Canadian Journal of Zoology
Revue canadienne de zoologie

Increased diet breadth of little brown bats (*Myotis lucifugus*) at their northern range limit: a multi-method approach

Journal:	<i>Canadian Journal of Zoology</i>
Manuscript ID	cjz-2017-0017.R2
Manuscript Type:	Article
Date Submitted by the Author:	02-Jun-2017
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Keyword:	Alaska, diet analysis, FORAGING < Discipline, fecal DNA, microhistology, bat guano, little brown bat

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1 **Increased diet breadth of little brown bats (*Myotis lucifugus*) at their northern range**
2 **limit: a multi-method approach**

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Increased diet breadth of little brown bats (*Myotis lucifugus*) at their northern range limit: a multi-method approach

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The distribution of small mammals is constrained by extreme environmental demands and variable food supplies that are commonly incurred at northern latitudes. Little brown bats (*Myotis lucifugus*, Le Conte, 1831) are at the northwestern limits of their range in Alaska, where environmental demands are higher and prey availability is more seasonal than elsewhere in their range. We hypothesized that the little brown bat in interior Alaska has adjusted to these constraints by broadening its foraging niche, relative to that of southern conspecifics. We analyzed arthropod fragments (microhistology) in guano to describe prey composition to Order. We compared the efficacy of evaluating diet by microhistology with DNA analysis and stable isotope analysis on guano and hair. Bats consumed aerial prey such as Lepidoptera (moths) and Diptera (flies and mosquitoes) as well as terrestrial arthropods including Araneae (spiders). Shifts in the proportion of aerial prey in the diet were closely linked to ordinal day. Values for $\delta^{15}\text{N}$ in hair indicated that bats were generalists in interior Alaska, coastal Alaska and Yukon but significant outliers indicated that some individuals have distinct diets. The little brown bat's flexibility in feeding strategies likely allows this species to sustain populations in arctic and subarctic regions.

Alaska, arthropods, diet analysis, foraging, bat guano, fecal DNA, generalists, little brown bat, microhistology, *Myotis lucifugus*, niche

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INTRODUCTION

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Generalist predators present a challenge for dietary analysis because they consume a variety of species that often includes multiple trophic levels. Small predators such as birds and bats may seasonally consume a wide diversity of invertebrates with a rich variety of life histories and environmental responses. Generalists may be better suited to range expansion and colonization of vacant habitats 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). This pattern has been observed in penguins of the Antarctic Peninsula, where the population of the generalist gentoo penguins (*Pygoscelis papua*, J.R. Forster, 1781) is expanding while the chinstrap penguin (*Pygoscelis antarcticus*, J.R. Forster, 1781) population which specializes on krill is declining (Polito et al. 2015). The ability of generalist predators to incorporate new dietary items as their range changes or expands may be an important factor in predicting extinction and colonization rates as habitats may shift rapidly with projected changes in climate (Boyles and Storm 2007).

Insectivorous bats are sensitive to air temperatures that affect both the energetic costs to the animal and availability of their prey (Moosman et al. 2012). Nocturnal foraging by many species of bats decreases predation risks and also reduces competition with diurnal insectivorous birds (Lima and O’Keefe 2013). Dietary studies from temperate regions suggests that little brown bats feed mainly on flying insects, and rarely glean prey from foliage (Belwood and Fenton 1976; Ratcliffe and Dawson 2003; Feldhamer et al. 2009; Clare et al. 2014). 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 (Kunz et al. 1998; Dzal and Brigham 2013). High-energy demands for little brown bats in northern latitudes may influence prey selection, but this theory has not yet been examined. Notably, northern populations of little brown bats may be expected to fill a wider niche than in southern regions where prey consists mostly of flying insects. Little brown bats are the only species present in interior Alaska (Parker et al. 1997) but >3 species of bats are present in coastal southeastern Alaska and Yukon (Slough et al. 2014). In southern regions, the gleaning niche is filled by congeners, such as the northern long-eared bat (*Myotis septentrionalis*, Trouessart, 1897) and the long-eared myotis (*Myotis evotis*, H. Allen, 1864) (Barclay 1991; Lausen et al. 2009). At high latitudes in northwestern North America, little brown bats may alter foraging to contend with prolonged day length and cool temperatures while possibly taking advantage of reduced competition from other species of bats (Whitaker and Lawhead 1992; Talerico 2008).

The little brown bat also is an ideal species for evaluating methods of diet analysis because it preys on several orders of arthropods. Microhistology of indigestible prey fragments in guano has served as a time-tested method for documenting the diet of insectivorous bats (Kunz and Whitaker 1983; Whitaker 2009). However, analysis of indigestible fragments may not detect soft-bodied prey, such as mayflies (Rabinowitz and Tuttle 1982), and it is labor intensive. In contrast to using microhistology to examine foraging, stable isotope analysis of guano or tissues, such as hair, can also be used to estimate dietary composition of bats when prey are isotopically distinct (Painter et al.

2009; Salvarina et al. 2013). Guano reflects prey consumed on the last foraging bout, as most indigestible fragments are voided within 45 minutes of consumption in insectivorous bats not entering torpor (Neuweiler 2000). Hair isotopes reflect diet over the period of molt, which in maternity colonies occurs after parturition and lactation but before fall migration (Sullivan et al. 2011). Amplification of DNA sequences in guano can also be used to identify a wide variety of prey in the diet (Whitaker 2009; Claire et al. 2014), but this method is more prone to identify the prey from lower trophic levels (e.g., insects consumed by predatory arthropods), than either stable isotopes or microhistology, which include degrees of quantification not possible in molecular analysis. Because of the lack of quantification, this identification of secondary predation could place an exaggerated weight on what the bat was consuming directly, and be misleading when looking at hawking vs gleaning behaviors. No known studies have compared the results of all 3 techniques.

We hypothesized that the population of little brown bats at a high latitude in interior Alaska are generalist predators that consume a variety of taxa, requiring flexibility in foraging strategies (i.e., aerial hawking and gleaning). We tested this hypothesis by examining a time series of guano samples to identify prey consumed through the active season from late spring through early fall. We used multiple methods, including microhistology, stable isotope analysis ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$), and DNA analysis to assess diet. 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 nearby Yukon, Canada. 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 predicted that interior Alaskan populations of little brown bats would have a broader diet than southern conspecifics, including Yukon and coastal Alaska, because flying insects might be scarce during cool nights, especially in the spring and fall.

MATERIALS AND METHODS

Guano sample collection

To examine changes in diet through the active season in interior Alaska (late spring to early fall), we collected guano samples at two little brown bat maternity roosts near Fairbanks, Alaska, U.S.A. (Fig 1). One roost was in a cabin on Harding Lake (64.434°; -146.884°; elevation 218 m) and the other was 45 km away in a barn on Moose Creek (64.646°; -147.143°; elevation 159.1 m). Both roosts were within the boreal forest and ≤ 100 m from water. The Harding Lake roost was on the shore of a 887.2 ha lake whereas the Moose Creek roost was adjacent to agricultural fields. Little brown bats have been sighted at the Harding Lake location for >30 years and at the Moose Creek location for >10 years. We secured clean plastic sheeting under 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 ($n = 12$ sampling periods 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 for comparison on an *ad hoc* basis during June and July, using the same method as above, from 5 little brown bat maternity colonies in bat houses that were ≤ 170 km from Whitehorse, Yukon, Canada (60.717°; -135.050°; elevation 670-1702 m). All samples were frozen and stored at -20°C in polyethylene bags until analysis.

136 *Hair sample collection*

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

142 We collected hair samples from live captured bats at the aforementioned colonies
143 in interior Alaska and southern Yukon, as well as from specimens in the collection of the
144 University of Alaska’s Museum of the North (Table S1). Museum specimens were
145 collected in the coastal zone of southeastern Alaska ($n=6$; Sitka, Alaska; elevation 8 m)
146 and Fairbanks ($n=1$). We captured little brown bats at the maternity roosts using
147 homemade harp traps (Tuttle 1974) and trimmed hair samples from the scapular region
148 following the American Museum of Natural History’s protocol (American Museum of
149 Natural History 2012). Hair samples were collected into cryovials and frozen for storage.

150 *Environmental Data*

151 We placed HOBO data loggers (Onset Computer Corporation, Cape Cod, MA)
152 outside of the entrance to the Moose Creek roost site to record temperature every 15
153 minutes for measures of daily minimum and maximum ambient air temperature. Local
154 monthly weather data were recorded at Eielson Air Force Base and hourly precipitation
155 data were recorded by the National Climatic Data Center in Fairbanks (NOAA). Weather
156 data were summarized by calendar month and by the period between the guano
157 collections (typically 2 weeks, but adjusted to exact dates of collection).

158 *Microhistology*

To identify consumed prey, we examined 3 guano pellets using microhistology from each of the two Alaskan little brown bat maternity roosts and for each collection date. 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 references on analysis of bat guano and a reference collection of arthropods (Lehmkuhl 1979; McAney et al. 1997; Collet 2008; Whitaker 2009; McGavin 2011). We used white cloth in a hoop to collect the reference aerial prey and picked spiders from webs in vegetation next to the maternity roosts sampled near Fairbanks, Alaska, and Whitehorse, Yukon, during summer 2013.

In the guano, Araneae were typically identifiable by their legs, Lepidoptera by wing scales, and Diptera by wing fragment. 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 correlated with 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 softening in isopropyl alcohol for thorough examination.

DNA analysis

Guano collected from Alaska was analyzed for the presence of DNA from arthropod prey at a commercial lab (Jonah Ventures LLC, Manhattan, KS).

Molecular Analyses:

DNA was extracted from swabs using the MoBio PowerSoils htp protocol. A fragment of the Folmer region of the Co1 gene was amplified using arthropod-specific

primers (Bohnmann et al. 2011; Zeale et al. 2011). Primers were modified for multiplex sequencing on the MiSeq platform, including illumina adapters, and unique error-correcting 12-basepair barcodes on the reverse primer. The 25 ul PCR cocktail included 3 ul gDNA, 12.5 ul Promega Master Mix, 1 uM each of the forward and reverse primers. Cycling parameters were modified from Pinot et al. (2011) (94 C for five min, followed by 45 cycles of 94 C 30 s, 45 C 45 s, 72 C 45 s and a final extension at 72 C for 10 min). Duplicate PCRs were cleaned and normalized using SequalPrep Normalization Plates before pooling and sequencing on a Miseq platform.

Sequence Processing:

Sequences were demultiplexed using ‘prep_fastq_for_uparse.py’ (<https://github.com/leffj/helper-code-for-uparse>). Read 2s were used for downstream analysis due to higher quality scores. The UPARSE pipeline (USEARCH 7) was used to filter sequences and select OTU (operational taxonomic units). Quality filtering included trimming sequences to the expected amplicon length (158 bp – only for 250 bp length reads), filtering by quality score (maxee value of 1.5), removing sequences below the minimum expected amplicon length (90 bp), and removing singletons. Sequences were clustered de novo at 99% similarity for selection of OTU. Taxonomic assignments were performed in QIIME, using the hierarchical naïve Bayesian classifier RDP, retrained with a custom reference database curated from the Barcode of Life Database (v3). Taxonomy was assigned at 99% similarity, with a 50% confidence threshold. Sequences were then further filtered to remove non-arthropod sequences and those sequences that were not resolved to at least the family level. Methods were provided by Joseph Craine and Noah

Feier (personal communications). To reduce potential issues with the high OTU threshold over-estimating diversity, this study only includes the results to the family level.

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. This was accomplished using 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 University of Alaska Fairbanks. Results were reported in delta notation and expressed in parts per thousand, relative to internationally accepted standards ($\delta = [(\text{isotope ratio sample/isotope ratio standard}) - 1] * 1000$) (Fry 2006; Gustine et al. 2014).

Statistical analysis

Shannon's Diversity Index (Magurran 1998) was used with the microhistology results to estimate 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 the independent covariates: minimum and maximum temperatures for period, and ordinal day. We used linear regression to examine the relationship between

We used one-way analysis of variance (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. For the guano samples from the Yukon, 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) in STATA 14.0 (StataCorp, College Station, TX) to detect outliers in the stable isotope values of hair.

Microhistology

Shannon's diversity index of prey items in the guano was related to minimum
daily temperature ($R^2 = 0.17$, $F_{1,20} = 2.94$, $P = 0.10$; Fig. 2). The results for maximum
daily temperature were similar ($R^2 = 0.14$, $F_{1,20} = 5.19$, $P = 0.03$; Fig 2). Other models

considered were ordinal day and maximum daily temperature ($R^2=0.17$, $F_{2,19} = 2.51$, $P = 0.11$), ordinal day, maximum and minimum daily temperatures ($R^2=0.25$, $F_{3,18} = 1.71$, $P = 0.20$), ordinal day and minimum daily temperature ($R^2=0.18$, $F_{2,19} = 1.69$, $P = 0.21$), and maximum and minimum daily temperatures ($R^2=0.24$, $F_{2,19} = 2.65$, $P = 0.10$).

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 $0.00 \text{ cm}\cdot\text{month}^{-1}$ for May 2013 to $5.89 \text{ cm}\cdot\text{month}^{-1}$ for July 2012 (Table 1). Shapiro Wilk test results indicated normal distribution in Lepidoptera ($P = 0.9$) while Araneae were not normally distributed ($P < 0.1$). We used an arcsine transformation on the Araneae data. Neither variation in the percentage of Araneae nor flying insects in the guano was significantly related to temperature or year and only ordinal day was included in the best-fit model. The transformed percent volume of Araneae in the guano increased through the season ($R^2 = 0.34$, $F_{1,21} = 9.64$, $P = 0.005$), while the percent volume of Lepidoptera decreased through the season ($R^2 = 0.30$, $F_{1,21} = 8.20$, $P = 0.01$; Fig. 3). For visual consistency, we have included the untransformed percent volume of Araneae through the season in the figures ($R^2 = 0.30$, $F_{1,21} = 5.71$, $P = 0.03$). There was no significant seasonal trend in percent volume of Diptera.

Stable Isotopes

Values for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of guano previously examined using microhistology did not vary with the proportion of different prey types in the sample in interior Alaska [Average percent volume Araneae: $\delta^{15}\text{N}$ ($R^2 = 0.03$, $F_{1,24} = 4.48$, $P = 0.04$) and $\delta^{13}\text{C}$ ($R^2 = 0.06$, $F_{1,24} = 3.14$, $P = 0.09$); Average percent volume Lepidoptera: $\delta^{15}\text{N}$ ($R^2 = 0.01$, $F_{1,24} = 0.24$, $P = 0.63$) and $\delta^{13}\text{C}$ ($R^2 = 0.00$, $F_{1,24} = 0.10$, $P = 0.75$)]. Pellets previously

dissected and identified as more than 50% Araneae ($n = 3$) had $\delta^{15}\text{N}$ values of -1.66 to 2.81 and $\delta^{13}\text{C}$ of -30.36 to -27.76, which overlapped values for $\delta^{15}\text{N}$ (0.61 to 1.83) and $\delta^{13}\text{C}$ (-31.53 to -29.46) in samples with more than 50% Diptera ($n = 3$). However, among samples from the Yukon, pellets containing Araneae, Diptera, and bat hair had the highest $\delta^{15}\text{N}$ at 4.74 ($n = 2$), while pellets containing Diptera and Lepidoptera had the lowest $\delta^{15}\text{N}$ at 2.61 ($n = 12$; Table 2). In a pairwise comparison of marginal linear predictions, the only significantly different isotopic signatures were Yukon pellets containing bat hair compared to those containing Diptera and Lepidoptera ($t = 2.52$, $P = 0.014$). Isotopic values of hair were significantly different between interior Alaska and the other sites in coastal Alaska and the Yukon for $\delta^{15}\text{N}$ ($F_{2,74} = 21.27$, $P = 0.000$) and $\delta^{13}\text{C}$ ($F_{2,74} = 13.77$, $P = 0.0001$; Fig 4). Significant outliers for $\delta^{15}\text{N}$ in hair from interior Alaska included 13 of 77 (16.9%) observations that were 2–5 units from the nearest value. Invertebrates captured ($n = 33$) and identified as potential prey items in the Fairbanks and Whitehorse areas had a wider range of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values than the residues from guano. Diptera ($n = 12$) had the widest range of $\delta^{15}\text{N}$ values from 1.48–12.84 that overlapped the range for Araneae ($n = 7$; range = 3.57–8.07).

DNA Analysis

DNA analysis detected more invertebrate orders than microhistology (11 vs. 9) (Table S2) and was better able to detect soft-bodied invertebrates. For example, DNA analysis detected mayflies (Ephemeroptera) in 2 of 26 samples and microhistology did not detect mayflies in any samples from the same dates. Detection rates for lacewings (Neuroptera) were 15 of 26 for DNA analysis and 4 of 26 for microhistology. Within orders that were identified using microhistology, DNA analysis was able to further

identify prey items to family, e.g. within the Order Araneae DNA analysis detected the families Araneidae, Linphiidae, Philodromidae, and Theridiidae.

DISCUSSION

Using multiple methods to determine dietary niche breadth of little brown bats at the northern edge of their range, we found that little brown bats at high latitudes in northwestern North America had a more diverse foraging strategy than conspecifics at more southern latitudes. 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 and also the timing of emergence. This is consistent with the theory that niche breadth increases with decreasing available resources (Pianka 1981). Others have reported Araneae in the diet of little brown bats from colonies at high latitudes in Alaska (Whitaker and Lawhead 1992) and the Yukon (Talerico 2008); however, Araneae have only occasionally been found in the guano of little brown bats at more southern latitudes, where Lepidoptera and Diptera are the main components of the diet (Belwood and Fenton 1976; Moosman et al. 2012; Clare et al. 2014). DNA analysis indicated that the majority of the spiders were orb-weavers (Araneidae), which supports the hypothesis that little brown bats are likely gleaning spiders from webs. Little brown bats are aerial hawkers over much of their range, but Ratcliffe and Dawson (2003) provided laboratory-based evidence that demonstrated this species is capable of gleaning prey in cluttered environments, similar to the northern long-eared bat, a species well adapted to gleaning as a foraging strategy. The large contribution (as high as 50% by microhistology on some sampled dates) of Araneae to the diet of little brown bats in

interior Alaska suggests that northern populations of this species have adapted their foraging behavior to exploit habitats with limited interspecific competition.

Individual bats (13 of 77; 16.9%) within the interior Alaskan 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 of little brown bats from Yukon ranged from 6.78 to 10.25‰, while those from coastal Alaska 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 specific foraging areas. Often, individuals that specialize within a generalist population are more efficient at a particular foraging method (Woo et al. 2008; Catry et al. 2014; Terraube et al. 2014) but distribution of prey may favor separation of foraging areas among individuals that share the same roost (Araujo et al. 2010). This spatial separation of individuals seems more likely than specialization in prey type because several prey items were always detected within each pellet. The suggestion that northern little brown bats spatially separate their foraging activities from a common roost would require repeated tracking of individuals during the summer. Populations of generalists with individual specialization may also be better able to colonize vacant or changing habitats by increasing intra-population diversity and genetic variation that favor adaptation to new environments (Bolnick et al. 2003).

Most of the little brown 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 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, consumption of Araneae may be higher in fall than in spring because early in the season other aerial prey are available, such as the mosquito species *Aedes communis* (De Greer, 1776), which emerges early in the spring from icy pools of water (Frohne 1954) and the moth species *Martyrhilda ciniflonella* (Hannemann, 1953) that overwinters as an adult (Miller 1982).

While the dietary analysis of generalist carnivores is challenging, understanding the ability of generalists to adapt their dietary niche breadth is an important factor in predicting the effect of habitat change. The dietary niche breadth of the little brown bat is greater at the northern limits of its range, compared to that at more southern latitudes. A broader dietary niche breadth among our sample of little brown bats may be because climatic conditions are harsher, aerial prey less predictably available, and there is no competition with other species of gleaning bats, in interior Alaska. Because of the dietary and foraging flexibility of little brown bats in interior Alaska, the species may be able to adjust to predicted shifts in climate at high latitudes.

ACKNOWLEDGMENTS

Bat Conservation International, Arctic Institute of North America, Yukon Department of Environment and Texas A&M University provided funding for this research. We thank field volunteers Brandon Elkins, Garrett Savory and Rachelle Ruffner. Piia Kukka assisted with sample collection in the Yukon. Nancy Hansen and

Dan Rees generously provided access to bat roosts on their properties. Dave Verbyla provided thoughtful comments on an earlier draft of this manuscript.

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491

Table 1. Mean daily temperature records from HOBO loggers located outside of the entrance to a little brown bat (*Myotis lucifugus*) maternity roosts in interior 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. Guano samples from little brown bats (*Myotis lucifugus*) from Yukon, Canada, categorized by the detected presence/absence of bat hair (molt), insect wings, and spider legs and the resulting average values for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in the washed residue.

Contents	N Pellets	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$
		Mean	Mean
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

Draft

1 Figure Legends

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3 Figure 1: Map of Alaska, USA and part of Yukon, Canada with interior Alaska roosts site
4 (black star), Whitehorse, Yukon (black square) and coastal Alaska (black diamond).

5

6 Figure 2: A. Linear regression of Shannon's Diversity Index of prey items in little brown
7 bat (*Myotis lucifugus*) guano from Moose Creek roost in interior Alaska, against the mean
8 maximum daily temperature during the preceding collection period (7-10 d) for Moose
9 Creek, Alaska [$n = 22$, $Y = 0.236 (\pm 0.104) X + 2.511 (\pm 2.848)$, $R^2 = 0.14$; $P = 0.03$]. B.

10 Linear regression of Shannon's Diversity Index of prey items in little brown bat (*Myotis*
11 *lucifugus*) guano from Moose Creek roost in interior Alaska, against the mean minimum
12 daily temperature during the preceding collection period (7-10 d) for Moose Creek,
13 Alaska [$n = 22$, $Y = 0.494 (\pm 0.288) X + 3.357 (\pm 3.240)$, $R^2 = 0.17$; $P = 0.10$].

14

15 Figure 3: Average percent volume by ordinal day with 95% confidence interval of
16 Lepidoptera (A) and Araneae (B) in little brown bat (*Myotis lucifugus*) guano from
17 Moose Creek in interior Alaska.

18

19 Figure 4: Isotopic values ($\bar{x} \pm SD$) of hair from little brown bats (*Myotis lucifugus*; $\delta^{15}N$,
20 $\delta^{13}C$) captured in interior Alaska, Yukon and coastal Alaska.

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23 Figure 1
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Figure 3

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