*Notes*

# Importance of Manually Vetting Acoustic Bat Call Files: A Case Study for Northern Long-Eared Bats

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## Abstract

Definitively identifying bats based on their acoustic calls is difficult and sometimes impossible. This is particularly true for *Myotis* species that can make similar calls, which could lead to false positive detections. This is problematic for conducting species presence or probable absence surveys using acoustic detection. Manual vetting of calls to reduce identification error by the automated programs is an option but not a requirement to survey for species listed as threatened or endangered pursuant to the Endangered Species Act. We conducted simultaneous mist net and acoustic surveys for bats in areas of Edgecombe and Johnston counties in eastern North Carolina where there are capture records of a common *Myotis* species, the southeastern myotis *Myotis austroriparius*, but not for the federally endangered northern long-eared bat *Myotis septentrionalis*. We caught southeastern myotis at six of the 12 sites surveyed. Although automated acoustics software produced a Maximum Likelihood Estimation value for probable presence of northern long-eared bats at three of the 12 sites surveyed, we did not catch any individuals or confirm the species acoustically through manual vetting. If we had used automated software alone without manual vetting, we would have incorrectly presumed presence of an endangered species at 25% of our sites. Therefore, manual vetting is highly recommended for northern long-eared bat acoustic surveys where southeastern myotis co-occur.

Keywords: acoustics; *Myotis septentrionalis*; *Myotis austroriparius*

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## Introduction

The northern long-eared bat *Myotis septentrionalis* is a small (5–8 g) insectivorous species (Caceres and Barclay 2000) that primarily roosts in cracks, crevices, and under exfoliating bark of a variety of tree species in the summer (Foster and Kurta 1999; Caceres and Barclay 2000; Lacki et al. 2009; Silvis et al. 2016). The species range encompasses the majority of the eastern United States and into Oklahoma north through portions of Canada (Caceres and Barclay 2000). Although the northern long-eared bat was abundant and widespread, the arrival of white-nose syndrome caused by the fungus *Pseudogymnoascus destructans* decimated populations throughout much of the range of the species (United States Fish and Wildlife Service [USFWS] 2022). This resulted in the species being listed as federally endangered as defined by the Endangered Species Act (ESA 1973). The reduction in the number of individuals available for capture increased the difficulty of surveying for the species with mist nets alone. Therefore, surveying for northern long-eared bats, along with other species of interest, could benefit from passive surveying using acoustic detectors.

The USFWS has developed, and updates yearly, the Range-wide Indiana Bat and Northern Long-Eared Bat

Survey Guidelines (USFWS 2023), hereafter referred to as the Guidance. The "Guidance is designed to determine whether [federally endangered] Indiana bats *Myotis sodalis* [ESA 1973] or northern long-eared bats are present or probably absent at a given site during the summer/active season. . ., within bridges and culverts. . ., or during the winter. . ." (USFWS 2023). The Guidance gives two options for determining presence or probable absence: acoustic surveys or mist net surveys. Although acoustic surveys can be more cost effective and detect some bat species that can avoid mist nets, they are also more likely to misidentify species based on similarities in echolocation call characteristics. Many bat species produce very similar echolocation calls, specifically when flying through spatially complex habitat (Tibbels 2000; Britzke et al. 2002), making species determination difficult. This is especially true when considering members of the *Myotis* genus (Krusic and Neefus 1995).

The USFWS, through a collaborative effort with the U.S. Geological Survey Virginia Cooperative Fish and Wildlife Research Unit, tests three software programs (Kaleidoscope Pro, BatCall ID, and Echoclass) to evaluate accuracy of species determination for the federally endangered Indiana bat and northern long-eared bat. However, the ability of these programs to correctly identify more common species is unknown. Specifically, "performance on species such as the southeastern myotis *Myotis austroriparius* continues to be poor" (USGS 2020). In addition, all these programs rely on libraries that contain calls typical of those created in "standard field recording conditions," and although USFWS guidelines provide instruction for how and where to place detectors, there is no guarantee that the calls collected during a given study will be represented by those in the known call library (Britzke et al. 2013).

In a pilot study comparing mist netting and acoustic data collection conducted in 2016 (Three Oaks Engineering 2016) with additional data not in the report collected in 2017, automated classifier programs Echoclass version 3.1 and BatCall ID version 2.7d were used to determine bat species presence in six counties in eastern North Carolina. Both programs identified the likely presence of northern long-eared bats at all sites, but only one site recorded a single mist net capture of this species. This site also captured over half of the total southeastern myotis on the project, suggesting they were the dominant *Myotis* species in the study area. False positive determinations (i.e., identifying that northern long-eared bats are likely present when they are not) are problematic when trying to conduct presence or probable absence surveys for listed species. Not only could false positives unnecessarily affect public and private land use, but they also lead to inaccurate species range maps and lead regulators to conclude that a species is more prevalent than it is.

Our goal was to determine if an acoustics study is an appropriate method to survey for northern long-eared bats in an area with southeastern myotis presence. To do this, we conducted our study in areas where previous mist net surveys had captured southeastern myotis but not northern long-eared bats (G. W. Jordan, USFWS, unpublished data). Our hypothesis was that acoustic surveys do not accurately represent the presence of northern long-eared bats when southeastern myotis are present. We expected likely presence determinations for northern long-eared bats and southeastern myotis using an acoustic survey approach, and we expected captures of southeastern myotis but not northern long-eared bats during mist net surveys. If the hypothesis was supported, it would suggest that the data used to create the guidelines for surveying for bats (USFWS 2023) were not extensive enough to encompass the entire range of northern long-eared bats, and where coexisting species such as southeastern myotis occur, accurate detection of northern long-eared bats should not rely solely on acoustic surveys.

## Study Site

We collected data at sites in Edgecombe and Johnston counties in eastern North Carolina. All sites were in the Environmental Protection Agency Level IV Ecoregion classified as Southeastern Floodplains and Low Terraces (Griffith et al. 2002). Sites in Edgecombe County were placed evenly in evergreen forest and woody wetlands as categorized by the National Land Cover Database (Dewitz 2019), but sites in Johnston County were all in woody wetlands. Both sample areas were on public lands. Ten tree species were recorded at the Edgecombe County sites, but they were dominated by sweetgum *Liquidambar styraciflua* and loblolly pine *Pinus taeda*. Seventeen tree species were recorded at the Johnston County sites, but sweetgum and red maple *Acer rubrum* were the most common species recorded.

Mist-net and acoustic detector sites were on dirt, gravel, or grass roads and paths within forest or on water features such as small ponds and creeks. Acoustic detector sites had little to no canopy cover. The average temperature throughout the survey period was 18.2°Celsius $\pm$ 0.3 (range: 13.9–25°Celsius), and there was no precipitation during the surveys.

## Methods

We surveyed for bats during the summer reproductive season at 12 sites in two areas of North Carolina: Lower Fishing Creek Game Land in Edgecombe County (4 sites) and Howell Woods in Johnston County (8 sites). Both areas have capture records for southeastern myotis and are in a part of the state where northern long-eared bats are not found (Figure 1). Figure 1 includes a buffer from actual capture locations (USFWS, unpublished data) based on extensive surveys conducted in central and eastern North Carolina (Jordan 2020). Although the Lower Fishing Creek Game Land location appears to be geographically close to the range of the northern long-eared bat, there is farmland and other landcover not conducive to the species roosting habitat needs between survey locations for this project, and the closest northern long-eared bat capture is 22 km to the northeast.

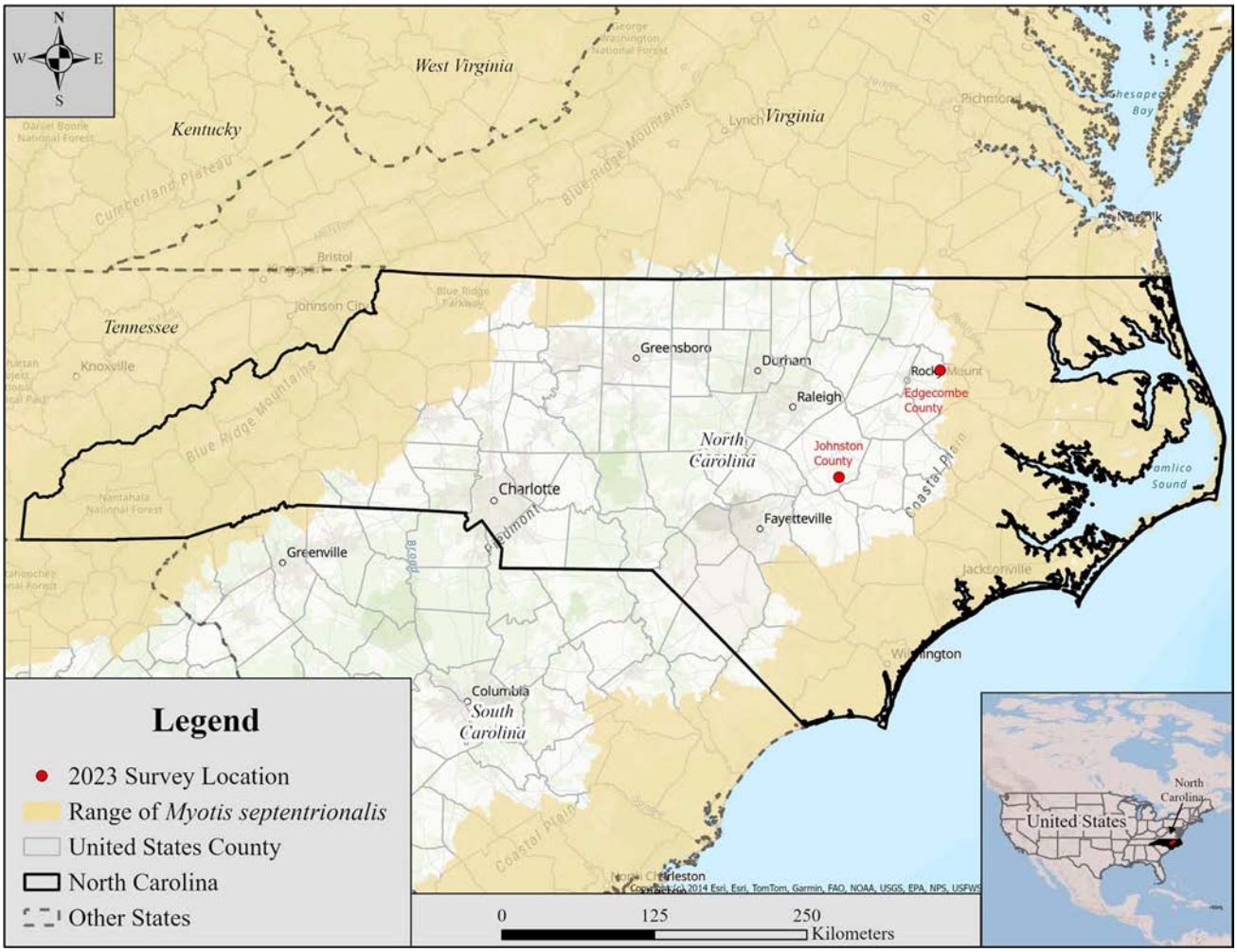


**Figure 1.** Range of northern-long eared bats *Myotis septentrionalis* in North Carolina as of September 2023. Red dots represent locations of a mist-net and acoustic study conducted from 17–21 May 2023 to determine the validity of using automated software to detect northern long-eared bats. Range provided by United States Fish and Wildlife Service.

## Mist net survey

Per USFWS guidelines (USFWS 2023), we set mist nets to maximize coverage of flight paths used by bats along suitable travel corridors, foraging areas, or drinking areas. We generally surveyed a single site for two nights using at least two net sets each night for a minimum of four net nights (one net in place for one night). However, because of low captures, we sampled two sites for one night and alternate sites for the other night to complete the total of four net nights. We conducted mist netting for five hours after sunset each night. We based the location of the mist net site on the extent of canopy cover, presence of an open flyway, and forest conditions near the site. We recorded the time of netting start and stop, GPS location of the net site, and hourly weather conditions.

We recorded biological and morphometric data for every individual captured (i.e., time of capture, capture net, species, sex, age class, reproductive condition, mass, and forearm length). We banded bats with uniquely numbered aluminum lipped bat bands (Porzana Ltd, Icklesham, East Sussex, United Kingdom) provided by Copperhead Consulting. We completed processing within 30 minutes from the time the bat was removed from the net and released captured bats near the point of capture. We recorded all data electronically using ArcGIS Survey123 software (ESRI, Redlands, CA, USA) and on pre-printed paper datasheets. We took special caution during survey activities to minimize the potential for transmission of white-nose syndrome by following the most recent decontamination protocols reducing the spread of white-nose syndrome (White-nose Syndrome Disease Management Working Group 2020). We evaluated bats for potential white-nose syndrome infection through wing scoring following Reichard (2008). We captured bats under USFWS permit number TE94849B-0 and followed the American Society of Mammalogists guidelines for the safe and proper handling of wild animals (Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016).

### Acoustic survey

Per USFWS guidelines (USFWS 2023), we deployed two AnaBat Swift (Titley Electronics Pty Ltd, Ballina, NSW Australia) acoustic detectors with a 5–250 kHz omnidirectional microphone vertically oriented at 0° and no weather proofing at each site at least 50 m from nets concurrent with mist-netting efforts. We deployed detectors for the duration of mist netting (i.e., at least 5 h). We set detectors to record in full spectrum mode and placed them in habitats where bats were likely to be foraging or traveling and in areas that increased the likelihood of recording high-quality calls (e.g., forest canopy openings, forest edges, road or stream corridors with open canopies, ponds, wetlands). We placed detector microphones on 3.5-m poles at least 3 m from vegetation to increase the sample area and reduce distortion from the ground or vegetation. We used the following detector settings: Hardware Rev 1.0 and 2.0, Software 1.6 (master dabb19a-mod), Trigger Freq 3kHz to 250kHz, Recording div 8 ZC files, Max file length 10s, Min event 2ms, and Analog HP filter On.

### Data analysis

We determined the presence of bat species using the manufacturer recommended settings in Kaleidoscope Pro (KPro) version 5.4.7 (Wildlife Acoustics, Inc. Maynard, MA). This software program has been approved by USFWS for the automated identification of frequency division call data of federally threatened and endangered bat species (USFWS 2019) as defined by the Endangered Species Act (ESA 1973, as amended). The KPro analysis settings we used are provided in Table S1. Based on species distribution maps and capture records (USFWS, unpublished data), we selected the species listed in Table S2 in the KPro 5.4.0 classifier. This list of species in Table S2 includes northern long-eared bats for the purpose of this study, although the area is considered outside the species range. The KPro software generated a Maximum Likelihood Estimation (MLE) for each species per site per night to determine presence ($P < 0.05$) or probable absence ($P \geq 0.05$). If southeastern myotis or northern long-eared bats were considered likely present, a qualified biologist visually examined all files from a site per detector per night to confirm those determinations. Additionally, to guard against false negative results for northern long-eared bats, we manually vetted all northern long-eared bat calls regardless of MLE score. We estimated Pearson's correlation to determine if there was a relationship between the number of calls of a given species of bat and the total bat activity (i.e., number of calls overall). We used Student's t-test to compare the number of calls produced by southeastern myotis at sites where the species was caught and at sites where it was not caught. We also used Student's t-test to compare the number of northern long-eared bat calls at sites where southeastern myotis were caught and sites where southeastern myotis were not caught. We set our level of statistical significance at $\alpha = 0.05$.

## Results

### Mist-net survey

We surveyed four sites each for two nights from 17–20 May 2023 at Lower Fishing Creek Game Land for a total of 16 net nights (Figure 2). At Howell Woods, we surveyed eight sites (four sites for two nights each and four sites for one night each) for a total of 34 net nights from 17–21 May 2023 (Figure 2, Table 1). We caught a total of 94 bats of seven species: 50 bats of seven species at Lower Fishing Creek Game Land and 44 bats of four species at Howell Woods (Table 1). We caught southeastern myotis at all four Lower Fishing Creek Game Land sites and at two of the eight sites surveyed at Howell Woods.

### Acoustic survey

We surveyed four sites each for two nights from 17–20 May 2023 at Lower Fishing Creek Game Land for a total of 16 detector nights (Figure 2). At Howell Woods, we surveyed eight sites (four sites for two nights each and four sites for one night each) for a total of 24 detector nights from 17–21 May 2023. One detector failed at Site 10 on 21 May 2023, so we completed a total of 23 detector nights. We collected 37,793 total files including 19,272 files that KPro identified as bat calls and 18,135 files that KPro identified to 11 species (Table 2). Of the files identified to species, 201 files were identified by the automated classifier as northern long-eared bat calls, 50 of which were at Site 2 at Lower Fishing Creek Game Land. KPro identified northern long-eared bat calls at every site, however, the species was only considered likely present (MLE $P < 0.05$) at three sites. Manual vetting determined that none of the 201 calls identified by KPro were likely made by northern long-eared bats (Table S4). Of the 201 northern long-eared bat calls manually vetted, 67.7% of them were identified as eastern red bats *Lasiurus borealis* ($n = 136$ calls), 23.3% were identified as southeastern myotis ($n = 47$ calls), and 9.0% were low-quality recordings (e.g., too few pulses, feeding buzzes, or multiple bats in a file) that did not contain enough diagnostic information to be assigned to a species ($n = 18$ calls).

The number of southeastern myotis calls collected at a given site was correlated with number of total calls collected at that site (Pearson's correlation coefficient $r[d.f. = 10)] = 0.67$, $P = 0.02$). The same was true for northern long-eared bats ($r[10] = 0.65$, $P = 0.23$) and tricolored bats *Perimyotis subflavus* ($r[10] = 0.76$, $P = 0.004$), but it was not true for the other eight species identified as likely present by KPro (all $P > 0.05$). When comparing the number of calls at sites that caught southeastern myotis versus sites that did not, there were significantly more southeastern myotis calls at sites that caught southeastern myotis (mean = 76.8 ± 18.5 [SE] calls, 95% CI 8.89–100.77; $P = 0.02$, Student's $t = 2.96$, $df = 10$), and there were also significantly more northern long-eared bat calls at sites that caught southeastern myotis (mean = 29.8 ± 6.0 calls, 95% CI 12.53–39.81; $P = 0.002$, $t = 4.27$, $df = 10$; Table 3). The effect size for both tests was large with a Cohen's *d* of 1.53 and 2.47, respectively.

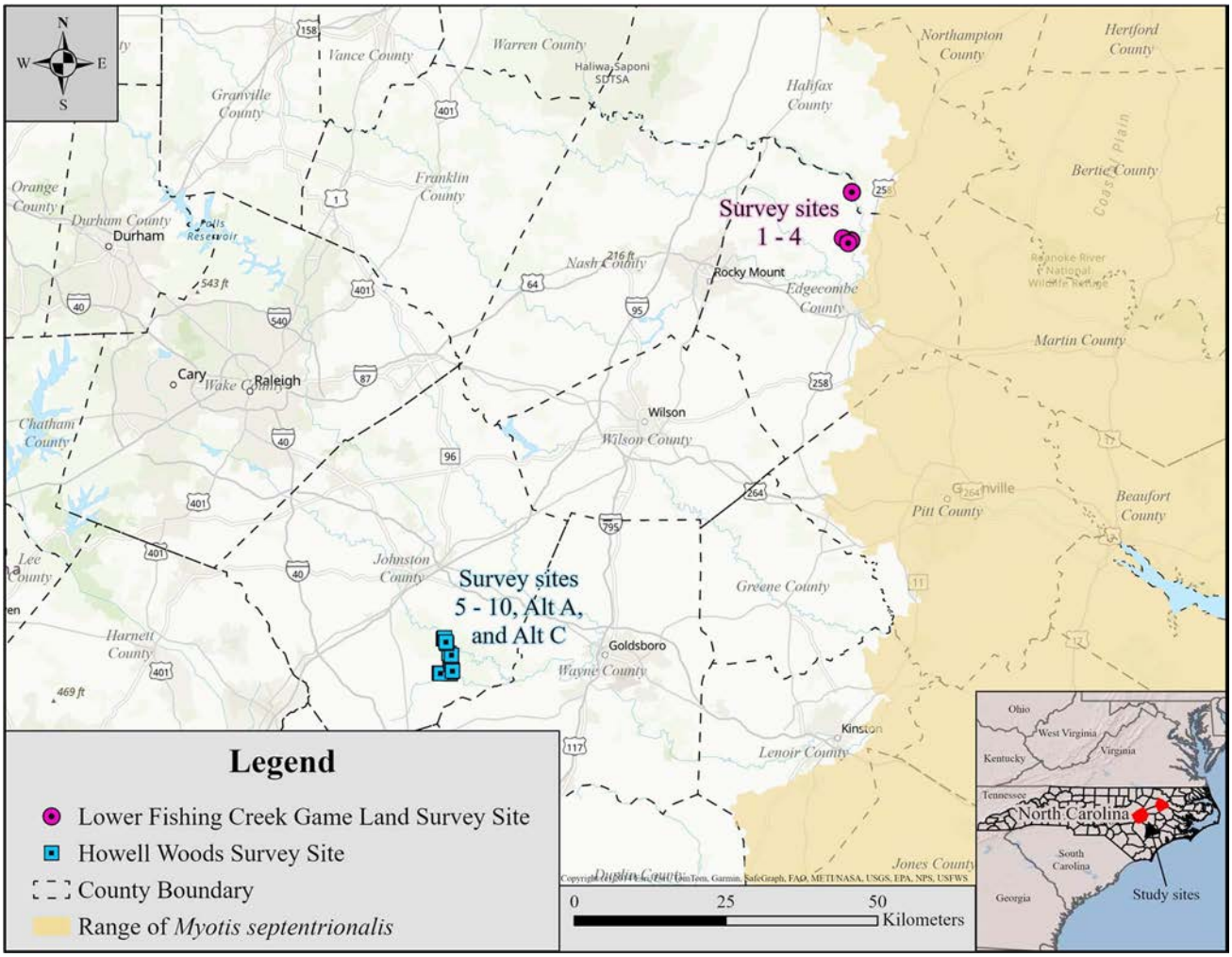


**Figure 2.** Mist-net and acoustic detector sites for bat surveys from 17–21 May 2023 at Lower Fishing Creek Game Land in Edgecombe County, North Carolina and at Howell Woods in Johnston County, North Carolina.

## Discussion

Although KPro did identify some files as northern long-eared bats, manual vetting revealed that those calls were not likely made by this species but more likely calls from eastern red bats in clutter (i.e., structurally complex space) or from congeneric southeastern myotis. Clutter can consist of vegetation near the bat when it is echolocating or of the acoustic detector, pole, and microphone. Eastern red bats can alter their call structure under these conditions (Obrist 1995), generally increasing the slope of each call pulse to obtain more information from their surroundings. Because higher slope calls are indicative of *Myotis* species, these eastern red bat calls are often confused for a *Myotis* species (Britzke et al. 2013; Nocera et al. 2019). Northern long-eared bats and southeastern myotis have similar morphologies and can produce the same type of call structure in similar situations. Given that these species are congenerics and that bats alter their call structure based environmental conditions (Kalko and Schnitzler 1993; Obrist 1995; Schnitzler et al. 2003; Schaub and Schnitzler 2006; Gillam and McCracken 2007; Schaub and Schnitzler 2007), a southeastern myotis in clutter is very capable of producing a large bandwidth, steep slope call indicative of a northern long-eared bat, which is what the automated identification programs are keying in on when they mis-identify them. Disambiguating clutter and approach-phase calls from eastern red bats and other *Myotis* species can be done by noting the calls-per-second metrics in the recordings. Calls that are greater than 12 are ambiguous for any 40 kHz bat in clutter, and those less than 12, but still steeply sloped with broad bandwidth, are more likely to be northern long-eared bats.

Early acoustic work and call library collection focused on distinguishing Indiana bats from three other *Myotis* species (Murray et al. 2001; Britzke et al. 2002), but southeastern myotis was not considered part of this comparison because they only overlap Indiana bats in a small portion of the species' ranges. Similarly, because southeastern myotis were not evaluated, there is insufficient data to distinguish between them and other *Myotis* species such as northern long-eared bats.

**Table 1.** Summary of bat captures 17–21 May 2023 at two areas in eastern North Carolina: Lower Fishing Creek Game Land in Edgecombe County and Howell Woods in Johnston County. Net night = 1 net in place for 1 night; CORA = *Corynorhinus rafinesquii*; EPFU = *Eptesicus fuscus*; LABO = *Lasiurus borealis*; LANO = *Lasionycteris noctivagans*; MYAU = *Myotis austroriparius*; NYHU = *Nycticeius humeralis*; PESU = *Perimyotis subflavus*.

| Area | Site (net nights) | Date 2023 | CORA | EPFU | LABO | LANO | MYAU | NYHU | PESU | Bats per site |
|---|---|---|---|---|---|---|---|---|---|---|
| *Lower Fishing Creek (Edgecombe County)* | 1 (4) | 17–18 May | 2 | 0 | 8 | 0 | 1 | 2 | 1 | 14 |
| | 2 (4) | 17–18 May | 0 | 2 | 6 | 1 | 2 | 0 | 1 | 12 |
| | 3 (4) | 19–20 May | 0 | 1 | 11 | 1 | 1 | 0 | 0 | 14 |
| | 4 (4) | 19–20 May | 0 | 0 | 4 | 1 | 4 | 0 | 0 | 9 |
| *Howell Woods (Johnston County)* | 5 (6) | 17–18 May | 1 | 0 | 11 | 0 | 3 | 0 | 0 | 15 |
| | 6 (6) | 17–18 May | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 |
| | 7 (2) | 19 May | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| | 8 (3) | 19 May | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 9 (6) | 20–21 May | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 7 |
| | 10 (6) | 20–21 May | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 8 |
| | Alt A (2) | 21 May | 2 | 0 | 2 | 0 | 1 | 1 | 0 | 6 |
| | Alt C (3) | 21 May | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 |
| *Total* | | | 5 | 3 | 65 | 3 | 12 | 3 | 2 | 93 |

The correlation of the number of calls from less common bat species (i.e., southeastern myotis, northern long-eared bats, and tricolored bats) with the overall number of calls may indicate that these species are more likely to be detected when the overall bat activity is higher. Also, the greater number of calls identified as northern long-eared bats by KPro at sites where southeastern myotis were caught supports our claim that southeastern bat calls are likely to be identified as northern long-eared bat calls by the auto classifier. However, manual vetting is also not an error-proof method for determining presence of bat species (Fritsch and Bruckner 2014; Rydell et al. 2017). Though automated software programs may identify files as a listed species, the MLE value is an important metric in the USFWS Guidance for presence or probable absence surveys. In this study, automated software identified call files as the federally endangered northern long-eared bat at every site. This study was conducted using one auto classifier program and does not address how other programs approved by USFWS would have performed (Nocera et al. 2019; Goodwin and Gillam 2021). Although KPro only produced a MLE value for probable presence at three of the 12 sites, had manual vetting not occurred, we would have incorrectly presumed presence of an endangered species at 25% of our survey sites. Since the study sites were outside the current range of the northern long-eared bat and extensive mist netting in Edgecombe and Johnston counties has never resulted in the capture of the species, it is highly unlikely that this species resides in these counties. Therefore, when acoustic surveys for northern long-eared

**Table 2.** Maximum likelihood estimation scores (MLE) as autoclassified by Kaleidoscope Pro (KPro) to determine presence ($P < 0.05$) or probable absence ($P \geq 0.05$) of bat species using calls recorded in North Carolina at Lower Fishing Creek Game Land in Edgecombe County and Howell Woods in Johnston County, 17–21 May 2023. MLE scores with $P < 0.05$ (bold text) indicated that bat species was considered present at the site by the auto classifier. MLE scores with $P \geq 0.05$ (open) were considered not present via acoustic recording. Southeastern myotis *Myotis austroriparius* were identified by KPro at every site except Site 7. Northern long-eared bats *Myotis septentrionalis* were identified by KPro at Sites 2, 3, and Alt C.

| Site | Date(s) surveyed in 2023 | CORA | EPFU | LABO | LACI | LANO | LASE | MYAU | MYSE | NYHU | PESU | TABR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 17–18 May | 1 | **<0.01** | **0** | 0.06 | **0.03** | 1 | **0** | 0.12 | 1 | 1 | **<0.01** |
| 2 | 17–18 May | **0** | **0** | **0** | 1 | 1 | 1 | **0** | **0** | 1 | 1 | 1 |
| 3 | 19–20 May | 1 | **0** | **0** | 1 | 1 | 1[a] | **0** | **0** | 1[a] | **0** | 0.23 |
| 4 | 19–20 May | 1 | 1 | **0** | 1 | 1 | 1 | **0** | 0.50 | 1 | **0** | **0** |
| 5 | 17–18 May | 1 | **0** | **0** | 1 | 0.85 | 1 | **0** | 1 | 1 | **0** | **0** |
| 6 | 17–18 May | **0** | **0** | **0** | 0.28 | 0.26 | 1 | **0** | 1 | 1 | **0** | **0.01** |
| 7 | 19 May | **0.01** | **0** | **0** | 1 | 1 | 1 | 0.06 | 1 | 1 | **<0.01** | 0.26 |
| 8 | 19 May | 0.10 | 1 | **0** | 1 | **0** | 1 | **0** | 1 | 1 | **0** | **<0.01** |
| 9 | 20–21 May | **0** | 0.19 | **0** | 1 | 1 | 1 | **0** | 1 | 1 | 1 | **0** |
| 10 | 20–21 May | 1 | **0.01** | **0** | **<0.01** | 1 | 1 | **0** | 1 | 1 | 0.84 | **0** |
| Alt A | 21 May | 0.33 | **<0.01** | **0** | 0.85 | 1 | 1 | **0** | 1 | 1 | 1 | **<0.01** |
| Alt C | 21 May | 0.15 | **0** | **0** | 1 | 0.07 | 1 | **0** | **0.03** | 1 | 1 | **0** |

[a] The MLE score for the site overall indicated that there were no LASE or NYHU calls collected, but there were calls collected on 20 May that KPro significantly identified as LASE and NYHU.

**Table 3.** Summary of the number of acoustic bat calls collected 17–21 May 2023 in North Carolina at Lower Fishing Creek Game Land in Edgecombe County and Howell Woods in Johnston County that did and did not result in the capture of southeastern myotis. MYAU = southeastern myotis *Myotis austroriparius*; MYSE = northern long-eared bat *Myotis septentrionalis*.

| | MYAU calls at sites with MYAU captures | MYAU calls at sites with no MYAU captures | MYSE calls at sites with MYAU captures | MYSE calls at sites with no MYAU captures | Total calls at sites with MYAU captures | Total calls at sites no MYAU captures |
|---|---|---|---|---|---|---|
| mean number of calls per site ± SE | 76.8 ± 18.5 | 22.0 ± 9.2 | 29.8 ± 6.0 | 3.7 ± 1.1 | 2,279.5 ± 549.7 | 932.5 ± 244.3 |
| range of number of calls per site | 34–149 | 3–65 | 7–50 | 1–9 | 601–4,278 | 350–1,592 |

bats are performed in areas where southeastern myotis co-occur, manual vetting is not only appropriate, but also essential. Moreover, as this study has illustrated, concurrent acoustic and capture surveys provide more insight into accurately assessing the auto classifier results from automated software programs and provide a more complete inventory of bat presence with less effort.

## Supplemental Material

Please note: The *Journal of Fish and Wildlife Management* is not responsible for the content of functionality of any supplemental material. Queries should be directed to the corresponding author.

**Table S1.** Analysis settings used for Kaleidoscope Pro version 5.4.7 using Bats of North America 5.4.0 classifier to automate bat vocalization identification. Bat vocalizations were recorded in North Carolina at Lower Fishing Creek Game Land in Edgecombe County and Howell Woods in Johnston County from 17–21 May 2023.
Available: https://doi.org/10.3996/JFWM-23-046.S1 (1 KB csv)

**Table S2.** Bat species included in the Kaleidoscope Pro analysis for the autoclassification of bat calls collected in North Carolina at Lower Fishing Creek Game Land in Edgecombe County and Howell Woods in Johnston County from 17–21 May 2023.
Available: https://doi.org/10.3996/JFWM-23-046.S2 (1 KB csv)

**Table S3.** All bats caught in mist nets in North Carolina at Lower Fishing Creek Game Land in Edgecombe County (Sites 1–4) and Howell Woods in Johnston County (Sites 5–10, Alt A, and Alt C) from 17–21 May 2023. Bands were provided by Copperhead Consulting and were stamped with 'CC' and a unique number.
Available: https://doi.org/10.3996/JFWM-23-046.S3 (6 KB csv)

**Table S4.** All bat call files autoclassified by Kaleidoscope Pro as a northern long-eared bat *Myotis septentrionalis* (MYSE) or southeastern myotis *Myotis austroriparius* (MYAU) collected from Edgecombe and Johnston counties, North Carolina from 17–21 May 2023. Species codes are found in Table S2. Fc = characteristic frequency.
Available: https://doi.org/10.3996/JFWM-23-046.S4 (66 KB csv)

**Reference S1.** Reichard JD. 2008. Wing-Damage Index used for characterizing wing condition of bats affected by white-nose syndrome. Provided on White-Nose Syndrome Response Team website https://www.whitenosesyndrome.org/mmedia-education/wing-damage-index-used-for-characterizing-wing-condition-of-bats-affected-by-white-nose-syndrome (March 2024).
Available: https://doi.org/10.3996/JFWM-23-046.S5

**Reference S2**. Silvis A, Perry RW, Ford WM. 2016. Relationships of three species of bats impacted by white-nose syndrome to forest condition and management. In: USDA, editor. 214 ed. Asheville, NC: Southern Research Station. p. 57.
Available: https://doi.org/10.3996/JFWM-23-046.S6

**Reference S3.** Three Oaks Engineering. 2016. Northern long-eared bat research project, eastern North Carolina. Report number TIP R-999, WBS No. 34634.1.4. Final Report submitted to NCDOT, Raleigh, NC. 212 pp.
Available: https://doi.org/10.3996/JFWM-23-046.S7

**Reference S4.** USFWS (United States Fish and Wildlife Service). 2022. Species status assessment report for the northern long-eared bat (*Myotis septentrionalis*), Version 1.2. August 2022. Bloomington, MN.
Available: https://doi.org/10.3996/JFWM-23-046.S8

**Reference S5.** USFWS (United States Fish and Wildlife Service). 2023. Range-wide Indiana bat & northern long-eared bat survey guidelines. U.S. Fish and Wildlife Service, Region 3, Bloomington, MN. 76 pp.
Available: https://doi.org/10.3996/JFWM-23-046.S9

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