# FORAGING STRATEGIES AND MORPHOMETRIC CHARACTERISTICS OF BATS IN NORTH AND SOUTH DAKOTA

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**Title**

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

**MASTER OF SCIENCE**

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# ABSTRACT

Insectivorous bats are an important group for studying links between dietary flexibility and associated impacts on foraging and morphology of individuals from different populations. Since different habitats generally have unique insect communities, populations across a species range will likely be adapted for most effective foraging within their local environment. I aim to 1) investigate the diets and associated foraging strategies of the eleven bat species found in North and South Dakota and 2) examine the morphological characteristics of *Eptesicus fuscus* (Big brown bat), *Myotis lucifugus* (Little brown bat), and *Myotis septentrionalis* (Northern long-eared bat). I predicted that 1) interspecific variation in foraging strategies would exist due to morphological variation and 2) any intraspecific variation in foraging strategies would correlate with population-level differences found in morphological characteristics. Understanding population-level differences can provide managers with critical information, keying in on the protection of resources that are important to local bat populations.

# ACKNOWLEDGEMENTS

I would like to thank my advisor Dr. Erin Gillam and Mandy Guinn for their support, feedback, and invaluable guidance offered. This project would never have happened without them. I would also like to thank my committee members Dr. Matthew Smith and Dr. Jason Harmon, as well as Dr. Emily Biggane, Kevin Cortes, Nick Johnson, Rebecca Jones, and the Gillam Lab for contributions to the development and implementation of this project. Shout out to Berlin West, Kimberlee Blevins, and Gillian MedicineCloud for their assistants with lab work. A big thanks goes to Dr. Jesse Alston for providing South Dakota fecal samples and capture data. Thank you, Dr. Patrick Beauzay and Dr. Darrell Ross, for allowing me to access your knowledge of entomology. A special thanks to my support system of friends and family who kept me sane. Finally, thank you to NDSU Biological Sciences, United Tribes Technical College, and NDSU College of Science and Mathematics for support and access to facilities.

# DEDICATION

I would like to dedicate this to my grandfather, Carl “Gilmore” Karevold.

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| | |
|---|---|
| ANOVA | Analysis of Variance |
| BHNF | Black Hill National Forest |
| BLAST | Basic Local Alignment Search Tool |
| COI gene | Mitochondrial cytochrome c oxidase subunit I gene |
| COTO | *Corynorhinus townsendii* (Townsend's Big-eared bat) |
| LABO | *Lasiurus borealis* (Eastern Red bat) |
| LACI | *Aeorestes cinereus* (Hoary bat, formerly *Lasiurus cinereus*) |
| LANO | *Lasionycteris noctivagans* (Silver-haired bat) |
| EPFU | *Eptesicus fuscus* (Big Brown bat) |
| MYCI | *Myotis ciliolabrum* (Western Small-footed *myotis*) |
| MYEV | *Myotis evotis* (Long-eared *myotis*) |
| MYLU | *Myotis lucifugus* (Little Brown bat) |
| MYSE | *Myotis septentrionalis* (Northern Long-eared bat) |
| MYTH | *Myotis thysanodes* (Fringed *myotis*) |
| MYVO | *Myotis volans* (Long-legged *myotis*) |
| NCBI | National Center for Biotechnology Information |
| PCR | Polymerase Chain Reaction |
| TRNP | Theodore Roosevelt National Park |
| WNS | White-Nose Syndrome |

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# CHAPTER 1: DIETARY COMPOSITION OF BAT SPECIES FOUND IN NORTH AND SOUTH DAKOTA

## 1.1. Abstract

Insectivorous bats often occupy particularly large geographic ranges. It is likely that availability and diversity of food resources will differ across these ranges, potentially leading to spatial variation in dietary preferences and foraging behaviors. This chapter aims to investigate the diet and associated foraging strategies of the eleven species of bats found in North and South Dakota. Species displaying morphology best suited for gleaning did not fit my initial prediction, having dietary profiles primarily composed of volant orders. However, species displaying morphology best suited for aerial hawking had dietary profiles primarily composed of volant orders, as predicted. This dataset provides a well-rounded picture of dietary preferences and foraging strategies used by bat species native to the Dakotas. Furthermore, understanding population-level differences in dietary needs can provide managers with critical information, allowing them to key in on the protection of resources that are important to local bat populations.

## 1.2. Introduction

Bats make up roughly 20% of all mammalian species and are present on all continents, except Antarctica. Due to the sheer number of species and their wide geographic distribution, it is not surprising that there is extensive variation in foraging niches occupied by different bat species. Looking at diet alone (a major component of a species' foraging niche), variation across the order can range from nectarivory to insectivory to sanguinivory. Because this taxon occupies so many different foraging niches, typical morphological expression is highly varied across species, making them most effective at obtaining a desired resource. For example, the Lesser Long-nosed bat (*Leptonycteris yerbabuena),* which is a specialized nectarivore and pollinator, displays a long, narrow muzzle and a long brush-tipped tongue that allows them to detect and reach nectar produced by various cacti species (Cole and Wilson 2006).

The vast majority (70%) of bat species are insectivores, with 42 of the 45 species found in the United States being classified as insectivores. Within the classification “insectivore”, species can be further split into groups based on specialization for specific insects and/or foraging strategies used to capture prey. Two of the most common foraging strategies used by insectivorous bats are gleaning and aerial hawking. Individuals using a gleaning strategy fly near the ground, within dense vegetative clutter, and listen for low frequency rustling noises made by insects on vegetative surfaces. Bat species using a gleaning strategy have long, narrow ears that help with the detection of low-frequency sounds (Obrist et al. 1993). They also have short wings with low wing loads that allow for greater maneuverability in the vegetative clutter (Norberg and Rayner 1987; Solick and Barclay 2006). Alternatively, an aerial hawking strategy involves using echolocation to seek out volant prey in open, relatively uncluttered spaces. Once detected, a chase is initiated to capture the targeted prey. Bat species that are most effective at aerial hawking have shorter, wider ears and longer wings with a larger wing load compared to gleaning species (Norberg and Rayner 1987).

Though direct observations of foraging behavior would be ideal to confirm the primary strategies used by a species, the volant, nocturnal nature of bats generally makes this infeasible. Instead, fecal samples can be collected and examined to quantify diet, thereby giving insight into the methods used by bats to capture their prey (Hamilton 1933; Whitaker et al. 1977; Ober and Hayes 2008). Bats using a gleaning strategy generally have diets composed of non-volant prey types, whereas aerial hawkers do not, as there is likely infrequent contact with non-volant prey in the open spaces where they typically forage. Before the advancement of DNA-based techniques, dissection of fecal pellets under a microscope and identification of arthropod parts, known as the Whitaker method (Whitaker et al. 2009) was the main technique used to quantify bat diet. However, a notable drawback of this method is the relative difficulty for researchers without extensive backgrounds in entomology to identify arthropods at a taxonomic level lower than order. Further, soft bodied arthropods are often not identifiable using the Whitaker method (Whitaker 2009) as they are more easily digested in comparison to prey types that have much harder exoskeletons and wings (i.e. beetles), which pass through and out of the digestive tract with minimal degradation. Fortunately, with the advancement of DNA technology, fecal composition can be readily quantified by bat researchers at a much finer taxonomic level and does not require an extensive background in entomology.

In the Northern Great Plains of the United States, bats have been less studied compared to other areas of the country, thus there are still basic questions about their regional ecology that remain unanswered. In North and South Dakota, the 11 native bat species are all classified as either gleaning or aerial hawking insectivores. This classification is based on typical ear and wing morphology expressed by each species, which theoretically makes them more effective at catching specific types of insect prey (e.g. volant or non-volant). While dietary information has been collected on these 11 bat species from other regions, prior to this study, no data on dietary composition or its relation to foraging strategy had been collected from the bat populations of North and South Dakota. Understanding the diets of these local bat populations could help managers key in on specific habitat and conservation needs, aiding the development of an informed management plan.

An additional reason that information about the diets and foraging strategies of bats in the Dakotas is needed relates to the impacts of the fungal pathogen, *Pseudogymnoascus destructans,* that causes White-Nose Syndrome (WNS). Specifically, the collection of information about regional dietary composition and foraging strategies provides a picture of how bats were using and consuming resources prior to the arrival of WNS in the study area. Unfortunately, near the end of this study, WNS was confirmed as present in both North Dakota (2019; USGS 2021) and South Dakota (2018; USGS 2021). Significant declines of certain bat populations in the Dakotas are anticipated and could have major impacts on various ecosystems across both states, making this study very timely.

The aim of this chapter is to determine the dietary composition and primary foraging strategies used by each of the 11 bat species found in North and South Dakota. These bat species are predicted to forage on a variety of volant and non-volant prey types, with unique dietary profiles for each species. Specifically, I hypothesized that the insectivorous bats of North and South Dakota use foraging strategies that align with their typical morphological expressions. Foraging strategy will be assessed by calculating the percent of volant or non-volant prey types found in the diet. If these species adhere to the foraging strategies their morphological characteristics appear to make them most effective for, expected dietary composition for North and South Dakota bat species should be as follows: *Corynorhinus townsendii, Myotis evotis,* and *M. septentrionalis* dietary compositions will contain higher percentages of non-volant prey orders (e.g. Araneae), and *Aeorestes cinereus* (formerly *Lasiurus cinereus*)*, Eptesicus fuscus, Lasionycteris noctivagans, Lasiurus borealis, M. ciliolabrum, M. lucifugus, M. thysanodes* and *M. volans* dietary compositions will contain higher percentages of volant prey orders (e.g. Lepidoptera). It is important to mention that past research on species that have geographic ranges spanning most of North America have found that some species use foraging techniques that do not align with those deemed to be the most effective according to morphological expression

(Belwood and Fenton 1976; Fenton and Morris 1976). Hence, it is possible that I may find no support for my hypothesis indicating that bats adjust their foraging strategies based on the availability of prey, making them opportunistic generalists. For example, research on a Canadian population of *M. lucifugus*, living in the northern part of this species' range showed the use of a gleaning strategy more often than conspecifics in southern Canada, presumably due to reduced availability of volant prey at cooler temperatures (Kaupas and Barclay 2017). It is possible that North and South Dakota bat species could similarly exhibit the use of potentially less effective strategies due to various environmental stressors (i.e. reduced prey availability due to cooler temperatures).

## 1.3. Methods

### *1.3.1. Ethics Statement*

All procedures followed a protocol approved by the North Dakota State University Animal Care and Use Committee (Protocol Number: A18040). No animals were euthanized during this study.

### *1.3.2. Study Area*

North and South Dakota fall directly within the Northern Great Plains ecoregion consisting of high-latitude land primarily dominated by grassland habitat types (Samson et al. 2004). However, it is important to note capture sites in South Dakota were all located within the Black Hills National Forest (BHNF), near Custer, SD, which is considered to be a part of the Middle Rockies ecoregion that consists of forested hills and mountains (Bryce et al. 1998; Bailey 2016).

Figure 1.1 outlines the five study areas sampled during the summer field seasons of 2016 – 2019. These five study areas are classified as being within the following ecoregions (Bryce et al. 1998; Bailey 2016): 1) Eastern (Pink) consisting of 3 sample sites within the Lake Agassiz Plain ecoregion, 2) Central (Yellow) consisting of 12 sample sites within the Northwestern Great Plains ecoregion, 3) Western (Green) consisting of 13 sample sites within the Northwestern Great Plains ecoregion, 4) Northern (Blue) consisting of 2 sample sites within the Northern Glaciated Plains ecoregion, and 5) Southern (Red) consisting of 14 sample sites within the Middle Rockies ecoregion of South Dakota. Additional information related to specific site names and locations are provided in Appendix A (Table A1).

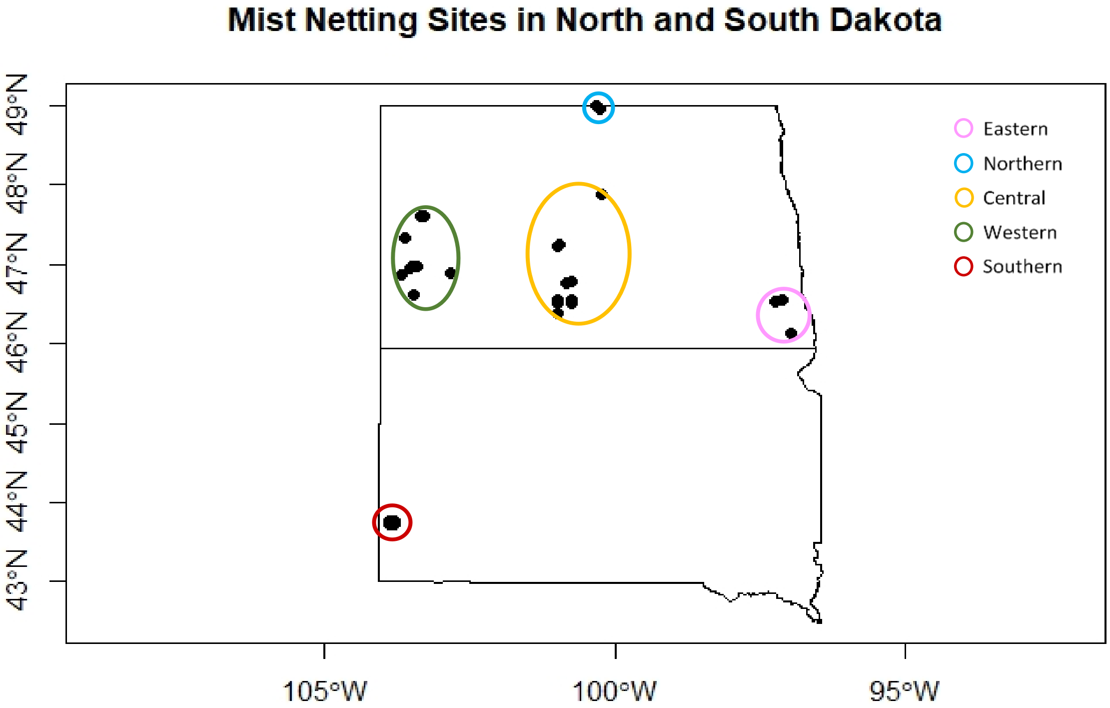

Figure 1.1: Mist netting sites used in North and South Dakota during the 2016-2019 field seasons.

### *1.3.3. Capture*

Mist nets and hand nets were the two methods used to capture bats. Mist nets were deployed along natural fly ways (e.g. trails, waterways, etc.). These nets are ideal for capture because they are difficult for bats to detect when placed among clutter (e.g. trees, buildings, etc.).

Bats that flew into mist nets would fall into a shelf, formed by the net, and remain until they were untangled and removed by the researcher and placed in a holding bag. Mist nets were checked every ten minutes to ensure the bats were removed quickly and without unnecessary strain. This is a standard method for capturing bats (Kunz and Parsons 2009). Mist nets used in one area were not used in another until they had been boiled and poles properly disinfected, as per USFWS White Nose Syndrome decontamination guidelines (USFWS 2011). This protocol was in place prior to the confirmation of WNS in either state.

Hand nets were used to capture bats found on the outside of buildings, under bridges, or any other manufactured structure on which a bat may rest in between foraging bouts. Capture required the researcher to place the hand net over a resting bat and hold it there until the bat attempted to fly away. The bat attempting to leave flies directly into the back of the net where it becomes stuck, at which point the researcher can lower the net and remove the bat. Similar methods have been used by researchers, but this method can be adapted to suit specific needs (Waldien et al. 1999).

### *1.3.4. Study Species*

Data was collected on the 11 species of bats native to North and South Dakota: *Aeorestes cinereus* (Hoary bat, formerly *Lasiurus cinereus*), *Corynorhinus townsendii* (Townsend's Big-eared bat), *Eptesicus fuscus* (Big brown bat), *Lasionycteris noctivagans* (Silver-haired bat), *Lasiurus borealis* (Eastern Red bat), *Myotis ciliolabrum* (Western Small-footed *myotis*), *M. evotis* (Long-eared *myotis*), *M. lucifugus* (Little brown bat), *M. septentrionalis* (Northern Long-eared *myotis*), *M. thysanodes* (Fringed *myotis*) and *M. volans* (Long-legged *myotis*).

### *1.3.5. Sample Collection*

North Dakota fecal samples were collected from captured individuals during the 2017-2019 field season (late May – late August/early September). Unused fecal samples and data collected by a previous graduate student during the 2016 North Dakota field season have also been included in the analysis. Captured individuals were temporarily held, no more than 3 hours, in cloth holding bags. Holding bags were checked every 30 minutes to look for any fecal droppings. Collected fecal samples were placed in a sterile 1.5 ml vial, labeled, and then stored in a freezer for later analysis. Once a fecal sample had been collected or holding time neared the 3-hour mark, captured individuals were released at or near the spot of capture. All holding bags were cleaned and properly disinfected, as per USFWS White Nose Syndrome decontamination guidelines (USFWS 2011). South Dakota fecal samples were collected by Dr. Jesse Alston and his team under protocols approved by the University of Wyoming and South Dakota GFP throughout the 2017-2019 field seasons (late May – late August/early September).

Table 1.1: Represents the total number of bats captured during all North and South Dakota field seasons (ND = 2016 – 2019; SD = 2017 – 2019). The number in parentheses represents the total number of fecal samples collected.

| Species | North Dakota (2016-2019) | South Dakota (2017-2019) | Total |
|---|---|---|---|
| *Aeorestes cinereus* | 30 (3) | 80 (14) | 110 (17) |
| *Corynorhinus townsendii* | 1 (1) | 5 (3) | 6 (4) |
| *Eptesicus fuscus* | 153 (83) | 219 (99) | 372 (182) |
| *Lasionycteris noctivagans* | 43 (9) | 36 (11) | 79 (20) |
| *Lasiurus borealis* | 22 (4) | 3 (1) | 25 (5) |
| *Myotis ciliolabrum* | 7 (1) | 17 (7) | 24 (8) |
| *Myotis evotis* | 3 (1) | 1 (1) | 4 (2) |
| *Myotis lucifugus* | 360 (82) | 45 (24) | 405 (106) |
| *Myotis septentrionalis* | 0 (0) | 93 (55) | 93 (55) |
| *Myotis thysanodes* | 4 (1) | 132 (81) | 136 (82) |
| *Myotis volans* | 0 (0) | 154 (102) | 154 (102) |
| Total | 623 (185) | 785 (398) | 1,408 (583) |

### *1.3.6. DNA-based Fecal Analysis*

Prey DNA was extracted from collected guano using the *Quick*-DNA Plant/Seed Miniprep Kit (Zymo Research, USA). No changes were made to the original DNA extraction protocol. To identify insect-prey from collected fecal samples, polymerase chain reactions (PCR) were performed in 25 µl reactions using PuReTaq Ready-To-Go™ PCR Beads from GE Healthcare. Each 25 µl reaction contained: 1 µl DNA, 1 µl of forward primer, 1 µl of reverse primer, 22 µl of molecular water, and 1 PCR bead. The primer set LCO1490/HCO2198 is a generic arthropod primer with wide taxonomic coverage that targets a 710-bp fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI) (Folmer et al. 1994). Additional information about the LCO1490 and HCO2198 primers are listed in Appendix A (Table A2). Thermocycling was completed using the following conditions: 94°C for 5 minutes, then 35 cycles of 94°C for 30 seconds, 60°C for 45 seconds and 72°C for 2 minutes, followed by a final extension at 72°C for 2 minutes, 12°C for 5 minutes, and a hold at 4°C for ∞. Following PCR amplification, 3 µl of PCR product was mixed with 2 µl of tracking dye and then transferred to 1-1.5% agarose gels and compared to a 100-bp ladder.

Due to the SARS-CoV-2 virus being introduced to the United States, resulting in the COVID-19 pandemic, the research facility being used was immediately shut down. At this point all samples were to be stored or packaged and shipped elsewhere before the lab and building would be sealed indefinitely. The crude PCR products containing the LCO1490 and HCO2198 primers were sealed with parafilm and shipped overnight on dry ice to Functional Biosciences, Inc. located in Madison, WI. Functional Biosciences completed PCR cleanup using ExoSAP-IT and sequenced using Big Dye V3.1, run-on ABI 3730xl instruments.

The immediate shut down of the research facility also did not allow the primary researcher time for protocol testing and PCR amplification with the additional primer set C_LepFolF/C_LepFolR. This primer set, along with all extracted DNA samples, and two false positive controls were individually sealed with parafilm and shipped standard overnight to Functional Biosciences, Inc., in Madison, WI. This resulted in protocol testing with the two extracted false positives, PCR amplification, PCR cleanup using ExoSAP-IT, and high throughput Sanger sequencing using Big Dye V3.1, run-on ABI 3730xl instruments all to be completed by Functional Biosciences, Inc., in Madison, WI. The false positive controls contained extracted DNA from an Araneae species and was used to verify PCR amplification of Araneae with the C_LepFolF/C_LepFolR primer set. During thermocycling, Functional Biosciences used a PCR annealing temp of 50°C.

The C_LepFolF/C_LepFolR primer set is another "universal" primer set that amplifies a 658-bp fragment region of the COI gene (DeWaard et al. 2019) but has been shown to specifically detect Arachnida. By using a second primer that can detect the non-volant invertebrates of Arachnida, DNA sequences missed by the generic arthropod primer are potentially amplified. This can also provide secondary confirmation of the LCO1490/HCO2198 primer set findings. Additional information about the C_LepFolF and C_LepFolR primers are listed in Appendix A (Table A2).

### *1.3.7. Data and Statistical Analysis*

Obtained sequencing results from the 583 collected fecal samples were entered into NCBI nt nucleotide sequence database using the Basic Local Alignment Search Tool (BLAST) (ncbi.nlm.nih.gov) to identify the orders present. This DNA-based approach is able to detect prey items that would not be visible under a microscope (i.e. soft bodied prey) as they are more readily broken down in the digestive tract. Orders identified using BLAST were only accepted if aligned DNA segments met a threshold of ≥94% sequence similarity. Of the 583 collected samples, 321 samples met this accepted standard. Dietary compositions for each species were first quantified by calculating the percent occurrence (%O) defined as the number of fecal samples containing an order divided by the total number of fecal samples x 100 (Hope et al. 2014). Second, dietary compositions for each species were quantified by calculating the percent frequency of occurrences (%FO) defined as the number of feces containing an order divided by the total number of all order occurrences x 100 (Razgour et al. 2011; Hope et al. 2014).

## 1.4. Results

### *1.4.1. Dietary Composition of North and South Dakota Bat Species*

Sequencing results from the 321 samples that were identified at the accepted standard of ≥94% sequence similarity represented 36 different orders. Of the 36 identified orders, 18 were orders that contain other organisms/materials that could potentially be found in the digestive tract (i.e. bacteria, nematodes, seeds, etc.) and do not fit the scope of this project. These 18 orders were removed from the dataset and therefore not included in any of the following analyses. The final dataset comprised of 18 orders (Table 1.2) represented across a total of 293 individual bats (Table 1.3). The dietary profiles for each of the 11 native bat species of North and South Dakota showed some variation between the species (Figure 1.2). An additional table with %FO values is found in Appendix A (Table A3.2).

Table 1.2: Eighteen orders represented in the DNA analysis that met the accepted standard and the common name for the species they contain.

| Order | Contains |
|---|---|
| Araneae | Spiders |
| Blattodea | Cockroaches and Termites |
| Coleoptera | Beetles |
| Diptera | Flies |
| Ephemeroptera | Mayflies |
| Hemiptera | Truebugs (Box-elder like) |
| Hymenoptera | Wasps, Bees, and Ants |
| Isopoda | Woodlice (Crustaceans) |
| Ixodida | Ticks |
| Lepidoptera | Moths and Butterflies |
| Neuroptera | Net-winged Insects |
| Odonata | Dragonflies |
| Orthoptera | Grasshoppers and Crickets |
| Plecoptera | Stoneflies |
| Siphonaptera | Fleas |
| Thysanoptera | Thrips |
| Trichoptera | Caddisflies |
| Trombidiformes | Mites |

Table 1.3: Fecal samples from the North and South Dakota field seasons (ND = 2016 – 2019; SD = 2017 – 2019) that were included in the genetic analysis and met the accepted standard.

| Species | North Dakota (2016-2019) | South Dakota (2017-2019) | Total |
|---|---|---|---|
| *Aeorestes cinereus* | 2 | 6 | 8 |
| *Corynorhinus townsendii* | 1 | 1 | 2 |
| *Eptesicus fuscus* | 49 | 40 | 89 |
| *Lasionycteris noctivagans* | 7 | 5 | 12 |
| *Lasiurus borealis* | 3 | 0 | 3 |
| *Myotis ciliolabrum* | 0 | 3 | 3 |
| *Myotis evotis* | 1 | 0 | 1 |
| *Myotis lucifugus* | 41 | 11 | 52 |
| *Myotis septentrionalis* | 0 | 27 | 27 |
| *Myotis thysanodes* | 1 | 35 | 36 |
| *Myotis volans* | 0 | 60 | 60 |
| Total | 105 | 188 | 293 |

The percent occurrence (%O) shows what percentage of captured individuals consumed an order (Figure 1.2). When combined, the total percentage can exceed 100% as some individuals may have consumed more than one order. The %O results show that individuals captured from the species *C. townsendii* (COTO; *n* = 2), *L. borealis* (LABO; *n* = 3), and *M. evotis* (MYEV; *n* = 1) did not have more than one order present in a fecal sample, as all three have %O equal to 100%. *M. septentrionalis* (MYSE; *n* = 27) has a dietary profile that shows the presents of five different orders, however the %O is equal to 103.7%. Though there is a variety of orders shown to be consumed this %O indicates almost all *M. septentrionalis* individuals only consumed one of the five orders represented. Whereas *L. noctivagans* having the highest %O at 183.4% indicates it is likely for an individual of this species to consume more than one order within the study region. For simplicity, all further result discussion will focus on the percent frequency of occurrence (%FO) which gives a proportional representation of the relative importance for each order and does not exceed 100% (Figure 1.3).

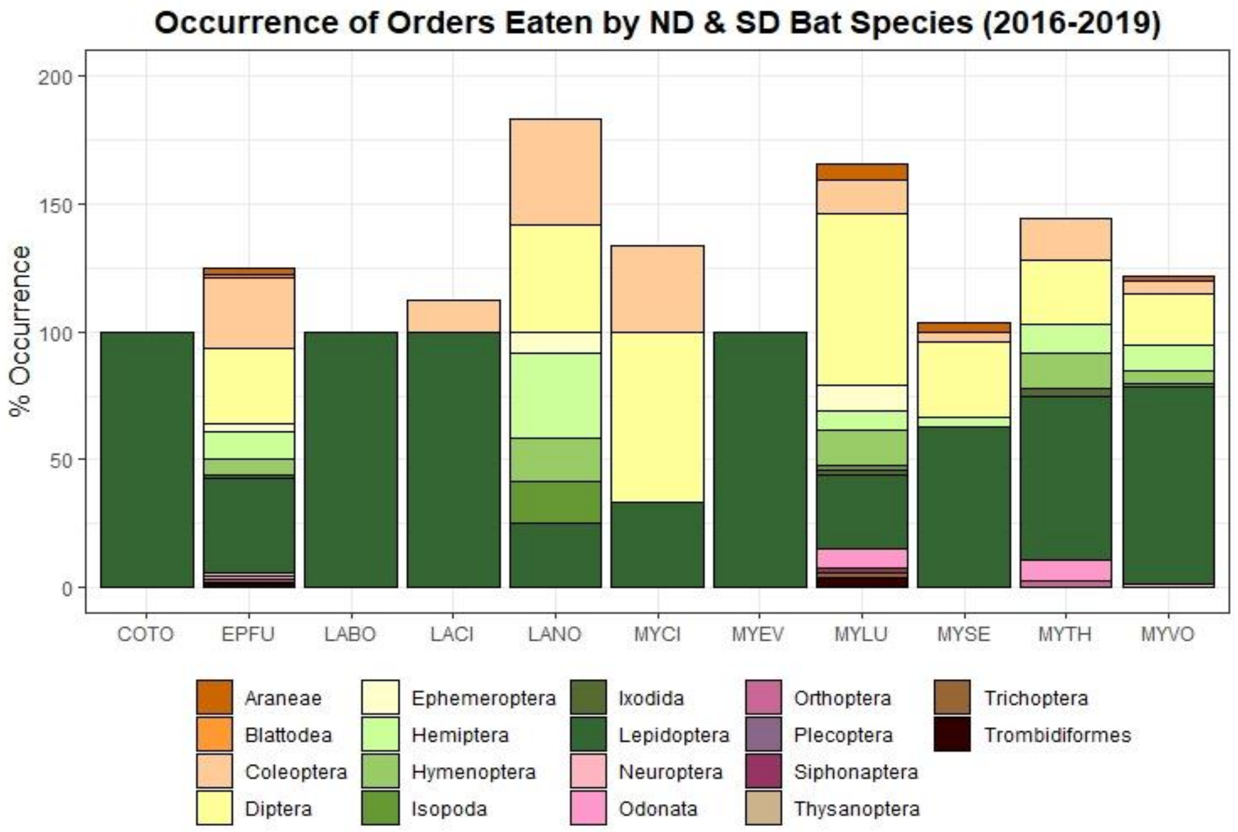

Figure 1.2: Dietary composition profiles at the ordinal level for the 11 native bat species of North and South Dakota. Dietary composition is represented as a percent occurrence (%O). Species abbreviations: *Aeorestes cinereus* (LACI; n = 8), *Corynorhinus townsendii* (COTO; n = 2), *Eptesicus fuscus* (EPFU; n = 89), *Lasionycteris noctivagans* (LANO; n = 12), *Lasiurus borealis* (LABO; n = 3), *Myotis ciliolabrum* (MYCI; n = 3), *M. evotis* (MYEV; n = 1), *M. lucifugus* (MYLU; n = 52), *M. septentrionalis* (MYSE; n = 27), *M. thysanodes* (MYTH; n = 36) and *M. volans* (MYVO; n = 60). See Appendix A Table A3 for specific values.

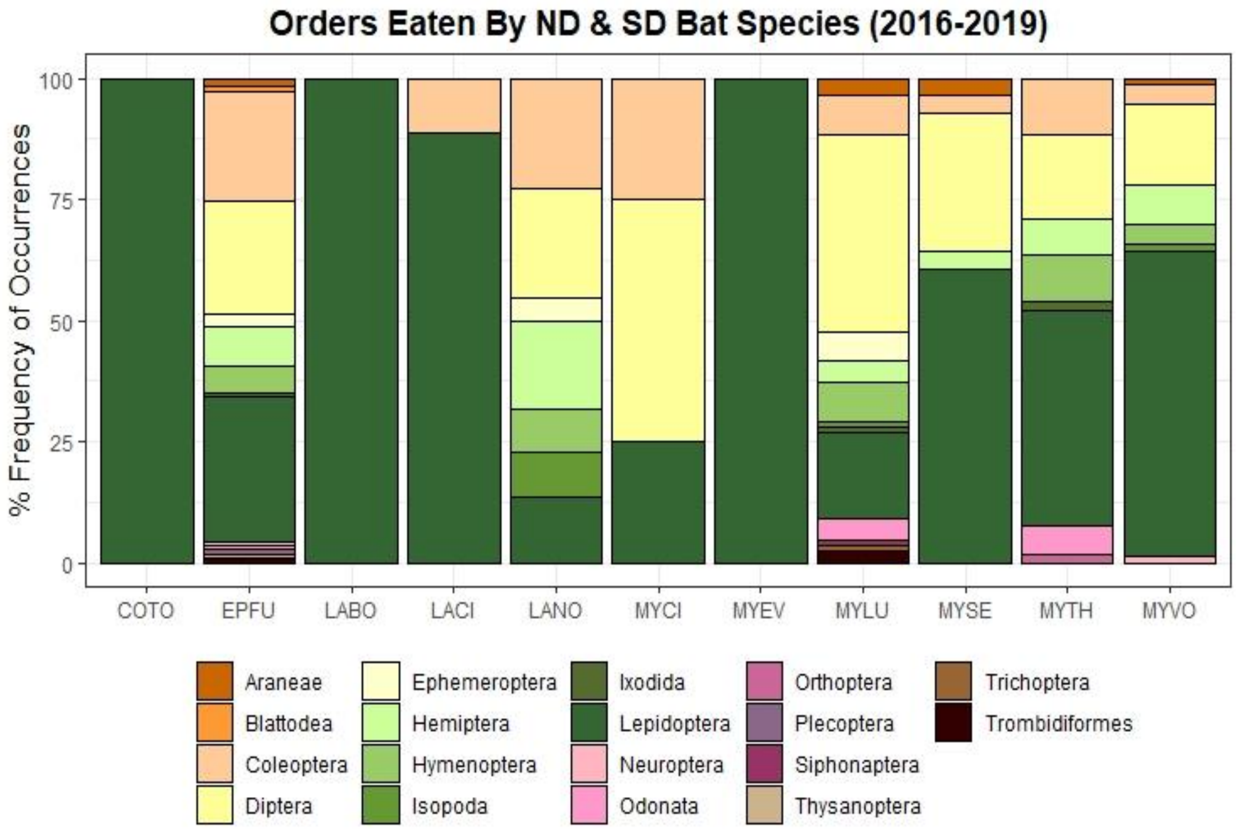

Figure 1.3: Dietary composition profiles at the ordinal level for the 11 native bat species of North and South Dakota. Dietary composition is represented as a percent frequency of occurrence (%FO). Species abbreviations: *Aeorestes cinereus* (LACI; n = 8), *Corynorhinus townsendii* (COTO; n = 2), *Eptesicus fuscus* (EPFU; n = 89), *Lasionycteris noctivagans* (LANO; n = 12), *Lasiurus borealis* (LABO; n = 3), *Myotis ciliolabrum* (MYCI; n = 3), *M. evotis* (MYEV; n = 1), *M. lucifugus* (MYLU; n = 52), *M. septentrionalis* (MYSE; n = 27), *M. thysanodes* (MYTH; n = 36) and *M. volans* (MYVO; n = 60). See Appendix A Table A5 for specific values.

My initial hypothesis predicted *C. townsendii* (COTO), *M. evotis* (MYEV), and *M. septentrionalis* (MYSE) dietary compositions to contain higher percentages of non-volant prey orders (e.g. Araneae). *C. townsendii* (COTO; *n* = 2) and *M. evotis* (MYEV; *n* = 1) have dietary profiles showing them to consume only the order Lepidoptera (%FO = 100%), which would indicate the use of an aerial hawking strategy. However, due to the small sample size of these two species, I was not able to draw robust conclusions about their diet. *M. septentrionalis* (MYSE; *n* = 27) had a dietary profile containing 5 of the 18 identified orders, only one of which, Araneae, contains non-volant prey types. *M. septentrionalis* fed most heavily on Lepidoptera (%FO = 60.7%) followed by Diptera (%FO = 28.6%). This finding indicates this species is using an aerial hawking strategy more frequently than a gleaning strategy within the study region. As *M. septentrionalis* has characteristics better suited for gleaning than aerial hawking, this finding was unexpected and does not support my initial hypothesis that *M. septentrionalis* would have a higher percentage of non-volant prey orders in their diet, indicating a gleaning strategy.

The second part of my hypothesis predicted *A. cinereus* (LACI), *E. fuscus* (EPFU), *L. noctivagans* (LANO), *L. borealis* (LABO), *M. ciliolabrum* (MYCI), *M. lucifugus* (MYLU), *M. thysanodes* (MYTH), and *M. volans* (MYVO) dietary compositions to contain higher percentages of volant prey orders (e.g. Lepidoptera). *A. cinereus* (LACI; *n* = 8) had a dietary profile that contained only 2 of the 18 found orders (Lepidoptera = 88.9%, Coleoptera = 11.1%) both of which are dominated by volant prey types. This finding aligns with my initial prediction of an aerial hawking strategy being used by *A. cinereus*. There was little variation of the diet seen across the eight *A. cinereus* sampled. This suggests that this species primarily feeds on prey within the order Lepidoptera, however, a larger sample size is needed to draw any strong conclusions.

*E. fuscus* (EPFU; *n* = 89) had a highly variable dietary profile that contained 14 of the 18 identified orders, 5 of which contain non-volant prey types. Coleoptera, Diptera, and Lepidoptera were the three most heavily fed on orders making up over 60% of the total dietary profile for *E. fuscus* (%FO = 22.5%, 23.4%, and 29.7% respectively). This finding agrees with my initial prediction of an aerial hawking strategy being used, which aligns with the morphological characteristics expressed by *E. fuscus*. However, with such high variation in the dietary profile (both volant and non-volant orders represented) and there being roughly equal feeding on three of the represented orders, it can be concluded that *E. fuscus* is an opportunistic generalist.

*L. noctivagans* (LANO; *n* = 12) had a dietary profile that contained 7 of the 18 identified orders, one of which consists of non-volant prey types. Coleoptera and Diptera were the two most heavily fed on orders, each with a %FO equal to 22.7% of the total dietary composition. This was followed by Hemiptera and Lepidoptera (%FO = 18.2% and 13.6% respectively). This finding agrees with my initial prediction of an aerial hawking strategy being used by this species in the study region. Interestingly, compared to the other 11 bat species, *L. noctivagans* has a %FO for Hemiptera that is roughly 10% higher than the next highest Hemiptera %FO. It is also the only species, besides *M. ciliolabrum,* to not have its highest %FO for the order Lepidoptera, coming in as the fourth highest order for *L. noctivagans*.

*L. borealis* (LABO; *n* = 3) had a dietary profile that showed consumption of only the order Lepidoptera (%FO = 100%) which would indicate the use of an aerial hawking strategy. Though this finding agrees with my initial hypothesis, it is important to note that my conclusion is drawn from a relatively small sample size.

*M. ciliolabrum* (MYCI; *n* = 3) showed little variation in their dietary profile, containing 3 of the 18 identified orders; Coleoptera, Diptera, and Lepidoptera (%FO = 25%, 50%, and 25%, respectively). This dietary profile indicates the use of an aerial hawking strategy which aligns with my initial hypothesis for this species. However, this is also a small sample size, which deters me from drawing any strong conclusion about *M. ciliolabrum* diet.

*M. lucifugus* (MYLU; *n* = 52) had a dietary profile that contained 13 of the 18 identified orders, 5 of which contain non-volant prey types. Diptera was the most heavily fed on order (%FO = 40.7%) with Lepidoptera being the second most heavily fed on order (%FO = 17.4%).

The other 11 orders that make up the *M. lucifugus* dietary profile all had a %FO $\leq$ 8.1%. This finding agrees with my initial prediction of an aerial hawking strategy being used, which aligns with the morphology of *M. lucifugus*. However, with such high variation seen in the dietary profile, and inclusion of both volant and non-volant orders, much like *E. fuscus*, it can be concluded that *M. lucifugus* is also an opportunistic generalist.

*M. thysanodes* (MYTH; $n$ = 36) had a dietary profile containing 8 of the 18 identified orders, one of which, Ixodida, contains only non-volant prey types. *M. thysanodes* fed most heavily on Lepidoptera (%FO = 44.2%) followed by Diptera (%FO = 17.3%). This finding indicates this species is using an aerial hawking strategy as predicted. Though one non-volant order, Ixodida, was found present in the dietary profile this does not indicate the use of a gleaning strategy as individual or social grooming of ectoparasites is likely the reason for the occurrence of Ixodida.

*M. volans* (MYVO; $n$ = 60) had a dietary profile that contains 8 of the 18 identified orders, 2 of which contain non-volant prey types. *M. volans* fed most heavily on Lepidoptera (%FO = 63%) followed by Diptera (%FO = 16.4%). This finding agrees with my initial prediction of an aerial hawking strategy being used, which aligns with the morphology of *M. volans*. There was some variation seen in the dietary profile, however, with Lepidoptera making up 63% of the total diet, I concluded that *M. volans*, within the study region, prefers to feed on prey within the order Lepidoptera.

### *1.4.2. A Closer Look at the Occurrence of Non-Volant Orders*

Non-volant orders are defined as orders entirely composed of wingless species (i.e. Araneae) or orders composed of species with extremely weak volant capabilities having wings that are unsuitable for conventional flight (i.e. Thysanoptera). Non-volant orders occurred in the dietary profiles of 6 bat species, *E. fuscus, L. noctivagans, M. lucifugus, M. septentrionalis, M. thysanodes,* and *M. volans* (Figure 1.4). However, these 7 non-volant orders made up less than 10% of each species total dietary profile with the highest combined total (%FO = 9.4) found in *M. lucifugus.* This particular result was surprising as *M. lucifugus* displays morphological characteristics better suited for an aerial hawking strategy. Additional tables with %O values and %FO values for each non-volant order are found in Appendix A (Table A4 and Table A6 respectively).

*L. noctivagans, M. septentrionalis,* and *M. thysanodes* are all species whose dietary profiles only contained one non-volant order (Isopoda, Araneae, and Ixodida respectively). *L. noctivagans* (LANO; $n$ = 12), though only consuming one non-volant order, has the second highest %FO total for non-volant orders (%FO = 9.1%). *E. fuscus* (EPFU; $n$ = 89) and *M. lucifugus* (MYLU; $n$ = 52) both had dietary profiles that contained 5 non-volant orders. *E. fuscus* and *M. lucifugus* both contained the orders Araneae, Ixodida, and Trombidiformes. *E. fuscus* is the only species that contained the orders Blattodea and Thysanoptera. The dietary profile of *M. lucifugus* also contained Isopoda and is the only species that contained the order Siphonaptera.

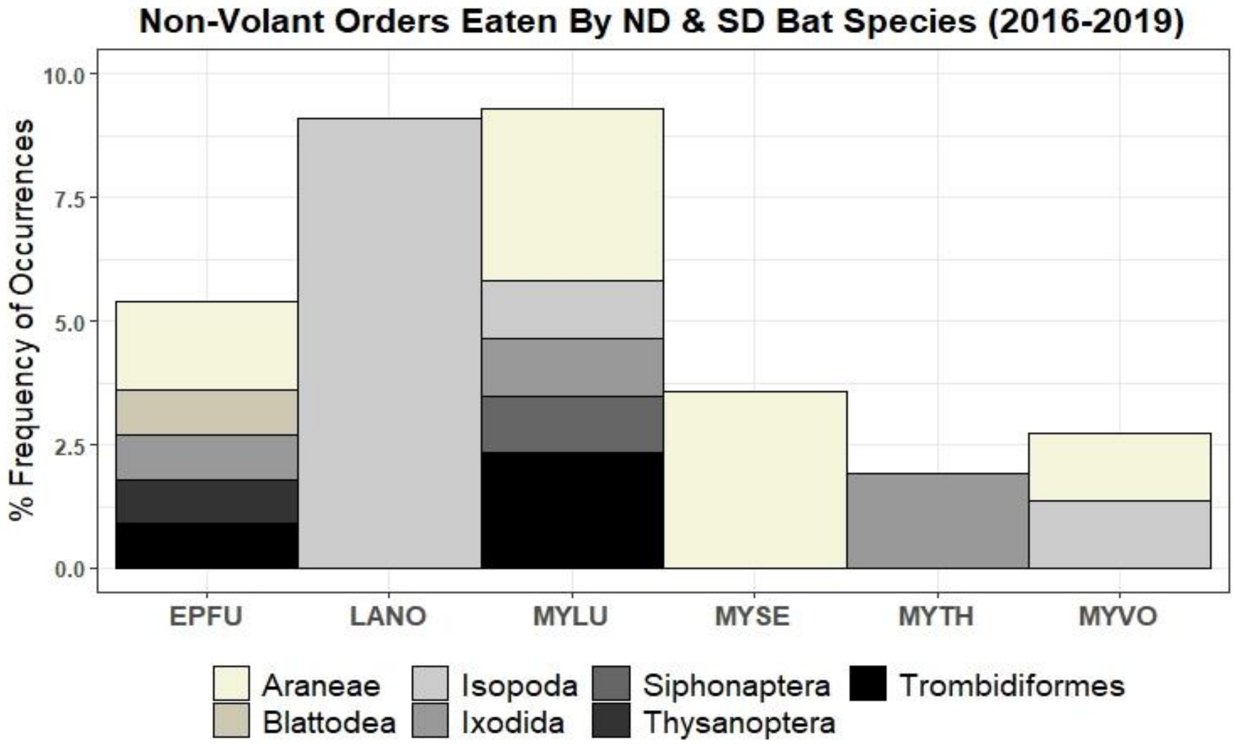

Figure 1.4: Non-volant order profiles for bat species found within North and South Dakota. Dietary composition is represented as a percent frequency of occurrence (%FO). Species abbreviations: *Eptesicus fuscus* (EPFU; n = 89), *Lasionycteris noctivagans* (LANO; n = 12), *M. lucifugus* (MYLU; n = 52), *M. septentrionalis* (MYSE; n = 27), *M. thysanodes* (MYTH; n = 36) and *M. volans* (MYVO; n = 60).

One possible reason for the orders Ixodida, Siphonaptera, and Trombidiformes (containing tick, flea, and mite species) showing up in the dietary profiles of bat species better suited for aerial hawking could be due to individual or social grooming of ectoparasites. Thysanoptera species were included as a non-volant order because though they possess wings, their flight capabilities are extremely weak. Araneae, an order I was specifically looking for, occurred in the dietary profiles of 4 species, *E. fuscus*, *M. lucifugus*, *M. septentrionalis,* and *M. volans* with its highest %FO occurring in *M. septentrionalis* (%FO = 3.6%) followed closely by *M. lucifugus* (%F0 = 3.5%). Overall, my findings show non-volant orders to be a prey type not likely to occur in the dietary profiles for the 11 native bat species of North and South Dakota and very seldom in the three species with morphological characteristics most effective for gleaning.

## 1.5. Discussion

Prior to this study, no regional information about the diet of bat species residing in North or South Dakota was available. Overall, I found species displaying morphology best suited for gleaning did not fit my initial prediction, having dietary profiles primarily composed of volant orders. However, I did find species displaying morphology best suited for aerial hawking to have dietary profiles primarily composed of volant orders, as predicted. Some aerial hawking species had non-volant orders present in their dietary profiles, suggesting they use both gleaning and aerial hawking strategies to capture prey. Three species predicted to use aerial hawking (*A. cinereus, L. borealis,* and *M. ciliolabrum*) had dietary profiles showing support for an aerial hawking strategy but their sample sizes (*n* = 8, 3, and 3 respectively) were too small for me to confidently draw any strong conclusions. This was also the case with two species predicted to use a gleaning strategy (*C. townsendii; n* = 3, and *M. evotis; n* = 1).

Lepidoptera, Coleoptera, and Diptera were the three most prevalent orders found in the dietary profiles for 10 of the 11 bat species, *L. noctivagans* being the only exception. *M. ciliolabrum, M. lucifugus,* and *L. noctivagans* were the only species to not have Lepidoptera as the most common prey in their diet. The most common type of prey in the diet of *M. lucifugus* was the order Diptera, which is similar to a northern Canadian population of *M. lucifugus* (Kaupas and Barclay 2017). Past research describes this species to typically forage over water, along riparian zones, or lake margins preying primarily on aquatic insects (Belwood and Fenton 1976; Anthony and Kunz 1977; Fenton and Barclay 1980). Clare et al (2013) documented Diptera as the second most common dietary item for *M. lucifugus* in Canada, particularly early in the summer. Future research examining temporal patterns of diet across the season would be valuable to better understand if this pattern holds in North and South Dakota.

Findings suggest *E. fuscus* and *M. lucifugus* to be opportunistic generalists who feed on a wide variety of volant orders and some non-volant orders potentially using both aerial hawking and gleaning. Though *M. lucifugus* showed preference for the order Diptera, *E. fuscus* showed no preference, as the orders Lepidoptera, Coleoptera, and Diptera occurred at roughly equal percentages. This supports the idea that *E. fuscus* are true opportunistic foragers in North and South Dakota. This does not align with findings from other dietary studies that show *E. fuscus* to have high occurrences of the order Coleoptera, suggesting a preference for beetles (Whitaker 2004; Moosman et al. 2012; Clare et al. 2014).

*M. volans* expresses morphological characteristics that makes them most effective at aerial hawking and, based off their dietary profiles, this is the strategy they are primarily using in North and South Dakota. This finding aligns with a previous dietary study on a population of *M. volans* captured in a habitat that also had high vegetative cover (Johnson et al. 2007). Interestingly, from 2016-2019, *M. volans* was never captured in North Dakota and only two were captured from 2009-2012 (Nelson et al. 2015) suggesting their distribution range may need to be reexamined.

A similar outcome was seen with *M. thysanodes,* another predicted aerial hawking species who had a small percentage of non-volant orders present in their dietary profile. The non-volant order present was Ixodida which was likely consumed through individual or social grooming of ectoparasites. This suggests that *M. thysanodes* is exclusively using an aerial hawking strategy. This finding fits with previously described foraging strategy and dietary composition studies (Bell 1982; Warner 1985).

*M. septentrionalis* was one species that expressed a dietary profile with unexpected results. This species has specifically been described as having morphology and echolocation call characteristics associated with the use of a gleaning strategy (Miller and Treat 1993; Caceres et al. 2000; Whitaker 2004). Furthermore, it has been shown to use a gleaning strategy through various dietary studies and has even been recorded picking insects from a backlit screen (Faure et al. 1993; Whitaker 2004; Kaupas and Barclay 2017). All *M. septentrionalis* individuals included in this study were captured in South Dakota, specifically within the Black Hill National Forest (BHNF) consisting of forested hills and mountains (Bryce et al. 1998; Bailey 2016). I expected non-volant orders to be more prevalent in the dietary profile not only because of morphological expression and previous research, but because they had been captured in an area with high vegetative cover. Instead, the *M. septentrionalis* dietary profile showed Lepidoptera, a volant order, to occur in the diet over 60% of the time. The distribution of this species may also need to be reexamined as only 23 were captured from 2009-2012 (Nelson et al. 2015) followed by no captures of *M. septentrionalis* from 2016-2019 in North Dakota. Yet, the captures from 2009-2012 were not genetically confirmed to be *M. septentrionalis,* so 100% confirmation of identity is not possible for those animals.

A large body of work has focused on understanding the dietary composition and foraging strategies used by a bat species across their range at various scales (i.e. entire range to localized populations). This large body of work includes studies on the 11 species studied here, but they did not explicitly occur in North and South Dakota. Given that some bat populations in the Dakotas are declining due to White-nose syndrome and other anthropogenic causes, it is critical to understand the local/regional resources needed by these populations to survive, including their prey base.

Though local ecosystem impacts are at the forefront of concern, one potentially overlooked impact from bat population declines could be to the local economy (Boyles et al.

2011). In 2006, using dietary information collected from Brazilian free-tailed bats (*Tadarida brasiliensis*) foraging in south-central Texas cotton fields, researchers developed an avoided-cost approach to analyze the economic value of Brazilian free-tailed bats to Texas cotton agriculture (Cleveland et al. 2006). In this model, avoided cost is defined as the cost humanity avoids due to the natural pest control services provided by these mammals (Woodward and Wui 2001; Cleveland et al. 2006). There are two components of cost; 1) the amount of a crops' market value lost due to the absence of bats and 2) the reduction of pesticide costs and applications when bats are present in the ecosystem (Woodward and Wui 2001; Cleveland et al. 2006). This avoided-cost approach was first used to model the economic value of wetlands (Woodward and Wui 2001) and has since been used as a method for assessing bats economic services in agroecosystems (Cleveland et al. 2006; Boyles et al. 2011). In 2011, the avoided cost economic analysis of Brazilian free-tailed bats in Texas cotton agriculture was used as a baseline to develop a model that estimated the economic value of bats to the agricultural industry, down to county, across the entire continental United States (Boyles et al. 2011). This large-scale model suggests that bats provide roughly a value of $22.9 billion per year to the U.S. agricultural industry, with the most conservative model suggesting $3.7 billion per year (Boyles et al. 2011). When scaling down to the study area of this project, this model estimated bats foraging in North and South Dakota could provide 33% of the annual market value of crop production. If these bat populations are in fact contributing a value equal to 1/3 of the total annual market value in terms of pest control services, it is imperative to have a better understanding of their interactions within agroecosystems.

Overall, no support was found for the use of a gleaning strategy by *M. septentrionalis* in North and South Dakota. *Corynorhinus townsendii* and *Myotis evotis* had samples sizes too small to draw any conclusions about diet and the use of a gleaning strategy. Support was found for the use of an aerial hawking strategy in *Eptesicus fuscus, Lasionycteris noctivagans, M. lucifugus, M. thysanodes* and *M. volans.* Support for the use of an aerial hawking strategy was also shown in *Aeorestes cinereus, Lasiurus borealis,* and *M. ciliolabrum* but their samples sizes were also too small to draw strong conclusions. Based on capture rates, *M. septentrionalis* and *M. volans* distribution ranges many need to be reexamined as both were not captured from 2016-2019 in North Dakota.

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# CHAPTER 2: FORAGING STRATEGIES AND MORPHOLOGICAL CHARACTERISTICS OF BAT SPECIES IN NORTH AND SOUTH DAKOTA

## 2.1. Abstract

Links between diet, resource availability, and morphology have been demonstrated to occur in various taxa as a result of selection and/or plasticity. Since bat populations are likely exposed to different prey fauna across their wide geographic ranges, they may be behaviorally or morphologically adapted for foraging in their local environment. This Chapter aims to examine the relationship between dietary and morphometric data from three bat species: *M. lucifugus, M. septentrionalis,* and *E. fuscus.* Specifically, I will 1) assess that ear length is not associated with body size, 2) assess state differences in ear morphology between conspecifics, and 3) assess morphology of the individuals who consumed non-volant orders. This study, to the best of my knowledge, is the first to examine intraspecific links between dietary preferences and morphological traits in bats at a local/regional level. Further investigation is still needed to understand why significant morphological variation is being seen on a rather small spatial scale.

## 2.2. Introduction

For species inhabiting a wide geographic distribution, it is likely that the availability and diversity of food resources will differ across their range, potentially leading to spatial variation in dietary preferences (Münzer et al. 2016), foraging behavior (Tremblay and Cherel 2003; Sih et al. 2004), and associated size and shape of morphological structures (Hill et al. 1999; Robbins et al. 1986; Solick and Barclay 2006). Dietary changes leading to morphological shifts can be driven by 1) selection invoking changes slowly over many generations due to environmental conditions placed on the previous generations, or 2) plasticity which involves an individual quickly modifying its phenotype in response to an experienced environmental condition.

One of the most well-known examples of diet-induced morphological and behavioral changes driven by selection are Darwin’s Galapagos finches. Beak shape and size are strongly selected for in these finches when seed type (i.e. small versus large, soft versus hard) availability varies over time (Grant 1985). Following changes in resource availability (e.g. seed type), individuals with a beak shape and size that allows maximum resource extraction will leave more offspring, shifting the average phenotypic state of the population (Boag and Grant 1981). Foster’s rule, a broad pattern in which body size differs based on resource availability, is another strong example of diet-induced morphological and behavioral changes (Foster 1964; Van Valen 1973).

Plastic morphological and behavioral shifts have been demonstrated experimentally in the laboratory and the field (Day and Mcphail 1996; Sampaio et al. 2013; McLean et al. 2016). Day and McPhail (1996) tested limnetic morphs of threespine sticklebacks, *Gasterosteus aculeatus*, in a diet switching experiment in which fish were given access to typical limnetic or benthic prey. They found that head depth, which is associated with foraging efficiency, was the only morphological characteristic that exhibited significant changes because of diet. Under natural field conditions, plastic morphometric changes have been seen in wild populations of the Barred tiger salamander, *Ambystoma mavoritum.* Specifically, it has been demonstrated that under conditions of high conspecific density, or when the prey available are large in size (i.e. fathead minnows), cannibalistic morphs will develop (McLean et al. 2016). Overall, these different studies demonstrate that examining the link between diet, resource availability and morphological traits can contribute to a greater understanding of phenotypic plasticity and specialization to local environments.

Insectivorous bats often exhibit particularly large geographic ranges, creating the potential for regional differences in behavioral and morphological traits that play important roles in prey detection and capture. Since the species composition of local insect communities is typically associated with spatial variation in temperature and precipitation (Kaupas and Barclay 2017), bat populations across the range of a species will likely be exposed to different prey fauna and therefore may be behaviorally or morphologically adapted for foraging in their local environment (Tremblay and Cherel 2003; Sih et al. 2004). Such differences in foraging behavior would involve the use of specific foraging strategies and prey detection methods best suited for the local environment. For example, individuals in montane temperate forests may rely on capturing insects from foliage, a strategy known as gleaning, compared to populations of the same species living primarily in open prairies where chase of volant prey (i.e. an aerial hawking strategy) is optimal (Simmons 1982).

A large body of work has focused on understanding the morphological adaptations of bats and their association with different foraging strategies. Ear morphology is one primary characteristic that has been identified as a key component to predicting habitat use and foraging behavior in bats. Long ears amplify sounds, sharpen directionality information, and improve detection of lower frequencies compared to short ears (Obrist et al. 1993). Not surprisingly, gleaning bat species, which rely on detection of low amplitude, prey generated sounds for final localization of prey on vegetative surfaces (Coles et al. 1989; Guppy and Coles 1988; Obrist et al. 1993; Simmons 1982; Solick and Barclay 2006) have longer ears than those species that are primarily aerial hawkers and exclusively use echolocation for prey detection and capture (Fenton and Bogdanowicz 2002). Yet, little is known about how locally adapted foraging strategies have impacted the evolution of morphological structures that play important roles in the processes of prey detection and capture.

Previous research has identified the bat species, *M. lucifugus,* as an aerial hawker (Norberg and Rayner 1987; Fenton and Bogdanowicz 2002). Yet, a northern Canadian population of *M. lucifugus* had a percent frequency of occurrence (%FO) of the order Araneae equal to 37.5% of the total dietary composition. Araneae %FO was highest in samples collected in early summer (%FO = 69.2%) and late summer (%FO = 42.3%), implying a switch to a gleaning strategy when volant prey were less available (Kaupas and Barclay 2017). It is plausible that the bat populations of North and South Dakota experience similar reductions in aerial prey availability during the cooler periods of early and late summer. If these populations are experiencing changes in prey availability, there is potential for morphological characteristics to shift (i.e. develop characteristics more effective for gleaning if aerial prey are less available).

While past work has focused on differences in foraging strategies and morphological characteristics across species of bats, it is plausible that intra-specific variation may also exist in these features, especially for species that occupy large and ecologically diverse geographic ranges. Simply looking at morphology across a species' distribution could potentially be misleading, as latitudinal changes in body size and extremity morphology are well documented in many species ("Bergmann's rule" - Bergmann 1847; Meiri and Dayan 2003; Symonds and Tattersall 2010; Salewski and Watt 2017), including bats (Burnett 1983; Bogdanowicz 1990). Thus, to accurately determine if populations are adapted to local environments and prey communities, both dietary information and morphometrics must be studied together.

This chapter aims to examine the relationship between dietary and morphometric data from three bat species: *M. lucifugus, M. septentrionalis,* and *E. fuscus.* Ear morphology has been strongly linked to foraging strategies used by a wide variety of bat species. Specifically, I will 1) assess that ear length is not associated to body size, using forearm length as a measurement of body size due to the fluctuating nature of weight, 2) assess differences in ear morphology between conspecifics occurring in North Dakota and South Dakota, and 3) assess the morphology expressed by the individuals whose dietary profiles contained non-volant orders. I predict 1) ear length and forearm length will not be associated with one another, 2) no state differences in ear morphology to occur between conspecifics and 3) individuals whose dietary profiles contained non-volant orders to express ear lengths at the upper most range that occur in that species.

## 2.3. Methods

### *2.3.1. Ethics Statement*

All procedures followed a protocol approved by the North Dakota State University Animal Care and Use Committee (Protocol Number: A18040). No animals were euthanized during this study.

### *2.3.2. Study Area*

North and South Dakota fall directly within the Northern Great Plains ecoregion consisting of high-latitude land primarily dominated by grassland habitat types (Samson et al. 2004). However, it is important to note capture sites in South Dakota were all located within the Black Hills National Forest (BHNF), near Custer, SD, which is considered to be a part of the Middle Rockies ecoregion that consists of forested hills and mountains (Bryce et al. 1998; Bailey 2016).

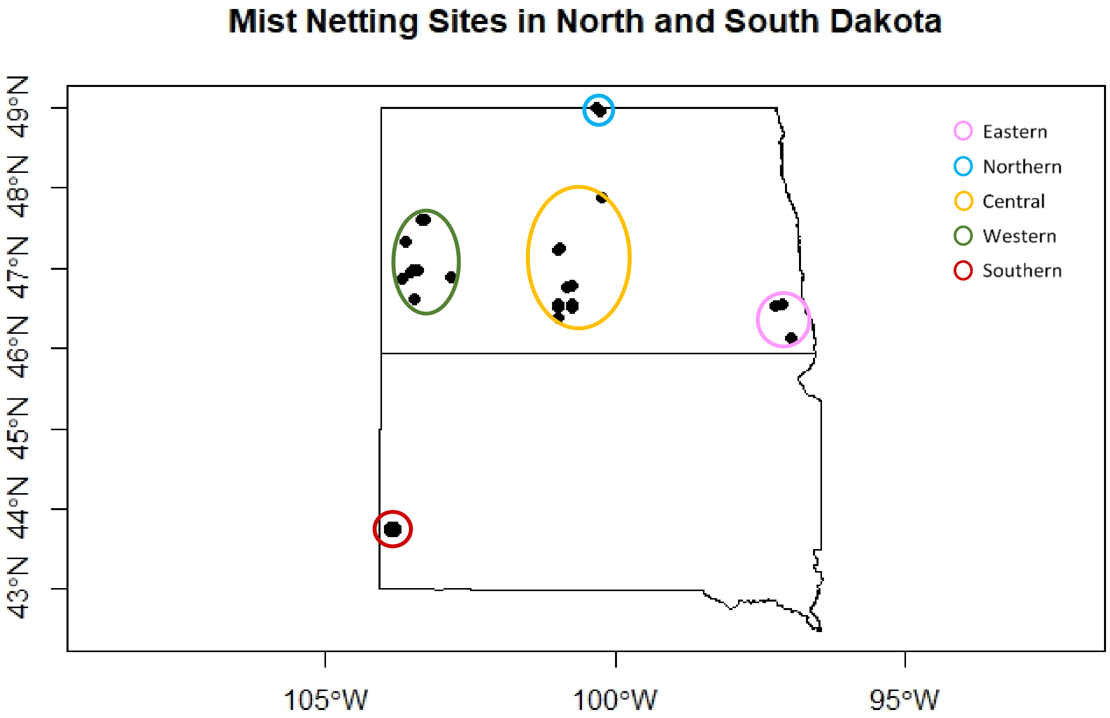

Figure 2.1: Mist netting sites used in North and South Dakota during the 2016-2019 field seasons.

Figure 2.1 outlines the five study areas sampled during the summer field seasons of 2016 – 2019. These five study areas are classified as being within the following ecoregions (Bryce et al. 1998; Bailey 2016): 1) Eastern (Pink) consisting of 3 sample sites within the Lake Agassiz Plain ecoregion, 2) Central (Yellow) consisting of 12 sample sites within the Northwestern Great Plains ecoregion, 3) Western (Green) consisting of 13 sample sites within the Northwestern Great Plains ecoregion, 4) Northern (Blue) consisting of 2 sample sites within the Northern Glaciated Plains ecoregion, and 5) Southern (Red) consisting of 14 sample sites within the Middle Rockies ecoregion of South Dakota. Additional information related to specific site names and locations are provided in Appendix A (Table A1).

### *2.3.3. Capture*

Mist nets and hand nets were the two methods used to capture bats. Mist nets were deployed along natural fly ways (e.g. trails, waterways, etc.). These nets are ideal for capture because they are difficult for bats to detect when placed among clutter (e.g. trees, buildings, etc.). Bats that flew into mist nets would fall into a shelf, formed by the net, and remain until they were untangled and removed by the researcher and placed in a holding bag. Mist nets were checked every ten minutes to ensure the bats were removed quickly and without unnecessary strain. This is a standard method for capturing bats (Kunz and Parsons 2009). Mist nets used in one area were not used in another until they had been boiled and poles properly disinfected, as per USFWS White Nose Syndrome decontamination guidelines (USFWS 2011). This protocol was in place prior to the confirmation of WNS in either state.

Hand nets were used to capture bats found on the outside of buildings, under bridges, or any other manufactured structure on which a bat may rest in between foraging bouts. Capture required the researcher to place the hand net over a resting bat and hold it there until the bat attempted to fly away. The bat attempting to leave flies directly into the back of the net where it becomes stuck, at which point the researcher can lower the net and remove the bat. Similar methods have been used by researchers, but this method can be adapted to suit specific needs (Waldien et al. 1999).

### *2.3.4. Study Species*

Data was collected on three species of bats native to North and South Dakota: *Eptesicus fuscus* (Big brown bat), *Myotis lucifugus* (Little brown bat), and *M. septentrionalis* (Northern Long-eared *myotis*).

### *2.3.5. Measurement Collection*

Ear and forearm measurements from captured individuals in North Dakota were collected during the 2016-2019 field season (late May – late August/early September). Ear and forearm measurements from captured individuals in South Dakota were collected by Dr. Jesse Alston and his team under protocols approved by the University of Wyoming and South Dakota GFP throughout the 2017-2019 field seasons (late May – late August/early September).

Ear length and forearm length were collected in the field from North Dakota bats using calipers (to the nearest 0.01 mm). Ear length is measured from the tip to the base of the ear. Forearm length is used as an indicator of body size due to the fluctuating nature of bats weight throughout the season. In South Dakota, ear length was collected (to the nearest 1 mm) by Dr. Jesse Alston and his team using a ruler. Forearm lengths were collected using a caliper (to the nearest 0.01 mm). All measurement equipment was cleaned and properly disinfected, as per USFWS White Nose Syndrome decontamination guidelines (USFWS 2011).

Table 2.1: Represents the total number of individual ear lengths and forearm lengths collected from *E. fuscus*, *M. lucifugus*, and *M. septentrionalis* during both North and South Dakota field seasons (ND = 2016 – 2019; SD = 2017 – 2019).

| Species | North Dakota (2016-2019) | South Dakota (2017-2019) | Total |
|---|---|---|---|
| *Eptesicus fuscus* | 71 | 213 | 284 |
| *Myotis lucifugus* | 192 | 45 | 237 |
| *Myotis septentrionalis* | 0 | 93 | 93 |
| Total | 263 | 351 | 614 |

### ***2.3.6. Data and Statistical Analysis***

All ear length measurements from South Dakota were recorded to the nearest whole millimeter, to standardize measurements between the states; in turn, ear length measurements from North Dakota were rounded to the nearest whole millimeter as well. These rounded measurements are used in all analyses that involve data from both states. All statistical analyses were run using RStudio Version 1.3.1 (RStudio Team, 2020).

To assess that ear length and body size are not associated with one another, I ran separate linear models (lm) for each bat species with ear length as the response variable and forearm length as the term variable. These linear models, for each bat species, were then statistically analyzed using an analysis of variance (ANOVA). For state comparisons of ear length measurements collected from each species, I ran a linear model (lm) with ear length as the response variable and state (i.e. North and South Dakota) as the categorical term variable. These two variables were then statistically analyzed for *E. fuscus,* and *M. lucifugus* using an analysis of variance (ANOVA). I was unable to do a state comparison for *M. septentrionalis* as none were captured in North Dakota from 2016-2019.

## 2.4. Results

### *2.4.1. State Comparisons (Eptesicus fuscus)*

A total of 284 *E. fuscus* captured from 2016-2019 had both ear and forearm length measurements collected (Table 2.2). The linear regression for ear length, the dependent variable, and forearm length, the independent variable ($R^2 = 0.014$; Figure 2.2A) contains all 284 captured *E. fuscus* and, using an ANOVA, is shown to be marginally significant ($P = 0.049^*$). However, because of the small group of outliers (circled in Figure 2.2A) and the very small $R^2$ value, this outcome is likely not biologically significant. When the small group of outliers were removed from the linear regression ($R^2 = 0.011$; Figure 2.2B) ear length and forearm length were no longer significant (ANOVA; $P = 0.078$). Finding no significance between ear and forearm length is important because if ear lengths were found to be associated with forearm lengths my methods would need to be adjusted to account for the size of the individual.

Table 2.2: Total number of *E. fuscus* captured split by state and sex.

| EPFU | North Dakota (2016-2019) | South Dakota (2017-2019) | Total |
|---|---|---|---|
| Female | 62 | 60 | 122 |
| Male | 9 | 153 | 162 |
| Total | 71 | 213 | 284 |

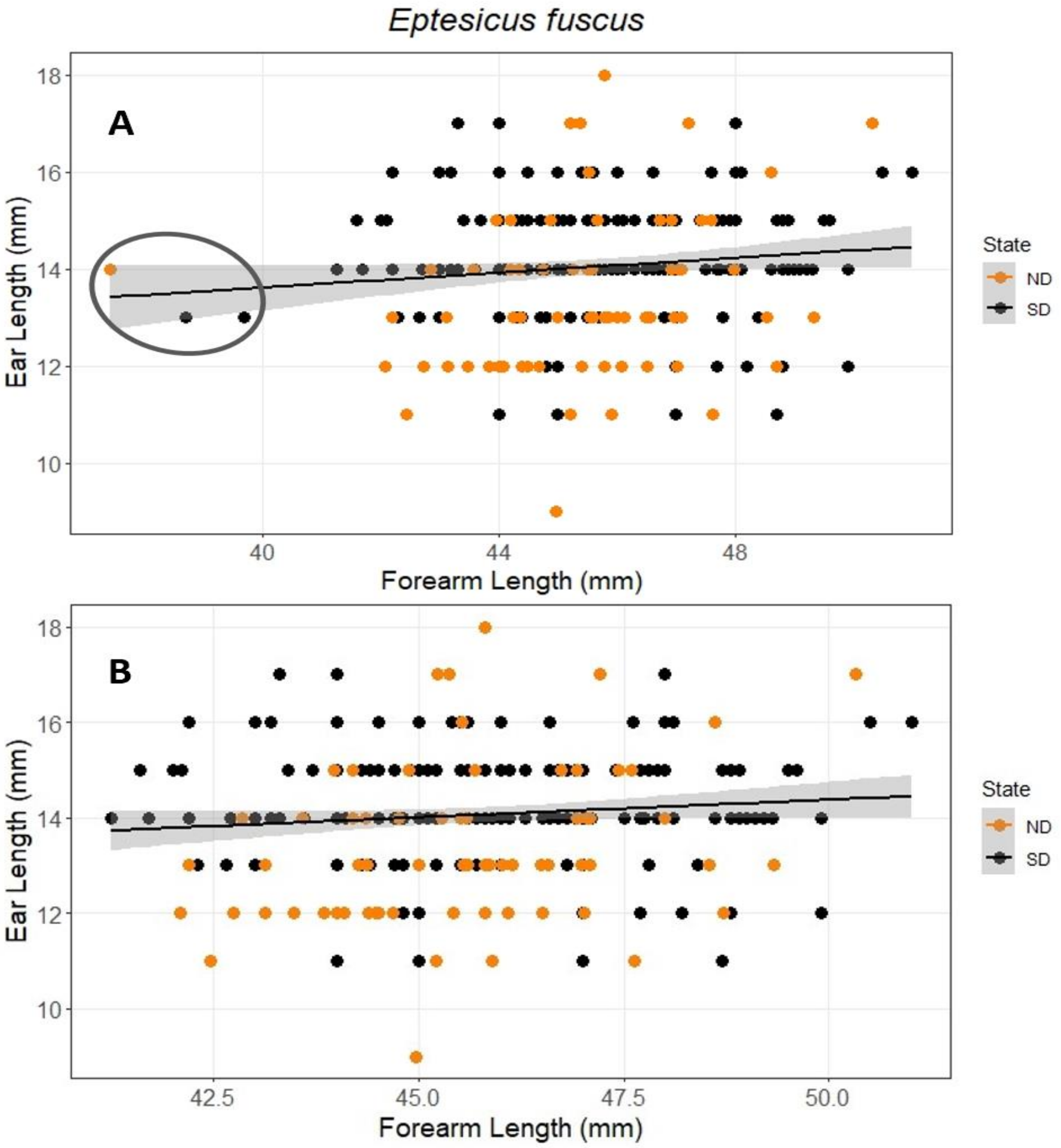

Figure 2.2: A linear regression showing the correlation between (A) all *E. fuscus* ear and forearm length measurements and (B) with outliers (circled in A) removed.

After confirming ear length and forearm length were not associated in *E. fuscus* (i.e. a bigger bat ≠ longer ears) data was assessed to determine if bats from North and South Dakota had similar distributions of ear length. Using all captured *E. fuscus* measurements, South Dakota ear lengths were found to be significantly longer, and this was statistically confirmed using an ANOVA ($P < 0.001$*). However, there was a potential sampling bias due to over 100 more *E. fuscus* being captured and measured in South Dakota than North Dakota (Table 2.2). To reduce sampling bias, male *E. fuscus* were removed from the analysis, as female *E. fuscus* had roughly equal sample sizes between the two states. Using only female *E. fuscus* measurements, ear lengths were still shown to be longer in *E. fuscus* captured and measured in South Dakota (Figure 2.3). This was found to be statistically significant using an ANOVA ($P < 0.001$*). Refer to Appendix A (Table A7-10) for all *E. fuscus* statistical outputs.

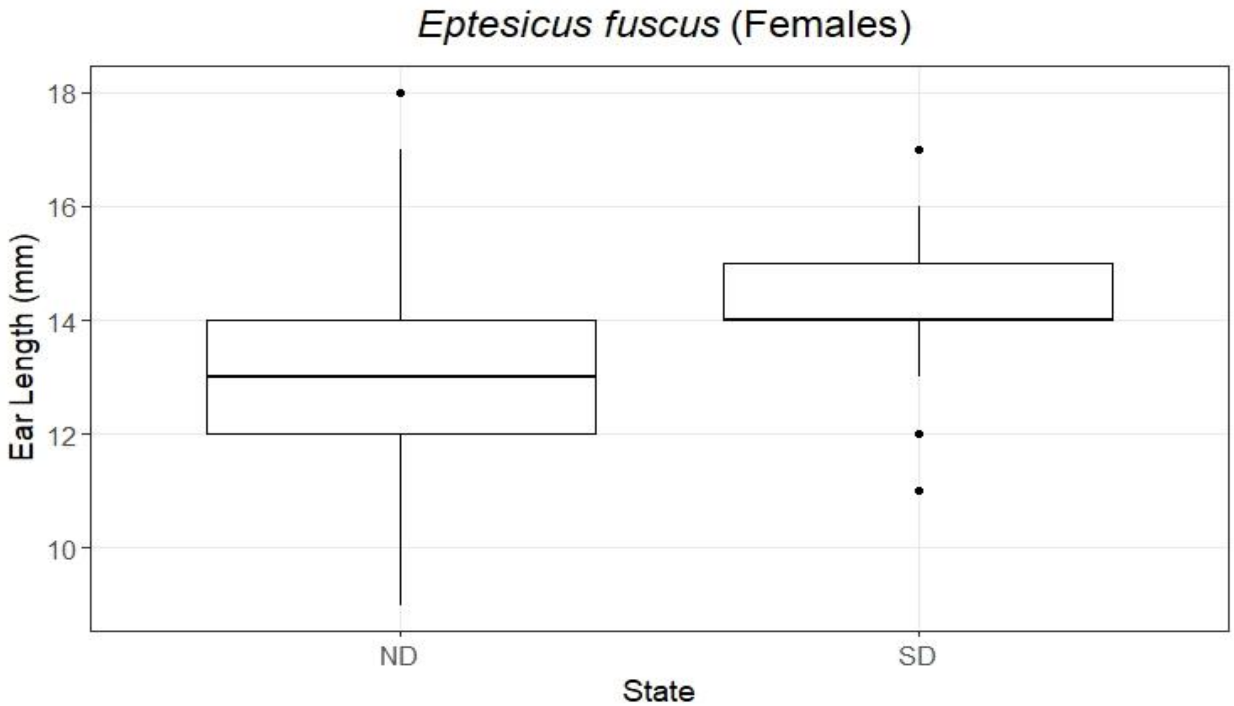

Figure 2.3: A comparison of the distribution of ear lengths shown between the North and South Dakota populations of female *E. fuscus*.

### *2.4.2. State Comparisons (Myotis lucifugus)*

A total of 237 *M. lucifugus* captured from 2016-2019 had both ear and forearm length measurements collected (Table 2.3). The linear regression for ear length, the dependent variable, and forearm length, the independent variable (Figure 2.4, $R^2 = 0.008$) containing all 237 individuals was confirmed be not statistically significant with an ANOVA ($P = 0.16$). Again, finding no significance between ear and forearm length is important because if ear length was found to be associated with forearm length my methods would need to be adjusted to account for the size of the individual.

Table 2.3: Total number of *M. lucifugus* captured split by state and sex.

| *MYLU* | North Dakota (2016-2019) | South Dakota (2017-2019) | Total |
|---|---|---|---|
| Female | 139 | 3 | 142 |
| Male | 53 | 42 | 95 |
| Total | 192 | 45 | 237 |

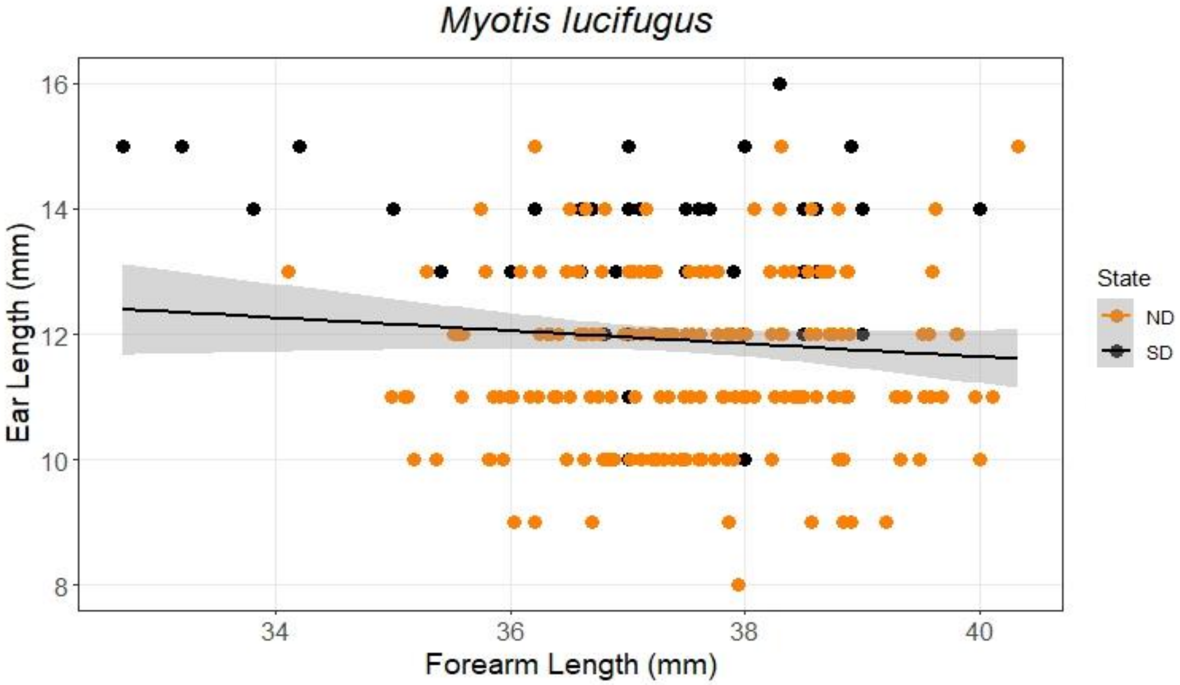

Figure 2.4: A linear regression showing the correlation between all *M. lucifugus* ear and forearm length measurements.

After confirming ear length and forearm length were not associated in *M. lucifugus* (i.e. a bigger bat ≠ longer ears) data was assessed to determine if bats from North and South Dakota had similar distributions of ear length. Using all captured *M. lucifugus* measurements, South Dakota ear lengths were found to be significantly longer, and this is statistically confirmed using an ANOVA ($P$ < 0.001*). However, there is again the potential for a sampling bias due to over 100 more *M. lucifugus* being captured and measured in North Dakota than South Dakota (Table 2.3). To reduce sampling bias, female *M. lucifugus* were remove from the analysis, as male *M. lucifugus* have roughly equal sample sizes. Using only male *M. lucifugus* measurements, ear lengths were still found to be longer in *M. lucifugus* captured and measured in South Dakota (Figure 2.5). This was found statistically significant using an ANOVA ($P$ < 0.001*). Refer to Appendix A (Table A11-13) for all *M. lucifugus* ANOVA outputs.

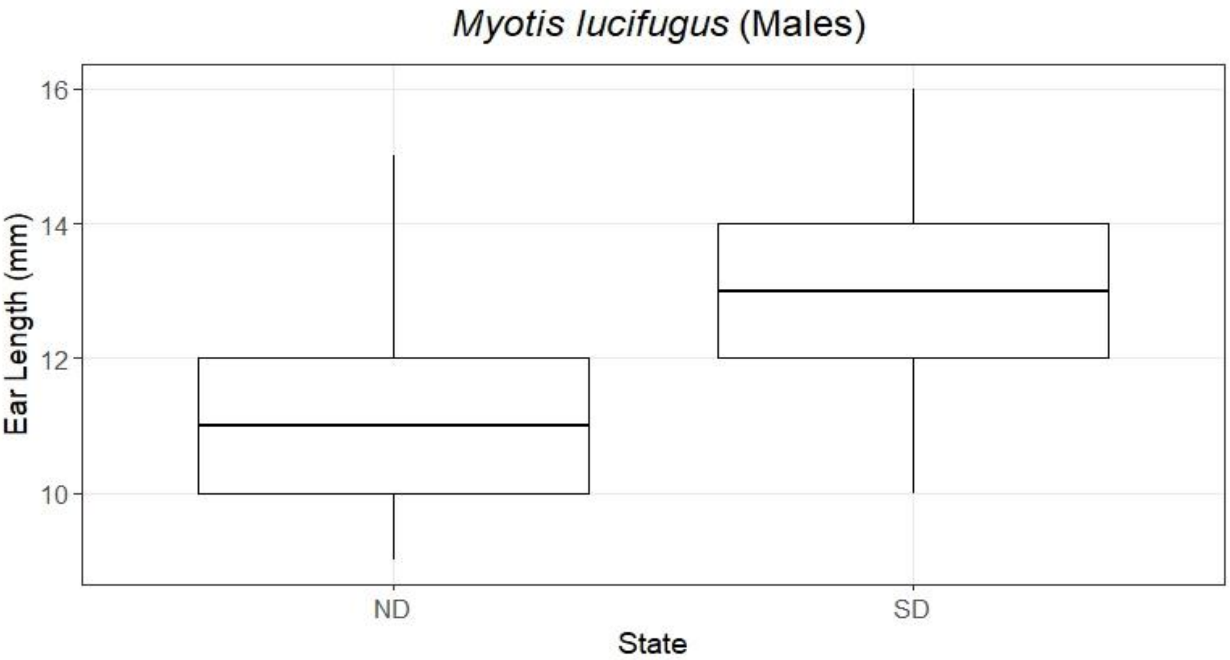

Figure 2.5: A comparison of the distribution of ear lengths shown between the North and South Dakota populations of male *M. lucifugus*.

### *2.4.3. State Comparisons (Myotis septentrionalis)*

A total of 93 *M. septentrionalis* captured from 2016-2019 had both ear and forearm length measurements collected (Table 2.4). The linear regression for ear length, the dependent variable, and forearm length, the independent variable (Figure 2.6, $R^2 = 0.004$), containing all 93 individuals was confirmed to be not statistically significant with an ANVOA ($P = 0.54$). Refer to Appendix A (Table A14) for statistical output of *M. septentrionalis.*

Table 2.4: Total number of *M. septentrionalis* captured split by state and sex.

| *MYSE* | North Dakota (2016-2019) | South Dakota (2017-2019) | Total |
|---|---|---|---|
| Female | 0 | 32 | 32 |
| Male | 0 | 61 | 61 |
| Total | 0 | 93 | 93 |

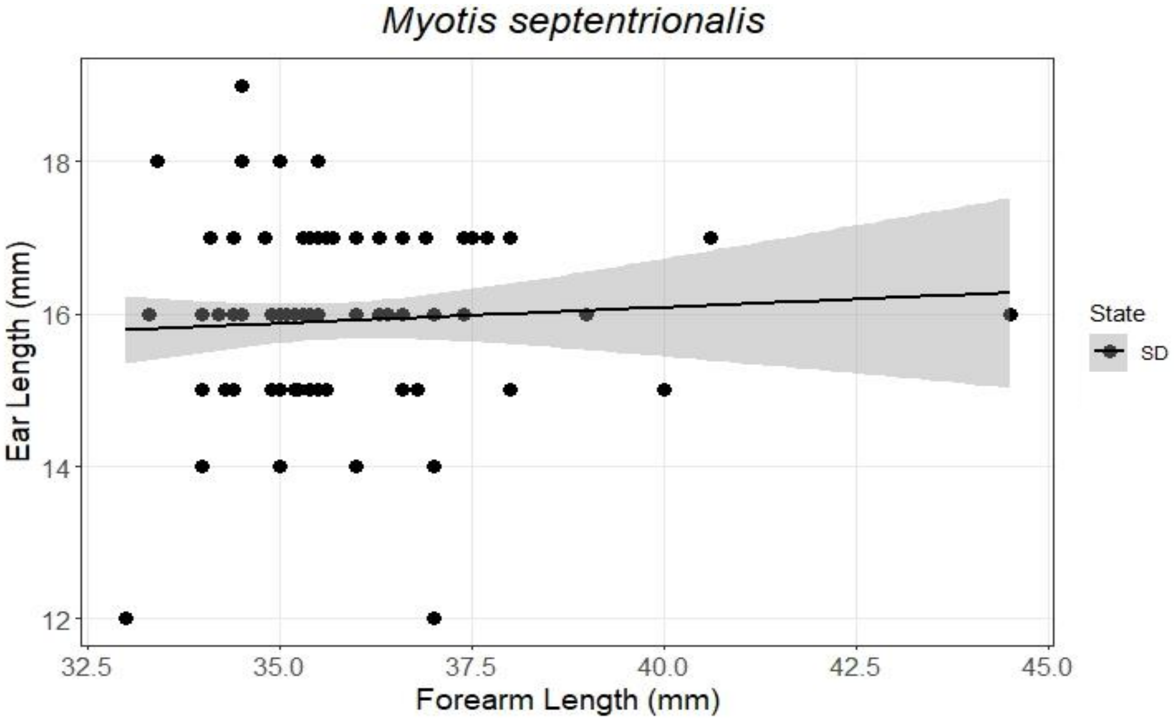

Figure 2.6: A linear regression showing the correlation between all *M. septentrionalis* ear and forearm length measurements.

Although ear length was not associated with forearm length, an assessment of variation between the states cannot be performed due to *M. septentrionalis* never being captured in North Dakota from 2017-2019.

### *2.4.4. Morphology and Diet*

In Chapter 1, I initially predicted *E. fuscus* and *M. lucifugus* to have dietary profiles primarily comprised of volant orders, having characteristics better suited for aerial hawking, and *M. septentrionalis* to have a dietary profile primarily comprised of non-volant orders, expressing characteristics better suited for gleaning. Examining the variation of *E. fuscus, M. lucifugus,* and *M. septentrionalis* morphology reveals some overlaps that occurs between the species (Figure 2.7). The major morphological overlap between the two *Myotis* species is seen with forearm length, which is used as a measurement for body size. However, these two *Myotis* species cluster out into distinct groups when looking at ear length measurements. *E. fuscus* clusters out into a distinct group when comparing forearm length (i.e. body size) to the two *Myotis* species, indicating this to be a larger bat species. However, there is an area of overlap occurring between the ear lengths of the *Myotis* species, with *E. fuscus* ear lengths spanning roughly the range of both *Myotis* species.

In Chapter 1, *E. fuscus*, *M. lucifugus*, and *M. septentrionalis* species had dietary profiles primarily composed of volant orders with the occurrence of some non-volant orders (%FO < 10%). A closer look showed there were 8 individuals (EPFU, $n$ = 2; MYLU, $n$ = 5; MYSE, $n$ = 1) that consumed a non-volant order (Figure 2.8). There does not seem to be a distinct pattern or clustering of the individuals who consumed a non-volant order (Figure 2.8). Coupling this finding with the fact that only a small percentage of individuals in the dietary study included non-volant orders (EPFU, $n$ = 2 out of 89; MYLU, $n$ = 5 out of 52; MYSE, $n$ = 1 out of 27), I would argue morphology is not driving the foraging strategy used for prey selection in the three native bat species of North and South Dakota. An additional table with ear and forearm length values for individual who consumed a non-volant order is found in Appendix A (Table A15).

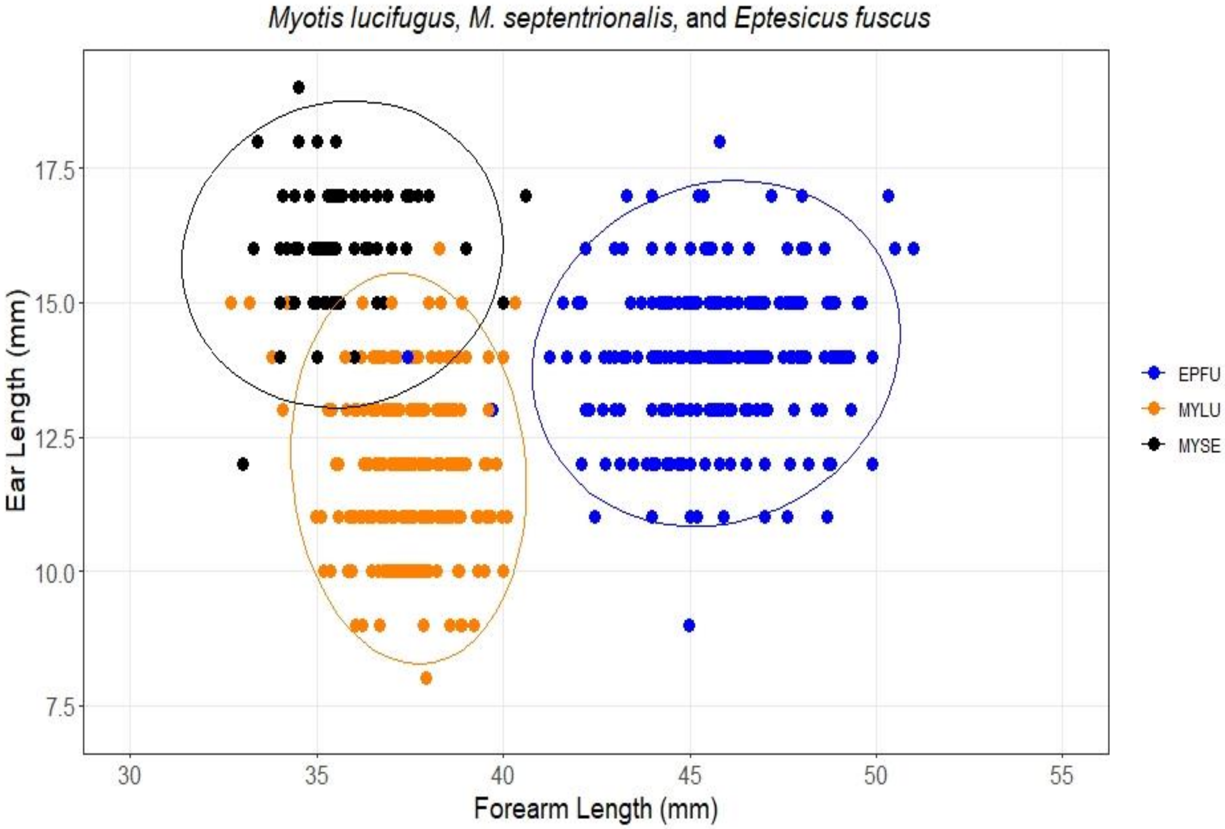

Figure 2.7: Ear and forearm length measurements for *E. fuscus* (EPFU), *M. lucifugus* (MYLU), and *M. septentrionalis* (MYSE) clustered by species to show overlaps of measured morphological characteristics.

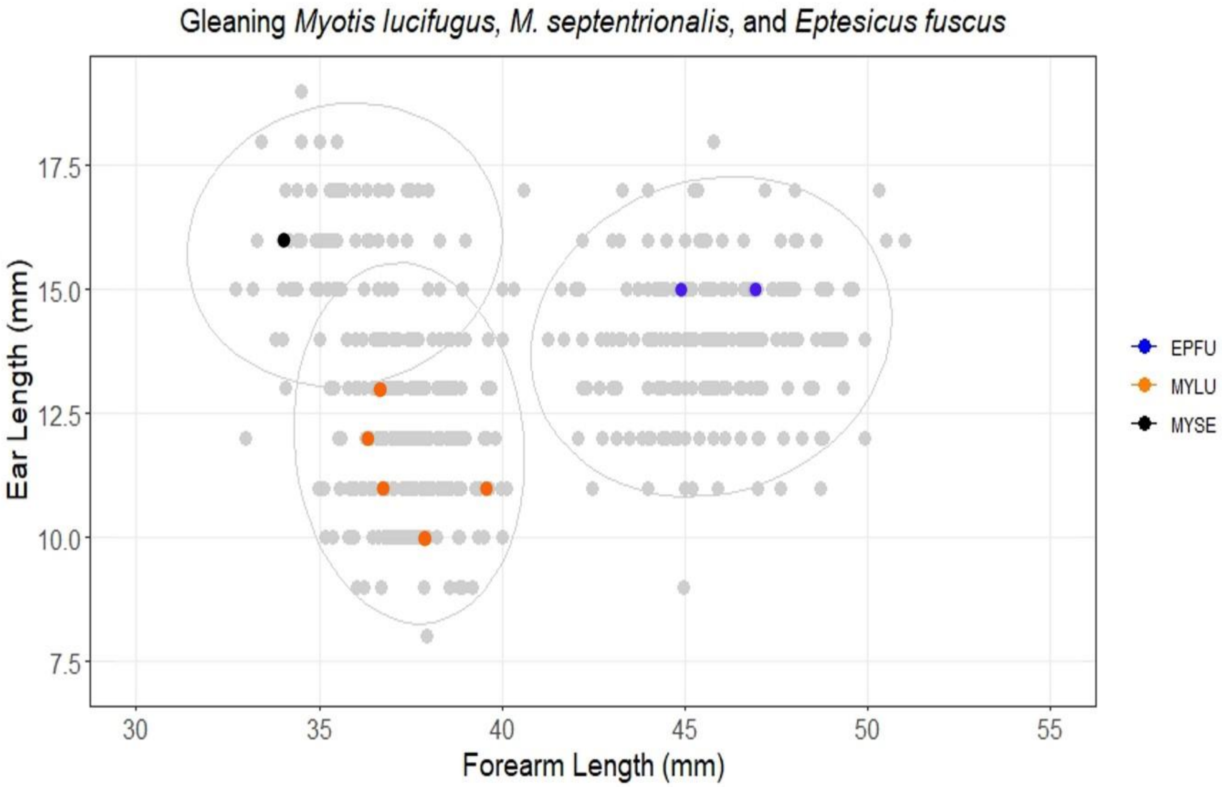

Figure 2.8: *E. fuscus* (EPFU), *M. lucifugus* (MYLU), and *M. septentrionalis* (MYSE) individuals that contained a non-volant order in their dietary profile (identified in Chapter 1).

## 2.5. Discussion

State comparisons of ear lengths found morphological differences between the conspecific populations of *E. fuscus* and *M. lucifugus* in North and South Dakota. Interestingly these findings suggest that neighboring populations of each species are potentially isolated enough to allow for the divergence of morphological characteristics. This is particularly interesting given the common assumption that some bats spending the summer in central and western North Dakota overwinter in the Black Hills of South Dakota. Alternatively, some bats do overwinter in western North Dakota (Barnhart and Gillam 2017), which could potentially lead to population isolation, but not enough is known about the proportion of the summer population overwintering in North Dakota to draw this conclusion.

For *M. lucifugus*, another reason for seen differences between the two states could be driven by the fact that the ranges of two subspecies (*M. l. lucifugus* and *M. l. carissima*) overlap within the study area (Fenton and Barclay 1980; Vonhof et al. 2015). This could also be the case for *E. fuscus*, as there are approximately 12 recognized subspecies, however studies identifying potential areas of range overlap are lacking.

My last question assessed an individual's morphology to its dietary composition to identify if morphology expression, known to make an individual more effective at a specific foraging strategy, aligned with dietary findings (see Chapter 1). I determined that ear morphology is not a driving factor in prey consumption for *E. fuscus, M. lucifugus* or *M. septentrionalis,* as there was no distinct pattern or clustering of the individuals who consumed a non-volant order (Figure 2.8).

I was not surprised to find a small number of *E. fuscus* and *M. lucifugus* individuals consuming non-volant orders as other studies have identified a small occurrence of these orders in their diets (Clare et al. 2014; Kaupas and Barclay 2017). The two *E. fuscus* individuals who consumed a non-volant order displayed ear lengths that were slightly above average and forearm lengths that were roughly average when compared to all individual *E. fuscus* captured (Figure 2.8; Appendix A: Table A15). Both *E. fuscus* individuals consumed the order Araneae so the occurrence of non-volant orders within the dietary profile was not simply due to individual or social grooming of ectoparasites. Interestingly, both *E. fuscus* individuals eating some non-volant prey were captured at sites located in South Dakota with high vegetative cover leading me to potentially conclude non-volant consumption is more likely associated with the primary habitat of an individual rather than morphological expression.

Five *M. lucifugus* individuals were identified to have consumed prey from a non-volant order and of these five, only one was captured in South Dakota. All but one of the individuals consumed a non-volant order that is not associated with individual or social grooming of ectoparasites. This confirms that four of the five individuals used a gleaning strategy at one point in time to capture prey. Looking at expressed morphology, all five *M. lucifugus* individuals had roughly average forearm lengths but ear length expression varied across the expressed range. However, none fell within the range of overlap that occurs between *M. lucifugus* and *M. septentrionalis* (Figure 2.8). Had all five *M. lucifugus* individuals expressed ear lengths that fell within the overlapping range between *M. lucifugus* and *M. septentrionalis* it could have been concluded that morphological structures, specifically ear length, potentially plays a role in determining foraging strategy. However, these individuals express morphological characteristics that are rather spread out across the range of all capture *M. lucifugus*, leading me to conclude morphology is not driving the foraging strategy used in these populations. Interestingly, because all but one individual was captured in North Dakota, primarily composed of open prairie, which is ideal for aerial hawking, habitat does not seem to be driving foraging strategy either.

I was very surprised that only one of the 27 *M. septentrionalis* captured had a dietary profile showing consumption of the order Araneae, a non-volant order. Multiple studies assessing *M. septentrionalis* have shown this species to feed on various Araneae species (Brack and Whitaker 2001; Whitaker 2004; Dodd et al. 2012; Kaupas and Barclay 2017). The *M. septentrionalis* individual who consumed Araneae displayed morphological characteristics that are roughly average when compared to all other *M. septentrionalis* individuals captured. Ultimately, due to only one individual consuming a non-volant order I am unable to make any conclusions as to how morphology, specifically ear morphology, is impacting *M. septentrionalis* dietary composition. Yet, this suggests that future work is needed on this species in South Dakota to better quantify how their diet and foraging strategy differ from other populations throughout their range.

Though this study assessed the diet and morphology of conspecifics at the population-level, further investigation is still needed. As all three species have rather wide geographic ranges, they need to be further examined to assess why significant morphological variation is being seen on a rather small spatial scale to identify if this is a similar trend seen across their ranges. Furthermore, there is a large difference in the sex-ratio of captured individuals seen between the two states which may need to be considered in future analyses.

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# CHAPTER 3: CONCLUSIONS

The objective of Chapter 1 was to determine the dietary composition and primary foraging strategies used by each of the 11 bat species found in North and South Dakota. These bat species were predicted to forage on a variety of volant and non-volant prey types, with unique dietary profiles for each species. Specifically, I tested the hypothesis that the insectivorous bats of North and South Dakota use foraging strategies that align with their typical morphological expressions. Foraging strategy was assessed by calculating the percent of volant or non-volant prey types found in the diet. My initial dietary composition expectations for North and South Dakota bat species was: *Corynorhinus townsendii, Myotis evotis,* and *M. septentrionalis* having dietary compositions containing higher percentages of non-volant prey orders (e.g. Araneae), and *Aeorestes cinereus* (formerly *Lasiurus cinereus*), *Eptesicus fuscus, Lasionycteris noctivagans, Lasiurus borealis, M. ciliolabrum, M. lucifugus, M. thysanodes* and *M. volans* having dietary compositions containing higher percentages of volant prey orders (e.g. Lepidoptera). My findings showed those initially predicted to have dietary profiles primarily containing non-volant orders (i.e. using a gleaning strategy) did not and those initally predicted to have dietary profiles primarily containing volant orders (i.e. using an aerial hawking strategy) did. However, some aerial hawking species also had non-volant orders present in their dietary profile, which suggests these species use both gleaning and aerial hawking strategies to capture prey.

The objective of Chapter 2 was to examine the relationship between dietary and morphometric data from three bat species: *M. lucifugus, M. septentrionalis,* and *E. fuscus.* Specifically, I 1) verified ear length was not correlated to body size in all three species, using forearm length as a measurement for body size, 2) assessed differences in ear morphology between North and South Dakota, and 3) assessed the morphology of individuals whose diet contained non-volant orders. My findings showed ear length and forearm length were not correlated in these three species of bats (i.e. a bigger bat ≠ longer ears). The initial state comparison between conspecific populations of *E. fuscus* and *M. lucifugus* found ear lengths to be longer in the South Dakota population for both species. However, due to a potential sampling bias driving this result, I reassessed by splitting these populations by sex and still found ear lengths to be longer in the South Dakota population. My assessment of individuals who consumed a non-volant order did not find ear morphology significantly different from those that did not consume non-volant prey.

These studies, to the best of my knowledge, are the first to examine intraspecific links between dietary preferences and morphological traits in bats. Not only is this a significant contribution in understanding plasticity and adaptation to local environments, but it will also provide key ecological information for development of effective conservation plans. From a management perspective, understanding the foraging preference of a species can provide important insight into habitat use (Clare et al. 2011), patterns of activity over space and time (Jones and Rydell 1994), metabolic rate (McNab 2003), risk of extinction (Boyles and Storm 2007), and even the ability to resist pathogens (Frank 2012). Bats also play a key role in regulating insect communities; if these prey communities are shifting, it is important to understand if local bat populations will be morphologically or behaviorally plastic.

Gaining an understanding of local bat populations dietary compositions could also be important to agroecosystems as roughly 47% of land in North and South Dakota is used for agricultural purposes (United States Department of Agriculture 2017). Findings could be relevant to farmers if bats were found to be primarily consuming agricultural pests. This information could help farmers decide when and whether to apply pesticides, potentially leading to identification of ideal spray intervals, preventing the overuse of chemical pesticides, and reduce insect resistance. From an economic perspective, this would cut chemical pesticide labor costs associated with pesticide application.

Finally, pressure from the fungus that causes White-Nose Syndrome makes this project crucial, contributing to our understanding of the ecological services and dietary needs of resident bat populations. Understanding population-level differences in foraging needs can provide managers with important details about how ecosystems with severely declining bat populations from White-Nose Syndrome will be impacted.

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# APPENDIX A. ADDITIONAL INFORMATION

Table A1: North and South Dakota sampling sites for 2016 - 2019 summer field seasons. Latitude and Longitude represent general locations sampled as multiple nets were set at each site.

| Site Name | State | Location | Latitude | Longitude |
|---|---|---|---|---|
| Ekre Ranch | ND | East | 46.55407 | -97.13239 |
| Hankinson Hills | ND | East | 46.12536 | -96.96165 |
| Mirror Pool WMA | ND | East | 46.53927 | -100.9996 |
| Cross Ranch State Park | ND | Central | 47.215199 | -100.998221 |
| Anamoose | ND | Central | 47.878643 | -100.243487 |
| Painted Woods Bridge | ND | Central | 47.241887 | -100.955413 |
| Painted Woods WMA | ND | Central | 47.249625 | -100.959455 |
| Ft. Lincoln State Park | ND | Central | 46.761272 | -100.843983 |
| United Tribes Technical College | ND | Central | 46.769881 | -100.758942 |
| Sheyenne Stock Pond | ND | Central | 46.37287 | -100.9996 |
| Ft. Mandan | ND | Central | 47.29843 | -101.08733 |
| Menoken Barn | ND | Central | 46.814823 | -100.4666 |
| Pigeon Point | ND | Central | 46.49915 | -100.9996 |
| McDowell Dam | ND | Central | 46.83024 | -100.63864 |
| Morton County (Wind Farm) | ND | Central | 46.9786 | -101.55173 |
| Metigoshe State Park | ND | Northern | 48.99584 | -100.334 |
| Homen State Forest | ND | Northern | 48.94658 | -100.252 |
| Peaceful Valley Ranch, South Unit, THRO | ND | Western | 46.959311 | -103.501229 |
| Juniper Camp Picnic Area, North Unit, THRO | ND | Western | 46.948131 | -103.530542 |
| Pond 3, North Unit, THRO | ND | Western | 47.594114 | -103.315617 |
| Culvert, North Unit, THRO | ND | Western | 47.602006 | -103.278864 |

Table A1: North and South Dakota sampling sites for 2016 - 2019 summer field seasons. Latitude and Longitude represent general locations sampled as multiple nets were set at each site. (continued)

| Site Name | State | Location | Latitude | Longitude |
|---|---|---|---|---|
| Badland Spur Trail, South Unit, THRO | ND | Western | 46.959685 | -103.407271 |
| Dickinson Funeral Home | ND | Western | 46.8806 | -102.8151 |
| Grassland River | ND | Western | 47.3189 | -103.6234 |
| Ponderosa | ND | Western | 46.6033 | -103.4722 |
| LMNG-E Reservoir | ND | Western | 46.8568 | -103.6628 |
| Jones Creek, South Unit, THRO | ND | Western | 46.96855 | -103.421 |
| Cottonwood Picnic Area, South Unit, THRO | ND | Western | 46.94761 | -103.531 |
| Hettinger County (Wind Farm) | ND | Western | 46.6574 | -102.7469 |
| Stark County (Wind Farm) | ND | Western | 46.6462 | -102.761 |
| 668 Minipond 1, USFS | SD | Southern | 43.76687 | -103.880109 |
| 668 Minipond 2, USFS | SD | Southern | 43.766251 | -103.886264 |
| 681 Stocktank, USFS | SD | Southern | 43.748001 | -103.781897 |
| Blacktail Spring, USFS | SD | Southern | 43.756401 | -103.864998 |
| Chokecherry Spring, NPS | SD | Southern | 43.721515 | -103.827325 |
| Lithograph Spring, USFS | SD | Southern | 43.732498 | -103.809998 |
| Lower Bat Pond, USFS | SD | Southern | 43.7178 | -103.831001 |
| Mann Pond, USFS | SD | Southern | 43.716491 | -103.876619 |
| Mud Spring, USFS | SD | Southern | 43.7687 | -103.859001 |
| Stock Pond 3, USFS | SD | Southern | 43.767201 | -103.877998 |
| Stock Pond 8, USFS | SD | Southern | 43.769908 | -103.879144 |
| Tepee Spring, USFS | SD | Southern | 43.7589 | -103.885002 |
| Upper Bat Pond, USFS | SD | Southern | 43.71719 | -103.829592 |
| Windmill Pond, USFS | SD | Southern | 43.762182 | -103.780919 |

Table A2: Additional primer information.

| Taxonomy Detection | Primer name | Sequence | Annealing Temp | Reference(s) |
|---|---|---|---|---|
| Wide taxonomic coverage of invertebrates | LCO1490 | 5’-GGT CAA CAA ATC ATA AAG ATA TTG G-3’ | 60°C | Herbert et al. 2003 |
| | HCO2198 | 5’-TAA ACT TCA GGG TGA CCA AAA AAT CA-3’ | | Folmer et al. 1994 |
| Diptera, Coleoptera, Arachnida, Collembola, and other small orders | C_LepFolF | 5’- ATT CAA CCA ATC ATA AAG ATA TTG G -3’ | 50°C | DeWaard et al. 2019 |
| | C_LepFolR | 5’- TAA ACT TCT GGA TCT CCA AAA AAT CA -3’ | | |

Table A3: Percent Occurrence (%O) of orders for North and South Dakota bat species. %O values = [number of fecal samples containing an order]/ [total number of individuals] x 100. *Combined %O values of Blattodea, Isopoda, Ixodida, Neuroptera, Orthoptera, Plecoptera, Siphonaptera, Thysanoptera, Tricoptera, Trombidiformes.*

| Bat Species | Abbrev. | *n* | Lepidoptera | Coleoptera | Diptera | Ephemeroptera | Hemiptera | Hymenoptera | Odonata | Araneae | Other* | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Aeorestes cinereus* | LACI | 8 | 100.0 | 12.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 112.5 |
| *Corynorhinus townsendii* | COTO | 2 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| *Eptesicus fuscus* | EPFU | 89 | 37.1 | 28.1 | 29.2 | 3.4 | 10.1 | 6.7 | 1.1 | 2.3 | 6.7 | 124.7 |
| *Lasionycteris noctivagans* | LANO | 12 | 25.0 | 41.7 | 41.7 | 8.3 | 33.3 | 16.7 | 0.0 | 0.0 | 16.7 | 183.4 |
| *Lasiurus borealis* | LABO | 3 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| *Myotis ciliolabrum* | MYCI | 3 | 33.3 | 33.3 | 66.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 133.3 |
| *Myotis evotis* | MYEV | 1 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100.0 |
| *Myotis lucifugus* | MYLU | 52 | 28.9 | 13.5 | 67.3 | 9.6 | 7.7 | 13.5 | 7.7 | 5.8 | 11.5 | 165.5 |
| *Myotis septentrionalis* | MYSE | 27 | 63.0 | 3.7 | 29.6 | 0.0 | 3.7 | 0.0 | 0.0 | 3.7 | 0.0 | 103.7 |
| *Myotis thysanodes* | MYTH | 36 | 63.9 | 16.7 | 25.0 | 0.0 | 11.1 | 13.9 | 8.3 | 0.0 | 5.6 | 144.5 |
| *Myotis volans* | MYVO | 60 | 76.7 | 5.0 | 20.0 | 0.0 | 10.0 | 5.0 | 0.0 | 1.7 | 3.3 | 121.7 |

Table A4: Percent Occurrence (%O) of non-volant order found in the dietary profiles for North and South Dakota bat species. %O values = [number of fecal samples containing an order]/ [ total number of individuals] x 100.

| Bat Species | Abbrev. | *n* | Araneae | Blattodea | Isopoda | Ixodida | Siphonaptera | Thysanoptera | Trombidiformes | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| *Eptesicus fuscus* | EPFU | 89 | 2.3 | 1.1 | 0.0 | 1.1 | 0.0 | 1.1 | 1.1 | 6.7 |
| *Lasionycteris noctivagans* | LANO | 12 | 0.0 | 0.0 | 16.7 | 0.0 | 0.0 | 0.0 | 0.0 | 16.7 |
| *Myotis lucifugus* | MYLU | 52 | 5.8 | 0.0 | 1.9 | 1.9 | 1.9 | 0.0 | 3.9 | 15.4 |
| *Myotis septentrionalis* | MYSE | 27 | 3.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.7 |
| *Myotis thysanodes* | MYTH | 36 | 0.0 | 0.0 | 0.0 | 2.8 | 0.0 | 0.0 | 0.0 | 2.8 |
| *Myotis volans* | MYVO | 60 | 1.7 | 0.0 | 1.7 | 0.0 | 0.0 | 0.0 | 0.0 | 3.3 |

Table A5: Percent Frequency Occurrence (%FO) of orders for North and South Dakota bat species. %FO values = [number of fecal samples containing an order]/ [total number of all order occurrences] x 100. **Combined %FO values of Blattodea, Isopoda, Ixodida, Neuroptera, Orthoptera, Plecoptera, Siphonaptera, Thysanoptera, Tricoptera, Trombidiformes.*

| Bat Species | Abbrev. | *n* | Lepidoptera | Coleoptera | Diptera | Ephemeroptera | Hemiptera | Hymenoptera | Odonata | Araneae | Other* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Aeorestes cinereus* | LACI | 8 | 88.9 | 11.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Corynorhinus townsendii* | COTO | 2 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Eptesicus fuscus* | EPFU | 89 | 29.7 | 22.5 | 23.4 | 2.7 | 8.1 | 5.4 | 0.9 | 1.8 | 5.5 |
| *Lasionycteris noctivagans* | LANO | 12 | 13.6 | 22.7 | 22.7 | 4.6 | 18.2 | 9.1 | 0.0 | 0.0 | 9.1 |
| *Lasiurus borealis* | LABO | 3 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Myotis ciliolabrum* | MYCI | 3 | 25.0 | 25.0 | 50.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Myotis evotis* | MYEV | 1 | 100.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| *Myotis lucifugus* | MYLU | 52 | 17.4 | 8.1 | 40.7 | 5.8 | 4.7 | 8.1 | 4.7 | 3.5 | 7.0 |
| *Myotis septentrionalis* | MYSE | 27 | 60.7 | 3.6 | 28.6 | 0.0 | 3.5 | 0.0 | 0.0 | 3.6 | 0.0 |
| *Myotis thysanodes* | MYTH | 36 | 44.2 | 11.5 | 17.3 | 0.0 | 7.7 | 9.6 | 5.7 | 0.0 | 3.8 |
| *Myotis volans* | MYVO | 60 | 63.0 | 4.1 | 16.4 | 0.0 | 8.2 | 4.1 | 0.0 | 1.4 | 2.8 |

Table A6: Percent Frequency Occurrence (%FO) of non-volant order found in the dietary profiles for North and South Dakota bat species. %FO values = [number of fecal samples containing an order]/ [total number of all order occurrences] x 100.

| Bat Species | Abbrev. | *n* | Araneae | Blattodea | Isopoda | Ixodida | Siphonaptera | Thysanoptera | Trombidiformes | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| *Eptesicus fuscus* | EPFU | 89 | 1.8 | 0.9 | 0.0 | 0.9 | 0.0 | 0.9 | 0.9 | 5.4 |
| *Lasionycteris noctivagans* | LANO | 12 | 0.0 | 0.0 | 9.1 | 0.0 | 0.0 | 0.0 | 0.0 | 9.1 |
| *Myotis lucifugus* | MYLU | 52 | 3.5 | 0.0 | 1.2 | 1.2 | 1.2 | 0.0 | 2.3 | 9.4 |
| *Myotis septentrionalis* | MYSE | 27 | 3.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 3.6 |
| *Myotis thysanodes* | MYTH | 36 | 0.0 | 0.0 | 0.0 | 1.9 | 0.0 | 0.0 | 0.0 | 1.9 |
| *Myotis volans* | MYVO | 60 | 1.4 | 0.0 | 1.4 | 0.0 | 0.0 | 0.0 | 0.0 | 4.2 |

Table A7: Analysis of Variance and fit linear model for all *E. fuscus* scatterplot.

| | Df | Sum Sq | Mean Sq | F value | *P*-value |
|---|---|---|---|---|---|
| Forearm | 1 | 6.649 | 6.649 | 3.91 | 0.04898 |
| Residuals | 282 | 479.6 | 1.701 | NA | NA |

*Fitting linear model: Ear Length ~ Forearm Length*

| Observations | Residual Std. Error | $R^2$ | Adjusted $R^2$ |
|---|---|---|---|
| 284 | 1.304 | 0.01368 | 0.01018 |

Table A8: Analysis of Variance and fit linear model for *E. fuscus* scatterplot with outliers removed.

| | Df | Sum Sq | Mean Sq | F value | *P*-value |
|---|---|---|---|---|---|
| Forearm | 1 | 5.374 | 5.374 | 3.133 | 0.07781 |
| Residuals | 279 | 478.6 | 1.715 | NA | NA |

*Fitting linear model: Ear Length ~ Forearm Length*

| Observations | Residual Std. Error | $R^2$ | Adjusted $R^2$ |
|---|---|---|---|
| 281 | 1.31 | 0.0111 | 0.00756 |

Table A9: Analysis of Variance table for all *E. fuscus* boxplot.

| | Df | Sum Sq | Mean Sq | F value | *P*-value |
|---|---|---|---|---|---|
| State | 1 | 44.63 | 44.63 | 28.5 | 1.928e-07 |
| Residuals | 282 | 441.6 | 1.566 | NA | NA |

Table A10: Analysis of Variance table for female *E. fuscus* boxplot.

| | Df | Sum Sq | Mean Sq | F value | *P*-value |
|---|---|---|---|---|---|
| State | 1 | 34.59 | 34.59 | 17.86 | 4.652e-05 |
| Residuals | 120 | 232.3 | 1.936 | NA | NA |

Table A11: Analysis of Variance and fit linear model for *M. lucifugus* scatterplot.

|  | Df | Sum Sq | Mean Sq | F value | *P*-value |
|---|---|---|---|---|---|
| Forearm | 1 | 4.174 | 4.174 | 1.921 | 0.1671 |
| Residuals | 235 | 510.6 | 2.173 | NA | NA |

*Fitting linear model: Ear Length ~ Forearm Length*

| Observations | Residual Std. Error | $R^2$ | Adjusted $R^2$ |
|---|---|---|---|
| 237 | 1.474 | 0.008108 | 0.003888 |

Table A12: Analysis of Variance table for all *M. lucifugus* boxplot.

|  | Df | Sum Sq | Mean Sq | F value | *P*-value |
|---|---|---|---|---|---|
| State | 1 | 96.68 | 96.68 | 54.34 | 2.86e-12 |
| Residuals | 235 | 418.1 | 1.779 | NA | NA |

Table A13: Analysis of Variance table for male *M. lucifugus* boxplot.

|  | Df | Sum Sq | Mean Sq | F value | *P*-value |
|---|---|---|---|---|---|
| State | 1 | 61.46 | 61.46 | 30.25 | 3.327e-07 |
| Residuals | 93 | 189 | 2.032 | NA | NA |

Table A14: Analysis of Variance and fit linear model for *M. septentrionalis* scatterplot.

|  | Df | Sum Sq | Mean Sq | F value | *P*-value |
|---|---|---|---|---|---|
| Forearm | 1 | 0.4891 | 0.4891 | 0.368 | 0.5456 |
| Residuals | 90 | 119.6 | 1.329 | NA | NA |

*Fitting linear model: Ear Length ~ Forearm Length*

| Observations | Residual Std. Error | $R^2$ | Adjusted $R^2$ |
|---|---|---|---|
| 92 | 1.153 | 0.004072 | -0.006994 |

Table A15: Forearm and ear length measurements for *E. fuscus*, *M. lucifugus*, and *M. septentrionalis* individual who consumed non-volant orders and the overall forearm and ear length measurements averages for all captured individuals of each species.

| | | Forearm Length | | | Ear Length | | |
|---|---|---|---|---|---|---|---|
| *ID* | *State* | *Eptesicus fuscus* | *Myotis lucifugus* | *Myotis septentrionalis* | *Eptesicus fuscus* | *Myotis lucifugus* | *Myotis septentrionalis* |
| EF1 | SD | 46.90 | - | - | 15 | - | - |
| EF2 | SD | 44.90 | - | - | 15 | - | - |
| ML1 | SD | - | 36.60 | - | - | 13 | - |
| ML2 | ND | - | 37.85 | - | - | 10 | - |
| ML3 | ND | - | 39.53 | - | - | 11 | - |
| ML4 | ND | - | 36.32 | - | - | 12 | - |
| ML5 | ND | - | 36.75 | - | - | 11 | - |
| MS1 | SD | - | - | 34.00 | - | - | 16 |
| *Overall Average:* | | 45.72 | 37.46 | 35.69 | 14.01 | 11.92 | 15.87 |