LONG-TERM MONITORING OF BAT POPULATIONS IN NORTH DAKOTA

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

By

Derek Tyler Krueger

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

Major Department:
Biological Sciences

April 2022

Fargo, North Dakota

North Dakota State University
Graduate School

Title

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

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Erin Gillam
Chair

Dr. Craig Stockwell

Dr. Torre Hovick

Approved:

4/18/2022
Date

Dr. Kendra Greenlee
Department Chair

ABSTRACT

Throughout the past few decades, North American bat species have experienced population declines due to White-Nose Syndrome, wind energy, climate change, and other factors. In North Dakota, the presence of wind energy, and the recent arrival of White-Nose Syndrome in 2019, pose serious threats to bat populations in the state. The objective of this study was to gather and analyze long term population data on the different bat species in North Dakota. In Summers of 2019-2021, we recorded bat echolocation call sequences at 60 grid cells established across North Dakota. We compared data across years to determine if any species showed changes in activity level. Occupancy modeling was also used to determine any link between occupancy/detectability and some environmental features for four bat species in 2020. Our results suggest a possible decline in regional populations of species in the *Myotis* genus. We found no link between occupancy and environmental factors.

ACKNOWLEDGEMENTS

I would like to thank my advisor Erin Gillam for the opportunities provided to me as an undergraduate, as well as the guidance and assistance as a graduate student. I'd also like to thank my committee members Craig Stockwell and Torre Hovick for their guidance/feedback with my thesis, and all the valuable lessons they taught me in graduate school. Furthermore, I'd like to acknowledge my colleagues Hanna Karevold, Lydia Nixon, and Brian Springall for their help and friendship through the years. I'd also like to express my gratitude toward my field assistants Daniel Syvertson and Devin Bush for all the help they provided during my field seasons.

Lastly, I'd like to thank my friends and family for all of their support throughout my entire academic experience.

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INTRODUCTION

Some North American bat populations are currently experiencing unprecedented population declines (Brooks 2011). Bats have been facing threats for many years due to habitat loss and fragmentation (Kitzes et al. 2014), pesticide contamination (Oliveira et al. 2018), and impacts of global climate change (Sherwin et al. 2013). In recent decades, the expansion of wind energy has caused a significant decline of some North American bat populations in a short period of time (Frick et al. 2017). Bats appear to be attracted to wind turbines, and it is estimated that over 500,000 bats are killed annually (Guest et al. 2022, Hayes 2013). It is predicted that further wind energy development may cause the extinction of certain bat species, such as the hoary bat (Frick et al. 2017) or red bat (Arnett et al. 2016).

The largest threat currently faced by bats in the United States and Canada is the spread of White-Nose Syndrome (WNS). White-Nose Syndrome is caused by the psychrophilic fungus *Pseudogymnoascus destructans*. Characterized by white fungal growth on the skin of its host, WNS disrupts the hibernation patterns of bats (Pettit et al. 2017, Hoyt et al. 2021). Infected individuals exhibit abnormal arousal patterns during hibernation that burn through fat reserves needed to survive winter (Reeder et al. 2012). Occasionally, afflicted bats will leave their hibernaculum too early and perish from the cold (Pettit et al. 2017). Bats that survive WNS may have difficulties surviving in the spring, as the fungus can cause major wing damage (Cryan et al. 2010). The disease has only been documented in North America for about 15 years (Foley et al. 2011). However, in that short period of time, the disease has had a profound impact on hibernating bat populations. It is estimated that over 5.5 million bats have died from White-Nose Syndrome (Pettit et al. 2017). The little brown bat (*Myotis lucifugus*) seems to be highly susceptible to the disease, along with other species of the genus *Myotis* (Dzal et al. 2011), although the reason for this increased susceptibility is not clear. As the disease continues to spread across the United States and Canada, more hibernating bats will die and population declines will continue.

The decline of North American bats is likely to have ecological and economic consequences. Bats help maintain ecosystems as a predator to insects (Kunz et al. 2011). Because of their long lifespans and sensitivity to environmental stressors, they have been used in the past as bioindicator species for assessing the stability of an ecosystem (Jones et al. 2009). As insectivores, bats act as natural pest control agents in human agricultural systems, valued at providing over 20 billion dollars of pest control services each year in the United States (Boyles et al. 2011). These potential benefits further emphasize concern over the decline of North American bat populations.

A critical tool for bat conservation efforts to succeed is long-term monitoring to track patterns of population growth and decline. Having reliable data on populations will greatly aid the decision making that has to be done to mitigate the various threats facing bats, as well as manage current populations (Loeb et al. 2015). However, gathering data on bat populations is challenging. Their ability to fly, coupled with difficulty recapturing individuals (i.e. trap-shy behavior) makes it difficult, if not impossible, to estimate population abundances (Britzke et al. 2013). Further, mist nets – the primary tool used to capture bats – are biased in that some species are particularly adept at avoiding them (MacSwiney 2008, Larsen et al. 2007).

Currently, the most effective and widely-used means of gathering population data on bats away from roosts is acoustic monitoring, which provides an index of abundance for comparisons over space or time (Kunz et al. 2009). This form of monitoring involves deploying ultrasonic detectors that can record the echolocation calls of passing bats. The North American Bat Monitoring Program (NABat) is a continent-wide effort to support the establishment of long-term acoustic monitoring programs and provide a repository for acoustic data (Loeb et al 2015), which can be used for regional and national conservation efforts.

Bat populations in the Northern Great Plains are less studied than populations in other regions of the United States. In 2019, White-Nose Syndrome was documented for the first time in North Dakota within Mercer county (USFWS 2022). Since then it has been documented in Billings and Burleigh county (USFWS 2022). Additionally, North Dakota currently has more than 2,200 wind turbines active in the state as of 2020 (Great Plains Energy Corridor 2021). It is critical that a long-term bat monitoring program be established and maintained in the state so that temporal trends can be assessed. Nelson et al. (2015) used acoustic monitoring with live capture data to determine the occurrence and distribution of bat species across the state of North Dakota from 2009-2012. However, this research was conducted before the arrival of WNS and the passive acoustic monitoring data was not collected using a standardized repeatable manner, such as the NABat program uses (Loeb et al. 2015).

In this study, our first objective was to quantify patterns of activity for individual bat species across the state of North Dakota from 2019-2021 with passive acoustic monitoring. Our second objective was to use occupancy modeling to better understand the distribution of species across the landscape and possible factors driving those patterns. We hypothesized that activity levels will have declined from 2019-2021 for certain species due to the arrival of WNS in the region. Our prediction is that species from the genus *Myotis* will experience decline compared to the other species, which will be stable over time. Previous research has shown that several species belonging to this genus experienced population declines following the arrival of WNS (Brooks et al. 2011, Ford et al. 2011). Additionally, we hypothesize that occupancy will be higher in areas that have relatively denser vegetation. These areas have high availability of insects, as well as opportunities for roosts.

METHODS

Study Area

Data was collected in the state of North Dakota from June 1 – August 15 during the summers of 2019 - 2021. The study followed the guidelines established by the NABat Monitoring Program (Loeb et al. 2015). We selected 30 grid cells in the state of ND that were identified as priority sampling areas by the NABat program. Grid cells were 10x10 km each, and were further divided into four 5x5 km sub-cells to identify sampling sites. It is recommended by NABat that 2-4 sub-cells are sampled per cell. Due to the size of North Dakota, we chose to sample 2 sub-cells in each cell. Sites were chosen based on their potential to attract bats, such as being near water, forest edge, or corridors. In total, 60 sampling sites across North Dakota were established in 2019 (Figure 1). To help identify population trends in different regions of the state, grid cells were categorized as belonging to either the East, West, or Central part of North Dakota based on longitude. East was classified as grid cells with a longitude greater than -98.9999 W. Central was defined as points between the longitudes -101.4999 W and -99.0000 W. West was classified as any grid cell with a longitude less than -101.5000 W.

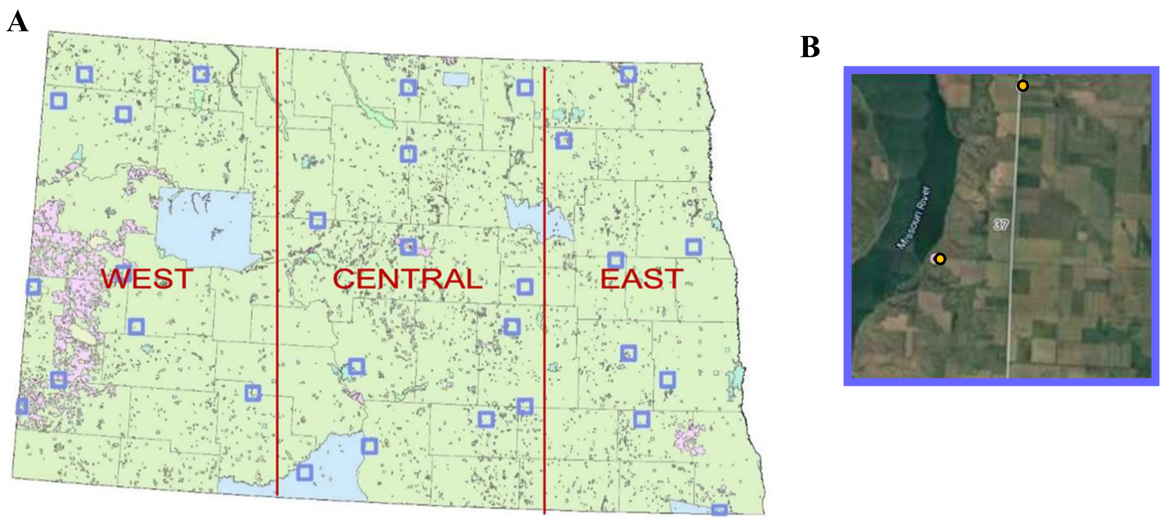

Figure 1. A) Map of North Dakota showing the West, Central, and East regions. Also shows the locations of the 30 10x10 km GRTS sampling cells (purple squares) selected for the North Dakota long-term bat monitoring program. Two sampling sites were established within each selected grid cell. B) Expansion of one sampling CELL (outlined in purple), within which are two sampling SITES (yellow circles).

Sampling sites were visited once for each year of the study. Each season, sampling would begin on the east side of the state and progressively move west until sampling concluded. To ensure all bats had emerged from hibernation, the sampling period was set up to overlap with the summer residency period for bat species in North Dakota. Permission to use the land was obtained primarily in advance via a written agreement; in some cases, permission was granted verbally on the day of equipment placement. If permission could not be obtained again, or if the site was deemed inaccessible, sites were relocated within the grid cell.

Field Methods

At a given site, data was collected for 4-7 nights. Data was obtained for the study via stationary acoustic monitoring devices. SM4BATFS Wildlife Acoustic bat detectors were used in this study (Figure 2). Each detector included a microphone attached to an extendable flag pole that could be adjusted to distance the microphone away from environmental clutter. When a detector was active, the calls of bats passing the microphone were recorded. Note that recording only occurred when a pre-set amplitude threshold was exceeded. Detectors were active from 6pm each evening until 6am the following morning. All settings were standardized across detectors, and microphones were tested intermittently to ensure high sensitivity.

Figure 2. Stationary monitoring setup at a typical site. The SM4BATFS acoustic bat detector is strapped to the tree, while a Wildlife Acoustics U2 microphone is attached to the end of the extendable flagpole (silver color)

Throughout the course of the study, six detectors were deployed simultaneously (one detector per site), allowing for multiple cells to be sampled at one time. Monitoring occurred at each site for a minimum of 4-5 nights. Deployment/pick up was extended or delayed on some occasions due to technical issues or harsh weather. At each site, the detector was deployed 1-3m above the ground depending upon the characteristics of the sampling site. For example, detector microphones would be positioned at a height and orientation that allowed the survey of open flight areas adjacent to a patch of trees. Precise data about the detector deployment location within each sampling site was gathered using a handheld GPS in 2019 and a Garmin device designed specifically to integrate with the bat detector in 2020 and 2021. The type of habitat in which the detector was deployed (e.g. forest, agricultural field, open water, etc.) was recorded for each site. Photos were taken of the area surrounding the detector, which also assisted in keeping the deployment location the same across seasons.

Sound Analysis

All data was stored on two hard drives, each of which was housed in a different location. In addition, all data and associated sound analysis was uploaded to the NABat online database. Sound analysis was done using Sonobat 4.4, an echolocation analysis program that allows automatic classification of call sequences to the species level using a region-specific call library (Szewczak 2018). Recordings were batch processed to: 1) identify and remove recordings of noise, and 2) identify and analyze recordings of bat echolocation. For each call within an echolocation recording, more than 70 parameter measurements were extracted; this call data was then compared to known recordings from different species to determine an identification. Decision algorithms within Sonobat aggregated species ID information for calls within a sequence to report the final species ID, as well as a measure of the program's confidence in that call sequence identification. Only identifications with a 90% or greater confidence score were included in data analysis. Call sequences were either identified to the species level, or were binned into one of four groups: HiF (high frequency echolocating species), LoF (low frequency echolocating species), HiLo (calls with both high and low frequency calls), and NoID (sequences where an ID could not be determined). The MT plains identification package was used for species ID analysis within Sonobat.

After species identification analysis, the Sonobat NABat Attributor was used to associate metadata with each recording, such as information about the sampling dates, location of collection, descriptors of the sampling site's habitat, and species ID assigned to the call sequence. Once data were attributed, both the metadata and the original call files were uploaded to the online NABat database.

Data Analyses

Activity patterns of individual species were compared across years to assess relative changes over time. Activity level is defined in this study as the number of bat passes recorded at a site divided by the number of deployment nights at that site. A bat pass is defined as the sequence of calls made by an individual bat as it flew by the microphone. Deployment nights are defined as the period of time when one detector was deployed, beginning from the evening of deployment to the morning of removal. Relative patterns of activity were assessed at the grid cell and regional level.

To assess the stated hypotheses regarding activity level, the R package lme4 was used to generate a linear mixed model. In the model, grid cell was the random effect. The fixed effects were species, region, year, and three interactions (species*year, species*region, and region*year). Only species and species groups with >15 identified call sequences per year were included in the model.

Species richness is defined as the total number of species in an assemblage or sampled area (Gotelli and Chao 2013). Species richness often reflects habitat quality, which bats are highly sensitive to (Jones et al. 2009). Similar to species activity, species richness was analyzed by region and year. Each survey year, the total number of detected species was recorded for each grid cell. Once collected, grid cells were grouped by region (East, Central, West), and analyzed using a linear mixed model. The grid cell was used as the random effect, while the fixed effects were region, year, and a region*year interaction.

Single-season, single-species occupancy modeling was used to assess occupancy and detectability of the four most common species found in the 2020 stationary monitoring dataset. Occupancy is defined as the probability that a species will be present at the study site. Detectability is the chance of detecting the species at the studied site if present. To ensure accuracy of detectability, multiple visits to each site are needed (MacKenzie et al. 2017). To account for this, we treated each night a detector was deployed as an independent visit/survey. Five nights were analyzed for each site.

The four species we examined included the big brown bat (*Eptesicus fuscus*), the little brown bat (*Myotis lucifugus*), the silver-haired bat (*Lasionycteris noctivagans)*, and the hoary bat (*Lasiurus cinereus)*. Analysis was conducted using the unmarked package within program R (R Core Team 2021). To examine the impact of environmental variables, two observational level covariates were examined: date and temperature. Three site-level covariates were also included in the model: vegetation, latitude, and longitude. Date refers to the start date on which detectors were deployed. Temperature references the background temperature at the time the detectors were deployed. All temperature data was collected using the R package RNCEP. This package uses data collected from the NCEP-DOE Reanalysis 1 and 2, a forecast system which utilizes past data from weather stations around the globe to estimate past/present weather conditions at a specified location/time based on historical data from nearby weather stations (Kemp et al. 2012; NOAA/OAR/ESRL PSD, Boulder, Colorado, USA; http://www.esrl.noaa.gov/psd/). Vegetation describes how dense the levels of foliage were surrounding the detectors. Vegetative clutter was ranked on a scale of zero to three, with zero being a completely open area around the microphone (e.g. open grassland), and three being heavily cluttered with trees and other vegetation (e.g. woodlands). Latitude and longitude provided information on where the detectors were deployed in the state.

RESULTS

Activity Level

All activity level data for the three-year sampling period can be found in Appendix 1. The fixed effects results from the activity level model can be found in Appendix 2. When modeling activity level, the only species/species groups that were significantly different in the model were the high frequency species group (HiF) and the group for sequences without an assigned ID (NoID) (Table 1; See appendix). For the Species*Year term (Figure 3), we found that activity level for the HiF group in 2021 was significantly lower compared to the two previous years. For the Species*Region interaction, the HiF group activity was significantly higher in the Central and West regions compared to the East, while the opposite pattern was seen for the LoF group and silver-haired bats (Figure 4).

Table 1. Fixed effects table from linear mixed model for species richness. In the model, Region, Year, and Region*Year were included as fixed effects, while grid cell was included as a random effect.

Species Richness Linear Mixed Model						
Fixed Effect	**Estimate**	**Std. Error**	**df**	**t value**	**Pr(>|t|)**	**Significance**
(Intercept)	4	0.3866	37.7681	10.346	1.41E-12	***
RegionEast	-0.4444	0.5618	37.7681	-0.791	0.43379	*n.s.*
RegionWest	**1.4545**	**0.5342**	**37.7681**	**2.723**	**0.00974**	**
Year2020	-0.5	0.2698	54	-1.853	0.06931	*n.s.*
Year2021	-0.5	0.2698	54	-1.853	0.06931	*n.s.*
RegionEast:Year2020	0.5	0.392	54	1.275	0.20759	*n.s.*
RegionWest:Year2020	**1.0455**	**0.3728**	**54**	**2.805**	**0.00699**	**
RegionEast:Year2021	0.6111	0.392	54	1.559	0.12485	*n.s.*
RegionWest:Year2021	0.1364	0.3728	54	0.366	0.71594	*n.s.*

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1 *n.s.*= Not Significant

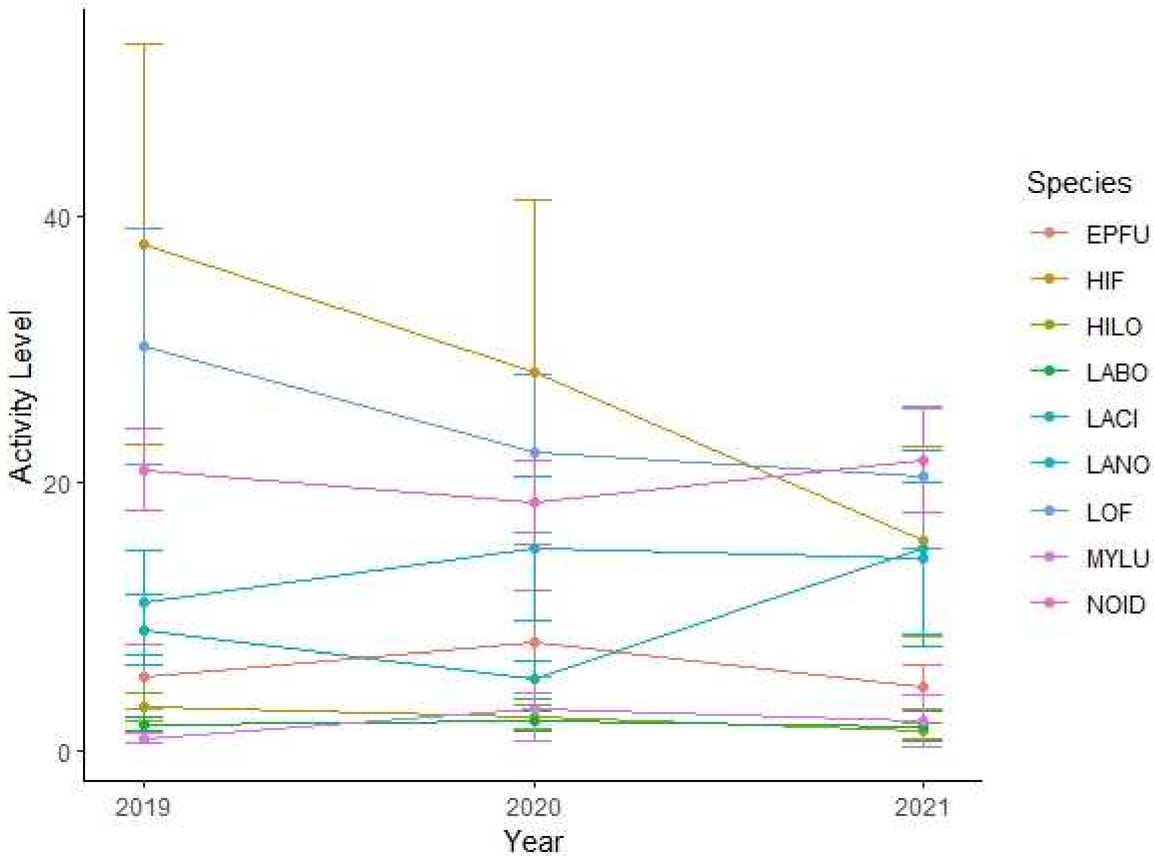

Figure 3. Activity level changes across a three-year period (2019-2021) for stationary acoustic monitoring collected across the state of North Dakota. Abbreviations for species/species group identification classifications from Sonobat are as following: EPFU = big brown bat, *Eptesicus fuscus*; HIF = high frequency echolocating bats; HILO = high or low echolocating bats; LABO = red bat, *Lasiurus borealis*; LACI = hoary bat, *Lasiurus cinereus*; LANO = silver-haired bat, *Lasionycteris noctivigans*; LOF = low frequency echolocating bats; MYLU = little brown bat, *Myotis lucifugus*; NOID = call sequences that could not be identified to species. Data for *Myotis evotis* (long-eared myotis; n = 11), *Myotis septentrionalis* (northern long-eared bat; n = 0), *Myotis thysanodes* (fringed myotis; n = 2), and *Myotis volans* (long-legged myotis; n = 81) were excluded due to very low sample sizes.

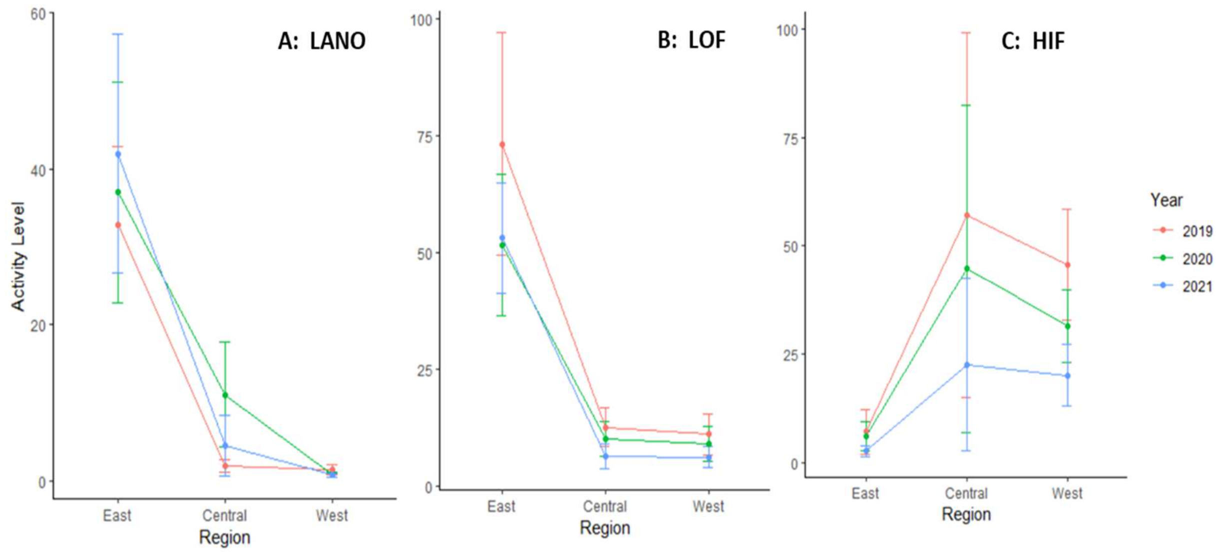

Figure 4. Activity level data divided by year (2019, 2020, 2021) and region (East, Central, West) for 1) silver-haired bats, *Lasionycteris noctavagans*, 2) LoF (species group for sequences with low frequency echolocation calls), and 3) HiF (species group for sequences with high frequency echolocation calls).

Species Richness

When examining the linear mixed model for species richness, the West region showed significantly higher levels of species richness compared to the Central or East regions (Table 2). For the Region*Year term, the west region exhibited significantly higher richness in 2020 compared to 2019 or 2021 (Table 1).

Occupancy and Detection

For the four species analyzed, the probability of a species occurring at a sampling site varied (Table 2), ranging from .49 (little brown bat) to 0.90 (hoary bat). Detectability ranged from .46 to .68 (Table 2). Of the four examined species, only *Myotis lucifugus* showed a significant difference in longitude, increasing in the West region, with a p-value of 0.006. No other covariates were significant for any of the species.

Table 2. Occupancy and detection values for each of the four common species in the data set, as well as the standard error, and upper/lower 95% confidence values. MYLU = little brown bat, *Myotis lucifugus*, EPFU = big brown bat, *Eptesicus fuscus*, LANO = silver-haired bat, *Lasionycteris noctivagans*, LACI = hoary bat, *Lasiurus cinereus*

Occupancy/Detection by Species								
Species	**Occupancy Estimate**	**SE**	**Lower**	**Upper**	**Detection Estimate**	**SE**	**Lower**	**Upper**
MYLU	0.49	0.07	0.35	0.62	0.46	0.05	0.37	0.56
EPFU	0.67	0.06	0.53	0.78	0.59	0.03	0.51	0.66
LANO	0.69	0.06	0.55	0.79	0.6	0.04	0.53	0.67
LACI	0.9	0.04	0.78	0.95	0.68	0.03	0.61	0.73

DISCUSSION

We predicted that due to WNS, species from the genus *Myotis* would experience significant declines in activity level. Furthermore, we also predicted that prey availability and roosting opportunities of densely vegetated areas would lead to high occupancy levels. Our results show evidence that *Myotis* populations have likely declined since 2019, but does not find any link between vegetation density and occupancy.

The activity level of the HiF group was significantly reduced in 2021 compared to 2019 or 2020. One possible cause of this decline could be the arrival of WNS in the Northern Great Plains. The genus *Myotis* most likely composes a large part of the HiF group, as it is well established that differentiating echolocation call sequences among species within the *Myotis* genus is difficult, hence the existence of this species group (Goodwin 2019). The most common *Myotis* species in North Dakota, the little brown bat, has been shown to be particularly vulnerable to this disease (Dzal et al. 2011, Frick et al. 2010). It seems likely that large numbers of little brown bats calls are being binned into the HiF group, and this reduction in activity in 2021 is indicative of population declines in the state, as has been seen in other states. For example, in Fort Drum, New York, three *Myotis* species (including *M. lucifugus*) showed significant decline in activity levels following the arrival of WNS (Ford et al. 2011). Similarly, central Massachusetts recorded a 72% decline in *Myotis* call activity after establishment of the disease (Brooks et al. 2011). Our data only shows significant decline in the HiF group, but note that few call sequences were classified as *Myotis* species. For example, the *Myotis lucifugus* classification makes up less than 1% of recorded calls in 2019, around 1.9% of calls in 2020, and approximately 3% of calls in 2021. It is likely that the ID algorithms are conservative, and commonly put MYLU sequences into the HiF group.

When examining the Species*Region interaction for the activity model, the East region had significantly different activity levels for HiF, LoF, and silver-haired bats when compared to the other regions. More specifically, the silver-haired bat and LoF group had significantly higher activity levels in the East, while the HiF group showed more significantly more activity in the Central and West regions. These trends are likely occurring due to the natural ranges of these bat species. Nelson et al. (2015) found that silver-haired bats, which also likely make up part of the LoF group, are more commonly found in the Central and East regions of North Dakota than in the West. Conversely, many of the species that likely compose the HiF group were found to be more common in the West and Central parts of North Dakota (Nelson et al. 2015).

Species richness was examined to determine if there was any variation in the number of species based on region or year. The West region showed significantly more species richness than the Central or East regions. This pattern is likely the result of differences in the number of bat species inhabiting each of the three regions. As previous studies have shown, more bat species are found in the West region than in the Central or Eastern regions of ND (Nelson et al. 2015). For example, six species belonging to the genus *Myotis* can be found in western North Dakota, while only one is found in eastern North Dakota (Nelson et al. 2015). Western North Dakota also had significantly higher richness in 2020 than in 2019 or 2021. While the reason for this is not completely clear, it is possible that this is a result of sampling error, as there is no guarantee that every species occupying a region will always be detected.

We assessed the occupancy of four bat species across the state. In addition, we examined if environmental factors had any effect on species occupancy or detectability. None of the tested observation or site-level covariates appeared to hold any significance over occupancy or detectability of the species. The only exception was for little brown bats, for which longitude predicted occupancy across the state. This is most likely a result of how rare the species is in eastern North Dakota. Previous capture and acoustic monitoring efforts found far substantially fewer little brown bats in the East region than in the Central and West regions (Nelson et al. 2015).

Hoary bats had the highest occupancy and detectability of all four tested species, with an occupancy of approximately 90% and detectability of 68%. These high rates could be a result of the low frequencies of their calls. Low frequency calls travel farther through the air than higher frequency calls, allowing them to be picked up at farther distances (Fenton 2003). Additionally, hoary bats are found in all parts of the state, and are capable of flying long distances in a single night (Nelson et al. 2015, Morningstar et al. 2019). Little brown bats had the lowest occupancy and detectability, with an occupancy of ~49% and detectability of 46%. It is possible this is due to the limited distribution of the species across the state. Previous capture and acoustic data show that little brown bats are rarely found in East ND, and are more prevalent in the Central and West regions (Nelson et al. 2015). Another factor to consider is that little brown bats have the highest frequency calls of the four examined species. High frequency calls attenuate quicker, and are less likely to be picked up by the detectors, reducing the likelihood that such species will have their calls recorded at moderate distances from the detector.

Several other studies have examined the impacts of environmental factors on bat species in similar ways. Bender et al. (2021) utilized acoustic data to determine if vegetative structure and/or insect abundance had any effect on occupancy in pine forest. They found a negative relationship between vegetation structure and occupancy, although occupancy was best explained by combining vegetation and insect abundance. Mena et al. (2020) used capture data to determine if elevation or forest cover had an impact on occupancy, as well as the effects of lunar illumination on detectability. While they found that elevation may have some impact on occupancy, forest cover did not. Unlike both of these studies, we did not find any links between environment and occupancy. It is possible that we did not find an effect of vegetation because of the coarseness of the scale we used, which was simpler than the methods used by Bender et al. (2021) and Mena et al. (2020). There is also the possibility that bats do not exhibit a strong preference in regards to vegetative clutter levels in North Dakota. However, this is unlikely as previous work from Trubitt et al. (2019) and Nelson et al. (2020) have found various bat species show a preference for cluttered areas. Future studies should incorporate the more detailed measures these studies used for assessing vegetative clutter, including tree density and canopy cover. Additionally, differences among study areas with different habitats should be noted. Occupancy patterns could be different in open plains compared to large areas of forests or elevated mountains.

Several limitations of this study should be considered. Passive acoustic monitoring has the potential for biased results. Factors such as echolocation call frequency, position, microphone sensitivity, or background noise, can potentially impact the number of call sequences at a site (Voigt et al. 2021). While these factors are inevitable when conducting passive acoustic monitoring, we believe that our use of a standardized protocol across sites and years minimizes the impacts these potential biases would have when making relative comparisons of activity over space and time.

We set out to quantify patterns of activity and occupancy for individual bat species across the state through acoustic monitoring. Our results suggest that since its arrival in 2019, WNS has reduced *Myotis* populations in North Dakota. No patterns were found linking occupancy or detectability with environmental covariates. The data collected provides useful insights into the activity patterns of various species of bats across the state. Wildlife managers will be able to use this data to assist with survey efforts in the future, knowing which parts of the state certain species are more active in. This is one of the first studies examining the potential impacts of WNS on the bat populations of North Dakota. However, more research, through acoustic monitoring and/or direct capture, will need to be conducted to better understand the impact of the disease as it continues to spread through the state and region.

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APPENDIX

Table A1. Data from long-term stationary acoustic monitoring in North Dakota from 2019 to 2021. Most grid cells included two sampling sites (data for sites within the same grid cell are pooled here). Abbreviations for species/species group identification classifications from Sonobat are as following: EPFU = big brown bat, *Eptesicus fuscus*; LABO = red bat; *Lasiurus borealis*, LACI = hoary bat, *Lasiurus cinereus*; LANO = silver-haired bat, *Lasionycteris noctivigans*; LoF = low frequency echolocating bats; Myci = western small-footed myotis, *Myotis ciliolabrum*; MYEV = long-eared myotis, *Myotis evotis*; MYLU = little brown bat, *Myotis lucifugus*; MYSE = northern long-eared myotis, *Myotis septentrionalis*; MYTH = fringed myotis, *Myotis thysanodes*; MYVO = *long-legged myotis*, Myotis volans; HIF = high frequency echolocating bats; HILO = high or low echolocating bats; LOF = low frequency echolocating bats; NOID = no identification to species.. Grid cells are arranged from east to west, and each grid cell was categorized into one of three regions in North Dakota. “West” was defined as all points with longitudes greater than -101.5000 W. “Central” was defined as all points with longitudes between -101.4999 W and -99.0000 W. “East” was defined as points with longitudes smaller than -98.9999W.

Year	Cell	Region	Epfu	Labo	Laci	Lano	Myev	Mylu	Myse	Myth	Myvo	HIF	HILO	LOF	NOID	Total
2019	309	East	685	88	390	246						84	82	2,129	435	4,139
2019	485	East	63	39	127	719						20	23	1,017	248	2,256
2019	741	East	11		68	60						2		160	139	440
2019	1333	East	137	2	584	343						5	7	808	439	2,325
2019	1765	East	107	1	27	489							3	926	566	2,119
2019	1893	East	3		18	237						8	2	168	177	613
2019	1509	East	44	21	63	359		17				383	26	436	343	1,692
2019	869	East			78	5						1		58	150	292
2019	821	East		29	50	3						34	65	61	140	382
2019	1637	Central			25	6								42	42	115
2019	997	Central		25	133	13						13	56	493	505	1,238
2019	1845	Central		6	8	7						9		41	113	184
2019	2021	Central		1	13							12	3	23	143	195
2019	1381	Central	1		31	47		1				25	6	22	49	182
2019	1077	Central		2	60			14			12	917	29	48	137	1,219
2019	613	Central	1	6	94	39		7				113	12	80	295	647
2019	37	Central	19	18	132	12		6			1	101	4	416	339	1,048
2019	1125	Central	18	80	200	105	1	16				377	38	388	430	1,653
2019	101	Central	3	84	200			79	1			5,990	408	72	550	7,387

Table A1. Data from long-term stationary acoustic monitoring in North Dakota from 2019 to 2021 (continued)

Year	Cell	Region	Epfu	Labo	Laci	Lano	Myev	Mylu	Myse	Myth	Myvo	HIF	HILO	LOF	NOID	Total
2019	2085	West	6	20	2	5		9				501	15	49	218	825
2019	357	West	5	16	15	11		5	2			1,311	25	47	186	1,623
2019	1653	West		7	2	2		9				52	2	8	12	94
2019	2005	West	76	46	81	22		42				484	73	142	83	1,049
2019	1749	West	223	38	80	20	3	22				476	22	484	333	1,701
2019	693	West	2	6	1			4				48	6	10	5	82
2019	1461	West	5	60	11	1		11			1	1,190	53	51	78	1,461
2019	981	West	9	4	5	6	4	2		1	2	162	25	55	44	319
2019	1205	West	2	11				5				350	37	13	89	507
2019	725	West	9		74	46	2	8		2		39	2	73	84	339
2019	1829	West	63	1	7	17	8	5		8		171	23	139	40	482
2019	**Total**		**1,492**	**611**	**2,579**	**2,820**	**18**	**262**	**3**	**11**	**16**	**12,878**	**1,047**	**8,459**	**6,412**	**36,608**
2020	309	East	135	212	103	131	0	0	0	0	0	295	72	796	580	2,189
2020	485	East	47	29	93	707	0	0	0	0	0	15	9	907	210	1,970
2020	741	East	25	2	23	49	0	0	0	0	0	3	1	105	72	255
2020	1333	East	94	1	379	151	0	0	0	0	0	1	1	365	360	1,258
2020	1765	East	2	0	21	194	0	0	0	0	0	0	0	74	107	396
2020	1893	East	46	6	45	1,172	0	0	0	0	0	5	0	1,030	521	2,779
2020	1509	East	40	0	35	844	0	1	0	0	0	144	76	1,152	442	2,694
2020	869	East	0	0	129	2	0	0	0	0	0	0	0	47	47	225
2020	821	East	0	167	24	3	0	0	0	0	0	111	72	27	123	527
2020	1637	Central	0	0	35	3	0	0	0	0	0	0	0	20	27	85
2020	997	Central	0	2	31	4	0	0	0	0	0	5	2	105	185	334
2020	1845	Central	0	0	12	0	0	0	0	0	0	1	0	66	208	287
2020	2021	Central	0	1	0	0	0	0	0	0	0	2	0	2	12	17
2020	1381	Central	20	1	26	647	0	6	0	0	0	14	2	233	198	1,127
2020	1077	Central	0	5	17	0	0	6	0	0	0	77	0	14	68	187
2020	613	Central	3	35	32	216	0	1	0	0	0	110	22	183	289	888

Table A1. Data from long-term stationary acoustic monitoring in North Dakota from 2019 to 2021 (continued)

Year	Cell	Region	Epfu	Labo	Laci	Lano	Myev	Mylu	Myse	Myth	Myvo	HIF	HILO	LOF	NOID	Total
2020	37	Central	88	19	14	0	0	37	0	0	0	264	6	34	52	426
2020	1125	Central	118	8	187	331	0	32	0	0	6	328	73	515	364	1,844
2020	101	Central	16	50	259	0	0	1,014	0	0	21	5,382	366	68	669	7,829
2020	2085	West	19	3	4	4	0	16	0	0	8	977	7	32	313	1,364
2020	357	West	6	6	13	16	0	1	0	0	2	392	13	54	123	620
2020	1653	West	4	24	6	0	0	22	0	0	2	90	2	14	33	193
2020	2005	West	1,012	39	24	11	0	76	0	0	7	646	73	369	161	1,406
2020	1749	West	312	18	79	16	4	5	0	0	0	266	14	151	239	792
2020	693	West	11	21	5	0	0	4	0	0	1	50	18	39	11	149
2020	1461	West	8	21	7	6	0	14	0	0	4	98	5	27	20	202
2020	981	West	22	16	7	5	5	5	1	0	0	116	12	21	51	239
2020	1205	West	9	31	7	5	0	9	0	0	0	219	6	25	22	324
2020	725	West	2	2	15	3	6	0	0	0	1	20	3	32	66	148
2020	1829	West	100	5	5	13	15	5	0	2	3	700	26	118	187	1,079
2020	**Total**		**2,139**	**724**	**1,637**	**4,533**	**30**	**1,254**	**1**	**2**	**55**	**10,331**	**881**	**6,625**	**5,760**	**31,833**
2021	309	East	483	12	90	85	0	0	0	0	0	50	23	993	765	2,501
2021	485	East	119	101	392	647	0	0	0	0	0	66	55	632	589	2,601
2021	741	East	15	2	167	190	0	0	0	0	0	8	5	327	305	1,019
2021	1333	East	145	8	2,966	320	0	0	0	0	0	6	49	1,047	719	5,260
2021	1765	East	21	4	35	612	0	0	0	0	0	2	1	537	360	1,572
2021	1893	East	49	0	45	778	0	0	0	0	0	1	0	638	358	1,869
2021	1509	East	41	5	209	1,052	0	5	0	0	0	74	20	825	554	2,785
2021	869	East	0	0	171	2	0	0	0	0	0	0	0	100	102	375
2021	821	East	0	2	41	1	0	0	0	0	0	3	1	45	106	199
2021	1637	Central	1	0	691	11	0	0	0	0	0	0	0	255	254	1,212
2021	997	Central	0	0	7	1	0	0	0	0	0	0	0	3	91	102
2021	1845	Central	0	11	13	0	0	0	0	0	0	19	1	22	98	164
2021	2021	Central	0	0	4	1	0	0	0	0	0	1	0	5	37	48

Table A1. Data from long-term stationary acoustic monitoring in North Dakota from 2019 to 2021 (continued)

Year	Cell	Region	Epfu	Labo	Laci	Lano	Myev	Mylu	Myse	Myth	Myvo	HIF	HILO	LOF	NOID	Total
2021	1381	Central	2	0	61	391	0	0	0	0	0	1	0	128	121	704
2021	1077	Central	0	0	22	5	0	6	0	0	1	113	1	21	82	251
2021	613	Central	0	0	19	6	0	2	0	0	0	3	0	50	124	204
2021	37	Central	59	4	5	0	0	13	0	0	1	55	0	19	40	196
2021	1125	Central	62	2	74	46	0	2	0	0	0	66	4	160	120	536
2021	101	Central	5	15	180	1	0	935	0	0	50	3,234	156	31	854	5,461
2021	2085	West	100	7	5	0	0	3	0	0	3	781	9	58	142	1,108
2021	357	West	8	4	10	11	0	0	0	0	17	470	6	37	115	678
2021	1653	West	1	9	5	0	0	3	0	0	0	23	3	3	21	68
2021	2005	West	297	15	52	17	0	6	0	0	0	153	22	261	282	1,105
2021	1749	West	73	8	58	18	0	4	0	0	2	230	2	70	425	890
2021	693	West	17	8	3	6	0	0	0	0	1	22	1	20	76	154
2021	1461	West	4	255	11	5	0	41	0	0	3	359	99	43	133	953
2021	981	West	18	1	26	7	0	2	0	0	3	64	0	35	50	206
2021	1205	West	1	2	4	0	0	0	0	0	0	31	3	14	43	98
2021	725	West	6	1	15	1	10	0	0	0	0	37	7	17	63	157
2021	1829	West	55	5	11	28	1	0	0	2	0	182	22	200	210	716
2021	**Total**		**1,582**	**481**	**5,392**	**4,242**	**11**	**1,022**	**0**	**2**	**81**	**6,054**	**490**	**6,596**	**7,239**	**33,192**

Table A2. Fixed effects table for the linear mixed model for activity. The model included species, year, region, species*year, species*region, and region*year as fixed effects, with grid cell as a random effect.

Fixed Effect	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	0.66	7.15	293	0.09	0.93
SpeciesHIF	**51.61**	**8.54**	**734**	**6.04**	**<0.001**
SpeciesHILO	3.27	8.54	734	0.38	0.70
SpeciesLABO	0.51	8.54	734	0.06	0.95
SpeciesLACI	6.10	8.54	734	0.71	0.48
SpeciesLANO	2.97	8.54	734	0.35	0.73
SpeciesLOF	15.23	8.54	734	1.78	0.08
SpeciesMYLU	3.37	8.54	734	0.39	0.69
SpeciesNOID	**17.25**	**8.54**	**734**	**2.02**	**0.04**
RegionEast	7.17	8.97	186	0.80	0.42
RegionWest	7.33	8.53	186	0.86	0.39
Year2020	3.78	7.32	734	0.52	0.61
Year2021	-2.57	7.32	734	-0.35	0.73
SpeciesHIF:Year2020	-12.22	9.36	734	-1.31	0.19
SpeciesHILO:Year2020	-3.35	9.36	734	-0.36	0.72
SpeciesLABO:Year2020	-2.40	9.36	734	-0.26	0.80
SpeciesLACI:Year2020	-6.32	9.36	734	-0.68	0.50
SpeciesLANO:Year2020	1.43	9.36	734	0.15	0.88
SpeciesLOF:Year2020	-10.57	9.36	734	-1.13	0.26
SpeciesMYLU:Year2020	-0.44	9.36	734	-0.05	0.96
SpeciesNOID:Year2020	-5.07	9.36	734	-0.54	0.59
SpeciesHIF:Year2021	**-21.53**	**9.36**	**734**	**-2.30**	**0.02**
SpeciesHILO:Year2021	-1.08	9.36	734	-0.12	0.91
SpeciesLABO:Year2021	0.51	9.36	734	0.06	0.96
SpeciesLACI:Year2021	6.68	9.36	734	0.71	0.48
SpeciesLANO:Year2021	4.01	9.36	734	0.43	0.67
SpeciesLOF:Year2021	-9.14	9.36	734	-0.98	0.33
SpeciesMYLU:Year2021	1.99	9.36	734	0.21	0.83
SpeciesNOID:Year2021	1.39	9.36	734	0.15	0.88
RegionEast:Year2020	-3.66	5.55	734	-0.66	0.51
RegionWest:Year2020	-0.18	5.28	734	-0.03	0.97
RegionEast:Year2021	5.03	5.55	734	0.91	0.36
RegionWest:Year2021	1.06	5.28	734	0.20	0.84

Table A2. Fixed effects table for the linear mixed model for activity (continued)

Fixed Effect	Estimate	Std. Error	df	t value	Pr(>|t|)
SpeciesHIF:RegionEast	**-43.84**	**9.62**	**734**	**-4.56**	**<0.001**
SpeciesHILO:RegionEast	-8.38	9.62	734	-0.87	0.38
SpeciesLABO:RegionEast	-5.93	9.62	734	-0.62	0.54
SpeciesLACI:RegionEast	6.65	9.62	734	0.69	0.49
SpeciesLANO:RegionEast	**23.76**	**9.62**	**734**	**2.47**	**0.01**
SpeciesLOF:RegionEast	41.97	9.62	734	4.36	<0.001
SpeciesMYLU:RegionEast	-12.48	9.62	734	-1.30	0.19
SpeciesNOID:RegionEast	9.19	9.62	734	0.96	0.34
SpeciesHIF:RegionWest	-16.68	9.14	734	-1.82	0.07
SpeciesHILO:RegionWest	-8.22	9.14	734	-0.90	0.37
SpeciesLABO:RegionWest	-6.04	9.14	734	-0.66	0.51
SpeciesLACI:RegionWest	-12.44	9.14	734	-1.36	0.17
SpeciesLANO:RegionWest	-12.46	9.14	734	-1.36	0.17
SpeciesLOF:RegionWest	-8.47	9.14	734	-0.93	0.35
SpeciesMYLU:RegionWest	-11.40	9.14	734	-1.25	0.21
SpeciesNOID:RegionWest	-12.33	9.14	734	-1.35	0.18