"A Comparison of Acoustic Bat Monitoring Survey Methods within the Ohio River Riparian Forest"

ABSTRACT

Bats fulfill several integral purposes within an ecosystem including insect control, pollination, seed dispersal, and forest health indication (Batcon.org). Bat populations are also threatened by climate change, habitat loss, dangerous pesticides, human disturbance, and wind turbines. There is a specific need to provide protected habitats. To do this, we need to understand where bat colonies are currently located and what species make up these colonies. The following study was conducted in June and July 2023 to determine what species of bats are present in three riparian forest sites and two inland sites in Boone and Campbell County, Kentucky. After week long stationary surveys and mobile acoustic monitoring of echolocation calls at five sites, a total of eleven species were detected: Big Brown Bat (*Eptesicus fuscus*), Eastern Red Bat (*Lasiurus borealis*), Evening Bat (*Nycticeius humeralis*), Hoary Bat (*Lasiurus cinereus*), Northern Long-eared Bat (*Myotis septentrionalis*), Silver-haired Bat (*Lasionycteris noctivagans*), Tricolored Bat (*Perimyotis subflavus*), Indiana Bat (*Myotis sodalis*), Small-footed Bat (*Myotis leibii*), Little Brown Bat (*Myotis lucifugus*), and Southeastern Bat (*Myotis austroriparius*).

Lillie Daniel, Thomas More University

August 4, 2023

INTRODUCTION

Bats are an important part of the ecosystem, contributing to the consumption of insects that disrupt agriculture (billions of dollars impact to US economy), pollination of plants, dispersal of seeds, and facilitation of forest restoration (Batcon.org). All these processes have significant economic value and are beneficial to the ecosystem. According to Annals of the New York Academy of Sciences, "Successfully conserving bats globally will require creative and collaborative efforts to tackle the urgent needs of already imperiled species while simultaneously working to improve understanding of the status and needs of a diverse fauna" (Frick et al. 2019). The bat populations and species present in an area are an important indicator to the health of the ecosystem (Tuneu-Corral et al 2020).

Two ecosystems were evaluated as part of this study: riparian forest and inland habitat. A riparian forest buffer includes an area near a wetland that can provide conservation benefits especially for wildlife habitat (USDAgov 2014). The inland habitats include areas defined by glacial deposits and old growth forest. The purpose of this study includes detecting the different species of bats present among the selected sites with stationary and mobile acoustic detectors. This is part of an ongoing project between the Boone County Conservation District and partners to learn more about habitat biodiversity and review the overall forest health in the county and nearby areas. This study is twofold: to provide baseline data about species diversity within Northern Kentucky and to evaluate the impact of unique habitat based on geology to this diversity.

Kentucky Fish and Wildlife details preferred habitats of the bat species typically located in the state. The following species prefer a forest habitat looking for hollow trees with loose bark, woodland edges with partially open areas to allow for hunting near ponds and waterways (wetlands):

- Big Brown
- Eastern Red
- Evening
- Hoary
- Indiana
- Little Brown
- Southeastern
- Tri Colored

Several species prefer areas that are more cave like with limestone karst areas, rock crevices and fissures, and cliff faces:

- Northern Long Eared
- Silver Haired
- Small Footed

(https://fw.ky.gov/Wildlife/Pages/Bats-of-Kentucky.aspx)

The **Perin Property** is 834 acres located on the Ohio River across from the protected Oxbow area in Lawrenceburg, Indiana. Second Creek runs through the property as an additional and valuable water source. Tree coverage on the property is mainly thick throughout the property with only a few open canopy spaces.

Figure 1: Perin Canopy

Figure 2: Perin Swift

Figure 3: Perin – Ohio River

Figure 4: Perin Property Google Earth Image

(Google, Retrieved July 3, 2023, from https://earth.google.com/web/

St. Anne Woods and Wetlands, managed by Campbell County Conservation, is 146 acres located in Eastern Campbell County running along the Ohio River and Four Mile Creek. It consists of open and closed canopy woods with old growth forest. Common trees are: beech, silver maple, cottonwood, sycamore, american elm, black willow, pin oak, and swamp white oak. Habitat protection is provided by a Kentucky Heritage Land Conservation Fund conservation easement. The North wetlands, where the device was placed, contain the Northern Kentucky University Research and Education Field Station and open canopy ponds where bats are frequently seen. Mobile devices were used in the South wetlands.

Figure 5: St. Anne North Wetlands

Figure 6: St. Anne Swift

Figure 7: St. Anne Woods and Wetlands Google Earth Image

(Google, Retrieved July 3, 2023, from https://earth.google.com/web/

Figure 8: Campbell County Conservation District: St. Anne Woods and Wetlands

(CCCD, Retrieved July 3, 2023, from https://campbellkyconservation.org/st-anne-woods-%26-wetlands

The **Thomas More Biology Field Station** is approximately 25 acres located in the Ohio River watershed in California, KY. As bats are a main predator of nocturnal insects, the nearby streetlights collect insect swarms as a food source. The forest consists of many snags and old growth forest (tree diameter greater than 10 inches). Additionally, there is a bat house attached to the Field Station Lab building. The area is managed by Thomas More University.

Figure 9: TMU Ohio River

Figure 10: TMU Swift

Figure 11: Thomas More Biology Field Station Google Earth Image

(Google, Retrieved July 3, 2023, from https://earth.google.com/web/

Two inland sites were selected to survey for comparison against the riparian sites. The **Boone Cliffs State Nature Preserve** is 74 acres of forest with cliffs formed from glacial deposits. The water source for this location is a tributary to Middle Creek.

Figure 12: Boone Cliffs Nature Preserve Google Earth Image

(Google, Retrieved July 3, 2023, from https://earth.google.com/web/

The **Dinsmore Woods State Nature Preserve** includes approximately 107 acres of old growth forest (never commercially logged), glacial deposits, and rich soil. This is mainly a maple-oak-ash forest. It is also home to running buffalo clover (a previously endangered species).

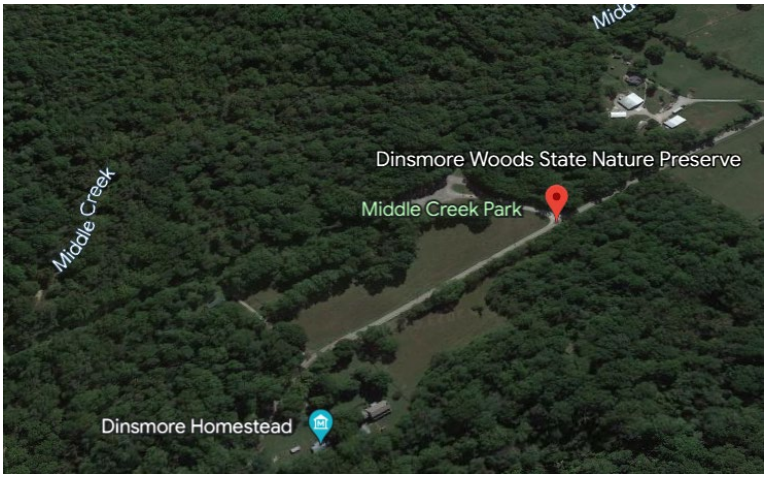

Figure 13: Dinsmore Woods State Nature Preserve Google Earth Image

(Google, Retrieved July 3, 2023, from https://earth.google.com/web/

MATERIALS AND METHODS

MATERIALS

Anabat Swift Acoustic Monitoring Stationary Device by Titley Scientific

Omni-directional Ultrasonic Microphone US-OV3

Anabat Swift Express Security Box by Titley Scientific

Anabat Walkabout Acoustic Monitoring Active Device by Titley Scientific

Microphone adapter for Anabat Walkabout by Titley Scientific

Anabat Stainless Steel Microphone by Titley Scientific

Car Mount for Anabat Detector by Titley Scientific

Echo Meter Touch 2 Pro for iOS by Wildlife Acoustics

Kaleidoscope Pro Analysis Software by Wildlife Acoustics (version 5.4.1)

Master Python Cable Locks

METHODS

The same experimental process, detailed below, was followed at each of the five determined sites. Devices and software selections were based on availability and past research at Boone County Conservation District. Researchers were thoroughly trained on how to use and deploy equipment. Indiana Bat Survey Protocol was followed as a generally accepted guideline.

Thomas More Biology Field Station

Northern Kentucky University Research and Education Field Station

Figure 14: TMU GIS

Credit: Jack Phillips

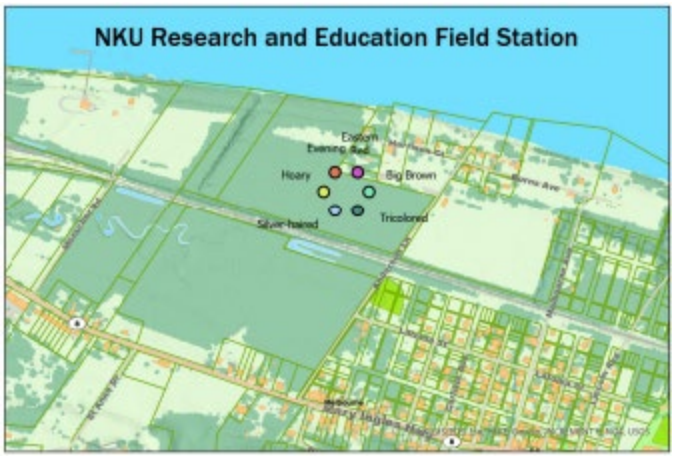

Figure 15: NKU GIS

Credit: Jack Phillips

Perin Property

Figure 16: Perin GIS

Credit: Jack Phillips

Inland Sites: Dinsmore and Boone Cliffs

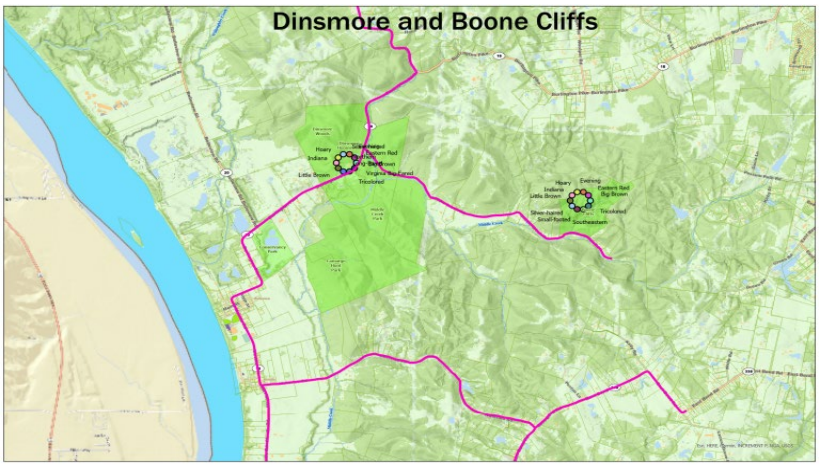

Figure 17: Inland GIS

Credit: Jack Phillips

After entering each location, researchers followed trails to an open area (forest canopy opening) with a nearby water source and snags. The Anabat Swift was hung on a tree, approximately five feet off the ground, with a Master Python cable lock and left there for a minimum period of seven days.

- Anabat Swift was set up at the Northern Kentucky University's Research and Education Field Station from June 21st -June 27th 2023. Set to record at dawn and dusk. Detector was placed on a sycamore tree behind a pond.
- Anabat Swift was set up at the Thomas More Biology Field Station from June 27th -July 5th 2023
- Anabat Swift was set up at Perin from July 6th -13th 2023

Figure 18: Anabat Swift

Mobile Acoustic Monitoring

The Walkabout is a hand held device used to walk around the selected area and collect live data.

- The Anabat Walkabout along with 3 Echo Touch Meters were used at St. Anne Woods and Wetlands on June 27th from 8:30 pm-10 pm
- An Echo Touch Meter was used at the Thomas More Biology Field Station on July 12th from 8:30pm-10pm.

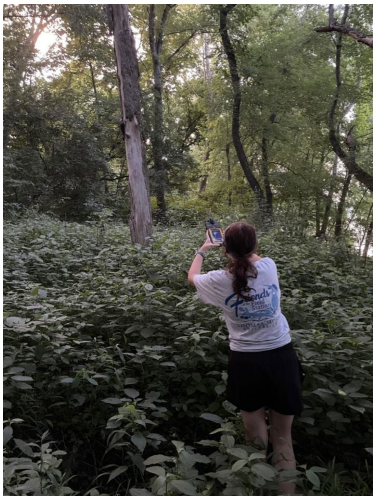

Figure 19: Anabat Walkabout, Lillie Daniel

Vehicle Acoustic Monitoring

The Anabat acoustic device with car mount was attached to a Boone County Conservation District vehicle. Researchers followed a pre-planned driving route from 9:18pm to 10:51pm on July 18th.

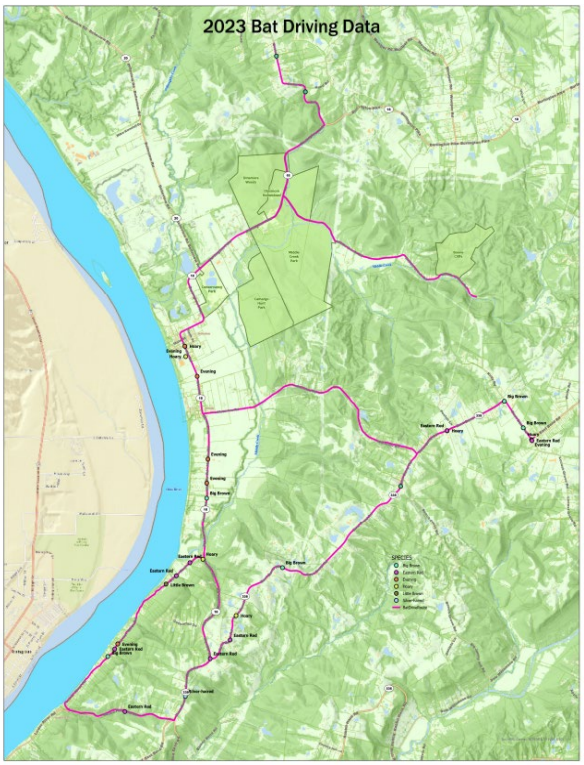

Figure 20: Driving Route GIS

Credit: Jack Phillips

RESULTS

Data threshold for results was set to a minimum of five high quality pulses and at least two call files to note a species. The study determined the presence of eleven species across the locations. The species found at each site are listed in Table 1. The Big Brown Bat, Evening Bat, Hoary Bat, and Tricolored Bat were the four species indicated at each of the survey locations. The greatest species diversity was found at the Inland locations of Boone Cliffs and Dinsmore.

Data Analysis

Acoustic devices generate .wav sound files containing echolocation pulses. All data collected was run through the Kaleidoscope software (version 5.4.1), an acoustic analysis program, to determine the species. Each bat species is assigned a six-character code in the software (Table 3, Appendix). Software is set to "Bat Mode" with default signal parameters and Auto ID set to North America and the state of Kentucky. Kaleidoscope provided an onscreen id (Figure 25, Appendix) and spreadsheet of species based on the detection of echolocation calls that are consistent with the structure of the species within acceptable confidence level (pulse match ratio). Software additionally provides on screen spectrograms of the echolocation calls (Figures 26 and 27, Appendix).

Photo: Hoary Bat

Photo: Evening Bat

Figure 21: Photos, Kentucky Fish and Wildlife

Table 1: Species detected chart per location (collective data from all survey methods)

	RIPARIAN			**INLAND**	
Bat Species	**Perin**	**St. Anne Woods and Wetlands**	**Thomas More Biology Field Station**	**Boone Cliffs**	**Dinsmore**
Big Brown Bat *(Eptesicus fuscus)*	X	X	X	X	X
Eastern Red Bat *(Lasiurus borealis)*	X	X		X	X
Evening Bat *(Nycticeius humeralis)*	X	X	X	X	X
Hoary Bat *(Lasiurus cinereus)*	X	X	X	X	X
Northern Long-eared Bat *(Myotis septentrionalis)*			X	X	X
Silver-haired Bat *(Lasionycteris noctivagans)*	X	X	X	X	X
Tricolored Bat *(Perimyotis subflavus)*	X	X	X	X	X
Southeastern Bat (Myotis austroriparius)				X	
Indiana Bat (*Myotis sodalis*)*			X	X	X
Little Brown Bat (*Myotis lucifugus*)				X	X
Small-footed Bat *(Myotis leibii)*				X	

**Endangered Species*

Driving Route Data

Weather: Rainy, 76 degrees F

Attendees: Lillie, Kamyrn, Brad, Susan

Driving acoustic survey included both vehicle mount (roof mounted) and handheld (device held in window) acoustic devices. Our driving route consisted of inland habitats and along the Ohio River starting at Boone Cliffs at 9:42 pm and ending on East Bend road at 10:51 pm.

Table 2: Driving Route Results

HANDHELD				*ON VEHICLE*		
Location	**Time**	**Bat**		**Location**	**Time**	**Bat**
Boone Cliffs Parking Lot				Kelly Elementary		
	9:18 PM				10:10 PM	Hoary
	9:42 PM	Hoary			10:14 PM	Evening
	9:43 PM	Eastern Red			10:17 PM	Evening
	9:43 PM	Tri Colored			10:17 PM	Evening
	9:44 PM	Eastern Red			10:17 PM	Big Brown
	9:45 PM	Tri Colored		Lower River Road		
	9:47 PM	Eastern Red			10:19 PM	Hoary
	9:47 PM	Little Brown			10:20 PM	Eastern Red
	9:48 PM	Eastern Red			10:21 PM	Easten Red
Botts Lane					10:22 PM	Little Brown
	9:54 PM	Eastern Red			10:26 PM	Evening
	9:54 PM	Eastern Red			10:16 PM	Eastern Red
	9:55 PM	Evening			10:26 PM	Big Brown
	9:55 PM	Eastern Red			10:32 PM	Eastern Red
	9:57 PM	Eastern Red			10:34 PM	Silver Haired
	9:57 PM	Tri colored			10:35 PM	Eastern Red
	9:59 PM	Big Brown		East Bend Road		
	9:59 PM	Evening			10:36 PM	Eastern Red
	10:00 PM	Evening			10:38 PM	Eastern Red
Burlington Pike					10:38 PM	Hoary
	10:01 PM	Evening			10:40 PM	Big Brown
	10:02 PM	Evening			10:49 PM	Big Brown
	10:07 PM	Evening			10:49 PM	Big Brown
	10:07 PM	Hoary			10:50 PM	Evening
					10:50 PM	Hoary
					10:51 PM	Evening
					10:51 PM	Eastern Red
				STOP		

DISCUSSION

During the months of June and July 2023, stationary, mobile, and vehicle acoustic monitoring was conducted to identify the bat species present in three riparian areas. Additionally, two inland areas were monitored for habitat comparison. Data was analyzed through the Kaleidoscope software identifying a total of eleven species across the surveyed areas: Big Brown Bat (*Eptesicus fuscus*), Eastern Red Bat (Lasiurus borealis), Evening Bat (*Nycticeius humeralis*), Hoary Bat (*Lasiurus cinereus*), Northern Long-eared Bat (*Myotis septentrionalis*), Silver-haired Bat (*Lasionycteris noctivagans*), Tricolored Bat (*Perimyotis subflavus*), Indiana Bat (*Myotis sodalis*), Small-footed Bat (*Myotis leibii*), Little Brown Bat (*Myotis lucifugus*), and Southeastern Bat (*Myotis austroriparius*). Based on the standard Indiana Bat Survey protocol as guidance, the minimum data threshold for species presence selected was five high quality pulses and at least two call files.

Sites were selected to survey a variety of habitats. The three riparian sites provided forest and wetland conditions. While surveying, bats were observed flying across the Ohio River at the Thomas More Biology Field Station. At this site there are streetlights that gather insects (food source), and there is a bat house providing additional roosting opportunities. There is also a nature trail with old growth; the detector was hung on a northern hackberry at the end of the trail loop adjacent to the Ohio River. At the North wetlands of St. Anne, there are multiple open canopy ponds (some with water) and open forest. The detector was hung on a sycamore tree behind the farthest pond and on the opposite side of the trail. Using the walkabout device, we walked the South wetlands and had consistent results. Perin also has a water source with Second Creek and the Ohio River as well as an open forest. The detector was placed in an open canopy area along the Ohio River. As anticipated the Big Brown and Hoary bats were at all sites, however, it was surprising that the Indiana bat was not in all the wetland habitats. This could be from noise disturbance on the Ohio River. Further research should be conducted for a longer period as this is a prime habitat for this species.

For the driving acoustic survey, rain delayed the start, pushing us past the peak batting hour of 9:30pm. An additional challenge with driving surveys and continuous recording is increased background noise potentially interfering with the echolocation call recording. There was still valuable data collected, as shown in Table 2, identifying many of the species that were also shown on the stationary monitors for the areas.

The inland sites provided additional habitat characteristics that are attractive to a variety of species. The geology of the area provides greater diversity due to the Cincinnati Arch. The Northern Kentucky area consists of primarily Ordovician bedrock. The State Nature Reserve, Boone Cliffs, is notable in Boone County for its Quaternary glacial conglomerates. The cliffs

were formed 250-500,000 years ago from the Pre-Illinoian glacier (Figure 22) creating cave-like structures and protected holes for bats to roost in (Stevenson 2023). Inland characteristics attractive to the more cave dwelling species include the glacial deposits, limestone, sinkholes, rock caves. Inland sites contained 11 species as compared to 6 or 7 at the riparian sites.

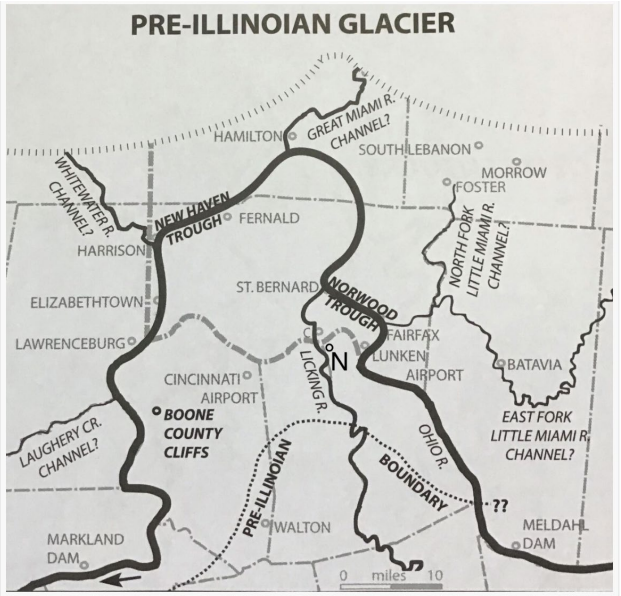

Figure 22: Pre-Illinoian Glacier Map for the Boone Cliffs Area

Throughout this research, Virginia Big-eared bat (*Corynorhinus townsendii virginianus)* calls were detected at both Dinsmore and Perin. This would have been an exciting discovery as their home range is restricted to an eleven-county area in Eastern, KY from Rowan down to Pulaski County. Although bats do move around and can always surprise us, these were false calls. Virginia Big-Eared bats are extremely habitat specific and only migrate short distances to overwintering sites. They use sandstone and limestone cliff line habitats and generally occur in quite isolated, scattered populations throughout this small range within Kentucky. Alexander Silvis (Wildlife Conservation Official in West Virginia) detailed that while Kaleidoscope is a great tool for automated bat acoustic identification, it lacks some useful features as a call viewer (Fraser et al., eds. 2020). This software is also not necessarily made to detect any of the *Corynorhinus* species like Virginia Big-eared or Rafinesque's Big-eared bat. Big-eared bats are also called “whispering bats” because their calls are so quiet. Running these calls through a different software, Anabat Insight, showed two important factors for differentiating the calls. The spectrograms are too intense of a call for a “whispering bat”. The second key point is the shape of the call. Big Brown bats have call shapes in the shape of “J” while Big-eared bats have small convex humps. Another example showed that within one call there were three different species, for example, Big brown – Hoary – Tri-colored. These could have been social calls or search face calls. Kaleidoscope can only show one species per call. Anabat Insight is helpful for species confirmation and determining a theory to what is happening in an acoustic.

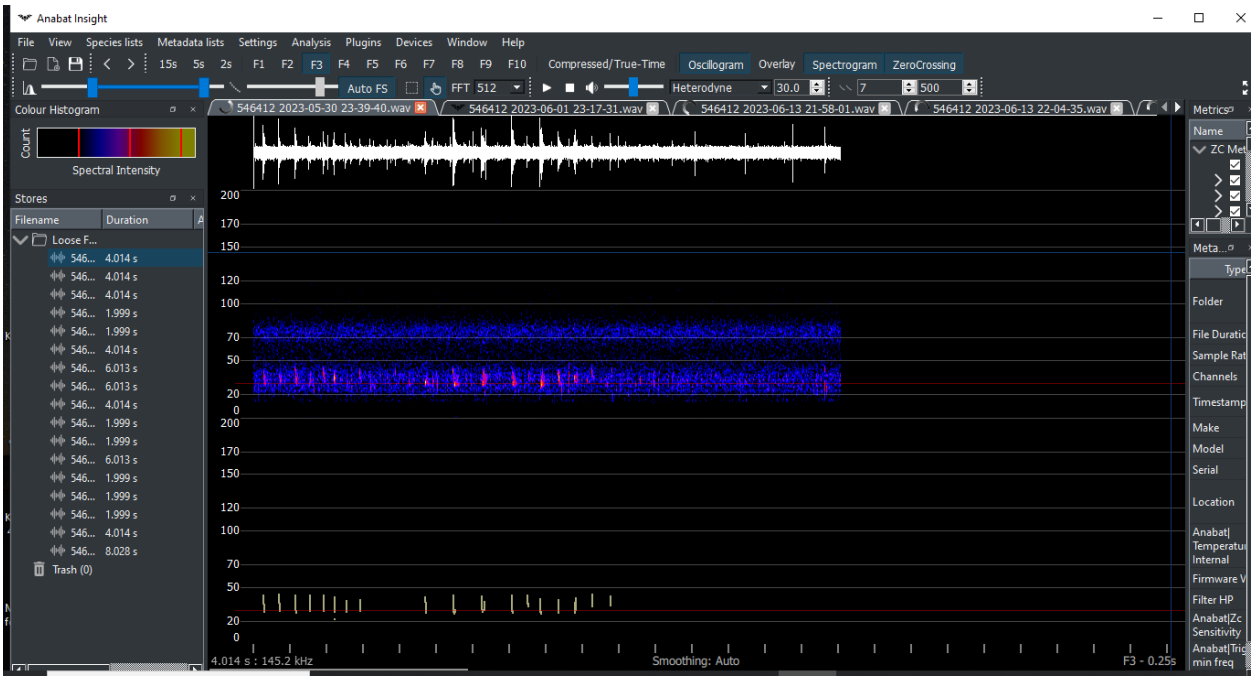

Figure 23: Anabat Insight Big Brown Bat

ACKNOWLEDGEMENTS

Photo: Big Brown Bat

Figure 24: Big Brown Bat, Cincinnati Museum Center

I would like to thank the following for supporting my research and inspiring my continued love of bats:

Dr. Durtsche for access and support on the St. Anne Woods and Wetlands property

Dr. Lorentz and Molly Williams for access and support at the Thomas More Biology Field Station property

Christy Noll and the Boone Conservancy for access and support on the Perin Property

Liz Fet at SD1 for data support and mentoring

The staff at Boone County Parks Natural Areas

A special note to Sarah Grote for teaching me about ecology

Jack Phillips for GIS map creation

The entire staff at Boone County Conservation District for sponsorship, encouragement, mentoring, and advice

A special note to Jasper Stevenson for teaching me about geology

To Kamryn Lee for partnership, support, and friendship

The Thomas More Biology Field Station Interns for helping me collect data

LITERATURE CITED

Bat Conservation International / Ending Bat Extinctions Worldwide. Bat Conservation International. https://www.batcon.org/. Accessed July 3, 2023.

Fraser et al., eds. 2020. Bat Echolocation Research: A handbook for planning and conducting acoustic studies. Second Edition. Bat Conservation International. Austin, Texas, USA.

Frick WF, Kingston T, Flanders J. 2019. A review of the major threats and challenges to global bat conservation. Annals of the New York Academy of Sciences. 1469(1): 1-21. doi:10.1111/nyas.14045.

Riparian Forest Buffers. 2014. Usdagov. https://www.fs.usda.gov/nac/practices/riparian-forest-buffers.php. Accessed July 3, 2023.

Stevenson J. Boone County, KY Geology Summary. 2023.

Tuneu-Corral C, Puig-Montserrat X, Flaquer C, Mas M, Budinski I, López-Baucells A. 2020. Ecological indices in long-term acoustic bat surveys for assessing and monitoring bats' responses to climatic and land-cover changes. Ecological Indicators. 110:105849. doi:https://doi.org/10.1016/j.ecolind.2019.105849.

Welcome - Kentucky Department of Fish & Wildlife. fwkygov. https://fw.ky.gov/Pages/default.aspx. Accessed July 3, 2023.

Appendix

Table 3: Kaleidoscope Codes for the Bat Species in Kentucky

Bat Species in Kentucky with Kaleidoscope Codes	
Common/Scientific Name	**Kaleidoscope Name**
Big Brown Bat *(Eptesicus fuscus)*	EPTFUS
Eastern Red Bat *(Lasiurus borealis)*	LASBOR
Evening Bat *(Nycticeius humeralis)*	NYCHUM
Gray Bat *(Myotis grisescens)* *	MYOGRI
Hoary Bat *(Lasiurus cinereus)*	LASCIN
Indiana Bat *(Myotis sodalis)* *	MYOSOD
Little Brown Bat *(Myotis lucifugus)*	MYOLUC
Northern Long-eared Bat *(Myotis septentrionalis)*	MYOSEP
Rafinesque's Big-eared Bat *(Corynorhinus rafinesquii)*	CORRAF
Southeastern Bat *(Myotis austroriparius)*	MYOAUS
Silver-haired Bat *(Lasionycteris noctivagans)*	LASNOC
Small-footed Bat *(Myotis leibii)*	MYOLEI
Tricolored Bat *(Perimyotis subflavus)*	PERSUB
Virginia Big-eared Bat *(Corynorhinus townsendii)* *	CORTOW
**Currently listed as Endangered*	

File Help

	FOLDER	IN FILE	OUT FILE FS	OUT FILE ZC	AUTO ID	PULSES	MATCHING	MATCH RATIO	MANUAL ID
1		546412 2023-06-28 22-35-46.wav			CORTOW	7	5	0.714000	
2		546412 2023-07-01 01-57-56.wav			CORTOW	4	4	1.000000	
3		546412 2023-07-02 03-37-09.wav			EPTFUS	35	21	0.600000	
4		546412 2023-06-27 22-31-29.wav			EPTFUS	25	20	0.800000	
5		546412 2023-07-02 00-12-43.wav			EPTFUS	26	19	0.731000	
6		546412 2023-06-27 23-14-05.wav			EPTFUS	18	16	0.889000	
7		546412 2023-06-30 23-04-52.wav			EPTFUS	8	6	0.750000	

Figure 25: Sample id output file by the analytical software Kaleidoscope

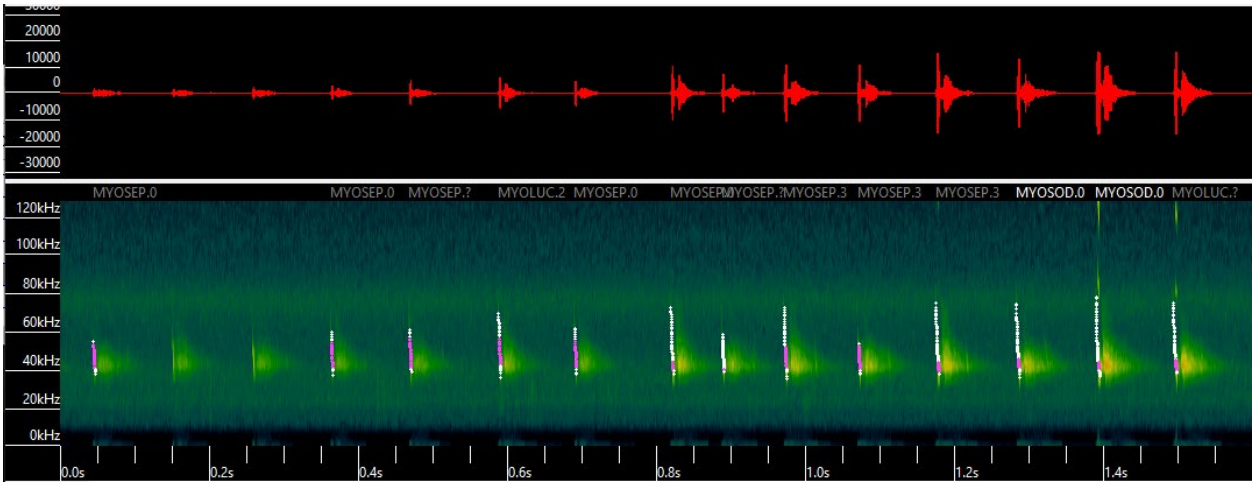

Figure 26: A spectrogram of an Indiana Bat call generated by the analytical software Kaleidoscope.

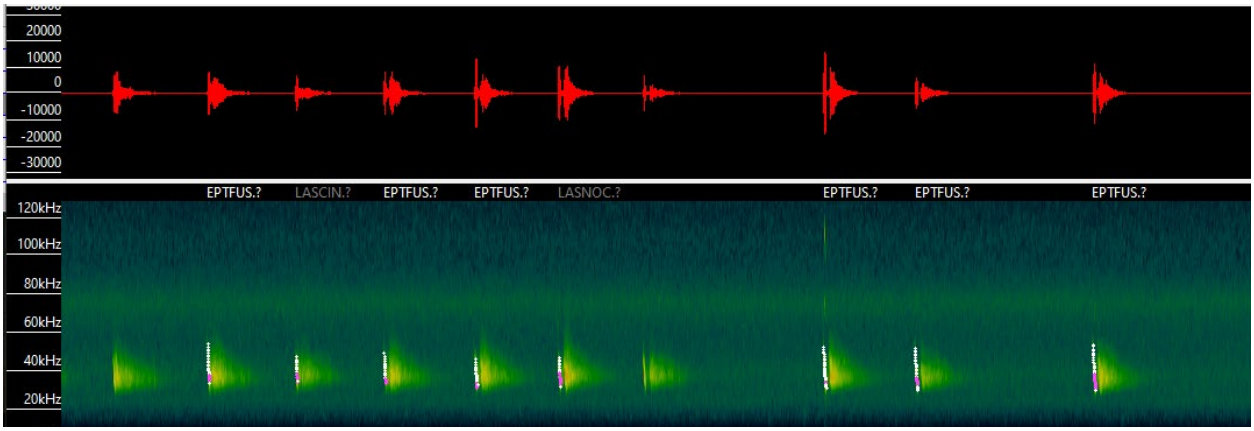

Figure 27: A spectrogram of Big Brown Bat call generated by the analytical software Kaleidoscope.

Research Permit Granted by the Northern Kentucky University: Research and Education Field Station (REFS) and Campbell County Conservation District (CCCD)