Acoustic Detection Reveals Fine-Scale Distributions of Myotis lucifugus, Myotis septentrionalis, and Perimyotis subflavus in Eastern Nebraska

Jeremy A. White  
*University of Nebraska at Omaha*, jeremywhite@unomaha.edu

Cliff A. Lemen  
*University of Nebraska-Lincoln*

Patricia W. Freeman  
*University of Nebraska-Lincoln*

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Acoustic detection reveals fine-scale distributions of *Myotis lucifugus*, *Myotis septentrionalis*, and *Perimyotis subflavus* in eastern Nebraska

Jeremy A. White  
*University of Nebraska at Omaha*, jeremywhite@unomaha.edu

Cliff A. Lemen  
*University of Nebraska–Lincoln*, clemen2@unl.edu

Patricia W. Freeman  
*University of Nebraska–Lincoln*, pfreeman1@unl.edu

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Currently, several species of bats in North America are undergoing large population declines due to white-nose syndrome (e.g., Frick et al. 2010). The disease has spread rapidly and has now been detected as far west as eastern Iowa, western Missouri, and western Arkansas (USFWS 2015). As white-nose syndrome (WNS) approaches the Great Plains, it is important to clarify distributions of bats in this region, particularly those species most susceptible to the disease. Three species of North American bats commonly diagnosed with WNS—the little brown myotis (Myotis lucifugus), the northern long-eared myotis (Myotis septentrionalis), and the tri-colored bat (Perimyotis subflavus)—all occur in Nebraska, in the central Great Plains. If WNS reaches the state, a better understanding of the distribution of these cave bats in eastern Nebraska will allow for a more accurate assessment of the impact of this disease on bats in the region.

Published accounts indicate Myotis lucifugus, Myotis septentrionalis, and Perimyotis subflavus share some similar distributional patterns in eastern Nebraska. All 3 species are known to inhabit mines in southeastern Nebraska (Cass and Sarpy Counties; Fig. 1a); all 3 species are largely absent from extreme southeastern Nebraska (Nemaha, Richardson, and Pawnee Counties), with only 1 record (for Perimyotis subflavus) in this region; and all 3 species have been captured in northeastern and north central parts of the state along the Missouri and Niobrara
Rivers, respectively (Figs. 1a, 2a, 3a; Jones 1964, Czaplewski et al. 1979, Benedict et al. 2000, Benedict 2004). Those surveys do leave important questions unanswered. First, Geluso et al. (2005) documented range extensions for *P. subflavus* in all states bordering Nebraska to the north, south, and west; thus, it might be concluded that *P. subflavus* is currently distributed throughout the state. Is *P. subflavus* actually widespread in eastern Nebraska but has avoided detection by previous surveys? Second, *M. lucifugus* and *P. subflavus* in southeastern Nebraska and *P. subflavus* in central Nebraska seem to be tightly clustered around mines (Figs. 1a, 3a). Is this clustering real or a product of increased sampling around areas with mines? Finally, east central Nebraska (Platte, Loup, and Elkhorn River systems), as well as extreme southeastern Nebraska (Nemaha, Richardson, and Pawnee Counties), have few published records for these bats, with the exception of *P. subflavus* at Happy Jack Mine (Figs. 1a, 2a, 3a). Both east central and extreme southeastern Nebraska

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**Fig. 1.** Distribution of the little brown myotis (*Myotis lucifugus*): a, distribution based on publications (Jones 1964, Czaplewski et al. 1979, Benedict et al. 2000, and Benedict 2004) and museum specimens (University of Nebraska State Museum); b, distribution based on expert analysis of acoustic recordings for a single night at each site from June to August 2012 and 2014; c, distribution based on Kaleidoscope probabilities for these same data. Gray circles show areas with known underground mines. Key to county names: B = Burt, C = Cass, D = Dakota, G = Greeley, H = Harlan, J = Johnson, L = Lancaster, N = Nemaha, O = Otoe, P = Pawnee, R = Richardson, S = Sarpy, T = Thurston, W = Washington, We = Webster.
seem to have suitable summer habitat for these species (forested areas along river systems), so are they really missing from these areas? Herein, we reassess and refine distributions of *M. lucifugus*, *M. septentrionalis*, and *P. subflavus* in eastern Nebraska using acoustic monitoring. We selected sites in eastern Nebraska that we considered ideal for those species to maximize the chance of detecting bats when present. Further, we attempted to place our sites across a wide area and several river systems to get a complete picture of the distributions of these bats in eastern Nebraska.

**METHODS**

From June to August 2012 and 2014, we deployed acoustic detectors (SM2Bat+ detector and SMX-US microphones, Wildlife Acoustics, Concord, MA) at 105 sites in eastern Nebraska. Microphones were affixed to poles and were about 2–3 m above the surface of the ground. Bat call sequences were recorded as full-spectrum in WAC0 (lossless compression) format and later converted to WAV format using Kaleidoscope Pro software (Wildlife Acoustics). Bat passes (i.e., a sequence of bat
calls) were separated into files with a maximum duration of 7 seconds.

We concentrated our efforts along wooded, riverine corridors because our species of interest often forage near water and trees. We recorded for a single night at each site. Naturally more species might have been detected if more nights were recorded at each site (Skalak et al. 2012), but recording multiple nights at each site would limit the number of different sites that could be sampled in our survey. Therefore, we opted to maximize the number of sites sampled by recording only 1 night at each site.

We used 2 methods to determine the presence of a species at each site. First we used visual inspection of call sequences by an expert (author CAL). The expert used Kaleidoscope Pro 2.2.2 (Wildlife Acoustics, Concord, MA) at the intermediate setting for accuracy/sensitivity to classify all call sequences. Then, all identifications of *M. lucifugus*, *M. septentrionalis*, and *P. subflavus* were verified by visual inspection based on the following criteria: For

![Fig. 3. Distribution of the tri-colored bat (*Perimyotis subflavus*): (a) distribution based on publications (Jones 1964, Czaplewski et al. 1979, Benedict et al. 2000, and Benedict 2004) and museum specimens (University of Nebraska State Museum); (b) distribution based on expert analysis of acoustic recordings for a single night at each site from June to August 2012 and 2014; (c) distribution based on Kaleidoscope probabilities for these same data. Gray circles show areas with known underground mines. Counties identified in Fig. 1.](image-url)
**P. subflavus** we looked for consistent, relatively flat calls that were above 40 kHz. Calls of *P. subflavus* can be similar to the evening bat (*Nycticeius humeralis*) and eastern red bat (*Lasiurus borealis*), but those species normally produce their flat calls below 40 kHz. For *M. septentrionalis*, we looked for vertical calls with the bottom frequency around 40 kHz. Some of the vertical calls in the sequence had to include a small tail at the end of the call when there is a quick drop in frequency. Indeed the vertical nature of the call makes the body and tail merge in one near-vertical pattern plunging from a high frequency (above the limit of the SMX-US microphone) to a low frequency around 35–40 kHz. Identification of *M. septentrionalis* is complicated because other species including *L. borealis, P. subflavus*, and *M. lucifugus* can produce calls that are almost identical (Broders et al. 2004). In such cases, the calls are either approach or clutter calls (truncated calls) that are typically only produced for a short period of time before the bat reverts to longer-duration, less vertical calls that are diagnostic for these 3 species. As a result, presence of any notes of suspiciously longer duration in a call sequence would result in rejection of the sequence as being that of *M. septentrionalis*. To identify *M. lucifugus* we looked for the characteristic small tail at the end of the call that is typical of many *Myotis*, coupled with a longer-duration and less vertical call than expected of *M. septentrionalis*. For *M. lucifugus*, the bottom frequency is normally around 40 kHz, particularly when the variable small tail at the end of the call is excluded.

Although several software programs are available, we feel that use of an expert is the best approach for analysis of acoustical data in eastern Nebraska. However, use of an expert has its drawbacks because of the subjective nature of such acoustical identifications and the variable skill of experts. Therefore, as a second approach, we also report the results of Kaleidoscope’s probability of presence at each site (bat was scored as present when probability of absence fell below 5%). The success and efficiency of either approach is dependent on the accuracy of the software program and, in the first case, the expert.

Accuracy is a crucial, yet difficult issue for both methods. Identification software often has associated literature that indicates the accuracy of identification by species (Lemen et al. 2015). These accuracy rates are typically high, often over 95%. We ran Kaleidoscope on a bat library (from Lynn Robbins) consisting of a mixture of calls from *M. lucifugus, M. septentrionalis, P. subflavus, L. borealis*, and *N. humeralis*. These species occur in our area and might be confused with one another. The accuracy of identification by Kaleidoscope was high for our target species: *M. lucifugus* (100% of 69 call sequences), *M. septentrionalis* (90% of 53 sequences), and *P. subflavus* (96% of 117 sequences). Our expert also identified these call sequences and had 100% accuracy for all target species. However, the expert declined to identify several sequences because they did not have the characteristics that he demanded for this study. By these criteria he could only identify 92% of *M. lucifugus, 84% of M. septentrionalis*, and 90% of *P. subflavus* sequences. Importantly, these call sequences are from a call library with high-quality calls and many calls in the sequences. As pointed out by Lemen et al. (2015), accuracies found while analyzing call libraries might be higher than those found for typical field-collected call sequences. Thus, for software programs and experts, accuracies might be lower than stated above.

**Results**

On the basis of acoustic recordings, distributions of *M. lucifugus, M. septentrionalis*, and *P. subflavus* are broadly consistent with published distributions in eastern Nebraska (Figs. 1, 2, 3). Calls of all 3 species were identified in the mining area of southeastern Nebraska, where individuals are known to use mines as hibernacula (Cass and Sarpy Counties). Moreover, all 3 species were recorded along the Missouri River in northeastern Nebraska and along the Niobrara River in north central Nebraska (by one acoustical method for *P. subflavus*), near areas where these species had been captured during past surveys (Figs. 1, 2, 3). However, because of the increased number of sites in this study, we have a more detailed picture of the species’ current distributions, including some important range extensions.

The expert and Kaleidoscope probability methods did not agree completely on the distribution of *M. lucifugus* in Nebraska (Fig. 1). Under the expert method, the little brown
myotis was tightly associated with the mining region in southeastern Nebraska and also occurred along the Missouri and Niobrara Rivers in northeastern and north central parts of the state, which corresponds to the distribution based on published records (Fig. 1). In contrast, Kaleidoscope predicted a far-wider-ranging M. lucifugus in the state (present at 37 sites versus 26 sites for the expert method), including the presence of this species in parts of east central, south central, and extreme southeastern Nebraska.

The results for M. septentrionalis from both acoustical methods strongly agreed with each other and with published records for this species (Fig. 2). The only major difference was that Kaleidoscope predicted the presence of M. septentrionalis in extreme southeastern Nebraska (Richardson County), unlike the expert method. Both acoustical methods did not record M. septentrionalis along the Loup and Elkhorn Rivers in central Nebraska. In general, acoustic evidence filled some gaps in the known distribution by connecting the northern tier records down the Missouri River through the mining area of Cass and Sarpy Counties to the Republican River along the southern tier of counties (Fig. 2).

Both expert and Kaleidoscope methods provided results that were largely consistent with the known distribution of P. subflavus (Fig. 3). One exception is that the Kaleidoscope method did not detect P. subflavus along the northern tier of the state, whereas the expert method predicted the presence of this species in this area. Also, both acoustical methods predicted the presence of P. subflavus in south central Nebraska (Harlan and Webster Counties), but there are no published, vouchered records from this area (Fig. 3).

**DISCUSSION**

In general, both acoustic methods showed similar distributional patterns for M. lucifugus, M. septentrionalis, and P. subflavus in eastern Nebraska and broadly corresponded to known distributions based on captures; however, several differences are notable. First, Kaleidoscope predicted the presence of M. lucifugus at sites in extreme southeastern Nebraska (Richardson County), south central Nebraska, and east central Nebraska, although the expert method did not find this species and no captures have been reported from these areas. Our expert maintains that the wider-ranging predictions of Kaleidoscope are a product of false positives. Second, Kaleidoscope predicted the presence of M. septentrionalis at one site in southeastern Nebraska (Richardson County), contrary to the expert method. Netting has been done repeatedly in this area, but this easy-to-capture species (Benedict 2004) has never been documented. We are skeptical that M. septentrionalis or M. lucifugus occurs in extreme southeastern Nebraska, but additional netting might be justified to resolve this issue. Lastly, the Kaleidoscope method did not predict the presence of P. subflavus in northern Nebraska, but the expert method found this species in that region. Perimyotis subflavus has been captured in northern Nebraska during recent surveys (Benedict 2004); thus, we suspect it still occurs there.

Due to the more rigorous requirements for identification, greater accuracy identifying known calls from a call library, and greater agreement with capture data, we prefer the expert method to Kaleidoscope for identification of calls in this study. Regardless of identification method, acoustical surveys are, in our opinion, not yet advanced enough to supplant in-hand identification of captured bats. However, acoustics supply a powerful tool in many cases to focus limited resources on netting at key sites to resolve presence/absence of target species.

**Possible Changes in Fine-Scale Distributions of Bats in Eastern Nebraska**

Our acoustical survey indicates P. subflavus is more widespread in eastern Nebraska than the 2 myotis species. Neither myotis species was found in east central or extreme southeastern Nebraska, whereas P. subflavus was detected in these areas. Although originally thought a short-distance migrant (Fujita and Kunz 1984), P. subflavus might be a latitudinal migrant in some areas as recent evidence suggests (Fraser et al. 2012). The more widespread distribution of P. subflavus from this study is consistent with either long-distance movements between hibernacula and summer grounds or undiscovered hibernacula in Nebraska. The only documented hibernacula in eastern Nebraska are human-made mines used by P. subflavus, as well as M. lucifugus, M. septentrionalis, and Eptesicus fuscus in Cass and Sarpy.
Counties (Jones 1964, Czaplewski et al. 1979, Benedict 2004). Additionally, *P. subflavus* inhabits Happy Jack Mine (Greeley County) in east central Nebraska where fewer than 100 individuals are known to overwinter (Damm and Geluso 2008).

In recent years, *P. subflavus* has expanded its range westward in the Great Plains (Geluso et al. 2005, White et al. 2006), but this species has been known to inhabit mines in Cass and Sarpy Counties since at least the early 1960s (Jones 1964). However, *P. subflavus* is infrequently captured in the state away from these hibernacula. For example, Geluso et al. (2004) captured only 2 *P. subflavus* out of 585 bats caught during 60 nights of netting from 1986 to 1999 at Fontenelle Forest in Sarpy County. We have recorded many calls of *P. subflavus* from Fontenelle Forest using acoustic detectors, yet we have only captured 1 individual during 20 nights of netting over the past 4 years at this site. We suspect that *P. subflavus* has avoided detection during previous mist net surveys at many sites in southeastern Nebraska. Given the relatively distinctive calls of *P. subflavus* in Nebraska, acoustic monitoring might be a more effective method to document presence/absence of this species in this region.

In contrast to southeastern Nebraska, presence of *P. subflavus* and *M. septentrionalis* along the Republican River Valley farther west than previously known (Harlan County) could represent recent range expansions. Past surveys have been conducted in these counties (Jones 1964, Czaplewski et al. 1979, Geluso et al. 2008), including a recent study in Harlan County (Geluso et al. 2008), as well as another study in counties immediately to the west (Serbousek and Geluso 2009) without capturing *P. subflavus* or *M. septentrionalis*. It is also possible that these species were present when those studies occurred but were just not captured. This might be the case for *P. subflavus* due to the difficulty in capturing it in Nebraska, but *M. septentrionalis* is captured readily at sites where we have detected it with acoustic monitoring. We suspect that *M. septentrionalis* and perhaps *P. subflavus* have expanded westward along gallery forests associated with the Republican River, similar to the westward movement of *Nycticeius humeralis* (Serbousek and Geluso 2009).

On the basis of acoustics, *P. subflavus* does not appear to be restricted to areas with mines; however, in southeastern Nebraska, *M. lucifugus* remains closely tied to this mining region. Given that *M. lucifugus* is known to travel over 600 km (Norquay et al. 2013) from hibernacula to summering grounds, it is unclear why *M. lucifugus* does not extend its range farther in southeastern Nebraska.

Recordings of *M. septentrionalis* at sites along the Missouri River Valley between known populations in southeastern and northeastern Nebraska might not reflect a recent colonization but rather undetected populations due to a lack of surveys in this region. Despite recent research on bats in Nebraska, parts of the northeastern edge of the state (such as Dakota, Thurston, Burt, and Washington Counties) have received little attention. The relationship between the distribution of *M. septentrionalis* and hibernacula appears to be intermediate between the restricted distribution of *M. lucifugus* and the widespread distribution of *P. subflavus*. *Myotis septentrionalis* has been considered a short-distance migrant (Caire et al. 1979, Caceres and Barclay 2000), but little is known about its behavior in the Great Plains. Absence of *M. septentrionalis* in extreme southeastern and east central Nebraska might be attributable to distance to hibernacula, because the necessary summer habitats seem to occur in both areas.

**Hibernacula in Eastern Nebraska**

We do not know of any hibernacula in eastern Nebraska other than the mines in Cass and Sarpy Counties. Benedict (2004) noted the importance of these mines for conservation of cave bats in the state, and with the spread of WNS, protection of this region is even more critical. WNS was recently discovered at a site in western Missouri (USFWS 2015), about 250 km from southeastern Nebraska’s mines. We are uncertain of the number of mines in this area because much of the land is owned by mining corporations that restrict access. Some of those mines have been sealed, including one that was closed in the 1980s (Benedict 2004). Prior to its closing, this mine contained colonies of *M. lucifugus*, *M. septentrionalis*, *P. subflavus*, and *E. fuscus* (Jones 1964, Czaplewski et al. 1979). We do not know whether this mine was completely sealed or whether bats can still access the mine to use it as a hibernaculum. Nevertheless, we suspect mines in this region are important...
to the continued presence of *M. lucifugus*, *M. septentrionalis*, and *P. subflavus* in eastern Nebraska. We are not aware of other hibernacula used by these 3 cave bats in this part of the state or in nearby areas of neighboring states. *Myotis lucifugus* once inhabited a gypsum mine in northeastern Kansas, but the mine is now closed, and this species has not been captured in the state recently (Sparks et al. 2011). Unless these species are using unknown hibernacula in eastern Nebraska, western Iowa, northwestern Missouri, or northeastern Kansas, mines in Cass and Sarpy Counties might be the only remaining hibernacula in this area. If these mines are closed, or invaded by WNS, we might see the disappearance of these species from southeastern Nebraska.

Besides occurring in the mining region of Cass and Sarpy Counties, these 3 species of cave bats also occur along the Niobrara River in the northern tier of the state. Rocky outcrops are common and might provide the necessary hibernacula along the Niobrara River Valley, or bats might migrate to caves in the Black Hills of South Dakota or to the mines in southeastern Nebraska. In a similar way, both *M. septentrionalis* and *P. subflavus* occur along the Republican River Valley in the southern tier of Nebraska. Perhaps there are hibernacula in this area as well, or bats migrate northeasterly to Cass and Sarpy Counties. Clearly, information on hibernacula in Nebraska is limited, and our work points to the need for further research in this area. We continue to work with mining corporations and landowners to protect mines that bats use in Cass and Sarpy Counties, to monitor those mines for the possible arrival of WNS, and to search for additional hibernacula in Nebraska.

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**Literature Cited**


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APPENDIX. Locations of acoustic recordings for a single night at each site during our survey (June–August 2012 and 2014). Numbers in brackets refer to presence [1] or absence [0] of Myotis lucifugus, Myotis septentrionalis, and Perimyotis subflavus, respectively. These data represent localities in maps a and c of Figs. 1, 2, and 3.