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Bats are one of the most economically important vertebrates. Anthropogenic disturbances such as deforestation, pesticide use, and urbanization have decimated bat populations around the world. Previous studies demonstrated that wetlands and riparian zones are heavily used habitats by bats. Many insects depend on water for part of their life cycles, making riparian and wetland habitats a potentially important foraging ground for bats. No study has specifically investigated how bats use wetlands. This study is one of the first to examine fine-scale wetland use by bats as foraging habitats. To address this research question, 10 wetlands and corresponding forest edges were acoustically monitored in the Piedmont region of NC. Using unidirectional microphones at each site, bat echolocation calls were recorded and classified into bat species and call types. This project had two aims: Aim 1 is to determine the species richness of bats at the sites. The independent variable is the site location, and the dependent variable is the number of species found at each site. Acoustic calls showed each species varied by site. Five out of the seven species showed significant differences between wetland and forest edges. Aim two is to determine if bats use open wetlands for foraging more than forest edges. Acoustic calls were used to monitor bat calls to quantify foraging calls versus commuting calls aimed over wetlands and along nearby forest edges. The seven focus species of this research had different activity levels than expected. The foraging activity over open wetlands versus forest edges also varied by bat species. This research could help implement wetland restoration and mitigation that will influence the proposed endangered Tricolored bat presence.

THE DIVERSITY AND BEHAVIOR OF BATS IN WETLANDS VERSUS FORESTED EDGE USING UNIDIRECTIONAL ACOUSTIC RECORDINGS IN THE PIEDMONT REGION OF NORTH CAROLINA

by

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CHAPTER I: INTRODUCTION

Wetlands Background

One general definition of wetland includes any land area surrounded by or saturated with groundwater (Richardson, 1994; Zedler, 2000). In the Piedmont region of North Carolina, riparian wetlands are those that lie adjacent to rivers, streams, swamps, marshes, and bogs, where the vegetation has adapted to live in saturated soil. Wetlands either continuously hold water or experience intermittent wet and dry periods. Water availability in riparian areas along rivers has been positively correlated with bat species diversity (McCain, 2009; Salvarina, 2016), and natural wetlands provide habitats during crucial stages of the life cycle in at least one migratory bat species, Nathusius' pipistrelle (*Pipistrellus nathusii*) (Flaquer et al., 2009).

Wetlands use to flourish, but over the last 200 years, the lower 48 states has lost approximately 53 percent of wetlands (Figure 1) (Dahl, 1990). North Carolina lost 49 percent of its wetlands since 1780 (Figure 2)(Dahl, 1990). In 1977, Executive Order No. 11990 Protection of Wetlands Section 404 of the Clean Water Act, was passed to protect wetlands, many of which were being drained to create agricultural fields (Dahl, 1990). Before the protection act, wetlands were considered wastelands that harbored disease and pests. Therefore, little research was conducted to understand the positive effects of wetlands on ecosystem function (Bierlein, 2007). Today, wetlands remain one of the most threatened ecosystems globally and continue to decline in many countries (Bolpagni & Piotti, 2016).

Figure 1. Wetland loss in the United States from 1780 to 1980 (Dahl, 1990).

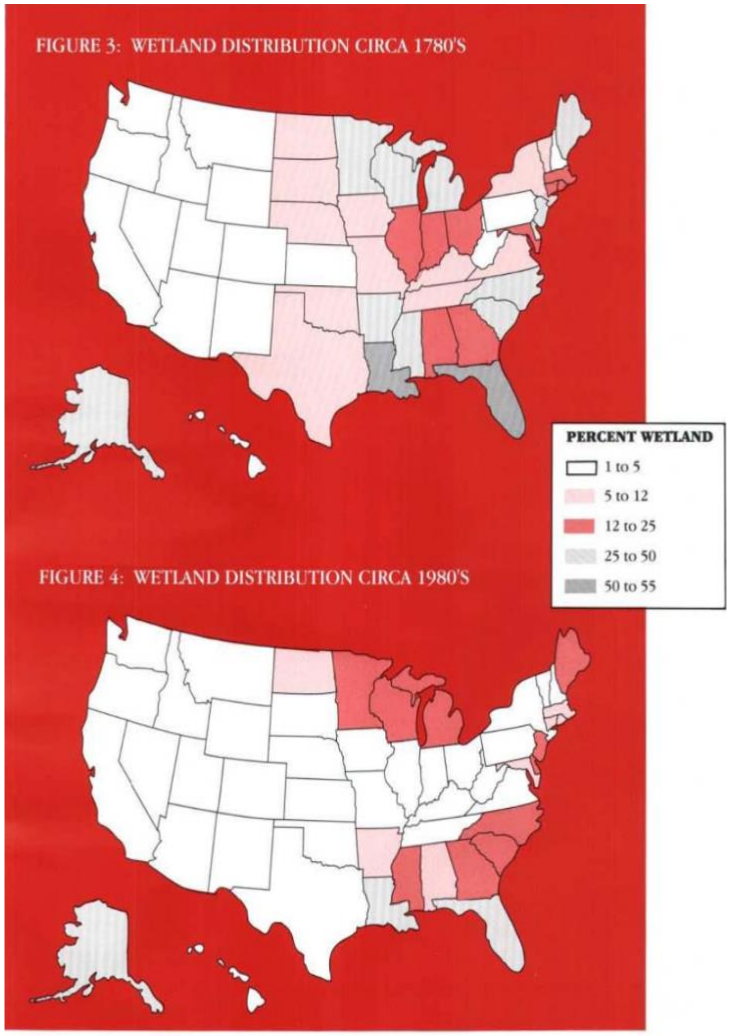
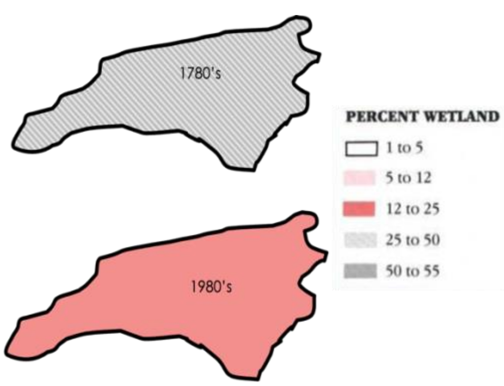


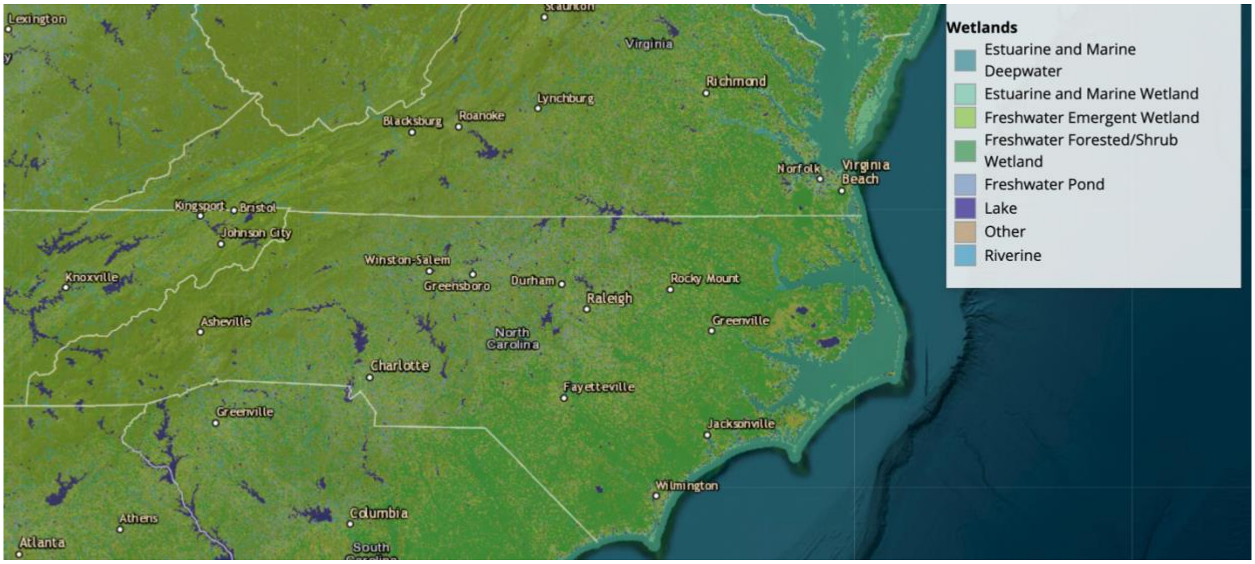
Figure 2. North Carolina has lost about 49% of its wetlands since the 1780's (Dahl, 1990).



Wetlands provide important habitat for many endangered species (Tiner, 1984). In 1991, the U.S. Fish and Wildlife Services published a list of 595 endangered plant and animal species, 256 of which rely on wetlands (Flynn, 1996). Wetlands provide myriad key spatial, climatic, and nutritional requirements for a wide-ranging assortment of terrestrial animals, including amphibians, reptiles, birds, and mammals (Jorgensen, 2009). Wetlands are among the most hydrologically diverse and productive ecosystems due to their location and functionality (Blackwell & Pilgrim, 2011). The term "wetland" encompasses a broad spectrum of variable water-influenced ecosystems. Wetland classification is therefore an important tool for scientists interested in the ecological properties of any wetland or wetland complex. Differing systems of classification have inspired a significant amount of research and debate. Current practices are to classify wetlands based on their hydrology, substrate, and vegetation (Bales & Newcomb, 1996).

In the Piedmont region of North Carolina, there are abundant natural and reconstructed wetlands (Figure 3) (Bales & Newcomb, 1996; Burchell & Hunt, 2019; Kristie, 2018). The Piedmont region has ongoing bat research; therefore, it is an ideal area to study the relative impact of wetlands on bat foraging behavior.

Figure 3. This imagine is from the National Wetlands Inventory and is a 40-year ongoing project depicting different types of wetlands across the United States. (National Wetlands Inventory | U.S. Fish & Wildlife Service, 2021).



Historically, the extent of natural wetlands in the United States has declined since the mid-1780s: existing wetlands are now broadly protected (Bierlein, 2007; Dahl, 1990). Restoration of wetlands has become a common practice to mitigate groundwater pollution and restore biodiversity, which is critical for healthy ecosystem function (Zedler, 2000). Since bats are a known keystone species, understanding the impact of wetlands on bat foraging activity, will provide insight and knowledge into the influence on species diversity and abundance (Gannon & Bovard, 2016; Russo et al., 2021). Bats may play an important role in assessing wetland functionality, due to their ecological association with wetlands and their status as a bioindicator of ecosystem health (Maslonek, 2010).

Studies have demonstrated that certain bat species exhibit activity patterns near riverine and calm bodies of water that are correlated with insect abundance, reduced noise interference, and lower habitat complexity (Li et al., 2021; Mackey & Barclay, 1989; Parker et al., 2019;

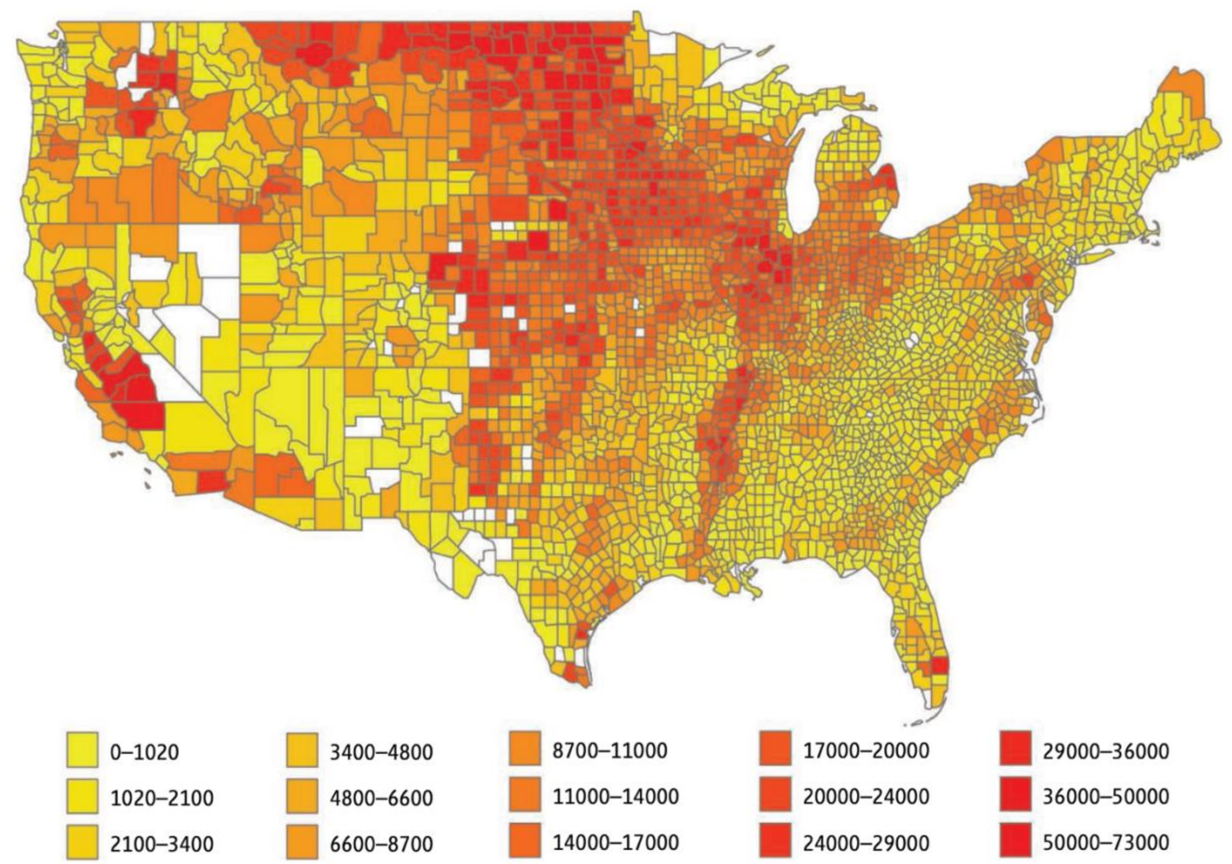
Siemers et al., 2001; Vindigni et al., 2009; Warren et al., 2000). Increasingly, researchers are using audio recordings of echolocation calls to identify bat species and quantify foraging behavior, navigation, and social behavior (Bohn & Gillam, 2018; Griffin, 1944; Moss & Schnitzler, 1995; Parker et al., 2019; Simmons et al., 1979; Wright et al., 2014).

Bat Background

Bats belong to the order *Chiroptera*, the second most diverse order of mammals, and are the only true flying mammals (Kasso & Balakrishnan, 2013). Comprising 1,440 known species, bats vary considerably in size, feeding habits, roosting habits, and behavior (Kunz & Fenton, 2003). Bat mortality rates are on the rise due to the acceleration of harmful anthropogenic activities such as deforestation, depletion of food resources via pesticide use, the proliferation of wind energy facilities, and caving, which contributes to the spread of diseases such as White Nose Syndrome, (Frick et al., 2010; Kunz & Fenton, 2003; Mickleburgh et al., 2002; O'Shea et al., 2016). Bats provide important ecosystem services in both natural and anthropogenically altered landscapes (Kunz et al., 2011).

Bats are considered one of the most economically important animals when it comes to reducing agricultural pests. Figure 4 shows the estimated annual value of insectivorous bats in agriculture. Diseases such as White-nose syndrome (*Pseudogymnoascus destructans*) and the increasing development of wind turbines have significantly decreased and threatened insectivorous bats in North America (Boyles et al., 2011). White-nose syndrome alone has been estimated to have killed millions of bats in North America since its first discovery in 2006. It can also wipe out up to 100% of hibernating bats in a colony (Hoyt et al., 2021). Bats are declining at rapid rates and so are wetlands. Protecting bat species has become a huge concern over the last 20 years because they offer great economic impacts. If we can link wetlands to important foraging grounds for these mammals it might lead to higher survivability of bats. If wetlands become protected areas for bats, especially endangered bat species that use these wetlands frequently for foraging then, this can help their numbers increase and provide stable foraging areas. It is important to think about bat foraging patterns and habitats to preserve these mammals and therefore preserve their economic importance, especially in agriculture. Foraging behavior varies by bat species and can be difficult to understand and study without disrupting their ability to forage.

Figure 4. Value of Insectivorous Bats to Agriculture. This figure shows the worth of insectivorous bats. This is an annual estimated representation of bats to the agriculture industry at the county level. The values are x$1000 per county. Bats have been estimate estimated to save billions of dollars annually for farmers and agriculture (Boyles et al., 2011).



Bats are highly significant vertebrates from an economic standpoint due to their impact on pest and pollination activities worldwide (Maslo et al., 2022). Consumption of insects by bats varies considerably by species, season, reproductive cycle, food availability, and location (Kunz et al., 2011). Insectivorous bats, being highly efficient generalist predators, play a vital role in consuming harmful agricultural pests, resulting in annual savings of billions of dollars to farmers in the United States alone (Maslo et al., 2022).

Seventeen species of insectivorous bats inhabit the state of North Carolina (N.C. Wildlife Resources Commission, 2017), seven of which have been recorded in the Piedmont region. Big Brown Bat (*Eptesicus fuscus*), Eastern Red Bat (Lasiurus borealis), Hoary Bat (*Lasiurus cinereus*), Silver-haired Bat (*Lasionycteris noctivagans*), Evening Bat (*Nycticeius humeralis*), Tricolored Bat (*Perimyotis subflavus*), and Mexican Free-tailed Bat (*Tadarida brasiliensis*).

The Big Brown bat (*E.fuscus) was* first described by a French biologist Palisot de Beauvois in 1796 (Palisot de Beauvois 1796). The Big Brown Bat went through several name changes from 1796 until 1918. It is in the *Vespertilionidae* family, and genus *Eptesicus* (Kurta & Baker, 1990). This is one of the larger species of bats in North Carolina, ranging in weight from 11 to 23 grams. It is widely distributed and found throughout most of North and Central America. (Kurta & Baker, 1990). Big Browns like to roost and live in man-made structures such as houses, barns, and bridges (*Big Brown Bat (Eptesicus fuscus) | North Carolina Bat Working Group*, 2013). This species is especially adapted to eating beetles but will eat many other insects including mosquitos, and moths. Big Brown bats emerge at dusk to forage on the same feeding ground every night (Kunz, 1973). Big Brown bats have a short bandwidth call, 20-35 kHz (Szewczak et al., 2011).

The Eastern Red bat (*L. borealis*) was first discovered and described by Philipp Ludwig Statius Müller, a German zoologist in 1776 (Shump & Shump, 1982a). The Eastern Red bat was first placed in the genus *Vespertilio* and later assigned to the *Lasiurus* genus in 1831. It is a medium-sized bat, 7 to 13 grams (Shump & Shump, 1982a) with a distinct rusty brick-red color and white underbelly. Its distribution ranges from southern Canada southward to the United

States, and as far south as Argentina (Shump & Shump, 1982a). The Eastern Red bat is capable of surviving low-temperature fluctuations by increasing their metabolism (Davis & Reite, 1967). They are a foliage roosting species compared to the *Eptesicus* and *Myotis* species due to their ability to insulate (Shump & Shump, 1982a). Eastern Red bats are solitary roosting bats, with a varying frequency call characterized by upturns at the start and end of their call to form a "U" structure (Szewczak et al., 2011).

The Hoary bat (*L. cinereus)* was first described by a French biologist Palisot de Beauvois in 1796 (Palisot de Beauvois 1796). It was originally classified as *Vespertilio cinereus* in 1796, and changed to *Vespertilio villosissimus* in1806, *A[talapha] Mexicana Saussure in 1861*, and finally *Lasiurus cinereus* in 1864 (Shump & Shump, 1982b). The Hoary bat is a large-sized bat in NC, weighing 20 to 30 grams with a distinctive grayish-brown color and frosted-looking tinge on the hair tips. This species is the most widespread of all other American bats and ranges from Canada to South America. Hoary bats tend to emerge late in the evening to forage approximately one hour and 40 minutes after sunset or right after the Eastern Red bat appears (Shump & Shump, 1982b). This species is a specialist similar to Eastern Red bats, with a diet preference for moths, beetles, grasshoppers, flies, wasps, and termites. The Hoary bat is a solitary roosting bat that is usually found among foliage in trees, they have been known to associate more with other species during the summer months while foraging (Mumford, 1953; Shump & Shump, 1982b). Hoary bats generally roost three to five meters above ground and tend to prefer the edges of clearings. Hoary bats have a low-frequency call of about 17 kHz. It is a very quick, sporadic call, never completely flat, and the power of the call tends to build in the middle and then gradually decline (Szewczak et al., 2011).

The Silver-haired bat (*L. noctivagans*) was first described by Le Conte in 1831 (Cuvier et al., 1827). This bat weighs around 8 to 11 grams and is found throughout most of North America (Kunz, 1982). Silver-haired bats tend to live as solitary tree-roosting bats in warmer months and are opportunistic insectivorous bats focused on moths, though will alter their dietary habits based on food availability (Jones, 1973). This bat species tends to forage after other bat species have already emerged for the night (Kunz, 1973). Silver-haired bats tend to have a shorter echolocation call with a distinct reverse J-shape and a flatter call ≥25 kHz (Solick, 2022; Szewczak et al., 2011)

The Evening bat (*N. humeralis*) was first described by Constantine Samuel Rafinesque in 1818 who studied several subjects including botany, zoology, ancient linguistics, and several other topics (Rafinesque & Boewe, 2005; Watkins, 1972). The Evening bat is a smaller bat weighing 7 to 15 grams, sometimes mistaken for younger big brown bats due to similarities in coat color (Watkins, 1972; Wilkinson, 1992). It prefers trees or buildings for roosting sites and eats beetles, moths, and leafhoppers as a main diet (Whitaker & Clem, 1992). Evening bats are absent in northern parts of its range during colder months indicating it is a migratory species (Humphrey & Cope, 1968). Evening Bat calls have a sweeping curved call that may lack inflection (pronounced change in the slope of the call), having a lower slope, and alternating frequencies toward end of the call (Szewczak et al., 2011).

The Tricolored bat (*P. subflavus*) and was first described by Jean Léopold Nicolas Frédéric, Baron Cuvier, a French naturalist and zoologist, in 1832. The Tricolored bat changed names several times. First known as the *Pipistrellus subflavus*, in 2006 it became recognized as the *Peromyotis subflavus* (Hoofer et al., 2006). It has distinct tricolored hairs, making it distinguishable from other *Myotis* species. The hairs are dark at the base, light in the middle, and dark again at the tip (Fujita & Kunz, 1984). Tricolored bats are found in most of Eastern and Central North America and in some parts of the Midwest. It ranges 4.6 to 7.9 grams in weight, with females weighing more than males (Myers, 1978). Tricolored bats hibernate in small numbers in caves, mines, or man-made structures. In summer, they tend to rooster solo unless part of a maternity roost. Studies demonstrate that Tricolored bats seem to be the first bats to enter hibernacula and the last to leave their hibernacula in the Spring (Fujita & Kunz, 1984). Foraging activity for Tricolored tends to be over waterways and near forest edges (Broders et al., 2001). This species is proposed as endangered and is under review for being officially added to the endangered species list due to the decline of the population from White Nose syndrome in the past 20 years. The Tricolored bat has a strongly inflected, hockey stick-shaped call, with a hook at the end of the call almost always around 42 kHz. The call is very similar to the Eastern Red bat call (Kaarakka et al., 2022; Szewczak et al., 2011).

The Mexican Free-tailed bat (*T. brasiliensis*) was first described by Isidore Geoffroy Saint-Hilaire, a French zoologist, in 1824. This medium-sized bat weight ranges from 11-14 grams, named for its long tail which can be almost half of its total body length (*Brazilian Free-Tailed Bat (Tadarida Brasiliensis)*, 2000). This animal roosts and hibernates in large numbers. Studies done by Lee and McCracken, showed evening Mexican Free-tailed diets varied from morning Mexican Free-tailed diets (Lee & McCracken, 2005). The diet of these bats varies indicating they are a generalist. Young Mexican Free-tailed bats are never attached to their mothers like most other species of bats. The young stay in the roost clinging to each other in large clusters for warmth while the mothers forage for food (Krutzsch, 1955). These bats tend to forage at sunset and continue to forage throughout the night. They are very fast flyers and have distinguishable long narrow straight wings that help with speed while flying (Krutzsch, 1955).

Their call upswings into the call and downswings out of the call, it also does not get above 40 kHz (Szewczak et al., 2011).

Bat activity is most concentrated in and around wetlands and riparian habitats (Brooks & Ford, 2005; Francl et al., 2004; Grindal et al., 1999; Maslonek, 2010; Owen et al., 2003; Zimmerman & Glanz, 2000). Riparian and wetland habitats could potentially serve as crucial foraging grounds for bats, as numerous insects they prey upon undergo an aquatic stage in their life cycles (Barclay, 1985a; Jung et al., 1999; J. M. Menzel et al., 2005). Despite the value of wetlands to bats, there is a lack of studies that examine whether bats use wetlands preferentially over other habitats for foraging.

Forest Edge impact on Bats

Wetland-adjacent forest edges act as corridors, homes, landmarks, and protection for migratory species (Jorgensen, 2009. Linear landscape features, like forested edges, deliver several benefits to bats in the form of navigational reference, protection from wind and predators, roosting site availability, as well as a positive contribution to insect abundance and availability (Estrada & Coates-Estrada, 2001; Hein et al., 2008, 2008; Verboom & Huitema, 1997). Species richness and abundance are also enhanced at forest edges, commonly known as the ‘edge effect’ (Morris et al., 2010; Potts et al., 2016). Bats use forested edges for commuting, foraging, and structure avoidance. Certain bat species, with wing morphologies adapted to flight in open areas rather than structurally complex habitats, also forage above forest canopies (Clark & Krynitsky, 1983; Crome & Richards, 1988; Fenton et al., 2008; Grindal et al., 1999; Hogberg et al., 2002; Kalcounis et al., 1999; M. A. Menzel et al., 2001; Pfalzer & Kusch, 2003; Walsh & Harris, 1996; Wunder & Carey, 1994). Bats also commonly use forests as roosting sites, especially during the summer months (Blakey et al., 2019; Taylor et al., 2020).

Research Study: How do the diversity and behavioral patterns of bats in open wetlands compare to those in forested edges?

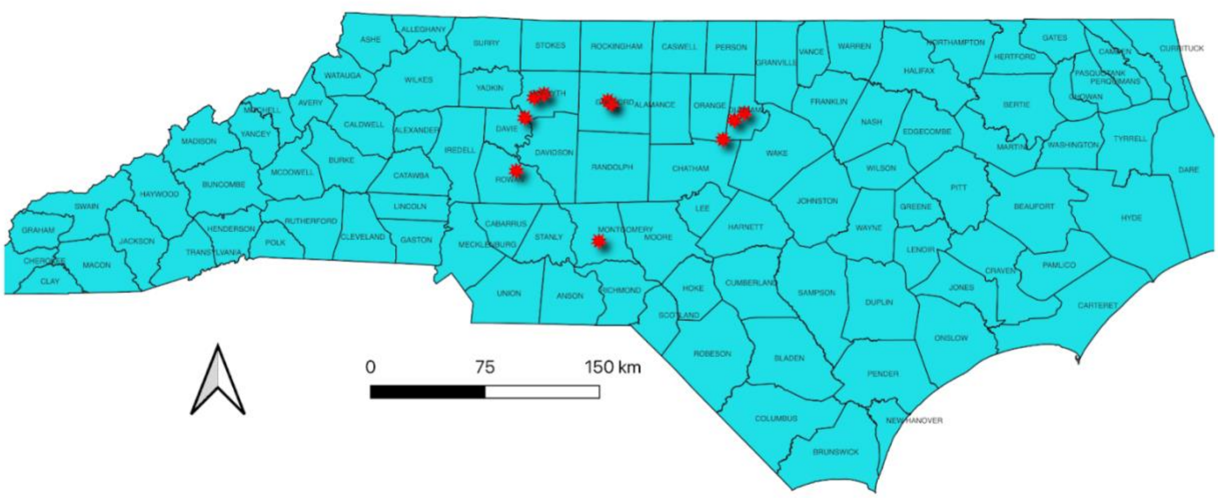
Aim 1 of this research is to test the hypothesis that bat species activity and diversity in ten open wetlands and corresponding forest edges are similar. Diversity for this study is defined as the number of bat species recorded at each site. I predict the presence of all seven insectivorous bat species at all sites due to their general similarities. I predict bat species diversity recorded by total activity to differ between wetlands and forest edges due to the habitat use varying by each species. This will help identify crucial bat habitats including the newly proposed endangered Tricolored bat and aid future research and conservation efforts.

Aim 2 of this research is to test the hypothesis that bats use open wetlands more often for foraging compared to forest edges. I will compare echolocation calls in wetland and forested edge habitats using unidirectional acoustic recordings in the Piedmont region of North Carolina. I will quantify foraging calls versus search phase echolocation calls focusing on wetlands and nearby forest edges. I predict a higher percentage of foraging calls at the open wetland site than at forest edge sites. I predict there to be a higher percentage of calls from each bat species at the open wetlands than at the corresponding forest edges, as open wetlands are easier for bats to access and use while foraging and flying compared to forest edges due to their wing morphology and the open space. Comparing audio recordings will determine the relative amount of foraging activity and search phase calls at each location. If significant foraging activity is found, these sites will be crucial for preserving endangered bat species.

CHAPTER II: METHODS

Fieldwork was conducted in the Piedmont of North Carolina wetlands and nearby forest edges located in Greensboro, Durham, Salisbury, Raleigh, Winston-Salem, and Troy from June to September 2022. Spring and Summer are ideal because bats are actively feeding on insects (Turbill, 2008). Ten sites were selected (Figure 5, Table 1). All are open wetlands with forested edges on at least one side.

Figure 5. A map of North Carolina counties. Marked with a red star are the ten sites where field research was conducted in the Piedmont regions of NC.



A general description of each wetland is as follows:

1. Lake Katharine in Winston Salem, NC is owned by Wake Forest University. Previously a sixteen-acre lake, 50 years of sediment and silt buildup turned it into a marsh with wetland vegetation, and it is now considered a wetland habitat.
2. Michael Burchell's wetland is behind a landowner's house in Winston Salem, NC, who graciously allowed the use of private land to study an ephemeral wetland. Even though mostly dry during the summer months, there is abundant wildlife and bat activity in the evenings.
3. Ellerbe Creek Beaver Marsh in Durham, NC is owned by the Ellerbe Creek Watershed Association who acquired this wetland in 2009. This wetland area is designed to purify drinking water from nearby Falls Lake that serves as a water source for over 425,000 people.
4. Bog Garden in Greensboro, NC contains seven acres of natural wetland and is primarily a City of Greensboro recreational and educational park with several boardwalks and stone pathways.
5. Uwharrie National Forest Pleasant Grove bog in Troy, NC is an upland pool surrounded by wetland plants. It is an isolated wetland surrounded by a longleaf pine forest located in an upland setting. Interestingly, this wetland is also home to a rare species of

salamander called *Ambystoma talpoideum* (mole salamander) (Seymour, 2011).

6. Mason Farm Biological Reserve, Chapel Hill, NC is owned by the University of North Carolina Chapel Hill who restored the wetlands within a Reserve.
7. University of North Carolina Greensboro has an open wetland constructed in 2017.
8. Catawba College, Salisbury NC has an 189-acre ecological preserve that contains diverse wetland habitats.
9. Tanglewood Park, Winston-Salem, NC has a wetland near the Yadkin River Nature Trail.
10. Duke SWAMP (Stream and Wetland Assessment Management Park), Durham, NC is a research and teaching facility on a restored section of the Sandy Creek stream and floodplain near Duke University's Campus. This site is used for research and was reconstructed in 2003 until all five phases of the building plans were completed in 2012.

Table 1. Characteristics of all 10 wetland study sites including geographic coordinates, size of the open wetland, city, and county, site visit dates, weather conditions, and urban population sizes. Bats are found in many urban environments throughout the world and thus should be present at all these sites (Li et al., 2021).

Site Name	Geographic Coordinates	Approximate size of wetland	Site location (City, county)	Site Visit Dates	Weather Conditions F°	Urbanization
Lake	36° 7’ 38” N.	7,270.2 m^2	Winston-	6/9/22	74 degrees, 8 MPH N	250,320 people
Katharine	80° 1’ 70” W		Salem,	7/24/22	82 degrees, 6 MPH SSE	
			Forsyth	9/7/22	75 degrees, 8 MPH N	
Michael	36° 6' 33"N.	N/A (ephemeral	Winston	6/12/22	81 degrees. 2MPH SSE	250,320 people
Burchell’s	80° 21’ 8" W	during summer)	Salem,	8/1/22	81 degrees, 6 MPH WSW	
Wetland			Forsyth	8/29/22	83 degrees, 6 MPH SE	

Ellerbe Creek Beaver Marsh	36° 01' 04"N 78° 88' 57" W	37,614.1m2	Durham, Wake	7/11/22	77 degrees, 2 MPH E/SE	285,527 people
				8/5/22	83 degrees, 3 MPH SSE	
				8/16/22	71 degrees, 4 MPH NNW	
Bog Garden	36° 05'24"N 7 9° 50'18"W	31,813.6 m^2	Greensboro, Guilford	6/14/22	85 degrees, 6 MPH N/NE	298,263 people
				7/20/22	82 degrees, 10 MPH SW	
				9/8/22	77 degrees, 5 MPH NE	
Uwharrie Pleasant Grove Bog	35° 17' 53"N 79° 53'37"W	75.6 m^2	Troy, Montgomery	6/15/22	83 degrees, 3 MPH SSE	2,913 people
				7/23/22	79 degrees, 1 MPH E	
				8/25/22	71 degrees, 1 MPH NNE	
Mason Farm Biological Reserve	35° 53'37" N 79° 01'01" W	28,435.0 m^2 (L)	Chapel Hill, Orange	6/20/22	70 degrees, Calm (No wind)	61,128 people
				8/3/22	82 degrees, 1 MPH S	
				8/21/22	75 degrees, 5 MPH S	

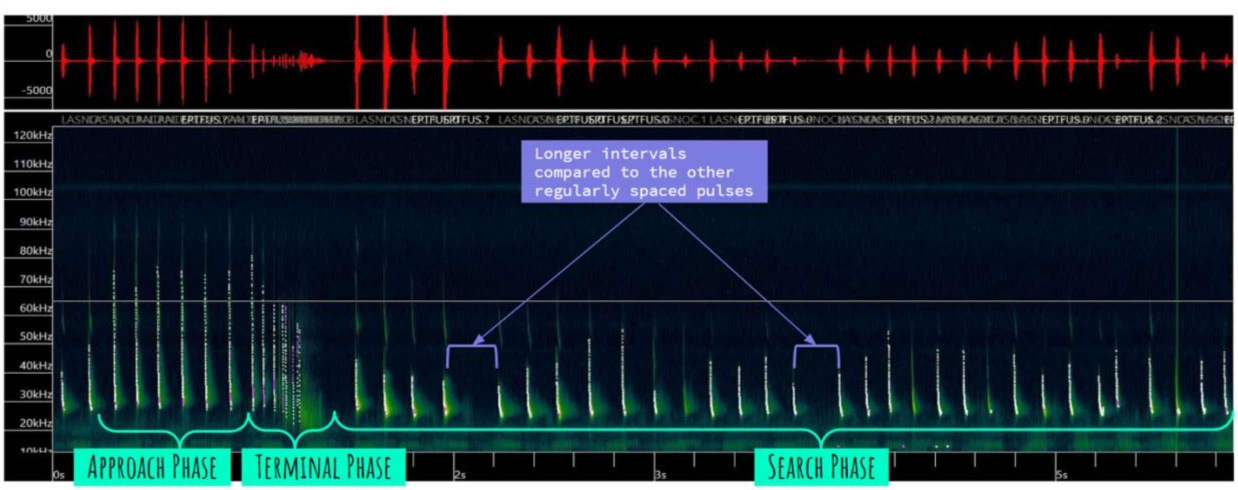
UNCG Open Wetland	36° 04' 21"N 79° 48’ 43” W	211.3 m^2	Greensboro, Guilford	7/31/22 8/17/22 9/14/22	78 degrees, 5 MPH W/SW 74 degrees, 2 MPH ENE 69 degrees, 2 MPH E	298,263 people
Catawba Open Wetland	35° 41’28” N 80° 28'45" W	21,768.4 m^2	Salisbury, Rowan	7/13/22 8/10/22 8/26/22	72 degrees, 1 MPH SW 77 degrees, 2 MPH WSW 71 degrees, Calm (No wind)	35,760 people
Tanglewood Park Wetland	35 °59'35" N 8 0° 25'09" W	3,398.9 m^2	Clemmons, Forsyth	7/14/22 7/22/22 8/22/22	77 degrees, 1 MPH W/SW 82 degrees, Calm (No Wind) 76 degrees, 1 MPH WNW	21517 people
Duke SWAMP	35° 59'20"N 78° 56'40' W	6,129.2 m^2	Durham, Wake	7/18/22 8/7/22 8/18/22	82 degrees, 5 MPH S 82 degrees, 2 MPH SSW 77 degrees, 2 MPH SSE	285,527 people

Acoustic Monitoring

Audio recorders (detectors) are a non-invasive method to record bat activity by identifying species-specific vocal behaviors (Murray et al., 1999; O'Farrell & Gannon, 1999). Microphones can be outfitted with a cone, so they record audio activity unidirectionally in one direction or without a cone, so they record audio activity 360 degrees. Unidirectional microphones detect and record bat calls at a longer distance than omnidirectional microphones which have shorter but broader detection ability (Limpens & McCracken, 2004). Unidirectional microphones also record lower background noise that may interfere with the interpretation of recordings focused on detecting bat calls (Kaiser & O'Keefe, 2015). Audio recorders can be permanently installed recording continuously, for example at the UNCG wetlands (Li et al., 2021) or are set up in the field for short periods of time to record bat activity (Loeb et al., 2020).

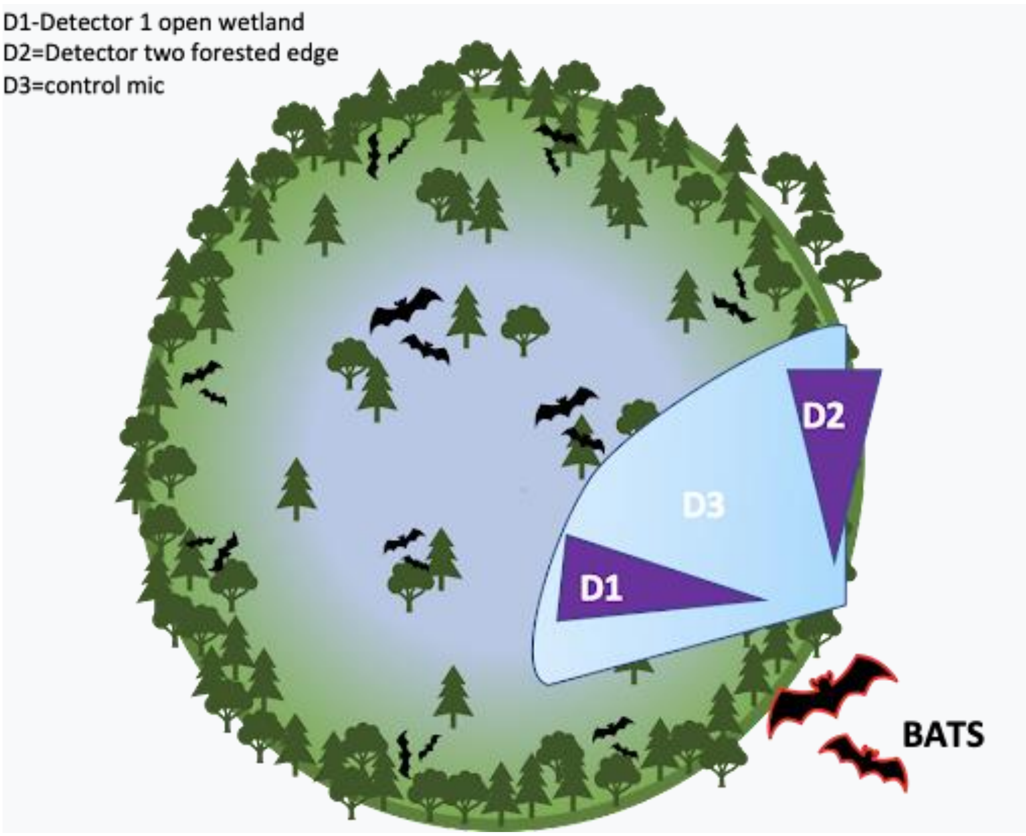
Echolocation represents a physiological process used for object location through the reflection of sound waves. Bats use a specific form of echolocation, emitting ultrasonic, high-frequency signals that bounce off objects or prey and return as signals enabling classification, characterization, and determination of object location (Schnitzler & Kalko, 2001). This type of echolocation allows bats to navigate and detect prey during the night (Surlykke et al., 2009). Bats emit species-specific calls, making acoustic monitoring a well-established method for monitoring bat activity patterns, changes in habitat use and general bat activity (Hayes, 2000). Acoustic monitoring facilitates the assessment of bat habitat usage. A benefit of acoustic monitoring is long-term deployment capability. Unlike other methods of recording and surveying bat data, acoustic monitors can be deployed for extended time without intervention (Frick, 2013). Figure 6 represents an echolocation call from *E. fuscus* as observed on Kaleidoscope Pro. There are some drawbacks to acoustic monitoring. For example, it does not directly provide information about the number of bats present, it may fail to capture all bat calls, and noise interference can complicate estimates of activity levels and species identification. Figure 6 shows examples of foraging and search phase echolocation calls that are distinctly different from one another. Visually analyzing bat echolocation recorded calls offers insights into behavior and type of call used. Acoustic monitoring represents a non-invasive method of studying bats compared to other methods such as mist netting by minimizing disruption and stress to the animals being studied.

Figure 6. Audiogram of a Big Brown bat (E. fuscus). The upper portion is the oscillogram that measures amplitude, and the lower portion is the spectrogram that measures frequency. The variation in call intervals, a common character of Big Brown bat calls, is indicated. Three primary phases of vocalizations used during foraging for prey are shown: search, approach, and terminal phases (Weichart & Clark, 2020). Search phase is featured by consistent call pattern as the bat is echolocating to search for prey. Approach phase is feedback from objects or prey as the bat approaches a potential food source. Terminal phase echolocation is featured by faster and shorter bursts of echolocation calls as the prey is identified and targeted. Longer intervals in the search phase represent transitions between echolocating calls.



Song Meter SM4BAT-FS ultrasonic recorders equipped with a SMM-U2 microphone (Wildlife Acoustics Inc., Maynard, Massachusetts, USA) were used as unidirectional detectors to record bat calls at forest edges and over open wetlands, and a SM4BAT-FS recorder equipped with a SMM-U1 microphone was used as an omnidirectional detector. Recorders were set up in two positions as follows: the SMM-U1 detectors had deflectors to make them unidirectional. Detector #1 was pointed towards an open wetland. Detector #2 was 30 feet 2 inches away from detector #3 also at a 25-degree angle and faced towards the forested edge near the wetland. Detector #3, the SMM-U2 microphone was set as the omnidirectional detector, placed 30 feet 2 inches away from Detector #1 at a 25-degree angle. Figure 7 demonstrates the study design that was replicated at all study sites. Each of the detectors were set to record for 125 minutes after sunset as most of the bat species are active right after sunset and tend to forage and fly. Research has demonstrated that directional detectors are more useful for identifying bat calls over specific landscape regions than omnidirectional detectors (Jakobsen et al., 2013).

Figure 7. A representation of the layout at each site. One unidirectional detector is pointing at the open wetland, the omnidirectional microphone is in the middle, and the second unidirectional detector is pointing toward the forested edge.

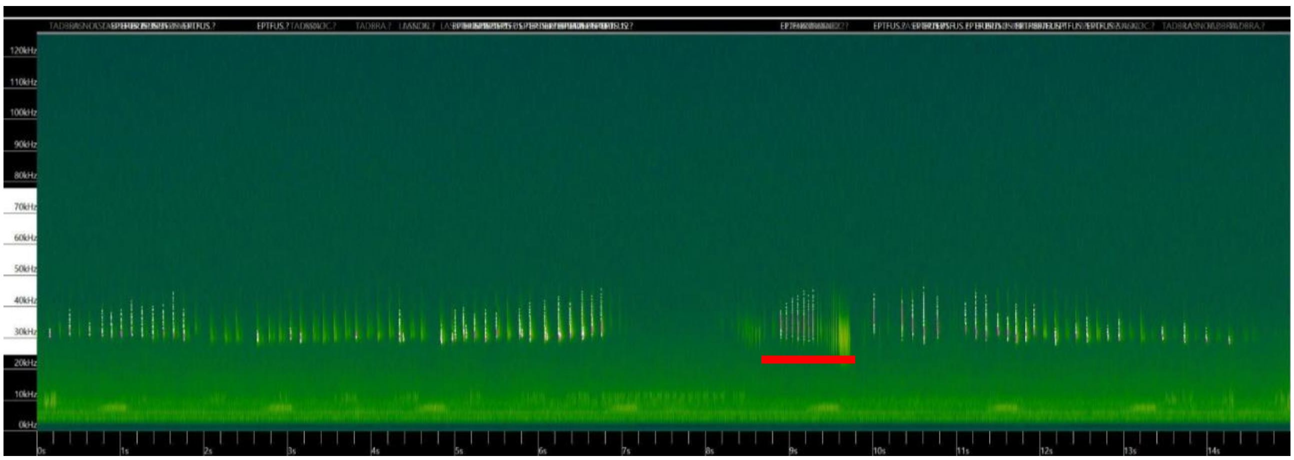


Audio files were downloaded after each recording session and analyzed using the automatic identification feature of Kaleidoscope Pro (version 4.4, Wildlife Acoustics Inc., Maynard, Massachusetts, USA). Each recording features a bat pass, defined as three or more complete bat echolocation calls within 0.5 seconds. Each of the ten sites was studied on three different nights to account for variations in nightly conditions including temperature, insect abundance, humidity, and weather (Hayes, 1997).

Automatic identification of species using Kaleidoscope Pro was used when possible for all recorded calls by using a match ratio of 60% or higher (Li et al., 2021; Li & Kalcounis-Rueppell, 2018; Parker et al., 2019; Schimpp et al., 2018). For identifications below the 60%

threshold calls were identified to species based on manual identification by reference to identified calls from Dr. Han Li’s previous research. If a bat call pass featured multiple foraging calls it counted as foraging call per bat call pass. Figure 8 shows an example of a bat call pass that contained a foraging call (marked with a red underline).

Figure 8. Foraging Call Example. The figure is an example of a collected acoustic call pass during the 2022 Summer Field season. The red line indicates the foraging call within a search phase's echolocation call.



Data was collected June 2022 through September 2022 at the ten study sites. Kaleidoscope Pro automatic identification feature was set to the seven local species. The identification accuracy was be set to neutral. These audio recordings of bat calls were used to quantify total bat activity, species-specific activity, any temporal changes in total activity and species-specific activity following Parker et al., 2019. All experiments were performed in the evenings when there was no rain or winds over 10 mph and an ambient temperature above 60°F (15.6°C) following the recommendations of Ford (Ford et al., 2005).

To test aim 1, a paired Wilcoxon rank sum test was performed by comparing all species by using open wetland sites as replicates comparing them with forest edges as replicates in a paired design. To further the analysis, the data were segregated by bat species. For statistical analysis, proportional values of acoustic calls per bat species for each of the ten study sites was created by calculating calls at the forest edge versus total calls for each species and the calls at the open wetlands versus total calls for each species. Given the greater bat calls per species at open wetlands than at forest edges proportion values were appropriate for analysis purposes. Proportions were derived by dividing each bat species per site at open wetland and forest edges to the total number of that species over all sites. This allowed for the assessment of proportional differences in call pass counts between open wetlands and forest edges.

To examine the data for aim 2, potential differences in foraging activity over wetlands compared to adjacent forest edges, an analysis was performed by using sites as replicates and comparing them with open wetlands as replicates in a paired design. Proportional activity was calculated as before by calculating the total foraging calls at each study site for each species at wetlands and at forest edges relative to the total echolocation calls recorded. To examine the data more deeply, the results for each species were examined.

The same design as aim 1 was replicated, testing for differences between forest edge and wetland foraging calls for each species independent of one another using sites as replicates in a paired design with the Wilcoxon sum rank test. To calculate the proportion, index all foraging bat call passes recorded at each research site were divided by the total foraging bat calls recorded at open wetlands and forest edges, respectively. This value was then multiplied by 100 to obtain the overall percentage. By subtracting one percentage from the other, the total difference between open wetlands and forest edges was determined.

A Test of Experimental Viability

To ensure the unidirectional microphones were recording bat acoustic calls in a directional cone pattern as expected, the omnidirectional microphone was placed between the two unidirectional microphones for each recording event. Because the omnidirectional microphones records bat acoustic calls in all directions it should capture more call passes than either of the unidirectional microphones alone. Thus, comparisons between the omnidirectional microphone and each unidirectional microphone should show fewer recordings at each of the unidirectional microphones than the omnidirectional microphone. Therefore, they can be used to determine if the experimental design aimed at comparing wetlands versus forest edges using the unidirectional microphones is valid.

CHAPTER III: RESULTS

There were 11,336 files recorded during the field season. The total recorded time was 3,750 minutes or 62.50 hours. After removing noise and N/A files, 5,176 calls remained to be analyzed. Of these remaining calls 1,385 were Big Brown bats, 939 were Eastern Red bats, 16 were Hoary bats, 85 were Silver-haired bats, 1,329 were Evening bats, 1,171 were Tricolored bats, and 251 were Mexican Free-tailed bat calls. Table 2 shows the calls recorded separately at each site for each bat species.

Each site had a difference in the number of bat species recorded and the species of bat with the largest number of calls (Figure 9). For Big Brown bats, the highest call pass count was site two. For Eastern Red bats the highest call pass count was at site three. For Hoary bats there was a single call count pass at site one. For Silver-haired bats, equal call count passes were at site one, four, and five. For Evening bats, the highest call count passes were at site three. For Tricolored bats the highest call pass count was at site five. For Mexican Free-tailed bats the highest call pass count was at site one and site three.

Table 2. Bat Species Activity Based on Recordings of Bat Calls Identified Manually and by Kaleidoscope at Each Research Site.

Site	Species						
	Big Brown	Eastern Red	Hoary	Silver-haired	Evening	Tricolored	Mexican Free-tailed
Bog Garden	104	24	11	25	122	12	69
Catawba College	303	198	2	12	191	5	114
Duke SWAMP	121	306	0	6	489	329	17
Ellerbe Creek	72	143	0	11	318	71	7
Lake Katharine	306	117	2	16	126	693	11
Mason Farm Biological Reserve	30	43	0	9	9	29	4
Michael Burchell	0	6	1	2	0	2	0
Tanglewood Park	218	83	0	2	26	22	2

UNCG Wetland	18	7	0	2	10	1	20
Uwharrie Pleasant Grove Bog	213	12	0	0	38	7	7
Total Sites Species is Recorded Present	**9/10**	**10/10**	**4/10**	**9/10**	**9/10**	**10/10**	**9/10**

Figure 9. Call pass counts for each species identified with over 60% accuracy using Kaleidoscope Pro are represented on the Y-axis, with each species and study site on the X-axis.

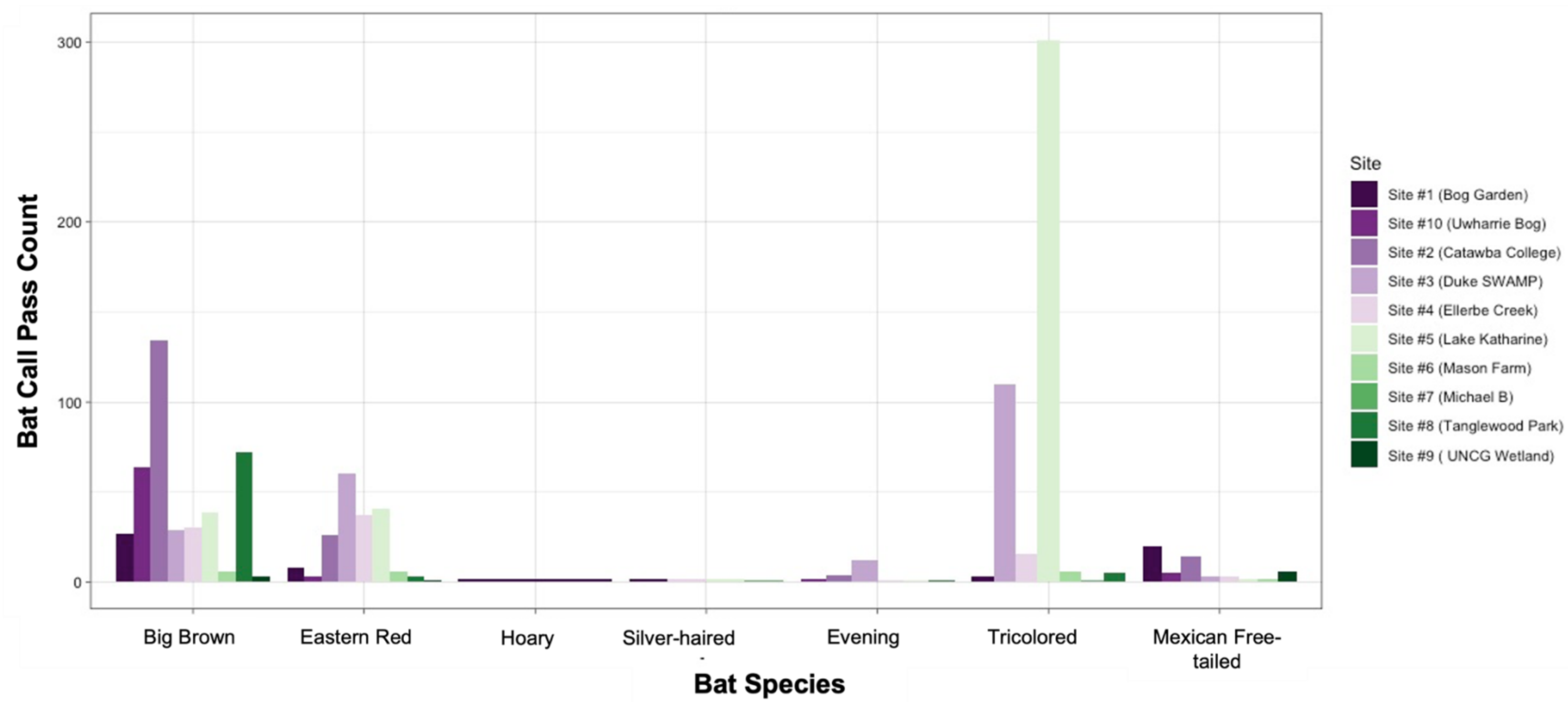
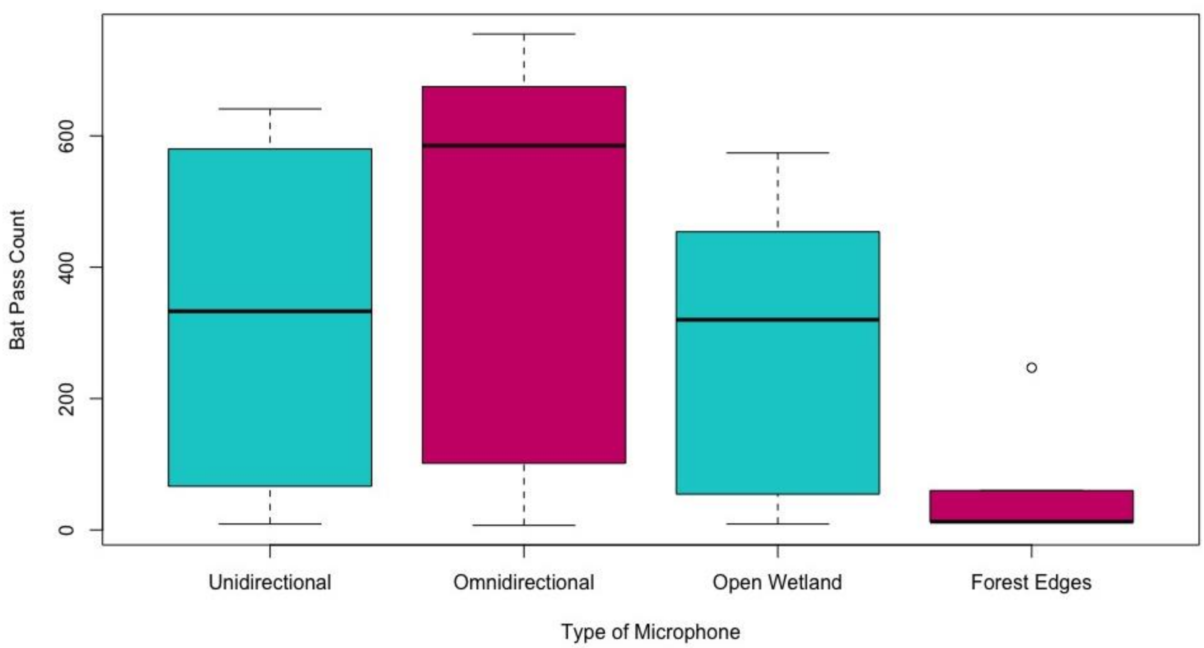


Figure 10 shows the number of calls at each microphone. The omnidirectional microphone versus the unidirectional microphones were tested first using a paired Wilcoxon sum rank test. and found no statistical significance among the detectors (*P*=0.47). Furthermore, the unidirectional microphone pointed over the wetlands compared to the omnidirectional microphones showed no significant difference (*P*=0.22). The unidirectional microphone pointed over the forest edge was statistically different than the omnidirectional microphone (*P*=0.03). Figure 10 shows the total bat call pass count recorded using unidirectional and omnidirectional microphones, and the total bat pass call count at open wetlands, and forest edges

Figure 10. Testing Equipment Viability. Omnidirectional, Unidirectional, Open Wetland and Forest Edge bat call pass count.



Aim 1 results: Test of bat diversity at wetlands versus forest edges.

Out of the seven bat species, only two species were recorded at all ten sites, the Eastern Red bat and the Tricolored bat. Three species were recorded at nine out of ten sites, Big Brown, Silver-haired, and Mexican Free-tailed. The hoary bat was recorded at only four of the ten sites (Table 2).

A significant difference in the use of open wetlands versus forest edges (*P*=0.03) was found. Figure 11 displays the bat call pass count for all sites at open wetlands compared to forest edges.

Figure 11. Open Wetland Bat Call Counts versus Forest Edge Bat Call Counts.

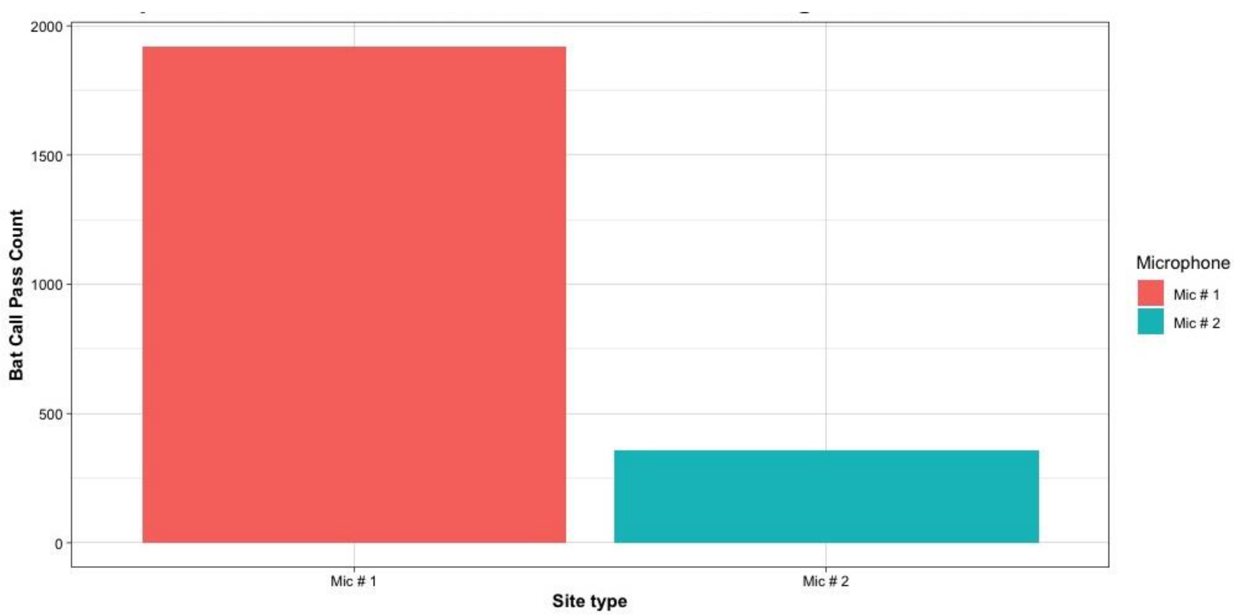


Figure 12 shows the data highlighting considerable variation among the bat species at open wetlands and forest edges across all sites. Bat call pass counts were 23x higher at open wetlands than at forest edges. Table 3 shows the total number of bat call passes for each species recorded at all research sites. The percentage column is representing the difference between the number of calls detected at each location (Open wetland versus Forest Edge).

The results showed variation in call activity levels and differences between open wetlands and forest edges for each species (Figure 12). Of the seven species analyzed, five showed statistically significant values, while two species exhibited differences in acoustic calls.

Call pass proportional count was higher at open wetlands relative to forest edges in all species except the Hoary bat where they were not recorded at forest edges.

Figure 12. Species Calls at Open Wetland versus Forest Edges. Graphical visualization of bat call passes at Open Wetland (Mic #1) versus Forest Edges (Mic #2) for each bat species combined for all study sites.

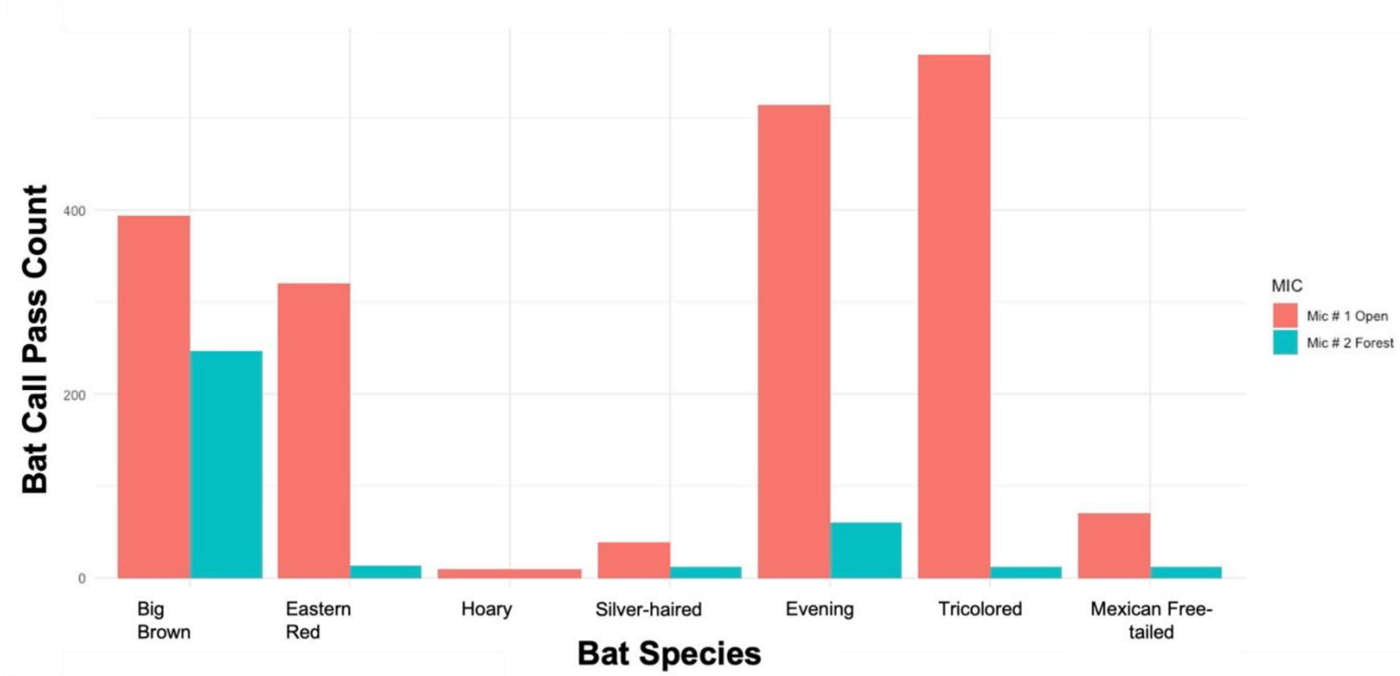


Table 3. Total Bat Species Call Passes for Open Wetland versus Forest Edge. The table shows the total number of bat call passes for each species recorded at all research sites. The percentage column represents the difference between the number of calls detected at each location (Open wetland versus Forest Edge).

Species	**Results**					
	Total Open Wetland Calls	**Total Forest Edge Calls**	**Total calls**	**Proportion of calls at Open Wetlands**	**Proportion of calls at Forest Edges**	**Percent Difference of Bat call Passes recorded**
Big Brown Bat	394	247	641	61%	39%	23% more calls at open wetland
Eastern Red Bat	320	13	333	96%	4%	92% more calls at open wetland
Hoary Bat	9	0	9	100%	0%	100% more calls at open wetland
Evening Bat	39	12	51	76%	24%	52% more calls at open wetland

Silver-haired Bat	514	60	574	90%	10%	80% more calls at open wetland
Tricolored Bat	574	12	586	98%	2%	96% more calls at open wetland
Mexican Free-tailed Bat	70	12	82	85%	15%	60 % more calls at open wetland

The results indicated that the Big Brown bats were not recorded differently at open wetlands vs forest edges (P= 0.7) but had 23 % more calls recorded at open wetlands than forest edges. Conversely, Eastern Red bats were recorded more often at open wetlands than at forest edges (P= 0.02), with 92% more calls at open wetlands than forest edges. Hoary bats showed no significant difference between open wetlands vs forest edges (P=0.17) due to lack of calls recorded, 100% of calls were recorded at open wetlands. In contrast, Silver-haired bats showed higher bat call passes at open wetlands relative to forest edges (P=0.02) with 80% more calls recorded at open wetlands than forest edges. Evening bats also showed higher activity over open wetlands relative to forest edges (P= 0.02) with 52% more calls recorded at open wetlands than forest edges. Similarly, Tricolored bats showed a difference in call passes between open wetlands and forest edges (P=0.05), with 96% more of their recorded calls coming from open wetlands. Finally, Mexican Free-tailed bats showed a higher call pass activity over open wetlands relative to forest edges (P=0.03) with 60% more recorded calls at open wetlands than forest edges. Five out of the seven species showed statistically significant values, whereas only two species did not show any significant differences among open wetland versus forest edges in acoustic calls proportionally. Figure 13 shows where the differences in bat calls fluctuate at each site and each microphone.

Figure 13. Bat call pass counts per site. Species found at open wetlands are represented on the left side. Species found at the forest edge are represented on the right of the graph.

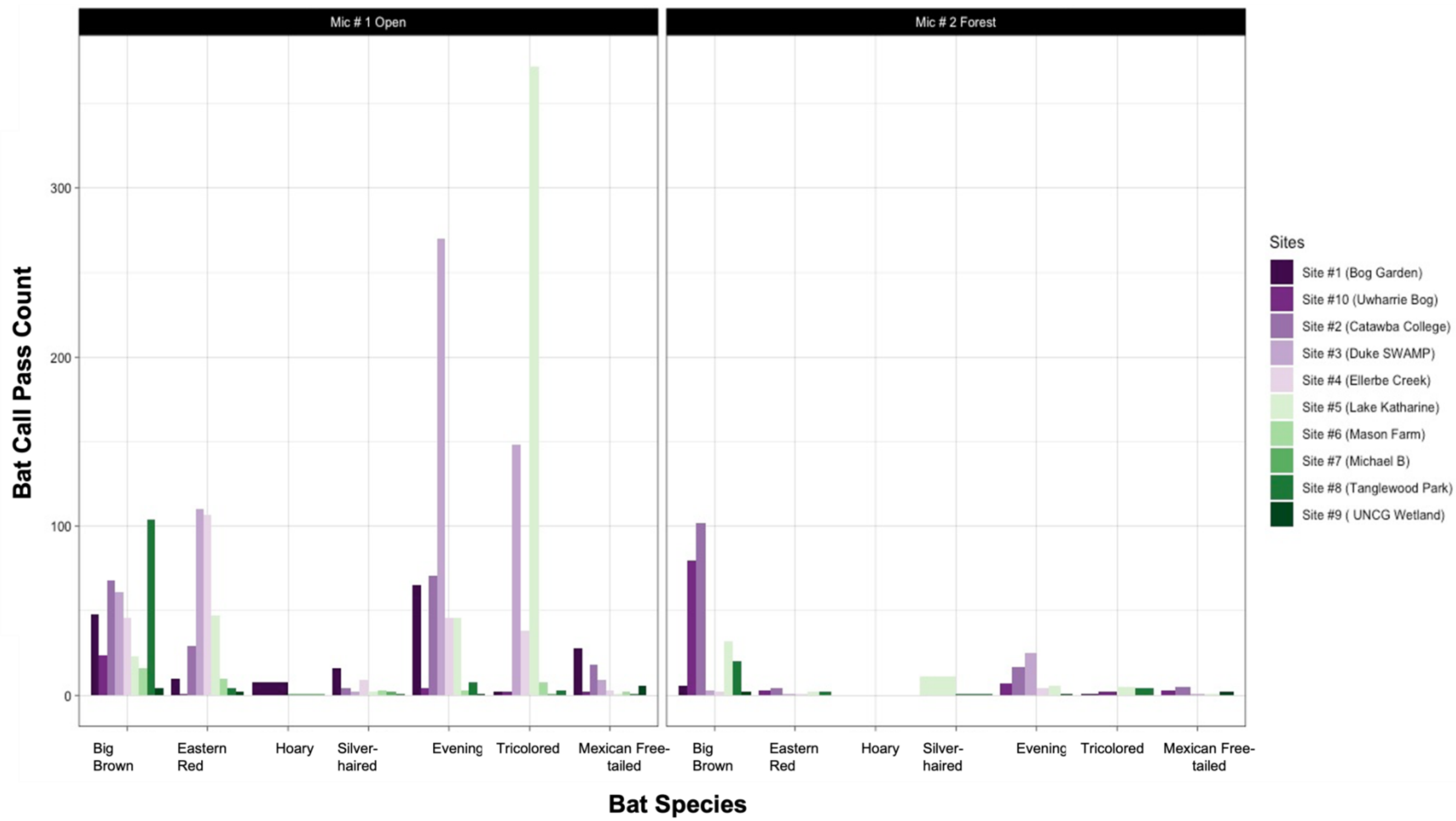


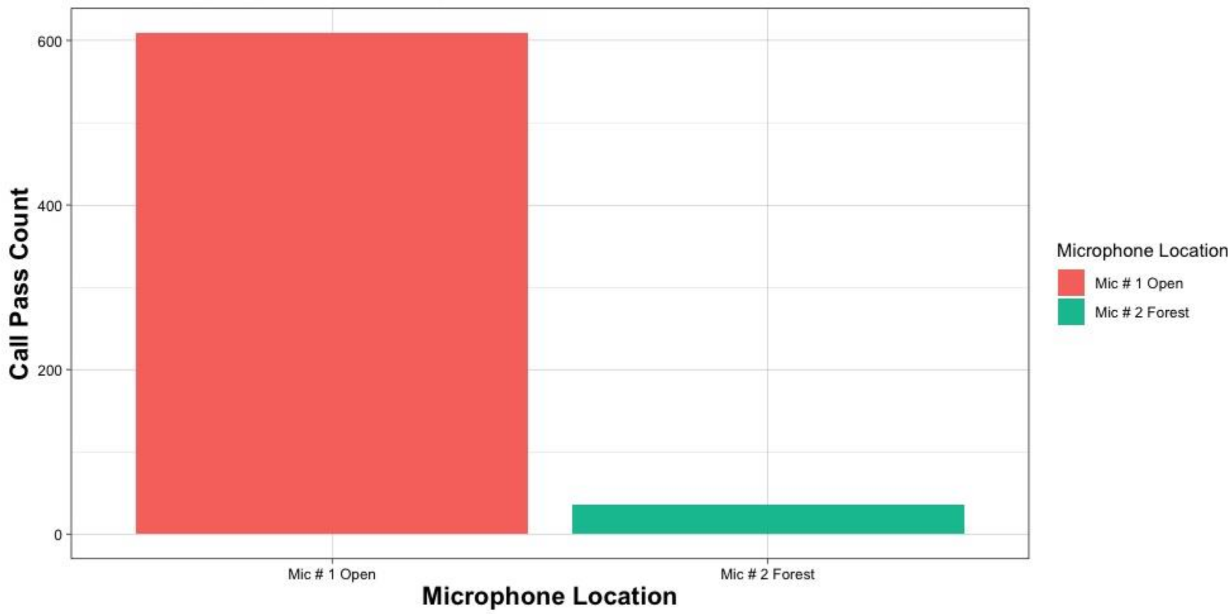
Table 4. Wetland Size. Bat call activity at each wetland is based on the size of the wetland in meters squared.

Site Name	Approximate size of wetland	Bat call activity
Lake Katharine	7,270.2 m^2	1273 calls
Michael Burchell's Wetland	N/A (ephemeral during summer)	10 calls
Ellerbe Creek Beaver Marsh	37,614.1m2	633 calls
Bog Garden	31,813.6 m^2	367 calls
Uwharrie Pleasant Grove Bog	75.6 m^2	551 calls
Mason Farm Biological Reserve	28,435.0 m^2	124 calls
UNCG Open Wetland	211.3 m^2	58 calls
Catawba Open Wetland	21,768.4 m^2	824 calls
Tanglewood Park Wetland	3,398.9 m^2	355 calls
Duke SWAMP	6,129.2 m^2	1267 calls

Aim II Results: Bat foraging activity at open wetlands compared to forested edges.

No significant difference among bat foraging calls between open wetlands and forest edges were found (P=0.14). Figure 14 shows bat foraging call pass counts for all sites at open wetlands compared to forest edges.

Figure 14. Foraging Calls at Open Wetlands versus Forest Edges for all seven bat species at the ten study sites.



Each wetland and forest edge study site were analyzed to identify differences in foraging calls across bat species (Figure 15). The data and results are in Figure 15 and Table 5. Across most species, the foraging call pass counts were significantly higher at the open wetland compared to forest edges, except for the Silver-haired bat.

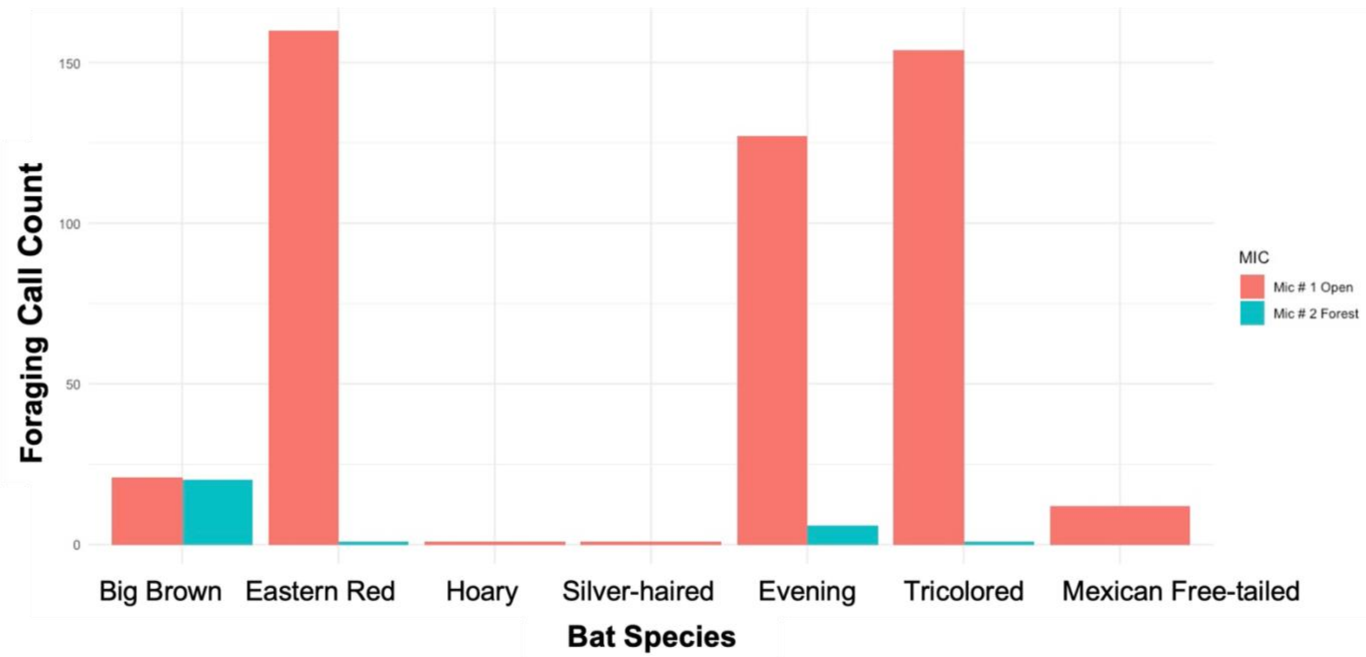
Table 5. Total Bat Species Foraging Call Passes for Open Wetland versus Forest Edge. The table shows the total number of foraging bat call passes for each species recorded at all research sites. The percentage column represents the difference between the number of foraging calls detected at each location (Open wetland versus Forest Edge).

Species	Results					
	Total Foraging calls at Open Wetland	**Total Foraging Forest Edge Calls**	**Total Foraging calls**	**Proportion of Foraging calls at Open Wetlands**	**Proportion of Foraging calls at Forest Edges**	**Percent Difference of Foraging Bat call Passes recorded**
Big Brown Bat	20	20	40	50%	50%	Even Number of Foraging Calls
Eastern Red Bat	162	1	163	99%	1%	99% more calls at open wetland
Hoary Bat	1	0	1	100%	0%	100% more calls at open wetland

Evening Bat	1	12	13	92%	8%	92% more calls at forest edges
Silver-haired Bat	125	6	131	95%	5%	95% more calls at open wetland
Tricolored Bat	154	1	155	99%	1%	99% more calls at open wetland
Mexican Free-tailed Bat	**12**	**0**	**12**	**100%**	**0%**	**100 % more calls at open wetland**

I next examined wetland and forest edge sites for differences in foraging calls by each species (Figure 15). Big Brown bats had an even number of foraging calls recorded at open wetlands versus forest edges. Eastern Red bats had a $P = 0.03$, demonstrating there was a statistical difference (99% more foraging calls at open wetlands) in open wetland foraging compared to forest edge foraging. Hoary bats had only one occurrence of a foraging call recorded, and it happened at Site 1 (Bog Garden) at the open wetland microphone. No other foraging calls were recorded for this species during the summer research. Silver-haired bats had 95% more foraging calls recorded at open wetlands compared to forest edges. Evening bats had 92% more foraging calls recorded at forest edges compared to open wetlands. Tricolored bats had 99% of their foraging calls recorded at open wetlands compared to forest edges. Mexican Free-tailed bats had 100% of recorded foraging calls occur at open wetlands compared to forest edges.

Figure 15. Foraging Calls at Open Wetlands versus Forest Edges for all seven bat species. Graphical representation of foraging calls at open wetlands (Microphone #1) versus forest edges (Microphone #2).



CHAPTER IV: DISCUSSION

Wetlands have historically declined since the mid-1780s and are now protected (Bierlein, 2007; Dahl, 1990). Restoration of wetlands is becoming a common practice to mitigate groundwater pollution and restore biodiversity which is important for ecosystem functioning (Zedler, 2000). Understanding the impact of wetlands on bat foraging activity will provide insight into the influence on species diversity and abundance in general because bats are a keystone species (Gannon & Bovard, 2016; Russo et al., 2021). Bats are an important bioindicator species and may be important to wetland functionality assessments due to their association with wetlands (Maslonek, 2010). A study done from 1998 to 2004 showed wetlands have begun to increase in numbers and exceed the losses that have happened over the last 200 years (Dahl, 2006). With wetland restoration on the rise, it is important to see the impacts it is having on keystone species such as bats. As created wetlands and wetland restoration continue to rise, bats should be considered as part of the design criteria and assessment due to wetland benefits for bats, especially with bat mortalities still on the rise (Maslonek, 2010).

While testing the experimental viability the results of the unidirectional microphone over wetland versus omnidirectional microphone showed (P=0.22). This could be due to several variables such as the omnidirectional and unidirectional microphones over the open wetlands had similar acoustic results meaning they picked up a lot of the same acoustic calls made by bats. Or that, since the unidirectional microphone pointed over the open wetland, had more open area for bats to fly and forage, it also allowed the omnidirectional microphone to pick up the same calls without any interference that the forest edge causes for the omnidirectional microphone to experience. Whereas the unidirectional microphone pointed over forest edges was statistically different compared to omnidirectional microphones (P=0.03). Reasons for this may include bat species using forest edges less than open wetlands and open spaces where the omnidirectional microphone was placed or that the trees in the forest blocked the omnidirectional ability to detect and trigger the detector to record bat calls due to interference by trees.

Examining open wetlands versus forested edges using unidirectional microphones for the diversity of bats in the Piedmont region of NC revealed different outcomes. Each bat species responded differently to each site monitored. Out of the seven species present, only two were recorded at all ten sites. There can be several reasons why this was the case. Each of the bat species found within the region has different habitat requirements, foraging habitats they prefer, avoiding predation risk, or avoiding competition among other bat species in the area (Surlykke et al., 2009). Four out of seven were recorded at nine sites, and one species was only recorded at four of the ten research sites. Some variables that could have affected the results found include wetland size (Straka et al., 2016), wetland type including the one ephermal site that had very little bat activity in general (Jung et al., 1999; Maslonek, 2010; Morris et al., 2010; Salvarina, 2016), and urbanization of the sites. (Li & Kalcounis-Rueppell, 2018; Russo & Ancillotto, 2015; Threlfall et al., 2012). Recognizing that each bat species has specific needs and responses to wetlands, it is important to consider the bat community and how interspecific interactions might affect the effects of open wetlands and forest edges on bats. Interaction between the interspecific seven bat species could lead to community molding and behaviors overtime (Chaverri et al., 2018; Culina & Garroway, 2019; Lewanzik et al., 2019; Li et al., 2021; Pfalzer & Kusch, 2003)

The size of the wetland was tested against overall bat activity and showed no correlation between wetland size and bat activity. Therefore, the size of the wetlands did not seem to affect the bat activity. In previous studies, the size of wetlands also did not affect the results of bat activity (Maslonek, 2010; Straka et al., 2016). Wetland type can be studied further in future studies and have multiple types of each wetland to represent wetland type to see if any results differ from this study and previous studies.

Examining differences in foraging activity over wetlands compared to adjacent forest edges in the Piedmont region of NC had various results depending on bat species. Overall, the bat species showed that open wetlands had higher call pass counts of foraging calls than corresponding forest edges. This information is important because it shows which bat species prefer foraging at open wetlands compared to forested edges. Although all bat species demonstrate higher echolocation calls at open wetland sites compared to the forest edge sites, some species chose to forage at both types of sites regardless of their activity levels at the locations. When broken down into individual species, the results showed different outcomes. The Hoary bat was not recorded as frequently compared to the other species. This could be because Hoary bats tend to emerge later in the night to forage than the other bat species recorded. This could be an influence to why microphones did not pick up as many foraging calls by Hoary bats compared to the other species (Shump & Shump, 1982b). The Hoary bat also tends to be a very high flyer, more than other bats, and could be foraging or echolocating higher than the unidirectional mics could have been able to record (Shump & Shump, 1982b). A study done by Corcoran and Weller, showed that Hoary bats can make different types of echolocation calls or no calls depending on their location and if other bat species are present (Corcoran & Weller, 2018).This can lead to further studies being done specifically on Hoary bats at open wetlands and what kind of calls they are emitting at open wetlands versus forest edges specifically. This is one of the downsides of only using acoustical data alone. The Eastern Red bat also tends to be a later flyer. According to Beilke et al, these bats tend to forage closer to their roost, which tends to be by forest openings. They also saw Eastern Red bats using foraging sites near roads, ponds, and ridges (Beilke et al., 2023). Foraging activity for tricolored bats tends to be over waterways and near forest edges (Broders et al., 2001), therefore this species showing no difference in foraging activity from open wetlands and forest edges makes sense. The Mexican Free-tailed bat tends to forage as soon as sunset and continue to forage throughout the night, they are very fast flyers and have distinguishable long narrow straight wings that help with speed while flying (Krutzsch, 1955). The speed of this bat makes unidirectional acoustical collection harder due to the recorders not being set off fast enough to capture this bats echolocation and foraging calls.

The Tricolored bat has recently (September 2022) been proposed endangered. Throughout this research and study, Tricolored bats were acoustically detected at all ten of the wetland sites. This may imply that wetlands and corresponding forest edges are especially important to this species as foraging grounds and high-activity sites. Conservation and preservation of these sites may be important for this species to thrive and increase its numbers especially for North Carolina.

Future research methods can include mist netting for this proposed endangered species at these sites and many other wetlands in the Piedmont area of NC. Some future research questions to investigate is why Tricolored bats are recorded higher at these wetlands and their forest edges if no significant difference was found in their foraging behavior. What other values could these wetlands and forest edges be offering the Tricolored bats if not for foraging behavior?

Another aspect to look at is the plant composition found in each wetland and forest edge. Most of the sites in this study had very similar plant compositions and therefore did not show any patterns specific to plants. Big Brown and Evening bats had higher call count passes at forest edges than the open wetlands. This can be due to many reasons, site ten was a longleaf pine forest that was recently controlled burned, which made the forest edges a lot less cluttered and might have allowed these bigger, less agile bat species to navigate easier through the forest edges. A study done by (Armitage & Ober, 2012) showed that prescribed fire may temporarily allow bigger bat species to forage more below the tree canopy due to the opening of space. There are no known specific studies that assess wetland vegetation impacts on the seven bat species in this study. Future studies of how plant life around wetlands can alter certain bat activity and the use of these wetlands needs to be studied.

Another thought-provoking question would be to test why Hoary bats and Evening bats were acoustically detected so little compared to the other species (Barclay, 1985b; Corcoran & Weller, 2018; Hein et al., 2009). To analyze this further, detectors can be left overnight to increase bat acoustics throughout the night rather than just a few hours after sunset. Another study would be to see the year-round use of these wetlands and forest edges to the seven bat species found in this region. Setting up mist net traps along with acoustic detectors would increase our understanding of the actual bat activity. Most of the research sites also had open fields nearby or around the wetland and forest edges, so it would be interesting to see the comparison of foraging at open fields, open wetlands, and forest edges. To get more information and further our understanding we could also set up omnidirectional microphones at all three instead of using unidirectional microphones. Omnidirectional microphones tend to capture a lot more bat activity and calls in general than unidirectional microphones, however, the same questions cannot be answered as there are limitations to be considered. Future studies can also look at social activity versus foraging activity at wetlands versus forest edges to get a better understanding of how bats are using these types of sites. The research would have to be ongoing to collect enough social and foraging calls to be able to quantify the activity levels at certain sites. This could answer and open more future directions for bat studies at open wetlands versus forest edges and give important data on how these lands are being used by different bat species.

Conclusion

Overall, this study's findings suggest that the different species of bats use wetlands and forest edges uniquely. Activity levels varied based on the species of bats, and foraging behavior also varied by species of bats. This study adds to the overall knowledge of bats using wetlands as foraging grounds but also indicates that species-specific interactions are important as well. This study shows the continued need for wetland protection and restoration to increase bat attraction and use. It also shows important conservation efforts are needed to help the Tricolored bat, especially at the research sites where it was detected more frequently. This study can help protect lands important to the proposed endangered species and continue focusing research on their preferred foraging grounds.

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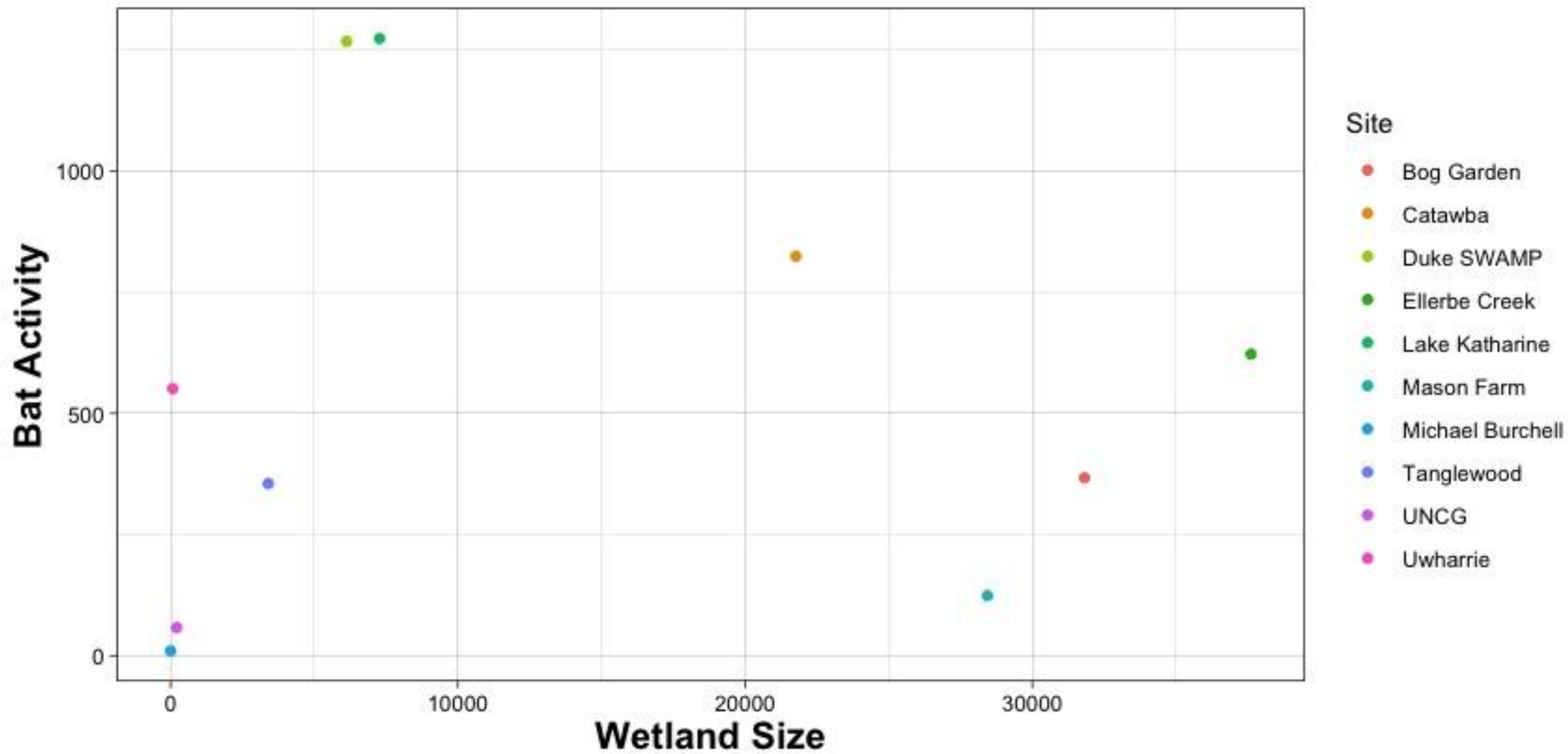
APPENDIX A: WETLAND SIZE

Since bat species have preferences for specific geographic features and resources, an examination of wetland size for potential impact on echolocation activity recorded in the data is appropriate to determine a potential impact on variation in species activity at the study sites. If wetland size has an impact on bat activity, then there should be a positive relationship between bat activity and the size of the wetlands in this study. The size of wetlands was determined by using Google Earth coordinates to estimate the length and width, and then converted into meters squared. Because there is no highly accurate method to take a size measurement, since wetlands are a variety of shapes and vary in water content throughout the year, a length and width were estimated on a single day for all study sites and multiplied to create a meter squared estimate of size (Table 4A). To analyze the data, the total bat call recordings for all study sites were then aggregated and divided by the bat call passes at each site. A Wilcox-sum rank test in a paired design showed a significant difference (P=0.013) indicating that the ranks of wetland size and amount of bat activity are not related. Appendix A shows there is no clear relationship between overall wetland size and bat activity.

Figure 16A. Wetland size in meters squared versus bat activity at the wetland sites.



APPENDIX B: PLANT COMPOSITION

Habitat similarities and differences among each wetland were examined to determine if plant composition (Table 6B) has an impact on bat activity. Big Brown bats and Evening bats had higher call count passes at the forest edges than the open wetlands compared to all the other species that had higher call count passes at open wetlands than forest edges. Since all the wetlands were in the Piedmont region of NC, they had similar characteristics and plant life. The one wetland that had major differences from the others was the upland bog found at Site #10, Uwharrie bog. This site was surrounded by longleaf pine trees. Normally it has a variety of plant life surrounding the wetland but had recently been exposed to a controlled burn to allow the long-leaf pines to flourish. Each of the sites is considered mesic to dry-mesic habitat types. Mesic is a habitat with a moderate or well-balanced supply of water or moisture (Gianopulos et al., 2022). These habitats can act like sponges and store water in a way that can be used in neighboring habitats as needed (Gianopulos et al., 2022).

The plant composition of wetlands can be important for many reasons. The longer a wetland has been around the more plants have a chance to adapt and grow. This can affect the water quality of the wetlands itself and therefore also affect the insect prey availability for bats in these wetlands (Li et al., 2021). Studies done on wetland plant diversity have been shown to be a key driver in macroinvertebrate diversity found (Hsu et al., 2011; Perron et al., 2021; Piña & Lougheed, 2022). The more diverse macroinvertebrates are the more likely bats are going to be attracted to the wetland based on prey abundance for these species. The open wetlands in this study provide food sources for bats, while the adjacent forest edges may provide roost options and protection for bats.

Trees such as oak, ash, and beech trees are suitable for bat roosts. These types of trees are found among and around all the wetland sites studied. Having roosting sites and feeding sites in such proximity can be beneficial to different bat species due to less exposure to predators and obstacles. Each bat species in this study responds and has different adaptations to wetlands and forest edges, but the habitat provided by common wetland plants found in this study tends to be known as proper roosting habitats for bats. Not only do they provide roosting habitats, but corresponding forest edges provide a different selection of macroinvertebrates available as prey for these bat species and therefore offer more food availability and selection.

Table 6B. Plants Found at Each Research Site. *Site #10 was recently controlled burned to allow the long leaf pines to obtain better growth, some plants may not be mentioned due to this. Site #10 was also the upland bog that was the rare, only site of its kind known in NC. * (Burchell & Hunt, 2019; Cronk & Fennessy, 2009; Gianopulos et al., 2022; *Piedmont and Mountain Upland Pools and Depressions*, n.d.)

Sites	*Plants (Most are mesic to dry mesic habitat types)*
Site #1 Bog Garden	Peltandra virginica, giant sunflower, New England aster, ironweed, swamp milkweed, railroad vine, jewelweed, coneflower, pink turtlehead, red cardinal flower, white boneset, ostrich fern, sensitive fern, cinnamon fern, and royal fern, bald cypress, wax myrtle, perennial marshmallow, black willow, spicebush, swamp dogwood, witch hazel, shrub elderberry, swamp roses, azaleas, iris pseudacorus.
Site #2 Catawba College	Asian knotweed plant, tree nettle, elderberry, bittersweet, solanum, buttonbush, Saururus cernuus (Lizards tail), Willow, silver birch, poplar tree, knotweed, southern wax myrtle, red maple (Acer rubrum), sweetgum (Liquidambar styraciflua), green ash (Fraxinus pennsylvanica), willow oak (Quercus phellos), possum haw viburnum (Viburnum nudum), cinnamon fern (Osmundastrum cinnamomeum), royal fern (Osmunda spectabilis), jewelweed (Impatiens capensis), false nettle (Boehmeria cylindrica).

Site #3 Duke SWAMP	Green Ash, Hazel Alcer,Boxalder, American Sycamore, common Buttonbush, Virginia iris, jewelweed, crimson eyed Rose mallow, Pickerelweed, dense flower knotweed, creeping Burhead, Floating Primrose-willow.
Site #4 Ellerbe Creek	Pine oak, red maple (Acer rubrum), overcup oak (Quercus lyrata),black gum (Nyssa sylvatica), ashes (Fraxinus spp.), American elm (Ulmus americana), cypress, (Taxodium spp.), and Atlantic white cedar (Chamaecyparis thyoides), swamp titi, (Cyrilla racemiflora), lizard's tail (Saururus cernuus), jewelweed (Impatiens capensis),giant cane (Arundinaria gigantea), cinnamon fern (Osmundastrum cinnamomeum), royal fern (Osmunda spectabilis), sensitive fern (Onoclea sensibilis), Virginia chain fern (Woodwardia virginica), netted chain fern (Woodwardia areolata), Canadian clearweed, (Pilea pumila), false nettle (Boehmeria cylindrica).
Site #5 Lake Katharine	Cattail (Typha spp.), rushes (Juncus spp.), sedges (Carex spp.; Cyperus spp.),beak rushes (Rhynchospora spp.), bulrushes (Scirpus spp.), spike rushes (Eleocharis spp.), black willow (Salix nigra), common buttonbush (Cephalanthus occidentalis), tag alder (Alnus serrulata), swamp rose (Rosa palustris), common wax myrtle (Myrica cerifera), swamp rose mallow (Hibiscus moscheutos), green arrow arum (Peltandra virginica), Virginia

iris (Iris virginica), bladderworts (Utricularia spp.), duckweed (Lemna spp.), yellow pond-lily (Nuphar lutea), American water-lily (Nymphaea odorata), American lotus (Nelumbo lutea).

Site#6
Mason Farm

Spear thistle, northern red oak, Maple plant, long-leaved wattle, scirpus, common alder, Potamogetron, scirpus cyperinus (wool grass), sweetgum, wingstem (verbesina, black eyed susan, ground elder, northern white cedar, grey angrove, eastern cotton wood, European spindle, rubus, sugar maple, wild carrot, Ruellia.

Site #7
Michael B

Pond pine (Pinus serotina), sweetbay (Magnolia virginiana), loblolly bay (Gordonia lasianthus), swamp bay (Persea borbonia), fetterbush lyonia (Lyonia lucida), swamp titi (Cyrilla racemiflora), hollies (Ilex spp.), laurel greenbrier (Smilax laurifolia).

Site #8
Tanglewood Park

A lot of native grasses: Big bluestem, little bluestem, gamagrass, switchgrass, Indian grass, broomsedge bluestem, blackberry, patridge pea, ragweed, annual sunflowers, Microstegium, easterm hemlock, eastern American black walnut, Oplismenus,American Elm, Cattail (Typha spp.), common wax myrtle (Myrica cerifera), swamp rose mallow (Hibiscus moscheutos), green arrow arum (Peltandra virginica), Virginia iris (Iris virginica).

Site #9 *UNCG*	Native wildflowers, lots of native grasses, Cattail, Red maple, eastern redbud, Asiatic dayflower (Commelina communis), bulbous buttercup (Ranunculus bulbosus), bull thistle (Cirsium vulgare), white clover (Trifoliu, repens), Crane-fly orchid (Tipularia discolor), Daffodil (Narcissis pseadonarcissus), Frost grape (Vitis vulpine), sweetgum, Sycamore, oak, pawpaw, red aok, white oak, Mimosa, white mulberry, Duck Potato (Sagittaria latifolia), Blue Flag (Iris virginica), Pickerelweed (Pontedaria cordata), Soft Rush (Jancus effuses), Swamp, Milkweed (Asclepias incarnate), Cardinal Flower (Lobelia cardinalis), Square-stem spike rush (Eleocharis quadrangulata), Sweet grass (Muhlenbergia capillaris), Little bluestem (Schizachyrium scoparium), Blazing star (liatris spicata).
Site #10 *Uwharrie Bog**	Long leaf pine forest, Byssa Biflora dominated, Cephalanthus occidentalis, Smilax walteri, Viburnum nudum, Vaccinium fuscatum, Alnus serrulata, Ilex verticillata, and Itea virginica. Smilax laurifolia, Toxicodendron radicans, Sassafras, northern oak fern, sweetgale, Carex crinite, acer rubrum, Liquidambar styraciflua.