

Maximum Likelihood Estimator and Nightly Acoustic Count Values as Weight of Evidence of Bat Maternity Activity

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Abstract: Since the spread of white-nose syndrome in North America, several bat species have shown precipitous declines in abundance and distribution. With lower netting detection probabilities for the currently threatened but proposed endangered northern long-eared bat (*Myotis septentrionalis*) and endangered Indiana bats (*Myotis sodalis*), determination of presence or absence for regulatory clearance often has shifted to the use of acoustic surveys. However, acoustic surveys are unable to differentiate between non-reproductive individuals versus a maternity colony. We used recorded nightly echolocation pass counts of bat species-specific probabilities with maximum likelihood estimator (MLE) scores to determine thresholds by cover type and reproductive period whereby the potential for northern long-eared bat or Indiana bat maternity colonies occurs. Where nightly MLE P -values were <0.05 , mean predicted nightly pass counts were significantly higher in areas of known northern long-eared bat and Indiana bat maternity colonies versus sites that were in either species' distribution but with no maternity activity known. Nightly pass counts (MLE $P < 0.05$) were higher for sites with observed maternity activity for both bat species across forest, forest-field edge, and riparian areas versus sites where no maternity activity was known. For northern long-eared bats, nightly pass counts were highest in the juvenile volancy period (after 15 July) whereas, for Indiana bats, nightly pass counts were highest in the lactation period (16 June to 15 July). Except for edge conditions for northern long-eared bats, a MLE $P < 0.05$ combined with nightly pass counts above thresholds developed from surveys at known maternity colony sites for both species may indicate potential presence of a maternity colony locally and provide a tool to more efficiently use targeted mist-netting for further determination.

Key words: acoustic sampling, echolocation pass count, maternity colony, *Myotis septentrionalis*, *Myotis sodalis*

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Following the precipitous decline of bats affected by white-nose syndrome (WNS), the U.S. Fish and Wildlife Service (USFWS) instituted an acoustic-based survey option and guidance for acoustically determining the presence or probable absence of the currently federally threatened but proposed endangered northern long-eared bat (*Myotis septentrionalis*) and federally endangered Indiana bats (*Myotis sodalis*; Armstrong et al. 2022, USFWS 2022). This shift in reliance on acoustic surveys largely was a function of declining mist-net detection probabilities whereby the minimum mist-net survey level of effort required to prove Indiana bat presence or probable absence exceeded 40 net-nights for portions

of the mid-Atlantic, central Appalachians and southern Appalachians (Niver et al. 2014). Although the general distribution of WNS-affected species from an acoustic-based occupancy perspective has not changed at the regional to landscape level (Nocera et al. 2020), reduction of mist-netting efforts has limited the ability of managers to collect demographic data such as sex, reproductive condition, and relative abundance or evidence of local maternity colony activity from capture and subsequent radio-tracking (Barr et al. 2021, Deeley et al. 2022).

Current USFWS guidance for conducting acoustic surveys for determining Indiana bat and northern long-eared bat presence

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seeks to strike a balance of avoiding false-negative data that comes with high potential conservation costs versus an acceptable level (20%) of false-positive occurrences for either species (USFWS 2019). Owing to the known misclassification rates from echolocation characteristic similarities among bats in the genus *Myotis* in the eastern United States (Britzke et al. 2002), the guidelines use a site-night maximum likelihood estimator (MLE) threshold of $P < 0.05$ for accepting the presence of Indiana bats and northern long-eared bats (USFWS 2022). Based on confusion matrices of bat species interspecific rates of misclassification, this test statistic is sensitive to both the bat community assemblage and the numbers of individual bat passes identified to species as components of species classification confidence (Britzke et al. 2002). For example, because of the relatively high rate of misclassification of Indiana bats as little brown bats (*Myotis lucifugus*) and vice versa, either species might be deemed present at the MLE threshold with few individual bat passes and none of the other species at the nightly level. Conversely, for a night where numerous bat passes are identified to one species and a significant MLE is returned, observations of just a few of the other species, while identified at the file level, may return a non-significant MLE score as numbers recorded are not able to overcome misclassification errors thereby indicating an ambiguous presence (Nocera et al. 2019a).

Periodic comparisons of occupancy and detection probability data for areas within ~ 8 km or outside of known Indiana bat and northern long-eared bat maternity colony occurrences since the advent of WNS have been used to determine necessary nightly total levels of effort for acoustic surveys (Barr et al. 2021, Armstrong et al. 2022). By comparing number of nightly acoustic passes of either species and returned MLE values between sites with known maternity colony presence and absence, it may be possible to move from simple Indiana bat and northern long-eared bat presence determinations, which may only be indicative of males or non-reproductive females, to potential identification of maternity colonies. Aside from regulatory clearance, using acoustic survey data as a screening tool before undertaking now logistically difficult and time-consuming mist-net sampling could be beneficial to resource managers and may provide a renewed impetus to locate maternity colonies of Indiana bats and northern long-eared bats. Assuming that bats could be caught, radio-tagged and tracked to day-roosts, improved efficiency in maternity colony location could facilitate understanding the status of either species in the post-WNS environment (St. Germain et al. 2017, Gorman et al. 2022a, Kalen et al. 2022). More importantly, these data could provide much-needed habitat association data, i.e., day-roost tree or snag type, forest condition, and forest configuration information that could be used

to predict habitat suitability and enhance habitat quality for maternity colonies (Ford et al. 2016a, Silvis et al. 2016, Ford et al. 2021, Gorman et al. 2022b).

With the objective of defining a nightly bat pass indicating maternity colony presence, we used acoustic data from 13 states collected for USFWS survey level-of-effort determination, 2020–2021 (Armstrong et al. 2022) to compare predicted site-night pass counts (bat passes recorded by one detector over one night) of Indiana bat and northern long-eared bat between known maternity colony areas with those where the presence of a maternity colony was unknown. We predicted that nightly pass counts would be greater at MLE threshold combinations of $P < 0.05$ where maternity activity was observed. Moreover, owing to the differences in bat activity on the landscape attributable to reproductive condition (Deeley et al. 2022), we predicted these differences would vary among the pregnancy, lactating, and volancy periods. Lastly, because of the species “swamping” phenomenon that occurs in conditions such as riparian zones and open water where other *Myotis* such as southeastern myotis (*Myotis austroriparius*), gray bats (*Myotis grisescens*), and little brown bats can be abundant (Britzke et al. 2011), we expected that higher necessary pass counts of Indiana bats and northern long-eared bats would be required to meet the MLE threshold in riparian areas as opposed to upland sites.

Study Area

Our study area across the eastern United States encompassed 25 acoustic survey sites occurring in the following 13 states: Alabama, Arkansas, Kentucky, Indiana, Illinois, New Jersey, New York, North Carolina, Ohio, Tennessee, Virginia, West Virginia, and Wisconsin within the Appalachian Plateau, Blue Ridge, Central Lowlands, Coastal Plain, Interior Low Plateau, Piedmont, and Ozark Plateau physiographic provinces (Figure 1). Depending on site and physiographic province, elevations ranged from a high of 860 m above sea level at the Fernow Experimental Forest, West Virginia to near sea level at Prince William Forest Park / Marine Corps Base Quantico, Virginia. Also variable across physiographic provinces, forest vegetation was classified as either cold temperate forest, e.g., Fort Drum Military Reservation, New York, cool temperate forest, e.g., Fort Knox Military Reservation, Kentucky, or warm temperate forest, e.g., Ouachita-St. Francis National Forest, Arkansas (Odom and Ford 2020). For more detailed descriptions of the sites listed in Figure 1, see: Strausberg and Hough (1997), Martin (2007), Kitchell (2008), Pauli et al. (2015), Stewart (2019), Hzyz et al. (2020), Reid et al. (2020), and Barr et al. (2021).

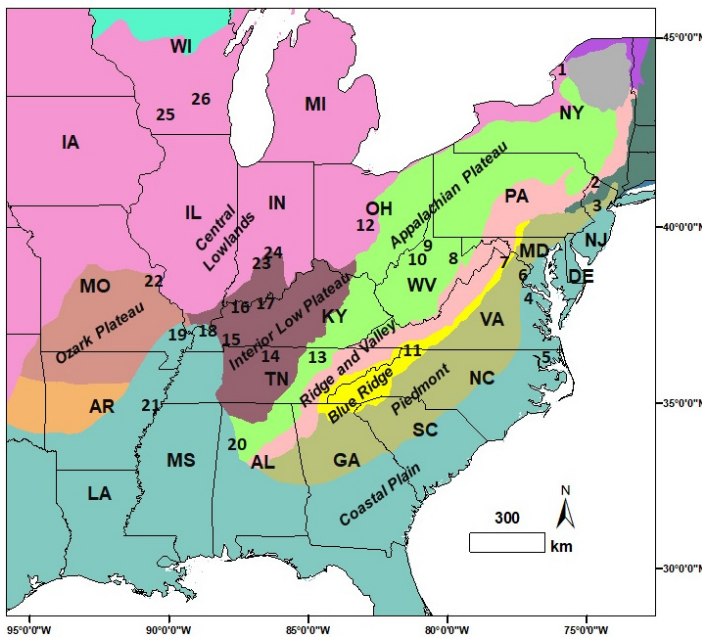


Figure 1. Acoustic survey sample sites (see text), 2020–2021 in the eastern United States across physiographic provinces (colors) by northern long-eared maternity colony (MYSE) and/or Indiana bat maternity colony (MYSO) versus within distribution but no current or recent (~5 years) active maternity colony known (MYSEU, MYSO): 1. Fort Drum Military Reservation (MR), New York (MYSEU, MYSO), 2. Wallkill National Wildlife Refuge (NWR), New Jersey (MYSE, MYSO), 3. Great Swamp NWR, New Jersey (MYSE, MYSO), 4. Fort A.P. Hill MR, Virginia (MYSEU, MYSO), 5. North River Gameland, North Carolina (MYSE, out of MYSO distribution), 6. Prince William Forest Park/Marine Corps Base Quantico, Virginia (MYSE, MYSO), 7. Sky Meadows State Park (SP), Virginia (MYSEU, MYSO), 8. Fernow Experimental Forest, West Virginia (MYSEU, MYSO), 9. Louis Wetzel Wildlife Management Area (WMA), West Virginia (MYSE, MYSO), 10. The Jug WMA, West Virginia (MYSE, MYSO), 11. Pineola Bog State Natural Area, North Carolina (MYSEU, MYSO), 12. Battelle Darby Metro Park, Ohio (MYSE, MYSO), 13. Catoosa WMA, Tennessee (MYSEU, MYSO), 14. Wilson County Artificial Roost, Tennessee (MYSEU, MYSO), 15. Fort Campbell MR, Kentucky-Tennessee (MYSEU, MYSO), 16. Yellowbank WMA, Kentucky (MYSE, MYSO), 17. Fort Knox MR, Kentucky (MYSE, MYSO), 18. Ballard WMA, Kentucky (MYSE, MYSO), 19. Cypress Creek NWR, Illinois, 20. Oakmulgee WMA, Alabama (MYSEU, MYSO), 21. Ouachita-St. Francis National Forest, Arkansas (MYSEU, MYSO), 22. Shaw Nature Center, Missouri (MYSEU, MYSO), 23. Beanblossom Bottoms Nature Preserve, Indiana (MYSE, MYSO), 24. Morgan-Monroe State Forest, Indiana (MYSE, MYSO), 25. Governor Dodge SP, Wisconsin (MYSE, out of MYSO distribution), 26. Horicon Marsh NWR, Wisconsin (MYSEU, out of MYSO distribution).

Methods

From approximately 15 June to 15 August, 2020 and 15 May to 15 August, 2021, to support acoustic survey minimum required level-of-effort determination for the USFWS's Range-wide Indiana Bat and Northern Long-eared Bat Survey Guidelines (USFWS 2022), we continuously deployed zero-crossing/frequency division acoustic bat detectors at 15 sites ($n=50$ detectors). Over the same period in 2021, we also added an additional 11 sites ($n=88$ detectors). Following the methods described by Barr et al. (2021), at each site, we placed 1–2 detectors in each of three broad cover types: forest, forest-field edge, and forested riparian. At each site, we cable-locked detectors to trees and mounted microphones on 3 m tall poles approximately 2–3 m from the bole of the tree. Most

detectors deployed were Wildlife Acoustic SM4 ZC with SMM U2 omni-directional microphones (Wildlife Acoustics Inc., Maynard, MA). However, at Fort Drum Military Reservation and Ouachita-St. Francis National Forest, we used Anabat SD2s with “Stainless” directional microphones (Titley Scientific, Columbia, MO) whereas those deployed at Oakmulgee Wildlife Management Area were Wildlife Acoustic SM2 ZCs with SMU U1 omni-directional microphones. We replaced detector batteries and downloaded data cards at 6 weeks intervals at each site. Each detector was set to default settings per USFWS recommendations (USFWS 2022).

Following summer recording and file compilation, we identified bat passes to species and recorded nightly pass counts and species-specific MLE probability of presence of known or potentially present bat species at each site on a nightly basis using Kaleidoscope version 5.1.0 (Wildlife Acoustics, Maynard, Massachusetts) and classifier 4.2.0 at the “0” setting (USFWS 2019). We used signal detection parameters of 8–120 kHz minimum and maximum frequency range, 2–500 ms minimum and maximum length of detected pulses, a 500 ms maximum inter-syllable gap and a minimum required five pulses for species assignment. Based on recent (<5 years) post-WNS records of bat maternity activity or proximity within 8 km to known northern long-eared bat or Indiana bat maternity colonies, sites were designated as either known maternity sites or as sites with unknown status but within the species' respective distributions (Armstrong et al. 2022). For analyses, we retained for each site, and detector location therein, all nights without precipitation from site or nearest Meteorological Terminal Aviation Routine records (<https://mesonet.agron.iastate.edu/request/download.phtml>) and where the returned MLE $P < 0.05$ for either northern long-eared bats, Indiana bats or both. Because within summer bat activity varies by female reproductive condition (Deeley et al. 2022), we further divided these acoustic data into three phases: pregnancy (15 May to 15 June), lactation (16 June to 15 July) and juvenile volancy (after July 15; Deeley et al. 2022). We used a generalized linear model with a negative binomial distribution and a log-link function in SAS 9.4 (PROC GENMOD; SAS Institute 2020) to assess the relationships of nightly bat passes by northern long-eared bats or Indiana bats between sites with three categorical variable sets: 1) maternity colony present or within-distribution designation as a pseudo-absence; 2) cover type; and 3) reproductive period. We checked each model for goodness-of-fit and over- and under-dispersion by examining residual plots and calculating the fit statistic $df/\text{deviance}$.

Results

Over 2020–2021, we collected 11,544 detector-nights where maternity colonies of the target species were known to exist and

Table 1. Mean nightly echolocation passes of northern long-eared bats and Indiana bats in the eastern United States, 2020–2021 by survey site cover type (edge, forest, and riparian; see text), reproductive phase (pregnancy, lactation and juvenile volancy; see text), and maternity colony status (unknown, known). See text for survey effort.

Colony	Phase	Cover Type	Northern long-eared bat			Indiana bat		
			<i>n</i>	Mean	SE	<i>n</i>	Mean	SE
Unknown	Pregnancy	Edge	84	0.81	0.30	84	0.24	0.30
		Forest	141	61.00	0.15	39	1.48	0.58
		Riparian	105	1.92	0.79	91	6.98	2.35
	Lactation	Edge	116	0.74	0.17	69	0.04	0.02
		Forest	240	5.26	0.90	54	0.24	0.21
		Riparian	121	2.76	0.65	78	13.85	3.89
	Volancy	Edge	109	0.74	0.33	85	0.11	0.05
		Forest	190	3.12	0.50	69	1.59	0.71
		Riparian	122	2.31	0.66	99	1.26	0.23
Known	Pregnancy	Edge	79	4.39	0.79	93	8.06	0.74
		Forest	134	7.84	0.99	148	12.19	1.48
		Riparian	139	13.98	1.58	185	22.85	2.95
	Lactation	Edge	109	4.98	0.63	175	13.67	1.19
		Forest	208	11.83	1.38	271	12.4	1.11
		Riparian	168	12.18	1.69	326	46.36	8.77
	Volancy	Edge	49	5.26	0.64	138	15.17	1.85
		Forest	158	10.17	1.48	245	9.66	0.69
		Riparian	179	30.4	4.73	337	35.1	4.11

Table 2. Generalized linear model parameter estimates (β) for predicted nightly echolocation passes of northern long-eared bats and Indiana bats in the eastern United States, 2020–2021 by survey site cover type (edge, forest, riparian), reproductive phase (pregnancy, lactation, volancy; see text), maternity colony status (known, unknown).

Species	Parameter	β	SE	Wald χ^2	<i>P</i>
Northern long-eared bat	Intercept ^a	2.99	0.01	983.99	< 0.0001
	Colony unknown	−1.65	0.08	410.99	< 0.0001
	Edge	−1.30	0.11	141.43	< 0.0001
	Forest	−0.25	0.09	7.47	0.0063
	Pregnancy	−0.59	0.10	34.98	< 0.0001
	Volancy	0.03	0.10	0.09	< 0.7661
Indiana bat	Intercept ^a	3.99	0.07	2,963.91	< 0.0001
	Colony unknown	−2.29	0.08	671.71	< 0.0001
	Edge	−1.49	0.09	5263.06	< 0.0001
	Forest	−1.32	0.08	248.84	< 0.0001
	Pregnancy	−0.30	0.09	10.63	0.0011
	Volancy	−0.33	0.08	16.82	< 0.0001

a. Intercept is for colony status Observed, Riparian cover type, and Lactation reproductive phase. Each parameter has *df* = 1.

another 12,983 detector-nights within their geographic range. For northern long-eared bats, we retained 1,223 rain-free nights with a returned MLE $P < 0.05$ across 12 sites where maternity colonies were known present versus 898 nights across 11 sites within the species' distribution but for which maternity colony presence was not known (Table 1). For Indiana bats, these values were 668 and

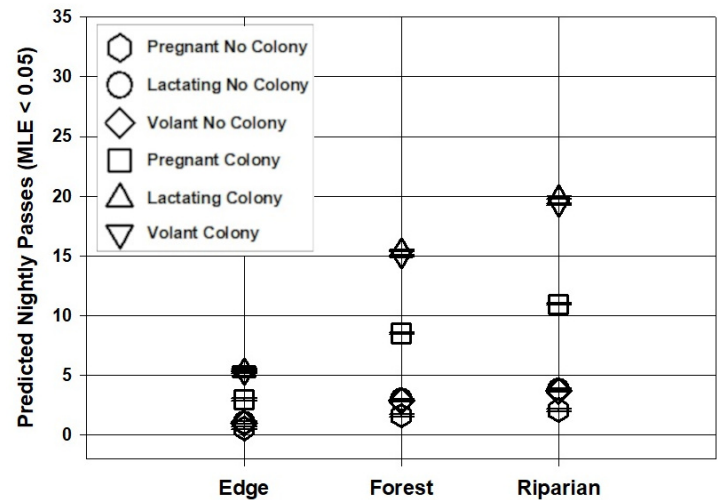


Figure 2. Mean and 95% confidence interval for predicted nightly echolocation passes of northern long-eared bats in the eastern United States, 2020–2021 by survey site cover type (edge, forest, and riparian; see text), reproductive condition (pregnancy, lactation and volancy; see text), and maternity colony status (unknown, known) where nightly maximum likelihood estimator (MLE) $P < 0.05$.

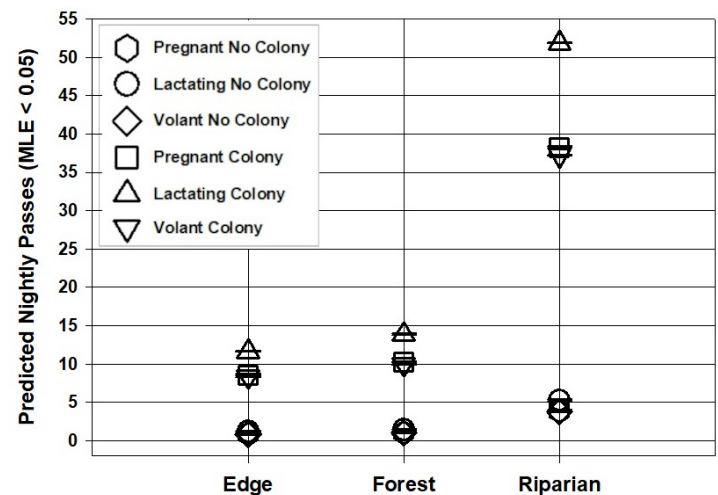


Figure 3. Mean and 95% confidence interval for predicted nightly echolocation passes of Indiana in the eastern United States, 2020–2021 by survey site cover type (edge, forest, and riparian; see text), reproductive condition (pregnancy, lactation and volancy; see text) and maternity colony status (unknown, known) where nightly maximum likelihood estimator (MLE) $P < 0.05$.

1,918 nights, across 14 and 9 sites, respectively (Table 1). For both species, we observed significantly greater mean predicted pass counts occurring where maternity colonies were known versus sites within the species' distributions but without known maternity colonies for all cover types and reproductive periods (Table 2, Figures 2–3). Nightly pass counts for both species were highest in riparian areas as opposed to forest and forest edge areas (Table 2, Figures 2–3). Highest predicted nightly pass counts for northern long-eared bats across all cover types occurred in the lactation and volancy seasons whereas for the Indiana bat, the highest pass counts occurred during the lactation period for the edge and forest

areas, whereas all reproductive seasons returned high pass counts in riparian areas (Table 1, Figures 2–3).

Discussion

During the summer maternity colony season, effective conservation measures to protect northern long-eared bats and Indiana bats require real-time knowledge of their presence on the landscape. Our findings show that nightly echolocation pass counts per detector when automated bat identification software returned an MLE score $P < 0.05$ varied significantly across cover types and reproductive season and between sites with known and unknown presence of maternity roosts for northern long-eared bats and Indiana bats. Subject to variation depending on reproductive period and site cover types, predicted nightly pass counts even at the lower 95% confidence limits where maternity colonies were known could serve as threshold values indicative of potential local maternity colony activity in future acoustic sampling efforts. Although requirements of the U. S. Endangered Species Act, relative to northern long-eared bats and Indiana bats, technically would not differentiate between the presence of males or non-reproductive females versus maternity colony areas, in practice, conservation measures typically are more expansive in scope where maternity colonies occur (Vermont Fish and Wildlife Department 2009). At a minimum, using nightly pass counts and MLE threshold scores could help define where northern long-eared bat and Indiana bat maternity activity on the post-WNS landscape is still occurring. Taken to its logical extent, this approach could enable resource managers to better engage in avoidance and minimization actions to prevent incidental take of northern long-eared bat and Indiana bat during the maternity season when the species are most vulnerable on the landscape, as well as better understand potential take from planned stewardship or development actions. Similarly, application of these thresholds could provide the rationale for engaging in proactive habitat management actions to benefit bats and provide data useful for more precise species distribution modeling (Ford et al. 2016b).

As these two bat species use forests for maternity colony day-roosts, resource managers can seek to prevent or minimize actions that may (or may not) have deleterious impacts from forest overstory removal through harvesting, highway construction, development, or surface mining (Silvis et al. 2015, Silvis et al. 2016). Conversely, managers can use maternity colony location data to engage in actions that maintain or modify forest conditions to facilitate day-roosting such as forest snag creation or prescribed fire that can benefit northern long-eared bats or Indiana bats (Ford et al. 2016a, Silvis et al. 2016, Schroder and Ward 2022). Outside of directed research or long-term monitoring, the shift to use of acoustics over

mist-netting, in most instances, has meant that opportunistic discoveries of northern long-eared bat and Indiana bat maternity colonies have been limited. For resource managers relying on acoustic surveys, the true conservation merit of actions taken after accepting the presence of either species from acoustic sampling is therefore unknown. Moreover, with the decline in mist-net detection probability, mist-netting efforts where species maternity status is unknown often are unproductive (Deeley et al. 2021). However, our findings linking high nightly pass counts where automated bat identification software returns MLE scores < 0.05 suggest managers could, where desirable, follow acoustic surveys with targeted mist-netting to capture, radio-tag, and track bats to day-roosts.

Nonetheless, we urge caution in the application of this approach absent field verification with mist-net effort comparisons where these nightly pass counts and MLE thresholds are met. For example, Kaiser and O’Keefe (2015) noted that even with known Indiana bat colonies in close proximity, inherently low acoustic detection probabilities could result in false negative results. With WNS impacts taking years to be fully realized on the landscape relative to observed bat activity (Johnson et al. 2013, Nocera et al. 2019b, Barr et al. 2021), data from our westernmost survey sites may not yet be reflective of the smaller post-WNS maternity colony numbers of bats that have been observed where WNS impacts occurred early (Kalen et al. 2022). Similarly, additional work examining larger landscape composition and configurations beyond the overly broad habitat designations used for acoustic level of effort determination for summer survey guidance (Armstrong et al. 2022) could be helpful in both pre- and post-WNS environments, as northern long-eared bat activity may be overly dispersed in completely forested landscapes (Gorman et al. 2022b) or exceedingly high in local patches for Indiana bats in fragmented landscapes (Jachowski et al. 2014, Silvis et al. 2014). Reliance on MLE-based acceptance of bat species presence has been criticized for being overly conservative in multi-*Myotis* spp. assemblages (Irvine et al. 2022). Additional work to determine how nightly pass count thresholds might change across more species-rich landscapes where Southeastern myotis, gray bats, and little brown bats are sympatric, i.e., the northern portions of the lower Mississippi Alluvial Valley versus where only northern long-eared bats and Indiana bats are extant, i.e., upper Chesapeake Bay, also are warranted.

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