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INVENTORY, ABUNDANCE, AND HABITAT SELECTION OF CHIROPTERANS AT FELSENTHAL NATIONAL WILDLIFE REFUGE IN SOUTHEAST ARKANSAS

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ABSTRACT

Chiropterans (bats) are the second largest taxonomic Order of Class Mammalia on earth, second only to rodents. Felsenthal NWR is a 65,000-acre refuge composed of three distinct habitat types: bottomland hardwood forest, cypress-tupelo swamp, and upland pine forest systems. This study was conducted from October 2015 through May 2017 to provide an inventory of bats on Felsenthal NWR, while also looking at seasonal abundance and habitat selection. Acoustic monitoring and mist netting was utilized to record bat species present throughout different habitat types in and around the refuge. All bats collected in mist nets were screened for white-nose lesions, though no signs of the disease or the fungus were observed. Ten of the eleven species documented by Connior (2010) in southern Arkansas were recorded either acoustically or via live capture. The sampling methods employed revealed habitat selection by species varied significantly.

A thesis and tabular data are provided as findings from a research project which is enclosed with this report as the final deliverable for FY15 I&M Project funding. The objective was to provide an inventory of Chiropterans present on the refuge including the northern long-eared bat. Through the graduate research project, the thesis provides additional information on Chiropteran abundance and habitat use on Felsenthal NWR.

DATA APPENDIX OR SUPPLEMENTAL INFORMATION

Document	File Type
Thesis	.pdf
Acoustic Tabular data	.xlsx
Capture Tabular data	.xlsx

INVENTORY, ABUNDANCE, AND HABITAT SELECTION OF CHIROPTERANS AT FELSENTHAL NATIONAL WILDLIFE REFUGE IN SOUTHEAST ARKANSAS

Tyler Joseph Porter

A thesis submitted to the graduate faculty of the College of Arts, Education, and Sciences at the University of Louisiana at Monroe in partial fulfillment of the requirements for the degree of Master of Science in Biology

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ABSTRACT

Tyler Joseph Porter

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(Major Professor: Kim Marie Tolson, Ph.D.)

Chiropterans (bats) are the second largest taxonomic Order of Class Mammalia on earth, second only to rodents. Felsenthal NWR is a 65,000-acre refuge composed of three distinct habitat types: bottomland hardwood forest, cypress-tupelo swamp, and upland pine forest systems. This study was conducted from October 2015 through May 2017 to provide an inventory of bats on Felsenthal NWR, while also looking at seasonal abundance and habitat selection. Acoustic monitoring and mist netting was utilized to record bat species present throughout different habitat types in and around the refuge. All bats collected in mist nets were screened for white-nose lesions, though no signs of the disease or the fungus were observed. Ten of the eleven species documented by Connior (2010) in southern Arkansas were recorded either acoustically or via live capture. The sampling methods employed revealed habitat selection by species varied significantly.

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LIST OF ABBREVIATIONS AND SYMBOLS

°C	degrees Celsius
AM	acoustic monitoring
ANOVA	analysis of variance
BLH	bottomland hardwood
cm	centimeters
CTB	cypress-tupelo bottom
FNWR	Felsenthal National Wildlife Refuge
g	gram
GIS	Geographic Information Systems
GPS	global positioning system
ha	hectares
km	kilometers
kph	kilometers per hour
m	meters
MABM	mobile acoustic bat monitoring
mm	millimeter
NWR	National Wildlife Refuge
PAM	passive acoustic monitoring
Pd	*Pseudogymnoascus destructans*
USFWS	United States Fish and Wildlife Service
WGCP	Western Gulf Coastal Plain
WNS	White-nose Syndrome

INTRODUCTION AND REVIEW OF THE LITERATURE

Introduction

The order Chiroptera (bats) represents the second largest mammalian order in regards to species number behind only Order Rodentia (rodents), consisting of 18 families, 192 genera, and over 1,300 species worldwide (Linzey 2001; Trani et al. 2007). Bats are cosmopolitan in their distribution, being absent only in polar regions and some oceanic islands. They fill a wide variety of ecological niches and have extensive taxonomic and functional diversity which makes them well suited as bioindicators for large-scale ecosystem health (Patterson et al. 2003, Simmons and Conway 2003). Many bats act as pollinators for plants, dispersal agents of seeds, predators of night-flying insects, and as prey for higher-level carnivores, as well (Findley 1993).

Around the world, bats act as voracious predators of nocturnal insects, including many crop and forest pests which may act as disease vectors to humans (Whitaker 1988). In the continental United States, there are 47 species of bats, representing four families and 19 genera which account for less than 5% of species worldwide. Bats are the only major predators of night-flying insects, and nearly all of the species in the United States are exclusively insectivores (Harvey et al. 2011). It is estimated that bats eat roughly half of their body mass or more in nocturnal insects (4-8 g) every night, and nursing females may eat up to their full body mass or more (8-16 g). Current estimates value bats saving the agricultural and farming industry in the United States anywhere from $3.7 to $53 billion per year in insect control (Boyles et al. 2011). Bats can travel long distances (several km per night) from roosting sites to foraging grounds which allows them the potential to suppress nocturnal insect populations and transport nutrients across a broad landscape (Pierson and Rainey 1998).

White-nose Syndrome (WNS) of bats, caused by a psychrophilic fungus known as *Pseudogymnoascus destructans* (*Pd*) (formerly *Geomyces destructans*), has decimated populations of insectivorous bats in eastern North America since its 2006 discovery in east-central New York (Minnis and Lindner 2013). WNS has been characterized as a condition of bats in hibernation and was named for the visually striking white fungal growth on muzzles, ears, and/or wing membranes of affected bats (Blehert et al. 2008). WNS initiates a cascade of physiologic disturbances after epidermal invasion of the skin which increases metabolic rate and decreases the bats ability to excrete carbon dioxide which leads to water and electrolyte loss and energy use during winter months (Verant et al. 2014). In turn, *Pd* affects bats by increasing the frequencies of arousal from the torpor of hibernation and directly alters their ability to survive during the winter months. Bats with this fungal infection exhaust their supply of stored energy long before the end of winter which causes them to starve due to lack of insect prey in mid-winter (Fenton 2012). Histological examination of infected bats shows that the fungus pervades the bats' tissues filling hair follicles and sebaceous glands. Yet, the fungus does not typically lead to inflammation or an immune response in the tissue of the hibernating bats. This factor makes it easy to transfer, hard to detect, and incredibly deadly (Meteyer et al. 2009). As of 2012, WNS has been estimated to have killed 5.7-6.7 million bats in the United States, with some estimates being higher (Froschauer and Coleman 2012).

Presently, seven bat species have been diagnosed with WNS, three of which are classified as Federally Endangered or Threatened: Big Brown Bat (*Eptesicus fuscus*; EPFU), Eastern Small-footed Myotis (*Myotis leibii*; MYLE), Gray Bat (*Myotis grisescens*; MYGR) (endangered), Little Brown Bat (*Myotis lucifugus*, MYLU), Indiana Bat (*Myotis sodalis*; MYSO) (endangered), Northern Long-eared Bat (*Myotis septentrionalis*; MYSE) (threatened), and Tri-colored Bat (*Perimyotis subflavus*, PESU). Five other species have been found to contain *Pd*, but have yet to develop the disease: Eastern Red Bat (*Lasiurus borealiu*;, LABO), Rafinesque's Big-eared Bat (*Corynorhinus rafinesquii*; CORA), Southeastern Myotis (*Myotis austroriparius*; MYAU), Silver-haired Bat (*Lasionycteris noctivagans*; LANO), and Townsend's Big-eared Bat (*Corynorhinus townsendii virginianus*; COTO) (Blehert et al. 2008; Bat Conservation International 2014). Each of these 12 species, along with four other species - Brazilian Free-tailed Bat (*Tadarida brasiliensis*; TABR), Evening Bat (*Nycticeius humeralis*; NYHU*)*, Hoary Bat (*Lasiurus cinerus*; LACI), and Seminole Bat (*Lasiurus seminolus*; LASE*)* - are found within the southeastern U.S. and the state of Arkansas (Connier 2010; Harvey et al. 2011).

The Western Gulf Coastal Plain (WGCP) is a 16 million hectare[2] geographic region falling within the states of Arkansas, Louisiana, Oklahoma, and Texas (Connor and Dickson 1997). Stroud and Hanson (1981) classified this region as a historically mixed-longleaf and shortleaf pine-dominated landmass with bottomland hardwood forests (BLH) in the region's floodplains. But, the WGCP is currently an area rich in agriculture, crude oil, natural gas, and logging timber worth billions of dollars that employees thousands of local residents (Connor and Dickson 1997). The WGCP harbors some of the most species-rich plant and animal communities in North America which has led it to be a major focus for research and conservation efforts (The Nature Conservancy 2003; Noss et al. 2015). Although the WGCP is heavily forested, it has been fragmented and dissected by logging practices, roads, pastures, cities, and reservoirs which have led to reduced forest productivity and natural diversity and abundance among aquatic and terrestrial species, including bats (USFWS 2009).

McPeake et al. (2012) identified 16 native and migratory bat species within the state of Arkansas, eleven of which have been documented within the southeastern part of the state (Baker and Ward 1967; Connior 2010) (Table 1). The Arkansas Game and Fish Commission lists five of these species as "vulnerable" within the state due to WNS and habitat degradation: Rafinesque's Big-eared Bat, Hoary bat, Silver-haired Bat, Seminole Bat, and Southeastern Myotis (Connior 2010). Southern Arkansas has few caves, but it is believed that tree cavities and road culverts that exemplify the same temperature and humidity characteristics as caves that hold migratory and native bats may be possible vectors for Pd and the development of WNS during winter months (Reynolds and Barton 2014; Russell et al. 2015). Performing monthly abundance measures of Chiropterans in areas that are at-risk, and areas affected by WNS and habitat degradation, could lead to better conservation protocols to protect bat populations at national and regional levels.

Table 1 Bats found in the state of Arkansas and their current statuses. Highlighted species have been documented in southern Arkansas. (Baker and Ward 1967; Connior 2010; McPeake et al. 2012)

Species	Common Name	IUCN Status	USFWS T&E Status	State of Arkansas Status
Corynorhinus rafinesquii	Rafinesque's Big-eared bat	Least Concern	Not Listed	Vulnerable (S3)
Corynorhinus townsendii	Townsend's Big-eared Bat	Least Concern	Endangered	Critically imperiled (S1)
Eptesicus fuscus	Big Brown Bat	Least Concern	Not Listed	Common (S4)
Lasionycteris noctivagans	Silver-haired Bat	Least Concern	Not Listed	Vulnerable (S3)
Lasiurus borealis	Eastern Red Bat	Least Concern	Not Listed	Common (S4)
Lasiurus cinereus	Hoary Bat	Least Concern	Not Listed	Vulnerable (S3)
Lasiurus seminolus	Seminole Bat	Least Concern	Not Listed	Vulnerable (S3)
Myotis austroriparius	Southeastern Bat	Least Concern	Not Listed	Vulnerable (S3)
Myotis grisescens	Gray Bat	Near Threatened	Endangered	Endangered (S2S3)
Myotis leibii	Small-footed Myotis	Least Concern	Not Listed	Critically imperiled (S1)
Myotis lucifugus	Little Brown Bat	Least Concern	Not Listed	Vulnerable (S3)
Myotis septentrionalis	Northern Long-eared Bat	Least Concern	Threatened	Common (S4)
Myotis. sodalis	Indiana Bat	Endangered	Endangered	Endangered (S1)
Nycticeius humeralis	Evening Bat	Least Concern	Not Listed	Common (S4)
Perimyotis subflavus	Tri-colored Bat	Least Concern	Not Listed	Common (S4)
Tadarida brasiliensis	Brazillian Free-tailed Bat	Least Concern	Not Listed	Common (S4)

Literature Review

Inventory, Monitoring, and Abundance

Inventory and monitoring (I & M) procedures are some of the most frequently conducted wildlife studies and are considered cornerstones in the management of natural and wildlife resources. The overall purpose of I & M efforts is to develop scientifically sound information on the current status and long-term trends in the composition, the structure, and the function of ecosystems and the species that inhabit them, while also determining how well current management practices are sustaining those ecosystems (Morrison et al. 2008; Fancy et al. 2009). Due to the declining population of bats in North America, natural resource agencies and academic institutions have employed I & M efforts annually to provide baseline information on bats at local and regional levels to establish such information for wildlife managers and policymakers (O'Shea and Bogan 2003; Loeb et al. 2015). Flaquer et al. (2007) noted that in the past, assessing the distribution and abundance of bats was difficult because of the technological barriers and the natural tendencies of bats: nocturnal behavior, large home ranges, and erratic aerial movement. Thus, a paucity of large-scale spatial data has been collected. In order for large-scale conservation planning of bats, it is critical to know the abundance of bats in different geographic locations and habitats. This type of information would result in the understanding of current populations and their trends over time (Ford et al. 2005). Loeb et al. (2015) stated that an efficient bat monitoring program would: (1) document the impact of stressors on populations, (2) identify priority species for conservations efforts and, maybe most importantly, (3) measure agencies' effectiveness in conservation and management to lessen stressors. Studies have been conducted with similar goals to achieve and expand on the premises established by Loeb et al. (2015) and their methods used. Both MacSwiney et al. (2008) and Furey et al. (2009) found that they increased inventory completeness using acoustic monitoring in addition to live capture efforts, which increased species richness of bats by nearly 30% at their study site. Many scientific studies of bats have inventory, monitoring, or both as one of their primary objectives since so little is known regarding their distribution and abundance (Seidman and Zabel 2001; Weller 2008; Meyer et al. 2010; Ethier and Fahrig 2011). Additionally, Meyer et al. (2010) stated that in order for I & M efforts to be accurate in depicting long-term trends of bat populations, studies would need to be extensive and long-lived, spanning decades and more. Thus, urgency in developing scientifically sound I & M efforts should be high-priority, extensively-funded, and long-lasting programs for agencies interested in bat conservation.

Animal abundance measures, the number of individuals found per sample (incidence), are a key parameter within I & M efforts that can be used as absolute or relative representations of a species in a particular ecosystem (Wright 1991; Magurran 2004). The simplest methods of estimating abundance of species are typically based on counting individuals or their signs through transects or quadrants, while more complex methods may delve into capture-recapture and/or radio telemetry efforts. Methods of measuring abundance depends on whether the species is in an “open” or “closed” system. (Schwarz and Seber 1999).

Methods for estimation of absolute abundance of bats from acoustical data have yet to be developed, but the number of calls per species along mobile transects can be used as an index of abundance (Loeb et al. 2015). Attempts to estimate bat abundance in the United States have relied heavily on use of indices at small, local sites through echolocation detectors and live capture. It is important for collecting efforts to be distributed throughout the year, but it is applicable to confine efforts to the season in which abundance peaks in some areas (Moreno and Haffter 2000). These efforts have documented dramatic changes in bat abundance and clear-cut causes that provided strong evidence for population gains and declines, however, shortcomings of those methods must be fully acknowledged (Anderson 2001; O'Shea and Bogan 2003). Knowing the relative number of individuals in an ecosystem is a crucial parameter that needs to be addressed before intense study and critical management decisions can be made (Magurran 2004).

Foraging and Habitat Use

Bats have long been identified as good indicators of the integrity of natural communities because they occupy a vast number of ecosystems, habitats, and ecological niches (e.g. roosting, watering, and feeding habits) (Hutson et al. 2001). However, current knowledge of foraging practices and habitat selection across widespread spatial scales are very limited for bat species. This creates a problem for those looking to effectively manage for Chiropterans. Ecological factors such as productivity, habitat diversity, and area of available habitat influence the structure of bat communities (Findley 1993). Wildlife and management practices may have profound effects on habitat, diversity, and abundance of bats in localized communities, thus making understanding of this subject of highest priority (Williams et al. 2006). Additionally, estimations of habitat preference and activity in those habitats for different bat species are of great importance when planning conservation efforts. (Ethier and Fahrig 2011; Palmqvist 2014).

Trani et al. (2007) recognized eleven species of bats that had ranges occurring within southern Arkansas, representing two families and eight genera. These species share the same ecosystem but maintain their own respective ecologic niches (Trani et al. 2007; Boyles et al. 2011). Many species have known roosting and foraging site preferences, but shared inhabitance and diet within communities may influence or even alter their behavior at localized levels (Kunz 1973; Findley 1993; Arlettaz et al. 1997). Within forested systems, bats utilize a variety of structures for diurnal roosts, including tree foliage, exfoliating bark, cracks or crevices in trees, and internal cavities of natural and anthropogenic structures (Brigham 2007).

Rafinesque’s Big-eared Bat and Southeastern Myotis are two species within the southeast that exhibit interspecific, communal roosting habits throughout the year and, often, the same foraging grounds. Southeastern Myotis are known to fly relatively great distances directly from roost sites to foraging grounds, usually near or above water. Rafinesque’s Big-eared Bat emerge later in the evening and forage in bottomland forests and sloughs (Reid 2006; Harvey et al. 2011). Due to habitat loss from logging and agriculture practices throughout most their range, these species are considered “at risk,” and efforts are being implemented to try and conserve the two species but little about their ecology is known. (Reid 2006; Carver and Ashley 2008; Trousdale 2011).

Other bat species that are considered foliage roosters and, sometimes cave roosters (where available), are the Big Brown Bat, the Eastern Red Bat, the Evening Bat, the Seminole Bat, and the Tri-colored Bat. These species are known to emerge shortly after dusk and forage on prey in open fields, edge communities, above vegetation canopy, and above water sources. These bats tend to occupy a variety of habitats, ranging from wet, deciduous timber stands to dry, coniferous forests across low-lying to moderate elevations in the United States (Reid 2006). Brooks and Ford (2005) demonstrated that bat species may choose foraging grounds based on relative body size due to weather conditions, nocturnal predators, and interspecific competition for insect prey. Although these bats share feeding habits that are relatively consistent with each other, many of these bats forage at different heights relative to the ground and forest canopy, which allows for coexistence and little infringement when resources are hearty (Menzel et al. 2005).

Silver-haired Bats and Hoary Bats are two other species that are occur concurrently in southern Arkansas and share similar foraging as species listed prior. But, their highly migratory nature only finds them in southern Arkansas during the colder months (Reid 2006; Connier 2010; Harvey et al. 2011). These species choose similar habitat types and foraging grounds as other “tree bats,” but the manner in which they locate prey and forage differs slightly. These species, along with other “tree bats” listed prior, have different strategies of foraging in regards to flight path, flight speed, and echolocation that may alter their habitat preference for foraging practices (Barclay 1985). Although much has been learned about bats’ foraging habits and the habitats they occupy, much is still unknown about their foraging ecology at localized levels throughout geographic ranges. It is safe to say, at best, that habitat preference is incredibly variable, in spatiotemporal regards, at different ecologic levels and should be studied extensively for better conservation practices (Walsh and Harris 1996; Agosta 2002).

Acoustic Monitoring

Acoustic monitoring (AM) has become a widely-utilized method of sampling in the field of wildlife conservation. Although relatively new, AM is beginning to be recognized as a potentially powerful, efficient, and non-intrusive tool in monitoring and surveillance studies since direct methods (e.g. visual observation, capture) are not always possible or practical (Miller 2001; Jones et al. 2013). AM is a dynamic means of long-term monitoring of the activity of a species and habitat use over time and space (Frick 2013). Bats are one such group whose presence or absence cannot be addressed by direct methods alone (Miller 2001). Bat acoustic detectors work by converting their ultrasonic echolocation calls to sounds that are clearly audible and identifiable to humans (Fenton 1992), which provides an outstanding means to uniquely identify, take inventory, and monitor them using direct counts or traditional estimates (USFWS 2012). Acoustic detectors and sampling methods have diverged significantly to accommodate specific research goals (Adams et al. 2012; Frick 2013). AM methodologies have been broadly categorized as either passive or active (also known as mobile) sampling. Active AM refers to surveying where the surveyor is present and able to change the orientation of the microphone relative to bats for optimal echolocation pass recording. Often times, the surveyor is moving on a mobile transect with the microphone and detector to cover a large spatial scale with multiple samples. Passive sampling refers to stationary, automatic recording without the user being present, and with the microphone fixed in a predetermined direction. Passive sampling often includes multiple sampling stations allowing sampling over wide spatial scales. This type of AM produces fewer independent samples but allows for longer periods of data collection and less time expenditure of the user (Coleman et al. 2014).

Mobile acoustic bat monitoring (MABM), a form of active AM, has become a widely-used technique utilized to record several different parameters such as abundance, occupancy, and diversity of species (Adams et al. 2012). USFWS (2012) concluded that MABM surveys along road-based transects provided an efficient method to sample bats across large areas in a relatively short time period. Whitby et al. (2014) tested MABM techniques (road-based versus water-based) and concluded that road-based transects yielded higher diversity indices and tended to show slightly higher species density, while also being the least time consuming. MABM surveys provide user-friendly, cost-effective means of gathering biodiversity data rapidly and sustainably across large spatial areas (Jones et al. 2013).

Many studies have utilized passive acoustic monitoring (PAM) in favor of, or in conjunction with, active methodologies because of its ability to gain large datasets across greater spatial and temporal scales (Frick 2013), while also requiring a relatively low-energy demand by the user (Coleman et al. 2014; Froidevaux et al. 2014). Coleman et al. (2014) compared PAM and MABM and found that there was no difference in species density between methods, although PAM accumulated species more quickly than their MABM efforts. Froidevaux et al. (2014) used different variations of PAM to optimize call capture while measuring activity, richness, and sampling effort. They found that it was necessary to sample across numerous microhabitats of given locations simultaneously, to generate the best outcome. Miller (2001) concluded that PAM provided an effective measure of bat activity while allowing comparisons between sites, times, and species that MABM cannot accomplish, practically. Although AM data, alone, cannot provide accurate estimates of individuals, this has not limited the uses of AM as an effective method to estimate numbers of bats. It may provide objectives and repeatable means to assess and compare relative activity of free-flying bats from this type of data (Fenton et al. 1987).

Mist Netting

The use of mist nets is a popular method for capturing bats away from their roost site while they are airborne (Kunz and Kurta 1988; Jones et al. 1996). Mist nets work by entangling flying bats that fail to detect their fine mesh until it is too late. Mist nets, although relatively low-costing and easily-portable, have several disadvantages. Mist nets are labor intensive, can be detrimental to bat's well-being if not removed quickly, and are unable to reach and capture bats high in the canopy where they may be foraging (Jones et al. 1996; Gukasova and Vlaschenko 2011; Fenton 2012). Several factors come into play when attempting to maximize mist net success such as net placement, proximity to roost sites, proximity to water, and in conjunction to known flyways. These factors cannot always be optimized and may pose bias in relation to the representativeness of a population (Kunz & Kurta 1988). At best, mist nets provide information on flight activity only at sites where actively netted, and may provide potentially misleading data. But, multiple studies have used the data obtained for inventory, monitoring, richness, diversity, and method comparison purposes (Kunz and Brock 1975; Ford et al. 2006; Larson et al. 2007; Gukasova and Vlaschenko 2011; Trevelin et al. 2017).

Several studies have utilized mist nets, alone, to achieve certain goals and reach specific outcomes. Gukasova and Vlaschenko (2011) found that mist netting in the midsummer months yielded the highest capture of bats because of peak bat activity and young bats are becoming volant. They also found that there was no correlation with the duration of mist netting for each month. Trevelin et al. (2017) also relied on mist netting to find optimum sample size. These researchers found that using mist nets, alone, during the first three to six hours of the night (optimal foraging hours) could not provide a complete sample and was not representative of the community. Bergallo et al. (2003) suggested distributing mist nets across various locations was more efficient than increasing net hours at a single location to accurately measure biodiversity of bats in a system. They also suggested having at least 1000 captures when sampling with mist nets to get an accurate representation of biodiversity and overall abundance. This could prove time-consuming and inefficient for the researcher. Biodiversity assessments and conservation guidelines based on short-term mist net surveys, alone, are not sufficient or reliable in regards to bats, thus they must be coupled with other means of capture over the long-term (Gukasova and Vlaschenko 2011).

Many studies have combined mist netting with other methods to optimize representativeness and decrease bias of different measures. Kuenzi and Morrison (1998) suggested combining mist net capture with ultrasonic detection to identify the presence of bat species, since acoustic detection can sometimes be unreliable or create false-positive detections. Hill and Greenway (2005) found that playing back echolocation calls near mist nets, as an acoustic lure, demonstrated an effective method which increased capture. Everette et al. (2001) documented that relative abundance based on mist net captures was similar to that revealed by their acoustic surveys, except that the latter revealed the likely presence of at least two additional species. Combining mist netting surveys with acoustic surveys will increase effectiveness of sampling efforts, regarding bats, and will, usually, both coincide in results (O'Farrel and Gannon 2001).

Objectives

Bat populations throughout North America have declined drastically within the past decade. This study was aimed to provide Felsenthal National Wildlife Refuge in southeast Arkansas, and surrounding lands with similar ecosystems, a better understanding of the bat species found there. The objectives of this study are as follows:

- Provide an inventory of bats found on Felsenthal National Wildlife Refuge, Arkansas.
- Measure seasonal abundance and biodiversity of bats at Felsenthal National Wildlife Refuge, Arkansas.
- Examine the preference and utilization of habitat for native bat species within and on lands surrounding the refuge using passive and mobile acoustic monitoring.
- Document the presence/absence of the fungal disease White-nose Syndrome in bat species caused by *Pseudogymnoascus destructans*.

MATERIALS AND METHODS

Study Site

The Felsenthal National Wildlife Refuge (FNWR) is located in Ashley, Bradley, and Union counties of southeast Arkansas, about 8 km west of Crossett, Arkansas on U.S. Highway 82 (Figure 1). FNWR is one of three refuges forming an administrative complex in southern Arkansas, which also includes Pond Creek NWR to the northwest and Overflow NWR to the east (Figure 2). Established in 1975 as a mitigation for the creation of the U.S. Corps of Engineers' Ouachita and Black Rivers Navigation Project and Felsenthal Lock and Dam, FNWR was named for a small community located at its southwest corner. It occupies a low-lying area dissected by an intricate system of rivers, creeks, sloughs, buttonbush swamps and lakes throughout a vast bottomland hardwood (BLH) forest that gradually rises to an upland forest community on its peripheral boundaries. FNWR parallels both the Ouachita River and the Saline River which pours into the Ouachita River near the refuge's geographic center. Historically, periodic flooding of the BLH areas during winter and spring provides excellent wintering waterfowl habitat, and these wetlands, in combination with the pine and upland hardwood forest on the higher ridges, support a wide diversity of native plants and animals (USFWS 2010).

The FNWR consists of ~26,305 hectares (ha) of total land. The refuge is broken up into 60% of BLH (~16,187 ha), 25% open water (~6,070 ha), and 15% upland area (~4,047 ha) (Figure 3). FNWR has the world's largest greentree reservoir. The

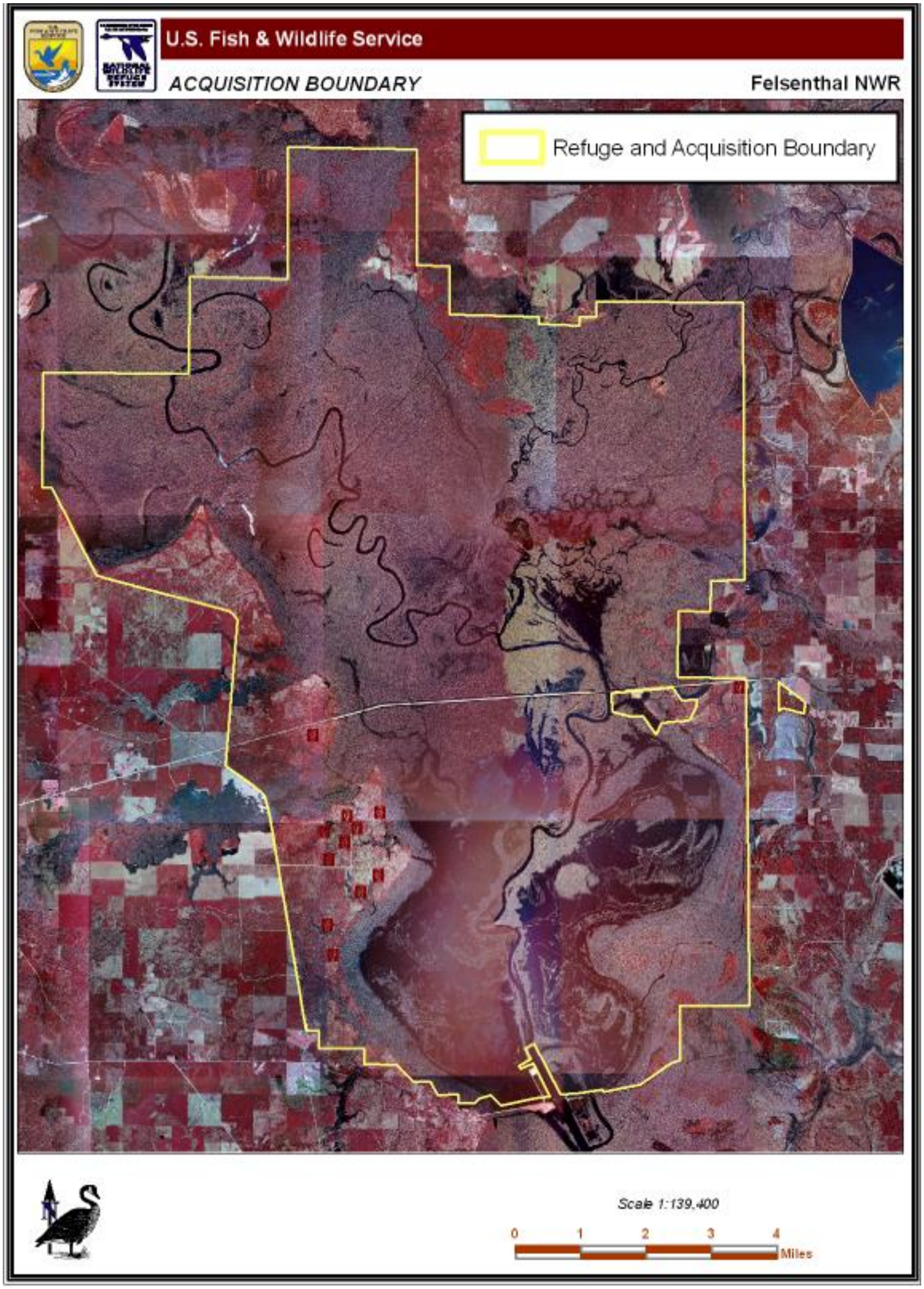


Figure 1 Felsenthal NWR and surrounding area (USFWS 2010)

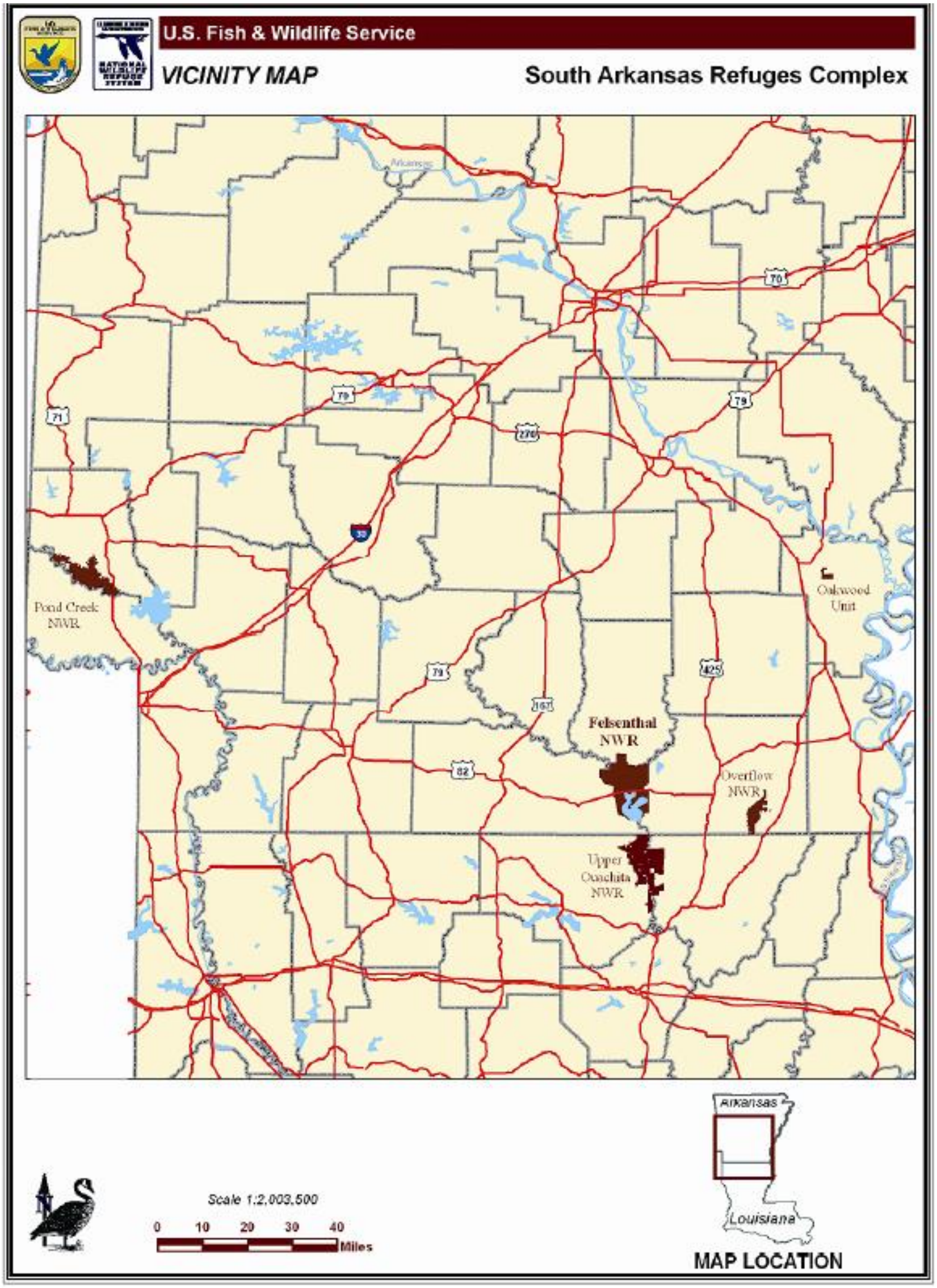


Figure 2 South Arkansas Refuge Complex: Felsenthal NWR, Overflow NWR, and PondCreek NWR (USFWS 2010)

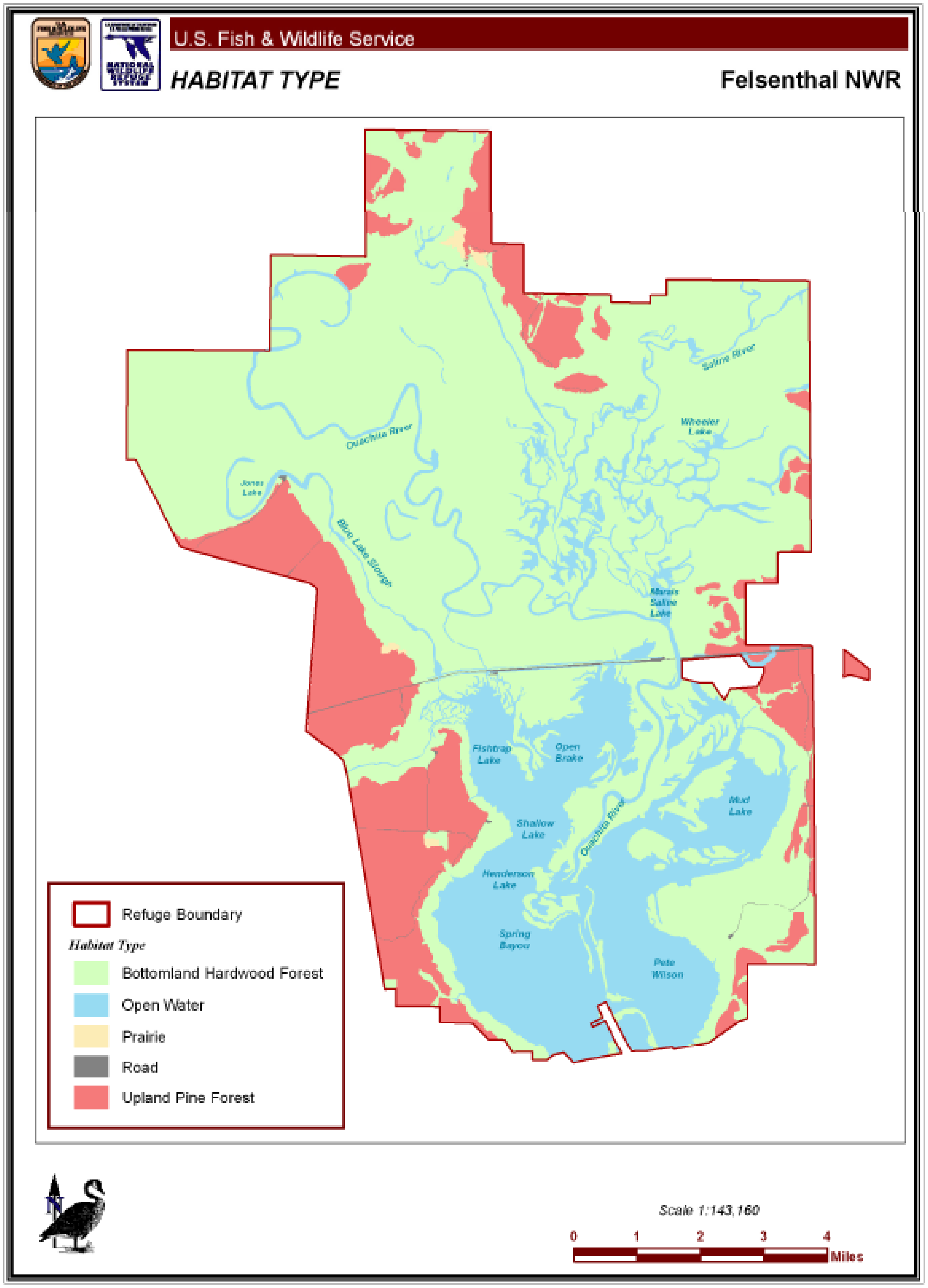


Figure 3 Felsenthal NWR with habitat characteristics (USFWS 2010)

6,070 ha Felsenthal Pool doubles in size to 14,569 ha during winter and spring flooding (USFWS 2010).

Mobile Acoustic Monitoring

Mobile acoustic bat monitoring (MABM) was conducted from November 2015-March 2017. MABM was done in accordance with USFWS (2012). Using an Anabat SD2 Active Bat Detector (5-250 kilohertz frequency range) (Titley Scientific, Columbia, Missouri), surveys were conducted two times a month to account for species abundance and habitat preference. Echolocation calls of bats were collected and georeferenced using a roof-mounted, vertically-pointed microphone and a global positioning system (GPS) attached to the Anabat detector while traveling approximately 32 kph in a vehicle. The Anabat detector was set to the maximum sensitivity setting of 7 so as to collect all species of bats and eliminate extra noise from insects, vehicles, etc. Audio division was set to 16, the mid-level setting, to hear bats calls through the loudspeaker, and data division was set to 8 to remove extra, unwanted data points and collect more bat calls (Figure 4). Transects were nonrandom and constructed around local road infrastructure, availability, and repeatability. Length of transect was determined by road constraints with target lengths of 24 to 40 km. Bat calls were recorded to a compact flash card inside the Anabat detector and initially filtered through CFCread Storage ZCAIM Interface (version 4.4n). To standardize call classifications, only call sequences consisting of five or more pulses were analyzed, and bat calls separated by greater than one second were treated as unique. Calls were auto-classified to species using the BCID Eastern USA software package (Bat Call Identification, version 2.7). To minimalize false-positive detections, call classifications were limited to bat species with seasonal geographic range overlap consistent with Felsenthal NWR and southeast Arkansas.

Figure 4 Anabat SD 2 unit interface with settings

Species, including the Brazilian Free-tailed Bat (*Tadarida braziliensis*) and the Seminole Bat (*Lasiurus seminolus*), that are present on the refuge but do not have classifiers within the BCID software, were not accounted for acoustically due to possible false-positive detections. All surveys began at least one-half hour after sunset. Surveys were only conducted during nights when no precipitation occurred or when ambient temperatures were above 7.2°C.

Passive Acoustic Monitoring

Passive acoustic bat monitoring (PAM) was conducted from November 2016-March 2017. Anabat detector sensitivity settings for PABM were consistent with the MABM protocol. Four Anabat SD2 Active Bat Detectors and two Anabat Express Passive Detectors (Titley Scientific, Columbia, Missouri) were utilized for this portion of the study to characterize bat species preference and utilization of habitats on the refuge. These habitats were upland pine forest, bottomland hardwood forest, and cypress-tupelo bottoms. A pseudo-random selection approach was implemented to select Anabat detector placement locations within pre-described habitat areas using ArcGIS (ArcMap, version 10.3.1). Locations were chosen from random GPS coordinates that were generated to place Anabat detectors in prime locations that were not submerged in water or beneath excessive mid-story vegetation. The six Anabat detectors were placed among the three habitat types classified on the refuge (two Anabat detectors per habitat type). Anabat SD2 detectors were placed inside weather-proof, custom holding-boxes mounted on t-posts. The Anabat Express detectors and their microphones were mounted to trees. All units were mounted approximately 2 m from the ground. The Anabat units' internal clocks were pre-programmed using CFCread, and data collection occurred from one half hour before sunset to one half hour after sunrise.

Mist Netting

Mist nets were utilized from October 2015 through May 2017 as another means to determine bat species present upon the refuge and confirm echolocation calls from the acoustic data. Mist netting occurred one to three times monthly during this time period when ambient air temperatures were greater than 7°C. Sampling started 15 minutes after sunset and lasted approximately two to four hours per survey. Sites and net placement were selected where a high level of bat activity was expected, and to account for factors that would increase capture rates including: accessibility, habitat type, forest canopy, travel corridors, and lentic or lotic water systems (Figure 5) (Gardner et al. 1989). Three to four polyester mist nets (38 mm mesh) (Avinet, Freeville, New York) were deployed per night with net width ranging from 6 m to 12 m. When applicable, nets were stacked to cover an entire flyway and increase capture rates (Jones et al. 1996). Nets ranged from 2.6 m to 7.8 m high when stacked. Data were recorded for all bats captured including: species, sex, age (adult or juvenile), forearm length (mm), ear length (mm), and thumb length (mm). The protocol described by the American Society of Mammalogists was followed for the handling of all bats that were captured during this study (Sikes et al. 2016). All bats captured were screened for White-nose syndrome using a 250 nanometer (nm) LED flashlight to check for wing and facial lesions. Mist nets, equipment, and clothing worn were decontaminated after every survey in accordance with USFWS (2016). Mist nets and processing bags were decontaminated using isopropanol (60% or greater) by submerging them for a minimum of one minute. Equipment was sterilized using Clorox® wipes after each use. All clothing was washed immediately after each survey using hot water and liquid detergent.

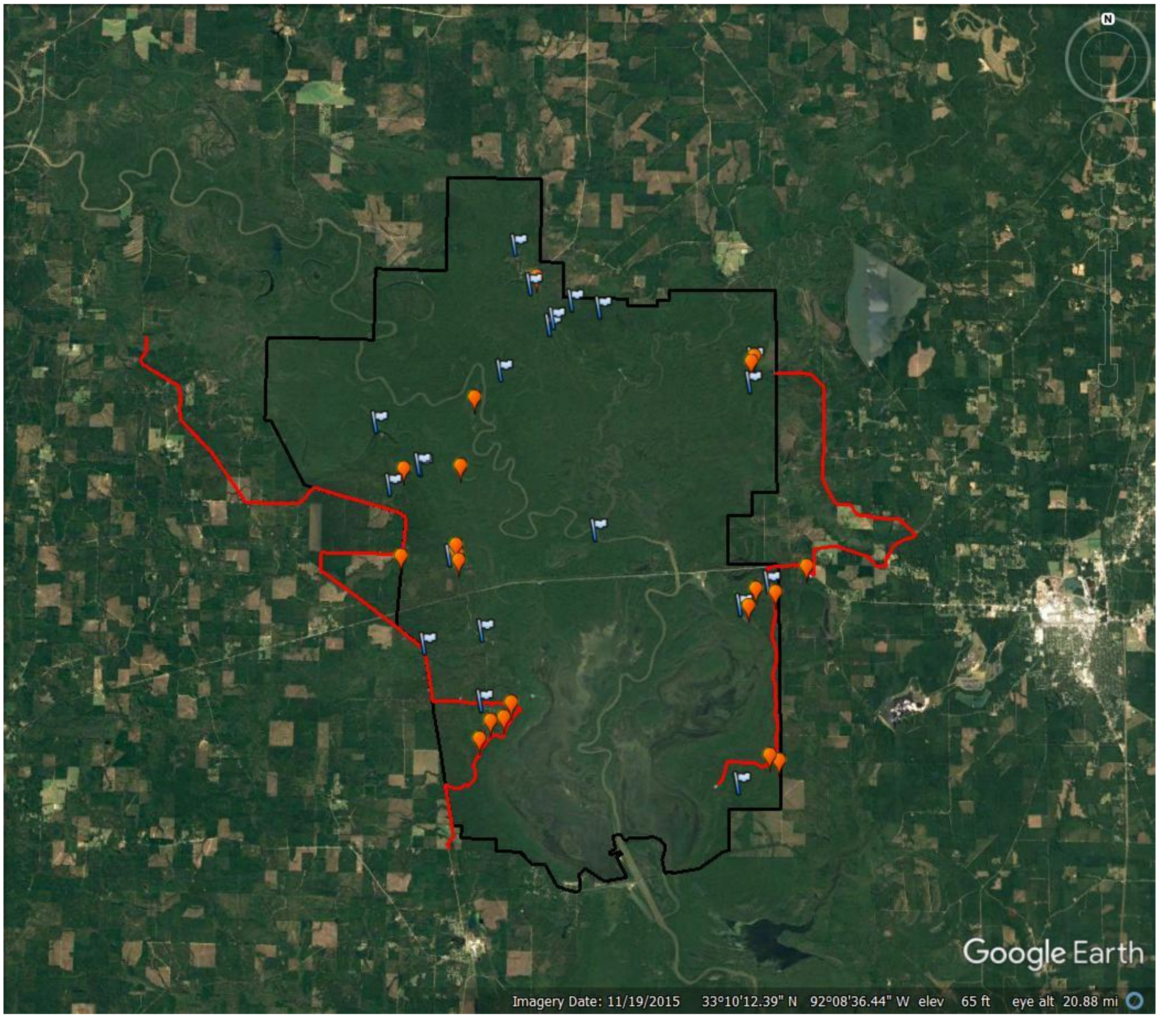


Figure 5 Felsenthal NWR with sampling locations and transects. Red lines = mobile acoustic routes, white flags = passive acoustic monitoring locations, orange balloons = mist-netting locations.

Data Analysis

All geospatial analysis was done in ArcMap 10.3 (ESRI 2009). Both MABM and PAM surveys were downloaded using CFCread Storage ZCAIM Interface and run through BCID program and transformed into call files to be uploaded to Arcmap. To evaluate habitat preference and geospatial location, call files were transposed onto satellite imagery of FNWR to be classified and grouped.

All data were analyzed using SAS 9.3 (SAS Institute Inc. 2017) and Microsoft Excel 2013 (Microsoft Corporation 2017). Richness, evenness, and diversity measurements for MABM and mist netting surveys were calculated using Microsoft Excel. MABM survey data were generated using number of individuals, species, habitat type, season, moon phase. Analysis of variance tests (ANOVA) were run to determine significance among parameters, and all were taken into consideration to answer research questions. A Proc GenMod was run to find significance among each species and the habitat types of FNWR and its peripheral lands. PAM surveys were analyzed using SAS 9.3 to determine habitat selection and activity by nighttime hours. This data set was generated using number of individuals, species, habitat type, and time. ANOVA tests were run to determine significance among these parameters as well. A Proc GenMod was conducted to find significance among each species and the habitat types of FNWR. A p value ≤ 0.05 was used to determine significance for all analyses.

RESULTS

Mobile Acoustic Monitoring

Thirty-four mobile acoustic surveys were conducted between November 2015 and April 2017, and a total of 919 individuals were collected and classified. Eight species were captured (Richness = 8) from the collected data, and a category of "unknown" was created for unidentifiable calls. Proportion of bats varied greatly: Evening Bat (31.01%), Tri-colored Bat (28.84%), and Eastern Red Bat (21.33%) were the most abundant, while all other species made up less than 25% of the sample. Other species included Big Brown Bat (7.94%), Hoary Bat (1.74%), Silver-haired Bat (5.88%), Southeastern Myotis (<1%), Northern Long-eared Myotis (<1%), and unknown species (2.29%) (Figure 6).

Of the biodiversity measures that were calculated, the Simpson's Diversity Index (0.7654) revealed moderately-high diversity among the sample. The calculated evenness value (0.4719) revealed a moderate evenness among the sample (Table 2)

An ANOVA test showed that there was significance among the number of individuals of each species found within the study area ($F_{8,36} = 5.30$, $p = 0.0002$) with significant values for the Eastern Red Bat, Evening Bat, and Tri-colored Bat (Table 3; Figure 7). An ANOVA was run to test for interaction of species by season and no significance was found ($F_{3,32} = 1.24$, $p = 0.279$), but the overall majority of bat activity (73%) was significantly greater during the spring and summer months (Figure 8; Table 4). A separate ANOVA found significance between moon phase and bat activity ($F_{5,48} =$

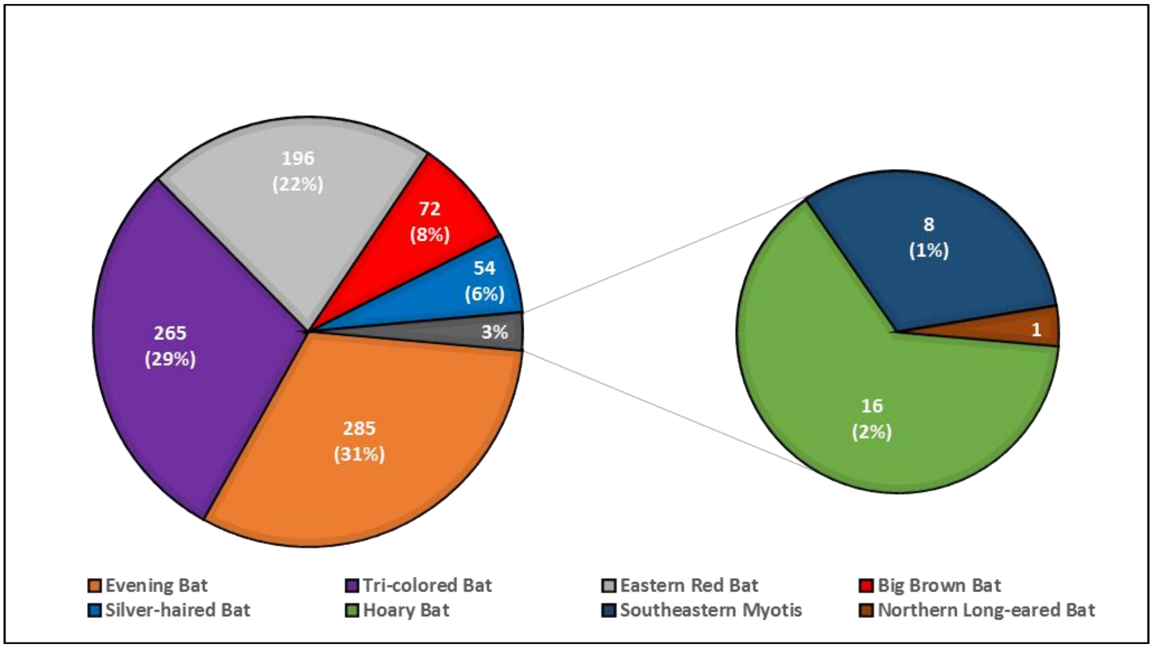


Figure 6 Summary of bat species from mobile acoustic surveys

Table 2 Biodiversity measures of mobile acoustic data

Spp	N	P^	P^2	Evenness	Ni(Ni-1)	N(N-1)	Ni(Ni-1)/N(N-1)	Diversity
EPFU	73	0.07943	0.00631	0.47186	5256	8.436E+05	0.00623	0.76536
LABO	196	0.21328	0.04549		38220	8.436E+05	0.04530	
LACI	16	0.01741	0.00030		240	8.436E+05	0.00028	
LANO	54	0.05876	0.00345		2862	8.436E+05	0.00339	
MYAU	8	0.00871	0.00008		56	8.436E+05	0.00007	
MYSE	1	0.00109	0.00000		0	8.436E+05	0.00000	
NYHU	285	0.31012	0.09617		80940	8.436E+05	0.09594	
PESU	265	0.28836	0.08315		69960	8.436E+05	0.08293	
UNKN	21	0.02285	0.00052		420	8.436E+05	0.00050	
	919		4.24674				0.23464	

Table 3 Significance of individuals relative to species from MABM data

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	4.20000000	B	9.95556796	0.42	0.6756
Species EPFU	10.40000000	B	14.07929922	0.74	0.4649
Species LABO	35.00000000	B	14.07929922	2.49	0.0177
Species LACI	-1.00000000	B	14.07929922	-0.07	0.9438
Species LANO	6.60000000	B	14.07929922	0.47	0.6421
Species MYAU	-2.60000000	B	14.07929922	-0.18	0.8545
Species MYSE	-4.00000000	B	14.07929922	-0.28	0.7780
Species NYHU	52.80000000	B	14.07929922	3.75	0.0006
Species PESU	48.80000000	B	14.07929922	3.47	0.0014
Species UNKN	0.00000000	B	.	.	.

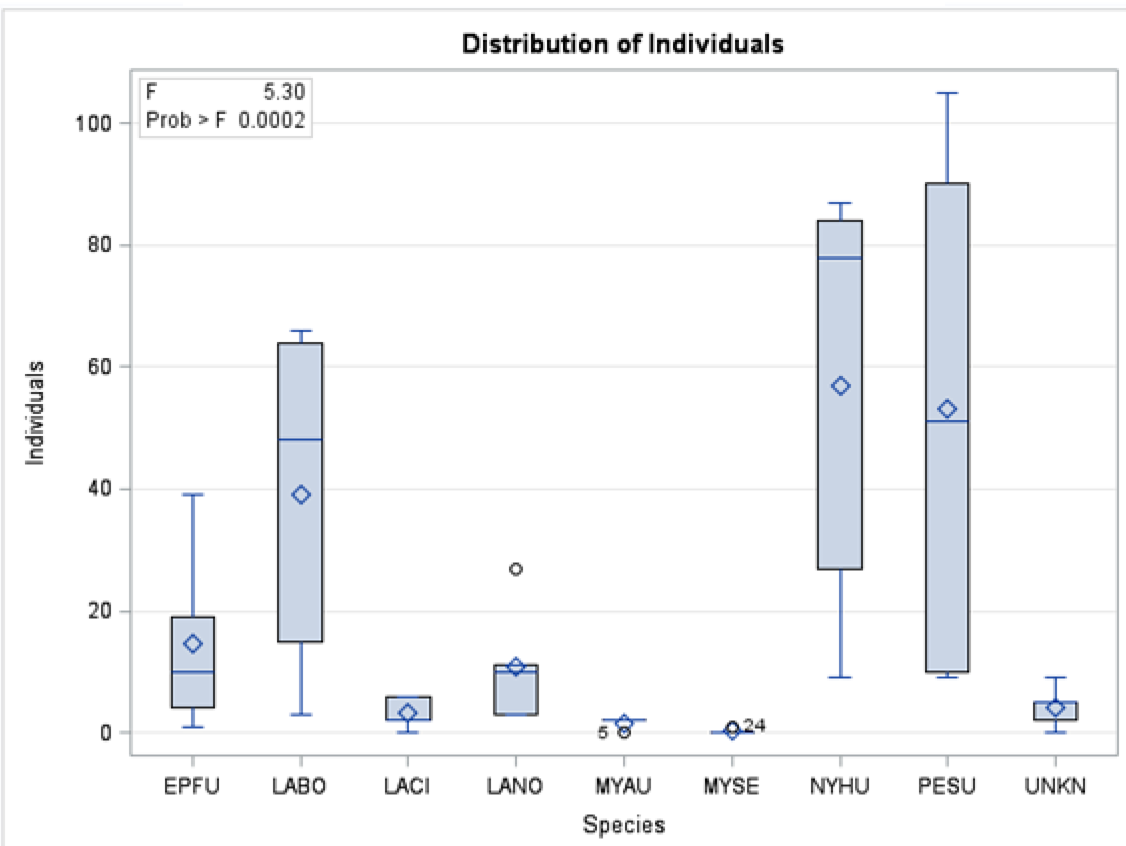


Figure 7 Distribution of individuals among bat species with 95% confidence intervals

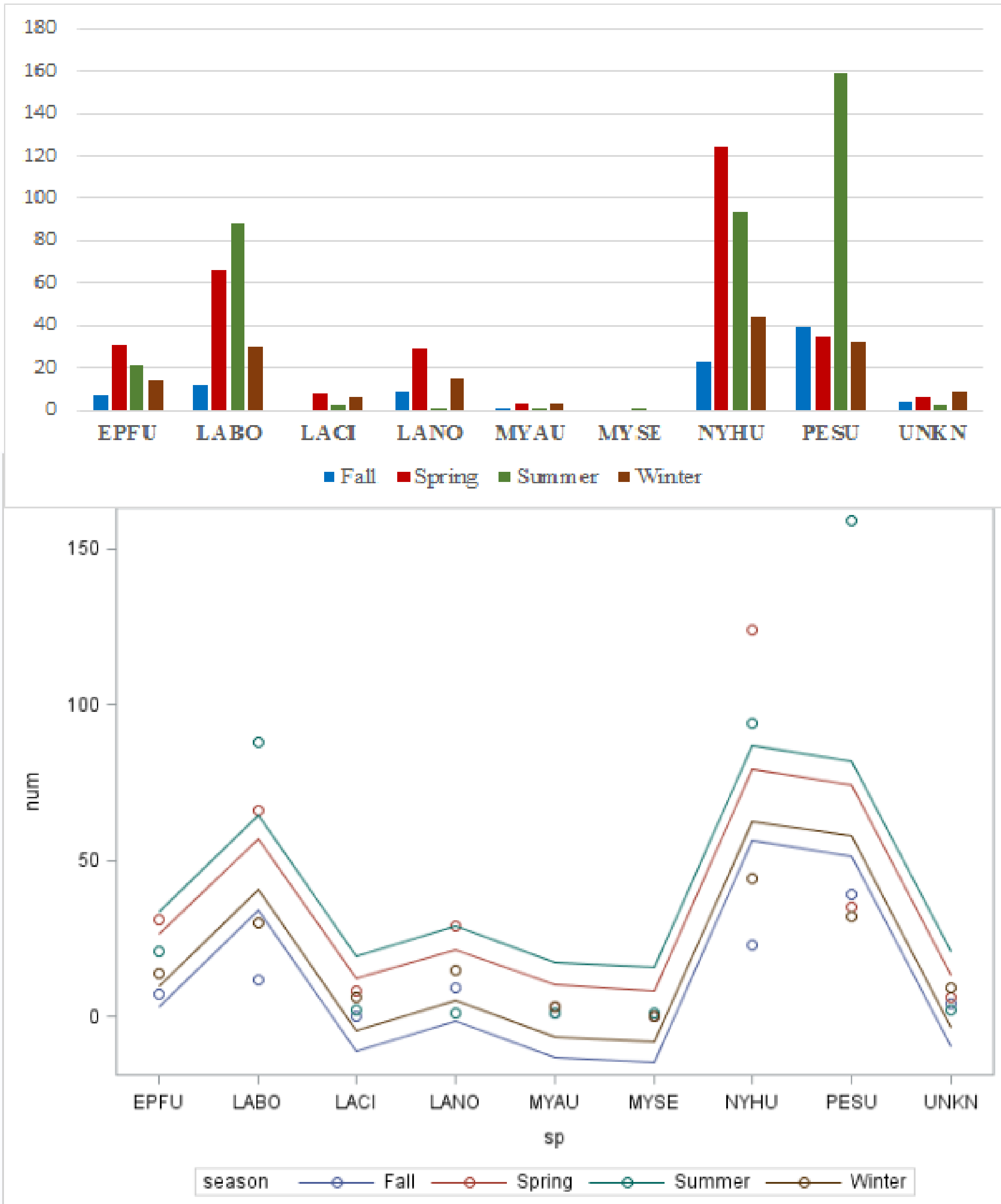


Figure 8 Seasonality of bat species from mobile acoustic surveys and ANOVA test results showing no interaction (y - axis = number of individuals)

Table 4 Occurrence of bat species from mobile acoustic surveys by season. Fall = September-November; Spring = March-May; Summer = June-August; Winter = December-February

BY SEASON						
	Fall	**Spring**	**Summer**	**Winter**		
EPFU	7	31	21	14		
LABO	12	66	88	30		
LACI	0	8	2	6		
LANO	9	29	1	15		
MYAU	1	3	1	3		
MYSE	0	0	1	0		
NYHU	23	124	94	44		
PESU	39	35	159	32		
UNKN	4	6	2	9		
Totals	95	302	369	153		
SUMMARY						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Fall	9	95	10.5556	167.27778		
Spring	9	302	33.5556	1591.7778		
Summer	9	369	41	3398		
Winter	9	153	17	225.75		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	5406.5278	3	1802.1759	1.3392	**0.2790**	2.9011
Within Groups	43062.4444	32	1345.7014			
Total	48468.9722	35				

2.89, $p = 0.02$; Table 5), with more detections occurring during the new, first quarter moon, and half-moon stages.

An ANOVA was run to test for interaction between individual occurrences of bats by habitat type and no significant difference was found ($F_{4,40} = 2.52$, $p = 0.056$; Table 6). Although, CTB and open field habitats showed significance among the individuals (Table 7; Figure 9). No interaction among individuals was found. A PROC GENMOD was run in SAS 9.3, and the maximum likelihood estimate found significance among many species and the habitat types where they were likely to be detected (Table 8). Thirty-one out of the 40 maximum likelihood estimates were significant.

Table 5 Occurrence of bat species from mobile acoustic surveys with phase of the moon

BY MOON PHASE						
	New	**First-Quarter**	**Half**	**Third-Quarter**	**Full**	
EPFU	16	21	19	14	3	
LABO	27	52	90	26	1	
LACI	4	3	3	6	0	
LANO	24	23	4	2	1	
MYAU	0	5	2	0	1	
MYSE	0	0	1	0	0	
NYHU	65	63	109	44	4	
PESU	29	52	143	35	6	
UNKN	3	10	2	5	1	
Totals	168	229	373	132	17	
SUMMARY						
Groups	**Count**	**Sum**	**Average**	**Variance**		
New	9	168	18.67	439.50		
First-Quarter	9	229	25.44	581.78		
Half	9	373	41.44	3170.78		
Third-Quarter	9	132	14.67	270.25		
Full	9	16	1.78	4.44		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	10746.09	5.00	2149.22	2.89	**0.02**	2.41
Within Groups	35734.89	48.00	744.48			
Total	46480.98	53.00				

Table 6 Occurrence of bat species from mobile acoustic surveys by habitat. CTB = Cypress-tupelo bottom; BLH = Bottomland hardwood forest; UP = Upland pine Forest; MP/REF = Mixed-pine reforested area; OF/LOG = Open field/ Logged area

BY HABITAT						
	CTB	**BLH**	**UP**	**MP/RF**	**OF/LOG**	
EPFU	1	19	39	10	4	
LABO	3	48	64	66	15	
LACI	0	6	6	2	2	
LANO	3	10	27	11	3	
MYAU	0	2	2	2	2	
MYSE	0	0	1	0	0	
NYHU	9	78	84	87	27	
PESU	9	51	90	105	10	
UNKN	0	5	5	9	2	
Totals	25	219	318	292	65	
SUMMARY						
Habitat	**Count**	**Sum**	**Average**	**Variance**		
CTB	9	25	2.8	13.9		
BLH	9	219	24.3	773.3		
UP	9	318	35.3	1291.5		
MP/RF	9	292	32.4	1723.3		
OF/LOG	9	65	7.2	77.7		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**F crit**
Between Groups	7809.644	4	1952.41	2.52	**0.056**	2.61
Within Groups	31037.33	40	775.93			
Total	38846.98	44				

Table 7 Significance of habitat type to individuals

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	35.33333333	B	9.28519331	3.81	0.0005
Habitat Bottomla	-11.00000000	B	13.13124631	-0.84	0.4072
Habitat Cypress-	-32.55555556	B	13.13124631	-2.48	0.0175
Habitat Mixed Pi	-2.88888889	B	13.13124631	-0.22	0.8270
Habitat Open Fie	-28.11111111	B	13.13124631	-2.14	0.0384
Habitat Upland P	0.00000000	B	.	.	.

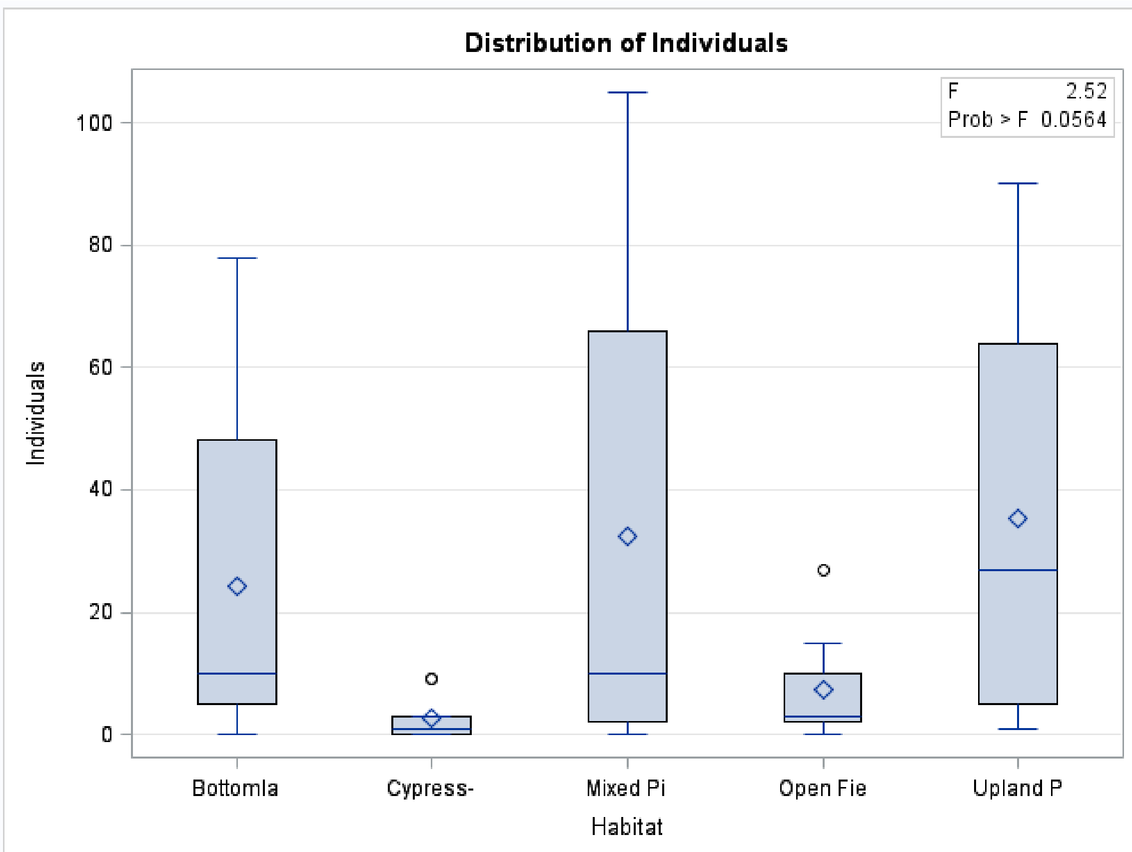


Figure 9 Distribution of individuals from mobile acoustic surveys by habitat type with 95% confidence intervals

Table 8 Maximum likelihood estimates of habitats and the species found within them from mobile acoustic surveys

Habitat*Species Least Squares Means					
Habitat	**Species**	**Estimate**	**Standard Error**	**z Value**	**Pr > |z|**
BLH	**EPFU**	19	1	19	<.0001
BLH	**LABO**	48	1	48	<.0001
BLH	**LACI**	6	1	6	<.0001
BLH	**LANO**	10	1	10	<.0001
BLH	**MYAU**	2	1	2	0.046
BLH	**MYSE**	-1.07E+14	1	0	1
BLH	**NYHU**	78	1	78	<.0001
BLH	**PESU**	51	1	51	<.0001
BLH	**UNKN**	5	1	5	<.0001
CTB	**EPFU**	1	1	1	0.317
CTB	**LABO**	3	1	3	0.003
CTB	**LACI**	1.42E-14	1	0	1
CTB	**LANO**	3	1	3	0.003
CTB	**MYAU**	7.11E-15	1	0	1
CTB	**MYSE**	-3.55E+15	1	0	1
CTB	**NYHU**	9	1	9	<.0001
CTB	**PESU**	9	1	9	<.0001
CTB	**UNKN**	0	1	0	1
MP	**EPFU**	10	1	10	<.0001
MP	**LABO**	66	1	66	<.0001
MP	**LACI**	2	1	2	0.046
MP	**LANO**	11	1	11	<.0001
MP	**MYAU**	2	1	2	0.046
MP	**MYSE**	-1.07E+14	1	0	1
MP	**NYHU**	87	1	87	<.0001
MP	**PESU**	105	1	105	<.0001
MP	**UNKN**	9	1	9	<.0001
OF/LOG	**EPFU**	4	1	4	<.0001
OF/LOG	**LABO**	15	1	15	<.0001

Table 8 (continued)

OF/LOG	**LACI**	2	1	2	0.046
OF/LOG	**LANO**	3	1	3	0.003
OF/LOG	**MYAU**	2	1	2	0.046
OF/LOG	**MYSE**	-2.13E+14	1	0	1
OF/LOG	**NYHU**	27	1	27	<.0001
OF/LOG	**PESU**	10	1	10	<.0001
UP	**MYAU**	2	1	2	0.046
UP	**MYSE**	1	1	1	0.317
UP	**NYHU**	84	1	84	<.0001
UP	**PESU**	90	1	90	<.0001
UP	**UNKN**	5	1	5	<.0001

Passive Acoustic Monitoring

Six Anabat units were deployed on FNWR in three different habitat types: upland pine forest, bottomland hardwood forest, and cypress-tupelo bottom. From 10 November 2016 to 24 February 2017, four different "tours" were conducted for a total of 31 nights of data collection. Tour one occurred from 10 November 2016 – 17 November 2016 with six units deployed. Tour two occurred from 12 January 2017 – 14 January 2017 with six units deployed. Tour three occurred from 23 January 2017 – 2 February 2017 with five units deployed. Tour four occurred from 16 February 2017 – 24 February 2017 with five units deployed. A total of 1,379 individual calls were collected and classified from the three habitat types; 518 calls were classified from cypress-tupelo bottom systems, 65 from bottomland hardwood systems, and 796 from upland pine systems. Number of Individual calls by species varied greatly. Eastern Bat (29.8%), Southeastern Myotis (22.7%), and Eastern Red Bat (13%) were the most abundant calls detected and represented almost two-thirds of the sample (Figure 10).

PROC GLM was utilized in SAS 9.3 for all ANOVA tests of PAM. An ANOVA of the PAM data revealed no significant difference among the number of individuals of each species ($F_{8,18} = 0.91$, $p = 0.53$; Table 9; Figure 11). There was no significance to distribution of individuals among the three habitat types ($F_{2,24} = 2.86$, $p = 0.07$; Figure 12; Table 10), and no interaction between species occurred. A PROC GENMOD was run in SAS 9.3, and the maximum likelihood estimate found significance among most species and the habitat types where they were likely to be detected (Table 11). Twenty of the 24 maximum likelihood estimates were significant.

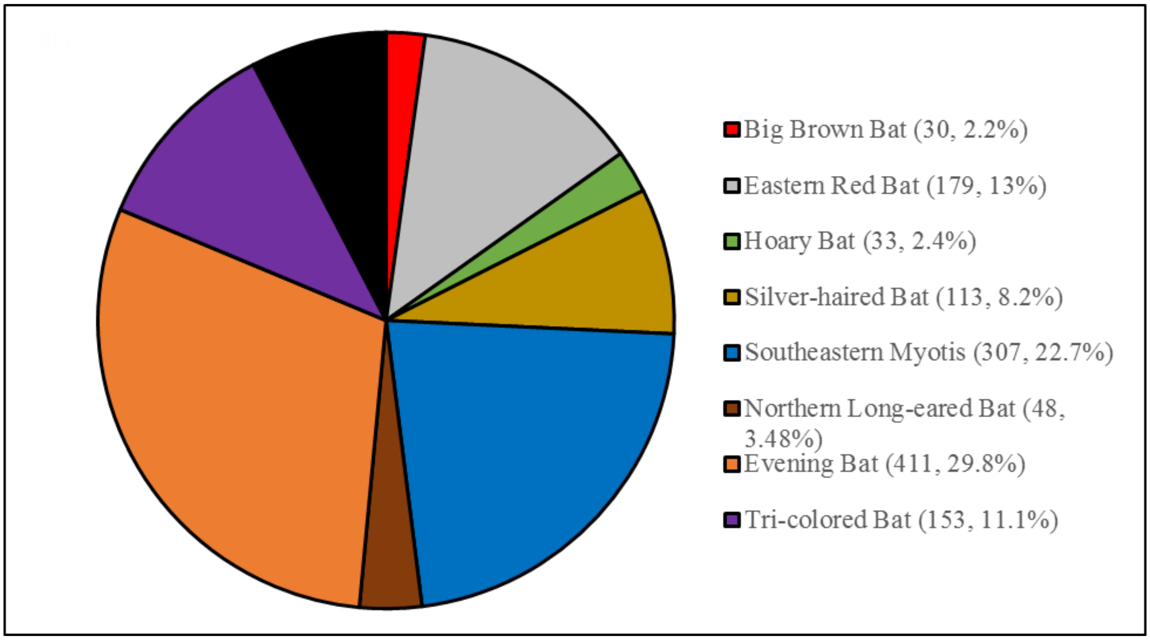


Figure 10 Summary of bat calls from passive acoustic surveys

Table 9 Significance of individuals relative to species from PAM data

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	35.0000000	B	45.54972862	0.77	0.4522
Species EPFU	-25.0000000	B	64.41704398	-0.39	0.7025
Species LABO	24.6666667	B	64.41704398	0.38	0.7063
Species LACI	-24.0000000	B	64.41704398	-0.37	0.7138
Species LANO	2.6666667	B	64.41704398	0.04	0.9674
Species MYAU	67.3333333	B	64.41704398	1.05	0.3097
Species MYSE	-19.0000000	B	64.41704398	-0.29	0.7714
Species NYHU	102.0000000	B	64.41704398	1.58	0.1307
Species PESU	16.0000000	B	64.41704398	0.25	0.8067
Species UNKN	0.0000000	B	.	.	.

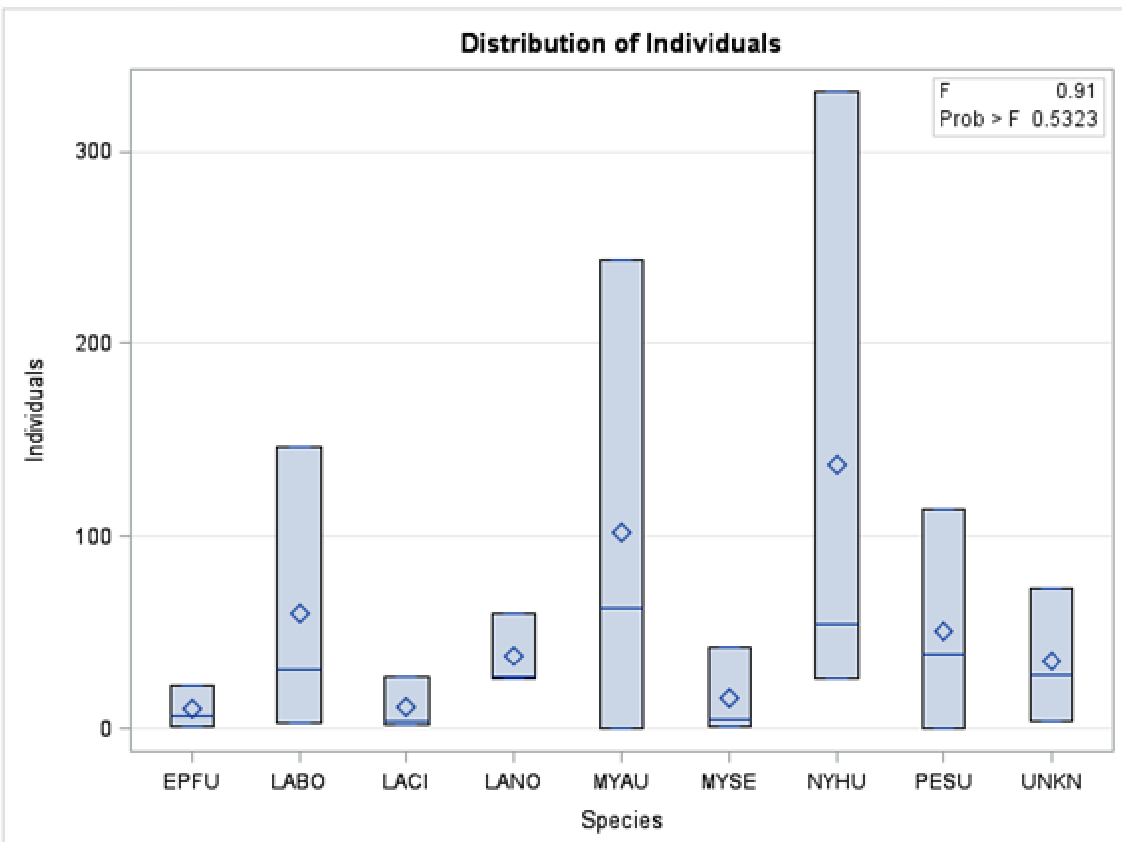
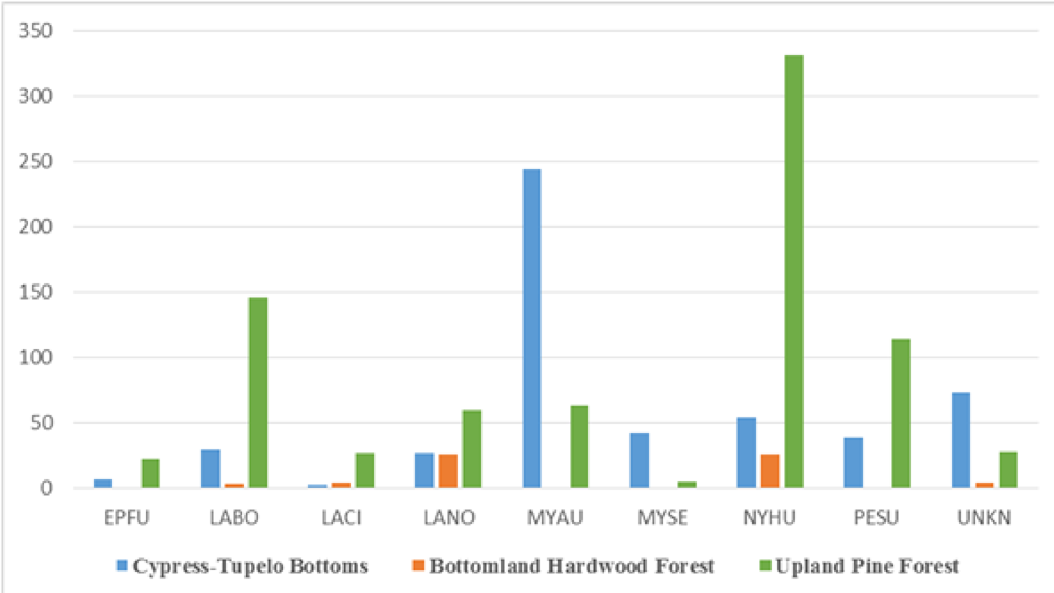


Figure 11 Distribution of individuals among bat species from PAM data



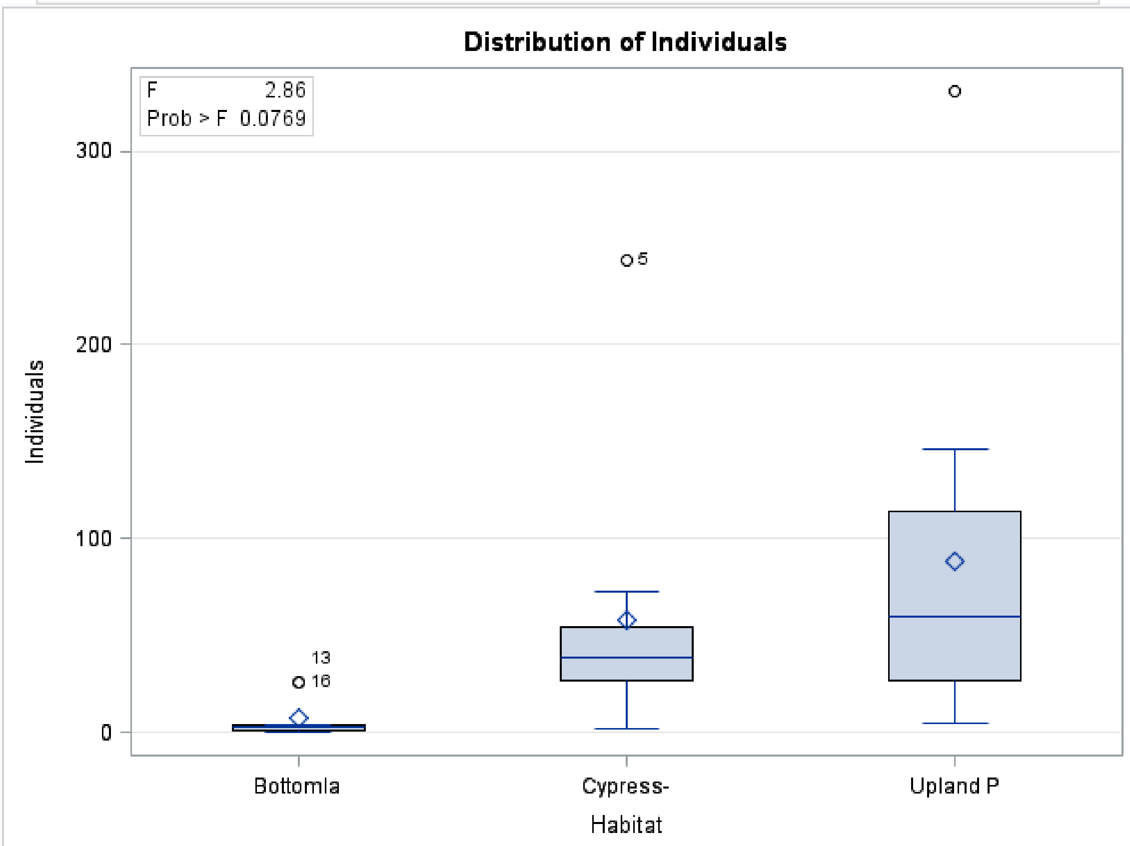


Figure 12 Distribution of bats among habitat type from PAM data (y - axis = number of individuals)

Table 10 Significance between species and habitat of PAM data

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	72.3703704	B	45.63405237	1.59	0.1323
Habitat Bottomla	-81.2222222	B	33.70298127	-2.41	0.0284
Habitat Cypress-	-30.8888889	B	33.70298127	-0.92	0.3730
Habitat Upland P	0.0000000	B	.	.	.
Species EPFU	-25.0000000	B	58.37527593	-0.43	0.6742
Species LABO	24.6666667	B	58.37527593	0.42	0.6782
Species LACI	-24.0000000	B	58.37527593	-0.41	0.6864
Species LANO	2.6666667	B	58.37527593	0.05	0.9641
Species MYAU	67.3333333	B	58.37527593	1.15	0.2657
Species MYSE	-19.0000000	B	58.37527593	-0.33	0.7490
Species NYHU	102.0000000	B	58.37527593	1.75	0.0997
Species PESU	16.0000000	B	58.37527593	0.27	0.7875
Species UNKN	0.0000000	B	.	.	.

Table 11 Maximum likelihood estimates of habitats and the species found within them from PAM surveys

Habitat*Species Least Squares Means					
Habitat	**Species**	**Estimate**	**Standard Error**	**z Value**	**Pr > |z|**
BLH	**EPFU**	1	1	1	0.3173
BLH	**LABO**	3	1	3	0.0027
BLH	**LACI**	4	1	4	<.0001
BLH	**LANO**	26	1	26	<.0001
BLH	**MYAU**	-1.42E-14	1	0	1
BLH	**MYSE**	1	1	1	0.3173
BLH	**NYHU**	26	1	26	<.0001
BLH	**PESU**	0	1	0	1
BLH	**UNKN**	4	1	4	<.0001
CTB	**EPFU**	7	1	7	<.0001
CTB	**LABO**	30	1	30	<.0001
CTB	**LACI**	2	1	2	0.0455
CTB	**LANO**	27	1	27	<.0001
CTB	**MYAU**	244	1	244	<.0001
CTB	**MYSE**	42	1	42	<.0001
CTB	**NYHU**	54	1	54	<.0001
CTB	**PESU**	39	1	39	<.0001
CTB	**UNKN**	73	1	73	<.0001
UP	**EPFU**	22	1	22	<.0001
UP	**LABO**	146	1	146	<.0001
UP	**LACI**	27	1	27	<.0001
UP	**LANO**	60	1	60	<.0001
UP	**MYAU**	63	1	63	<.0001
UP	**MYSE**	5	1	5	<.0001
UP	**NYHU**	331	1	331	<.0001
UP	**PESU**	114	1	114	<.0001
UP	**UNKN**	28	1	28	<.0001

Bat activity by time peaked at sunset and slowly declined as the night progressed (Figure 13). An ANOVA was conducted to find significance between bat activity and time of night. The ANOVA revealed there was not a significant distribution of individuals ($F_{9,90} = 1.61$, $p = 0.12$), but there was significance among species and their activity relative to time ($F_{12,87} = 1.61$, $p = 0.0002$; Figure 13). The ANOVA also revealed no relationship between species and its activity relative to time ($F_{21,78} = 3.68$, $p = <0.0001$; Table 12).

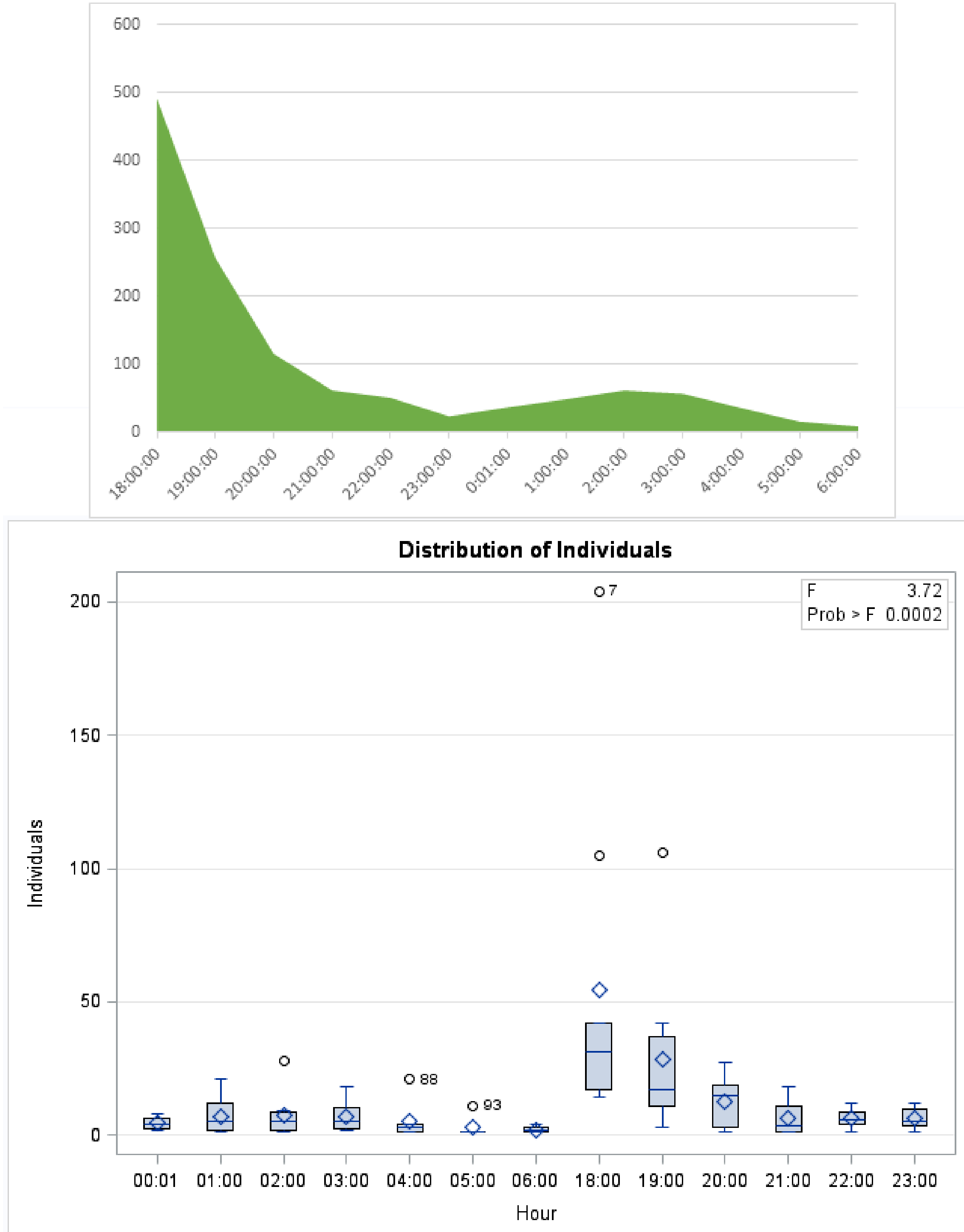


Figure 13 Summary of bat activity over nighttime hours from PAM data (x – axis = time, y – axis = number of individuals)

Table 12 ANOVA showing significance of time of night and individual bats detected

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	1.70977538	B	8.96871532	0.19	0.8493
Hour 00:01	-2.00577805	B	10.24962840	-0.20	0.8454
Hour 01:00	-1.47197172	B	10.56648903	-0.14	0.8896
Hour 02:00	1.37500000	B	10.15224476	0.14	0.8926
Hour 03:00	0.49422195	B	10.24962840	0.05	0.9617
Hour 04:00	1.38014298	B	10.64726698	0.13	0.8972
Hour 05:00	-2.82774535	B	11.71866276	-0.24	0.8100
Hour 06:00	-8.36298400	B	12.63543428	-0.66	0.5100
Hour 18:00	49.80639752	B	9.90298636	5.03	<.0001
Hour 19:00	23.69528640	B	9.90298636	2.39	0.0191
Hour 20:00	8.02861974	B	9.90298636	0.81	0.4200
Hour 21:00	1.91750863	B	9.90298636	0.19	0.8470
Hour 22:00	-0.25577805	B	10.24962840	-0.02	0.9802
Hour 23:00	0.00000000	B	.	.	.
Species CORA	-2.62728401	B	22.08063717	-0.12	0.9056
Species EPFU	-11.01357743	B	10.28529733	-1.07	0.2876
Species LABO	5.76923077	B	7.96407602	0.72	0.4710
Species LACI	-7.96735302	B	9.29442553	-0.86	0.3940
Species LANO	0.74807467	B	8.39383587	0.09	0.9292
Species MYAU	15.77889928	B	8.16902091	1.93	0.0570
Species MYSE	-4.80443405	B	8.16902091	-0.59	0.5581
Species NYHU	24.99744985	B	8.37743151	2.98	0.0038
Species PESU	3.84615385	B	7.96407602	0.48	0.6305
Species UNKN	0.00000000	B	.	.	.

Mist Netting

Mist netting surveys were conducted year-round (when weather conditions permitted). Thirty-one mist netting surveys were completed from October 2015-May 2017, amounting to a total of 91 man-hours. A relatively low number of bats were captured during this time period (n = 28), and a total of seven species were identified through capture (Richness = 7) (Table 13). Biodiversity measures were calculated for the mist net data as they were for the MABM data. Simpson's Index of Diversity (0.7725) revealed moderately high diversity among the sample. Evenness (0.5490) of the sample was found to be moderate for the mist netting data.

Eastern Red Bat (25.29%) and Evening Bat (28.57%) were the two most abundant species, while all other species made up less than 33% of the sample. These species included Rafinesque's Big-eared Bat (10.71%), Big Brown Bat (7.14%), Seminole Bat (3.57%), Southeastern Myotis (7.14%), and Tri-colored Bat (3.57%). Two other bat species were captured in mist nets that were not recorded with the acoustic monitoring, these species were Rafinesque's Big-eared Bat and Seminole Bat. All bats captured were sexually mature adults. Thirteen females were captured, four of which were captured in July and lactating, while one was pregnant that was captured in May. Thirteen males were captured, four of which had descended testes, captured in August and September. Two bats captured were of unknown sex due to escape before processing. No bats captured exhibited signs of harboring *Pd* or lesions from WNS on the wing and tail patagia or muzzle when subjected to UV light screening.

Table 13 Results of mist netting surveys

Date	Species	Sex	Age	Forearm (mm)	Weight (g)	Ear (mm)	Thumb (mm)	Time
01-31-2016	LABO	M	A	41.7	10	7.9	8.2	18:04:00
4/28/2016	LABO	F	A	41	8.5	4.1	8.5	20:37:00
4/28/2016	LABO	M	A	36.8	8.8	5	8.8	21:05:00
4/28/2016	LABO	M	A	41.4	6.8	4.4	6.8	22:15:00
4/28/2016	LABO	F	A	42	8.3	4.5	8.3	22:16:00
6/6/2016	MYAU	M	A	36.5	7	7.7	7	21:30:00
6/6/2016	MYAU	F	A	37.3	8	8.6	7.2	23:00:00
6/22/2016	NYHU	M	A	NA	NA	NA	NA	21:45:00
7/7/2016	NYHU	NA	A	NA	NA	NA	NA	23:03:00
7/15/2016	LABO	F	A	41.8	14	9.9	7	20:45:00
7/15/2016	LABO	F	A	42.1	11	10	8	21:45:00
7/15/2016	LABO	F	A	40.9	16	9.4	6.6	22:28:00
7/15/2016	PESU	F	A	39.2	6	11.9	7.6	22:54:00
7/15/2016	LABO	F	A	40.8	13	9.1	7.2	23:27:00
8/26/2016	NYHU	M	A	34	8.5	11	5.5	21:10:00
8/26/2016	LASE	F	A	37.5	12.5	9	6.5	23:19:00
08/28/2016	NYHU	M	A	35	10.5	10	5.5	21:05:00
08/28/2016	NYHU	M	A	34.5	9.5	8.5	5	21:05:00
10/6/2016	CORA	M	A	43.2	10	28.8	7	19:05:00
10/6/2016	EPFU	M	A	47	23	10.05	6	20:10:00
10/28/2016	NYHU	F	A	38.7	10	8.8	5.5	19:23:00
10/28/2016	NYHU	M	A	37.3	12	7	5.9	19:23:00
11/17/2016	CORA	F	A	44.7	11	29	6.7	18:12:00
11/17/2016	CORA	M	A	42.2	11	23.4	7.1	18:12:00
5/7/2017	NYHU	M	A	35.9	11	6.7	5.6	21:40:00
5/14/2017	LABO	F	A	41.6	14	8.2	5.6	20:47:00
5/14/2017	EPFU	NA	A	NA	NA	NA	NA	20:47:00
5/21/2017	LABO	F	A	40.2	20	8.9	7.8	20:47:00

DISCUSSION

Mobile Acoustic Monitoring

Loeb et al. (2015). Said, “Mobile acoustic monitoring for bats has become an efficient way to collect relatively large samples across large spatial and temporal ranges, while taking into account a number of parameters.” This study was able to successfully collect data year-round, including the winter months, to account for species on and around FNWR, annually. The three most abundant bat species recorded were the Evening Bat (n = 285), Tri-colored Bat (n= 265), and Eastern Red Bat (n = 196), which closely coincides with the Baker and Ward study’s (1967) most abundant species. These species made up over 80% of the sample, which entailed a moderately high diversity of species (0.7654), although evenness (0.47186) of the sample was moderate. In contrast, Baker and Ward (1967) found the Big Brown Bat as one of the most abundant species, which was the fourth most abundant bat sampled (n = 73) in the present study. Eight of the eleven species found in southern Arkansas listed by Connier (2010), Baker and Ward (1967) and the Arkansas Game and Fish Commission were recorded from the MABM surveys. The only species that were not recorded were Seminole Bat, Rafinesque’s Big-eared Bat, and Brazilian Free-tailed Bat. This was most likely due to the Anabat SD 2 unit’s settings that would classify false-positive recordings of bat species or the frequency of these bat species’ calls were outside the unit’s range. Reid (2006) and Harvey et al. (2011) said that bats like Rafinesque’s Big-eared Bat and Southeastern Myotis typically forage over water, in swampy areas. The transects of the MABM surveys crossed very few of these ecosystems, which may have been the cause for such low occurrence of one or both of these species. Unknown species were species captured but not classified to a species or group by the BCID software. These unknown species could have had an altered echolocation call due to ambient noise recorded, or a filter for a different species may not have been activated while processing echolocation calls.

Seasonal abundance varied by species, but the trend was consistent for each species. Bat activity appeared to be highest during the summer months (n = 369) of June through August, which is extremely common because of high ambient temperatures and rearing of young is occurring, as found by Trani et al. (2007). Bat activity did occur during the fall and winter months (n = 397) when temperatures were greater than 10°C, although activity and number of species decreased significantly. There was not a significant relationship between individual species and their seasonal foraging patterns. Most species shared the same activity patterns, annually.

Moon phase played a significant role in the activity of bats. The distribution of bats was evenly spread between moon phases, but a full moon appeared to be a time of little bat activity (n = 17) which coincides with Ethier and Fahrig's (2011) study which found bat activity was minimal when the moon was full. This study revealed bat activity was highest when the moon was at a half moon phase or below, which accounted for nearly 84% of the sample.

Habitat selection of all species from MABM surveys approached significance (p = 0.056). Bats varied across habitat types, the most common being the upland pine forests (n = 318). The Big Brown Bat seemed to prefer upland pine forests (n = 39) over all other habitat types ($p < 0.001$), which is consistent with Brooks and Ford (2005). Other species like the Eastern Red Bat, Hoary Bat, Evening Bat, and Tri-colored Bat were spread fairly evenly across bottomland hardwood, upland pine, and open field/edge habitats. Cypress-tupelo bottoms and open fields / edge habitats most likely yielded relatively low numbers of bats because the MABM transects did not coincide or cover a significant amount of these habitats. There were no species that were exclusively found in one type of habitat that was significant.

Passive Acoustic Monitoring

PAM has become an efficient method to capture species occurrence across different landscapes for longer periods of data collection (Coleman et al. 2014). This study utilized PAM as another means to determine habitat selection of bats, and the times they are utilizing their habitats to forage. The results for species occurrence were similar to the MABM surveys. Evening Bat (n = 411), Tri-colored Bat (n = 153), and Eastern Red Bat were three of the most common species, but Southeastern Myotis (n = 307) was the second most common. Most Southeastern Myotis were recorded over the span of a few nights in November, and thereafter were very uncommon. The Big Brown Bat seemed exclusive to upland pine habitats, as also highlighted in the MABM data. Distribution of individuals among species was not significant because capture rates were skewed. Species to habitat type was not a significant model. But, individual species were run by habitat type, and this proved to have significance. Upland pine forests most commonly harbored the Big Brown Bat, Eastern Red Bat, Evening Bat, Tri-colored Bat, and the Hoary Bat ($p < 0.001$). This coincided with our MABM data. Cypress-tupelo bottom habitats showed significance among Southeastern Myotis most of all, which is concurrent with Carver and Ashley (2008). Barclay (1985) found Silver-haired Bats were habitat generalists that utilized a variety of habitats because of their migratory behavior. This study mirrored Barclay (1985) in finding Silver-haired Bats spread evenly across the three habitat types of FNWR. A significant portion of PAM coincided with the MABM in terms of habitat selection and occurrence of bats.

Bat activity by time proved to be significant. Activity peaked at the beginning hours of darkness and slowly declined in the following hours, except for a slight increase during the early morning hours. There was no relationship between species of bat and specific activity hours, except Southeastern Myotis and Evening Bat which showed to have highest activity during the beginning hours of the night. Trends stayed static for all species among hours of the night.

Mist Netting

Although mist netting capture yielded a low total capture, seven out of the eleven listed species found in southern Arkansas by Connier (2010), Baker and Ward (1967) and the Arkansas Game and Fish Commission were captured. Two of the species captured, Rafinesque's Big-eared Bat and Seminole Bat, were captured exclusively by mist net and not by acoustic monitoring, which increased inventory. Richness and evenness measures were similar between mist netting data and MABM data.

Bergallo et al. (2003) suggested having a sample size of n = 1000 from mist netting to have a representative sample of the population. This study failed to reach that number, but mist netting in combination with acoustic results may have given a more representative sample of the ecosystem. Most bats were captured within the first hour of surveying time. No signs of *Pd* or WNS were found on any bats that were captured and processed. Few bats contained parasitic mites, which is common among many species.

Management Implications

Ten out of the eleven bat species listed by Connier (2010), Baker and Ward (1967) and the Arkansas Game and Fish Commission were captured acoustically or physically during this study. The most abundant species were ones listed as “least concern” by the International Union for Conservation of Nature, and they were not listed under the U.S. Fish and Wildlife Service’s Threatened and Endangered list. These species include the Big Brown Bat, Silver-haired Bat, Eastern Red Bat, Evening Bat, and the Tri-colored Bat. The Seminole Bat, the Hoary Bat, and Rafinesque’s Big-eared Bat were captured, but they were not common in the sample. These species are either listed as “vulnerable” in the state of Arkansas or of “special concern” under the USFWS Threatened and Endangered list. Long-term I & M surveys have been occurring throughout the Southeastern United States since 2010 and will continue into the future by the USFWS Region 4 I & M Office. Long-term monitoring is the best way to view population and occurrence trends over time, while also monitoring rare species that are at-risk.

Habitat conservation on FNWR should also be a priority due to species that are at-risk because of habitat degradation like Rafinesque’s Big-eared Bat and Southeastern Myotis. Mature upland pine stands on the refuge are burned to promote habitat for the Red-cockaded Woodpecker. These burn cycles seem to have little effect on bat activity, long-term, and seem to promote foraging of bats in these areas because of open mid-story. Although BLH forest and CTB habitats did not produce a majority of bat calls, these habitats are still important for roosting sites and production of nocturnal insects on which bats forage.

Although signs of *Pd* or WNS were not found on bats captured upon the refuge, measures should be taken to closely monitor for the fungus and disease. The refuge harbors culverts that could potentially provide hospitable conditions in which the fungus could thrive and infect bats that are roosting within them.

Conclusions

In conclusion, the four objectives presented at the beginning of this document were addressed by the researcher. First, an inventory of bats at Felsenthal NWR was generated as a result of sampling over the course of 20 months, while utilizing several sampling methods. Ten out of eleven species were captured that have been found historically in southern Arkansas. Second, seasonal abundance and biodiversity of bats on and around the refuge was measured by sampling year-round. Biodiversity measures showed moderate evenness and moderately-high diversity of the sample. Bats were most abundant during the warmer months of the year. Third, habitat preference and utilization was examined while using acoustic monitoring. Bats on and around the refuge appeared to be distributed across all habitat types, and one species was not exclusive to one habitat type. Lastly, no signs of *Pd* or WNS were not found on any bats captured by mist net.

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VITA

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