BAT DIVERSITY, ACTIVITY, AND HABITAT USE IN A MIXED DISTURBANCE LANDSCAPE

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ABSTRACT

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Bat species face multiple threats. One such threat, white-nose syndrome (WNS) has drastically reduced many bat populations. Also, habitat loss and fragmentation often forces bats to concentrate in remnant natural areas, or utilize habitats that are not as suitable. Both of these threats, while threaten bats in a general sense, also affect species differentially. The Oak Openings Region of Northwest Ohio is a biodiversity hotspot with a landscape composed of remnant natural areas within a matrix of agriculture and urban areas. This area, which provides crucial summer foraging habitat, has experienced declines in bat activity, shifts in bat assemblages, and some in diversity, in recent years, especially since WNS introduction. To study bats in this diverse landscape, we sampled bats acoustically from May – August 2016. We sampled mobile transects along roads along with stationary sites within the Oak Openings Preserve within the region. We identified calls to species and ran analyses investigating total bat activity, species-specific activity and presence, and bat diversity compared to. We compared bats to environmental, vegetation, road, and landcover parameters. Our results show that certain parameters influence bats as a whole, while others only affect one or a few species. We found that savanna stationary sites had more species-specific activity and bat diversity than forested sites (Rank Sums, $p<0.05$). Parameters that affected most bat species most prevalently were temperature and forest cover, both reflecting positive relationships with total bat activity and diversity (Chi-square; Rank Sums, $p<0.05$). When looking at species specific relationships, we focused on the least active species, as they may be more in need of management than more active species. Parameters that most influenced our least active species were humidity and open/savanna vs. forested sampling areas. Humidity had positive relationships with the likelihood of presence of our rarer species, while habitat type relationships depended on species specific life history traits (Chi-Square; Rank Sums, $p<0.05$). Our research suggests managing for forest cover across the landscape for all native bats; however, encourages managers to consider heterogeneity by maintaining both dense and open forest stands, along with open areas to benefit certain species.

This is dedicated to my mother and fiancé, who have given me nothing but unconditional love and support before and throughout this journey, and to the rest of my friends and family who have done so much for me throughout my life.

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GENERAL INTRODUCTION

Bat Importance

Bats species provide services to humans, which can equate to a large amount of economic value. One study estimates that 1 billion dollars in corn damage is prevented in the U.S. per year due to bats eating herbaceous insects (Maine and Boyles 2015). They also speculate that bat foraging also reduces fungal growth and toxic compound build up on corn (Maine and Boyles 2015). Boyles et al. (2011) estimates that bats prevent over 3 times the monetary damage to crops overall; 3.7 billion dollars or an average of $74/acre. In addition to eating crop pests, bats also eat other insects. For example, a colony of 150 big brown bats (*Eptesicus fuscus*) inn Indiana was found to eat 1.3 million pest insects a year (Boyles et al. 2011). Also, a single little brown bat (*Myotis lucifugus)* can eat 4 – 8g of insects a night (Boyles et al. 2011). The insects consumed by these bats can include mosquitos that are vectors for infectious diseases, such as malaria and the newly discovered Zika virus (Yakob and Walker 2016), both of which are deadly (Bourtzis et al. 2016). Reduction of mosquitos means a decreased chance of these diseases infecting the human population.

Bats also play a crucial role in the habitats they live in. Bats may have a top-down effect in the communities in which they thrive related to nutrient cycling. Guano from bats can support vertebrate communities in roost caves (Pape 2014) and help fertilize the trees they roost in the summer (Voigt et al. 2015). Bats also may serve as an indicator species. Studies have shown potential in using bats as indicators of heavy metal (Zukal et al. 2015). Also, many bats, with sensitivity to fragmentation and agricultural or urban expansion, could be indicators of ecosystem health (Jones et al. 2009; Park 2015; Russo and Ancillotto 2015).

Threats to Bats

Bats currently face several threats to survival that have caused significant declines in various bat populations. Wind turbines are one such threat (Hein and Schirmacher 2016; Boyles et al. 2011). There are some hypotheses as to why bats are attracted to turbines. Bats may interpret turbines as tree-like structures, and have actually been observed foraging around them, even trying to land on them (Hein and Schirmacher 2016). Another hypothesis is they may be attracted to the gaps formed by turbine corridors; a feature they are attracted to on the landscape level (Hein and Schirmacher 2016). Many estimates have been made on how many bats are killed in the U.S. as a result of wind turbines, and they range from 200,000 to over 800,000 individuals killed annually (Hein and Schirmacher 2016).

Bats are also battling a deadly pathogen known as white-nose syndrome (WNS). First discovered in the U.S. in 2006, WNS is a fungus that infects bats (Blehert et al., 2009) and disrupts their hibernating cycle. Bats infected with WNS have shown increases in arousal during hibernation (Reeder et al. 2012; Warnecke et al. 2012), including flights out of hibernacula during the winter (Turner et al 2011). This increases energy usage and often causes the infected individuals to die due to depleted fat reserves saved for hibernation(Turner et al 2011). Millions of bats were killed within the first five years of the infection (Turner et al 2011; Reeder et al. 2012) and WNS is responsible for devastating population crashes of certain groups of bats (Boyles et al. 2011; Frick et al. 2010).

Finally, as mentioned previously, bats have shown evidence of being sensitive to fragmentation from agricultural and or urban expansion (Russo and Ancillotto 2015; Park 2015; Jones et al. 2009). Fragmentation, and habitat loss, are responsible for the decline of many wildlife populations (Allendorf et al. 2009). Some research has found evidence indicating that urbanization and fragmentation may negatively impact bats (Kurta and Teramino 1992; Johnson et al. 2008). Others, however, have found results suggesting that bats may not be as affected by fragmentation as once thought (Gehrt and Chelsvig 2003; 2004). One specific type of anthropogenic fragmentation that is gaining more interest in ecology and conservation biology is the breaking up of landscape by roads. It is estimated that 83% of the Unites States' land area is 1 km away from a road (Riiters and Wicham, 2003). Since roads fragment many natural areas and preserves (Ramp et al. 2006), combined with the discrepancies about the impact that fragmentation may have on bats, there is a gap in our knowledge about these potential effects that may provide critical information for bat conservation.

The Oak Openings Region

The Oak Openings Region is an area of more than 476 km² spanning Fulton, Henry, and Lucas counties in northwestern Ohio (Brewer and Vankat 2006). Since European settlement, urban sprawl, agricultural expansion, hunting, fire suppression, and other anthropogenic influences have altered the region and reduced the amount of natural areas (Mayfield 1969). It is a complex mixed disturbance landscape of urban sprawl, agricultural expanses, and remnant natural ecosystems. A study in 2011 found that approximately 40% of the region was dominated by urban expansion, 27% was cropland, and the remaining was natural areas (Schetter and Root 2011). The Oak Openings Region contains many ecosystems, some of which are rare, including wet prairie and oak savannas (Brewer and Vankat 2004) (Figure 1.1). The region is considered a biodiversity hotspot, encompassing only 0.5% Ohio's total land area, but containing 1/3 of the state's rare plant and animal species (Schetter et al. 2013).

CHAPTER I. DIVERS OF BAT ACITIVTY AND DIVERSITY IN A BIODIVERSITY HOTSPOT

Introduction

Where conducting wildlife management, managers must consider the goals they wish to achieve with their efforts. Conservation often revolves around a single, often imperiled, species (Soulé and Wilcox 1980; Kohm 1990). One big reason for this type of management strategy is budget. Budget constraints often limit managers to managing only a priority, or select few species (Teeffelen and Moilanen 2008). For this reason, especially for organizations with small budgets, finding management strategies that can benefit multiple species may not only be more cost effective, but also benefit multiple species at once (possibly including species that normally would not have received any aid).

Managing a suite of species is something that can be easily done for similar taxa, especially if these individuals occur in similar habitats or play similar roles in the ecosystem. This approach has been utilized for a number of taxa including arthropods (Braman and Pendley 1993; Swengel 1996), fish (Gratwicke and Speight 2005; Hudy et al. 2011), birds (Swengel and Swengel 2001; Cerezo et al. 2011; Quinn et al 2012), and more.

Grouping species into a single management plan may hold precedence over single-species models if you are interested in increasing abundancies of multiple species. A good example of this is found in Swengel and Swengel's (2001) research on grassland birds. Tallgrass prairie has been drastically reduced from its native range, and continues experiencing loss from factors such as urbanization (Swengel and Swengel 2001). Grassland birds that live in these habitats have followed these same trends, with select species seeing a decline in abundance of over 90% (Swengel and Swengel 2001). Due to declines in many grassland birds, this study chose to look at a suite of species, rather than a single one, and investigated what influences their abundance and thus aiding management of these birds (Swengel and Swengle 2001).

This type of multispecies management is better for fulfilling a common, and arguably one of the most important, goals of most management plans: encouraging biodiversity (Margules and Pressey 2000; Nicholson et al. 2006). While increasing the number of individuals may be important, such as with the previous study, sometimes management looks, instead, to promote the largest number of species possible. A study conducted by Cerezo et al. (2011) was interested in discovering what factors were associated with the highest richness of birds in an agricultural landscape. Their goal looked mostly into bird diversity because declines in grassland/farmland birds have largely been attributed to fragmentation and habitat loss from agriculture. Cerezo et al.'s (2011) research , therefore, informed managers what they should do to encourage the largest number of bird species possible in a less-than-favorable landscape.

Bats in the eastern United States have been declining in recent years from a number of threats. Habitat loss and fragmentation (Russo and Ancillotto 2015; Park 2014; Jones et al. 2009), wind turbines (Boyles et al. 2011; Hein and Schirmacher 2016), and White-nose Syndrome (WNS) (Boyles et al. 2011; Frick et al. 2010) are responsible for dramatic reduction in bats. While bat assemblages respond differently to forest loss, research has shown that this is a strong influence on most bat species (Garcia-Morales et al. 2013). MYLU is a bat species that has been especially impacted by WNS and their numbers have been drastically reduced across much of their range (Dzal et al 2010). One study predicted a 99% chance of regional extinction of little brown bats by 2026 if mortality rates remain the same (Frick et al. 2010). Bats are also affected differentially by these threats. For example, WNS syndrome affects certain bat species more than others (Blehert et al 2009); little brown bats, tri colored bats, and northern long-eared bats are considered particularly susceptible. The threats highlight the need for research investigating influences on bat presence, activity, and diversity.

The Oak Openings Region of Northwest Ohio is a biodiversity hotspot that is home to a large number of native species (see Table 1.1 for species names and abbreviations), including many rare ones (Schetter et al. 2013). It is a landscape that has remnant natural areas within a matrix of agriculture and urban areas. This area, which is a crucial summer foraging location, has experienced declines in bat activity, and some in diversity, in recent years, especially since WNS introduction (Sewald 2012; Janos 2013; Nordal 2016). We have also seen evidence of shifts of bat assemblages in our region (Sewald 2012; Janos 2013; Nordal 2016). These, along with the multiple threats that bats face, especially habitat loss for bats in the Oak Openings Region, validates researching a suite of species, rather than just the rarer bats in our study area. Human development continues in the region with intensification of agriculture and urban sprawl from the city of Toledo.

With an interest in boosting bat numbers and richness in the region, this study set out to identify factors that influence activity of the full suite of native species of bats, along with bat diversity, through the use of acoustic monitoring. We hypothesized that certain environmental and landscape elements would affect total activity and diversity, independent species-specific requirements. We predicted that temperature and amount of forest cover would be positively related to bat activity and diversity. Alternatively, we expected to find that cropland coverage and distance to tree features and water would have negative influences on bats in the Oak Openings Region. Finally, we expect that bat activity will be higher in areas of savanna compared to other habitats in both preserves and edge-habitats.

Methods

Acoustic Sampling

Bat calls were collected using an Anabat SD2 acoustic detector (Titley Electronics, Ballina, New South Wales, Australia). An Anabat is a device that records with an omnidirectional microphone, and saves to a memory card, high frequency sounds emitted by bats. The Anabat was set to a sensitivity of 5. Acoustic sampling was not conducted on nights with wind speed over 24kph, temperatures below 10°C, or with a high probability of rain.

We chose to perform large scale acoustic sampling across the region using road transects. Twelve 10km transects were created at random across the study area using public roads. The very northern part of the study area was excluded from the transect layout as it is highly urban and not conducive to the survey method chosen for this study, and most of the study species are not well-adapted to urban-dominated areas. Six transects ran in a north-south direction, while the other six went in an east-west direction. Transects were placed at least 2km apart from other transects running in the same direction to reduce autocorrelation. Transects running in opposite directions did not always meet this rule. However, transects that were not 2km apart were never surveyed one after the other. Transects were surveyed via a car driven approximately 30kph. Up to five transects were surveyed in a single night. To record the calls, the detector was secured to a painter's pole and extended out the car window. A handheld GPS (Garmin eTrex) was attached to the Anabat detector, allowing us to obtain GPS coordinates of each bat call as they were recorded by the detector. Transects were surveyed from ½ hour after sunset for three hours. Each transect was sampled twice a month from May – August 2016 and took approximately 20 minutes.

Stationary overnight acoustic monitoring (hereafter stationary sites) was conducted to collect data from core habitat and compare to edge habitat data obtain from transect sampling. We set up 16 stationary sampling points in the Oak Openings preserve in pairs, one point in oak savanna and one nearby in an oak forest. All of these sites have been sampled previously by former researchers (Sewald 2012; Janos 2013; Nordal 2016) and have been categorized as being either “savanna” or “forested” sites. Eight of each type were included in our sampling. All stationary sites were located at least 100m apart, which is beyond the 30 m radius of the detectors. Bat calls were collected using an Anabat SD2 acoustic detector held in a weatherproof station secured to a tree. Detectors were left at two sites (one pair) each night overnight. Stationary sites were run monitored continuously all evening, but we only included bats recorded from 21:00 – 01:00 in analyses to compare to facilitate comparison to transect sampling. Each sampling point was monitored at least once per month from May – August 2016.

Bat Identification

Bat calls were analyzed to species by the author using the software Analook (version 4.1). The calls were double-checked using the software called BATcall ID (BCID) (Allen, version 2.7c). If a discrepancy was encountered, the author made the final determination of the species.

Bat calls were identified to species by looking at various features of the sonogram and comparing to known call libraries (Sewald 2012). Sonograms of the bat calls resembled vertical slashes that spread across a range of frequencies (Figure 1.2). Identifiable features of a sonogram included, but are not limited to, call frequency, overall shape of the call (such as hooked bottoms), and slope of the call (see Appendix II for species-specific sonogram diagnostic characteristics).

Explanatory Variables

Temperature (°C), wind speed (kph), cloud cover (%), relative humidity (%), and starting time was recorded at the beginning of each transect survey. For any night of sampling, either overnight or transect, nightly hourly weather conditions (temperature (°C), wind speed (kph)), relative humidity (%), and incidence of precipitation were recorded from a weather station at Toledo Express Airport (http://w1.weather.gov/data/obhistory/KTOL.html). Moon illumination (%) was recorded via a website tracking moon phases (http://aa.usno.navy.mil/cgi-bin/aa_moonill2.pl?form=1&year=2016&task=00&tz=-05). Nightly average, maximum, minimum, and range of temperatures, humidity, and wind were the specific atmospheric parameters used in analyses.

Environmental characteristics were measured at fixed sampling points at 1km increments along each transect (hereafter transect points), equaling 11 points along each of the 12 transects. Vertical clutter, which is the density of vegetation at various heights, hereafter clutter, was measured using a 6.5m cloth profile board at 15m (half the range distance of the Anabat detectors) from the edge of both sides of the road. Measures of clutter were done for low (understory; 0 – 3m) and high (midstory; 3 – 6.5m) levels, along with total clutter by taking a photograph of the profile board (Figure 1.3). Canopy cover was also measured at each transect point using a camera held at approximately 1.5m pointing up into the tree canopy (Figure 1.4). Any nearby non-permanent water presence was recorded as well. These three parameters were recorded once a month (May – August) for each transect. Clutter was measured as a percent by using the picture taken in the field to estimate the amount of the profile board that was covered by vegetation.

Canopy cover was also measured as a percent, and the photograph was analyzed using ImageJ. The photograph was converted to a 32-bit greyscale image. A histogram was then made to count the number of different colored pixels. The range of values was 0 – 250, with 0 being 100% black and 250 being almost white. The number of pixels falling in the range of 0 – 100 was calculated and divided by the total number of pixels in the picture to obtain the percent canopy cover. We chose 100 as the threshold as it was the point where darker pixels (representing canopy cover) transitioned to lighter pixels (representing open space).

Clutter and canopy measures were not always obtainable for each transect point, as it would have often required measuring on private land. Therefore, clutter and canopy measures were also estimated via observation. These observations were categorized on a scale from 1 – 6, 1 being no/little cover and 6 being much/full cover. A category was selected based on how many sixths the profile board was covered by vegetation or how much canopy cover was in the picture. Therefore, category 1 was 0 – 16.7% coverage, category 2 was 16.8 - 33.3%, category 3 was 33.4 – 50%, category 4 was 50.1 – 66.%, category 5 was 66.7 – 83.3, and category 6 was 83.4 – 100. All measures that could be calculated as a percent were also assigned a rank to maintain consistency. Clutter and canopy at each transect point were eventually averaged between both sides of the road since we could not detect from which side of the road a bat was detected. This average was on a scale of 1 – 6, but in increments of 0.5 to produce a finer scale.

Clutter and canopy measures were also taken once per month at each of the stationary sites. The only difference was that these parameters were measured 15 m from the detector in four directions: northeast, northwest, southeast, southwest. Also, analyses with these parameters were performed only with percentages, rather than categories, since we were able to successfully sample all sites.

At each transect point, distance to closest natural features and water, along with the slope of any ditch that was present was recorded on either side of the road. Slope of ditches were recorded for both sides of the road using a level app, called Bubble Level (Version 3.12) on a cell phone. Natural features recorded included single trees (trees standing alone), tree lines (a single thin line of trees), or tree stands (a group of trees of considerable depth). Distance was recorded to each of these features up to 300m in the field using a range finder (Nikon Prostaff 3; 6x zoom, measured range 10 – 500m). The maximum, minimum, and average of each measure on either side of the road (average distance to natural features and average slope) were chosen as parameters for analysis. Presence of water was recorded using Google maps (www.google.com/maps). Each transect point was plotted on the map and a measuring tool was used to measure distances to any source of water within 300m. These parameters were not measured at stationary sites.

To get an estimate of land use, both sides of the road at each transect point were categorized as either natural, agricultural, or urban. We calculated the percent of each land use type along each transect. To expand this, we also categorized our sampling points based on land use on a scale from 1 – 6, to take into account total land use at the point rather than evaluating either side of the road individually. Category 1 indicated that both sides of the road were considered residential, category 2 indicated that one side of the road was considered residential and the other agricultural, category 3 indicated that both sides of the road were considered agricultural, category 4 indicated that one side of the road was considered residential and the other natural, category 5 indicated that one side of the road was considered agricultural and the other natural, category 6 indicated that both sides of the road were considered natural. Land cover types were also evaluated at each transect point.

Insect sampling was conducted via sweep netting at our stationary sites. We performed 20 sweeps at heights of 3m and 5m in all directions, sweeping throughout the air for any flying insects, along with sweeping any vegetation, within 15 meters of the overnight site. Because of the very low number of insects collected, these data were not utilized further in the study. However, we did try to quantify the amount of insects in an area utilizing the sonograms recorded by our Anabat detectors. Insects produce a sonogram distinguishable from bats. We therefore counted the number of sonograms produced by insects and used this as measure of relative abundance. Any nearby non-permanent water presence was recorded as well. These three parameters were recorded once a month for each stationary site.

Using ArcGIS (10.2.2) a land cover map of the Oak Openings Region (Schetter and Root 2011). was overlaid with the coordinates of each sampling point. This map consisted of 15 habitat types (turf, wet prairie, residential, asphalt, pond, savanna, shrub/scrub, swamp forest, conifers, upland forest, floodplain forest, barrens, Eurasian meadow, prairie, and cropland). We created 300m buffers around each transect sampling point, and the percent of each land cover type was calculated. These percentages from the 11 transect points were also averaged to estimate the overall percent of each land cover type for a transect. We also counted the number of each land cover type to get a measure of heterogeneity at each point. Using these data, we also determined which transect point had the largest percent of open and forest land cover types. Similarly, we created 100m buffers around our stationary sites to obtain the same measure of heterogeneity.

Model Creation/Testing

Statistical analyses were conducted using JMP relating total activity (i.e., total number of calls) or diversity (i.e., number of species detected) to individual or sets of the various environmental or landscape variables.

We began by conducting a correlation analysis using a Spearman's rank correlation coefficient test in JMP for nonparametric measures of statistical dependence our explanatory variables. Variables highly correlated with each other ($r > 0.7$, $p<0.05$) were assessed and a subset of variables selected were chosen *a priori.*

Transect-related parameters were assessed in two groups: transect level explanatory variables (instantaneous, maximum, minimum, average, and ranges of temperature, humidity, and wind, moon phase, and % of each of the 15 land cover types at the transect level) and point level explanatory variables (overall, low, and high clutter percent and category, canopy percent and category, land use proportion and categories, water distance, maximum, minimum, and average slope, distance to water, average, minimum, and maximum distance to single trees, tree lines, and tree stands, and % of each of the 15 land cover types at the point scale). Our correlation analysis found a high correlation between maximum, minimum, and average temperature, humidity, wind, slope, and distance to single trees, tree lines, and tree stands, and we choose to use the average values of these parameters. Average, high, and low clutter were also correlated with each other. Here, we chose to use the low clutter parameter (0 – 3m), as it can more easily be manipulated for management practices.

Overnight site variables were run all together since they were all on the same scale. As in our transects, our correlation analysis found a high correlation between maximum, minimum, and average temperature, humidity, and wind. Again, we retained the average values of these parameters. However, unlike our transects, low and high clutter at the stationary sites were not correlated with each other, but both were correlated with average clutter. Therefore, we retained average clutter as a parameter for further analysis.

We also used a stepwise logistic regression in JMP the relationships between bat diversity and combinations of environmental and landscape variables. We conducted tests of the two groups of transect explanatory variables, along with a suite of all of the explanatory variables, with the point-level variables averaged to produce transect-level values (except the point-level land use %, which was already an average at the transect-level) versus the number of bat species detected. Stepwise logistic tests were also conducted for stationary sites. As in the correlation tests, overnight parameters were only organized into the single group of explanatory variables to assess versus bat diversity. We conducted these tests for bats for each month of the sampling period and for the whole sampling period. We also computed principal component analyses (PCA) to visualize the relationships between bat diversity and our explanatory variables. Analyses were conducted separately for transects and stationary sites.

To test for autocorrelation in point level transect parameters (e.g., land cover %, distance to tree features) we conducted a Moran's I test in ArcGIS (10.2.2) for the 300m transect point buffers. If $p<0.05$, and the z-value was positive, it was determined that that parameter was spatially autocorrelated. To combat this problem, the data for the parameters deemed spatially autocorrelated were then subsampled. Instead of using the entirety of the data, three out of the 11 points along each transect were randomly chosen, for our statistical analyses.

To analyze bat activity, nonparametric one-way analyses (Wilcoxon Kruskal-Wallis test) and logistic regression were utilized to analyze the relationships between bat diversity or activity using JMP. These analyses were conducted for each month and for the entire sampling session. We used a Bonferroni correction to account for multiple comparisons, focusing on models that obtained a p-value of 0.0025 or less (considered "highly significant", but reporting all models under a significance value of $p \leq 0.05$.

For atmospheric variables, transect samples were considered independent. Therefore, each month had a total of 24 samples (2 samples per transect) per month (or 96 samples for the entire field season) when creating models for atmospheric parameters. For land cover at the transect level, transect samples were combined into one, creating 12 samples with the total calls from either a single month or over the entire field season. Calls were summed in these analyses since land cover did not change throughout the sampling session. Calls from point level parameters were summed within months and over our sampling session, again, because these parameters did not change during the field season. The only exception was with clutter and canopy measures. The calls were still summed within months, but these measures were only run at the monthly scale since these parameters did change throughout the season.

Bat activity and diversity for stationary sites were analyzed individually, as sites were only sampled once a month. Sites were also regarded as independent when combining data for the whole sampling period (making for 64 total samples).

Results

General Results

Over the moths of May, June, July, and August of 2016, we identified 795 acoustic bat calls on our transects (Table 1.2). Total activity increased as the season continued, May had 54 calls, June had 151 calls, July had 225 calls, and August had 365 calls (Table 1.2). Looking at transects, transect V4 had the largest proportion of calls at 95 (~11%), while H1 had the lowest proportion at 33 calls (~4%) (Table 1.3). Species diversity on transects ranged from 0 – 7 at any single transect sampling period; the average number of species detected on transects ranged from 1.87 species/sampling session (transect H1) to 3.875 species/sampling session (transects V4 and H4) (Table 1.3). The lowest species diversity detected on a transect throughout the sampling session was 5 (transects V3 and H1), while the highest diversity was 7 (transects V4, V5, and H4) (Table 1.3).

Over the moths of May, June, July, and August of 2016, we identified 2856 acoustic bat calls at our stationary sites (Table 1.4). Activity in May was lowest at 229 calls, August was next with 410, July had the second highest activity with 1063 calls, and June had the highest activity with 1154 calls (Table 1.4). The site with the largest proportion of these total calls, and thus the highest amount of bat activity, was GR2 with 517 (~18%) (Table 1.5). The site with the lowest bat activity was SD1, with 4 total calls (<1%). The total diversity of stationary sites ranged from 1 – 7 species (low: RD2; high:CR5, CR6) (Table 1.5). Minimum number of species detected at any overnight on any sampling session was 0 – 4 (low: GR1, RD1, RD2, SD1; high: SD3) species (Table 1.5). Maximum number of species detected at any overnight on any sampling session was 1 – 6 (low: RD1; high: SD3, JF1, CR3, CR6) species (Table 1.5). Average amount of species detected at any overnight site was highest at SD3 (4.8 species/sampling session) and lowest RD2 (0.5 species/sampling session) (Table 1.5).

We found that transect samples with the most forest coverage had a higher amount of bat activity and diversity that those with the most open area. Total bat activity and diversity for August, and over our sampling session was higher in forested as compared to open points (Chi-Square; Rank Sums, $p<0.05$). We also found June activity and July diversity was higher at forested sites (Chi-Square; Rank Sums, $p<0.05$). Stationary sites activity followed this same trend, with total activity in July being higher in forested rather than savanna/open sites (Rank Sums, $p<0.04$). However, diversity was the opposite. We found that diversity in May and August was significantly higher in savanna sites compared to forested ones (Figure 1.5).

Atmospheric parameters

Temperatures ranged from 7.2 to 29.4 degrees Celsius and the wind speed varied from 2.4 to 49.9 kph. See Table 1.7 for atmospheric information over the field season.

We only detected two clear responses in bat activity to temperature (instantaneous, nightly average, and range). Instantaneous and nightly average temperatures significantly affected all total bat activity for the entire field season. Both relationships were positive, indicating increased bat activity at higher temperatures (Rank Sums, $p<0.05$) (Figure 1.6). No significant relationships were detected at the month-long time scale or with temperature ranges.

Similar trends were observed when analyzing bat diversity relationships to temperature factors. Significant effects of temperature on activity were not common at the shorter month-long time scale, but were observed with data from the entire field season. Also, bats diversity was not affected by nightly temperature ranges.

We found bat diversity was positively related to instantaneous temperatures (Chi-Square, $p<0.04$). Similarly, we observed a positive significant relationship between nightly average temperatures and bat diversity (Chi-Square, $p<0.04$).

We found effects of humidity (instantaneous, nightly average, and range) on bat activity and diversity were largely insignificant. The single relationship we identified was with diversity throughout our field season, which had a significant positive relationship with instantaneous humidity (Chi-Square, $p<0.02$).

Wind responses (instantaneous, nightly average, and range) had a single significant on overall bat activity. Over our sampling period, we found that bat activity decreased with increased instantaneous wind speed (Rank Sums, $p<0.05$). Diversity was more affected by wind parameters. First, we observed a significant relationship with total diversity in May, which was positively related to the range of nightly wind speed (Chi-square, $p<0.04$). Alternatively, a decrease in diversity was observed in August (Chi-Square, $p<0.02$)with increasing wind speeds. We found diversity decreased over the field season as instantaneous wind increased (Chi-Square, $p<0.05$). Nightly wind average had the same effect on all season and fell below our Bonferroni correction threshold (Chi-Square, $p<0.0025$).

We did not observe any significant relationships between moon phase and total bat activity or diversity.

Vegetation

Clutter was divided into three categories: low (0 – 3m), high (3 – 6.5m), overall (0 – 6.5m) and scored on a 1 – 6 scale from lowest to highest percent clutterf. Our data analysis found that these categories were highly correlated with each other. Therefore, we will only report on the results from low clutter category. We found that means of bat activity were highest at the lowest clutter category, category 1, for all months sampled. We also found that category 3, the middle category, had the next highest mean (except in June, in which categories 2 and 3 means were the same). Points with a clutter category of 2 was the category with the 3rd highest mean (except in August, which category 4 was slightly higher than 2). Next highest means were recorded at points with clutter category of 4 (except in August, which category 4 was slightly higher than 2). Points with clutter ranked at a category 6 had the next highest means, with the category with the lowest means were points with a clutter of 5 (except in June, in which category

6 was slightly higher than 5). Therefore, the overall trend we saw were means were highest at points with a clutter category of 1, with a decrease in bat activity means at points at a category 2, followed by an increased mean at category 3 points. After that, means decrease again at points with a clutter category of 4, decrease more at category 5 points, and increase slightly at points categorized at a clutter 6 ranking. Trends at clutter categories 5 and 6 may be slightly skewed as samples of these categories were fewer than that of 1 – 4.

Canopy had very little consistent trends total bat activity, but trends were significant for all months (Rank Sums, $p<0.05$). When analyzing canopy at the broad 1 – 6 category scale (without the 0.5 increments), generally, with the lowest canopy category had the highest means, while there were no occurrences of points with a canopy cover measure of 6. It did appear, however, that there was an implied trend of decreased bat activity mean at higher canopy categories. The one significant relationship we found at the more specific scale was with total bat activity in August. These results were almost the opposite of what we found at the broader scale. Average activity of bats increased with larger amounts of canopy (but there is still no occurrence of clutter categories 5.5 or 6).

Roadside Characteristics

Distance to single trees had a single significant relationship with total bat activity and diversity. We found a decrease in overall bat diversity in July (Chi-Square, $p<0.05$) as distance to single tree increased.

All relationships for bat activity and diversity we found in regards to distance to tree lines were negative. August total activity decreased with increased distance to tree lines (Rank Sums,

$p<0.05$). Also, overall diversity in June, August, and over all months decreased as tree lines were situated farther from sampling points (Chi-Square, $p<0.05$).

Our data showed that bat activity was not associated with distance to tree stands. Bat presence and diversity, in contrast, were significantly influenced by distance to the nearest tree stand. Diversity in May, August, and over all months also had negative relationships with tree stand distance (Chi-Square, $p<0.05$).

We found no significant relationships with average slope and with distance to water were not evaluated with overnight data.

Unfortunately, none of these models fell below our Bonferroni correction threshold.

Land Use/Cover

We found total bat activity followed similar trends throughout our field season. We found that bat activity was highest when one side of the road was natural, with the other side being either natural or residential, but not agricultural. June and all season activity was highest at category 4 (one side of the road natural, the other residential), while July and August total activity was highest at category 6 (both sides of the road natural). Whether 6 or 4 was the most active category, the other category was always the second most active. As for the next most active category, June was the only month that had agriculture as part of the category, with one side being agriculture and one residential. The rest of the trends showed that the third most active category for bats was 1, which was both sides of the road categorized as residential. This suggests that bats may avoid agriculture (Figure 1.7).

We found that certain land cover types did influence total bat activity and bat diversity. The percentage of turf in an area, however, was not one of them.

Asphalt, savanna, barren, Eurasian meadow, prairie, and residential percentages, like turf, also had no associations with bat activity or diversity.

Wet prairie percentage, on the other hand, did have one significant relationship. At the point- level, percentage of wet prairie had a significant positive association with total activity in August, and fell below our Bonferroni correction (Rank Sums, $p<0.0025$).

Local amounts of pond had a highly significant negative relationship with total activity during August and over our field season (Rank Sums, $p<0.0025$). We found no relationships with bat diversity or at the transect scale.

We found August total activity increased with increased shrub/scrub coverage at the smaller sampling scale, and was highly significant (Rank Sums, $p<0.0025$). Shrub/Scrub at the transect scale had no significant trends with bats, nor were any trends with bat diversity observed.

Bat diversity did not show any significant relationships with the amount of conifer stands at the point or transect scale. We found, though, that June total activity, August total activity, and all-season total activity was significantly related to this feature at the point scale (Rank Sums, $p<0.04$). Bats in June had a positive relationship while bats in August and from the entire sampling period had more of a negative trend.

Relationships between bats and cropland percentage at the transect level were not observed. However, we did find significant relationships with cropland amount at the point scale, both of which were negative. We found that total activity in August and all-season activity decreased as cropland increased at the point scale (Rank Sums, $p<0.04$; Figure 1.8).

Relationships between bats our three forest cover types had the highest amount of significant relationships between total bat activity and diversity. First, we found, at the point level, swamp forest amount and overall diversity in June were positively significantly related to each other (Chi-Square, $p<0.05$). Also at this scale, total activity in June, August, over our whole sampling period all increased, like with bat presence, with increases in swamp forest coverage (Rank Sums, $p<0.05$). Total activity over the entire field season fell below our Bonferroni threshold (Rank Sums, $p<0.0025$). Oppositely, at the transect scale, our models found bat diversity in August and over our field season decreased with increased swamp forest at this scale (Chi-Square, $p<0.02$).

Next, we observed positive significant relationships between bats and the amount of floodplain forest at the point scale. We found total activity in August and throughout our sampling period increased with more floodplain forest present (Rank Sums, $p<0.05$). The only significant relationship we observed at the transect level in response to floodplain forest amount was August diversity, which exhibited a negative trend (Chi-Square, $p<0.02$).

Finally, our models showed many significant relationships between bats and upland forest percentage, especially at the point scale. At this scale, all relationships we positive. Responses included diversity in June, and total activity in July and over our entire sampling period (Chi-square, Rank Sums, $p<0.04$) We did find one significant negative association with upland forest amount, which was at the transect level, and that was with diversity over the field season (Chi-Square, $p<0.01$). July activity was the only model that was not significant after our Bonferroni correction (Rank Sums, $p<0.04$).

Multivariate Analysis

Our multivariate analyses were only partially successful in in identifying sets of variables that influenced diversity, probably as a result of the heterogeneity within our data. However, our results did suggest that canopy cover and land use were the most influential factors for bat diversity, as they were the factors that were significant most often in our models (Table 1.6).

Principal component analysis (*PCA*)

We conducted a PCA analysis on bat diversity, and chose to evaluate what parameters were associated with high diversity areas (having five bat species). Our PCA for transects showed that principal component 1 explained 18.0% and was negatively related to single tree distance, while principal component 2 explained 23.6% and was positively related to barrens and asphalt (Figure 1.9A). Our PCA for stationary sites showed that principal component 1 explained 18.8% and was negatively related to shrub/scrub coverage and Eurasian meadow, while principal component 2 explained 33.0% and was positively related to swamp forest and floodplain forest (Figure 1.9B).

Discussion/Management Implications

Investigating overall activity or diversity of a group of organisms can give a broad prospective into management of those individuals. This is a technique that may be favorable if the desire is to increase numbers of a group of organisms, or to conserve multiple species that occupy similar guilds or niches. Investigating overall bat activity and diversity in the Oak Openings Region can help inform influences bats in mixed disturbance landscape.

We found a total of 795 calls with our transect sampling and 2856 calls with our overnight sampling. Activity was lowest for both sampling types in May. Transect abundance was highest in August while overnight abundance was highest in June. Overnight sampling detected all eight of our native species, while transects only detected seven.

Total bat activity and diversity throughout the field season increased as the temperature at the beginning of the sampling sessions and average nightly temperature increased (Figure 1.6). This trend is supported by previous studies (Walsh and Mayle 1991; Rydell 1991, Coleman and Barclay 2012), including ones conducted previously in our study area (Sewald 2012; Nordal 2016). We were also able to sample a wide range of temperatures (8.9 – 26.7°C for transects; 7.2 -29.4°C for stationary sites; Table 1.7). Bats are known to be sensitive to lower ambient temperatures, often staying in their roosts when temperatures are lower than 10°C (Sattler et al. 2007; Britzke and Herzog 2009; Townsend 2014), which may explain why bat numbers and richness increased with higher temperatures. Increases in temperature may lead to increased amount of insect activity (Rydell 1991, Coleman and Barclay 2013) and hatches, providing bats increases in foraging opportunities (Musolin and A. Kh. Saulich 2012). Similar trends were found by previous studies in the Oak Openings Region (Sewald 2012, Nordal 2016). Diversity of bats over all months also increased with the relative humidity recorded before each sampling session. This, much like temperature may be a function of easier thermoregulation at these higher humidity levels (Range: 30.0 – 100.0% for transects and stationary sites; Table 1.7). (Willis and Brigham 2007; Reichard et al. 2010; Ben-Hamo et al. 2013). These results were interesting as temperature and humidity were not significantly correlated with each other. This was even more interesting because temperature and humidity are usually correlated (Barreca 2012), including with research in our study area (Nordal 2106). This variability in response is likely to reflect the year to year variability in local climate.

We found four instances were diversity decreased as with increases in wind variables (Range: 2.4 – 49.9kph for transects; 2.4 – 22.5kph for stationary sites; Table 1.7). Being volant organisms, bats can have a hard time maneuvering in high winds, which often leads to a decrease in bat activity when winds are too high (Rydell 1991). In fact, commonly, monitoring of bats only occurs on nights with low (~50 kph) wind to avoid this problem (Britzke and Herzog 2009). We found interesting result, though, that diversity of bats in May increased as nightly wind ranges increased. Bats seem to be less influenced by ranges of atmospheric conditions overnight (there was no significant relationships with temperature or humidity ranges), which may indicated that bats may be opportunistic, being active when conditions are ideal.

We detected only one significant relationship between total bat activity or diversity and our explanatory variables in our overnight sampling within the Oak Openings Preserve, with the only noteworthy models including insects, barrens, and Eurasian meadow (Table 1.5).

We found sampling points with higher amounts of forest areas had higher total bat activity and diversity compared to sites with more open areas. Bats have an affinity for forested areas, so this can explain the trend that we saw (Russ and Montgomery 2002; Fuentes-Montemayor et al 2013; Kalda et al. 2015 (1)). Surprisingly, we found this same trend, with activity at stationary sites, which counters previous findings (Sewald 2012; Janos 2013; Nordal 2016). However, diversity did increase at savanna stationary sites compared to forested ones (Figure 1.5). Sewald (2012) detected a similar trend of bats utilizing the open areas of savanna habitat, including the most abundant species. Sewald (2012), specifically, found that savanna/open stationary sites had more than twice the total activity, had higher activity of 5 of the 8 native species, and, while not significant, a trend towards more species richness. Janos (2013) similarly found that 60% and 89% of all calls occurred in savanna sites depending on sampling method, and up to 7 of the 8 native species used savanna sites preferentially over forest sites. The habitats that made up the open areas on transects versus stationary sites were different, though; open areas at stationary sites were mostly savanna, while transects were mostly cropland. This result suggests that bats will likely respond to these “open” areas differently.

The explanatory variable that had the largest impact on bats was percent forest cover both at our sampling point level and transect level. Our three forest types (i.e., swamp, floodplain, and upland forest) all had more influence than any other parameter we analyzed. We also found that bat activity was highest in natural land covers. This stresses the importance of forests to bats, even if not critical for foraging, directly (Russ and Montgomery 2002; Fuentes-Montemayor et al 2013; Kalda et al. 2015 (1)).

Conifer stands tended to be less utilized bats than deciduous stands, as total activity of our study and in the month of August decreased with amount of conifer coverage. Studies have found that conifer stands may not provide enough roosting opportunities for bats, thus decreasing their activity in these habitats, although certain species may use conifers more than others (Vonhof et al. 2007; Yoshikura et al. 2011). We are unsure as to why June bat activity increased in relation to conifer coverage, but it may have been the result to the types and number of bats recorder during this month compared to August and over our field season.

We found that total activity decreased with increases in the amount of cropland (Figure 1.10). We also found that average total bat activity was lowest at our land use categories associated with agriculture (i.e., categories 2,3, 5; at least one side of the road was agriculture; Figure 1.7). Previous research has suggested that cropland may not be beneficial to bats, as there is an overall lack of natural features (Gehrt and Chelsvig 2003; 2004). However, studies rarely look strictly at bat activity in agriculture, and concentrate their research in the isolated tree stands in these landscapes. These studies, though, encourage habitat management of these forests, as they are used intensely by bats, and to reduce homogeneity of the landscape (Russ and Montgomery 2002; Fuentes-Montemayor et al 2013; Kalda et al. 2015 (1)) Further, different types of agriculture, such as less intensive systems, may help encourage use by bats (Park 2015, Mendes 2017). It is important to note, however, that Nordal (2016) also found an negative trend with agriculture presence/amount and bat activity.

Heterogeneity of habitats at the sample point showed a positive influence on bat activity, but not with bat diversity. One explanation for these results may be that heterogeneity did not differ much between transect points. We found that transect points did, generally, all but four transect points with at least five habitat types in close proximity. High heterogeneity along our transects means that we are sampling bats in a suite of habitat types. While we did not detect a significant relationship between bat diversity and habitat heterogeneity, we can say that it is likely that diversity would increase with increased amounts of habitats in an area, supported by a number of studies that found this trend (Hovestadt et al. 1999; Fahr and Kalko 2011; López Gonzále et al. 2015). While diversity was not significant, more diversity in a landscape can provide more opportunities for different species, or simply more opportunities for survival for individuals (such as different foraging options) (Mendes et al. 2014). However, we cannot eliminate the possibility that the fragmentation of the landscape, and decreased size of patches, may force bats to use "less desirable" portions of the landscape, rather than doing so by choice (Mendes et al. 2014). These explainations are further supported by bats utilizing a mix of natural and urban land use categories, suggesting they favor heterogeneity (Figure 1.7;9).

Bat activity and diversity at the transect level did show any significant relationships with slope of roadside verges. While literature on slope influence on bats is scarce, previous research in our study area found that slope had a high number of, mainly negative, relationships with bats (Nordal 2016). Nordal theorized that slope along roadsides may affect bats as a result of slope impact on water flow (and thus vegetation characteristics) (Forman and Alexander, 1998; Gilbert, E.H., 2002; Toman, 2004), and insect dynamics (Samways et al, 1997; Noordijk et al, 2009). While some roads were sampled in both Nordal's (2016) and our study, we sampled a larger range of roads, so this may explain why we did not find the same results with slope and bats.

The distance to water also did not significantly impact either total bat activity or bat diversity along transects. This is unexpected since previous research, specifically research done in semi-urban areas, like our study area, has found bats often associate with water (Gehrt and Chelsvig 2003; Johnson et al. 2008; Dixon 2012; Li and Wilkins 2014). Also, many of the bat species in the area prefer utilizing areas that are near water (Barbour and Davis 1969; Kurta 1995; Whitaker and Mumford 2009). We hypothesize that a lack of relationship between bats and water distance may a result of the frequent occurrence of water along our transects in our study area. About 88% of our sampling points had permanent water sources within 300m, offering many opportunities for bats.

We found that vertical clutter from 0 – 3m produced a differential response in bats. Looking at the broad 1 – 6 categorical scale (rather than the specific scale with 0.5 increments), average total activity was highest when associated with the lowest category (i.e., 1; low clutter coverage), and vice versa. This suggests that bats prefer more open space to maneuver while foraging, especially since it seems fewer bats (for example, 3 of 8 native species in our area) are specifically adapted to more closed habitats (Sewald 2012). This is especially true for big brown bats (Silvis et al. 2016). This same study, though, found that thinning of a forest, while decreasing bat activity initially, increased activity after a short recovery as compared to unaltered sites (Silvis et al. 2016). Other studies have reported seeing this preference for lower amounts of clutter, especially in lower strata (Titchenell, et al 2011; Kalda et al. 2015 (2), Marques et al 2016).

It would make sense that bat activity and diversity would decrease with increased amounts of canopy. As with vertical clutter, having a thinner canopy would allow bats who are not as well adapted to foraging in closed canopy, like LABO, to better forage in these areas (Elmore et al. 2005). In fact, Sewald (2012), as mentioned previously, determined that more bats native to our area tended to forage in more open areas than in forested areas. Also in our study area, Nordal (2016) found that activity of most species of bats decreased with amount of overstory in an area. These findings support our finding at our broad categorical scale, but not at our specific scale, which exhibited the opposite trend. Breaking our canopy categories into more classes means smaller sample sizes per group. This could contribute to the skew in our results; the higher canopy categories had few samples, but happened to have a high average. This is an area of our study that could use more improvement. Our sampling technique, taking pictures, did not translate well in analysis, sometimes producing images that were too dark or to light to get a very accurate reading on the amount of canopy cover. We would suggest either the use of a light meter or densiometer to increase the accuracy of canopy measurements.

Despite the fact that our models were not highly significant, bat response to tree features has been shown in the literature. Our findings showed that distance to tree stands had more relationships with bats (3) than either distance to tree lines (1) or single trees (1). All parameters had negative relationships with diversity, meaning that diversity decreased as the distance to tree features increased. Research has shown bats are strongly influenced by trees and vegetation structure, including bats in urban areas; a number of studies found strong correlations between bats habitat usage and proximity of forest edge, utilizing areas with closer forests (Gehrt and Chelsvig 2003; Johnson et al. 2008; Dixon 2012; Li and Wilkins 2014). We hypothesize that bats will have more relationships with features that are less prevelant across the landscape. This may explain the difference in the number of relationships between bats and tree lines, stands, and single trees; tree stands were less prevalent than tree lines or single trees across our study area. Tree stands may also be a more valuable feature for bats than either tree lines or single trees; perhaps meaning bats require patches be a certain size before being utilized.

In our study, total bat activity and diversity did not differ between transect, likely because our transects were on a large enough scale (e.g., 10km) that each single transects would likely have had both ideal and non-ideal habitat conditions for bats. Total activity and diversity did, however, did differ at the more local scale sampling points (e.g., 1km). This suggest that both monitoring and management need to be conducted at multiple scales to best understand how features change, and thus their relationships to wildlife.

Our findings have positive implications for managing bats in general in a mixed disturbance landscape. Management suggestions differ slightly when working in preserves, such as the Oak Openings Preserve, or along roadways or in urban/agricultural areas. Our overnight data suggests that bat richness and activity increases in savanna sites, therefore management in these areas should focus on this habitat type, especially as these areas rare and required disturbance for persistence. However, our research clearly shows that bats do use edge habitat, so management should focus on heterogeneity in successional states to provide a variety of edges with both savanna and forest. As an example, outside of the preserve, more bats were more active in forested, less open areas, thus indicating a shift in focus based on landscape context and something that management can be applied to. In other words not all “open areas” are equivalent; agriculture is not likely a suitable substitute for savanna, for example. These results further emphasize that, especially in areas of intense agriculture, habitat heterogeneity, specifically forest fragments (as tree lines and single trees did not appear to be as beneficial to bats as tree stands), should be maintained or integrated within landscapes to benefit bats. In fact, it has been found that bats will actively utilize “urban” forests as much as they do “pristine” forests (Kalka et al. 2015 (2)). Modifying canopy and clutter for wildlife conservation are easy strategies for land managers to employ. Management of low strata clutter would not only benefit bats in the region, but is an especially easy technique to utilize, as the desired results can come from removal of small trees, rather than old growth trees. Generally, we encourage management that promotes heterogeneity across the landscape. We also encourage acoustic monitoring of bats along roads, at least in our study area, continue, as a wide array of habitat types are sampled using this method, and it is clear from our results that bats do utilize roads for foraging. Finally, our results suggest that bats may be able to tolerate high temperatures. This is good news as climate is predicted to change, specifically with increases with thermal ranges. Climate change models predict that maximum daily temperatures in the Midwest may increase 2 - 9°C by 2100, likely in the higher end for summer months, and 20 to 50 more days a year exceeding 32°C (Wuebbles and Hayoe 2004). If bats can tolerate this predicted increase in daily temperatures, bats may be more likely to survive as the climate shifts, thus, more focus can be put towards more crucial threats, such as habitat loss.

Figures

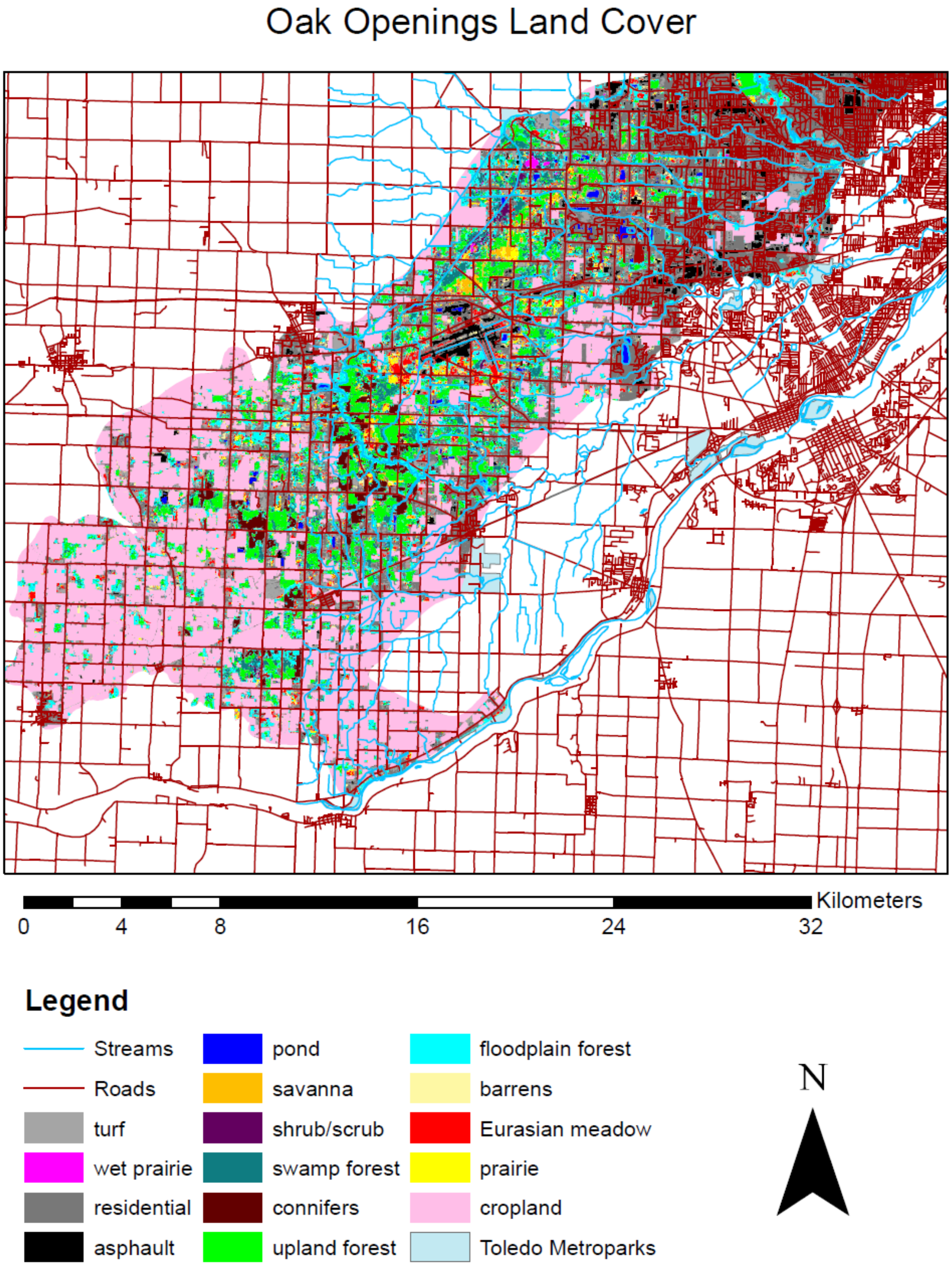


Figure 1.1. Map of the Oak Openings Region land cover, based on a supervised classification by Schetter and Root 2011, showing the 15 land cover classes, streams and roads.

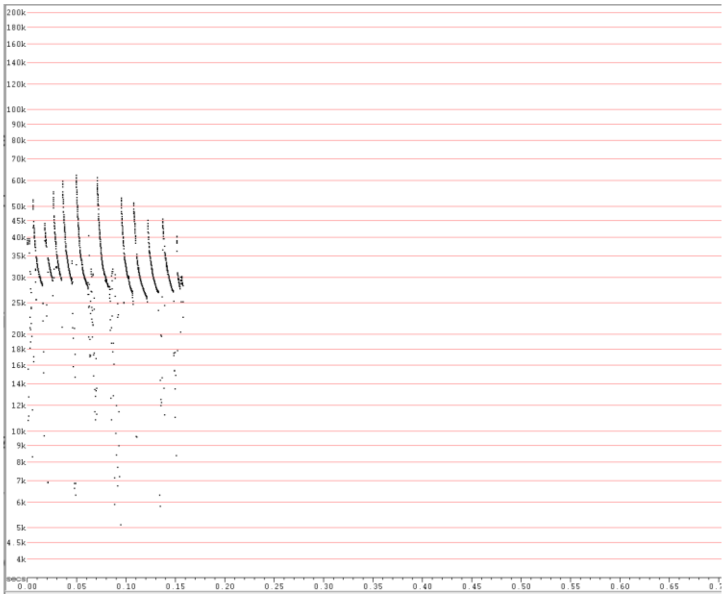


Figure 1.2. Sonogram of a *E. fuscus*. X-axis is time (in seconds), and the y-axis in frequency (in kilohertz).

Figure 1.3. An example of a picture taken in the field to measure vertical clutter.

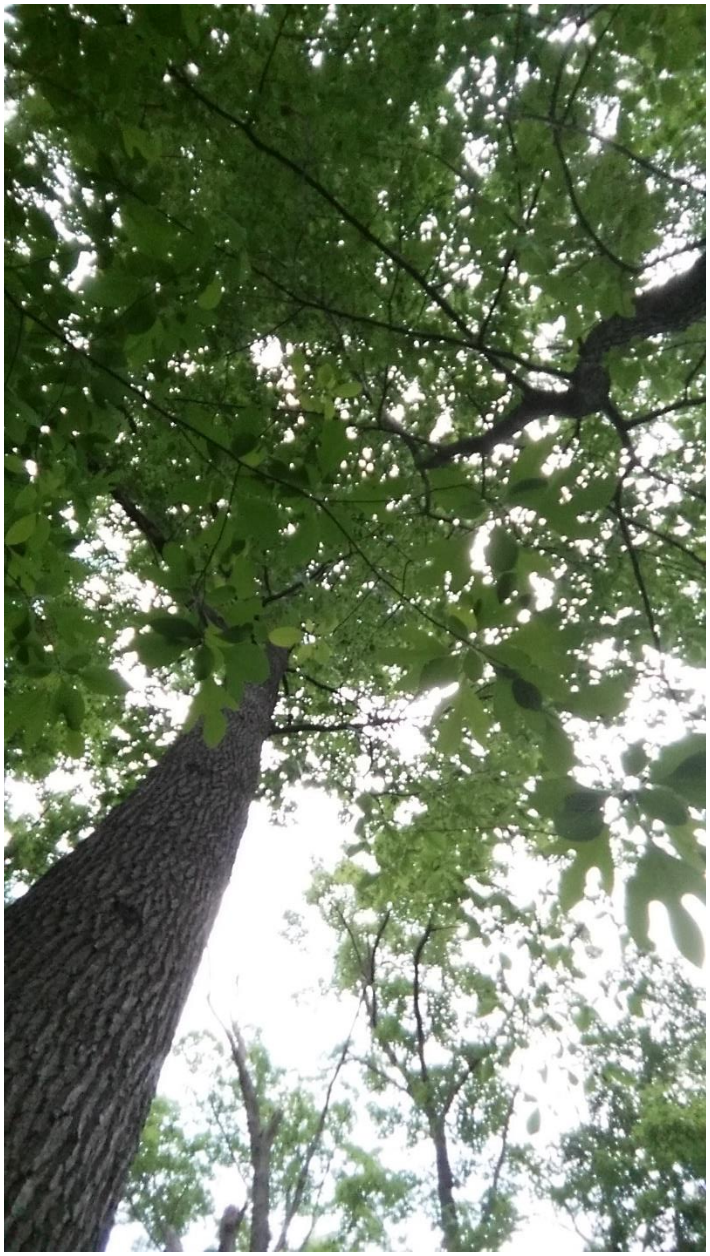


Figure 1.4. An example of a picture taken in the field to measure canopy.

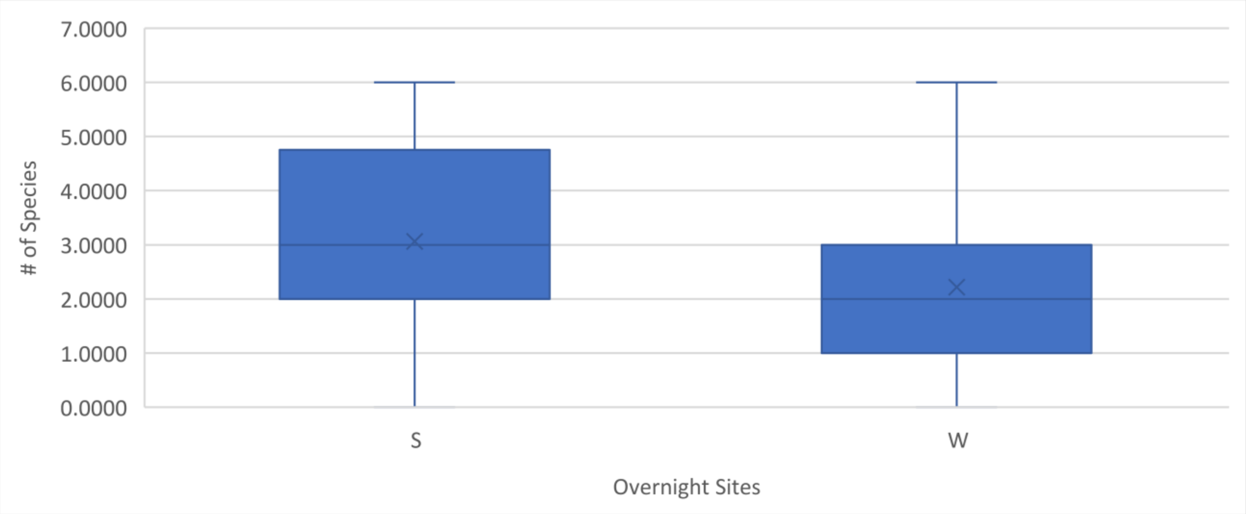


Figure 1.5. Graph showing differences in diversity between savanna and wooded stationary sites. Diversity was generally higher at savanna sites, likely due to the large amount of edge-adapted species in the Oak Openings Region.

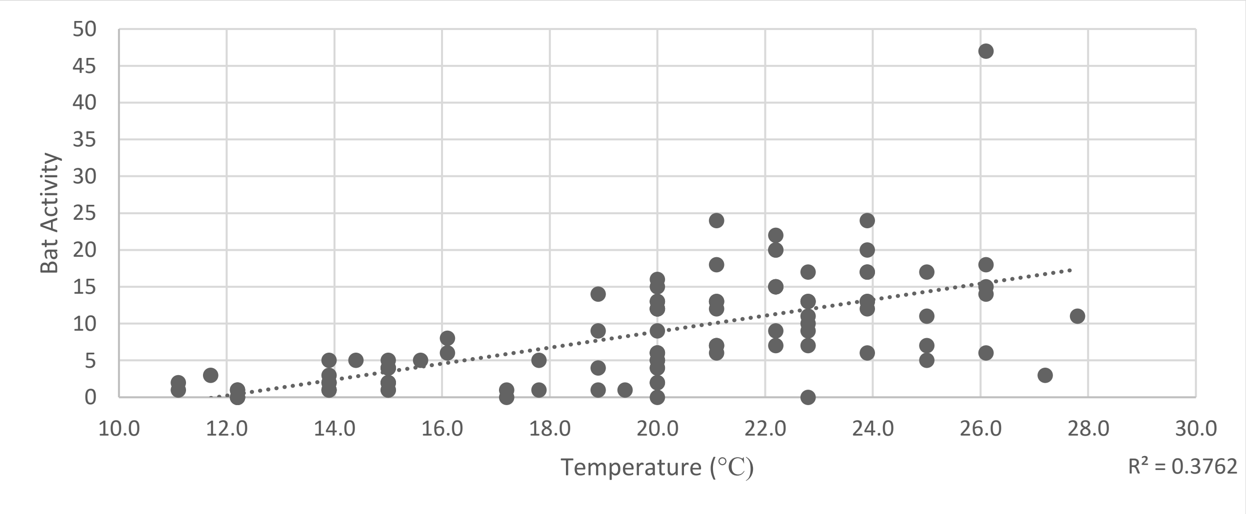


Figure 1.6. Graph showing total bat activity from the entire field season compared to instantaneous temperature. This graph shows the significant positive trend we observed between bats and temperature variables, indicating bats are more active at higher temperatures, possibly due to easier thermoregulation.

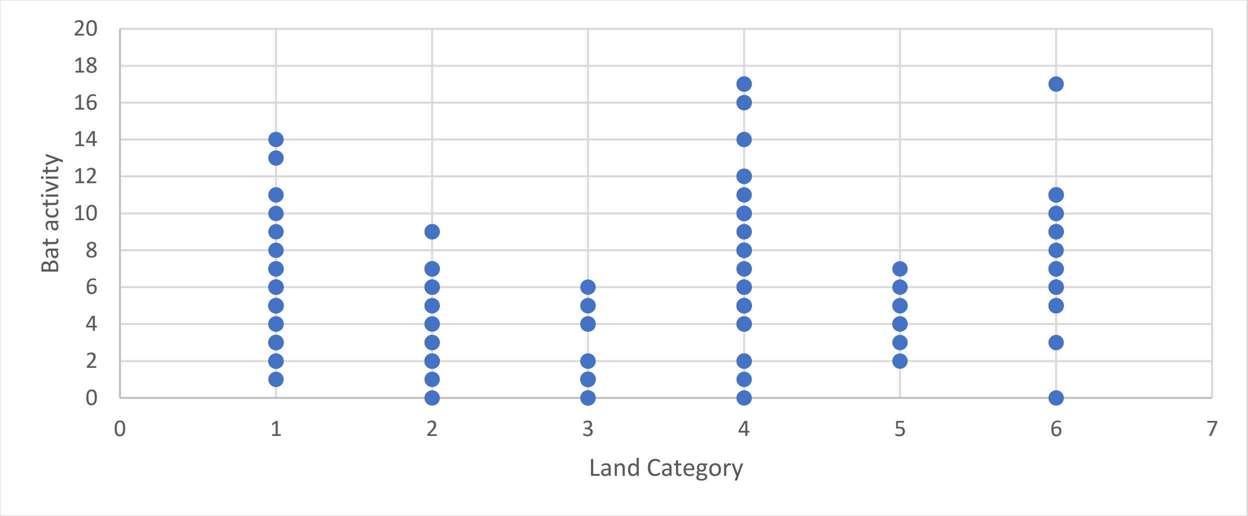


Figure 1.7. Graph showing total bat activity complied from all months at each land use category. Categories are as follows: 1 – both sides of the road urban; 2 – one side urban, one side agricultural; 3 – both sides of the road agricultural; 4 – one side urban, one side natural; 5 – one side agricultural, one side natural; 6 – both sides of the road natural. The graph clearly shows that all three categories associated with cropland (2, 3, and 5) had the lowest activity, with the lowest of those being category 3. In this particular example, activity was actually highest at category 4, rather than 6, indicating bats may prefer heterogeneity between natural and urban habitat.

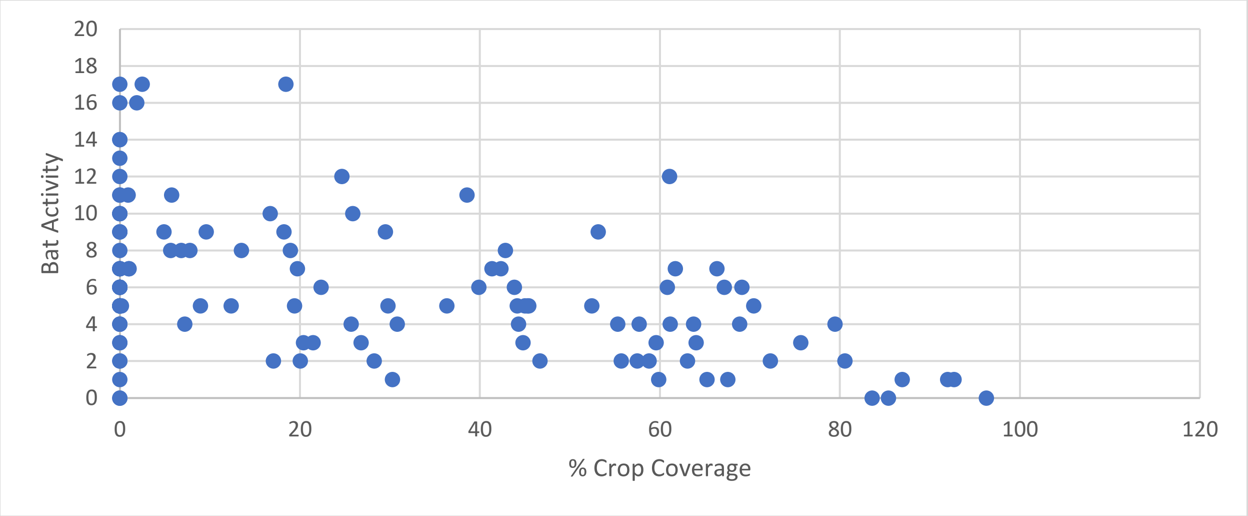
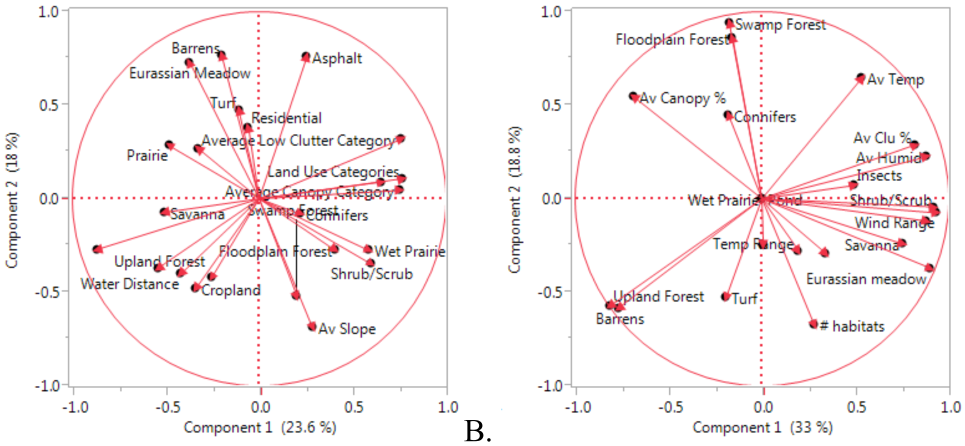


Figure 1.8. Graph showing total bat activity from the entire field season compared to cropland coverage at transect points. This graph shows the significant negative trend we observed between bats and agriculture. This supported our hypothesis that bats don't utilize agricultural areas due to a lack of natural features and resources, like trees and water.



A. B.

Figure 1.9. A. Principal Components Analysis of bat diversity along all transects over the entire field season. B. Principal Components Analysis of bat diversity over all stationary sites over the entire field season. Explanatory variable associations are represented by the line position within the diagram. Explanatory variables included atmospheric and environmental factors.

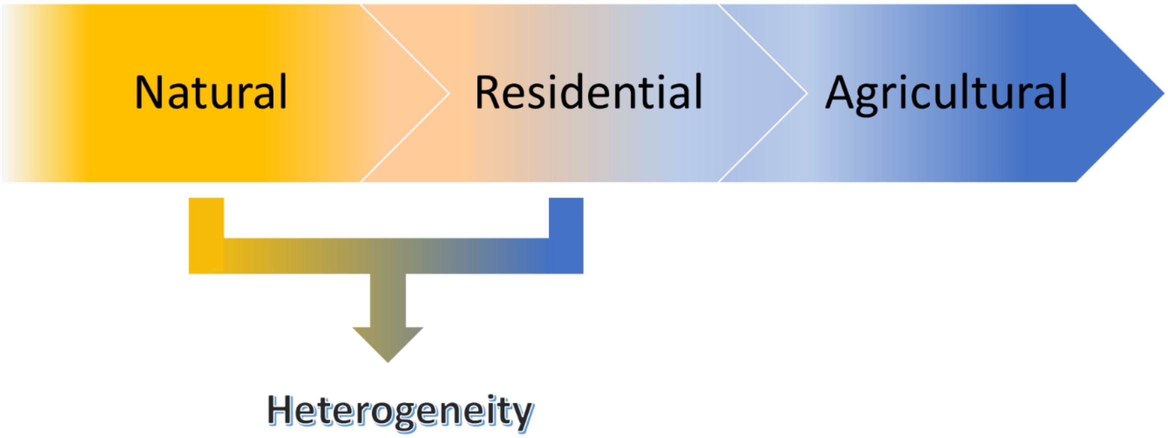


Figure 1.10. Diagram representing bat habitat presence in the Oak Openings Region across a gradient of habitats. Bats most often preferred natural and avoided agriculture. Bats used residential differentially. Some results suggest that bats utilized residential areas more than natural areas. Therefore, we theorized than an element of heterogeneity may be in play with bat natural and residential habitat use.

Tables

Table 1.1. List of the native bat species in the Oak Openings Region, including common and scientific name, their code, and rarity across the region.

Species Common Name	Species Scientific Name	Species Code	Region Rarity
Hoary	*Lasiurus cinereus*	LACI	Common
Big Brown	*Eptesicus fuscus*	EPFU	Very Common
Silver-haired	*Lasionycteris noctivagans*	LANO	Common
Evening	*Nycticeius humeralis*	NYHU	Common
Eastern Red	*Lasiurus borealis*	LABO	Common
Tricolored	*Perimyotis subflavus*	PESU	Uncommon
Little Brown	*Myotis lucifugus*	MYLU	Rare
Long-eared	*Myotis septentrionalis*	MYSE	Rare (Federally Threatened)

Table 1.2. Total number of calls of species and overall bat activity per month and over the entire sampling period for transects. Also shown is the proportion of sampling sessions that had each bat species present.

	LACI	EPFU	LANO	NYHU	LABO	PESU	MYLU	Total
May Activity	4	29	13	0	7	0	1	54
June Activity	27	62	21	8	33	0	0	151
July Activity	44	81	44	30	21	4	1	225
August Activity	73	91	114	38	31	15	3	365
Total Activity	148	263	192	76	92	19	5	795
% Detection	57.3	81.3	64.6	39.6	49.0	14.6	5.2	93.8

Table 1.3. Total number of calls of species and overall bat activity per transects. Div. is the number of species detected on the transect over the entire sampling period. Min Sp. Indicates the fewest number of species detected on that transect at any individual sampling period. Max Sp. Indicates the largest number of species detected on that transect at any individual sampling period. Av. Sp. is the average number of species detected during sampling sessions of a particular transect.

	LACI	EPFU	LANO	NYHU	LABO	PESU	MYLU
V1	16	29	15	3	8	1	0
V2	14	19	15	5	4	1	0
V3	10	32	14	4	6	0	0
V4	9	36	21	10	16	2	1
V5	17	24	23	6	10	1	1
V6	16	25	14	9	10	2	0
H1	8	15	6	3	1	0	0
H2	8	14	8	2	5	3	0
H3	12	17	12	3	7	2	0
H4	12	30	23	7	4	2	3
H5	12	8	11	9	6	3	0
H6	14	14	30	15	15	2	0

	Total	Div.	Min Sp.	Max Sp.	Av Sp.
V1	72	6	1	5	3.375
V2	58	6	1	6	3.000
V3	66	5	1	5	3.000
V4	95	7	0	5	3.875
V5	82	7	0	6	3.375
V6	76	6	0	5	3.250
H1	33	5	0	4	1.875
H2	40	6	2	4	2.750
H3	53	6	0	4	2.750
H4	81	7	1	7	3.875
H5	49	6	0	6	3.000
H6	90	6	1	6	3.250

Table 1.4. Total number of calls of species and overall bat activity per month and over the entire sampling period for stationary sites. Also shown is the proportion of sampling sessions that had each bat species present.

	LACI	EPFU	LANO	NYHU	LABO	PESU	MYLU	MYSE	Total
May Activity	0	91	104	11	20	3	0	0	229
June Activity	13	635	416	44	41	0	4	1	1154
July Activity	29	498	200	231	89	3	9	4	1063
August Activity	18	186	119	32	48	2	4	1	410
Total Activity	60	1410	839	318	198	8	17	6	2856
% Detection	15.6	79.7	70.3	29.7	35.9	12.5	15.6	6.3	85.9

Table 1.5. Total number of calls of species and overall bat activity per overnight site. Div. is the number of species detected on the transect over the entire sampling period. Min Sp. Indicates the fewest number of species detected on that transect at any individual sampling period. Max Sp. Indicates the largest number of species detected on that transect at any individual sampling period. Av. Sp. is the average number of species detected during sampling sessions of a particular transect.

	S/W	LACI	EPFU	LANO	NYHU	LABO	PESU	MYLU	MYSE
GR1	S	5	66	26	0	7	0	1	0
GR2	W	0	300	213	1	1	1	1	0
MON1	W	0	108	75	0	3	1	0	0
MON2	S	17	7	15	2	4	1	0	0
CR7	S	16	18	66	5	5	0	0	0
SD2	W	0	148	29	22	17	0	5	0
SD3	S	0	88	70	67	99	2	2	0
RD1	S	1	51	32	2	5	1	0	0
RD2	W	0	5	0	0	0	0	0	0
JF1	S	6	20	23	1	11	1	0	0
JF2	W	0	136	8	30	1	0	0	0
CR2	S	0	146	193	0	0	0	0	0
CR3	W	0	187	18	73	14	0	4	1
CR5	W	0	59	3	111	27	1	2	4
CR6	S	15	70	66	3	4	0	2	1
SD1	W	0	1	2	1	0	0	0	0

	Total	Div.	Min Sp.	Max Sp.	Av Sp.
GR1	105	5	0	5	2.3
GR2	517	6	2	5	3.0
MON1	187	4	1	3	2.3
MON2	46	6	0	5	3.0
CR7	110	5	2	5	2.8
SD2	221	5	3	5	3.5
SD3	328	6	4	6	4.8
RD1	92	6	2	5	3.3
RD2	5	1	0	1	0.5
JF1	62	6	0	6	2.5
JF2	175	4	1	4	1.8
CR2	339	2	2	2	2.0
CR3	297	6	1	6	3.8
CR5	207	7	1	5	2.3
CR6	161	7	3	6	4.0
SD1	4	3	0	2	0.8

Table 1.6. Results of Stepwise multivariate tests for bat diversity. If the relationship occurred at overnight sites (O) or on transects (T) (specifically looking at transect-level parameters), model parameters ((-) in front indicates a negative relationship), degrees freedom, p-value, RSquare value, and AICc value. Following is the result of the stepwise model looking at transect point-level parameters.

Diversity	Time	T or O	Model	DF	P<0.05	R^2	AICc
	May	T	Av Low Clutter(2); Av Canopy(2); Land Use; Av TL Dist; Av TS Dist; Turf; Asphalt; Shrub/Scrub; Barrens	11		1.00	36.0
		O	Insects; Barrens	2	0.002	0.31	65.4
	June	T	Av Canopy; Land Use(2); Av Slope; Upland Forest	5		0.21	123.3
		O	-	-	-	-	-
	July	T	Av Canopy; Land Use, Av TL Dist; Wet Prairie	4		0.18	147.0
		O	-	-	-	-	-
	August	T	Land Use	1		0.10	156.1
		O	(-)Eurasian Meadow	1	0.0059	0.13	72.5

Transect Point	Model	DF	P<0.05
Diversity	Day #; Temp Range; Inst Wind	3	<0.0001

Table 1.7. Max, Min, and SD of nightly temperature, humidity, and wind of all months at transects and stationary sites.

	Transects				Stationary sites			
	May	June	July	August	May	June	July	August
Temperature Max (°C)	16.7	26.1	26.7	26.1	16.7	26.1	28.9	29.4
Temperature Min (°C)	8.9	12.8	17.8	18.9	7.2	13.3	15.0	16.1
Temperature SD (°C)	2.4	3.2	2.5	2.8	3.1	4.3	3.5	3.5
Humidity Max %	93.0	97.0	90.0	100.0	93.0	97.0	100.0	100.0
Humidity Min %	42.0	30.0	49.0	48.0	42.0	30.0	53.0	48.0
Humidity SD %	19.0	18.8	11.5	18.3	18.3	16.7	13.1	14.5
Wind Max (kph)	20.9	19.3	14.5	49.9	20.9	22.5	19.3	16.1
Wind Min (kph)	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Wind SD (kph)	6.0	6.0	4.3	11.4	6.1	4.2	5.1	4.4

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CHAPTER II. SPECIES-SPECIFIC RESPONSES OF BATS IN A MIXED DISTURBANCE LANDSCAPE.

Introduction

Traditionally, wildlife management has operated on smaller scales, such as focusing on a single, imperiled species (Soulé and Wilcox 1980; Kohm 1990). Since then, more and more, wildlife management plans have shifted to trying to manage multiple species, or even entire landscapes (Hobbs 1994; Tracy and Brussard 1994; Lambeck 1997). This shift is supported by a number of factors (Lindenmayer et al. 2002). Likely the biggest factor, however, is assuming that managing a single species will ultimately benefit the entire ecological community (Lindenmayer et al. 2002; Fischer et al. 2004). This is especially assumed when managing for an "umbrella species"; a species who's needs, when managed for, will benefit not only that species but many associated species in the community (Simberloff 1998). However, it is not guaranteed that all species of the community will benefit, or that all management goals can be achieved, using the umbrella species concept (Simberloff 1998; Rubinoff 2001; Suter et al. 2002). Therefore, while the umbrella species concept may be partially successful in some situations (Suter et al. 2002; Caro 2003), it is not valid for all situations, especially with when conservation involves varied taxa groups (Simberloff 1998; Rubinoff 2001; Suter et al. 2002).

Despite the problems with managing a single species, there are challenges with management on a large scale, e.g., landscapes or suites of species. Focusing generally on landscapes or ecosystem services and processes can hide the complexity within ecological management (Fischer et al 2004). For example, a species can suffer in the interest of maintaining certain landscape features or functions (Lindenmayer et al 2002). While these large-scale process do impact species, influences of local factors are also present, and must be considered Mendes et al. 2017). Managing multiple species can also mean that single species who do, indeed, need more management, may be ignored. Therefore, managers should be encouraged to target multiple scales; local and landscape, focal and suites of species (Lindenmayer et al 2002).

Dornak et al. (2013) provides a good example of studying multiple species, yet keeping in mind the vulnerable species in the area. In this study, Dornak et al. (2013) measured resettlement of Henslow's Sparrows (*Ammodramus henslowii*), but compared it to two other sparrow species. While the focus was on Henslow's Sparrows, a species that has experienced great declines in its population, they were able to compare data from the other birds (Dornak et al. 2013). For example, their management recommendations suggested a focus on Henslow's sparrows, as the abundance of the other two sparrows were significantly higher than that of Henslow's sparrows and they were more generalist in their preferences, making them more responsive to management (Dornak et al 2013).

Research on bats in the eastern United States has combined both single/dual and multi-species approaches. Single or dual-species approaches (Limpert et al. 2007; Baerwald and Barclay 2010), have often revolved around bat species who have experienced dramatic declines in recent years (Frick et al 2010; Dzal et al. 2011; Ehlman et al. 2013). Studies that investigate multiple species often utilize acoustic monitoring (e.g. Gehrt and Chelsvig 2003; 2004). By recording bat calls, Gehrt and Chelsvig (2003; 2004) were able to sample up to eight species in their study area, and then determine total activity and diversity from these measures. Their research revealed important information regarding bats in Midwest USA, such as increased bat diversity, total activity, and certain species' activity in urban areas (Gehrt and Chelsvig 2003; 2004). They also determined that bat activity increased with amounts of forest cover and, at least certain species, of bats were negatively associated with agriculture (Gehrt and Chelsvig 2003; 2004).

Bat acoustic monitoring, as demonstrated above, is an efficient method of surveying bats; providing a way that you can evaluate both rare and more abundant bat species. Analysis of bat call recordings provides identification of bats to species; this analysis is alsooften better at identifying a full suite of species that mist netting may not capture (O'Farrel and Gannon 1999; Ochoa et al. 2000). Acoustic monitoring has several advantages over mist netting, a common bat monitoring technique, including no stress on the bats through physical handling, no altering behavior of bats, and no possibility of disease transfer since the individuals will not be forced into close contact with one another (this is especially important in regards to white-nose syndrome (WNS) (Loeb et al. 2015). Acoustic monitoring provides the opportunity for optimal management: investigating imperiled species, but also gaining knowledge on the other species that will be affected by management, thus ensuring management practices are fully informed to have the lowest negative impact for all organisms involved. This is especially important considering the number of threats bats are facing.

The Oak Openings Region of Northwest Ohio is a biodiversity hotspot that is home to a large number of native species, including many rare ones (Schetter et al. 2013). It is a landscape that has remnant natural areas within a matrix of agriculture and urban areas. Human development continues in the region with intensification of agriculture and urban sprawl from the city of Toledo. This region hosts eight native species of bats for summer foraging, including one threatened species, another with confirmed drastic population declines (Dzal et al 2010), and another with documented declines within our study area (Janos 2013; Nordal 2016). This area has experienced declines in bat activity, and some in diversity, in recent years, especially since

WNS introduction (Sewald 2009; Janos 2013; Nordal 2016). We have also seen evidence of shifts of bat assemblages in our region. This, along with the multiple threats that bats face, especially habitat loss for bats in the Oak Openings Region, validates researching a suite of species, rather than just the rarer bats in our study area.

The goals of this study were to look at native bat species to identify environmental and landscape factors that affect the activity of each of the eight native species. We focused on parameters that affect our most imperiled species (tricolored, little brown, and northern long-eared bats), while keeping in mind the needs of the other bat species in the region. We predicted that species would respond differentially to various response variable within their environment, e.g., canopy cover, due mostly to differences based on life history traits. For example, we predicted that open-adapted species, such as tri-colored bats, would have negative relationships with amount of forest coverage, while forested-adapted species, such as little brown bats, would be positively related. We also predicted that our individual bat species would continuing showing the population trends that we have seen in recent years (Janos 2013; Nordal 2016). For example, we expected tri-colored, little brown, and northern long-eared bats will continue to decline, while big brown bats will still have high activity.

Methods

Acoustic Sampling

Bat calls were collected using an Anabat SD2 acoustic detector (Titley Electronics, Ballina, New South Wales, Australia). An Anabat is a device that records with an omnidirectional microphone, and saves to a memory card, high frequency sounds emitted by bats. The Anabat was set to a sensitivity of 5. Acoustic sampling was not conducted on nights with wind speed over 24kph, temperatures below 10°C, or with a high probability of rain.

We chose to perform large scale acoustic sampling across the region using road transects. Twelve 10km transects were created at random across the study area using public roads. The very northern part of the study area was excluded from the transect layout as it is highly urban and not conducive to the survey method chosen for this study, and most of the study species are not well-adapted to urban-dominated areas. Six transects ran in a north-south direction, while the other six went in an east-west direction. Transects were placed at least 2km apart from other transects running in the same direction to reduce autocorrelation. Transects running in opposite directions did not always meet this rule. However, transects that were not 2km apart were never surveyed one after the other. Transects were surveyed via a car driven approximately 30kph. Up to five transects were surveyed in a single night. To record the calls, detector was secured to a painter's pole and extended out the car window. A handheld GPS (Garmin eTrex) was attached to the Anabat detector, allowing us to obtain GPS coordinates of each bat call as they were recorded by the detector. Transects were surveyed from ½ hour after sunset for three hours. Each transect was sampled twice a month from May – August 2016.

Stationary overnight acoustic monitoring (hereafter stationary sites) was conducted to collect data from core habitat provide an estimate of bat activity and diversity in core foraging habitat as opposed to roadside edges. We set up 16 stationary sampling points in the Oak Openings Preserve in pairs, one point in oak savanna and one nearby in an oak forest. All of these sites have been sampled previously by former researchers (Sewald 2012; Janos 2013; Nordal 2016) and have been categorized as being either "savanna" or "forested" sites. Eight of each type were included in our sampling. All stationary sites were located at least 100m apart, which is beyond the 30 m radius of the detectors. Bat calls were collected using an Anabat SD2 acoustic detector held in a weatherproof station secured to a tree. Detectors were left at two sites (one pair) each night overnight. Stationary sites were run monitored continuously all evening, but we only included bats recorded from 21:00 – 01:00 in analyses to facilitate comparison to transect sampling. Each sampling point was monitored at least once per month from May – August 2016.

Bat Identification

Bat calls were analyzed to species by the author using the software Analook (version 4.1). The calls were double-checked using the software called BATcall ID (BCID) (Allen, version 2.7c). If a discrepancy was encountered, the author made the final determination of the species.

Bat calls were identified to species by looking at various features of the sonogram and comparing to known call libraries (Sewald 2012). Sonograms of the bat calls resembled vertical slashes that spread across a range of frequencies (Figure 2.1). Identifiable features of a sonogram included, but were not limited, to call frequency, overall shape of the call (such as hooked bottoms), and slope of the call (see Appendix II for species-specific sonogram diagnostic characteristics).

Explanatory Variables

Temperature (°C), wind speed (kph), cloud cover (%), relative humidity (%), and starting time was recorded at the beginning of each transect survey. For any night of sampling, either overnight or transect, nightly hourly weather conditions (temperature (°C), wind speed (kph)), relative humidity (%), and incidence of precipitation were recorded from a weather station at

Toledo Express Airport (http://w1.weather.gov/data/obhistory/KTOL.html). Moon illumination (%) was recorded via a website tracking moon phases (http://aa.usno.navy.mil/cgi-bin/aa_moonill2.pl?form=1&year=2016&task=00&tz=-05). Nightly average, maximum, minimum, and range of temperatures, humidity, and wind were the specific atmospheric parameters used in analyses.

Environmental characteristics were measured at fixed sampling points at 1km increments along each transect (hereafter transect points), equaling 11 points along each of the 12 transects. Vertical clutter, which is the density of vegetation at various heights, hereafter clutter, was measured using a 6.5m cloth profile board at 15m (half the range distance of the Anabat detectors) from the edge of both sides of the road. Measures of clutter were done for low (understory; 0 – 3m) and high (midstory; 3 – 6.5m) levels, along with total clutter by taking a photograph of the profile board (Figure 2.2). Canopy cover was also measured at each transect point using a camera held at approximately 1.5m pointing up into the tree canopy (Figure 2.3). Any nearby non-permanent water presence was recorded as well. These three parameters were recorded once a month (May – August) for each transect. Clutter was measured as a percent by using the picture taken in the field to estimate the amount of the profile board that was covered by vegetation.

Canopy cover was also measured as a percent, and the photograph was analyzed using ImageJ. The photograph was converted to a 32-bit greyscale image. A histogram was then made to count the number of different colored pixels. The range of values was 0 – 250, with 0 being 100% black and 250 being almost white. The number of pixels falling in the range of 0 – 100 was calculated and divided by the total number of pixels in the picture to obtain the percent canopy cover. We chose 100 as the threshold as it was the point where darker pixels (representing canopy cover) transitioned to lighter pixels (representing open space).

Clutter and canopy measures were not always obtainable for each transect point, as it would have often required measuring on private land. Therefore, clutter and canopy measures were also estimated via observation. These observations were categorized on a scale from 1 – 6, 1 being no/little cover and 6 being much/full cover. A category was selected based on how many sixths the profile board was covered by vegetation or how much canopy cover was in the picture. Therefore, category 1 was 0 – 16.7% coverage, category 2 was 16.8 - 33.3%, category 3 was 33.4 – 50%, category 4 was 50.1 – 66.%, category 5 was 66.7 – 83.3, and category 6 was 83.4 – 100. All measures that could be calculated as a percent were also assigned a rank to maintain consistency. Clutter and canopy at each transect point were eventually averaged between both sides of the road since we could not detect from which side of the road a bat was detected. This average was on a scale of 1 – 6, but in increments of 0.5 to produce a finer scale.

Clutter and canopy measures were also taken once per month at each of the stationary sites. The only difference was that these parameters were measured 15 m from the detector in four directions: northeast, northwest, southeast, southwest. Also, analyses with these parameters were performed only with percentages, rather than categories, since we were able to successfully sample all sites.

At each transect point, distance to closest natural features and water, along with the slope of any ditch that was present was recorded on either side of the road. Slope of ditches were recorded for both sides of the road using a level app, called Bubble Level (Version 3.12) on a cell phone. Natural features recorded included single trees (trees standing alone), tree lines (a single thin line of trees), or tree stands (a group of trees of considerable depth). Distance was recorded to each of these features up to 300m in the field using a range finder (Nikon Prostaff 3; 6x zoom, measured range 10 – 500m). The maximum, minimum, and average of each measure on either side of the road (average distance to natural features and average slope) were chosen as parameters for analysis. Presence of water was recorded using Google maps (www.google.com/maps). Each transect point was plotted on the map and a measuring tool was used to measure distances to any source of water within 300m. These parameters were not measured at stationary sites.

To get an estimate of land use, both sides of the road at each transect point were categorized as either natural, agricultural, or urban. We calculated the percent of each land use type along each transect. To expand this, we also categorized our sampling points based on land use on a scale from 1 – 6, to take into account total land use at the point rather than evaluating either side of the road individually. Category 1 indicated that both sides of the road were considered residential, category 2 indicated that one side of the road was considered residential and the other agricultural, category 3 indicated that both sides of the road were considered agricultural, category 4 indicated that one side of the road was considered residential and the other natural, category 5 indicated that one side of the road was considered agricultural and the other natural, category 6 indicated that both sides of the road were considered natural. Land cover types were also evaluated at each transect point.

Insect sampling was conducted via sweep netting at our stationary sites. We performed 20 sweeps at heights of 3m and 5m in all directions, sweeping throughout the air for any flying insects, along with sweeping any vegetation, within 15 meters of the overnight site. Because of the very low number of insects collected, these data were not utilized further in the study. However, we did try to quantify the amount of insects in an area utilizing the sonograms recorded by our Anabat detectors. Insects produce a sonogram distinguishable from bats. We therefore counted the number of sonograms produced by insects and used this as measure of relative abundance. Any nearby non-permanent water presence was recorded as well. These three parameters were recorded once a month for each stationary site.

Using ArcGIS (10.2.2) a land cover map of the Oak Openings Region (Schetter and Root 2011) was overlaid with the coordinates of each sampling point. This map consisted of 15 habitat types (turf, wet prairie, residential, asphalt, pond, savanna, shrub/scrub, swamp forest, conifers, upland forest, floodplain forest, barrens, Eurasian meadow, prairie, and cropland). We created 300m buffers around each transect sampling point, and the percent of each land cover type was calculated. These percentages from the 11 transect points were also averaged to estimate the overall percent of each land cover type for a transect. We also counted the number of each land cover type to get a measure of heterogeneity at each point. Using these data, we also determined which transect point had the largest percent of open and forest land cover types. Similarly, we created 100m buffers around our stationary sites to obtain the same measure of heterogeneity.

Model Creation/Testing

Statistical analyses were conducted using JMP relating activity (i.e., total number of calls) and presence/absence (presence represented by a 1, absence a 0) of each to individual or sets of the various environmental or landscape variables.

We began by conducting a correlation analysis using a Spearman’s rank correlation coefficient test in JMP for nonparametric measures of statistical dependence our explanatory variables. Variables highly correlated with each other ($r > 0.7$, $p<0.05$) were assessed and a subset of variables selected were chosen *a priori.*

Transect-related parameters were assessed in two groups: transect level explanatory variables (instantaneous, maximum, minimum, average, and ranges of temperature, humidity, and wind, moon phase, and % of each of the 15 land cover types at the transect level) and point level explanatory variables (overall, low, and high clutter percent and category, canopy percent and category, land use proportion and categories, water distance, maximum, minimum, and average slope, distance to water, average, minimum, and maximum distance to single trees, tree lines, and tree stands, and % of each of the 15 land cover types at the point scale). Our correlation analysis found a high correlation between maximum, minimum, and average temperature, humidity, wind, slope, and distance to single trees, tree lines, and tree stands, and we choose to use the average values of these parameters. Average, high, and low clutter were also correlated with each other. Here, we chose to use the low clutter parameter (0 – 3m), as it can more easily be manipulated for management practices.

Overnight site variables were run all together since they were all on the same scale. As in our transects, our correlation analysis found a high correlation between maximum, minimum, and average temperature, humidity, and wind. Again, we retained the average values of these parameters. However, unlike our transects, low and high clutter at the stationary sites were not correlated with each other, but both were correlated with average clutter. Therefore, we retained average clutter as a parameter for further analysis.

We also used a stepwise logistic regression JMP to test relationships between bat activity, and presence and combinations of environmental and landscape variables. We conducted tests of the two groups of transect explanatory variables, along with a suite of all of the explanatory variables, with the point-level variables averaged to produce transect-level values (except the point-level land use %, which was already an average at the transect-level) versus the number of bats detected. Stepwise logistic tests were also conducted for stationary sites. As in the correlation tests, overnight parameters were only organized into the single group of explanatory variables. We conducted these tests for bats for each month of the sampling period and for the whole sampling period.

To test for autocorrelation in point level transect parameters (e.g., land cover %, distance to tree features) we conducted a Moran's I test in ArcGIS (10.2.2) for the 300m transect point buffers. If $p<0.05$, and the z-value was positive, it was determined that that parameter was spatially autocorrelated. To combat this problem, the data for the parameters deemed spatially autocorrelated were then subsampled. Instead of using the entirety of the data, three out of the 11 points along each transect were randomly chosen, for our statistical analyses.

To analyze bat activity, nonparametric one-way analyses (Wilcoxon Kruskal-Wallis test) and logistic regression were utilized to analyze the relationships between bat presence or activity and each of the explanatory variables using JMP. These analyses were conducted for each month and for the entire sampling session. We used a Bonferroni correction to account for multiple comparisons, focusing on models that obtained a p-value of 0.0025 or less (considered "highly significant", but reporting all models under a significance value of $p\leq0.05$.

For atmospheric variables, transect samples were considered independent. Therefore, each month had a total of 24 samples (2 samples per transect) per month (or 96 samples for the entire field season) when creating models for atmospheric parameters. For land cover at the transect level, transect samples were combined into one, creating 12 samples with the total calls from either a single month or over the entire field season. Calls were summed in these analyses since land cover did not change throughout the sampling session. Calls from point level parameters were summed within months and over our sampling session, again, because these parameters did not change during the field season. The only exception was with clutter and canopy measures. The calls were still summed within months, but these measures were only run at the monthly scale since these parameters did change throughout the season.

Stationary sites run individually, as sites were only conducted once a month. Sites were also kept independent when combining data from all months (making for 64 total samples).

Tests investigating differences in bat activity and presence between individual stationary sites, individual transect sites, and between stationary sites and transects were conducted. Activity of bats was compared between transects using a non-parametric one-way analysis. Activity and presence of bats was compared between open and forested transect sites. Likewise, comparisons were conducted between savanna and forested stationary sites to compare presence and activity of bats at these to habitat types. Finally, transect points and stationary sites were compared to each other by pairing transect points classified as “open” and savanna stationary sites, and then pairing transect points classified as “forested” and forested stationary sites. Overnight data was divided by 12 to obtain a 20-minute average activity value, to match the time length of sampled transect.

Results

General Results

Over the moths of May, June, July, and August of 2016, we identified 795 acoustic bat calls. MYSE was not detected on transects, therefore, has been eliminated from transect analyses. Total activity at the species level ranged from 263 calls (~33%; EPFU) to 5 calls (<1%; MYLU) (Table 2.1). The number of bat calls increased throughout our sampling period, with the least amount of calls in May and the most amount of calls in August (the only outlier being LABO, who had the highest amount of calls in June) (Table 2.1). EPFU was detected most often, being recorded ~81% of all sampling sessions with MYLU only being detected ~5% of the time. Only about 7% of sampling sessions had no bat recorded (Table 2.1). LACI, EPFU, LANO, and LABO were detected during all months. NYHU was not detected in May, PESU was not detect in May or June, and MYLU was not detected in June (Table 2.1). There was no transect that dominated over the others in regards to the number of species calls (Table 2.2). In fact, we found that across transects species specific activity did not differ significantly. The only exception to this was MYLU activity, where 3/5 calls were recorded on one transect (Rank Sums, $p<0.03$). LACI, EPFU, LANO, NYHY, and LABO were present at least once on every transect throughout the sampling period. PESU was detected on all but 2 transects and MYLU was only detected on 3 transects (Table 2.2).

Over the moths of May, June, July, and August of 2016, we identified 2856 acoustic bat calls at our stationary sites. Again, we found that EPFU had the highest activity with 1410 calls (~49%) and the lowest was MYSE with 6 calls (<1%) (Table 2.3). Trends throughout months was less defined than for the transects. However, we found that, generally, May had the lowest amount of activity and July had the highest (Table 2.3). Our two most prevalent species, EPFU and LANO, had their highest activity in June. (Table 2.3). EPFU was detected at ~79% of sampling sessions, while MYSE was only detected ~6% of the time. Approximately 15% of sampling sessions had no bats calls. EPFU, LANO, NYHU and LABO were detected in all months (Table 2.3). LACI, MYLU, and MYSE were not detected in May, while PESU was not detected in June (Table 2.3). EPFU was detected at all stationary sites, while MYSE was only detected at three stationary sites, most species were present at seven or more of the stationary sites (Table 2.4). No noticeable trends in activity were observed, with the highest activity of species distributed across various stationary sites. However, differences were observed when comparing savanna sites to forested sites.

Activity of LABO in May and LACI in August and over the field season was higher in savanna sites that forested sites with the LACI relationship falling within our Bonferroni correction(Rank Sums, $p<0.05$). We found the opposite trend in July with EPFU, where they were higher in forested sites as compared to savanna sites (Rank Sums, $p<0.04$). Savanna also had an increased likelihood of species presence. We found LANO (highly significant) and LABO in May, LACI (highly significant) and LANO in August and LACI over the entire field season were more likely to be present in savanna sites (Rank Sums, $p<0.05$; Figure 2.4).

While we found no difference between transects in general for bat activity and presence, when looking at sampling points dominated by either forest or open habitats, we found the opposite of what we did with stationary sites. We found abundance of bats, specifically EPFU in August, and EPFU, LANO, and LABO throughout our sampling period, increased in forested sites as compared to open ones (Rank Sums, $p<0.05$) along transects. Presence showed a similar relationship. We found EPFU, LANO, and LABO, over all months, were more likely to be present in forested transect points rather than open ones (Rank Sums, $p<0.03$).

Atmospheric Parameters

Temperatures ranged from 7.2 to 29.4 degrees Celsius and the wind speed varied from 2.4 to 49.9 kph. See Table 2.7 for atmospheric information over the field season.

We only detected one clear response in bat activity to temperature (instantaneous, nightly average, and range), at smaller time scales, such as monthly, on our transects, but we did observe some significant trends over the entire sampling period. The only significant relationship on a month-long time scale was in June. EPFU activity was positively related to instantaneous temperature (Rank Sums, $p<0.05$). When looking at bats from all months, nightly temperature ranges had little impact on bats. LACI was the only species that had a response to nightly temperature range; LACI were more likely to be active on nights with a lower range of (e.g., more constant) temperatures (Rank Sums, $p<0.02$). Instantaneous and nightly average temperatures significantly affected all bat species, except for LABO and MYLU. All relationships were positive, indicating increased bat activity at higher temperatures, most of which were highly significant (Rank Sums, $p<0.05$). We found no relationships at our stationary sites with bat species and nightly average temperatures or ranges.

Similar trends were observed when analyzing transect bat presence and diversity relationships to temperature factors. Significant effects of temperature were not common at the shorter month-long time scale, but were observed with data from the entire field season, and LABO and MYLU presence exhibited fewer significant relationships. Also, nightly temperature ranges influenced the fewest number of species and had little effect on overall bat activity. In August, the presence of LABO (Chi-Square, $p<0.03$) and PESU (Chi-Square, $p<0.02$) were positively significantly related to nightly temperature ranges; LABO activity was positively related to nightly temperatures ranges relationship while PESU activity was negatively related.

We found more significant relationships when we examined the entire field season compared to looking at specific months. The likelihood of bat species presence, except LABO and MYLU, was positively related to instantaneous temperature, many of which were highly significant (Chi-Square, $p<0.04$). Similarly, positive significant relationships were observed with nightly average temperatures but here, LABO likelihood of presence also had a significant response, along with the other bat species with, again, many having a significant value below our Bonferroni correction (Chi-Square, $p<0.04$). It is here that we found our one significant overnight species trend with temperature; when considering data from all months, LACI was highly significant more likely to be present with increased nightly average temperatures (Chi-Square, $p<0.01$). Finally, the likelihood that PESU probability of presence decreased as the range of temperatures increased over the field season (Chi-Square, $p<0.02$).

We found effects of humidity (instantaneous, nightly average, and range) on bat activity, presence/absence, and diversity on transects mostly occurred during August and when analyzing the entire field season's data. In August, MYLU activity (Rank Sums, $p<0.03$) and likelihood of PESU presence (Chi-Square, $p<0.02$) were both positively related to instantaneous humidity. Total data from the entire field season showed highly significant relationships with PESU activity (Rank Sums <0.01) and likelihood of being present (Chi-Square, $p<0.01$), MYLU activity (Rank Sums, $p<0.04$) and likelihood of being present (Chi-Square, $p<0.02$) all had significant positive relationships with instantaneous humidity. MYLU activity in August responded to average humidity positively (Rank Sums, $p<0.04$) along with PESU activity from the entire field season (which was highly significant) (Rank Sums, $p<0.0025$). Likelihood of LABO presence (Chi-Square, $p<0.02$) in August, along with the likelihood of all season PESU presence (Chi-Square, $p<0.01$) was also significantly positively related. We also observed a negative relationship between MYLU activity (Rank Sums, $p<0.04$; Figure 2.5), likelihood of PESU presence (Chi-Square, $p<0.02$) and with humidity ranges in August. Alternatively, we saw likelihood of LABO presence (Chi-Square, $p<0.02$) increased with a wider range of nightly humidity. Similarly, LACI had lower activity (Rank Sums, p<0.01), but a highly significant lower probability of presence (Chi-Square, p<0.0025), overall, with increased nightly humidity ranges when considering all months. Bat species or presence at stationary sites were not significant with nightly average humidity and humidity ranges.

Similar to the response for humidity, we only observed bats on transects respond to wind (instantaneous, nightly average, and range) in August and for the complete field season. NYHU activity and likelihood of being present in August (Rank Sums, p<0.03; Chi-Square, p<0.01, respectively) and over the sampling period (Rank Sums, p<0.01; Chi-Square, p<0.01, respectively) was negatively correlated with instantaneous wind. A decrease in probability of presence with LABO (Chi-Square, p<0.02) was also observed in August. We found LACI, LANO, LABO likelihood of presence and EPFU activity decreases over the field season as instantaneous wind increased, with LACI and LANO falling below our Bonferroni correction threshold (Chi-Square/Rank Sums, p<0.05). Negative associations with average nightly wind over the sampling period were seen with the probability of presence of LACI (highly significant), LANO, NYHU and EPFU presence likelihood and activity (both highly significant) (Chi-Square/Rank Sums, p<0.01). Nightly wind range had a differential effect between activity and the likelihood of bat presence. We observed bat activities decreases (August – LAB0, MYLU (Rank Sums, p<0.05)) and presence probability increased with data from the entire sampling period (Chi-Square, p<0.02). We also observed NYHU presence probabilities at stationary sites sites in July and over all months decreased as nightly wind ranges increased (Chi-Square, p<0.02).

We only observed one, not highly, significant relationship between moon phase and bat activity. We found the probability of LABO presence in August decreased the larger the % of the moon that was illuminated (Chi-Square, $p<0.05$). Overnight bats had no significant associations with moon illumination.

Vegetation

Clutter was divided into three categories: low (0 – 3m), high (3 – 6.5m), overall (0 – 6.5m). Our data analysis found that these categories were highly correlated with each other. Therefore, we will only report on the results from low clutter category. When looking at the general scale (without 0.5 increments) there were no significant trends with transect bat species and horizontal clutter. When looking at a more specific scale, we were able to detect a few relationships. LANO bats in June were most active at middle clutter categories (2, 2.3, and 3) (Rank Sums, $p<0.01$). NYHU in July, generally, were more active with higher clutter categories, we saw the same with LANO in August (Rank Sums, $p<0.05$). Clutter percentages had a negative association with all season LACI, EPFU, and LANO present probability at stationary sites; higher clutter percentages decreased the likelihood of presence of these species (Chi-Square, $p<0.02$). No associations were found with overnight bat activity.

Like horizontal clutter, canopy category had no significant relationships with bat species along transects when broken into more general groups, but were noticeable at the finer categorical scale. We found June LANO had above average activity at categories 1.5, 2, 2.5 and especially at 3.5 and 4 (Rank Sums, $p<0.02$), with similar trends of LABO in June (Rank Sums, $p<0.01$). July NYHU activity peaked at canopy categories 2.5, 3.5, and especially 4.5; LABO in July was similar (Rank Sums, $p<0.02$). PESU in July had slightly above average activity at categories 1 and 1.5, but had the highest activity at category 5 (Rank Sums, $p<0.02$). EPFU in August were most active higher clutter categories, but was also above average at medium levels of canopy (Rank Sums, $p<0.03$). LANO activity in August, generally, increased with increased canopy (Rank Sums, p<0.01). The single relationship observe with clutter percentage with overnight bats was a decrease in August LACI activity with increased canopy (Rank Sums, p<0.03).

We found a single association between overnight bats and insect amount; LABO activity in June increased with more insects detected (Rank Sums, p<0.02).

Road Parameters

We detected little influence of slope on bat activity or probability of presence (except for LABO). LABO activity over the entire sampling period increased with increased averaged slope of either side of the road of transect sampling points. (Rank Sums, p<0.04).

Distance to single trees did not have a significant effect on bat activity. However, we found that the likelihood of the presence of EPFU over the whole sampling period decreased with increased distance to single trees, along with a decrease in overall bat diversity in July (Chi-Square, p<0.05).

Distance to tree lines had a greater influence than distance to single trees. All relationships, for bat presence, activity, and diversity, we found were negative. Activity of May LANO and LABO, August LACI, LANO, and total activity, and all season LANO and NYHU activity decreased with increased distance to tree lines (Rank Sums, p<0.05). We also found the likelihood of presence for May LANO, June EPFU, LANO, and LABO, August LACI and EPFU, and EPFU, LANO, and LABO from the whole sampling period decreased with increased tree line distance (Chi-Square, p<0.05). Finally, overall diversity in June, August, and over all months decreased as tree lines were situated farther from sampling points.

Our data showed that, largely, bat activity was not related to distance to tree stands. The single relationship between bat activity and tree stand distance was LABO bats in July (Rank Sums, $p<0.04$) which exhibited a negative trend. Bat presence and diversity, in contrast, was significantly influenced by distance to the nearest tree stand. LANO bats in May, LABO bats in July, LANO bats in August, and LABO and LANO over the entire sampling period had were less likely to be present with increased distance to tree stands (Chi-Square, $p<0.05$)

We did not detect a general effect of distance to water on bat activity on transects. Although, we did observe one negative relationship with the probability of EPFU presence over the entire sampling period (Chi-Square, $p<0.01$).

Land Use/Cover

When evaluating our land cover categories (1 – 6) at our sampling points, we found that bats preferred natural area on at least one side of the road. However, preference differed concerning what the other side of the road was categorized as; some bats preferred natural on the second side as well, others preferred residential. Activity was highest for August LANO and NYHU, along with all season LANO when both sides of the road where categorized as natural (Rank Sums, $p<0.04$). Alternatively, activity was highest at sites with one natural side and one residential side in June for EPFU and all seasons for EPFU and NYHU (Rank Sums, $p<0.03$). It was evident, also, from our data that bats mostly avoided agricultural areas, as agricultural categories were rarely included in the top half of bat preferred sites, with the third most activity category often being 1, which is residential on both sides of the road.

We found a large number of relationships between bats and percent land cover types. Turf at a local (sampling point) scale had no significant associations with bat activity. However, the likelihood of LACI presence in July and from the whole sampling period decreased with increased percentages of turf in an area (Chi-Square, $p<0.04$). At the large (transect) level turf percentage also did not affect activity. However, LACI presence in July showed an opposite relationship at the transect level than it did at the point scale, showing that LACI probability of being present increased with increased turf levels (Chi-Square, $p<0.01$). Alternatively, the likelihood of EPFU presence in June and LABO in August had a positive relationship with percentage of turf along an entire transect. Bats detected at stationary sites had no significant relationships with turf.

Point- level percentage of wet prairie had a significant positive association with NYHU and LABO probability of presence and activity in August, along with their activity over all months (Chi-Square; Rank Sums, $p<0.05$). Most of those relationships were highly significant. Similarly, LANO activity in August and from the entire field season was highly significant (Rank Sums, $p<0.025$). The percentage of wet prairie did significantly influence at the transect scale. No wet prairie habitat occurred within the buffers of our stationary sites.

Asphalt percentages at the point scale showed no associations with bat activity. We did find that LACI in June were more presence, while LANO, overall, were less present with increases in the amount of local asphalt (Chi-Square, $p<0.03$). At the transect scale, NYHU likelihood of being present in July and LACI likelihood of presence in August both had a significant negative relationship with asphalt percentage (Chi-Square, $p<0.01$), and still no significant relationships with bat activity. No asphalt occurred in the area surrounding our stationary sites.

Local amounts of pond had a large number of significant relationships. Bat activity increased for LACI in July, LANO in August and LACI, LANO, and NYHU over our field season, with LANO over the field season falling uder our Bonferroni correction (Rank Sums, $p<0.04$) at the point scale. However, total activity during August and over our field season, overall, had the opposite relationship (Rank Sums, $p<0.02$). All relationships with presence and pond percentage at the smaller scale were positive – LANO in May and NYHU in July and over our whole sampling period were more likely to be present with increased levels of pond coverage. Pond coverage at the transect level showed decreased amount of LABO activity and likelihood of presence in July along with NYHU likelihood of presence in the same month, along with probability of LACI being present in August (Rank Sums; Chi-Square, $p<0.04$). Our stationary sites had no pond coverage within their buffers.

Point-level savanna percentage had significant positive trends with probability of LANO presence in June and NYHU in August (Chi-Square, $p<0.04$). Likewise, activity of LANO in June and NYHU and LABO over our sampling period increased with the amount of savanna in the local area (Rank Sums, $p<0.05$). EPFU activity in June showed mixed trends in response to savanna percentage, with 0, 2, and 4 having lower means, while 1 and 3 had higher means. We only saw a single significant relationship with savanna percentage at the transect level, which was a positive relationship in of LABO probability of being present in June (Chi-Square, $p<0.01$). Savanna coverage had an positive association with PESU activity in July at the stationary sites (Rank Sums, $p<0.04$).

We found NYHU were more present in August and over our field season with increases in the shrub/scrub land cover type at our point scale (Chi-Square, $p<0.0025$). Bat activity followed this positive trend – August activity of LANO, NYHU, LABO, MYLU along with NYHU activity across our entire sampling season, increased with increased shrub/scrub coverage (Rank Sums, $p<0.04$). In all cases, NYHU relationships werestill significant after utilizing the

Bonferroni corrections. Shrub/Scrub at the transect scale had no significant influence on bats. Shrub/Scrub was not associated with overnight bats.

Bat presence was not significant influenced by the amount of conifer stands at the point scale. We found, though, that activity of June EPFU, August LANO, and all-season EPFU and LANO activity was significantly related to this feature (Rank Sums, $p<0.04$). Bats in June had a positive relationship while bats in August and from the entire sampling period had more of a negative trend. The only significant relationship between transect level conifer coverage and bats was a positive association of likelihood of MYLU presence in June (Chi-Square, $p<0.01$). Overnight bats had no significant relationships with conifer coverage.

EPFU probability of being present in August was highly significant and showed a positive trend relating to the amount of barren land cover at our point scale (Chi-Square, $p<0.0025$). LABO bat activity in May showed this same trend (Rank Sums, $p<0.04$). This trend was also seen at the transect level – LABO were more present in June with increases in barren percentage (Chi-Square,, $p<0.01$). However, we observed significant negative relationships with likelihood of LANO presence and PESU activity in July (Chi-Square; Rank Sums, $p<0.05$). We also observed a highly significant relationship for LABO probability of presence at stationary sites which increased with more barren in the area with bats in May (Chi-Square, $p<0.01$).

Our data shows that the number of bats measured over a whole transect was influence more by the proportion of Eurasian meadow than by bats at the point scale. We found, in June, EPFU and LABO and LABO in August were more present with increases Eurasian meadow amount (Chi-Square, $p<0.01$). We did find, at the point level, a positive association with August NYHU probability of presence with Eurasian meadow percentages (Chi-Square, $p<0.4$). No significant trends were observed for bat activity. We found no correlations with Eurasian meadow with overnight bat species.

We observed positive trends with probability of NYHU presence and activity in August and with LABO activity in July in relationship to prairie percentage at our smaller sampling scale (Chi-Square; Rank Sums, $p<0.05$). Alternatively, LABO were less present in August decreased with increased prairie percentages at the transect level (Chi-Square, $p<0.01$). EPFU at stationary sites in July had a highly significant decreased likelihood of presence with more prairie coverage in the area (Chi-Square, $p<0.0025$).

Amount of the residential land cover type at smaller scales was our only parameter to have a significant amount of autocorrelation. To account for this, we subsampled our data rather than creating models of the entire data set. Our subsampled models showed that bat presence was not significantly influenced by amounts of residential land cover. Bat activity only had one significant relationship with residential amount, and that was LANO over our entire sampling period. LANO acitivity showed a differential response depending on the number of LANO detected – 0, 2, and 6 all had slightly lower means, while 1, and especially 3, were above the mean (Rank Sums, $p<0.03$). The only significant relationship with transect-level amount of residential is with likelihood of LABO presence in August, exhibiting a negative trend (Chi-Square, $p<0.01$). We observed an increase in PESU activity with increased residential areas near stationary sites (Rank Sums, $p<0.04$).

Relationships between bats and cropland percentage at the transect level were not observed. However, we did find significant relationships with cropland amount at the point scale, all of which were negative. Likelihood of EPFU presence in June (highly significant), EPFU and NYHU in July, LACI, EPFU, LANO (highly significant), and NYHU in August, and likelihood of EPFU, LANO, NYHU, and LABO presence over our whole sampling session (with LANO and NYHU being highly significant) all decreased with more cropland present (Chi-Square, $p<0.04$). Similarly, we found that activity of EPFU in June, LANO in August, and LANO and NYHU from the entire field season decreased as cropland increased at the point scale (Rank Sums, $p<0.04$; Figure 2.6). There was no cropland cover near the stationary sites.

Relationships between bats our three forest cover types, by far, were the highest in number. First, we found, at the point level, swamp forest amount and the probability of LANO presence in June, likelihood of LANO (highly significant), NYHU, and MYLU presence in August, and probability of LANO, NYHU (highly significant), LABO, and MYLU presence over our entire sampling period were positively significantly related to each other (Chi-Square, $p<0.05$). Bat activity was significant at this scale as well. We observed EPFU and LANO in June, LANO, NYHU and LABO in August, and LANO, NYHU and LABO over our whole sampling period (with LANO and NYHU being highly significant) all increased, like with bat presence, with increases in swamp forest coverage (Rank Sums, $p<0.05$). Our data also showed that NYHU activity in June increased with increases in swamp forest at the transect scale (Rank Sums, $p<0.05$). Alternatively, we found all-season PESU activity at stationary sites decreased with increased amount of swamp forest (Rank Sums, $p<0.03$; Figure 2.6).

Next, we observed positive significant relationships between bats and the amount of floodplain forest at the point scale. NYHU in July, LANO, NYHU, and MYLU in August, and EPFU, LANO NYHU, LABO, and MYLU over our full sampling period were more present with larger percentages of floodplain forest (Chi-Square, $p<0.05$). We also found activity of LANO in May and June, NYHU in July, LANO and NYHU in August and throughout our sampling period increased with more floodplain forest present (Rank Sums, $p<0.05$). In all cases, except NYHU in July, LANO and NYHU model p-values fell below our Bonferroni correction threshold. PESU activity, found for swamp forest coverage, decreased at stationary sites over the sampling period as floodplain forest coverage increased in the area (Rank Sums, $p<0.03$).

Finally, our models showed many significant relationships between bats and upland forest percentage, especially at the point scale. At this scale, all relationships were positive. Responses included likelihood of EPFU (highly significant) and LANO presence in June, likelihood of LACI and LANO presence in July, LANO in August, and LANO and NYHU over our full field season (Chi-square, $p<0.04$). Responses of bat activity included EPFU, LANO, NYHU, and LABO in June, EPFU and LANO in July, LANO in August, and EPFU and LANO for the entire sampling session (Rank Sums, $p<0.04$). At the transect level, we continued observing positive relationships. We found EPFU were more present in June, as well as LABO in August, with upland forest percentage (Chi-Square, $p<0.01$). MYLLU at stationary sites were also positively related to upland forest; MYLU had an increased presence probability in July as upland forest increased (Chi-Square, $p<0.02$).

Heterogeneity did not affect bats detected at stationary sites. Transects, on the other hand, had a number of significant relationships with bat species and number of habitats in an area. All relationships were positive. June presence probability of EPFU (highly significant) and LANO increased with more habitats (Chi-Square, $p<0.02$). LANO activity and likelihood of presence (highly significant), along with EPFU and NYHU (highly significat) presence probability in August also increased (Chi-Square; Rank Sums, $p<0.03$). Finally, all season data indicated that LANO and NYHU activity and likelihood of presence of EPFU and LABO activity all increased as habitat heterogeneity increased, all of which were below our Bonferroni correction threshold expect EPFU (Chi-Square; Rank Sums, $p<0.03$).

Multivariate Analysis

Our multivariate analyses for our transect parameters showed that the amount of clutter and canopy were often significant influences on bat species in general (Table 2.5). Forest cover types were also important for many species, including the at-risk species (Table 2.5). At-risk species also showed some influences from humidity and water distance (Table 2.5). The day number did not have a large influence overall (Table 2.5). Our models for our stationary data had few consistent trends; showing strong influence from a wide array of both atmospheric and environmental explanatory variables (Table 2.5). However, we observed humidity and upland forest having impacts on our declining bat species (Table 2.5).

Principal component analysis (*PCA*)

Table 2.6 shows the first and second principle components of our PCA analysis. Principal component one was often influenced by average clutter and or average canopy for many bat species, while principal component 2 was often related to residential coverage. For example, EPFU presence on transects had average clutter and canopy contributing to principal component 1 (23.6%), and residential contributing to principal component 2 (12.1%) (Figure 2.8A). Focusing on our at-risk species, we found different types of forest cover in an area affected these bats, but largely not our more common species. For example, PESU presence on transects principle component 2 was largely influenced by swamp forest (Figure 2.12A). Finally, we found humidity only affected MYSE (Figure 2.14A).

Discussion/Management Implications

Researching bats in the Oak Openings Region of Northwest Ohio provides insight into bat activity, presence, and diversity in an understudied landscape; one composed of habitats with a range of disturbance levels.

We found a total of 795 calls with our transect sampling and 2856 calls with our overnight sampling. Activity of bat species on our transects were as follows, in decreasing order: 263 EPFU calls, 192 LANO calls, 148 LACI calls, 92 LABO calls, 76 NYHU calls, 19 PESU calls, 5 MYLU calls, and MYSE had no calls. Activity of bat species at our stationary sites were as follows, in decreasing order: 1410 EPFU calls, 839 LANO calls, 318 NYHU calls, 198 LABO calls, 60 LACI calls, 17 MYLU calls, 8 PESU calls, and 6 MYSE calls.

The species that had the highest amount of activity was EPFU, with 2205 of our 3651 total calls for stationary sites and transects. EPFU is known to be a generalist bat species, able to utilize a number of habitat types and foraging strategies (Clare et al. 2014). The prominence of EPFU was similar to what was found in previous research in our area; Sewald (2012), Janos(2013), and Nordal (2016) reported EPFU as their most active species.

The lowest activity from any of our native bat species was from MYLU, with only 5 calls over the entire field season. MYLU used to be a very common species, including in our study area. Sewald (2012) detected a substantial number MYLU both years of her study, and lists it as the second most prevalent species in her 1st sampling year. Janos (2013) and Nordal (2016) found results similar to ours; both found that LANO was the second most prevalent species, like we found, and they also found that MYLU had few calls. MYLU is a bat species that has been especially impacted by WNS and their numbers have been drastically reduced across much of their range (Dzal et al 2010). One study predicted a 99% chance of regional extinction of little brown bats by 2026 if mortality rates remain the same (Frick et al. 2010). WNS has not only affected MYLU, though, but also greatly affects MYSE, our bat species with the fewest calls recorded during our study, and PESU (Blehert et al 2009), the species with the lowest amount of calls in our study area after MYLU. Janos (2013) and Nordal (2016) also found that Myotis species, not MYLU, were low in numbers, declining from Sewald's (2012) numbers. Bat call numbers seem to have dramatically declined at stationary sites, evident when comparing our total overnight calls (2856 calls over 64 nights of sampling) compared to Sewald's (2012) (5455 calls over 28 nights of sampling). These results indicate that, even though not all bats are affected by WNS, all bat species have experienced declines in activity in our study area since 2009 (Sewald 2012; Janos 2013; Nordal 2016), especially in the core protected natural areas. This suggest that it is critical to continue monitoring bats both within and outside of protected areas.

Transects did not differ from each other when considering bat activity, but we found significant relationships when comparing points with more forest or more open habitat. EPFU, LANO, and LABO had increased activity and were more likely to be present in forested sites over more open sites. Open areas along these road transects may not have been ideal for foraging bats, as "open areas" generally consisted of monoculture agriculture. Agriculture has been found to be detrimental to bats. Research has shown that in agriculture-dominated landscapes bats preferentially utilize forest fragments in the area over other land cover types, especially agricultural (Russ and Montgomery 2002; Gehrt and Chelsvig 2003; 2004; Fuentes-Montemayor et al 2013; Kalda et al. 2015). The significant relationships with LANO and EPFU that were detected may have been a result of these species having the highest number of calls. However, it is documented that these species actively use forested areas, with LANO specifically using forest edge, which is more characteristic of what we sampled along roads (Barbour and Davis 1969; Kurta 1995; Whitaker and Mumford 2009). LABO, while also documented as utilizing forest habitats, including forest edge (Barbour and Davis 1969; Kurta 1995; Whitaker and Mumford 2009), are designated as clutter-adapted species (Menzel et al. 2005), and tend to select sites with a large amount of forests (Limpert et al. 2007).

In contrast, we found increased activity and presence of a number of species in savanna stationary sites as compared to forested sites. Significant relationships of activity and presence with savanna were found with LACI, LANO and LABO (Figure 2.4). It is not surprising that we found more LACI in savanna sites, as it is well documented as an open-adapted bat species (Barclay et al. 1985; Menzel et al. 2005). LANO has also been documented using clearings for foraging (Barclay et al. 1985). While LABO actively utilized forest areas, as stated previously, another study found that LABO forage in areas that have low vegetation density (Loeb et al, 2006). Previously, Sewald (2012), Janos (2013), and Nordal (2106) all found that LACI and LANO were more active in savanna sites. Results for LABO were less clear. Sewald (2012) found LABO more in savanna sites, Janos (2013) only found this with one of the survey methods, and Nordal (2016) found no relationship. We did find one bat species more prevalently in forested as compared to savanna sites, which was EPFU. Nordal (2016) found no difference with EPFU and site type, but Sewald (2012) and Janos (2013) did find more EPFU in savanna sites. The difference we found with EPFU may be because, as stated, is a generalist species (Clare et al. 2014), and therefore may utilize sites used less by other species to avoid competition. These trends, in general, may also be attributed to shifts in bat communities from threats such as WNS and from continued loss of habitat in the region.

With WNS having the largest impact on PESU, MYLU, and MYSE, coupled with the fact that these bats were detected the least often in the lowest amounts on both transects and stationary sites, it is important that we explore in depth what parameters influenced these species.

Since MYSE had so few calls, it is not surprising that few relationships were significant. Our PCA, however, showed that MYSE activity was positively associated with humidity. This could be of importance when considering monitoring and the future of MYSE in the face of climate change. The positive significant association MYSE activity and likelihood of presence had with nightly wind range should be taken with caution. While our p-value was significant, the parameter estimates for the model were not. However, this may suggest a trend between nightly wind and MYSE. Therefore, it is something to keep in mind and possibly investigate further. This suggestion is further supported by the fact that MYLU, another critical bat species, activity had a significant, albeit negative, relationship with nightly wind ranges. These findings suggest that management of tree densities should encourage heterogeneity, but mostly favoring thicker stands to reduce wind, to favor bats.

Instantaneous and average temperatures had a positive effect on PESU. However, all bat species, except MYLU and MYSE had similar relationships, indicating that this was less a species-specific trait, but rather something that affects bats in general. Humidity largely positively affected PESU and MYLU activity and likelihood of presence (Figure 2.5), with some, mostly negative, relationships with LABO and LACI activity and likelihood of presence. MYLU, specifically, has been shown to increase activity in response to relative humidity (Lack 1984). Humidity can help with thermoregulation, especially water loss (Willis and Brigham 2007; Reichard et al. 2010; Ben-Hamo et al. 2013). These trends may indicate that select species may have challenges with staying warm and hydrated. Ehlman et al. (2013) found that evaporative water loss, which may be increased if humidity levels decrease, may be a key component in what causes individuals infected with WNS to die. In fact, they state that this water loss, along with slight changes in relative humidity levels in caves, offset increases in survivability that comes when bats cluster (Ehlman et al. 2013). Another study found similar results (Willis et al. 2011). This may be important to PESU and MYLU (and MYSE) who are greatly affected by WNS, as this may be a remnant effects of the disease if they are surviving individuals. Positive relationships with instantaneous and nightly average humidity, but negative ones with nightly humidity ranges, may also suggest that these bats prefer nights experiencing only high humidity levels. This sort of trend was also seen with temperatures for PESU, showing further that, PESU in particular, may be particularly sensitive to lower temperatures (and humidity levels) than other bat species.

Many of the relationships we saw with PESU and MYLU can be explain by their life history traits. PESU is a bat more commonly found foraging in more open areas, while MYLU, in contrast is a more forest adapted bat (Carter et al 1999; Patriquin and Barclay 2003; Broders et al. 2006; Poissant et al. 2010; Ethier and Fahrig 2011; Fabianek et al. 2011). Research has shown that PESU prefer open areas (Carter et al 1999; Poissant et al. 2010; Ethier and Fahrig 2011) while MYLU is more forest adapted (Patriquin and Barclay 2003; Broders et al. 2006 Fabianek et al. 2011). Sewald (2012) found this in her research in our study area. Janos (2013) found no significant relationships with these two species and their habitat preference. However, he found that MYLU analyses suggested a preference with forested sites, and PESU had no preference (Janos 2013). Nordal (2016) could not confirm MYLU preference, but did find that PESU were detected more in open sites than forested ones.

Significant relationships between our explanatory variables and PESU made sense when considering PESU and their preferred habitat. PESU activity was negatively associated with various forest types (Figure 2.6). Ethier and Fahrig's (2011) study on Canadian bats found that PESU, indeed, was less associated with large amounts of forest coverage. Also, PESU activity in our study were negatively related to clutter and canopy amounts. Poissant et al. (2010) also found that PESU tend to roost in areas with lower canopy closure. These relationships favor the idea that PESU are a more open-adapted bat species (Carter et al 1999; Poissant et al. 2010; Ethier and Fahrig 2011).

It is noteworthy that a exception occurred when evaluating PESU activity and clutter; the highest activity occurred at a higher canopy category (category 5). This may have been an artifact of the small sample size in this canopy category. Also, we found that our method of measuring canopy (taking pictures in the field) did not always give accurate results during analysis. Therefore, we suggest that a better methodology be applied to measuring this parameter. One suggestion may be to use a light meter to measure light penetration at sampling sites.

PESU activity was positively related to residential cover at stationary sites. Residential coverage near the preserve consisted of houses with a large number of trees spread throughout the property. It is possible that these areas were open enough for PESU use, while still maintaining the resources needed by PESU (such as insects on the remaining trees). Next, PESU activity had a negative relationship with the amount of barren habitat. This is surprising since barren habitats are characterized as being an open habitat, however, the quality of barrens may differ depending on region. In our study area, barren areas can mean oak barrens, characterized as open stands of oak trees, which can be related to the oak savannas in the area (Kost et al 2007). In this case, PESU may avoid these barrens since there are a number of trees associated with it. However, in contrast to the relationship with residential land cover, barren habitat, while open, may not have other vital features needed by PESU.

MYLU likelihood of presence had positive relationships with all of our forest habitat types. Again, this is not surprising as MYLU is a forest adapted species (Patriquin and Barclay 2003; Broders et al. 2006; Fabianek et al. 2011). Specifically, Fabianek et al. (2011) found this same relationship with *Myotis sp.* In general; activity increased with more forest coverage. We also found positive relationships with MYLU likelihood of presence with conifer coverage, and MYLU activity with scrub/shrub cover amount. This may indicate that MYLU are more generalists in their requirements of tree cover; they may utilize any area that has a number of trees. This is supported by research conducted by Pedro and Simonetti (2013), which showed bats will actively, rather than just passively, utilize commercial pine stands, even when natural forest stands were available. This is similar to our results showing increased MYLU activity with conifer coverage.

Some of our explanatory variables had relationships with a majority of our species. Temperature and wind parameters, distance to tree features (mainly distance to tree lines), and relationships with forests coverage impacted five or more of the region's native species. Habitat heterogeneity, shrub/scrub coverage, and wet prairie coverage also affected four of the eight native species.These results indicate that these relationships are less likely a species level response but something that likely affects eastern North American bat species.

Our results, firstly, reveal the importance to monitoring a suite of species, rather than one or two select species. Mainly, our study showed that, while rare species remained rare, more acommon species, like EPFU, remained more common. It also reinforced the shift in bats that we have seen in recent years (Sewald 2012; Janos 2013; Nordal 2016). Mainly, that MYLU has gone from once being a very common species (Sewald 2012) to a not as common one (Janos 2013; Nordal 2016). Also, that bats that used to not be as active (e.g, LANO) are not very common (Sewald 2012; Janos 2013; Nordal 2016). These shifts are likely attributable to threats to bats, like WNS, forest alterations, and habitat loss.

Humidity levels within bat habitat may be manageable with canopy control within utilized tree stands. Studies have shown that stands with intact canopies, rather than those thinned by management, have higher humidity levels than the alter stands (Sharma and Singh 2006; Brooks and Kyker-Snowman 2008). While some bat species may benefit from a thinning of canopy, keeping thinning to a minimum, or concentrating thinning in patches less used by imperiled bat species, may help aid in their recovery. Heterogeneity in the amount of clutter and canopy would foster a diversity of bat species.

Savanna sites, both in preserves and within their matrix, should be maintained for use by PESU. Savanna sites, especially within the Oak Openings Preserve, may easily be maintained by natural browse or through thinning. McPherson (1997) notes in his book that savannas can be managed by both natural and domestic herbivores, such as prairie dogs and livestock, respectively. Other studies support this claim, both with natural grazers ((small rodents, Weltzin et al 1997) and domestic grazers (cattle – Dumont et al. 2012). Fire, a common management tool in the Oak Openings Preserve for the rare oak savanna, is also a valid tool for maintaining savanna (McPherson 1997; Peterson et al. 2001). Management should focus on quality savanna sites, such as the ones within the preserve, that have more suitable habitat surrounding them. Again, though, heterogeneity in successional states would be likely to favor a wider variety os species.

MYLU utilized forest both in core and edge habitats. Maintaining forest is especially important in areas dominated by agriculture, as these forest patches are sources of refuge in an otherwise mostly unusable landscape. While forest stands are most preferred, especially near large amounts of agriculture, MYLU has shown it will use a variety of tree features, such as shrub/scrub habitats or even conifer stands. MYLU may not seek out specific type of tree features, but instead utilize any that are available to them, meaning that management can be more flexible when it is easier or more within budget constraints.

The differences we detected in PESU and MYLU habitat preference was expected due to differences in the bats physiology and behavior. Management of one species, in a sense, conflicts with management of another. However, this reinforeces the idea that heterogeneity across a landscape is important in supporting multiple species, including those who are imperiled. Using management to integrate both open/savanna and forested sites throughout the region, especially through thinning or maintaining horizontal clutter, will encourage PESU and MYLU use and recovery, along with provide a diversity of opportunities for both other bat species and a wide array of other taxa. Overall, it is important to consider the habitat neds of bats over multiple temporal and spatial scales to increase their viability in the future in missed disturbance landscapes.

Figures

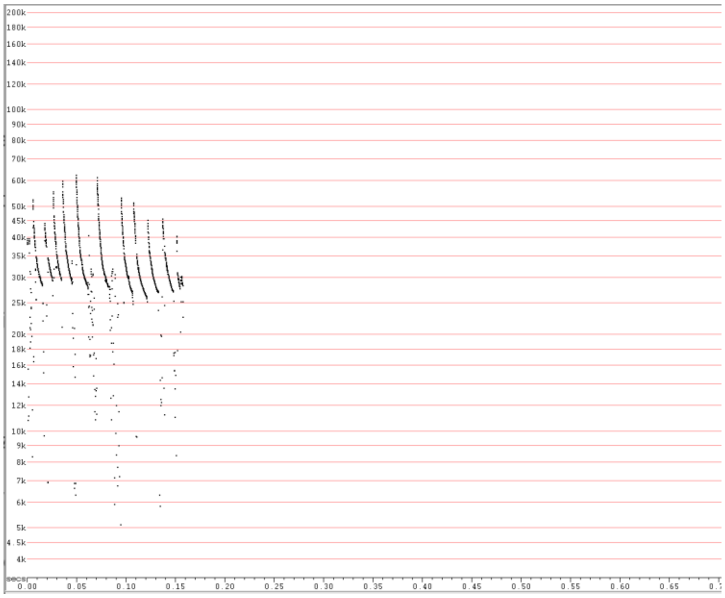

Figure 2.1. Sonogram of a *E. fuscus*. X-axis is time (in seconds), and the y-axis in frequency (in kilohertz).

Figure 2.2. An example of a picture taken in the field to measure vertical clutter.

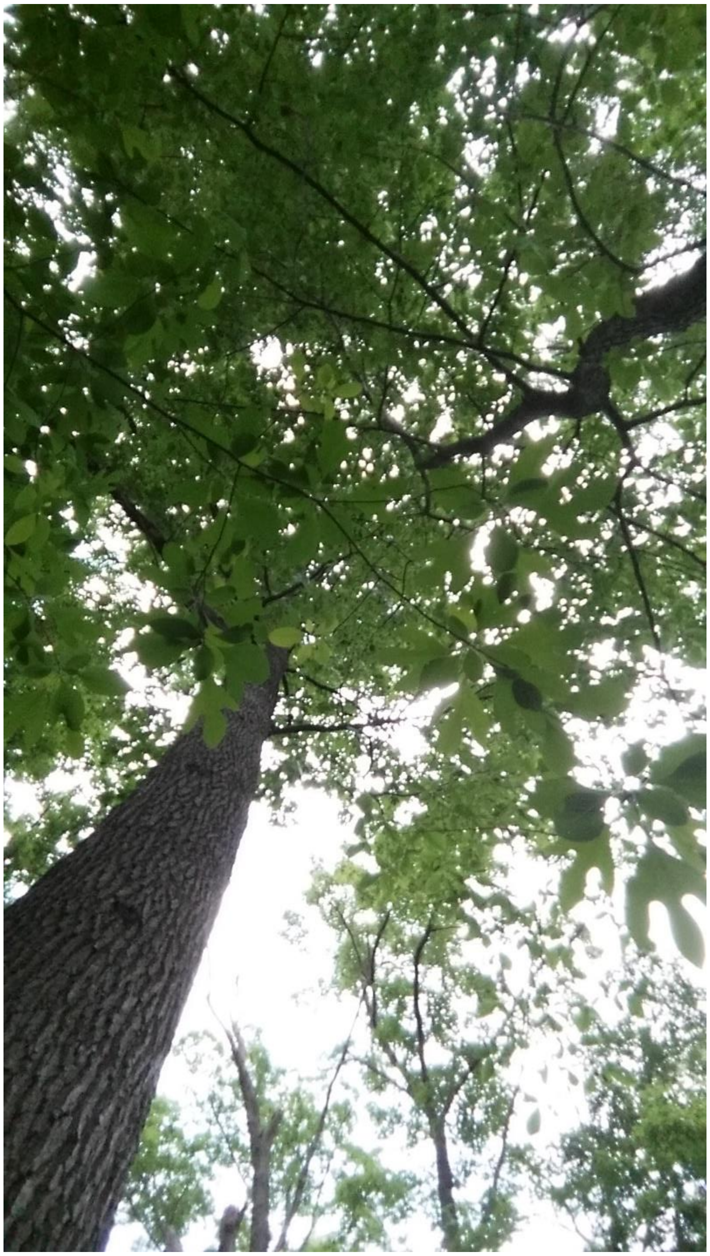

Figure 2.3. An example of a picture taken in the field to measure canopy.

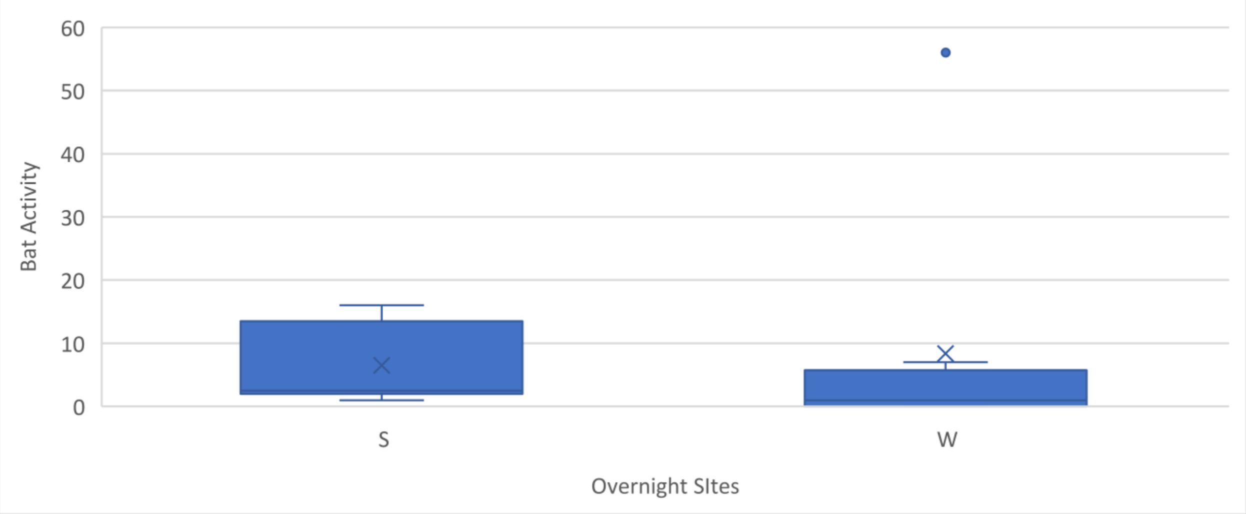

Figure 2.4. Graph showing silver-haired bat activity differences between savanna and wooded stationary sites in June. Activity was generally higher at savanna sites, likely due to the large amount of edge-adapted species in the region, along with a possibility of increased insects.

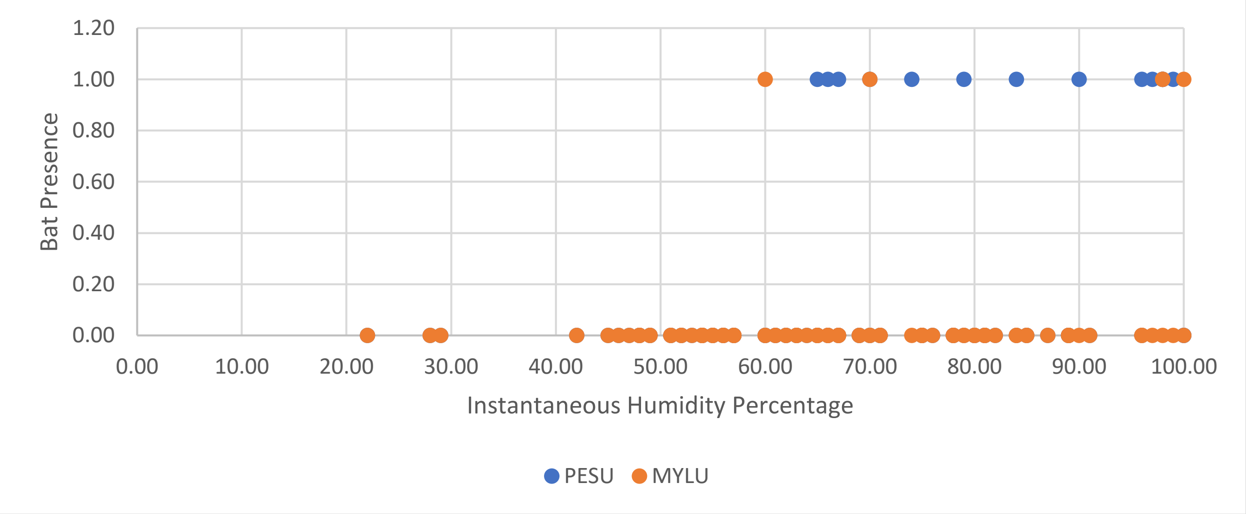

Figure 2.5. Graph showing tricolored bat and little brown bat presence probability compared to instantaneous humidity. This graph shows the significant positive trend we observed tricolored and little brown bats and humidity variables. These results indicate that these species are more active at higher humidity levels, possibly due to easier thermoregulation, which may also be a remnant effect of having once been infected by WNS.

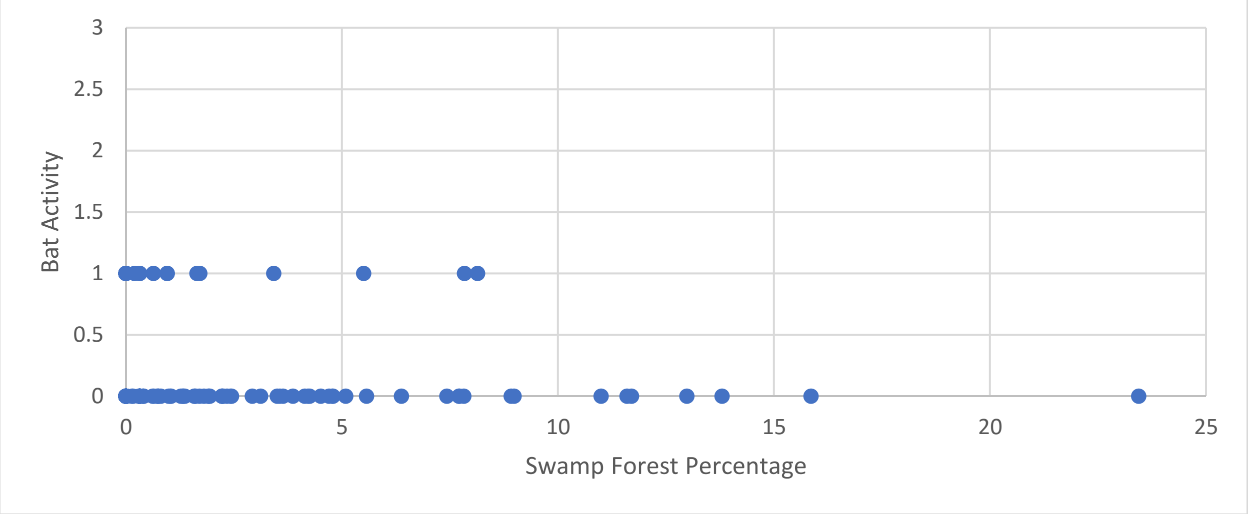

Figure 2.6. Graph showing tricolored bat activity from the entire field season compared to swamp forest coverage at transect points. This graph shows the significant negative trend we observed between PESU and forest coverage, in general. This supports the hypothesis that PESU, an open-adapted species, dose not utilize forested areas as much as open areas, such as savannas.

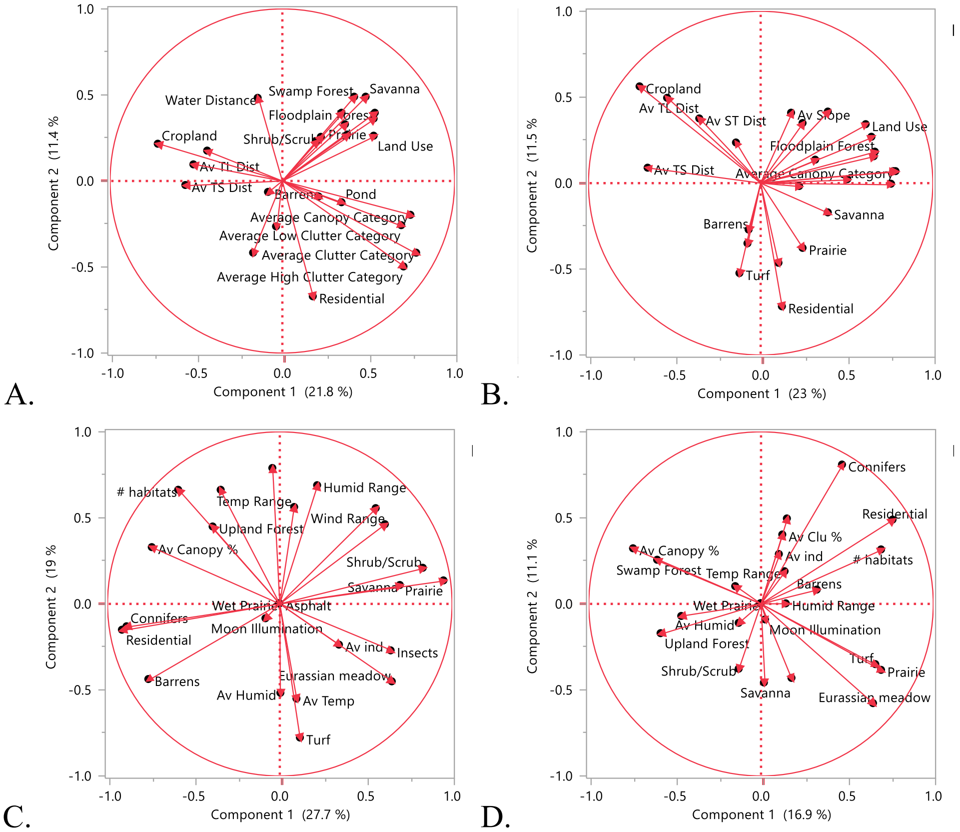

Figure 2.7. A. Principal Components Analysis of LACI presence along all transects over the entire field season. B. Principal Components Analysis of LACI absence along all transects over the entire field season. C. Principal Components Analysis of LACI presence among all overnight sites over the entire field season. D. Principal Components Analysis of LACI absence among all overnight sites over the entire field season. Explanatory variable associations are represented by the line position within the diagram. Explanatory variables included atmospheric and environmental factors.

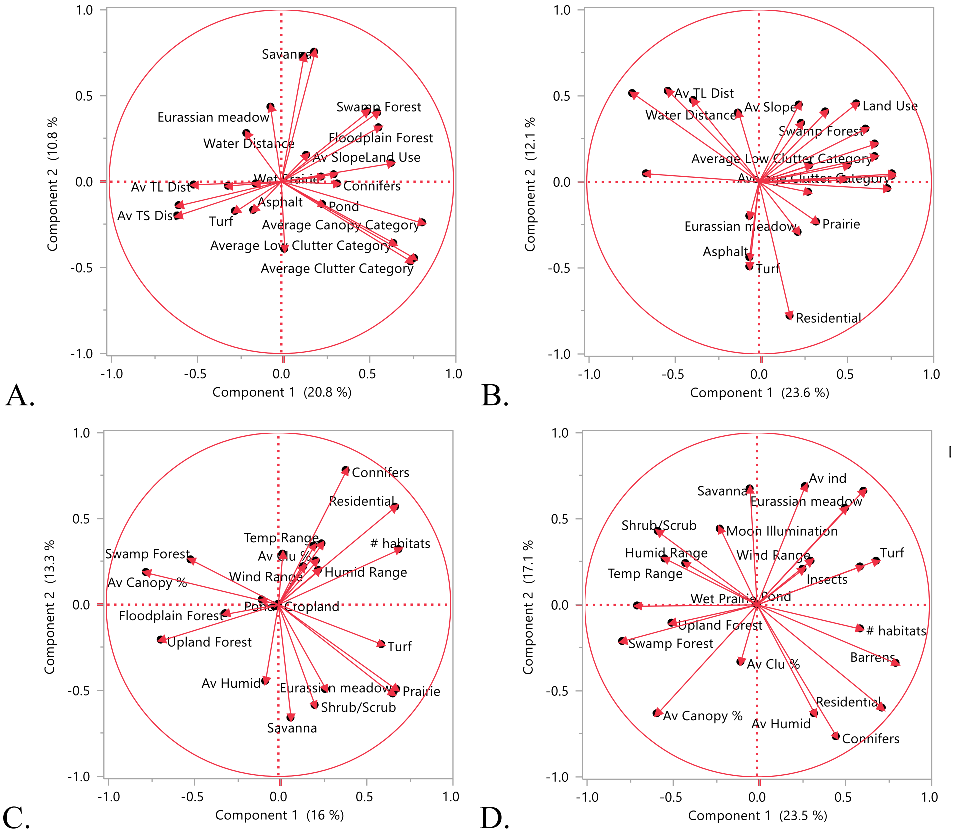

Figure 2.8. A. Principal Components Analysis of EPFU presence along all transects over the entire field season. B. Principal Components Analysis of EPFU absence along all transects over the entire field season. C. Principal Components Analysis of EPFU presence among all overnight sites over the entire field season. D. Principal Components Analysis of EPFU absence among all overnight sites over the entire field season. Explanatory variable associations are represented by the line position within the diagram. Explanatory variables included atmospheric and environmental factors.

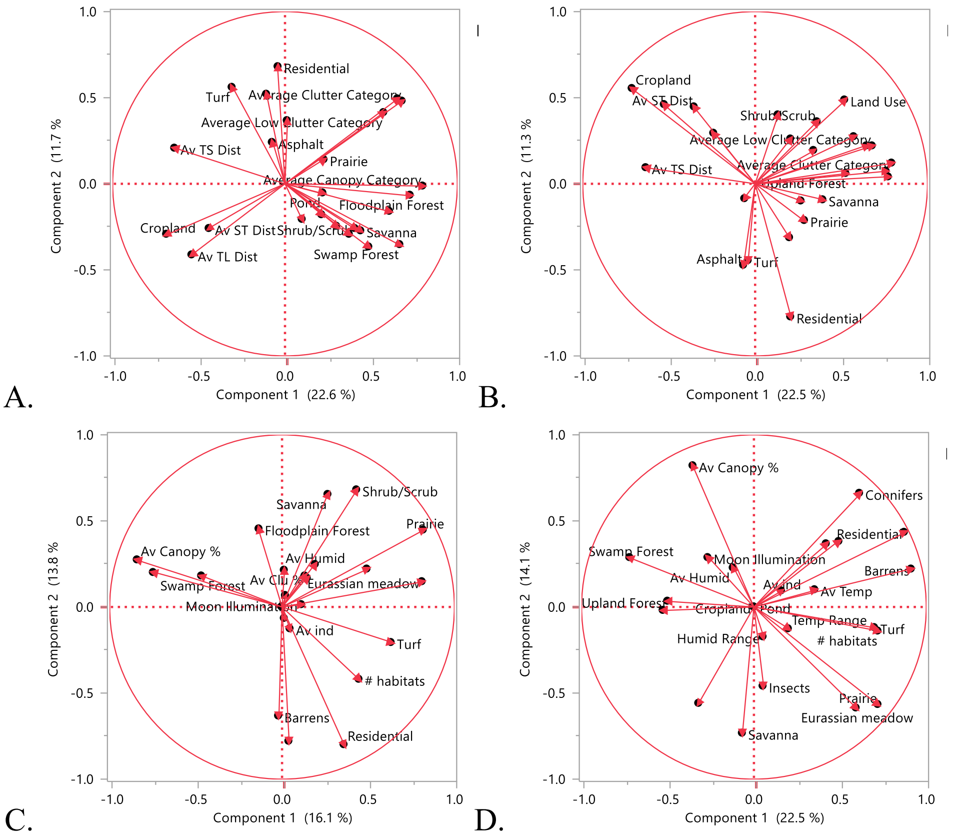

Figure 2.9. A. Principal Components Analysis of LANO presence along all transects over the entire field season. B. Principal Components Analysis of LANO absence along all transects over the entire field season. C. Principal Components Analysis of LANO presence among all overnight sites over the entire field season. D. Principal Components Analysis of LANO absence among all overnight sites over the entire field season. Explanatory variable associations are represented by the line position within the diagram. Explanatory variables included atmospheric and environmental factors.

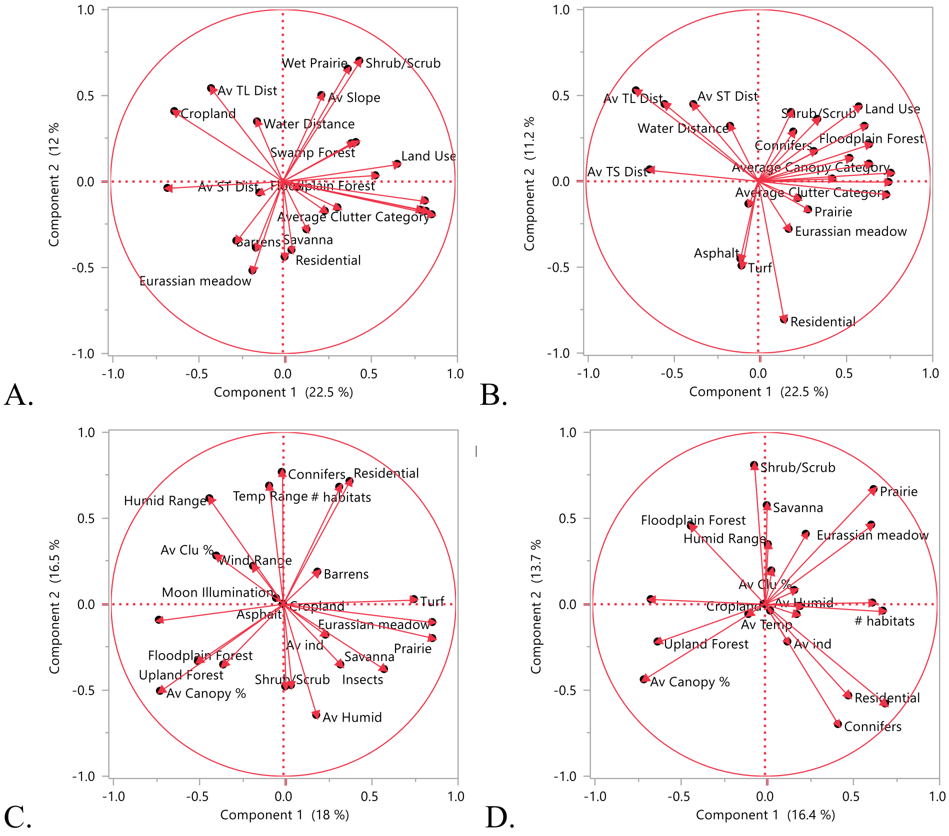

Figure 2.10. A. Principal Components Analysis of NYHU presence along all transects over the entire field season. B. Principal Components Analysis of NYHU absence along all transects over the entire field season. C. Principal Components Analysis of NYHU presence among all overnight sites over the entire field season. D. Principal Components Analysis of NYHU absence among all overnight sites over the entire field season. Explanatory variable associations are represented by the line position within the diagram. Explanatory variables included atmospheric and environmental factors.

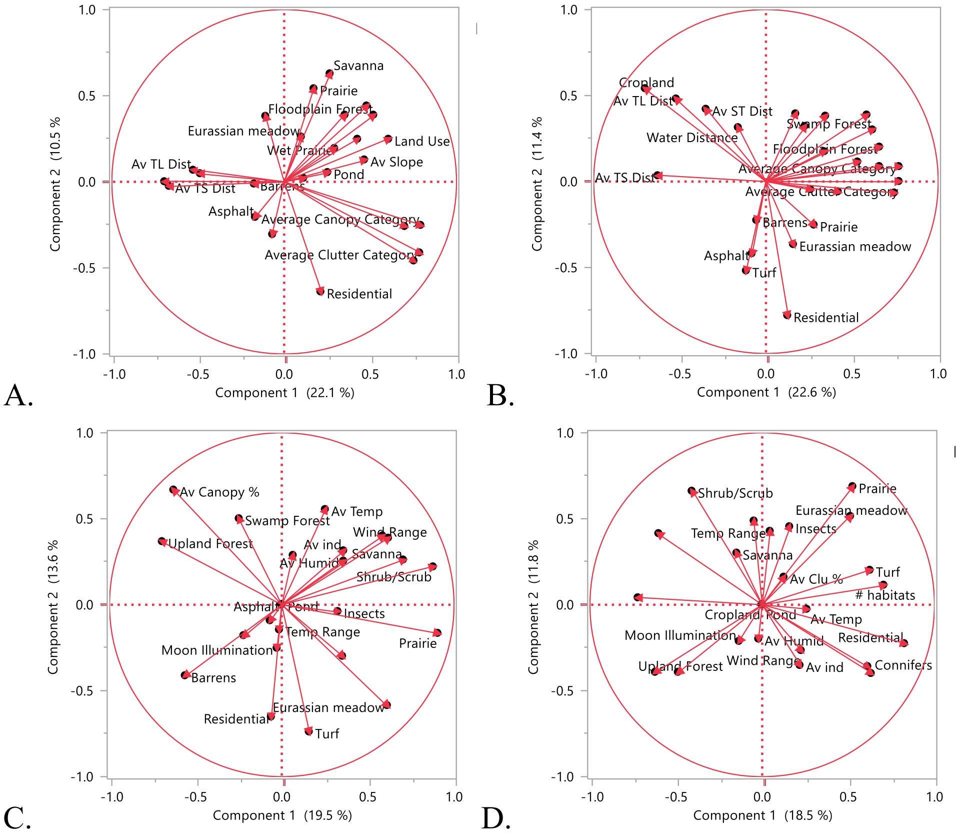

Figure 2.11. A. Principal Components Analysis of LABO presence along all transects over the entire field season. B. Principal Components Analysis of LABO absence along all transects over the entire field season. C. Principal Components Analysis of LABO presence among all overnight sites over the entire field season. D. Principal Components Analysis of LABO absence among all overnight sites over the entire field season. Explanatory variable associations are represented by the line position within the diagram. Explanatory variables included atmospheric and environmental factors.

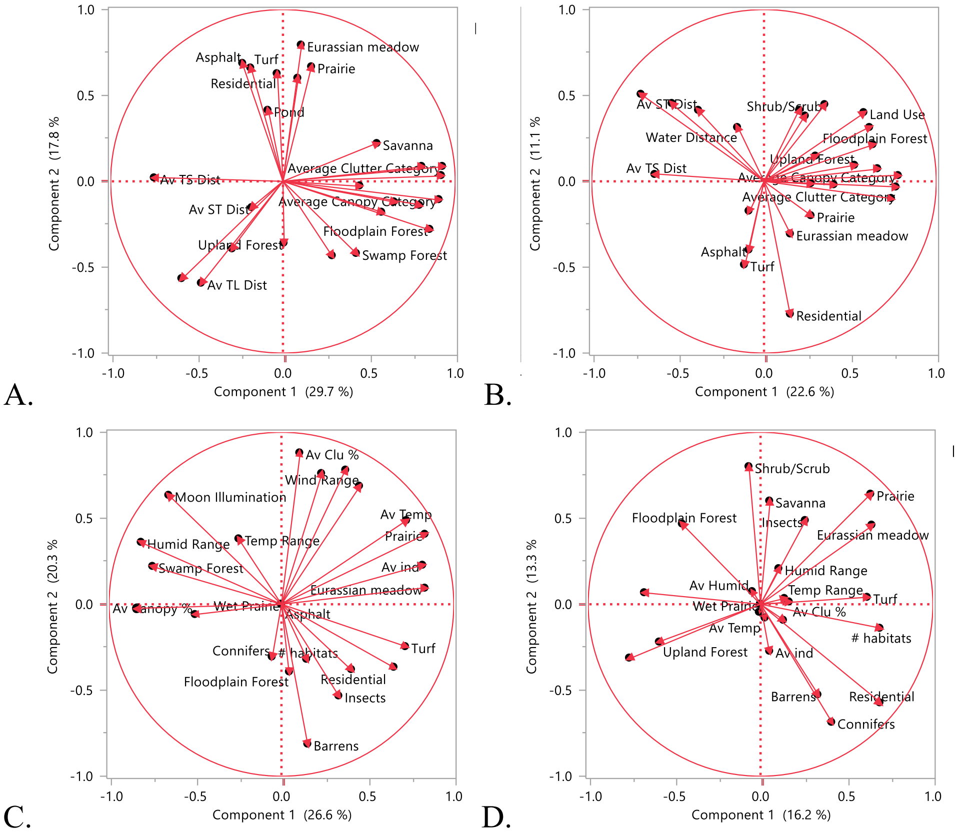

Figure 2.12. A. Principal Components Analysis of PESU presence along all transects over the entire field season. B. Principal Components Analysis of PESU absence along all transects over the entire field season. C. Principal Components Analysis of PESU presence among all overnight sites over the entire field season. D. Principal Components Analysis of PESU absence among all overnight sites over the entire field season. Explanatory variable associations are represented by the line position within the diagram. Explanatory variables included atmospheric and environmental factors.

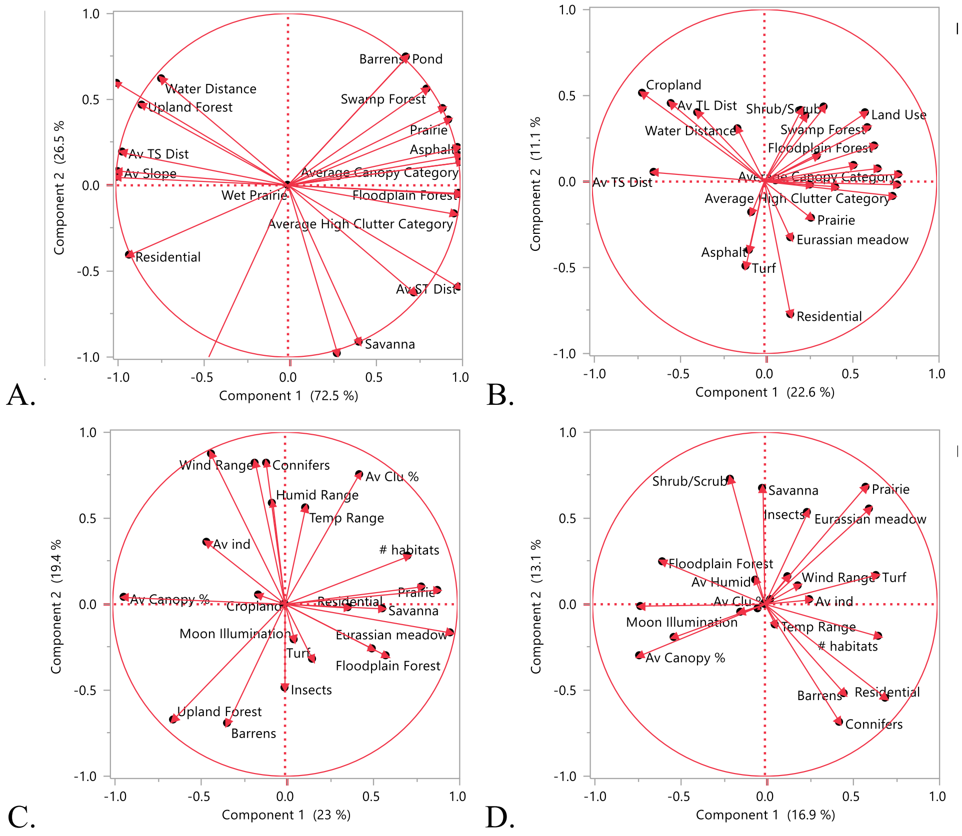

Figure 2.13. A. Principal Components Analysis of MYLU presence along all transects over the entire field season. B. Principal Components Analysis of MYLU absence along all transects over the entire field season. C. Principal Components Analysis of MYLU presence among all overnight sites over the entire field season. D. Principal Components Analysis of MYLU absence among all overnight sites over the entire field season. Explanatory variable associations are represented by the line position within the diagram. Explanatory variables included atmospheric and environmental factors.

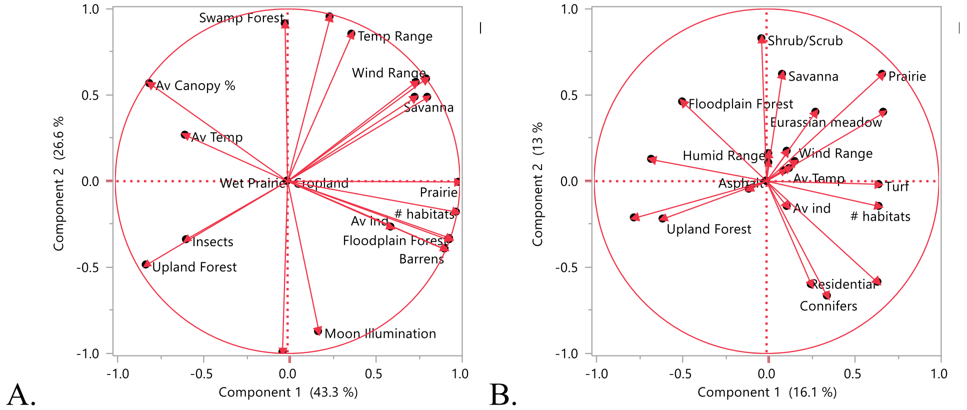

Figure 2.14. A. Principal Components Analysis of MYSE presence among all overnight sites over the entire field season. B. Principal Components Analysis of MYSE absence among all overnight sites over the entire field season. Explanatory variable associations are represented by the line position within the diagram. Explanatory variables included atmospheric and environmental factors.

Tables

Table 2.1. Total number of calls of species and overall bat activity per month and over the entire sampling period for transects. Also shown is the proportion of sampling sessions that had each bat species present.

	LACI	EPFU	LANO	NYHU	LABO	PESU	MYLU	Total
May Activity	4	29	13	0	7	0	1	54
June Activity	27	62	21	8	33	0	0	151
July Activity	44	81	44	30	21	4	1	225
August Activity	73	91	114	38	31	15	3	365
Total Activity	148	263	192	76	92	19	5	795
% Detection	57.3	81.3	64.6	39.6	49.0	14.6	5.2	93.8

Table 2.2. Total number of calls of species and overall bat activity per transects. Div. is the number of species detected on the transect over the entire sampling period. Min Sp. Indicates the fewest number of species detected on that transect at any individual sampling period. Max Sp. Indicates the largest number of species detected on that transect at any individual sampling period. Av. Sp. is the average number of species detected during sampling sessions of a particular transect.

	LACI	EPFU	LANO	NYHU	LABO	PESU	MYLU
V1	16	29	15	3	8	1	0
V2	14	19	15	5	4	1	0
V3	10	32	14	4	6	0	0
V4	9	36	21	10	16	2	1
V5	17	24	23	6	10	1	1
V6	16	25	14	9	10	2	0
H1	8	15	6	3	1	0	0
H2	8	14	8	2	5	3	0
H3	12	17	12	3	7	2	0
H4	12	30	23	7	4	2	3
H5	12	8	11	9	6	3	0
H6	14	14	30	15	15	2	0

	Total	Div.	Min Sp.	Max Sp.	Av Sp.
V1	72	6	1	5	3.375
V2	58	6	1	6	3.000
V3	66	5	1	5	3.000
V4	95	7	0	5	3.875
V5	82	7	0	6	3.375
V6	76	6	0	5	3.250
H1	33	5	0	4	1.875
H2	40	6	2	4	2.750
H3	53	6	0	4	2.750
H4	81	7	1	7	3.875
H5	49	6	0	6	3.000
H6	90	6	1	6	3.250

Table 2.3. Total number of calls of species and overall bat activity per month and over the entire sampling period for stationary sites. Also shown is the proportion of sampling sessions that had each bat species present.

	LACI	EPFU	LANO	NYHU	LABO	PESU	MYLU	MYSE	Total
May Activity	0	91	104	11	20	3	0	0	229
June Activity	13	635	416	44	41	0	4	1	1154
July Activity	29	498	200	231	89	3	9	4	1063
August Activity	18	186	119	32	48	2	4	1	410
Total Activity	60	1410	839	318	198	8	17	6	2856
% Detection	15.6	79.7	70.3	29.7	35.9	12.5	15.6	6.3	85.9

Table 2.4. Total number of calls of species and overall bat activity per overnight site. Div. is the number of species detected on the transect over the entire sampling period. Min Sp. Indicates the fewest number of species detected on that transect at any individual sampling period. Max Sp. Indicates the largest number of species detected on that transect at any individual sampling period. Av. Sp. is the average number of species detected during sampling sessions of a particular transect.

	S/ W	LACI	EPFU	LANO	NYHU	LABO	PESU	MYLU	MYSE
GR1	S	5	66	26	0	7	0	1	0
GR2	W	0	300	213	1	1	1	1	0
MON1	W	0	108	75	0	3	1	0	0
MON2	S	17	7	15	2	4	1	0	0
CR7	S	16	18	66	5	5	0	0	0
SD2	W	0	148	29	22	17	0	5	0
SD3	S	0	88	70	67	99	2	2	0
RD1	S	1	51	32	2	5	1	0	0
RD2	W	0	5	0	0	0	0	0	0
JF1	S	6	20	23	1	11	1	0	0
JF2	W	0	136	8	30	1	0	0	0
CR2	S	0	146	193	0	0	0	0	0
CR3	W	0	187	18	73	14	0	4	1
CR5	W	0	59	3	111	27	1	2	4
CR6	S	15	70	66	3	4	0	2	1
SD1	W	0	1	2	1	0	0	0	0

	Total	Div.	Min Sp.	Max Sp.	Av Sp.
GR1	105	5	0	5	2.3
GR2	517	6	2	5	3.0
MON1	187	4	1	3	2.3
MON2	46	6	0	5	3.0
CR7	110	5	2	5	2.8
SD2	221	5	3	5	3.5
SD3	328	6	4	6	4.8
RD1	92	6	2	5	3.3
RD2	5	1	0	1	0.5
JF1	62	6	0	6	2.5
JF2	175	4	1	4	1.8
CR2	339	2	2	2	2.0
CR3	297	6	1	6	3.8
CR5	207	7	1	5	2.3
CR6	161	7	3	6	4.0
SD1	4	3	0	2	0.8

Table 2.5. Results of Stepwise multivariate tests. Lists bat species associate with the model and time frame. Lists if the relationship occurred at overnight sites (O) or on transects (T) (specifically looking at transect-level parameters). Lists model parameters ((-) in front indicates negative relationship), degrees freedom, p-value, RSquare value, and AICc value. Following is the species specific results of the transect point-level parameters.

Species	Time	T or O	Model	DF	$P<0.05$	R^2	AICc
LACI	May	T	-	-	-	-	-
		O	-	-	-	-	-
	June	T	Av Low Clutter(2); Land Use	3		0.37	37.9
		O	(-)Temp Range; (-)Prairie	2	0.0024	1.00	8.0
	July	T	Av Low Clutter; Av Canopy(2((-)1)); (-)Av TL Dist; (-)Swamp Forest; (-)Eurasian (-)Meadow; Prairie	7	0.0020	0.65	39.8
		O	Temp Range	1	0.0107	0.54	10.5
	August	T	Av Low Clutter (2); Av Canopy (2); Av TL Dist; Turf; Wet Prairie; Residential; Asphalt; Upland Forest	10	0.0003	0.34	117.1
		O	Av Canopy	1	<0.0001	1.00	4.9
EPFU	May	T	Av Canopy; Land Use; Av TL Dist; (-)Turf	4	0.0536	0.45	28.9
		O	Temp Range; (-)Barrens	2	0.0001	1.00	8.0
	June	T	Av Low Clutter (2((-)1)); Av Canopy (4); Land Use; (-)Av Slope; (-)Turf; (-)Swamp Forest	10	0.0796	1.00	29.5
		O	Eurasian Meadow	1	0.0005	1.00	4.9
	July	T	Av Low Clutter (4((-)2)); Av Canopy (2((-)1)); Land use (2); (-)Turf; Wet Prairie; (-)Savanna; (-)Barrens; Eurasian Meadow	13	0.5198	1.00	41.1
		O	Shrub/Scrub; Eurasian Meadow	2	0.0001	1.00	8.0
	August	T	Av Low Clutter; Av Canopy; Land Use (2); Av TL Dist	5	0.0003	0.39	53.5
		O	(-)Wind Range; (-)Moon Illumination; Av Clutter	3	0.0004	1.00	11.1

LANO	May	T	Av Canopy; (-)Pond; Conifers; Cropland	4	<0.0001	0.874	14.9
		O	(-)Savanna; (-)Barren	2	0.0003	0.73	13.9
	June	T	Av Low Clutter; (-)Land Use (2); Asphalt; (-)Pond	5	0.0013	0.65	27.9
		O	Av Temp; Savanna	2	0.0009	0.78	11.9
	July	T	Av Low Clutter; Av Canopy (2); Land Use (3((-)2)); Water Dist; (-)Turf; Conifer; Barrens	10	0.2201	0.86	37.5
		O	Av Humid; Av Canopy; Floodplain Forest	3	0.0004	1.00	11.6
	August	T	Av Low Clutter (3); Av Canopy (5); Land Use, Av TS Dist; Swamp Forest	11	-	0.75	49.2
		O	(-)Wind Range; Av Clutter; Av Canopy	3	0.0004	1.00	11.6
NYHU	May	T	-	-	-	-	-
		O	(-)Temp Range; (-)Barrens	3	0.0004	1.00	8.0
	June	T	(-)Upland Forest	1	0.0505	0.22	29.2
		O	Wind Range; (-)Insects	2	0.0096	0.77	10.8
	July	T	Av Canopy (3); (-)Shrub/Scrub; Floodplain Forest	5	0.0035	0.67	26.1
		O	Wind Range; Shrub/Scrub	2	0.0015	0.59	16.9
	August	T	Av Canopy (2); Pond; (-)Upland Forest; (-)Floodplain (-)Forest; (-)Eurasian Meadow	6	0.0087	0.67	32.2
		O	(-)Swamp Forest; Conifer; (-)Barrens; (-)Eurasian Meadow	4	0.0002	1.00	16.0
LABO	May	T	Eurasian Meadow	1	0.9787	1.00	4.3
		O	Av Temp; (-)Savanna; (-)Barrens	3	<0.0001	1.00	11.6
	June	T	Av Canopy (2); Shrub/Scrub; (-)Floodplain Forest	4	0.0018	0.91	16.0
		O	Wind Range; (-)Insects	2	0.0018	0.82	10.8
	July	T	Av Low Clutter (2); Av Canopy (2); (-)Water Dist; Av ST Dist; Av TL Dist; Av TS Dist	8	<0.0001	1.00	22.9
		O	-	-	-	-	-
	August	T	Av Low Clutter (2); Av Slope; Av TL Dist; Turf;	10	0.0422	1.00	29.5

			Wet Prairie; (-)Savanna; Swamp Forest; (-)Conifers; (-)Upland Forest				
		O	(-)Humid Range; Insects; (-)Eurasian Meadow	3	<0.0001	1.00	11.6
PESU	May	T	-	-	-	-	-
		O	Av Clutter	1	0.0982	0.18	17.6
	June	T	-	-	-	-	-
		O	-	-	-	-	-
	July	T	Av Low Clutter (2((-)1)); (-)Av TL Dist	3	0.0279	1.00	8.95
		O	Av Temp; (-)Insects; (-)Savanna; (-)Upland Forest	4	0.0039	1.00	16.0
	August	T	Av Canopy; Wet Prairie; (-)Pond	3	0.5626	0.83	12.8
		O	Av Temp; (-)Insects; (-)Turf	3	0.0072	1.00	11.6
MYLU	May	T	Water Dist	1		1.00	4.3
		O	-	-	-	-	-
	June	T	-	-	-	-	-
		O	-	-	-	-	-
	July	T	-	-	-	-	-
		O	(-)Humid Range; Wind Range; (-)Upland Forest	3	0.0004	1.00	11.6
	August	T	(-)Water Dist; (-)Shrub/Scrub	2	0.4266	0.61	13.1
		O	(-)Moon Illumination; (-)Swamp Forest; Floodplain Forest	3	0.0072	1.00	11.6
MYSE	May	T	N/A	N/A	N/A	N/A	N/A
		O	-	-	-	-	-
	June	T	N/A	N/A	N/A	N/A	N/A
		O	(-)Upland Forest	1	0.0062	1.00	4.9
	July	T	N/A	N/A	N/A	N/A	N/A
		O	(-)Av Humid; Prairie	2	0.0024	1.00	8.0
	August	T	N/A	N/A	N/A	N/A	N/A
		O	(-)Wind Range	1	0.0300	.63	7.7

Species Transect Point	Model	DF	P<0.05	R^2	AICc
LACI	(-)Av Temp; (-)Temp Range; Inst Wind	3	<0.0001	0.32	98.2

EPFU	(-)Av Temp; (-)Inst Wind; Av Wind; (-)Wind Range	4	0.0003	0.22	82.4
LANO	(-)Day #; Av Temp; Inst Wind	3	<0.0001	0.24	103.8
NYHU	(-)Av Temp; Av Humid; Humid Range; Inst Wind; (-)Wind Range; (-)Av Wind; (-)Moon Illumination	7	<000.1	0.34	102.1
LABO	Av Humid; Humid Range; Inst Wind; (-) Wind Range	4	0.0028	0.12	127.5
PESU	(-) Day #; (-)Av Temp; (-)Temp Range; Av Humid; Humid Range; Av Wind; (-) Wind Range; Moon Illumination; Inst Temp; (-) Inst Humidity	10	<0.0001	0.74	46.3
MYLU	(-)Temp Range, Wind Range, (-)Inst Humidity	3	0.0016	0.39	32.4

Table 2.6. Results of our principal component analysis for each species at our transect (T) and overnight (O). Includes the explanatory contribution (%) of the first two principal components of our analysis. Also includes the one or two explanatory variables most correlated with the principal components 1 and 2.

Species	T or O	P/A	P1 %	P1 Components	Eigenvalues	P2 %	P2 Components	Eigenvalues
LACI	T	P	21.8	Av Clutter; Av Canopy	0.333; 0.320	11.4	Residential	-0.399
		A	23.0	Av Canopy; Av Clutter	0.322; 0.328	11.5	Residential	-0.425
	O	P	27.7	Prairie; Residential	0.355; 0.341	19.0	Av Clutter; Turf	-0.354; -0.0351
		A	16.9	Av Canopy; Prairie	-0.354; 0.334	11.1	Conifers	0.473
EPFU	T	P	20.8	Av Canopy; Av Clutter	0.361; 0.341	10.8	Savanna; Prairie	0.456; 0.441
		A	23.6	Av Clutter; Av Canopy	0.319; 0.318	12.1	Residential	-0.446
	O	P	16.0	Av Canopy	0.377	13.3	Conifers	0.421
		A	23.5	Barrens; Swamp Forest	0.326; 0.317	17.1	Conifers	0.363
LANO	T	P	22.6	Av Canopy	0.337	11.7	Residential	0.396
		A	22.5	Av Clutter; Av Canopy	0.334; 0.321	11.3	Residential	-0.461
	O	P	16.1	Av Canopy; Prairie	-0.412; 0.402	13.8	Residential; Conifers	-0.424; -0.413
		A	22.5	Barrens; Residential	0.379; 0.361	14.1	Av Canopy	0.428
NYHU	T	P	22.5	Av Clutter	0.365	12.0	Shrub/Scrub	0.404
		A	22.5	Av Canopy; Av Clutter	0.325; 0.321	11.2	Residential	-0.484
	O	P	18.0	Eurasian Meadow; Prairie	0.399; 0.399	16.5	Conifers	0.371
		A	16.4	Residential; Av Canopy	0.340; 0.339	13.7	Shrub/Scrub	0.425
LABO	T	P	22.1	Av Canopy; Av Clutter	0.338; 0.335	10.5	Residential; Savanna	-0.393; 0.389
		A	22.6	Av Canopy; Av Clutter	0.325; 0.324	11.4	Residential	-0.461
	O	P	19.5	Prairie; Shrub/Scrub	0.402; 0.389	13.6	Turf	-0.392
		A	18.5	Residential	0.377	11.8	Prairie; Shrub/Scrub	0.393; 0.0378

PESU	T	P	29.7	Av Clutter; Av Canopy	0.340; 0333	17.8	Eurasian Meadow	0.375
		A	22.6	Av Canopy; Av Clutter	0.327; 0.323	11.1	Residential	-0.466
	O	P	26.6	Upland Forest; Eurasian Meadow	-0.319; 0.317	20.3	Av Clutter	0.385
		A	16.2	Av Canopy	0.371	13.3	Swamp Forest	0.430
MYLU	T	P	72.5	Av Canopy; Land Use	0.234; 0.234	26.5	Turf	-0.391
		A	22.6	Av Canopy; Av Clutter	0.327; 0.323	11.1	Residential	-0.465
	O	P	23.0	Eurasian Meadow; Av Canopy	0.392, -0.382	19.4	Swamp Forest; Wind Range	0.388; 0.364
		A	16.9	Av Canopy; Swamp Forest	-0.346, -0.345	13.1	Shrub/Scrub, Conifers	0.394; 0.373
MYSE	T	P	N/A	N/A	N/A	N/A	N/A	N/A
		A	N/A	N/A	N/A	N/A	N/A	N/A
	O	P	43.3	Prairie; # habitats	0.296; 0.293	26.6	Av Humid; Humid Range	0.376; 0.364
		A	16.1	Av Canopy	-0.375	13.0	Shrub/Scrub	0.448

Table 2.7. Max, Min, and SD of nightly temperature, humidity, and wind of all months at transects and overnight sites.

	Transects				Overnight Sites			
	May	June	July	August	May	June	July	August
Temperature Max (°C)	16.7	26.1	26.7	26.1	16.7	26.1	28.9	29.4
Temperature Min (°C)	8.9	12.8	17.8	18.9	7.2	13.3	15.0	16.1
Temperature Average (°C)	13.4	18.7	22.0	22.6	12.6	18.7	21.2	22.9
Temperature SD (°C)	2.0	2.5	2.5	2.4	3.9	3.7	5.7	3.2
Humidity Max (%)	93.0	97.0	90.0	100.0	93.0	97.0	100.0	100.0
Humidity Min (%)	42.0	30.0	49.0	48.0	42.0	30.0	53.0	48.0
Humidity Average (%)	70.6	61.8	74.1	83.4	69.5	65.9	76.4	86.2
Humidity SD (%)	37.7	16.8	22.2	16.0	15.8	14.0	19.1	12.0
Wind Max (kph)	20.9	19.3	14.5	49.9	20.9	22.5	19.3	16.1
Wind Min (kph)	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Wind Average (kph)	12.6	9.7	7.2	15.1	6.8	9.2	7.6	8.7
Wind SD (kph)	11.0	5.2	4.4	28.6	3.6	5.9	5.3	4.0

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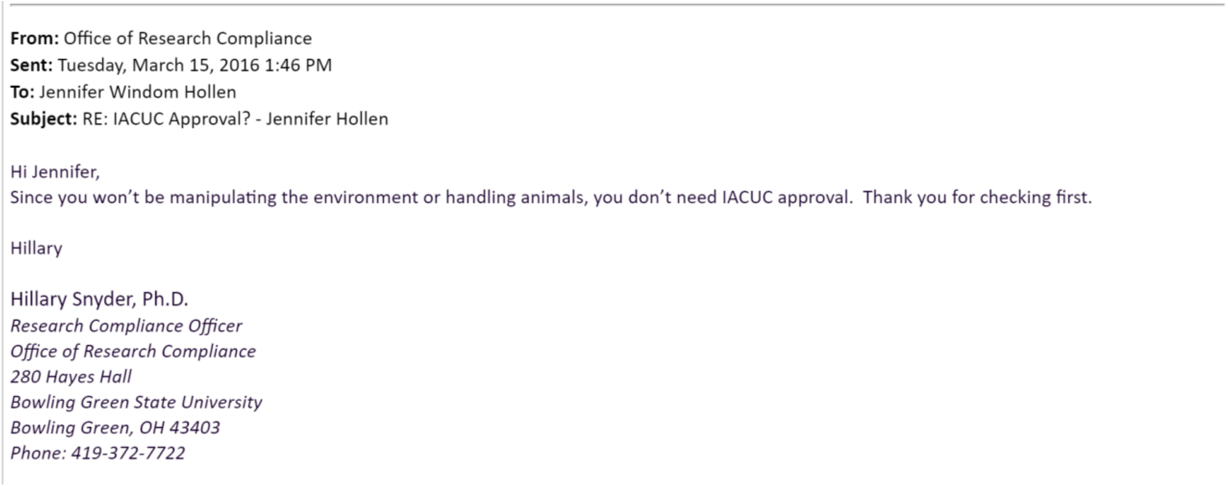
APPENDIX A. INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL



From: Office of Research Compliance
Sent: Tuesday, March 15, 2016 1:46 PM
To: Jennifer Windom Hollen
Subject: RE: IACUC Approval? - Jennifer Hollen

Hi Jennifer,
Since you won't be manipulating the environment or handling animals, you don't need IACUC approval. Thank you for checking first.

Hillary

Hillary Snyder, Ph.D.
Research Compliance Officer
Office of Research Compliance
280 Hayes Hall
Bowling Green State University
Bowling Green, OH 43403
Phone: 419-372-7722

APPENDIX B. SPECIES-SPECIFIC SONOGRAM DIAGNOSTIC CHARACTERISTICS

L. cinereus is characterized as the bat with the lowest call frequency, between 18 and 30kHz, with some pulses always under 25kHz. The pulses are often hooked at the bottom, without a distinct pattern, and can appear flat at lower frequencies. *E. fuscus* are the next lowest frequency calling bat, have calls that can peak as high as 60kHz but usually end between 25 and 30kHz, but can go lower. Pulses generally have a steady pattern without hooked bottoms, but can have sloped ends. *L. noctivagans* have calls that don't usually peak over 45 or 50kHz and usually end between 25 and 30kHz. *L. noctivagans* calls rarely go below 25kHz, have a steady pattern, and have moderately hooked bottoms. Some calls may be flat closer to 25kHz. *L. borealis* calls can have a great deal of variety. Calls often end between 30 to 40kHz, and pulses jump around sporadically (i.e., no specific patterns). Calls can sometimes be hooked, somewhat flat near 30kHz, and can resemble high frequency "40kHz" *Myotis sp.* bats with high peaks. *N. humeralis* have calls that generally end at about 35kHz. Calls often have hooks and have an alternating pattern of higher and lower pulses. *P. subflavus* have calls that end between 40 and 45kHz. They produce long sequences of similar looking pulses. Some calls can be hooked. *M. lucifugus* is a high frequency "40 kHz" *Myotis sp.* Their calls end at about 40kHz, but can go down to 35kHz. They often peak near 70kHz, and often have a distinct, gradually sloping pulse with noticeable space between pulses. *M. septentrionalis*, another 40kHz *Myotis sp.*, have calls that end between 35 and 40kHz and peak as high as 100kHz. The pulses are close together and often very straight with little sloping and no hooks. *M. sodalist*, also a 40kHz *Myotis sp.*, ends at about 40kHz, but can go down to 35kHz and peaks between 70 and 80kHz. The pulses are straight at the top but have a slight slope at the bottom, with no hooks.