UTILIZING ACOUSTIC METHODS TO CLASSIFY BAT SPECIES, AND TO ASSESS THEIR HABITAT USE AND PERCEPTION OF OWLS

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ABSTRACT

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The Oak Openings Region of Northwest Ohio is important because this area is home to more rare plant and animal species than any other similarly sized area in Ohio. Increasing our knowledge about bats, a vulnerable taxon, in this diverse region is critical due to a decline in bat populations caused by habitat loss and fragmentation, wind energy, and White-nose Syndrome. To address this need my thesis research was focused on: 1) comparing the effectiveness of bat acoustic identification programs; 2) monitoring habitat utilization; and 3) assessing how bats perceive owls.

I acoustically surveyed for bats using an Anabat detector to determine the presence of bat species within a protected area of the Oak Openings Region. I identified the files to species then compared my identification to those of two identification programs. Each program was consistent in its identification and they performed similarly. The identification programs are effective in speeding up analysis, but can produce false positives for the endangered Indiana bat (*Myotis sodalis*). Therefore, files should be double checked by an expert before identification is made final.

For the second aspect of my research, I utilized the files I recorded with the Anabat system and analyzed the type of habitat (open or forest) in which each species was most often recorded. Overall bat activity was greatest in open sites, but this varied by species. Additionally, I conducted a few surveys from sunset to sunrise to monitor changes in activity levels during the night as well as potential habitat changes throughout the night.

Lastly, I used Anabat detectors to record the amount of bat activity in responses to owl calls. Little research has been done on this interaction and it is thought that foraging bats compose a small portion of owl's diets. From this study, I found bat activity to be unchanged by any of owl species calls or a treatment of ambient nocturnal noise, suggesting that bat activity is not altered by the presence of owls.

This work is dedicated to my family who has supported

me through all of my endeavors.

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GENERAL INTRODUCTION

The Oak Openings Region of Northwest Ohio is a unique landscape comprised of a heterogeneous mix of oak savanna, oak woodland, and wet prairies (Higgins 2003, Brewer and Vankat 2006). Despite our knowledge about the region, only recently has there been research conducted on its bat populations. On a worldwide scale bat populations are declining and thus we need to better understand them if conservation is to be successful (Lane et al. 2006, Winhold et al. 2008, Bat Conservation International 2012). Conducting surveys in the Oak Openings Region not only improves our understanding of bats, but also assists in the management of this diverse set of ecosystems.

This thesis had three major objectives that are each written in as a stand-alone chapter, with a fourth chapter of general conclusions. The first chapter focused on a comparison of bat acoustic identification programs. As acoustic surveys become more common, identification programs are an effective way to quickly collect large quantities of data. The challenge becomes how to efficiently analyze these data. This chapter is formatted for publication in *The Journal of Wildlife Management*.

The goal of the second chapter was to monitor the habitats different bat species were using and to assess if bat activity fluctuated throughout the night. Additionally, I analyzed if bats altered their habitat use during the night. This chapter is also formatted for submission to *The Journal of Wildlife Management*.

The final chapter assessed how bats respond to the presence of a potential predator. Owl calls were broadcast in order to evaluate any influence they may have on bat activity. This is an area where data is lacking and of great interest in areas where these species overlap. This chapter is formatted for submission to *Ecology*.

CHAPTER I

COMPARISON OF METHODS FOR ACOUSTIC IDENTIFICATION OF BAT SPECIES USING ECHOLOCATION CALLS

ABSTRACT

Acoustic devices are becoming a more common method to survey for bats, replacing the traditional method of capture using mist nets. The limitations of mist netting include: bats fly around or over nets, net detectability, nets may facilitate the spread of White-nose Syndrome, and it is labor intensive. Acoustic devices record bat echolocation calls and are effective at detecting many species difficult to capture with mist nets. Though identification of bat species by their echolocation calls can be difficult, the U.S. Fish and Wildlife Service is modifying their Indiana bat survey guidelines to include acoustic surveys as the primary method of monitoring. We recorded bat activity in Northwest Ohio using Anabat™ detectors and identified the recorded calls manually and using two identification programs (BCID East and EchoClass). The programs agreed with expert identification of files 61% and 69% of the time, but agreed with each other's identification for 50% of the files. This variation between programs may be caused by: the habitat, the variation in calls produced by species and individuals, and the quality of the calls. No Indiana bat calls were confirmed, but there were 22 false positives. Acoustic identification programs are a valuable research tool but caution should be taken before replacing mist netting as the primary method of Indiana bat surveying. We recommend using mist nets and acoustic devices simultaneously.

INTRODUCTION

Bats are very important species within ecosystems. They fill a similar role at night that birds fill during the day. They are crucial in controlling insect populations, pollination, and providing seed dispersal (Fleming 1988, Fujita and Tuttle 1991, Hodgkison et al. 2003, Boyles et al. 2011). The nocturnal nature and mobility of bats make them difficult to study (O'shea et al. 2003). Due to the ecological importance of bats there is a concern about declines in bat populations resulting from White-nose Syndrome (WNS) (Cohn, 2008, Thogmartin et al. 2012), a cold dwelling fungus, which has been confirmed in 21 states (Bat Conservation International 2012*a*) and has greatly impacted *Myotis* species such as the Eastern small-footed bat (*Myotis leibii*), little brown bat (*Myotis lucifugus*), Northern long-eared bat (*Myotis septentrionalis*), and Indiana bat (*Myotis sodalis*) (Frick et al. 2010, Thogmartin et al. 2012, USGS 2012).

Traditionally, mist netting has been the primary method to survey for bats (O'Farrell and Gannon 1999, USFWS 2012). This survey method involves attaching fine mesh nets to poles and erecting them, often across pools of water, wooded corridors, streams, or roads, and capturing bats as they fly through the area. While this is an effective way to sample bat populations (USFWS 2012), some species tend to fly high (e.g. hoary bats (*Lasiurus cinereus*), Rafinesque's big-eared bats (*Corynorhinus rafinesquii*)) and foraging bats can often detect nets, causing mist nets to only sample a small proportion of the bat community (O'Farrell and Gannon 1999).

Another method to survey bats is to use acoustic devices that record the echolocation calls bats emit while flying and feeding (Betts 1998, O'Farrell and Gannon 1999, Broders et al. 2004, Adams et al. 2010, Britzke et al. 2011). Acoustic surveys are not new (Broders 2004), but this approach is becoming more common as a research tool as the portable field devices have become more effective at recording calls and the increasing availability of programs that identify calls to species. Identification programs are very useful because they can quickly analyze large quantities of files (call sequences are saved as 15 second files) in a shorter period of time than manual analysis of every individual file (Jennings et al. 2008, Adams et al. 2010, USFWS 2012, personal experience). Identification programs (e.g. BCID, EchoClass, AnaScheme, SonoBat^{TM}) are developed using large call libraries and it is the characteristics (e.g. frequency, slope, shape, duration etc.) of these calls that the program compares with unknown calls and decides to which species the unknown calls are most similar.

Many species have unique calls that allow them to be identified. However, several species produce calls that overlap with those of other species (Betts 1998, Adams et al. 2010) and individual bats of the same species can call at different frequencies (Obrist 1995, Krusic and Neefus 1996, Broders et al. 2004, Britzke et al. 2011), which can make identifying some species difficult (e.g. silver-haired bats (*Lasionycteris noctivagans*), evening bats (*Nycticeius humeralis*), and *Myotis* species). Acoustic devices record insect noise, which can complicate identification of bat calls. Often acoustic devices only record one species at a time (the species calling at the highest frequency) and bats are known to alter their echolocation calls when there are other bats echolocating (Obrist 1995), making identification more difficult. Complicating the problem further is the fact that there are many devices that vary in how they record echolocation calls and the quality with which they record (Allen et al. 2011).

With the increased reliability of identification programs, the United States Fish and Wildlife Service (USFWS) plans to implement a new Indiana bat protocol for the 2013 summer season. The Indiana bat (*Myotis sodalis*) is a federally endangered species. This species is an insectivorous bat that spends the summers roosting under the exfoliating bark of trees and hibernates in large colonies in caves (Britzke et al. 2003, Carter and Feldhamer 2005, USFWS 2007). Due to the endangered status of the Indiana bat, any project (e.g. road, pipeline, wind farm, strip-mining, etc.) that involves the destruction of potential habitat within the species range requires surveying to be conducted to determine if the Indiana bat is present (USFWS 2012). The Indiana bat protocol is important to the protection of this endangered species because it attempts to minimize direct impacts to the species (e.g. cutting down occupied maternity roosts). The new protocol is an attempt to more effectively survey for Indiana bats and to protect their habitat by putting more restrictions on projects if Indiana bats are detected.

The current USFWS protocol for surveying Indiana bats requires mist netting (USFWS 2007) which does have the advantage over acoustic surveys in that data can be collected on sex, age, weight, and reproduction status (O'Farrell and Gannon 1999). However, one concern is that mist netting may facilitate the spread of WNS, which has caused a great decline in bat populations in eastern North America (Turner et al. 2011, Bat Conservation International 2012*a*). Acoustic surveys alleviate this concern and thus the draft of the new protocol (released February 2012) shifts the emphasis to acoustic monitoring and identification of species, with follow up surveys of mist netting if there is a detection of an Indiana bat (USFWS 2012). In areas where Indiana bat populations have been heavily reduced by WNS, acoustic surveys may make it easier to record the presence of an Indiana bat rather than attempting to catch them via mist net surveys.

The new protocol has led us to ask: how effective are identification programs? To address this question, we compared the species identification of bat calls recorded in a field study using three different methods: the software programs BCID East and EchoClass, and expert opinion. The objective of this research was to compare the effectiveness of acoustic identification programs to each other and the expert. We expected bat acoustic identification programs would not be as accurate at identifying the species of a bat call as an experienced bat researcher due to the variability with which a single bat species and individual can call. We also expected that identification would vary for the same call because of the algorithms of each program and the knowledge of the expert.

STUDY AREA

The Oak Openings Region consists of 476,000 hectares and is a heterogeneous landscape of oak savanna, oak woodland, and wet prairies (Higgins 2003, Brewer and Vankat 2006) located in Lucas, Fulton, and Henry counties in Ohio. The Oak Openings Region comprises <0.5% of the total land in Ohio (Schetter and Root 2011), yet many rare plant (ODNR Div. of Natural Areas & Preserves 2011) and animal species (ODNR Div. of Wildlife 2012) are found here. Our surveys took place in the Oak Openings Preserve located in Swanton, Ohio. This park is 1,523.6 hectares and a recent study of bats (Sewald 2012) found eight different species to occur here including: big brown bats (*Eptesicus fuscus*), eastern red bats (*Lasiurus borealis*), hoary bats (*Lasiurus cinereus*), silver-haired bats (*Lasionycteris noctivagans*), northern long-eared bats (*Myotis septentrionalis*), little brown bats (*Myotis lucifugus*), evening bats (*Nycticeius humeralis*), and tri-colored bats (*Perimyotis subflavus*). The Indiana bat (*Myotis sodalis*) can be found in Ohio (U.S Fish & Wildlife Service 2007), but has not been confirmed as present in the Oak Openings Region.

METHODS

We surveyed 12 sites throughout the Oak Openings Preserve. Eight sites were selected based on high bat activity observed by Sewald (2012). The four additional sites were selected based on areas that appeared to be good foraging habitat for bats. Six of the sites were located in open grasslands, prairies, or savanna while the other six sites were located in forested areas. Two of these sites were located on the edge of ponds. These sites were selected to sample different habitats and increase the possibility of recording a diversity of species. Each site was surveyed 4 times a month. Surveys were done around the new moon phase of June, July, and again in early September. June and July have been shown to have the greatest amount of bat activity (Hayes 1997, Sewald 2012) and some studies have shown bat activity to increase around the new moon or move into shadowed areas on moonlit nights (Reith 1982, Adam et al. 1994, Hecker and Brigham 1999, Lang et al. 2006).

Acoustic surveys began a half hour after sunset and concluded within three hours of sunset, which is when bat activity is at its highest (Hayes 1997). Six surveys were conducted each night by two surveying teams. Surveys were not conducted during inclement weather such as high wind or precipitation (Kunz 1973, Adam et al 1994). Sites were surveyed for twenty minutes (Ford et al. 2006, Johnson and Gates 2008, Brooks 2009) using an Anabat™ SDII acoustic detector (Titley Electronics, Ballina, NSW, Australia) to record bat echolocation calls. Each Anabat unit was attached to a one meter pole to focus the Anabat microphone towards the bats and decrease the amount of insect noise the device recorded, which is concentrated near the ground.

We utilized two different identification programs, Bat Call Identification East (BCID, Inc., Kansas City, MO) and EchoClass (U.S. Army Engineer Research and Development Center, Vicksburg, MS), and expert opinion to identify bat calls recorded in the field to species. Our expert opinion was developed by consulting with other bat researchers, studying call libraries, and reviewing notes from an Anabat workshop. BCID was written by Allen (2007) and released in 2012, and EchoClass was produced by Britzke and released in 2012. BCID makes an identification of call files based on call sequence characteristics, individual calls, and pairwise discrimination using Mahalanobis distance (Allen 2012). EchoClass analyzes call sequences in a similar manner similar to BCID. It is important to note that EchoClass is a draft. These programs were chosen for this study because both programs are under consideration for recommendation in the new survey protocol.

The amount of bat activity at each site was quantified by the number of files recorded by the Anabat (Hayes 1997). We considered a call to be a single sound emission produced by a bat and a call sequence to be a series of calls separated by <1 second (Fenton 1999). These call sequences are saved by the Anabat as 15 second files. The recorded files were loaded onto AnalookW (version 3.8v, Chris Corban), a program that projects calls on a sonogram allowing the user to view recorded bat calls and to measure call characteristics. Files were identified based on visual inspection of frequency, shape, and slope of the calls. We considered this identification as correct with which to compare the identification programs. Only files that had at least three calls were identified and included in analysis. Files containing a sequence of calls that could not be identified to species or files that had fewer than three calls were labeled as "unknown" and were excluded from analysis. Files that contained call sequences from more than one bat species were identified as the species with the most numerous calls. If the number of calls were equal for both species then the file was labeled as "unknown". This was done to be consistent with the programs, which identify files based on the species with the most abundant calls.

For comparison, all files were processed with BCID (version 2.4p, 2012) using the Ohio species list (excluding the gray bat (*Myotis grisescens*) and Eastern small-footed bat because they are not found in this area of Ohio (BCI 2012*c, d*)) and through EchoClass (version 1.0, 2012) using species set one. Species set one includes 12 potential species a file can be identified as, with no option to reduce this number. Due to few recorded northern long-eared and little brown bat files and the difficulty of identifying *Myotis* species (Obrist 1995, Krusic and Neefus

1996, Tibbels and Kurta 2003, Hays 1997), these two species were labeled as *Myotis* for statistical analysis.

After identifying all files, we compared the species identification for each file across the three different methods across all sites and surveys. Agreement of identification with the expert across all files was estimated as a percentage for BCID and EchoClass identifications. Using JMP® 9 (SAS Institute, Inc., Cary, NC) a matched pair Wilcoxon signed-rank test was applied to all species-specific percentages to test for differences between the two programs. A matched pair Wilcoxon signed-rank test was also utilized using the percentage of files the programs labeled as "unknown" to assess if one program was more or less likely than the other program to identify a call. We took into consideration that the programs are not right or wrong when they label a file as "unknown." We took the number of files identified as each species by the expert, and for each program subtracted the number of those files the program labeled as "unknown" (i.e. the expert identified a file to species but the program labeled it as "unknown"). This left us with the number of files both the expert and the programs identified to species. We then calculated the percent of files correctly identified by each program for each bat species and used a matched pair Wilcoxon signed-rank test to evaluate if one program was better than the other. Furthermore, the percentage of calls attributed to each species was compared to those data collected in another study of bats in the Oak Openings Region in 2010 and 2011 (Sewald 2012) to evaluate differences over time.

RESULTS

A total of 2,216 files were qualitatively identified to species; 33 files had sequences of two different species, and 329 files were labeled as "unknown." Of these 2,216 identified files, 1,951 were identified to species by BCID and 1,863 were identified to species by EchoClass.

Eight bat species were identified from these surveys. All methods agreed that big brown bats and silver-haired bats were the most common species. Following these species, there were discrepancies amongst the identification methods as to the order or rank of species activity (Table 1). EchoClass agreed with the expert identification that red bats were the third most common, whereas BCID ranked hoary bats as the third most common. EchoClass agreed with the expert on the order of the four most abundant species sequences (Table 1). The expert identified few sequences attributed to little brown bats, northern long-eared bats, evening bats, and the tri-colored bat. Matched pair Wilcoxon signed-rank tests indicated no difference from previous survey data of 2010 or 2011 ($S = 5.50$, $df = 6$, $p = 0.3125$).

All three methods were in agreement on the identification of 44% of the files. BCID agreed with expert opinion on 53% of the files, whereas EchoClass agreed on 58% of the files (Table 2). The programs agreed with each other on 38% of the files. Both programs preformed equally well on big brown files (BCID agreed on 55% of files and Echoclass agreed on 57%) and *Myotis* files (22% for BCID and 26% for EchoClass). BCID performed better on identifying evening (58% vs. 21%), and tri-colored bats (44% vs. 6%), whereas EchoClass performed better on red (66% vs. 33%), hoary (69% vs. 34%), and silver-haired bats (69% vs. 55%). Matched pairs Wilcoxon signed-rank tests found no difference between the programs on the number of files that were in agreement with the expert ($S = -0.50$, $df = 6$, $p = 1.00$).

The programs performed better on all levels when subtracting out the files labeled "unknown" (265 for BCID and 353 for EchoClass). BCID agreed with the expert on 61% of the files and EchoClass agreed on 69%. The two programs were in agreement on 50% of the files. Matched pairs Wilcoxon signed-rank tests found no difference between the programs in the percent of files correctly identified ($S = 4.00$, $df = 6$, $p = 0.5781$) or in their ability to assign an identification to files (S = 7.00, df = 6, p = 0.2969). Figure 1 shows some of the common disagreements between the expert and the programs, and the percent of files correctly identified for each species.

The expert did not identify any files as Indiana bats. We classified three files as "unknown," which were labeled as the Indiana bat by BCID or EchoClass. We could not confirm these were Indiana bat calls so the files were labeled as "unknown" and were not included in analyses. EchoClass identified 19 additional files as the Indiana bat, but we identified most of these files as big brown bats and therefore were used in analysis. BCID was more accurate in the sense that it did not produce as many false positives of Indiana bats as EchoClass. EchoClass also identified one file as the southeastern bat (*Myotis austroriparius*) and six files as the gray bat (*Myotis grisescens*). Neither of these two species occurs in Ohio (USFWS 2009, Bat Conservation International 2012*b*). EchoClass had the potential to identify files as the Eastern small-footed bat and was successful in that it did not identify any files as this species.

DISCUSSION

With the implementation of the new Indiana bat protocol set for the 2013 summer season, it is important to note that acoustic identification programs may identify bat calls differently. Compared to expert identification, BCID overestimated the amount of files attributed to silver-haired and hoary bats, whereas EchoClass overestimated red, silver-haired, hoary, and Indiana bats. The calls of big brown and silver-haired bats can be difficult to distinguish (Betts 1998) and that is likely why both programs underestimated big brown bats and overestimated silver-haired bats. Any call that had a smaller amplitude (the distance between the highest and lowest frequency of a call) than a typical big brown call, BCID identified as a silver-haired bat.

EchoClass was a little more selective when identifying silver-haired files, but still overestimated silver-haired files. BCID also identified any call that had a frequency below 25 kHz as hoary bats, but big brown bats can also call at a frequency below 25 kHz (Obrist 1995, Livengood et al. 2010). Many of these sequences closely resembled big brown calls (call were consistently at the same frequency, unlike hoary bats which irregularly call at different frequencies) and were therefore identified as big brown bats.

EchoClass likely overestimated the abundance of Indiana bat files because most of the calls in these files were fragmented, meaning the calls were not high quality. If this fragmentation occurred at the right frequency then it is possible the program identified the sequence as an Indiana bat based on the fragment of the call that resembled the Indiana bat. This approach explains why the program over-estimated red bats, since many of those calls appeared to be fragmented big brown bat calls. Fragmented calls can lead to misidentification by acoustic programs (Jennings et al. 2008, Adams et al. 2010) because the programs are developed using only high quality calls (e.g. Parsons and Jones 2000, Gannon et al. 2004, Preatoni et al. 2005). Many studies only use high quality unfragmented calls in analysis (Broders et al. 2004, Britzke et al. 2011); fragmented calls were used in this study and in Adams et al. (2010) because low quality calls are common in the field and are likely to be recorded when conducting surveys under the Indiana bat protocol.

Additionally, EchoClass identified seven files as species that are not found within the state: the southeastern and gray bats (USFWS 2009, BCI 2012*b, d*). Every file identified as a gray bat by EchoClass was identified as a tri-colored bat by the expert. These two bats exhibit similar calls (Livengood et al. 2010). Combating this issue will require knowledge of the current range of native bat species and will be more complicated during initial surveys in areas where these species are likely absent. BCID helps alleviate this issue by allowing the user to select to which species a file can be identified, an option not available in EchoClass.

Perhaps of greater concern is a program's ability to successfully identify the endangered Indiana bat since this is the focal species of required protocols. Between the two programs, 22 files were identified as Indiana bats. Three of these files were excluded from analysis because we could not confidently identify them to species and were thus labeled as "unknown." The remaining 19 files were identified as Indiana bats by EchoClass whereas the expert identified 18 of the files as big brown bats and one file as an evening bat. Again, most of the calls in these files were fragmented, which can lead to misidentification (O'Farrell and Gannon 1999, Adams et al. 2010). From these results, EchoClass appears to be more sensitive to Indiana bats and may produce more false positives than BCID.

Based on this study, the two programs performed equally well overall; however, the programs performed differently at a species level. This difference is likely because each program is developed using different call libraries. Some programs perform better or worse on a given species than do other programs, as shown by our study. When disregarding the files the programs labeled as "unknown," the programs performed decent (61% and 69%). If the programs are considered incorrect if they misidentify a file or label it "unknown," then for approximately half of the species, neither program exceeded a performance better than 50%. Regardless, programs need to improve in their overall ability to correctly identify calls before they can be heavily relied on. Based on BCID's consistency at identifying *Myotis* species, we believe it to be better suited for the new Indiana bat protocol than EchoClass. BCID also has the advantage of rapid file processing and the ability to narrow down the number of possible species that a file can be identified as.

Our decision on what species to label these files was not likely 100 percent correct but our data was comparable to acoustic and mist netting surveys of the past (Sewald 2012). Experts may disagree with the identification of the same files, as Betts (1998) and Jennings et al. (2008) found in their studies, because many species overlap in the frequency at which they call, making identification difficult. Regardless, bat acoustic identification programs need be more consistent with expert opinion than 69% if they are to replace mist-netting as the primary method of surveying for bats. The identification programs speed up the process of identification but until programs become more accurate, the identification of each file must be double checked before a final decision is made. If bat species identification is critical to the conservation of a species, then we need to have assurance that the standardized methods are effective at providing that identification. Programs should also become more consistent with one another and include low quality calls in their libraries. Adams et al. (2010) retained poor quality calls to construct and test the keys they used in AnaScheme (another acoustic identification program) and found their keys to increase in consistency with expert opinion. Only using high quality calls to develop and test programs may increase the accuracy of the program (Broders et al. 2004, Obrist et al. 2004) but will limit the program's ability to identify lower quality calls often recorded during research studies. Regardless of these drawbacks, acoustic surveys are an effective method to survey for bat activity. Conducting acoustic surveys to determine where bats are most active can be helpful in identifying the most productive areas for mist net surveys.

MANAGEMENT IMPLICATIONS

Misidentification could have big implications for a project if researchers do not have the expertise or time to correctly identify the species in an area. If software programs mistakenly identify a call as the Indiana bat, this will limit development and management activities, which could have broad consequences. At times Indiana bats can be difficult to identify when they are captured (personal experience, NYS Department of Environmental Conservation 2012, ODNR Div. of Wildlife 2012). If a program identifies a file as an Indiana bat, we recommend confirmation by an expert before further actions take place, rather than simply assume that the program has correctly identified the species. Acoustic programs may be very accurate when using high quality calls found in call libraries, but the accuracy of these programs decreases with field data as suggested by this study and others (Jennings et al. 2008). These data also show that files are identified as different species depending on which program is used, which can complicate matters when the disagreement is over an endangered species. As suggested by O'Farrell and Gannon (1999) and Murray et al. (1999), we recommend surveying for Indiana bats using mist nets and acoustic detectors simultaneously.

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Table 1. Percentage of call sequences attributed to each species by the three methods of identification across all sites and surveys. Dashes mean those species were not considered as an identification option.

Species	Expert (%)	BCID (%)	EchoClass (%)
Big brown	80.60	50.90	55.68
Silver-haired	9.75	31.15	19.13
Eastern red	5.28	2.51	14.31
Hoary	1.31	11.95	6.11
Evening	1.08	1.59	0.80
Northern long-eared	0.81	0.10	2.47
Tri-colored	0.77	0.56	0.05
Little brown	0.41	0.26	0.05
Indiana	0.00	0.00	1.02
Gray	-	-	0.32
Southeastern	-	-	0.05
Eastern small-footed	-	-	0.00

Table 2. Percent of files in agreement with the expert by the two acoustic identification programs.

Species	**BCID/Expert**	**EchoClass/Expert**
Big brown	55.26	57.28
Silver-haired	56.02	69.44
Eastern red	38.48	75.21
Hoary	34.48	68.97
Evening	58.33	20.83
Myotis species	22.22	25.93
Northern long-eared	5.56	27.78
Little brown	44.44	0.00
Tri-colored	47.06	5.88
Overall % of agreement	**53.70**	**58.30**

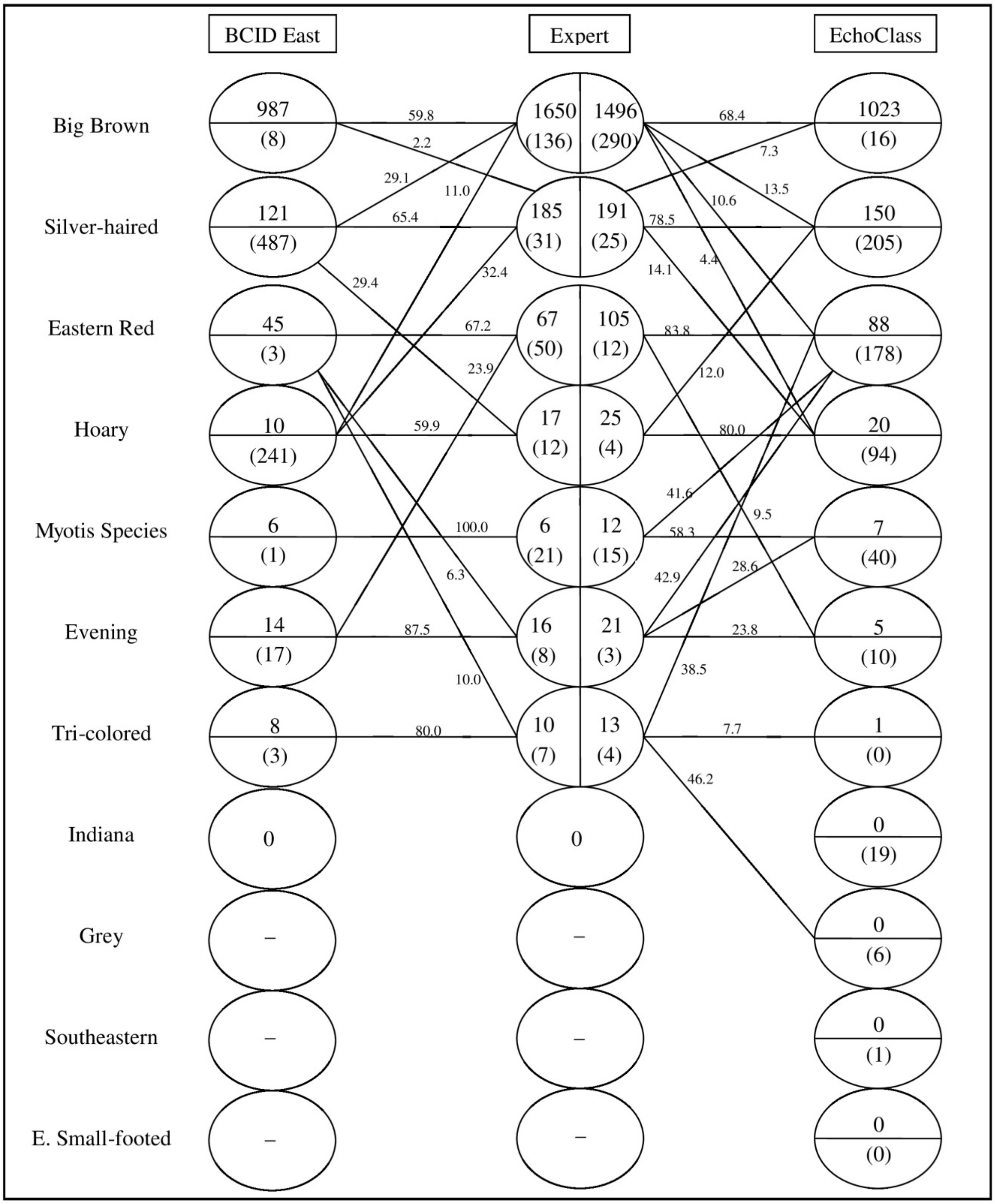

Figure 1. The percent of files identified to each species by the expert and the two programs. The expert column is divided for each program. The top numbers are the number of files identified to species by the expert and the program. The number in parentheses is the number of files the expert identified to species but the program labeled as “unknown.” In the program columns, the top number is the number of files identified to species correctly and the parentheses are files incorrectly identified. The percent of files in agreement or disagreement with the expert are signified by the lines leading from the expert to each program.

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CHAPTER II

MONITORING OF BAT HABITAT UTILIZATION IN THE OAK OPENINGS REGION OF OHIO

ABSTRACT

A number of bat species are declining in the United States with the spread of White-nose Syndrome, habitat loss, and wind energy. Nine bat species are of concern in Ohio with most of these species found in the Oak Openings Region of Northwest Ohio. Until recently, not a lot of bat research has occurred in this ecosystem, which is characterized by oak savannas, oak woodlands, and wet prairies. We acoustically surveyed for bats in the Oak Openings Region to monitor changes in activity from a recent study. We analyzed the amount of activity in forest versus savanna habitats and assessed how activity varies throughout the night. We found a continued decline in *Myotis* species, which is of great concern and is likely an effect of White-nose Syndrome and habitat loss. Surveys found conflicting results in habitat use for red and evening bats but big brown, hoary, and silver-haired bats were consistently more active in savanna habitats. *Myotis* species were consistently more active at forested sites. Bat activity was greatest after sunset (2100-0000) and before sunrise (0300-0600). The suite of bat species utilized different habitats, emphasizing the importance of managing for both savanna and forest. Continued surveys are recommended to monitor changes in bat activity and to better understand changes in bat activity throughout the night.

INTRODUCTION

Recently there has been a decline in bat populations worldwide (Lane et al. 2006, Winhold et al. 2008, Frick et al. 2010, Lametti 2010, Bat Conservation International 2012). This decline is important because bats play a vital role in ecosystems such as controlling insect populations, pollination, and providing seed dispersal (Boyles et al. 2011, Fleming 1988, Fujita and Tuttle 1991, Hodgkison et al. 2003). In addition to habitat loss in the United States (Krusic and Neefus 1996, Smith and Gehrt 2010), White-nose Syndrome, caused by a cold-loving fungus *Geomyces destructans*, is a more recent threat to bats. It is believed that well over a million bats have died due to this fungus since 2006 (Dzal et al. 2010, Turner et al. 2011) which, if this trend continues, could disrupt ecosystems and negatively impact the agricultural industry (Boyles et al. 2011).

As with many other states, bat populations are of concern in Ohio. In Ohio, nine bat species are listed as species of concern, one is of special interest, and the Indiana bat (*Myotis sodalis*) is a federally endangered species (ODNR Division of Wildlife 2012*c*). Eight of these listed species can be found in the Oak Openings Region of Northwest Ohio. This area is a 476,000 hectare heterogeneous mix of oak savanna, oak woodland, and wet prairies (Higgins 2003, Brewer and Vankat 2006). The region is an important landscape because it is home to many state listed and rare plant and animal species (ODNR Division of Natural Areas and Preserves 2011, ODNR Division of Wildlife 2012*c*).

Survey methods for bats include: mist nets, harp traps, hibernacula counts, and acoustic surveys (O'Farrell and Gannon 1999, Broders et al. 2003, Kingston et al. 2003, Tuttle 2003, Britzke 2011, USFWS 2012). Historically, mist netting was the primary method of surveying, but acoustic surveys have become more common (O'Farrell and Gannon 1999, USFWS 2012). Ultrasonic devices record the echolocation calls of bats, which can then be loaded onto a computer where the calls are projected on a sonogram for the researcher to analyze. Acoustic surveys are advantageous because they can be used in areas difficult for netting (e.g. open fields, corridors with high canopy, ponds or lakes) and it avoids the stress bats encounter when captured and handled via mist net surveys. Some bats are difficult to mist net (e.g. hoary bats (*Lasiurus cinereus*), Rafinesque's big-eared bats (*Corynorhinus rafinesquii*)) because of their flight behavior (fast and high) or because they can detect nets; thus, acoustic surveys often better sample the full species assemblage (Murray et al. 1999, O'Farrell and Gannon 1999, MacSwiney et al. 2008). Acoustic surveys can also be used to estimate the amount of bat activity in an area (Hayes 1997).

Bats vary in their wing morphology and their flight patterns which influence where they forage (Aldridge and Rautenbach 1987, Norberg and Rayner 1987). Hoary bats are a large species with low maneuverability and are often found foraging in open areas (Barclay 1985), whereas northern long-eared bats (*Myotis septentrionalis*) are small and highly maneuverable and often associated with forests (Brooks and Ford 2005). The other bat species in Ohio utilize varying amounts of open and forested habitats (Sewald 2012).

The objective of this research was to compare the amount of bat activity to that of the past few years. We expected big brown bats to be the most common because they are a widespread species and have been the most abundant in the past. We also compared habitat utilization for each of the species. A recent study found big brown, red, hoary, silver-haired, and tri-colored bats to be most active in savanna sites (Sewald 2012) and this is what we expected for this study. Likewise, we expected long-eared, little brown, and evening bats to be more active in forested habitats.

STUDY AREA

Our surveys took place in the Oak Openings Metropark located in Swanton, Ohio, USA. This park is 1,523.6 hectares and is part of the 467,000 hectare Oak Openings Region of Lucas, Fulton, and Henry counties in Ohio. The Oak Openings Region comprises <0.5% of the total land in Ohio (Schetter and Root 2011), yet it has more state listed species than any other similarly sized area in Ohio (ODNR Division of Wildlife 2012*b*). A recent study of bats at this park (Sewald 2012) found eight different species to occur here including: big brown bats (*Eptesicus fuscus*), eastern red bats (*Lasiurus borealis*), hoary bats (*Lasiurus cinereus*), silver-haired bats (*Lasionycteris noctivagans*), northern long-eared bats (*Myotis septentrionalis*), little brown bats (*Myotis lucifugus*), evening bats (*Nycticeius humeralis*), and tri-colored bats (*Perimyotis subflavus*). The Indiana bat (*Myotis sodalis*) can be found in Ohio (U.S Fish & Wildlife Service 2007), but has not been confirmed as present in the Oak Openings Region.

METHODS

Twelve sites were surveyed throughout the Oak Openings Preserve. Eight sites were selected based on high bat activity data that were collected by Sewald (2012). The four additional sites were visually selected based on areas that appeared to be good foraging habitat for bats. Six of the sites were located in open grasslands, prairies, or savanna and were classified as open because these sites had little to no canopy cover. The other six sites were located in forested areas and were classified as forest. Two of these sites were located on the edge of ponds: one small pond in interior forest and the other a large recreation pond (walking paths around it and fishing access) that is surrounded by forest. These sites were selected to sample different habitats and increase the possibility of recording a diversity of species. Each site was surveyed four times a month. Surveys were done around the new moon phase of June and July 2012. June and July have been shown to have the greatest monthly bat activity (Hayes 1997) and some studies have shown bat activity to increase around the new moon or bats move into shadowed areas on moonlit nights (Reith 1982, Adam et al. 1994, Hecker and Brigham 1999, Lang et al. 2006).

Surveys began a half hour after sunset and concluded within three hours of sunset, the period in which bat activity is at its highest (Hayes 1997). Each survey was 20 minutes in length (Francl et al. 2004, Brooks and Ford 2005, Ford et al. 2006, Johnson and Gates 2008, and Brooks 2009), which allowed six surveys to be conducted each night by two surveying teams. Surveys were not conducted during inclement weather such as strong wind or precipitation (Kunz 1973, Adam et al 1994). Start time, temperature, cloud cover, precipitation, wind speed, and noise level was recorded at each site. Noise was estimated using a scale of 0-4, a similar scale to the Beaufort scale used for estimating wind speed (Takats et al. 2001). Sites were surveyed using an Anabat^{TM} SDII acoustic detector (Titley Electronics, Ballina, NSW, Australia) to record bat echolocation calls. Each Anabat unit was attached to a one meter pole to focus the Anabat microphone towards the bats and decrease the amount of insect noise (which concentrated near the ground) the device recorded.

The amount of bat activity at each site was quantified by the number of call sequences recorded by the Anabat (Hayes 1997). We considered a call to be a single sound emission produced by a bat and a call sequence to be a series of calls separated by <1 second (Fenton 1999). Typically a single call sequence is saved as a 15 second file. The recorded files were loaded into AnalookW (version 3.8v, Chris Corban), a program that projects calls on a sonogram allowing an expert to view recorded bat calls and to measure call characteristics. Files were identified based on visual inspection of frequency, shape, and slope of the calls by the author. Only files that had at least three calls were identified and included in analysis. Files containing a sequence of calls that could not be identified to species or a file that had fewer than three calls were labeled as "unknown" and were not included in analysis. Identification of files was aided by call libraries, notes from an Anabat workshop (Livengood et al. 2010), and the identification programs of Bat Call Identification 10 (BCID) version 2.4 (Bat Call Identification, Inc., Kansas City, MO) and EchoClass version 1.0 (U.S. Army Engineer Research and Development Center, Vicksburg, MS). The identification programs compare unknown bat calls to a library of known bat calls and label the unknown files as the species that a majority of the calls most closely resemble (e.g. frequency, slope).

After identifying all call files, the percentage of files attributed to each species was calculated from the total number of files. Due to few recorded northern long-eared and little brown bat files and the difficulty of identifying *Myotis* species (Obrist 1995, Krusic and Neefus 1996, Tibbels and Kurta 2003, Hays 1997), these two species were combined and labeled as *Myotis* for statistical analysis. Using JMP® 9 (SAS Institute, Inc., Cary, NC), matched pair Wilcoxon signed-rank tests were utilized across all percentages to test for a difference between species-specific activity levels from this year's data and data collected the past two years. Mann-Whitney U tests were used to examine if any species were more active at open or forested sites.

Overnight Data Collection

In addition to the nightly surveys we conducted, overnight surveys were set up at six sites. These sites were paired together, one a forest site and the other a savanna site; thus, there were three sets of paired sites. Each pair of sites was located 100m from one another to avoid potential overlap in detection by the Anabat (Livengood et al. 2003). Two Anabats, one at a forested site and the second at its paired savanna site, were placed in a weather proof container and programmed to begin recording at sunset and turn off at sunrise. The following day the Anabats were collected and moved to another pair of sites for the night. The data collected from these surveys were used to look at habitat and temporal differences in activity across species. The night was divided into three 3 hour segments: 2100-0000 (early night), 0000-0300 (midnight), and 0300-0600 (early morning). All the files were identified then placed into one of the three time periods based on the time the calls were recorded. Due to low sample size no statistical analyses were conducted.

RESULTS

A total of 1,623 files were identified to species from the nightly surveys. We identified eight species of bats with a majority of the files being attributed to big brown and silver-haired bats (80.6% and 9.7%, respectively). The next most common species were red bats (5.3%), hoary bats (1.3%), and evening bats (1.1%). Few calls were attributed to *Myotis* species and tri-colored bats (1.2%, and 0.8%). Matched pair Wilcoxon signed-rank tests indicated that these data were not different from what Sewald (2012) found over the past two years ($S = 5.50$, $df = 6$, $p = 0.3125$). There was an increase in the percentage of files identified as silver-haired bats from the past two years (1.5% in 2010 and 4.4% in 2011), but we also found a continued decline in the number of *Myotis* files (6.3% in 2010 and 2.7% in 2011).

On average bat activity was higher at open sites than at forested sites ($z = 2.27$, $p = 0.0230$). Sixty percent of all files were recorded in open habitats. All species were recorded in both open and forested habitats, but some species were more active (i.e. number of files recorded were more abundant) in one over the other. A Mann-Whitney U test was performed across all species and the results indicated that big brown ($z = 2.53$, $p = 0.0113$), silver-haired ($z = 2.71$, $p = 0.0068$), and hoary bats ($z = 2.03$, $p = 0.0426$) were significantly more active at open sites than forested (Figure 1). Though not significant, more red bat (z 0.14, $p = 0.8887$), evening bat ($z = -1.41$, $p = 0.1586$), and *Myotis* species ($z = -.79$, $p = 0.4289$) calls were recorded in forested sites (Figure 1). The amount of tri-colored bat activity was equal at forested and open sites.

Overnight Data

A total of 2,617 files were identified to species from the overnight surveys. Again, all eight species were recorded and big brown bats accounted for 76% of all files. Silver-haired bats and tri-colored bats each accounted for 7%. Few files were identified as red, hoary, little brown, long-eared, and evening bats. Nineteen files were identified as *Myotis* species. Most calls (89%) were recorded in savanna habitats (Figure 2). All species of bats were found to be more active at savanna sites with the exception of *Myotis* species, which were more active at forested sites.

Peak activity was early in the night (2100-0000) and early in the morning (0300-0600), with most activity recorded in the early morning (Figure 3). This double peak was true for most of the species (Figure 2). Big brown, red, hoary, evening, and tri-colored bats, were more active early in the morning. Silver-haired bats were most active early in the night and *Myotis* species were equally active through the night.

DISCUSSION

Our surveys found eight bats species utilize the Oak Openings Preserve. It was not surprising that big brown bats were the most common bat recorded. These results are comparable to Sewald's (2012) findings from the past two years in the Oak Openings Region. Of interest is the increase in the number of files identified as silver-haired bats. There could be some error in identifying these calls because silver-haired calls can resemble big brown bat calls (Betts 1998). However, we surveyed a pond that was not surveyed in the past and it is at this pond where a majority of the silver-haired bat calls were recorded. As with many bat species, silver-haired bats are known to forage quite often over ponds and streams (Walsh and Harris 1996, Vaughan et al. 1997). Silver-haired bats were one of the most captured species by Seidman and Zabel's (2001) study of bat activity over streams.

Also of major concern is the decline in the abundance of files attributed to little brown and northern long-eared bats. This is the second year in a row that there has been a documented decline for these two species in the Oak Openings Region. Both our 20 minute nightly surveys and the overnight surveys yielded fewer *Myotis* species than in past surveys. This decline may be credited to habitat loss (Krusic and Neefus 1996, Smith and Gehrt 2010) and more recently to White-nose Syndrome which has had a big impact on *Myotis* species (Dzal et al. 2010, Frick 2010, Thogmartin 2012). It is also possible that big brown bats, who are generalists, are outcompeting other species.

We expected big brown, red, hoary, silver-haired, and tri-colored bats to be most active in open sites. Our 20 minute surveys found big brown, silver-haired, and hoary bats to be most active at these sites and the overnight surveys found similar results. Tri-colored bats did not show a preference for either habitat during our 20 minute surveys, but were found to be much more active at savanna sites and early in the morning based on the overnight surveys. In addition, we expected *Myotis* species to be more active in forested habitats. Long-eared bats are forest specialists (Lacki and Hutchinson 1999, Broders et al. 2003) and forest sites are where we found *Myotis* species activity to be the greatest for both types of surveys.

Interestingly, our 20 minute nightly surveys and overnight surveys disagreed on the habitat in which red bats and evening bats were most often found. Red bats were slightly more active at forested sites during the 20 minute surveys but more active at savanna sites during the overnight surveys. This discrepancy between the 20 minute surveys and the overnight surveys may be due to one of the forested sites that was part of the 20 minute surveys. This site had a high thin canopy created by management activities. This site had the greatest red bat activity of all the forested sites suggesting that the openness of the site was favorable for red bats (Jung et al. 1999, Elmore et al. 2005).

The discrepancy in evening bat habitat use occurred when looking at the time of night. The 20 minute surveys found evening bat activity to be greatest at forested sites whereas overnight surveys found evening bat activity to be equal in both habitat types early in the night (2100-0000). However, most evening bat activity was recorded early in the morning (0300-0600) and occurred in savanna habitats. This shift in activity and habitat use may coincide with competition or resource availability (Kunz 1973, Reith 1980, Arlettaz 1996). It is also possible that these calls could have been red bats because the two species have similar calls (Livengood et al. 2010) and evening bats are not common in Ohio (Kurta et al. 2005, ODNR Division of Wildlife 2012*a*).

There was a peak in activity early in the night and early in the morning, similar to that of Kuenzi and Morrison (2003). Results in the savanna habitats followed this same pattern with the greatest activity in the morning. Forested habitats had the greatest activity early in the night with greatly reduced activity during midnight and early morning. Early night activity in forested habitats might be attributed to bats leaving their roost in the forest to forage in savannas. Furthermore, bat activity is often positively correlated with insect abundance (Swift 1980, de Jong 1994, Hayes 1997, Tibbels and Kurta 2003), which often declines during the night (Rydell et al 1996). Our data were consistent with what Sewald (2012) found; however, we do recommend more overnight surveys to build upon these findings and to make stronger conclusions.

Acoustic sampling is a critical tool in better understanding bats, especially in a time when bat populations are on the decline (Cohn 2008, Winhold et al. 2008, Turner et al. 2011, Thogmartin et al. 2012). These surveys allow researchers to determine which species are present and to estimate the amount of activity (Hayes 1997). Acoustic devices can be easily deployed in a variety of habitats and for long periods of time, allowing large quantities of data to be collected. However, it must be considered that the detectablity of bats varies on the amount of clutter surrounding the Anabat, as well as other factors (e.g. frequency of calls, loudness of calls, atmospheric conditions) (Hourigan and Corben 2010, Corben 2003). It is possible that we found a greater amount of bat activity in open sites because bats were easier to detect in the open habitats rather than in forested habitats. Acoustic surveys are useful to monitor population trends over several years, as are models used to predict where bats may occur. We found models created for the bats of the Oak Openings Region (Sewald 2012) to vary in accuracy from species to species; but nevertheless, to be a useful tool in complementing survey results.

MANAGEMENT IMPLICATIONS

Based on these results, we recommend that surveys be continued within the Oak Openings Region to monitor the decline in *Myotis* species, which are important forest foraging species. If these species continue to decline we may see an increase in more generalist species (e.g. big brown and red bats) to fill the niche. Managers need to maintain savanna and open habitats since that is where a majority of the bats were detected. However, forest is still required for *Myotis* species and evening bats. Heterogeneous habitats are clearly important to maintain the full suite of native bat species. We also recommend more overnight surveys to help better understand any temporal/habitat association species may have. This would aid land managers in making management decisions and increase our understanding of bats.

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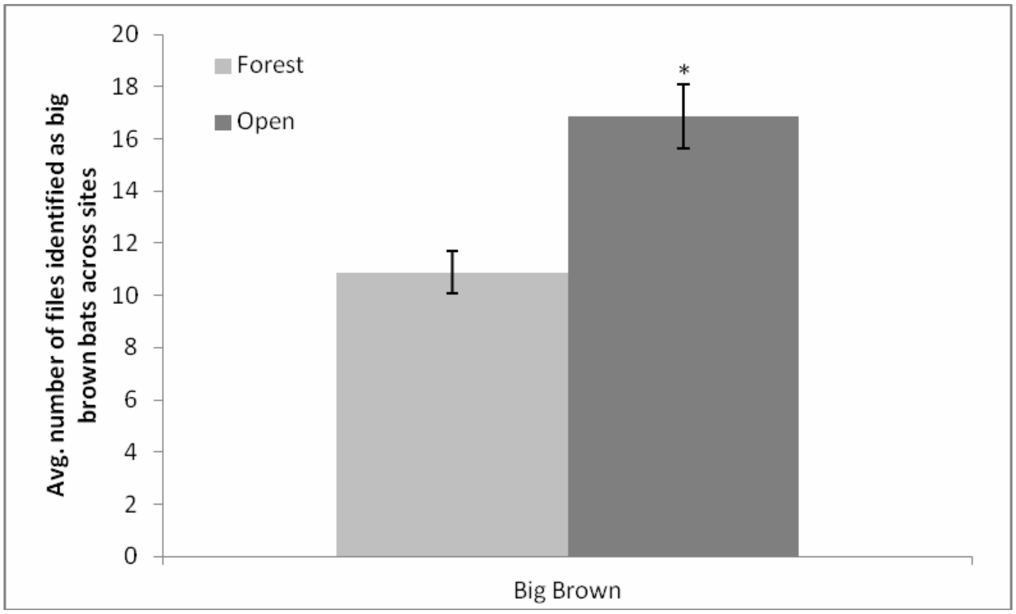

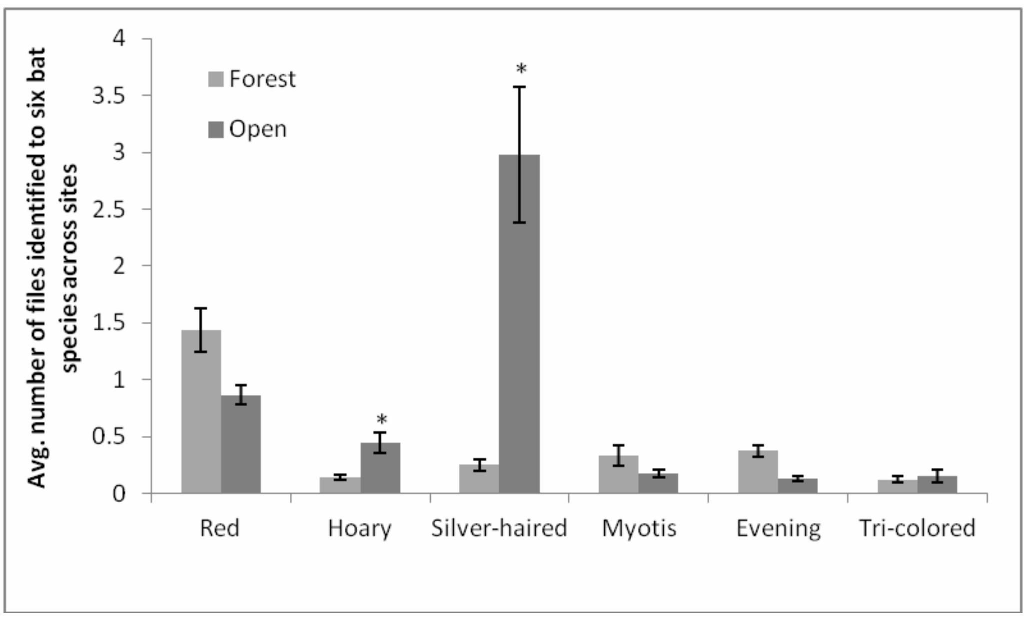

Figure 1: Difference in the average amount of activity for each species (based on the 20 minute surveys) at forested and open sites. Big brown bat are separate because the average is much larger than the other species. Total sample size of 1,623 files. Significance indicated by *.

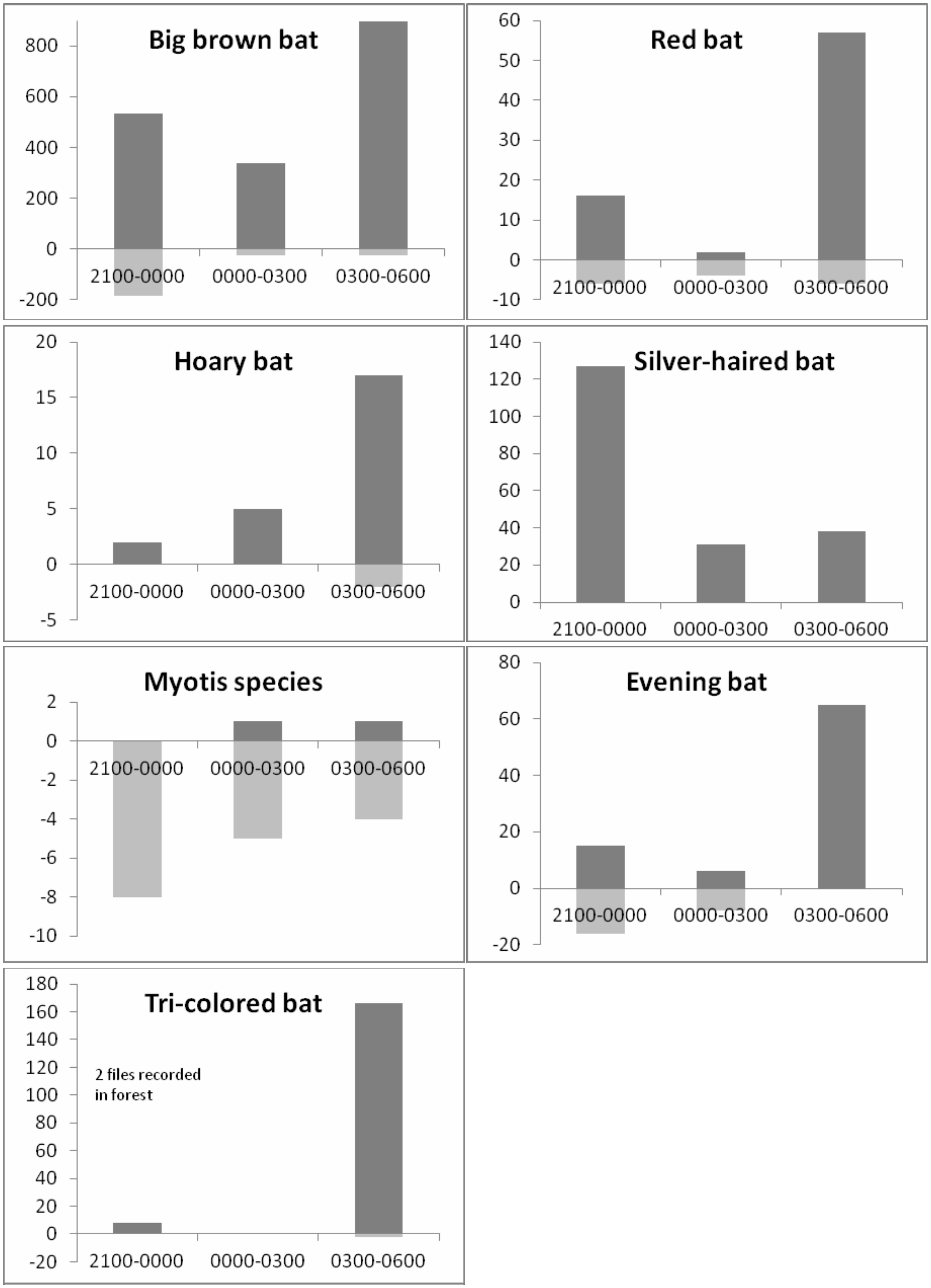

Figure 2: Difference in the average amount of activity for each species (based on the overnight surveys) during different times of night at forested and open sites. Time of night is represented on the x-axis and the number of recorded files on the y-axis. Dark grey bars indicate files recorded at savanna sites and light grey bars for forested sites. Forested sites are shown as negative numbers to visualize the difference in activity between habitats.

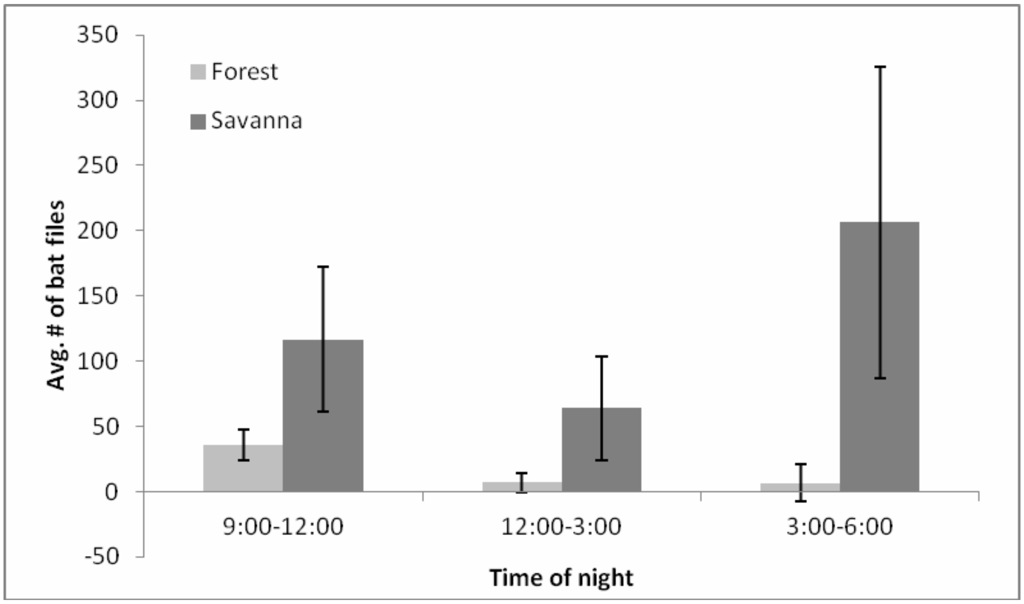

Figure 3: The overall average number of files recorded in forest and savanna sites at three different times of the night.

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CHAPTER III

BATS DO NOT ALTER THEIR FORAGING ACTIVITY IN RESPONSE TO OWL CALLS

ABSTRACT

A large emergence of bats from a roost tree or more commonly a cave, provide birds of prey with an improved opportunity to capture bats. Bats that are dispersed throughout their foraging area make it more difficult for birds of prey, especially owls, to capture them. Away from large bat communities, bats are thought to make up an insignificant portion of owl diets. Little research has been done on how bats may perceive owls, which do pose as a potential predatory threat. We conducted acoustic bat surveys to assess if bat activity was altered in the presence of owl calls or ambient nocturnal noise. Bat activity was recorded 10 minutes before and after broadcasting owl calls and nocturnal noises. Our surveys found no difference in the amount of bat activity recorded during the control, owl calls, and nocturnal noise. It is possible bats do not respond to calling owls because bats do not perceive owls as a threat. Bats are highly maneuverable and likely can escape if pursued by owls. It is also likely the need for bats to forage is greater than the predation risk they face from owls. Bat populations are threatened by habitat loss, wind energy, and White-nose Syndrome, but owls do not appear to be a major threat.

INTRODUCTION

Owls are considered top predators in many ecosystems (Manley et al. 2006), taking a variety of prey animals such as: rabbits, small mammals, reptiles, amphibians, and birds (ODNR Division of Wildlife 2007). Owls do occasionally take bats as prey but this is not common (Ritchison and Cavanagh 1992, Swengel and Swengel 1992, Marti and Kochert 1996). Owls may opportunistically take bats but they make up an insignificant portion of owl diets (Fenton and Fleming 1976, Duncan and Sidner 1990, García et al. 2005).

Most reports of owls depredating bats have been observations made near large roosts and caves (Twenty 1954, Baker 1962, Fenton et al. 1994). There is often a lot of bat activity over bodies of water (Walsh and Harris 1996, Vaughan et al. 1997) and this too potentially could increase predation risk by owls. García et al. (2005) studied Long-eared Owls (*Asio otus*) in Europe and found bats made up approximately 2% of the owl's diets. However, they believe that these bats were congregated in an area when captured by the owls because the distribution of bats per pellet was aggregated. It is understandable that hawks and owls would hunt near roosts and caves where large quantities of bats reside. Predators can wait for the bats to emerge in large numbers and this improves the predator's chances of capturing a bat. Nevertheless, Baker (1962) found owls, specifically Great Horned Owls (*Bubo virginianus*), to be one of the least effective avian predators near a cave in New Mexico. The owls cannot outmaneuver the bats so they captured bats with their talons by flying into dense streams of bats exiting the cave. Owls do have the advantage over hawks in that they can forage throughout the night.

Large aggregations of bats could be an important food source for hawks and owls but once bats are dispersed throughout their foraging area they appear to be a less common food source for birds of prey (Fenton and Fleming 1976, Speakman 1991, Garcia et al. 2005). However, it is uncertain if bats perceive owls as a threat and alter their behavior when owls are present. Studies have found bats to be more active around the new moon or to move into shadowed areas on moonlit nights (Reith 1982, Adam et al. 1994, Hecker and Brigham 1999, Lang et al. 2006), which could be in response to predation risk or prey availability. Petrželková and Zukal (2001) found that a predator model placed outside of a roost had no effect on bat emergence. Conversely, Baxter et al. (2006) found a decrease in bat activity in the presence of owl calls but could not distinguish if this decrease was in response to the owl calls or noise in general. When conducting bat surveys for other projects, we have noticed that when an owl was heard calling or flew into the area, bat activity did not appear to be altered (personal observation). At other, times few bats were captured in mist nets when owls were observed roosted near the nets (personal observation).

Both predators and prey are important in maintaining a healthy ecosystem and ecologists have long been interested in the interactions between the two. Prey may alter their behavior in response to predators and therefore, the predators respond to the changes made by the prey (Lima 1998, Lima 2003). Prey are often faced with a tradeoff between energy intake and predation risk; thus, they may alter their habitat use and level of activity to limit costs and increase benefits. Predators can influence the density of prey in an area (Harvey 1991, Doncaster 1994) and have a strong "top-down" effect on ecosystems (Power 1992, Carpenter and Kitchell 1993, Ripple and Beschta 2003).

The potential predation risk bats face from avian predators is small and is likely outweighed by the need to forage. The energy demand for female bats greatly increases during the time of pregnancy through lactation (Kunz 1987, Kurta et al. 1989). It was found that female hoary bats (*Lasiurus cinereus*) increase the time spent foraging when they had more than one offspring and as lactation progressed (Barclay 1989). Additionally, bats need to store fat for both migration and hibernation. Fat deposition in the fall is likely more for hibernation because it is longer than migration and bats can "refuel" during migration (Fleming and Eby 2003). Considering the energetic demands of reproduction, migration, and hibernation, the risk of not foraging is likely to be more costly than the risk of predation associated with foraging.

Bats are an uncommon prey item for owls and little research has been done on how bats perceive owls. Understanding if bats perceive owls as a predation threat can be important in understanding where bats forage and have important implication on bat habitat conservation (Rydell et al. 1996). The goal of this project was to assess how bats respond to the presence of owl calls. Initially it was thought that bat activity would decrease in the presence of owl calls because owls can be a potential predator; however, after personal observations and literature review, we predicted bat activity to be minimally altered by calling owls. If owls do not pose a great enough risk to bats, then their foraging activity would not be greatly altered.

METHODS

Study Area

Our surveys took place in the Oak Openings Preserve located in Swanton, Ohio, USA. This park is 1,523.6 hectares and is part of the 467,000 hectare Oak Openings Region of Lucas, Fulton, and Henry counties in Ohio. The Oak Openings Region comprises less than 0.5% of the total land in Ohio (Schetter and Root 2011); yet it has more state listed species than any other similarly sized area in Ohio (ODNR Division of Wildlife 2012). A recent study of bats at this park (Sewald 2012) found eight different species to occur here including: big brown (*Eptesicus fuscus*), eastern red (*Lasiurus borealis*), hoary (*Lasiurus cinereus*), silver-haired (*Lasionycteris noctivagans*), northern long-eared (*Myotis septentrionalis*), little brown (*Myotis lucifugus*), evening (*Nycticeius humeralis*), and tri-colored bats (*Perimyotis subflavus*). The Indiana bat (*Myotis sodalis*) can be found in Ohio (U.S Fish & Wildlife Service 2007) but has not been confirmed as present in the Oak Openings Region.

There are four resident species of owls in Ohio (Department of Natural Resources 2006) and three of those species typically can be seen or heard calling in the Oak Openings Region (personal observation, Karen Menard Metroparks of the Toledo Area, pers. comm.). Great Horned Owls (*Bubo virginianus*), Barred Owls (*Strix varia*), and Eastern Screech-Owls (*Megascops asio*) can all be found within the Oak Openings Preserve (personal observation, Karen Menard, pers. comm.). The fourth resident owl species in Ohio is the Northern Saw-whet Owl, which is more often found in the Oak Openings Regions during the winter (Karen Menard, Sherrie Duris Toledo Naturalist Association, pers. comm.). Based on auditory cues, Barred Owls are thought to be the most common, but this could be misleading because they are more vocal than the other two species (Mazur 2000).

Survey Methods

Sixteen sites, a minimum of 400 meters apart, were surveyed throughout the Oak Openings Preserve in September 2012. A buffer of 400 meters was chosen so multiple sites could be surveyed in an area and so that the auditory treatments could not be heard at more than one site. Twelve sites were selected based on high bat activity data collected from surveys conducted during a previous study (Sewald 2012) and from additional surveys conducted in June and July 2012. The four additional sites were selected based on areas that appeared to be suitable foraging habitat for bats. Half of the sites were located in open grasslands, prairies, or savannas while the other eight sites were located in forested areas. Two of these sites were located on the edge of ponds: one small pond in interior forest and the other a large recreation pond (walking paths around it and fishing access) that is surrounded by forest. A diversity of sites was selected to sample different habitats, increase the possibility of recording a diversity of species, and to maximize the amount of bat activity recorded.

Surveys began a half hour after sunset and concluded within three hours of sunset which is when bat activity is at its highest (Hayes 1997). Surveys began with 10 minutes of recording bat activity using an Anabat^{TM} SDII acoustic detector (Titley Electronics, Ballina, NSW, Australia). Each Anabat unit was attached to a one meter pole to focus the Anabat microphone towards the bats and decrease the amount of insect noise the device recorded, which was concentrated near the ground. The initial 10 minutes served as a control to monitor bat activity before treatments. After 10 minutes of recording, a treatment was randomly selected to be played through a boombox (Naxa NPB-250) located underneath the Anabat for an additional 10 minutes. There were 4 possible treatments a site could be given: Great Horned Owl calls, Barred Owl calls, Eastern Screech-owl calls, or noise. These treatments were 20 seconds in length and were broadcasted once every minute for 10 minutes while the Anabat continued to record bat activity. The boombox was pointed in a different cardinal direction every time a call was broadcast so as to uniformly distribute the calls/noises. Based on owl surveying protocols (Takats et al. 2001, Manley 2006) and personal observations, the calls were naturally spaced and broadcasted at a natural volume to accurately represent a calling owl.

The noise treatment consisted of a mix of American toad calls (*Bufo americanus*), Common Nighthawk calls (*Chordeiles minor*), and a train horn, all of which are common ambient noises heard at the park. The purpose of the noise treatment was to help distinguish if bats responded to a perceived predation risk or noise in general. Treatments were broadcast at a volume that did not travel more than 200 meters to prevent them from being heard at more than one site. All four treatments were randomly selected for a given survey at a given site. Sites received each treatment once and no more than one treatment on a given night. Sites were surveyed in the reverse order during the final two surveys so that sites were not always surveyed at the same time.

Each survey was a total of 20 minutes in length (Francl et al. 2004, Brooks and Ford 2005, Ford et al. 2006, Johnson and Gates 2008, Brooks 2009), which allowed eight surveys to be conducted each night by two surveying teams. Surveys were not conducted during inclement weather such as strong wind, precipitation, or cold temperatures (Kunz 1973, Adam et al 1994). Start time, temperature, cloud cover, precipitation, wind speed, and noise level was recorded at each site. Noise was estimated using a scale of 0-4, a similar scale to the Beaufort scale used for estimating wind speed (Takats et al. 2001).

The amount of bat activity at each site was quantified by the number of call sequences recorded by the Anabat (Hayes 1997). We considered a call to be a single sound emission produced by a bat and a call sequence to be a series of calls separated by less than one second (Fenton 1999). Files were 15 seconds in length and typically contained one call sequence. After the first two surveys at every site, the number of files recorded at each site was quantified. Many sites yielded little to no bat activity so only sites (n=8) where bats were the most active were selected to be surveyed two additional times.

The recorded files were loaded into AnalookW (version 3.8v, Chris Corban), a program that projects calls on a sonogram allowing an expert to view recorded bat calls and to measure call characteristics. Files were identified based on visual inspection of frequency, shape, and slope of the calls by the author. Only files that had at least three calls were identified. Files containing a sequence of calls that could not be identified to species or a file that had fewer than three calls were labeled as “unknown.” All bat files were analyzed together (not by individual species) because most files were attributed to a single species. Identification of these files was aided by call libraries, notes from an Anabat workshop (Livengood et al. 2010) and the identification programs of Bat Call Identification 10 version 2.4 (Bat Call Identification, Inc., Kansas City, MO) and EchoClass version 1.0 (U.S. Army Engineer Research and Development Center, Vicksburg, MS). These programs compare unknown bat calls to a library of known bat calls and label the unknown files as the species that a majority of the calls most closely resemble. All the files were identified then placed into one of the four treatment categories (Great Horned Owl, Barred Owl, Screech-owl, or noise) based on the time the calls were recorded.

For each species, the average number of files per survey was calculated and a Wilcoxon signed-rank test was used to compare the average number of files recorded during the summer with those recorded in September. For sites that received all four treatments (n=8), the number of files recorded during each treatment was subtracted from the number of files recorded during the control. An ANOVA was utilized to look for a difference in the number of bat files recorded during the four treatments.

RESULTS

A total of 674 files were recorded during our surveys (n=48). Most of the bats recorded were big brown bats (74% of all files) and 13% of the files could not be identified to species. Files attributed to silver-haired bats made up 10% with few files being identified to red, hoary, *Myotis* species, and tri-colored bats. No evening bats were recorded.

When the average number of files recorded in September was compared to those of the summer (Table 1) we found there to be a significant decrease in activity during September (S=14.00, df=6, p=0.0156). The average number of files recorded during the owl treatments (10.96) was slightly higher than the control (9.22) and noise treatment (9.88) (Figure 1). However, we found no statistically significant difference in the number of bat files recorded during any of the treatments ($F_{(3,28)}$=1.57, p=0.2177). The average number of bat files recorded was highest during the Barred Owl treatment and lowest for the Great Horned Owl treatment (Figure 2).

DISCUSSION

The number of call sequences recorded in September was significantly lower than the number recorded during the summer. This was expected since bat activity peaks during the summer months (Hayes 1997). Many bat species migrate south to hibernate in caves over the winter and it appears that this migration begins sometime in August. Sewald (2012) found bat activity in the Oak Openings Region to decrease in August, so it makes sense that activity would be further reduced in September (Davis and Hitchcock 1965, Organization of Bat Conservation). Red and hoary bats are known to hibernate in trees and have been found hibernating as far north as the Ohio River Valley (Tuttle 1991) and will forage for insects when the weather is warm (Whitaker and Gummer 1992). The only species we found to be very active was big brown bats. Big brown bats are a sedentary species (Barbour and Davis 1969) that often overwinter in barns or houses (Tuttle 1991).

Few studies have examined bats' perception of predators. Baxter et al. (2006) conducted a similar study to ours and they found bat activity to be altered by an owl treatment, but could not confidently conclude that bats were responding to perceived predation risk versus general auditory noise. We utilized a noise treatment in an attempt to avoid this issue and contrary to their findings, our study found bat activity to be unaltered during any owl treatment or the noise treatment. It does not appear that bats associate calling owls with a predator.

We cannot say if owl calls affect individual bat species differently because we had few bat files attributed to species other than big brown bats. We recommend repeating this study during the summer months when there is a greater diversity of bats to assess if different bat species respond to owl calls. However, we can say that the average number of bat files recorded did not statistically differ between treatments and the control. More bat calls were recorded during the Barred Owl treatment than any other treatment or the control. All treatments were conducted at different times of the night so that there was no bias between the treatment and the time of night. We do not believe Barred Owl calls stimulated bat activity but rather bat activity happened to be greater those nights and/or during that time of night when Barred Owl calls were played. Furthermore, Barred Owls are very vocal (Mazur 2000) and bats appear to have become accustomed to their calls. Once a bat leaves its roost to begin foraging their only potential predators are owls, but this risk is still thought to be low (Speakman 1991, Smith et al. 1999). Bats are more likely at risk to predation by raccoons or snakes when they are roosting, but these are probably infrequent threats as well (Gillette and Kimbrough 1970, Saunders 1989).

Bat activity was not altered by owls most likely because owls do not regularly take bats as prey (Ritchison and Cavanagh 1992, Swengel and Swengel 1992, Marti and Kochert 1996), and therefore, bats do not perceive owls as a threat. While conducting acoustic bat surveys for another project, we observed that when owls flew into the survey area and started calling, bat activity did not appear to be altered. Bats unresponsiveness to owl calls is likely a result of the benefits of foraging outweighing the cost of avoiding predators. There are no specialized bat predators in North America so the risk of predation is low. Of greater importance for bats is meeting the high energy demands of reproduction (Kunz 1987, Barclay 1989, Kurta et al. 1989), migration, and hibernation.

Furthermore, insectivorous bats are highly agile (Norberg and Rayner 1987) and likely can out-maneuver owls. This is probably why most observations of owls taking bats as prey have occurred near large roosts when bats exit in large numbers and owls have a better chance of catching a bat (Twenty 1954, Baker 1962, Barclay et al. 1982). Catching an individual bat in the woods or open field would be more difficult. Boinski et al. (2003) found that habitats associated with a predation risk may be more of a determining factor whether to forage or not, than the actual presence of predators.

Another possible explanation for the lack of response by bats is that like most predators, owls are quiet when they hunt. If an owl is hunting it is not likely to be calling because it will give its location away to the prey. Owls call to establish and defend territories and to attract mates (Johnsgard 1988, Duncan and Duncan 1997). When bats hear an owl calling, they may perceive it as another typical nocturnal noise. Our results concur as we found the amount of activity to be the same between the owl treatments and the noise treatment. Owls and noise do not seem to negatively influence bat activity although there is likely a noise level threshold that will impact bat activity. Of greater concern for bats are habitat loss, wind energy, and White-nose Syndrome (Krusic and Neefus 1996, Smith and Gehrt 2010, Johnson 2005, Kunz et al 2007, Frick et al. 2010, Lametti 2010) and these issues need to be the focus of bat conservation.

ACKNOWLEDGEMENTS

This research was part of my Master's thesis work which was in part funded by the Root Lab and the Toledo Naturalist Association. We would like to thank Dan Wiegmann and Verner Bingman for serving as committee members. This project would not have been possible without the support of the Root Lab: Karen Root (advisor) and members Matt Cross, Jessica Sewald, Kat Baczynski, Amanda Kuntz, and Christine Whorton. Many thanks to my dedicated field assistants: Brad Crim, Margaret Vogel, Rob Baker, Nicholas Kuns, and Kathryn Mehlow for their efforts in data collection. We are also appreciative of the Metroparks of the Toledo Area and Karen Menard for allowing me to survey within the parks.

Table 1. The average number of files recorded during summer (June and July) and fall (September) surveys. Bat activity was significantly lower in September than in June and July.

Species	**Summer Avg.**	**September Avg.**
Big brown	13.77	10.44
Silver-haired	1.56	1.46
Red	1.16	0.19
Hoary	0.29	0.04
Myotis species	0.26	0.06
Evening	0.26	0.00
Tri-colored	0.14	0.08

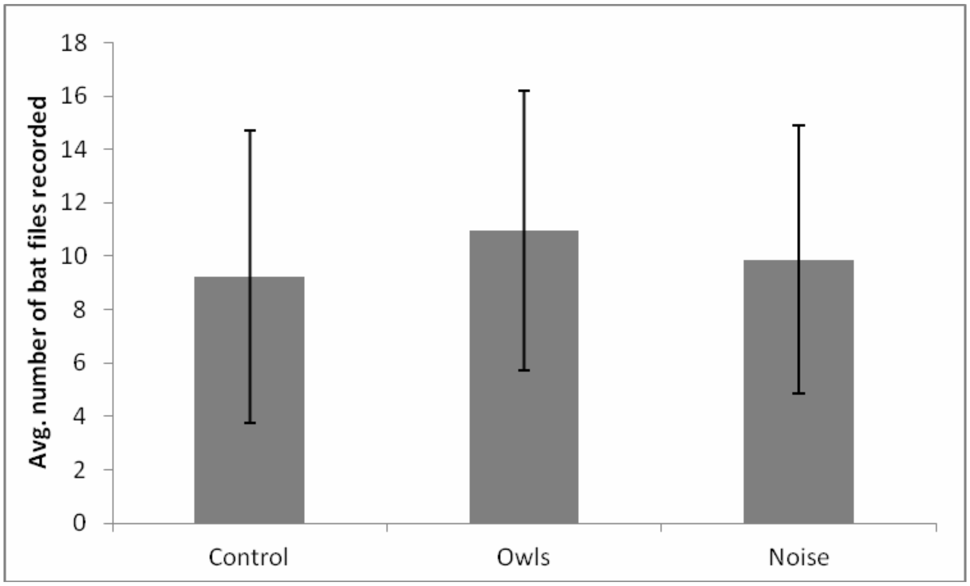

Figure 1. The average number of bat files recorded during the control, all owl treatments pooled together, and the noise treatment.

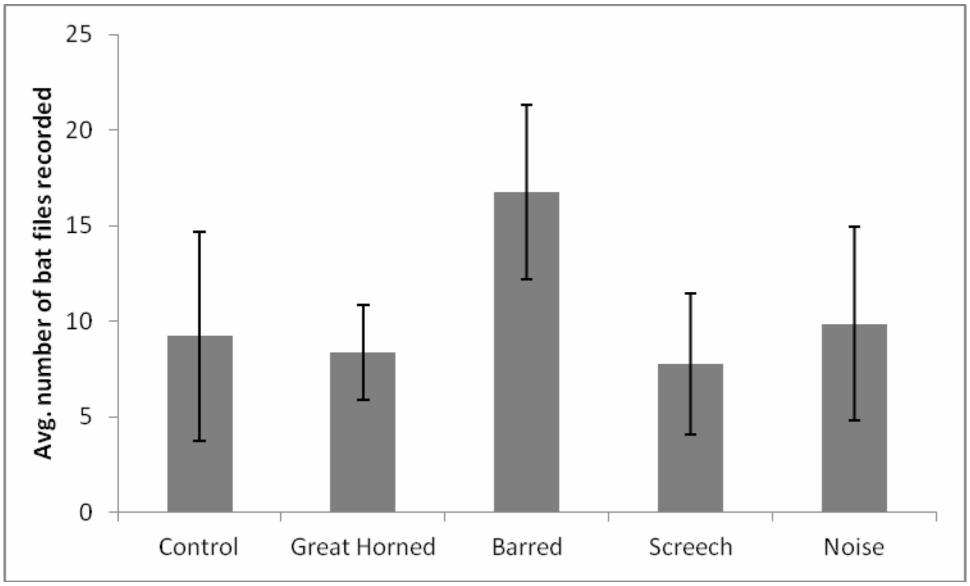

Figure 2. The average number of bat files recorded during the control and the four treatments. There was no statistical difference between any of the treatments.

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CHAPTER IV

CONCLUSIONS

In chapter I, I conducted acoustic surveys to record bat species in order to compare the identifications of two acoustic identification programs to one another as well as to an expert's identification. The two programs selected for this experiment were chosen because they are under consideration by the U.S. Fish and Wildlife Service for use when conducting bat surveys. The results indicated that the two programs agreed with the expert's identification of a given call file 61 percent and 69 percent of the time. Comparing the two programs to one another, they agreed on the identification of a particular file about 50 percent of the time. They also produced a varying number of false positive identifications of the endangered Indiana bat. These programs are important in analyzing large quantities of acoustic bat files but the identifications need to be checked by an expert before a final decision is made on a file.

For chapter 2, acoustic surveys were again utilized to assess which habitats were more often used by different bat species and to determine if the amount of bat activity changed throughout the night. Bat activity, in general, was greatest at open sites, but activity did vary by species. Big brown, red, hoary, and silver-haired bats were more active in at open sites, while *Myotis* species were more active at forest sites. These results highlight the importance of managing for a heterogeneous landscape. Bat activity was greatest a few hours after sunset and a few hours before sunrise. The evening bat appears to use different habitats during different times of the night and I suggest additional surveys be done to see if this is the case for other species.

Finally, in chapter 3 bat activity was acoustically recorded before and after broadcasting owl calls to evaluate if bats were influenced by owls. Owls occasionally take bats as prey, but little research has been done on how bats perceive this potential threat. My surveys found that bat activity was unaltered in the presence of calling owls, which suggests that owls pose little threat to bats. This is likely because owls do not vocalize when hunting, but rather to defend their territory and attract mates. Bats have a high energy demand and the necessity to meet this demand likely outweighs the predation risk posed by owls. Bat populations worldwide are declining for multiple reasons, but based on these results owls are not a major threat to the conservation of bats.

In summary, the major take home points from this thesis are these: 1) acoustic programs speed up the analysis of recorded bat calls but the identification needs to be checked by the researcher before a final decision is made. 2) Bat species utilize different habitats in the Oak Openings Region and some species may adjust their habitat use throughout the night. This emphasizes the importance of managing for a heterogeneous landscape. 3) Owls appear to have no influence on the foraging behavior of bats.

APPENDEX I

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL

Bowling Green State University

Office of Research Compliance
309A University Hall
Bowling Green, OH 43403-0183
Phone: (419) 372-7716
Fax: (419) 372-6916
E-mail: hsrb@bgnet.bgsu.edu

September 4, 2012

Dr. Karen Root
Biological Sciences
Bowling Green State University

Re: IACUC Protocol 12-009

Title:
Assessing if bats alter their foraging behavior in the presence of owl calls

Dear Dr. Root:

On **September 4, 2012** the above referenced protocol was **approved** after review by the IACUC. This approval expires on September 3, 2013, by which time renewal must be requested if you wish to continue work on the protocol. The Office of Research Compliance will send notification reminding you of the need for renewal in advance of that date.

Please have all members of your research team read the approved version of the protocol.

Sincerely,

Hillary Harms, Ph.D.
IACUC Administrator

Comments:

Consider consulting with the Attending Veterinarian to discuss research methods (e.g., natural frequency of owl calls).