Virginia Journal of Science
Volume 66, Issue 4
Winter 2015

A Comparison of Survey Methods for Documenting Presence of *Myotis leibii* (Eastern Small-Footed Bats) at Roosting Areas in Western Virginia

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ABSTRACT

Many aspects of foraging and roosting habitat of *Myotis leibii* (Eastern Small-Footed Bat), an emergent rock roosting-obligate, are poorly described. Previous comparisons of effectiveness of acoustic sampling and mist-net captures have not included Eastern Small-Footed Bat. Habitat requirements of this species differ from congeners in the region, and it is unclear whether survey protocols developed for other species are applicable. Using data from three overlapping studies at two sampling sites in western Virginia's central Appalachian Mountains, detection probabilities were examined for three survey methods (acoustic surveys with automated identification of calls, visual searches of rock crevices, and mist-netting) for use in the development of "best practices" for future surveys and monitoring. Observer effects were investigated using an expanded version of visual search data. Results suggested that acoustic surveys with automated call identification are not effective for documenting presence of Eastern Small-Footed Bats on talus slopes (basal detection rate of 0%) even when the species is known to be present. The broadband, high frequency echolocation calls emitted by Eastern Small-Footed Bat may be prone to attenuation by virtue of their high frequencies, and these factors, along with signal reflection, lower echolocation rates or possible misidentification to other bat species over talus slopes may all have contributed to poor acoustic survey success. Visual searches and mist-netting of emergent rock had basal detection probabilities of 91% and 75%, respectively. Success of visual searches varied among observers, but

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detection probability improved with practice. Additionally, visual searches were considerably more economical than mist-netting.

INTRODUCTION

There has been an estimated mortality of more than 6 million bats in the genus *Myotis* in White-Nose Syndrome (WNS) affected areas (Blehert et al. 2009; Ford et al. 2011; Francl et al. 2011; Minnis and Lindner 2013; Puechmaille et al. 2011). This disease has continued to spread across the Northeast into the Appalachians, Midwest and mid-South (Francl et al. 2012), and now is present throughout much of the eastern United States and Canada (U.S. Fish & Wildlife Service 2016a). Undoubtedly, this increased geographic footprint has led to higher overall mortality than original estimates.

Biologists have long relied on capture methods such as mist-netting near roosts or water sources and along flyways to document presence of bats (Kunz et al. 2009). Declines in bat populations due to WNS have made previous standard capture methods largely ineffective for some bat species of conservation concern in WNS-impacted areas (Coleman et al. 2014; Ford et al. 2011). As early as 1994, long before the WNS emergence, the U.S. Geological Survey (USGS) acknowledged a need to resolve questions about bat population status, recognizing that data available from state and federal agencies were insufficient to provide population estimates and assess trends, thereby recommending new sampling strategies (Loeb et al. 2015). Threats of additional population declines and regional extirpation of some bat species from WNS have heightened the need to effectively monitor long-term trends in population status, distribution, and structure of species assemblages within both WNS and presumed future WNS-impacted areas.

The distribution, use of hibernacula, and foraging and roosting habits during the maternity season by *Myotis leibii* (Eastern Small-Footed Bat) were poorly documented prior to WNS, compared to its congeners (Krutzsch 1966; Best and Jennings 1997; Chapman 2007; Johnson et al. 2011). In Virginia, lack of targeted survey efforts and research has led to considerable variability in conclusions about the species' conservation status; including designations as locally abundant in western Virginia (Dalton 1987), uncommon in Virginia (Webster et al. 2003), and greatest conservation need, Tier I Virginia Wildlife Action Plan (Virginia Department of Game and Inland Fisheries 2016). Moreover, reports of declines in population sizes associated with WNS vary among bat species (Hayes 2012). It has been difficult to precisely document declines for Eastern Small-Footed Bats because they often hibernate alone, in small groups, and often in obscure locations opposed to aggregative hibernators such as *Myotis lucifugus* (Little Brown Bats) and *Myotis sodalis* (Indiana Bats; Veilleux 2007:Turner et al. 2011; Francl et al. 2012).

In 2013, the U.S. Fish & Wildlife Service (USFWS) was petitioned to consider listing Eastern Small-Footed Bat as threatened or endangered under the Endangered Species Act (U.S. Fish & Wildlife Service 2014). After reviewing the available scientific information, USFWS (U.S. Fish & Wildlife Service 2013) determined that listing the Eastern Small-Footed Bat was not warranted; however, numerous data gaps were noted that need to be addressed to better understand Eastern Small-Footed Bat ecology and true conservation status.

For most *Myotis* in WNS-impacted areas, acoustic monitoring has emerged as an increasingly-used method to detect presence. Acoustic monitoring requires less effort and mitigates the higher costs, low detection probabilities, and potential false negatives from surveying with mist-nets (Coleman et al. 2014). Accordingly, USFWS now allows acoustic surveys to document presence or presumed absence of the endangered Indiana Bat (Niver et al. 2014) and is currently developing similar guidelines for the threatened *Myotis septentrionalis* (Northern Long-Eared Bat; Mike Armstrong, U.S Fish & Wildlife Service, personal communication). Although mist-netting allows gathering of information on sex ratios, body condition, and reproductive condition (Kunz et al. 2009), acoustic detectors are an attractive alternative sampling tool because they are relatively simple to operate and can collect large amounts of data for extended periods (Morris et al. 2011). Acoustic detectors also are capable of sampling a much larger area than nets (O'Farrell and Gannon 1999), and detection should be less sensitive to abundance, adding to the technique's utility. Even prior to WNS, a combination of sampling methods had been proposed as the most effective monitoring strategy, as this maximized information collected and leveraged the strengths of each method (O'Farrell and Gannon 1999; Patriquin et al. 2003; Flaquer et al. 2007; Robbins et al. 2008). Although acoustic monitoring is effective for many species, a post-WNS study on bat detection probabilities in northwestern New York using opportunistic capture and acoustic methods found that Eastern Small-Footed Bats had substantially lower detection probabilities than other species in that area (Coleman et al. 2014). Because Coleman et al. (2014) focused on Indiana and Little Brown Bats' foraging habitats, the efficacy of acoustic surveys in habitats more likely to be used by Eastern Small-Footed Bats (i.e., emergent rock formations and nearby 1st and 2nd order streams) largely is unknown.

To address the lack of comparisons of detection methods within Eastern Small-Footed Bat roosting areas in the central Appalachians and to aide in the development of "best practices" for future surveys and monitoring, a *post-hoc* comparison of detection probabilities of three survey methods was performed: acoustic surveys with automated identification of calls, visual searching for roosts on emergent rock formations, and mist-netting at sites where Eastern Small-Footed Bats were known to occur. Secondary benefits of each survey method also were considered.

MATERIALS AND METHODS

This *post-hoc* study used Eastern Small-Footed Bat detection data collected during three separate studies from sites in Virginia where Eastern Small-Footed Bats were known to occur. To maximize comparability, the original datasets were reduced to two local sites utilized by all three studies and where Eastern Small-Footed Bats previously had been detected (Moosman et al. 2015). The study sites were post-Pleistocene colluvial fields (talus slopes) in western Virginia. Sites differed in their specific geology and physical setting. Site one, Devil's Marbleyard (hereafter DMY), is a 3.0 ha field of large Antietam quartzite boulders located in the George Washington and Jefferson National Forest in Rockbridge County (37.581332°N, 79.471420°W, datum WGS 84). The DMY is surrounded by a mixed deciduous forest predominated by *Quercus prinus* L. (Chestnut Oak), *Quercus rubra* L. (Northern Red Oak), *Quercus coccinea* (Scarlet oak), *Pinus virginiana* (Virginia Pine), and *Acer rubrum* L. (Red Maple) (Mengak and Castleberry, 2008). Site two is a 3.34 ha talus slope of smaller scree composed of quartzite with some larger boulders located within the Sherando Lake's Recreation Area (hereafter Sherando) of the George Washington and Jefferson National Forest in Augusta County (37.929370°N, -79.004356°W, datum WGS 84). Sherando is surrounded by a mixed deciduous forest similar to that surrounding DMY.

As a capture baseline, mist-net data were collected during June 2009 and July 2014 (Moosman et al. 2015), and visual search and acoustic data were collected between June and August 2014. Mist-nets were deployed with 38-mm mesh in two manners. Two 12-m-long x 3-m-high nets end to end directly on the talus slope were deployed at DMY because the location lacked corridors conventionally considered suitable for surveys with mist-nets. Mist-nets were placed perpendicular to the forest edge extending toward the center of the boulder field. Mist-nets were deployed 15 min before sunset for a duration of 1.5 hours. Mist-nets at Sherando followed conventional placements. Two stacked 6-9-m-long nets were placed more than 30 m apart adjacent to the talus slope where Eastern Small-Footed Bats had access to the stream corridor. Mist-nets were deployed 15 min before sunset for a duration of 4.25 hours. Captured Eastern Small-Footed Bats were individually weighed to the nearest 0.1 g using a spring scale (Pesola AG, Baar, Switzerland[1]). Sex, age, and reproductive state were recorded for each Eastern Small-Footed Bat and a numbered aluminum band (Porzana Limited, East Sussex, UK) was placed on the forearm of each Eastern Small-Footed Bat and then subsequently released.

Occurrence data were gathered using visual surveys. The survey team visually searched for Eastern Small-Footed Bats in crevices using penlights (Energizer Holdings, Inc., St. Louis, Missouri) over the length and width of the survey area by means of belt transects. Belt transects followed a defined azimuth between two points, yet were adapted to allow transects to be bent in response to impassable areas (e.g. large gaps, rock faces, dangerous footing). The survey team performed simultaneous visual searches on different transects separated by 3 m and walked laterally across slopes from tree edge to adjacent edge. Once the adjacent edge was reached, the survey team started a new transect 3 m above or below the outmost completed transect. This was repeated until the entire rock slope was surveyed. Eastern Small-Footed Bats were not handled during visual surveys.

Passive acoustic surveys were conducted using Song Meter SM2BAT+ detectors set on zero-crossing/frequency division recording (Wildlife Acoustics Inc., Maynard, Massachusetts). Recordings were started an hour before dusk, and ended an hour after dawn. Talus slopes at DMY and Sherando were acoustically sampled and independence was maintained among detectors. Two detectors were placed on DMY. Both detectors were placed on the forest edge each fastened to a tree at a height of 2 m using bungee cords (The Original Bungee Cord Company, Anaheim, California). One detector was placed on the southeast forested edge of DMY with the microphone facing northwest towards the talus slope. A second DMY detector was placed on the western forested edge facing northeast towards the talus slope. Five detectors were placed at Sherando. Three detectors were placed adjacent to the colluvial field on the forest edge each fastened to a tree at a height of 2 m using bungee cords. One Sherando detector was

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[1] Use of trade, product, or firm names does not imply endorsement by the US government.

placed on the northeastern edge of the talus slope facing southwest towards the talus slope. A second detector was placed on the southernmost edge of the talus slope facing northwest towards the talus slope. The third detector was placed on the southwestern edge with the microphone facing east towards the talus slope. Two additional Sherando detectors were placed on their sides secured with bungee cords to boulders directly on the talus slope. One was placed within the northern one-third of northeastern talus slope roughly 50 m from either forest edge facing east. The other was placed within the middle of the northeastern talus slope 20 m from either edge facing south.

Bat calls were analyzed using Kaleidoscope Pro 2.2.2 software (Wildlife Acoustics Inc., Maynard, Massachusetts) using the U.S. Fish & Wildlife (Ford 2014) standards with sensitivity set at negative 1, signal parameters at 5-120 kHz and 2-500 ms. Minimum pulses were 3 with species classifier pool set to include Eastern Small-Footed Bat, Northern Long-Eared Bat, Little Brown Bat, Indiana Bat, *Eptesicus fuscus* (Big Brown Bat), *Lasiurus borealis* (Eastern Red Bat), *Lasiurus cinereus* (Hoary Bat), and *Perimyotis subflavus* (Tri-colored Bat).

Detection methods were compared by calculating detection probability for each data type using a single-season, single-species occupancy model; detection probability models were fit using program PRESENCE version 8.0 (Hines and McKenzie 2002; MacKenzie et al. 2002). Considering Eastern Small-Footed Bats were known to occur at the two study areas, occupancy (Φ) was fixed to one. An exploratory analysis of an expanded version of the visual detection dataset was performed to examine interpersonal variance in detection rates, also using a single-season, single-species occupancy model.

RESULTS

Visual surveys found 62 Eastern Small-Footed Bats, 10 at Sherando and 52 at DMY during the summer of 2014. No other bat species were found by visual searches in rock crevices at Sherando and DMY. The three-person survey team visually searched 13.5 hours at Sherando and 37.8 hours at DMY for a total of 51.33 hours. Mist-netting efforts captured a total of 39 Eastern Small Footed-Bats between the two sites between the summers of June of 2009 and July of 2014. At Sherando, mist-netting efforts captured 6 Eastern Small-Footed Bats, 10 Northern Long-Eared Bats, 2 Big Brown Bats, 4 Eastern Red Bats, 1 Hoary Bat, 1 Tri-Colored Bat and 2 *Lasionycteris noctivagans* (Silver-haired Bats). At DMY, 33 Eastern Small-Footed Bats were captured. No other bat species were captured at DMY. The time spent mist-netting was 43.22 hours at Sherando and 12.24 hours at DMY for a total of 55.46 hours mist-netting with two people netting (Moosman et al. 2015). Lastly, analysis of the calls recorded by the 5 detectors at Sherando and the 2 detectors at DMY did not yield definitive detection of Eastern Small-Footed Bats in 392 total detector-hours over 7 nights per accepted USFWS acoustic monitoring guidance (U.S. Fish & Wildlife Service 2016b). A total of 4446 echolocation passes at 7 survey points between DMY and Sherando were recorded including 15 Big Brown Bat passes, 183 Eastern Red Bat passes, 21 Hoary Bat passes, 9 Little Brown Bat passes, 927 Northern Long-Eared Bat passes, 24 Tri-Colored Bat passes, and 3267 passes not identified because of poor call quality or insufficient call duration.

Detection probabilities varied among sampling methods. Basal detection probabilities of 91% for visual searches, 75% for mist-netting, and 0% for acoustic surveys were found (Figure 1). Visual detection probability varied among surveyors, but all improved with each subsequent site visit.

DISCUSSION

Visual surveys produced the highest detection probability of any of the sample methods used. It should be noted that mist-netting on the rocks was conducted for 1.5 hours at Sherando and 4.25 hours at DMY rather than sitting with nets open for multiple hours as is typical protocol at both sites when mist-netting corridors.

Prior to this study, research by Coleman et al. (2014) suggested that passive acoustic sampling was more efficacious than active acoustic sampling or mist-netting when surveying for Indiana Bats or Little Brown Bats. Similarly, Murray et al. (1999) noted that passive detection using bat detectors to determine site-level species richness values was typically more effective than mist-netting and generally documented more extant species at a location. Although accurate for the species detected, the general recommendation of passive acoustics by Coleman et al. (2014) and Murray et al. (1999) clearly is not supported for Eastern Small-Footed Bats, at least in or near emergent rock habitats. Eastern Small-footed Bats are challenging to detect acoustically as supported by the lack of acoustic detection at known occupied roosts and the lack of detection by Coleman et al. (2014).

Misidentification by Kaleidoscope Pro 2.2.2 software also could have occurred. There was a large number of Northern Long-eared bat calls identified by Kaleidoscope. Northern Long-eared bats have similar echolocation call characteristics to Eastern Small- footed Bat calls, and it is possible that some of these calls were Eastern Small-Footed Bat calls that were misidentified as Northern Long-eared bats. However, Ford (2014) showed that overall correct classification rates of Eastern Small-Footed Bats generally exceed 90% with low mis-classification overlap for Northern Long-eared Bats – the species we would presume from our findings to have been the plausible source for errors of omission.

A suite of reasons is likely to have contributed to the lack of acoustic detection including variability among detector sites (e.g. vegetative clutter, wind), atmospheric attenuation, frequency and amplitude of the bat, and the directionality of the bat call itself (Griffin 1971; Lawrence and Simmons 1982; Fricke 1984; Fenton et al. 1998; Larson and Hayes 2000; Murray et al. 2001; Scott et al. 2010; Adams et al. 2012). The high frequency echolocation calls of this species (Mukhida et al. 2004) increase the difficulty of its detection, as high frequency echolocation calls attenuate more than lower frequency calls (Griffin 1971; Lawrence and Simmons 1982; Fricke 1984), and emergent rock habitats with complex and angular shapes probably promoted signal reflection that degraded call quality (Winkler and Murphy 1995; Agranat 2014). Approximately 42% of echolocation passes recorded were unable to be assigned to bat species which is strongly indicative poor call quality. Moreover, it also is unknown if Eastern Small-Footed Bats engage in search-phase echolocation over rocks when they emerge. There is ample evidence that bats employ visual cues when navigating (Ellins and Masterson 1974; Horowitz et al. 2004), so it may be that Eastern Small-Footed Bats navigate primarily by sight or memory when exiting roosts and commuting over the large open expanses of talus/colluvial fields to forest edges to commence foraging activity. Because talus slopes are relatively more reflective and less shaded at night as compared to the surrounding forest edge, there may be less need to

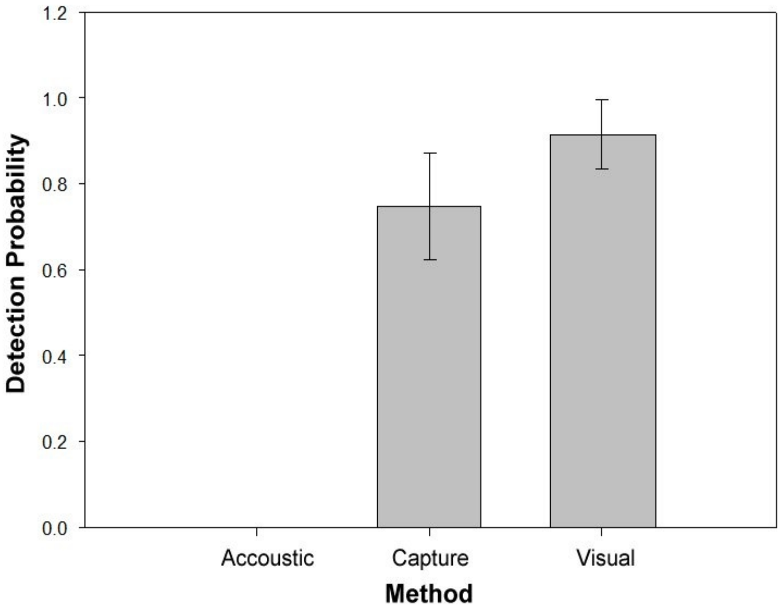

FIGURE 1. Detection probability estimates by method from single-season, single species models for Eastern Small-Footed Bat (*Myotis leibii*) at 2 sites in western Virginia. ‘Acoustic’ refers to passive sampling using acoustic detection meters. ‘Capture’ refers to mist-netting conducted June 2009 and July 2014. Visual searches and acoustic recordings were conducted June-August 2014. Overlapping error bars between ‘Capture’ and ‘Visual’ depict no significant difference between these two methods. Occupancy (Ψ) was fixed to 1 because Eastern Small-Footed Bat were known to exist at the 2 study areas.

echolocate to navigate around the large rock obstacles although this merits additional work to fully demonstrate.

Detection of rare bat species often requires considerable efforts and incurs substantial monetary costs (Weller 2008). Detection of changes in population status of bats also is difficult due to the limited recapture rates (Schorr et al. 2014). Although acoustic monitoring is a more efficient and cost effective tool for estimating occupancy and detection probability than traditional netting, these results strongly suggest that acoustic monitoring Eastern Small-Footed Bat and automated call identification software such as Kaleidoscope may not be the most accurate technique for determining Eastern Small-Footed Bat presence in these habitat types.

Mist-netting is an adaptable bat survey technique, but it is necessary to consider roosting habits, movement and bat ecology to choose the correct deployment strategy that maximizes the chance of capture (Carroll et al. 2002; Brack Jr et al. 2004; Kunz et al. 2009). In the eastern United States, bat assemblages often have been documented using Indiana Bat survey protocols (Winhold and Kurta 2008) leading to possible netting bias. Using Indiana Bat survey protocols reduces the chances of collecting other bat species with disparate foraging habits or habitat associations (Larsen et al. 2007). Currently no such standardized protocol exists for documenting Eastern Small-Footed Bat occurrence.

Visual surveys in this study had the highest detection probability, and had an added utility in that it is relatively non-invasive to examine crevices to determine whether Eastern Small-Footed Bats are present. Visual surveys likely reduce the stress to individual bats because they are not handled. Likewise, visually confirming Eastern Small-Footed Bat presence at roosting sites provides an opportunity to accrue additional data about Eastern Small-Footed Bat day-roost ecology and habitat that otherwise would be impossible to obtain without radio-tracking subsequent to mist-net capture. In addition, visual searches provide the potential for development and deployment of population size estimation and mark-recapture efforts (Moosman and Warner 2014). Success during visual searches varied among observers, but detection probability during visual searches improved with additional site visits. As is supported by cognitive theory, visual searchers become more proficient and efficient with practice (Lawson and Shen 1998). Techniques used in this study are similar to avian nest-searching methods described widely in the literature (Nichols et al. 1986). For example, ornithologists became more efficient at finding nests over time (Powell et al. 2005; Gervasi et al. 2014). Furthermore, increasing the skill of visual searchers would improve cost effectiveness of the technique through reduction in-person hours necessary to denote occurrence at a given location.

CONCLUSION

The results suggest that visual searches are an efficient way to detect and monitor Eastern Small-Footed Bats. The utility of visual searches depends on specific monitoring needs, with visual searches potentially offering a more efficient method, particularly if the objective is to document occurrence and habitat associations of this species. However, detection probabilities for this species probably will vary with the size, configuration and accessibility of the talus slope. Because many aspects of the roosting behavior of Eastern Small-Footed Bats have not been extensively studied, numerous questions remain. Visual searches were effective for the talus slopes we surveyed, but many emergent rock formations in the Appalachians are not conducive to this survey method. For instance, using visual searches of cliffline habitats in the central Appalachians, e.g., New River Gorge in West Virginia, will not be possible without specialized rock climbing equipment, and thus a change in method and additional personnel training. These results highlight the need to continue to refine Eastern Small-Footed Bat survey protocols. Since the use of acoustic monitoring has gained acceptance, and Eastern Small-Footed Bats were listed as a species of greatest conservation need Tier I rank in the Virginia Wildlife Action Plan (Virginia Department of Game and Inland Fisheries 2016), this is particularly relevant for managers relying on acoustics to understand potential biases resulting from false negatives in their surveys.

ACKNOWLEDGMENTS

Funding was provided by the Virginia Department of Game and Inland Fisheries and U.S. Geological Survey cooperative agreement to Virginia Polytechnic Institute and State University, Department of Fish and Wildlife Conservation. We thank the U.S. Forest Service for allowing us to conduct this study. Furthermore, we are grateful to B. Hyzy, E. Thorne, and L. Austin, for the help they provided with the research and data collection. Capture and handling protocol followed the guidelines of the American Society of Mammalogists and was approved by the Virginia Polytechnic Institute and State University Institutional Animal Care and Use Committee (protocol number 11-040-FIW).

Statement of Responsibility: John K. Huth was the primary investigator and involved in all aspects of the project. Alexander Silvis was involved in data collection and running statistical analyses. Paul R. Moosman, Jr. provided mist-net data for comparison with the other methods and editing. W. Mark Ford provided advice about study design, statistical analysis and editing. Sara Sweeten provided guidance and analysis with program PRESENCE.

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