

MIGRATORY ECOLOGY AND WING MORPHOLOGY OF NORTH DAKOTA BATS

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Kevin Michael Cortes

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Program:
Environmental and Conservation Science

January 2019

Fargo, North Dakota

North Dakota State University
Graduate School

Title

MIGRATORY ECOLOGY AND WING MORPHOLOGY OF NORTH
DAKOTA BATS

By

Kevin Michael Cortes

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

SUPERVISORY COMMITTEE:

Erin Gillam

Chair

Ned Dochtermann

Torre Hovick

Approved:

January 9, 2019

Date

Kendra Greenlee

Department Chair

ABSTRACT

Understanding links between a species' migratory behavior, wing morphology, and foraging strategy can provide insight into factors driving the evolution of behavioral and ecological responses. The first chapter of this thesis examines the use of a river corridor as a migratory guide for bats. Results indicated no seasonal movement along the Missouri River corridor, indicating that the river was not being used as a key feature for migration. These results align with other recent studies. The second chapter examined the impacts of foraging habitat on wing morphology in two bat species. Expected differences between species were found in the wing shapes of the study species. No biologically significant differences were identified within species across different habitats. High gene flow and preferential habitat use may explain this. The results of these studies build upon past work on bats in North Dakota and provide insight into the behavior and morphology of bats.

ACKNOWLEDGEMENTS

The author thanks Dr. Erin Gillam, Dr. Ned Dochterman, Dr. Torre Hovick, Dr. JJ Nelson, Dr. Raphael Royaute, Nick Johnson, Monica Anderson-Berdal, and Courtney Garrison. Thanks to NDSU Biological Sciences, NDSU ECS program, and NDSU College and Science and Mathematics for support.

DEDICATION

I would like to dedicate this to my parents for their love and support and all of the wonderful people I've met at North Dakota State University who have supported me with insight, mentorship, and friendship over the last three years. This work would never have been completed without them.

TABLE OF CONTENTS

ABSTRACT iii

ACKNOWLEDGEMENTS iv

DEDICATION v

LIST OF TABLES viii

LIST OF FIGURES ix

LIST OF ABBREVIATIONS x

LIST OF APPENDIX TABLES xi

CHAPTER 1: IMPORTANCE OF MIGRATORY CORRIDORS FOR TEMPERATE
BATS 1

 Abstract 1

 Introduction 1

 Methods 4

 Study Area 4

 Acoustic Detection 5

 Analysis of Directionality 6

 Statistical Methods 7

 Results 8

 Discussion 10

 References 13

CHAPTER 2: INFLUENCE OF FORAGING HABITAT AVAILABILITY ON WING
SHAPE IN TWO NORTH DAKOTA BAT SPEICES 19

 Abstract 19

 Introduction 19

 Methods 23

 Results 26

Species-comparison	27
<i>M. lucifugus</i>	27
<i>E. fuscus</i>	28
Discussion	30
References	33
CHAPTER 3: CONCLUSIONS	38
References	40
APPENDIX. ADDITIONAL INFORMATION.....	43

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1.1. AIC table of a priori models. The GLMM with Species and Season was ranked as the top model.	10

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1.1. Map of study sites along the Missouri River. Five sampling sites from 2016 (two sites, black dots) and 2017 (three sites, white dots) are displayed.	4
1.2. Graphical representation of methods adapted and modified for this study. See Jarzembowski (2003) and Furmankiewicz and Kucharska (2009) for original methods.	6
1.3. Chi-Square tests found significant differences in seasonal directional patterns for <i>Lasionycteris noctivagans</i> and <i>Lasiurus borealis</i> in the Spring. These species more southward than northward in the Spring. No other significant differences were found.....	9
2.1. Capture sites across North Dakota from which morphological data were collected. Western sites are shown with black dots (nine), and central sites are shown with white dots (six). The Missouri River is shown in black.....	23
2.2. Landmark (filled circles) and semilandmark (open circle) placement on a bat wing. Landmarks are highly repeatable, while semilandmarks may vary in relative placement.....	25
2.3. Deformation grids of mean wing shapes of compared groups as they differ from the overall mean shape. A. depicts how <i>M. lucifugus</i> (left) and <i>E. fuscus</i> (rights); B. depicts <i>M. lucifugus</i> from central sites (left) and western sites (right); C. depicts <i>E. fuscus</i> from central sites (left) and western sites (right).....	29

LIST OF ABBREVIATIONS

AIC.....	Akaike's Information Criterion
ANOVA.....	Analysis of Variance
GLMM.....	Generalized Linear Mixed Effect Model
GPS.....	Global Positioning Systems
MANCOVA.....	Multivariate Analysis of Covariance
RW.....	Relative Warp
SD.....	Secure Digital

LIST OF APPENDIX TABLES

<u>Table</u>	<u>Page</u>
A1. Landmark and semilandmarks descriptions used to assess bat wing shape.....	43
A2. MANCOVA results for between species analysis (combined dataset of <i>M. lucifugus</i> and <i>E. fuscus</i>).	44
A3. MANCOVA results for within species analysis of <i>M. lucifugus</i>	44
A4. MANCOVA results for within species analysis of <i>E. fuscus</i>	45

CHAPTER 1: IMPORTANCE OF MIGRATORY CORRIDORS FOR TEMPERATE BATS

Abstract

Investigating landscape level movement patterns of migratory animals can often be challenging, yet this is a major component of an animal's life history and behavior. In particular, migration by bats has historically been difficult to characterize, yet research in recent decades has progressed much in the field of bat migratory ecology. It has been largely accepted that rivers and other linear landscape features may be important migratory corridors for bats during both long and short distance migrations. However, this idea is primarily based on a handful of studies throughout the world, and recent studies have questioned the importance of linear features in bat migration, warranting further evaluation. The goal of this work was to assess the migratory behavior of multiple temperate bat species along a major river corridor by using seasonal directional passes as an estimate for migratory behavior. Count comparisons within species and across seasons demonstrated significant differences in directionality in one season for two species. However, the variation explained, as estimated from generalized linear mixed effects models found, that the effects of season and species explained less than one percent of the variation of directional passes, potentially indicating an absence of season-specific movement patterns along the studied river corridor.

Introduction

Migratory behavior has evolved independently in a variety of taxa including insects, reptiles, birds, and mammals and plays a critical role in mate and resource acquisition. Although migration is generally high risk and incurs considerable energetic costs, these are outweighed by the benefits of reaching habitat rich in forage, mates, and shelter (Avgar et al. 2014). How and

via what pathways migratory animals travel during migration are questions researchers have only recently been able to answer with precision, with modern technologies being largely responsible for this progress. Most notably, advancements in global positioning systems (GPS) satellite telemetry have led to a greater understanding of the mechanisms and routes taken by some large birds during migration (García-Ripollés et al. 2010; Bouten et al. 2013). However, many animals are too small to bear the additional costs of carrying current GPS tracking devices (Aldridge and Brigham 2014). Despite the relative difficulties of investigating the migratory ecology of such animals, it is essential that these species be given due attention to further understand the mechanics of seasonal migration, especially with the rise of introduced diseases across the globe (Kilpatrick et al. 2010; Froschauer and Coleman 2012).

Many species of temperate bats regularly undertake seasonal migrations. The scale of these migrations is largely linked to species variation in roosting requirements and hibernation behavior (Fleming and Eby 2003). Sedentary bats typically roost in trees or geologic cavities during the summer and utilize nearby hibernacula during the winter. In the absence of nearby hibernacula, these bats can exhibit short-range migratory behavior, migrating distances in the hundreds of kilometers to suitable hibernacula. These bats are classified as “regional migrants (Griffin 1970; Krauel and McCracken 2013.” Exclusively foliage roosting bats tend to migrate greater distances (>1000km) for winter roosting habitat; these bats are known as “long-distance migrants” and do not typically engage in seasonal hibernation (Fleming and Eby 2003). Understanding these different strategies is essential for effective species-specific management and for identification of potential threats during migration

Population genetic studies (Kerth et al. 2003; Moussy et al. 2013; Johnson et al. 2014), and stable isotope analyses (Cryan et al. 2004; Sullivan et al. 2012; Fraser et al. 2017) have

provided information about broad-scale patterns of movement, yet the specific pathways bats take while migrating remain largely unclear (Cryan 2003; Baerwald et al. 2014). Evidence has supported the hypothesis that some tropical species, particularly nectar and fruit bats, migrate along forage corridors where fruits and flowers become sequentially abundant along a geographic gradient (Fleming et al. 1993). However, this sort of behavior has not been observed in temperate insectivorous bats. Temperate bats are instead thought to rely on linear landscape features, in addition to magnetic compasses, as primary guides during seasonal migration (Wang et al. 2007; Holland et al. 2008; Furmankiewicz and Kucharska 2009; Ijäs et al. 2017). Several studies have supported the hypothesis that bats use linearly oriented bodies of water (i.e. coasts and rivers) as migratory corridors (Jarzembowski 2003; Furmankiewicz and Kucharska 2009; Rydell et al. 2014; Ijäs et al. 2017). However, recent work in North America using GPS trackers and broad spatial scale telemetry have suggested geographic corridors may not be as important to bats during migration as once thought (Weller et al. 2016; Krauel et al. 2017).

Investigations of temperate bat migratory corridors, especially rivers, are extremely limited; there is only one study that is frequently cited that directly assesses the use of rivers as migratory corridors by temperate bats (Furmankiewicz and Kucharska 2009). In this study, we aimed to document mass seasonal movements of sedentary/regional and long-distance migratory bats along a major river corridor in North America. We hypothesized that both /regional and long distance migratory bats residing in riparian habitat with little to no surrounding natural geologic hibernacula would display season-specific movements along a river (i.e., northward in Spring, southward in Fall). Although these bats typically exhibit different migratory strategies, the absence of known hibernacula paired with the proximity of a major riparian corridor suggests both regional migrants and long-distance migrants may result in convergent migratory strategies.

We used paired microphones of high-frequency acoustic detectors at multiple sites along a major river corridor to determine directionality of bat flight, with the goal of investigating the role of a riparian corridors in seasonal migration.

Methods

Study Area

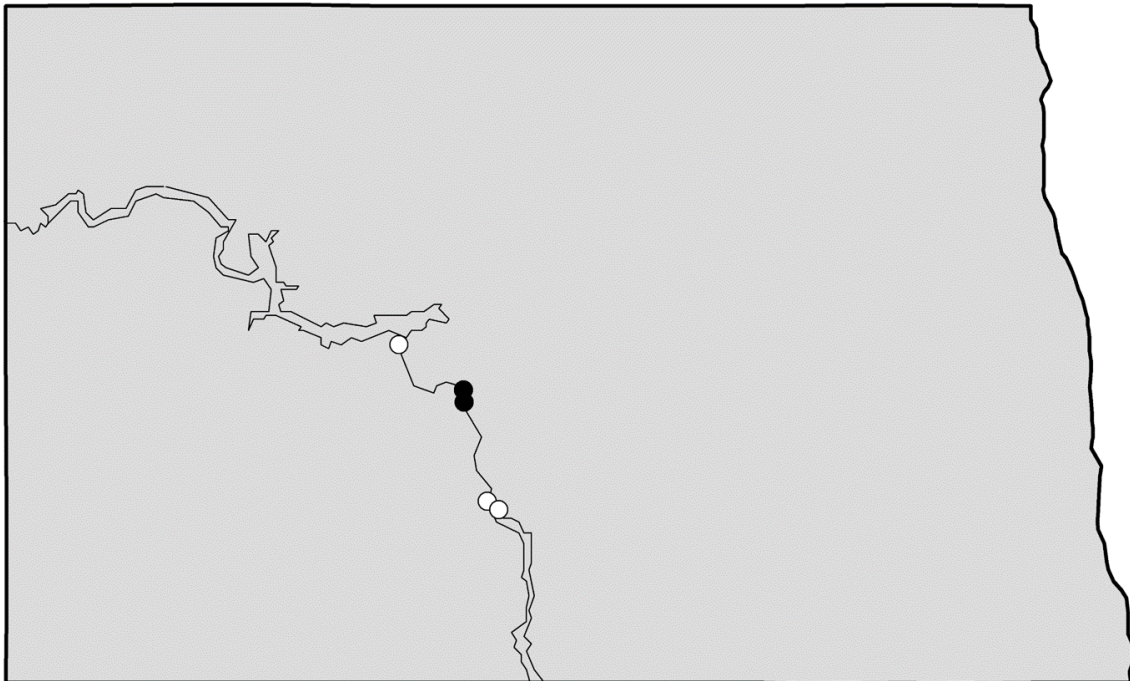


Figure 1.1. Map of study sites along the Missouri River. Sampling sites from 2016 (two sites, black dots) and 2017 (three sites, white dots) are displayed.

The riparian areas along the North Dakota segment of the Missouri River are comprised mainly of cottonwood stands, grasslands, and wetlands, and house many resident bat species (Johnson et al. 1976, 2012; Nelson et al. 2015). These riparian areas are surrounded by agriculture and prairie, creating a relatively narrow corridor of optimal bat habitat. Although the Missouri River Valley offers habitat rich in summer foraging and roosting resources, the absence of natural hibernacula or widely used anthropogenic alternatives, such as mines, necessitates bats vacate this area for the winter months. Since the Missouri River Valley is the largest natural

North-South running landscape feature in the region, it has the potential to act as a migratory corridor for bats.

Acoustic Detection

We used acoustic detectors to continuously monitor the passage of bats along a riparian corridor of the Missouri River Valley in central North Dakota. Two detectors were deployed from March 25th until October 22nd 2016, and three detectors were deployed from April 7th until October 20th in 2017. Sampling was continuous with the exception of sporadic gaps due to equipment malfunction. The end of the first week of June and the beginning of the last week of August were set as dates for the beginning and end of Summer, respectively. All calls recorded prior to the end of the first week of June were classified as Spring, and all calls recorded after the beginning of the last week of August were classified as Fall. Sites spanned a ~100km along the Missouri River, which varied in width from 300-1000 meters at any individual site. Specifically, sites were located within Cross Ranch State Park, Fort Lincoln State Park, Riverdale Wildlife Management Area, and Sibley Nature Park (Fig. 1).

Acoustic monitoring methods were adapted and modified from past studies to assess migratory behavior (Jarzembowski 2003; Furmankiewicz and Kucharska 2009). At each site, SM3BAT (Wildlife Acoustics, Concord, Massachusetts) acoustic detectors were used to record calls of passing bats and, when possible, determine their flight direction. Detectors were set to record from 30 minutes prior to sunset until 30 minutes after sunrise based on each unit's specific GPS coordinates. Two microphones linked to these detectors were mounted on tripods 0.5m from the ground, aimed away from each other, angled slightly towards the river, and were spaced 10m apart parallel with the river (Fig. 1). When one of the microphones was triggered, both began recording simultaneously. Recordings from these microphones were automatically

combined into a single stereo file with two channels. To maximize battery life and allow continuous nightly sampling, the detectors were connected to 12V car batteries and charged daily with 10W solar panels. Memory SD cards (64 GB) were exchanged every two to three weeks. The bat classification software, SonoBat (version 3.03 MT plains, SonoBat, Arcata, CA), was used to analyze and identify bat calls to the species level with 0.95 confidence.

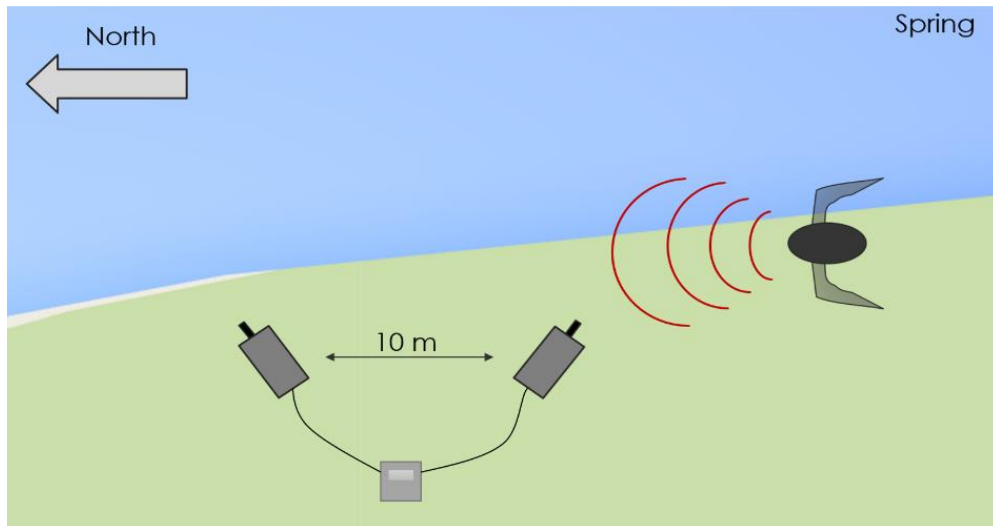


Figure 1.2. Graphical representation of methods adapted and modified for this study. See Jarzembowski (2003) and Furmankiewicz and Kucharska (2009) for original methods.

All research conducted on live animals adhered to the guidelines of the American Society of Mammalogists (Sikes 2016). This research was approved by the North Dakota State University Institutional Animal Care and Use Committee (Protocol #A16056).

Analysis of Directionality

Calls from each stereo channel were batch analyzed using the Pulse Train Analysis function in the acoustics software, SASLab Pro (Avisoft Bioacoustics, Berlin, Germany). A baseline amplitude threshold of 0.15V was chosen to ensure nearness of assessed animals. Due to numerous insect calls overlapping with those of bat calls in both amplitude and frequency, a pulse duration maximum of 0.65s was used to exclude recordings with insect calls.

Directionality was assigned by assessing differences in time of call arrival at each microphone. The Pulse Train Analysis output from each stereo channel was used to obtain the time at which a call was first detected based on the 0.15V threshold of each microphone. Directional movements were defined by occurrences when calls were recorded on both channels with a 0.89—1.82 second difference, with microphone sequence determining whether the movements were northward or southward. Movements with calls on a single channel or detection differences of less than 0.89s or greater than 1.82s were considered noninformative, as it could not be inferred whether bats were flying east/west or performing some other form of behavior. The “directional” range was chosen based on the theoretical minimum and maximum flight speeds of *Myotis sodalis* calculated in another migratory study (Krauel et al. 2017) and is comparable to what others have found when examining foraging and commuting flight speeds in other species (Salcedo et al. 1995; Grodzinski et al. 2009). Each microphone was consistently pointed either northward or southward. The difference between the timing of first pulse detection for each microphone was used to assess direction: positive values were classified as northward movements and negative values were classified as southward.

Statistical Methods

Chi-square tests were used to assess whether differences existed in species-specific seasonal directionality counts (five 2x3 tables). A Bonferroni correction was applied to adjust for potential family-wise error, and Pearson residuals were then calculated for each contingency table to evaluate significant sources of deviance from expected values. Observed values were expected to differ from the expected during the Spring and Fall seasons, as this would indicate nonrandom, directional movement patterns.

Three *a priori* binomial generalized linear mixed effects models (GLMMs) and a null GLMM were generated with the R package “lme4” using fixed effects to compare species grouping hypotheses. These hypotheses varied by whether all species behaved the same (all species grouped together), if migratory behavior varied by species (all species independently grouped), or if species varied by traditional migratory type classification (species grouped by type of migrant: sedentary/regional or long distance). All models included season as a fixed effect as well as site and year as random effects. Akaike’s Information Criterion (AIC) was used to select the best model, and the top model was chosen for further analysis.

To further assess the suitability of the top model, variation explained was calculated. The variation explained by a model is used to assess a model’s ability to explain the data irrespective of p-values (Nakagawa and Schielzeth 2010). Variation explained for the fixed and random effects were calculated using the rpt function of the R package, “rptR,” set for binary datatype, logit link, and using 1000 bootstraps. All statistical analyses were carried out using R (version 3.4.3; R Core Team, 2017)

Results

In 2016 and in 2017, 254,520 and 287,919 stereo recordings were collected, respectively, and nine species were identified by Sonobat. After filtering for classifications made with at least 95% confidence, removing recordings with insect calls, removing files identified as species with <300 occurrences, and excluding noninformative calls, our final dataset was composed of 12,811 observations of five species: two regional migrants, *Myotis lucifugus* and *Eptesicus fuscus*, and three long-distance migrants, *Lasionycteris noctivagans*, *Lasiurus cinereus*, and *Lasiurus borealis*.

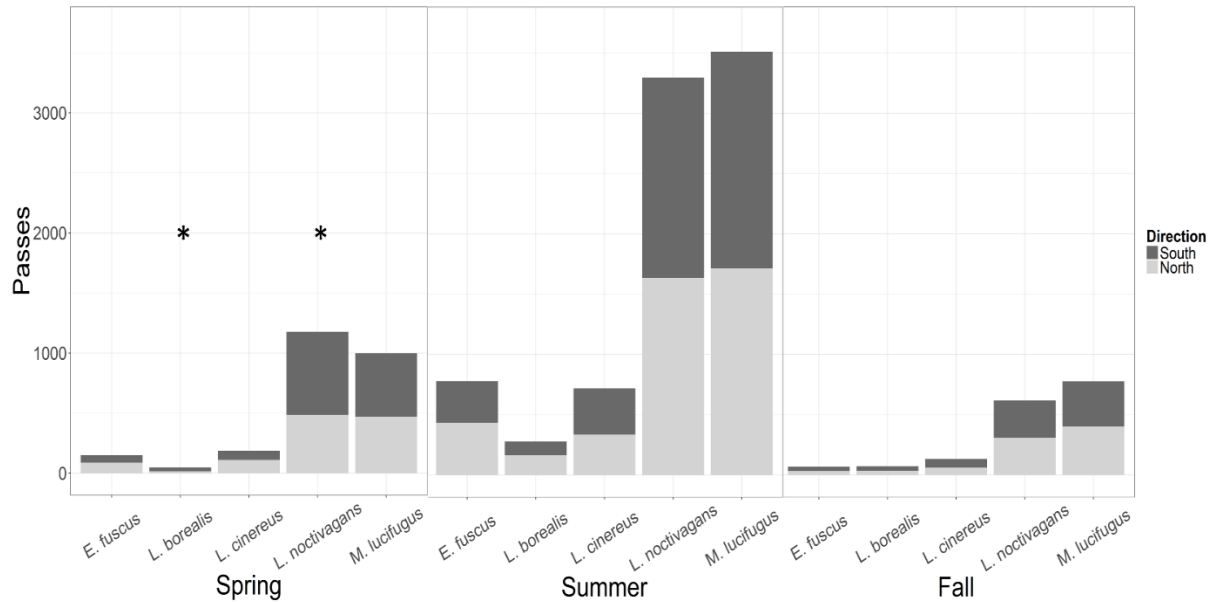


Figure 3.1. Chi-Square tests found significant differences in seasonal directional patterns for *Lasionycteris noctivagans* and *Lasiurus borealis* in the Spring. These species more southward than northward in the Spring. No other significant differences were found.

Significant differences (Bonferroni correction; $\alpha = 0.01$) in seasonal directional movements were found for *L. noctivagans* ($\chi^2 = 25.84$, d.f. = 2, $P < 0.001$) and *L. borealis* ($\chi^2 = 13.04$, d.f. = 2, $P < 0.01$). For both species, a greater number of southward movements and fewer northward movements than expected were observed in the Spring. No other differences were observed in *L. cinereus* ($\chi^2 = 8.91$, d.f. = 2, $P > 0.01$), *M. lucifugus* ($\chi^2 = 3.38$, d.f. = 2, $P > 0.01$), nor *E. fuscus* ($\chi^2 = 2.84$, d.f. = 2, $P > 0.1$).

Among the three candidate models, the species-level differences model was the best supported ($\Delta AIC = 0$), which also carried an AIC weight of one; as a result, inferences from other models nor model averaging were considered further (Table 1.1). Variation explained for all effects included in our top model were near zero. The random effects of Site ($R = 0.008$, confidence interval [CI] = 0 - 0.021) and Year ($R = 0.006$, CI = 0-0.014), as well as the combined fixed effects ($R = 0.003$; CI = 0.002-0.005) of season and species, were all near zero. Overall, the top model explained less than 2% of the variation in our dataset, suggesting that,

despite significant differences in some species and by seasons, very little of the observed variation in the directionality of recorded passes of bats was explained by species, migratory category or season differences.

Table 1.1. AIC table of a priori models. The GLMM with Species and Season was ranked as the top model.

Model	K	AICc	DAICc	w
Species+Season	9	17556.46	0	1
Migratory Type + Season	6	17569.63	13.17	0
Season	5	17577.76	21.3	0
Null	3	17583.05	26.59	0

Discussion

In contrast to past studies using the same methodology for assessing migratory movement along linear landscape features, this study found no evidence to support the hypothesis that temperate bats extensively use rivers as migratory corridors. Seasonal differences in counts of directional passes were found only to differ in spring for *L. noctivagans* and *L. borealis*. However, further analysis using GLMMs failed to support the hypothesis that any variation in directional movements could be explained by seasonal and species-specific factors. The absence of expected directional patterns along the study river may suggest that the role major rivers play in bat migration has been overstated (Popa-Lisseanu and Voigt 2009).

Although it has largely been accepted that some species of temperate bats fly along rivers and coasts during migration, many details of bat migration remain unclear. Recently, GPS tagging of the long-distance migrant, *L. cinereus*, during the migratory period documented one male making several, fairly long-distance multidirectional movements during the migratory period, apparently without following any linear landscape features (Weller et al. 2016).

Likewise, a study on the Indiana bat, *Myotis sodalis*, found little evidence that animals followed rivers during their regional migrations to hibernacula (Krauel et al. 2017). Overall, there appears to be little observational evidence that migratory bats actually follow rivers during the migratory period, with only one study directly supporting this hypothesis (Furmankiewicz and Kucharska 2009). Evidence of directional migratory movements along coasts is much more well documented (Jarzembowski 2003; Johnson et al. 2011; Rydell et al. 2014; Ijäs et al. 2017). However, *Pipistrellus nathusii*, another long-distance migrant (Petersons 2004), is known to migrate both along the coast of the Baltic Sea (Ijäs et al. 2017) and over the open Baltic Sea (Ahlén et al. 2009). This may suggest that rivers and coasts can vary in their importance as migratory corridors even within a species or population.

It may be the case that rivers primarily serve as navigational landmarks used for orientation as a component of a larger cognitive map rather than as corridors to be continuously followed (Serra-Cobo et al. 2000). Egyptian fruit bats (*Rousettus aegyptiacus*) displaced from their caves by 44 or 84km and released either deep within a natural crater or at the top of the crater were documented returning either to their caves or to remote fruit trees they were known to feed at (Tsoar et al. 2011). It has been proposed this was possible through the use of “large-scale cognitive maps” likely paired with other forms of navigation (Tsoar et al. 2011). As bats are known to orient themselves using internal magnetic compasses based on Earth’s magnetic field (Holland et al. 2006, 2010; Wang et al. 2007), it is feasible that some bats may use rivers primarily for facultative orientation or foraging along their migratory journey, and not necessarily as an obligate corridor that they must follow to maintain an appropriate heading.

Internal magnetic compasses are also known to play a role in the navigation of a number of other animals during migration (Burda et al. 1990; Lohmann and Lohmann 1993; Durif et al.

2013; Munro and Wiltschko 1993). However, the extent to which these animals also rely on linear landmarks, or landmarks in general, is unclear. Some migratory birds, for instance, have long been thought to follow linear landmarks, often referred to as “leading lines,” in avian literature (Geyer Von Schweppenburg 1963). However, the importance of these leading lines has been called into question by a study on inland migratory ducks, which concluded that ducks effectively ignored a major river during their migration (O’Neal et al. 2015). Migrating passerines have also been found to display little reliance on leading lines in the form of peninsular coasts (Nilsson et al. 2014), and others have found low within-individual repeatability in migratory routes, suggesting route familiarity may not inherently be necessary for effective migration (Catry et al. 2004; Stanley et al. 2012).

While this study does not support the hypothesis that bats follow rivers during migration, the possibility remains that the data collected were not sufficient to fully capture this behavior. A number of recent studies have characterized migratory timing and departure through the use of weather variables, most notably atmospheric pressure, wind speed, and temperature (Smith and McWilliams 2016; Dechmann et al. 2017; Pettit and O’Keefe 2017). However, most of these focused on how these variables drove phenology rather than direction along a route. Hence, it is unclear whether the inclusion of these variables would necessarily improve the models of the current study. Two studies tracking directional movements of bats along a coast (Jarzembowski 2003) and a river (Furmankiewicz and Kucharska 2009) found evidence of migratory movements while using the same methods as this study without the inclusion of weather variables.

This study did find seasonal differences in gross movement patterns of two species normally classified as long-distance migrants, but our model did not explain much of the variation in our data, and, thus, these differences could not be attributed to season. These

findings highlight the importance of calculating the variation explained by models before drawing superficial conclusions from these models. The absence of any variation explained in directional passes by seasonal and species differences calls the role of rivers as migratory corridors into question. Recent studies on individual migration of bats suggest some North American bats make nonlinear movements in unpredictable directions during the migratory period (Weller et al. 2016; Krauel et al. 2017), and the results of this study are in agreement with those findings.

References

- AHLÉN, I., H. J. BAAGØE, AND L. BACH. 2009. Behavior of Scandinavian bats during migration and foraging at sea. *Journal of Mammalogy* 90:1318–1323.
- ALDRIDGE, H. D. J. N., AND R. M. BRIGHAM. 2014. Load carrying and maneuverability in an insectivorous bat: a test of the 5% “rule” of radio-telemetry. *Journal of Mammalogy* 71:3–6.
- AVGAR, T., G. STREET, AND J. M. FRYXELL. 2014. On the adaptive benefits of mammal migration¹. *Canadian Journal of Zoology* 92:481–490.
- BAERWALD, E. F. F., W. P. PATTERSON, R. M. R. BARCLAY, AND A. R. M. R. BARCLAY. 2014. Origins and migratory patterns of bats killed by wind turbines in southern Alberta: evidence from stable isotopes. *Ecosphere* 5:1–17.
- BOUTEN, W., E. W. BAAIJ, J. SHAMOUN-BARANES, AND K. C. J. CAMPHUYSEN. 2013. A flexible GPS tracking system for studying bird behaviour at multiple scales. *Journal of Ornithology* 154:571–580.
- BURDA, H., S. MARHOLD, T. WESTENBERGER, R. WILTSCHKO, AND W. WILTSCHKO. 1990. Magnetic compass orientation in the subterranean rodent *Cryptomys hottentotus* (Bathyergidae). *Experientia* 46:528–530.

- CATRY, P. ET AL. 2004. Are long-distance migrant passerines faithful to Their stopover sites?
Journal of Avian Biology 35:170–181.
- CRYAN, P. M. 2003. Seasonal distribution of migratory tree bats (*Lasiurus* and *Lasionycteris*) in
North America. Journal of Mammalogy 84:579–593.
- CRYAN, P. M., M. A. BOGAN, R. O. RYE, G. P. LANDIS, AND C. L. KESTER. 2004. Stable hydrogen
isotope analysis of bat hair as evidence for seasonal molt and long-distance migration.
Journal of Mammalogy 85:995–1001.
- DECHMANN, D. K. N., M. WIKELSKI, M. ELLIS-SOTO, K. SAFI, AND M. T. O'MARA. 2017.
Determinants of spring migration departure decision in a bat. Biology Letters 13:0–3.
- DURIF, C. M. F., H. I. BROWMAN, J. B. PHILLIPS, A. B. SKIFTESVIK, L. A. VØLLESTAD, AND H. H.
STOCKHAUSEN. 2013. Magnetic compass orientation in the European Eel. PLoS ONE 8:1–8.
- FLEMING, T. H., R. A. NUÑEZ, AND S. LOBO. 1993. Seasonal Changes in the diets of migrant and
non-migrant nectarivorous bats as revealed by carbon stable isotope analysis. Oecologia
94:72–75.
- FLEMING, T. H., AND P. EBY. 2003. Ecology of bat migration. Pp. 156– 208 in Bat ecology (T. H.
Kunz and M. B. Fenton, eds.). University of Chicago Press, Chicago, Illinois.
- FRASER, E. E., D. BROOKS, AND F. J. LONGSTAFFE. 2017. Stable isotope investigation of the
migratory behavior of silver-haired bats (*Lasionycteris noctivagans*) in eastern North
America. Journal of Mammalogy 98:1–11.
- FROSCHAUER, A., AND J. COLEMAN. 2012. North American bat death toll exceeds 5.5 million
from white-nose syndrome. USFWS News Release1–2.
- FURMANKIEWICZ, J., AND M. KUCHARSKA. 2009. Migration of bats along a large river Vvley in
Southwestern Poland. Journal of Mammalogy 90:1310–1317.

- GARCÍA-RIPOLLÉS, C., P. LÓPEZ-LÓPEZ, AND V. URIOS. 2010. First description of migration and wintering of adult Egyptian Vultures *Neophron percnopterus* tracked by GPS satellite telemetry. *Bird Study* 57:261–265.
- GRODZINSKI, U., O. SPIEGEL, C. KORINE, AND M. W. HOLDERIED. 2009. Context-dependent flight speed: Evidence for energetically optimal flight speed in the bat *Pipistrellus kuhlii*? *Journal of Animal Ecology* 78:540–548.
- HOLLAND, R. A., I. BORISSOV, AND B. M. SIEMERS. 2010. A nocturnal mammal, the greater mouse-eared bat, calibrates a magnetic compass by the sun. *Proceedings of the National Academy of Sciences* 107:6941–6945.
- HOLLAND, R. A., J. L. KIRSCHVINK, T. G. DOAK, AND M. WIKELSKI. 2008. Bats use magnetite to detect the earth’s magnetic field. *PLoS ONE* 3:1–6.
- HOLLAND, R. A., K. THORUP, M. J. VONHOF, W. W. COCHRAN, AND M. WIKELSKI. 2006. Bat orientation using Earth’s magnetic field. *Nature* 444:702.
- IJÄS, A., A. KAHILAINEN, V. V. VASKO, AND T. M. LILLEY. 2017. Evidence of the migratory bat, *Pipistrellus nathusii*, Aggregating to the coastlines in the Northern Baltic Sea. *Acta Chiropterologica* 19:127–139.
- JARZEMBOWSKI, T. 2003. Migration of the Nathusius’ pipistrelle *Pipistrellus nathusii* (*Vespertilionidae*) along the Vistula Split. *Acta Theriologica* 48:301–308.
- JOHNSON, J. B., J. E. GATES, AND N. P. ZEGRE. 2011. Monitoring seasonal bat activity on a coastal barrier island in Maryland, USA. *Environmental Monitoring and Assessment* 173:685–699.

- JOHNSON, J. B., J. H. ROBERTS, T. L. KING, J. W. EDWARDS, W. M. FORD, AND D. A. RAY. 2014. Genetic structuring of northern myotis (*Myotis septentrionalis*) at multiple spatial scales. *Acta Theriologica* 59:223–231.
- JOHNSON, W. C. ET AL. 2012. Forty years of vegetation change on the Missouri River floodplain. *BioScience* 62:123–135.
- JOHNSON, W. C., R. L. BURGESS, AND W. R. KEAMMERER. 1976. Forest overstory vegetation and environment on the Missouri River floodplain in North Dakota. *Ecological Monographs* 46:59–84.
- KERTH, G., A. KIEFER, C. TRAPPMANN, AND M. WEISHAAR. 2003. High gene diversity at swarming sites suggest hot spots for gene flow in the endangered Bechstein's bat. *Conservation Genetics* 4:491–499.
- KILPATRICK, A. M., C. J. BRIGGS, AND P. DASZAK. 2010. The ecology and impact of chytridiomycosis: an emerging disease of amphibians. *Trends in Ecology and Evolution* 25:109–118.
- KRAUEL, J. J., L. P. MCGUIRE, AND J. G. BOYLES. 2017. Testing traditional assumptions about regional migration in bats. *Mammal Research* 20 Novembe.
- LOHMANN, K. J., AND C. LOHMANN. 1993. A light-independent magnetic compass in the Leatherback Sea Turtle. *The Biological Bulletin* 185:149–151.
- MOUSSY, C., D. J. HOSKEN, F. MATHEWS, G. C. SMITH, J. N. AEGERTER, AND S. BEARHOP. 2013. Migration and dispersal patterns of bats and their influence on genetic structure. *Mammal Review* 43:183–195.

- MUNRO, U., AND W. WILTSCHKO. 1993. Magnetic compass orientation in the Yellow-faced Honeyeater, *Lichenostomus chrysops*, a day migrating bird. *Behavioral Ecology and Sociobiology* 32:141–145.
- NAKAGAWA, S., AND H. SCHIELZETH. 2010. Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. *Biological Reviews* 85:935–956.
- NELSON, J. J., P. R. BARNHART, AND E. H. GILLAM. 2015. Distribution and occurrence of bat species in North Dakota. *The Prairie Naturalist* 47:83–92.
- NILSSON, C., J. BÄCKMAN, AND T. ALERSTAM. 2014. Are flight paths of nocturnal songbird migrants influenced by local coastlines at a peninsula? *Current Zoology* 60:660–669.
- O’NEAL, B. J., J. D. STAFFORD, AND R. P. LARKIN. 2015. Migrating ducks in inland North America ignore major rivers as leading lines. *Ibis* 157:154–161.
- PETERSONS, G. 2004. Seasonal migrations of north-eastern populations of Nathusius’ bat *Pipistrellus nathusii* (Chiroptera). *Myotis* 41–42:29–56.
- PETTIT, J. L., AND J. M. O’KEEFE. 2017. Day of year, temperature, wind, and precipitation predict timing of bat migration. *Journal of Mammalogy* 98:1236–1248.
- POPA-LISSEANU, A. G., AND C. C. VOIGT. 2009. Bats on the move. *Symposium A Quarterly Journal In Modern Foreign Literatures* 90:1283–1289.
- RYDELL, J. ET AL. 2014. Phenology of migratory bat activity across the Baltic Sea and the South-Eastern North Sea. *Acta Chiropterologica* 16:139–147.
- SALCEDO, H., M. FENTON, M. HICKEY, AND R. BLAKE. 1995. Energetic consequences of flight speeds of foraging red and hoary bats (*Lasiurus borealis* and *Lasiurus cinereus*; Chiroptera: Vespertilionidae). *The Journal of Experimental Biology* 198:2245–2251.

- SERRA-COBO, J., M. LÓPEZ-ROIG, T. MARQUÈS-BONET, AND E. LAHUERTA. 2000. Rivers as possible landmarks in the orientation flight of *Miniopterus schreibersii*. *Acta Chiropterologica* 45:347–352.
- SIKES, R. S. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy* 97:663–688.
- SMITH, A. D., AND S. R. MCWILLIAMS. 2016. Bat activity during autumn relates to atmospheric conditions: implications for coastal wind energy development. *Journal of Mammalogy* 97:1565–1577.
- STANLEY, C. Q., M. MACPHERSON, K. C. FRASER, E. A. MCKINNON, AND B. J. M. STUTCHBURY. 2012. Repeat tracking of individual songbirds reveals consistent migration timing but flexibility in route. *PLoS ONE* 7:5–10.
- SULLIVAN, A. R., J. K. BUMP, L. A. KRUGER, AND R. O. PETERSON. 2012. Bat-cave catchment areas: using stable isotopes (dD) to determine the probable origins of hibernating bats. *Ecological Applications* 22:1428–1434.
- TSOAR, A., R. NATHAN, Y. BARTAN, A. VYSSOTSKI, G. DELL, AND N. ULANOVSKY. 2011. Large-scale navigational map in a flying mammal : Evidence from GPS tracking of Egyptian fruit bats large-scale navigational map in a mammal. *PNAS* 108:718–724.
- WANG, Y., Y. PAN, S. PARSONS, M. WALKER, AND S. ZHANG. 2007. Bats respond to polarity of a magnetic field. *Proceedings. Biological sciences / The Royal Society* 274:2901–2905.
- WELLER, T. J., K. T. CASTLE, F. LIECHTI, C. D. HEIN, M. R. SCHIRMACHER, AND P. M. CRYAN. 2016. First direct evidence of long-distance seasonal movements and hibernation in a migratory bat. *Scientific Reports* 6:34585.

CHAPTER 2: INFLUENCE OF FORAGING HABITAT AVAILABILITY ON WING SHAPE IN TWO NORTH DAKOTA BAT SPECIES

Abstract

Foraging success contributes to an animal's fitness and is often impacted by how well an animal is adapted to foraging. Foraging adaptations come in a variety of forms, such as morphology and behavior. Wing shape among species is commonly known to differ among bird and bat species based on foraging behavior, with animals adapted to maximize efficiency in specific foraging habitats. While intraspecific variation in wing shape related to habitat occupation and use has been documented in several bird species, this concept is much less studied in bats. This study used geometric morphometrics to assess wing shape in two species that differ in foraging strategy, as well as to assess within-species variation in wing shape based on differences in available habitat types. Wing shape between species differed as predicted by foraging strategy, while no biologically significant differences in wing shape were identified within species. Interspecific variation in wing shape remain apparent in bats, yet potential drivers leading to differences in wing shape within a species remain unconfirmed.

Introduction

The ability to be a successful forager can have extensive impacts on fitness, as investment in bodily growth and repair, as well as production and care of offspring, is inextricably linked to an individual's rate of energy intake (e.g. Ritchie 1990; Lemon 1991). Further, foraging success is highly influenced by the relationship between morphology and environment. Variation of homologous structures among taxonomic groups, even closely related ones, can be diverse and often exhibit notable specialization based on differences in foraging (Norberg and Rayner 1987; Ehlinger and Wilson 1988; Forstmeier and Keßler 2001). A

traditional example would be the variation found amongst species of Darwin's finches, which differ notably in beak morphology based on dietary specialization; this is particularly clear among species hybrids, which exhibit reduced foraging success compared to either parent species (Grant and Grant 1996, 2002).

Wing shape in many insectivorous birds is also known to be influenced by both foraging behavior (which is linked to foraging environment) and migratory behavior (Lockwood et al. 1998a)—both bear significant energetic consequences and an appropriate wing shape can maximize flight performance while minimizing energetic costs. Much of the variation in wing shape of birds has been assessed through the use of morphometrics based largely on basic measurements and ratios, such as wing area, aspect ratio, and wing loading, among others (Busse 1967; Hedenström and Pettersson 1986), although there have been criticisms of using such measurements (Chandler and Mulvihill 1998). In regard to foraging behavior, species hunting in cluttered habitats that require frequent sharp turns have rounder, broader wings that permit greater maneuverability. Alternatively, those with narrower, more pointed wings are more suited to unobstructed and open foraging habitats, as well as flying greater distances (Norberg 1990:243). In regards to migratory behavior, studies broadly suggest that migrants have more pointed wings and lower wing loadings (narrower wings relative to body mass) compared to non-migratory individuals (Lo Valvo et al. 1988; Marchetti et al. 1995; Bowlin 2007). These migrant-specific wing characteristics are known to increase flight efficiency over longer distances (Lockwood et al. 1998b; Bowlin and Wikelski 2008). Overall, it is plausible that both foraging habitat and migratory behavior can impact wing shape. For example, the *Phylloscopus* genus of warblers exhibits variation in wing elongation and pointedness related to migratory distance, yet these changes are also linked to foraging habitat. Species with shorter, rounder wings migrated

shorter distances and demonstrated a preference for arboreal habitat for foraging while those with longer, pointed wings spent more time in open habitat (Marchetti et al. 1995).

Traditional measures of wing shape have historically been used in the assessment of bat wings, leading to similar conclusions about wing shape as it relates to both foraging behavior and migratory behavior across species (Findley et al. 1972; Norberg et al. 1986; Norberg and Rayner 1987). However, the use of geometric morphometrics analysis, which is used to assess and visualize size-independent shape variation in wings, has begun to be used more extensively in biomechanical research. Birch (1997) found that landmark-based geometric morphometrics was a superior tool to traditional measures for characterizing statistical variation between and among species and visualizing shape variation. To date, geometric morphometric analysis in bats has been primarily limited to interspecific comparisons (Birch 1997; Schmieder et al. 2015), with, to our knowledge, only two studies having examined intraspecific variation in wing shape of bats using this method. O'Mara et al. (2016) found that in *Nyctalus noctula*, males and females exhibit significant differences in migration distance and body size but no differences in wing shape, which may be due to limitations imposed by their ecological niche or genetic constraints. This may suggest that the wing shape of bats is less impacted by migratory flight efficiency and more by effectively navigating foraging habitat. Adams (1996) found that juvenile *Myotis lucifugus* with larger and broader wings exploited a wider range of foraging habitats (differing degrees of clutter) compared to those with smaller wings, which foraged predominantly in open habitat. This may suggest that wing shape is susceptible to selection if a certain mean wing morph is superior in a given habitat type.

The overarching objective of this study was to examine the relationship between wing morphology and primary foraging habitat in two widely distributed bat species. The little brown

bat, *Myotis lucifugus*, is one the most abundant bat species in North America. The big brown bat, *Eptesicus fuscus*, is another highly abundant insectivorous species found across much of the same range as *M. lucifugus*. While *M. lucifugus* is known to be an arboreal forager with short, rounded wings and forages in cluttered habitat, *E. fuscus* typically forages above trees in relatively uncluttered, open environments, and, thus, exhibits more elongated and pointed wings (Norberg and Rayner 1987). Our work focused on bats from central and western North Dakota, which are part of the Northern Great Plains ecoregion. The central part of the state displays a large degree of cluttered habitat along a major riparian corridor, and the western part of the state is much more open and uncluttered by comparison. Both of these species are commonly found in central and western North Dakota, with *Myotis lucifugus* having previously been shown to forage in a variety of habitat types in the study area (Nelson et al. 2015).

The specific aims were to 1) find expected differences in wing shape between *M. lucifugus* and *E. fuscus* with geometric morphometrics; and 2) determine whether the wing shape of the two study species varies predictably based on regionally expected-foraging habitat preferences. Potential effects of sex and year on wing shape are also considered. We predict that *M. lucifugus*, which primarily forages in cluttered habitat, will display typical wing shapes for this species, while those primarily using open habitat types will exhibit wing shapes similar to *E. fuscus* (more elongated and pointed wings). *E. fuscus* primarily forages in habitat independent of clutter and is not predicted to exhibit intraspecific variation.

Methods

A total of 1,856 images were collected from 182 individuals in 2017 and 2018, however, only images of 138 individuals (88 *M. lucifugus* and 50 *E. fuscus*) from 15 different sites across the state of North Dakota were of sufficient quality for morphometric analysis. These sites were grouped into two categories: bats from western and central ND (Fig. 1.2). Samples collected from western sites were primarily from animals captured in or near the North Dakota Badlands and Little Missouri National Grasslands, while samples collected from central sites were primarily captured along the Missouri River Valley in North Dakota. Western habitat was generally characterized by riparian habitat surrounded by dry, irregular geology of steep bluffs with low, shrub-like vegetation. Central habitat was characterized by denser riparian habitat with lower understory cover, higher ground vegetation, and surrounded primarily by open agricultural fields or ranchland.

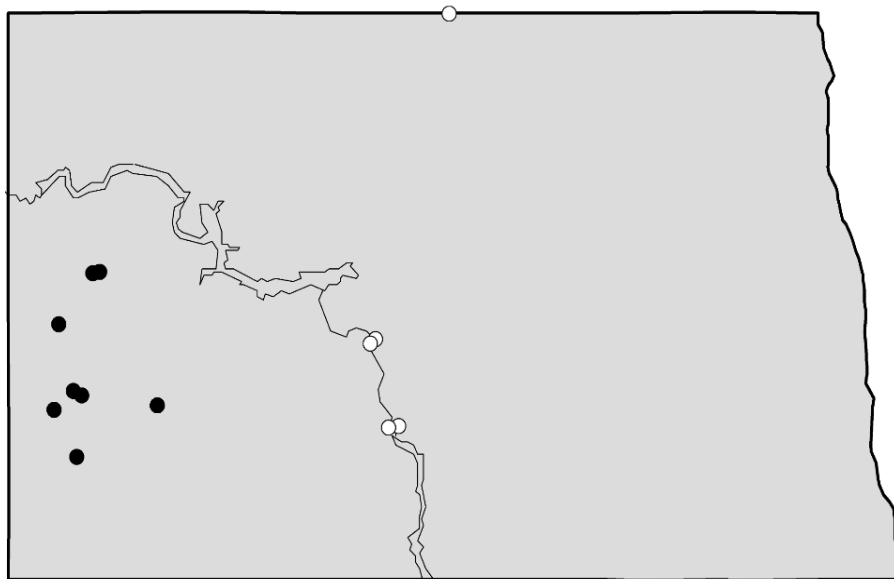


Figure 2.1. Capture sites across North Dakota from which morphological data were collected. Western sites are shown with black dots (nine), and central sites are shown with white dots (six). The Missouri River is shown in black.

Individuals were live-captured using standard mistnetting techniques along likely flyways. Upon capture, individuals were placed in small cloth bags until processing occurred. Standard measurements (mass and forearm length), sex, and reproductive condition were assessed and recorded. Following these measurements, bats were fixed to a small piece of plexiglass using a strip of Scotch tape that was placed across the thumb and fifth digit of each wing (Schmieder et al. 2015). Only non-pregnant, adult bats were used. Individuals were then placed on a custom photographing stand with a gridded background scale and a fixed camera position. The right wing of each individual was quickly photographed (Nikon D3400), after which a wing biopsy sample was collected as part of another ongoing study. Following these steps, bats were released back into the wild at the point of capture. All handling methods were conducted in accordance with the North Dakota State University Institutional Animal Care and Use Committee under protocol (A16056).

Eleven landmarks and 41 semilandmarks (Fig. 2.2) were chosen to assess the shape of the handwing i.e. the skeletal portion of the wing and wing membrane comprised of the 5 distal digits and surrounding membrane. The landmarks represent repeatable points along the structural skeleton of the handwing and the semilandmarks represent curvature along wing edges and bones (Fig. 2.2). During the morphometric analysis, all of which was conducted using the tps series software (Rohlf 2004), individuals from both years and species were combined and the order of analysis randomized. Landmarks were manually placed using the geometric morphometrics software tpsDig and semilandmarks were identified using tpsUtil. Landmark 1 was placed in the center of the wrist. Landmarks 7, 9, 21, 23, 37, and 39 were placed at the joints of fingers 3-5. Landmarks 10, 19, and 35 were placed at the fingertips of fingers 3-5. Semilandmarks 2-6, 8, 11-18, 20, 22, 24, 25-34, 36, 38, 40-47, and 48-52 all were placed along

the between-wing curvature between fingers as well as non-fixed points along the finger bones (see Appendix, Table A1).

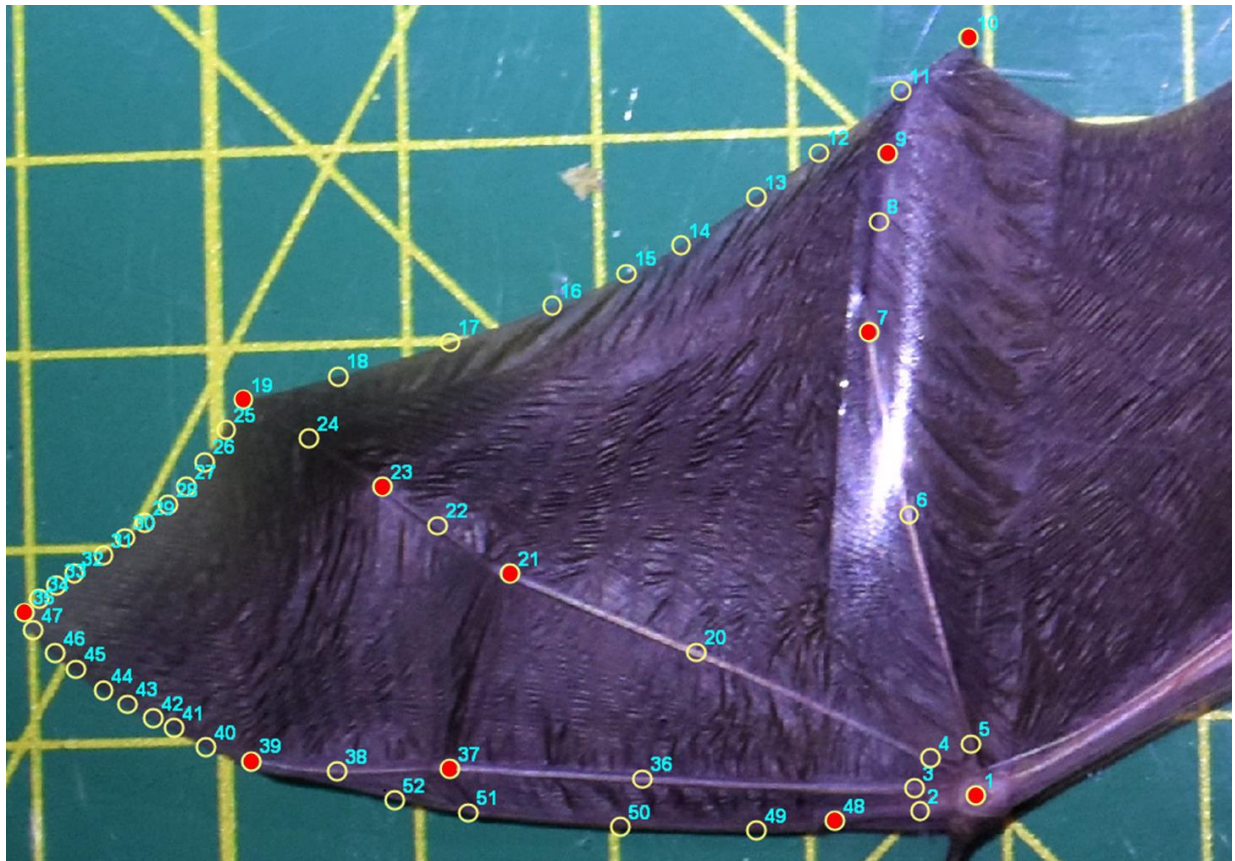


Figure 2.2. Landmark (filled circles) and semilandmark (open circle) placement on a bat wing. Landmarks are highly repeatable, while semilandmarks may vary in relative placement.

Using tpsRelw version 1.69, size and orientation differences were accounted for by transforming landmarks using a partial Procrustes superimposition, and data were further transformed into shape variables, on which a relative warp analysis was conducted. Relative warp analysis is synonymous with principal component analysis and, thus, percent variation explained by each axis of the relative warps (RW) was extracted. RW scores and centroid size, an approximate for wing size, were calculated for all individuals; data was also combined with other individual metadata for further analysis. Photographs were subsetted by species using

tpsUtil. Percent variation explained by each RW, RW scores, and centroid sizes were then calculated for within-species analyses of wing shape by location and sex.

Centroid size variation was assessed between and within species using one-way analysis of variance (ANOVA). Three multivariate analyses of covariance (MANCOVA) were also used to assess variables potentially influencing wing shape in the combined species dataset, within the *M. lucifugus* dataset, and within the *E. fuscus* dataset (as represented by RW1-RW10). The MANCOVA of the combined dataset of the two species included centroid size as a covariate, species, location, sex, a location by year interaction, and a species by location interaction. The MANCOVAs for each species used identical variables, excluding those related to species: centroid as a covariate, location, and a location by year interaction, and sex. Mean RW scores were calculated for the RW affecting variation of variables of focus. Using tpsRelw, these scores were used to create thin plate spline deformation grids based on mean RW scores, which can be used to visualize variations in shape across groups. Analysis of Covariance (ANCOVA) including the same variables identified for the MANCOVAs were used to determine which variables significantly affected each RW. The RW which a variable was first found to have a significant effect on was used to describe how that variable influenced wing shape also using deformation grids. The following RW was also used in these comparisons to characterize as much of the variation in these groups as possible.

Results

A total of 138 individuals were used for analysis: 88 *M. lucifugus* (n=47 from central ND; n=41 from western ND) and 50 *E. fuscus* (n=17 for central ND; n= 33 for western ND). RW1-RW10 in each relative warp analysis accounted for at least 90% of the variation (combined species: 92.03%; *M. lucifugus*: 90.88%; *E. fuscus*: 94.04%).

Species-comparison

RW1-RW10 for the combined species dataset accounted for 92.03% of the variation. RW1 (34.28%) and RW2 (22.0%) accounted for 56.28% of the total variation explained, where RW3-RW10 each accounted for <10% of the variation explained. One-way ANOVA of centroid size indicated significant differences based on species ($F_{1,137} = 663.28.11$, $P < 0.0001$). MANOVA identified significant effects of centroid size ($F_{1,137} = 78.780$, $P < 2.2e-16$), location ($F = 2.532$, $P < 0.01$), year ($F_{1,137} = 4.303$, $P < 0.0001$) and species ($F_{1,137} = 7.987$, $P < 0.0001$). Sex ($F_{1,137} = 0.822$, $P = 0.62$), a location by year interaction ($F_{1,137} = 1.833$, $P = 0.062$), and a location by species interaction ($F_{1,137} = 1.667$, $P = 0.096$) were not found to significantly affect wing shape (see Appendix, Table A2). Mean RW scores for RW1 and RW2 were used to assess overall wing shape differences between species based on the two axes explaining the most variation. These values were multiplied by two in the deformation plots to more clearly visualize shape variation. ANCOVA determined centroid size ($F_{1,137} = 33.85$, $P < 0.0001$), year ($F_{1,137} = 7.18$, $P < 0.01$), and species ($F_{1,137} = 32.77$, $P < 0.0001$) significantly affected RW1. Location ($F = 4.85$, $P < 0.0001$) was found to be significant on RW3 which explained 8.65% of the total variation. RW1 and RW2 were used to characterize overall species differences using deformation grids. *M. lucifugus* had an overall shorter wing, with overall less concavity along the edges of the wing membrane, resulting in a broader, rounder mean wing shape. *E. fuscus* is clearly more elongated with deeper concavity of the wing membranes, resulting in a narrower, more pointed wing shape (Fig. 2.3A)

M. lucifugus

RW1-RW10 for the dataset solely containing *M. lucifugus* accounted for 90.88% of the total variation. RW1 (31.41%) and RW2 (16.86%) accounted for 48.28% of the variation. RW3

accounted for 11.64% while RW4-RW10 each accounted for <10% of the variation. One-way ANOVA of centroid size found no significant effect of location on centroid size within *M. lucifugus* ($F_{1,87} = 1.68$, $P = 0.20$). MANOVA found centroid size ($F_{1,87} = 5.18$, $P < 0.0001$) and location ($F_{1,87} = 3.42$, $P < 0.01$) to significantly affect wing shape. Year ($F_{1,87} = 1.70$, $P = 0.096$), sex ($F_{1,87} = 0.817$, $P = 0.61$) and an interaction of location by year ($F_{1,87} = 1.31$, $P = 0.24$) were not found to vary significantly. ANCOVA determined only centroid size ($F_{1,87} = 9.59$, $P < 0.01$) significantly affected RW1 (see Appendix, Table A3). Location ($F_{1,87} = 15.28$, $P < 0.01$) was found to be significant at RW5, which only explained 5.28% of the total variation. Mean RW scores for RW5 and RW6 were used to assess overall wing shape differences between locations. These values were multiplied by ten in the deformation plots to more clearly visualize shape variation. Variation in shape along these axes was likewise explored using deformation grids along RW5 (5.8% variation explained) and RW6 (4.82% variation explained) to encompass as much of the location-based variation in shape as possible. No discernible differences are readily apparent between *M. lucifugus* sampled at central versus western sites. Overall broadness of wing shape and pointedness appear largely the same (Fig. 2.3B).

E. fuscus

RW1-RW10 for the dataset solely containing *E. fuscus* accounted for 94.04% of the total variation. RW1 (43.5%) and RW2 (17.27%) accounted for 60.77% of the total variation. RW3 accounted for 11.32% of the variation, while RW4-RW10 accounted for <10% of the variation. One-way ANOVA found no effect of location on centroid size ($F_{1,49} = 0.002$, $P = 0.96$). MANOVA found a significant effect of centroid size ($F_{1,49} = 4.46$, $P < 0.001$), location ($F_{1,49} = 2.62$, $P < 0.05$), and year ($F_{1,49} = 3.83$, $P < 0.01$) on wing shape, while sex ($F_{1,49} = 0.1664$, $P = 0.130$), and an interaction of location by year ($F_{1,49} = 1.25$, $P = 0.30$) were not found to have

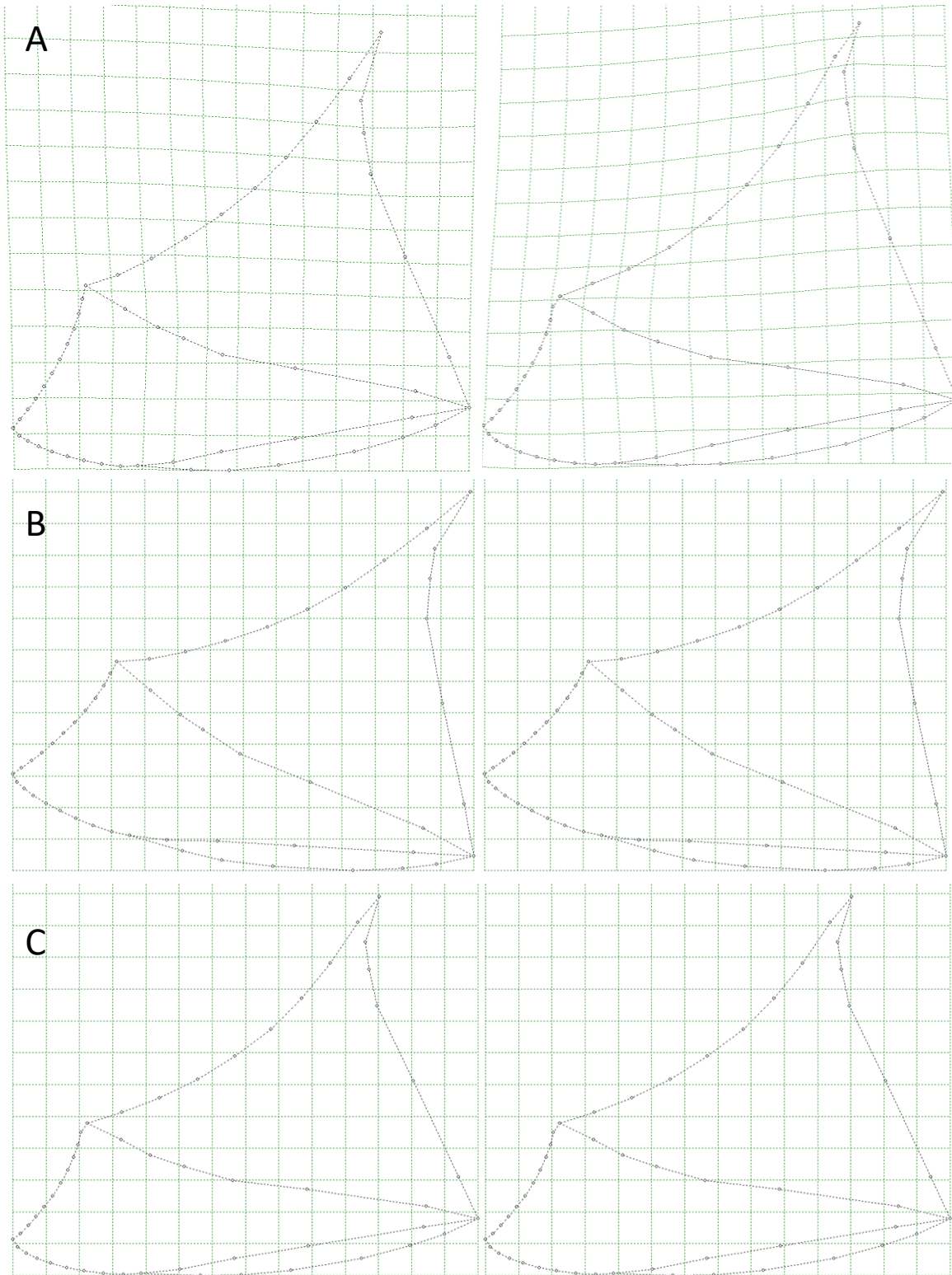


Figure 2.3. Deformation grids of mean wing shapes of compared groups as they differ from the overall mean shape. A. depicts how *M. lucifugus* (left) and *E. fuscus* (rights); B. depicts *M. lucifugus* from central sites (left) and western sites (right); C. depicts *E. fuscus* from central sites (left) and western sites (right).

wing shape differences between locations. These values were multiplied by ten in significant effect on wing shape (see Appendix, Table A4). ANCOVA determined centroid size ($F_{1,49} = 5.96, P < 0.001$) and year ($F_{1,49} = 6.79, P < 0.05$) significantly affected RW1; Location ($F_{1,49} = 4.09, P < 0.05$) was significant for RW4, which only explained 6.47% of the total variation. Mean RW scores for RW4 and RW5 were used to assess overall the deformation plots to more clearly visualize shape variation. Variation in shape along these axes was likewise explored using deformation grids along RW4 (6.47% variation explained) and RW5 (4.01% variation explained) to encompass as much of the location-based variation in shape as possible. No discernible differences are readily apparent between *E. fuscus* sampled at central versus western sites. As with *M. lucifugus*, overall broadness of wing shape and pointedness appear largely the same (Fig. 2.3C).

Discussion

Geometric morphometric methods have been used in many different contexts, including assessing variation among phylogenetically distant species (Birch 1997), differentiating between closely related species (Schmieder et al. 2015), and investigating the effect of behavioral dimorphism on wing structure in temperate bats (O'Mara et al. 2016); it remains one of the best tools for shape assessment. Variation in wing shape between *M. lucifugus* and *E. fuscus*, as assessed with geometric morphometrics, varied as predicted based on known foraging strategy and previous studies (Norberg and Rayner 1987; Birch 1997). While impacts of habitat on wing shape achieved statistical significance, assessment of deformation grids calls the ecological relevance of these results into question. For both *M. lucifugus* and *E. fuscus* within-species analyses, no distinction could be made using the deformation grids to visualize differences between habitat types, leading to the conclusion that there is no discernible functional adaptation

of wing shape to these two environments by these species. This is not surprising given the RWs for which location was significant explained little of the variation in either species.

High levels of gene flow may account for the lack of observed localized adaptation to habitat. Although there is a paucity of population structure information from bats in the Northern Great Plains, there is little overall differentiation of nuclear DNA across the range of *M. lucifugus*, with no apparent strong barriers to gene flow (Lausen et al. 2008; Vonhof et al. 2015). Females of this species, the majority in our study, are highly philopatric (Dixon 2011) and, thus, are likely to be exposed to consistent summer foraging habitat from year to year. However, *M. lucifugus* is capable of making migrations of over 500km (Norquay et al. 2013), so it is possible that there is interbreeding among bats sampled in central and western sites separated by ~200km. If gene flow is occurring freely, localized adaptive divergence may be constrained (Hendry et al. 2001; Bolnick and Nosil 2007; Moore et al. 2007).

Less information is known regarding the population structure and overall movement patterns of *E. fuscus*. There are two known distinct mitochondrial lineages of *E. fuscus* (eastern and western) thought to have diverged during the Pleistocene. However, these two lineages are known to currently occur sympatrically, appear to be hybridizing, and lack apparent divergence in morphology (Nebaum et al. 2007). Further, *E. fuscus* is largely thought to exhibit migratory behavior similar to *M. lucifugus* (Fleming and Eby 2003), where individuals traveling a variety of distances meet and mate at common hibernacula during the winter (Kurta and Baker 1990). As with *M. lucifugus*, it is possible that a high degree of gene exchange among populations reduces the potential for habitat-specific adaptation in this system.

Conflict of selective forces on wing shape also may contribute to an absence of observed differences in wing shape within species. In some birds, differences in migratory tendency and/or

distance is associated with population-level differentiation in wing shape (Milá et al. 2008; Baldwin et al. 2010). Yet, this relationship between morphology and migration was not found in *Nyctalus noctula*, the common noctule bat, a species with sexually dimorphic migratory behavior (O'Mara et al. 2016). However, there has been only one within-species geometric morphometrics study of wing shape as related to migration (O'Mara et al. 2016), so these results should not be considered all-inclusive. Thus, it is possible that differing migratory behavior among the sampled populations acts to oppose selection related to habitat types. As with the population structure of *M. lucifugus* and *E. fuscus* in the study area, little information is known about the seasonal movement patterns of these bats. *E. fuscus* and *M. lucifugus* have been documented overwintering in the geologic crevices in the western region of North Dakota (Barnhart and Gillam 2017), confirming that at least some individuals in this area do not migrate. However, it is possible that migratory tendency varies among individuals. The lack of natural hibernacula available in other parts of the state suggests migration should be necessary, however, *E. fuscus* has been observed overwintering in anthropogenic structures in North Dakota (personal observations), whereas *M. lucifugus* has not been observed overwintering outside of western North Dakota. Due to this lack of information it is difficult to determine whether there may be conflicting selective forces on wing shape.

A third potential explanation for the lack of intraspecific differentiation is that differences in habitat availability may not coincide with predicted differences in habitat use. These bats may be seeking out habitats specific to their preferences and, therefore, may not be under the selective pressures of a sub-optimal habitat type. While the overall landscapes of the study areas differed, there were still areas in the western habitat type that displayed similarity to the central habitat type, especially in riparian zones. Bats in Britain were found to preferentially select certain

habitat types across a variety of landscape types, irrespective of availability (Walsh and Harris 1996). Further, *M. lucifugus* is known to modulate habitat use based on wing loading (mass to wing area ratio) (Kalcounis and Brigham 1995). If alteration of foraging behavior shifts significantly with such a temporally labile trait (Baker et al. 1968; Kunz et al. 1998), any potential effects of habitat availability affecting habitat use and, further, wing shape may be constrained by this mass-linked variation in behavior.

This study further supports the known shape characterization of *M. lucifugus* and *E. fuscus*, using geometric morphometrics. We did not find population-level differentiation in wing shape among two populations in the Northern Great Plains occupying different types of summer habitat that differ in levels of vegetative clutter. High gene flow, variation in migratory behavior, and intra-individual variation in foraging behavior may be responsible for the lack of expected differences in wing shape. To date, there remains no evidence for intra-specific variation in size-independent wing shape as affected purely by migratory behavior or foraging strategy. However, the limited sampling of bat species for these investigations constrains our ability to draw broad conclusions. Future studies examining the effect of foraging behavior on wing shape should investigate populations on a broader geographic scale, that are genetically isolated, and in habitats more distinctly different than the ones sampled in this study. Studies incorporating genetic information, habitat characterization, and large sample sizes may uncover this pattern of intraspecific variation largely confirmed in birds, yet unresolved in bats.

References

BAKER, W. W., S. G. MARSHALL, AND V. B. BAKER. 1968. Autumn fat deposition in the Evening Bat (*Nycticeius humeralis*). *Journal of Mammalogy* 49:314–317.

- BALDWIN, M. W., H. WINKLER, C. L. ORGAN, AND B. HELM. 2010. Wing pointedness associated with migratory distance in common-garden and comparative studies of stonechats (*Saxicola torquata*). *Journal of Evolutionary Biology* 23:1050–1063.
- BARNHART, P. R., AND E. H. GILLAM. 2017. Documentation of overwintering bat species presence and hibernacula use in the Badlands of North Dakota. *Northwestern Naturalist* 98:48–56.
- BIRCH, J. M. 1997. Comparing wing shape of bats: the merits of principal-components analysis and relative-warp analysis. *Journal of Mammalogy* 78:1187–1198.
- BOLNICK, D. I., AND P. NOSIL. 2007. Natural selection in populations subject to a migration load. *Evolution* 61:2229–2243.
- BOWLIN, M. S. 2007. Sex, wingtip shape, and wing-loading predic arrival date at stopover site in the Swainson's Thrush (*Catharus ustulatus*). *The Auk* 124:1388–1396.
- BOWLIN, M. S., AND M. WIKELSKI. 2008. Pointed wings, low wingloading and calm air reduce migratory flight costs in songbirds. *PLoS ONE* 3:1–8.
- BUSSE, P., 1967. Application of the numerical indexes of the wing shape. *Notatki Ornitol.* 8:1-8. Polish with English summary.
- CHANDLER, C. R., AND R. S. MULVIHILL. 1998. The use of wing shape indices: An evaluation. *Ornis Scandinavica* 19:212–216.
- DIXON, M. D. 2011. Population genetic structure and natal philopatry in the widespread North American bat *Myotis lucifugus*. *Journal of Mammalogy* 92:1343–1351.
- EHLINGER, T. J., AND D. S. WILSON. 1988. Complex foraging polymorphism in bluegill sunfish. *Proceedings of the National Academy of Sciences* 85:1878–1882.

- FINDLEY, J. S., E. H. STUDIER, AND D. E. WILSON. 1972. Morphologic properties of bat wings. *Journal of Mammalogy* 53:429–444.
- FLEMING, T. H., AND P. EBY. 2003. Ecology of bat migration. Pp. 156–208 in *Bat ecology* (T. H. Kunz and M. B. Fenton, eds.). University of Chicago Press, Chicago, Illinois.
- FORSTMEIER, W., AND A. KEBLER. 2001. Morphology and foraging behaviour of Siberian *Phylloscopus* warblers. *Journal of Avian Biology* 32:127–138.
- GRANT, B. R., AND P. R. GRANT. 1996. High survival of Darwin's Finch Hybrids: Effects of beak morphology and diets. *Ecological Society of America* 77:500–509.
- GRANT, P. R., AND R. B. GRANT. 2002. Unpredictable evolution in a 30-year study of Darwin's finches. *Science* 296:707–711.
- HEDENSTROM, A. AND PETTERSSON, J., 1986. Differences in fat deposits and wing pointedness between male and female Willow Warblers caught on spring migration at Ottenby, SE Sweden. *Ornis Scandinavica*, 17:182-185.
- HENDRY, A. P., T. DAY, AND E. B. TAYLOR. 2001. Population mixing and the adaptive divergence of quantitative traits in discrete populations: A theoretical framework for empirical tests. *Evolution* 55:459–466.
- KALCOUNIS, M. C., AND R. M. BRIGHAM. 1995. Intraspecific variation in wing loading affects habitat use by little brown bats (*Myotis lucifugus*). *Canadian Journal of Zoology* 73:89–95.
- KUNZ, T. H., J. A. WRAZEN, AND C. D. BURNETT. 1998. Changes in body mass and fat reserves in pre-hibernating little brown bats (*Myotis lucifugus*). *Ecoscience* 5:8–17.
- KURTA, A., AND R. H. BAKER. 1990. *Eptesicus fuscus*. *Mammalian Species* 356:1–10.

- LAUSEN, C., I. DELISLE, R. M. R. BARCLAY, AND C. STROBECK. 2008. Beyond mtDNA: nuclear gene flow suggests taxonomic oversplitting in the little brown bat (*Myotis lucifugus*):700–713.
- LEMON, W. C. 1991. Fitness consequences of foraging behaviour in zebra finch. *Nature* 352:152–155.
- LOCKWOOD, R., J. P. SWADDLE, AND J. M. V. RAYNER. 1998a. Avian wingtip shape reconsidered: Wingtip shape indices and morphological adaptations to migration. *Journal of Avian Biology* 29:273–292.
- MARCHETTI, K., T. PRICE, A. RICHMAN, K. MARCHETTI, T. PRICE, AND A. RICHMAN. 1995. Correlates of wing morphology with foraging behaviour and migration distance in the genus *Phylloscopus*. *Journal of Avian Biology* 26:177–181.
- MILÁ, B., R. K. WAYNE, AND T. B. SMITH. 2008. Ecomorphology of migratory and sedentary populations of Yellow-rumped Warbler (*Dendroica Coronata*). *The Condor* 110:335–344.
- MOORE, J. S., J. L. GOW, E. B. TAYLOR, AND A. P. HENDRY. 2007. Quantifying the constraining influence of gene flow on adaptive divergence in the lake-stream threespine stickleback system. *Evolution* 61:2015–2026.
- NEBAUM, M. A., M. R. DOUGLAS, M. E. DOUGLAS, AND T. J. O'SHEA. 2007. Molecular ecology of the big brown bat (*Eptesicus fuscus*). *Journal of Mammalogy* 88:1230–1238.
- NELSON, J. J., P. R. BARNHART, AND E. H. GILLAM. 2015. Distribution and occurrence of bat Species in North Dakota. *The Prairie Naturalist* 47:83–92.
- NORBERG, U. M. 1986. Evolutionary convergence in foraging niche and flight morphology in insectivorous aerial-hawking birds and bats. *Ornis Scandinavica (Scandinavian Journal of Ornithology)* 17:253–260.

- NORBERG, U. M., AND J. M. V. RAYNER. 1987. Ecological morphology and flight in bats (Mammalia ; Chiroptera): Wing adaptations, flight performance, foraging strategy and echolocation. The Royal Society, The Royal Transactions, Philosophical Society, Royal Sciences, Biological 316:335–427.
- NORQUAY, K. J. O., F. MARTINEZ-NUÑEZ, J. E. DUBOIS, K. M. MONSON, AND C. K. R. WILLIS. 2013. Long-distance movements of little brown bats (*Myotis lucifugus*). Journal of Mammalogy 94:506.
- O'MARA, M. T., K. BAUER, D. BLANK, J. W. BALDWIN, AND D. K. N. DECHMANN. 2016. Common noctule bats are sexually dimorphic in migratory behaviour and body size but not wing shape. PLoS ONE 11:1–13.
- RITCHIE, M. E. 1990. Optimal foraging and fitness in Columbian ground squirrels. Oecologia 82:56–67.
- SCHMIEDER, D. A., H. A. BENÍTEZ, I. M. BORISSOV, AND C. FRUCIANO. 2015. Bat species comparisons based on external morphology: A test of traditional versus geometric morphometric approaches. PLoS ONE 10:8–13.
- LO VALVO, F., G. LO VERDE, AND M. LO VALVO. 1988. Relationships among wing length, wing shape and migration in Blackcap *Sylvia atricapilla* populations. Ringing & Migration 9:51–54.
- VONHOF, M. J., A. L. RUSSELL, AND C. M. MILLER-BUTTERWORTH. 2015. Range-wide genetic analysis of little brown bat (*Myotis lucifugus*) populations: Estimating the risk of spread of white-nose syndrome. PLoS ONE 10:1–23.
- WALSH, A. L., AND S. HARRIS. 1996. Foraging habitat preferences of Vespertilionid bats in Britain. Journal of Applied Ecology 33:508–518.

CHAPTER 3: CONCLUSIONS

The objective of Chapter 1 was to use acoustic monitoring of echolocation to assess directional movements of bats along a major river corridor in Spring, Summer, and Fall. Two out of five bats were found making more southward movements in the Spring than expected, however, this could not be attributed to overall seasonal movement trends, based on variation explained using generalized linear mixed effect models. Further, these statistically significant patterns did not align with the species' life history, as findings suggested these two bats moved in the opposite direction to their known migratory pattern. Overall, no evidence was found for linear movement of bats along the Missouri River during migratory periods, suggesting the bats in this study system do not obligately follow the river.

The objective of Chapter 2 was to use geometric morphometrics to assess differences between two sympatric species differing in foraging behavior (open versus cluttered foraging) as well as examine population differences within species in regard to predominant available habitat type. Wing shape differences between species were found, aligning with past research using traditional morphometrics and known differences in foraging strategies. Specifically, the species foraging in open habitat had narrow, elongated, pointed wings, while the clutter forager had broader, shorter, rounder wings as predicted. Within species, statistically significant differences in wing shape were found between locations, yet exaggerated visualizations of wing shape for within species comparisons displayed no discernible differences.

Population genetics, stable isotopes, and banding have long been used to infer broad-scale movement patterns of bats and are responsible for much of what is known about bat migration (Fleming and Eby 2003). While these studies are valuable, they are limited by their inability to answer questions about the fine-scale movement patterns of bats. More recently,

researchers have attempted to use acoustics and traditional radiotelemetry to investigate landscape-level aspects of bat migration (e.g. Neubaum et al. 2006; Furmankiewicz and Kucharska 2009; Johnson et al. 2011). However, there are relatively few of these studies, and some of the inferences made are widely cited, yet rarely replicated. The first chapter of this thesis specifically aimed to test the claim that temperate bats use rivers as migratory corridors as found in the one study, widely cited testing this (Furmankiewicz and Kucharska 2009). The results of this did not find evidence in support of this claim.

As with bat migration, the study of wing morphology is undergoing a shift in methodology. Wing shape historically has been assessed using traditional morphometrics, which relied heavily on physical measurements and ratios, tracings, and was most easily conducted on museum or euthanized specimens (e.g. Norberg et al. 1986). These methods yielded many important insights regarding wing shape as it relates to foraging behavior, migration, and overall flight efficiency. Among species, birds and bats were found to vary in wing shape (narrow/pointed versus broad/rounded) largely as impacted by how they forage and aspects of their migratory ecology (Norberg et al. 1986; Norberg and Rayner 1987; Lo Valvo et al. 1988). Geometric morphometrics, a method of analyzing the shapes of structures using repeatable points placed on images (Rohlf 2015), has been proven to be more effective at characterizing closely related species (Schmieder et al. 2015). Thus, geometric morphometrics is expected to more effectively characterize intraspecific variation than traditional morphometrics. Intraspecific in wing shape in bats, in respect to migratory behavior, has been assessed using geometric morphometrics (O'Mara et al. 2016), yet this has not been applied intraspecifically to bat species occupying different habitat types. The second chapter of this thesis aimed to test potential differences in wing shape within species based on predominant available habitat, while further

validating differences between two species differing in foraging strategy. No differences were found within species, but the predicted differences between species were validated.

Increases in technological advances as well as reassessment of methods will likely contribute to considerable improvements in assessing the migratory ecology and morphology of bats. The first study ever using GPS tags to track a temperate bat was conducted recently and determined that migratory temperate bats are capable of making large movements over relatively short time spans without following any conspicuous landscape features (Weller et al. 2016). Further, a large scale study designed to test traditional assumptions of a common regionally migratory bat found no strong evidence that these bats closely followed rivers, as commonly accepted (Krauel et al. 2017). While these studies are limited by small sample size, logistic issues, and technological limitations, the evidence found offers significant insights into aspects of migratory behavior previously unknown. As technologies continue to improve in the coming years, and the possibility of long-term sampling with remote data logging approaches, our understanding of bat migratory behavior is expected to improve drastically. Although morphological studies will undoubtedly benefit from continued technological improvements, conclusions of these studies may become more powerful by increasing sample size, increased sampling areas across a greater geographical range, and incorporating genetic data to ensure isolation, all of which will provide a finer resolution from which to assess morphological impacts by environment.

References

FURMANKIEWICZ, J., AND M. KUCHARSKA. 2009. Migration of bats along a large river valley in Southwestern Poland. *Journal of Mammalogy* 90:1310–1317.

- JOHNSON, J. B., J. E. GATES, AND N. P. ZEGRE. 2011. Monitoring seasonal bat activity on a coastal barrier island in Maryland, USA. *Environmental Monitoring and Assessment* 173:685–699.
- KRAUEL, J. J., L. P. MCGUIRE, AND J. G. BOYLES. 2017. Testing traditional assumptions about regional migration in bats. *Mammal Research*. 1-9
- NEUBAUM, D. J., T. J. O'SHEA, AND K. R. WILSON. 2006. Autumn migration and selection of rock crevices as hibernacula by big brown bats in Colorado. *Journal of Mammalogy* 87:470–479.
- NORBERG, U. M. 1986. Evolutionary convergence in foraging niche and flight morphology in insectivorous aerial-hawking birds and bats. *Ornis Scandinavica (Scandinavian Journal of Ornithology)* 17:253–260.
- NORBERG, U. M., AND J. M. V. RAYNER. 1987. Ecological morphology and flight in bats (Mammalia ; Chiroptera): Wing adaptations, flight performance, foraging strategy and echolocation. *The Royal Society, The Royal Transactions, Philosophical Society, Royal Sciences, Biological* 316:335–427.
- O'MARA, M. T., K. BAUER, D. BLANK, J. W. BALDWIN, AND D. K. N. DECHMANN. 2016. Common noctule bats are sexually dimorphic in migratory behaviour and body size but not wing shape. *PLoS ONE* 11:1–13.
- ROHLF, F. J. 2015. The tps series of software. *Hystrix* 26:1–4.
- SCHMIEDER, D. A., H. A. BENÍTEZ, I. M. BORISSOV, AND C. FRUCIANO. 2015. Bat species comparisons based on external morphology: A test of traditional versus geometric morphometric approaches. *PLoS ONE* 10:8–13.

LO VALVO, F., G. LO VERDE, AND M. LO VALVO. 1988. Relationships among wing length, wing shape and migration in Blackcap *Sylvia atricapilla* populations. *Ringing & Migration* 9:51–54.

WELLER, T. J., K. T. CASTLE, F. LIECHTI, C. D. HEIN, M. R. SCHIRMACHER, AND P. M. CRYAN. 2016. First direct evidence of long-distance seasonal movements and hibernation in a migratory bat. *Scientific Reports* 6:34585.

APPENDIX. ADDITIONAL INFORMATION

Table A1. Landmark and semilandmarks descriptions used to assess bat wing shape.

Landmark	Description	Type
1	Center of wrist	Landmark
2	Base of 2nd finger	Semilandmark
3	Base of 3rd finger	Semilandmark
4	base of 4th finger	Semilandmark
5	base of 5th	Semilandmark
6	Between the base of 5th finger and 1st knuckle of 5th finger	Semilandmark
7	Base of 1st knuckle on 5th finger	Landmark
8	Between 1st and 2nd knuckle on 5th finger	Semilandmark
9	Base of 2nd knuckle on 5th finger	Landmark
10	Wingtip of 5th finger	Landmark
11	Along wing edge between 5th and 4th	Semilandmark
12	Along wing edge between 5th and 4th	Semilandmark
13	Along wing edge between 5th and 4th	Semilandmark
14	Along wing edge between 5th and 4th	Semilandmark
15	Along wing edge between 5th and 4th	Semilandmark
16	Along wing edge between 5th and 4th	Semilandmark
17	Along wing edge between 5th and 4th	Semilandmark
18	Along wing edge between 5th and 4th	Semilandmark
19	Wingtip of 4th finger	Landmark
20	Between the base of 4th finger and the 1st knuckle of the 5th finger	Semilandmark
21	Base of 1st knuckle on 4th finger	Landmark
22	Between 1st and 2nd knuckle on 4th finger	Semilandmark
23	Base of 2nd knuckle on 4th finger	Landmark
24	Between base of 2nd knuckle on 4th finger and wintip of 4th finger	Semilandmark
25	Along wing edge between 4th and 3rd	Semilandmark
26	Along wing edge between 4th and 3rd	Semilandmark
27	Along wing edge between 4th and 3rd	Semilandmark
28	Along wing edge between 4th and 3rd	Semilandmark
29	Along wing edge between 4th and 3rd	Semilandmark
30	Along wing edge between 4th and 3rd	Semilandmark
31	Along wing edge between 4th and 3rd	Semilandmark
32	Along wing edge between 4th and 3rd	Semilandmark
33	Along wing edge between 4th and 3rd	Semilandmark
34	Along wing edge between 4th and 3rd	Semilandmark
35	Wingtip of 3rd finger	Landmark
36	Between base of 3rd finger and 1st knuckle of 3rd finger	Semilandmark
37	Base of 1st knuckle of 3rd finger	Landmark
38	Between base of 1st knuckle and base of 2nd knuckle on 3rd finger	Semilandmark
39	Base of 2nd knuckle of 3rd finger	Landmark
40	Wing edge along 3rd finger	Semilandmark
41	Wing edge along 3rd finger	Semilandmark
42	Wing edge along 3rd finger	Semilandmark
43	Wing edge along 3rd finger	Semilandmark
44	Wing edge along 3rd finger	Semilandmark
45	Wing edge along 3rd finger	Semilandmark
46	Wing edge along 3rd finger	Semilandmark
47	Wing edge along 3rd finger	Semilandmark
48	Along the outer edge of the 2nd finger.	Semilandmark
49	Along the outer edge of the 2nd finger.	Semilandmark
50	Along the outer edge of the 2nd finger.	Semilandmark
51	Along the outer edge of the 2nd finger.	Semilandmark
52	Along the outer edge of the 2nd finger.	Semilandmark

Table A2. MANCOVA results for between species analysis (combined dataset of *M. lucifugus* and *E. fuscus*).

Variable	Df	Pillai	approx F	num Df	den Df	Pr(>F)	
Centroid Size	1	0.86699	78.87	10	121	< 2.2e-16	***
Location	1	0.17306	2.532	10	121	0.008334	**
Year	1	0.26233	4.303	10	121	3.81E-05	***
Species	1	0.39756	7.985	10	121	8.77E-10	***
Sex	1	0.06361	0.822	10	121	0.608181	
Location:Year	1	0.13154	1.833	10	121	0.061755	.
Location:Species	1	0.12108	1.667	10	121	0.096161	.
Residuals	130						

Table A3. MANCOVA results for within species analysis of *M. lucifugus*.

Variable	Df	Pillai	approx F	numDf	denDf	Pr(>F)	
Centroid Size	1	0.41518	5.1824	10	73	1.05E-05	***
Location	1	0.31934	3.4249	10	73	0.00101	**
Year	1	0.18923	1.7038	10	73	0.09606	.
Sex	1	0.10064	0.8169	10	73	0.61334	
Location:Year	1	0.152	1.3085	10	73	0.24224	
Residuals	82						

Table A4. MANCOVA results for within species analysis of *E. fuscus*.

Variable	Df	Pillai	approx F	numDf	denDF	Pr(>F)	
Centroid Size	1	0.56056	4.4648	10	35	0.00044	***
Location	1	0.42795	2.6184	10	35	0.01715	*
Year	1	0.52237	3.8279	10	35	0.00147	**
Sex	1	0.27334	1.3165	10	35	0.26007	
Location:Year	1	0.2632	1.2503	10	35	0.29524	
Residuals	44						