DIET AND HABITAT OF THE LITTLE BROWN BAT (*MYOTIS LUCIFUGUS*) IN INTERIOR AND NORTHERN ALASKA

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DIET AND HABITAT OF THE LITTLE BROWN BAT (*MYOTIS LUCIFUGUS*) IN INTERIOR AND NORTHERN ALASKA

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Abstract

Little brown bats are sensitive to cold winters but consistent records of roosts in interior Alaska for 30 years indicate that the range of this species expands into the subarctic. We hypothesized that the little brown bat in interior and northern Alaska has adapted to high environmental demands by shifting foraging strategies. We analyzed guano to describe prey composition by microhistology, DNA analysis, stable isotope analysis, and image fragment recognition software. Alaskan bats consumed moths and flies, which was similar to the diet of southern conspecifics. However, bats in Alaska also consumed spiders. The stable isotopes of N and C in hair from bats in interior Alaskan bats were significantly different from bats in Yukon and coastal Alaska, which indicated the use of a separate habitat through summer. We used citizen science to collect reports of bats that ranged over most of Alaska and included sightings in the Arctic during autumn. Alaskan bats stored similar amounts of body fat to southern bats in autumn but unlike southern bats that migrate over 200 km, radio tracked bats in Alaska migrated short distances (<100km) to hibernacula in human structures. Expansion of the range of the little brown bat is apparently associated with a shift in foraging behavior to include gleaning of arthropods from surfaces. Overwintering at the extremely low air temperatures in interior Alaska is unlikely. Consequently, the persistence of bats in interior and northern Alaska may be related to consistent availability of human structures.

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Chapter 1: General Introduction

Baseline knowledge of a species' diet, range and habitat are essential for management of populations especially when considering the effects of changing climate and disease. The little brown bat (*Myotis lucifugus*) is facing regional extinction in eastern North America due to the spread of the fungal disease White Nose Syndrome (Frick et al., 2010a). Conversely, reported sightings of little brown bats are increasing in interior and northern Alaska, which suggests the presence of a growing population. The little brown bat is an ideal species to examine responses in diet and habitat selection to shifts in climate at the northern edge of a generalist's range. Developing a baseline of information about its northern populations will guide future directions for monitoring shifts resulting from changes in the climate or the spread of disease.

The little brown bat is widely distributed from the mountainous areas in Mexico to the northern regions of Canada and Alaska (Fenton and Barclay, 1980). This species preys on many species of arthropods. Bat diets can be used as an index of insect diversity while the animal indirectly provides a valuable service as a consumer of insect pests (Jones et al., 2009). Bats can live up to 35-40 years and are the longest-lived small mammal in the world (Wilkinson and Smith, 2002). Bat life span is much longer than expected given their small body size, and this may be associated with their resistance to protein oxidation and enhanced protein homeostasis (Salmon et al., 2009). Temperate populations of the little brown bat migrate 200 to 800 km between summer foraging ranges and their winter hibernacula (Fenton, 1969; Norquay et al., 2013). During the fall migration, bats aggregate in mating swarms. In the spring, reproductive females disperse to maternity colonies, while males and non-reproductive individuals disperse more widely, often maintaining smaller social groups (Fenton and Barclay, 1980).

Diet studies at more southern latitudes have found that little brown bats feed mainly on flying insects including moths and flies and rarely glean prey such as spiders from foliage as the gleaning niche is filled by other species of bats (Belwood and Fenton, 1976; Clare et al., 2014; Feldhamer et al., 2009). Bats feed intensively during the summer as females support their pups through pregnancy and lactation and as both sexes deposit fat stores for winter (Kunz et al., 1998). On cooler nights when foraging costs may be high, bats may enter torpor to conserve energy, but pregnant and lactating females have high metabolic demands that are associated with shorter and shallower bouts of torpor than males (Dzal and Brigham, 2013; Kunz et al, 1998).

Predicted changes in climate for the southern regions of the western United States are likely to cause declines in bat populations due to water scarcity (Adams, 2010), but warming climate in Alaska may enable bats to extend their ranges and water scarcity is less likely to be an issue in Alaska. Fecundity of the little brown bat population increases with warm weather patterns that allow females to begin foraging earlier in spring and give birth earlier in the summer (Frick et al., 2010b). In northern regions, a warming trend in the climate would increase prey availability in the spring for pregnant females and likely lead to earlier births. Earlier births that allow a longer window of summer for growth and development are likely to increase the survivorship of offspring.

We hypothesized that the population of little brown bats in interior Alaska are generalists with varied foraging strategies. We tested this hypothesis through diet analysis by using a time series of guano samples to identify prey species through the summer. We used microhistology, stable isotope analysis (∂^{15}N. ∂^{13}C, and ∂^{34}S), DNA analysis, and image analysis software as alternative methods for diet evaluation. We used isotopic markers in both guano and hair to indicate diet. We also compared stable isotope values from sites in interior Alaska to coastal

Alaska and the Yukon. Comparing isotopic signatures of multiple populations can provide information about how those populations may differ in feeding strategy or in movements across the landscape (DeNiro and Epstein, 1981; Fry, 2006).

We assessed the geographic distribution of the little brown bat in Alaska with a combination of traditional ecological knowledge, habitat surveys, captures and telemetry. We also examined the hypothesis that human structures aid in the persistence of this species in interior and northern Alaska where temperatures are too low for suitable natural hibernacula. We compared bat-sighting locations to an annual mean temperature and compared the fall body condition of interior and northern Alaskan bats with records of southern populations that have a longer foraging season and warmer temperatures. We combined information about the population's diet, habitat and range to begin developing a baseline to inform future management decisions.

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Chapter 2: Evaluating the diet of a generalist carnivore: the little brown bat at its northern range limit[1]

Abstract

Small mammals are sensitive to environmental demands and changes in their food supplies. Little brown bats (*Myotis lucifugus*) are at the limits of their range in interior Alaska where environmental demands are high and prey availability is highly dependent on seasonal changes. We hypothesized that the little brown bat in interior and northern Alaska has adjusted to high environmental demands by broadening its foraging strategies and the prey it consumes. We analyzed fragments (microhistology) in guano to describe prey composition to taxonomic order. Along with examining diet breadth using microhistology, we compared the efficacy of diet evaluation using DNA analysis, stable isotope analysis on guano and hair, and image recognition software. Alaskan bats consumed aerial prey such as Lepidopteran (moths) and Dipteran (flies and mosquitoes) insects as well as terrestrial arthropods including Araneae (spiders). Shifts in aerial prey consumption were closely linked to Julian day. Values for ∂^{15}N in hair indicated that Alaskan bats were generalist carnivores but significant outliers also indicated that some individuals are consuming distinct diets. The interior Alaskan bats had significantly different isotopic signatures from bats in Yukon and coastal Alaska. The little brown bat's flexibility in feeding strategies is likely to allow this species to tolerate changing environments. Fecal microhistology and DNA gave similar results as far as orders of prey consumed, but microhistology provided quantitative information and DNA provided presence/absence data to the scale of family. Fecal microhistology may be the most effective tool to continue monitoring

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diet in these bats, which could be supplemented with DNA analysis if greater taxonomic resolution is desired.

Introduction

Generalist carnivores present a challenge for dietary analysis because they consume a variety of prey species that often includes multiple trophic levels. Small predators such as birds and bats may consume a wide diversity of invertebrates with a rich variety of life histories and environmental responses. Generalists may be better suited to range expansion than specialists if they are able to further diversify or shift their diet to fulfill their needs in different habitats (Angert et al., 2011; Betzholtz et al, 2012; Zielinski et al., 2005). The ability of generalist carnivores to incorporate new dietary items as their range changes or expands may be an important factor in predicting extinction rates as habitats may shift rapidly with projected changes in climate (Boyles and Storm, 2007).

Small mammals are sensitive to environmental factors such as temperature and precipitation (Walther et al., 2002; Root et al, 2003; Rexstad and Kielland, 2006). Insectivorous bats are sensitive to air temperatures that affect both the energetic costs to the animal and also affect their prey availability (Moosman Jr. et al., 2012). Nocturnal foraging in many species of bats decreases predation risks and also reduces competition with diurnal insectivorous birds (Kunz, 1974). The little brown bat (*Myotis lucifugus*) in the interior and northern regions of Alaska and the adjacent Yukon Territory forage where prolonged day length, cool temperatures, and reduced competition from other species of bats may affect feeding behaviors (Whitaker and Lawhead, 1992). We hypothesized that the relatively harsh environmental conditions in interior Alaska may cause this species to diversify their foraging strategies compared to lower latitude conspecifics.

The little brown bat is also an ideal species for evaluating methods of diet analysis because it is a voracious predator on several orders of arthropods. The little brown bat is not

sensitive to human disturbance during the summer (Bunkley et al., 2015) and is often found roosting in human structures, allowing minimally invasive collection of fecal samples at roosts without altering feeding habits.

The little brown bat is widely distributed from the mountainous areas in Mexico to the northern regions of Canada and Alaska (Fenton and Barclay, 1980). Diet studies at more southern latitudes have found that little brown bats feed mainly on flying insects including moths and flies and rarely glean prey such as spiders from foliage (Belwood and Fenton, 1976; Clare et al., 2014; Feldhamer et al., 2009). Bats feed intensively during the summer as females support their pups through pregnancy and lactation and as both sexes deposit fat stores for winter (Kunz et al., 1998). On cooler nights when foraging costs may be high, bats may enter torpor to conserve energy, but pregnant and lactating females have high metabolic demands and shorter and shallower bouts of torpor than males (Dzal and Brigham, 2013; Kunz et al, 1998). High-energy demands for little brown bats in northern latitudes may influence prey selection. Northern little brown bats may fill a wider niche than in more southern regions where prey consists mostly of flying insects. In southern regions, the gleaning niche is filled by other species such as the northern long-eared bat, *M. septentrionalis (*Lausen et al., 2009).

Currently, microhistology of indigestible prey fragments in guano is the best method available for evaluating the diet of insectivorous bats (Kunz and Whitaker, 1983). However, analysis of indigestible fragments may not detect soft-bodied prey, such as mayflies (Rabinowitz and Tuttle, 1982). Stable isotope analysis of guano or other tissues, such as hair, can also be used to estimate diet composition whenof prey are isotopically distinct (Painter et al., 2009; Salvarina et al, 2013). Amplification of DNA sequences in guano can be used to identify a wider variety of prey in the diet (Whitaker, 2009), but this method may also identify the prey from lower trophic

levels, such as the insects consumed by the spider that was consumed by the bat. Image analysis software may provide an alternative quantitative analysis if software is able to reliably identify fragments of prey items in images of dissected guano pellets.

We hypothesized that the population of little brown bats in interior Alaska are generalists with varied foraging strategies. We tested this hypothesis through diet analysis by using a time series of guano samples to identify prey species through the summer. We used microhistology, stable isotope analysis (∂^{15}N. ∂^{13}C, and ∂^{34}S), DNA analysis, and image analysis software as alternative methods for diet evaluation. We used isotopic markers in both guano and hair to indicate diet. We also compared stable isotope values from sites in interior Alaska to coastal Alaska and the Yukon, Canada. Guano reflects prey consumed on the last foraging bout, as indigestible fragments are voided within 45 minutes of consumption in insectivorous bats (Neuweiler, 2000), while hair isotopes reflect diet over the period of molt, which in maternity colonies occurs after parturition and lactation before fall migration (Sullivan et al., 2011). ∂^{15}N varies with trophic level, with approximately 3‰ enrichment with each increase in trophic level. Enriched ∂^{15}N can also indicate nitrogen from marine sources. ∂^{13}C is more depleted in terrestrial than freshwater aquatic food chains. ∂^{34}S varies with geology, which may make it a good option for developing an isoscape in Alaska where the coastal and mountainous geography can complicate spatial patterns of other isotopes. Comparing isotopic signatures of multiple populations can provide information about how those populations may differ in feeding strategy or in movements across the landscape (DeNiro and Epstein, 1981; Fry, 2006).

Materials and Methods

Animals were captured and handled in accordance with the guidelines of the American Society of Mammalogists (Sikes et al., 2011) and the White-Nose Syndrome Decontamination

Protocol (WNS Decontamination Team, 2012) under permit #14-138 from the State of Alaska and under protocol #341381-1 from the Institutional Animal Care and Use Committee of the University of Alaska Fairbanks.

Guano sample collection

To examine changes in diet through the season, we collected guano samples at two maternity roosts in the Fairbanks area. One roost was in a cabin on Harding Lake (64°26'00.52"N 146°53'08.79"W) and the other was in a barn on Moose Creek (64°38'44.90"N 147°08'33.56"W). Both roosts were within the boreal forest and within 100 m of water. The Harding Lake roost was on the shore of a lake whereas the Moose Creek roost was adjacent to agricultural fields. Bats have been sighted at the Harding Lake location for over 30 years and at the Moose Creek location for over 10 years. We secured clean plastic sheeting at the main entrance points of the roosts and collected the accumulated guano every week from the arrival of the bats in late May through their departure in late August at Moose Creek (n=12 in 2012 and n=11 in 2013), and from late July through late August at Harding Lake (n=4 in 2013). In 2013 additional guano samples (n=67) were collected opportunistically from captured bats near Whitehorse, Yukon (60°43'0"N 135°3'0"W) at Chadburn Lake, Dalton Post, Drury Creek, Little Attlin, Salmo Lake, and Squanga Lake. All samples were frozen and stored at -20°C in polyethylene bags until analysis.

Hair sample collection

We collected hair samples from live captured individuals in Alaska and Yukon as well as from *M. lucifugus* specimens in the collection of the University of Alaska's Museum of the North (Appendix 2.1). Museum specimens (n=7) were collected mostly in coastal southeastern Alaska, providing samples from a location with expected differences in isotopic values, such as

higher $\partial^{15}N$, from the interior samples. We captured bats using homemade harp traps (Tuttle, 1974) and trimmed hair samples from the scapular region of the back following the America Museum of Natural History's protocol (American Museum of Natural History, 2012). Hair samples were collected into cryovials and frozen for storage.

Environmental data

We placed HOBO data loggers (Onset Computer Corporation, Cape Cod, MA) in the Moose Creek roost site to record temperature every 15 minutes for measures of daily minimum and maximum ambient air temperature. Local monthly weather data were recorded at Eielson Air Force Base and hourly precipitation data were recorded by the National Climatic Data Center in Fairbanks (NOAA). Weather data were summarized by calendar month and by the period between the guano collections.

Microhistology

In order to identify consumed prey, from each of the two Alaska roosts and for each collection date we examined 3 pellets of guano for microhistology. We soaked individual pellets in 99% isopropyl alcohol for 6 hours to soften the material prior to dissection. Pellets were dissected under 45x magnification (Bausch and Lomb Student Stereo Microscope, Rochester, NY). We identified prey items in each pellet to order or family by using images from published field guides and articles on analysis of bat guano and a reference collection of arthropods (Lehmkuhl, 1979; McAney et al., 1997; McGavin, 2011; Whitaker, 2009). We used white cloth in a hoop to collect the reference aerial prey and picked spiders from webs in the summer of 2013 near roosts in Fairbanks and Whitehorse. In the guano, Araneae were typically identifiable by their legs, Lepidoptera by wing scales, and Diptera by wing fragments (Fig. 2.1). The contribution of prey from a given family or order was estimated visually as the percent volume

of identified fragments within each pellet. Safi and Kerth (2004) found that percent volume was directly related to percent frequency. Guano samples collected from roosts in the Yukon were examined only for presence/absence of Araneae fragments, Diptera wings, and bat hair prior to stable isotope analysis without going through the process of being softened in isopropyl alcohol for thorough examination.

Stable isotope analysis

Soluble materials were filtered from guano to remove endogenous components from the digestive tract as well as any microbial growth on the pellet. We used polyester filter bags to individually boil guano samples in separate beakers of deionized water (F57 filter bags, Ankom Technology, Macedon, NY) for 20 minutes followed by 3 rinses with water. Hair was washed in a 2:1 mixture of chloroform:methanol to remove surface oils (Cryan et al., 2012).

We air-dried collected arthropods, hair samples, and guano samples, which were weighed into tins for isotope analysis. We assayed ^{13}C and ^{15}N by continuous flow isotope ratio mass spectrometry by using a Finnigan Delta V plus mass spectrometer (Thermo Scientific, Waltham, MA) combined with a Costech Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA) at the Alaska Stable Isotope Facility at UAF in Fairbanks, Alaska. Analysis of ^{34}S from interior and coastal Alaskan samples was performed separately at the USGS Federal Center in Denver, Colorado. Results were reported in delta notation and expressed in parts per thousand, relative to internationally accepted standards (δ=[(isotope ratio sample/isotope ratio standard)-1]*1000) (Fry, 2006; Gustine et al., 2014).

DNA analysis

Guano collected from Alaska was analyzed for the presence of DNA from arthropod prey by Jonah Ventures LLC (Manhattan, KS). DNA was extracted using the MoBio PowerSoils

protocol and amplification followed the methods of Zeale et al. (2011) and Bohmann et al (2011). Taxonomic assignment was performed in QIIME and sequences not resolved to at least the family level were removed.

Image fragment recognition

In order to test image fragment recognition as a potentially more efficient method for diet analysis, we took 2000 photos of microscopic views from dissected guano pellets during the microhistology analysis (25 fields of view from each of n=80 pellets) using a Celestron (Model #44104, Torrance, CA) compound microscope on the 40x setting with a Celstron Digital Microscope Imager with a 15x lens (Celestron, Torrance, CA). The microscope imager decreased the field of vision, making it more difficult to estimate proportional volumes from the images. We used ImageJ (U.S. National Institutes of Health, Bathesda, Maryland) to assess the feasibility of using images for identifying and quantifying Lepidoptera scales, which were uniform fragments commonly found in guano samples making them an ideal candidate for automated image fragment recognition.

Statistical analysis

Shannon's Diversity Index (Magurran, 1998) was used with the microhistology results to estimate the diversity of prey in guano as the number of orders of prey detected. We used linear regression to examine the relationship between Shannon's Diversity Index and minimum temperature for period, maximum temperature for period, precipitation and Julian day. We used linear regression to examine the relationship between minimum temperature for period, maximum temperature for period, precipitation and Julian day and the proportion of each prey type. The corrected Akaike information criterion (AICc) was used to select the best set of explanatory factors in each regression model (Anderson, 2008). We used One-way ANOVA with

Bonferroni's adjustment for multiple comparisons of stable isotope values of hair among sites, which included interior Alaska, coastal Alaska, and the Yukon. On Whitehorse guano samples, we used a pairwise comparison of marginal linear variables to test for a significant difference in isotopic signatures of pellets based on the observed presence of moth scales, spider legs, fly wings, and bat hair. We used command BACON (package st0197) to detect outliers in the stable isotope values of hair (STATA 14.0, StataCorp, College Station, TX).

Results

Environmental data

At Moose Creek, temperature ranged from a low of 0.8°C in May 2013 to a high of 39.0°C in July 2013. Rainfall ranged from 0.00 cm•month^{-1} for May 2013 to 5.89 cm•month^{-1} for July 2012 (Table 2.1).

Microhistology

Guano samples contained items from 8 orders of arthropods (Araneae, Lepidoptera, Diptera, Trichoptera, Formicidae, Neuroptera, Coleoptera, and Hemiptera) including items within the Dipteran families Culicidae, Tipulidae, Simulidae, Chironomidae. Each guano pellet contained between 4 and 10 individual invertebrates as estimated from the number of legs identified. The most abundant fragments were from the orders Diptera, Lepidoptera and Araneae. Out of 82 pellets from the Moose Creek and Harding Lake roosts, Diptera were present in 66 (80%), Lepidoptera in 62 (76%), and Araneae in 27 (33%).

Shannon's diversity index of prey items in the guano was directly proportional to the maximum daily temperature (Y = 0.236 (± 0.104) X + 2.511 (± 2.848), R^2 = 0.14, $F_{1,20}$ = 5.19, P = 0.03; Fig. 2.2) but was not related to the daily minimum temperature, Julian Day, or precipitation.

Neither variation in the percentage of Araneae nor flying insects in the guano was significantly related to daily precipitation or temperature and these effects were not included in the best-fit model. The presence of Araneae in the guano increased through the season ($Y = 0.21X - 32.72$, $R^2 = 0.30$, $F_{1,21} = 5.71$, $P=0.03$), while the presence of Lepidoptera decreased through the season ($Y = -0.13X + 37.15$, $R^2 = 0.30$, $F_{1,21} = 8.20$, $P=0.01$; Fig. 2.3). There was no significant seasonal trend of Diptera.

Stable isotope analysis

Values for $\delta^{15}N$ and $\delta^{13}C$ of guano previously examined using microhistology did not vary with the proportion of different prey types in the sample in interior Alaska. Pellets previously dissected and identified as more than 50% Araneae (n=3) had $\delta^{15}N$ values of -1.66 to 2.81 and $\delta^{13}C$ of -30.36 to -27.76, which overlapped values for $\delta^{15}N$ (0.61 to 1.83) and $\delta^{13}C$ (-31.53 to -29.46) in samples with more than 50% Diptera (n=3). However, among Whitehorse samples, pellets containing Araneae, Diptera, and bat hair had the highest $\delta^{15}N$ at 4.74 (n = 2), while pellets containing Diptera and Lepidoptera had the lowest $\delta^{15}N$ at 2.61 (n = 12) (Table 2.2). In a pairwise comparison of marginal linear predictions, the only significantly different isotopic signatures were Whitehorse pellets containing bat hair compared to those containing moth and fly wings ($t = 2.52$, $P = 0.014$).

Isotopic values of hair were significantly different between interior Alaska and the other sites in coastal Alaska and the Yukon for $\delta^{15}N$ ($F_{2,74} = 21.27$, $P= 0.000$) and $\delta^{13}C$ ($F_{2,74} = 13.77$, $P = 0.0001$) (Fig 2.4). The $\delta^{34}S$ values of hair samples were not significantly different between interior Alaska ($\bar{x} = 5.11$, $SD = 2.83$) and coastal Alaska ($\bar{x} = 7.69$, $SD = 3.16$) locations ($F_{1,38} = 2.93$, $p = 0.095$). Significant outliers for $\delta^{15}N$ in hair from interior Alaska included 13 of 77 observations that were 2 to 5 units from the nearest value.

Invertebrates captured (n = 33) and identified as possible prey items in the Fairbanks and Whitehorse area had a wider range of $\delta^{15}N$ and $\delta^{13}C$ values than the residues from guano. Diptera (n = 12) had the widest range of $\delta^{15}N$ values from 1.48 to 12.84 that overlapped the range for Araneae (n = 7, 3.57 to 8.07).

DNA analysis

DNA analysis detected a similar number of invertebrate orders as microhistology, with an increased detection of soft-bodied invertebrates such as mayflies (Ephemeroptera) and lacewings (Neuroptera). We detected 91 taxa to the level of genus from DNA sequences whereas microhistology only distinguished prey to order or family.

Image fragment recognition

ImageJ was unable to reliably recognize moth scales. The software could identify moth scales when they were isolated from all other material if they were lying flat in the image. In most instances, scales are piled upon each other, or at least touching other material. ImageJ was unable to find the edges of the scales in these cases.

Discussion

Little brown bat diet in Interior Alaska

The northern little brown bat had a more diverse foraging strategy than southern conspecifics, which feed mainly on flying arthropods (Moosman Jr. et al., 2012). Prey consumption by the little brown bat is probably related to availability, which is driven by the rise of air temperature above a threshold for emergence or activity of arthropods. Araneae have occasionally been found in the guano of southern little brown bats, but Lepidoptera and Diptera are the main components of the diet (Belwood and Fenton, 1976; Clare et al., 2014; Moosman Jr. et al., 2012). The large contribution (as high as 50% on some sampled dates) of Araneae to the

diet of northern little brown bats suggests that foraging behavior has changed in response to prey availability.

Individual bats (13 of 77) within the northern populations had hair stable isotope signatures that significantly departed from the group. Isotopes in hair also varied within location by more than 3 units, which is associated with a shift in diet by one trophic level (DeNiro and Epstein, 1981), e.g. δN^{15} values in Whitehorse ranged from 6.78 to 10.25‰ and δS^{34} values in Sitka ranged from 4.55 to 12.08‰. This pattern of isotopic variation suggests that while the population has a generalist feeding strategy, individuals with distinctly different isotopic signatures, such as the outliers in the interior Alaskan colonies, could be specialists either on specific prey types or spatially. Often, individuals that specialize within a generalist population are more efficient at foraging (Catry et al., 2014; Terraube et al., 2014; Woo et al, 2008). Populations of generalists with individual specialization may also be better able to expand their ranges or adjust to changing habitats by increasing intrapopulation diversity and genetic variation that improves adaptation to changing environments (Bolnick et al., 2003).

Most of the bats in the monitored colonies were lactating females that attain daily food intakes equivalent to 150% of their body mass each night to meet the demands of milk production and fat deposition for winter hibernation (Neuweiler, 2000). These individuals may have shifted their feeding strategy to include gleaning in order to meet the high-energy demands in the cooler climate, because fewer Lepidoptera (moths) and Diptera (flies) are available in cold and rainy conditions (Taylor, 1963). In spite of similarly cool temperatures in the spring and the fall, consumption of Araneae may be higher at the end of the season than at the beginning of the season because early in the season other aerial prey are available, such as the mosquito species *Aedes communis* which emerges early in the spring in still icy pools of water (Frohne, 1954).

The little brown bat fills a larger dietary niche in the northern limits of its range. The population has adjusted to the conditions in the north to be able to survive over a large range of northern Alaska. Because of this flexibility, the species may be better able to adjust to predicted shifts in climate at high latitudes.

Methods of diet analysis for generalist carnivores

Fecal microhistology is currently the most reliable quantitative method for diet analysis of a generalist carnivore. Although the supplies required for microhistology are inexpensive, sample processing is time consuming even after the initial investment in mastering insect identification from reference samples. Microhistology results in a bias towards identification of prey with indigestible fragments, while soft-bodied digestible prey items may not be identified.

Another quantitative method using image analysis software, such as ImageJ, has potential to automate some of the process and recognize fragments such as moth scales or Diptera wings. Unfortunately, the software could not distinguish the edges of fragments especially when items overlapped in the field of view. This might be overcome by preparing the fragments more completely before imaging by spreading the sample with a slide cover. The process of automated pollen grain classification may be well applied to moth scales and other arthropod fragments (Koutsoukos, 2013; Pozo-Banos et al., 2012). Image analysis is rapidly improving, and more sophisticated programs capable of recognizing the fragment edges may become available to the wildlife community.

Qualitative methods of diet analysis through fecal samples include DNA analysis and stable isotopes. Carnivores that prey on multiple trophic levels present a problem in DNA analysis, because items from lower trophic levels may be detected in the gut contents of prey (Sheppard and Harwood, 2005). Stable isotopes offer the possibility of developing a mixing

model but prey items must be isotopically distinct. The difficulty with this method of analysis for dietary generalists is the variation in isotopic signatures of some of the prey groups and an overlap in isotopic signatures among groups. For example, Diptera are a diverse group of prey feeding across multiple trophic levels from predators to phytophagous forms with high variation in isotopic signatures. We had difficulty categorizing prey items by stable isotope signatures. Because Lepidoptera in the area feed on plants, they had little variation in isotopic values as a group (Collet, 2008). This was not true for Araneae or Diptera. We looked at the isotopic signature of pellets that were already dissected, but because these samples had been soaked in isopropyl alcohol prior to analysis, the isotopic signatures had changed. Isopropyl alcohol can result in some tissues becoming more ^{15}N enriched while others become more depleted (Correa, 2012).

DNA analysis was helpful in identifying some prey items at a finer taxonomic scale including the detection of spiders to the level of family. DNA analysis indicated that the majority of the spiders were orb-weavers, which supports our hypothesis that little brown bats are gleaning spiders from webs close to the ground.

While the dietary analysis of generalist carnivores is challenging, understanding the ability of generalists to adapt their diet breadth is an important factor in predicting the effect of habitat change. The little brown bat is one generalist carnivore that has adapted its feeding strategy in northern climates to include gleaning nonflying arthropods. Fecal microhistology may be the most effective tool to continue monitoring diet in these bats, which could be supplemented with DNA analysis if prey diversity is an important metric. Bats may be a useful study species to test software for analysis of microhistology images because these small animals consume diets

that pass quickly through the digestive system. Improved applications of image analysis software could be useful in assessing the diet of generalist carnivores with greater efficiency.

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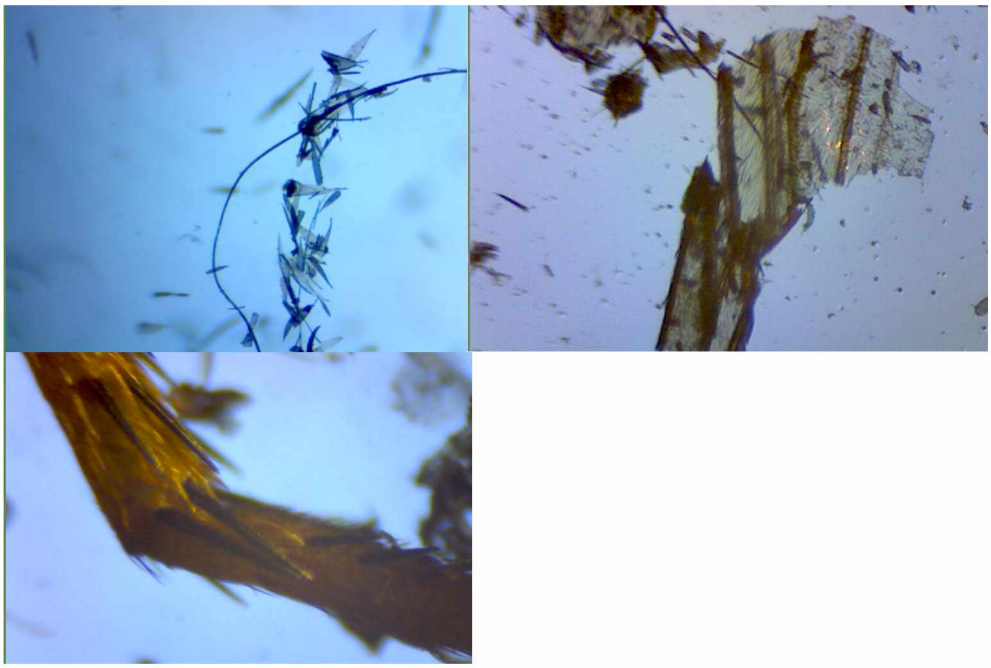
Table 2.1. Mean daily temperature records from HOBO loggers inside roosts in Alaska with precipitation data from the weather station at Eielson Air Force Base (64°40'59.88"N, 147°4'58.80"W).

Date	Mean High (°C)	Mean Low (°C)	Total Precipitation (cm)
May 2012	14.9	1.2	2.24
June 2012	21.4	9.6	5.49
July 2012	20.7	9.8	5.89
August 2012	18.9	6.3	3.84
May 2013	13.9	-0.4	0.00
June 2013	24.7	10.6	0.51
July 2013	22.1	11	2.06
August 2013	21.4	8.1	2.69

Table 2.2. Whitehorse guano samples categorized by the detected presence/absence of bat hair (molt), insect wings, and spider legs and the resulting average values for $\delta^{15}N$ and $\delta^{13}C$ in the washed residue.

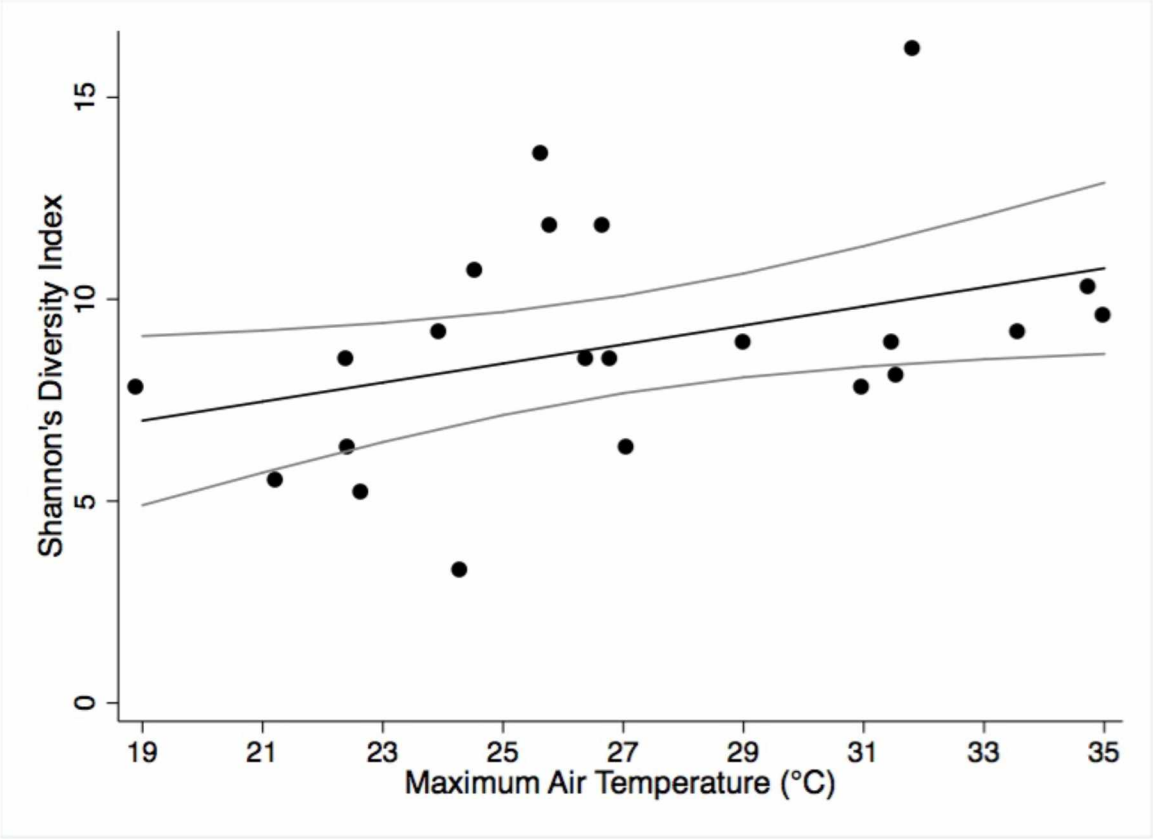
		$\delta^{15}N$		$\delta^{13}C$	
Contents	N Pellets	Mean	SD	Mean	SD
Moth	6	3.96	0.95	-30.42	2.02
Spider	9	4.09	1.49	-28.89	1.42
Fly + Moth	12	2.61	1.53	-29.71	1.17
Fly + Spider	4	3.79	1.00	-30.49	1.15
Moth + Molt	17	4.00	1.44	-29.98	2.65
Spider + Molt	2	4.64	2.25	-29.68	0.01
Fly + Moth + Molt	15	3.16	1.63	-29.36	1.90
Fly + Spider + Molt	2	4.74	0.05	-27.74	3.36

Figure 2.1



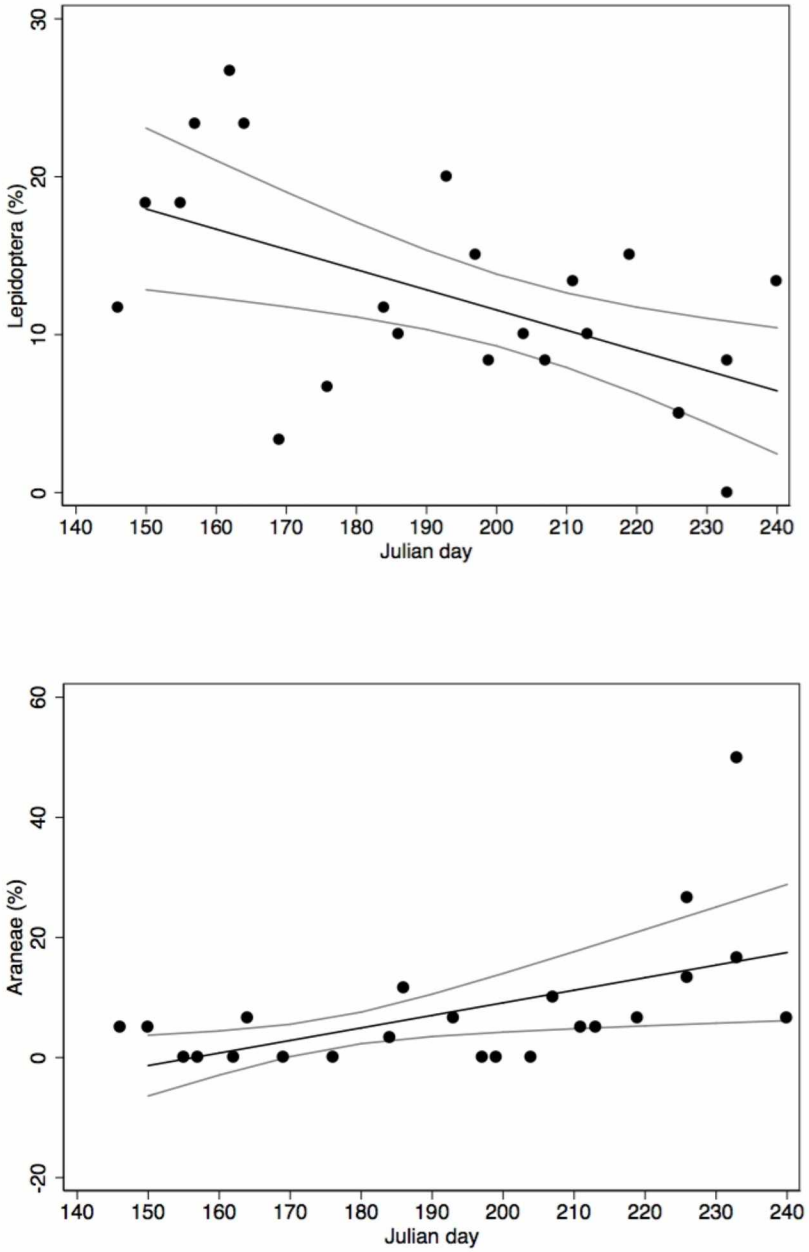
Examples of identifiable fragments of prey in bat guano. A. fine scales from the wing of a moth (Lepidoptera) with a strand of bat hair, B. veined wing from a fly (Diptera) (C), hairy leg from a spider (Araneae).

Figure 2.2



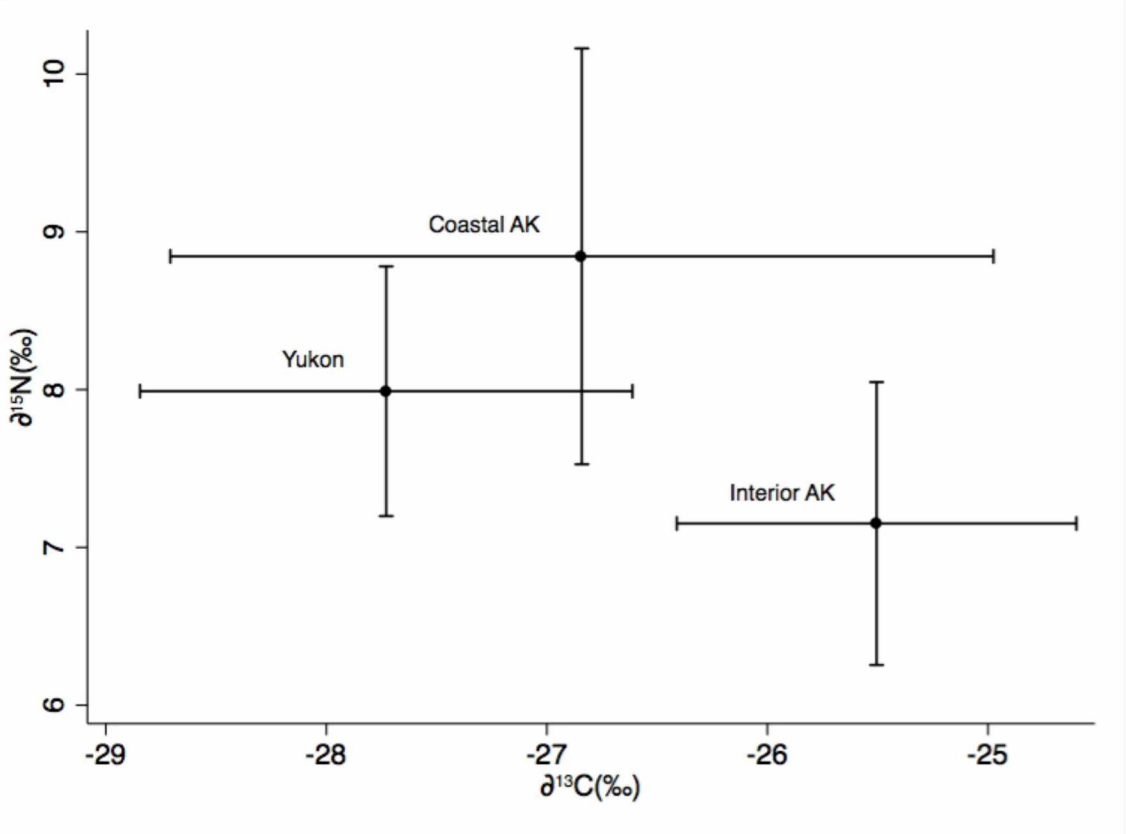
Linear regression of Shannon's Diversity Index of prey items against the mean maximum daily temperature during the preceding collection period (7-10 d) for Moose Creek, Alaska [n = 22, Y = 0.236 (± 0.104) X + 2.511 (± 2.848), R^2 = 0.14; *P* = 0.03].

Figure 2.3



Average percent volume by Julian Day of Lepidoptera (A) and Araneae (B) in bat guano.

Figure 2.4



Isotopic values ($\bar{x}$ ± SD) of hair from bats ($\delta^{15}N$, $\delta^{13}C$) captured in interior Alaska, Yukon and Costal Alaska.

Appendix 2.1. Specimens sampled from the University of Alaska Museum of the North's Mammalogy Collection as catalogued in the Arctos Database (http://arctos.database.museum)

Museum ID	Sex	Location	Latitude	Longitude	Date of Collection
AF74096	M	Chena Hills Dr., Fairbanks	64°49'46.99"	-147°56'42.35"	4/27/10
AF71189	M	Takatz Bay, Sitka	57°3'7.5"	-135°19'35.4"	2/17/11
AF74091	M	Gibson Place, Sitka	57°3'58.5"	-133°21'41.4"	2/1/12
AF74093	M	Finn Alley, Sitka	57°3'7.5"	-135°19'35.4"	9/5/12
AF74094	M	Finn Alley, Sitka	57°3'7.5"	-135°19'35.4"	9/5/12
AF74097	M	Finn Alley, Sitka	57°3'7.5"	-135°19'35.4"	2/17/11
AF74099	M	Finn Alley, Sitka	57°3'7.5"	-135°19'35.4"	2/17/11

Chapter 3: Habitat and ecology of the little brown bat, *Myotis lucifugus*, in interior and northern Alaska[1]

Abstract

The range of small mammals is constrained by environmental conditions such as temperature and precipitation. Very little is known about the range of the little brown bat in Alaska and how the species is surviving in a region where temperatures fall below their predicted threshold for winter survival. Establishing a baseline of current distribution is critical to monitoring potential population shifts. We hypothesized that the persistence of the little brown bat in interior and northern Alaska is dependent on the availability of human structures for roosting sites in areas where temperatures are too low for natural roosting sites and that persistence is also due to the northern population's ability to gain sufficient mass over a short summer with limited darkness. We used outreach through citizen science and traditional ecological knowledge (TEK) combined with roost studies and telemetry to describe the habitat of bats in interior and northern Alaska. We compared current known habitats to landscapes across the state. The length of nightly activity outside of the roost was positively related to the length of time between sunset and sunrise ($Y = 0.54X + 64$, $R^2 = 0.34$). Little brown bats in interior Alaskan roosts had a mean estimated fat mass of 21% of total mass in the fall prior to dispersal. We radio-tracked bats migrating short distances (<100km) to assumed hibernacula in human structures. The persistence of bats in interior Alaska may be related to consistent availability of human structures. Bats in interior and northern Alaska are surviving in spite of temperatures below their predicted tolerance.

[1] Rachel Shively, Perry Barboza. 2016. Prepared for submission to Northwestern Naturalist.

Introduction

The range of bats is extending in Alaska even though northern and interior Alaska are typically too cold for bats to overwinter. Very little is known about the habitat and range of the little brown bat (*Myotis lucifugus*) in interior and northern Alaska and how the species is surviving in a region where temperatures fall below their predicted threshold for winter survival. Establishing a baseline of current distribution is a vital step in monitoring potential shifts due to climate change. Establishing a baseline may become more critical as threats to the eastern population of the species continue towards regional extinction. Previously reported locations from museum specimens and citizen science programs include only 44 sites in interior Alaska and 35 sites in western Alaska (Parker et al., 1997; Tessler et al., 2014).

The little brown bat is widely distributed in North America from the mountainous areas in Mexico to the northern regions of Canada and Alaska and from the east coast to the west coast (Fenton and Barclay, 1980). This species provides a valuable service through pest control and is a potential bioindicator (Jones et al., 2009). It survives the winter through a combination of migration and hibernation, often migrating between 200 and 800 km to a suitable hibernaculum (Fenton, 1969; Norquay et al., 2013). During the fall migration, this species forms mating swarms, although fertilization is delayed until the following spring or summer. In the spring, reproductive females disperse to maternity colonies, while males and non-reproductive individuals disperse more widely (Fenton and Barclay, 1980).

Maternity colonies use warm roosts and individuals often cluster together to retain body heat and raise the microclimate to a mean hourly temperature of 35°C (Burnett and August, 1981). Little brown bats have been observed basking in the sun near maternity roosts in the Yukon (Slough, 2009). Little brown bats tolerate humans and it is common for this species to use

attics and barns for maternity roosts. These locations are likely to have a gradient of microclimates that bats can use as temperatures shift throughout the season. Bats have a high fidelity to these maternity roosts (Norquay et al., 2013). No known maternity colonies in interior or western Alaska exist outside of man-made structures (Tessler et al., 2014). Anthropogenic disturbance has not been found to alter the feeding rates of the little brown bat (Bunkley et al., 2015). The species has also been found to have the best body conditions in transition zones between urban and rural prairie areas, as urban areas provide more man-made roosting sites and rural areas lower bat population densities that decreases competition (Coleman and Barclay, 2011). Bats in Alaska may be relying on human structures for roosts that allow them to persist in areas that would otherwise be inhospitable.

Winter survival times of bats are directly related to metabolic rate in torpor, ambient temperature and the store of fat (Burles et al., 2014; Thomas et al., 1990). The optimal temperature for hibernacula of the little brown bat is 2°C (Hock, 1951; McManus, 1974). Winter energy costs increase above and below this temperature, and hibernacula temperatures that vary too much from 2°C could cause the bats to deplete their winter fat reserves before spring (Humphries et al., 2002). Bats with adequate fat stores may be able to choose warmer microclimates and decrease the amount of time spent in torpor, thus minimizing the physiological costs of hibernation including reduced motor function and reduced immune response and protein synthesis (Boyles et al., 2007; Humphries et al., 2003). Cooler microclimates will deplete fat even while bats remain in torpor, so hibernation below the ideal temperature range (0°C to 10°C based on a winter length of 193 days) cannot be sustained over long winters (Humphries et al., 2002).

A fungal disease of hibernating bats, White Nose Syndrome (WNS), is rapidly spreading across eastern North America where little brown bats often hibernate in large congregations in caves and mines. In contrast, little brown bats in the Washington and Oregon area may hibernate in smaller groups and use tree cavities and small rock crevices for hibernacula, both of which could reduce the transmission and virility of the disease (Burles et al., 2014; Neubaum et al., 2006). WNS often affects the majority of individuals within a hibernaculum, which has a large impact on local populations when bats are grouping together in large numbers to hibernate. Smaller and more dispersed hibernacula may slow the spread and effect of WNS if the disease reaches the northwest. If conditions of Alaska hibernacula vary enough from conditions of hibernacula in the east, Alaska may provide a refuge from this pathogen for the species.

While predicted climate changes in more southern regions of the western US are likely to cause declines in bat populations due to water scarcity (Adams, 2010), shifts in the climate in Alaska may enable bats to extend their ranges. Warming trends in the north may reduce the duration of winter and allow females to give birth earlier in the summer. Because timing of delayed fertilization is linked to prey availability, in northern regions an earlier spring melt could shift both the phenology of prey availability and bat parturition (Frick et al., 2010). Earlier births increase the survivorship of offspring by extending the window of time for the young to increase fat mass before hibernation.

Citizens are often the first to report new and unusual species in their area. Knowledge of cryptic species in ranges with small and dispersed human populations usually relies on historical reports and traditional ecological knowledge (TEK). TEK is useful for developing a baseline in cases where there is a lack of historical data (Huntington, 2000). Additionally, citizen science is helpful for projects covering large spatial areas, particularly for monitoring species occurrence

(Bonney et al., 2009). Citizen science is beneficial to the ecosystems being monitored as well as to the participants by increasing scientific literacy and community involvement (Conrad and Hilchey, 2011; Cooper et al., 2007). Alaska Department of Fish and Game has successfully used citizen science to increase the documented locations of bats in Alaska using a web-based reporting system (Tessler et al., 2014).

We assessed the geographic distribution of the little brown bat in Alaska with a combination of TEK, habitat surveys, captures and telemetry. We examined the hypothesis that this species is surviving the cold temperatures of Alaska in part by relying on human structures and that this population is able to gain similar amounts of body fat to southern conspecifics in spite of shorter nightly foraging times and a shorter season.

Materials and Methods

Animals were captured and handled in accordance with the guidelines of the American Society of Mammalogists (Sikes et al., 2011) and the White-Nose Syndrome Decontamination Protocol (WNS Decontamination Team, 2012) under permit #14-138 from the State of Alaska and under protocol #341381-1 from the Institutional Animal Care and Use Committee of the University of Alaska Fairbanks.

Captures

To assess body fat and collect measurements we captured bats in August of 2012, 2013, and 2014. We selected capture sites based on location of museum specimens collected from the Fairbanks area, as well as sightings and collection sites reported by Parker et al. (1997) for interior Alaska. Capture sites included two established maternity roosts that were accessible by road in the Fairbanks area. One roost was in a cabin on Harding Lake (64°26'00.52"N 146°53'08.79"W) and the other was in a barn on Moose Creek (64°38'44.90"N

147°08'33.56"W). Both roosts were within the boreal forest and within 100 m of water. The Harding Lake roost was on the shore of a lake whereas the Moose Creek roost was adjacent to agricultural fields. Bats have been sighted at the Harding Lake location for over 30 years and at the Moose Creek location for over 10 years.

We captured bats using homemade harp traps (Tuttle, 1974). Bats were collected within 10 minutes of capture by checking traps every 5-10 minutes. Each animal was held in a clean cotton bag for up to 40 minutes to allow for defecation and weighed to the nearest 0.1 g on an electronic scale. Body fat was estimated based on total body mass (Kunz et al., 1998). We recorded sex, age class and reproductive status before measuring size including the lengths of the tragus, ear, and forearm.

Radio telemetry

We attached glue on radio transmitters (A2414 0.3g 14 pulses per minute (Advanced Telemetry Systems, Isanti, MN)) to the backs of individuals with a total body mass of ≥6 g using Skin Bond (Smith and Nephew Inc., Mississauga, ON) after trimming hair to expose the skin. We tracked radio-tagged bats using fixed wing aircraft to determine a general location and then attempted to pinpoint the locations where possible on foot, using a TR-5K scanning receiver with RA-2AHS Antenna (Telonics, Mesa, AZ).

Temperature and activity

We placed HOBO data loggers (Onset Computer Corporation, Cape Cod, MA) inside and outside of the Moose Creek roost to record temperature every 15 minutes for measures of daily minimum and maximum ambient air temperature. Local monthly weather data, along with sunrise and sunset data, was recorded at Eielson Air Force Base and hourly precipitation data was recorded by the National Climatic Data Center in Fairbanks (NOAA). Nightly emergence

times were recorded from visual observations and using an AnaBat II and ZCaim (Titley Scientific, Columbia, MO) at the roost. Bat activity at sites away from the maternity roosts was detected and recorded using an AnaBat. Nightly activity duration at the roost was determined using the AnaBat to be the time between the first recorded bat activity for the evening until the last recorded bat activity in the morning.

Surveys/Traditional Ecological Knowledge

Interviews were conducted under permit #373251-4 from the Institutional Review Board of the University of Alaska Fairbanks. We interviewed community members throughout the Fairbanks and Delta area as well as along the Yukon River and Delta following traditional ecological guidelines developed by the Alaska Native Knowledge Network. We used information from community members interviewed in villages along the Yukon River and in the Fairbanks North Star Borough region to develop a map of known locations of bats. Some community members voluntarily completed written surveys to assess local opinions of bats and science (Appendix 3.1).

Data analysis

We mapped citizen bat sightings from our interviews in ArcGIS (Fig. 3.1) (Version 10.3.1, ESRI, Redlands, CA), along with museum collection sites of little brown bats (Arctos Database, http://arctosdb.org/home/data), human population data (http://dnr.alaska.gov/lrisservices/ls_proxy/email?layerid=14), and rivers from Alaska Department of Natural Resources (http://dnr.alaska.gov/lrisservices.ls_proxy/email?layerid=30).

We tested for a difference in the mass and size of animals recaptured in subsequent years by t-test using STATA 14.0 (College Station, TX). We used linear regression to examine the effect of minimum daily temperature, maximum daily temperature, and length of night between

sunset and sunrise on nightly activity duration outside of the roosts. The corrected Akaike information criterion (AICc) was used to select the best set of explanatory factors in each regression model (Anderson, 2008).

Results

Captures

We captured 68 individuals from two roosts over 3 years with 5 individuals recaptured in a subsequent year. Measurements of ear, tragus, forearm, body mass and the estimated body fat of individuals was similar to measurements from other studies of southern conspecifics (Table 3.1). Fifteen ear and tragus measurements were censored for incorrect technique. There was no significant difference from initial measurements in individuals that were recaptured in subsequent years ($t = 0.425$, $df = 3$, $P = 0.699$). We captured between Julian date 215 and 239 (August 3rd through August 27th). Lactating females were captured on every date ($n = 54$, $\bar{X} = 222$, $SD = 4.91$), which overlapped with the capture of a small number of pregnant females ($n = 3$, $\bar{X} = 221$, $SD = 1.73$) and juveniles ($n = 16$, $\bar{X} = 224$, $SD = 6.68$).

Radio telemetry

We were unable to track animals in 2013 because dispersal started on Julian Day 237 (25 August), which preceded the delivery of our radio-tags. We tagged six bats from Julian day 226 – 239 (14 – 27 August, 2014) and tracked five of those frequencies on flights and on foot from Julian day 246 – 251 (3 – 8 September). Flights followed the river and the highway north and south of the capture site. No tags were detected after 8 September, which aligns with the expected battery life of the tags. Two frequencies were located within 1 km of the roost at Harding Lake where the animals were tagged. The final locations of the remaining three frequencies were 70 – 81 km south of the roost along the Delta River.

Temperature and activity

At Eielson AFB, air temperature at the weather station ranged from a low of 0.8°C in May to a high of 39.0°C in July. Rainfall ranged from 0.00 cm for May 2013 to 5.89 cm for July 2012. In 2012, 2013, and 2014, bats departed for migration the day after the minimum daily temperature fell below 0°C at the roost. Duration of activity (1.3 to 8 hours) was directly related to the length of time between sunset and sunrise ($Y = 0.54X + 64$, $R^2 = 0.34$), which ranged from 2.5 hours on Julian Day 164 (13 June) to 8.75 hours on Julian Day 239 (26 August) (Fig. 2). The outlier in this set was on August 26, 2012 when the length of night was 527 minutes and the activity duration was only 63 minutes. The daily maximum temperature for that date was 13.6°C, so it may have been too cool outside to make a long night of foraging energetically cost-efficient. Models that included Julian Day and temperature did not rank as well in predicting activity duration as the selected model, which included only the length of time between sunset and sunrise. Temperatures in the roost during summer ranged from -2°C to 39°C. Bats were not observed in the maternity roost when maximum daily temperatures exceeded 36°C for a period of 8 days in June 2013.

Bats observed in the summer not associated with maternity colonies or human structures were typically in small groups (<10) along rivers in densely forested areas. In the fall, bats were observed swarming in large numbers adjacent to a cliff face in east Fairbanks and among rocky outcroppings in the White Mountains north of Fairbanks. One fall swarming site had no bat activity observed during the summer, but high bat activity (>100 bats) every year at the end of August and early September. The bats were observed exiting an attic at this site, but it is unknown if this residence is used as a hibernaculum.

Surveys/Traditional Ecological Knowledge

We visited 8 communities to present information on bats to ~ 400 people and also interviewed 60 community members. Presentations were Video Teleconferenced into an additional 8 communities. Questionnaires to assess familiarity with scientific inquiry and natural history of bats were completed by 23 participants. Questionnaire responses were based on a ranking system of 1 to 5. Only a few participants were aware of citizen science (4 of 23 respondents with rank ≤2 where 1 indicated they had heard of citizen science) but all participants agreed that scientists should share their data with the public and that science can apply to everyday life (rank ≤2 where 1 indicated they agreed with the statement). Participants correctly answered questions about bats including common misconceptions about blindness and their distant relationship to rodents (13 of 23 response ≥3 where 5 indicated that they did not agree that bats were blind or related to mice). However, written surveys are less effective than direct interviews for these communities. Community members reported bat sightings as far north as Wainwright and as late in the year as October (Table 3.2) (Fig. 3.1). Most sightings were near or within human structures (i.e. barns, schools, aircraft hangars), but some were away from villages near steep riverbanks with trees. The largest roost (>500 individuals) was located at a mine in Sleetmute (Table 3.2).

Discussion

The range of little brown bats in Alaska extends into the north and the species is common throughout the interior. The little brown bat in interior Alaska was not observed migrating long distances (>200 km) to hibernacula. Combining bat sightings from AnaBat surveys and TEK presented a clear trend of the bat's presence along major rivers, which coincides with the locations of towns in the state. Bats are possibly using rivers to navigate as they disperse each

summer and are able to use human structures with suitable roosting sites along the way. Males and non-reproductive individuals may be using steep riverside cliff areas or tree bark as day roosts in summer where the dense forest may provide some cover from predators. Low activity at these locations on cooler nights indicates that these non-reproductive individuals may be using torpor in these satellite roosts. Bats returned to these locations over multiple years and some individuals may develop a fidelity to a variety of roosting locations.

The activity of the bats was directly proportional to the length of the night between sunset and sunrise even though nights in interior Alaska are short during the summer (2.5 to 9h; Fig. 3.3). The bats had a very short window for activity around summer solstice when it was never completely dark. In spite of these constraints on foraging time, the bats at these roosts had healthy ranges of body mass (7-8 g) at the end of summer in August (Table 3.1) (Kunz et al., 1998). Bats still achieved a body mass similar to that of other populations at the end of summer even when the summer was further reduced by a late spring in 2012 that delayed returns to the maternity roosts until 20 May. Bats at these roosts were apparently more sensitive to high temperatures than more southern colonies (Burnett and August, 1981) because when temperatures exceeded 36°C, there was no activity at either maternity roost, while bats in Wisconsin have been observed to roost at 40°C. Alaskan colonies of little brown bats may have adjusted to cooler temperatures, increased day lengths and to shorter foraging windows by shifting foraging activity to cooler temperatures while gaining mass over a shorter period of time. In Alaska, little brown bats may be gleaning spiders inside and outside the roosts when temperatures are too low for flying insects (Shively et al., 2016).

The estimated fat content of the adult bats at the maternity roosts before fall dispersal was $\bar{X}=1.86 \pm 0.43$ g (Table 3.1), which is greater than that reported for this species at the same stage

for a maternity roost in New Hampshire ($\bar{X}$ = 0.52 ± 0.06) (Kunz et al., 1998). Fall fat content of bats at the maternity roost in Alaska is more similar to those reported for bats at a post-migration swarming site in Vermont in mid-September ($\bar{X}$ = 2.49 ± 0.40) when bats have usually completed mass gain for winter. The bats at the interior Alaska roost may be putting on more mass pre-migration than southern conspecifics. Although Alaska bats appear to be migrating shorter distances they have a shorter window post-migration to regain mass when rapidly declining temperatures decrease prey availability.

Predicted body fat of juvenile bats at roosts in interior Alaska ($\bar{X}$=1.47 ± 0.53) was also greater than those reported for juveniles of this species at the Vermont swarming site ($\bar{X}$ = 0.57 ± 0.06) and the New Hampshire maternity roost ($\bar{X}$ = 0.67 ± 0.14) (Kunz et al., 1998). In interior Alaska, juveniles were first observed learning to fly on 31 July. Dispersal from the roosts occurred each year at the end of August the day after temperatures reached <0°C. Consequently, juvenile bats in Alaska have a short window between learning to fly and leaving the roost to forage and gain sufficient mass for the winter. Bats at high latitudes may put on more mass to survive low winter temperatures, as do northern songbirds (Sharbaugh, 2001).

Low annual mean temperatures (-10 to -3°C) and the presence of permafrost across much of the interior and northern portion of Alaska indicate that natural winter hibernacula are unlikely north of the Alaska Range (akclimate.org). Cave temperatures tend to be very close to the annual mean temperature for an area (Wigley and Brown, 1976), so if there are caves large enough to have a stable temperature in interior and northern Alaska, they are likely too cold to make suitable hibernacula. However, as some citizens reported, bats have been found overwintering in this region in human structures including utility corridors, attics, aircraft hangers, and school buildings. For example, bats were observed inside a school building in Wainwright where winter

temperatures are too low for bats to survive without a supplemental heat source. Hibernating in buildings where temperatures are warmer introduces the possibility that prey items, such as spiders, are available to these bats throughout the winter. Little brown bats hibernating in interior Alaska buildings tend to occur in small groups. Because they are hibernating in small numbers the risk to the greater population due to buildings losing their heat source or being demolished is lower than if they hibernated in large numbers (Whitaker and Gummer, 1992).

The persistence of bats in interior Alaska may be related to consistent availability of human structures for overwintering population that are within a potentially short range from summer roosts (<100km). Low external temperatures and low densities of bats in hibernacula may also reduce the prevalence of infectious diseases. For example, the fungus WNS tends to occur where bats hibernate in large (>50) groups and its optimal temperature for growth is 12°C (Verant et al., 2012).

The window for foraging in summer probably limits the range of this population, which is further affected by changes in rainfall, minimum air temperature and the phenology of insect abundance. Little brown bats in interior and northern Alaska face mountain barriers to a coastal migration. Although one of the colonies we studied has persisted for 30 years, expanding segments of the population may be vulnerable to delays in spring food availability and early onset of winter.

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Table 3.1. Morphological measures of *M.lucifugus* in interior Alaska at Harding Lake Moose Creek.

Parameter	n	Minimum	Maximum	$\bar{X}$	SD
Body Mass (g)					
pregnant	3	9.78	11.14	10.52	0.69
juvenile	16	5.90	8.85	7.54	0.94
lactating adult	53	6.45	9.71	8.21	0.76
Estimated Fat Mass (g)					
lactating adults	53	0.86	2.71	1.86	0.43
juveniles	16	0.54	2.21	1.47	0.53
Forearm Length (mm)					
adults	58	35	41	38.02	1.14
juvenile	16	34	40	37.31	1.74
Ear Length (mm)					
adults	47	10	15	12.11	1.06
juveniles	11	10	13	11.18	0.98
Tragus length (mm)					
adults	47	5	8	5.70	0.83
juveniles	11	4	6	5.09	0.83

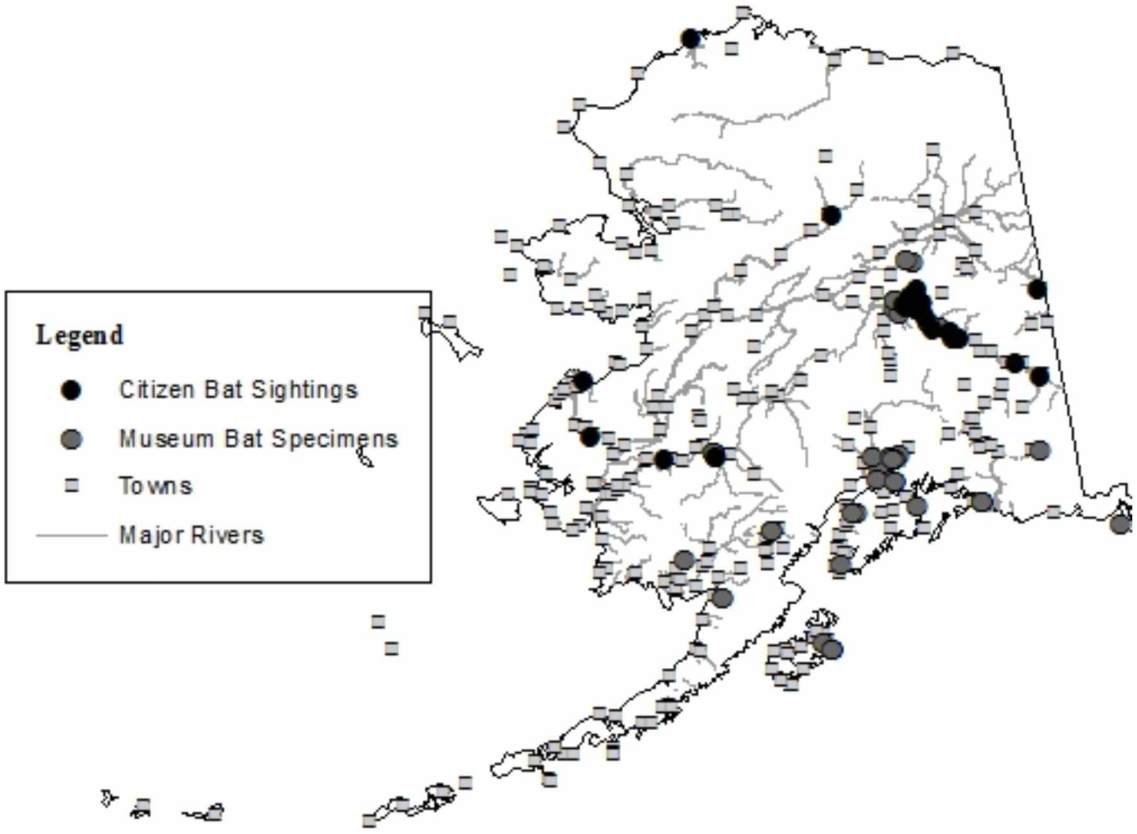
Table 3.2. Bat sightings reported by citizen scientists

Location	Latitude	Longitude	Season	Notes
Tok, Alaska	63.3366667	-142.9855556	Summer	
Kotlik, Alaska	63.0341667	-163.5544443	Summer	
Northway, Alaska	62.9616667	-141.9372222	Summer	Maternity
Eagle, Alaska	64.7880556	-141.2	Summer/ Winter	
Wainwright, Alaska	70.647222	-160.016111	Fall	
Eielson AFB	64.63211545	-147.0684814	Winter	
Salcha, AK	64.54017653	-146.986083	Summer/ Fall	Maternity
Harding Lake	64.312539	-146.6619873	Summer	Maternity
Quartz Lake	64.20398689	-145.8242798	Summer	
Rika's Rd	64.15374189	-145.827026	Winter	
Delta Junction	64.037351	-145.7226563	Fall	
Delta Clearwater	64.05297838	-145.4356384	Summer	No human structure
Chena Point	64.805681	-147.9401401	Fall	Swarms
CHSR	64.892093	-147.42279	Summer	
Upper Chatanika Campground	65.19225085	-147.255249	Summer	No human structure
Grapefruit Rocks	65.06536437	-147.6640606	Fall	No human structure

Table 3.2 continued

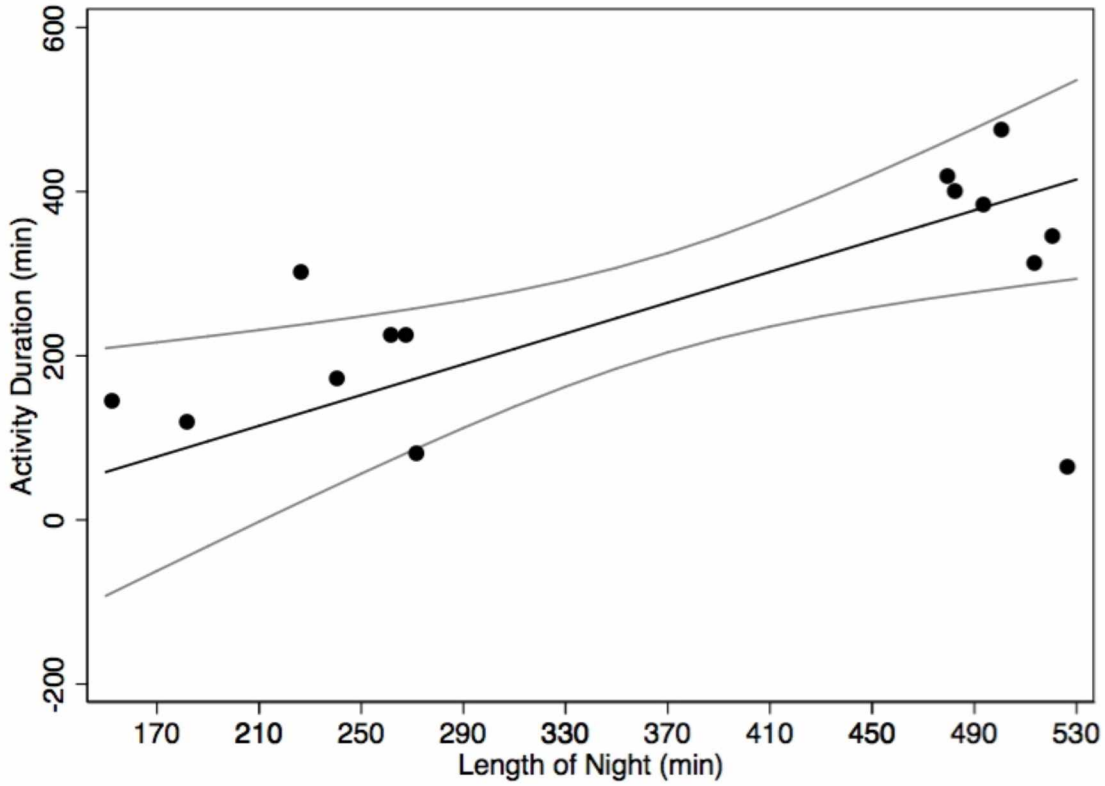
Fishing hole near Pilot Station	61.882048	-162.960205	Summer	No human structure
Peat Yard, College Rd, Fairbanks	64.841855	-147.8155517	Summer	
Dale Rd, Fairbanks	64.8238073	-147.8695443	Summer/ Fall	
Sleetmute, AK	61.7025	-157.1697222	Summer	
Aniak, AK	61.5828109	-159.5407139	Summer	
Fort Wainwright	64.8277871	-147.642915	Summer/ Fall	Swarms
Moose Creek	64.7099999	-147.14361	Summer	
Eielson Farm Rd	64.6901972	-147.208546	Summer	Maternity
Pearl Dr, Fairbanks	64.8742549	-147.1574158	Summer	
Badger Rd, North Pole	64.8136088	-147.4359306	Summer	Maternity
KJNP, North Pole	64.8788888	-148.052778	Fall	
Bettles, AK	66.9188889	-151.516111	Fall	

Figure 3.1



Map of bat sightings reported through citizen science/TEK outreach (black circle), and from collection sites of museum specimens (gray circle) with towns (white squares), and major rivers (gray lines) in Alaska.

Figure 3.2



Duration of bat activity (min) plotted against the length of night (min) from sunset to sunrise (solid circles). The solid line indicates the predicted relationship between activity duration and length of night (min) with daily maximum temperature as a covariate (range of temps used). Gray lines indicate the 95% confidence interval for the regression. ($Y = 0.53(\pm 0.21)\ X + 64.5(\pm 84)$; $R^2 = 0.34$).

Appendix 3.1

Bats and Alaska Questionnaire

The purpose of this study is to learn more about bats in interior Alaska and to test citizen science as another method of science education beyond the classroom. All responses are anonymous. You are free to choose not to participate at any time. Questions or comments may be directed at any time to Rachel Shively, PO Box 750347, Fairbanks, AK 99775 email: rdshively@alaska.edu. Concerns may also be sent to the University of Alaska Fairbanks Office of Research Integrity, Suite 212 WRRB, PO Box 757270, Fairbanks, AK 99775 email: fycomp@uaf.edu . This contact information will be left with you on a card. By completing this questionnaire I agree to participate in the study.

Please circle your selection on the color scale:

1. There are bats in Alaska.

   Agree Disagree

   ☐ ☐ ☐ ☐ ☐

2. Bats go south for the winter.

   Agree Disagree

   ☐ ☐ ☐ ☐ ☐

3. How often do scientific theories change?

Very often Never

☐ ☐ ☐ ☐ ☐

4. Scientists should share their data with the public.

Agree Disagree

☐ ☐ ☐ ☐ ☐

5. Doing a scientific experiment is difficult.

Agree Disagree

☐ ☐ ☐ ☐ ☐

6. Bats are related to mice.

Agree Disagree

☐ ☐ ☐ ☐ ☐

7. Bats are blind.

Agree Disagree

☐ ☐ ☐ ☐ ☐

8. The results of a scientific experiment will be the same each time it is repeated.

Agree Disagree

☐ ☐ ☐ ☐ ☐

9. Science can be applied to everyday life.

Agree Disagree

☐ ☐ ☐ ☐ ☐

10. I have heard of citizen science.

Agree Disagree

☐ ☐ ☐ ☐ ☐

Bats and Alaska Interview Question

The purpose of this study is to learn more about the habitat use of bats in interior Alaska. Participating in this study will provide an opportunity to share your knowledge of bats with others. All responses are anonymous and will be recorded by pen and paper. You are free to choose not to participate at any time. Questions or comments may be directed at any time to Rachel Shively, PO Box 750347, Fairbanks, AK 99775 email: rdshively@alaska.edu. Concerns may also be sent to the University of Alaska Fairbanks Office of Research Integrity, Suite 212 WRRB, PO Box 757270, Fairbanks, AK 99775 email: fyirb@uaf.edu. This contact information will be left with you on a card.

What memories do you have of seeing bats or hearing about bats being present in this area in the past?

Chapter 4: Conclusion

Little brown bats are persisting at the limits of their range in northern Alaska. We hypothesized that the little brown bat in interior Alaska is altering its feeding strategy to include a wider variety of prey than southern conspecifics. We analyzed feces collected each week from a maternity roost and compared our estimates of diet composition among four methods: microhistology, DNA sequence analysis, stable isotopes and image recognition of prey parts. We also hypothesized that the little brown bat in Alaska is expanding their range by utilizing human structures as an effective buffer against extremely cold temperatures resulting in inhospitable conditions for natural roosts. We tested this hypothesis using a combination of citizen science, acoustic surveys, captures, and radio telemetry.

Diet Analysis

In Chapter 2 we analyzed guano samples collected at maternity roosts in the Fairbanks area to examine changes in diet throughout the active season. Prey items included aerial insects as well as terrestrial arthropods. Diptera (flies) and Lepidoptera (moths) were the most common prey items (present in 80% and 76% of samples, respectively) followed by Araneae (spiders; present in 33% of samples). Shifts in prey consumption were linked to Julian day. The presence of spiders in the guano increased through the season, while the presence of moths decreased through the season. The northern little brown bats had a more diverse foraging strategy than their southern conspecifics, which feed mainly on flying arthropods (Moosman et al., 2012). The large contribution of spiders to the diet of northern little brown bats suggests a change in foraging behavior that may be associated with cooler temperatures.

We used isotopic markers (∂^{15}N. ∂^{13}C, and ∂^{34}S) in both guano and hair to indicate diet, and compared stable isotope values from sites in interior Alaska to those from coastal Alaska and

the Yukon. Isotopic values of hair, which reflect the diet over the entire molt period, were significantly different between interior Alaska and the other sites in coastal Alaska and the Yukon for $\delta^{15}N$ and $\delta^{13}C$. Values for $\partial^{15}N$ vary with trophic level, with an increase of approximately 3‰ for each trophic level. Values for $\partial^{15}N$ are also more enriched in marine tissues whereas values for $\partial^{13}C$ vary with vegetation and can be more enriched in terrestrial food chains than those from freshwater. Differences between the interior Alaska population and the Yukon and coastal populations are most likely a combination of diet and proximity to the marine environment. Significant outliers for $\delta^{15}N$ in hair included 13 of 77 observations from interior Alaska that were 2 to 5 ‰ from the nearest value. This pattern of isotopic variation suggests that while the population has a generalist feeding strategy, individuals with distinctly different isotopic signatures could be specialists that prey on carnivorous arthropods such as spiders. Alternatively, outliers may also indicate a small number of individuals that could have dispersed to interior Alaska from Yukon or coastal Alaskan populations.

Fecal microhistology is currently the most reliable quantitative method for diet analysis of a generalist carnivore. Image analysis software has potential in this area, but is currently unable to reliably distinguish fragment edges. Qualitative methods of diet analysis through fecal samples include DNA analysis and stable isotopes. DNA analysis was helpful in identifying some prey items at a finer scale including the detection of spiders to the level of family. Stable isotopes offer the possibility of developing a mixing model but prey items must be isotopically distinct. We were not able to distinguish consumption of Areanae from Diptera because the flies include a wide variety of trophic levels that overlap with the predominantly carnivorous spiders. Fecal microhistology may be the most effective tool to continue monitoring diet in these bats, which could be supplemented with DNA analysis if prey diversity is an important metric.

Habitat

In Chapter 3 we assessed the geographic distribution of the little brown bat in Alaska with a combination of traditional ecological knowledge, habitat surveys, captures and telemetry. We compared known locations to a map of habitat features and compared the body condition of Alaskan bats in fall with published reports on southern conspecifics.

Knowledge of obscure species in ranges with small and dispersed human populations usually relies on historical reports and traditional knowledge. Traditional ecological knowledge is useful for developing a baseline in cases where there is a lack of historical data (Huntington, 2000). We visited 8 communities to present information on bats to ~ 400 people and interviewed 60 community members. Community members reported bat sightings as far north as Wainwright and most of these sightings were near or within human structures (i.e. barns, schools, aircraft hangars).

Winter survival times of bats are directly related to metabolic rate in torpor, ambient temperature and the store of fat (Burles et al., 2014; Thomas et al., 1990). We captured 68 individuals from two roosts over 3 years. We estimated that adult bats at the maternity roosts before fall dispersal had a mean of 21% body fat by mass, which is only slightly lower than reports for bats at southern swarming sites, when bats have already gained mass for winter. Bats at high latitudes may put on more mass to survive low winter temperatures, as do populations of songbirds that overwinter in Alaska (Sharbaugh, 2001).

The little brown bat in interior Alaska was not observed migrating long distances (>200 km) to hibernacula. We tagged six bats in August 2014 and tracked five of those frequencies. Two frequencies were located within 1 km of the roost at Harding Lake where the animals were tagged. The remaining three frequencies were located 70 – 81 km south of the roost along the

Delta River. The persistence of bats in interior Alaska may be related to consistent availability of human structures that are within the apparently short range of migration (<100km), which is limited by mountain ranges on each side of the Yukon drainage.

Implications

The little brown bat fills a broader dietary niche in the northern limits of its range. The population has adapted to the conditions in the north to be able to survive over a large range of northern Alaska. Because of this adaptability, the species may be better able to adjust to predicted shifts in climate at high latitudes. The persistence of bats in interior Alaska may be related to consistent availability of human structures that are within the apparently short range of migration (<100km). While natural hibernacula may be likely as far north as Whitehorse, Yukon, where annual mean temperatures are 0°C and bats are observed in the spring as early as late April, the cooler temperatures (-10 to -3°C annual mean temperatures) in interior and northern Alaska where the bats are not observed in the spring before late May is not likely to have suitable natural wintering sites without supplemental heating, either human sourced or geothermal. Low external temperatures and low densities of bats in hibernacula may also reduce the prevalence of infectious diseases such as WNS where bats hibernate in large (>50) groups.

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Appendix 4.1 Acknowledgments

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All of my committee members provided valuable guidance along the way and helped me through several drafts of papers. I could not have done this without the support of my advisor, Perry Barboza. He not only kept me employed throughout the process, he tolerated my sleep-deprived state during field season and always knew when I needed encouragement the most. He was kind enough to take me on as a student in spite of my interest in the smaller, non-ungulate, flying mammals. There's no accounting for taste.

Finally, a huge thank you to my family and friends. My parents raised me with an appreciation of nature and science, and even encouraged me when I wanted a bat box to attract bats to our yard at the age of 8. They helped me discover the path I wanted to take at a young age. I couldn't have completed this project without the support of everyone involved.

Appendix 4.2 Institutional Animal Care and Use Committee letter of approval

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Institutional Animal Care and Use Committee

909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

July 16, 2012

To:	Perry Barboza Principal Investigator
From:	University of Alaska Fairbanks IACUC
Re:	[341381-2] Diet and habitat for bats in interior Alaska

The IACUC reviewed and approved the Protocol Revision referenced above by Designated Member Review.

Received:	July 12, 2012
Approval Date:	July 13, 2012
Initial Approval Date:	July 13, 2012
Expiration Date:	July 13, 2013

This action is included on the July 26, 2012 IACUC Agenda.

The PI is responsible for acquiring and maintaining all necessary permits and permissions prior to beginning work on this protocol. Failure to obtain or maintain valid permits is considered a violation of an IACUC protocol, and could result in revocation of IACUC approval.

The PI is responsible for ensuring animal research personnel are aware of the reporting procedures on the following page.

Appendix 4.3 Institutional Review Board letter of approval

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Institutional Review Board

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September 26, 2012

To:	Perry Barboza Principal Investigator
From:	University of Alaska Fairbanks IRB
Re:	[373251-1] Bat habitat and diet in interior Alaska citizen science

Thank you for submitting the New Protocol referenced below. The submission was handled by Expedited Review under the requirements of 45 CFR 46.110, which identifies the categories of research eligible for expedited review.

Title:	Bat habitat and diet in interior Alaska citizen science
Received:	August 30, 2012
Expedited Category:	7
Action:	APPROVED
Effective Date:	September 26, 2012
Expiration Date:	September 26, 2013

This action is included on the October 4, 2012 IRB Agenda.

No changes may be made to this project without the prior review and approval of the IRB. This includes, but is not limited to, changes in research scope, research tools, consent documents, personnel, or record storage location.