-Aug-09	S14-09	M	S	-87		6.3	-24.9
L. noctivagans	Long Point	27-Aug-09	S15-09	M	S	-69			
L. noctivagans	Long Point	29-Aug-09	S20-09	M	S	-80			
L. noctivagans	Long Point	29-Aug-09	S23-09	M	S	-94		6.9	-26.4
L. noctivagans	Long Point	29-Aug-09	S24-09	M	S	-94			
L. noctivagans	Long Point	30-Aug-09	S25-09	M	S	-98		8.3	-29.2
L. noctivagans	Long Point	30-Aug-09	S27-09	M	S	-78			
L. noctivagans	Long Point	31-Aug-09	S29-09	M	S	-86		7.8	-25.5
L. noctivagans	Long Point	31-Aug-09	S30-09	M	S	-86			
L. noctivagans	Long Point	11-Sep-09	S33-09	M	S	-81		5.1	-25.6
L. noctivagans	Long Point	11-Sep-09	S34-09	M	S	-90			-27.9
L. noctivagans	Long Point	11-Sep-09	S35-09	M	S	-84			
L. noctivagans	Long Point	11-Sep-09	S36-09	M	S	-90		9.0	-26.5

Species	Location	Date	Bat	Sex	Age	δD (dorsal)	δD (ventral)	δ¹⁵N	δ¹³C
L. noctivagans	Long Point	11-Sep-09	S37-09	M	S	-95			
L. noctivagans	Long Point	11-Sep-09	S39-09	M	S	-88			
L. noctivagans	Long Point	12-Sep-09	S45-09	M	S	-94			
L. noctivagans	Long Point	12-Sep-09	S51-09	M	S	-90			
L. noctivagans	Long Point	16-Sep-09	S59-09	M	S	-76		7.0	-28.8
Lasiurus borealis	Long Point	22-Aug-09	R6-09	F	A	-25		7.4	-21.8
L. borealis	Long Point	23-Sep-08	125-08	M	A	-76		7.5	-23.8
L. borealis	Long Point	20-Aug-09	R1-09	M	A	-23		10.1	-21.6
L. borealis	Long Point	21-Aug-09	R2-09	M	A	-69		11.0	-25.1
L. borealis	Long Point	21-Aug-09	R4-09	M	A	-29		7.2	-22.7
L. borealis	Long Point	22-Aug-09	R5-09	F	S	-67		8.6	-23.7
L. borealis	Long Point	22-Aug-08	97-08	M	S	-82		10.5	-25.3
L. borealis	Long Point	3-Sep-08	115-08	M	S	-84		12.3	-24.2
L. borealis	Long Point	4-Sep-08	116-08	M	S	-81		10.3	-24.9
L. borealis	Long Point	4-Sep-08	117-08	M	S	-83		11.6	-24.2
L. borealis	Long Point	17-Sep-08	122-08	M	S	-83		9.7	-25.0
L. borealis	Long Point	21-Aug-09	R3-09	M	S	-76		7.0	-23.2
L. borealis	Long Point	4-Sep-09	R7-09	M	S	-79		9.4	-25.4
L. borealis	Long Point	8-Sep-09	R8-09	M	S	-25		8.7	-21.0
L. borealis	Long Point	24-Aug-08	100-08	F	U	-81		9.3	-24.5
L. borealis	Pinery	26-Jun-07	15-07	F	A	-69		8.1	-23.8
L. borealis	Pinery	28-Jun-07	17-07	F	A	-62	-60	6.3	-24.1
L. borealis	Pinery	2-Jul-07	19-07	F	A	-62	-66	8.2	-24.3
L. borealis	Pinery	2-Jul-07	20-07	F	A	-65	-59	4.8	-23.4
L. borealis	Pinery	7-Jul-07	29-07	F	A	-77	-67	6.9	-24.0
L. borealis	Pinery	7-Jul-07	30-07	F	A	-74	-71	8.6	-24.0

Species	Location	Date	Bat	Sex	Age	δD (dorsal)	δD (ventral)	δ^{15}N	δ^{13}C
L. borealis	Pinery	7-Jul-07	31-07	F	A	-71	-61	6.6	-24.0
L. borealis	Pinery	12-Jul-07	42-07	F	A	-54	-51	5.9	-22.7
L. borealis	Pinery	25-Jul-07	62-07	F	A	-23	-37	8.0	-22.5
L. borealis	Pinery	28-Jul-07	63-07	F	A	-51	-53	5.2	-23.2
L. borealis	Pinery	29-Jul-07	64-07	F	A	-67	-64	7.7	-23.8
L. borealis	Pinery	8-Aug-07	88-07	F	A	-69	-59		
L. borealis	Pinery	8-Aug-07	89-07	F	A	-61	-59	6.6	-23.2
L. borealis	Pinery	12-Aug-07	92-07	F	A	-44	-40		-23.4
L. borealis	Pinery	28-Jun-07	18-07	M	A	-49	-32	6.2	-22.6
L. borealis	Pinery	12-Jul-07	43-07	M	A	-45	-50	7.1	-23.2
L. borealis	Pinery	4-Aug-07	75-07	F	S	-24	-16		
L. borealis	Pinery	5-Aug-07	76-07	F	S	-37	-34		
L. borealis	Pinery	5-Aug-07	77-07	F	S	-37	-36		
L. borealis	Pinery	5-Aug-07	78-07	F	S	-31	-25		
L. borealis	Pinery	8-Aug-07	87-07	F	S	-71	-71		
L. borealis	Pinery	15-Aug-07	103-07	F	S	-44	-30		
L. borealis	Pinery	15-Aug-07	105-07	F	S	-71	-70		
L. borealis	Pinery	25-Jul-07	61-07	M	S	-67	-65		
L. borealis	Pinery	29-Jul-07	65-07	M	S	-66	-63		
L. borealis	Pinery	12-Aug-07	90-07	M	S	-65	-55		
L. borealis	Pinery	12-Aug-07	91-07	M	S	-35	-27		
L. borealis	Pinery	12-Aug-07	93-07	M	S	-62	-66		
L. borealis	Pinery	15-Aug-07	104-07	M	S	-39	-56		
L. borealis	Pinery	5-Aug-07	79-07	F	U	-28	-33		
Lasiurus cinereus	Cypress Hills	7-Jul-08	c40-08	F	A	-117			
L. cinereus	Cypress Hills	22-Jul-08	c36-08	F	A	-87		8.6	-23.9

Species	Location	Date	Bat	Sex	Age	δD (dorsal)	δD (ventral)	δ¹⁵N	δ¹³C
L. cinereus	Cypress Hills	31-Jul-08	c44-08	F	A	-112		9.3	-25.4
L. cinereus	Cypress Hills	31-Jul-08	c45-08	F	A	-104		9.4	-24.1
L. cinereus	Cypress Hills	1-Aug-08	c35-08	F	A	-88		7.9	-23.5
L. cinereus	Cypress Hills	1-Aug-08	C6-08	F	A	-101		11.1	-23.2
L. cinereus	Cypress Hills	1-Aug-08	C7-08	F	A	-90		10.3	-22.8
L. cinereus	Cypress Hills	1-Aug-08	C8-08	F	A	-58		8.3	-22.7
L. cinereus	Cypress Hills	4-Aug-08	c41-08	F	A	-87		10.1	-23.1
L. cinereus	Cypress Hills	4-Aug-08	c42-08	F	A	-107		9.4	-24.3
L. cinereus	Cypress HIlls	22-Jul-09	CCLF4-09	F	A	-106		10.4	-23.0
L. cinereus	Cypress HIlls	22-Jul-09	CCLF5-09	F	A	-117		10.0	-23.0
L. cinereus	Cypress Hills	26-Jul-08	c43-08	M	A	-113		9.8	-25.7
L. cinereus	Cypress Hills	30-Jul-08	C5-08	M	A	-59		9.3	-21.9
L. cinereus	Cypress Hills	1-Aug-08	C9-08	M	A	-112		10.3	-23.6
L. cinereus	Cypress HIlls	22-Jul-09	CC12-09	M	A	-108		10.4	-23.0
L. cinereus	Cypress HIlls	22-Jul-09	CC15-09	M	A	-95		8.1	-24.0
L. cinereus	Cypress HIlls	25-Jul-09	CC13-09	M	A	-66		10.1	-23.6
L. cinereus	Cypress HIlls	28-Jul-09	CC14-09	M	A	-59		8.3	-23.4
L. cinereus	Cypress Hills	20-Jul-08	C2-08	F	S	-98		10.6	-23.0
L. cinereus	Cypress Hills	21-Jul-08	c19-08	F	S	-123		10.3	-24.1
L. cinereus	Cypress Hills	22-Jul-08	c22-08	F	S	-100		10.5	-23.4
L. cinereus	Cypress Hills	28-Jul-08	c25-08	F	S	-126		10.2	-23.6
L. cinereus	Cypress Hills	1-Aug-08	c14-08	F	S	-118		10.5	-23.3
L. cinereus	Cypress Hills	1-Aug-08	c15-08	F	S	-129		10.2	-23.3
L. cinereus	Cypress Hills	1-Aug-08	c20-08	F	S	-127		10.5	-23.7
L. cinereus	Cypress Hills	1-Aug-08	c28-08	F	S	-125		10.4	-23.4
L. cinereus	Cypress Hills	1-Aug-08	c29-08	F	S	-124		10.5	-23.6

Species	Location	Date	Bat	Sex	Age	δD (dorsal)	δD (ventral)	δ¹⁵N	δ¹³C
L. cinereus	Cypress Hills	3-Aug-08	c13-08	F	S	-119		10.2	-23.9
L. cinereus	Cypress Hills	3-Aug-08	c27-08	F	S	-123		10.0	
L. cinereus	Cypress Hills	4-Aug-08	c12-08	F	S	-121		10.4	-23.9
L. cinereus	Cypress Hills	4-Aug-08	c21-08	F	S			9.9	-23.8
L. cinereus	Cypress HIlls	22-Jul-09	CCSAF15-09	F	S	-125		10.3	-23.7
L. cinereus	Cypress HIlls	28-Jul-09	C-CSAF-16-09d	F	S	-137		10.5	-23.6
L. cinereus	Cypress HIlls	2-Aug-09	CCSAF17-09	F	S	-129		10.1	-23.9
L. cinereus	Cypress Hills	21-Jul-08	c34-08	M	S	-121		10.5	-23.7
L. cinereus	Cypress Hills	22-Jul-08	c16-08	M	S	-113		10.0	-23.6
L. cinereus	Cypress Hills	1-Aug-08	c18-08	M	S	-124			
L. cinereus	Cypress Hills	1-Aug-08	c26-08	M	S	-123		10.4	-23.9
L. cinereus	Cypress Hills	2-Aug-08	c17-08	M	S	-120		10.4	-23.6
L. cinereus	Cypress Hills	3-Aug-08	c24-08	M	S	-122		9.8	-23.6
L. cinereus	Cypress HIlls	20-Jul-09	CCSAM11-09	M	S	-122		10.4	-23.4
L. cinereus	Cypress HIlls	25-Jul-09	CCSAM12-09	M	S	-130		10.5	
L. cinereus	Cypress HIlls	30-Jul-09	CSAM13-09	M	S	-125		10.4	-23.7
Myotis lucifugus	Benmiller	29-Jul-09	501a-09	F	A	-80		10.9	-21.8
M. lucifugus	Benmiller	29-Jul-09	502a-09	F	A	-92		9.6	-20.9
M. lucifugus	Benmiller	29-Jul-09	503a-09	F	A	-84		9.6	-19.4
M. lucifugus	Benmiller	29-Jul-09	504a-09	F	A	-81		10.4	-23.7
M. lucifugus	Benmiller	29-Jul-09	505a-09	F	A	-94		10.9	-22.8
M. lucifugus	Benmiller	29-Jul-09	506a-09	F	A	-101		11.7	-23.8
M. lucifugus	Benmiller	29-Jul-09	507a-09	F	A	-76		10.2	-23.1
M. lucifugus	Benmiller	29-Jul-09	508a-09	F	A	-85		10.5	-22.6
M. lucifugus	Benmiller	29-Jul-09	509a-09	F	A	-76		10.5	-23.2

Species	Location	Date	Bat	Sex	Age	δD (dorsal)	δD (ventral)	δ¹⁵N	δ¹³C
M. lucifugus	Benmiller	31-Jul-09	512-09	F	A	-96			
M. lucifugus	Benmiller	31-Jul-09	513-09	F	A	-101		10.4	-22.6
M. lucifugus	Benmiller	31-Jul-09	515-09	F	A	-94			
M. lucifugus	Benmiller	31-Jul-09	517-09	F	A	-86			
M. lucifugus	Benmiller	31-Jul-09	519-09	F	A	-94			
M. lucifugus	Benmiller	31-Jul-09	520-09	F	A	-94		10.0	-22.4
M. lucifugus	Benmiller	31-Jul-09	522-09	F	A	-95		10.4	-23.0
M. lucifugus	Benmiller	31-Jul-09	518-09	M	A	-91		10.3	-22.9
M. lucifugus	Benmiller	29-Jul-09	502b-09	F	S	-92		10.5	-21.3
M. lucifugus	Benmiller	29-Jul-09	503b-09	F	S	-94		10.3	-20.6
M. lucifugus	Benmiller	31-Jul-09	510-09	F	S	-96			
M. lucifugus	Benmiller	31-Jul-09	511-09	F	S	-98			
M. lucifugus	Benmiller	31-Jul-09	514-09	F	S	-85		11.4	-25.5
M. lucifugus	Benmiller	31-Jul-09	521-09	F	S	-95			
M. lucifugus	Benmiller	31-Jul-09	523-09	F	S	-94		11.4	-24.3
M. lucifugus	Benmiller	31-Jul-09	524-09	F	S	-101			
M. lucifugus	Benmiller	29-Jul-09	501b-09	M	S	-90		11.7	-22.7
M. lucifugus	Benmiller	29-Jul-09	504b-09	M	S	-96		12.4	-24.9
M. lucifugus	Benmiller	29-Jul-09	505b-09	M	S	-92		11.1	-24.5
M. lucifugus	Benmiller	29-Jul-09	506b-09	M	S	-97		12.2	-24.7
M. lucifugus	Benmiller	29-Jul-09	507b-09	M	S	-92		10.6	-21.0
M. lucifugus	Benmiller	31-Jul-09	508b-09	M	S	-92		11.1	-22.9
M. lucifugus	Benmiller	31-Jul-09	509b-09	M	S	-95		10.6	-21.4
M. lucifugus	Benmiller	31-Jul-09	516-09	M	S	-97			
M. lucifugus	Kananaskis	12-Jul-08	10-08	F	A	-113			
M. lucifugus	Kananaskis	12-Jul-08	12-08	F	A	-114			

Species	Location	Date	Bat	Sex	Age	δD (dorsal)	δD (ventral)	δ¹⁵N	δ¹³C
M. lucifugus	Kananaskis	12-Jul-08	14-08	F	A	-105			
M. lucifugus	Kananaskis	19-Jul-08	18-08	F	A	-110			
M. lucifugus	Kananaskis	19-Jul-08	19-08	F	A	-115			
M. lucifugus	Kananaskis	19-Jul-08	20-08	F	A	-109			
M. lucifugus	Kananaskis	19-Jul-08	21-08	F	A	-112			
M. lucifugus	Kananaskis	19-Jul-08	22-08	F	A	-109			
M. lucifugus	Kananaskis	19-Jul-08	23-08	F	A	-125		7.8	-26.0
M. lucifugus	Kananaskis	19-Jul-08	25-08	F	A	-114		7.5	-25.5
M. lucifugus	Kananaskis	19-Jul-08	26-08	F	A	-113		7.4	-25.0
M. lucifugus	Kananaskis	19-Jul-08	27-08	F	A	-113		7.8	-25.0
M. lucifugus	Kananaskis	19-Jul-08	28-08	F	A	-119		7.2	-25.5
M. lucifugus	Kananaskis	19-Jul-08	29-08	F	A	-117		8.1	-25.2
M. lucifugus	Kananaskis	19-Jul-08	30-08	F	A	-123		7.1	-25.5
M. lucifugus	Kananaskis	19-Jul-08	31-08	F	A	-112		7.8	-26.2
M. lucifugus	Kananaskis	19-Jul-08	32-08	F	A	-115		7.4	-24.8
M. lucifugus	Kananaskis	19-Jul-08	33-08	F	A	-113		7.4	-24.8
M. lucifugus	Kananaskis	19-Jul-08	34-08	F	A	-115		8.0	-25.4
M. lucifugus	Kananaskis	19-Jul-08	35-08	F	A	-121		7.4	-26.5
M. lucifugus	Kananaskis	19-Jul-08	36-08	F	A	-117		7.4	-24.4
M. lucifugus	Kananaskis	12-Jul-08	11-08	M	A	-96			
M. lucifugus	Kananaskis	12-Jul-08	13-08	F	S	-123		7.9	-25.2
M. lucifugus	Kananaskis	19-Jul-08	24-08	F	S	-119		8.2	-25.4
M. lucifugus	Pinery	24-Jun-07	8-07	F	A	-71			
M. lucifugus	Pinery	24-Jun-07	9-07	F	A	-73		7.3	-19.5
M. lucifugus	Pinery	24-Jun-07	10-07	F	A	-69			
M. lucifugus	Pinery	24-Jun-07	11-07	F	A	-62			

Species	Location	Date	Bat	Sex	Age	δD (dorsal)	δD (ventral)	δ¹⁵N	δ¹³C
M. lucifugus	Pinery	24-Jun-07	12-07	F	A	-64			
M. lucifugus	Pinery	24-Jun-07	13-07	F	A	-58		6.9	-21.1
M. lucifugus	Pinery	3-Jul-07	21-07	F	A	-73	-68		-20.6
M. lucifugus	Pinery	3-Jul-07	22-07	F	A	-75	-76		
M. lucifugus	Pinery	3-Jul-07	23-07	F	A	-85	-81		
M. lucifugus	Pinery	3-Jul-07	24-07	F	A	-63		6.9	-23.5
M. lucifugus	Pinery	3-Jul-07	25-07	F	A	-67		7.3	-21.2
M. lucifugus	Pinery	8-Jul-07	36-07	F	A	-80	-79		
M. lucifugus	Pinery	8-Jul-07	37-07	F	A	-68			
M. lucifugus	Pinery	8-Jul-07	38-07	F	A	-65			
M. lucifugus	Pinery	8-Jul-07	39-07	F	A	-76	-71	8.4	-21.4
M. lucifugus	Pinery	8-Jul-07	40-07	F	A	-74			
M. lucifugus	Pinery	13-Jul-07	44-07	F	A	-82	-78	8.8	-22.8
M. lucifugus	Pinery	13-Jul-07	45-07	F	A		-76	7.9	-25.7
M. lucifugus	Pinery	24-Jul-07	51-07	F	A	-72	-73		
M. lucifugus	Pinery	30-Jul-07	66-07	F	A	-76	-68		
M. lucifugus	Pinery	30-Jul-07	67-07	F	A	-74	-76		
M. lucifugus	Pinery	30-Jul-07	68-07	F	A	-83			
M. lucifugus	Pinery	30-Jul-07	69-07	F	A	-67	-64		
M. lucifugus	Pinery	6-Aug-07	82-07	F	A	-81	-84	6.6	-22.5
M. lucifugus	Pinery	6-Aug-07	83-07	F	A	-77	-76		
M. lucifugus	Pinery	6-Aug-07	84-07	F	A	-85			
M. lucifugus	Pinery	6-Aug-07	85-07	F	A	-66			
M. lucifugus	Pinery	15-Aug-07	98-07	F	A	-88	-84	12.6	-27.1
M. lucifugus	Pinery	15-Aug-07	99-07	F	A	-69	-70		
M. lucifugus	Pinery	15-Aug-07	100-07	F	A	-83			

Species	Location	Date	Bat	Sex	Age	δD (dorsal)	δD (ventral)	δ^{15}N	δ^{13}C
M. lucifugus	Pinery	24-Aug-07	198-07	F	A	-76	-74		
M. lucifugus	Pinery	24-Aug-07	200-07	F	A	-67	-64		
M. lucifugus	Pinery	24-Aug-07	201-07	F	A	-82	-81		
M. lucifugus	Pinery	29-Aug-07	208-07	F	A	-81	-78		
M. lucifugus	Pinery	24-Jun-07	14-07	M	A	-40			
M. lucifugus	Pinery	7-Jul-07	28-07	F	S	-79			
M. lucifugus	Pinery	8-Jul-07	32-07	F	S	-63	-60		
M. lucifugus	Pinery	13-Jul-07	46-07	F	S	-68	-66		
M. lucifugus	Pinery	13-Jul-07	47-07	F	S	-54		12.5	-24.1
M. lucifugus	Pinery	13-Jul-07	49-07	F	S	-60			
M. lucifugus	Pinery	24-Jul-07	52-07	F	S	-75			
M. lucifugus	Pinery	24-Jul-07	55-07	F	S	-67			
M. lucifugus	Pinery	24-Jul-07	57-07	F	S	-70			
M. lucifugus	Pinery	24-Jul-07	58-07	F	S	-70	-76	6.8	-22.8
M. lucifugus	Pinery	30-Jul-07	70-07	F	S	-76	-77		-21.9
M. lucifugus	Pinery	6-Aug-07	80-07	F	S	-82	-74	8.1	-21.4
M. lucifugus	Pinery	6-Aug-07	81-07	F	S	-73			
M. lucifugus	Pinery	15-Aug-07	94-07	F	S	-74	-69	8.8	-21.1
M. lucifugus	Pinery	24-Aug-07	197-07	F	S	-71			
M. lucifugus	Pinery	24-Aug-07	199-07	F	S	-83	-85	11.8	-25.0
M. lucifugus	Pinery	8-Jul-07	33-07	M	S	-71		6.0	-23.6
M. lucifugus	Pinery	8-Jul-07	34-07	M	S	-65			
M. lucifugus	Pinery	8-Jul-07	35-07	M	S	-57	-52		
M. lucifugus	Pinery	13-Jul-07	48-07	M	S	-72	-74		
M. lucifugus	Pinery	13-Jul-07	50-07	M	S	-68			
M. lucifugus	Pinery	24-Jul-07	53-07	M	S	-84			

Species	Location	Date	Bat	Sex	Age	δD (dorsal)	δD (ventral)	δ¹⁵N	δ¹³C
M. lucifugus	Pinery	24-Jul-07	54-07	M	S	-70			
M. lucifugus	Pinery	24-Jul-07	56-07	M	S	-76	-74	6.1	-23.6
M. lucifugus	Pinery	24-Jul-07	59-07	M	S	-77			
M. lucifugus	Pinery	24-Jul-07	60-07	M	S	-75			
M. lucifugus	Pinery	30-Jul-07	72-07	M	S	-74	-71		
M. lucifugus	Pinery	30-Jul-07	74-07	M	S	-66		7.6	-22.7
M. lucifugus	Pinery	6-Aug-07	86-07	M	S	-83	-76	9.2	-23.0
M. lucifugus	Pinery	15-Aug-07	95-07	M	S	-60	-61	9.2	-21.1
M. lucifugus	Pinery	15-Aug-07	95-07	M	S	-70		9.3	-21.1
M. lucifugus	Pinery	15-Aug-07	97-07	M	S	-70			
M. lucifugus	Pinery	15-Aug-07	101-07	F	U	-56			
M. lucifugus	Pinery	15-Aug-07	102-07	F	U	-73			

Appendix D: Stable isotope data reported in Chapter 4.

Table D.1 Each individual tri-coloured bat (*Perimyotis subflavus*) is identified with a museum-specific abbreviation (CU - Cornell University Museum of Vertebrates, Ithaca, NY; LSU - Louisiana State University Museum of Natural Science, Baton Rouge, LA; MCZ - Museum of Comparative Zoology, Harvard University, Cambridge, MA; ROM - Royal Ontario Museum, Toronto, ON) and its associated catalog number. Collection coordinates for each specimen are included in parentheses in decimal degrees. For some specimens, exact collection coordinates were available. For specimens where exact collection data were not available, coordinates for the centroid of the county of collection were used and reported below (obtained from the United States Geological Survey Geographic Names Information System [http://geonames.usgs.gov/domestic/]). Growing season meteoric water stable hydrogen isotope compositions were obtained from waterisotopes.org (Bowen and Revenaugh 2003; Bowen 2011). All stable isotope data are reported in ‰ relative to VSMOW. F=female; M=male.

Bat	State/ Province	Sex	Year	Julian date	δD_{fur}	Lat	Long	δD_{precip}
LSU 18633	Alabama	F	1974	332	-19	34.24	-87.20	-36
LSU 11582	Alabama	M	1966	221	-26	34.00	-87.32	-36
LSU 18632	Alabama	M	1974	235	-25	34.24	-87.20	-36
LSU 761	Alabama	M	1935	227	-14	34.52	-85.67	-39
LSU 11579	Arkansas	F	1966	200	-6	33.36	-91.96	-25
LSU 11574	Arkansas	F	1966	198	-19	33.49	-92.29	-25
LSU 11571	Arkansas	F	1966	199	-11	33.49	-91.65	-26
MCZ 29938	Arkansas	F	1933	293	-30	36.33	-94.33	-38
LSU 11578	Arkansas	M	1966	200	-30	33.36	-91.96	-25
LSU 11576	Arkansas	M	1966	198	-31	33.49	-92.29	-25
LSU 20389	Arkansas	M	1976	297	-18	34.49	-93.63	-29

Bat	State/ Province	Sex	Year	Julian date	δD_fur	Lat	Long	δD_precip
LSU 11570	Arkansas	M	1966	203	-20	34.49	-93.63	-29
LSU 19710	Arkansas	M	1973	320	-30	35.75	-92.92	-36
LSU 15179	Arkansas	M	1969	2	-34	35.78	-91.41	-35
ROM 999999	Arkansas	M	1934	89	-35	36.15	-93.68	-37
ROM 3412090003	Arkansas	M	1934	89	-33	36.15	-93.68	-37
MCZ 29937	Arkansas	M	1933	293	-20	36.33	-94.33	-38
ROM 76294	Florida	F	1959	186	-20	30.42	-84.47	-24
LSU 11142	Florida	F	1966	100	-36	30.70	-85.08	-25
MCZ BANGS-5598	Florida	M	1894	247	-23	28.52	-82.28	-20
ROM 76292	Florida	M	1951	12	-9	28.55	-81.35	-20
ROM 76293	Florida	M	1963	334	-4	29.67	-82.63	-22
LSU 11141	Florida	M	1966	100	-26	30.70	-85.08	-25
ROM 76295	Florida	M	1959	205	-16	30.79	-85.17	-25
CU 163	Georgia	F	1921	150	-11	30.83	-82.13	-25
MCZ 46625	Illinois	F	1947	316	-42	42.35	-90.18	-47
MCZ 46626	Illinois	M	1947	316	-58	42.35	-90.18	-47
ROM 76298	Indiana	F	1974	234	-34	38.25	-86.25	-41
ROM 76304	Indiana	F	1974	234	-32	38.25	-86.25	-41
ROM 76297	Indiana	F	1973	71	-51	39.05	-86.82	-43
ROM 76296	Indiana	F	1974	248	-38	39.05	-86.82	-43
ROM 45577	Indiana	F	1965	178	-38	41.12	-87.47	-40
ROM 45578	Indiana	M	1968	32	-41	38.22	-86.28	-41
ROM 76299	Indiana	M	1974	234	-35	38.25	-86.25	-41
ROM 45579	Indiana	M	1967	328	-48	38.67	-86.78	-41
ROM 45585	Indiana	M	1967	328	-19	38.67	-86.78	-41
ROM 45591	Indiana	M	1967	328	-36	38.67	-86.78	-41
ROM 45621	Indiana	M	1956	360	-31	38.75	-85.55	-43
ROM 45580	Indiana	M	1967	26	-62	38.86	-86.49	-42
ROM 76300	Indiana	M	1974	248	-36	39.05	-86.82	-43
ROM 76301	Indiana	M	1974	248	-32	39.05	-86.82	-43
ROM 45581	Indiana	M	1966	44	-56	39.17	-86.52	-43
ROM 45582	Indiana	M	1966	44	-32	39.17	-86.52	-43
ROM 45583	Indiana	M	1966	44	-33	39.17	-86.52	-43
ROM 45584	Indiana	M	1966	44	-44	39.17	-86.52	-43
CU 881	Kentucky	F	1925	196	-25	37.53	-83.32	-45
CU 10375	Kentucky	F	1939	45	-35	38.18	-83.43	-43
MCZ 46624	Kentucky	F	1940	82	-29	38.30	-83.03	-43
MCZ 40976	Kentucky	M	1940	82	-38	38.30	-83.03	-43

Bat	State/Province	Sex	Year	Julian date	δD$_{fur}$	Lat	Long	δD$_{precip}$
MCZ 40977	Kentucky	M	1940	82	-27	38.30	-83.03	-43
MCZ 46623	Kentucky	M	1940	82	-30	38.30	-83.03	-43
ROM 22955	Kentucky	M	1950	106	-27	38.35	-83.13	-43
ROM 22956	Kentucky	M	1950	106	-20	38.35	-83.13	-43
LSU 11599	Louisiana	F	1966	192	-12	30.41	-90.98	-20
LSU 11600	Louisiana	F	1966	228	-22	30.41	-90.98	-20
LSU 3321	Louisiana	F	1949	4	-28	30.62	-91.23	-20
LSU 11138	Louisiana	F	1966	149	-38	30.62	-91.23	-20
LSU 34147	Louisiana	F	1986	333	-28	30.62	-90.14	-20
LSU 6776	Louisiana	F	1951	259	-16	30.62	-91.23	-20
LSU 3671	Louisiana	F	1949	235	-14	30.77	-89.97	-22
LSU 29877	Louisiana	F	1972	279	-33	30.97	-91.86	-20
LSU 11597	Louisiana	F	1966	179	-22	31.26	-91.66	-21
LSU 11598	Louisiana	F	1966	281	-30	31.26	-91.66	-21
LSU 25413	Louisiana	F	1981	234	-33	31.49	-92.32	-20
LSU 1973	Louisiana	F	1941	16	-28	31.75	-93.14	-21
LSU 6479	Louisiana	F	1954	168	-20	32.04	-91.27	-23
LSU 11595	Louisiana	F	1966	194	-32	32.44	-92.19	-22
LSU 10551	Louisiana	F	1965	254	-30	32.54	-93.39	-22
LSU 26732	Louisiana	M	1983	7	-34	29.30	89.48	-21
LSU 11601	Louisiana	M	1966	228	-24	30.41	-90.98	-20
LSU 34146	Louisiana	M	1986	333	-24	30.62	-90.14	-20
LSU 3320	Louisiana	M	1948	346	-32	30.62	-91.23	-20
LSU 9299	Louisiana	M	1963	208	-23	30.62	-91.23	-20
LSU 9300	Louisiana	M	1963	215	-18	30.62	-91.23	-20
LSU 2494	Louisiana	M	1947	285	-6	30.62	-91.23	-20
LSU 25224	Louisiana	M	1981	311	-18	31.08	-93.09	-20
LSU 10552	Louisiana	M	1965	163	-43	31.20	-92.42	-20
LSU 1423	Louisiana	M	1939	301	-12	31.75	-93.14	-21
LSU 1109	Louisiana	M	1938	356	-27	31.75	-93.14	-21
LSU 11594	Louisiana	M	1966	183	-22	32.54	-93.39	-22
MCZ 55622	Maine	F	1903	258	-67	43.85	-70.33	-56
MCZ 55627	Maine	F	1912	152	-69	44.47	-69.77	-57
MCZ 55624	Maine	F	1915	177	-64	44.47	-69.77	-57
MCZ 55625	Maine	F	1915	177	-60	44.47	-69.77	-57
MCZ 55630	Maine	F	1914	178	-67	44.47	-69.77	-57
MCZ 55628	Maine	F	1913	182	-73	44.47	-69.77	-57
MCZ 55629	Maine	F	1909	240	-58	44.47	-69.77	-57
MCZ 55626	Maine	M	1916	182	-58	44.47	-69.77	-57
MCZ 55631	Maine	M	1914	182	-64	44.47	-69.77	-57

Bat	State/ Province	Sex	Year	Julian date	δD_fur	Lat	Long	δD_precip
MCZ 55633	Maine	M	1916	255	-63	44.47	-69.77	-57
MCZ 55623	Maine	M	1913	252	-70	45.80	-69.30	-61
MCZ BANGS-5596	Massachusetts	F	1878	224	-54	41.67	-70.25	-56
MCZ 34654	Massachusetts	F	1937	51	-66	42.17	-72.58	-54
MCZ 34580	Massachusetts	F	1936	327	-54	42.17	-72.58	-54
CU 7869	Massachusetts	M	1953	217	-39	41.67	-70.25	-56
CU 7870	Massachusetts	M	1953	217	-56	41.67	-70.25	-56
MCZ 34653	Massachusetts	M	1937	51	-69	42.17	-72.58	-54
MCZ 37489	Massachusetts	M	1937	121	-62	42.17	-72.58	-54
MCZ 34553	Massachusetts	M	1936	284	-63	42.33	-73.25	-58
LSU 8727	Mississippi	F	1960	359	-27	31.71	-88.63	-26
LSU 10956	Mississippi	F	1966	22	-20	32.03	-88.55	-27
LSU 11589	Mississippi	F	1966	219	-33	34.58	-88.15	-35
LSU 11591	Mississippi	M	1966	151	-50	31.13	-90.66	-22
LSU 11593	Mississippi	M	1966	160	-35	31.71	-88.63	-26
LSU 8728	Mississippi	M	1960	359	-28	31.71	-88.63	-26
LSU 11590	Mississippi	M	1966	215	-22	31.97	-89.54	-26
LSU 11588	Mississippi	M	1966	219	-28	34.58	-88.15	-35
LSU 11561	Missouri	F	1966	251	-30	36.88	-89.36	-39
LSU 6155	Missouri	F	1950	365	-33	38.91	-92.18	-40
LSU 6156	Missouri	M	1950	365	-25	38.91	-92.18	-40
CU 7836	New Jersey	F	1940	183	-48	40.83	-74.53	-53
CU 7837	New Jersey	F	1940	204	-44	40.83	-74.53	-53
CU 7838	New Jersey	F	1940	204	-59	40.83	-74.53	-53
CU 7833	New Jersey	F	1940	205	-46	40.83	-74.53	-53
CU 7835	New Jersey	F	1940	205	-50	40.83	-74.53	-53
CU 7839	New Jersey	F	1940	205	-43	40.83	-74.53	-53
CU 7841	New Jersey	F	1940	205	-59	40.83	-74.53	-53
CU 7834	New Jersey	M	1940	205	-31	40.83	-74.53	-53
CU 7840	New Jersey	M	1940	205	-57	40.83	-74.53	-53
CU 822	New York	F	1926	44	-91	42.25	-76.47	-55
CU 7831	New York	F	1929	62	-44	42.25	-76.47	-55
CU 880	New York	F	1924	130	-76	42.25	-76.47	-55
CU 7827	New York	F	1907	145	-77	42.25	-76.47	-55
CU 702	New York	F	1924	150	-67	42.25	-76.47	-55
CU 9599	New York	F	1953	229	-73	42.25	-76.47	-55
CU 9600	New York	F	1953	229	-65	42.25	-76.47	-55
MCZ 41941	New York	F	1896	219	-72	44.10	-73.82	-64
CU 878	New York	M	1924	130	-80	42.25	-76.47	-55

Bat	State/ Province	Sex	Year	Julian date	δD_{fur}	Lat	Long	δD_{precip}
CU 701	New York	M	1924	150	-61	42.25	-76.47	-55
MCZ 48533	New York	M	1941	305	-74	42.60	-74.00	-54
MCZ 48535	New York	M	1949	348	-57	42.60	-74.00	-54
CU 19679	New York	M	1973	83	-47	42.73	-77.77	-55
CU 12875	New York	M	1967	286	-85	42.73	-77.77	-55
CU 7740	New York	M	1953	226	-68	44.05	-75.90	-57
ROM 22950	North Carolina	F	1950	103	-24	36.05	-81.87	-49
ROM 22951	North Carolina	F	1950	103	-25	36.05	-81.87	-49
ROM 22954	North Carolina	F	1950	103	-30	36.05	-81.87	-49
MCZ 11399	North Carolina	M	1912	103	-13	35.87	-82.67	-52
ROM 22952	North Carolina	M	1950	103	-31	36.05	-81.87	-49
ROM 22953	North Carolina	M	1950	103	-50	36.05	-81.87	-49
LSU 11562	Oklahoma	F	1966	204	-15	34.27	-95.16	-26
ROM 19677	Oklahoma	F	1948	351	-35	35.83	-94.62	-33
LSU 11566	Oklahoma	M	1966	205	-34	33.89	-94.64	-25
LSU 11563	Oklahoma	M	1966	204	-31	34.27	-95.16	-26
ROM 19676	Oklahoma	M	1948	351	-21	35.83	-94.62	-33
ROM 83037	Ontario	F	1966	79	-82	44.43	-78.13	-58
ROM 3305150002	Ontario	M	1933	125	-75	43.25	-79.07	-53
ROM 33660	Ontario	M	1957	358	-65	43.43	-79.90	-52
ROM 21809	Ontario	M	1952	103	-71	43.47	-79.92	-52
ROM 20948	Ontario	M	1951	106	-88	43.52	-79.88	-52
ROM 14338	Ontario	M	1940	56	-93	43.62	-80.13	-54
ROM 14339	Ontario	M	1940	56	-75	43.62	-80.13	-54
ROM 22498	Ontario	M	1952	348	-83	43.75	-79.92	-54
ROM 14850	Ontario	M	1941	17	-70	44.30	-77.18	-56
ROM 16062	Ontario	M	1942	93	-77	44.30	-77.18	-56
ROM 83035	Ontario	M	1966	79	-76	44.43	-78.13	-58
CU 3945	Pennsylvania	F	1940	335	-71	40.75	-77.50	-50
ROM 24686	Pennsylvania	M	1932	169	-35	40.12	-78.28	-47
CU 7298	Pennsylvania	M	1947	98	-56	40.25	-77.67	-49
ROM 24685	Pennsylvania	M	1932	161	-40	40.37	-78.28	-51
ROM 24683	Pennsylvania	M	1931	223	-61	40.37	-75.88	-51
ROM 24684	Pennsylvania	M	1931	228	-43	40.37	-78.28	-51
MCZ 48534	Pennsylvania	M	1941	307	-74	40.75	-77.50	-50
CU 3942	Pennsylvania	M	1940	335	-63	40.75	-77.50	-50
CU 725	Pennsylvania	M	1924	306	-76	41.25	-77.90	-51
ROM 14849	Quebec	M	1941	18	-68	45.63	-75.93	-61
ROM 22948	Quebec	M	1946	347	-78	45.63	-75.93	-61

Bat	State/ Province	Sex	Year	Julian date	δD_{fur}	Lat	Long	δD_{precip}
ROM 22949	Quebec	M	1946	347	-93	45.63	-75.93	-61
LSU 3669	Tennessee	F	1949	361	-29	35.32	-87.69	-39
LSU 3670	Tennessee	M	1949	361	-46	35.32	-87.69	-39
LSU 19498	Tennessee	M	1950	217	-42	36.07	-87.28	-40
MCZ 11398	Tennessee	M	1911	357	-20	36.13	-83.28	-44
LSU 19500	Tennessee	M	1949	352	-36	36.16	-86.68	-40
MCZ 37490	Vermont	F	1935	93	-64	43.55	-72.57	-58
MCZ 59115	Vermont	F	1914	326	-71	43.62	-73.02	-58
MCZ 59113	Vermont	M	1913	124	-60	43.62	-73.02	-58
MCZ 59117	Vermont	M	1934	312	-73	43.62	-73.02	-58
MCZ 59116	Vermont	M	1915	332	-77	43.62	-73.02	-58
MCZ 59114	Vermont	M	1914	279	-65	43.62	-73.02	-58
MCZ 35435	Vermont	M	1937	285	-68	43.62	-73.02	-58
CU 591	Virginia	F	1923	278	-39	38.87	-78.60	-48
CU 1082	West Virginia	M	1931	187	-39	38.33	-81.57	-44
CU 1078	West Virginia	M	1931	222	-52	38.68	-79.35	-48
ROM 10388	West Virginia	M	1936	357	-38	39.63	-79.95	-45

References

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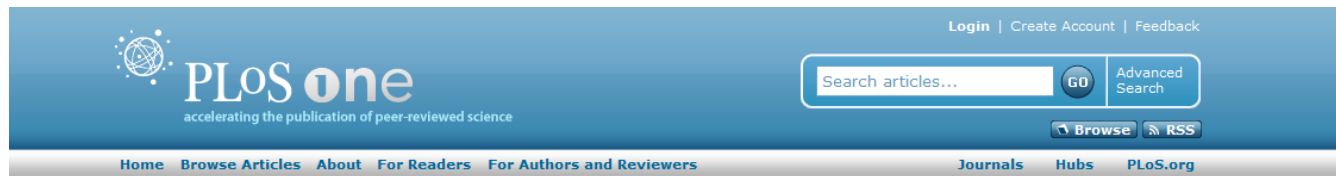
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Appendix E: Open-access license agreement for Public Library of Science ONE (PLoS ONE)

Accessed December 16, 2011: http://www.plosone.org/static/license.action. See this link for further information on the PLoS ONE open-access license agreement.

Appendix F: Animal use protocol approval documentation

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

The University *of* Western Ontario
Animal Use Subcommittee / University Council on Animal Care
Health Sciences Centre, ● London, Ontario ● CANADA – N6A 5C1
PH: 519-661-2111 ext. 86770 ● FI 519-661-2028 ● www.uwo.ca / animal

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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.

c.c.   L McGuire, J Wasylenko

The University *of* Western Ontario
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Western

Date: 05.28.07

Dear Dr. Fenton:

A **MAJOR MODIFICATION** to your "Animal Use Protocol" entitled:

Behavioural Ecology of Bats has been approved.

The protocol **2004-027-03** and yearly expiry date of **03.31.08** remain unchanged.

Please ensure that individual(s) performing procedures, as described in this protocol, are familiar with the contents of this document.

c.c. Approved Modification   - B. Fenton, J. Wasylenko, D. Cheshuk
      Approval Letter   - J. Wasylenko, D. Cheshuk

The University of Western Ontario
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Curriculum Vitae

Name: Erin Fraser

Post-secondary Education and Degrees:
University of Toronto
Toronto, Ontario, Canada
2000-2003 B.Sc.

The University of Western Ontario
London, Ontario, Canada
2004-2006 M.Sc.

The University of Western Ontario
London, Ontario, Canada
2006-2011 Ph.D.

Honours and Awards:
Province of Ontario Graduate Scholarship (OGS)
2004-2005, 2005-2006

Province of Ontario Graduate Scholarship in Science and Technology (OGSST)
2007-2008

Natural Sciences and Engineering Research Council Canadian Graduate Scholarship (NSERC CGS)
2008-2009, 2009-2010

Bat Conservation International Student Scholarship
2007-2008, 2008-2009, 2010-2011

Related Work Experience
Teaching Assistant
The University of Western Ontario
2004-2010

Teaching Assistant Training Program Instructor and Coordinator
The University of Western Ontario
2007-2011 (Instructor); 2008-2011 (Coordinator)

Publications:

Peer-reviewed journal publications

Submitted
Fraser, E.E., McGuire, L.P., Eger, J.L., Longstaffe, F.J. and Fenton, M.B. *Submitted.* Evidence of latitudinal migration in Tri-colored bats, *Perimyotis subflavus.* Submitted October 11 to *PLoS ONE* (submission #: PONE-D-11-20148v).

Published
Clare, E.L., **Fraser, E.E.**, Braid, H.E., Fenton, M.B. and Hebert, P.D.N. 2009. Species on the menu of a generalist predator, the eastern red bat (*Lasiurus borealis*): using a molecular approach to detect arthropod prey. *Mol. Ecol.* **18(11):** 2532-2542.

Murray, K.L., **Fraser, E.E.,** Davy, C., Fleming, T.H. and Fenton, M.B. 2009. Characterization of the echolocation calls of bats from Exuma, Bahamas. *Acta Chiropterol.* **11(2):** 415-424.

Fraser, E.E. and Miller, J.F. 2008. Diurnal, above-ground movement in Hairy-tailed Moles, *Parascalops breweri. Can. Field-Nat.* **122(3):** 267.

Davy, C.M. and **Fraser, E.E.** 2007. Observation of foliage-roosting in the little brown bat, *Myotis lucifugus. Can. Field-Nat.* **121(4):** 420.

Fraser, E.E. and Fenton, M.B. 2007. Age and food hardness affect food handling by insectivorous bats. *Can. J. Zool.* **85:** 985-993. (Summary of M.Sc. thesis)

Books:
Fraser, E.E., MacKenzie, A. and Davy, C. 2007. Photo field guide to the bats of Ontario. St. Thomas Field Naturalist Club Incorporated.