The ecology of eastern small-footed bats (*Myotis leibii*) at Shenandoah National Park, Virginia

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ABSTRACT

Several aspects of rock-dwelling eastern small-footed bat (*Myotis leibii*) ecology remain poorly resolved including the species' local distributions and abundance within their range, maternity habits, and thermoregulatory strategies. Broadly rare, eastern small-footed bats appear to be largely localized to mountainous habitats that feature their preferred roosting habitat, emergent rock features and talus slopes. Following initial reports of mortality from the bat epidemic white-nose syndrome, eastern small-footed bats were reviewed for protection under the endangered species act but were rejected due to lack of significant evidence of declines and overall understanding of their broadscale abundance. In response, a novel method of conducting visual searches of rock outcrops and talus slopes was developed to detect the presence of summer day-roosting eastern small-footed bats. I explored a novel methodology expanding on this technique, whereby I conducted visual searches of talus slopes at Shenandoah National Park, Virginia, modeled bat abundance by topographic variables, and predicted bat abundance to talus slopes throughout the park to talus slopes classified with algorithms utilizing high-definition imagery and a topographic index measuring terrain ruggedness. Eastern small-footed bats were widespread and relatively abundant at Shenandoah National Park, occurring in the majority of sampled talus slopes and topographic conditions. The top abundance model included covariates aspect, elevation, topographic exposure index (TEI), vector ruggedness measure (VRM), and talus area and had a pseudo R-squared of 0.58. Aspect, elevation, VRM, and area positively influenced bat abundance, whereas TEI negatively influenced abundance. Eastern small-footed bats were most abundant in positions with increased solar exposure, which drove model performance. The abundance model estimated 1,330 eastern small -footed bats roosting in talus slopes throughout Shenandoah National Park. I also documented the phenology, roost use, thermoregulatory patterns, and roost and social network dynamics and of a maternity colony of eastern small-footed bats. Eastern small-footed bat females formed a cohesive social network that used rock crevice roosts in a fission-fusion dynamic similar to tree-roosting bat species, exhibiting similar network dynamics centralized to a primary, central node roost. Natality was high and all females were either pregnant or lactating. Parturition occurred synchronously in mid-June. The maternity colony was philopatric to a large talus slope receiving high solar exposure and featuring large boulders and a dense concentration of large crevices. To evaluate thermoregulatory ecology and foraging activity periods, I used temperature-sensing radio transmitters to record bat skin temperatures pregnant and lactating eastern small-footed bats. All bats engaged in torpor every day but pregnant and lactating eastern small-footed bats engaged in distinctly different thermoregulatory patterns, in which pregnant bats generally used shallower torpor and shorter torpor periods compared to lactating bats that more frequently engaged in deep torpor. These regulating and conforming thermoregulatory strategies were similar to observations of little brown bats (*M. lucifugus*) and suggest respective strategies offer energetic trade-offs supportive of each reproductive phase. Pregnant and lactating eastern small-footed bats also exhibited significantly differing foraging periods, in which pregnant bats left day roosts for longer continuous periods, whereas lactating bats left for short, sometimes multiple forage bouts returning to day roosts in between. Differences in foraging periods may reflect the foraging efficiency, maternal responsibilities, and energetic requirements associated with thermoregulatory strategies of each reproductive phase. Both pregnant and lactating eastern small-footed bats passively rewarmed in day roosts, supporting the importance of solar exposure to abundance model. Energetic savings from daily torpor and that likely contributes to their maternity habits, day roost selection, and spatial abundance.

The ecology of eastern small-footed bats (*Myotis leibii*) at Shenandoah National Park, Virginia

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GENERAL AUDIENCE ABSTRACT

Eastern small-footed bats (*Myotis leibii*) are rare, rock-dwelling species whose occurrence and population numbers are not well understood within their range. They were considered for protection as an endangered species following early impacts of a bat disease white-nose syndrome, but they were not listed due to lack of evidence of population declines and overall understanding of the bat's populations and habits. To investigate a means of estimating eastern small-footed bats populations, I searched their preferred habitat, boulder fields, or talus slopes, at Shenandoah National Park, Virginia, counting eastern small-footed bats at each and used these count results to predict how many bats occurred in talus slopes throughout the entire park based on landscape characteristics. I used computer algorithms to automate the classification of talus slopes to facilitate predicting bat numbers to all talus slopes in the park. I found eastern small-footed bats at most talus slopes and was able to predict their population in the park. I found that greater numbers of eastern small-footed bats occurred in talus slopes that received a lot of sunlight and used this relationship to help estimate their numbers. I also observed eastern small-footed bat reproductive habits and found that females group together during their pregnancy and nursing periods of reproduction and used a collection of rock crevices throughout these periods in a similar manner to bats that live in trees, with the group being oriented around a single, most important roost. To save energy during pregnancy and nursing periods, these bats used torpor, or daily periods of light hibernation. They used this strategy differently during these two different times of reproduction to save energy while preventing negative effects on successful reproduction that may go along with using torpor. I also found that eastern small-footed bats relied on the warmth of the sunlight on the rocks in which they dwelled to warm their body temperature. This showed that talus slope locations receiving a lot of sunlight are important to eastern small-footed bats and that this relationship can be helpful in predicting where they occur in greater numbers.

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Chapter 1: Abundance modeling and population estimation of talus-roosting eastern small-footed bats utilizing visual search and talus classification

Abstract

The broadscale abundance and distribution of eastern small-footed bats (*Myotis leibii*) remain unresolved due to the relative ineffectiveness of existing bat censusing methodology given the species' unique habits. Visual searches of their preferred summer day roost habitat, talus slopes, hold promise for informing their occupancy, but have thus far been limited in scope. I explored using visual search results to calibrate a spatial abundance model and geospatial algorithms to classify talus slopes for the purposes of estimating the talus-roosting population of eastern small-footed bats in Shenandoah National Park, Virginia. I observed 144 eastern small-footed bats at 18 of 23 (78%) searched talus slopes. I conducted acoustic surveys at talus slopes in which eastern small-footed bat call activity was identified at all sampled slopes, but call activity results did not correlate with visual search counts ($p = 0.36$) and identification of calls to species was problematic. Talus classification with deep learning algorithms yielded a classification accuracy of 79% and classified 382 talus slopes with *post-hoc* refinement. The top abundance model included covariates aspect, elevation, topographic exposure index (TEI), vector ruggedness measure (VRM), and talus area and had a pseudo R-squared of 0.58. Aspect, elevation, VRM, and area positively influenced bat abundance, whereas TEI negatively influenced abundance. Distance to perennial streams was not significant for predicting abundance. Eastern small-footed bats occupied a variety of topographic conditions but were most abundant in positions with increased solar exposure, which drove model performance. The abundance model estimated 1,330 eastern small footed bats roosting in talus slopes throughout Shenandoah National Park. This novel methodology has potential for quantifying eastern small-footed bat populations using talus slopes and warrants further investigation.

Introduction

Quantifying abundance and spatial variation are essential components of wildlife management and conservation. Topography and landscape characteristics are significant predictors of species presence and abundance for a variety of taxa (Mazerolle and Villard 1999). The relationships between landscape variables and species abundance may be used to calibrate models to predict species abundance at large scales (Forsyth et al. 2009). This technique has potential for improving population estimation and understanding of spatial ecology for elusive species.

The eastern small-footed bat (*Myotis leibii*) is a small, insectivorous, primarily rock-roosting bat that ranges from Ontario and New England south to Georgia and Alabama and west to Oklahoma (Best and Jennings 1997). Often, the local to regional abundance and distribution of eastern small-footed bat populations within this range remain relatively unresolved due to shortcomings of existing survey methodology given the species' unique habits, i.e., summer day-roosting in emergent rock and talus slopes and use of non-traditional hibernacula (Best and Jennings 1997, Moosman et al. 2017, Moosman et al. 2020). Hibernacula surveys, the primary method of estimating bat populations, have historically recorded eastern small-footed bats in very low numbers compared to other species, leading to the conclusion that they are rare in eastern North America (Best and Jennings 1997, Turner et al. 2011). Yet, they have been found to be locally abundant in the Appalachian Mountains during the summer (Beilke et al. 2021, Best and Jennings 1997, Johnson et al. 2011, Johnson and Gates 2008, Kearny et al. 2022, Moosman et al. 2023). Furthermore, emerging research has found eastern small-footed bats do not always overwinter in traditional hibernacula such as caves and mines, and that they may continue to use emergent rock features through the winter as has been documented in Pennsylvania, Virginia, West Virginia, and Maine (Heilakka 2023, Moosman et al. 2017, USFWS 2013), or anthropogenic structures such as a stone culvert as observed in Arkansas (Sasse et al. 2013). If this behavior is common in eastern small-footed bats, hibernacula surveys may not be appropriate for evaluating their broadscale abundance.

An alternative survey method, mist-netting, is generally conducted during the summer maternity season and may inform occupancy and relative abundance trends (Francl et al. 2012, Moosman et al. 2013, Reynolds et al. 2016). However, mist-netting capture results do not allow for abundance estimates.

Attempts to do so are complicated by high variability in probability of detection caused by factors including environmental conditions, net placement, and potential net avoidance resulting from the requisite high sample effort (Francl et al. 2012, Geluso and Geluso 2012, Larson et al. 2007, Marques et al. 2013, Moosman et al. 2020). Moreover, mist-netting in the eastern United States, and specifically in the Appalachians, has been primarily focused on associated summer day-roost forest habitats of the federally endangered Indiana bat (*Myotis sodalis*) and northern long-eared bat (*Myotis septentrionalis*). As such, there has been survey bias against eastern small-footed bats that are most associated with emergent rock features and are rarely encountered in areas without such geologic features (Best and Jennings 1997, Francl et al. 2012, Kearny et al. 2022, Moosman et al. 2015, Whitby et al. 2013). Also, eastern small-footed bats may be particularly effective at detecting and avoiding mist nets, potentially owing to their slow flight speed and high maneuverability (USFWS 2013). Alternatively, acoustic surveys rely on the recording and identification of bat echolocation calls and are now commonly used to identify bat species occupancy and activity trends but may not provide abundance estimates due to complications in associating bat passes with abundance (Ford et al. 2005). Additionally, bat echolocation calls are prone to misidentification by automated software, especially in the case of *Myotis* spp. Moreover, eastern small-footed bats are often not included in call classification analysis due to their unresolved distribution (Britzke et al. 2002, Moosman et al. 2020, Russo et al. 2018).

The eastern small-footed bat was petitioned for listing as an endangered species following initial reports of declines from the bat disease white-nose syndrome (WNS) in light of their historically low hibernacula count numbers, but their listing was rejected based on lack of evidence of significant population decline (USFWS 2013). This led to increased focus on the roosting and occurrence of the species, as well as the development of a novel survey technique, visual search of its rock outcrop and talus slope day-roosting habitat in the summer (Whitby et al. 2013). This technique has been performed in several locations and has proven effective for documenting the presence of eastern small-footed bats in summer day roosts and determining desirable roosting conditions (Kearny et al. 2022, Moosman et al. 2020, Whitby et al. 2013). Most researchers have utilized relatively small plot-based sampling (Kearny et al. 2022, Moosman et al. 2020, Moosman et al. 2023), whereas others have searched entire talus slopes (Whitby et al. 2013). Key findings identified use of rock crevices with narrow openings, preference for talus slopes lacking forest canopy cover, and apparent selection for west- and south-facing slopes and warm roost temperatures (Johnson et al. 2011, Kearny et al. 2022, 2023, Moosman et al. 2023, Whitby et al. 2013). The status of eastern small-footed bat populations following WNS remain unknown throughout much of their range as indications of eastern small-footed bat mortality have been limited and conflicting, whereas other species have experienced population declines exceeding 90% (Francl et al. 2012, Moosman et al. 2013, Turner et al. 2011). Expanding the capabilities of visual searches through the use of abundance models for population estimation may help resolve this knowledge gap and provide for better conservation and management of the species.

For many bat species, including the eastern small-footed bat, day-roost selection for suitable microclimate is critical for thermoregulation (Barclay and Kurta 2007, Chruszcz and Barclay 2002, Lausen 2007, Moosman et al. 2023, Silvis et al. 2016, Solick and Barclay 2006). Solar exposure is a primary contributor to bat day-roost microclimate, particularly in more northern latitudes or in mountainous areas with altitudinal zonation and cooling (Lausen and Barclay 2002, Kunz and Lumsford 2003, O'Keefe 2009). Because the timing and intensity of daily solar exposure are determined by aspect, elevation, topographic exposure, and slope in locations with topographic relief (Evans et al. 2014, Ferry et al. 2024), these conditions have potential to be used as predictors of eastern small-footed bat habitat suitability and broadscale abundance if selection for microclimate is significant.

To investigate relationships of topographic conditions with eastern small-footed bat abundance and evaluate their potential for estimating bat populations, I conducted visual searches for eastern small-footed bats in talus slopes at Shenandoah National Park to develop a generalized linear model with landscape variables to predict their abundance in talus slopes. I leveraged remote sensing algorithms to classify available talus slopes to then predict and estimate the talus-roosting population of eastern small-footed bats throughout the park. I predicted that eastern small-footed bats would be abundant based on recent observations in Virginia, and that greater abundance would occur in west- to south-facing aspects reflecting preference for solar exposure.

Study Area

Shenandoah National Park encompasses approximately 80,600 hectares spanning a narrow stretch along approximately 100 km of the Blue Ridge Mountains in north-central Virginia. The Blue Ridge Mountains were formed, uplifted and then eroded to their present configuration and height over a long and complex geologic history (see Southworth et al. 2009 and Thornberry-Erlich 2014). Erosion resistant gneisses and granites from Mesoproterozoic, greenstones and metasedimentary rocks of the Neoproterozoic, and quartzites and metasedimentary rocks from the Cambrian predominate (Southworth et al. 2009). Shenandoah National Park features an abundance of emergent rock and talus slopes throughout that make it an ideal study site for eastern small-footed bats. Talus slopes at Shenandoah National Park consist of debris fields of rock fractured by periglacial frost-weathering cycles during the Pleistocene ice age (Thornberry-Erlich 2014). Although occur throughout, the southwestern portion of the park features the greatest concentration of rock outcrops and talus consisting of exposed Chilhowee Formation metasedimentary rock (Southworth et al. 2009). Elevation ranges from 170 m to over 1200 m, with the majority of peaks between 600 m and 1200 m (Plummer et al. 2001, Southworth et al. 2009). Local topographic relief averages 600 m and exceeds 900 m in some locations (Southworth et al. 2009, Plummer et al. 2001). More than 95% of the park is forested, with oak-hickory associations comprising the majority of cover, in which dominant tree species include chestnut oak (*Quercus montana*), northern red oak (*Q. rubra*), white oak (*Q. alba*), red hickory (*Carya ovalis*), pignut hickory (*C. glabra*), and red maple (*Acer rubrum*; Young et al. 2009). Cove forests with tulip poplar (*Liriodendrom tulipifera*), sweet birch (*Betula lenta*), and white ash (*Fraxinus americana*) are common in mesic aspects and low elevations. Highest elevations contain northern hardwood and montane northern red oak forests (Young et al. 2009). The crest of the Blue Ridge in Shenandoah National Park divides the Shenandoah River drainage flowing to the west and the James and Rappahannock River systems flowing southeast; perennial streams, as well as ephemeral streams, springs, and seeps are abundant (Plummer et al. 2001, Southworth et al. 2009). Annual temperature at the centrally located Big Meadows (1080 m) averages 8.0° C annually and average annual precipitation is 140 cm (Gawtry and Stenger 2007).

Methods

I used ortho-photo imagery (1 m; NRCS 2006) to manually identify visual search sample locations of talus slope sites in Shenandoah National Park using ArcPro software version 3.2.0 (ESRI, Redlands, CA; Figure 1.1). I sampled talus sites that represented the range of available topographic conditions (i.e. aspect, elevation, etc.) to reduce bias in model calibration (Ford et al. 2016, Waldock et al. 2022). This was accomplished *ad hoc*, in which I selected from talus slopes that were relatively accessible (< 3 km from roads or trails) and opportunistically chose sites of varying covariate conditions. I then surveyed talus sites, whereby two or more researchers systematically navigated up and down transects approximately 5 meters wide, visually searching all rock crevices in the entire area of the slope for roosting eastern small-footed bats with handheld LED flashlights (LuxPro LP600V3, Luxpro, Bluffdale, UT; Moosman et al. 2015; Figure 1.2). I searched 23 talus slopes during the summer (May-August) over the course of 2018-2021. At bat roosts, I recorded point locations with a handheld GPS (Garmin GPSmap 64st, Garmin International, Inc., Olathe, KS), identified the number of bats in each roost, measured the greatest width of crevice openings, and measured the internal roost temperature adjacent to bat position using an infrared thermometer (General IRT102, General Tools and Instruments, Secaucus, NJ).

I deployed two SM4BAT zero-crossing, frequency division acoustic bat detectors with omnidirectional SMM-U1 microphones (Wildlife Acoustics, Concord, MA) at each talus site to record the ultrasonic echolocation calls of bats for a minimum of seven nights to serve as a second method of identification of eastern small-footed bats and allow for comparison of acoustic activity in calls/night with visual search results. I used Kaleidoscope Pro software version 5.1 (Wildlife Acoustics, Concord, MA) using a maximum likelihood estimator threshold of $p \leq 0.05$ to identify bat species nightly activity from recorded echolocation calls (Britzke et al. 2002). I validated selections of calls identified as eastern small-footed bats, as well as other *Myotis* spp., with visual inspection from each talus site to evaluate accuracy of identifications. I used Spearman's rank correlation coefficient in Program *R* (Version 4.2.3, R Core Team 2023) to compare acoustic survey results from each site with visual search results to accommodate non-normal distribution of data

To calculate topographic variables of talus slopes that may influence bat abundance, I calculated mean values of: aspect, elevation, slope, topographic exposure index (TEI; Evans et al. 2014, Ford et al. 2016), and vector ruggedness measure (VRM; Sappington et al. 2007) from a 1-meter resolution digital elevation model (DEM; USGS 2023). Area (m^2) and distance (m) to nearest perennial stream (USGS 2019) were calculated with Geometry Attributes the Zonal Statistics tools in ArcPro, respectively. Elevation of sampled talus slopes ranged from 472 – 1196 m. I measured aspect in degrees azimuth and slope in degrees decline of terrain surfaces as expressed in the DEM (USGS 2023). To linearize aspect from circular degrees, I used a transformation of [1- cosine(aspect)] + [1-sine(aspect)] to depict most mesic northeasterly (45°) aspects as lowest values and most xeric southwesterly (225°) aspects as the highest values (Ford et al. 2002). To calculate TEI, I subtracted the mean elevation of a 1 km circular area surrounding a cell (1 m resolution) from the elevation of that cell (Evans et al. 2014, Ford et al. 2016). The resulting index reflected the exposure of a location relative to its surrounding topography and serves as an indicator of a position's exposure to sunlight, wind, and other biological and climatic conditions (Odom and McNab 2000). Topographic exposure index ranged from -181.3 - 243.2 at Shenandoah National Park and from -99.2 – 172.4 at sampled talus slopes. I used VRM as a measure of fine scale terrain ruggedness and based on the 3-dimensional dispersion of vectors normal to planar grid cell facets calculated at a 15 m neighborhood using Arc Hydro build (version 2.6.26) in ArcPro (Sappington et al. 2007). Vector ruggedness measure at the park ranged from 0.000219 - 0.0018 at sampled talus slopes. Lastly, I measured distance of talus slopes to perennial streams using the National Hydrology Dataset in ArcPro (USGS 2019).

I considered candidate variables aspect, elevation, slope, TEI, VRM, distance to steams, talus slope area, and distance to stream to model bat abundance by individual talus slope using generalized linear models (GLM) with a Poisson distribution. Prior to model building, I tested for collinearity among continuous candidate variables using Pearson's correlation coefficient with package *corrplot* in Program *R* version 3.6.0 (R Core Team 2023, Wei and Simko 2017) to avoid inclusion of highly correlated ($r \geq 0.6$) predictors in the model that may bias model performance (Barr et al. 2021). I centered and scaled all covariates and created GLM models using package *glmmTMB* (Brooks et al. 2017). I selected the top model with backwards-stepwise selection, evaluating models with AIC with the *AICcmodavg* package (Mazerolle 2023) and McFadden's R-squared in Program R (R Core Team 2023). To evaluate induced collinearity in the model, I calculated variance inflation factors for covariates using the package *car* (Fox 2002).

To classify available talus slope habitat in the entirety of Shenandoah National Park, I automated identification in ArcPro using HRNet and MMSegmentation within a deep learning model utilizing high-definition 3-band orthophotography (0.305 m resolution; VGIN 2022) and VRM (15 m; Smith and Heinemeyer 2017). This model was trained with a composite 4-band raster of 50 manually digitized talus slope polygons, including 22 of the visually searched sites. The learning rate utilized a stochastic gradient descent and training included 50 epochs. To eliminate paved and human-developed areas that were misidentified as talus, I removed identified polygons that were within 10 m of roads and that intersected with buildings as identified with Virginia Base Mapping road and buildings layers (VGIN 2024). I assessed accuracy of the identified talus polygon layer by generating 100 random points and manually assessing accuracy of each point as talus or non-talus with high resolution imagery (VGIN 2022). To avoid bat abundance prediction inaccuracies (Waldock et al. 2022), I trimmed identified talus polygons whose landscape covariate values were outside the range of one standard deviation above or below covariate values observed in sampled, visually searched talus slopes.

For all resulting classified talus slopes, I calculated landscape variables using the same methods for model calibration and used the top bat abundance GLM to predict number of bats for each identified talus slope by significant covariates to achieve an estimate of talus-roosting eastern small-footed bats at Shenandoah National Park.

Results

I observed 144 eastern small-footed bats at 18 of 23 (78%) searched talus slopes (Figure 1.3; Table 1.1). Mean number of bats per talus site was 6.26 ± 6.42 and mean density was 22.25 ± 30.78 bats/ha. Bats were located in a total of 66 roosts, with 49 roosts of individual bats, and the other 17 roosts containing 2-15 bats (mean = 5.59 ± 3.80 bats). Mean roost crevice width was 1.43 ± 0.55 cm. Mean roost temperature was 30.66 ± 5.76 °C (87 ± 10.37 °F) and ranged from 14.4 – 45.3 °C (57.9 -113.5°F).

Acoustic surveys recorded echolocation calls identified as eastern small-footed bats at all 23 talus slopes with calls per night averaging 25.08 ± 27.02 (Table 1.1). Detectors recorded bat echolocation calls for 416 detector-nights (mean = 9.7 ± 0.8) from 44 sample sites, two per talus slope with the exception of two sites in which detectors either malfunctioned or were disturbed. Nightly echolocation call activity did not correlate with visual search counts ($p = 0.36$) and varied greatly between individual detectors within the same slope. Automated bat echolocation software identified numerous calls as other *Myotis* spp., i.e., the northern long-eared (*Myotis septentrionalis*), little brown (*Myotis lucifugus*), and Indiana bat (*Myotis sodalis*). However, upon visual review, the majority of these were in fact, attributable to eastern small-footed bats.

The talus classification model identified 498 talus polygons, excluding polygons removed that were adjacent to roads and intersecting buildings. Precision of polygons with respect to talus borders were highly accurate, with some exceptions (Figure 1.4). The accuracy assessment of classified talus polygons resulted in a pixel classification accuracy rate of 79%. Removal of polygons that contained topographic conditions outside of one standard deviation above and below the minimum and maximum of covariate values observed in sampled talus resulted in the removal of an additional 116 polygons. This step was only applicable in terms of slope and VRM and yielded a final classification of 382 talus slope polygons.

The top model for bat abundance included aspect, elevation, TEI, VRM, and talus area and had a McFadden's R-squared of 0.58 (Table 1.2). Slope was not included in the top model but was significant in competing models. Distance to stream was not significant ($p = 0.86$). Aspect, elevation, VRM, and area positively influenced abundance, whereas TEI had negatively influenced bat abundance (Figure 1.5; Table 1.3). Linear fits performed best for all covariates. Variance inflation factors were low for all covariates, indicating low induced collinearity (Fox 2002).

Predicting abundance to the 382 classified talus slopes using the GLM yielded an overall estimate of 1,330 eastern small-footed bats in Shenandoah National Park. Mean number of bats per slope was 3.48 ± 3.85, with the number of bats per talus slope ranging from 0-44 (Figure 1.6).

Discussion

Visual search of talus slopes, talus slope classification, and bat abundance modeling and prediction appear effective and may represent important steps forward in quantifying eastern small-footed bat distribution and abundance. Eastern small-footed bats were widespread and relatively abundant in talus slopes at Shenandoah National Park and their abundance was influenced by aspect, elevation, TEI, and VRM, and talus slope area. These relationships allowed for an ecologically informed population estimation. Abundances of eastern small-footed bats recorded with visual searches and the accompanying population estimate suggest impacts from WNS have not been severe at Shenandoah National Park relative to other *Myotis* species in the eastern United States (Turner et al. 2011), although prior data are lacking.

Visual searches successfully documented the presence of eastern small-footed bats in talus slopes similar to findings of others (Moosman et al. 2015, Kearny et al. 2022, Whitby et al. 2013). Bat abundance varied greatly by talus site and roosts were often located in clusters, indicating that eastern small-footed bats tended to roost in close proximity to other bats even when not sharing roosts (Moosman et al. 2023). This suggests an advantage of searching entire talus slopes when feasible for the purposes of population estimation as opposed to plot-based searches that may produce more biased results (i.e., over- or underestimating populations depending on if such aggregations are observed in plots; Moosman et al. 2015). Observed bats occupied very narrow cracks amid rocks and crevices between rocks (Kearny et al 2023, Moosman et al. 2023; Figure 1.3). Use of narrow rock roosts has been attributed to predator avoidance but also facilities solar thermoregulation (Chruszcz and Barclay 2002, Moosman et al. 2023). I observed that bats inhabiting narrow roosts maintain close contact with rock surface, often dorsally and ventrally, which supports efficient heat transfer. Internal roost temperature was generally high, but searches took place during different times of day, which greatly affects roost temperature (Chruszcz and Barclay 2002, Moosman et al. 2023).

Acoustic detectors recorded eastern small-footed bat echolocation activity at every talus slope sampled, and activity was not correlated with search counts. Bat call activity can be attributable to a variety of behaviors including foraging or commuting and not necessarily an indication of day-roosting, which may account for activity where bats were not found day-roosting during visual searches. Of course, it is also likely visual searches did not identify observable bats and/or bats that were unobservable because they were either roosting below the surface layer of rocks or inhabiting microsites of cracks that were not visible (Moosman et al. 2020, Solick and Barclay 2006), thereby contributing to discrepancies between visual search and acoustic results. During radio telemetry tracking for Chapter 2 objectives, I found that several eastern small-footed bats roosted below the surface layer of rocks that would have been unobservable with visual searching. Acoustic activity varied significantly between individual detector locations in the same slope. Some detectors that recorded high activity were close to observed roosts containing multiple bats, so proximity to occupied roosts may have been the greatest contributor to call activity. To further complicate acoustic survey results, echolocation call identification can be problematic with high rates of misidentification for the genus *Myotis* (Britzke et al. 2002, Russo et al. 2018). This was apparent during manual review of calls in which I found eastern small-footed bat call activity to be greatly underrepresented due to misidentification as other *Myotis* species. Northern long-eared and little brown bats do occur at Shenandoah National Park but are captured at much lower rates compared to eastern small-footed bats, whereas the presence of Indiana bats remains possible but is yet unconfirmed by physical capture (Kalen et al. 2022, Kalen et al. 2024). Considering the classification errors encountered during manual review, acoustic results should be interpreted with caution in regard to eastern small-footed, little brown, and federally endangered northern long-eared and Indiana bats, especially in mountainous areas where they are sympatric.

Classification of talus slopes resulted in relatively high accuracy, which improved with *post-hoc* refinement, and these methods have potential applicability in other locations with similar geology in the context of eastern small-footed bats or other species that utilize talus slopes (Smith and Heinemeyer 2017). Initial results included misclassification of paved parking lots and similar structures, which required removal. Other sources of error included misidentification of non-talus rocky areas in coves and stream channels and, oddly, portions of grasslands. Conveniently, both scenarios were largely resolved by trimming classified talus for which covariate values exceeded one standard deviation of sampled talus distributions. This step was only relevant to two covariates, slope and VRM. This improved classified talus accuracy by removing errors associated with misidentified grasslands that inherently had lower slopes and non-talus rocky cove sites that had higher VRMs than this threshold.

The top abundance model fit sample data reasonably well, explaining just over half (58%) of bat variance observed in searched talus slopes. Landscape covariates aspect, elevation, TEI, VRM, and talus slope area were highly significant. Aspect had the strongest positive effect on bat abundance in the model resulting from many sampled slopes from south- to west-facing aspects (~180-270°) having higher bat abundances compared to those facing north to southeast (Figure 1.6). Linear transformation of aspect degrees facilitated linear modeling of this relationship (Ford et al. 2002). Preference for more xeric aspects by eastern small-footed bats corresponds with findings of others in the central Appalachians of Virginia and West Virginia (Johnson and Gates 2008, Johnson et al. 2011, Moosman et al. 2023) as well as the Ouachita Mountains of Arkansas (Kearny et al. 2022). However, associations observed by others were somewhat confounded by the availability of talus slope aspects, which were reported as predominately south- or west-facing (Johnson et al. 2011, Kearny et al. 2022, Moosman et al. 2023). Classified talus slopes at Shenandoah National Park indicate they are more evenly distributed in terms of aspect (Figure 1.7). Greater availability of a variety of aspect positions and inclusion of a range of these aspects in the visual search sample set provide a more equitable comparison and support abundance model results. Preference for day-roosting in south- and west-facing slopes has been attributed to thermal benefits of pronounced and extended solar exposure, especially in rock-roosting species (Moosman et al. 2023, Solick and Barclay 2007). Radiant warming of roosts not only reduces energetic cost of thermoregulation in metabolically active bats, but temperature fluctuations of rock roosts support torpor as cool temperatures facilitate entering torpor, then increasing temperatures passively warm bats, thereby providing normothermic body temperatures throughout much of the day at minimal energetic expense (Chruszcs and Barclay 2002, Moosman et al. 2023). Selection for such roost microclimates regimes appears to be nuanced and complex, with bats seeking optimal ranges of conditions to balance energy savings via torpor and evaporative water loss with requisite body temperature and water for biological processes, which vary greatly depending on sex and reproductive condition (i.e., pregnant, lactating, post-lactating; Chruszcs and Barclay 2002, Moosman et al. 2023, Solick and Barclay 2006, Solick and Barclay 2007). Furthermore, intensity, timing, and duration of solar exposure are also influenced by elevation, slope, and topographic exposure in mountainous localities (Ferry et al. 2024). So, it is reasonable that greater abundances occurred in a range of aspects with increased solar exposure.

Eastern small-footed bat abundance at Shenandoah National Park was positively associated with elevation, similar to what others have observed with regard to occupancy (Kearny et al. 2022, Moosman et al. 2015, Thomson et al. 2013). This also implies selection for solar radiation, which can be greater in higher elevations amid topographic relief (Blumthaler et al. 1997). Paradoxically, TEI was negatively associated with bat abundance as greatest abundances occurring in semi-sheltered slopes. This was unexpected as more sheltered locations generally receive less sunlight (Evans et al. 2014, Ferry et al. 2024). These combined results may be indicative of selection for roost conditions that are sheltered enough to delay solar exposure early in the day, potentially supporting low, torpid body temperatures and inhibiting overheating and evaporative water loss (Moosman et al. 2015, Solick and Barclay 2007), but high enough in elevation to receive sunlight and thermal benefits in the afternoon and evening leading up to nightly arousal and activity. Talus slopes at Shenandoah National Park do appear to be limited to moderately sheltered exposures as indicated by the TEI of predicted talus slopes, so availability of exposure may have contributed bias to the observed relationship with abundance (Figures 1.8 and 1.9). It is logical that colluvial depositions that comprise talus slopes would be largely limited to moderately exposed, often side slope positions, considering the geological process of talus slope formation which includes falling and tumbling of eroded rocks (Thornberry-Erlich 2014).

Bat abundance trended positively with VRM. Higher VRM values are indicative of more uneven surfaces and associated with the presence of larger rocks (Smith and Heinemeyer 2017). Larger rocks provide larger roost crevices that support greater groups of eastern small-footed bats (i.e. maternity colonies) and are more thermally stable (Moosman et al. 2023). Conversely, males most often roost solitarily and prefer smaller roosts crevices more aligned with smaller rocks (Moosman et al. 2023), more even surfaces, and lower VRM values.

Area of talus slope was positively associated with bat abundance, but I did observe high variance in this relationship. Nevertheless, area was highly significant and coarsely represents the number of available day roosts (Moosman et al. 2020). Larger talus slopes with more available roosts likely support larger social groups (i.e. maternity colonies) and frequent roost switching of eastern small-footed bats (Johnson and Gates 2008, Johnson et al. 2011, Moosman et al. 2023).

Slope was not included in the top model but was negatively associated with bat abundance in competing models, with greater abundances occurring on more gradual slopes. Slope gradient determines the solar angle of incidence on surfaces, with cumulative daily solar radiation increasing with decreasing slope during summer between latitudes of 30-40°N, which would translate to overall warmer roost temperatures (Buffo et al. 1972, Ferry et al. 2024, Lausen 2007).

Distance to streams was not a significant predictor. Kearny *et al.* (2022) also considered proximity to perennial streams in the context of predicting eastern small-footed bat occupancy and attributed the lack of significance to both positive correlations with high elevations and access to closer ephemeral water sources as posited by Johnson *et al.* (2011). This is likely also the case at Shenandoah National Park, where I observed selection for higher elevations, and upland water sources are abundant, including springs, seeps, and small intermittent streams that are not represented in hydrology data (Plummer et al. 2001, USGS 2019). Distance to perennial streams may have greater significance in more xeric climates (Solick and Barclay 2007).

Conventionally, species abundance models based on environmental conditions are believed to rely on the assumptions of niche theory, in which gradients of the environmental space drive species abundance through mechanisms that affect population demographics (Holt 2009, Waldock et al. 2022). It appears the eastern small-footed bat abundance model reflects a top-down perspective in that this highly mobile species variably selects environmental conditions, specifically topographic positions. I believe this is driven by sex-based roost temperature regime selection evidenced by greater presence of social groups in conditions favorable to increased solar exposure. Searched talus slopes with higher abundances (≥ 7 bats) always included groups of three or more bats, indicative of maternity colonies that select for roosts with higher and more stable temperatures (Moosman et al. 2023). Roost selection is widely recognized as one the most important factors affecting bat species distribution, although most work has concerned arboreal species (Barclay and Kurta 2007, Bellamy and Altringham 2015, Ford et al. 2016, Kunz 1982, Silvis et al. 2016). Others have suggested the importance of roost selection for thermal conditions by eastern small-footed bats (Johnson et al. 2011, Kearny et al. 2023, Moosman et al. 2023), and my results suggest this relationship may be used to predict their abundance in topographically heterogenous landscapes.

Limitations to this methodology include an overall low sample size for training the abundance model, an inherent assumption of closed populations within individual talus slopes, and lack of consideration for imperfect probability of detection or interannual fluctuations of slope abundances. There is most certainly movement between talus slopes that affects individual talus slope abundances that I used to calibrate the model. I expect that individual slopes are not closed populations *per se*, but eastern small-footed bats do exhibit high fidelity to day-roosting areas, moving only short distances between roosts, and occupying small home ranges, so the effects of movement between slopes on my results may not be great (Johnson et al. 2011, Johnson and Gates 2008, Moosman et al. 2023). My sample size of 23 talus slopes represents approximately 16% of the 382 talus slopes classified at Shenandoah National Park, and a larger sample set would improve model and prediction accuracy. Although I found bats to be generally observable often roosting close to crevice openings, undoubtedly bats were missed during searches (Moosman et al. 2020). In a brief single-blind examination of probability of detection for visual search, Moosman *et al.* (2020) found researchers positively identified 7 of 11 (64%) known eastern small-footed bats tracked with radio telemetry. Of missed bats, two were overlooked and two roosted in locations that were not visible. I expect that I had a similar probability of detection that skewed visual search results yielding an underestimate of the population. Visual searches took place over four summers so interannual fluctuations in abundance likely biased my prediction as well. Moosman *et al* (2020) observed relatively low interannual abundance variation within plot-based surveys, but this was based on very limited data. Because demographic rates of birth, death, immigration, and emigration were not addressed in this study, their effects on abundance estimates are unknown.

Abundance predictions to classified talus polygons were similar to the range of observed abundances in the sample set and appear to be reasonable considering the visual search results (Figure 1.10). Individual slope estimates are likely relatively imprecise considering limitations, but collectively they provide an informed estimate of the talus-roosting summer population of eastern small-footed bats at Shenandoah National Park for which there is presently no alternative. This estimate is likely conservative considering factors such as imperfect detection of visual search and talus slopes that may not have been classified. It is important to note that my population estimate considers only bats day-roosting in talus slopes and does not consider bats utilizing non-talus rock roosts such as cliffs or other emergent rock formations or anthropogenic structures that would be additive to the overall population (Best and Jennings 1997, Johnson and Gates 2008, O'Keefe and LaVoie 2011). I did observe eastern small-footed bats using cliffs and other non-talus roosts while radio-tracking bats for other objectives, further indicating that the total population at Shenandoah National Park invariably is greater than my estimate for talus-roosting bats.

This methodology may offer a useful alternative for quantifying eastern small-footed bat distribution and abundance, yet further research is needed. Visual searches and abundance modeling should be conducted in other locations with differing geologies to evaluate their effectiveness beyond this study site and improve understanding of eastern small-footed bat distributions in other portions of their range. Investigating interannual fidelity to individual slopes and various spatial scales may reveal implications on estimating populations from visual search results. Investigating eastern small-footed bat occurrence in non-talus roosts such as cliffs and other emergent rock formations would inform population estimates beyond those utilizing talus slopes and reveal roosting ecology useful in other portions of Appalachians and elsewhere within its range where talus is rare. Also, evaluating wintering behavior of eastern small-footed bats outside of traditional hibernacula and the extent to which this occurs is necessary, as their population estimates are currently based on hibernacula counts (Heilakka 2023, Moosman et al. 2017, USFWS 2013).

Eastern small-footed bats are globally endangered according to the International Union for Conservation of Nature and are considered species of conservation interest in several states (Solari 2018) However, they have not received federal protection in the United States because their populations and subsequent impacts of WNS have not been sufficiently quantified (USFWS 2013). Yet they continue to face threats from WNS, as well as habitat loss and degradation from mining, quarrying, shale gas extraction, and other development that may impact talus habitats on which they are reliant (Francl et al. 2012, Moosman et al. 2013, Kearny et al 2022, Turner et al. 2011, USFWS 2013). Large, high elevation, semi-sheltered talus slopes of more xeric aspects that large boulders appear to support increased abundances of eastern small-footed bats and constitute ideal habitat. Rarity of eastern small-footed bats throughout their range increases the importance of identifying and conserving locally abundant populations, and emerging tools in geospatial science can support existing techniques to accomplish this and warrant further investigation.

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Table 1.1. Visual search results of eastern small-footed bats (*Myotis leibii*) observed at talus slopes, number of predicted bats by spatial abundance model, talus slope area in hectares, density in bats per hectare, and nightly bat activity in mean calls per night from acoustic bat detectors from 2018-2021 in Shenandoah National Park, Virginia. Detectors with no values either malfunctioned or were disturbed. Bat calls were identified using Kaleidoscope Pro version 5.1 requiring a nightly MLE of ≤ 0.05 to qualify call identification.

Site	Observed	Predicted	Area (ha)	Bats/ha	Detector 1	Detector 2
TALUS01	4	5	0.35	11.4	24.3	9.7
TALUS02	12	10	0.39	30.8	20.9	12.3
TALUS03	0	3	0.24	0	16.3	22.3
TALUS04	14	12	0.26	54.7	15.7	117.2
TALUS05	9	13	0.17	53.9	17.7	126.3
TALUS06	21	19	0.15	140.6	25.1	6.9
TALUS07	23	15	0.81	28.2	9.8	11.1
TALUS08	8	14	1.16	6.9	2.2	5.4
TALUS09	6	1	0.08	73.2	8.4	8.7
TALUS10	3	2	0.13	23.1	3.5	2.3
TALUS11	0	3	0.19	0	-	1.3
TALUS12	0	1	0.47	0	2.2	2.1
TALUS13	2	4	0.53	3.7	0.86	4.3
TALUS14	5	1	0.32	15.8	1.4	2.3
TALUS15	4	2	0.54	7.4	29.9	38.3
TALUS16	14	9	0.44	32.0	5.3	12.9
TALUS17	0	4	0.44	0	13.7	16.2
TALUS18	3	2	0.45	6.7	21.1	15.1
TALUS19	1	5	0.11	8.9	43	96
TALUS20	7	7	0.28	24.9	86.3	41.3
TALUS21	0	1	0.21	0	4.3	2.25
TALUS22	5	4	0.71	7.0	3.7	-
TALUS23	3	4	0.24	12.2	84.1	91.5

Table 1.2. Comparison of generalized linear models for predicting eastern small-footed bat (*Myotis leibii*) abundance at Shenandoah National Park, Virginia from visual search results conducted from 2018-2021. Generalized linear models are ranked by Akaike Information Criterion (AIC) and AIC weight. Model covariates are talus slope mean aspect (linear transformation), elevation, vector ruggedness measure (VRM), slope, topographic exposure index (TEI), and talus slope area.

Model	**K**	**AIC**	**ΔAIC**	**Model Likelihood**	**AIC Weight**	**LL**	**Weight**
Area + Aspect + Elevation + TEI + VRM	6	145.7	0.0	1.000	0.565	-64.23	0.565
Area + Aspect + TEI + VRM	5	147.4	1.68	0.430	0.243	-66.94	0.808
Area + Aspect + Elevation + Slope + TEI + VRM	7	148.6	2.86	0.239	0.135	-63.56	0.943
Area + Aspect + Slope + TEI + VRM	6	150.9	5.19	0.075	0.042	-66.83	0.985
Area + Elevation + Slope + TEI + VRM	6	153.9	8.14	0.017	0.010	-68.30	0.995
Null	1	217.6	71.9	<0.0001	<0.0001	-107.72	1.000

Table 1.3. Parameters coefficient, coefficient standard error, *Z*-value, variance inflation factor (VIF), and *P*-value for the top generalized linear model of eastern small-footed bat (*Myotis leibii*) abundance at talus slopes in Shenandoah National Park, Virginia based on visual search results from 2018-2021. Covariates are centered and scaled and include talus slope mean aspect, elevation, vector ruggedness measure (VRM), topographic exposure index (TEI), and talus slope area.

	Estimate	**Std Error**	***Z* - value**	**VIF**	***P* - value**
Intercept	1.46	0.11	12.7	-	<0.00001
Aspect	0.457	0.12	3.90	1.26	<0.00001
Area	0.330	0.08	4.06	1.54	<0.00001
VRM	0.301	0.08	3.55	1.31	0.0004
Elevation	0.282	0.12	2.29	1.95	0.021
TEI	-0.612	0.13	-4.57	2.82	<0.00001

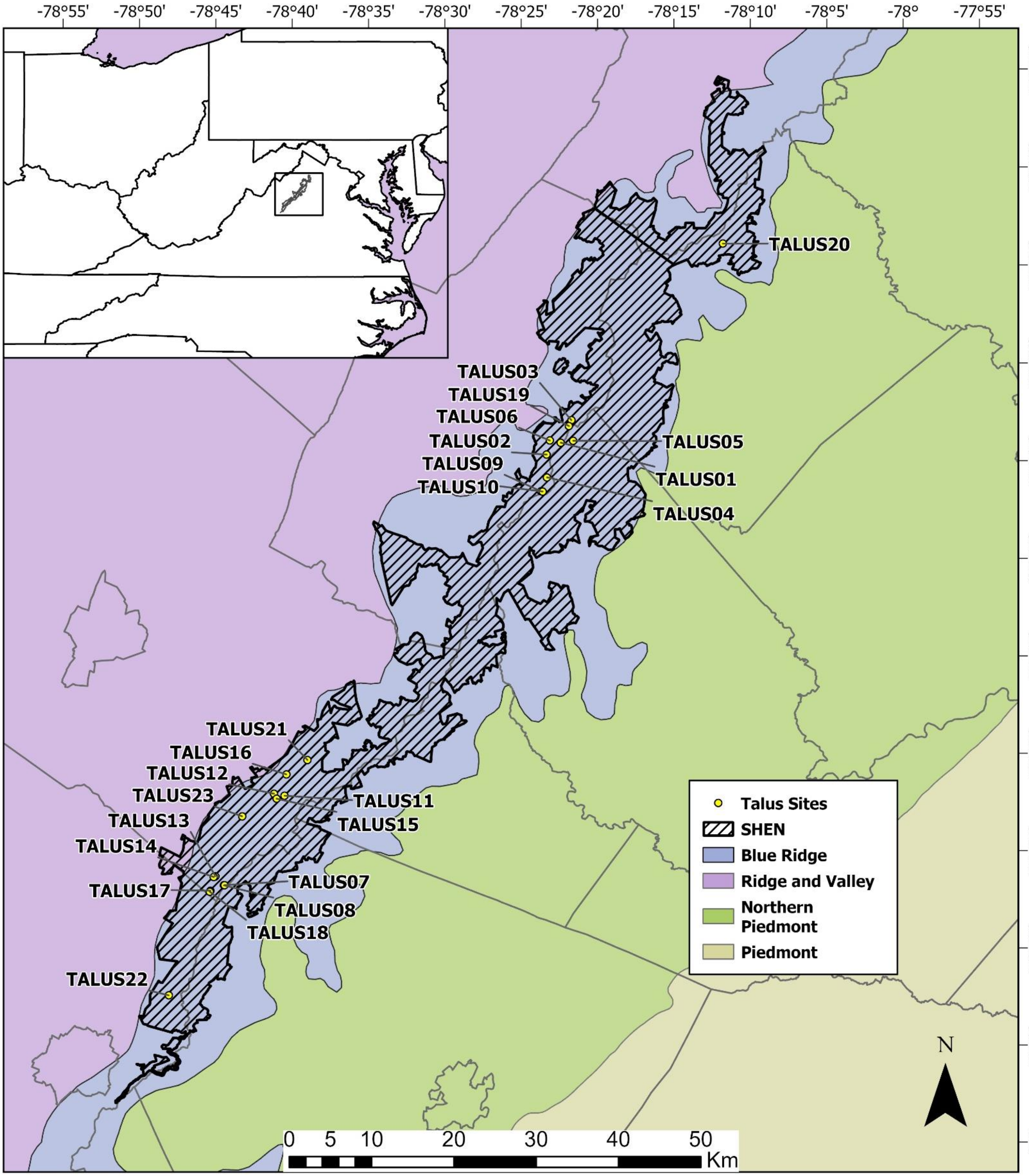

Figure 1.1. Talus slope sites at Shenandoah National Park, Virginia where visual searches for eastern small-footed bats (*Myotis leibii*) were conducted from 2018-2021.

Figure 1.2. Representative talus slope surveyed with visual searches for eastern small-footed bats (*Myotis leibii*) at Shenandoah National Park, Virginia from 2018-2021.

Figure 1.3. Day-roosting eastern small-footed bats (*Myotis leibii*) at Shenandoah National Park, Virginia observed during visual searches on July 2, 2019.

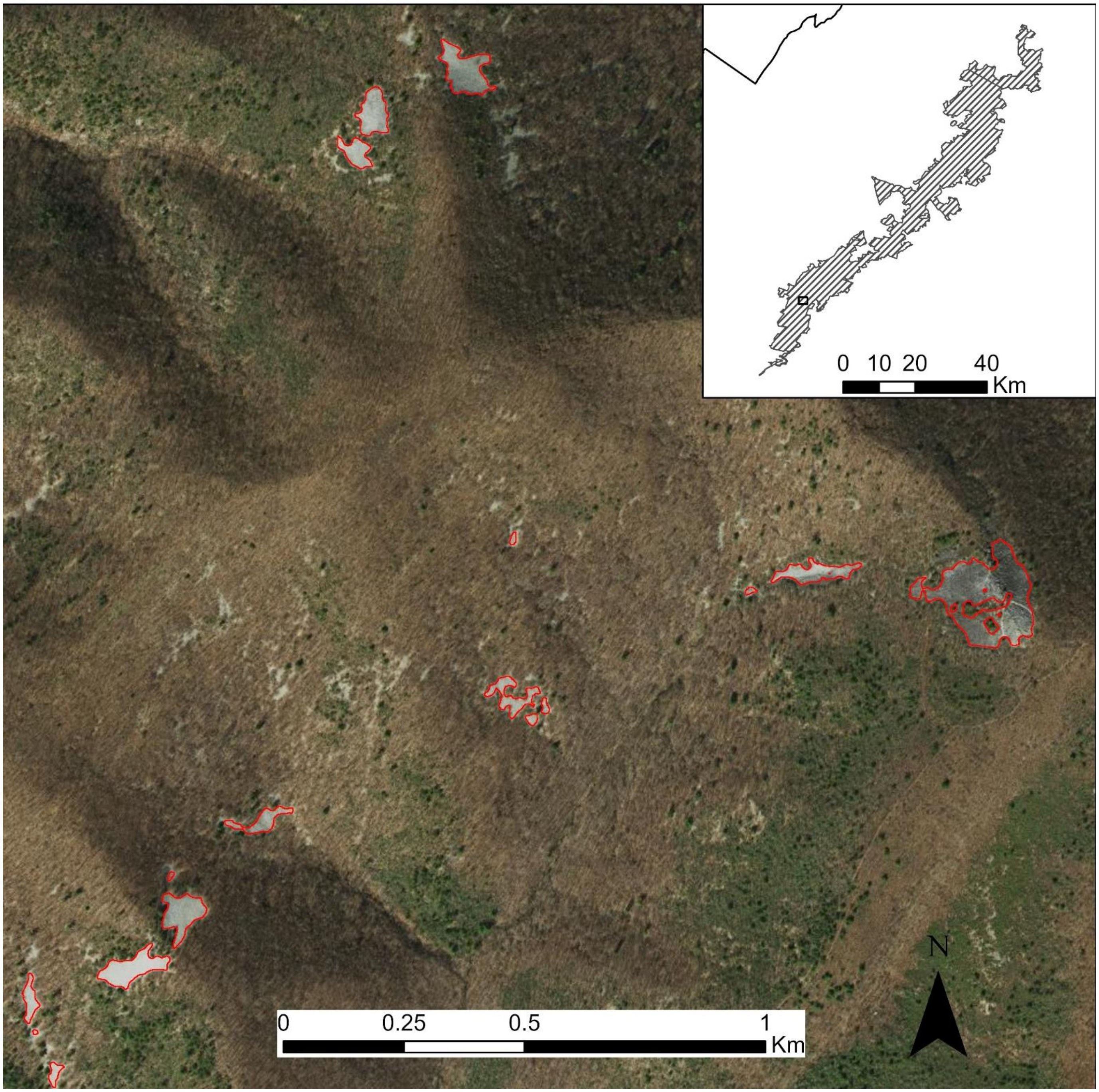

Figure 1.4. Talus slope polygons (in red border) classified with ESRI deep learning in ArcPro software version 3.2.0 (ESRI, Redlands, CA) trained with high resolution imagery (0.305 m; VGIN 2024) and vector ruggedness measure at Shenandoah National Park, Virginia.

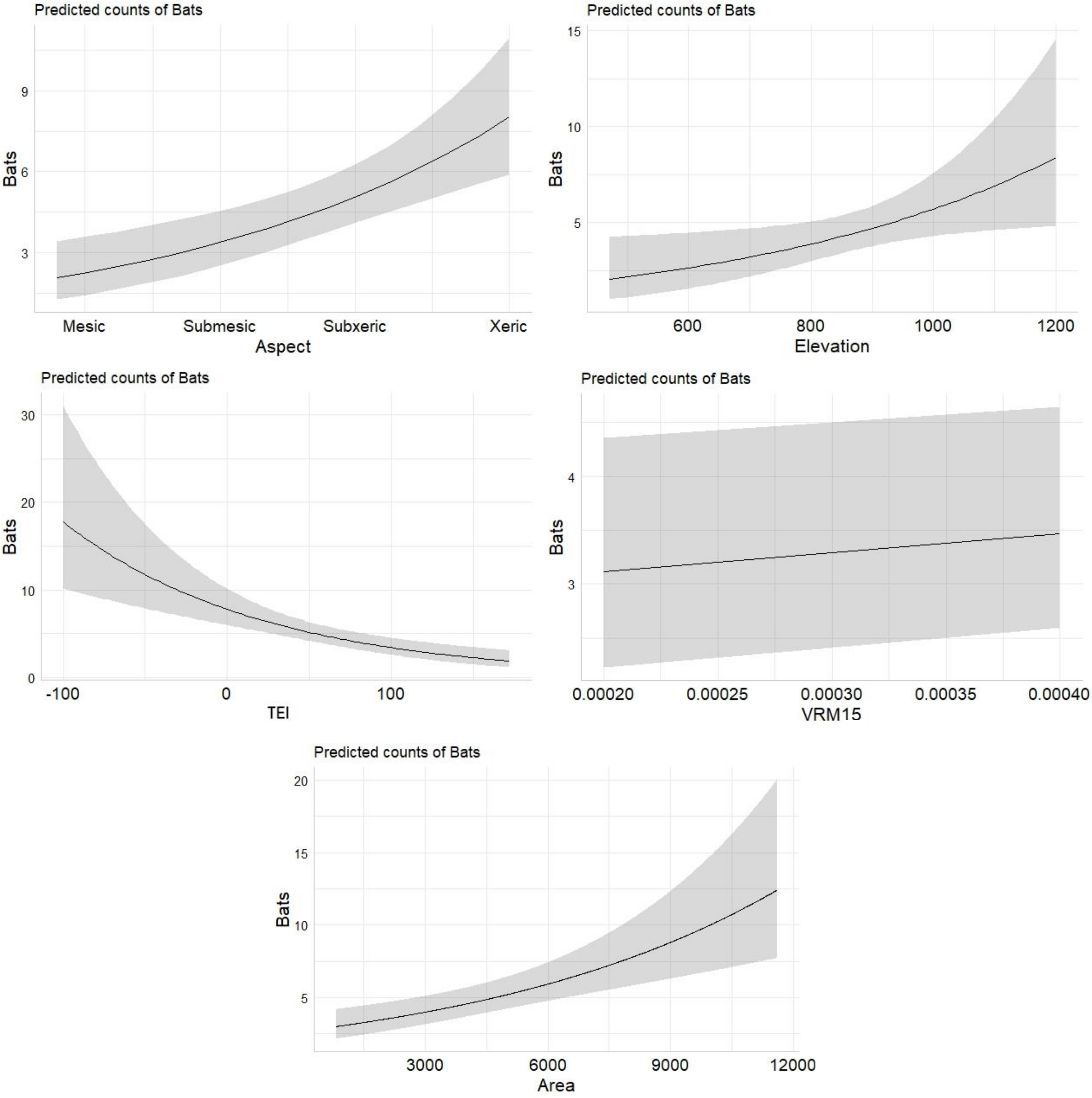

Figure 1.5. Fixed effects plots of eastern small-footed bat (*Myotis leibii*) abundance per talus slope for the top generalized linear model by covariates aspect (linearized transformation), elevation (m), topographic exposure index (TEI), vector ruggedness measure (VRM), slope (degrees), and area (m^2) based on visual search observations at Shenandoah National Park, Virginia from 2018-2021. Aspect is transformed with a sinusoidal function to linearize the relationship of most mesic (45°) to most xeric (225°) exposures.

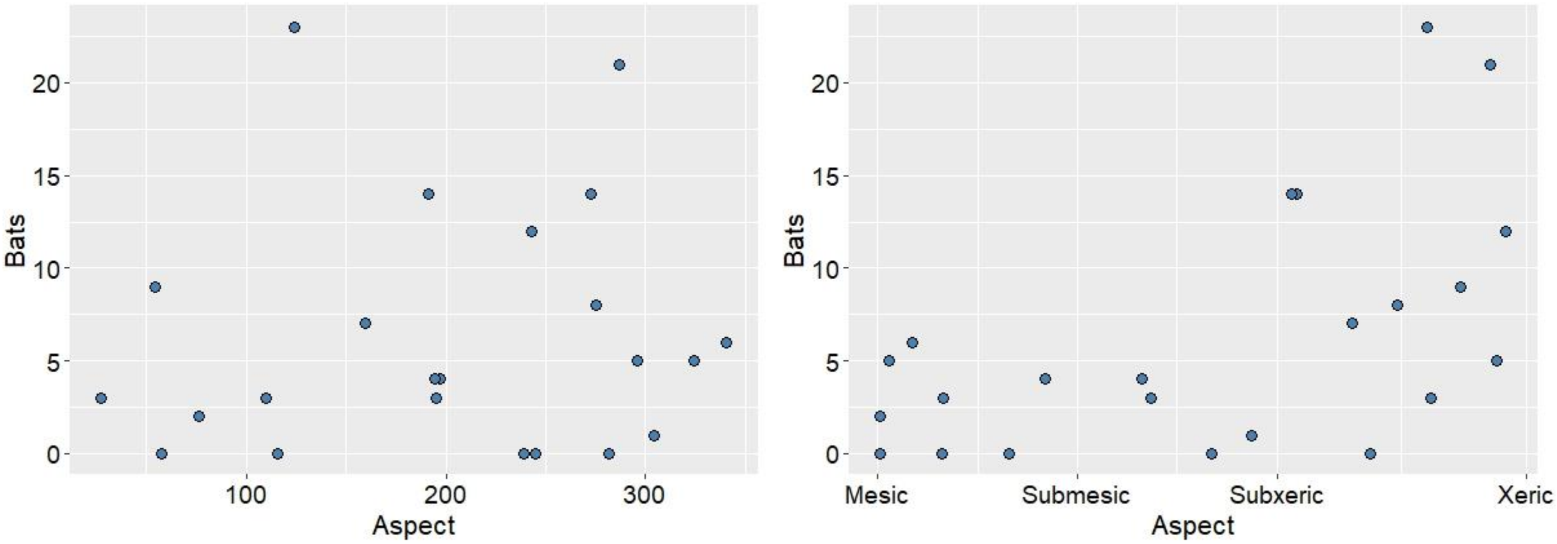

Figure 1.6. Visual search results of number of eastern small-footed bats (*Myotis leibii*) by aspect in degrees and linearized transformation at talus slopes from 2018-2021 at Shenandoah National Park, VA. Aspect is transformed with a sinusoidal function to linearize the relationship of most mesic (45°) to most xeric (225°) exposures.

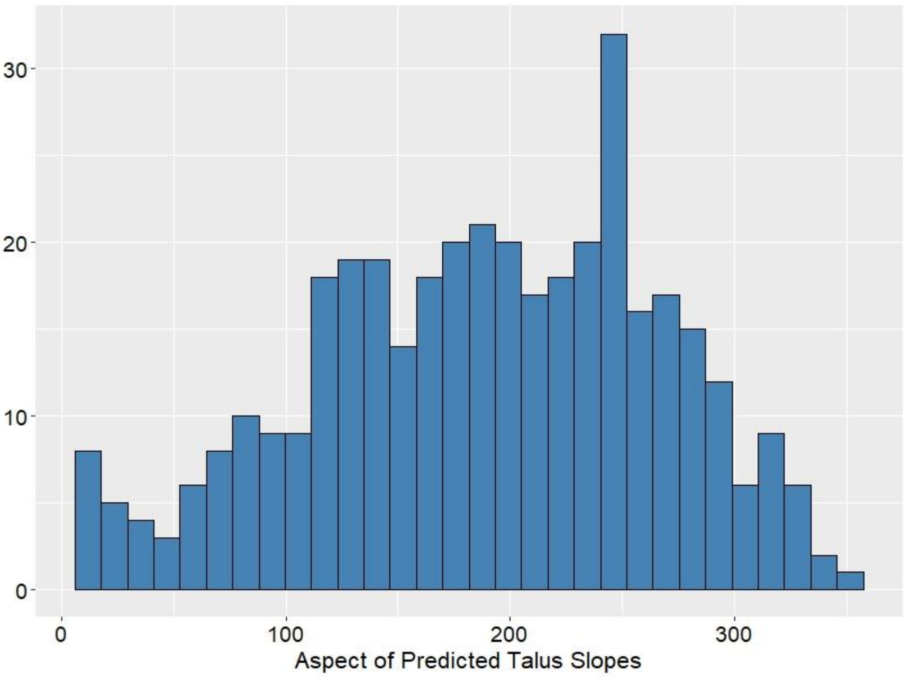

Figure 1.7. Histogram of aspects in degrees of talus slopes predicted with at Shenandoah National Park, Virginia.

Figure 1.8. Visual search results of number of eastern small-footed bats (*Myotis leibii*) by topographic exposure index (TEI) in talus slopes from 2018-2021 at Shenandoah National Park, Virginia.

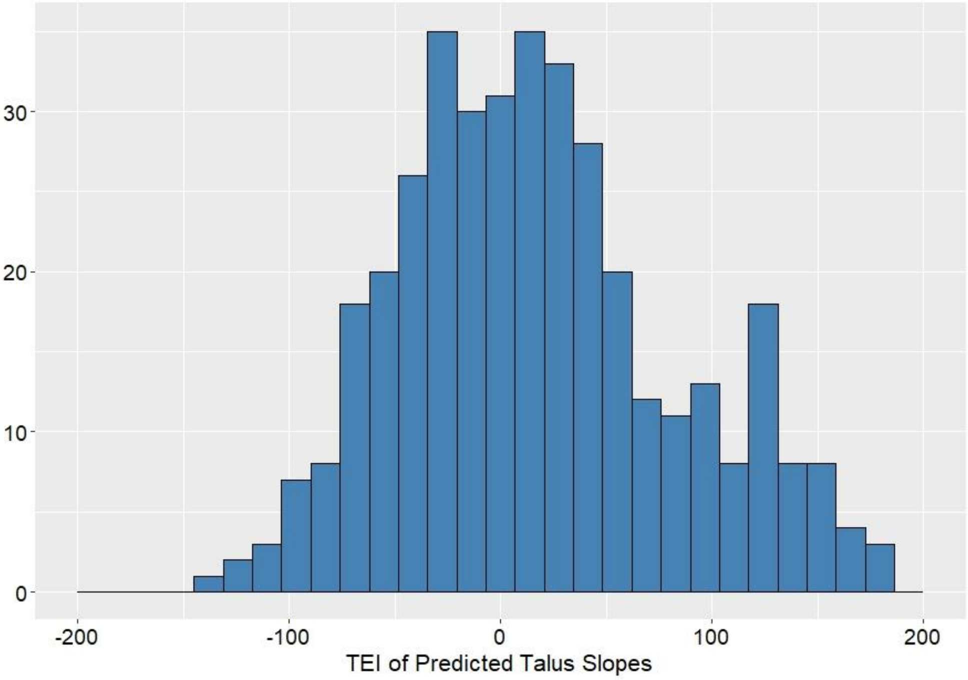

Figure 1.9. Histogram of topographic exposure index (TEI) of talus slopes predicted at Shenandoah National Park, Virginia.

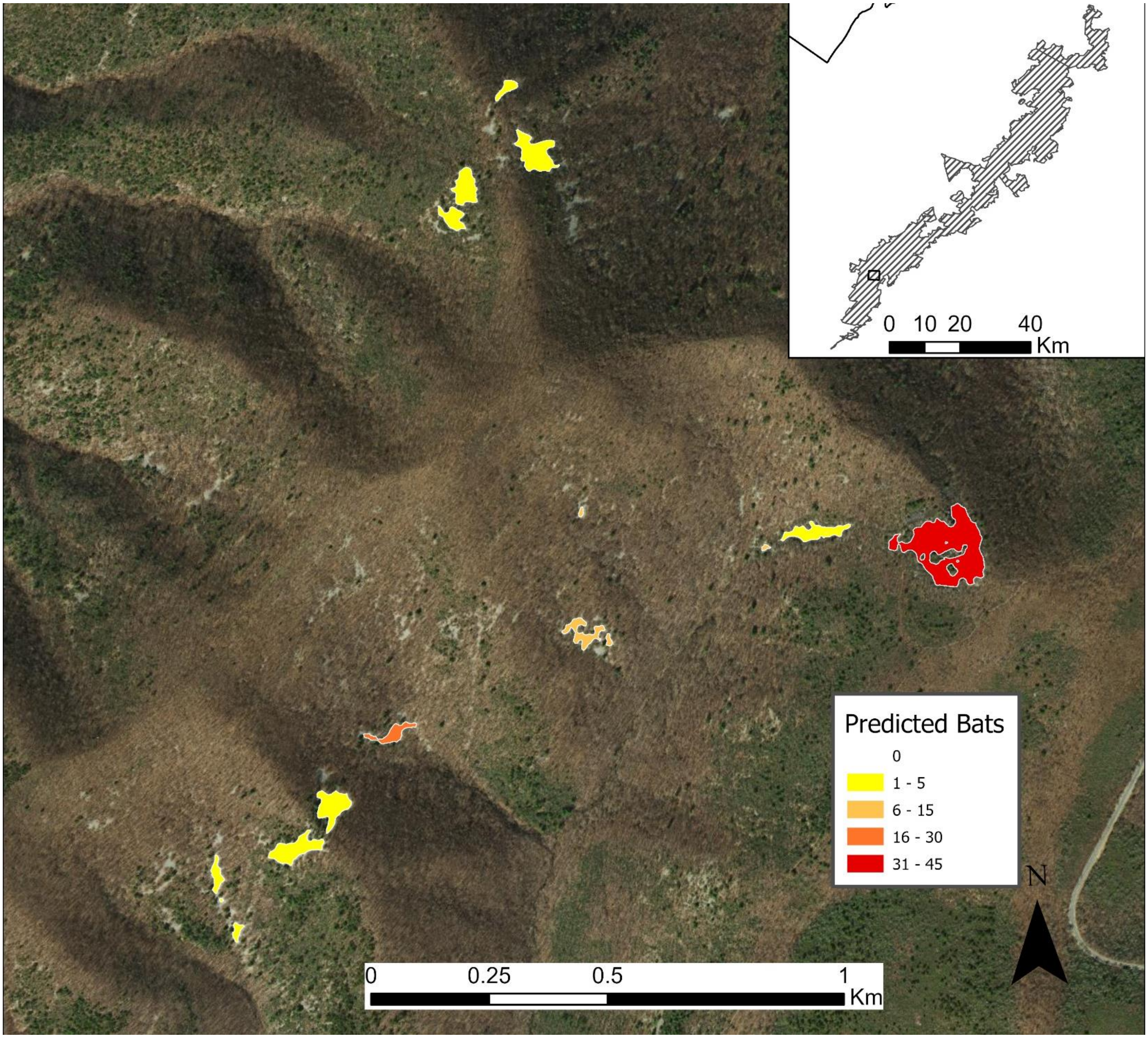

Figure 1.10. Representative eastern small-footed bat (*Myotis leibii*) abundance predictions to talus slopes based on the generalized linear model created from visual search results conducted from 2018-2021 at Shenandoah National Park, Virginia.

Chapter 2: Ecology of eastern small-footed bat maternity colonies

Abstract

Many bat species form colonies of social networks during the summer maternity period in which reproductive females utilize roosts in a fission-fusion roost network dynamic. Bat maternity colonies and roost-switching behaviors can impart physiological and ecological benefits that include social thermoregulation, communal care of young, and decreased susceptibility to predation and parasitism. Effective management and conservation of bat species requires understanding of maternity ecology and reproductive requirements. Thus far, observations of eastern small-footed bat maternity colonies are limited to reports of colonies using anthropogenic structures and records of individual groups cohabiting rock crevice roosts during visual searches, with no indications of potential roost and social network characteristics that have been well-documented in tree-roosting bat species. I observed two eastern small-footed bat maternity colonies at Shenandoah National Park by tracking radio-tagged females during pregnancy and lactation periods, focusing on one colony in the South District to characterize their roost use, social networks, and spatial utilization distribution. Eastern small-footed bats formed a cohesive social network that used rock crevice roosts in a fission-fusion dynamic similar to tree-roosting species. I radio-tagged and tracked 23 eastern small-footed bat females to 47 rock day roosts with 144 relocations during June in 2019 and 2021 at the focal South District colony that included at least 32 adults. The colony was philopatric to a single talus slope and used a 95% kernel density utilization distribution area of 0.46 ha. All captured eastern small-footed bats were either pregnant or lactating and parturition occurred in mid-June at both colonies. Bats day-roosted in rock crevices with narrow openings and similar dimensions to those reported by others. Largest individual roost groups were recorded following parturition and early lactation, as has been reported in tree-roosting bat species, but a generalized linear model of group size by date only received marginal support ($p = 0.01$, $\beta = 0.017$, $SE = 0.007$). Network analysis results of the South District colony were similar to those reported for tree-roosting bat maternity colony. The roost use network mean degree was 1.87, degree centralization was 0.38, density was 0.08, and clustering was 0.11. The bat social network mean degree was 7.13, degree centralization was 0.39,

density was 0.32, clustering was 0.71, mean shortest path was 1.91, and modularity was 0.26 and suggested three subgroups. Also similar to tree-roosting bats, eastern small-footed bats used a primary roost that comprised the central node of the roost network as indicated by greatest degree and degree centrality, as well as greatest number of uses and use by largest group. My findings provide novel evidence of fission-fusion maternity colony network use by eastern small-footed bats, as well as indications spatial and roost requirements in talus slopes that help inform the reproductive ecology of this broadly rare species.

Introduction

Many bat species form maternity colonies whereby females congregate during the gestation, parturition, and lactation periods during the summer reproductive season (Barclay and Kurta 2007, Kerth 2008). These social colonies can provide significant physiological benefits including social thermoregulation that can hasten gestation and juvenile development while reducing energetic costs to individual females (Altringham 2011, Racey and Swift 1981, Trune and Slobodchikoff 1978, Willis and Bringham 2007), communal care and nursing of young (Beck and Rudd 1960, Kerth 2008, Wilkinson 1992a, Wilkinson et al. 2016), and information sharing about forage sites and roosts (Wilkinson 1992b, Wilkinson and Boughman 1999, Wright 2016). Tree-roosting *Myotis* species such as the northern long-eared bat (*Myotis septentrionalis*), Indiana bat (*M. sodalis*), and little brown bat (*M. lucifugus*) form non-random assorting maternity colonies that inhabit a network of day roosts used in a fission-fusion dynamic, whereby individuals form groups of variable sizes within the collective over the period of reproduction (Garroway and Broders 2007, Hyzy et al. 2020, Olson and Barclay 2013, Silvis et al. 2014a, Silvis et al. 2014b). Group sizes within colonies may vary with reproductive condition, and some have observed greatest aggregations occurring following parturition during the early lactation phase (Garroway and Broders 2007, Olson and Barclay 2013, Patriquin et al. 2010). Some bats utilize two-tiered hierarchical structure with distinctly associating subgroups within the colony network (Garroway and Broders 2007, Patriquin et al. 2010, Silvis et al. 2014b). Roost networks are often centralized with “primary” roosts being used more often by more bats than “secondary” roosts, and some have observed “central” roosts that are most degree-central among network associations, used most often, and used by the greatest number of bats, even in subsequent years (Johnson et al. 2012, Silvis et al. 2014b).

Relatively little is known about the ecology of eastern small-footed bat maternity colonies. Relevant findings have either related to visual search surveys focused on determining presence and roosting characteristics, or consisted of limited observations of colonies in anthropogenic structures rather than natural roosts (Hitchcock 1955, Johnson et al. 2011, Moosman et al. 2023, O'Keefe and LaVoi 2011). Hitchcock (1955) documented a maternity colony in Renfrow County, Ontario that consisted of approximately a dozen bats roosting in an outbuilding. O'Keefe and LaVoi (2011) reported a colony in a historic cabin in Swain County, North Carolina where they captured 22 lactating or post-lactating adult females and ten juvenile eastern small-footed bats in mid-July. However, eastern small-footed bat day roosts in the summer maternity season are most commonly associated with emergent rock or analogous structures (Best and Jennings 1997, Moosman et al. 2023). Groups of up to 25 have been reported roosting together in rock crevices in talus slopes from May through July in Virginia, West Virginia, Illinois, and Arkansas (Johnson et al. 2011, Kearny et al. 2023, Moosman et al. 2023, Whitby et al. 2013). Observations of lactating females in late May, early June, and early July in Virginia, West Virginia, North Carolina, respectively, suggest parturition occurs as early as late May in these locations coinciding with larger roosting group observations (Johnson et al. 2011, Moosman et al. 2023, O'Keefe and LaVoi 2011). Such findings indicate that eastern small-footed bat females form maternity colonies during summer similar to other bat species, but colony dynamics beyond the occurrence of individual roost groups is unknown and phenology of reproduction remains unclear. Johnson *et al.* (2011) conducted radio-tracking of eastern small-footed bats in the central Appalachians of West Virginia and reported that females switched roosts every 1.1 days during summer, and mean distance traveled was only 66.5 m. Frequent roost switching over a small area could be indicative of colony networks of roosting groups that are socially connected, as is common in other bat species (Johnson et al. 2013, Kerth 2008, Silvis et al. 2014b, Willis and Brigham 2004). Frequent roost switching and fission-fusion colony dynamics allow bats the ability to reduce probability of predator detection and exposure of parasites or to utilize roosts of different sizes and positions, therefore, microclimates (Barclay and Kurta 2007, Gikas et al. 2011, Lewis 1995, Moosman et al. 2023, Reckardt and Kerth 2007). Varying group sizes also allow bats to opportunistically increase their capacity for social thermoregulation and communal care (Garroway and Broders 2007, Olson and Barclay 2013, Patriquin et al. 2010).

The roosting behavior and potential social network dynamics of eastern small-footed bats needs to be investigated beyond individual group observations to better understand their reproductive habits and requirements, especially following potential impacts from the bat disease white-nose syndrome (WNS). Eastern small-footed bats have been affected by WNS, but mortality estimates remain unclear as limited results are conflicting (Francl et al. 2012, Moosman et al. 2013, Turner et al. 2011). Other bat species appear to have suffered severe population declines, reduced reproductive success, and evidence of maternity colony failure following WNS (Francl et al. 2012, Kalen et al. 2022, Reynolds et al. 2016, Turner et al. 2011). Persistence of eastern small-footed bats at Shenandoah National Park, which features an abundance of talus slopes, their preferred day roost habitat, presents an opportunity to evaluate their maternity colony ecology.

I located and observed two maternity colonies of eastern small-footed bats at Shenandoah National Park, Virginia to document their state of reproduction following WNS and to better understand their roost use, phenology, and colony networks. By capturing and radio-tracking pregnant and lactating eastern small-footed bats, I observed their daily roost use and social network. I predicted that eastern small-footed bats would utilize rock roosts in talus slopes in a fission-fusion network similar to their tree-roosting congeners, that group size would be greatest following parturition when young are most vulnerable, and that parturition would occur in June based on findings by Moosman *et al*. (2023) in Virginia.

Study Area

Maternity colony observations took place at two talus slope sites at Shenandoah National Park. The focal colony for which roost characteristics, roost switching, network dynamics, and phenology were observed inhabited a talus slope located in the South District of the park and was approximately 3.77 ha. The South District talus slope featured a downslope field of regularly-sized blocks and an upslope summit accumulation of larger blocks of fractured and toppled sub-horizontal bedding planes and tor composed of quartz sandstone and quartzites of the Lower Cambrian Harpers Formation (Southworth et al. 2009). Mean elevation was 930 m, mean aspect was 275°, and mean slope was 23°. A second colony in which roost characteristics, group sizes, and phenology were observed occupied a smaller talus slope of approximately 0.36 ha in the Central District and consisted of a sideslope accumulation of smaller metabasalt blocks of the Catoctin Formation (Southworth et al. 2009). Mean elevation was 800 m, mean aspect was 286°, and mean slope was 29°. Both talus slope sites are surrounded by dense chestnut-oak and oak-hickory forest dominated by chestnut oak (*Quercus montana*), northern red oak (*Q. rubra*), red hickory (*Carya ovalis*), pignut hickory (*C. glabra*), and sweet birch (*Betula lenta*).

Methods

In May and June of 2019 and 2021, I located eastern small-footed bat maternity roosts during talus slope visual searches and used mist nets (38 mm, low-bag, Avinet Research Supplies, Portland, ME) set adjacent to and surrounding maternity roosts in talus slopes to capture bats during both the pregnancy and lactating phases as they emerged from roosts at dusk. Restrictions on bat handling relating to SARS-CoV-2 prevented work in 2020. For each bat captured, I recorded sex, age, reproductive condition, mass, forearm length, and wing damage index and placed 2.4 mm uniquely numbered aluminum bands on the forearms of monitored bats (Porzana Ltd., Icklesham, East Sussex, UK; Menzel et al. 2002). I assessed age (i.e., adult or juvenile) by examining epiphyseal-diaphyseal fusion and reproductive condition in females by palpating the abdomen and examining mammaries (Menzel et al. 2002). Radio transmitters (VHF LB-2, 0.22 g; and LB-2XT, 0.28g Holohil Systems Ltd., Woodlawn, Ontario, CA) were attached between the scapulae by trimming fur and affixing them to skin with Perma-Type surgical cement (Perma-Type Company Inc., Plainville, CT; Silvis et al. 2012). I then released bats near the site of capture within 20 minutes. Capture and handling of bats adhered to the guidelines of the Virginia Polytechnic Institute and State University Institutional Animal Care and Use Committee Protocol #16-049, Virginia Department of Wildlife Resources Threatened and Endangered Species Permit #055802, and National Park Service Research and Collection Permit #SHEN-2019-SCI-0004.

I located radio-tagged female eastern small-footed bats using TRX1000S receivers and three-element Yagi antennas (Wildlife Materials Inc., Carbondale, IL), and upon discovery, georeferenced locations with a handheld GPS (Garmin GPSmap 64st, Garmin International Inc., Olathe, KS). Once located, I identified the number of visible adults and juveniles cohabiting the day roost with radio-tagged bats. To evaluate rock day roost characteristics where bats were observed, I recorded measurements of crevice width and length; length, width, and height of the rock or combination rocks above and below the crevice to the nearest 0.1 cm using a tape measure; and internal roost temperature adjacent to bats using an infrared thermometer (General IRT102, General Tools and Instruments, Secaucus, NJ). I calculated mean and standard deviation of these measures, as well as number of bat roosts used, tracking periods, relocations, and roost-switching frequency for radio-tagged bats in base Program R (Version 4.2.3, R Core Team 2023). I relocated radio-tagged eastern small-footed bats while transmitters remained active and attached. I evaluated number of adults per roost by crevice length and width as well as roost rock volume using generalized linear models with a Poisson distribution in base Program R to identify if larger groups used larger rocks and/or crevices. These same methods were used for group size by date to discover if aggregation changed over the maternity period. I considered a quadratic fit for date if group size had a negative parabolic distribution over the maternity period, with larger groups being centered near parturition (Olson and Barclay 2007, Patriquin et al. 2010). Bat per roost data also included roosts previously, but not presently, used by radio-tagged bats and chance observations while tracking.

To evaluate space use by the colony, I used packages *sp* and *adehabitat* (Pebesma and Bivand 2005; Calenge 2006) in R to calculate mean distance traveled between roosts and utilization distributions at 25%, 50%, 75%, and 95% using bivariate normal fixed-kernel methodology (Gorman et al. 2023, Silvis et al 2014b). Utilization distributions were calculated with weighted roost locations to reflect use frequency by tracked bats (Silvis et al. 2014b).

To assess and visualize the eastern small-footed bat maternity roost network, I used a bipartite, two-mode network with bat and roost nodes and projected this into a unipartite, single-mode network of bat nodes in Program R using packages *igraph* (Csardi and Nepusz 2006) and *tnet* (Opsahl 2009; Gorman et al 2023, Johnson et al. 2013, Silvis et al. 2014a). I used the two-mode, bat and roost network to evaluate roost use and the single-mode network of bats only to evaluate the bat social network as it provides a more generalized depiction of bat association (Silvis et al 2014b). To evaluate networks, I calculated mean degree, network degree centralization, network density, and clustering coefficient (Borgatti and Everett 1997, Gorman et al. 2023, Opsahl 2009, Silvis et al. 2014b). Edge weights (i.e., repeated uses of roosts) were omitted in these calculations to reduce bias from uneven tracking periods and incomplete observation of all bats in the colony (Silvis et al. 2014b). Additionally, I calculated shortest path length and leading eigenvector modularity for the bat social network to further evaluate sociality and presence of subgroups (Newman 2006, Opsahl 2009). Degree is the number of connections, or edges, a node has with other nodes with mean degree reflecting the average number of connections per node in the network. Network degree centralization, density, clustering coefficient, and modularity range between 0 and 1 (0 = low, 1 = high) and reflect network structure in terms of individual nodes and the distribution of their connections. Network degree centralization measures the difference between individual node centrality with the greatest degree and the centralities of the other nodes in the network, with low values representing more equitable interconnectedness, and high values indicating greater importance on individual ‘hub’ nodes (Borgatti and Everett 1997, Dong and Horvath 2007). Density compares the number of observed node connections considering the number of possible connections reflecting node interconnectedness (Dong and Horvath 2007). Clustering coefficient represents the extent to which nodes are densely connected with high values indicating strong cliques or subgroups (Opsahl 2009). Alternatively, leading eigenvector modularity designates subgroups by measuring the segregation of network nodes (Newman 2006). Mean shortest path length is the average minimum number of steps between all possible pairs of the network with implications on information transfer (Opsahl 2009). I used software Gephi (WebAtlas, Paris, France) to generate two-mode and single-mode bat and roost network visualizations, and included edge weights in the two-mode bat-roost network to depict number of roost uses by bats.

Results

I captured maternity colonies of eastern small-footed bats at two talus slope locations at Shenandoah National Park during June of 2019 and 2021. At the Central District site, on June 11, 2019, I captured nine lactating and five pregnant eastern small-footed bats at a single rock roost with mist nets draped over the entrance. Of these, four had neonates clinging under their wings that appeared just days old, so were immediately returned to the roost. I wing-banded the remaining 10 and radio-tagged four pregnant and four lactating females. Tracking was limited for this colony, and results consisted of just 23 relocations and six roosts over four days, including the initial roost capture event. Bats could not be located following this period either because they removed their radio tags and/or left the talus slope in one to three days following capture. This Central District colony contained at least 19 bats (14 adults and five juveniles) based on the capture event. Data from this colony was insufficient for network analysis and were not included in roost use, roost-switching frequency, or distance traveled summary statistics.

At the South District site, I radio-tagged and tracked 23 eastern small-footed bat females to 47 rock day roosts with 144 relocations during June in 2019 and 2021 (Figure 2.1). On June 24, 2019, I captured and radio-tagged three lactating female eastern small-footed bats in mist nets set over two rock day roosts. The following day, I tracked two of these bats to a large colony roost, later identified as a primary roost, where I visually identified 32 adults and approximately 20 juveniles (Figure 2.2). There, I captured an additional 18 lactating females, radio-tagging five, by setting mist nets in a square perimeter of around the roost (Figure 2.3). I tracked these eight radio-tagged females for 2 -7 days yielding a total of 43 relocations and 15 day roosts in 2019. In 2021, I returned to South District site where I captured eight pregnant females on June 2, including three that were captured and wing-banded in 2019. I radio-tagged and tracked seven of these bats for 2-8 days for a total of 32 relocations to 16 roosts, four of which were previously used in 2019. On June 14, 2021, I captured eight lactating females at two roosts at the South District site and radio-tagged and tracked all eight bats for 5-10 days totaling 69 relocations to 25 roosts, five of which were used previously including the primary roost. Collectively, mean number of relocations per bat for this South District colony was 6.26 ± 2.88 (range 2-10). Bats used a mean of 3.83 ± 1.95 roosts (range 1-7) and their mean roost-switching frequency was 1.89 ± 1.17 days (range 1-5.5). Roosts were used an average of 3.06 ± 3.16 times (range 1-15). Mean distance traveled between roosts was 26.4 ± 17.3 m (range 1.5 – 93.5 m). The estimated 95% utilization distribution of the roosting area was 0.46 ha and the core area of 50% was 0.12 ha. At minimum, the South District colony included at least 52 bats (32 adults and 20 juveniles) based on visual observation of the primary roost on June 25, 2019.

Of all 60 rock roosts measured at both sites, including roosts that did not contain radio-tagged bats, mean crevice length was 60.3 ± 39.89 cm (range 14.5 -148.6), mean crevice width was 2.4 ± 1.8 cm (range 0.5 – 6.35), mean rock length was 128.3 ± 58.2 (range 40.6 - 254.0), mean rock width was 73.7 ± 39.1 cm (range 14.0 – 158.8), mean rock volume was 1.1 ± 1.3 m^3 (range 0.01 – 6.1). Mean roost temperature was 30.5 ± 8.2°C (range 20.5 – 44.1). Some roosts consisted of multiple crevices that were used by bats at different times, in which case I considered the rock to be a single roost from a network and roost use perspective, but included measurements of each crevice used.

Mean number of adult bats per roost from all colony roost observations (n = 97) was 3.89 ± 4.35 (range 1-32). Group size was weakly positively correlated with day number with a linear fit performing best ($p = 0.01$, $\beta = 0.017$, SE = 0.007; Figure 2.4). Group size was also weakly positively correlated with rock volume ($p < 0.0001$, $\beta = 0.17$, SE = 0.03; Figure 2.5) and crevice width ($p = 0.002$, $\beta = 0.08$, SE = 0.03), but was not significant for crevice length (p = 0.50)

All bats and roosts of the South District colony were connected components via coincident roost use constituting a single bat-roost maternity colony network (Figure 2.6). Despite disparate tracking periods, repeated observations of bats and close proximity of all roosts were consistent with being characterized as a single colony (Gorman et al. 2023). The roost use network mean degree was 1.87, degree centralization was 0.38, density was 0.08, and clustering was 0.11. The primary roost had the greatest number of uses (15), number of occupying bats (32 adults, 20 juveniles), degree (10), and degree centrality (0.43) in the roost use network, indicating it was also the central node roost in the network (Johnson et al. 2012, Silvis et al. 2014b). The bat social network mean degree was 7.13, degree centralization was 0.39, density was 0.32, clustering was 0.71, mean shortest path was 1.91, and modularity was 0.26 and suggested three subgroups.

Discussion

Eastern small-footed bats formed a fission-fusion maternity colony network using rock roosts similarly to arboreal roosting *Myotis* species (Ford et al. 2016, Garroway and Broders 2007, Gorman et al. 2023, Hyzy et al 2020, Johnson et al. 2011, Olson and Barclay 2013, Silvis et al. 2014a, Silvis et al. 2014b). Parturition took place synchronously during the second week of June in both colonies in both years, which was slightly later than phenology inferred by others in Virginia, West Virginia, and North Carolina (Johnson et al. 2011, Moosman et al. 2023, O'Keefe and LaVoie 2011). All females captured were reproductive and juveniles were frequently encountered, indicating that natality was high. This is significant as other *Myotis* species have demonstrated declines in reproductive success following WNS in the form of lower proportions of juveniles (Francl et al. 2012), and northern long-eared bats have shown signs of colony failure at Shenandoah National Park (Kalen et al. 2022). Estimates of mortality for eastern small-footed bats from WNS have been low (12%) compared to other species based on hibernacula surveys (Turner et al. 2011), but the effectiveness of these surveys for censusing eastern small-footed bats has been questioned (Moosman et al. 2015), and greater declines in mist-netting rates (68–84%) in West Virginia and New Hampshire suggest more substantial impacts in those areas (Francl et al. 2012, Moosman et al. 2013). Persistence of high natality in eastern small-footed bats at Shenandoah National Park may be attributable to using hibernacula unaffected by WNS including anthropogenic structures, hibernacula less amenable to maintaining a reservoir of *Pseudogymnoascus destructans,* the fungus that causes WNS, or hibernacula where this fungus has not been introduced. Alternatively, their resilience could be wholly or partially due to their habits of engaging in relatively short hibernation periods and/or use of atypical microsites near entrances of karst hibernacula that may be unconducive to *Pseudogymnoascus destructans* in terms of temperature or relative humidity (Barbour and Davis 1969, Best and Jennings 1997, Langwig et al. 2012). However, they may be wintering outside of traditional hibernacula entirely, potentially remaining in talus slopes or other rock structures as has been observed in Virginia and Maine (Heilakka 2023, Moosman et al. 2017). In any case, eastern small-footed bats appear to be fecund at Shenandoah National Park based on these limited results, suggesting their reproduction there has not been affected by WNS. Annual survival of adult females and juvenile eastern small-footed bats have been estimated as 0.64 and 0.47, respectively, based on the monitoring of populations roosting in concrete guardrails in Arkansas (Sasse and Perry 2023). If survivorship at Shenandoah National Park is similar, results suggest recruitment is sufficient for replacement.

Eastern small-footed bats day-roosted exclusively in the crevices amid and between talus rocks with narrow openings similar to observations in Virginia, West Virginia, Illinois, and Arkansas (Johnson et al. 2011, Kearny et al. 2023, Moosman et al. 2023, Whitby et al. 2013). Most bats were readily observable in surface level crevices, but on several occasions, bats roosted beneath the surface layer of rocks, in which case no roost measures were taken but bat-roost locations were recorded for the network. Roost crevice and rock dimensions were highly variable except for crevice width, which was consistently narrow. Roost measures were comparable to other observations in Virginia and Arkansas, where crevice width was also consistently narrow (Kearny et al. 2023, Moosman et al. 2023). The model of bat number by crevice width had a low coefficient and relatively high standard error, so the positive relationship was considered uninformative. There was greater evidence supporting larger groups using larger roosts in terms of rock volume, as was observed by Moosman *et al.* (2023). Similarly, Olson and Barclay (2013) found that largest group sizes of little brown bats coincided with use of the largest roost trees, with the peak occurring during parturition. Larger groups of eastern small-footed bats generally occupied large rocks, but this relationship also had relatively weak support from the model because individuals and small groups frequently used large roosts as well, in many cases the same roosts used by greater numbers on other occasions. Nonetheless, larger roosts and crevices provide more space for large groups and greater rock volume provides greater thermal inertia that may better support daily torpor by buffering ambient temperature changes, providing a lag in roost warming by day and cooling at night (Chruszcz and Barclay 2002). Thermal buffering would support torpor leading to passive rewarming as rocks heated, which has been documented in eastern small-footed bats and the rock-roosting western long-eared myotis (*Myotis evotis*; Chruszcz and Barclay 2002, Moosman et al. 2015). Thermal buffering may also serve to mitigate potential effects of overheating and evaporative water loss by slowing warming during the day, whereas warmer temperatures at night could be beneficial to juveniles in early development (Chruszcz and Barclay 2002, Olson and Barclay 2013). Selection for narrow widths has been attributed to protection such roosts would provide from small mammal or snake predators that may otherwise easily access them at ground level (Moosman et al. 2023). Narrow roosts may also provide efficient heat transfer, as bats often had ventral and dorsal contact with rock surface. Efficient conduction would facilitate thermal buffering of temperature regimes. Roost temperatures were generally high, but had considerable variance, similar to observations by others who related roost temperature regimes to ambient temperatures (Kearny et al. 2023, Moosman et al. 2023). Hourly temperature variance confounded examination of relationships between rock volume or group size and roost temperature because they were measured at different times of day. Maximum roost temperature (44.1°C) was well above and the minimum (20.5°C) was well below reported normothermic body temperature of eastern small-footed bats, indicating wide thermal tolerance and supportive of the torpor-related hypotheses (Kearny et al. 2023, Moosman et al. 2015, Moosman et al. 2023).

Roosting at South District colony was largely concentrated to the 75% utilization distribution area of 0.22 ha located at the summit of the slope that encompassed 91% of roosts. This summit comprised a concentration of large rocks with an abundance of roosting opportunities relative the rest of the slope. This coincides with use of upslope concentrations of large rocks by Moosman *et al.* (2023) at Sherando, Virginia, approximately 30 km southwest of the South District site. The entire roosting area was < 0.5 ha, much smaller than roosting areas reported for tree-roosting northern long-eared and Indiana bats (Gorman et al 2023, Kalen et al. 2022, Silvis et al. 2014a, Silvis et al 2014b). Talus slopes provide spatially dense concentrations of roosting opportunities for maternity colonies compared to forest areas large enough to include enough suitable roost trees that are selected for by species, decay stage, height, dbh, and presence of cavities or sloughing bark (Johnson et al. 2009, Kalcounis-Ruppell 2005, Kunz and Lumsford 2003, Moosman et al. 2020, Silvis et al. 2016). So, it is unsurprising that talus-roosting eastern small-footed bats used a much smaller area compared to tree-roosting species. Roost-switching distances were very low, and mean and maximum were less than half of those observed for eastern small-footed females by Johnson *et al.* (2011) in West Virginia, although their results were not from a single colony. Roost-switching in the South District colony was frequent but not as frequent as observations in West Virginia (Johnson et al. 2011). Frequent roost-switching behavior has been attributed to its potential to reduce incidence of parasitism and predation, or to provide daily optimal thermal conditions in tree-roosting species (Lewis 1995, Olson and Barclay 2007, Reckardt and Kerth 2007, Silvis et al. 2014b). These factors are likely also relevant for rock-roosting bats that have been found to have more frequent relocation rates (Chruszcz and Barclay 2002, Johnson et al. 2011, Moosman et al. 2023). Ectoparasites such as the bat bug (*Cimex adjunctus*), a variety of chiggers (larval mites), and several helminth and coccidian endoparasites are known to parasitize eastern small-footed bats, which may exacerbate the need to switch roosts frequently (Gikas et al. 2011, McAllister et al. 2017, Sasse et al. 2016). Chiggers were commonly present on the ears of eastern small-footed bats at Shenandoah National Park. Chiggers were not collected and identified but may have been *Leptotrombium myotis* which was found on the majority of a dozen eastern small-footed bats in a talus slope in West Virginia that were sampled for ectoparasites (Gikas et al. 2011).

The South District colony was larger than other eastern small-footed bat maternity colonies and group sizes reported by others, constituting the largest colony of eastern small-footed bats yet observed (Hitchcock 1955, Johnson et al. 2011, Kearny et al. 2023, Moosman et al. 2023, O'Keefe and LaVoi 2011). Both colonies may have included additional members beyond observations of greatest cumulative numbers. Fission-fusion dynamics of the South District colony were apparent from variance in group size. Mean group size was slightly lower than that reported from other locations in Virginia by Moosman *et al.* (2023; 5 ± 6 bats), however, their result did not include bats roosting individually. Greatest group sizes were observed from June 11 to June 24, coinciding with parturition and early lactation, giving some support to a trend of greater aggregations during these periods similar to northern long-eared and little brown bats in Canada (Olson and Barclay 2013, Patriquin et al. 2010). The model only supported this weakly with a positive linear relationship. A negative parabolic relationship potentially would be more demonstrative of this trend, but a quadratic relationship did not improve model fit. This was likely because small groups and individuals were observed throughout these periods as well. This may at least partially be a result of bats dispersing from and not returning to primary roosts at both sites following capture events. They did not appear to collectively relocate, but rather "fission" in to smaller groups following disturbance. Bats returned to the primary roost after dispersing at the South District a week after capture, but did not return to the Central District roost during the brief tracking period. Extended periods and more relocations with radio telemetry or, potentially, visual searches of known colony roosts without disturbance during parturition and early lactation may provide less biased indications of group dynamics over the maternity period. While larger aggregations potentially pose increased risk on the collective being discovered and predated (Barclay et al. 1982), individual risk is diluted by manner of the "selfish herd effect" and larger groups may be more vigilant of threats (Bohn et al. 2009). Though unknown, it is possible that predation risk might be low for eastern small-footed bats while roosting owing to their selection for narrow crevices (Kearny et al. 2023, Moosman et al. 2023), but they are more vulnerable upon emergence to avian predators, such owls (Bergstrom and Smith 2017), or possibly small and medium-size mammalian predators, i.e., raccoons (*Procyon lotor*) or mustelids. There are no reports of predation on eastern small-footed bats specifically, but predators of other bat species include snakes and birds of prey, specifically owls (Barbour and Davis 1969, Barclay et al. 1982). A bat attributed to an unknown *Myotis* species was found in a gut analysis of timber rattlesnakes (*Crotalus horridus*; Keenlyne 1972). Timber rattlesnakes are common at Shenandoah National Park, especially in rock habitats as they derive similar thermal benefits as bats, even during pregnancy (Keenlyne 1972). Shared preference for rock and talus slopes suggests timber rattlesnakes are a potential predator of eastern small-footed bats and driver for their occupance of narrow crevices that exclude snakes. Barred owls (*Strix varia*) also prey on bats, with southeastern bats (*Myotis austroriparious*) constituting a majority of their diet in Georgia (Bergstrom and Smith 2017), and are common at Shenandoah National Park, thereby being potentially a predator affecting eastern small-footed bat maternity roosting behavior. In fact, dispersal from primary roosts (not returning for a week or more) following the capture disturbance may reflect a predator avoidance behavior. Barclay et al. (1982) reported that approximately 1/3 of a large maternity colony of little brown bats left an attic roost following predation attempts by a screech owl (*Otus asio*) and did not return.

Others have posited that larger group sizes confer advantages to maternity colony by increasing capacity for social thermoregulation and communal care, being most important when juveniles are most vulnerable and energetic demands on mothers are high (Garroway and Broders 2007, Kerth 2008, Olson and Barclay 2013, Patriquin et al. 2010). Communal care may have been observed in the South District colony, as 18 of the 32 identified adult females emerged and were captured, while at least 20 juveniles and 14 adults remained in the roost, indicating some juveniles were left in the roost by their mothers while they emerged to forage. I also observed several incidences in which one female roosted with two or more juveniles. Disturbance may have influenced behavior at the primary roost, and observations in which juveniles exceeded number of adults in roosts could be an artifact of twin births. However, communal care has been recorded in other bat species, so it is reasonable that it occurs in eastern small-footed bat and is a factor on maternity colony behavior and group size (Bohn et al. 2009, Kerth 2008, Wilkinson et al. 2016).

The roost use network was also indicative of a fission-fusion dynamic and corresponding statistics were within the range of observations for northern long-eared and Indiana roost use and social networks, (Gorman et al. 2023, Silvis et al. 2014a, Silvis et al. 2014b). As with others, network statistics were based on a portion of the colony and limited tracking periods, so were not fully representative of cumulative associations and roost uses of either the roost use or social network. Nevertheless, results provide novel findings of eastern small-footed bat maternity colony roost use and social networks by indicating network structure and social association. Assuming the South District colony consisted of only 32 adults, the network included results for approximately 2/3 of the collective, but observations were spread over two summers with only eight bats being tracked in 2019. Also, tracking periods were uneven and relatively short, with some bats only having two locations. These factors likely underrepresent network connectivity significantly in terms of the roost use and, to a lesser degree, social association that considers more generalized association (Silvis et al. 2014b). Degree centralization of the two-mode roost use network was moderate, indicating the importance of the central node, primary roost for network connectivity, similar to observations in northern long-eared and Indiana bats (Gorman et al. 2023, Silvis et al. 2014a, Silvis et al. 2014b). However, the network appeared highly structured and mean degree was relatively low because bats most commonly roosted in smaller groups, several with the same individuals, resulting in many bats having a low degree from direct coincident roost use among radio-tagged bats. This dynamic also resulted in a low density, identifying that a low portion of potential network connections were observed. Dispersal following capture events likely influenced results, skewing them towards decreased network connectivity. Similar disturbance to northern long-eared bats using an artificial bat house as primary roost has been reported (Gorman et al. 2023), so it is unsurprising this occurred in eastern small-footed bats occupying ground-level rock roosts.

Interconnectivity was greater among the single-mode bat social network (Silvis et al. 2014a, Silvis et al. 2014b). This was supported by higher mean degree and density, as well as low shortest path. This suggests that bats were highly socially connected despite separation in the roost use network, with coincident use of the primary roost being a driving factor. Greater sociality facilitates efficient information transfer concerning forage sites and roosts and would be beneficial to maintaining colony cohesion among the large number of roosts occupied in this study (Wilkinson 1992b, Wilkinson and Boughman 1999, Wright 2016). Cohesive social networks are also more likely to engage in communal care of young supporting potential observations (Kerth 2008). Clustering coefficient in the social network was somewhat high and modularity suggested three subgroups, providing some evidence of non-random two-tiered hierarchical association (Garroway and Broders 2007, Patriquin et al. 2010, Silvis et al. 2014b). Several dyads repeatedly roosted together, but uneven tracking periods did not allow for full investigation of social index analysis among individuals (Silvis et al. 2014a). Longer tracking periods and more complete observations of eastern small-footed bat maternity colonies would provide greater insight to potential two-tiered hierarchy.

These results signify successful reproduction by eastern small-footed bats at Shenandoah National Park, Virginia following WNS, and continued efforts should be made to evaluate maternity colonies in other locations in terms of natality, roosting, and network dynamics for comparison, especially where declines have been reported. Eastern small-footed bat maternity colony roosting should be explored in locations devoid of talus slopes to evaluate roost use beyond talus and anthropogenic structures. Colony network observations demonstrate that eastern small-footed bat colonies are highly social, utilize variable group sizes, may include communal care, and are philopatric to primary roosts that constitute central nodes in roost and social networks. Fission-fusion group dynamics may serve to balance costs such as risks of parasitism and predation with benefits like social thermoregulation and communal care. Concentrations of large rocks in talus slopes appear to constitute preferred roosting conditions where they are present, as they may provide beneficial thermal conditions, adequate space for large groups, and spatially dense roosting opportunities. If so, conservation of such areas among talus slopes may benefit eastern small-footed bat populations. Mining, quarrying, shale gas extraction, and other development activities could threaten eastern small-footed bat maternity colonies in talus habitats in other locations, as they are currently unprotected at the federal level. Further study of eastern small-footed bat distribution, abundance, and maternity colony habitat selection may elucidate whether protection of eastern small-footed bats is warranted. If so, presence/absence surveys at talus slopes prior to disturbance could be performed similar to those required for federally protected tree-roosting species to prevent mortality or negative impacts to colonies.

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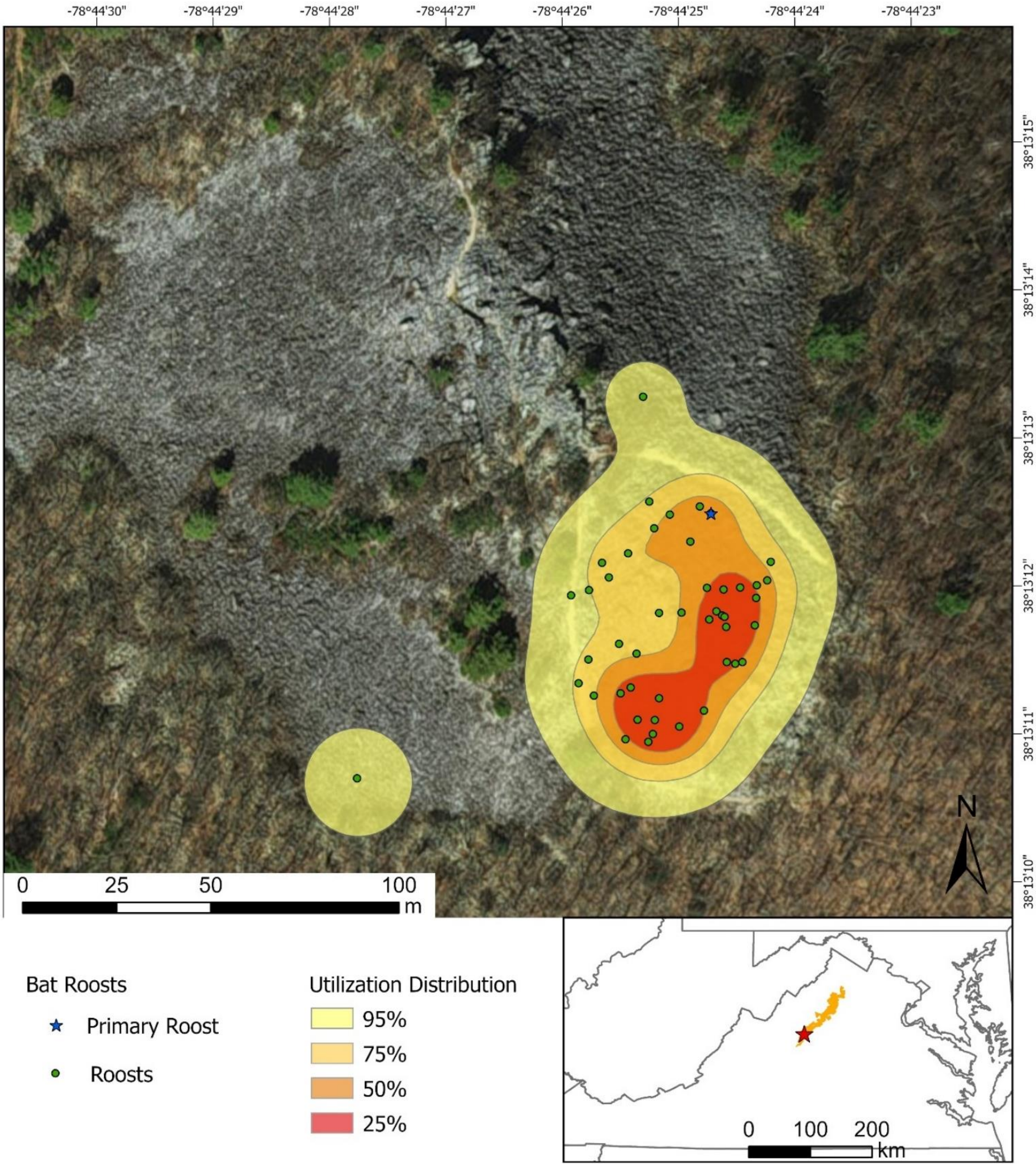

Figure 2.1. Eastern small-footed bat (*Myotis leibii*) maternity colony roosts and roosting area fixed kernel density utilization distribution estimates at Shenandoah National Park, Virginia in 2019 and 2021. Contour lines represent 25%, 50%, 75% and 95% utilization distributions reflecting the percent of day roosts in the roost area with lower percentage areas indicating core use areas. Bat roosts are shown as green points and the primary roost as a blue star.

Figure 2.2. The primary and central node roost of eastern small-footed bats (*Myotis leibii*) at the South District site, Shenandoah National Park, Virginia.

Figure 2.3. Mist net arrangement for capturing eastern small-footed bats (*Myotis leibii*) at the primary roost at the South District site Shenandoah National Park, Virginia on June 25, 2019.

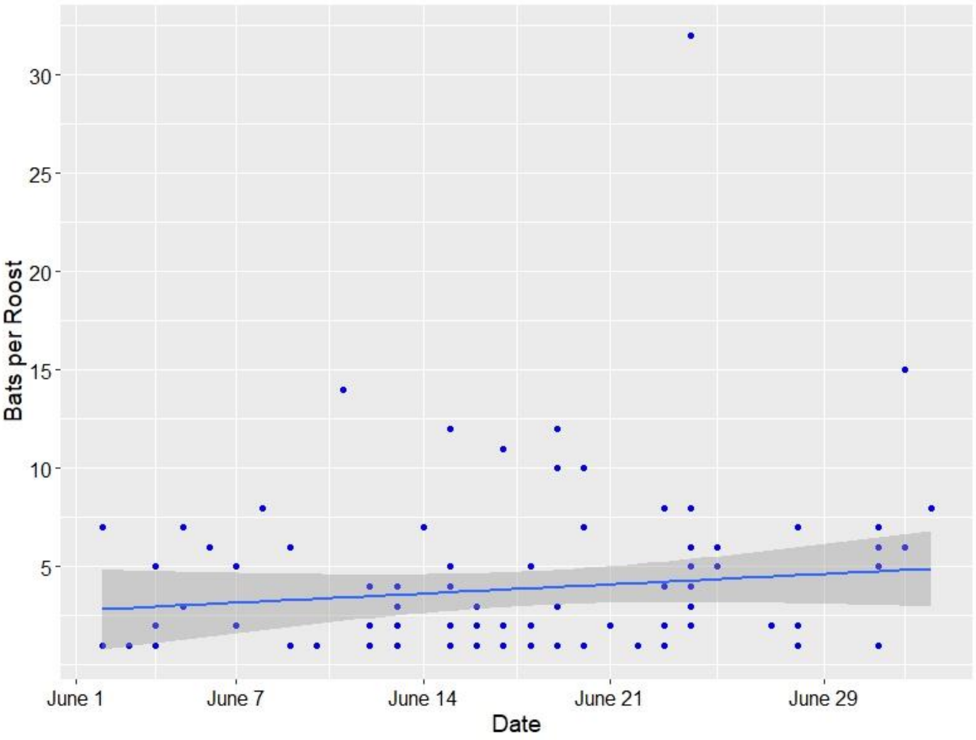

Figure 2.4. Number of eastern small-footed bats (*Myotis leibii*) per roost by day number for all colony roost observations at Shenandoah National Park, VA during the summers of 2019 and 2021.

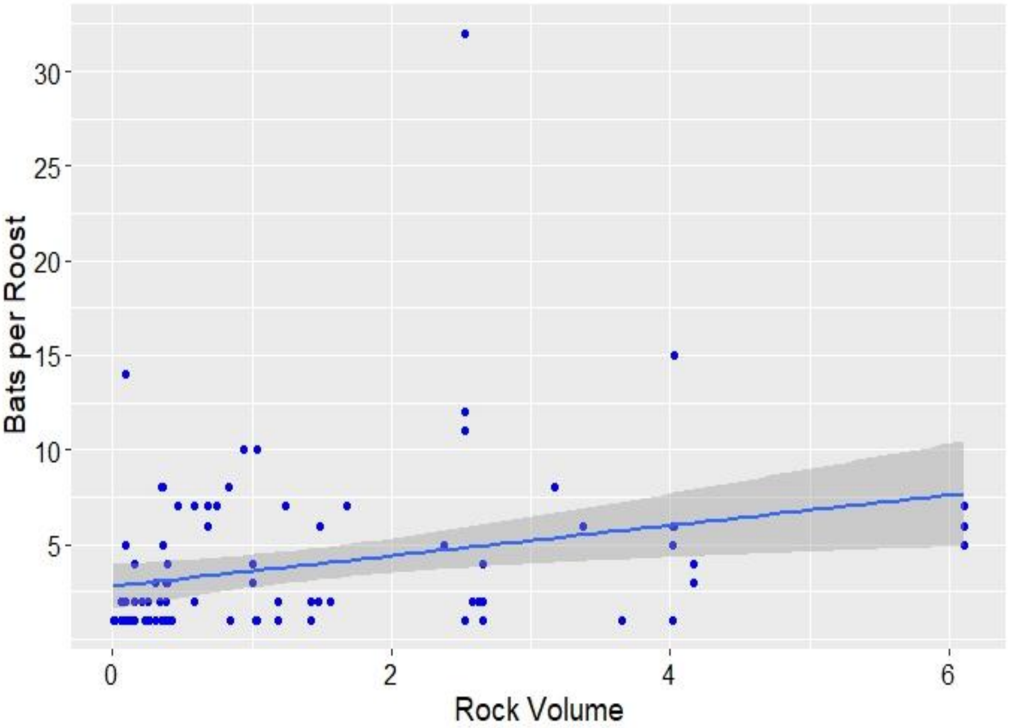

Figure 2.5. Number of eastern small-footed bats (*Myotis leibii*) per roost by roost rock volume in cubic meters (m^3) for all colony roost observations at Shenandoah National Park, VA during the summers of 2019 and 2021.

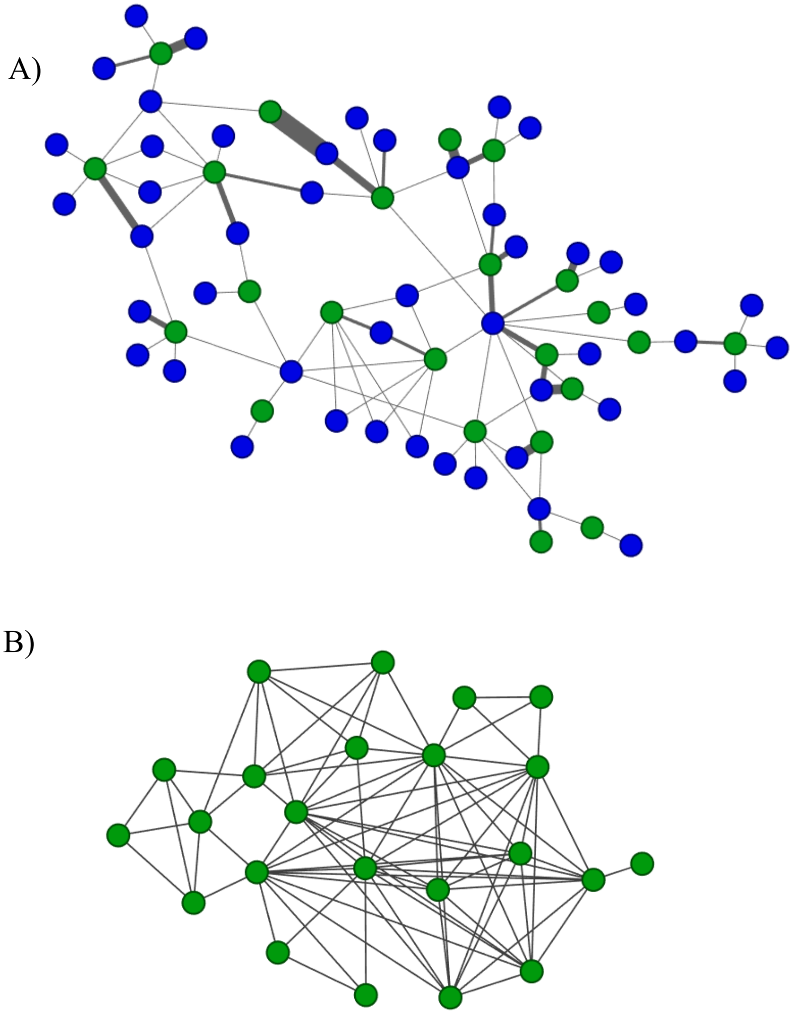

Figure 2.6. (A) Two-mode bat roost-use network visualization of the South District eastern small-footed bat (*Myotis leibii*) maternity colony with bats depicted in green, roosts depicted in blue, and roost uses depicted by gray lines weighted by number of uses (B) single-mode bat social network with bats depicted in green with unweighted associations depicted by gray lines. Both networks are based on radio telemetry tracking observations during June of 2019 and 2021 at the South District site, Shenandoah National Park, Virginia.

Chapter 3: Thermoregulatory patterns and foraging behavior of reproductive eastern small-footed bats

Abstract

Daily torpor provides significant energetic savings to mammals, but reduced body temperatures and metabolic rates can result in reproductive costs to reproductive bats. Bat species appear to utilize variable thermoregulatory strategies depending on reproductive condition to balance tradeoffs of torpor and reproduction. Torpor patterns may also relate to roost selection and foraging activity. To evaluate thermoregulatory ecology in eastern small-footed bats (*Myotis leibii*), I used temperature-sensing radio transmitters to record bat skin temperatures of three pregnant and four lactating eastern small-footed bats over 31 bat-days at Shenandoah National Park, Virginia. All bats engaged in torpor every day but pregnant and lactating eastern small-footed bats engaged in distinctly different thermoregulatory patterns, whereby pregnant bats generally used shallower torpor with higher daily minimum temperatures ($W = 197$, $p = 0.0002$) and shorter torpor periods ($W = 35$, $p = 0.01$) compared to lactating bats that more frequently engaged in deep torpor ($T_{act} - 10$ °C). Regulating and conforming thermoregulatory strategies by pregnant and lactating eastern small-footed bats were similar to observations of little brown bats (*M. lucifugus*) and further demonstrated the importance of daily torpor in reproductive *Myotis* spp. Pregnant and lactating eastern small-footed bats also exhibited significantly differing foraging periods, where pregnant bats left day roosts for much longer continuous periods ($W = 169$, $p < 0.0001$), whereas lactating bats left for short, sometimes multiple forage bouts returning to day roosts in between. Differences in foraging periods may reflect the foraging efficiency, maternal responsibilities, and energetic requirements associated with thermoregulatory strategies of each reproductive phase. Both pregnant and lactating eastern small-footed bats passively rewarmed in day roosts. Substantial energetic savings of daily torpor by reproductive eastern small-footed bats likely contributes to their maternity habits, day roost selection, and spatial abundance.

Introduction

A wide variety of heterothermic mammals use torpor, a state of suppressed metabolic rate, as a strategy to conserve energy and tolerate environmental stressors throughout the year in addition to winter hibernation (Hock 1951, Johnson and Lacki 2013, Wang and Wolowyk 1988). Among temperate zone bats, daily torpor is common but has been found to slow gestation (Racey 1973, Racey and Swift 1981) and decrease milk production (Wilde et al. 1999) in some bat species, suggesting that it is ecologically costly to reproductive females. In temperate regions, adequate prey to sustain insectivorous bats is only available for approximately 4-5 months, so delays in juvenile development and late parturition can reduce survivorship in both mothers and juveniles if they are unable store sufficient fat reserves prior to winter hibernation (Hamilton and Barclay 1994, Kunz 1987). Despite apparent ecological costs, daily torpor appears to be a useful strategy for bats to meet energetic demands of reproduction, the greatest of which occurs during lactation (Dzal and Brigham 2013, Johnson and Lacki 2013). Regular torpor may also serve to reduce evaporative water loss while day-roosting (Chruszcz and Barclay 2002, Webb et al. 1995). In several bat species, reproductive females have been found to regularly engage in torpor but to a lesser degree than male and unreproductive females (Chruszcz and Barclay 2002, Dzal and Brigham 2013, Hamilton and Barclay 1994, Johnson and Lacki 2013, Solick and Barclay 2006). It appears bats demonstrate variable torpor patterns by frequency (i.e. days entering torpor), duration (i.e. hours torpid), and depth (i.e. change in body temperature) to balance energetic savings with ecological costs and do so in relation reproductive condition (i.e. non-reproductive, pregnant, lactating, or post-lactating; Dzal and Brigham 2013, Johnson and Lacki 2013, Solick and Barclay 2006). Ambient temperature, roost characteristics, and social thermoregulation also likely contribute to torpor patterns (Chruszcz and Barclay 2002, Johnson and Lacki 2013, Solick and Barclay 2006).

Solick and Barclay (2006) found that female, solitary rock crevice-roosting western long-eared bats (*Myotis evotis*) in Alberta, Canada entered torpor frequently, but non-reproductive females did so for longer periods and entered deep torpor more than reproductive females. Pregnant and lactating females similarly either maintained relatively normothermic temperature with occasional shallow torpor bouts, or underwent torpor in the morning and appeared to passively rewarm throughout the day to an active body temperature (Solick and Barclay 2006). They observed consistent use of torpor as well as deep torpor during the pregnancy phase (Solick and Barclay 2006). Dzal and Brigham (2013) reported that, in New York, pregnant little brown bats (*Myotis lucifugus*) used torpor less frequently and for shorter durations compared to lactating individuals. The only published observation of torpor in several eastern small-footed bats (*Myotis leibii*) was by Moosman et al. (2015) who reported similar torpor use among sexes in Virginia, including pregnant and lactating females (Moosman et al. 2015). During summer, bats routinely entered torpor each morning upon return to roosts, then passively rewarmed through the day similar to observations of Solick and Barclay (2006; Moosman et al. 2015). Rewarming is the most energetically costly phase of torpor use (Chruszcz and Barclay 2002, Hamilton and Barclay 1994), so ameliorating this expenditure by utilizing solar-radiated rock crevice roosts would provide these bats with an important ecological advantage in balancing the high energetic demands of reproduction, in which bats derive the benefits by avoiding energetic expenses of endothermy.

To further investigate the thermoregulatory ecology of reproductive eastern small-footed bats, I monitored skin temperature of female eastern small-footed bats during the pregnancy and lactation phases with temperature-sensitive radio transmitters to observe torpor use by frequency, duration, and depth. The daily torpor patterns of eastern small-footed bats have important implications regarding their spatial distribution, maternity roost selection, and reproductive ecology as indicated previously. Data from radio-transmitters also convey nightly foraging periods, which have relevance to torpor patterns (Dzal and Brigham 2013). Evaluating torpor patterns of eastern small-footed bats will supplement understanding regarding the prevalence of torpor use by reproductive bats and thermoregulatory strategies of eastern small-footed bats that influence their maternity roosting habits. I expected that both pregnant and lactating eastern small-footed bats would utilize torpor to some degree and take advantage of passive rewarming conferred by rock crevice roosts, based on the observations of others and hypotheses regarding use of torpor from Chapters 1 and 2. I anticipated that lactating eastern small-footed bats would utilize longer periods and deeper torpor similar to observations of little brown bats.

Methods

I captured eastern small-footed bats at a known maternity colony using methods detailed in Chapter 2 at the South District maternity colony talus slope and used temperature-sensitive radio transmitters (LB-2XT; 0.29-g, Holohil Systems Ltd., Woodlawn, Ontario, CA) to transmit skin temperature of free-ranging pregnant and lactating eastern small-footed bats (Moosman et al. 2015, Solick and Barclay 2006). I deployed three Lotek SRX-DL data-logging receivers (Lotek Wireless, Inc., New Market, Ontario, Canada), one with a 6 m omnidirectional antennae, and two with two three-element directional Yagi antennas each, powered by 12V LiFePO deep cycle batteries (Expertpower EP1250, Energy Storage Systems Inc., Wilsonville, OR) stationed within and along the edges of the talus slope used by the maternity colony. Dataloggers received pulses from radio transmitters that transmitted at an interval of 5 minutes. Pulses from transmitters corresponded with transmitter-specific calibration curves provided by the manufacturer that translated pulse rates to temperatures. Because rock roosts offer thermal buffering to ambient air temperatures (Chruszcs and Barclay 2002), I represented ambient roost temperature by placing a Thermochron iButton (iButtonLink LLC, Whitewater, WI) in an unoccupied rock crevice roost used previously by other members of the maternity colony. I tracked radio-tagged bats daily using a handheld TRX1000S receiver and three-element Yagi antennas (Wildlife Materials Inc., Carbondale, IL) to confirm radio transmitters were attached to bats.

Skin temperature (T_{sk}) recorded by external transmitters accurately reflects core body temperature of active and torpid bats (Barclay et al. 1996). Bat temperature data were used for evaluating torpor patterns during periods when bats were located daily, transmitters were confirmed attached, and temperatures were consistently recorded from the time of return from nightly foraging through emergence for foraging the following evening for at least two days. Torpor was defined as in other studies with bats being considered torpid when its T_{sk} declined below its active body temperature (T_{act}; Chruszcz and Barclay 2002, Hamilton and Barclay 1994, Solick and Barclay 2006). Active temperature was defined as the lowest T_{sk} of an individual bat recorded immediately (< 10 min) before flight to forage. Deep torpor was defined as when T_{sk} declined 10 °C below T_{act}. I calculated frequency of torpor in bat-days, duration of torpor periods in minutes, and duration of periods in deep torpor (i.e., torpor: $T_{act} – T_{sk} < 10$ °C , deep torpor: $T_{act} – T_b \geq 10$ °C) for each bat. To evaluate foraging activity behavior, times in which otherwise consistent temperature data ceased and resumed were used to interpret when bats emerged nightly to forage and returned to day roosts, respectively, which I interpreted as foraging periods. I used the Wilcoxon rank sum test to compare daily time in torpor, daily minimum temperatures, and nightly foraging periods by pregnancy and lactation phases in Program R (Version 4.2.3, R Core Team 2023).

Results

I radio-tagged seven pregnant and eight lactating eastern small-footed bats with temperature-sensitive radio transmitters during June of 2021. On June 2, 2021, I captured eight pregnant females at the South District maternity colony, radio-tagging and tracking seven bats (see Chapter 2). On June 14, I captured and radio-tagged eight lactating eastern small-footed bats at this same colony. Of bats that were fitted with radio transmitters, temperature data were analyzed for three pregnant (18 bat-days) and four lactating (13 bat-days) eastern small-footed bats. Temperature data used for the pregnancy phase were recorded from June 3 through June 10, 2021, and data used for the lactation phase were recorded from June 16 through June 20, 2021.

All seven eastern small-footed bats entered torpor during all 31 bat-days and mean daily time in torpor for both reproductive phases was 561 ± 185 min (range = 232 - 1055). However, pregnant and lactating eastern small-footed bats engaged in two distinct thermoregulatory patterns (Figure 3.1). Pregnant bats generally utilized shallower torpor with higher daily minimum temperatures ($W = 197$, $p = 0.0002$; mean = 25.6 ± 3.5 °C; range 18.73 – 29.59; Figure 3.2) and, on average, engaged in torpor for shorter periods ($W = 35$, $p = 0.01$; mean = 476 ± 139 min.; range 232 – 765; Figure 3.3). Two pregnant bats entered deep torpor for brief periods (95, 140, and 145 min.) on three of 18 bat-days (16.6%). Lactating eastern small-footed bats utilized deeper torpor with lower daily minimum temperatures (mean = 18.5 ± 3.7 °C; range 14.43 – 27.1) for generally longer periods (mean = 680 ± 170 min.; range 365 - 1055). Lactating bats entered deep torpor during 10 of 13 bat-days (76.9%) for longer periods (mean =

418 ± 116 min; range 150 – 595). Overall, bats in both phases of reproduction entered torpor within an hour of returning to day roosts following foraging bouts and appeared to passively rewarm with warming ambient temperatures. This was most apparent in lactating bats in which warming from deep torpor temperatures closely aligned with unoccupied ambient roost temperatures (Figure 3.1B). On several occasions, total torpor time included two periods, a morning bout and an evening bout before resuming T_{act} to depart to forage separated by a period in which their temperature exceeded their T_{act} in the afternoon during the warmest times of day. This occurred during three bat-days in pregnant bats and during four bat-days in lactating bats. These instances accounted for the longest times in torpor by pregnant bats, but the longest daily torpor period in lactating bats consisted of a continuous period (1055 min) in which the bat did not reach T_{act} until it just prior to emerging to forage.

All eastern small-footed bats left day roosts to forage nightly on all 31 bat-days, and mean time of nightly foraging was 400 ± 136 min (range = 125 – 559). Pregnant and lactating bats also exhibited different foraging activity patterns (Figure 3.4). Pregnant bats left to forage for much longer continuous nightly periods ($W = 169$, $p < 0.0001$; mean = 504 ± 27 min.; range 475 – 578). Pregnant bats reliably emerged from day roosts within 30 minutes of 21:00, approximately 30 minutes after sunset (20:30), on all but one night and returned to the day-roosting site within 20 minutes of 5:20, approximately 20 minutes before sunrise, on all mornings, with most returns occurring within just a 5-minute window of 5:20. Lactating eastern small-footed bats engaged in shorter cumulative nightly forage periods (mean = 272 ± 105 min.; range 120 – 445), with foraging activity sometimes including two or three individual forage bouts. Lactating bats similarly departed to forage at consistent times on most occasions but slightly earlier. On all but two nights, initial departure from day roosts by lactating bats occurred within 30 minutes of 21:00 and on most occasions within just a 5-minute window of 21:05, also 30 minutes after sunset which occurred at 20:35. Intervals for radio transmissions of bat T_{sk} were 5 minutes, indicating notable consistency of initial departures during both phases of reproduction. Return times to day roosts by lactating bats were highly variable, and nightly activity included single forage and multiple bouts.

Discussion

Eastern small-footed bats regularly engaged in torpor during both pregnancy and lactation but used distinctly different thermoregulatory strategies. In pregnant bats, thermoregulation most often followed a regulating pattern in which bats entered shallow torpor, regulating their body temperature as indicated by higher daily minimum temperatures, shorter torpor periods, and relatively few incidences of deep torpor compared to lactating bats. Conversely, lactating eastern small-footed bats generally followed a conforming, more heterothermic thermoregulatory pattern evidenced by lower minimum daily temperatures, longer torpor periods, and more frequent use of deep torpor (Studier and O'Farrell 1972). Results suggest that shallow daily torpor utilized by eastern small-footed bats represent a thermoregulatory optimum allowing energetic savings of torpor while maintaining fetal growth, and that body temperatures below ~20 °C may represent a threshold of this balance (Racey 1973, Studier 1981). Studier et al. (1972) found that little brown bats similarly exhibited comparative increased homeothermy during pregnancy when they had increased thermoregulatory ability that peaked during mid-pregnancy but did not continue through lactation. Racey and Swift (1981) hypothesized that high concentrations of the pregnancy hormone progesterone, which is greatest mid-pregnancy, may provide for greater homeothermic ability in common pipistrelles (*Pipistrellus pipistrellus*) because its metabolites are highly pyrogenic. Heat generated through metabolization of progesterone may explain differences of thermoregulatory patterns observed in eastern small-footed bats (Racey 1973, Racey and Swift 1981). External factors such as social thermoregulation and selection roost thermal properties, and of course, ambient temperatures affect bat's ability to maintain body temperature as well (Chruszcz and Barclay 2002, Kerth 2008, Solick and Barclay 2006), which may have contributed to exceptions to this trend in which pregnant bats entered deep torpor and lactating bats did not. Because lactation requires significantly more daily energy than pregnancy, it is energetically economical to restrict homeothermy during this time (Dzal and Brigham 2013, Studier et al. 1972). This substantial energy savings of deeper torpor during lactation are likely coincide with the cost of decreased milk production at colder temperatures (Wilde et al. 1999). However, decreased milk production would bear a lower energetic and nutrient burden, so low body temperatures may doubly benefit bats by allowing them to theoretically use energy saved from maintaining homeothermy to support lactation at a lowered rate and therefore expense, which would be ecologically beneficial provided the resulting milk supply is sufficient to nourish neonates. Increased torpor use also directly supports lactation by reducing evaporative water loss (Webb et al. 1995).

Time spent foraging was also significantly different among pregnant and lactating eastern small-footed bats. Surprisingly, pregnant bats spent much longer periods away from day roosts. However, it is likely that pregnant bats did not forage the entire time they were absent and took breaks from foraging to rest and digest prey in night roosts during extended periods away from day roosts (Ormsbee et al. 2007). Nevertheless, consistent and protracted periods away from day roosts by all pregnant bats do suggest greater overall time foraging compared to lactating bats that left day roosts for short forage bouts and returned to day roosts in between. More time spent foraging by pregnant eastern small-footed bats may be indicative of greater nutritional requirements to support increased homeothermy of their regulating torpor strategy (Racey 1973, Racey and Swift 1981). Also, the greater wing load of pregnant bats increases already costly energetic expenses of flight and foraging (Hock 1951, Studier et al. 1972). Therefore, they may have to consume more prey to achieve net energetic gain compared to lactating bats. Furthermore, essentially foraging all night, with occasional breaks at opportunistic night roosts would be beneficial in mountainous terrain where elevation gain associated with returning to high elevation day roosts in talus slopes would be more energetically costly. On the other hand, lighter, more maneuverable lactating females would be more energetically efficient while foraging, thereby achieving net energetic gain in shorter time and more capable of traversing elevational gradients to return to high elevation roosts to care for juveniles in between bouts. Also, decreased thermoregulatory energy consumption in lactating bats is likely to reduce prey requirements contributing to shorter forage periods (Racey 1973). In fact, shorter foraging bouts and longer, deeper torpor periods are mutually supportive as earlier return to day roosts facilitate longer torpor periods at through prolonged roosting in colder temperatures. Conversely, later return observed in pregnant bats delays homeothermic costs and shortens inactive exposure to cold early morning temperatures.

Eastern small-footed bats demonstrated very similar patterns of thermoregulation as those observed in little brown bats in New York in which reproductive females entered torpor regularly and exhibited differing patterns between pregnant and lactating phases with pregnant bats generally engaging in significantly shorter, shallower bouts than lactating bats (Dzal and Brigham 2013). Minimum temperatures in pregnant females were very similar (25.4 ± 1.2 °C) while minimum temperatures of lactating little brown bats were slightly higher (21.2 ± 1.0 ° C) than eastern small-footed bats in this study (Dzal and Brigham 2013). Torpor duration was much shorter in both pregnant (133.1 ± 33.7 min.) and lactating (334.1 ± 50.8 min.) little brown bats compared to eastern small-footed bats (Dzal and Brigham 2013). Torpor was characterized in a different manner by Dzal and Brigham (2013), but temperature thresholds of torpor were similar to my results, so differences in torpor duration were more likely to be attributable to differences in climate, day roost thermal properties, or species behavior and physiology. Dzal and Brigham (2013) also evaluated time spent foraging and found that pregnant and lactating little brown bats spent similar time foraging 270.8 ± 34.1 min. and 231.1 ± 24.2 min., respectively, which corresponded to my observations of lactating eastern small-footed bats but differed with those of pregnant eastern small-footed bats despite exhibiting similar thermoregulatory patterns (Dzal and Brigham 2013). Anthropogenic structure roosts used by little brown bats likely had different thermal properties than rock crevice roosts used by eastern small-footed bats and the location appeared to lack the high elevational gradient of the day roost location at Shenandoah National Park, both of which may have contributed to disparities in foraging strategies between the species.

Chruszcz and Barclay (2002) also observed regulating and conforming thermoregulatory patterns in western long-eared bats in the badlands of southeastern Alberta, Canada, but only in pregnant females, whereas lactating bats used torpor but did not comply with either strategy. Contrary to eastern small-footed bats, pregnant western long-eared bats used deep torpor more frequently than lactating females and minimum temperatures were similar to opposite reproductive phases of eastern small-footed bats. They cited variable availability of insect prey in the semiarid climate as a possible explanation of differences in torpor patterns between reproductive phases (Chruszcz and Barclay 2002). Prey availability has been shown to directly affect thermoregulation and torpor in reproductive bats (Racey 1973, Racey and Swift 1981), so this may also explain differences between western long-eared bats and eastern small-footed bats that occupied a humid climate that most likely supports greater insect abundance. Solick and Barclay (2006) also reported frequent torpor in western long-eared bats in the Rocky Mountains of Alberta, Canada, but did not observe differences among time in torpor or deep torpor of pregnant and lactating bats. Thermoregulation in lactating eastern small-footed bats more closely aligned with non-reproductive western long-eared bats in terms of torpor and deep torpor use (Solick and Barclay 2006). The much colder temperatures in the higher elevation and higher latitude of the Canadian Rockies compared to Shenandoah National Park as well as climate-related prey availability may account for these disparities.

Similar thermoregulatory patterns among pregnant and lactating eastern small-footed and little brown bats suggest other *Myotis* species may utilize torpor patterns to save energy and balance energetic requirements of reproduction, counter to observations of genera of bats (Grinevitch et al. 1995, Hamilton and Barclay 1994, Vaughn and O’Shea 1976). Similarities in thermoregulatory strategies between reproductive little brown and eastern small-footed bats also support my results in light of my relatively low sample size. Frequent use but differing patterns of torpor in western long-eared bats indicate thermoregulatory strategies may vary due to environmental factors such as roost structure, prey availability, climate, and elevation (Chruszcz and Barclay 2002, Solick and Barclay 2006).

As anticipated, eastern small-footed bats passively rewarmed taking advantage of solar radiation of rock crevice roosts in agreement with the observations of others and supporting Chapter 1 hypotheses regarding spatial abundance and solar exposure (Chruszcz and Barclay 2002, Moosman et al. 2015, Solick and Barclay 2006). During both phases of reproduction, eastern small-footed bat body temperature dropped abruptly upon return to day roosts, with bats quickly entering torpor facilitated by cold roost temperatures. Pregnant bats generally regulated minimum body temperatures above 20 °C, whereas lactating bats most often dropped below this threshold, appearing to conform to ambient roost temperature. The body temperatures of bats in both reproductive phases slowly increased at rates similar to ambient temperatures of an unoccupied roost. Bat body temperatures cooled with evening temperatures sometimes dropping below T_{act} constituting a second torpor period and further demonstrating poikilothermy and reliance on the radiant heat of rock roosts (Chruszcz and Barclay 2002, Hock 1951, Solick and Barclay 2006). Passive rewarming undoubtedly serves to relieve homeothermic burden in pregnant bats maintaining temperatures to support fetal growth as well as balance low body temperatures of lactating bats that must produce adequate milk for offspring by providing normothermic temperatures and metabolic rates at reduced energetic cost. Behavioral poikilothermy enabling substantial energy savings through daily torpor and passive rewarming may represent critical aspects of thermoregulation, reproduction, foraging behavior, roost selection, and the overall ecology of rock-dwelling eastern small-footed bats.

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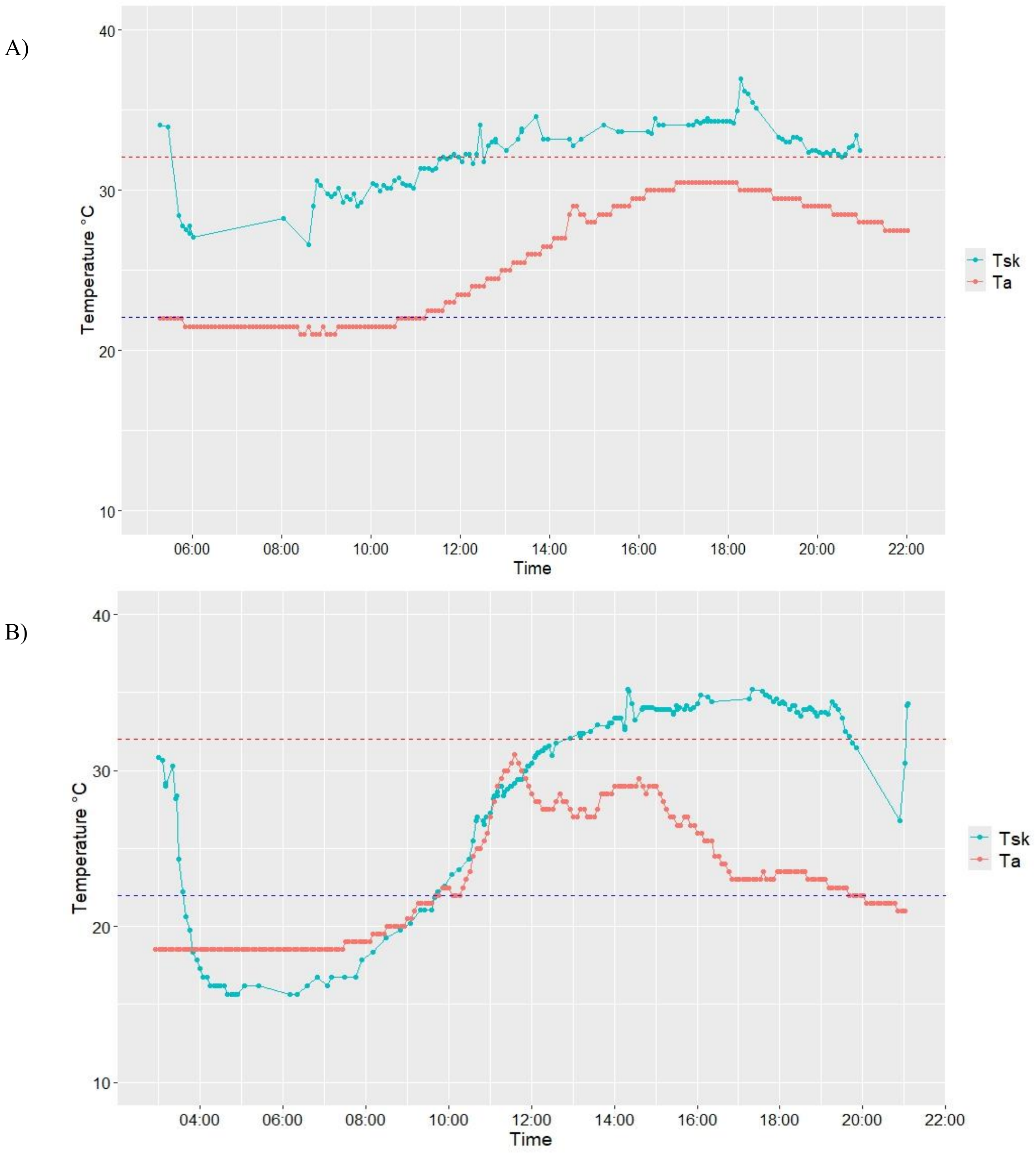

Figure 3.1. Representative thermoregulatory patterns of (A) pregnant and (B) lactating eastern small-footed bats (*Myotis leibii*) as indicated by temperature-sensing radio transmitters during June 2021 in the South District of Shenandoah National Park, Virginia. Bat skin temperature (T_{sk}) is represented by blue solid lines with points indicating individual temperature readings. Unoccupied roost ambient temperatures (T_a) are shown by the solid red lines with dots indicating individual temperatures readings. Active temperature (T_{act}) of bats is represented by red dashed lines and deep torpor temperature (T_{act} – 10 °C) is represented by the blue dashed lines.

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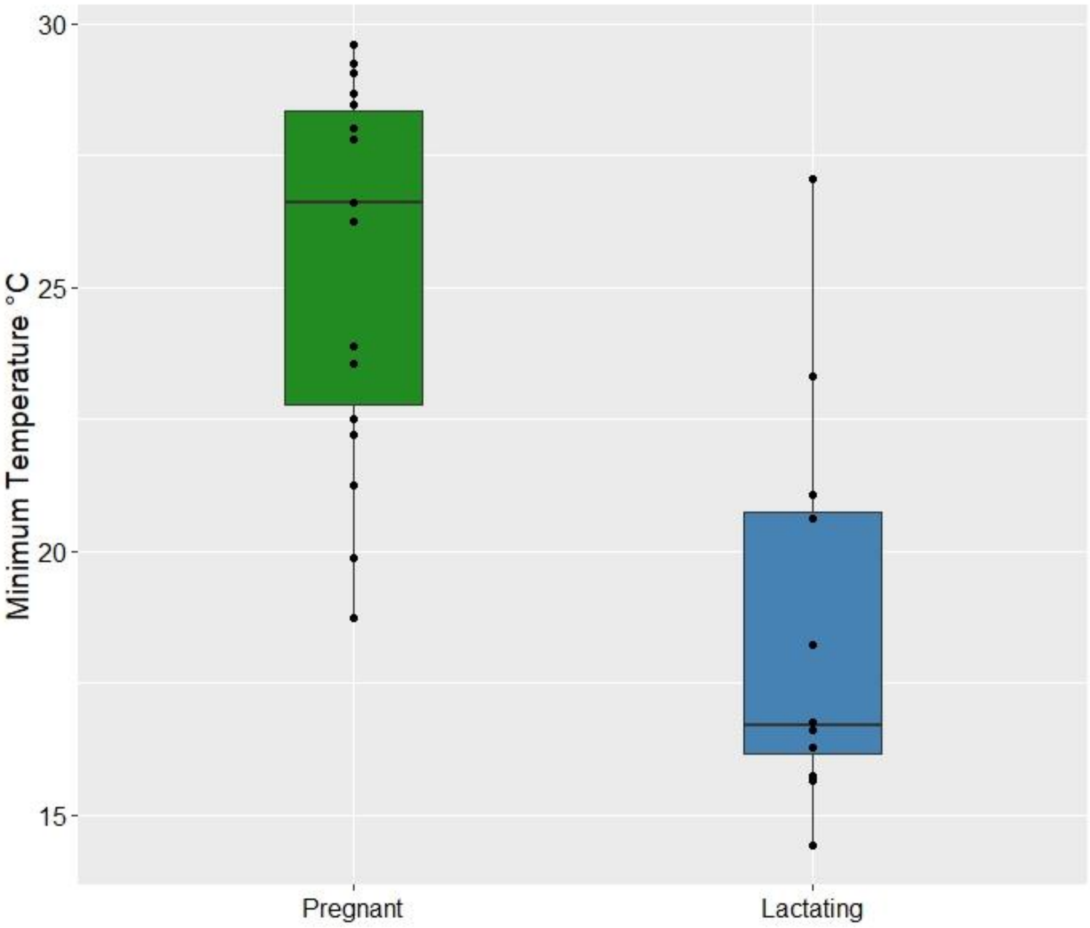

Figure 3.2. Daily minimum temperature of pregnant and lactating eastern small-footed bats (*Myotis leibii*) as indicated by temperature-sensing radio transmitters during June 2021 in the South District of Shenandoah National Park, Virginia.

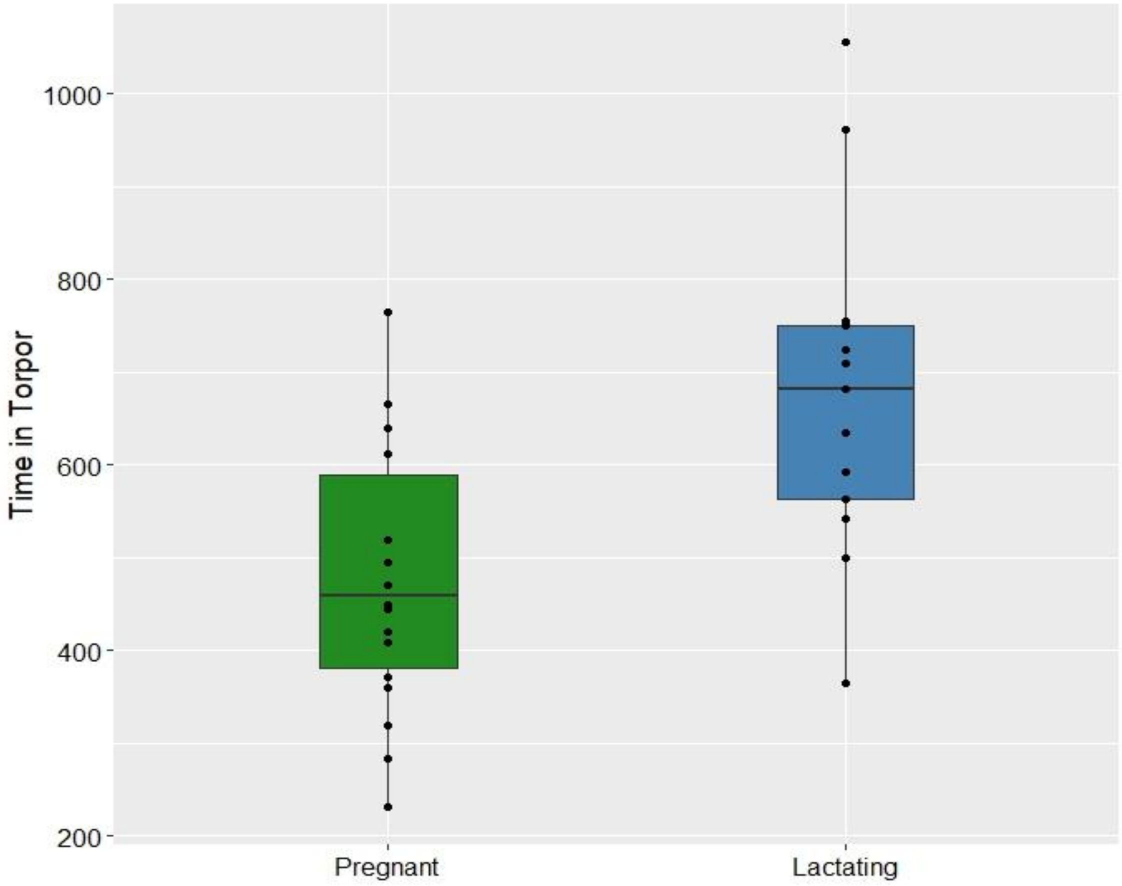

Figure 3.3. Daily time in torpor in minutes by pregnant and lactating eastern small-footed bats (*Myotis leibii*) as indicated by temperature-sensing radio transmitters during June 2021 in the South District of Shenandoah National Park, Virginia.

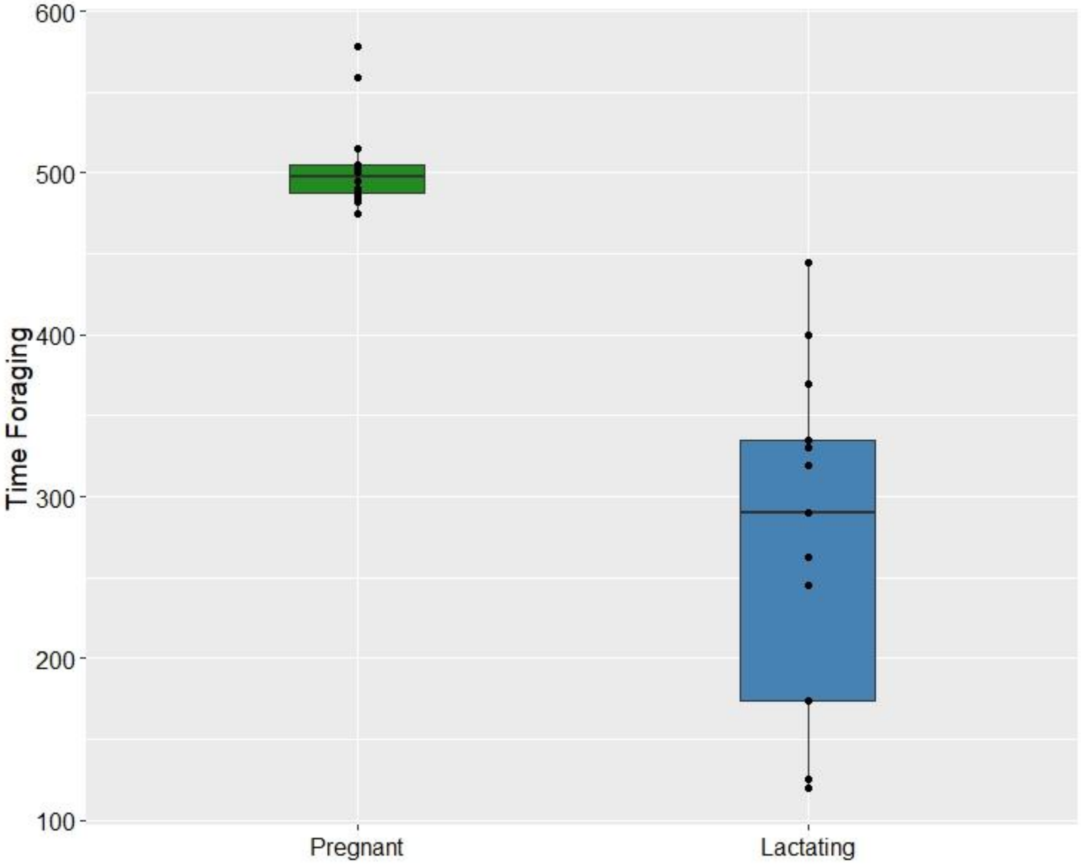

Figure 3.4. Nightly total time spent foraging in minutes by pregnant and lactating eastern small-footed bats (*Myotis leibii*) as indicated by temperature-sensing radio transmitters during June 2021 in the South District of Shenandoah National Park, Virginia.

Chapter 4 Conclusions regarding eastern small-footed bat ecology

Eastern small-footed bats at Shenandoah National Park represented an ideal scenario to observe many novel findings that will serve to better understand their spatial abundance, maternity colony habits, and reproductive thermoregulation. They have thus far remained an underrepresented species in bat research, and my objective was to better elucidate their ecology and contribute to methods that can be applied to quantifying their populations and habits in other portions of their range. As a habitat specialists mostly limited mountainous landscapes in the eastern United States and Canada, eastern small-footed bats are widely considered rare but have not received federal protection that would help conserve their populations in summer habitats. It is my hope that these findings and methods can be applied to this unique species throughout its range so that its habits, distributions, and range-wide abundance can be better understood to inform and guide its conservation.

The bat epidemic white-nose syndrome (WNS) has resulted in the mortality of millions of bats in the United States, and it continues to spread. Eastern small-footed bats at Shenandoah National Park remain abundant following this disease and were recorded at most talus slopes using the visual search technique. They occupied a broad range of topographic conditions and were not restricted to south- or west-facing aspects as hypothesized but were more abundant in a range of aspects that received high solar exposure, relatively high elevations, and semi-sheltered positions. Greater abundances related to the presence of maternity colonies that also appeared to prefer talus slopes containing large boulders that support large colony groups and offer thermal benefits important to torpor use and energy balancing in reproductive females.

Geospatial tools using image classification algorithms constitute an important means of identifying discrete wildlife habits and were effective in classifying talus slopes. Prediction from spatial abundance model calibrated with visual search observations and corresponding topographic variables to classified talus slopes yielded a useful landscape-scale population estimate of eastern small-footed bats. This novel method has potential for repeatability in other areas of their range to predict populations and may streamline the identification of quality habitat that support eastern small-footed bat maternity colonies.

The maternity colony in the South District of Shenandoah National Park utilized structured, highly connected fission-fusion roost and social networks that appeared to balance aspects of colonial reproduction such as social thermoregulation, threats from predators, parasitism, and communal care. The colony exhibited site fidelity over several years, indicating the importance of high-quality maternity roost habitats that contain a dense concentration of variable rock crevice roosts, as well as the continuity of matrilineal social networks to successful reproduction. Natality was high in this colony, suggesting recruitment has not been compromised by WNS at Shenandoah National Park as has been reported in other species.

Thermoregulation in this maternity colony revealed that torpor use was important to reproduction in eastern small-footed bats, as it was used consistently. Differing thermoregulatory patterns among pregnant and lactating bats provided insights to the tradeoffs of these strategies in terms of energy conservation and reproductive success. Torpor patterns and passive warming of eastern small-footed bats in rock crevice roosts confirmed the importance of solar exposure to the reproductive ecology of eastern small-footed bats.

The ecology of eastern small-footed bats at Shenandoah National Park demonstrates an elegant example of the interrelationships of behavior and physiology in which a species may take advantage of the unique properties of a niche to thrive.