ABSTRACT

Title of Dissertation:	INFORMING CONSERVATION OF THREATENED BAT SPECIES USING GENOMICS AND ACOUSTICS
	Juliet Joy Nagel, Doctor of Philosophy, 2022
Dissertation directed by:	Professor David Nelson, Marine, Estuarine, and Environmental Science

Bats are vital to healthy ecosystems, providing billions of dollars of services in the form of forest and crop pest control. Unfortunately, North American bat populations have faced novel pressures during the past decade that may threaten their persistence. First, several species of tree-roosting bats (primarily hoary [*Lasiurus cinereus*], eastern red [*L. borealis*], and silver-haired [*Lasionycteris noctivagans*] bats) are experiencing large numbers of fatalities at industrial wind-energy facilities. Second, several species of cave-dependent bats have experienced large-scale mortality as the result of infection by a fungal pathogen that causes white-nose syndrome (WNS). As bats are generally long-lived and have low reproductive rates, such increases in mortality can cause significant population declines from which they may be unable to recover. Basic questions about population trends, size and structure remain largely unanswered for these species because of challenges in applying traditional wildlife monitoring approaches to bats. This lack of understanding impedes conservation and management efforts. In my dissertation, I use genomic and acoustic survey techniques to investigate questions related to the threats that wind-energy development and WNS are posing to bat species in North America. In my first chapter, I evaluate range-wide

population structure and effective population size (N_e) for hoary, eastern red, and silver-haired bats. Using genotyping-by-sequencing (GBS), I genotyped single-nucleotide polymorphism (SNP) data from 173 hoary, 113 eastern red, and 89 silver-haired bats from multiple locations spread across their geographic distributions. Hoary bats and eastern red bats showed no geographic structure in genetic diversity, whereas silver-haired bats displayed longitudinal population variation. Coalescent modeling suggested that eastern red bats have the largest evolutionary N_e, followed by hoary bats, then silver-haired bats. In my second chapter, I used GBS to assess the population structure of two federally endangered cave bat species: Indiana bats (*Myotis sodalis*) and gray bats *(M. grisescens*). Using tissue samples from 45 Indiana bats and 47 gray bats spread across their ranges, I showed that Indiana bats display no geographic genetic structure, whereas gray bats exhibit east–west population variation across the Mississippi River Valley. In my final chapter, I used acoustic surveys across the State of Maryland to investigate bat community changes in the decade following the arrival of WNS. From 2010 through 2019, I conducted annual mobile acoustic routes each summer, for a total of 344 completed routes resulting in 426 hours of recordings and 24,375 identified bat passes. I detected massive (> 92%) declines of little brown bats (*M. lucifugus*), northern long-eared bats (*M. septentrionalis*), eastern small-footed bats (*M. leibii*), and tricolored bats (*Perimyotis subflavus*), with no evidence of recovery in recent years. Trends in hoary bats and eastern red bats were non-significant during this period. Bat community composition varied among Maryland's physiographic regions, with eastern red bats comprising a larger percentage in the east. Species composition across the state likely reflects the

impact of several factors, including mortality from WNS and wind-energy development, and perhaps reduced interspecific competition. Overall, my results illustrate the unique insights, but also distinct limitations, that genomic and acoustic data can provide regarding the conservation of bats in North America.

INFORMING CONSERVATION OF THREATENED BAT SPECIES USING GENOMICS AND ACOUSTICS

by

Juliet Joy Nagel

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2022

Advisory Committee:
Professor David Nelson, Chair
Professor Emeritus J. Edward Gates
Dr. Paul Gugger
Assistant Professor Jennifer Mullinax
Associate Professor Louis Plough
Professor Reginal Harrell, Dean's Representative

Dedication

With gratitude and love, I dedicate this dissertation to my family. My Dad, who showed me the outdoors and told me I could do and be anything. I believed you, and set off to chase bats. My sisters, three incredibly kind, intelligent, and creative women. I could not have completed this without your support, laughter, and love. I will forever be thankful to have each of you in my life. And to my Mom. You were a spark of joy and positivity, always seeing the best in people and the world around you. You gave me the courage to follow my own path. I will love and miss you always.

Acknowledgements

They say it takes a village to raise a child, and while I am no longer a "child," the same is certainly true of earning a PhD. First and foremost, I thank my advisor Dave Nelson for giving me this chance and sticking with me through a rough road. I am grateful to have you as a guide on this journey. I also thank Paul Gugger for teaching me conservation genetics, and answering my many "Paul, why isn't this working?" questions while learning to write code. Thank you also to my committee members: Ed Gates, Jennifer Mullinax, and Louis Plough; your expertise has been invaluable. Thank you to Regina Trott for teaching me all I know about lab work; I would not have made it far without your help and friendship. Thank you to Caitlin Campbell, a fellow bat friend, for your consistent encouragement and extensive modeling help. Thanks to Shannon Pederson and Dana Limpert for driving around Maryland's roads in the dark, recording bat calls. Many thanks to the several dozen people who sent me tissue samples from across North America, the genomics chapters would not have been possible without all of you. And a big thank you to the staff at the Appalachian Lab, especially Heather Johnson, who was always in my corner, fighting for whatever I needed. I appreciate you all.

Table of Contents

List of Tables

Chapter 2

Chapter 3

Chapter 4

List of Figures

Chapter 2

List of Abbreviations

AIC	Akaike Information Criterion
BIC	Bayesian Information Criterion
CI	Confidence Interval
DAPC	Discriminant Analysis of Principal Components
GBS	Genotyping-By-Sequencing
IUCN	International Union for Conservation of Nature
K	Number of genetic clusters
LD	Linkage-Disequilibrium
m	Minimum stack depth
M, n	Distance allowed between stacks
N_c	Census population size
N_e	Effective population size
PCA	Principal Components Analysis
PC1	Principal Component 1
RADseq	Restriction site-Associated DNA sequencing
SFS	Site Frequency Spectrum
SNPs	Single-Nucleotide-Polymorphisms
WNS	White-Nose Syndrome

Chapter 1: Introduction

Background

In western culture, bats often have a negative connotation as organisms to be feared, which has frequently led to persecution by humans (Sieradzki and Mikkola 2022). Such fears are largely unfounded, as bats provide numerous services to humans and ecosystems including insect predation, pollination, and seed dispersal (Fenton 2001; Kunz et al. 2011). In North America, nearly all bat species are insectivorous. These bats consume a wide range of forest and crop pests, a service estimated at billions of dollars to North American agriculture (Boyles et al. 2011), and help protect forest seedlings from insect herbivory (Beilke and O'Keefe, 2022).

As insects are not available year-round in colder parts of the continent, bats living in temperate regions of North America have developed two main strategies to survive winter: hibernate or migrate to warmer regions. Species that use the former strategy are referred to as cave bats. These species make use of stable winter temperatures in caves or mines, gathering at hibernacula each winter that may be 100s of kilometers from their summering habitat. Mating usually occurs in fall; in spring, they disperse across the landscape where they may use a variety of roosts (e.g., trees, human structures, rock outcroppings) depending on specific species' preferences. Many species form maternity colonies where females give birth and raise their young (Fenton 2001). Cave bats are often censused through winter hibernacula counts, a valuable tool to monitor population changes in the species. Indiana bats (*Myotis*

sodalis) for example, a federally endangered cave bat species, have been surveyed biannually at known hibernacula since the early 1980s (USFWS 2018).

Species that do not usually hibernate in caves are termed tree bats, so called because they typically roost in trees year-round. Often roosting alone in tree canopies, these species do not form summer colonies or gather in groups at winter hibernacula (i.e., cave) sites. Instead, they use annual long-distance migration, flying to warmer regions in the fall and cooler regions in the spring (Fenton 2001). Thus, they cannot be censused in the same way as cave bats are censused via winter hibernacula counts. Traditional methods such as mark-recapture are also poorly suited for tree bats, as these species are cryptic, highly mobile, and difficult to capture (O'Shea and Bogan 2003). Consequently, basic population demographics, such population size, growth rate, and structure, remain largely unknown.

Both cave and tree bat species are at risk from a changing climate and habitat loss (Jones et al. 2009; Rebelo et al. 2010). Each group has faced additional recent, but distinct, threats to their persistence during the past several decades. Many cave-dependent bats have been experiencing mass mortality caused by the fungal disease white-nose syndrome (WNS) (Frick et al. 2015), and hundreds of thousands of long-distance migratory tree bats are being killed at utility-scale wind-energy farms annually (Arnett and Baerwald 2013). As bats are generally long-lived and have low reproductive rates, this increased adult mortality can cause significant population declines from which they may be unable to recover (Barclay and Harder 2003; Podlutsky et al. 2005; Frick et al. 2017).

White-nose syndrome, caused by the pathogen *Pseudogymnoascus destructans*, first appeared in North America in 2006 (Blehert et al. 2009). A cold-loving fungus, it grows on the epidermis of cave-dwelling bats during hibernation, causing lesions (Meteyer et al. 2009), disrupting hibernation patterns, and ultimately leading to death (Warnecke et al. 2013; Frick et al. 2015; Bohn et al. 2016). Massive declines (> 90%) have been documented for multiple cave bat species across eastern North America, especially little brown bats (*Myotis lucifugus*), northern long-eared bats (*M. septentrionalis*), and tricolored bats (*Perimyotis subflavus*) (R. T. Brooks 2011; Dzal et al. 2011; Francl et al. 2012; Powers et al. 2015; Pettit and O'Keefe 2017; O'Keefe et al. 2019). Some cave bat species, such as big brown bats (*Eptesicus fuscus*) and gray bats (*Myotis grisescens*) are not experiencing the same declines, even though the fungus has been detected on them (Frank et al. 2014; Cheng et al. 2021; Jackson et al. 2022).

While the fungus that causes WNS has been detected on tree bats, so far they have displayed no symptoms of the disease (Bernard et al. 2015; Campbell et al. 2022). Tree bats, however, and three species in particular, are most at risk of mortality from rotating turbine blades at wind-energy facilities. Hoary (*Lasiurus cinerieus*), eastern red (*L. borealis*), and silver-haired bats (*Lasionycteris noctivagans*) are killed in large numbers during fall migration each year (Arnett et al. 2008; Arnett and Baerwald 2013). One approach that has proven most effective in mitigating these fatalities involves raising the wind speed at which turbines begin operating (Baerwald et al. 2009; Arnett et al. 2011; Arnett and May 2016). However,

such mitigation is largely voluntary, and it remains unknown whether wind-energy mortality is causing population level impacts (O'Shea et al. 2016; Frick et al. 2020).

These threats disproportionally affect different species, and thus declines or extirpation of some species may allow others with similar prey or habitat requirements to increase in abundance once being released from the pressure of interspecific competition (Morningstar et al. 2019). Given the importance of bats to a functioning ecosystem, it is important to understand how species composition and abundances are changing in the face of these multiple impacts.

While many traditional wildlife techniques are challenging to apply to bats, two promising tools for bat research are genetic studies and acoustic surveys (Hale et al. 2022). Population genetics can offer insights into a species genetic diversity, population structure, and effective population size, which are metrics useful for management and conservation. Understanding gene flow and identifying genetically distinct subpopulations is essential to preserving a species' adaptive genetic variance, and can guide delineation of management units (Crandall et al. 2000; Fraser and Bernatchez 2001). A shortcoming of population genetics is that it can be difficult to assess short-term changes in population demographics that may be relevant to conservation. These short-term changes may be better assessed with other methods, such as acoustic monitoring. Acoustic monitoring (i.e., recording bats' echolocation calls) is a repeatable survey method that does not require bat handling, can cover a broad geographic area, surveys for multiple species simultaneously, and is relatively low expense (Whitby et al. 2014; Fisher-Phelps et al. 2017; Neece et al. 2019; Hale et al. 2022). In my dissertation, I applied these tools to investigate questions related to

the threats that wind-energy development and WNS are posing to tree-roosting and cave bats, respectively, in North America.

Research Overview

In chapter 2, I evaluate range-wide population structure and effective population size (N_e) for hoary, eastern red, and silver-haired bats. Using genotyping-by-sequencing (GBS), I genotyped single-nucleotide polymorphisms (SNPs) from 173 hoary, 113 eastern red, and 89 silver-haired bats from multiple locations spread across their geographic distributions. I assessed population structure with ADMIXTURE, principal component analyses, and gradient forest modeling. I calculated evolutionary N_e using coalescent modeling, and contemporary N_e using the linkage-disequilibrium method. Hoary bats and eastern red bats showed no geographic structure in genetic diversity, while silver-haired bats displayed longitudinal population variation. Coalescent modeling of N_e suggested that eastern red bats have the largest evolutionary N_e, followed by hoary bats, then silver-haired bats.

In chapter 3, I use GBS to assess the population structure and N_e of two federally-endangered cave bat species: Indiana bats (*Myotis sodalis*) and gray bats (*M. grisescens*). Both species are reliant on a limited number of winter hibernacula sites, placing them at risk of human disturbance and habitat loss. As these species are relatively well-censused through winter hibernacula counts, I sought to calculate an N_e/N_c ratio, using linkage-disequilibrium (LD N_e) to calculate a contemporary measurement of N_e and relating it to the census counts. Doing so would be valuable for estimating census population sizes in at-risk, but hard-to-monitor, related species

like the hoary bat. Using tissue samples from 45 Indiana bats and 47 gray bats spread across their ranges, I show that Indiana bats display no genetic structure, while gray bats exhibit east-west population variation across the Mississippi River Valley. LD N_e estimates had confidence intervals including infinity for both species, thus I was unable to calculate N_e/N_c ratios.

In Chapter 4, I use acoustic surveys across the State of Maryland to investigate bat community changes in the decade after arrival of WNS. From 2010, immediately after the first detection of WNS in the state on 5 March 2010, through 2019, I conducted annual mobile acoustic routes each summer, for a total of 344 completed routes resulting in 426 hours of recordings and 24,375 identified bat passes. I detected precipitous (> 92%) declines of little brown bats, northern long-eared bats, eastern small-footed bats, and tricolored bats. Population trends in hoary bats and eastern red bats were non-significant during this period.

Chapter 2: Range-wide population genetic structure and effective sizes of three long-distance migratory tree bat species impacted by wind-energy development in North America

Abstract

Long-distance migratory bat species are killed in large numbers across utility-scale wind-energy facilities each year, but basic population demographic information (e.g., size and structure) necessary for understanding potential population-level impacts are unknown. Particularly impacted are hoary (*Lasiurus cinereus*), eastern red (*L. borealis*), and silver-haired bats (*Lasionycteris noctivagans*). Previous genetic studies with these species used samples from a fraction of each species' range, sometimes just a single location. Here I evaluate range-wide population structure and effective population size for these species, using genotyping-by-sequencing (GBS). Specifically, I genotyped single-nucleotide polymorphism (SNP) data from 173 hoary, 113 eastern red, and 89 silver-haired bats from multiple locations spread across the species' ranges. All samples were from bats caught or collected during their summering period, defined by published studies that used fur stable hydrogen isotope data to determine migration periods. Population structure for each species was assessed using ADMIXTURE, DAPC, PCA, and Gradient Forest analyses. Hoary bats and eastern red bats showed no geographic structure in genetic diversity, suggesting panmictic population structure for these species. In contrast, the Principal Component Analysis of GBS data for silver-haired bats revealed longitudinal population variation along PC1, which accounted for 2.06% of all variation. Geographically, this results in an apparent east–west separation near the Rocky Mountains, though with some spatial overlap. In terms of effective sizes, coalescent

modeling suggest that eastern red bats have the largest N_e (126,142–131,153) followed by hoary bats (101,355–106,128), then silver-haired bats (49,551–64,801). The best-fit model for all three species included long-term increasing populations on an evolutionary timescale. Regions with high mortality may function as population sinks for the panmictic eastern red and hoary bats, negatively impacting the species range-wide, thus managers should consider the entire population when considering conservation measures. Silver-haired bats, with alleles varying in abundance across the landscape, may be more vulnerable to local population declines and loss of rare alleles, and may be best managed by considering both eastern and western populations.

Introduction

Bats provide a range of vital ecosystem services including insect predation, pollination, and seed dispersal (Fenton 2001; Kunz et al. 2011; Beilke and O'Keefe 2022). However, many bat species, and the services they provide, are under threat from factors such as habitat loss, climate change, pesticide use, and mortality at utility-scale wind-energy facilities. In the United States alone, it is estimated that wind-energy facilities kill hundreds of thousands of bats annually, the majority of which are three tree-roosting, insectivorous, and migratory species: hoary (*Lasiurus cinereus*), eastern red (*Lasiurus borealis*), and silver-haired (*Lasionycteris noctivagans*) bats (Arnett et al. 2008; Arnett and Baerwald 2013). These species also make up the majority of bats killed at wind-energy facilities in Canada (Zimmerling and Francis 2016). Since these estimates were published, wind-energy capacity in North America has more than doubled ("CanWEA" 2022; ACP 2022). As bats are

generally long-lived and have low reproductive rates, increased adult mortality can cause significant population declines from which they may be unable to recover (Barclay and Harder 2003; Podlutsky et al. 2005). Mitigation approaches such as raising the cut-in speeds of turbines have proven effective at reducing bat mortality rates at wind-energy fatalities (Baerwald et al. 2009; Arnett et al. 2011; Arnett and May 2016; Weaver et al. 2020). However, in the absence of mandated widespread application of effective mitigation, there remains the need to assess whether wind energy impacts have population-level implications (O'Shea et al. 2016; Frick et al. 2020).

Unfortunately, estimating census population size (N_c) of those species most affected by wind turbine mortality is not possible using traditional methods, such as mark-recapture, as they are nocturnal, cryptic, highly mobile, and do not gather in groups that can be censused (O'Shea and Bogan 2003). Thus, basic population demographics, such as population size, growth rate, and structure, remain largely unknown. Given the lack of empirical estimates, expert elicitation and data from related taxa have been used to model the potential effect of long-term mortality at wind-energy facilities on continent-wide population viability of hoary bats (Frick et al. 2017). After extrapolating fatality rates of hoary bats to North American wind-energy capacity in 2014, population projection models suggested a 90% decline by 50 years and a 22% risk of extinction by 100 years. Subsequent expansion to account for projected buildout of wind-energy facilities through 2050 suggest that significant population declines may have already occurred if the initial population size of hoary bats was below 3 million individuals (Friedenberg and Frick 2021). No such analyses

have been performed for eastern red or silver-haired bats, to my knowledge. These expert elicitation methods are limited by the gaps in fundamental knowledge of the species.

Population genetics can offer insights into a species' genetic diversity, population structure, and effective population, which are metrics useful for management and conservation. Evolutionary events in a species' history, such as bottlenecks or population growth, range expansion or contraction, and migration or isolation, all influence the abundance and distribution of alleles on the landscape (Ellegren and Galtier 2016). If genetically unique subpopulations experience unusually high local mortality, rare alleles may be lost from the species (Pannell and Charlesworth 2000). A summary of genetic diversity in a population, N_e, represents the number of individuals in a theoretical ideal population that would experience the same amount of genetic drift observed in the actual population (Wright 1931). At low values of N_e, genetic drift can threaten a population's viability through loss of alleles, reduced fitness, and inbreeding, thus limiting the species' potential to respond to selection and environmental change (Frankham 2005).

Various methods are used to estimate N_e. Some methods, such as coalescent modeling, reflect the combined effects of population demographics in past and present generations, providing a long-term evolutionary N_e. Other methods, such as linkage-disequilibrium (LD N_e), measure contemporary N_e and can detect recent population declines, although estimating contemporary trends is particularly challenging (Tallmon et al. 2012). LD N_e is most accurate for very small populations with a census size under 100 (Waples et al. 2016), and confidence intervals of LD N_e

are often large for species with large census population sizes, even encompassing infinity at the upper bound (Do et al. 2014). A confidence interval bounded by infinity does not imply a very large population; rather it is an artifact of the method failing to constrain the estimate with precision.

Several microsatellite studies have been previously conducted on tree-roosting bats. Using six microsatellites and mitochondrial DNA, hoary and eastern red bats collected in Texas and Minnesota showed no evidence of population structure, and had LD N_e values with upper confidence bound limits reaching infinity (Korstian et al. 2015). Another study used 14 microsatellites of eastern red and hoary bats found at wind-energy facilities in Maryland, West Virginia, and Pennsylvania, and found no evidence of population structure (Pylant et al. 2016). Using coalescent modeling, they reported an N_e of ~335,000 for eastern red bats (95% CI 0.06–2.61 million), but a much smaller N_e for hoary bats (~1,600, 95% CI 662–4697).

Recent advances now allow the analysis of thousands of genetic loci per individual in the form of single-nucleotide polymorphisms (SNPs), even for non-model organisms, with little or no prior existing genomic data (Gayral et al. 2013). This development provides more accurate and precise inferences of population structure and N_e than those based on microsatellites (Liu et al. 2005). Using RADseq, a reduced-representation sequencing method to analyze SNPs, samples collected at a wind-energy facility in Ohio showed no population structure in silver-haired, hoary, or eastern red bats, with coalescent models estimating current N_e of 189,288 (CI 155,354–321,689), 830,623 (CI 730,470–884,046), and 1,600,183 (CI 1,241,280–1,692,354), respectively (Sovic et al. 2016). Hoary bats from a wind-energy facility in

Indiana were also analyzed with RADseq, demonstrating no population structure once low-data samples were removed, with LD N_e estimates including confidence intervals bounded by infinity (Cornman et al. 2021). Finally, a recent study sequenced whole mitochondrial genomes from three silver-haired bats (one each from California, Idaho, and West Virginia), and found that the individuals from California and Idaho clustered separate from the individual from West Virginia (Monopoli et al. 2020).

Unfortunately, previous studies cannot be readily integrated and compared, as they used different genetic and genomic methods, with varying microsatellites or different primers and genomic analyses. Additionally, previous studies used samples sourced from a small portion of the species' ranges, sometimes only a single location. As a result, we lack a clear understanding of range-wide structure and confidence estimates for N_e for tree-roosting bats. This information is urgently needed to inform conservation and management of these species.

Using genotyping-by-sequencing to analyze 375 samples from multiple locations spread across the species' ranges, I addressed the following questions: What is the genetic population structure of hoary bats, eastern red bats, and silver-haired bats across North America? What are the effective sizes of genetically distinct populations, or the population as a whole if no structure exists? Secondarily, if population structure is found, what landscape variables explain the structure?

Methods

Tissue Collection

I obtained tissue or extracted DNA from hoary, eastern red, and silver-haired bats that were sampled across their ranges by state and federal natural resources agencies, private companies, museums, and universities. Most (76.5%, Table 1) sequenced samples were from bat carcasses collected at wind-energy facilities; the remainder were live captures (21%) or were acquired through rabies labs or museums (4%). Samples consisted of wing, tail, ear, or liver tissue, or were DNA that had already been extracted. Tissue samples were preserved in either ethanol or silica pellet desiccant upon collection and stored at −80 °C. All samples used in the following analyses were from bats caught or collected during their summering period, as defined primarily by stable hydrogen isotope data: 20 June to 23 August for hoary bats (Cryan et al. 2014), 14 June to 7 August for eastern red bats (Pylant et al. 2014), and 20 June to 26 August for silver-haired bats (Fraser et al. 2017).

DNA Extraction and Sequencing

I extracted DNA from tissue samples using DNeasy Blood & Tissue Kits (Cat no. 69504 or 69506; QIAGEN GmbH, Germany). I followed all kit protocol, with the addition of a 6-minute centrifugation at 17,000 x g following tissue digestion to pellet out pigments and fur. Extracted DNA was quantified using an Invitrogen Qubit 1.0 Fluorometer. Samples with a quantification at or above 100 ng/μL were diluted with nuclease-free water before further processing. Samples between 3 and 7 ng/μL were

concentrated using a vacufuge. Samples with a concentration below 3 ng/μL were excluded from further analyses.

DNA libraries were prepared using a genotyping-by-sequencing (GBS) approach (Elshire et al. 2011), following the modifications applied by Gugger et al. (2021). Starting with 100 ng of DNA per sample, bat DNA was first digested with the restriction enzyme *Pst*I. Sequencing adapters containing unique barcodes for each sample were then ligated to the resulting fragments. These barcoded samples were pooled in batches of 48 and amplified with PCR; each batch of 48 was considered one "library." Samples were sequenced single-end, 50-bp on an Illumina HiSeq4000 v3 at the Coates Genomics Sequencing Laboratory at the University of California Berkeley. Before sequencing, each library was checked with Bioanalyzer for quality and fragment size. I sequenced 13 libraries, including five for hoary bats, three for eastern red bats, four for silver-haired bats, and one consisting of multiple species. Three or four technical replicates were included in each library to assist in Stacks parameter optimization (see below). Individuals sampled at different locations were spread across libraries (i.e., samples originating from any given location were sequenced on multiple libraries) to prevent potential biases from individual sequencing runs.

SNP Discovery and Filtering

Illumina reads were processed using the Stacks 2.41 bioinformatics pipeline (Catchen et al. 2011, 2013) and analyzed for single-nucleotide polymorphisms (SNPs). Stacks can identify SNPs *de novo*, even when a reference genome is unavailable. The *process_radtags* function was used to demultiplex and quality-filter

the Illumina data, after which individual samples with low read counts (< 30% of the median number of reads) were removed from analyses. To identify the optimal parameters for Stacks, I used Principal Component Analyses (PCA) of the samples repeated across libraries, following methods outlined by Gugger et al. (2018). For each species, I tested seven combinations of parameters (minimum stack depth, distance allowed between stacks, and bound-high values), and the combination that resulted in the best clustering of technical replicates was used for further analyses (Figure 1); this protocol was repeated for each species. Differences between parameter settings were small, suggesting the results are likely robust to changes in parameters.

Using the optimal parameters selected, the Stacks pipeline identified loci, and then the Stacks *populations* function were used to apply filters and output the data for use in other programs. First, a SNP had to have < 20% missing data. For population structure analyses, I also removed SNPs with a minor allele frequency less than 0.05, and I used the write-single-SNP option to keep only one SNP per stack/fragment, thus reducing the number of physically linked SNPs and the effects of linkage disequilibrium. For estimation of effective population size, I produced a site frequency spectrum (SFS) using easySFS (Overcast [2016] 2021). When filtering SNPs for the SFS, the write-single-SNP option was included, but SNPs were not filtered for minor allele frequency.

Genomic Analyses

Population structure for each species was assessed using multiple methods, including ADMIXTURE (Alexander et al. 2009), discriminant analysis of principal

components, PCA, and gradient forest modeling. ADMIXTURE uses the same likelihood model-based estimation of ancestry as the program STRUCTURE (Pritchard et al. 2000) used by Pylant et al. (2016), but ADMIXTURE integrates a faster algorithm that is better able to handle the large SNP datasets produced by GBS. By modeling the probability of the observed genotypes using population allele frequencies, ADMIXTURE estimates *K*, the number of genetic clusters ("populations"), without *a priori* knowledge of geographic sampling. Multiple values of *K* are tested, with the most likely value of *K* selected to minimize cross-validation error. ADMIXTURE assigns individuals, either wholly or partially, to each cluster, and therefore, it assumes discrete populations exist or have existed, followed by possible admixture among those populations.

Discriminant analysis of principal components (DAPC) optimizes variance between groups, seeking to describe the differences between them. As such, this method also assumes discrete populations, or clusters. In addition, DAPC requires that these prior groups be defined. The *adegenet* package (Jombart 2008) in R allows for first identifying groups with the *find.clusters* function using the k-means method. These clusters are then used in the DAPC, which outputs membership probabilities for each sample.

A limitation of the above methods is the assumption of discrete populations, which is unlikely to be true of highly mobile species of bat. Therefore, population structure was also assessed using PCA to identify potentially continuous gradients in allele frequency shifts across each species' range. Although PCA can identify gradients of population structure, it does not account for additional geographic or

environmental variables which may be influencing the pattern of alleles on the landscape. An additional benefit of gradient forest over a PCA is the ability of gradient forest to detect nonlinear responses in allele frequency to environmental variables. To test whether landscape variables correlate with population structure, I applied gradient forest analysis in R version 4.04 (*gradientForest* package [(Ellis et al. 2012)]). In the gradient forest models I included latitude, longitude, and 4 bioclimatic variables from WoldClim version 2.1 (Fick and Hijmans 2017): elevation, max temperature of the warmest month, minimum temperature of the coldest month, and seasonal precipitation. WorldClim climate data are an average for the years 1970-2000. I used a spatial resolution of 2.5 minutes, approximately 4.5 km at the equator.

I used *fastsimcoal2* (Excoffier and Foll 2011) to estimate coalescent effective population size (N_e) and NeEstimator V2 (Do et al. 2014) to calculate LD N_e. The package *fastsimcoal2* uses coalescent modeling to estimate parameters that would produce the given SFS under a modeled evolutionary scenario. For each species, I fit two models. The first model assumed a constant population size and contained only a single variable, the estimated N_e. The second model contained additional variables: a changing population size, the rate of change, the number of generations previous to when the population size began to change, and the ancestral N_e. The two models were compared with Akaike information criterion (AIC). For all species, the model that included changing population size had a lower AIC, indicating a better fit, and I selected that model for subsequent analyses.

I used bootstrapping to calculate the confidence interval for N_e, following the methods outlined in Excoffier and Foll (2011). First, I performed 100 independent

runs of *fastsimcoal2* (30 cycles and 50,000 simulations per run) and selected the run with the highest likelihood. The parameter values from the best run were used to simulate 50 datasets, and each simulated SFS was run through *fastsimcoal2*. The minimum and maximum values from the 50 runs were retained as the bounds of the confidence intervals.

I estimated contemporary N_e with the linkage disequilibrium method (LD N_e) using the program NeEstimator V2 (Do et al. 2014). The LD method is among the most robust and accurate methods to estimate contemporary N_e (Wang et al. 2016; Waples and Do 2010); additionally it only requires a single sample in time, unlike other methods that need samples separated by generations, difficult to collect with a long-lived bat species. With large SNP datasets like those presented here, failure to account for physically linked SNPs can substantially underestimate N_e and cause confidence intervals to be estimated too narrowly (Waples et al. 2016). In addition, using 4000 SNPs only added a slight benefit over 1000 SNPs, suggesting that quality is preferable to quantity (Waples et al. 2016; Marandel et al. 2020). As I did not have reference genomes for these species, and therefore could not ascertain if SNPs are physically linked, I used a random subset of 1000 SNPS to calculate LD N_e. I also assumed random mating and screened out alleles with frequencies below 0.05. Rare alleles are more likely to be sequencing errors and have been show to upwardly bias N_e in simulated datasets (Waples and Do 2010).

Results

I sequenced DNA from an initial dataset of 417 summering bats, comprising 195 hoary, 129 eastern red, and 93 silver-haired bats (Table 1). Twenty-four samples

had low read counts and were removed from further analyses (12 hoary, 8 eastern red, and 4 silver-haired bats). Ten additional hoary bats and eight eastern red bats were also removed from analyses due to outlier status, as described below. Final datasets used in structure analyses include 375 samples (173 hoary bats, 113 eastern red bats, and 89 silver-haired bats [Figure 2]).

To test for potential species misidentification, the 393 samples with sufficient data were combined in a PCA. Missing data for each sample were replaced with average values, thus samples with fewer reads fell towards the origin. Two individuals field-identified as hoary bats (one from New Mexico and one from Maryland) fell between hoary and eastern red bats, suggesting either hybridization or sample contamination. Another individual field-identified as a hoary bat (from Oregon) fell in line with the silver-haired bats (Figure 3). These three bats were removed from the dataset, resulting in 180 hoary bat samples (the initial 195, minus 12 with low read counts and the three removed here).

Population Structure

The 180 hoary bat samples were analyzed in a PCA, resulting in four "outlier" bats that fell far outside the cluster of the others (Figure S1). As these four samples showed no geographic pattern, they were removed from the dataset, and the PCA was rerun. This second dataset of 176 samples showed three new outliers (Figure S2), again with no geographic pattern. When these three were removed, the final hoary bat dataset of 173 samples showed a scattershot pattern, with no geographic structure based on longitude. Graphing the PC1 score geographically by sample origin also did not result in any clear population structure. Eastern red bats showed a similar pattern

of outliers. The initial PCA of 121 bats had six outliers. When these were removed, two new outliers emerged. After these were also removed, a PCA of the final dataset of 113 samples showed no geographic pattern. The PCA for silver-haired bats revealed longitudinal population structure along PC1, which accounted for 2.06% of all variation. Geographically, this indicates an apparent east–west separation near the Rocky Mountains, though with some spatial overlap (Figure 4).

In addition to the PCA analyses, I also used ADMIXTURE and DAPC to assess potential population structure. Both methods indicated a best fit of $K = 1$ (Figures S3 and S4). These methods, however, are unlikely to detect gradual changes in allelic frequencies.

Gradient forest analyses of silver-haired bats indicated that longitude had the greatest R^2 weighted importance (0.013), followed by latitude (0.011). The cumulative importance graph for longitude showed a rapid change around −110°N (Figure 5). The gradient forest model for hoary bats had even lower R^2 values, ~0.005 for latitude and longitude (Figure 6). The eastern red bat gradient forest model had R^2 values highest for precipitation and elevation (0.013), but lower for latitude (0.011) and longitude (0.008) (Figure 7).

Effective Population Size

Using coalescent modeling with *fastsimcoal2*, the best-fit model for all three species included long-term increasing populations on an evolutionary timescale. Eastern red bats had the largest N_e (126,142–131,153) followed closely by hoary bats (101,355–106,128). When silver-haired bats were split into an eastern group (11,687–

90,707) and western group (103–124,461), the confidence intervals were large. When analyzed as a single population, N_e was 49,551–64,801.

LD N_e point estimates were much smaller than those estimated by coalescent modeling, but the 95% confidence interval included infinity in the upper bounds. For hoary bats, N_e was 1445 (95% CI: 958–∞). Eastern red bat LD N_e was 771 (95% CI: 312–∞), and silver-haired bat LD N_e was 426 (95% CI: 173–∞).

Discussion

My range-wide sampling that included dozens of sites across North America indicates that eastern red and hoary bats are panmictic, whereas silver-haired bats display a generally east–west gradient in genetic population variation. The data show valuable information for wildlife managers in the context of conserving these species from threats, such as fatalities from wind turbines. Species with genetic structure and distinct subpopulations require efforts to conserve each population to maintain genetic diversity; under panmixia, declines in regions of a species' range is less concerning from a genetic conservation viewpoint (Pinsky et al. 2010).

Gradient forest modeling allows us to evaluate the association of additional environmental variables that may influence genetic pattern on the landscape beyond simply spatial distance. For example, genetic structure of a European bumble bee, *Bombus terrestris*, was influenced by temperature, vegetation, and topography, but not by geographic distance (Glück et al. 2022). Along with latitude and longitude, I included the variables elevation, maximum temperature of the warmest month, minimum temperature of the coldest month, and seasonal precipitation (WorldClim, all averages of the years 1970–2000), as these will influence habitat characteristics

important to bats, such as tree species and insect abundance. For silver-haired bats, longitude had the greatest R^2 weighted importance (0.013), supporting the east–west gradient detected with the PCA. For eastern red bats, seasonal precipitation and elevation had the highest R^2 weighted importance (0.012). These findings indicate these variables have a small direct or indirect influence on the two species' genetics. However, the calculated R^2 values are low, and no structure was detected with any other method in eastern red bats. For hoary bats, maximum temperature had the greatest R^2 weighted importance of 0.006; all other variables were even lower, thus supporting a panmixia hypothesis.

I greatly increased the sampling coverage across each species' range compared to prior studies, though sampling in some regions was sparse. Some of my samples came from surveys in which bats were live trapped; however, most (76.5%) were from bats killed at wind-energy facilities. While I do not expect sampling method to influence structure or N_e results, it does mean there were fewer samples in regions where fewer wind-energy facilities currently operate, such as in the southeastern USA. I also obtained relatively few samples from the North American mid-continent. Adding samples from the middle of the hoary bat range would be unlikely to change the conclusion of a panmictic population, as I detected no genetic difference in hoary bats in the more western and eastern extents of their range. For silver-haired bats, however, samples in the mid-continent could more precisely characterize the longitudinal of the split or gradient in population differentiation.

I excluded a relatively low number of samples (24 out of 417, or 5.8%) from the initially sequenced dataset because of either low coverage or outlier status. It is

common for genetic studies to exclude samples with low coverage, which can occur because of degraded DNA (such as might happen with a decomposing bat carcass). The "outlier" bats were more perplexing, and occurred in both *Lasiurus* species, but not silver-haired bats. It is possible that these samples were hybrids or had been cross-contaminated either in the field or lab. It is also possible that the genus *Lasiurus* contains previously undescribed diversity, perhaps including hybridization or introgression among the seven North American lasiurids. When I analyzed all three species together in a PCA, two samples did fall halfway between eastern red bats and hoary bats (Figure 3). These could potentially be hybrids or contaminated samples. However, this explanation cannot easily explain the remaining "outlier" samples, as they clustered with the others of their species when all species were analyzed together. When each species was analyzed by PCA separately, a handful of eastern red bats and hoary bats fell far outside the rest of the individuals for each species. While I do not know why this occurred, there was no geographic pattern to the outlier bats. Thus, since my goal was to detect population structure and removing these samples did not affect my interpretation, I decided to exclude those outliers as described above.

As with previous studies with these species (Korstian et al. 2015; Cornman et al. 2021), I was not able to precisely estimate LD N_e as infinity was included in the upper confidence interval. This known limitation of this method occurs when census population sizes are large (Do et al. 2014), however increasing sample size has been shown to increase precision (Waples and Do 2010). I decided to attempt this analysis in the event that the relatively large sample sizes and the increased power of SNP data

could overcome this limitation. Coalescent modeling of N_e, on the other hand, encompasses a much longer, evolutionary, time-frame. My coalescent model indicates that eastern red, silver-haired, and hoary bat populations have been increasing in size in recent evolutionary history.

Census size (N_c) is the number of most conservation relevance in the context of the impact of fatalities of tree-roosting bats at wind-energy facilities because this number affects the impact of such fatalities on population viability (i.e., Frick et al. 2017; Friedenberg and Frick 2021). The N_e/N_c ratio for the three species of tree-roosting bats considered in this study is unknown, though some ratios have been published for other mammals. A summary of 192 published N_e/N_c ratios across multiple taxa reported an average of 0.11 in natural populations, though this varied widely: 30 were non-human mammals, and ranged from 0.069 in bison (*Bison bison*) to 0.83 in prairie dogs (*Cynomys ludocicianus*), with an average mammal ratio of 0.44 (Frankham 1995). This review was later expanded to include 508 published N_e/N_c ratios; only 31% correctly linked N_e to N_c, with an average ratio of 0.23 (Palstra and Fraser 2012). Few published N_e/N_c ratios exist for bats. Captive colonies of Rodrigues fruit bats (*Pteropus rodricensis*) had N_e/N_c ratios between 0.18 and 0.43 (Carroll and Mace 1988). Using demographic measurements, short-nosed fruit bats (*Cynopterus sphinx*) had N_e/N_c ratios of 0.42 to 0.56, depending on whether the population was promiscuous or polygynous (Garg and Ramakrishnan 2017). Using LD N_e to calculate N_e/N_c, Ozark big-eared bats (*Corynorhinus townsendii igens*) had ratios between 0.177 and 1.76 for five different caves, and N_e confidence intervals included infinity for some caves (Lee et al. 2015).

Using these methods, I cannot estimate the current census size for tree-roosting bats. My coalescent N_e estimates were slightly smaller than those reported by Sovic et al. (2016), though with the same pattern across species: eastern red bats had the largest N_e, followed by hoary bats, and finally silver-haired bats. Sovic et al. (2016) used different methods both for library preparation (RadSeq vs GBS) and bioinformatic processing (iPyrad vs Stacks), which likely influences downstream results (Shafer et al. 2017). My coalescent N_e estimates do not exclude the possibility of a population size within the range that published models (i.e., Frick et al. 2017; Friedenberg and Frick 2021) indicate could put hoary bats at risk of decline and perhaps extirpation or extinction. However, methods other than genetics, such as standardized acoustic surveys from which occupancy can be inferred (Loeb et al. 2015), are likely required to better detect recent population changes in these species.

Overall, my results suggest that managers should consider the entire population when choosing conservation measures for hoary and eastern red bats. Silver-haired bats, which have some spatial patterns of genetic variation, may be more vulnerable to local population declines and loss of alleles, and may be best managed by considering both eastern and western populations.

Data Availability

Demultiplexed FASTQ files will be submitted to the National Center for Biotechnology Information Sequence Read Archive.

Acknowledgements

I would like to thank the many people and organizations who contributed samples: Erin Baerwald, Charles Brown, Caitlin Campbell, Jeff Clerc, Duke Energy Renewables, Georgia Department of Natural Resources, Great Basin Institute, Dana Green, Amanda Hale, Carlyle Herzog, Hannah Holmes, Idaho Department of Fish and Game, Becky Johnson, Jan Just, Meghan Lout, Carlyle Meekins, Museum of Comparative Zoology, Museum of Southwestern Biology, Nevada Department of Wildlife, Sarah Nguyen, New York Department of Environmental Conservation, Scott Osborn, Pennsylvania Game Commission, Lori Pruitt, Scott Pruitt, Cortney Pylant, Rhode Island Department of Environmental Management, Schoener Environmental, Beth Stevenson, SWCA Environmental Consultants, US Fish and Wildlife Service, Anna Weaver, West Inc., J. Paul White, Wisconsin Department of Natural Resources, and Jason Williams. This research was funded by the U.S. Fish and Wildlife Service, and the Maryland Department of Natural Resources Power Plant Research Program.

Tables

Table 1. Individual bats sequenced (Seq) and included (Inc) in the structure analyses, by sampling method. Samples were excluded due to low read counts or outlier status. Turbine-killed individuals are those sampled through wind-energy fatality monitoring, live-captures through mist-netting and harp-trapping for population monitoring, and "other" includes submissions from rabies labs, museum collections, and non-turbine carcass salvage. All individuals were sampled during their summering period.

	Turbine-killed		Live-captures		Other		Total	
Species	Seq	Inc	Seq	Inc	Seq	Inc	Seq	Inc
Hoary	166	148	25	22	4	3	195	173
Eastern red	96	88	25	24	8	1	129	113
Silver-haired	52	50	36	35	5	4	93	89
Total	314	287	86	81	17	8	417	375

Table 2. Bootstrapped effective population size (N_e) confidence intervals calculated with fastsimcoal2. After 100 runs, the parameter values from the run with the highest likelihood was used to simulate 50 datasets. Each simulated site frequency spectrum was analyzed using fastsimcoal2, and confidence intervals are the minimum and maximum values for each parameter. Silver-haired bats were analyzed both with and without east and west groups divided at longitude -110 ^{o}N. Ancestral N_e is the effective population before growth, and time of growth start is reported in number of previous generations.

	N_e		Ancestral N_e		Growth Began	
Species	Min	Max	Min	Max	Min	Max
Hoary	101,355	106,128	10	20,028	50,971	273,647
Eastern Red	126,142	131,153	10	23,402	55,249	301,328
Silver-haired (all)	49,551	64,801	13	332,463	6,965	466,429
Silver-haired (east)	11,687	90,707	10	106,762	4,389	484,844
Silver-haired (west)	103	124,461	11	777,589	802	604,647

Figures

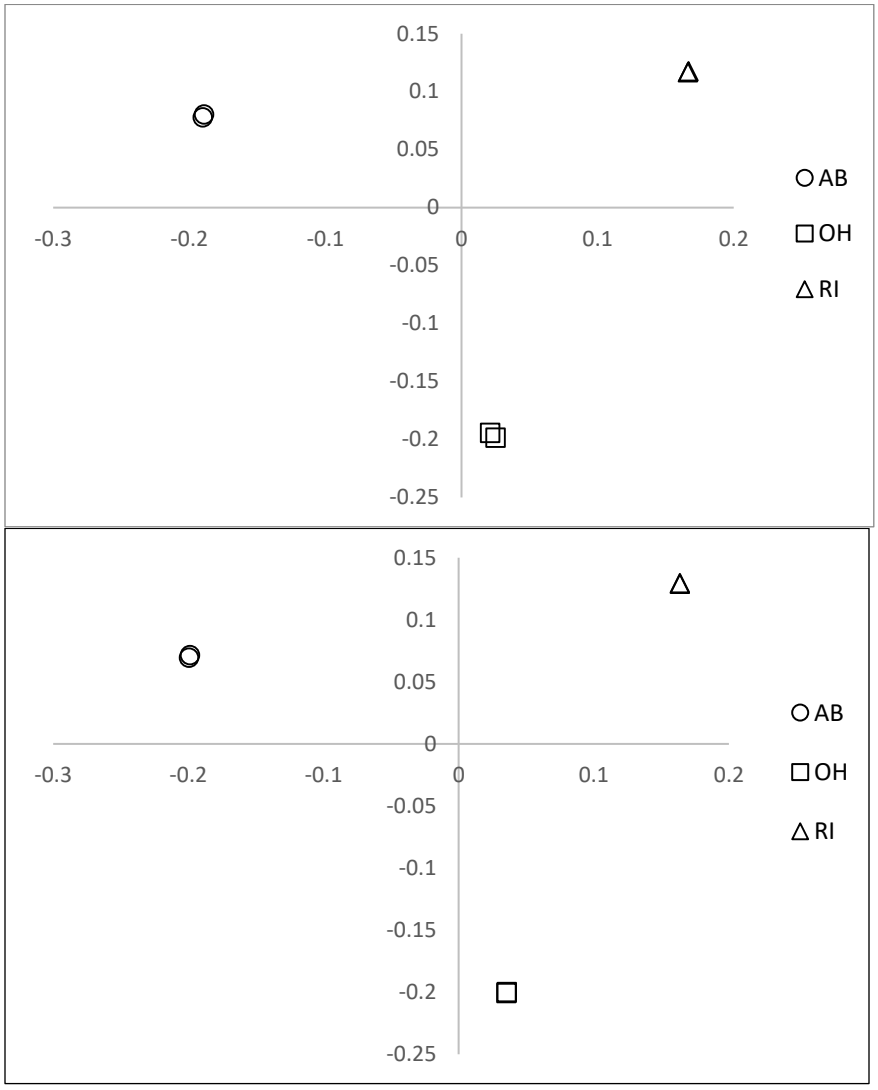

Figure 1. Principal component analyses (PCA) of three silver-haired bat samples replicated across libraries and processed through Stacks using varying parameter levels (minimum stack depth, distance allowed between stacks, and bound-high values). AB is a bat from Alberta, OH from Ohio, and RI from Rhode Island. Top graph is an example of parameters resulting in samples not aligning (m = 4, M/n = 1, bound-high = 0.05). Bottom graph shows the PCA results from the parameters resulting the best alignment (m = 3, M/n = 2, bound-high = 0.1), used in final analyses.

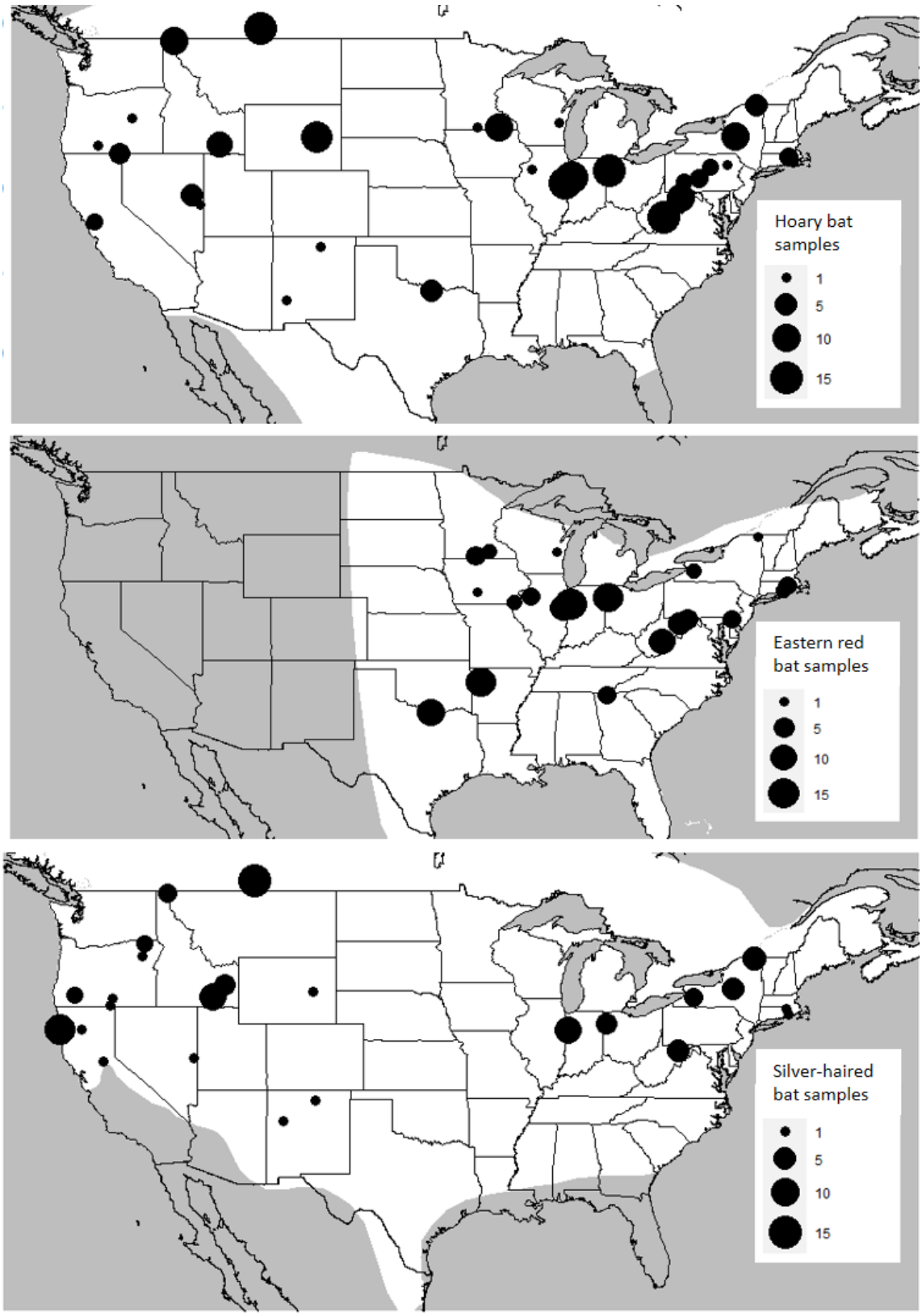

Figure 2. Hoary (n = 173), eastern red (n = 113), and silver-haired (n = 89) bat sample locations. White region is IUCN species range.

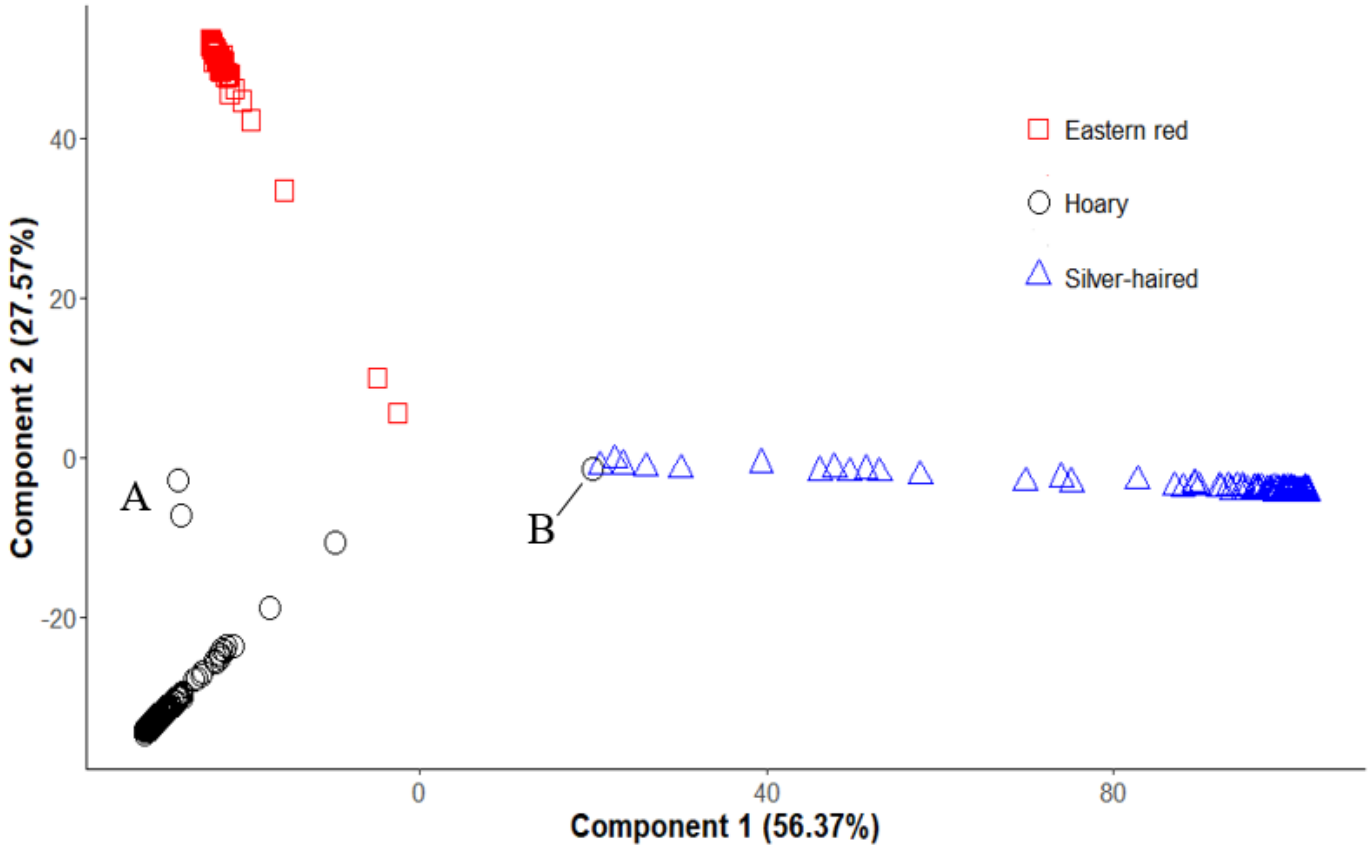

Figure 3. Principal component analysis of 393 samples of hoary, eastern red, and silver-haired bats used in the present study. Two samples identified as hoary bats fell between hoary bat and eastern red bat samples (A), suggesting either hybridization or sample contamination. Another field-identified hoary bat clustered with the silver-haired bats (B). Missing data were replaced with average values, thus samples with higher amounts of missing data (i.e., lower read counts) fell towards the origin.

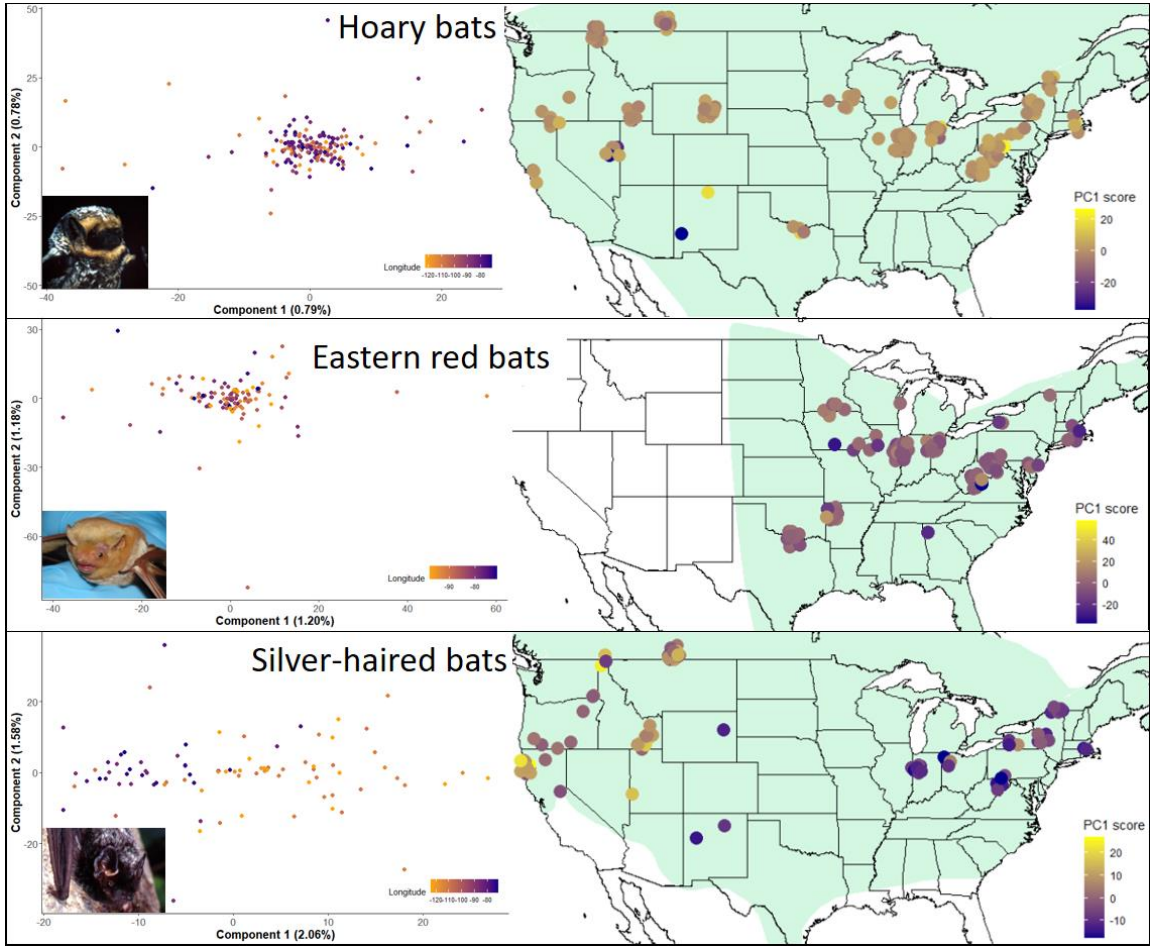

Figure 4. Principal component analyses of hoary bats (n = 173 samples, 10 outliers removed), eastern red bats (n = 113, 8 outliers removed), and silver-haired bats (n = 89). Plots on left are the first two principal components, colored by sample longitude. Plots on the right are colored by PC1 score, with jitter added to sample locations to reduce overlap of data. Green region is IUCN species ranges.

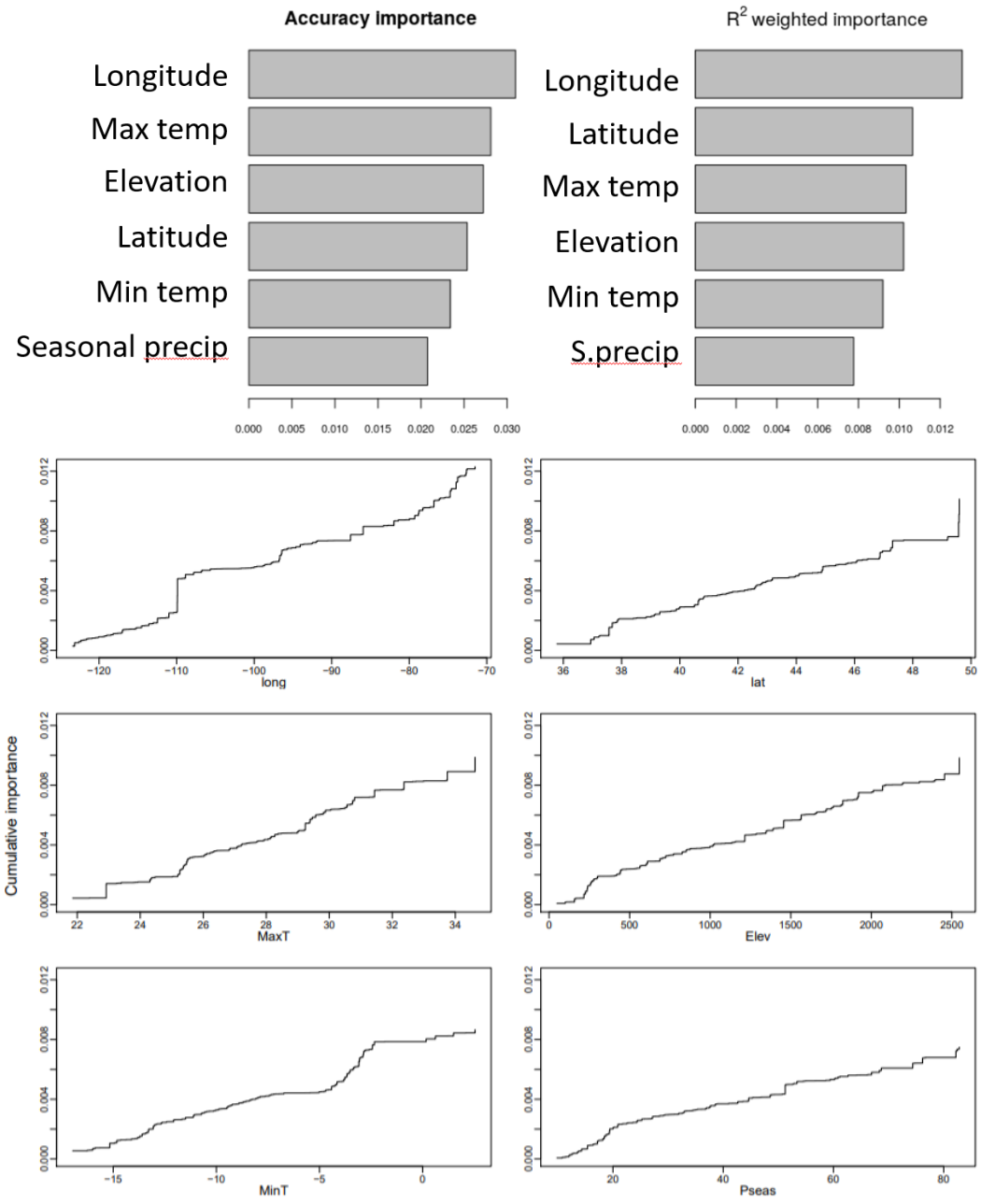

Figure 5. Results of gradient forest model of silver-haired bats, by variable importance (top) and cumulative importance (bottom).

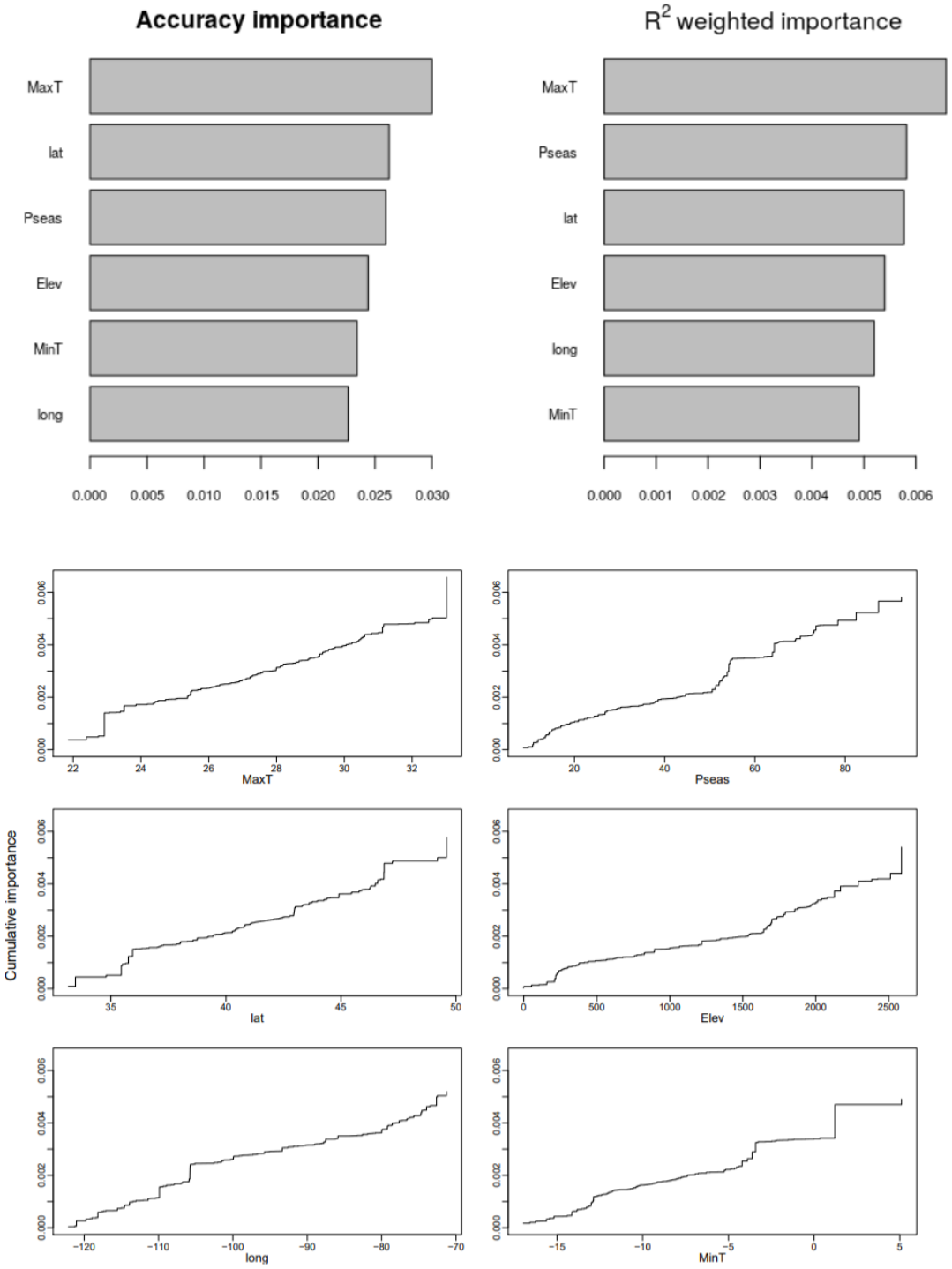

Figure 6. Results of gradient forest model of hoary bats, by variable importance (top) and cumulative importance (bottom).

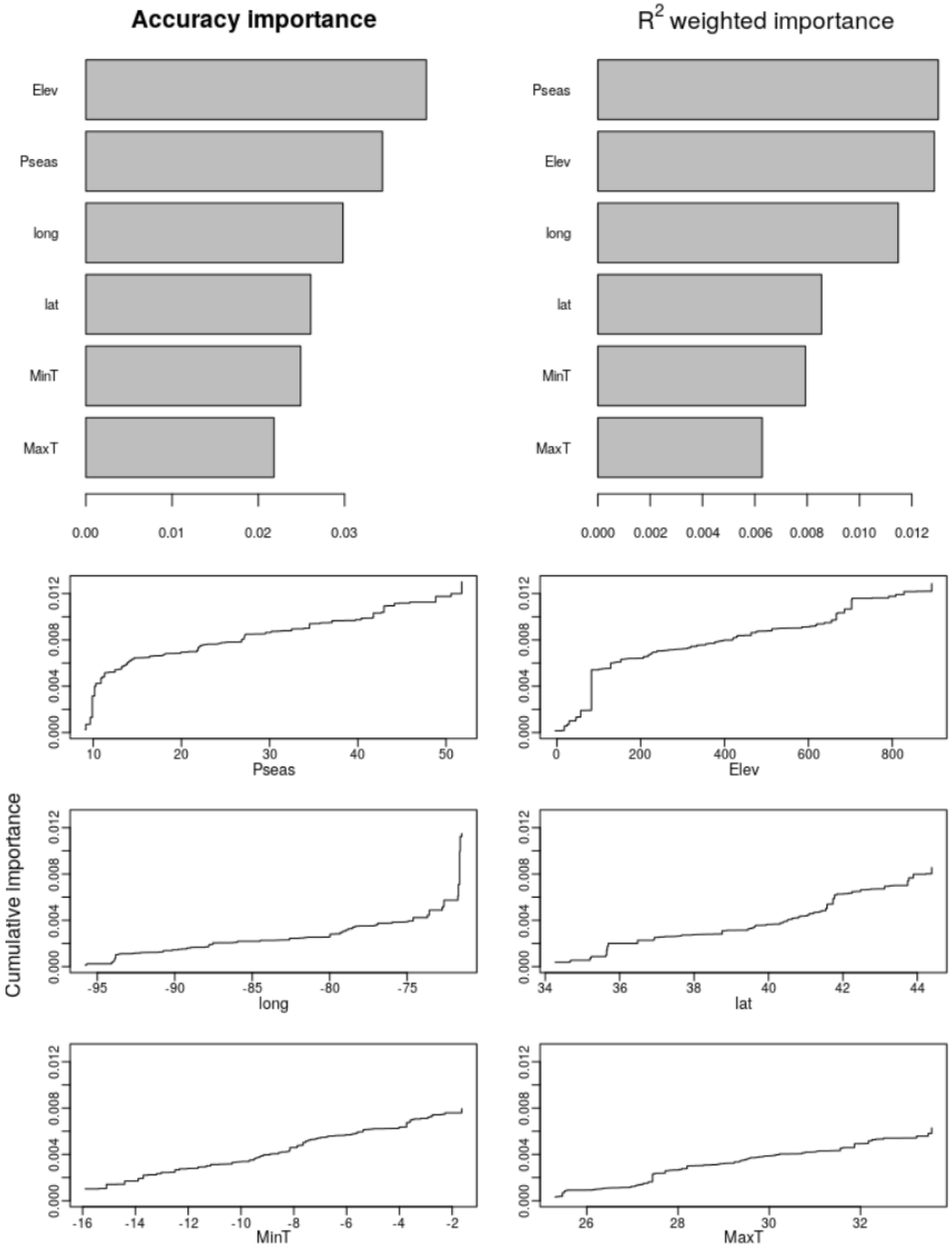

Figure 7. Results of gradient forest model of eastern red bats, by variable importance (top) and cumulative importance (bottom).

Supplementary Figures

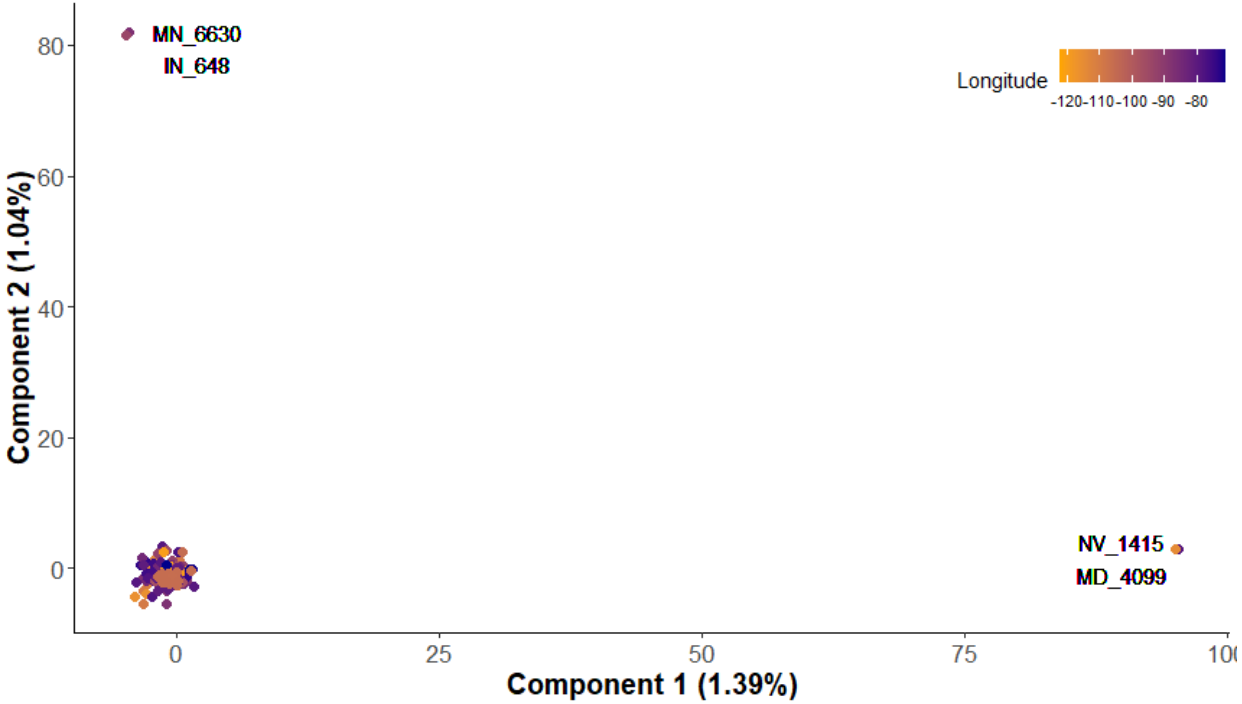

Figure S1. Principal component analysis of 180 samples of hoary bats, colored by sample longitude. The four labeled samples fall far outside the other 176 and are therefore called "outliers" in this study. The two letters in the sample labels of the outliers refer to state of origin (i.e., Minnesota, Indiana, Nevada, and Maryland).

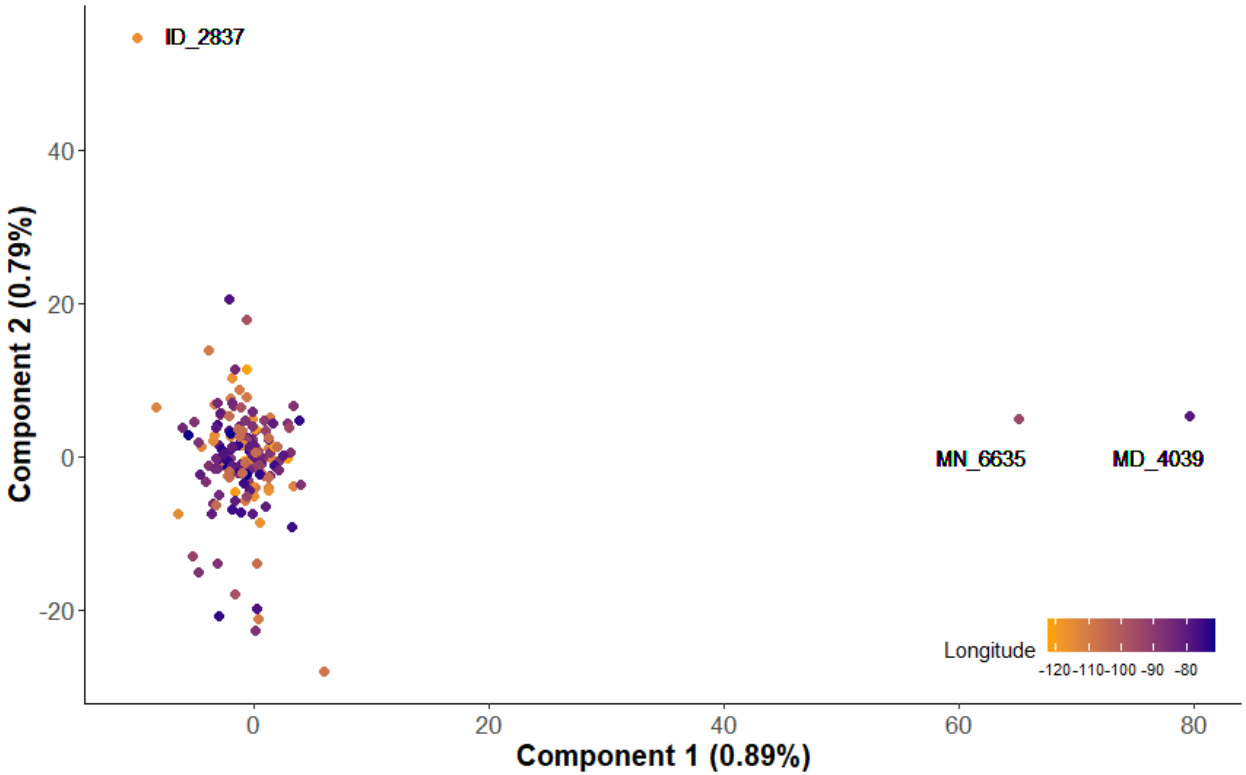

Figure S2. Principal component analysis of 176 samples of hoary bats (i.e., with low read and initial outlier samples removed), colored by sample longitude. The three labeled samples fall far outside the other 174 and are therefore called "outliers" in this study. The two letters in the sample label refer to state of origin (i.e., Idaho, Minnesota, Maryland).

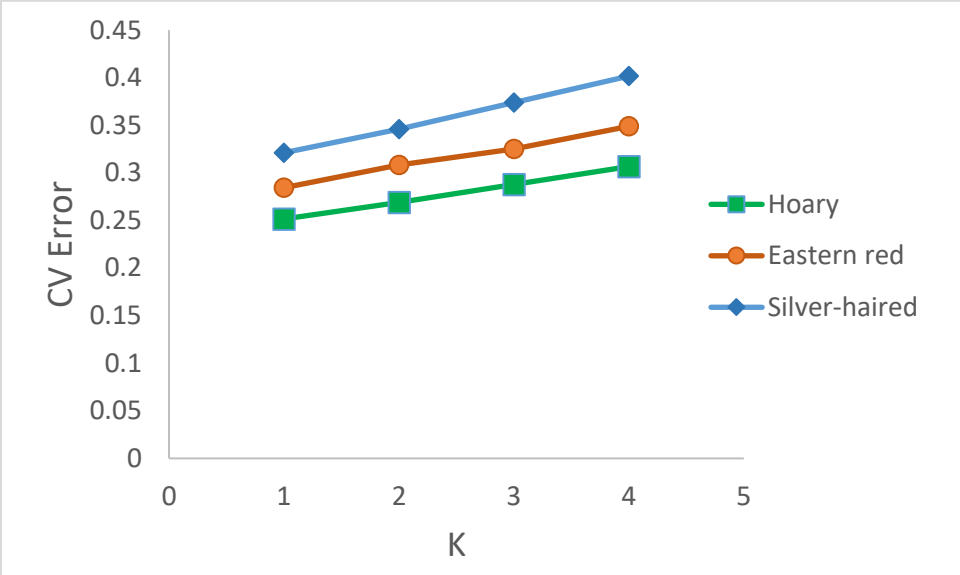

Figure S3. ADMIXTURE results for hoary, eastern red, and silver-haired bats, indicating *K* (number of genetic clusters) = 1 for all species.

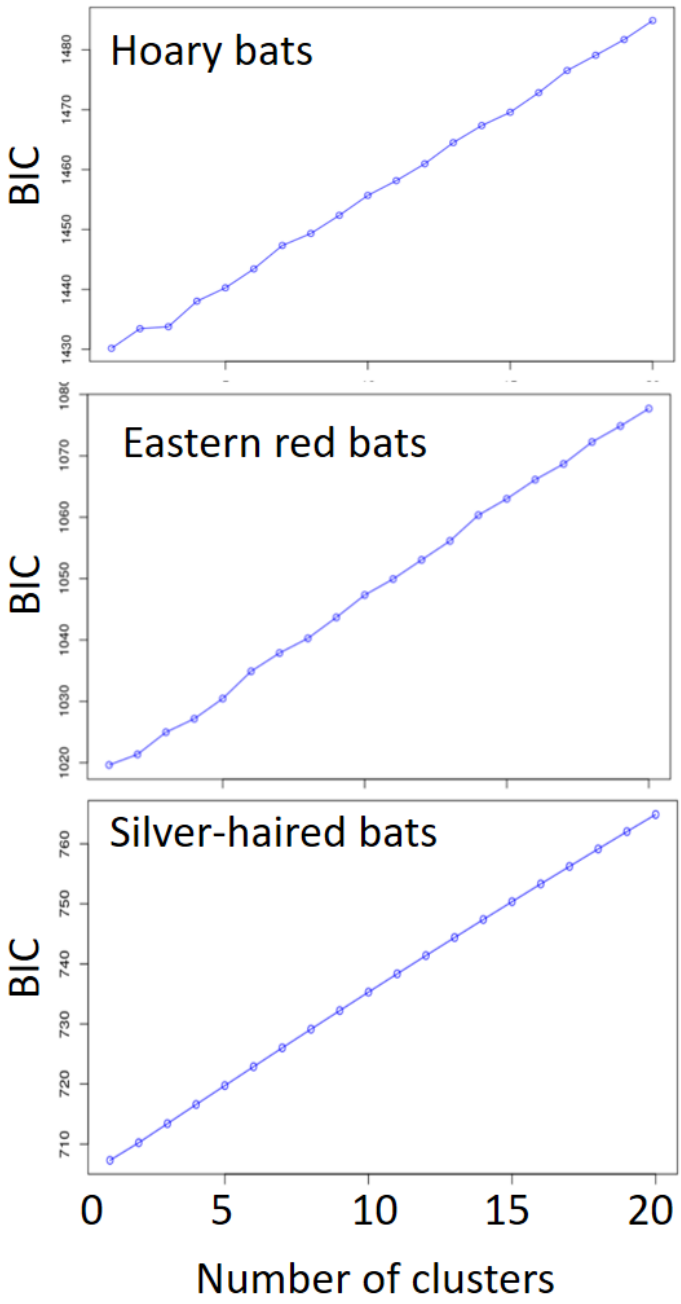

Figure S4. Discriminant Analyses of Principal Components results for hoary, eastern red, and silver-haired bats. BIC (Bayesian Information Criterion) values indicate that the most probable number of clusters is 1 for all species.

Chapter 3: Population genetic structure and effective size of two endangered cave bat species

Abstract

Indiana (*Myotis sodalis*) and gray bats (*Myotis grisescens*) are federally endangered species that use a limited number of hibernacula, placing them at risk of extirpation and/or extinction due to numerous threats. Understanding the genetic structure and diversity of a species is essential to long-term management and preservation of adaptive genetic variation. Here, I used next-generation sequencing to analyze the genetic structure and effective sizes of Indiana and gray bats. Using genotyping-by-sequencing, I genotyped single-nucleotide polymorphism (SNP) data from 45 Indiana and 47 gray bats spread across their ranges. I assessed population structure using ADMIXTURE and Principal Component Analyses (PCA). Indiana bats showed no genetic structure, whereas gray bats displayed east–west population variation, with differential east and west of the Mississippi River Valley. These genomic results support the conclusions of earlier published microsatellite studies. From a genetic conservation standpoint, Indiana bats may best be managed as a single population, while gray bats should be managed with consideration to eastern and western populations.

Introduction

Indiana bats (*Myotis sodalis*) and gray bats (*Myotis grisescens*) are federally endangered species that rely on a small number of hibernacula in the eastern United States. Like many insectivorous bat species, they face a range of threats, including habitat loss, climate change, disturbance of hibernacula, and declines in prey

populations. In addition, white-nose syndrome (WNS), a fungal disease caused by the pathogen *Pseudogymnoascus destructans*, has caused large and rapid declines in the population size of many cave-hibernating bat species in North America since its appearance in 2006 (Blehert et al. 2009; Frick et al. 2015). As bats are generally long-lived with low reproductive rates, increased adult mortality can cause significant population declines (Barclay and Harder 2003; Podlutsky et al. 2005).

Indiana bats were originally listed under the Endangered Species Preservation Act of 1966 and listed as endangered under the Endangered Species Act of 1973, largely due to their high vulnerability to disturbances at hibernacula: 97% of the known population at the time hibernated in just four caves (Humphrey 1978). The Fish and Wildlife Service currently manages Indiana bats in four Recovery Units: Ozark-Central, Midwest, Appalachian, and Northeast. These units are designed with a goal of redundancy and resiliency, and are based in part on preliminary mitochondrial DNA studies and band recovery patterns, as well as differences in population trends and broad-level macrohabitats and land use (USFWS 2007).

Indiana bats have been censused biennially since the early 1980s. Winter censuses are conducted by entering hibernacula and counting each visible bat, most often using digital photography. The most recent survey was during the winter of 2019, with a range-wide population count of 537,297 bats in 223 hibernacula across 16 states, which represented a 19% decline since the 2008 arrival of WNS (USFWS 2019a, b).

Indiana bats mate in fall, congregating at swarming sites outside hibernacula. In spring, Indiana bats disperse from hibernacula, migrating to maternity colonies

primarily located in dead trees, where each female gives birth to a single pup (Kurta et al. 1993). Stable hydrogen isotope and banding data show that bats may migrate more than 600 kilometers between summering habitat and hibernacula, although most migrate shorter distances. Most Indiana bats exhibit fidelity to summer habitat and hibernacula, although some use different hibernacula in different years (Kurta and Murray 2002; Britzke et al. 2013).

Gray bats were listed as endangered in the United States in 1976 due to their declining numbers, high susceptibility to human disturbance, and reliance on a handful of caves both for winter hibernacula and summer maternity colonies (Brady et al. 1982). They use caves year-round, seasonally migrating up to several hundred kilometers (Tuttle 1976) between cold, deep, vertical caves in winter and warmer caves close to water during summer months, where females give birth to a single pup. Banding records show bats are philopatric to both winter caves and summer ranges, but may use multiple caves during summer (Tuttle 2003). The Mississippi River Alluvial Plain, a broad area devoid of caves, separates the eastern and western karst regions used by gray bats. Winter surveys of all known hibernacula in 2006 estimated the total population at 3,377,100 bats (Martin 2007), which is a large increase since a 1982 estimate of 1,575,000 (Brady et al. 1982). Gray bats appear to be less susceptible to WNS than many other cave-dwelling bat species (Powers et al. 2016; Jackson et al. 2022).

While population counts of both species are monitored through winter hibernacula counts, a thorough understanding of the population genetic structure and effective population size is important for long-term conservation and management.

Understanding gene flow and identifying genetically distinct subpopulations is necessary to help inform delineation of management units, with the goal of preserving a species' adaptive genetic variance (Crandall et al. 2000; Fraser and Bernatchez 2001).

To date, few peer-reviewed studies have been published on these species. Using five microsatellites from Indiana bat guano samples as a mark-recapture method was more accurate than fly-out counts to measure maternity colony size (Oyler-McCance et al. 2017). Indiana bats collected from 18 hibernacula showed no genetically distinct populations using nuclear DNA (10 microsatellites), though mitochondrial DNA indicated the presence of five matrilineal clusters (Vonhof et al. 2016). Using eight microsatellites and mitochondrial DNA, guano at eight gray bat hibernacula indicated east–west genetic structure separated by the Mississippi River Valley, though evidence of some gene flow was present between groups (Lindsay et al. 2015).

Recent advances now allow the analysis of thousands of genetic loci per individual in the form of single-nucleotide polymorphisms (SNPs), even for non-model organisms, with little or no prior existing genomic data (Gayral et al. 2013). This development provides more accurate and precise inferences of population structure and N_e than those based on microsatellites (Liu et al. 2005).

A summary of genetic diversity in a population, N_e represents the number of individuals in a theoretical ideal population that would experience the same amount of genetic drift observed in the actual population (Wright 1931). At low values of N_e, genetic drift can threaten a population's viability through loss of alleles, reduced

fitness, and inbreeding, thus limiting the species' potential to respond to selection and environmental change (Frankham 2005).

Various methods can be used to estimate N_e. Some methods, such as coalescent modeling, reflect the combined effects of population demographics in past and present generations, thus providing a long-term evolutionary N_e covering several thousand years. Evolutionary N_e can provide insight into species' historical population levels (for example, estimating the pre-whaling population abundance of gray whales, *Eschrichtius robustus* [Alter et al. 2007]). Other methods, such as those based on linkage disequilibrium (LD N_e), measure a contemporary N_e. This includes the most recent generations and can detect recent population declines, although estimating contemporary trends is particularly challenging (Tallmon et al. 2012). The LD N_e is most accurate for very small populations with a census size under 100 (Waples et al. 2016), and confidence intervals of LD N_e are often large for species with large census population sizes, even encompassing infinity at the upper bound (Do et al. 2014). A confidence interval bounded on the upper end by infinity does not suggest the existence of a very large population; rather, it is an artifact of the method failing to constrain the estimate with precision. As increasing the number of markers has been shown to increase precision (Waples and Do 2010), I attempted this analysis in hopes that the increased power of SNP data could overcome this limitation.

Typically N_e is much smaller than census size, N_c, and is reduced by population characteristics including fluctuations in population size, bottlenecks, unequal sex ratio, and non-random number of offspring (Frankham 1995). Many studies have compared N_e and N_c, and in most cases researchers are interested in

estimating N_e in populations with a known N_c (Nunney 1995; Shrimpton and Heath 2003). Frankham (1995) reviewed over 100 studies on animals and plants and reported an average N_e/N_c of 0.11 in natural populations. Of those, 30 were non-human mammals, and ranged from 0.069 in bison (*Bison bison*) to 0.83 in prairie dogs (*Cynomys ludocicianus*), with an average mammal ratio of 0.44. A review of studies published since 1995 found a median N_e/N_c of 0.14 for plants and animals combined (average 0.16 ± 0.13 standard deviation; Palstra and Fraser 2012). Few published N_e/N_c ratios exist for bats. Using demographic measurements to calculate N_e, short-nosed fruit bats (*Cynopterus sphinx*) had a ratio of 0.42 to 0.56, depending on whether the population was promiscuous or polygynous (Garg and Ramakrishnan 2017). Using linkage disequilibrium to assess population structure in Ozark big-eared bats (*Corynorhinus townsendii ingens*) across five caves resulted in N_e/N_c ratios between 0.177 and 1.76 , with the confidence intervals of some caves including infinity (Lee et al. 2015).

The objectives of this study were to apply genotyping-by-sequencing (GBS) to assess the 1) effective population size and 2) evidence of population structure in Indiana and gray bats. To the first aim, I hoped to leverage the well-established census sizes of these species to increase the number of estimates of insectivorous bat N_e/N_c. Doing so would be valuable for estimating census population sizes in at-risk but hard-to-monitor related species such as the hoary bat (*Lasiurus cinereus*). For this species, establishing a census population estimate would be invaluable for assessing the potential demographic impact of the large numbers of fatalities this species is experiencing at wind-energy facilities in North America (Frick et al. 2017;

Friedenberg and Frick 2021). In calculating such a ratio, it is necessary to use a contemporary measurement such as LD N_e to accurately compare the current census size with N_e. To the second aim, I applied next-generation sequencing to assess the reliability of conclusions related to the occurrence of population structure of existing studies (i.e., Lindsay et al. 2015, Vonhof et al. 2016) that were based on small numbers of microsatellites and mitochondrial DNA, as new sequencing-based approaches are expected to be more sensitive to such structure.

Methods

Tissue Collection

I obtained tissue from 48 Indiana and 48 gray bats that were sampled or captured for other studies by the US Fish and Wildlife Service, Kentucky Department of Fish and Wildlife, New York Department of Environmental Conservation, National Park Service, and Missouri State University. Indiana bat samples covered all four USFWS Indiana bat Recovery Units, and gray bat samples were from both the eastern and western karst regions.

Sample collection was permitted by the United States Fish and Wildlife Service (permit numbers TE02365A-4, TE02373A-10, TE134817-1, TE13487-1, TE237549-0, TE-62313A-0, TE-697830-10 sub-permit 16-01), Arkansas Game and Fish, Indiana Department of Natural Resources, Missouri Department of Conservation, New York Department of Conservation, Ohio Department of Natural Resources, and Tennessee Wildlife Resources Agency. Samples consisted of wing or tail tissue preserved in either ethanol or silica pellet desiccant upon collection and

were stored at −80 °C once received. Indiana bat tissue samples were collected between August 2002 and January 2013. Gray bat tissue samples were collected between February 2009 and July 2017. Samples were collected during winter and summer.

DNA Extraction and Sequencing

I extracted DNA from tissue samples using DNeasy Blood & Tissue Kits (Cat no. 69504 or 69506; QIAGEN GmbH, Germany). Kit protocols were followed with the addition of a 6-minute centrifugation at 17,000 x g following tissue digestion to pellet out pigments and fur. Extracted DNA was quantified using an Invitrogen Qubit 1.0 Fluorometer. Samples with a quantification at or above 100 ng/μL were diluted with nuclease-free water before further processing. Samples between 3 and 7 ng/μL were concentrated using a vacufuge. Samples with a concentration below 3 ng/μL were excluded from further analyses.

DNA libraries were prepared using a GBS approach (Elshire et al. 2011), following the modifications applied by Gugger et al. (2021). Starting with 100 ng of DNA per sample, DNA was first digested with the restriction enzyme *Pst*I. Sequencing adapters containing unique barcodes for each sample were ligated to the resulting fragments. These barcoded samples were pooled in batches of 48 and amplified with PCR. Each batch of 48 was considered one "library." Samples were sequenced single-end, 50-bp on an Illumina HiSeq4000 v3 at the Coates Genomics Sequencing Laboratory at the University of California Berkeley. Before sequencing, each library was checked with Bioanalyzer for quality and fragment size. I sequenced one library each of Indiana and gray bats.

SNP Discovery and Filtering

Illumina reads were processed using the Stacks 2.41 bioinformatics pipeline (Catchen et al. 2011, 2013) and analyzed for single-nucleotide polymorphisms (SNPs). Stacks can identify SNPs *de novo*, even when a reference genome is unavailable. The *process_radtags* function was used to demultiplex and quality-filter the Illumina data, after which individual samples with low read counts (< 30% of the median number of reads) were removed from analyses. The Stacks pipeline identified loci, and then the Stacks *populations* function was used to apply filters and output the data for use in other programs.

For use, a SNP had to have < 20% missing data. For population structure analyses SNPs with a minor allele frequency less than 0.05 were removed, and I used the write-single-snp option to keep only one SNP per stack/fragment, thus reducing the number of physically-linked SNPs and the effects of linkage disequilibrium.

For estimation of effective population size, I produced a site frequency spectrum (SFS) using easySFS (Overcast [2016] 2021). When filtering SNPs for the SFS, the write-single-SNP option was included, but SNPs were not filtered for minor allele frequency.

Genomics Analyses

Population structure for each species was assessed using ADMIXTURE (Alexander et al. 2009) and Principal Component Analysis (PCA). ADMIXTURE uses the same likelihood model-based estimation of ancestry as the program STRUCTURE, but ADMIXTURE uses a faster algorithm that is better able to handle the large SNP datasets produced by GBS. By modeling the probability of the

observed genotypes using population allele frequencies, ADMIXTURE estimates *K*, the number of genetic clusters ("populations"), without *a priori* knowledge of geographic sampling. Multiple values of *K* are tested, with the most likely value of *K* selected to minimize cross-validation error. ADMIXTURE assigns individuals, either wholly or partially, to each cluster, and therefore, it assumes discrete populations exist or have existed, followed by possible admixture among those populations. A limitation of the above methods is the assumption of discrete populations, which may be untrue of highly mobile species of bat. Therefore, population structure was assessed using PCA to identify potentially continuous gradients in allele frequency shifts across each species' range.

Using *fastsimcoal2* (Excoffier and Foll 2011), I estimated coalescent effective population size (N_e) and NeEstimator V2 (Do et al. 2014) to calculate LD N_e. The package *fastsimcoal2* uses coalescent modeling to estimate variables that would produce the given SFS under a modeled evolutionary scenario. For each species, I fit two models. The first model assumed a constant population size and contained only a single variable, the estimated N_e. The second model contained additional variables: a changing population size, the rate of change, the number of generations previous when the population size began to change, and the ancestral N_e. Under this model, population size is a dynamic predictor; a negative rate of change reflects an increasing population. The two models were compared with Akaike information criterion (AIC). For all species, the model that included changing population size had a lower AIC, which indicates a better fit, and was selected as the model for subsequent analyses.

I used bootstrapping to calculate the confidence interval for N_e, following the methods outlined in Excoffier and Foll (2011). First, I performed 100 independent runs of *fastsimcoal2* (30 cycles and 50,000 simulations per run) and selected the run with the highest likelihood. The parameter values from the best run were used to simulate 50 datasets, and each simulated SFS was run through *fastsimcoal2*. The minimum and maximum values from the 50 runs were retained as the bounds of the confidence intervals.

I estimated contemporary N_e with the linkage disequilibrium method (LD N_e) using the program NeEstimator V2 (Do et al. 2014). The LD method is among the most robust and accurate methods to estimate contemporary N_e (Wang et al. 2016; Waples and Do 2010). Additionally this method only requires a single sample in time, unlike other methods that need samples separated by generations, which are difficult to collect for long-lived bat species. With large SNP datasets like those presented here, failure to account for physically-linked SNPs can substantially underestimate N_e and datasets above 1000 SNPs can cause confidence intervals to be estimated too narrowly (Waples et al. 2016). I did not have reference genomes for these species, and therefore could not ascertain if SNPs were physically linked. Instead, I used a random subset of 1000 SNPS to calculate LD N_e. I also assumed random mating and screened out alleles with frequencies below 0.05. Rare alleles are more likely to be sequencing errors and have been shown to upwardly bias N_e in simulated datasets (Waples and Do 2010).

Results

I sequenced DNA from 48 Indiana bats and 48 gray bats. Three Indiana bats and one gray bat were excluded from further analysis because of low read counts, resulting in final datasets of 45 Indiana bats and 47 gray bats spread across their ranges (Figure 1).

ADMIXTURE resulted in a best fit of $K = 1$ for both species (Figure 2). The PCA of Indiana bat samples also showed no clear geographic genetic structure. In contrast, the PCA for gray bat samples revealed longitudinal population variation along PC1, which accounted for 2.82% of all variation (Figure 3). Geographically, this indicated east-west differentiation across the Mississippi River Valley.

Using coalescent modeling with *fastsimcoal2*, the best-fit model for both species included long-term changing populations on an evolutionary timescale, increasing for Indiana bats and the eastern group of gray bats, and decreasing for the gray bat western group. Parameter estimates suggested an Indiana bat current (haploid) effective population size of 349,488–368,414 that grew from an ancestral population of 10–19,230 bats beginning 58,134–207,875 generations ago. The eastern group of gray bats (sample size = 34) had an ancestral effective population size of 13–106,762 growing to 2,078–66,935, beginning 1,651–746,822 generations ago. Conversely, the western group (sample size = 13) had an ancestral population size between 32,178–575,868 that shrunk to 22,863–31,350, beginning 682–1,147,364 generations ago (Table 1). LD N_e point estimates with NeEstimator returned estimates of infinity for both species.

Discussion

This study represents the first use of next-generation sequencing on Indiana and gray bats. I was able to genotype thousands of loci in both species, including samples taken from 3mm wing punches collected from live bats, which clearly indicates sufficient quality and quantity of DNA for genomics can be acquired from these small samples. Overall, my genomic results support the conclusions of prior microsatellite studies on both species (Lindsay et al. 2015; Vonhof et al. 2016), with Indiana bats displaying no genetic structure and gray bats displaying east-west genetic variation across the Mississippi River Valley. However, I did not detect evidence of matrilineal clades indicated previously using Indiana bat mitochondrial DNA (Vonhof et al. 2016). High nuclear gene flow in these species likely masks any structure present in the mitochondrial DNA. Such high gene flow, even among species exhibiting female philopatry to breeding sites, is common with temperate bats and has also been demonstrated in brown long-eared bats in Scotland (*Plecotus auritus* [Burland et al. 1999]), Bechstein's bats in Germany (*Myotis bechsteinii* (Kerth et al. 2000; Kerth et al. 2002)), the greater mouse-eared bat in Europe (*Myotis myotis* [Castella et al. 2001]), big brown bats in the United States (*Eptesicus fuscus* [Vonhofet al. 2008]), and little brown bats in North America (*Myotis lucifugus* [Dixon 2011; Vonhof et al. 2015]).

Unlike many migratory bird species that mate in spring at their breeding grounds, Indiana and gray bats do not mate in their summer ranges. Rather, they gather at swarming sites each fall, which may be hundreds of kilometers from summer habitats (Kurta and Murray 2002; Gardner and Cook 2002). These

movements facilitate gene flow, and are likely to contribute to the spread of WNS (Miller-Butterworth et al. 2014; Vonhof et al. 2016).

The coalescent modeling of N_e indicated that Indiana bats historically had a much larger population size than gray bats. Evolutionarily, gray bat populations may have been restricted in their dispersal due to their reliance on caves year-round, unlike Indiana bats, which migrate into forest habitat each summer. In congruence with several previous bat studies on other species (Korstian et al. 2015; Cornman et al. 2021), I was not able to estimate LD N_e without including infinity in the upper confidence interval. Unfortunately, as I could not accurately estimate a current N_e, I cannot calculate an N_e/N_c ratio in relation to the current census size.

Overall, these results provide additional evidence to confirm the genetic structure previously reported for these species. This is valuable information for conservation managers, as it further supports previous recommendations that Indiana bats may best be managed as a single unit from a genetic viewpoint (Vonhof et al. 2016). Recovery Units may be useful for other aspects of Indiana bat management, however, such as tracking regional population trends or WNS impacts. Conversely, gray bats should be managed in consideration of both eastern and western groups to preserve the species' genetic variation.

Data Availability

Demultiplexed FASTQ files will be submitted to the National Center for Biotechnology Information Sequence Read Archive.

Acknowledgements

I would like to acknowledge the following people and organizations that assisted with sample contribution: Environmental Solutions and Innovations Inc., Heather Fenton, Carl Herzog, Kentucky Department of Fish and Wildlife, Missouri State University, National Park Service, New York Department of Environmental Conservation, US Fish and Wildlife Service, and Michelle Willis. This research was funded by the Maryland Department of Natural Resources Power Plant Research Program.

Tables

Table 1: Bootstrapped effective population size (N_e) confidence intervals calculated with *fastsimcoal2*. After 100 runs, the parameter values from the run with the highest likelihood was used to simulate 50 datasets. Each simulated SFS was analyzed using *fastsimcoal2*, and confidence intervals are the minimum and maximum values for each parameter. Ancestral N_e reflects the effective population before growth, and time of growth start is reported in number of previous generations. A model of population growth was supported in Indiana bats and the eastern group of gray bats; a model of population decline was supported in the western group of gray bats.

	Sample	N_e		Ancestral N_e		Change Began	
Species	Size	Min	Max	Min	Max	Min	Max
Indiana	45	349,488	368,414	10	19,230	58,134	207,875
Gray (east)	34	2,078	66,935	13	106,762	1,651	746,822
Gray (west)	13	22,863	31,350	32,178	575,868	682	1,147,364

Figures

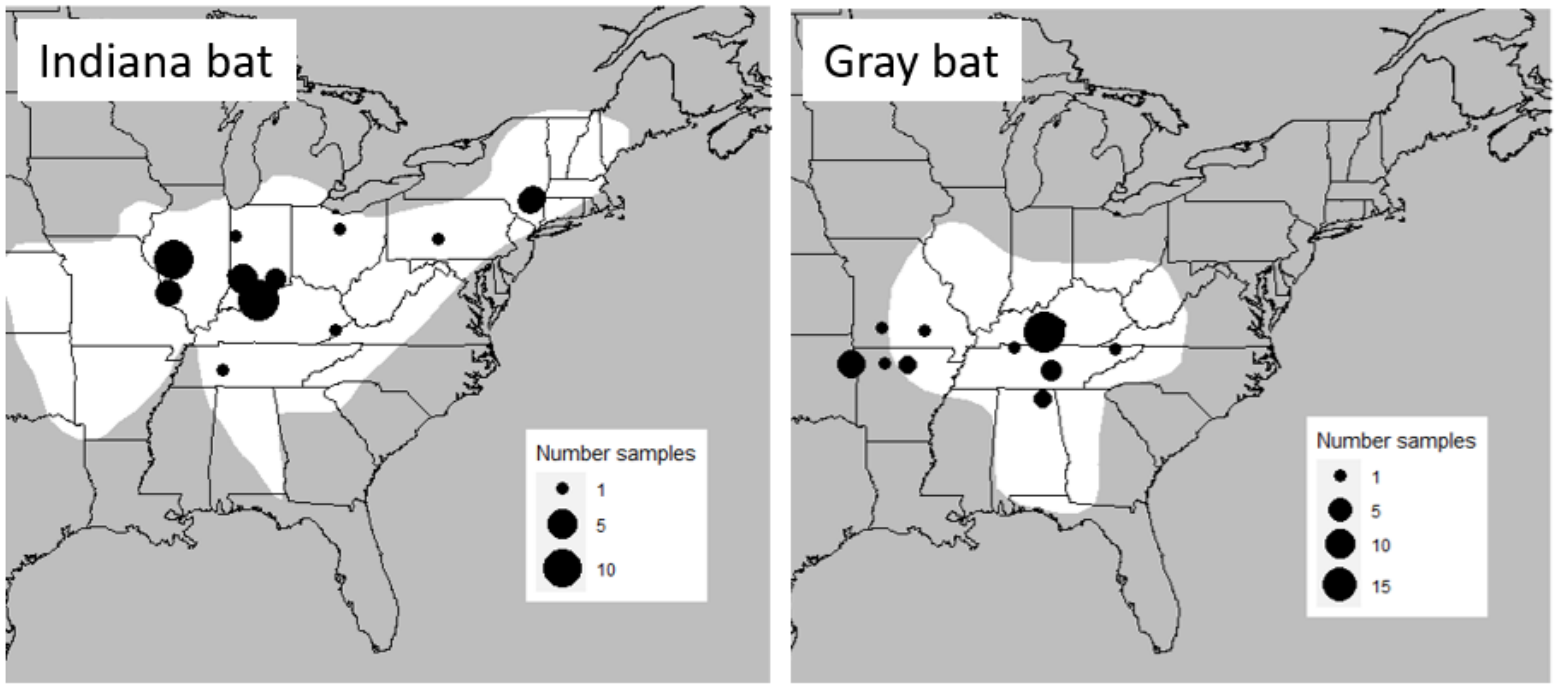

Figure 1. Indiana (n = 45) and gray bat (n = 47) sample locations. White regions are IUCN species ranges.

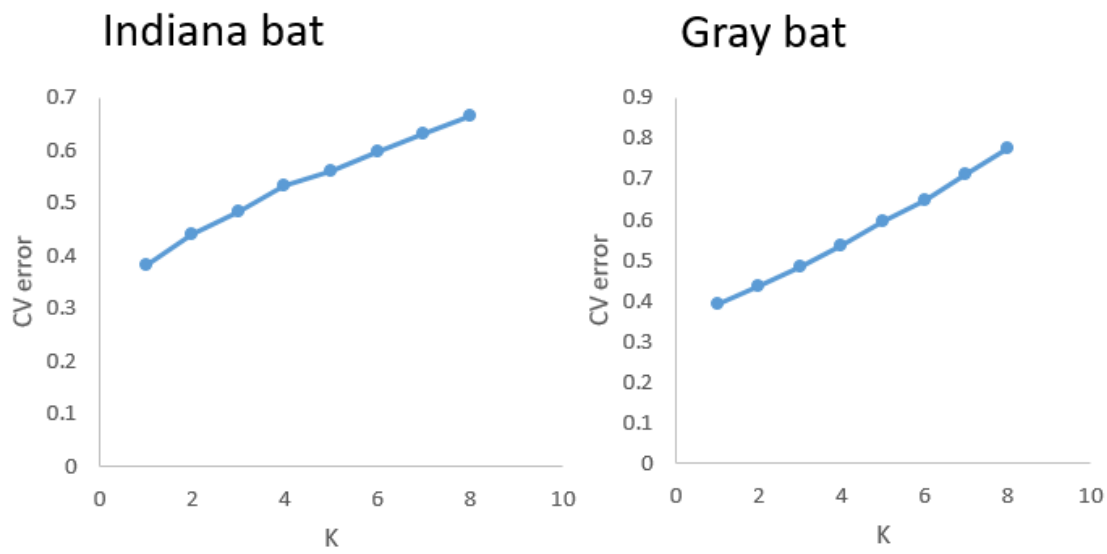

Figure 2. Indiana (n = 45) and gray bat (n = 47) ADMIXTURE results. CV error was lowest for *K* = 1, indicating no discrete populations for either species.

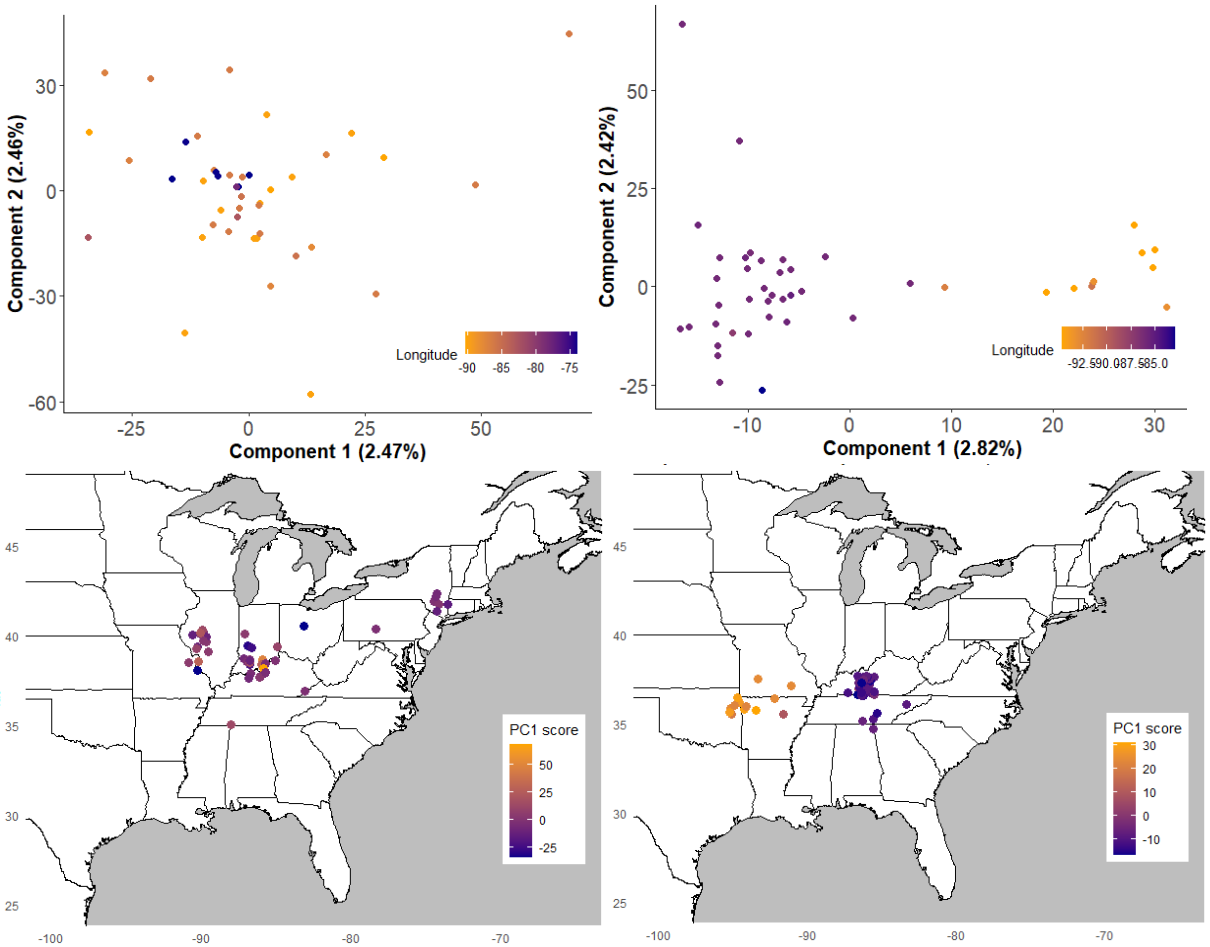

Figure 3. Principal component analyses of Indiana (left, n = 45) and gray bats (right, n = 47). Top row: samples colored by sample longitude. Bottom row: samples colored by PC1 score, with jitter added to reduce sample overlap.

Chapter 4: Trends in Maryland bat populations in the decade following the arrival of white-nose syndrome

Abstract

Several species of cave-dependent bats in eastern North America have been decimated by a fungal pathogen that causes white-nose syndrome (WNS). For species unaffected by WNS, this may be an opportunity to expand into newly vacant niche space. However, several such species are themselves experiencing large numbers of fatalities at industrial wind-energy facilities. Given these impacts, it is unclear how bat species composition is shifting on the post-WNS landscape. To monitor changes in Maryland's bat species, I conducted a decade of annual mobile acoustic surveys beginning in summer 2010, immediately after the first detection of white-nose syndrome in the state in March 2010. Using an ultrasonic microphone attached to the roof of a vehicle, routes were driven between 1 June and 15 July, two to four times per year. From 2010 to 2019, 344 routes were completed, resulting in 426 hours of recordings and 24,375 identified bat passes. I fit generalized linear mixed models using a tweedie distribution with a log-link function. The top-performing model included activity (bat passes per minute) as the response variable, a year-by-group interaction, a region-by-group interaction, species and route as random intercept terms, and species as the random slope. I detected declines of little brown bats (*Myotis lucifugus*), northern long-eared bats (*M. septentrionalis*), eastern small-footed bats (*M. leibii*), and tricolored bats (*Perimyotis subflavus*), at 98.9%, 100%, 92.8%, and 94.1%, respectively. Trends in hoary (*Lasiurus cinereus*) and eastern red (*L. borealis*) bats were nonsignificant. Bat community composition varied among

Maryland's physiographic regions, with eastern red bats comprising a larger percentage in the east. The present-day species composition of bats across the state likely reflects the impact of mortality from white-nose syndrome and wind-energy on different groups of bats.

Introduction

North American insectivorous bats are integral to a healthy ecosystem, providing invaluable services in the form of crop and forest pest control (Kunz et al. 2011; Beilke and O'Keefe 2022). Unfortunately, bats today face a wide range of threats. Like all wild species, they must contend with a changing climate and habitat loss associated with human activities (Jones et al. 2009; Rebelo et al. 2010). In addition, bats face two specific dangers: 1) several cave-dependent bat species have been decimated by an invasive fungal pathogen that causes white-nose syndrome (WNS) (Blehert et al. 2009; Frick et al. 2015), and 2) long-distance migratory tree bats face mortality due to impacts from industrial scale wind-energy turbines (Arnett et al. 2008; Arnett and Baerwald 2013). These threats disproportionately affect different species, and thus declines or extirpation of some species may allow others with similar prey or habitat requirements to increase in abundance following release from the pressure of interspecific competition (Morningstar et al. 2019; Clare et al. 2009). Given the importance of bats to a functioning ecosystem, it is important to understand how species composition and abundance are changing in the face of these multiple forces.

WNS was first observed on a Maryland bat in March 2010, and is caused by the pathogen *Pseudogymnoascus destructans*. A cold-loving fungus that grows on the

epidermis of cave-dwelling bats during hibernation, it causes lesions (Meteyer et al. 2009), disrupts hibernation patterns, and triggers unusual behavior (such as winter flights and roosting at cave entrances) and death (Bohn et al. 2016; Warnecke et al. 2013). Since the discovery of WNS in New York State in February 2006 and its spread throughout North America, bat surveys indicate that populations of North America's insectivorous cave-dwelling bat species have plummeted by millions of individuals, an ecological disaster of staggering proportions (Blehert et al. 2009). Massive declines (> 90 %) have been documented for several cave bat species across eastern North America, especially little brown bats, northern long-eared bats, and tricolored bats (R. T. Brooks 2011; Dzal et al. 2011; Francl et al. 2012; Powers et al. 2015; Pettit and O'Keefe 2017; O'Keefe et al. 2019). Yet, there is some evidence that remaining individuals of some of these species may have resistance to WNS and could serve to found new populations (Maslo et al. 2015; Gignoux-Wolfsohn et al. 2021). Other cave bat species, such as big brown bats (*Eptesicus fuscus*) and gray bats (*Myotis grisescens*) seem better able to withstand WNS, and have shown population growth in some WNS-infected caves (Frank et al. 2014; Jackson et al. 2022).

Cave bat species are often censused through winter hibernacula counts, a valuable tool to monitor changes in these species. Indiana bats (*Moyis sodalis*), for example, a federally-endangered cave-bat species, have been surveyed biannually at known hibernacula since the early 1980s (USFWS 2018). Hibernacula surveys are labor-intensive and may miss large numbers of bats hibernating in unknown sites. Indeed, after the arrival of WNS, several previously unknown hibernacula were discovered due to the presence of dead bats outside of cave entrances (personal

communication, NYDEC wildlife biologist). Additionally, winter hibernacula counts cannot directly measure changes in summer bat populations, especially in regions with no caves or mines, nor do they offer any information on species that do not use caves.

In contrast to cave-dwelling bat species, WNS does not appear to affect insectivorous, tree-roosting, long-distance migratory bat species that typically do not use caves (Bernard et al. 2015). However, these species, and three in particular, are those most at risk of mortality at industrial wind-energy facilities. Estimates from nearly a decade ago are that hundreds of thousands of hoary (*Lasiurus cinereus*), eastern red (*Lasiurus borealis*), and silver-haired (*Lasionycteris noctivagans*) bats are killed during migration each fall across the United States (Arnett et al. 2008; Arnett and Baerwald 2013; ACP 2022). Mitigation approaches such as raising the cut-in speeds of turbines have proven effective at reducing bat mortality rates at wind-energy fatalities (Baerwald et al. 2009; Arnett et al. 2011; Arnett and May 2016). However, such mitigation is largely voluntary, and it remains unknown whether mortality at wind-energy facilities is causing population-level impacts (O'Shea et al. 2016; Frick et al. 2020).

Currently, all on-shore industrial wind-energy facilities in Maryland are located in the western region of the state in Garrett County, along the ridges of the Allegheny Mountain Range (Figure 1, Hoen et al. 2018). The first industrial-scale wind energy project in Maryland, Criterion, came online in 2010 and consists of 28 turbines. The Roth Rock project followed shortly afterwards in 2011, with an additional 20 turbines. The 16 turbines of the Fourmile Ridge project came online in

2014, followed by an additional 12 turbines in 2015 with the Fair Wind project. These 76 turbines are all rated at 2.5MW, with hub heights of either 90 or 100 m. Additional wind-energy projects were completed nearby during the same timeframe in Pennsylvania and West Virginia (Hoen et al. 2018).

While the presence of wind turbines on the landscape might lead to a decline in tree bats, a potential positive influence on these species is the release of interspecific competition for prey and habitat resources as cave bats decline. Dietary studies have shown that insectivorous cave-dwelling and tree-roosting bats in North America overlap greatly in the species they prey upon (Brack and Whitaker 2001; Carter et al. 2003; Clare et al. 2009; Dodd et al. 2012; Feldhamer et al. 2009). A dietary shift post-WNS has been recorded in at least one species; big brown bats in Ontario, Canada, consumed a wider range of prey post WNS, including many insect species once eaten by little brown bats before their decline (Morningstar et al. 2019). Whether such dietary shifts have occurred in hoary, eastern red, or silver-haired bats remains unknown.

Unfortunately, estimating census population size of the species most affected by wind turbine mortality is not possible with traditional methods, as these species are nocturnal, cryptic, highly mobile, and do not gather in groups that can be censused (O'Shea and Bogan 2003). One survey method that has been used with bats is ultrasonic recording of their echolocation calls. Acoustic monitoring is a repeatable survey method that does not require bat handling and has a relatively low expense. In addition, acoustic surveys can cover a broad geographic area and survey for multiple species simultaneously.

Acoustic surveys can be conducted at passive stationary points or along moving routes. Stationary acoustic methods are best used for presence/absence studies and are often set up to record for long periods. Stationary surveys are useful in areas with few roads; however, this method can only measure bat activity, not abundance or density, as it is impossible to determine whether bat passes are from multiple individuals or from a single individual flying by multiple times (Whitby et al. 2014; Fisher-Phelps et al. 2017; Neece et al. 2019; Hale et al. 2022). In contrast, mobile acoustic surveys travel along a route at a set speed faster than bats will normally travel. Thus, each bat detection should represent a unique individual, and relative abundance can be measured (Roche et al. 2011). Mobile routes can maximize the geographic coverage of surveys, although they are mostly limited to areas where roads can safely be traveled at a slow constant speed (typically 32 kph [20 mph]).

Maryland covers a broad range of habitats across a mountains-to-sea gradient (Figure 2). Western Maryland includes portions of the heavily-forested Appalachian Plateau and is also where all of Maryland's onshore wind turbines are located. Moving east, agriculture and urban areas become more common in the Ridge and Valley Province, the Blue Ridge Mountains, and the hills of the Piedmont. The Atlantic Coastal Plain borders the Chesapeake Bay, which includes urban areas and the mostly flat Delmarva Peninsula where agriculture is the predominate land use. These different physiographic regions contain different habitats, which influence the bat communities present. Over 90% of Maryland's caves are located in the Appalachian Plateau, Ridge and Valley Province, and Blue Ridge Mountains (Gates et al. 1984). The west-to-east gradient presents an opportunity to investigate potential

spatial variation in bat populations following the arrival of WNS and during a period of wind-energy development in western Maryland.

With the interacting forces of white-nose syndrome mortality, wind-energy mortality, and interspecific competition, it is important to monitor the changes in bat communities across various habitats to inform management and conservation decisions. Using mobile acoustic routes across the state of Maryland, I addressed the following questions: First, in the decade post WNS, do any cave bat species show signs of recovery? Second, what is the status of hoary bats and eastern red bats on the landscape?

Methods

To monitor changes in the activity of bat-species populations in Maryland, eighteen acoustic survey routes were designed throughout the state; eight were established in western Maryland in 2010 (Garrett, Allegany, and Washington counties), and ten more were added in 2014 that covered the rest of the state (Figure 3). In collaboration with Ed Gates and Maryland DNR personnel Dan Feller and Dana Limpert, some routes were established based on Breeding Bird Survey routes used to monitor bird populations in North America and Canada. Others we designed to pass through forests, along rivers, or near habitat where bats are likely to congregate. Routes 1-5 are in the Appalachian Plateau physiographic province, routes 6-9 are in the Ridge and Valley, route 10 is in the Blue Ridge, routes 11-13 are in the Piedmont, and routes 14-18 are in the Coastal Plain. As there is only a single route in the Blue Ridge province, I combined this with the Ridge and Valley routes for analyses (hereafter referred to as the Ridges region). Routes were between 30.8 km (19.1 mi)

and 45.1 km (28.0 mi) in length, with a mean of 37.5 km ± 3.7 SD (23.3 mi ± 2.3 SD; Table 1).

Data Collection

From 2010 to 2013, the eight western routes were surveyed four times annually. Survey direction was switched (starting from the opposite end) with each repetition. From 2014 to 2019, all 18 routes were surveyed twice annually, once in each direction. To control for day-of-year effects, all surveys were completed during 1 June – 15 July each year to target local adult bats in their summering habitat, before young of the year are volant. My field crews and I sampled between one and three routes simultaneously per night. To control for time-of-night effects, all surveys began 0.5 to 1 hour after local sunset. Each route was driven without stopping (except where required to make turns or navigate intersections), traveling as close to 32 kph (20 mph) as possible the entire distance, and typically lasting between 60 and 90 minutes. Surveys were not conducted during precipitation and only occurred in the temperature range following Fish and Wildlife Service Indiana bat guidelines (> 10°C).

To record bat calls, Binary Acoustic's ultrasonic microphone AR125 (Binary Acoustic Technology, Tucson, Arizona) was mounted magnetically, pointing up, on the roof of a vehicle (Figure 4). GPS locations were recorded simultaneously each second with a DeLorme GPS receiver and DeLorme's Street Atlas USA 2010 (DeLorme, Yarmouth, Maine).

Call Identification

Each echolocation pulse was considered to be one "bat call;" multiple calls from the same individual make up one "bat pass" as the bat flies by the microphone. To identify bat passes, I used CallViewer18 (Skowronski and Fenton 2008) to visually inspect the spectrogram of each bat pass (Figure 5). Using the minimum and maximum frequencies along with call duration and shape, each pass was identified to one of the following categories: unknown, hoary bat (*Lasiurus cinereus*), eastern red bat (*Lasiurus borealis*), big brown bat/silver-haired bat (*Eptesicus fuscus/Lasionycteris noctivagans*), tricolored bat (*Perimyotis subflavus*), little brown bat/Indiana bat (*Myotis lucifugus/Myotis sodalis*), northern long-eared bat (*Myotis septentrionalis*), or eastern small-footed bat (*Myotis leibii*). I did not attempt to separate big brown bats and silver-haired bats as they have overlapping call characteristics, as do little brown bats and Indiana bats (Lemen et al. 2015; Russo and Voigt 2016). As Indiana bats are very rare in Maryland, I assumed that the majority of those calls are from little brown bats. Bat passes with less than three echolocation pulses, those that were too faint to obtain all call characteristics, and those that did not fit into expected species categories, were classified as unknown. I calculated the location of each bat pass along the route using Global Positioning System (GPS) data with time stamps and the start time of each bat pass. I used GPSBabel (http://www.gpsbabel.org/index.html, accessed 29 March 2016) to convert the DeLorme GPS data to a format compatible with Excel software.

Data Analysis

For each night, I standardized the results as bat passes per minute for each category. The total time of recording for each night was calculated as the time from beginning to end of the route, minus any time spent on detours, wrong turns, and pauses lasting over one minute (e.g., at a stoplight). Based on their time stamp, any bat passes recorded during detours or stops, based on their time stamp, were excluded.

To test for the significance of changing bat activity, I fit generalized linear mixed models using the glmmTMB package v 1.1.4 (Brooks et al. 2017) (Brooks et al. 2017) in R version 4.2.1. A histogram showed that 34% of the values were zero (Figure S1), thus I used the tweedie distribution, which performs well with zero-inflated data (Shono 2008). I included a log-link function as acoustic data are a form of count data will never be negative in value. As I expected similar population trends in the WNS-affected species, I grouped all WNS-affected species (little brown bats, northern long-eared bats, small-footed bats, and tricolored bats) together as one functional group. Thus, groups included in the models were: WNS-affected species, eastern red bats, hoary bats, and big brown/silver-haired bats. I also rescaled the 'year' predictor to center on 0. I included a 'region' term as a categorical variable based on the physiography defined by the Maryland Geological Survey (Figure 2).

I applied model selection to models subsetted from a global model, which was constructed with bat activity (passes per minute) as the response variable, and a three-way interaction between group, region, and route. Random intercept terms were

species and route; the random slope term was species. After colinearity checks, I performed iterative model selection on subsetted models with and without interaction terms, removing models with high multi-collinearity and selecting models with the lowest AIC. The top-performing model included activity (bat passes per minute) as the response variable, a year-by-group interaction, a region by group interaction, species and route as random intercept terms, and species as the random slope (Table 2).

Results

From 2010 to 2019, 344 surveys were completed, resulting in a total of 426 hours of recordings and 24,375 identified bat passes. I was able to identify 85.8% of the calls, leaving 14.2% classified as unknown.

The top-performing model contained interactive effects of functional group and year and functional group and region, respectively. The model predicted that, on average, bat activity during the study period was highest in big brown/silver-haired bats (prediction of 0.30 passes/minute), followed by eastern red bats (0.14 passes/minute), hoary bats (0.11 passes/minute), and WNS-affected species (0.01 passes/minute). Estimates of passes/minute were significantly different between the WNS-affected group and the other species (Table 3).

I found evidence of decline in the WNS-affected species. In the initial survey year, little brown bats were the highest recorded species, comprising nearly a third (31.4%) of all recorded bat passes. However, they declined dramatically in the following years. By 2019, bat passes of little brown bats had declined by 98.9% from

their 2010 levels. Between 2010 and 2019, eastern small-footed bats, tricolored bats, and northern long-eared bats, which are also WNS-susceptible species, had declined 92.8%, 94.1%, and 100%, respectively. None of the other species (hoary bats, eastern red bats, or the big brown/silver-haired bat group) showed significant change in bat passes over the course of my study (Figure 7, Table 3).

The bat communities varied among the four physiographic regions (Figures 8, S2, and S3). In the Appalachian Plateau, the big brown/silver-haired bat species group was most recorded from 2011 onward, after little brown bats declined by 44% in the first year. In the Ridges region, eastern red bats were the highest recorded species for most years, although this varied year to year; the big brown/silver-haired group was occasionally higher. Although the Piedmont was not surveyed until 2014, the big brown/silver-haired bat grouping was over a third of all bat passes, at 38% during the first year. Their passes dropped during 2016-2018 then increased to 49% of recorded passes in 2019. Eastern red bats in this region were recorded much more frequently than hoary bats across all survey years. On the Coastal Plain, eastern red bats comprised between 43% and 50% of all bat passes each year, far more than any other species. Overall, bat abundances across the study period were not significantly different between the Appalachian Plateau and the Ridges regions. Bat numbers were significantly higher in the Piedmont Plateau, and lower in the Coastal Plain in comparison to the Appalachian Plateau (Table 3).

Discussion

My results indicate a major shift in bat species composition in the decade following the arrival of WNS in Maryland, due to a near complete loss of four cave bat species. In the summer of 2010, following the first detection of WNS in Maryland, nearly a third (31.4%) of all bat passes were little brown bats. Ten years later, little brown bats were detected very rarely, at only 0.47% of all bat passes. This resulted in a higher percent of overall calls coming from the remaining species, though population trends for hoary, eastern red, and big brown/silver-haired bats did not significantly change.

By conducting mobile surveys while moving at a relatively constant 32 kph, each bat pass should be a unique bat, as the vehicle speed is faster than the flight speeds of most bats. Reported average flight speeds include 18 kph for little brown bats, 17.2 kph for big brown bats (Hayward and Davis 1964). 24.1 kph for hoary bats, and 27.7 for eastern red bats (Salcedo et al. 1995). However, I acknowledge that multiple detections of a single bat are possible where a route doubles back near itself (e.g., the southeastern section of the Hancock-Clear Spring route; Figure 3), or when driving slowly through intersections or along lengths of rough road which necessitated slower travel.

Another potential bias is the limitation of surveying along roadways. Some species, such as big brown bats, may use roads to travel and/or feed, concentrating activity and increasing detections (Hein et al. 2009). Conversely, the northern long-eared bat is a forest-interior species (Kurta 1995) and may be less likely to feed along forest edges created by roads. Combined with this species' relatively quiet call (Faure

et al. 1993), these factors could contribute to relatively fewer detections. Finally, the AR125 only detects bats flying within approximately 100 m of the microphone (http://binaryacoustictech.com/Web%20design%202/3%20Downloads/Doc/AR125%20Product%20Specification.pdf, accessed 1 October 2022). The exact limit of detection distance varies based on wind speed, humidity levels, and direction the bat is facing relative to the detector. High-flying bats, including hoary bats which often fly above tree top height (Kurta 1995), are less likely to be detected, and therefore may be underrepresented in surveys conducted along routes with heavy forest cover. Despite these potential biases, trends in calls of individual species through time are likely to be robust at a given location, as the same roads were used in each survey year.

The observed decrease in cave bats was expected and is almost certainly indicative of mass mortality following the arrival of WNS. Unfortunately, there was no evidence of recovery during this period, only a continual decline for these species.

The echolocation group of big brown bats and silver-haired bats showed no significant population changes over the decade post arrival of WNS. However, as the two species' calls are combined in this dataset, I cannot say whether either one experienced any change in population. Big brown bats are susceptible to WNS, though at lower levels than other species (Frank et al. 2014). Thus, it is likely they are maintaining a presence on the landscape. Alternatively, a decrease in big brown bats could be masked by an increase in silver-haired bats, which are not susceptible to WNS, but are one of the species most often killed at wind turbines (Arnett et al. 2008). Conducting hibernacula surveys of big brown bats may be one potential way

to disentangle the acoustic survey results. If big brown bats are surviving in hibernacula, they are also likely present on the landscape during the summer.

Several factors may be influencing the eastern red bat and hoary bat populations, including the presence of wind turbines and decreasing interspecific competition for prey. The same year that WNS was first detected in Maryland also saw the first industrial-scale wind-energy facility come online in the state (though there were already wind farms active in nearby states beginning as early as 2002). Turbines on the forested ridges of the Appalachian Mountains have been documented to have among the highest mortality rates, up to 70 bats per turbine per year (Arnett et al. 2008). Most of the mortality, however, happens during fall migration, while these surveys were conducted during bats' summering season. Based on stable hydrogen isotope data from bat hair, ~43% of eastern red bat mortality at western Maryland turbines was from individuals that summered locally, compared to ~99% of hoary bat mortalities coming from individuals that summer locally (Pylant et al. 2016). Even with these high local mortality levels, hoary bat population levels did not decrease across the study period.

A change in prey abundance and competition may explain why eastern red bats and hoary bats were able to maintain stable populations, even with wind energy mortality of individuals summering locally. Dietary studies have shown the bats of North America overlap greatly in their insect prey (Brack and Whitaker 2001; Carter et al. 2003; Clare et al. 2009; Dodd et al. 2012; Feldhamer et al. 2009). With the disappearance of the cave bat species, the remaining bats on the landscape may be experiencing reduced interspecific competition for food sources. Alternatively, it is

possible that insect prey are not more abundant even with the decrease in cave bats. Due to causes such as climate change, pesticide use, and habitat loss, declines in overall insect abundance have been reported around the world (Goulson 2019), and could certainly be the case in Maryland.

Other acoustic studies have measured changes in bat activity after the arrival of WNS. Mobile surveys across Ohio detected no significant change for hoary bats, but unlike my findings, eastern red bats decreased (Simonis et al. 2020). Stationary surveys in Massachusetts documented a 72% decline in overall bat activity in the four years post arrival of WNS, with no significant changes in hoary or eastern red bats (R. T. Brooks 2011). Stationary acoustic surveys in New York detected an increase of hoary bat activity post arrival of WNS, but no significant change in eastern red bat activity (Jachowski et al. 2014; Nocera et al. 2019). Conversely, eastern red bats increased significantly during mist-net surveys in Kentucky post arrival of WNS (Thalken et al. 2018). The conflicting results of various surveys in different states could indicate a regional effect of habitat, differing interspecific competition, local effects of wind-energy mortality, or other unknown variables. Overall, many interesting questions remain about the interspecific species interactions and specific influences of habitat, including distance to turbines. Regular re-surveying of the same routes would also be valuable in understanding and monitoring long-term changes in Maryland's bat populations.

Acknowledgements

I would like to acknowledge the following people that assisted in collection of the acoustic data or aided us in securing road access: Mark Beals, Beau Emerson, Dan

Feller, Grace Hummell, Dana Limpert, Becky Loncosky, Sara Nehemiah, Kelly Pearce, Shannon Pederson, and Rachel Schadegg. Our monitoring effort was supported by the Maryland Department of Natural Resources, Natural Heritage Program.

Tables

Table 1. Length and location of each bat acoustic survey route in Maryland.

Route	Length		County	Physiographic Region
	Kilometers	Miles		
1.) Friendsville-Grantsville	38.8	24.1	Garrett	Appalachian Plateau
2.) Cranesville-Bittinger	33.9	21.0	Garrett	Appalachian Plateau
3.) Swallow Falls-Swanton	41.0	25.5	Garrett	Appalachian Plateau
4.) Southernmost Garrett	39.0	24.3	Garrett	Appalachian Plateau
5.) Finzel-Westernport	38.8	24.1	Garrett	Appalachian Plateau
6.) Cumberland-Flintstone	32.7	20.3	Allegany	Ridge and Valley
7.) Green Ridge-Hancock	39.5	24.5	Allegany/Washington	Ridge and Valley
8.) Hancock-Clear Spring	32.8	20.4	Washington	Ridge and Valley
9.) Hagerstown	37.9	23.5	Washington	Ridge and Valley
10.) Catoctin Mountain	36.3	22.6	Frederick	Blue Ridge
11.) Prettyboy Reservoir	30.8	19.1	Baltimore	Piedmont Plateau
12.) Rocks-Susquehanna	45.1	28.0	Harford	Piedmont Plateau
13.) Poolesville	32.3	20.0	Montgomery	Piedmont Plateau
14.) Huntingtown	41.5	25.8	Calvert	Coastal Plain
15.) Leonardtown	39.4	24.5	St Mary’s	Coastal Plain
16.) Kent County	39.0	24.2	Kent	Coastal Plain
17.) Federalsburg	38.0	23.7	Caroline/Dorchester	Coastal Plain
18.) Pocomoke City	38.6	24.0	Somerset/Worcester	Coastal Plain

Table 2. Competing bat activity (passes per minute) models for predicting species population changes, 2010-2019. I ranked models based on Akaike's Information Criterion (AIC).

Model	AIC	ΔAIC
(0 + year|species) + (1|species) + (1|route) + year*group + region*group	-2526.14	0
(0 + year|species) + (1|species) + (1|route) + year*group + region	-2294.68	231.456
(0 + year|species) + (1|species) + (1|route) + year*group	-2286.6	239.54
(0 + year|species) + (1|species) + (1|route) + year + group + region	-2286.55	239.592

Table 3. Generalized linear mixed model results. WNS-affected bats include little brown bat, northern long-eared bat, small-footed bat, and tricolored bat. Model includes a year-by-group interaction and a region-by-group interaction. Random intercept terms were species and route; the random slope term was species. The big brown/silver-haired bat group was the reference species, and reference region was the Appalachian Plateau.

	Estimate	Std. Error	z-value	p-value	Significance
Intercept	-1.190	-0.625	-1.906	0.057	
Year	-0.001	0.217	-0.004	0.997	
Eastern red bat	-0.767	0.878	-0.873	0.383	
Hoary bat	-1.024	0.879	-1.166	0.244	
WNS-affected	-3.593	0.698	-5.148	0.000	***
Coastal Plain	-0.429	0.134	-3.209	0.001	**
Piedmont Plateau	0.569	0.141	4.019	0.000	***
Ridges	-0.088	0.118	-0.743	0.457	
Year:Eastern red bat	0.098	0.307	0.319	0.750	
Year:Hoary bat	-0.001	0.308	-0.004	0.997	
Year:WNS-affected	-1.139	0.249	-4.580	0.000	***
Eastern red bat:Coastal Plain	1.609	0.123	13.103	<0.001	***
Hoary bat:Coastal Plain	0.109	0.156	0.698	0.485	
WNS-affected:Coastal Plain	-0.125	0.206	-0.609	0.543	
Eastern red bat:Piedmont Plateau	0.314	0.131	2.403	0.016	*
Hoary bat:Piedmont Plateau	-0.311	0.156	-1.998	0.046	*
WNS-affected:Piedmont Plateau	0.038	0.184	0.206	0.837	
Eastern red bat:Ridges	0.893	0.097	9.169	<0.001	***
Hoary bat:Ridges	0.359	0.108	3.316	0.001	***
WNS-affected:Ridges	0.270	0.100	2.701	0.007	**

Figures

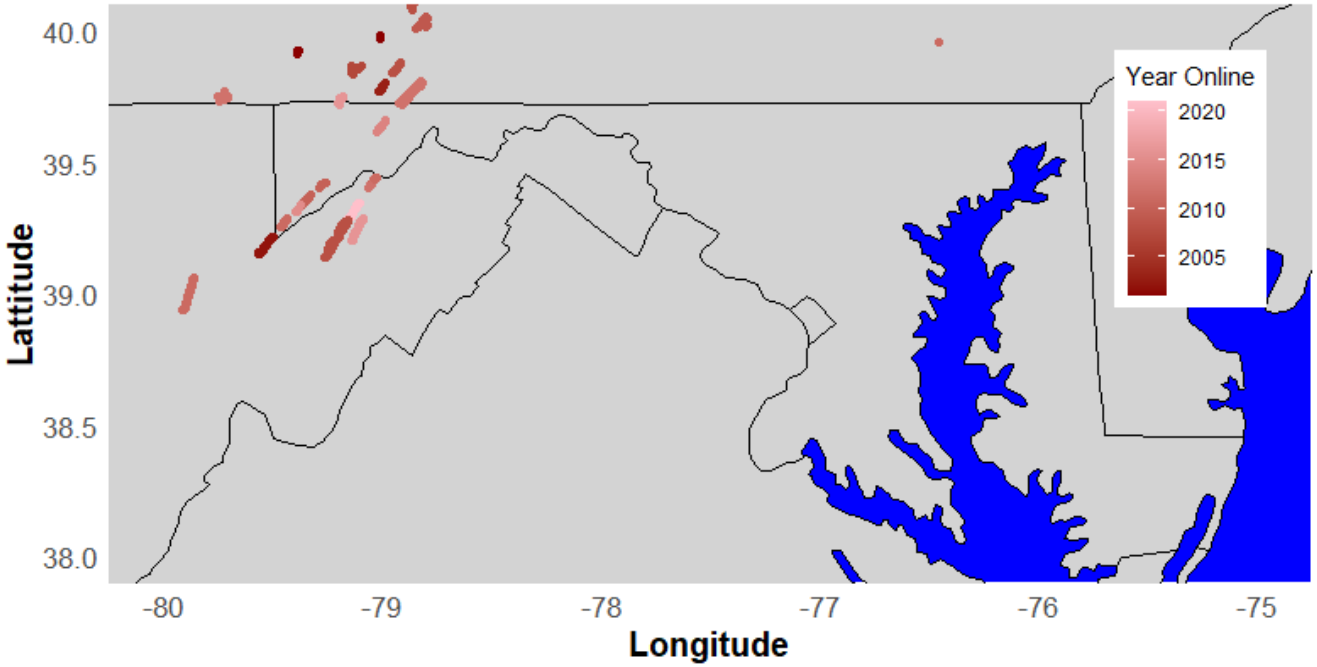

Figure 1. Current on-shore industrial wind-energy projects in and near Maryland (Hoen et al. 2018, version 5.2, August 2022).

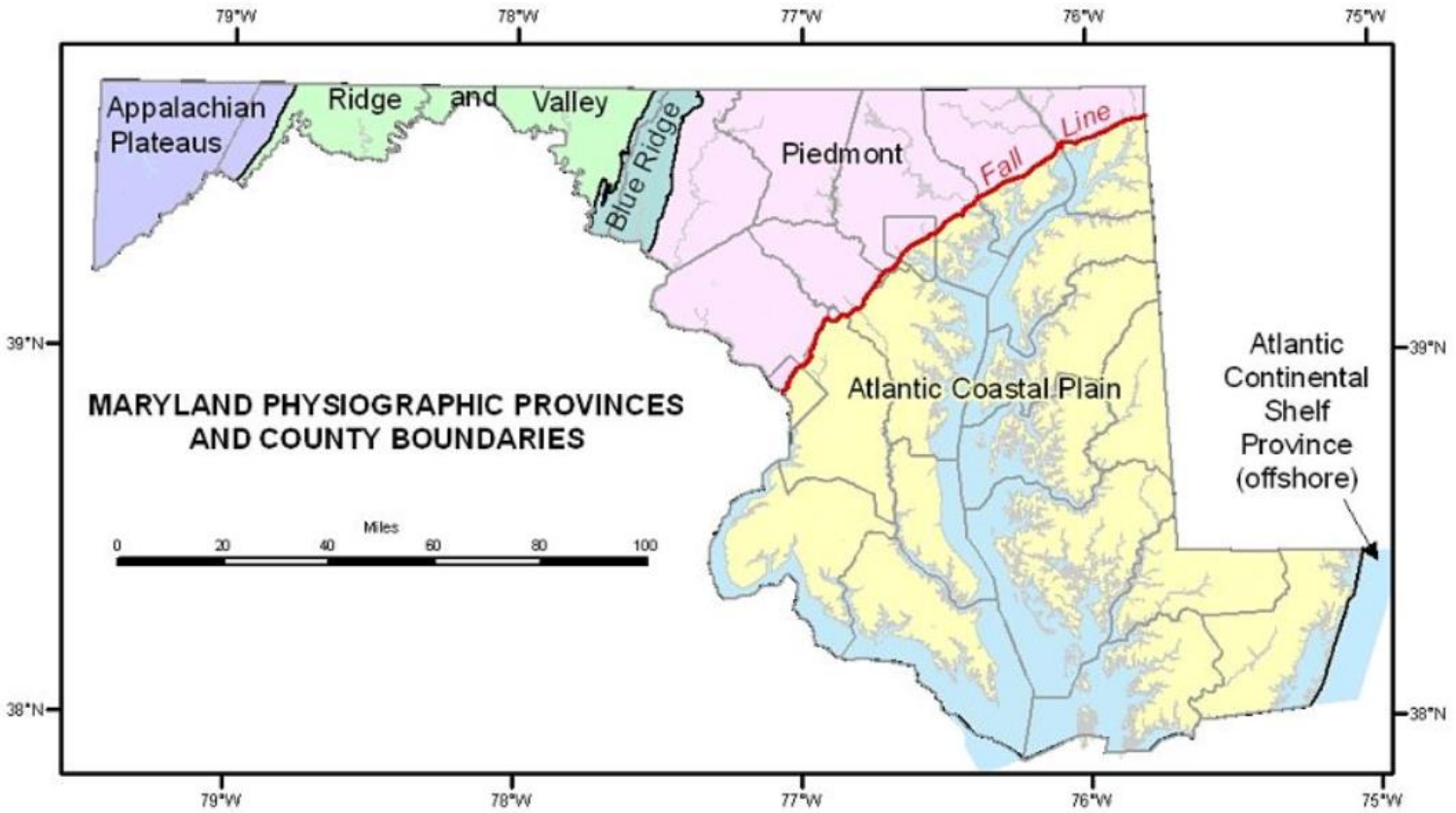

Figure 2. Maryland's Physiographic Regions. Map source: http://www.mgs.md.gov/geology/index.html, accessed 1 October 2022.

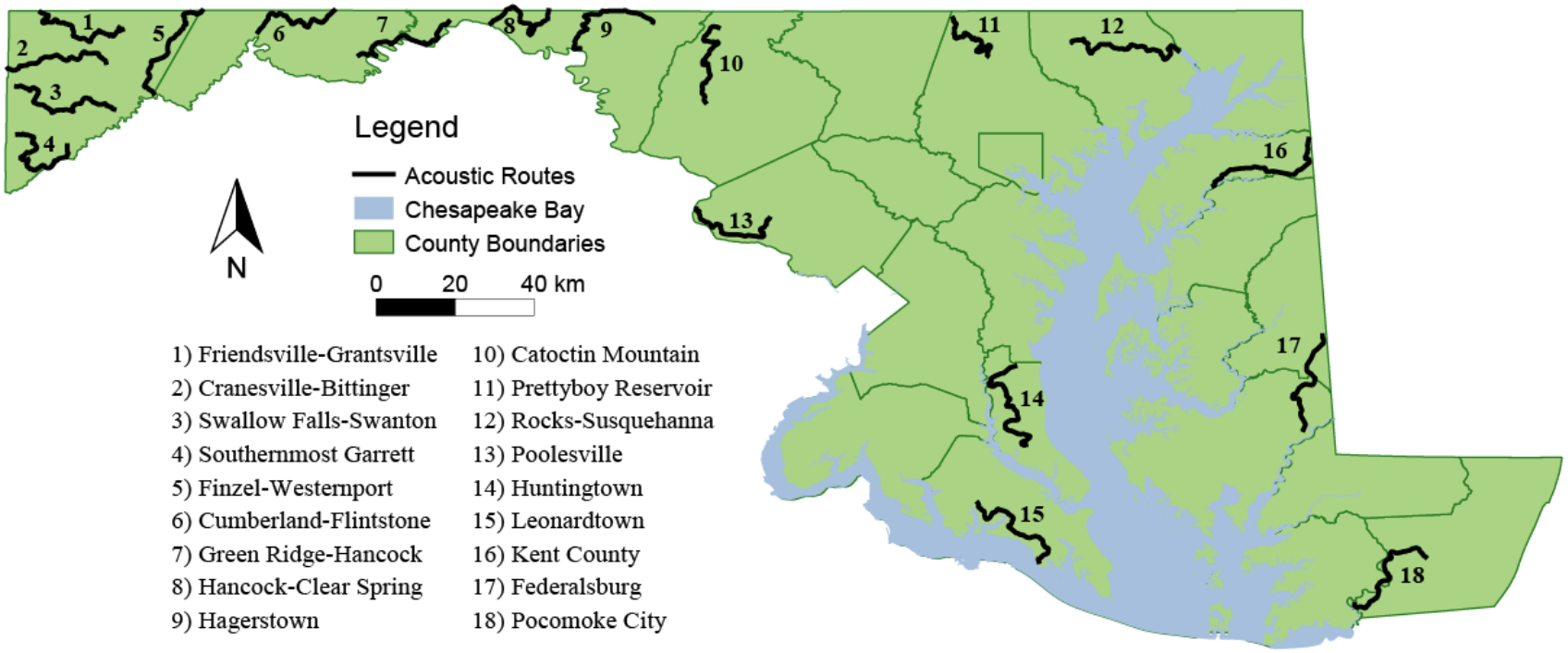

Figure 3. Locations of eighteen bat survey acoustic routes located throughout Maryland.

Figure 4. Photo of an ultrasonic microphone and safety light, magnetically mounted on the roof of a vehicle.

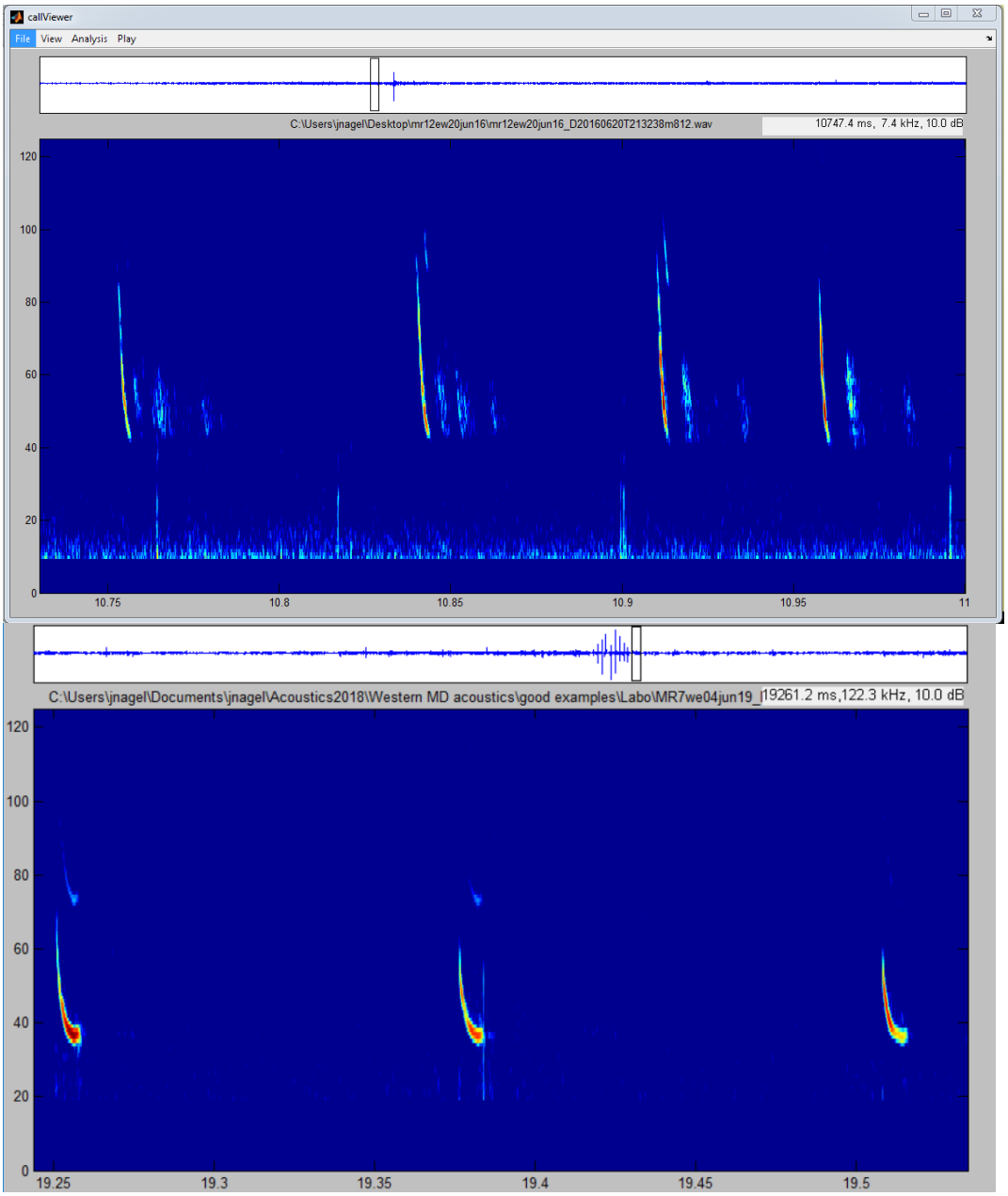

Figure 5. Example spectrograms of bat calls. Above: northern long-eared bat (*Myotis septentrionalis*) recorded along the Rocks-Susquehanna Route. Below: eastern red bat (*Lasiurus borealis*) recorded along the Green Ridge Route. X-axis is time in milliseconds, Y-axis is frequency in kHz.

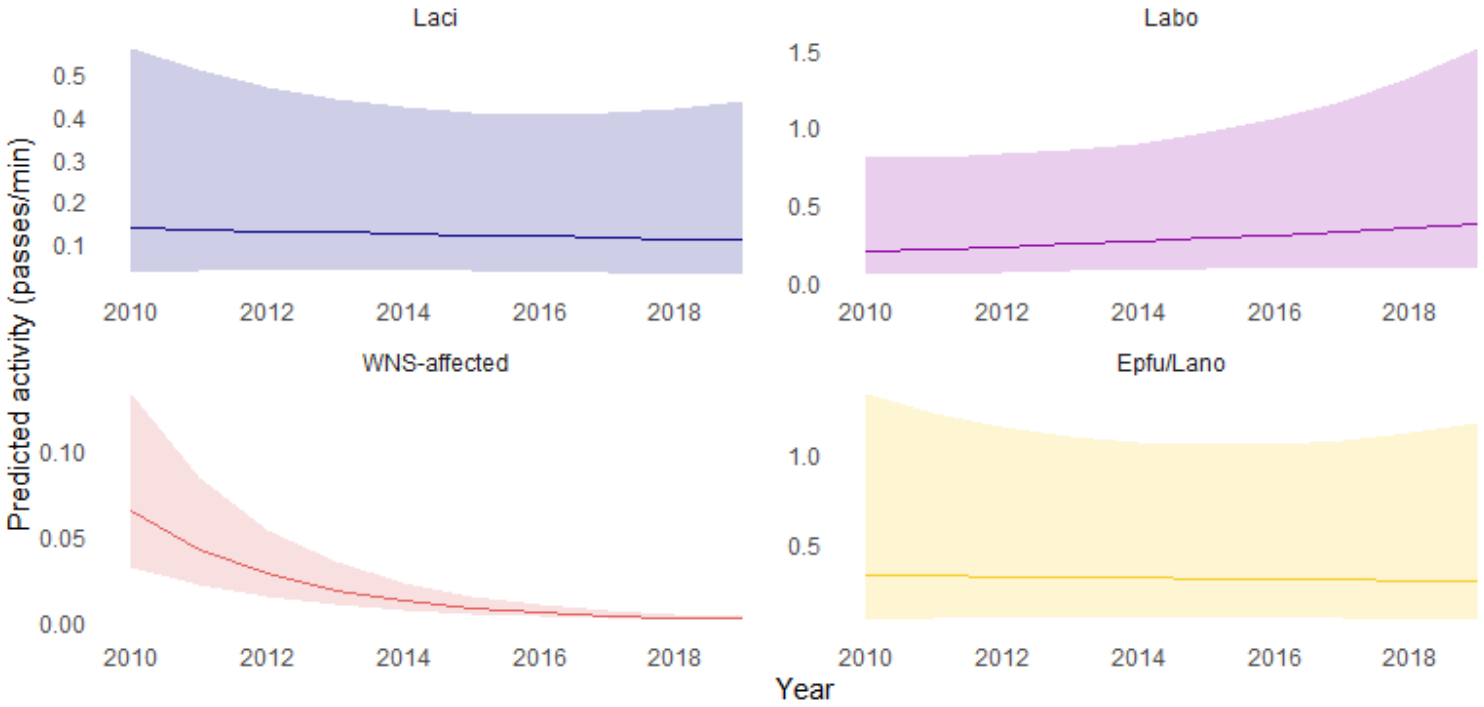

Figure 6. Predicted activity (bat passes per minute), by species group, using a generalized linear mixed model. Shaded areas represent 95% confidence intervals. WNS-affected species include little brown bats, eastern small-footed bats, northern long-eared bats, and tricolored bats.

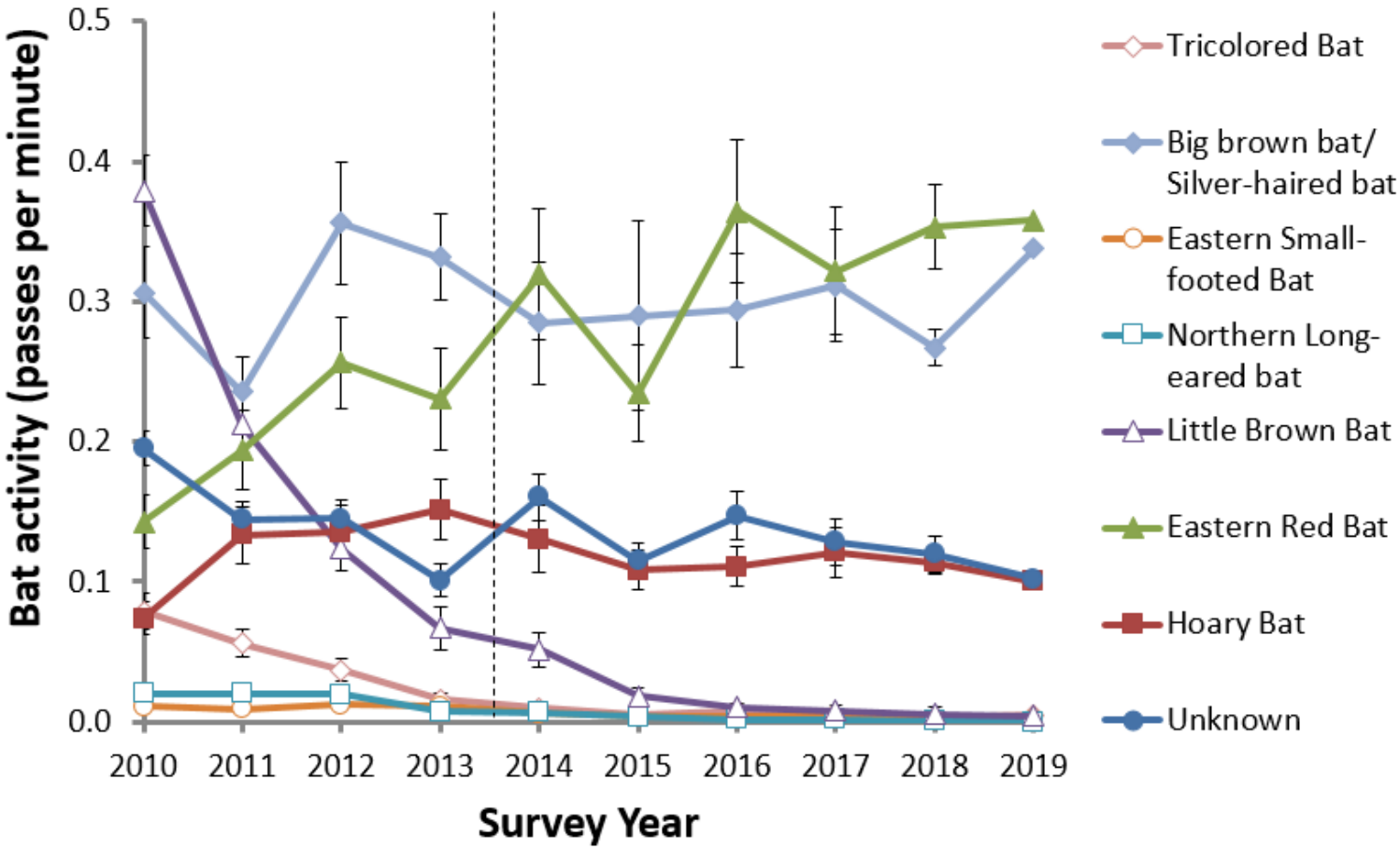

Figure 7. Bat passes per minute (mean and standard error) across Maryland for all 18 routes combined, 2010–2019. The vertical dotted line indicates the that year routes 9–18 were added.

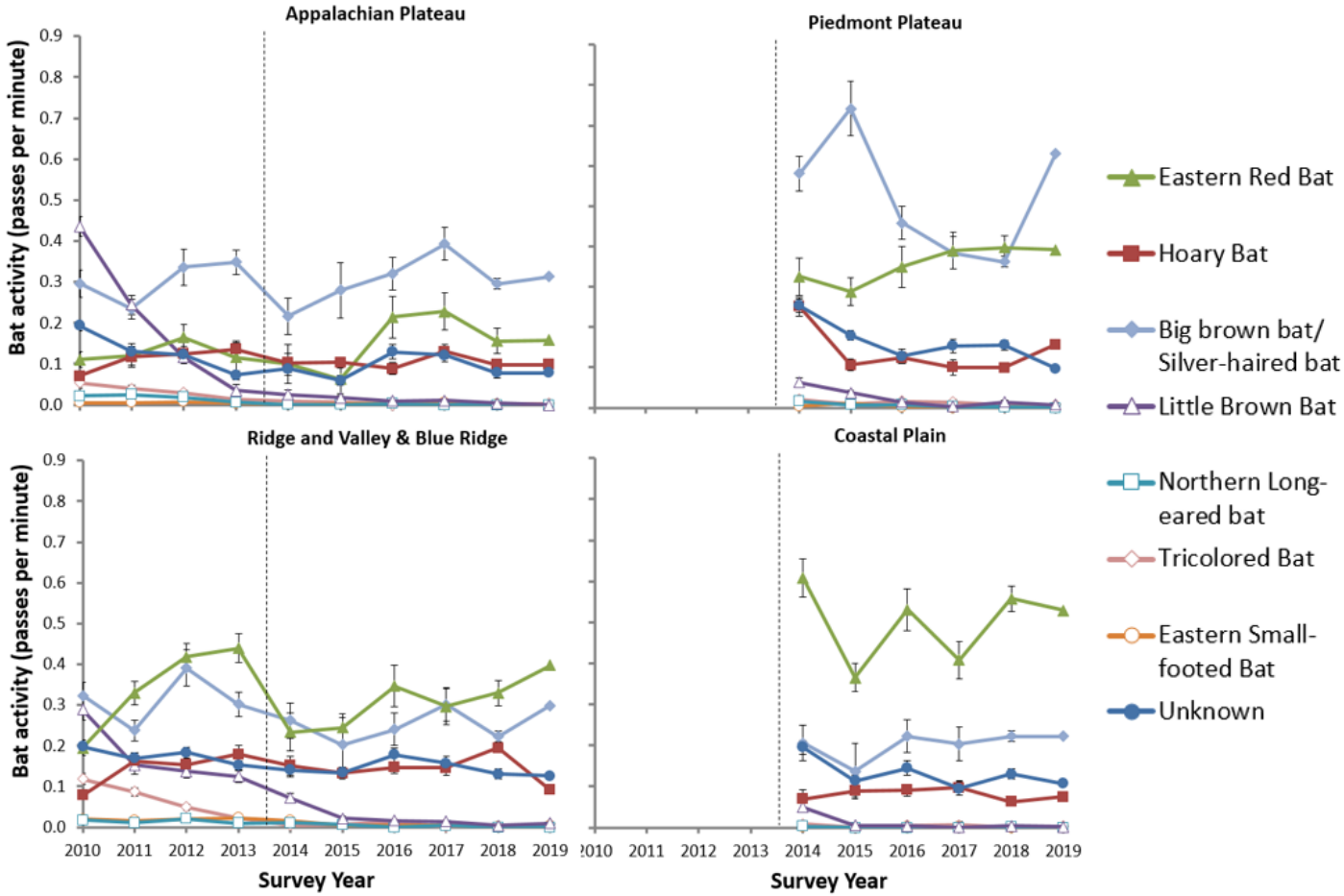

Figure 8. Bat passes per minute (mean and standard error) in the four physiographic regions of Maryland, 2010–2019. The vertical dotted line indicates the year that routes 9–18 were added.

Supplementary Figures

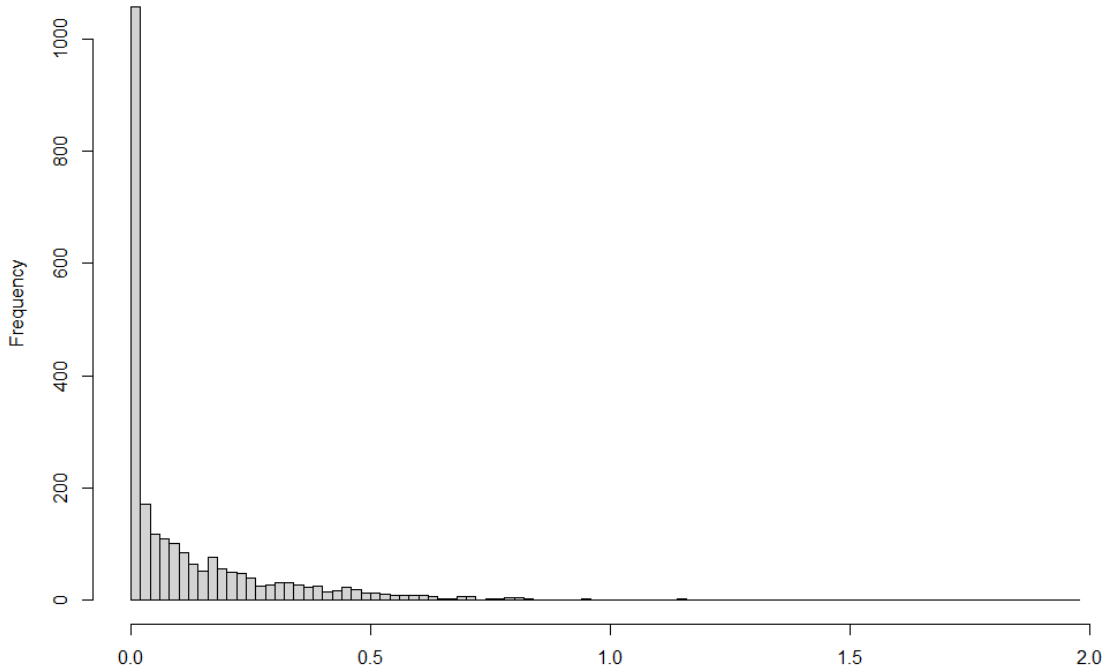

Figure S1. Histogram of bat passes per minute for all 10 years combined, showing a zero-inflated (34 % zeroes) dataset. Thus, a Tweedie distribution was used with the generalized linear mixed models.

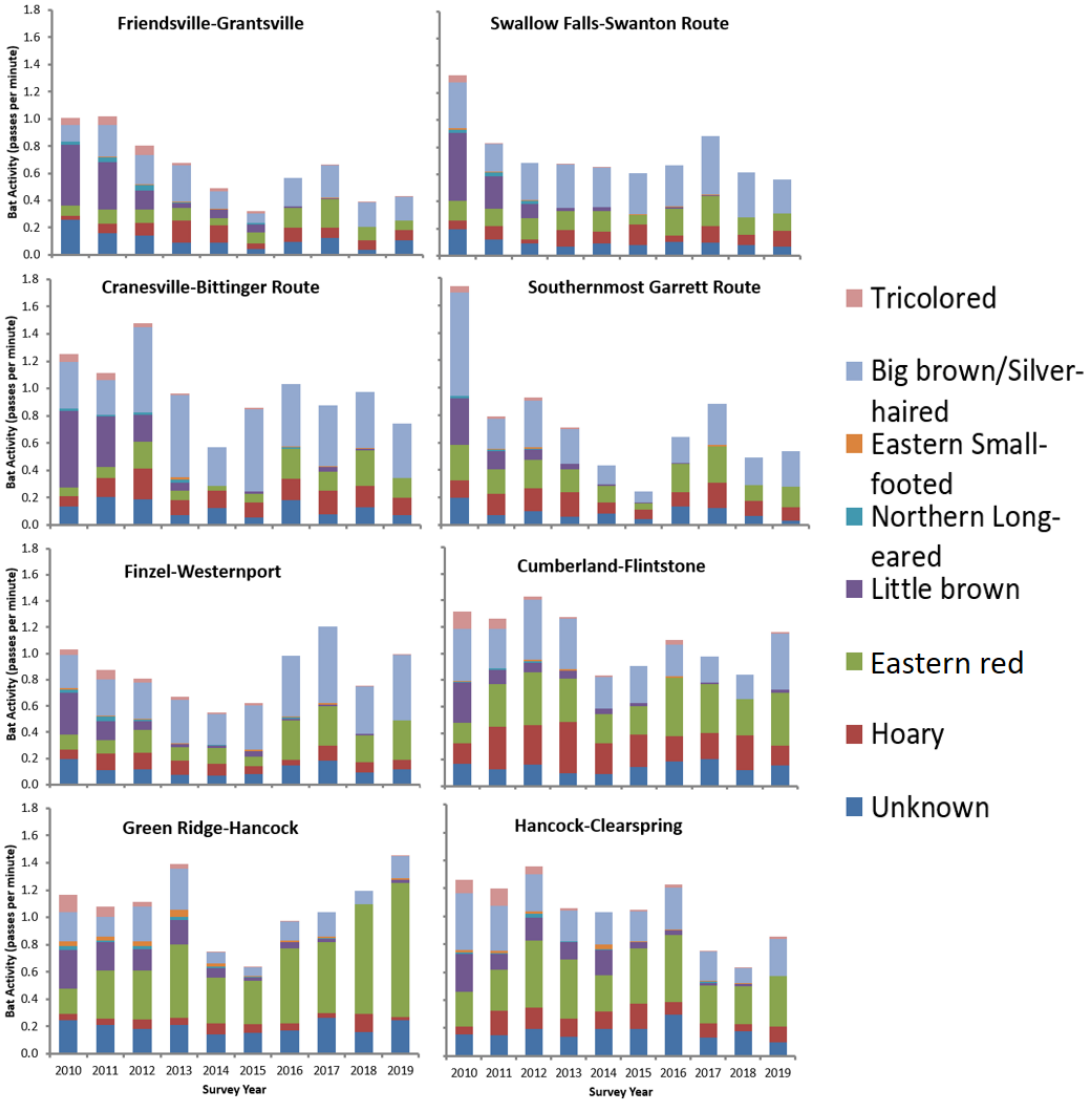

Figure S2. Bat passes per minute by year for the eight western routes, 2010 – 2019.

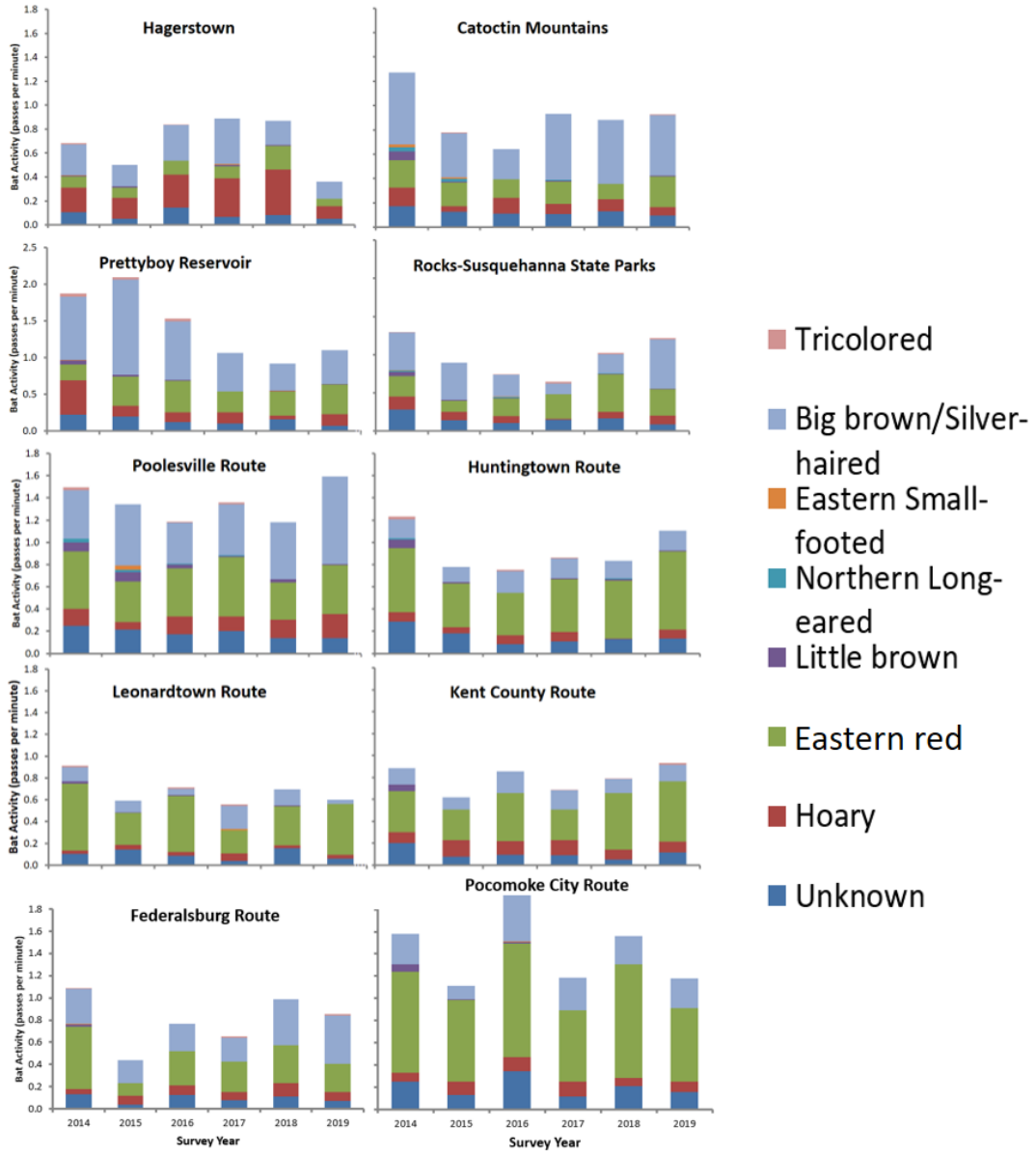

Figure S3. Bat passes per minute by year for routes 9-18, 2014 – 2019. Note the larger y-axis for Prettyboy Reservoir and Rocks-Susquehanna State Parks routes.

Chapter 5: Conclusions

Bat populations face multiple threats to their continued survival. White-nose syndrome has devastated several species of cave bats (Blehert et al. 2009), and long-distance migratory tree bats are killed in great numbers each year at industrial wind-energy sites (Arnett et al. 2008; Arnett and Baerwald 2013). Despite this, the true risk to bat populations cannot be quantified without knowledge of basic demographic information, which is lacking in many species (Frick et al. 2017). In this dissertation, I used genotyping-by-sequencing and acoustic surveys to address the following questions: What is the range-wide population structure and effective population size of hoary bats (*Lasiurus cinereus*), silver-haired bats (*Lasionycteris noctivagans*), eastern red bats (*Lasiurus borealis*), Indiana bats (*Myotis sodalis*), and gray bats (*Myotis grisescens*)? Can a current N_e/N_c ratio be calculated for the two cave bat species (Indiana and gray bats), given their relatively well-censused populations? Finally, what are the trends in abundance and community composition of bats in Maryland in the decade post-WNS?

In Chapter 2, I evaluated range-wide population structure and effective population size for three tree bat species. I used genotyping-by-sequencing (GBS) to genotype SNPs from 173 hoary, 113 eastern red, and 89 silver-haired bats from locations spread across the species' ranges. Hoary bats and eastern red bats showed no geographic structure in genetic diversity, suggesting a panmictic population structure. In contrast, silver-haired bats displayed longitudinal genetic variation. The best-fit coalescent model included long-term increasing populations on an

evolutionary timescale and suggested that eastern red bats had the largest N_e, followed by hoary bats, and then silver-haired bats.

In Chapter 3, I applied the same GBS methods to two federally-endangered cave bat species: Indiana bats and gray bats. These species have been censused at winter hibernacula sites, so census population size is better understood than for most bat species. I genotyped SNPs from 45 Indiana and 47 gray bats from across their ranges. Linkage-disequilibrium methods were unable to successfully calculate a current N_e (confidence intervals included infinity), thus I could not generate an N_e/N_c ratio. Indiana bats showed no genetic structure, whereas gray bats displayed east-west population variation, separated by the Mississippi River Valley.

In Chapter 4, I designed, conducted, and analyzed a decade of annual mobile acoustic surveys in Maryland, immediately after the first detection of white-nose syndrome in the state. From 2010 to 2019, my field crews and I completed 344 routes, resulting in 426 hours of recordings and 24,375 manually identified bat passes. To test the effect of year on species abundance, I fit a generalized linear mixed model using the tweedie distribution and a log-link function. I detected massive declines of little brown bats (*Myotis lucifugus*), northern long-eared bats (*M. septentrionalis*), eastern small-footed bats (*Myotis leibii*), and tricolored bats (*Perimyotis subflavus*), at 98.9%, 100%, 92.8%, and 94.1%, respectively. There were no trends in hoary or eastern red bat abundances, though eastern red bats comprised a larger portion of the bat community in eastern Maryland.

Overall, this dissertation informs conservation of these threatened bat species by:

1. Determining that hoary bats and eastern red bats display no range-wide geographic genetic structure (i.e., panmixia), whereas silver-haired bats have longitudinal population structure.
2. Confirming that genomics did not detect any population structure in Indiana bats, despite previously published evidence of mitochondrial clades, indicating that high nuclear gene flow exists in this species. Conversely, gray bats show east-west differentiation in genetic alleles, separated by the Mississippi River.
3. Monitoring the population changes of tree and cave bats across the state of Maryland in the decade post arrival of WNS. The decline of multiple cave bat species did not result in an observable increase of tree bats to fill the vacant niche space.

Future Research

Given the limitations of effective population size methodology, such as linkage-disequilibrium, to constrain a current N_e accurately and precisely, genomics is likely not the answer to quantifying recent population declines in bats. It is valuable in other ways, however, such as detecting subpopulations and informing the management and conservation of genetic diversity. Future genetic work on silver-haired bats could fill in the mid-continental gap in this dissertation, helping to better understand the longitudinal population structure in this species.

Another potentially informative line of genetic bat research could be a continent-wide investigation into all *Lasiurus* species. The two species researched here, hoary bats (*Lasiurus cinereus*) and eastern red bats (*Lasiurus borealis*),

contained unexplained outlier samples, as well as two potential hybrids. While these may simply be contaminated or degraded samples, this did not occur with the three non-*Lasiurus* species in this dissertation. A study incorporating all seven North American *Lasiurus* spp. may uncover patterns of hybridization or introgression useful to conservation of these species.

Perhaps the most promising method to monitor bat population changes is through standardized continent-wide acoustic surveys, such as NABat (Loeb et al. 2015). Acoustic surveys across several states currently present conflicting reports of increasing or decreasing trends in tree bat populations (Brooks 2011; Jachowski et al. 2014; Thalken et al. 2018; Nocera et al. 2019; Simonis et al. 2020). With multiple species of cave bats rapidly declining across North America, the fate of other bat species remains unclear. Given the vital importance of bats to a healthy functioning ecosystem and their role in protecting our agriculture from insect pests, we all have a stake in understanding and protecting these nocturnal winged mammals.

Bibliography

ACP. 2022. “American Clean Power.” ACP. 2022. https://cleanpower.org/facts/wind-power/.

Alexander, D. H., J. Novembre, and K. Lange. 2009. “Fast Model-Based Estimation of Ancestry in Unrelated Individuals.” *Genome Research* 19 (9): 1655–64. https://doi.org/10.1101/gr.094052.109.

Alter, S. E., E. Rynes, and S. R. Palumbi. 2007. “DNA Evidence for Historic Population Size and Past Ecosystem Impacts of Gray Whales.” *Proceedings of the National Academy of Sciences* 104 (38): 15162–67. https://doi.org/10.1073/pnas.0706056104.

Arnett, E. B., W. K. Brown, W. P. Erickson, J. K. Fiedler, B. L. Hamilton, T. H. Henry, A. Jain, G. D. Johnson, J. Kerns, R. R. Koford, C. P. Nicholson, T. J. O'Connell, M. D. Piorkowski, R. D. Tankersley Jr. 2008. “Patterns of Bat Fatalities at Wind Energy Facilities in North America.” *The Journal of Wildlife Management* 72 (1): 61–78.

Arnett, E. B., M. P. Huso, M. R. Schirmacher, and J. P. Hayes. 2011. “Altering Turbine Speed Reduces Bat Mortality at Wind-Energy Facilities.” *Frontiers in Ecology and the Environment* 9 (4): 209–14. https://doi.org/10.1890/100103.

Arnett, E. B., and E. F. Baerwald. 2013. “Impacts of Wind Energy Development on Bats: Implications for Conservation.” In *Bat Evolution, Ecology and Conservation; Adams, R.A., Pedersen, S.C., Eds.*, 435–56. Springer: New York, NY, USA.

Arnett, E. B., and R. May. 2016. “Mitigating Wind Energy Impacts on Wildlife: Approaches for Multiple Taxa.” *Human–Wildlife Interactions* 10 (1). https://doi.org/10.26077/1jeg-7r13.

Baerwald, E. F., J. Edworthy, M. Holder, and R. M. R. Barclay. 2009. “A Large-Scale Mitigation Experiment to Reduce Bat Fatalities at Wind Energy Facilities.” *The Journal of Wildlife Management* 73 (7): 1077–81. https://doi.org/10.2193/2008-233.

Barclay, R.M.R., and Harder, L.D. 2003. “Life Histories of Bats: Life in the Slow Lane.” In *Bat Ecology; Kunz, T.H., Fenton, M.B., Eds.*, 209–53. The University of Chicago Press: Chicago, IL, USA.

Beilke, E. A., and J. M. O’Keefe. 2022. “Bats Reduce Insect Density and Defoliation in Temperate Forests: An Exclusion Experiment.” *Ecology*: e3903. https://doi.org/10.1002/ecy.3903.

Bernard, R. F., J. T. Foster, E. V. Willcox, K. L. Parise, and G. F. McCracken. 2015. “Molecular Detection of the Causative Agent of White-Nose Syndrome on Rafinesque’s Big-Eared Bats (*Corynorhinus rafinesquii*) and Two Species of Migratory Bats in the Southeastern USA.” *Journal of Wildlife Diseases* 51 (2): 519–22. https://doi.org/10.7589/2014-08-202.

Blehert, D. S., A. C. Hicks, M. Behr, C. U. Meteyer, B. M. Berlowski-Zier, E. L. Buckles, J. T. Coleman JT, S. R. Darling, A. Gargas , R. Niver, J. C. Okoniewski, R. J. Rudd, W. B. Stone. 2009. “Bat White-Nose Syndrome: An

Emerging Fungal Pathogen?" *Science (New York, N.Y.)* 323 (5911): 227. https://doi.org/10.1126/science.1163874.

Bohn, S. J., J. M. Turner, L. Warnecke, C. Mayo, L. P. McGuire, V. Misra, T. K. Bollinger, and C. K. R. Willis. 2016. "Evidence of 'Sickness Behaviour' in Bats with White-Nose Syndrome." *Behaviour* 153 (8): 981–1003. https://doi.org/10.1163/1568539X-00003384.

Boyles, J. G., P. M. Cryan, G. F. McCracken, and T. H. Kunz. 2011. "Economic Importance of Bats in Agriculture." *Science* 332 (6025): 41–42. https://doi.org/10.1126/science.1201366.

Brack, V. and J. O. Whitaker, Jr. 2001. "Foods of the Northern Myotis, *Myotis septentrionalis*, from Missouri and Indiana, with Notes on Foraging." *Acta Chiropterolgica* 3 (January): 203–10.

Brady, J. T., T. H. Kunz, M. D. Tuttle, and D. E. Wilson. 1982. *Gray Bat Recovery Plan*. U.S. Fish and Wildlife Service, Denver, CO.

Britzke, E. R., S. C. Loeb, C. S. Romanek, K. A. Hobson, and M. J. Vonhof. 2013. "Variation in Catchment Areas of Indiana Bat (*Myotis sodalis*) Hibernacula Inferred from Stable Hydrogen (δ^2H) Isotope Analysis." *Can. J. Zool. 90:1243–1250* 90: 1243–50.

Brooks, M. E., K. Kristensen, K. J. van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Mächler, and B. M. Bolker. 2017. "GlmmTMB Balances Speed and Flexibility Among Packages for Zero-Inflated Generalized Linear Mixed Modeling." *The R Journal* 9 (2): 378–400.

Brooks, R. T. 2011. "Declines in Summer Bat Activity in Central New England 4 Years Following the Initial Detection of White-Nose Syndrome." *Biodiversity and Conservation* 20 (11): 2537–41. https://doi.org/10.1007/s10531-011-9996-0.

Burland, T. M., E. M. Barratt, M. A. Beaumont, and P. A. Racey. 1999. "Population Genetic Structure and Gene Flow in a Gleaning Bat, *Plecotus auritus*." *Proceedings of the Royal Society of London. Series B: Biological Sciences* 266 (1422): 975–80. https://doi.org/10.1098/rspb.1999.0732.

Campbell, C. J., D. M. Nelson, J. E. Gates, H. L. Gibbs, E. R. Stevenson, B. Johnson, J. Nagel, R. Trott, J. G. Wieringa, and H. B. Vander Zanden. 2022. "White-Nose Syndrome Pathogen *Pseudogymnoascus destructans* Detected in Migratory Tree-Roosting Bats." *Journal of Wildlife Diseases* 58 (3): 652–57. https://doi.org/10.7589/JWD-D-21-00160.

"CanWEA." 2022. 2022. https://canwea.ca/wind-energy/installed-capacity/.

Carroll, J. B., and G. M. Mace. 1988. "Population Management of the Rodrigues Fruit Bat." *International Zoo Yearbook* 27 (1): 70–78. https://doi.org/10.1111/j.1748-1090.1988.tb03198.x.

Carter, T. C., M. A. Menzel, S. F. Owen, J. W. Edwards, J. M. Menzel, and W. M. Ford. 2003. "Food Habits of Seven Species of Bats in The Allegheny Plateau and Ridge and Valley of West Virginia." *Northeastern Naturalist* 10 (1): 83–88. https://doi.org/10.1656/1092-6194(2003)010[0083:FHOSSO]2.0.CO;2.

Castella, V., M. Ruedi, and L. Excoffier. 2001. "Contrasted Patterns of Mitochondrial and Nuclear Structure among Nursery Colonies of the Bat *Myotis myotis*." *Journal of Evolutionary Biology* 14 (5): 708–20.

Catchen, J. M., A. Amores, P. Hohenlohe, W. Cresko, and J. H. Postlethwait. 2011. "Stacks: Building and Genotyping Loci de Novo from Short-Read Sequences." *G3 (Bethesda, Md.)* 1 (3): 171–82. https://doi.org/10.1534/g3.111.000240.

Catchen, J., P. A. Hohenlohe, S. Bassham, A. Amores, and W. A. Cresko. 2013. "Stacks: An Analysis Tool Set for Population Genomics." *Molecular Ecology* 22 (11): 3124–40. https://doi.org/10.1111/mec.12354.

Cheng, T. L., J. D. Reichard, J. T. H. Coleman, T. J. Weller, W. E. Thogmartin, B. E. Reichert, A. B. Bennett, H. G. Broders, J. Campbell, K. Etchison, D. J. Feller, R. Geboy, T. Hemberger, C. Herzog, A. C. Hicks, S. Houghton, J. Humber, J. A. Kath, R. A. King, S. C. Loeb, A. Masse, K. M. Morris, H. Niederriter, G. Nordquist, R. W. Perry, R. J. Reynolds, D. B. Sasse, M. R. Scafini, R. C. Stark, C. W. Stihler, S. C. Thomas, G. C. Turner, S. Webb, B. J. Westrich, W. F. Frick. 2021. "The Scope and Severity of White-Nose Syndrome on Hibernating Bats in North America." *Conservation Biology* 35 (5): 1586–97. https://doi.org/10.1111/cobi.13739.

Clare, E. L., E. E. Fraser, H. E. Braid, M. B. Fenton, and P. D. N. Hebert. 2009. "Species on the Menu of a Generalist Predator, the Eastern Red Bat (*Lasiurus borealis)*: Using a Molecular Approach to Detect Arthropod Prey." *Molecular Ecology* 18 (11): 2532–42. https://doi.org/10.1111/j.1365-294X.2009.04184.x.

Cornman, R. S., J. A. Fike, S. J. Oyler-McCance, and P. M. Cryan. 2021. "Historical Effective Population Size of North American Hoary Bat (*Lasiurus cinereus*) and Challenges to Estimating Trends in Contemporary Effective Breeding Population Size from Archived Samples." *PeerJ* 9 (April): e11285. https://doi.org/10.7717/peerj.11285.

Crandall, K. A., O. R. P. Bininda-Emonds, G. M. Mace, and R. K. Wayne. 2000. "Considering Evolutionary Processes in Conservation Biology." *Trends in Ecology & Evolution* 15 (7): 290–95. https://doi.org/10.1016/S0169-5347(00)01876-0.

Cryan, P. M., C. A. Stricker, and M. B. Wunder. 2014. "Continental-Scale, Seasonal Movements of a Heterothermic Migratory Tree Bat." *Ecological Applications* 24 (4): 602–16. https://doi.org/10.1890/13-0752.1.

Dixon, M. D. 2011. "Population Genetic Structure and Natal Philopatry in the Widespread North American Bat *Myotis lucifugus*." *Journal of Mammalogy* 92 (6): 1343–51. https://doi.org/10.1644/10-MAMM-A-426.1.

Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillett, and J. R. Ovenden. 2014. "NeEstimator v2: Re-Implementation of Software for the Estimation of Contemporary Effective Population Size (Ne) from Genetic Data." *Molecular Ecology Resources* 14 (1): 209–14. https://doi.org/10.1111/1755-0998.12157.

Dodd, L. E., E. G. Chapman, J. D. Harwood, M. J. Lacki, and L. K. Rieske. 2012. "Identification of Prey of *Myotis septentrionalis* Using DNA-Based Techniques." *Journal of Mammalogy* 93 (4): 1119–28. https://doi.org/10.1644/11-MAMM-A-218.1.

Dzal, Y., L. P. McGuire, N. Veselka, and M. B. Fenton. 2011. "Going, Going, Gone: The Impact of White-Nose Syndrome on the Summer Activity of the Little

Brown Bat (*Myotis lucifugus*)." *Biology Letters* 7 (3): 392–94. https://doi.org/10.1098/rsbl.2010.0859.
Ellegren, H., and N. Galtier. 2016. "Determinants of Genetic Diversity." *Nature Reviews Genetics* 17 (7): 422–33. https://doi.org/10.1038/nrg.2016.58.
Ellis, N., S. Smith, and C. Pitcher. 2012. "Gradient Forests: Calculating Importance Gradients on Physical Predictors. Ecology. 93: 156--168." *Ecology* 93 (January): 156–68. https://doi.org/10.2307/23144030.
Elshire R. J., J. C. Glaubitz, Q. Sun, J. A. Poland, K. Kawamoto, E. S. Buckler, and S. E. Mitchell. 2011. "A Robust, Simple Genotyping-by-Sequencing (GBS) Approach for High Diversity Species." *PloS One* 6 (5): e19379. https://doi.org/10.1371/journal.pone.0019379.
Excoffier, L., and M. Foll. 2011. "Fastsimcoal: A Continuous-Time Coalescent Simulator of Genomic Diversity under Arbitrarily Complex Evolutionary Scenarios." *Bioinformatics (Oxford, England)* 27 (9): 1332–34. https://doi.org/10.1093/bioinformatics/btr124.
Faure, P. A., J. H. Fullard, and J. W. Dawson. 1993. "The Gleaning Attacks of the Northern Long-Eared Bat, *Myotis septentrionalis*, Are Relatively Inaudible to Moths." *Journal of Experimental Biology* 178 (1): 173–89.
Feldhamer, G. A., T. C. Carter, and J. O. Whitaker. 2009. "Prey Consumed by Eight Species of Insectivorous Bats from Southern Illinois." *The American Midland Naturalist* 162 (1): 43–51. https://doi.org/10.1674/0003-0031-162.1.43.
Fenton, M. B. 2001. *Bats, Revised Edition*. Ontario, Canada: Fitzhenry and Whiteside.
Fick, S. E., and R. J. Hijmans. 2017. "WorldClim 2: New 1-Km Spatial Resolution Climate Surfaces for Global Land Areas." *International Journal of Climatology* 37 (12): 4302–15. https://doi.org/10.1002/joc.5086.
Fisher-Phelps, M., D. Schwilk, and T. Kingston. 2017. "Mobile Acoustic Transects Detect More Bat Activity than Stationary Acoustic Point Counts in a Semi-Arid and Agricultural Landscape." *Journal of Arid Environments* 136 (January): 38–44. https://doi.org/10.1016/j.jaridenv.2016.10.005.
Francl, K. E., W. M. Ford, D. W. Sparks, and V. Brack Jr. 2012. "Capture and Reproductive Trends in Summer Bat Communities in West Virginia: Assessing the Impact of White-Nose Syndrome." *Journal of Fish and Wildlife Management* 3 (1): 33–42. https://doi.org/10.3996/062011-JFWM-039.
Frank, C. L., A. Michalski, A. A. McDonough, M. Rahimian, R. J. Rudd, and C. Herzog. 2014. "The Resistance of a North American Bat Species (*Eptesicus fuscus*) to White-Nose Syndrome (WNS)." *PLOS ONE* 9 (12): e113958. https://doi.org/10.1371/journal.pone.0113958.
Frankham, R. 1995. "Effective Population Size/Adult Population Size Ratios in Wildlife: A Review." *Genetics Research* 66 (2): 95–107.
Frankham, R. 2005. "Genetics and Extinction." *Biological Conservation* 126 (2): 131–40. https://doi.org/10.1016/j.biocon.2005.05.002.
Fraser, D. J., and L. Bernatchez. 2001. "Adaptive Evolutionary Conservation: Towards a Unified Concept for Defining Conservation Units." *Molecular Ecology* 10 (12): 2741–52. https://doi.org/10.1046/j.0962-1083.2001.01411.x.

Fraser, E., D. Brooks, and F. Longstaffe. 2017. “Stable Isotope Investigation of the Migratory Behavior of Silver-Haired Bats (*Lasionycteris noctivagans*) in Eastern North America.” *Journal of Mammalogy* 98 (October): 1225–35. https://doi.org/10.1093/jmammal/gyx085.

Frick, W. F., E. F. Baerwald, J. F. Pollock, R. M. R. Barclay, J. A. Szymanski, T. J. Weller, A. L. Russell, S. C. Loeb, R. A. Medellin, and L. P. McGuire. 2017. “Fatalities at Wind Turbines May Threaten Population Viability of a Migratory Bat.” *Biological Conservation* 209 (May): 172–77. https://doi.org/10.1016/j.biocon.2017.02.023.

Frick, W. F., T. Kingston, and J. Flanders. 2020. “A Review of the Major Threats and Challenges to Global Bat Conservation.” *Annals of the New York Academy of Sciences* 1469 (1): 5–25. https://doi.org/10.1111/nyas.14045.

Frick, W. F., S. J. Puechmaille, J. R. Hoyt, B. A. Nickel, K. E. Langwig, J. T. Foster, K. E. Barlow, T. Bartonicˇka, D. Feller, A. Haarsma, C. Herzog, I. Horácˇek, J. van der Kooij, B. Mulkens, B. Petrov, R. Reynolds, L. Rodrigues, C. W. Stihler, G. G.Turner, and A. M. Kilpatrick. 2015. “Disease Alters Macroecological Patterns of North American Bats.” *Global Ecology and Biogeography* 24 (7): 741–49. https://doi.org/10.1111/geb.12290.

Friedenberg, N. A., and W. F. Frick. 2021. “Assessing Fatality Minimization for Hoary Bats amid Continued Wind Energy Development.” *Biological Conservation* 262 (October): 109309. https://doi.org/10.1016/j.biocon.2021.109309.

Gardner, J. E., and E. A. Cook. 2002. “Seasonal and Geographic Distribution and Quantification of Potential Summer Habitat.” In *The Indiana Bat: Biology and Management of an Endangered Species*. Austin, TX: Bat Conservation International.

Garg K. M., and Ramakrishnan U. 2017. “Variance in Female Reproductive Success Differentially Impacts Effective Population Size in the Short-Nosed Fruit Bat, *Cynopterus sphinx*.” *Evolutionary Biology* 44 (3): 366–73. https://doi.org/10.1007/s11692-017-9414-y.

Gates, J. E., G. A. Feldhamer, L. A. Griffith, and R. L. Raesly. 1984. “Status of Cave-Dwelling Bats in Maryland: Importance of Marginal Habitats.” *Wildlife Society Bulletin (1973-2006)* 12 (2): 162–69.

- Gayral, P., J. Melo-Ferreira, S. Glémin, N. Bierne, M. Carneiro, B. Nabholz, J. M. Lourenco, P. C. Alves, M. Ballenghien, N. Faivre, K. Belkhir, V. Cahais, E. Loire, A. Bernard, and N. Galtier. 2013. “Reference-Free Population Genomics from Next-Generation Transcriptome Data and the Vertebrate–Invertebrate Gap.” *PLOS Genetics* 9 (4): e1003457. https://doi.org/10.1371/journal.pgen.1003457.

Gignoux-Wolfsohn, S. A., M. L. Pinsky, K. Kerwin, C. Herzog, M. Hall, A. B. Bennett, N. H. Fefferman, and B. Maslo. 2021. “Genomic Signatures of Selection in Bats Surviving White-Nose Syndrome.” *Molecular Ecology* 30 (22): 5643–57. https://doi.org/10.1111/mec.15813.

Glück, M., J. C. Geue, and H. A. Thomassen. 2022. “Environmental Differences Explain Subtle yet Detectable Genetic Structure in a Widespread Pollinator.”

BMC Ecology and Evolution 22 (1): 8. https://doi.org/10.1186/s12862-022-01963-5.

Goulson, D. 2019. "The Insect Apocalypse, and Why It Matters." *Current Biology* 29 (19): R967–71. https://doi.org/10.1016/j.cub.2019.06.069.

Gugger, P. F., S. T. Fitz-Gibbon, A. Albarrán-Lara, J. W. Wright, and V. L. Sork. 2021. "Landscape Genomics of *Quercus lobata* Reveals Genes Involved in Local Climate Adaptation at Multiple Spatial Scales." *Molecular Ecology* 30 (2): 406–23. https://doi.org/10.1111/mec.15731.

Gugger, P. F., C. T. Liang, V. L. Sork, P. Hodgskiss, and J. W. Wright. 2018. "Applying Landscape Genomic Tools to Forest Management and Restoration of Hawaiian Koa (*Acacia koa*) in a Changing Environment." *Evolutionary Applications* 11 (2): 231–42. https://doi.org/10.1111/eva.12534.

Hale, A. M., C. D. Hein, and B. R. Straw. 2022. "Acoustic and Genetic Data Can Reduce Uncertainty Regarding Populations of Migratory Tree-Roosting Bats Impacted by Wind Energy." *Animals* 12 (1): 81. https://doi.org/10.3390/ani12010081.

Hayward, B., and R. Davis. 1964. "Flight Speeds in Western Bats." *Journal of Mammalogy* 45 (2): 236–42.

Hein, C. D., S. B. Castleberry, and K. V. Miller. 2009. "Site-Occupancy of Bats in Relation to Forested Corridors." *Forest Ecology and Management* 257 (4): 1200–1207.

Hoen, B., J. E. Diffendorfer, J. Rand, L. A. Kramer, C. P. Garrity, A. D. Roper, and H. Hunt. 2018. "United States Wind Turbine Database (Ver. 5.2, August 2022)." U.S. Geological Survey. https://doi.org/10.5066/F7TX3DN0.

Humphrey, S R. 1978. "Status, Winter Habitat, and Management of the Endangered Indiana Bat, *Myotis sodalis*." *Florida Scientist* 41 (2): 65–76.

Jachowski, D. S., C. A. Dobony, L. S. Coleman, W. M. Ford, E. R. Britzke, and J. L. Rodrigue. 2014. "Disease and Community Structure: White-Nose Syndrome Alters Spatial and Temporal Niche Partitioning in Sympatric Bat Species." *Diversity and Distributions* 20 (9): 1002–15. https://doi.org/10.1111/ddi.12192.

Jackson, R. T., E. V. Willcox, and R. F. Bernard. 2022. "Winter Torpor Expression Varies in Four Bat Species with Differential Susceptibility to White-Nose Syndrome." *Scientific Reports* 12 (1): 5688. https://doi.org/10.1038/s41598-022-09692-x.

Jombart, T. 2008. "Adegenet: A R Package for the Multivariate Analysis of Genetic Markers." *Bioinformatics* 24 (11): 1403–5. https://doi.org/10.1093/bioinformatics/btn129.

Jones, G., D. S. Jacobs, T. H. Kunz, M. R. Willig, and P. A. Racey. 2009. "Carpe Noctem: The Importance of Bats as Bioindicators." *Endangered Species Research* 8 (1–2): 93–115. https://doi.org/10.3354/esr00182.

Kerth, G., F. Mayer, and E. Petit. 2002. "Extreme Sex-Biased Dispersal in the Communally Breeding, Nonmigratory Bechstein's Bat (*Myotis bechsteinii*)." *Molecular Ecology* 11 (8): 1491–98. https://doi.org/10.1046/j.1365-294X.2002.01528.x.

Kerth, G., F. Mayer, and B. König. 2000. “Mitochondrial DNA (MtDNA) Reveals That Female Bechstein’s Bats Live in Closed Societies.” *Molecular Ecology* 9 (6): 793–800. https://doi.org/10.1046/j.1365-294x.2000.00934.x.

Korstian, J. M., A. M. Hale, and D. A. Williams. 2015. “Genetic Diversity, Historic Population Size, and Population Structure in 2 North American Tree Bats.” *Journal of Mammalogy* 96 (5): 972–80. https://doi.org/10.1093/jmammal/gyv101.

Kunz, T. H., E. Braun de Torrez, D. Bauer, T. Lobova, and T. H. Fleming. 2011. “Ecosystem Services Provided by Bats.” *Annals of the New York Academy of Sciences* 1223 (1): 1–38. https://doi.org/10.1111/j.1749-6632.2011.06004.x.

Kurta, A. 1995. *Mammals of the Great Lakes Region*. Ann Arbor, Michigan: University of Michigan Press.

Kurta, A., D. King, J. A. Teramino, J. M. Stribley, and K. J. Williams. 1993. “Summer Roosts of the Endangered Indiana Bat (*Myotis sodalis*) on the Northern Edge of Its Range.” *The American Midland Naturalist* 129 (1): 132–38. https://doi.org/10.2307/2426441.

Kurta, A., and S. W. Murray. 2002. “Philopatry and Migration of Banded Indiana Bats (*Myotis sodalis*) and Effects of Radio Transmitters.” *Journal of Mammalogy* 83 (2): 585–89. https://doi.org/10.1644/1545-1542(2002)083<0585:PAMOBI>2.0.CO;2.

Lee, D. N., R. C. Stark, W. L. Puckette, M. J. Hamilton, Jr. L. David M., and R. A. Van Den Bussche. 2015. “Population Connectivity of Endangered Ozark Big-Eared Bats (*Corynorhinus townsendii ingens*).” *Journal of Mammalogy* 96 (3): 522–30. https://doi.org/10.1093/jmammal/gyv057.

Lemen, C., P. W. Freeman, J. A. White, and B. R. Andersen. 2015. “The Problem of Low Agreement among Automated Identification Programs for Acoustical Surveys of Bats.” *Western North American Naturalist* 75 (2): 218–25. https://doi.org/10.3398/064.075.0210.

Lindsay, D. L., X. Guan, H. L. Farrington, M. D. Blake, N. D. Barker, M. R. Carr, E. Navarro P., and R. F. Lance. 2015. “Genetic Structure Among Hibernacula of the Endangered Gray Bat (*Myotis grisescens*).” *Acta Chiropterologica* 17 (2): 293–306. https://doi.org/10.3161/15081109ACC2015.17.2.005.

Liu, N., L. Chen, S. Wang, C. Oh, and H. Zhao. 2005. “Comparison of Single-Nucleotide Polymorphisms and Microsatellites in Inference of Population Structure.” *BMC Genetics* 6 (1): S26. https://doi.org/10.1186/1471-2156-6-S1-S26.

Loeb, S. C., T. J. Rodhouse, L. E. Ellison, C. L. Lausen, J. D. Reichard, K. M. Irvine, T. E. Ingersoll, J. T. H. Coleman, W. E. Thogmartin, J. R. Sauer, C. M. Francis, M. L. Bayless, T. R. Stanley, and D. H. Johnson. 2015. “A Plan for the North American Bat Monitoring Program (NABat).” *Gen. Tech. Rep. SRS-208. Asheville, NC: U.S. Department of Agriculture Forest Service, Southern Research Station.* 208: 1–100. https://doi.org/10.2737/SRS-GTR-208.

Marandel, F., G. Charrier, J. Lamy, S. Le Cam, P. Lorance, and V. M. Trenkel. 2020. “Estimating Effective Population Size Using RADseq: Effects of SNP Selection and Sample Size.” *Ecology and Evolution* 10 (4): 1929–37. https://doi.org/10.1002/ece3.6016.

Martin, C. O. 2007. “Assessment of the Population Status of the Gray Bat (*Myotis grisescens*); Status Review, DoD Initiatives, and Results of a Multi-Agency Effort to Survey Wintering Populations at Major Hibernacula, 2005-2007.” ENGINEER RESEARCH AND DEVELOPMENT CENTER VICKSBURG MS ENVIRONMENTAL LAB.

Maslo, B., M. Valent, J. F. Gumbs, and W. F. Frick. 2015. “Conservation Implications of Ameliorating Survival of Little Brown Bats with White-Nose Syndrome.” *Ecological Applications* 25 (7): 1832–40. https://doi.org/10.1890/14-2472.1.

Mastretta-Yanes, A., N. Arrigo, N. Alvarez, T. H. Jorgensen, D. Piñero, and B. C. Emerson. 2015. “Restriction Site-Associated DNA Sequencing, Genotyping Error Estimation and de Novo Assembly Optimization for Population Genetic Inference.” *Molecular Ecology Resources* 15 (1): 28–41. https://doi.org/10.1111/1755-0998.12291.

Meteyer, C., Uphoff, E. L. Buckles, D. S. Blehert, A. C. Hicks, D. E. Green, V. Shearn-Bochsler, N. J. Thomas, A. Gargas, and M. J. Behr. 2009. “Histopathologic Criteria to Confirm White-Nose Syndrome in Bats.” *Journal of Veterinary Diagnostic Investigation* 21 (4): 411–14. https://doi.org/10.1177/104063870902100401.

Miller-Butterworth, C. M., M. J. Vonhof, J. Rosenstern, G. G. Turner, and A. L. Russell. 2014. “Genetic Structure of Little Brown Bats (*Myotis lucifugus*) Corresponds with Spread of White-Nose Syndrome among Hibernacula.” *Journal of Heredity* 105 (3): 354–64. https://doi.org/10.1093/jhered/esu012.

Monopoli, M., J. G. Wieringa, J. Nagel, D. M. Nelson, and H. L. Gibbs. 2020. “Sequencing Whole Mitochondrial Genomes to Assess Genetic Divergence between Proposed Silver-Haired Bat (*Lasionycteris noctivagans*) Populations.” *Mitochondrial DNA Part B* 5 (4): 3838–39. https://doi.org/10.1080/23802359.2020.1841577.

Morningstar, D. E., C. V. Robinson, S. Shokralla, and M. Hajibabaei. 2019. “Interspecific Competition in Bats and Diet Shifts in Response to White-Nose Syndrome.” *Ecosphere* 10 (11): e02916. https://doi.org/10.1002/ecs2.2916.

Neece, B. D., S. C. Loeb, and D. S. Jachowski. 2019. “Implementing and Assessing the Efficacy of the North American Bat Monitoring Program.” *Journal of Fish and Wildlife Management* 10 (2): 391–409. https://doi.org/10.3996/092018-JFWM-087.

Nocera, T., W. M. Ford, A. Silvis, and C. A. Dobony. 2019. “Patterns of Acoustical Activity of Bats Prior to and 10 Years after WNS on Fort Drum Army Installation, New York.” *Global Ecology and Conservation* 18 (April): e00633. https://doi.org/10.1016/j.gecco.2019.e00633.

Nunney, L. 1995. “Measuring the Ratio of Effective Population Size to Adult Numbers Using Genetic and Ecological Data.” *Evolution* 49 (2): 389–92. https://doi.org/10.1111/j.1558-5646.1995.tb02253.x.

O’Keefe, J. M., J. L. Pettit, S. C. Loeb, and W. H. Stiver. 2019. “White-Nose Syndrome Dramatically Altered the Summer Bat Assemblage in a Temperate Southern Appalachian Forest.” *Mammalian Biology* 98 (1): 146–53. https://doi.org/10.1016/j.mambio.2019.09.005.

O'Shea, T. J., and M. A. Bogan. 2003. "Monitoring Trends in Bat Populations of the United States and Territories: Problems and Prospects." U. S. Geological Survey, Biological Resources Division, Information and Technology Report: Washington, DC, USA.

O'Shea, T. J., P. M. Cryan, D. T.S. Hayman, R. K. Plowright, and D. G. Streicker. 2016. "Multiple Mortality Events in Bats: A Global Review." *Mammal Review* 46 (3): 175–90. https://doi.org/10.1111/mam.12064.

Overcast, I.. (2016) 2021. *EasySFS*. Python. https://github.com/isaacovercast/easySFS.

Oyler-McCance, S. J., J. A. Fike, P. M. Lukacs, D. W. Sparks, T. J. O'Shea, and J. O. Whitaker Jr. 2017. "Genetic Mark–Recapture Improves Estimates of Maternity Colony Size for Indiana Bats." *Journal of Fish and Wildlife Management* 9 (1): 25–35. https://doi.org/10.3996/122016-JFWM-093.

Palstra, F. P., and D. J. Fraser. 2012. "Effective/Census Population Size Ratio Estimation: A Compendium and Appraisal." *Ecology and Evolution* 2 (9): 2357–65. https://doi.org/10.1002/ece3.329.

Pannell, J. R., and B. Charlesworth. 2000. "Effects of Metapopulation Processes on Measures of Genetic Diversity." *Philosophical Transactions of the Royal Society B: Biological Sciences* 355 (1404): 1851–64.

Pettit, J. L., and J. M. O'Keefe. 2017. "Impacts of White-Nose Syndrome Observed During Long-Term Monitoring of a Midwestern Bat Community." *Journal of Fish and Wildlife Management* 8 (1): 69–78. https://doi.org/10.3996/102016-JFWM-077.

Pinsky, M. L., S. D. Newsome, B. R. Dickerson, Y. Fang, M. Van Tuinen, D. J. Kennett, R. R. Ream, and E. A. Hadly. 2010. "Dispersal Provided Resilience to Range Collapse in a Marine Mammal: Insights from the Past to Inform Conservation Biology." *Molecular Ecology* 19 (12): 2418–29. https://doi.org/10.1111/j.1365-294X.2010.04671.x.

Podlutsky, A. J., A. M. Khritankov, N. D. Ovodov, and S. N. Austad. 2005. "A New Field Record for Bat Longevity." *The Journals of Gerontology: Series A* 60 (11): 1366–68. https://doi.org/10.1093/gerona/60.11.1366.

Powers, K. E., R. J. Reynolds, W. Orndorff, W. M. Ford, and C. S. Hobson. 2015. "Post-White-Nose Syndrome Trends in Virginia's Cave Bats, 2008-2013." *KIP Articles*, January. https://digitalcommons.usf.edu/kip_articles/4255.

Powers, K. E., R. J. Reynolds, W. Orndorff, B. A. Hyzy, C. S. Hobson, and W. M. Ford. 2016. "Monitoring the Status of Gray Bats (*Myotis grisescens*) in Virginia, 2009–2014, and Potential Impacts of White-Nose Syndrome." *Southeastern Naturalist* 15 (1): 127–37. https://doi.org/10.1656/058.015.0114.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. "Inference of Population Structure Using Multilocus Genotype Data." *Genetics* 155 (2): 945.

Pylant, C. L., D. M. Nelson, M. C. Fitzpatrick, J. E. Gates, and S. R. Keller. 2016. "Geographic Origins and Population Genetics of Bats Killed at Wind-Energy Facilities." *Ecological Applications* 26 (5): 1381–95. https://doi.org/10.1890/15-0541.

Pylant, C. L., D. M. Nelson, and S. R. Keller. 2014. “Stable Hydrogen Isotopes Record the Summering Grounds of Eastern Red Bats (*Lasiurus borealis*).” *PeerJ* 2 (October): e629. https://doi.org/10.7717/peerj.629.

Rebelo, H., P. Tarroso, and G. Jones. 2010. “Predicted Impact of Climate Change on European Bats in Relation to Their Biogeographic Patterns.” *Global Change Biology* 16 (2): 561–76. https://doi.org/10.1111/j.1365-2486.2009.02021.x.

Roche, N., S. Langton, T. Aughney, J. M. Russ, F. Marnell, D. Lynn, and C. Catto. 2011. “A Car-Based Monitoring Method Reveals New Information on Bat Populations and Distributions in Ireland.” *Animal Conservation* 14 (6): 642–51. https://doi.org/10.1111/j.1469-1795.2011.00470.x.

Russo, D., and C. C. Voigt. 2016. “The Use of Automated Identification of Bat Echolocation Calls in Acoustic Monitoring: A Cautionary Note for a Sound Analysis.” *Ecological Indicators* 66 (July): 598–602. https://doi.org/10.1016/j.ecolind.2016.02.036.

Salcedo, H. D. L. C., M. B. 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).” *Journal of Experimental Biology* 198 (11): 2245–51.

Shafer, A. B. A., C. R. Peart, S. Tusso, I. Maayan, A. Brelsford, C. W. Wheat, and J. B. W. Wolf. 2017. “Bioinformatic Processing of RAD-Seq Data Dramatically Impacts Downstream Population Genetic Inference.” *Methods in Ecology and Evolution* 8 (8): 907–17. https://doi.org/10.1111/2041-210X.12700.

Shono, H.. 2008. “Application of the Tweedie Distribution to Zero-Catch Data in CPUE Analysis.” *Fisheries Research* 93 (1): 154–62. https://doi.org/10.1016/j.fishres.2008.03.006.

Shrimpton, J. M., and D. D. Heath. 2003. “Census vs. Effective Population Size in Chinook Salmon: Large- and Small-Scale Environmental Perturbation Effects.” *Molecular Ecology* 12 (10): 2571–83. https://doi.org/10.1046/j.1365-294X.2003.01932.x.

Sieradzki, A., and H. Mikkola. 2022. “Bats in Folklore and Culture: A Review of Historical Perceptions around the World.” In *Bats-Disease-Prone but Beneficial*, 3–20.

Simonis, M. C., B. K. G. Brown, and V. Bahn. 2020. “Mobile Bat Acoustic Routes Indicate Cavity-Roosting Species Undergo Compensatory Changes in Community Composition Following White-Nose Syndrome.” *Acta Chiropterologica* 22 (2): 315–26. https://doi.org/10.3161/15081109ACC2020.22.2.007.

Skowronski, M., and B. Fenton. 2008. *Model-Based Automated Detection of Echolocation Calls Using the Link Detector*. Vol. 124. https://doi.org/10.1121/1.2924122.

Sovic, M. G., B. C. Carstens, and H. L. Gibbs. 2016. “Genetic Diversity in Migratory Bats: Results from RADseq Data for Three Tree Bat Species at an Ohio Windfarm.” *Peerj* 4: e1647. https://doi.org/10.7717/peerj.1647.

Tallmon, D. A., R. S. Waples, D. Gregovich, and M. K. Schwartz. 2012. “Detecting Population Recovery Using Gametic Disequilibrium-Based Effective

Population Size Estimates." *Conservation Genetics Resources* 4 (4): 987–89. https://doi.org/10.1007/s12686-012-9689-3.
Thalken, M. M., M. J. Lacki, and J. S. Johnson. 2018. "Shifts in Assemblage of Foraging Bats at Mammoth Cave National Park Following Arrival of White-Nose Syndrome." *Northeastern Naturalist* 25 (2): 202–14. https://doi.org/10.1656/045.025.0203.
Tuttle, M. D. 1976. "Population Ecology of the Gray Bat (*Myotis grisescens*): Philopatry, Timing, and Patterns of Movment, Weight Loss during Migration, and Seasonal Adaptive Strategies." *Occasional Papers of the Museum of Natural History, The University of Kansas, Lawrence*, 54: 1-38.
Tuttle, M. D. 2003. "Estimating Population Sizes of Hibernating Bats in Caves and Mines." In *Monitoring Trends in Bat Populations of the United States and Territories: Problems and Prospects (T. J. O'Shea and M. A Bogan, Eds.)*. U.S. Geological Survey, Biological Resources Discipline, Information and Technology Report, USGS/BRD/ITR 2003-0003.
USFWS. 2007. *Indiana Bat (Myotis sodalis) Draft Recovery Plan: First Revision.* U.S. Fish and Wildlife Service, Fort Snelling, MN.
USFWS. 2018. *2017 Indiana Bat (Myotis sodalis) Population Status Update*. Retrieved from: https://www.fws.gov/midwest/endangered/mammals/inba/pdf/2017_Population_Stats_Indiana_Bat_Revised_%2013Nov2018.pdf.
USFWS. 2019a. "Indiana Bat (*Myotis sodalis*) 5 Year Review: Summary and Evaluation." U.S. Fish and Wildlife Service, Bloomington, Indiana. https://ecos.fws.gov/docs/tess/species_nonpublish/2918.pdf.
USFWS. 2019b. https://www.fws.gov/sites/default/files/documents/2019_IBat_Pop_Estimate_6_27_2019a.pdf.
Vonhof, M. J., S. K. Amelon, R. R. Currie, and G. F. McCracken. 2016. "Genetic Structure of Winter Populations of the Endangered Indiana Bat (*Myotis sodalis*) Prior to the White Nose Syndrome Epidemic: Implications for the Risk of Disease Spread." *Conservation Genetics* 17 (5): 1025–40. https://doi.org/10.1007/s10592-016-0841-6.
Vonhof, M. J., C. Strobeck, and M. B. Fenton. 2008. "Genetic Variation and Population Structure in Big Brown Bats (*Eptesicus fuscus*): Is Female Dispersal Important?" *Journal of Mammalogy* 89 (6): 1411–20. https://doi.org/10.1644/08-MAMM-S-062.1.
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 (7): e0128713. https://doi.org/10.1371/journal.pone.0128713.
Wang, J., E. Santiago, and A. Caballero. 2016. "Prediction and Estimation of Effective Population Size." *Heredity* 117 (4): 193–206. https://doi.org/10.1038/hdy.2016.43.
Waples, R. K., W. A. Larson, and R. S. Waples. 2016. "Estimating Contemporary Effective Population Size in Non-Model Species Using Linkage

Disequilibrium across Thousands of Loci." *Heredity* 117 (4): 233–40. https://doi.org/10.1038/hdy.2016.60.
Waples, R. S., and C. Do. 2010. "Linkage Disequilibrium Estimates of Contemporary Ne Using Highly Variable Genetic Markers: A Largely Untapped Resource for Applied Conservation and Evolution." *Evolutionary Applications* 3 (3): 244–62. https://doi.org/10.1111/j.1752-4571.2009.00104.x.
Warnecke, L., J. M. Turner, T. K. Bollinger, V. Misra, P. M. Cryan, D. S. Blehert, G. Wibbelt, and C. K. R. Willis. 2013. "Pathophysiology of White-Nose Syndrome in Bats: A Mechanistic Model Linking Wing Damage to Mortality." *Biology Letters* 9 (4): 20130177. https://doi.org/10.1098/rsbl.2013.0177.
Weaver, S. P., C. D. Hein, T. R. Simpson, J. W. Evans, and I. Castro-Arellano. 2020. "Ultrasonic Acoustic Deterrents Significantly Reduce Bat Fatalities at Wind Turbines." *Global Ecology and Conservation* 24 (December): e01099. https://doi.org/10.1016/j.gecco.2020.e01099.
Whitby, M. D., T. C. Carter, E. R. Britzke, and S. M. Bergeson. 2014. "Evaluation of Mobile Acoustic Techniques for Bat Population Monitoring." *Acta Chiropterologica* 16 (1): 223–30. https://doi.org/10.3161/150811014X683417.
Wright, S. 1931. "Evolution in Mendelian Populations." *Genetics* 16 (2): 97–159.
Zimmerling, J. R., and C. M. Francis. 2016. "Bat Mortality Due to Wind Turbines in Canada." *The Journal of Wildlife Management* 80 (8): 1360–69.