

**Movement dynamics of the little brown bat (*Myotis lucifugus*) and
the northern long-eared bat (*Myotis septentrionalis*)
in Nova Scotia**

by
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Abstract

Movement dynamics of the little brown bat (*Myotis lucifugus*) and the northern long-eared bat (*Myotis septentrionalis*) in Nova Scotia

By Jordi L. Segers, June 23, 2014

Two resident bat species in Nova Scotia are the little brown bat (*Myotis lucifugus*), an aerial hawker, and the northern long-eared bat (*M. septentrionalis*), a forest gleaner. Little is known about their local movement patterns and response to deforestation in summer, and their migration between summering sites and swarming sites in fall. A study was conducted near a newly constructed wind farm in Nova Scotia, for which forest was cleared, to make inferences on variable, inter-specific effects of ecosystem alterations. A second study was conducted to make inferences on migration between summer- and swarming sites, using stable isotopes of carbon and nitrogen in bat fur. The results indicate that both species are impacted differently by deforestation due to their different foraging and roosting requirements. Migration in fall appears to be uniform in both species, where swarming sites are congregations of bats from several summering sites.

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Chapter 1

Movement dynamics of the little brown bat (*Myotis lucifugus*) and the northern long-eared bat (*Myotis septentrionalis*) in Nova Scotia: Introduction

Six species of bat are known to occur in Nova Scotia for at least part of the year. The little brown bat (*Myotis lucifugus*), northern long-eared bat (*M. septentrionalis*) and tri-coloured bat (*Perimyotis subflavus*) are year-round resident, short-distance migratory species that generally migrate from their cave or mine hibernaculum to a summering site, generally within 300 km (Faure et al. 1993; Fenton and Barclay 1980; Owen et al. 2002; Ratcliffe and Dawson 2003; Broders et al. 2004). The hoary bat (*Lasiurus cinereus*), red bat (*L. borealis*) and silver-haired bat (*Lasionycteris noctivagans*) are long-distance migratory species that may travel >500 km between southern wintering sites and northern summering sites (Fleming and Eby 2003). For both short and long-distance migrants little is known about migration dynamics. In Nova Scotia few captures and acoustic detections of long-distance migrants are described in the literature (Shump and Shump 1982; Van Zyll de Jong 1985; Lucas and Hebda 2011; Segers et al 2013), but extensive capture and sampling studies have been done in the province on short-distance migrants, mainly *M. lucifugus* and *M. septentrionalis* (Broders et al 2003). Though several swarming, hibernation and summering sites of these species are known, it is unclear how individuals or colonies move or mix between these. In addition, little is known about summer movement patterns and how these may be affected by anthropogenic alterations of bat habitat. The research in this thesis was conducted to answer movement-related questions in bats at two different scales: a local and regional (provincial) scale.

1.1 Impact of anthropogenic alterations

A major cause for anthropogenic habitat alteration, that may affect bats at a local scale, is deforestation. In Nova Scotia logging is a major industry and expansion of

suburban communities contributes to non-regenerative culling of forests (Davis and Browne 1996). Deforestation creates fragmented forests that have more forest edge habitat and open spaces (Soule et al. 1992). *Myotis lucifugus* and *M. septentrionalis* have different life-history traits and their response to deforestation may vary (Loehle and Li 1996; Swihart et al. 2003; Marvier et al. 2004; Webala et al. 2011).

In recent years, the fragmentation of natural areas is often attributed to the development of wind farms. Wind generated energy has been utilized as early as 1000 BC by the ancient Egyptians, who used wind mills to pump water onto the land to irrigate agricultural land (Hau 2000). In 1887, in Scotland, wind power was used to generate electricity for the first time (Price and Blyth 2005) and over the next 50 years this industry developed rapidly with 600,000 units installed in the United States of America in the 1930's (Hepbasli and Ozgener 2004; Deng 2008). In the 1980's and 1990's the demand for renewable energy sources, such as wind power, increased dramatically worldwide, with a 30% yearly increase in Gigawatt production (Leung and Yang 2012). To meet the Canadian Government's commitment to reduce the emission of greenhouse gases the target was set to increase wind power capacity from 444 megawatts in 2005 to 4000 megawatts in 2010 (Guha et al. 2005). By 2025 the Canadian wind energy association wants 20% of Canada's electrical energy demand to be supplied by the wind energy industry by adding another 55000 megawatts of generating capacity (CanWEA 2008).

The major direct threat of wind turbines to bats is direct strikes with rotating turbine blades causing lethal blunt force trauma that may either result in instantaneous death or severe internal and external injuries resulting in death at a later time (Rollins et

al. 2012). A second direct threat may be caused by pulmonary barotrauma; tissue damage to the respiratory system due to rapid air-pressure changes near moving turbine blades (Baerwald et al. 2008). It is suggested that migratory bat species are more affected by wind turbines than short distance migrants (Johnson et al. 2003; USGS Collins Science Center 2012), but Arnett et al. (2008) detected high mortality of *Myotis* species at wind farms, estimating thousands of bat casualties per year in the eastern United States alone. However, secondary impacts on bats, such as the effects of forest clearing for wind farm construction, are not well understood.

1.2 Migration

Migration is the act of making regular, seasonal movements between places to maximize resource utilization and energy efficiency. Many species migrate, well known examples are the great migration of terrestrial mammals on the Serengeti, where millions of wildebeests and other grazers travel hundreds of kilometers to find water and food (Thirgood et al. 2004; Harris et al. 2009), and the migration of birds, some of which travel 80,000 km annually (Egevang 2010; Elewa 2010). Many bat species in temperate zones also migrate between wintering and summering sites. Long-distance migrants winter at low latitudes and fly to temperate areas for the summer, while short-distance migrants often migrate between wintering hibernacula and nearby summering roosts sites (Griffin 1936; Davis and Hitchcock 1965; Popa-Lisseanu and Voigt 2009; Fraser 2012). While both bats and birds migrate to use ephemeral resources at temperate areas during the summer, their life-history characteristics differ dramatically. In many bird species, males benefit from early migration to be the first to arrive at northern summering sites and

establish a breeding territory (Verhulst and Tinbergen 1991; Kokko 1999). Bats however, mate in fall at swarming sites and sperm is stored by females over the winter to start fertilization in the spring (Racey 1979). In bats, females might benefit from early migration to provide their pups with a longer summer season, whereas males only need to make it through summer to mate again at swarming sites in fall.

There is much interest in studying migratory patterns of animals and many methods have been applied on a variety of species and taxa. Direct methods to study migration are capture-mark-recapture, radio- or satellite-tracking or using geolocators. However, recaptures in bats are rare and many species are too small to successfully track long distances (Rubenstein and Hobson 2004; Holland and Wikelski 2009). Indirect methods for studying migration are through using molecular techniques or stable isotopes. Inferences on migration movement can be made by using genetic markers to detect genetic connectivity between populations (Hellberg et al. 2002; Boulet et al. 2007; Lowe and Allendorf 2010). Stable isotopes provide additional opportunities to study migratory movement.

1.3 Stable isotopes

Isotopes are naturally occurring components of elements found in nature. The nucleus of an atom contains neutrons and protons in variable abundance. The number of neutrons in the nucleus defines the isotope type of that element. Having too many or too few neutrons results in unstable- or ‘radio-isotopes’ which decay over time into stable forms. Stable isotopes do not decay. While many elements exist as numerous different stable isotopes, some elements exist as only a light and heavy isotope (Fry 2006). Heavy

isotopes contain more neutrons and are often far less abundant (e.g.: ~1:100) than their lighter counterpart (Rubenstein and Hobson 2004). Atoms with heavy isotopes have a stronger chemical bond which results in slower reactions and isotopic separation, called 'fractionation'. Fractionation of heavy and light stable isotopes causes variation in heavy-light ratio, which can be measured in organic tissue. The most commonly used stable isotopes in biological research are hydrogen ($^2\text{H}/^1\text{H}$ or D for Deuterium), carbon ($^{13}\text{C}/^{12}\text{C}$), nitrogen ($^{15}\text{N}/^{14}\text{N}$), oxygen ($^{18}\text{O}/^{16}\text{O}$) and sulphur ($^{34}\text{S}/^{32}\text{S}$) (Hobson 1999; Rubenstein and Hobson 2004; Fry 2006).

Stable isotopes can be measured through mass-spectroscopy, in which the sample is converted to a gas, magnetically charged to separate lighter and heavier molecules and finally counted. Abundance of stable isotopes is expressed as the ratio of the heavy and light isotopes of the sample compared to an international standard. For this the 'delta' (δ) notation is used. The difference between the measured ratio and the standard are very small and are therefore expressed as parts per thousand (per mil; ‰) (Coplen 1996; Fry 2006). The international standard for $\delta^{13}\text{C}$ is Vienna Pee Dee Belemnite (VPDB) and for $\delta^{15}\text{N}$ it is air (Werner and Brand 2001).

Isotopes are transported from the environment (e.g., water, atmosphere) to consumers (plants and animals) and from prey to predator through consumption. Tracing stable isotopes to their origin organism or location allows biologists to make inferences on animal migration and trophic dynamics. Extensive data of global δD are available and can be used to study long-distance migration. δC , δN and δS are most commonly used to study food webs, and δO is used both for food web studies and migration (Hobson 1999; Rubenstein and Hobson 2004; Fry 2006).

1.4 White-nose syndrome

In most of the eastern United States and Canada bats are in serious decline due to the fungus *Pseudogymnoascus destructans* (formerly *Geomyces destructans*), that causes white-nose syndrome (WNS) and major mortality amongst hibernating bats (Blehert et al. 2009; Minnis and Lindner 2013). First discovered in Albany, N.Y. in 2006, WNS has spread to 25 states and 5 Canadian provinces in 2014 and has since wiped out over 5.7 million bats. However, long-distance migrants might not be affected by WNS (Dzal et al. 2011). White-nose syndrome was first detected in Nova Scotia in the winter of 2011-2012 and in the following winter a decrease of 90-99% was detected amongst *M. lucifugus* and *M. septentrionalis* in known hibernacula (H.G Broders and L.E. Burns, unpublished data). White-nose syndrome puts additional pressure on many bat populations that might already be impacted by habitat fragmentation and wind farm mortality. Never before has it been this important to understand bat ecology, such as migratory patterns and local movement response to forest fragmentation, for the conservation of impacted species.

1.5 Research objectives

Chapters 2 and 3 describe my conducted studies on bats' habitat fragmentation response and regional migration. In more detail chapter 2 addresses habitat fragmentation response of summering *M. lucifugus* and *M. septentrionalis* in Nova Scotia. The objectives, addressed in chapter two, were:

1. To investigate which landscape features predict bat presence in a fragmented landscape;
2. To characterize the variation in habitat of summering bats; and

3. To determine whether there are differences in magnitude of bat activity between fragmented and less-fragmented landscapes.

A newly constructed wind farm in Nova Scotia provided the right conditions to conduct this study and collect important information on anthropogenic effects on bats.

Chapter 3 focusses on using stable isotopes to make inferences on regional migration of *M. lucifugus* and *M. septentrionalis* between summering and swarming sites.

The objectives addressed in chapter 3 were:

1. To investigate whether $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in bat fur can effectively be used to make inference on the migration patterns of regionally migrating bats between summering and swarming sites.
2. To characterize the spatial variability in the stable isotope patterns of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in bats throughout Nova Scotia in the summer and fall; and

Availability of bat fur samples, collected throughout Nova Scotia over the last decade, provided a unique opportunity to study site- and seasonal-variability in isotopic signatures of populations.

Finally, chapter 4 provides an overall conclusion, focussing on local-scale summer movement patterns and provincial-scale movement patterns from summering sites to swarming sites of *M. lucifugus* and *M. septentrionalis*. Here, associated threats are discussed based on the results of the studies described in chapters 2 and 3, to suggest an effective conservation response.

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Chapter 2
Interspecific effects of forest fragmentation on bats

2.1 Abstract

Wind farm development may be an important contributor to forest fragmentation but how such developments impact bats is poorly understood. We hypothesized that bat activity at a wind farm would be explained, at least in part, by attraction and avoidance behaviour caused by deforestation. We tested predictions of this hypothesis via a landscape-level acoustic, capture and radio telemetry survey of *Myotis lucifugus* and *M. septentrionalis*. Acoustic and capture data indicated no significant difference in magnitude of activity between the fragmented wind farm and the less-fragmented surrounding areas. However, only two of 19 radio-tracked bats were ever located inside the wind farm despite being captured adjacent to it. Bat locations were compared against randomly generated locations within the same area in a logistic regression framework to rank landscape variables in order of association with bats. A multi-criteria evaluation of forest metrics showed that, over a three-year period, there was an increase of suitable habitat inside the wind farm for *M. lucifugus* and a decrease for *M. septentrionalis*. These results support the contention that, at this level of disturbance, *M. lucifugus* may use the cleared areas while *M. septentrionalis* is negatively impacted by increased deforestation caused by wind farm development.

Key words: Little brown bat, *Myotis lucifugus*, Northern long-eared bat, *Myotis septentrionalis*, forest fragmentation, distribution, movement, multi-criteria evaluation, radio telemetry, logistic regression

2.2 Introduction

Anthropogenic alteration of natural environments can have major impacts on populations of local species (Tilman et al. 1994). The specific impacts may vary by species life history traits, where some species benefit and others are negatively affected (Loehle and Li 1996; Ricklefs 2001; Marvier et al. 2004; Webala et al. 2011). In addition to community composition, fragmentation of natural landscapes may reduce food-chain length and the rate of predation or parasitism (Kruess and Tscharntke 1994). Edge effects and cumulative area loss following isolation of remnant populations have been correlated with loss of species diversity (Soule et al. 1992). Many studies have focussed on landscape alteration and its direct and indirect effects on population's distribution and animal's home ranges. To explain why animals have home ranges and how they use them Spencer (2012) hypothesized that animals use cognitive maps they have acquired over long-term use of an area. Barchi et al. (2013) showed the principle of cognitive maps in a study on spatial memory in big brown bats, *Eptesicus fuscus* (Beauvois, 1796), where individuals remembered obstacle configuration from an experiment conducted one month earlier. Home ranges exist because knowledge about places increases the fitness of an individual (Spencer 2012), therefore a home range is defined as: a foraging and roosting area in which an animal regularly exploits and updates spatial information.

An animal must respond to alterations within its home range to maintain fitness, but drastic, acute alterations often inhibit this response. Swihart et al. (2003) found that species exhibit considerable interspecific variation in their response to fragmentation of natural areas. They concluded that niche breadth is the primary determinant of a species' adaptability to fragmentation where species with the greatest niche breadth were most

tolerant of anthropogenic disturbance. For example, western roe deer, *Capreolus capreolus* (Linnaeus, 1758), show a high behavioural plasticity in habitat selection in partially deforested agricultural areas (Morellet et al. 2011), whereas the least weasel, *Mustela nivalis* (Linnaeus, 1766), is classified as being sensitive to a fragmented home range due to a narrow niche breadth (Swihart et al. 2003), resulting in increased vulnerability to extinction. However, species vagility may also be important factors affecting their ability to adapt to anthropogenic alterations (Blem 1980; Robinson and Wilcove 1994; Moore et al. 1995).

Little is known about how fragmentation impacts local bat populations. A recent cause of fragmentation in forested landscapes, that is the increase of open areas and forest edge habitat, is attributable to the development of commercial wind farms in forested areas. Since the 1990's, the demand for renewable energy sources, amongst which is wind power generated energy, increased dramatically worldwide, with an expected yearly increase of 30% (Leung and Yang 2012). To meet the Canadian Government's commitment to reduce the emission of greenhouse gases, the target was set to increase wind power capacity ten-fold from 2005 to 2010 (Guha et al. 2005). The Canadian Wind Energy Association has set a target of 20% of Canada's electrical energy demand to be supplied by wind by 2025, another tenfold increase since 2010 (CanWEA 2008). Besides the positive contribution that wind energy makes to the environment, negative impacts to wildlife have been detected as the industry expands.

Direct threats of wind turbines to wildlife are strikes with rotating turbine blades causing lethal blunt force trauma that results in instantaneous death or severe injuries resulting in death at a later time (Rollins et al. 2012) and pulmonary barotrauma

(Baerwald et al. 2008). Studies suggest that migratory bat species (e.g., hoary bats, *Lasiurus cinereus* (Beauvois, 1796), eastern red bats, *Lasiurus borealis* (Müller, 1776), silver-haired bats, *Lasionycteris noctivagans* (Le Conte, 1831)) are significantly more affected by wind turbines than short-distance migrants (e.g. *Myotis* sp.) (Johnson et al. 2003; USGS Collins Science Center 2012). Nonetheless, *Myotis* species have also been shown to be dramatically impacted by the increasing number of wind turbines and wind farms, causing thousands of bat casualties per year in the eastern United States alone (Arnett et al. 2008). In addition, wind farm development in a forested landscape causes forest fragmentation, a secondary impact beyond direct mortality. In this study we investigated how forest fragmentation, typical of a wind farm, may impact local bat species.

Two resident, short distance migratory bat species in Nova Scotia are the northern long-eared myotis, *Myotis septentrionalis* (Trouessart, 1897), and the little brown bat, *Myotis lucifugus* (Le Conte, 1831). Females of both species are highly social, roosting in trees (*M. septentrionalis*) or houses (*M. lucifugus*) in maternity colonies in summer to give birth to a single pup in July, whereas males are mostly solitary in their roosting habits. *Myotis septentrionalis*, a forest-dwelling, gleaning bat, is known to have different echolocation tactics from *M. lucifugus* during foraging (Faure et al. 1993; Ratcliffe and Dawson 2003). Studies by Owen et al. (2002) and Broders et al. (2004) suggest that *M. septentrionalis* has a shorter perceptual range than *M. lucifugus*, presumably due to its specialization for foraging in forested (cluttered) areas. Forest fragmentation may negatively impact this species due to this specialization.

Females have additional energetic constraints, compared with males, during pregnancy and lactation. A higher wing loading during pregnancy could make female bats less manoeuvrable and less effective at catching prey in cluttered habitats (Kalcounis and Brigham 1995). During lactation, females are limited in the distance that they can fly to forage because they have to return to the roost to feed their pup regularly (Anthony and Kunz 1977). Henry et al. (2002) showed that *M. lucifugus* females had a 50% smaller home range during lactation in July compared with their pregnancy period. In addition, Broders et al. (2006) showed that female *M. septentrionalis* have a larger minimum forage area and minimum roost area than conspecific males, possibly increasing risks when roosting in an area near forest fragmentation.

The life-history characteristics of both *M. lucifugus* and *M. septentrionalis* may serve as a model to assess risks and to help formulate better management plans to mitigate forest fragmentation. Although many studies have focussed on movement patterns of bats (e.g., Williams and Williams 1970; Henry et al. 2002; Owen et al. 2003; Broders and Forbes 2004; Broders et al. 2006; Henderson and Broders 2008), impacts of deforestation on bats (Grindal and Brigham 1999) and the impact of wind turbine facilities on bats (Fenton and Barclay 1980; Johnson et al. 2003; Williams 2003; Nicholson et al. 2005; Barclay et al. 2007; Baerwald et al. 2008; Horn et al. 2008; Orbach and Fenton 2010; Rollins et al. 2012), no published data are available on their specific movement patterns or any changes in behaviour that may result from these anthropogenic environmental changes.

The objectives of this study were: 1) to investigate which landscape features predict bat presence in a landscape fragmented for a wind farm, 2) to characterize the

inter- and intra-specific variation in habitat of summering bats, and 3) to determine whether there are differences in the magnitude of activity between fragmented and less-fragmented landscapes. We hypothesized that bat activity in a fragmented forest landscape is, at least in part, an effect of the attraction and avoidance behaviour caused by deforestation due to the construction of a wind farm. Specifically, we predicted that the probability of presence of the forest-dwelling *M. septentrionalis* would be lower in fragmented, open areas while the probability of presence of *M. lucifugus* will be higher there. To test this prediction we conducted a radio telemetry survey to quantify use of fragmented and less-fragmented areas. Similarly, we also predicted that the magnitude of bat activity would be different between fragmented and less-fragmented areas. This prediction was tested with an acoustic survey to compare activity levels among site types. Movement patterns for *M. lucifugus* were expected to indicate use of forest clearings and other open areas like ponds, whereas *M. septentrionalis* was expected to avoid these features and instead use more cluttered habitats.

2.3 Materials and methods

2.3.1 Study area

This study was conducted in and around the Nuttby Mountain wind farm in Nova Scotia, Canada (UTM 20, 482000E, 5045000N) (Fig. 2-1). Nuttby Mountain is the highest point in the Cobequid Mountains ranging in elevation from 250 to 360 meters above sea level and has been subjected to forest fragmentation over at least the past nine years. The wind farm, constructed in 2008, consists of 22 wind turbines with the capacity to generate 25 megawatts of energy (Nova Scotia Power 2012). Vegetation ranges from

bare or grass-covered to regrowth and semi-mature forest. Surrounding the wind farm are areas, ranging in elevation from 150 to 300 meters above sea level, that are less-fragmented and consist of hardwood climax forest with predominantly pure and mixed stands of sugar maple (*Acer saccharum* Marsh.), yellow birch (*Betula alleghaniensis* Britton), and American beech (*Fagus grandifolia* Ehrh.) with patches of spruce (genus *Picea* A. Dietr.) plantations and open agricultural land (blueberry (genus *Vaccinium* L.) and strawberry (genus *Fragaria* L.) fields) (Davis and Browne 1996). The fragmented area is characterized by a 5.2% decrease in forest canopy cover and a 19 km or 22.77 m/hectare increase of forest edges between 2008 and 2011, whereas the less-fragmented surrounding area has lost less than 2.9% of forest cover and forest edges increased by 4.7 m/hectare in the same time. Bats from maternity roosts of *M. lucifugus* in at least two houses 2–5 kms from the study area were known to commute either through or around the wind farm (H.G. Broders, unpublished data).

2.3.2 Acoustic survey

We compared the magnitude of bat activity within and among site types through passive bat echolocation detection, using at least 3 Anabat II systems (Titley Electronics, Ballina, New South Wales, Australia) to test for differences in the magnitude of bat activity between the fragmented and less-fragmented site. Detectors were placed in tubs on the ground and directed parallel to forest and stream edges and perpendicular to pond edges. Microphones were oriented 45° above horizontal using a PVC elbow joint (O'Farrell 1998). Bat activity was quantified by the number of files recorded (Broders et al. 2006). Only files containing at least two discrete bat call pulses were counted. Data

were tested for normality using a Shapiro-Wilk test and further analyzed with Mann-Whitney *U* tests (Sokal and Rohlf 1994) to determine whether there was any significant difference in magnitude of activity of bats among sites and site types.

Acoustic monitoring was carried out from June to August 2012 (70 nights) and 2013 (48 nights). In 2012, twelve sites were monitored including nine outside (three forest edges, three ponds, three streams) and three inside (one forest edge, one pond, one stream) the wind farm. In 2013, six sites previously monitored in 2012, and two additional sites were monitored including three outside (two forest edges, one stream) and five inside (three forest edges, one pond, one stream) the wind farm. Activity at fragmented and less-fragmented sites was recorded simultaneously to control for seasonal effects and weather conditions. Three sites outside and two sites within the wind farm were monitored both years at the same time of year for at least 12 nights to test for differences in magnitude of bat activity between years. No attempts were made to identify *Myotis* to species level therefore the acoustic survey was used to study the difference in overall magnitude of bat activity between sites. During the winter of 2012-2013 the psychrophilic fungus, *Pseudogymnoascus destructans* (formerly *Geomyces destructans*), that causes white-nose syndrome and was responsible for killing millions of bats in North America (WNS; Blehert et al. 2009; Minnis and Lindner 2013) caused major mortality of bats in Nova Scotia, including our study site. Acoustic data from 2013 was therefore not used in the analysis of magnitude of activity between the fragmented and less-fragmented sites.

2.3.3 Capture survey

Myotis septentrionalis and *M. lucifugus* were captured in the summer of 2012 using mist nets (Avinet Inc, Dryden, New York, USA) and harp traps (Austbat Research Equipment, Lower Plenty, Victoria, Australia) placed at linear features in the landscape (streams, pond edges, forest edges and trails) or near known *M. lucifugus* house roosts. Nets and traps were set up one hour before sunset and left open for at least three hours. When catching with mist nets, constant net monitoring and bat extractions were done until midnight, while harp traps were left unmonitored for a maximum of two hours. Species, sex, age, body mass and reproductive status were recorded. Reproductive status was assigned by gently examining the belly for a fetus and assessing nipples for signs of lactation (Racey, 1988). Only capture data of free-flying bats, caught in the forest, were used for analysis. Habitat use was quantified by comparing capture success (catch result per unit effort) within and outside the fragmented area.

2.3.4 Radio telemetry survey

Throughout the summer of 2012, female bats were equipped with radio transmitters (0.35 g, model LB-2N; Holohil Systems Ltd., Carp, Ontario, Canada) affixed between the scapulae using surgical adhesive (Skin-Bond; Smith and Nephew United, Largo, Florida). Bats were tracked daily by homing in on their signals to locate roosts. Foraging bats were tracked using handheld receivers (R-1000; Communications Specialists Inc., Orange, California; and R2000; Advanced Telemetry Systems, Inc., Isanti, Minnesota) and 3-element yagi antennas (AF Antronics, Urbana, Illinois, USA) starting at sunset of the night after a transmitter was attached and continued until the

transmitter fell off or battery power ran out (Anthony and Kunz 1977; Anthony et al. 1981; Barclay 1982). Triangulations of bats were done at least 30 minutes apart and were obtained using a handheld GPS receiver (Garmin (E-trex Legend), Olathe, Kansas City, Kansas, USA) and signal bearings were taken with a compass (Suunto MC-2, Vantaa, Finland). Variable signals were interpreted as bats foraging or commuting and constant signals were interpreted as bats being stationary (Henderson and Broders 2008). Radio tracking was conducted at an equal number of early nights (sunset to 1 am) and late nights (1 am to sunrise). Location fixes from triangulations were calculated in Locate III (Pacer Computing, Tatamagouche, Nova Scotia, Canada; Nams 2006) and only intersects between 30° and 150° were used. A minimum convex polygon around all observed bat fixes was created in ArcMaps 10.1 (ESRI Inc, Redlands, California, USA) within which 200 random fixes were generated to simulate a uniform distribution model in the landscape for comparison with fixes collected through tracking bats. Intersecting features (dominant forest type, canopy cover, elevation) and proximity of fixes to landscape features (waterways, ponds, trails, roads, forest edges) were calculated. Forest data from 2008 were taken from the Nova Scotian Forest Inventory (1:10,000, Nova Scotia Department of Natural Resources). Forest data changes in 2011 (most recent forest data available) were obtained using Bing satellite imagery maps (ESRI's ArcGIS Online base map service).

2.3.5 Analysis and modeling of area use

Geographic information system (GIS) based multi-criteria evaluations (MCE) were used to characterize changes in area suitability for bats before and after wind farm

development to quantify the impact of increased forest fragmentation. Multi-criteria evaluations were applied through the process of assigning values to pixels (10x10m) within map layers of data by weighting all layers (assigning a value of importance) to define and categorize cells on a geographic map (Phua and Minowa 2005; Wood and Dragicevic 2007; Razgour et al 2011).

Potentially important landscape variables were selected following review of the literature, obtained from ArcMaps and included in *a priori* chosen balanced models (Table 2-1) for both species to calculate second order Akaike's Information Criteria (AIC_c; Burnham and Anderson 2002) and parameter estimates. Models were ranked according to their AIC_c, and only independent variables in the 95% confidence set were used and ranked using relative Akaike weights (w_i). Odds ratios (the odds that a bat can be found at x meters from landscape variables, compared to the odds for 0 meters) and w_i were used in the MCE to create two maps per species: area suitability before (2008) and after (2011) the construction of the wind farm and its related impacts of forest composition. Suitability of areas was represented as a raster, where every pixel holds a value from 1 (lowest suitability) to 5 (highest suitability). Pixel values of the pre-wind-farm raster were subtracted from the post-wind-farm raster to calculate changes in area suitability. Polygons were drawn around the outer wind turbines and bat fixes to represent land area covered by the wind farm and observed ranges of both bat species individually. Raster properties were used to calculate the percentage of increase or decrease of suitable area within the polygons.

2.4 Results

2.4.1 Acoustic survey

In 2012, 19,130 bat echolocation sequence files were recorded over 196 detector nights (number of detectors * number of nights recording) and in 2013 there were 171 bat echolocation sequence files recorded over 175 detector nights. In 2012, all but four files were attributable to *Myotis* spp. with the remaining four attributable to *L. cinereus* and these were recorded at the pond site within the wind farm. In 2013, all but one file were *Myotis* species with the remaining one attributable to *L. cinereus*, which was recorded on the edge of Nuttby Mountain. Data were non-normally distributed (Shapiro-Wilk test: df 350, $P < 0.001$). Overall for 2012, Mann-Whitney U tests indicated that there was no significant difference in the magnitude of echolocation activity between fragmented areas and less-fragmented areas ($U = 2229$, $P = 0.802$) (Table 2-2).

Separate analyses showed a significantly higher magnitude of activity at ponds in less-fragmented areas compared to the fragmented area ($U = 225.5$, $P = 0.037$). However, activity at the stream was significantly higher in the more fragmented area ($U = 62$, $P < 0.001$). In 2013, significantly higher activity was observed in less-fragmented areas overall ($U = 1920$, $P < 0.001$) and in forested areas ($U = 838$, $P = 0.011$) and stream areas ($U = 12$, $P < 0.001$). Acoustic activity was significantly lower in 2013 than 2012 ($U = 1394.5$, $P < 0.001$), where a 99.15% decrease in activity was recorded.

2.4.2 Capture survey

Over 20 nights from June 7 to July 30 2012, 33 bats were captured including 13 female *M. septentrionalis*, 10 male *M. septentrionalis*, 8 female *M. lucifugus* and 2 male

M. lucifugus. Of these, three bats (one female *M. septentrionalis*, one male *M. septentrionalis* and one female *M. lucifugus*) were caught in the fragmented area and the other 30 were caught in the forest and near houses (22 and 8, respectively) in less-fragmented areas. No bats were recaptured during the study period. Capture success was non-normally distributed (Shapiro-Wilk: $df = 75$, $P < 0.001$) and was not significantly different between the more fragmented areas (within the wind farm) and the less-fragmented areas (Mann-Whitney: $U = 372$, $P = 0.328$).

2.4.3 Radio telemetry survey

Nineteen adult bats were radio tracked in 2012 including 10 female *M. septentrionalis* (8 pregnant, 2 lactating) and 9 female *M. lucifugus* (2 pregnant, 2 lactating, 5 non-reproductive). The 193 location fixes collected from *M. septentrionalis* (observed range = total polygon size of 21.71 km²) and the 207 location fixes collected from *M. lucifugus* (observed range = total polygon size of 35.38 km²) were used to rank models (Table 2-1) and variables (Table 2-3) in order of their association with - and thus how these may predict - presence of bats. *Myotis lucifugus* was associated with the presence of ponds, roads and low crown closures, whereas *M. septentrionalis* was associated with waterways and a crown closure of 51-75%. Pixel count of MCE maps (Fig. 2-2) showed 6.4% increase in suitable areas for *M. lucifugus* within the more fragmented area (total polygon size of 5.72 km²) and a 0.05% increase in the bat's observed range (polygon based on radio telemetry observations) between 2008 and 2011. For *M. septentrionalis* a decrease of 2.18% within the more fragmented area of the wind farm and an increase of 0.32% in the bat's observed range were calculated (Table 2-4).

2.5 Discussion

In line with our predictions and previous studies (Owen et al. 2002; Broders and Forbes 2004; Henderson and Broders 2008), *M. septentrionalis* presence was positively associated with forest-covered streams, whereas *M. lucifugus* presence was positively associated with ponds and roads. In addition, both species showed a specific affinity to forest stands with particular crown closures. *Myotis septentrionalis* was associated with crown closures between 51% and 75%, although 0%–25% scored marginally higher and almost doubled compared with 26%–50% and 76%–100% respectively. *Myotis lucifugus* was associated with lower crown closures from 0% to 50%.

Over three years the extent of suitable habitat for *M. septentrionalis* inside the wind farm decreased by 2.2% due to deforestation. Furthermore, given that the potential direct effects of wind turbine attraction or avoidance were not tested in this study this may be a conservative estimate. In contrast, extent of habitat for *M. lucifugus* in the wind farm increased by 6.4%, likely caused by an increase in edges and openings created by deforestation. Despite the deforestation inside the wind farm, area suitability maps show more suitable area overall for *M. septentrionalis* than for *M. lucifugus* over the study period. This may be explained by the abundance of forests and forested streams and paucity of ponds in the landscape (Belwood and Fenton 1976; Jung et al. 1999; Owen et al. 2003; Broders and Forbes 2004; Ford et al. 2005; Henderson and Broders 2008). The extent of decrease in landscape suitability for *M. septentrionalis* in just three years is important, especially considering logging occurred in the area before 2008, but it has been subjected to more intensive deforestation when the wind farm was constructed. In addition, in an area between 1 and 3 km southwest of the study site's wind farm an even

greater effect has been observed with a decrease in suitability for *M. septentrionalis* and an increase in area suitability for *M. lucifugus*, likely due to the deforestation of a large area of forest between 2008 and 2011.

Neither the acoustic survey nor the capture survey showed any significant difference in magnitude of bat activity at forest edges and trails between the fragmented area and the less-fragmented areas. However, acoustic data collected in 2012 suggest a significantly lower activity at ponds and higher activity at streams in fragmented areas. Both these landscape features were limited within the area and only one of each site type could be sampled. All sampled *M. lucifugus* maternity roosts were in close proximity to ponds, and considerable acoustic activity was recorded at each. The low activity at the pond site in the fragmented area is likely a result of the absence of nearby buildings, which female *M. lucifugus* typically use as maternity roosts (Anthony et al. 1981; Furlonger et al. 1987). The higher activity at the stream site may be due to it being one of very few waterways near the top of Nuttby Mountain and hence acting as an important, isolated feeding site or commuting lane for *M. septentrionalis* and possibly *M. lucifugus*. Alternatively, differences in activity may be caused by the topography of Nuttby Mountain, such as a higher elevation. Studies showed that elevation can be a predictor of bat presence (Cryan et al. 2000; Russo 2009) and reproductive females may be energetically constrained at elevated areas (Fenton et al. 1980; Barclay 1991; Bogan et al. 1996; Cockrum et al. 1996). However, small elevation differences, as found on Nuttby Mountain, may not be biologically meaningful in this study, elevation was not found to be an important predictor of bat presence for either species.

Between 2012 and 2013 a dramatic decrease in acoustic activity of 99.15% was observed (Table 2-2). Hibernaculum surveys conducted in Nova Scotia in 2012–2013 showed similar trends in observed mortality during hibernation (between 91% and 99.88%), impacting all resident species; *M. lucifugus*, *M. septentrionalis* and the tri-coloured bat, *Perimyotis subflavus* (Hooper and Van Den Bussche, 2003), (H.G Broders and L.E. Burns, unpublished data). This is most certainly attributable to the spread of the fungus *Pseudogymnoascus destructans* (Minnis and Lindner, 2013), which has caused severe bat mortality throughout eastern Canada and the United States (Blehert et al. 2009; Meteyer et al. 2009; Dzal et al. 2011).

Closely related species with similar morphology can fill different niches within the same area (Arlettaz 1999) and therefore show a different response to anthropogenic alterations of their home range (Webala et al. 2011). Indeed, the results from our study suggest that the development of Nuttby Mountain wind farm and its resultant increased forest fragmentation impacts resident bat populations to a different extent. Forest fragmentation creates linear elements that *M. lucifugus* is known to use but may hinder *M. septentrionalis* (Broders et al. 2004; Broders et al. 2006). Observed foraging ranges of bats did not show major overlap with the wind farm and the MCEs suggest a low suitability of the fragmented area even before wind farm development.

Peripherally, many studies have found major mortality rates of bats and birds colliding with man-made structures like communication towers (Kemper 1996), high tension lines (Koops 1994), buildings (Dunn 1993; Ogden 1996) and wind turbines (Stienen et al. 2008; Martin 2011; Martínez-Abraín et al. 2012). Causes of bats collisions with wind turbines are not fully understood but one hypothesis is that insects may be

attracted to them (Long et al. 2011). In addition to the increasing number of wind turbines per facility and the increasing number of wind turbine facilities, newer turbines tend to be higher than older turbines, which increases bat fatalities considerably (Barclay et al. 2007). Chamberlain et al. (2006) argue that collision risk models used to predict mortality of animals colliding with wind turbines may be less accurate than expected. In addition, secondary threats like habitat fragmentation need more research to assess risks for different species.

It is essential that field data on attraction and avoidance behaviour be collected to make accurate estimates of risks to animals living in or near fragmented forest areas. The use of MCEs, as demonstrated in this study, may be a useful strategy in the decision-making process for predicting area suitability to wildlife before or after alterations and will give invaluable insight in wildlife management implications. Indeed, our study indicates that *M. septentrionalis* may be much more negatively impacted by increased forest fragmentation than *M. lucifugus* is, and these life history traits need to be well documented and understood for other populations and species that are living in fragmented habitats.

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Table 2-1. Akaike weight and rank of each model in the candidate set of models to explain landscape use of female *M. lucifugus* and *M. septentrionalis* in and around a fragmented forest caused by a wind farm in Nova Scotia. Bold indicates that the model is included in the 95% confidence set of models for that species and parameter estimates and standard errors from only these models were used for calculations of model-averaged parameter estimates and estimated unconditional standard errors*.

Model statement	w_i	Rank
<i>Myotis lucifugus</i>		
1. waterw ^a	2.99E-14	7
2. roads ^b	3.64E-27	10
3. ponds ^c	0.001	3
4. ponds + roads	0.357	2
5. crncl ^d	7.41E-23	8
6. fedge ^e	4.77E-23	9
7. elev ^f	1.29E-08	5
8. waterw + elev	1E-05	4
9. ponds + crncl	0.641	1
10. roads + fedge + waterw	7.9E-11	6
<i>Myotis septentrionalis</i>		
1. crncl	3.6E-07	6
2. waterw	0.045	2
3. crncl + waterw	0.928	1
4. trails ^g	3.47E-12	11
5. fedge	3.88E-11	8
6. trails + fedge	1.63E-11	10
7. height ^h	2.03E-11	9
8. elev	1.13E-09	7
9. height + elev + fedge	1.33E-05	4
10. crncl + trails + fedge	1.19E-06	5
11. waterw + fedge + elev	0.027	3

* Parameter estimates were consistent among the models within the 95% confidence sets

^a Proximity to nearest waterway

^b Proximity to nearest forest covered trail or open road

^c Proximity to nearest pond

^d Percentage of forest crown closure

^e Proximity to nearest forest edge

^f Elevation of land

^g Proximity to nearest forest covered trail

^h Average forest height in forest patch

Table 2-2. Magnitude of echolocation activity among sites and site-types in 2012 and 2013 at Nuttby Mountain, NS, showing number of nights sampled, mean number of files recorded, standard deviation of number of files and the results of Mann-Whitney *U* tests.

	Less-fragmented area			Fragmented area			Mann-Whitney <i>U</i> test	
	detector	Mean	SD	detector	Mean	SD	<i>U</i>	<i>P</i>
	nights			nights				
2012								
Forest (<i>n</i> =4)	39	90.31	136.45	9	13.56	17.64	118.5	0.132
Pond (<i>n</i> =4)	47	112.36	123.22	15	29.47	16.67	225.5	0.037
Streams (<i>n</i> =4)	53	115.08	172.59	9	407.11	300.02	62	<0.001
Total (<i>n</i> =12)	139	107.21	144.23	33	128.12	229.89	2229	0.802
2013								
Forest (<i>n</i> =5)	42	1.31	2.09	54	0.57	1.24	838	0.011
Pond (<i>n</i> =1)	*	*	*	34	0.68	1.65		
Stream (<i>n</i> =2)	14	3.86	2.57	34	0.18	0.46	12	<0.001
Total (<i>n</i> =8)	56	1.95	2.46	122	0.49	1.23	1920	<0.001
		2012			2013			
Overall	195	111.22	163.54	155	0.95	1.84	1394.5	<0.001

* site type not sampled

Table 2-3. Model averaged parameter estimates ($\beta \pm SE$) and odds ratios ($\pm 95\% CI$) for variables from the top-ranked models from logistic regression analyses to rank landscape variables in order of their association with *M. lucifugus* and *M. septentrionalis* females. For biological relevance odds ratios for ponds, waterways and roads represent 300 m versus 0 m proximity and odds ratios for forest crown closure are referenced to 0–25% forest crown closure.

Model terms	β (SE)		Odds ratio (95% CI)	
<i>Myotis lucifugus</i>				
ponds	-0.0043	(0.0042)	0.280	(0.987-1.004)*
roads	-0.0032	(0.0009)	0.388	(0.995-0.999)*
crncl25 ^a	0		1	(1.000-1.000)
crncl50 ^b	0.5154	(0.4289)	1.674	(0.722-3.881)
crncl75 ^c	-0.2485	(0.2952)	0.78	(0.437-1.391)
crncl100 ^d	-1.224	(0.3509)	0.294	(0.148-0.585)
<i>Myotis septentrionalis</i>				
waterw	-0.0055	(0.0064)	0.190	(0.982-1.007)*
crncl25	0		1	(1.000-1.000)
crncl50	-0.1515	(0.4589)	0.859	(0.350-2.113)
crncl75	0.406	(0.2715)	1.501	(0.881-2.555)
crncl100	-0.5738	(0.3222)	0.563	(0.300-1.059)

^a crown closure of 0–25%

^b crown closure of 26–50%

^c crown closure of 51–75%

^d crown closure of 76–100%

* CI given for odds ratio of a one-unit increase in distance

Table 2-4. Pixel count and percentage of suitable area difference for *M. lucifugus* and *M. septentrionalis* between 2008 and 2011 in the study site's fragmented area and the range in which radio tagged bats were found.

Polygon	Change in pixel value	Pixel count	% of surface	% increase suitable area
<i>Myotis lucifugus</i>				
Wind farm	-2	0	0.000	6.40
	-1	79	0.138	
	0	53382	93.324	
	1	3196	5.587	
	2	522	0.913	
	3	22	0.038	
	Observed range	-2	0	
	-1	10	0.003	
	0	353560	99.940	
	1	201	0.057	
	2	0	0.000	
	3	0	0.000	
<i>Myotis septentrionalis</i>				
Wind farm	-2	394	0.689	-2.18
	-1	1013	1.771	
	0	55636	97.264	
	1	158	0.276	
	2	0	0.000	
Observed range	-2	0	0.000	0.32
	-1	1584	0.730	
	0	213205	98.218	
	1	1921	0.885	
	2	363	0.167	

Figure 2-1. Topographic representation of Nuttby Mountain and its wind turbine and its surrounding areas, Nova Scotia, Canada.

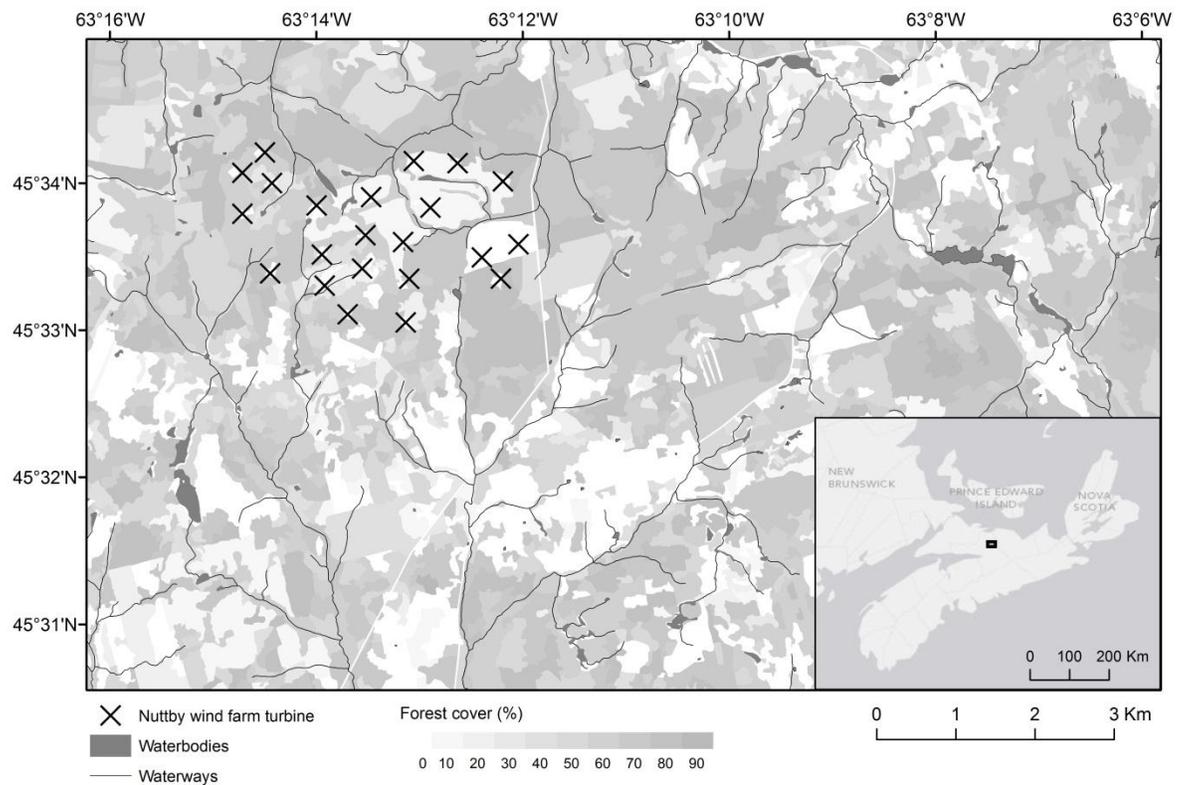
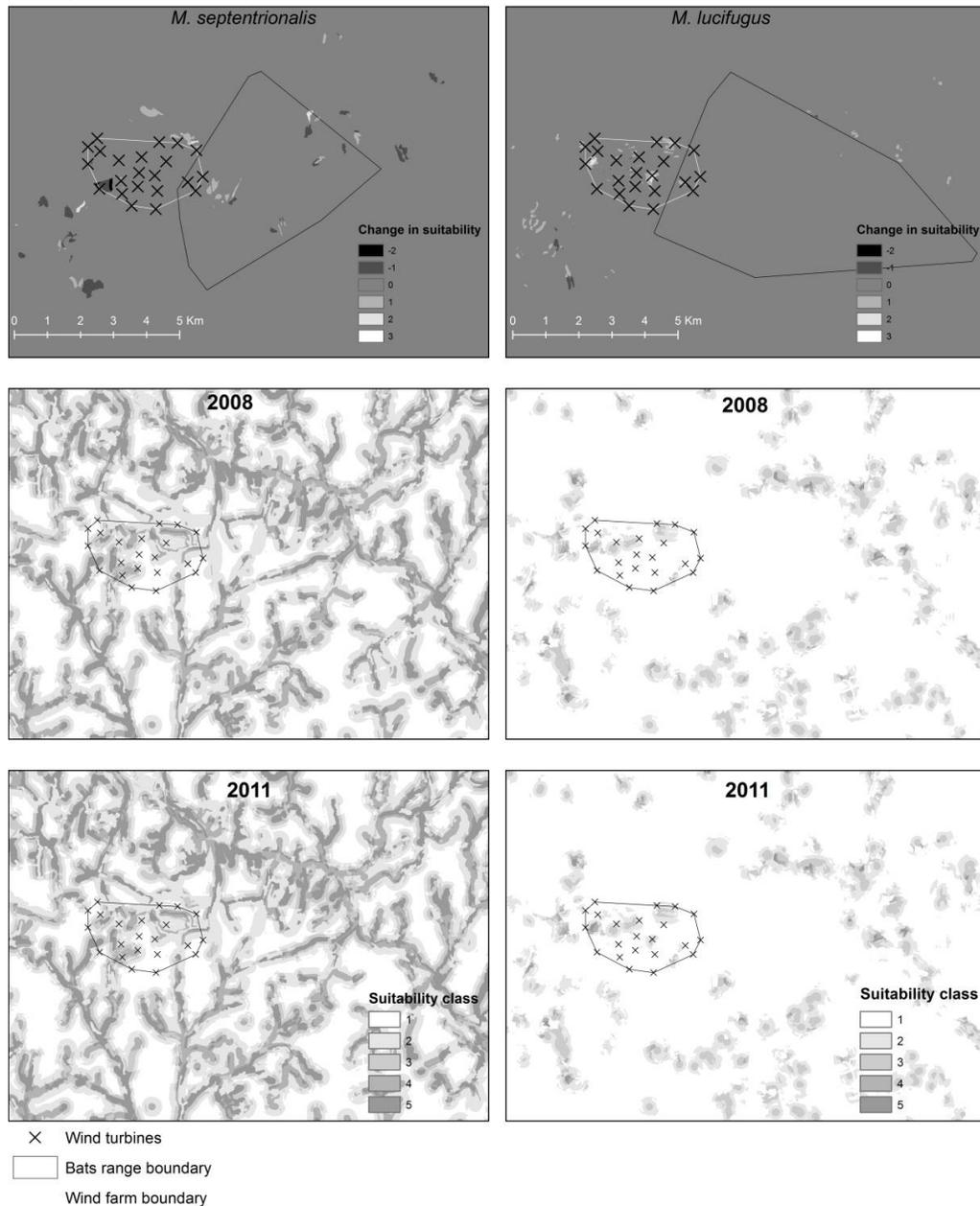


Figure 2-2. Suitable area maps for *M. septentrionalis* (left) and *M. lucifugus* (right) pre wind farm development (2008) and post wind farm development (2011) as a result of a MCE. Top two maps show the change in suitable areas per bat species between 2008 and 2011, including boundary of the wind farm and bat's telemetry range, where darker areas represent a decrease and lighter areas represent an increase of suitability class. Bottom four maps show the suitability classes of the landscape before and after the fragmentation associated with the wind farm development, classes are represented from white to dark grey as; 1: lowest suitability, 2: low suitability, 3: average suitability, 4: high suitability, 5: highest suitability.



Chapter 3

Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope signatures in bat fur indicate swarming sites are catchment areas for bats from different summering areas

3.1 Abstract

Migratory patterns of bats are not well understood and traditional methods to study this, like capture-mark-recapture, often do not provide enough detail. Stable isotope profiles of many animal species have been studied to make inferences on migration. *Myotis lucifugus* and *M. septentrionalis* migrate every year from summering roosts to swarming caves and mines in the fall, but how bats move between these sites is not well understood. In this study, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopes of 305 *M. lucifugus* and 200 *M. septentrionalis* fur samples were analyzed to make inferences on migration patterns between summering and swarming sites in Nova Scotia, Canada. We expected that there would be greater variability in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among individuals at swarming sites because it was believed that these sites are used by individuals originating from many summering sites. There was extensive overlap in the standard ellipse area, corrected for small sample sizes, (SEA_c) of bats at swarming sites, whereas there was much less overlap in SEA_c among summering sites. For *M. lucifugus*, swarming sites had larger SEA_c than summering sites and predictive discriminant analysis assigned swarming bats to several summering sites, supporting the contention that swarming bats are mixed aggregations of bats from several summering sites. *Myotis septentrionalis* SEA_c was much smaller than *M. lucifugus* indicating a more narrow dietary niche breadth. Isotopic profiles of *M. lucifugus* varied among summering sites and the data support the contention that swarming sites are catchment areas for bats from multiple summering sites. These data suggest that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ profiling of bat fur offer some potential to make inference on regional migration in bats.

Keywords: Little brown bat, *Myotis lucifugus*, Northern long-eared bat, *Myotis septentrionalis*, stable isotopes, migration, SIAR

3.2 Introduction

Migration is a key life history strategy among animals to cope with seasonal variation in resource abundance (Elewa 2010; Wilcove and Wikelski 2008). Migratory animals depend on multiple ranges (e.g., wintering ground, summering ground and stop-over places) and therefore these species may be more vulnerable to environmental perturbations than sedentary species (Forman and Godron 1986; Kelly et al. 2005; Wilcove and Wikelski 2008). For example anthropogenic alteration within a species' breeding range may cause decreased breeding success (Robinson and Wilcove 1994) and disturbance at stop-over sites may affect the ability to replenish fat reserves (Blem 1980; Moore et al. 1995). Hence, there is great interest in understanding the dynamics of migration (Berger et al. 2008; Sanderson et al. 2006).

Traditionally, direct methods of capture-mark-recapture were used to study migration (Darroch 1958; Uchida 1932). More recently, advances in DNA profiling technology and population genetic theory are being applied to indirectly study migration (Boulet et al. 2007; Hellberg et al. 2002; Lowe and Allendorf 2010) as population level data may be used to characterize patterns of gene flow and estimate effective population size (Hellberg et al. 2002; Lowe and Allendorf 2010). However, different markers often yield conflicting results (Hellberg et al. 2002) and often do not provide sufficient geographic resolution (Lovette et al. 2004).

Another indirect means to study animal migration may be the spatiotemporal characterization of stable isotopes of elements. Stable isotopes are naturally occurring forms of elements that vary in atomic weights. For example, carbon exists in the stable forms of ^{12}C and ^{13}C and nitrogen exists in the stable forms of ^{14}N and ^{15}N (Fry 2006). The lighter atoms (^{12}C and ^{14}N) naturally occur in much larger quantities than their heavier counterparts (about 100:1) (Hobson 1999). Unlike radioactive atoms, which decay at a known rate, stable isotopes do not decay. However, as molecules are produced in plants through photosynthesis some of the lighter atoms are converted into heavy atoms due to a difference in reaction speed (fractionation) caused by external climatic factors (e.g., sun exposure, temperature, humidity). Stable isotope profiles of organisms are, in part, a function of the chemical composition of the lower trophic level from which they derive their diet. When these move along the food chain they can potentially be traced back to their origins (e.g., habitat type, climatic condition) (Fry 2006; Hobson 1999; Rubenstein and Hobson 2004). Hence, in a way, they may be used as “fingerprints” representing, at least in part, the time and location of origin (Rundel et al. 1989). In biology, stable isotopes are most often used to understand trophic dynamics and diet (Crawford et al. 2008; Phillips 2001; Polis and Hurd 1996). However, recently some types of stable isotopes have been used to make inference on migration patterns (Fraser et al. 2012; Hobson 2005; Knick et al. 2013; Pain et al. 2004).

Typically, when using stable isotopes to study movement of animals, hydrogen (δD) or sulfur ($\delta^{34}\text{S}$) isotopes are used. Hydrogen isotopes can be useful for making inferences on long-distance migration because precipitation patterns cause worldwide latitudinal gradients in meteoric water for which extensive isotopic maps are available

(Hobson 1999). Also, δD patterns differ seasonally and between marine and terrestrial sites (Rubenstein and Hobson 2004). Sulphur isotopes are used primarily to make inference on marine and marsh food webs (Krouse 1988), but Zazzo et al. (2011) demonstrated their use for terrestrial animals, finding a correlation between $\delta^{34}S$ values and distance from the coast. Scale of movement of short-distance migratory species is likely too small to use δD for identifying movement patterns. Sulphur isotopes may be effective, especially in coastal areas, but require a labour and cost intense baseline study prior to analysis (Zazzo et al. 2011). Most often, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes are used in studies on terrestrial or freshwater food webs (Crawford et al. 2008; Fry 2006; Phillips 2001), but multivariate analysis has been applied to these to study movement of marine species (Carlisle et al. 2010; Harrod et al. 2005; Litvin and Weinstein 2004) and birds (Coulton et al. 2010).

Though it is known that many bat species migrate (Fleming and Eby 2003), details of the dynamics for most species are unavailable. Traditional methods to study migration have many limitations for small volant animals like bats. Recaptures over vast distances are sparse, radio telemetry often has limited broadcasting range (Rubenstein and Hobson 2004), satellite tags are too heavy for most species (Richter and Cumming 2008) and geolocators rely on daylight for geo-referencing locations (Åkesson et al. 2012). Some studies however, have made inferences on bat migration ecology using mark-recapture techniques with a large number (3000 – 73000) of captured animals (Davis and Hitchcock 1965; Griffin 1936; Norquay et al. 2013).

Bat migration can be classified, depending on species, as long-distance migration (>500 km) and regional migration (100 – 500 km) (Boulet et al. 2007; Fleming and Eby

2003; Gómez-Díaz and González-Solís 2007). Examples of long distance migrants in North America are the hoary bat (*Lasiurus cinereus*) and red bat (*L. borealis*), which are believed to fly > 1000 km between their temperate summering site and their southern wintering site (Fleming and Eby 2003). Straw-coloured fruit bats (*Eidolon helvum*) from Africa, large enough for satellite tracking studies, migrate over 2000 km (Richter and Cumming 2008). The little brown bat (*Myotis lucifugus*) and the northern long-eared bat (*M. septentrionalis*) are regional migrants from North America that migrate from hibernacula to summering sites. Davis and Hitchcock (1965) described movements of *M. lucifugus* up to 275 km from hibernacula to summer colonies and Norquay et al. (2013) report recaptures of *M. lucifugus* as far as 569 km from their initial capture site.

Between September and April *M. lucifugus* and *M. septentrionalis* use natural caves and abandoned mines to hibernate (hibernacula) and after leaving their hibernacula they migrate to summering sites (Broders et al. 2004; Faure et al. 1993; Fenton and Barclay 1980; Owen et al. 2002; Ratcliffe and Dawson 2003) to which they may have long-term fidelity (Fenton and Barclay 1980; Lewis 1995). Bats spend most of their foraging-time at summering sites, thus energy-intake occurs mostly here and intraspecific variation in isotopic signatures should largely be a function of diet and environmental variation among summer sites. From August to October these bats migrate and congregate at the entrance of caves and mines (swarming) before going into hibernation. This swarming behaviour may serve multiple purposes including mating and information transfer (Fenton 1969).

Abundance of different stable isotopes in nature varies because of biological (terrestrial vs. aquatic) and anthropogenic (e.g., agriculture, pollution) factors. Through

spatial variation in stable isotope profiles in the environment, inferences may be made on population-level movements (Hobson 1999; Rubenstein and Hobson 2004). Stable isotopes of carbon and nitrogen are typically used for dietary studies and there can be both interspecific and intraspecific variation. For example, because the diet of *M. lucifugus* is composed mainly of insects from aquatic systems their profiles may be different from *M. septentrionalis* which mainly feeds on insects from a terrestrial origin (Broders et al. 2004; Faure et al. 1993; Owen et al. 2002; Ratcliffe and Dawson 2003; Randall and Broders, in press). Fractionation of stable isotopes differ in the aquatic and terrestrial ecosystems and therefore both species may be exposed to site specific isotopic signatures (Britzke et al. 2009).

Several summering sites and hibernacula for *M. lucifugus* and *M. septentrionalis* have been identified in Nova Scotia, Canada (Moseley 2007; Randall and Broders, in press), but little is known on where animals that summer in one place go at the end of the season for swarming. The goal of this study was to test whether $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the fur of bats may be used to make inference on their migration patterns. Specifically, we were interested in characterizing the spatial variability in the stable isotope patterns of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in bats at summering and swarming sites in Nova Scotia to make inference on the origin of bats at swarming sites. Since $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ data are generally used to make inference on diet, and *M. lucifugus* and *M. septentrionalis* are thought to occupy different dietary niches (Faure et al. 1993; Ratcliffe and Dawson 2003; Broders et al. 2004; Owen et al. 2002), it was predicted that there would be little or no overlap in isotopic signatures between the species.

3.3 Materials and methods

3.3.1 Sample collection

Myotis septentrionalis and *M. lucifugus* were captured from 1999 to 2013 using mist nets (Avinet Inc, Dryden, New York, USA) and harp traps (Austbat Research Equipment, Lower Plenty, Victoria, Australia). Fur samples were collected by cutting a small amount (≈ 1.4 mg) from between bats' scapulae with cuticle scissors and stored in 1.5 ml eppendorf tubes and archived at -20 °C. Summering bats were captured between May and August and swarming bats were captured between August and October. Nets and traps were set one hour before sunset and left open for at least three hours. Samples for analysis were selected to represent a wide geographic area within Nova Scotia (Fig. 3-1) with variability among environment types (e.g., terrestrial, aquatic, marine, agriculture, forests) and individuals were selected to represent the breadth of variation in forearm length at each site in the event there may be an effect of body size on diet. For *M. lucifugus* we selected between 5 and 28 (mean 17) adult females from each of 9 swarming sites and between 10 and 14 (mean 12) adult females from 9 maternity roosts for analysis. For *M. septentrionalis* we selected between 4 and 28 (mean 14) adult females from each of 9 swarming sites and between 12 and 15 (mean 14) adult females from 3 summering sites (Table 3-1). In addition, 34 *M. lucifugus* and 31 *M. septentrionalis* males captured at one swarming site were analyzed and 7 *M. lucifugus* males at one summering site to assess intersexual variability.

3.3.2 Stable isotope extraction

Samples were analyzed at the Stable Isotopes in Nature Laboratory at the University of New Brunswick where they were washed by soaking them in 2:1 (v/v) chloroform:methanol for 10-15 minutes. With clean tweezers samples were stirred and removed from the vial. This method was repeated three times before samples were left to air-dry under a fume hood overnight. Once dried samples were ground to a fine powder, placed in tin capsules and weighed to the nearest 0.001 mg. Samples were then combusted at 1000 °C in ThermoQuest CE Instruments NC2500 Element Analyser (ThermoQuest Italia, Rodano, Italy) and subjected to mass spectrometry with a Thermoquest Finnigan-Mat Delta Plus Continuous Flow Mass Spectrometer (ThermoFinnigan, Bremen, Germany).

3.3.3 Stable isotope analyses and metrics

Data were tested for normality using a Shapiro-Wilk test (Shapiro and Wilk, 1965) and further analyzed with either independent t-test or Mann-Whitney U test. Isotopic metrics of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were calculated using the Stable Isotope Analysis in R (SIAR) package (Parnell et al. 2010) and the integrated Stable Isotope Bayesian Ellipses in R (SIBER) package (Jackson et al. 2011) using R version 3.0.2 (The R Foundation for Statistical Computing 2013). Several metrics (Layman et al. 2007) were used to make inference on migration dynamics. $\delta^{13}\text{C}$ range (CR) and $\delta^{15}\text{N}$ range (NR) provide information on isotopic range that a population occupies. Small sample size corrected standard ellipse area (SEA_c) represents 40% of the total area (TA), which is the convex hull area encompassing a population in CR and NR bi-plot space and is a measure of total

amount of niche space occupied. The degree of overlap between populations' SEA_c is a function, at least in part, of overlap in prey and environment of origin (Jackson et al. 2011; Layman et al. 2007). Mean distance to centroid (CD) is the estimate of the average degree of trophic diversity within a population, providing information on niche width and species spacing. Mean nearest neighbour distance (MNND) is the measure of the overall density of individual spacing and the standard deviation of nearest neighbour distance (SDNND) is the measure of the trophic evenness of a population in $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ space.

Through descriptive discriminant analyses summering bats were reassigned to summering sites and swarming bats were reassigned to swarming sites to make inferences on degree of isotopic separation between sites. In addition, predictive discriminant analyses (Thomas and Cahoon 1993; Litvin and Weinstein 2004; Harrod et al. 2005) were computed with Systat 12 (Systat Software, Inc. 2007) to determine the extent to which animals captured at swarming sites could be statistically assigned to summering sites using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ profiles. Overlap of SEA_c were calculated in SIAR and spatial distance between sampling sites was calculated in ArcGIS 10.1 (ESRI Inc, Redlands, California, USA), using spatial analyst's point distance tool. These data were output as matrices and compared using a Mantel test.

3.4 Results

Fur samples were analyzed from 115 *M. lucifugus* from 9 summering sites, 190 *M. lucifugus* from 9 swarming sites, 42 *M. septentrionalis* from 3 summering sites and 158 *M. septentrionalis* from swarming 9 sites (Table 3-1). For both *M. lucifugus* and *M. septentrionalis* there was no significant association between forearm length and $\delta^{13}\text{C}$

(linear regression, $\beta_{M.lucifugus} = 0.005$, $r^2 < 0.001$, $P = 0.933$; $\beta_{M.septentrionalis} = -0.052$, $r^2 = 0.003$, $P = 0.463$) or $\delta^{15}\text{N}$ (linear regression, $\beta_{M.lucifugus} = 0.034$, $r^2 = 0.001$, $P = 0.549$; $\beta_{M.septentrionalis} = 0.024$, $r^2 = 0.001$, $P = 0.736$).

Descriptive discriminant analyses correctly reassigned 65% of *M. lucifugus* summering site samples to their origin (Table 3-2) and only 16% of the swarming samples to their capture site (Table 3-3). Bats from the maternity roost at West Chezzetcook were correctly reassigned most often (92% correct) and bats from Gore were most often miss assigned (17% correct). For *M. septentrionalis* 57% of summering bats were correctly reassigned to their origin (Table 3-4) but only 21% of swarming bats were correctly reassigned to their capture site (Table 3-5). Bats from the maternity roost at Earltown were most often correctly reclassified (75% correct) and bats from Kejimkujik were most frequently misclassified (40% correct).

Predictive discriminant analysis suggested that *M. lucifugus* captured at swarming sites originated, on average, from 6.3 (out of 9) sampled summering sites. Minasville ice cave and Glenelg mine swarming populations were assigned to originate from fewer sampled summering sites (3 and 4 respectively) than bats from other swarming sites (ranging from 5 to 9) (Table 3-6). *Myotis septentrionalis* swarming populations originated on average from 2.8 (out of 3) sampled summering sites. Donkin mine and Glenelg mine swarming populations were only classified originating from Kejimkujik and Earltown maternity roosts, whereas bats from all other swarming sites were classified originating from all 3 sampled summering sites (Table 3-7).

For *M. lucifugus* the overall CR was 32.14 (30.70 for swarming sites and 30.79 for maternity roosts) but there was considerable variation among sites (Table 3-1). For

example, the CR among maternity sites ranged from 1.81 (Tatamagouche) to 22.93 (Mill Village). Overall NR was 14.82 (10.84 for swarming sites and 14.82 for maternity roosts) and there was less variability among maternity roosts ranging from 1.21 (Christmas Island) to 9.12 (Annapolis Royal). For *M. septentrionalis* the overall CR was 5.82 (3.56 for swarming sites and 5.82 for maternity roosts) and variability in CR among summering sites ranged from 1.02 (Earltown) to 5.82 (Kejimikujik). The overall NR was 5.82 for *M. septentrionalis* (5.57 for swarming sites and 2.77 for maternity roosts) and there was little variation among the summering sites (Table 3-1).

For *M. lucifugus* SEA_c were not normally distributed (Shapiro-Wilk test: $P = 0.022$, $df = 18$) and were significantly larger at swarming sites than summering sites (Mann-Whitney: $U = 7.000$, $P = 0.003$, $Z = -2.958$; Fig. 3-2 and supplementary material 3-A). For *M. septentrionalis* SEA_c were normally distributed (Shapiro-Wilk test: $P = 0.213$, $df = 12$) and showed no significant difference between summering and swarming sites (Independent t-test: $t = -0.784$, $P = 0.451$, $df = 10$; Fig. 3-2 and supplementary material 3-B).

There was no correlation between SEA_c overlap and distance between sites for *M. lucifugus* (Mantel test: 999 replicates, $R^2: -0.067$, $P = 0.649$) or *M. septentrionalis* (Mantel test: 999 replicates, $R^2: 0.336$, $P = 0.509$). However, a negative correlation was detected between *M. lucifugus* CR ranges and latitude of sampling sites (linear regression: $\beta = -0.899$, $P = 0.001$, $r^2 = 0.808$)

3.5 Discussion

Stable isotopes in keratinous tissues are arguably the best for studying seasonal movement patterns of animals (Rubenstein and Hobson 2004). Unfortunately, few studies on the moult of bats exist and for many species moult time is not known or data are sporadic and inconsistent. Jones and Genoways (1967) describe one record of a male *M. lucifugus* moulting early July, but no others showed any signs of moulting during that study. Fraser et al. (2012) suggested that tri-coloured bats (*Perimyotis subflavus*) moult between June and October. Other bat species have been observed to moult between July and mid-August (Constantine 1957; Constantine 1958; Tiunov and Makarikova 2007). Our data suggest that *M. lucifugus* and *M. septentrionalis* moult some time during the summer, as summering bats were more often correctly reassigned to their summering catchment site than swarming bats were to their swarming catchment site. Thus, summering bat colonies are more distinct in isotopic composition, energy is acquired there and moulting happens before bats arrive at swarming sites. Perhaps more precise estimates on moult time can be made for long-distance migratory bats by using δD isotopes from fur and δD gradient maps, but these methods likely will not work for short-distance migrants.

Body size, especially in predatory animals, is often correlated with niche breadth where larger species are capable of eating both large and smaller prey and smaller species can only consume smaller prey items (Barclay and Brigham 1991; Scharf et al. 2000). Not only is this the case inter-specifically but also on an intra-specific level where larger or older individuals may have a larger or different niche width than smaller or younger individuals (Polis 1984). However, no significant correlation between body size (forearm

length) and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were detected in this study, therefore we were not able to detect variation in niche width as an effect of body size.

Samples from summering sites for both *M. lucifugus* and *M. septentrionalis* were correctly reassigned at a higher frequency than individuals sampled at swarming sites, although this effect was stronger for *M. lucifugus* (65% correct between 9 summering sites; 16% correct between 9 swarming sites) than for *M. septentrionalis* (57% correct between 3 summering sites; 21% correct between 9 swarming sites). This suggests that the isotopic profiles of bats at summering sites were more distinct than those at swarming sites, likely because swarming sites consist of individuals originating from multiple summering sites. *Myotis lucifugus* sampled from swarming sites at Minasville and Glenelg were assigned to fewer summering sites which may suggest that individuals from fewer summering sites congregated there. However, the low sample size (5) at Minasville likely distorts these data. For *M. septentrionalis* fewer connections to summering sites were detected for Donkin and Glenelg, but low sample sizes (4 and 6, respectively) may again result in spurious conclusions. Furthermore, predictive discriminant analysis will always assign all cases to one of the provided groups, even if it fits none of these groups very well. These results therefore permit inference on the best fit amongst the sampled sites, but cases may fit non-sampled sites better and therefore bats may originate from non-sampled locations. Nonetheless, stable isotopes in fur permit some inference on regional migration, even more so when combined with other assignment techniques. Several studies have demonstrated a high rate of assignment accuracy by combining morphometric-, isotopic- and molecular data to assign populations to their place of origin (Gómez-Díaz and González-Solís 2007; Hansen et al. 2001).

For *M. lucifugus* SEA_c was significantly larger at swarming sites than at individual summering sites and there was more variation in profiles among summering sites than swarming sites. These results support the contention that congregations of bats at swarming sites consist of bats that had originated from multiple summering areas. No such significant differences were detected for *M. septentrionalis* in this study, but only three summering sites were sampled and there was much less variability among sites (summering and swarming). Molecular and mark-recapture methods have provided similar evidence for mixing at swarming sites in other bat species. Sexual interactions between individuals from separate colonies occurs at swarming sites and because of this, colony borders break down and genetic diversity is greater there than within summering colonies (Rivers et al. 2006; Veith et al. 2004).

There was no relationship between distance between sampling sites and extent of SEA_c overlap. The SEA_c of Annapolis Royal stands out from all other summering sites due to high $\delta^{15}N$ values. Annapolis Royal, in the Annapolis valley is an area with intensive agriculture (Davis and Browne 1996). Fertilizer is high in nitrogen and run-off from agriculture leaks into the environment and thus $\delta^{15}N$ enters the food chain (Kohl et al. 1971; Heaton 1986; Hebert and Wassenaar 2001; Kellman and Hillaire-Marcel 2003). The sampled swarming site nearest Annapolis Royal is Vault Cave and interestingly this site, located in a diverse environment of coastal forest and agriculture (Davis and Browne 1996), showed a higher NR than other swarming sites. Despite this observation more individuals from Vault Cave were assigned to summering sites other than Annapolis Royal (Antigonish, Mill Village and Gore), likely because this broad NR did not cover the higher range found at Annapolis Royal.

Carbon range decreased with increasing latitude of summering sites (from Mill Village; lat. 44.147630 to Christmas Island; lat. 45.958440, is 365 km). Studies have found that $\delta^{13}\text{C}$ decreases with increasing latitude on a continental scale (Kelly 2000; Körner et al. 1991), but no mention of isotopic range correlation with latitude has been found in the literature and more sampling would have to be done to determine if this effect is related to a latitudinal effect or whether there are more local factors, such as air pollution or forest composition (Martin et al. 1988; Wunderlich et al 1995), that may explain this trend.

Together, these results indicate that individuals sampled at swarming sites originated from many different summering sites. Females of *M. lucifugus* and *M. septentrionalis* are known to show high fidelity to summering sites which could result in isolation of populations and thus low genetic variation (Fenton and Barclay 1980; Arnold 2007). However, the mixing that occurs at swarming sites may help prevent isolation of populations and result in a higher genetic variability (Webster et al. 2002). High fidelity towards summering sites and mixing at swarming sites is advantageous to bats and because bats mate at swarming sites in the fall, instead of in spring as many other species with high fidelity do, isolation of populations may not occur. Bat movement dynamics appear tortuous due to the mixing of summering individuals at swarming sites. Bats may travel tens to hundreds of kilometers (Davis and Hitchcock 1965; Griffin 1936; Norquay et al. 2013) to reach swarming sites, even if other swarming sites are closer. Although there is still much to learn about fur moulting in bats, our data indicate that bat fur can be used to make inferences on local migration, using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$.

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3.7 References

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Table 3-1. Population level stable isotope metrics derived from $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of fur samples of *M. lucifugus* and *M. septentrionalis* at summering sites and swarming sites in Nova Scotia.

Summering	<i>Myotis lucifugus</i>								<i>Myotis septentrionalis</i>							
	N	NR	CR	SEA _c	TA	CD	NND	SDNND	N	NR	CR	SEA _c	TA	CD	NND	SDNND
Annapolis Royal	12	9.12	9.67	19.62	37.52	2.86	1.48	1.59	0							
Antigonish	12	4.29	5.45	5.02	9.40	1.45	0.85	1.00	0							
Christmas Island	10	1.21	3.04	1.16	1.69	0.70	0.49	0.41	0							
Dollar Lake	0								15	2.03	4.20	1.86	4.44	0.80	0.42	0.82
Kejimikujik	14/7*	4.93	20.44	10.63	30.19	4.69	0.72	0.73	15	2.14	5.25	3.29	7.73	1.19	0.69	0.71
Millvillage	12	4.62	22.93	9.71	21.03	3.53	2.04	4.05	0							
Earltown	12	2.04	5.28	3.56	6.34	1.24	0.66	0.94	12	1.44	1.02	0.59	1.13	0.52	0.28	0.09
Gore	12	4.98	2.04	2.31	4.40	1.37	0.59	0.33	0							
Tatamagouche	12	1.33	1.81	1.14	2.05	0.74	0.38	0.18	0							
West Chezzetcook	12	5.13	17.76	6.24	11.50	2.78	1.68	3.66	0							
Swarming																
Cave of the Bats	7	3.23	17.79	22.94	30.60	4.04	2.97	3.59	20	2.97	2.10	1.58	4.02	0.94	0.31	0.15
Cheverie	20	4.52	12.65	11.66	26.00	3.15	0.87	0.36	17	3.58	2.35	1.82	4.86	0.94	0.44	0.31
Donkin	0								4	0.72	2.35	0.66	0.42	0.72	0.77	0.42
Glenelg	16	2.90	11.74	6.84	17.69	2.14	1.08	0.88	6	1.16	1.50	0.68	0.74	0.61	0.45	0.33
Hayes Cave	20	7.82	25.93	26.09	75.13	4.47	1.63	1.82	19	4.45	3.13	2.32	7.12	0.94	0.50	0.54
Lake Charlotte	20	4.53	16.48	9.03	26.26	3.63	0.74	0.44	7	1.08	2.63	1.63	1.96	1.03	0.49	0.24
Lear Shaft	20	7.79	30.37	36.53	124.27	4.63	1.81	1.94	19	2.97	2.44	1.84	4.24	0.96	0.37	0.16
Minasville	5	4.77	13.90	39.26	32.93	4.45	3.41	2.45	7	2.10	1.53	1.37	1.79	0.79	0.66	0.18
Rawdon	28/34*	7.30	30.28	23.31	126.40	3.86	0.82	1.08	28/31*	4.06	3.01	1.68	7.98	0.92	0.23	0.19
Vault	20	10.38	21.37	44.48	123.30	5.63	1.80	1.22	0							

* Number of males sampled

Table 3-2. Classification matrix of *M. lucifugus* correctly assigned to their known summering site based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of the populations.

To → From ↓	N	Annapolis Royal	Antigonish	Christmas Island	Earltown	Keji mkujik	Mill village	Gore	Tatama gouche	West Chezzet cook	% Correct
Annapolis Royal	12	9	2	0	0	0	0	0	0	1	75
Antigonish	12	0	7	0	2	1	0	2	0	0	58
Christmas Island	10	0	0	8	0	1	1	0	0	0	80
Earltown	12	0	0	3	6	0	0	2	1	0	50
Kejimkujik	21	0	0	0	2	12	0	0	7	0	57
Millvillage	12	0	0	1	0	1	10	0	0	0	83
Gore	12	1	5	0	4	0	0	2	0	0	17
Tatamagouche	12	0	0	0	2	0	0	0	10	0	83
West Chezzetcook	12	0	0	0	0	1	0	0	0	11	92

Table 3-3. Classification matrix of *M. lucifugus* correctly assigned to their swarming capture sites based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of the populations.

To → From ↓	N	Cave of the Bats	Cheverie	Glenelg	Hayes Cave	Lake Charlotte	Lear Shaft	Minas ville	Rawdon	Vault Cave	% Correct
Cave of the Bats	7	3	0	0	1	0	2	0	0	1	43
Cheverie	20	1	5	6	3	0	2	1	1	1	25
Glenelg	16	1	4	7	0	0	0	0	1	3	44
Hayes Cave	20	4	3	5	2	1	3	2	0	0	10
Lake Charlotte	20	7	2	4	3	1	0	1	2	0	5
Lear Shaft	20	3	3	0	3	0	6	2	0	3	30
Minasville	5	1	1	0	0	0	0	2	0	1	40
Rawdon	62	10	7	8	5	0	6	18	1	7	2
Vault Cave	20	2	0	6	1	1	0	5	1	4	20

Table 3-4. Classification matrix of *M. septentrionalis* correctly assigned to their known summering site based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of the populations.

From ↓ To →	N	Dollar Lake	Earltown	Keji mkujik	% Correct
Dollar Lake	15	9	5	1	60
Earltown	12	3	9	0	75
Kejimkujik	15	4	5	6	40

Table 3-5. Classification matrix of *M. septentrionalis* correctly assigned to their swarming capture sites based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic signatures of the populations.

From ↓ To →	N	Cave of the bats	Cheverie	Donkin	Glenelg	Hayes Cave	Lake Charlotte	Lear Shaft	Minasville	Rawdon	% Correct
Cave of the Bats	20	5	6	0	1	2	2	2	1	1	25
Cheverie	17	3	8	0	1	0	2	1	2	0	47
Donkin	4	0	0	3	1	0	0	0	0	0	75
Glenelg	6	0	0	0	5	0	1	0	0	0	83
Hayes Cave	19	2	4	2	2	0	2	5	1	1	0
Lake Charlotte	7	0	2	0	0	0	4	1	0	0	57
Lear Shaft	19	2	3	1	4	1	3	3	0	2	16
Minasville	7	2	0	0	1	1	2	1	0	0	0
Rawdon	59	3	6	5	19	5	6	9	1	5	8

Table 3-6. Number of *M. lucifugus* from swarming sites assigned to summering sites with predictive discriminant analysis.

From ↓	To →	N	Anna polis Royal	Anti gonish	Christ mas Island	Keji mkujik	Mill village	Earl town	Gore	Tatama gouche	West Chezzet cook	# sites assigned to
Cheverie		20		3	4		6		2	5		5
Cave of the Bats		7		1	2			1	1	1	1	6
Glenelg		16		2			8		2	4		4
Vault Cave		20	3	6		1	6		4			5
Lake Charlotte		20		2	2		6	1	4	3	2	7
Lear Shaft		20	1	1	4	4	3	1	1	3	2	9
Minasville		5		3			1				1	3
Rawdon		62	4	19	7	6	12	2	7	4	1	9
Hayes Cave		20	1	1	1	4	5	1	3	2	2	9

Table 3-7. Number of *M. septentrionalis* from swarming sites assigned to summering sites with predictive discriminant analysis.

From ↓	To →	N	Dollar Lake	Keji mkujik	Earl town	# sites assigned to
Cheverie		17	9	4	4	3
Donkin		4		2	2	2
Cave of the Bats		20	11	4	5	3
Glenelg		6		5	1	2
Lake Charlotte		7	2	1	4	3
Lear Shaft		19	5	8	6	3
Minasville		7	1	3	3	3
Rawdon		59	7	37	15	3
Hayes Cave		19	4	8	7	3

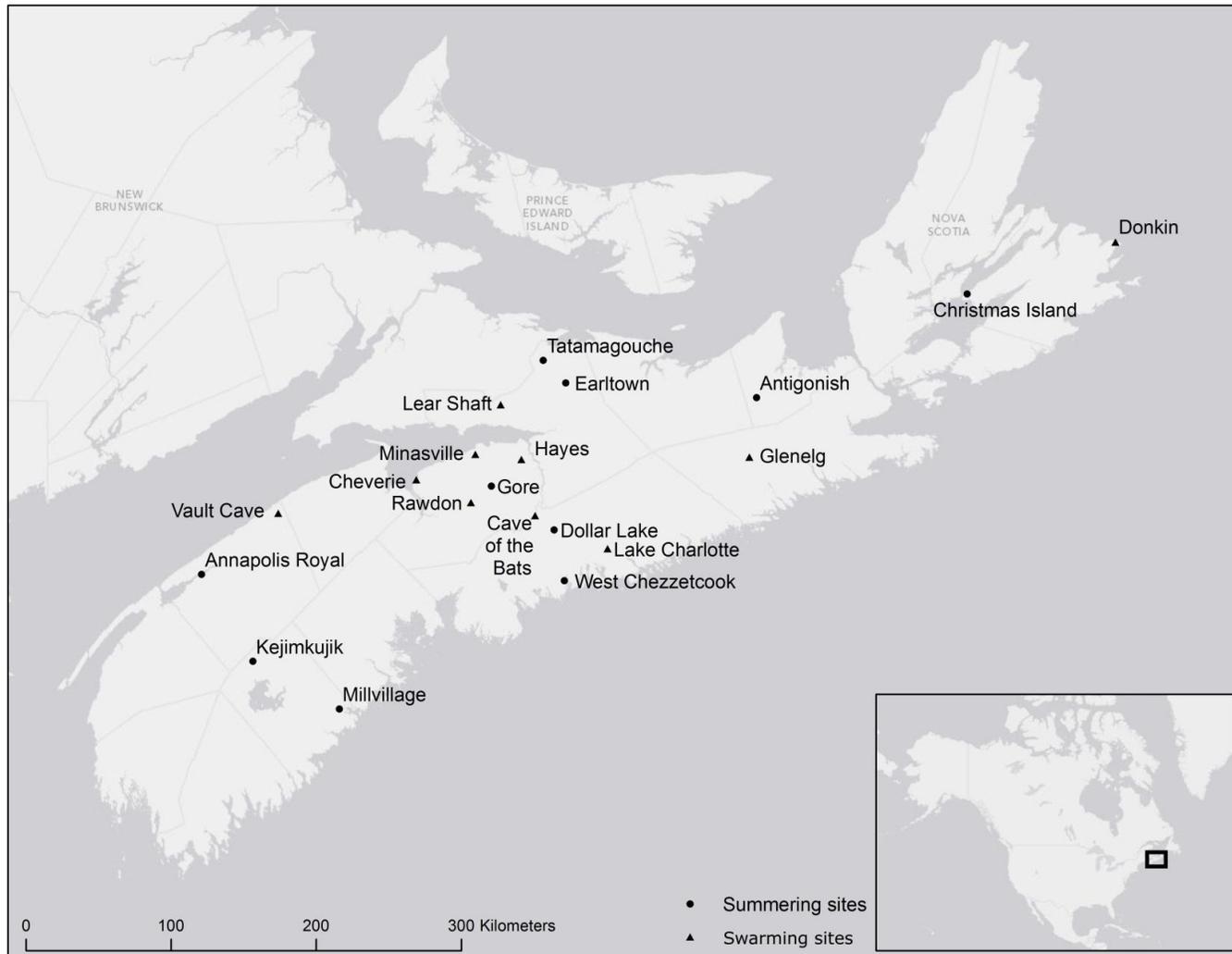


Figure 3-1. Sampling sites in Nova Scotia; circles indicate summering sites and triangles indicate swarming sites.

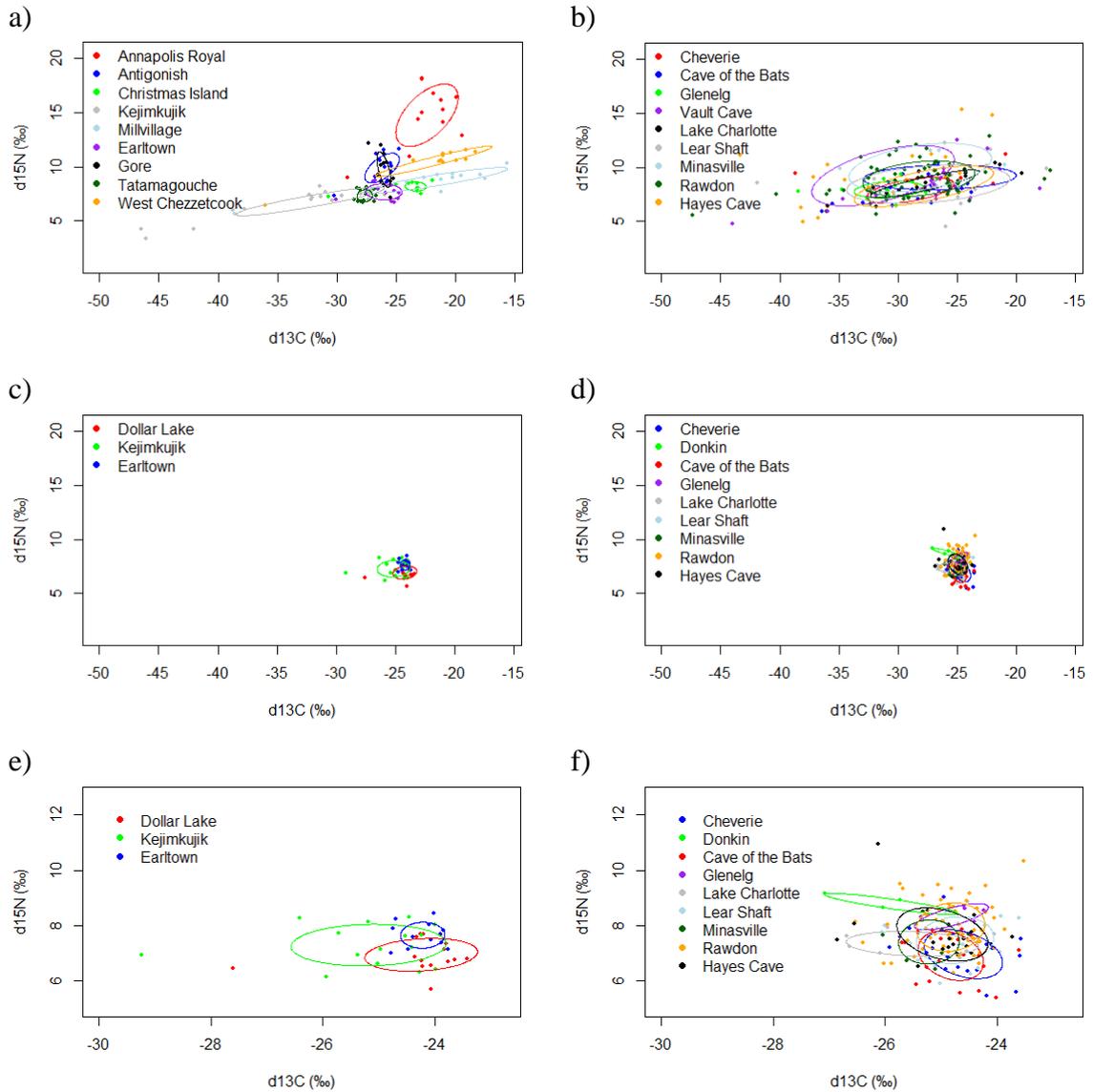


Figure 3-2. Scatter plots of $\delta^{13}\text{C}$ ‰ and $\delta^{15}\text{N}$ ‰ values of *M. lucifugus* (a: summering) (b: swarming) and *M. septentrionalis* (c: summering, e: detailed) (d: swarming, f: detailed) fur samples in Nova Scotia where ovals enclose the small sample size standard ellipse area (SEA_c; 40%). Bins c and d are presented on the same scale as a and b for comparison between species, bins e and f present the same data on a more detailed level.

Supplementary material 3-A. Matrix of SEA_c overlap between sites (area of overlap/area of larger ellipse) and spatial distances (in kilometers: lower half) comparing all *M. lucifugus* summering and swarming sites in Nova Scotia.

	Summering									Wintering								
	Anna polis Royal	Antigo nish	Christ mas Island	Kejim kujik	Mill village	Earl town	Gore	Tatama gouche	West Chezze tcook	Cave of the Bats	Glenelg	Vault Cave	Lake Char lotte	Lear Shaft	Minas ville	Raw don	Hayes Cave	
Summering																		
Annapolis Royal		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.000	
Antigonish	283		0.000	0.000	0.000	0.000	0.321	0.000	0.094	0.056	0.000	0.000	0.080	0.149	0.088	0.128	0.170	0.101
Christmas Island	395	112		0.000	0.098	0.000	0.000	0.000	0.000	0.000	0.027	0.000	0.000	0.032	0.000	0.000	0.019	
Kejimkujik	49	278	389		0.061	0.068	0.000	0.053	0.000	0.422	0.014	0.156	0.081	0.143	0.138	0.006	0.106	0.240
Millvillage	94	254	365	47		0.170	0.009	0.017	0.000	0.097	0.188	0.000	0.000	0.003	0.162	0.000	0.068	0.136
Earltown	199	92	197	204	194		0.028	0.069	0.000	0.133	0.042	0.000	0.000	0.038	0.097	0.006	0.063	0.107
Gore	145	137	247	144	131	62		0.000	0.057	0.038	0.069	0.000	0.032	0.091	0.043	0.056	0.080	0.052
Tatamagouche	195	104	205	204	197	15	67		0.000	0.067	0.000	0.000	0.000	0.002	0.031	0.000	0.017	0.041
West Chezzetcook	177	129	239	158	127	95	57	106		0.000	0.089	0.000	0.004	0.093	0.053	0.070	0.066	0.073
Swarming																		
Cheverie	113	170	279	120	118	84	36	83	86		0.216	0.420	0.147	0.553	0.318	0.137	0.364	0.447
Cave of the Bats	165	122	234	155	135	65	24	75	35	59		0.100	0.255	0.286	0.585	0.414	0.725	0.569
Glenelg	272	28	132	263	234	95	125	110	108	161	107		0.147	0.423	0.187	0.099	0.228	0.235
Vault Cave	47	239	349	75	101	152	104	147	143	69	125	230		0.116	0.388	0.625	0.361	0.262
Lake Charlotte	199	104	212	183	152	81	63	95	26	98	37	82	162		0.247	0.183	0.363	0.346
Lear Shaft	166	124	229	174	168	32	39	29	91	54	56	122	121	86		0.451	0.561	0.669
Minasville	145	139	249	149	141	55	17	55	76	30	42	133	101	78	26		0.493	0.316
Rawdon	133	148	260	132	119	73	12	76	60	28	32	137	94	69	50	23		0.606
Hayes Cave	165	119	228	164	150	41	19	47	63	51	29	111	100	59	26	22	33	

Supplementary material 3-B. Matrix of SEAc overlap between sites (area of overlap/area of larger ellipse) and spatial distances (in kilometers: lower half) comparing all *M. septentrionalis* summering and swarming sites in Nova Scotia.

	Summering			Swarming								
	Dollar Lake	Keji mkujik	Earl town	Cheverie	Donkin	Cave of the Bats	Glenelg	Lake Charlotte	Lear Shaft	Minasville	Rawdon	Hayes Cave
Summering												
Dollar Lake		0.298	0.101	0.642	0.000	0.456	0.000	0.166	0.381	0.139	0.087	0.255
Kejimkujik	161		0.131	0.383	0.000	0.340	0.031	0.474	0.482	0.391	0.103	0.505
Earl town	71	204		0.100	0.000	0.020	0.000	0.026	0.218	0.000	0.126	0.121
Swarming												
Cheverie	71	120	84		0.000	0.700	0.000	0.328	0.604	0.315	0.307	0.439
Donkin	303	465	273	355		0.000	0.072	0.000	0.000	0.000	0.058	0.051
Cave of the Bats	12	155	65	59	309		0.000	0.409	0.535	0.436	0.287	0.385
Glenelg	100	263	95	161	203	107		0.000	0.112	0.123	0.357	0.182
Lake Charlotte	27	183	81	98	286	37	82		0.392	0.517	0.281	0.368
Lear Shaft	65	174	32	54	305	56	122	86		0.461	0.602	0.686
Minasville	52	149	55	30	324	42	133	78	26		0.403	0.461
Rawdon	43	132	73	28	334	32	137	69	50	23		0.578
Hayes Cave	38	164	41	51	303	29	111	59	26	22	33	

Chapter 4

Movement dynamics of the little brown bat (*Myotis lucifugus*) and the northern long-eared bat (*Myotis septentrionalis*) in Nova Scotia: Synthesis

4.1 Synthesis

The research in this thesis was conducted to answer movement-related questions in bats on a local and regional (provincial) scale. Chapter 2 showed that anthropogenic alterations, in this case deforestation for the development of a wind farm, have variable effects on different bat species. The distribution of *M. lucifugus* and *M. septentrionalis* are driven by the distribution and abundance of landscape features such as forests, creeks and clearings. The top predictor variables for the presence of *M. lucifugus* were proximity to ponds and roads, and the presence of this species was associated with forests characterized by semi-open tree crown closure. In contrast, *M. septentrionalis* was associated with close proximity to forest-covered streams and forests with dense tree crown closures. Applied to our study site, these data indicate that *M. lucifugus* has gained suitable habitat inside the boundaries of the wind farm, but *M. septentrionalis* has lost a considerable amount of suitable habitat since the wind farm was built. While the total forest loss of the fragmented area only accounts for a small percentage, this results in a considerable increase in forest edge habitat. Besides the direct negative effects that forest fragmentation has on *M. septentrionalis*; a decrease in suitable habitat (Faure et al. 1993; Owen et al. 2002; Ratcliffe and Dawson 2003; Broders et al. 2004), *M. lucifugus*' increase in suitable habitat may cause for additional risks for this species. In many species of bat, including *M. lucifugus*, high mortality rates have been observed at wind farms (Johnson et al. 2003; Arnett et al. 2008; Baerwald et al. 2008; Rollins et al. 2012). Several studies have focussed on hypotheses related to the attraction-effect; is high mortality detected at wind farms because bats are more attracted to wind farm associated features than would be expected in a random situation? Some studies hypothesize that bats are

attracted to wind farms because: spinning turbines may produce high frequency noises (Horn et al. 2008), insects might be attracted to turbines (Long et al. 2011), high efficiency areas for wind farms may overlap with major migratory routes (Johnson et al. 2003; Nicholson et al. 2005). Any, or a combination of these, may contribute to high mortality of bats at wind farms, but in addition to these, our study suggests that an increase in favourable habitat may attract certain species that have a preference towards open and fragmented forest types.

While *M. lucifugus* and *M. septentrionalis* differ in their summer habitat requirements and behaviour, come time for migration the behaviour appears to be more uniform between species. Long-distance migratory bats and birds move vast distances on a latitudinal plane and studies have linked wind farm mortality to major migratory routes (Johnson et al. 2003; Nicholson et al. 2005). Migratory routes of short-distance migratory *Myotis* spp. appear more omnidirectional, as was demonstrated in chapter 3. Bats acquire energy during the summer and moulting happens some time before bats arrive at swarming sites, thus summering bat colonies are more distinct in isotopic composition. Our data indicated that both *M. lucifugus* and *M. septentrionalis* at swarming sites have broad $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ranges, suggesting that bats at swarming sites are aggregations of bats that migrated from various summering areas. Though these bats show high fidelity to summering sites (Fenton and Barclay 1980; Arnold 2007), which decreases genetic diversity in some species (Lewis 1995), the mixing and breeding of bats at swarming sites from several summering sites may prevent loss of genetic diversity and potentially increase diversity (Webster et al. 2002).

It is unclear whether short-distance migratory bats use major migratory routes and, if so, what landscape features are predictors for this. *Myotis* spp. mortality at wind farms is not documented as extensively as mortality of *Lasiurus* spp. (Johnson et al. 2003; Arnett et al. 2008) and one explanation for this may be that *Myotis* spp. use different tactics and routes for migration between summering and swarming sites. However, these observations may be explained by something different altogether. For example, shorter migration distance may mean that *Myotis* spp. are exposed to fewer wind farms than long-distance migrants. Alternatively, *Myotis* spp. may simply be less abundant than long-distance migrants. Searcher efficiency may be biased towards finding more larger, non-*Myotis* spp. during wind farm ground surveys due to visibility or scavenger effects (Kunz et al. 2007; Arnett et al. 2008).

Ecological traits of short-distance migratory bats, like *M. lucifugus* and *M. septentrionalis*, may however subject these species to risks which long-distance migrants are not affected by (Blehert et al. 2009; Turner et al. 2011; Minnis and Lindner 2013). Females of these bats are highly social; roosting in large colonies in summer and congregating and interacting with many other individuals during swarming. In addition bats form passive aggregations during winter by hibernating in large, dense clusters (Fenton and Barclay 1980; Faure et al. 1993; Owen et al. 2002; Ratcliffe and Dawson 2003; Broders et al. 2004). This high degree of direct contact with other individuals from numerous places exposes a species to many transmittable pathogens. Mixing of bats from different summering sites at swarming sites can spread pathogens at a fast rate (Turner et al. 2011). This process was demonstrated with the discovery of white-nose syndrome (WNS) in North American hibernating bats. Millions of bats have died in many states and

provinces as a result of the spread of WNS, and Nova Scotia is no exception (Blehert et al. 2009; Minnis and Lindner 2013; H.G Broders and L.E. Burns, unpublished data). The high fidelity that populations of these bats show to summering sites and possibly hibernacula (Fenton and Barclay 1980; Arnold 2007) combined with mixing of individuals at swarming sites is likely a major cause for the rapid spread of WNS.

The studies conducted and described in this thesis demonstrate the effectiveness of studying 1) local movement patterns and habitat selection using radio telemetry and combining logistic regression with a multi-criteria evaluation, and 2) regional movement patterns using stable carbon and nitrogen isotopes in combination with discriminant analyses, when animals are too small and their ranges too large to successfully study through traditional tracking methods (mark-recapture, radio-, gps- or satellite tracking).

In addition to allowing us to make inferences on regional migration, stable isotope analysis can provide insight in inter-specific and geographical variation in diet, large-scale movement patterns, habitat composition and sources of pollution in the environment (Hobson 1999; Rubenstein et al 2004). For example, in chapter 3 we discussed populations with increased $\delta^{15}\text{N}$ values and implied that these are related to nitrogen runoff, resulting from agricultural activity (Kohl et al. 1971; Heaton 1986; Hebert and Wassenaar 2001; Kellman and Hillaire-Marcel 2003). Peripherally, small sample size corrected standard ellipse areas (SEA_c) of our studied populations were very different between summering sites and swarming sites, but also between *M. lucifugus* and *M. septentrionalis*. For *M. lucifugus* we detected a much greater SEA_c than for *M. septentrionalis*, and these data might suggest that the latter either has a narrower dietary niche or forages in a less diverse habitat and thus is less of a generalist than *M. lucifugus*

(Martin et al. 1988; Parnell et al. 2010; Jackson et al. 2011). Conducting these studies on a larger spatial scale and with larger sample sizes may provide important insight in the variability of dietary needs, habitat requirements and movement patterns amongst regions and species (Syväranta et al. 2013).

Our studies demonstrate that ecosystem diversity is essential to sustain species diversity and therefore, in landscape planning, the ecological needs of different native species need to be considered. *Myotis septentrionalis* is dependent on forests with mature stands for foraging, roosting and raising pups, whereas *M. lucifugus* has adapted to roosting in houses and is overall less dependent on the forest.

4.2 Literature cited

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