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Abstract: Few studies have investigated autumn migration of the northern long-eared myotis (*Myotis septentrionalis*). We conducted a two-year radio-tracking study of *M. septentrionalis* in southeastern Nebraska to document migration dates, activity in autumn, and movements to wintering sites. We observed that at least some *M. septentrionalis* migrate in late October and early November. Prior to migration, cold nights curtailed nighttime volant activity outside of roosts for *M. septentrionalis*. Two bats in this study did not emerge from their roost tree for seven consecutive nights during a period of colder weather. We monitored one bat leaving our research area on the night of 20 October 2015 and detected it 2.8 h later near a mine 41 km away. This observation likely represents a migratory movement, but more research is needed to have a better understanding of autumn migration in *M. septentrionalis*.

Keywords: *Myotis septentrionalis*, migration, northern long-eared myotis, radio tracking, autumn activity, distance.

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Introduction

In eastern Nebraska, the northern long-eared myotis (*Myotis septentrionalis*) occurs in forested areas (Jones 1964, Czaplewski et al. 1979, Benedict 2004, White et al. 2016). It has been predicted that individuals roosting in forests in southeastern Nebraska move to nearby mines in the Louisville – Weeping Water area in Cass and Sarpy counties for winter (Jones 1964, Czaplewski et al. 1979, Benedict 2004, Geluso et al., 2004; White et al., 2016). This prediction follows because *M. septentrionalis* is known to use these mines to hibernate (Jones, 1964; Czaplewski et al., 1979; Cliff Lemen unpublished data), and there are no other mines or caves known in eastern Nebraska (White et al., 2016). However, recent work shows that *M. septentrionalis* also uses cracks in rock faces (Lemen et al. 2016). Rock and cliff faces are

not common in Nebraska, but are much more common and widespread than mines or caves in the state (Lemen et al. 2016). Such sites, and other alternative types of hibernacula as yet unknown, might offer *M. septentrionalis* of southeastern Nebraska other opportunities for hibernacula and question the absolute importance of mines and caves for the species.

Our main objective in this study was to test the hypothesis that *M. septentrionalis* migrates from a forested region along the Missouri River (Fontenelle Forest, Bellevue, Nebraska) to mines in the Louisville–Weeping Water area of southeastern Nebraska (Fig. 1). To document migratory timing and distance, we captured and radio-tagged bats near the time when individuals migrate from the study area. A long-term survey based on mist netting at our research site documented that 16 October was the last date of capture for *M. septentrionalis* (Geluso et al. 2004). Based

on that study, we initiated our project in early October to maximize the chance that radio-tagged bats would migrate before failure of the transmitter battery or glue securing the transmitter to the bat.

Methods

All *M. septentrionalis* in this study were captured by mist netting in Fontenelle Forest (1111 Bellevue Boulevard North, Bellevue, Sarpy County, NE) in southeastern Nebraska. Fontenelle Forest is a 6 km² deciduous forest preserve along the Missouri River that was founded about 100 years ago. About half of the preserve is on the floodplain of the Missouri River. Dominant species of trees in the floodplain forest included eastern cottonwood (*Populus deltoides*), sycamore (*Platanus occidentalis*), hackberry (*Celtis occidentalis*), white mulberry (*Morus alba*), rough-leaved dogwood (*Cornus drummondii*), and box elder (*Acer negundo*). The lowlands also contained marshy grasslands with scattered downed trees and large snags. The remaining property was primarily an upland forest that starts abruptly at the edge of the floodplain where loess hills steeply rise about 60 m above the floodplain. The upland forest was dominated by bur oak (*Quercus macrocarpa*), red oak (*Q. rubra*), shagbark hickory (*Carya ovata*), American linden (*Tilia americana*), green ash (*Fraxinus pennsylvanica*), hackberry, and ironwood (*Ostrya virginiana*) (Gelusio et al. 2004).

At the time of capture, all individuals were weighed and their gender determined. In late autumn it was not possible for us to determine the age of bats with the result that we could not distinguish differences in behavior between young of year and older bats. During this project *M. septentrionalis* was not yet listed as threatened under the endangered species act. We were working under Nebraska Game and Park permit # 280, and IACUC permit 15-047-07-EP from the University of Nebraska Medical Center.

We used Holohil transmitters (Model: LB-2X, 0.31g, <http://www.holohil.com>) in the 2014 season and Lotek NanoTags (Model: NTQB-1, 0.29 g, <http://www.lotek.com>) in the 2015 season. These small tags were chosen to reduce the weight added to bats. The weights of radio transmitters were < 5% of the body weights of individuals. Both types of transmitters were similar externally, had similar weights, and were attached by glue to the dorsum of the bat (Carter et al. 2009). The first year we used Perma Type glue (Perma-Type Company Inc. Plainville, Connecticut 06062 USA), and the second year we used Perma Type on 10 bats and Gluture glue (Abbott Laboratories, North Chicago, IL 60064 USA) on 11 bats.

Holohil transmitters produced a short pulse every two

sec on a unique frequency for each bat, and the range of frequencies for these transmitters varied from about 151-152 MHz. The high rate of pulsing facilitated tracking the transmitter, but at the cost of battery life. Holohil estimated those transmitters should last 10 to 14 d. We used eight days as the cutoff for battery failure. Any loss of signal after eight days was considered as ambiguous between leaving the area and battery failure.

Lotek transmitters broadcasted a coded signal for unique identification of each bat on a frequency of 166.380 MHz. Lotek transmitters were set to broadcast 12 h a day and with 10 s intervals between pulses to deliver a battery life of about 40 d. This meant that loss of detection within 35 d of this study was probably not due to battery failure. The 12 h period of time selected was from 1600 to 0400 h.

In 2014, we used R410 and R2000 receivers from ATS (Advanced Telemetry Systems, <https://atstrack.com>). We conducted 10 daytime surveys by vehicle using three-element yagi antennas attached to the roof of the vehicle but rotatable to direct towards wooded areas where the bats were likely to roost during the day. We concentrated our vehicular surveys in areas known to have mines (Weeping Water and Louisville, NE) and along wooded corridors of the Platte and Missouri Rivers and Weeping Water Creek between Fontenelle Forest and Weeping Water, NE (Fig. 1). Vehicular surveys did not follow formal routes or times but were added to our schedule as time allowed. Because we could not get access to the land of the corporations owning the large mines, we could only drive nearby roads. This and the problem of monitoring such a large area by road caused us to view these surveys as an augment to aerial surveys. Three nighttime surveys were conducted by plane (Cessna Skyhawk) to search a rough rectangle defined by Fontenelle Forest in the northeast to Weeping Water in the southwest (Fig. 1). We used 2 rigid, 3-element yagi antennas (Advanced Telemetry Systems) mounted on the struts on each side of an airplane to conduct aerial searches for radio-tagged bats. Our tests with the airplane indicated a detection distance of at least 5 km. We flew an east-west grid over our survey area with spacing of about 3.2 km between flight paths. The survey flights were on 25 and 28 October and 4 November 2014 and were timed to begin at Fontenelle Forest about 30 m after sunset. This allowed us to fly over the mine sites about 60 to 110 minutes after sunset. We scheduled our flights for nights with no rain, low winds, and mild temperatures at sunset (about 10 °C for all three flights).

In 2015, we used 6 stationary towers with antennas and dataloggers (sensorgnome receivers <https://sensorgnome.org>) and did not use road or aerial surveys. To analyze our data for the coded signals transmitted by the Lotek transmitters, we wrote our own software and

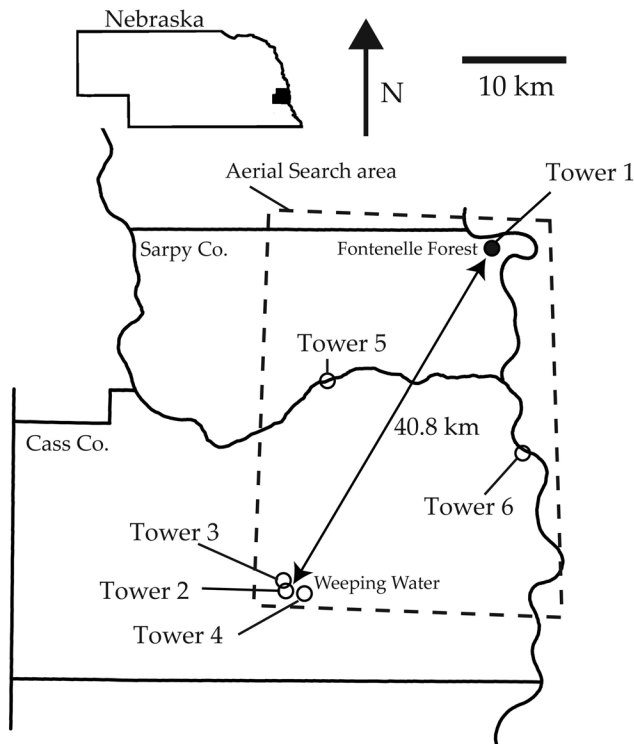


Figure 1. Map of the study area in southeastern Nebraska. Circles represent the tower sites in 2015 with the scale radius of these circles being roughly the detection distance of receivers (0.75 km). However, yagi antennas are directional such that the actual reception patterns are not circular. The dashed line bounds the area searched during 10 vehicle and 3 aerial surveys in 2014.

submitted our data to Motus (www.motus.org) for analysis. The Motus Wildlife Tracking System is a coordinated network of radio-telemetry arrays managed under a common database that facilitates tracking movements and behaviors of small organisms at local, regional, or even hemispheric scales. Motus is a program of Bird Studies Canada in partnership with collaborating researchers and organizations. Results from analyses of data from receivers by both our software and Motus's software were the same.

All dataloggers recorded data 24 h a day and batteries (6 v Energizer GC2 golf cart batteries rated at 107 min @ 75 A) were charged every 5 d. At each of our 6 tower locations, we used 2 yagi antennas affixed to the towers (Go Vertical USA <https://go-vertical-usa.myshopify.com>). We typically raised the top antenna to 7.4 m and the bottom antenna was placed 1.2 m below to avoid interference between antennas. Most of our antennas had 9 elements to increase detection range; however, in 2

situations where range was shortened because of topography, we opted for 5-element yagi's with broader angles of detection.

Effective distance of detection was tested with the tower system in and out of the forest. Yagi antennas are directional so that the direction the antenna is pointing has a large impact on detection distance. For tests reported here, we pointed an antenna (9-element yagi mounted at 7.4 m) directly at the transmitter (held on a pole about 2.5 m above the ground) with the transmitter's whip antenna oriented parallel to elements of the yagi. This represents a best-case test for distance of detection. We observed that detection distances varied depending upon topography and/or intervening forest. Our longest successful test of detection distance was about 3.0 km. Greater distances of 14 km have been reported in more open habitats such as over water or for taller towers and bats flying at higher altitudes (Taylor et al. 2011). We suspect our shorter detection distances are the most relevant for our study of this forest species.

In 2015, we placed tower #1 at Fontenelle Forest in the lowlands (41.17738, -95.891162; Fig. 1) near the site where most *M. septentrionalis* were captured and tagged. This lowland site was flat and no hills blocked the tower from the lowland forest. However, sites where bats were netted and tagged in the uplands (Mormon Hollow and Child's Hollow) did not have a direct line of site to the tower.

We concentrated our effort on detecting radio-tagged bats near mines of the Weeping Water and Louisville area (Cass County, NE). There are 5 known mines in the Weeping Water area and 4 in the Louisville area. Corporate mine owners declined access to their mine sites and property so the mines only were surveyed from nearby property where other landowners granted access. This limited monitoring to 2 of the 5 mines in the Weeping Water area. One private landowner with a small mine in the Louisville area allowed us access to that mine.

Weeping Water Area: A large inactive mine west of the town of Weeping Water, was monitored with 2 towers. Tower #2 (40.870426, -96.166122; Fig. 1) was located 0.32 km south of a mine entrance. This tower had two 9-element yagi antennas. One pointed directly north towards the mine entrance but without line of sight to the entrance because of topography. The second lower yagi antenna was pointed south in the direction of Weeping Water Creek. Tower #3 was placed north of the mine 1.0 km away (40.885101, -96.163566; Fig. 1). This tower was equipped with two 9-element yagi antennas. Tower #3 was not in direct line of sight of the mine entrance. We pointed the high antenna south towards the mine entrance and the low antenna north. We placed tower #4 about 0.33 km from an active mine east of Weeping

Water (40.861108, -96.130271; Fig. 1). A line of site view to the mine entrance was not possible from this tower. Because topography limited the effective range of the antenna pointed towards the mine entrance, we used a 5-element yagi pointed towards the entrance to take advantage of its lesser gain but broader width of detection. The second antenna (9-element) was pointed southeast along Weeping Water Creek, which might serve as a foraging area for bats.

Louisville Area: We placed tower #5 on a bluff above a small inactive mine just south of the Platte River near Cedar Creek, Nebraska (exact location withheld by request of landowner, Fig. 1). One antenna pointed northeast across the Platte River. The second antenna pointed northwest, also across the Platte River. This tower had a dual purpose to monitor potential bat activity near the mine and to detect possible movements of bats along the Platte River.

Missouri River: We placed tower #6 about 1 km west of the Missouri River and about 20 km south of Fontenelle Forest (40.983523, -95.855351; Fig. 1) with one antenna pointed east and one west. There was no mine at this site, but we placed a receiver here in case radio-tagged individuals moved south along the forested corridor of the Missouri River.

Recordings from the datalogger at Tower #1 in Fontenelle Forest were used to determine when bats were actively flying outside of roosts in 2015. When a bat was stationary the signal strength was fairly constant, but when the bat was flying signal strength varied widely. Thus, documenting periods of activity and inactivity through the night was simple for transmitters with a strong signal to the receiver. However, some bats only occasionally flew within range of our tower, especially individuals captured in the upland sites. Therefore, our analysis was restricted to 13 bats that remained within detection distance of the tower. Another issue with measuring activity or timing of migration is the problem of static tags (e.g., a tag that has dropped off a bat but continues to be recorded) versus active tags (tags still on bats). To avoid this problem, we only used data for bats until their last known flight was recorded.

Flight time in minutes per night was determined for 58 bat flights from 13 different individuals in the 2015 field season with dataloggers. Temperature (°C at sunset) and wind speed (m/s at sunset) were obtained from the closest weather station to the study site (Offutt Air Force Base, 5 km away). Dates are recorded as sequential days of 1 to 23 with 18 October 2015 as day one.

We used multilinear regression analysis to determine whether temperature, temperature², wind speed, date, and their interaction terms could predict flight time (lm(),

R Core Team 2013). Temperature² was included to test (using anova() for comparison of models with and without temperature²) for a curvilinear relationship between activity and temperature. Model selection was performed using the step() procedure in R. The model was checked for assumptions by viewing the residuals versus fitted plot, the normal Q-Q plot, and the residual versus leverage plot. The problem of collinearity of variables was also investigated using the variance inflation factor (VIF). A maximal VIF value of 10 is frequently used (Kutner et al 2004), but Allison (1999) states that values of VIF as low as 2.5 are concerning.

Results

We radio-tagged a total of 36 *M. septentrionalis* at Fontenelle Forest during the 2-year study. In 2014, we radio-tagged 15 bats (7 females and 8 males), all from the lowland forest (Table 1). In 2015, we radio-tagged 21 bats (11 females and 10 males). Thirteen bats (6 females and 7 males) were captured in lowland forest, and 8 individuals were captured in upland forest: 6 in Mormon Hollow (4 females and 2 males) about 1.0 km southwest of the lowland site and 2 in Child’s Hollow (1 female and 1 male) about 1.8 km northwest of the lowland site (Table 2). Across all sites and both years, weights of bats averaged 8.6 g with a standard deviation of 0.69 g.

Table 1. Data from 15 northern long-eared myotis (*Myotis septentrionalis*) captured in lowland forest and radio tagged in 2014. Dates list the date of radio-tagging and the last date transmitter was detected for each bat. Distance refers to the distance from where bat was radio-tagged to its detection location outside of Fontenelle Forest.

ID	Sex	Dates	Distance
1	f	2-18 Oct	
2	m	2-13 Oct	
3	f	2-12 Oct	
4	m	2-7 Oct	1.5 km
5	f	7-31 Oct	
6	m	7-18 Oct	
7	f	7-15 Oct	
8	f	7-15 Oct	
9	m	7-25 Oct	2.5 km
10	f	17-17 Oct	
11	m	17-29 Oct	
12	m	17-18 Oct	
13	m	22 Oct-1 Nov	
14	m	24-Oct-4 Nov	
15	f	25 Oct-1 Nov	

Table 2. Data from 21 northern long-eared myotis (*Myotis septentrionalis*) radio-tagged in 2015 from Fontenelle Forest, Sarpy County, Nebraska. Net site refers to area where bats were captured and radio-tagged. Dates list the date of radio-tagging and the last date bat was detected flying. Distance refers to the distance from the point each bat was radio-tagged to its detection location outside of Fontenelle Forest. Status shows the transmitters that were still transmitting but considered static at end of study (9 November).

ID	Sex	Net site	Dates	Distance	Status
1	f	Lowland	6-23 Oct		Static
2	f	Lowland	6-19 Oct		
3	m	Lowland	6 Oct		Static
4	f	Lowland	10-24 Oct		Static
5	f	Lowland	10 Oct-5 Nov		Static
6	m	Lowland	10-20 Oct	41 km	
7	f	Lowland	10 Oct		
8	m	Lowland	10 Oct-5 Nov		Static
9	m	Lowland	10-18 Oct		
10	m	Lowland	10-19 Oct		
11	m	Lowland	18-26 Oct		
12	f	Lowland	18-20 Oct		
13	m	Lowland	22 Oct-4 Nov		
14	m	Upland	20-21 Oct		
15	f	Upland	20 Oct		
16	f	Upland	20 Oct		
17	f	Upland	20 Oct		
18	f	Upland	20 Oct-4 Nov		
19	m	Upland	20 Oct-5 Nov		
20	m	Upland	21 Oct		
21	f	Upland	21-29 Oct		

Nightly activity patterns in autumn

In 2015, bats usually began to fly about 30 min after sunset (sunset was about 2000 CDT on 15 October at our research site) and stopped flying before midnight. The flight time averaged 2.5 h with considerable variation (Fig. 2). The Lotek transmitters turned off at about 0400 for battery conservation; therefore, we cannot quantify activity patterns after that time. But only one individual bat was detected flying in the early morning hours after 0100 (on the nights of 18, 19 and 20 October).

Testing for possible curvilinear effects of temperature on flight time determined that multiple regression including both temperature and temperature² was significantly better than the model with only temperature (using anova()) comparison of two models, $P < 0.00001$). Temperature, temperature² and date were statistically significant, but not wind speed and the interaction terms in the

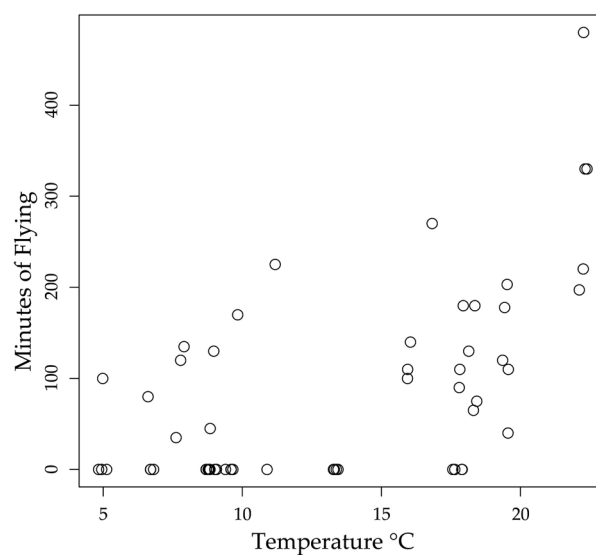


Figure 2. Temperature and minutes of flying per night for northern long-eared myotis (*Myotis septentrionalis*) in Fontenelle Forest, Sarpy County, Nebraska during autumn 2015. Jitter has been added to the temperature variable to see overlapping points.

regression predicting flight time for bats (Intercept: Estimate = 64.5, Std. Error = 76.5, $P < 0.40$; Temperature: -36.1, 11.1, 0.0021; Temperature²: 2.01, 0.41, 1.0×10^{-5} ; Date 10.12, 2.80, 0.0007; Adjusted $R^2 = 0.57$). There is a highly significant positive regression coefficient for date. This might indicate that at later dates the bats were more active at the same temperature. However there is a correlation of temperature and date of -0.76. The VIF for date is 2.5, which is marginal and may indicate problems for interpreting regression coefficients (Allison 1999). Therefore the analysis was run excluding date. Temperature and temperature² still were highly significant and there was little change in the regression coefficients for Temperature (-36.1 versus -35.0) and Temperature² (2.01 versus 1.71). Checking for deviations by the data from the assumptions of linear regression revealed no substantial problems. The only issue was that flight time could not fall below zero minutes no matter how cold the night (Fig. 2). This gave a hard boundary to the lower limit of flight time, which might impact a linear regression. We felt this problem was not sufficient to invalidate the regression analysis.

One consequence of the effect of temperature on activity was that during a cold period from 24 to 30 October 2015, two bats remained detected but there was no indication of flight (during our monitoring period of 1700 until 0400) for 7 nights in a row. Later with warming temperatures, these two bats were detected flying again on 31 October and 1 November.

Timing of migration

In 2014, we lost signals of 4 transmitters within 8 days of being deployed (our criterion for a bat leaving Fontenelle in 2014). The signal from one bat (#4 Table 1) was lost after 6 days on 7 October 2014. Two other bats left within 2 days of being tagged. These bats (#10 and #12, Table 1) were marked on 17 October. One signal was lost almost immediately the same night. The other signal was lost the following day on 18 October. Finally the signal of bat #15 was lost on 1 November, 8 days after it was radio-tagged.

In 2015, we affixed 10 bats with radio transmitters in the lowlands of Fontenelle Forest on 6 and 10 October (Table 2), and we started the datalogger at Tower #1 in Fontenelle Forest on 18 October. Over that time period 1 tagged bat permanently left the study site. Over the next 3 nights, 19-21 October 2015, signals from 5 of 11 active transmitters in the lowland were lost (Table 2). Over the next 19 days, from 22 October to 9 November, signals from 2 lowland active transmitters were lost whereas the other 5 remained and were eventually defined as static (Table 2).

Detection of bats outside of Fontenelle Forest

In 2014, we detected two radio-tagged bats outside of Fontenelle Forest; both were detected in the adjacent suburb of Bellevue, Sarpy County, Nebraska. The first bat, radio-tagged on 2 October (last detected in Fontenelle on 7 October) was detected by vehicular survey 1.5 km from its initial capture point on 8 October in a large tree in the backyard of a suburban home. This transmitter remained static in this tree until its signal became weak and failed on 15 October. A second bat was detected by aerial survey in Bellevue on 28 October (bat initially tagged on 7 October and last detected in Fontenelle on 25 October). This bat was located about 2.5 km from where it was initially tagged. The next day, a ground survey failed to detect this bat at that site in Bellevue.

In 2015, of the five towers outside Fontenelle Forest, only tower #2 at Weeping Water detected a radio-tagged bat. This male bat was last detected by tower #1 in Fontenelle Forest at 2010 on 20 October 2015, and was detected a second time that night at 2259 for 50 s at tower #2 in Weeping Water. The distance between these 2 towers is about 41 km, which means the bat averaged about 14.6 km/h for 2.82 h in transit.

Discussion

Our analysis of the 2015 data suggests that in October to early November, temperatures had an influence on how long a bat would be volant outside of roosts at the study site (Fig. 2). At the extreme, 2 bats remained in their roosts for 7 consecutive nights before flying again. Due to the influence of temperature on bat activity outside of roosts in autumn, researchers need to consider temperature as a factor when determining presence and/or absence of *M. septentrionalis* at this time of year.

In both years, *M. septentrionalis* still was present in Fontenelle Forest in October to early November. This late timing of activity at the summer grounds extends the known period of activity for *M. septentrionalis* in this region prior to autumn migration and hibernation. Geluso et al. (2004) observed that *M. septentrionalis* remained at Fontenelle Forest at least until mid-October. Moreover, a maternity roost of *M. septentrionalis* in an unheated cabin near Nebraska City, Otoe County (60 km south of our study site) was monitored during autumn of 2015. Individuals were seen in the roost on 8 October but were not present on 6 November (Stein and White 2016). These observations, along with results from our study, indicate that migration of *M. septentrionalis* in southeastern Nebraska might extend later than expected at this latitude, especially on years with warmer autumn temperatures. Swarming behavior at hibernacula in Indiana (similar latitude to our study site) was documented from early August until the first week of October (Whitaker and Rissler 1992). Indeed, some *M. septentrionalis* might have left Fontenelle Forest in September or even August before our project began. More research will be needed to determine a more complete view of both the beginning and end of migration in this area.

The five transmitters still being detected at the end of the 2015 field season (9 November) were all classified as static. We tracked all five transmitters to trees; none had been shed on the ground. Tags might still be on bats that were inactive, transmitters might have fallen off inside the roost, or bats might have died in place. Unfortunately, given the large size of these trees, we could not recover the tags to determine their fate.

In 2014, we tracked 2 bats to the adjacent town of Bellevue. Both bats detected in Bellevue had moved south of Fontenelle Forest, in the general direction of the mining region. However, these individuals might also have been moving between roosts. In summer, Henderson and Broders (2008) found most movements by *M. septentrionalis* between roosts were relatively short, but rare longer movements of over 1 km were documented, which is similar to the distance traveled by the 2 bats in our study (1.5 and

2.5 km). Cryan et al. (2001) found an average movement from capture location to roost site of 2.2 km. Therefore; it is not known whether movements to Bellevue were part of migration or simply longer movements to new roosts.

We were not able to detect bats in the mining region with aerial surveys in 2014. Airplane flight data indicated we could detect the transmitters at Fontenelle Forest at a distance of 5 km. By flying a grid pattern over the research area, we should have detected any bat transmitting above ground. Part of the reason we did not detect bats in the mining area might be that relatively few bats left Fontenelle Forest in 2014 with transmitters still broadcasting. Also given the small number of radio-tagged bats we were searching for, if they did not emerge from the mines on the nights of our aerial surveys, then they would not be detected.

In 2015, documentation of a bat (bat #6, Table 2) moving 41 km from Fontenelle Forest to Weeping Water offers support for the hypothesis that mines of Cass and Sarpy counties provide hibernacula for some *M. septentrionalis* in eastern Nebraska. However this is only a single detection and many questions remained unanswered. For example, this bat might have only been at the mine for swarming and moved later to another hibernaculum (Whitaker and Rissler 1992).

Given that *M. septentrionalis* is a common woodland species, we would predict there are many thousands of these bats in eastern Nebraska during the summer. Where are all these bats going for winter? There are no estimates of the number of bats in any of the large mines in Nebraska. The three smaller mines we have been able to enter had less than 100 total *M. septentrionalis* hibernating in them (Cliff Lemen unpublished data). The question remains whether most *M. septentrionalis* of southeastern Nebraska use these mines for hibernation, migrate much farther to other hibernacula, or overwinter in unknown hibernacula such as cracks in rock faces that are closer to their summering grounds (Lemen et al. 2016). More research will be needed to answer these questions.

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