Seasonal Activity Patterns of Northern Long-eared Bats at Hibernacula in Western Virginia

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Abstract: Understanding the relationships of biotic and abiotic factors to seasonal activity at hibernacula is important for the conservation of bats impacted by white-nose syndrome (WNS). Research on the relative and probable activity patterns of the federally endangered northern long-eared bat (*Myotis septentrionalis*) primarily has focused on summer maternity colonies, whereas surveys at hibernacula have traditionally relied on external capture and internal counts. We used passive acoustic monitoring to assess the relative and probable activity of northern long-eared bats at 13 hibernacula in western Virginia, from August 2020 to May 2022. Northern long-eared bats were most active near hibernacula during warmer weeks of the fall swarm and spring emergence, when rainfall was low. Similarly, the probability of northern long-eared bat activity was highest near hibernacula during the spring/summer season. However, unlike relative activity, the likelihood of recording northern long-eared bats was associated with more heterogeneous, interior forests. Our results suggest that northern long-eared bat activity largely follows the described pre-WNS hibernation phenology of the species. Therefore, acoustical surveys to monitor northern long-eared bat populations at hibernacula should focus on entrances during peak activity periods (mid-April and early September), rather than the nearby landscape. Finally, management to promote resource-rich foraging habitat adjacent to hibernacula for use during swarming and emergence may increase survival during hibernation, fitness for spring migration, and ultimately, improve the reproductive success of northern long-eared bats in western Virginia.

Key words: *Myotis septentrionalis*, hibernacula, seasonal, activity

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Northern long-eared bats (*Myotis septentrionalis*) were recently listed as federally endangered (USFWS 2022) under the U.S. Endangered Species Act of 1973 due to extreme population declines resulting from the spread of white-nose syndrome (WNS) caused by the fungal pathogen *Pseudogymnoascus destructans* (*Pd*; Cheng et al. 2021). Northern long-eared bats, once among the most commonly captured species in the central Appalachians (Ford et al. 2006), have declined by 95% in total captures and limited reproductive success among surviving bats has been documented (Francl et al. 2012, Reynolds et al. 2016). Recent observation of maternity roosts in the Ridge and Valley and Blue Ridge Mountains of Virginia (Figure 1) provide evidence, based on early abandonment of roosts and absence of juvenile captures, for ongoing maternity colony collapse and failed recruitment (Kalen et al. 2022). Moreover, in the nearby High Appalachian Plateau of the Central Appalachians in West Virginia (Figure 1), an area characterized by colder winters with longer hibernation periods, northern long-eared bat populations declined rapidly after *Pd* invasion of karst hibernacula (Johnson et al. 2013, Ford et al. 2016, Austin et al. 2018).

The breakup of summer colonies often is associated with the conclusion of the summer reproductive season and departure of bats to hibernation sites (Pfeiffer and Mayer 2013). Preceding hibernation, northern long-eared bats, along with other hibernating species, engage in swarming behavior, gathering at cave and mine entrances to mate and select suitable hibernation sites (Caceres and Barclay 2000, Van Schaik et al. 2015, Fraser and McGuire 2023). During this period in the early fall, bats may enter their selected hibernaculum during the night to mate but exit to forage and then will typically roost in trees during the day (Caceres and

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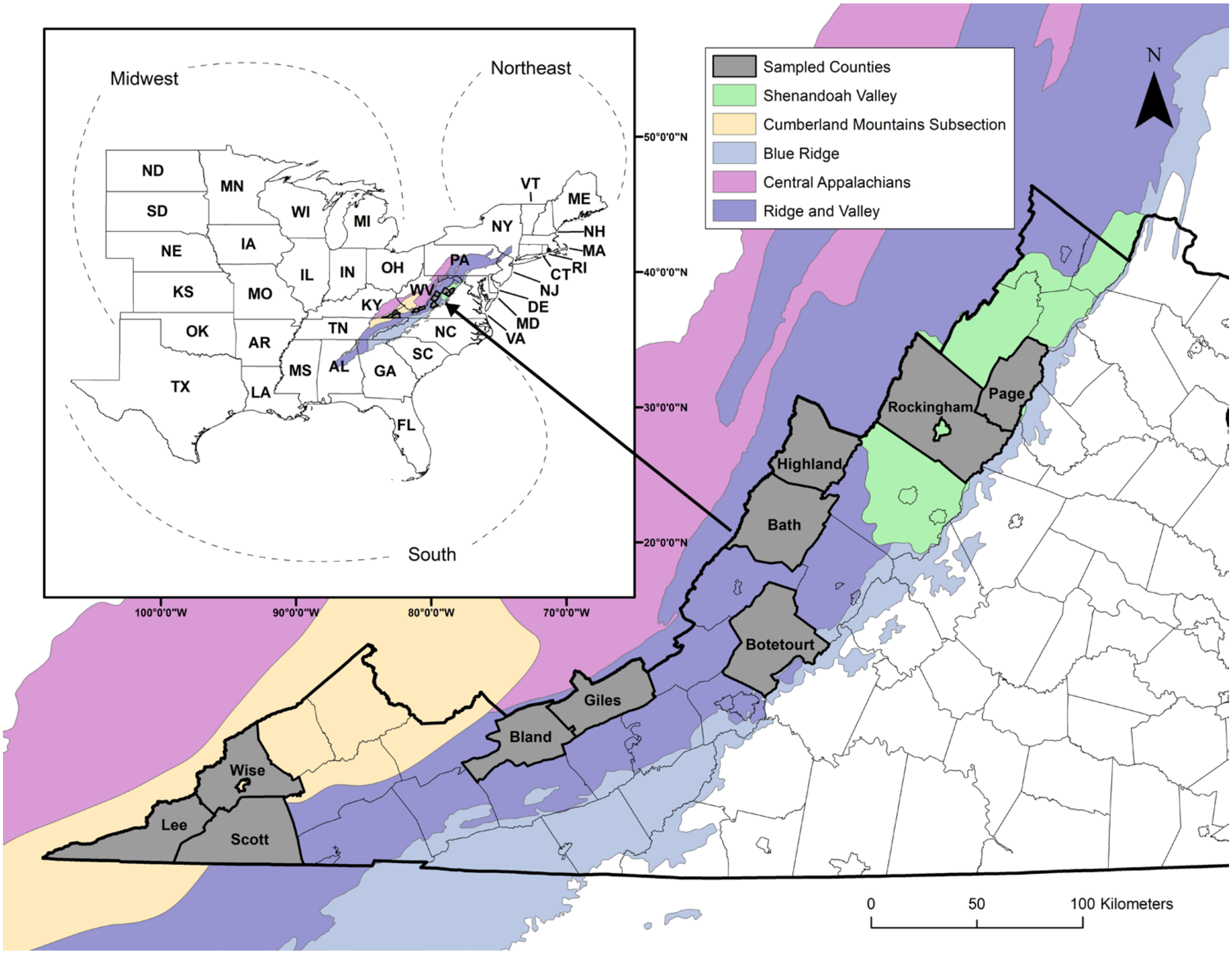

Figure 1. Ten county boundaries (gray fill denotes sampled area) for 13 hibernacula in western Virginia surveyed for northern long-eared bat (*Myotis septentrionalis*) calls, 2020–2022.

Barclay 2000). Successful foraging during the swarming period to increase fat deposits is essential for survival during hibernation, especially in the post-WNS era (Frick et al. 2017, Cheng et al. 2019, Cheng et al. 2021). This need is constrained by summer breeding, the energetic costs of fall migration, and mating (Fraser and McGuire 2023). Therefore, it is beneficial that bats select hibernacula near highly productive foraging habitat to promote fat deposition prior to hibernation (Jackson et al. 2022). The identification and management of high-quality habitat for fall roosting and foraging for many species remains a critical conservation data gap (Muthersbaugh et al. 2019). For example, prescribed fire near hibernacula may benefit northern long-eared bats by reducing forest clutter, increasing day-roost suitability, and enhancing foraging opportunities (Ford et al. 2016), thereby potentially leading to increased body mass going into hibernation (Lacki et al. 2015).

Following swarming, temperate zone bats may hibernate as long as 200 days (Speakman and Thomas 2003). Despite the high energetic costs (Thomas and Geiser 1997), northern long-eared bats frequently arouse during winter (Whitaker and Rissler 1992). Since the invasion of *Pd*, such episodes are thought to reduce bat susceptibility to *Pd* invasion, and therefore possibly disease related mortality (Bernard et al. 2017, Reynolds et al. 2017, Jackson et al. 2022). During these arousal events, bats may reduce *Pd* loads by grooming and therefore potentially increase the likelihood of survival so long as fat reserves are not dangerously depleted (Brownlee-Bouboulis and Reeder 2013, Reynolds et al. 2017).

Additionally, research in Tennessee has shown that some bats, including northern long-eared bats, are actively and successfully foraging on the winter landscape (Bernard et al. 2021), regardless of disease status (Bernard and McCracken 2017). It appears likely that the mild climate of the southeastern U.S. provides an insect prey base even during the hibernation period (Bernard et al. 2021). These conditions likely reduce both the physiological (e.g., weight loss) and disease (e.g., WNS severity) related stresses of hibernation compared to more northern regions (Bernard and McCracken 2017). Although arousal during hibernation may offer some survival benefits (e.g., grooming, foraging) for WNS-affected bats, abnormal behaviors such as daytime activity or excursions in subfreezing temperatures probably are detrimental to bats whether in the mild Southeast (Bernard and McCracken 2017) or colder areas of the Northeast (Reynolds et al. 2017) or Midwest (Langwig et al. 2021).

During the spring emergence period, northern long-eared bats leave hibernacula and disperse across the landscape (Caceres and Barclay 2000). During this time, reproductively active females undertake short- to mid-range (5–150 km), but energy-demanding, migrations from hibernation sites to summer roosting sites to form maternity colonies (Whitaker and Hamilton 1998, Gumbert et al. 2002). After emergence and an initial foraging session, bats may stage or begin migrating immediately, alternating thereafter between foraging bouts and migration flights (Roby et al. 2019). Conversely, male bats typically emerge after females (Cope and Humphrey 1977) and often remain near the hibernacula far into summer (Ford et al. 2005). Roby et al. (2019) found that bats reduce foraging or even cease migration when air temperatures drop below 10 C. Similarly, staging female northern long-eared bats in Kentucky utilized diurnal torpor while roosting on the landscape under adverse conditions (Thalken et al. 2018). While northern long-eared bats may use torpor to preserve energy reserves, prey availability and foraging efficiency near hibernacula during emergence are critical determinants of successful migration and reproduction (Meyer et al. 2016). However, successful foraging prior to migration is dictated not only by habitat availability and quality adjacent to hibernacula, but also by weather conditions at emergence (Meyer et al. 2016, Roby et al. 2019).

To assess the drivers of seasonal relative (i.e., weekly counts of echolocational recordings) and probable (i.e., likelihood to collect ≥1 recording) activity of northern long-eared bats at hibernacula, we initiated a multi-season survey in 2020 at 13 bat hibernacula in western Virginia. Our objectives were to use passive acoustic monitoring to evaluate the effect of 1) seasonality; 2) weather covariates; 3) proximity to hibernacula; and 4) habitat factors on relative and probable activity estimates of northern long-eared bats. We hypothesized that northern long-eared bat activity would be greatest during the fall swarm and spring emergence, nearer hibernacula, during warmer, dryer periods, and in interior portions of heterogenous forest habitats. Additionally, we hypothesized that the likelihood to record northern long-eared bat activity would follow similar trends.

Study Area

We collected acoustic data at hibernacula located in the Ridge and Valley and Appalachian Plateau physiographic regions of Bath, Bland, Botetourt, Giles, Highland, Lee, Page, Rockingham, Scott, and Wise counties in western Virginia (Figure 1). The Ridge and Valley is a series of long, narrow valleys and high ridges, with elevations ranging from 350 m to 1460 m. The Ridge and Valley has a temperate climate, with average annual temperatures ranging from 7–14 C in the north to 13–17 C in the south. Precipitation ranges from 80–150 cm therein (USDA NRCS 2022). The relatively dry climate of the Ridge and Valley supports xeric oak (*Quercus* spp.)-hickory (*Carya* spp.) or oak-pine (*Pinus* spp.) forests on ridges and side slopes. On mesic, north-facing slopes, yellow poplar (*Liriodendron tulipifera*), American beech (*Fagus grandifolia*), and sugar maple (*Acer saccharum*) occur (Braun 1950). In the Cumberland Mountain subsection of the Appalachian Plateau, more mesic oaks, such as northern red oak (*Q. rubra*), typically dominate the diverse second- and third-growth side-slope forests, and rich, mixed mesophytic hardwood forests are present in sheltered coves and north-facing slopes (Braun 1950). Additionally, this region contains significant legacy below-ground coal mines, that can provide important overwintering sites for bats (Lituma et al. 2021). Regional topography here is characterized by steep slopes and narrow valleys ranging from approximately 240 m to 1130 m in elevation. The average annual temperature and precipitation of the Cumberland Mountain subsection are cooler and wetter than in the Ridge and Valley, at 13 C and 120 cm, respectively (USDA NRCS 2022).

Methods

Acoustic Monitoring

We conducted acoustic monitoring at 13 hibernacula (Big Salt, Breathing, Church Mountain, Cove Creek Mine, Cudjos, Hupmans Saltpeter, Kelly, New River, Newberry-Bane, Peerys, Rocky Hollow, Starr Chapel Saltpeter, and Woods Cave) within the 10 above-mentioned western Virginia counties from August 2020 to May 2022 (Figures 1, 2). We established on average $\bar{x} = 3 \pm 2$ (± SD) acoustic sampling sites at each hibernaculum, with recorders deployed near the opening feature to 550 m ($\bar{x} = 170 \pm 187$ m) beyond openings, to assess both relative and probable activity of northern long-eared bats. We used ultrasonic recorders to collect

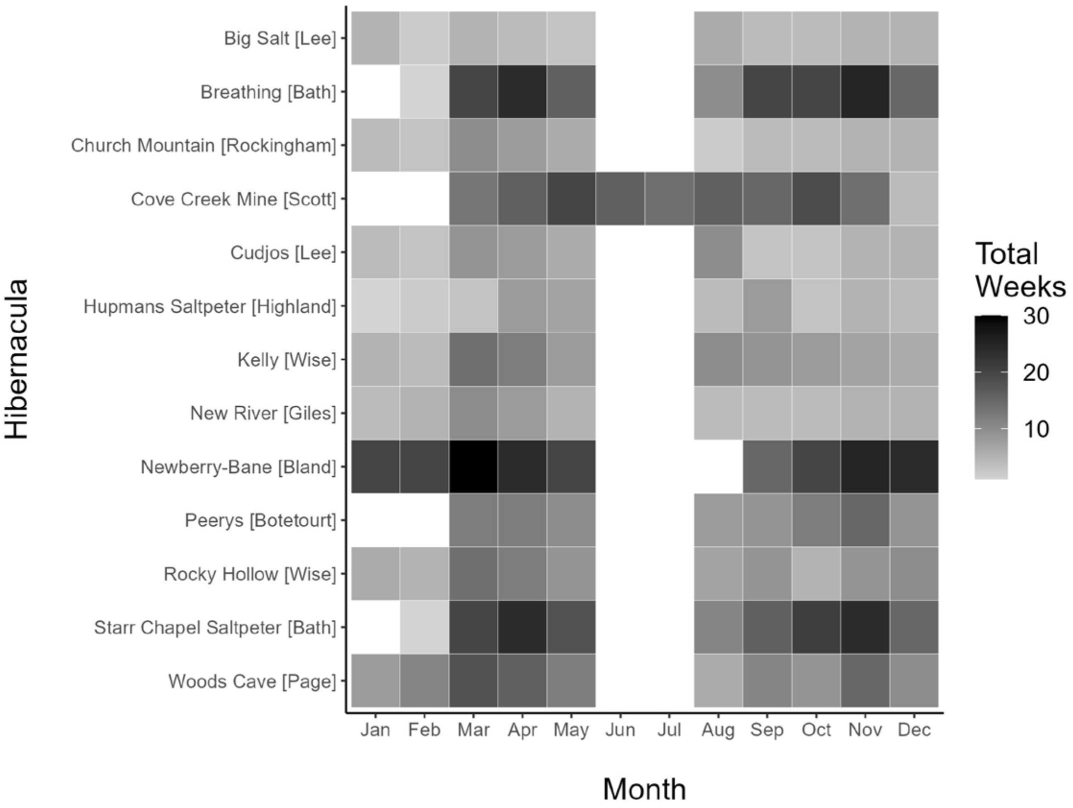

Figure 2. Sampling effort as represented by a monthly heatmap (white areas represent unsampled months) of the total number of acoustic monitoring weeks conducted at 13 hibernacula in western Virginia, 2020–2022 (county name in brackets; see Figure 1 for county locations).

acoustic data (Song Meter (SM) Mini, SMZC, and SM4; Wildlife Acoustics, Maynard, Massachusetts). We attached recorders to trees at an approximate height of 1.5 m above the ground, ensuring microphones extended beyond the diameter of the bole, or on 3-m poles as dictated by site conditions (Muthersbaugh et al. 2019). We deployed recorders perpendicular to hibernacula entrances and foraging/flyway features (e.g., forest roads/trails, streams, and rivers; Britzke et al. 2010). We programmed detectors to record from 1.5 hr prior to sunset to 1.5 hr after sunrise. We checked functionality of recorders and downloaded and processed data at approximately 30- to 60-day intervals. We used an automated acoustic software (Kaleidoscope Pro software; v. 5.4.7; Wildlife Acoustics) to identify echolocational recordings to species using default signal detection parameters and the 5.4.0 classifier set to 'Balanced/Neutral' (USFWS 2020). We categorized assemblages based on a north-south gradient, with a transition zone in Giles County. In southern sites, we examined data for 12 species: Virginia big-eared bats (*Corynorhinus townsendii virginianus*), big brown bats (*Eptesicus fuscus*), eastern red bats (*Lasiurus borealis*), hoary bats (*Lasiurus cinereus*), silver-haired bats (*Lasionycteris noctivagans*), gray bats (*M. grisescens*), eastern small-footed bats (*M. leibii*), little brown bats (*M. lucifugus*), northern long-eared bats, Indiana bats (*M. sodalis*), evening bats (*Nycticeius humeralis*), and tri-colored bats (*Perimyotis subflavus*). In northern sites, we excluded gray and evening bats based on their limited distribution in the region. However, due to recent range expansions into the New River Valley, we included gray bats in Giles County (Reynolds and Fernald 2021, Taylor et al. 2023).

Predictor Variables

Following recent research in eastern West Virginia (De La Cruz et al. 2023), we examined relative and probable activity of northern long-eared bats in relation to landscape richness (i.e., total number of local cover types within a 500-m moving window) using the 2019 National Land Cover Database (NLCD) raster dataset (Dewitz and USGS 2021). Also using the NLCD layer, we incorporated a measure of distance from forest edge (0) into forest interior (–) and from forest edge into (+) non-forest cover (De La Cruz et al. 2023). This layer was created using forest/non-forest reclassifications of NLCD raster data and the distance function in the raster package in R (R Core Team 2020, Hijmans 2023). We standardized both our landscape richness and forest edge raster layers to a 100-m resolution. We also examined the effect of the weather variables weekly mean of daily average temperature (C) and weekly mean of daily total precipitation (mm; Muthersbaugh et al. 2019). We obtained matching nightly weather data for sites from the Parameter-elevation Regressions on Independent Slopes Model (PRISM) Data Explorer, using the inverse-distance squared weighting interpolation option for the standard 4 km PRISM grid cell (PRISM Climate Group 2022). To account for likely bimodal activity patterns associated with swarming and emergence before and after hibernation, we incorporated a fourth-order polynomial of week of hibernation in models. Additionally, we assessed course temporal trends using seasonal (i.e., summer/fall, winter, and spring/summer) covariates (Muthersbaugh et al. 2019, Gorman et al. 2021, Deeley et al. 2022, Taylor et al. 2023). Finally, we evaluated the effect of proximity to hibernacula (≤200 m or >200 m; Muthersbaugh et al. 2019).

Statistical Analysis

To account for known rates of misclassification and to minimize false positive and false negative errors, we assumed presence of northern long-eared bats only if a statistically significant maximum likelihood estimate (MLE; $P < 0.05$) was returned for any given night/site (Britzke et al. 2002). We aggregated nightly data by hibernaculum, recording site, and year, then totaled counts for northern long-eared bats across each corresponding week of the year (Straw et al. 2022). We used these counts (i.e., weekly relative activity) as the response variable in all modeling efforts. We used a Shapiro-Wilk test to evaluate weekly relative activity totals for non-normality and found that the data were not normally distributed ($P < 0.01$). Therefore, we used zero-inflated negative binomial generalized linear mixed models (GLMMs) in the glmmTMB package of R (Brooks et al. 2017) for all statistical analyses. All GLMMs included two sub-models: 1) a conditional count sub-model to model relative activity; and 2) a zero-inflation sub-model to model probable activity. Prior to modeling, we assessed variables for collinearity using pairwise correlation (threshold = $|0.8|$) and centered and scaled continuous variables. We developed and compared seven multivariate GLMMs and a null model. We included in all conditional sub-models the random effect of hibernaculum location (Muthersbaugh et al. 2019, Taylor et al. 2023). We ranked models using Akaike Information Criterion corrected for small sample sizes (AIC_c) and considered models within two ΔAIC_c as competing (Burnham and Anderson 2002). We assessed goodness-of-fit and over- and under-dispersion of our top models using a quantile-quantile plot, residual plot, and a one-sample Kolmogorov-Smirnov test with the R DHARMa package (Hartig 2020). Additionally, we assessed ecological relevance of our top models by measure of conditional Nakagawa R^2 (i.e., proportion of variance explained by the fixed and random effects) using the performance package in R (Nakagawa and Schielzeth 2013, Lüdecke et al. 2021). We interpreted R^2 values as follows: ≤0.20, very low; 0.21–0.40, low; 0.41–0.60, medium; 0.61–0.80, high; and 0.81–1.00, as very high proportions of variance explained (Gorman et al. 2021, Hill et al. 2024, Torre et al. 2022). Finally, we created model-averaged partial effect plots using AIC_c weights to examine the relationship between relative and probable activity and predictor variables found to be significant.

Results

During 2020–2022, we sampled for 1267 weeks and collected 6,622,598 files of acoustic data from 13 hibernacula in western Virginia. A significant ($P < 0.05$) nightly MLE identified 712,221 (11%) files from our recordings to species, with 17,492 (2.4%) passes identified as northern long-eared bats. We recorded northern long-eared bats at all hibernacula sampled, with the greatest number of passes collected at Woods Cave ($n = 5031$; three sampling sites) and the least at Hupmans Saltpeter ($n = 58$; one sampling site; Figure 1).

We had two competing models within two ΔAIC_c units explaining drivers of relative and probable activity of northern long-eared bats (Table 1). Our top models passed all DHARMa goodness-of-fit tests and explained a medium proportion of variance (Model 1: $R^2 = 0.49$; Model 2: $R^2 = 0.47$), suggesting moderate ecological relevance. We observed that northern long-eared bat relative activity was greatest near hibernacula, and that activity was bimodal as expected, with distinct peaks related to the fall swarm (early-September) and spring (mid-April) emergence (Table 2; Figure 3). Furthermore, we found that northern long-eared bat relative activity was greatest during drier, warmer weeks (Table 2; Figure 3). However, we observed no influence of distance from forest edge (0) into forest interior (–) and from forest edge into (+) non-forest cover or total landscape richness on relative activity (Table 2). Our top-ranking models indicated that the probability for northern long-eared bat activity was highest near hibernacula during the spring/summer season (Table 2; Figure 4). However, results suggest that probable northern long-eared bat activity was highest in interior, heterogenous forest settings (Table 2; Figure 4). Finally, we observed no relation between probable activity of northern long-eared bats and the weekly mean of daily average temperature or precipitation (Table 2).

Discussion

We observed significant peaks in relative activity of northern long-eared bats coinciding with both fall swarming and spring

Table 1. Variables included in both conditional count and zero-inflation sub-models of generalized linear mixed models, number of full-model parameters (K), Akaike's information criterion (AIC_c) units, ΔAIC_c units, full-model weights (w_i), and full-model log-likelihood (LL), predicting weekly relative and probable activity of northern long-eared bats (*Myotis septentrionalis*) at 13 hibernacula in western Virginia, 2020–2022.

Model[a]	K[b]	AIC_c	ΔAIC_c	w_i	LL
TEMP + PRECIP + PROX + FOREST + RICHNESS	20	5256.95	0.00	0.71	−2608.14
TEMP + PRECIP + PROX	16	5258.72	1.77	0.29	−2613.14
PROX	12	5274.10	17.15	0.00	−2624.92
DIST + FOREST + RICHNESS	16	5274.23	17.28	0.00	−2620.90
TEMP + PRECIP + FOREST + RICHNESS	18	5437.04	180.10	0.00	−2700.25
FOREST + RICHNESS	14	5450.65	193.70	0.00	−2711.16
TEMP + PRECIP	14	5487.72	230.78	0.00	−2729.69
Null	3	5704.79	447.84	0.00	−2849.39

a. TEMP: weekly mean of daily average temperature (C); PRECIP: weekly mean of daily total precipitation (mm); PROX: proximity to hibernacula (<200 m or >200 m); FOREST: distance from forest edge (0) into forest interior (–) and from forest edge into (+) non-forest cover (m); RICHNESS: number of local cover types.

b. All models include a single dispersion parameter and intercepts in both the conditional and zero-inflation sub-models. Excluding the Null model, each conditional sub-model contained the fourth-order polynomial effect of week of hibernation and random effects of hibernacula location, while zero-inflation sub-models contained the fixed effect of season.

Table 2. Predictor variables, β estimates, and SE estimates for the top two generalized linear mixed models predicting weekly relative (i.e., counts of echolocational recordings; conditional sub-model) and probable (i.e., likelihood to collect ≥1 recording; zero-inflation sub-model) activity of northern long-eared bats (*Myotis septentrionalis*) at 13 hibernacula in western Virginia, 2020–2022.

		Model 1			Model 2		
Sub-model	Variable[a]	β	SE	P	β	SE	P
Conditional	Intercept	2.76	0.29	<0.01	2.34	0.31	<0.01
	TEMP	0.55	0.19	<0.01	0.65	0.20	<0.01
	PRECIP	−0.38	0.08	<0.01	−0.38	0.09	<0.01
	PROX – >200 m	−1.81	0.28	<0.01	−1.13	0.23	<0.01
	HIB	0.67	3.25	0.84	3.60	3.50	0.30
	HIB^2	7.22	5.64	0.20	5.27	6.15	0.39
	HIB^3	−1.96	2.95	0.51	−0.74	3.06	0.81
	HIB^4	−25.19	3.36	<0.01	−22.82	3.41	<0.01
	FOREST	0.62	0.34	0.07	–	–	–
	RICHNESS	−0.28	0.36	0.43	–	–	–
Zero-inflation	Intercept	0.89	0.27	<0.01	2.27	0.95	0.02
	TEMP	−0.20	0.16	0.20	−0.33	0.22	0.12
	PRECIP	−0.02	0.12	0.88	0.02	0.15	0.88
	PROX – >200 m	−1.69	0.23	<0.01	−2.90	0.82	<0.01
	SEASON – Winter	−0.25	0.34	0.47	−0.61	0.49	0.21
	SEASON – Spring/Summer	0.59	0.25	0.02	0.74	0.35	0.04
	FOREST	−0.55	0.16	<0.01	–	–	–
	RICHNESS	0.53	0.15	<0.01	–	–	–

a. TEMP: weekly mean of daily average temperature (C); PRECIP: weekly mean of daily total precipitation (mm); $HIB + HIB^2 + HIB^3 + HIB^4$: terms of fourth-order polynomial of week of hibernation; PROX: proximity to hibernacula (<200 m or >200 m); FOREST: distance from forest edge (0) into forest interior (–) and from forest edge into (+) non-forest cover (m); RICHNESS: number of local cover types; - denotes variables absent from model ; conditional sub-models contained the random effects of hibernacula location.

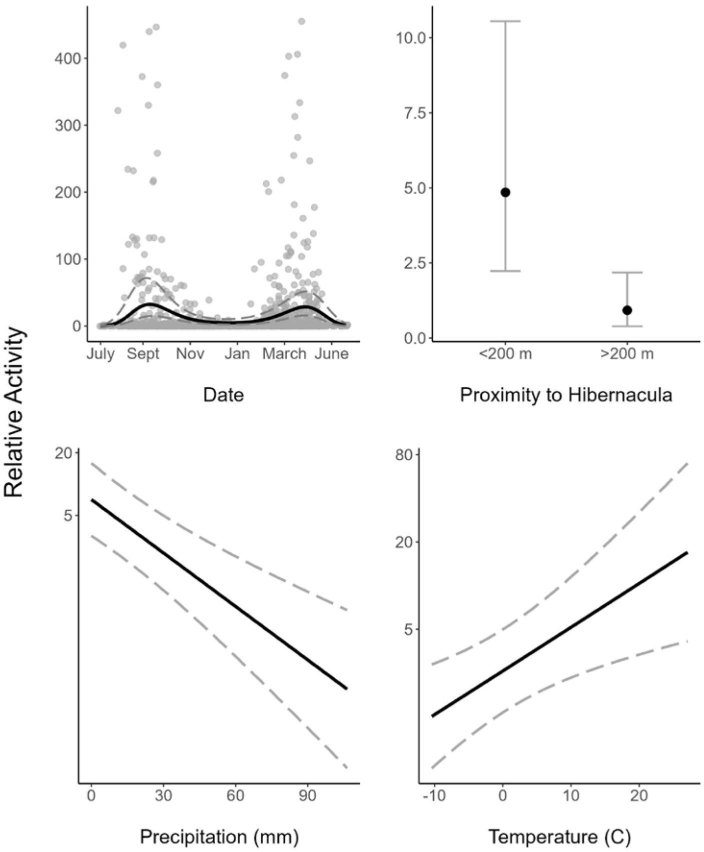

Figure 3. Model-averaged partial effect plots of predicted weekly relative activity (and 95% CI) of northern long-eared bats (*Myotis septentrionalis*) at 13 hibernacula in western Virginia, 2020–2022.

emergence, with activity concentrated near hibernacula entrances. Similarly, the probability of recording northern long-eared bats was highest near hibernacula during the spring/summer season and suggest that prioritizing sampling efforts at these entrances, rather than the broader landscape, could be a more efficient approach for monitoring northern long-eared bats at hibernacula. The increased likelihood of documenting northern long-eared bats during spring/summer raises questions about the composition and behavior of remnant populations. For example, an extended emergence period could be indicative of remnant populations represented by high proportions of resident males. However, similar to the varied emergence behavior observed in Indiana bats by Roby et al. (2019), some northern long-eared bats in our study may immediately migrate to summer maternity grounds whereas other individuals stage longer near hibernacula to replenish fat reserves and repair WNS-induced damage prior to migration. Additionally, we observed a higher probability to record northern long-eared bats in both interior forests and areas of greater landscape richness. This result, coupled with documented winter predation of insect prey (Bernard et al. 2021), may provide support for management that provides for the interspersion of high-quality foraging

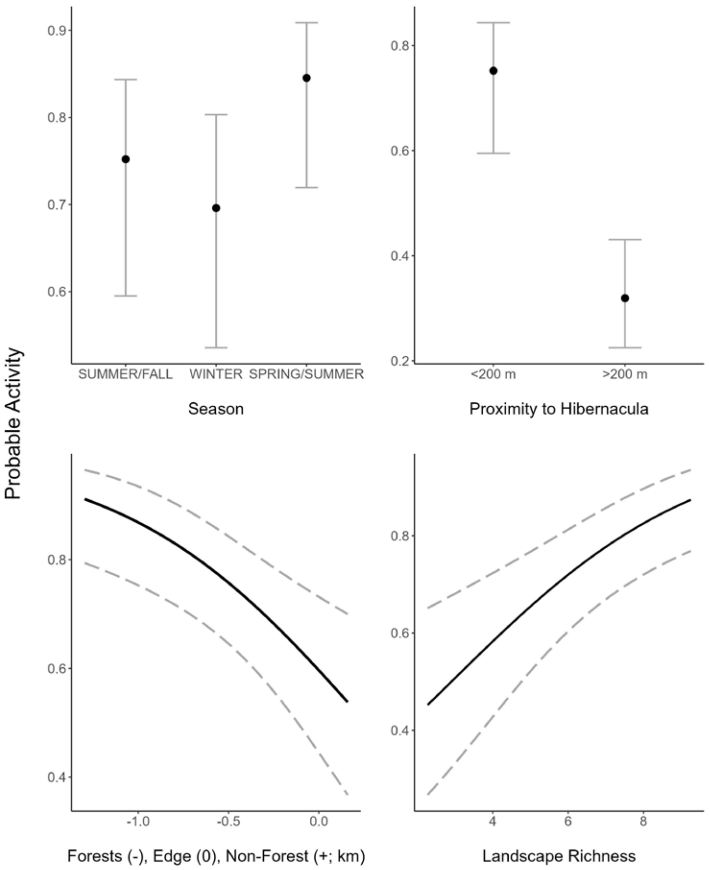

Figure 4. Model-averaged partial effect plots of weekly predicted probability of activity (and 95% CI) of northern long-eared bats (*Myotis septentrionalis*) at 13 hibernacula in western Virginia, 2020–2022.

habitat near hibernacula. Such management may improve the survival, migratory fitness, and ultimately, reproductive success of the northern long-eared bat.

Prior to our study, only Muthersbaugh et al. (2019) had assessed any facet of the post-WNS relative and probable activity of northern long-eared bats at hibernacula during the overwintering period in the Central Appalachians and our study was far more expansive in duration and sample-size. Still, our results are largely consistent with this previous work and suggest that northern long-eared bat activity at hibernacula is associated with the described phenology of the species, with bats arriving at hibernacula in the fall to mate and build fat stores before hibernation, and emerging in the spring to forage before migrating to summer roosting sites. Importantly, these results suggest that habitat alterations near hibernacula during specific timeframes (15 August–1 November and 15 March–15 May) may have a negative impact on northern long-eared bats, even within the context of the post-WNS landscape. Although relative activity estimates indicated peak periods during fall swarming and spring emergence, the probability of recording northern long-eared bat activity exceeded 50% during winter. This high probability for activity during winter, despite low relative activity, reveals that mid-winter arousal events are not uncommon. While these events may be partially driven by the detrimental impacts of WNS, causing bats to exit hibernacula under abnormal conditions (i.e., subfreezing temperatures, during daylight; Bernard and McCracken 2017), they also may be associated with active foraging, drinking, and grooming (Brownlee-Bouboulis and Reeder 2013, Reynolds et al. 2017, Bernard et al. 2021), roost switching within hibernacula (Ryan et al. 2019), or even movement between hibernacula (Langwig et al. 2021). Therefore, the persistence of northern long-eared bats in western Virginia may be due to their behavioral and physiological mitigation of advanced WNS, as evidenced by recent summer captures of the species in the Ridge and Valley of Virginia nearly 10 years post-WNS invasion (Kalen et al. 2022).

Our results indicate that weather also was a significant driver of northern long-eared bat activity at hibernacula in western Virginia. Specifically, relative activity increased with greater weekly means of daily average temperature; however, the probability of recording northern long-eared bats was not significantly impacted by temperature. Activity and temperature are typically linked to insectivorous prey availability and cost to an animal's energy budget to procure prey in cold weather (Bernard and McCracken 2017). The apparent decoupling of temperature for some species may signify WNS arousal, particularly for *Pd*-sensitive species such as northern long-eared bats. Our results support both observations. Northern long-eared bats in western Virginia were significantly more active during the warmer weeks of the year, when insect availability to bats is presumably greater. Nevertheless, the probability of recording the species remained relatively high despite low temperatures during winter, coinciding with the likely progression of WNS. Unlike Muthersbaugh et al. (2019), we observed a significant negative effect of weekly means of daily average precipitation on relative activity of northern long-eared bats. High precipitation may reduce hibernation arousals and exiting flights in northern long-eared bats in western Virginia, as the energy deficits from WNS and flight are unlikely to be offset by behavioral or physiological mechanisms. This hypothesis is supported by research showing that precipitation increases the energy cost of flight for bats (Voigt et al. 2011), maternity colonies avoid exposure to inclement weather (Patriquin et al. 2016), and torpor length in little brown bats is positively linked with precipitation totals (Dzal and Brigham 2013).

Our observed northern long-eared bat hibernacula associations derived from passive acoustic monitoring occasionally differ from those of site-specific internal counts conducted by the Virginia

Department of Wildlife Resources and the Virginia Department of Conservation and Recreation, Division of Natural Heritage. Although some automated acoustic misidentification of northern long-eared bats cannot be fully discounted (Nocera et al. 2019), this disparity may simply be attributed to roost selection within hibernacula, as the species often roosts in small clusters or singly in fissures and crevices of mines and caves that are overlooked or unapparent to observers during surveys (Caceres and Barclay 2000). Rather than using these sites simply as overwintering habitat, northern long-eared bats also may be attracted to hibernacula for short periods due to elevated social calls, for feeding opportunities, as migratory stopovers, and for mating (Lacki et al. 2015). Additionally, the seemingly high frequency of mid-winter arousals, potentially exacerbated by climate change-induced disruptions to normal hibernation periods (McClure et al. 2022), raises concerns about the adequacy of single-day internal surveys for monitoring of northern long-eared bats, given potential biases associated with imperfect detection (Cheng et al. 2021). Implementation of several methods (i.e., repeated external and internal surveys, long-term acoustic sampling) may increase monitoring confidence for northern long-eared bats at hibernacula in Virginia and elsewhere.

Northern long-eared bats appeared very active at Woods Cave. This cave, despite being farther north than other sampled hibernacula, is located in the Shenandoah Valley (Figure 1), which is significantly warmer during winter than the Ridge and Valley/Appalachian Plateau border to the west where northern long-eared bats were abundant pre-WNS (Johnson et al. 2013). Additionally, Woods Cave is in a mosaic of diverse forest types, moderately fragmented by agriculture, and is adjacent to the South Fork of the Shenandoah River. The post-WNS persistence of northern long-eared bat populations at Woods Cave may be attributed to the favorable combination of mild winter conditions and a diverse habitat assemblage characteristic of the Shenandoah Valley. Although Woods Cave and Hupmans Saltpeter, our most and least productive locations, respectively, are only 113 km apart, they may differ in their suitability for northern long-eared bats due to geophysical variation, suggesting that research assessing how factors such as elevation, structure, airflow, and ambient temperature affect northern long-eared populations post-WNS could be contributory. Additionally, because activity during winter may be associated with hibernacula switching, relating density of mine/cave openings near known hibernacula may be of value as well. Due to the relatively high probability of recording northern long-eared bats during winter that we observed, additional research related to prey availability and foraging of cave-hibernating bats in western Virginia may be warranted. Finally, the combination of passive monitoring and active capture may assist in acoustic monitoring validation and reveal whether relative activity correlates with trapping counts from the fall swarm and spring emergence (Whiting et al. 2022). Our research suggests that managers may benefit from scheduling active capture surveys for northern long-eared bats during periods of peak relative activity in spring (April) and early fall (September). Additionally, internal counts may benefit from multiple surveys between 28 October and 23 February, when activity was lowest, to mitigate bias associated with estimates of abundance and imperfect detection. Repeated hibernacula entry can negatively affect WNS-impacted species, however, research has shown that up to three surveys per year had no detectable impact on populations of Indiana bats, little brown bats, or tri-colored bats (Kilpatrick et al. 2020). Passive acoustics surveys may serve as either the basis for hibernacula surveys or, at minimum, provide useful additive information for inclusion in integrated models to support the management of rare, threatened, and endangered bats at hibernacula in western Virginia.

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