Northern Long-eared Bats in the Central Appalachians Following White-nose Syndrome: Failed Maternity Colonies?

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Abstract: Northern long-eared bat (*Myotis septentrionalis*) populations have experienced severe declines in eastern North America from white-nose syndrome (WNS), yet potential secondary effects on maternity roosting and recruitment remain largely unknown. We documented female day-roosting at two locations in the central Appalachians of Virginia, Back Creek Mountain (BCM) and Rapidan Camp (RC), during 2015 and 2016, approximately six years after the regional onset of WNS. We compared roost characteristics with available trees and roosts recorded prior to WNS at the Fernow Experimental Forest (FEF), West Virginia, in 2007 and 2008. Roosts at BCM were smaller than pre-WNS roosts but were otherwise similar in terms of stand condition and species use, though bats selected for red maple (*Acer rubrum*) at BCM rather than black locust (*Robinia pseudoacacia*) as at FEF. At RC, bats roosted almost exclusively in large eastern hemlock (*Tsuga canadensis*) snags (dbh $\bar{x} = 50.13$ cm, SD = 23.1) with high solar exposure that had been killed by the hemlock woolly adelgid (*Adelges tsugae*). The two observed strategies, selection of smaller, midstory trees at BCM and of dominant, exposed roosts at RC, correspond with pre-WNS observations of female northern long-eared bat roost use at similar sites. However, our results suggest reliance on smaller roosts and canopy-dominant positions that better accommodate solitary individuals and small groups associated with smaller post-WNS colonies in terms of space and thermoregulatory benefits. Despite some observations of pregnant and lactating individuals, all three post-WNS colonies vacated roost networks in early June, and we observed no juveniles. Potential colony failure at BCM and RC is consistent with predicted secondary physiological effects from WNS-induced population collapses, suggesting, if recruitment failed, northern long-eared bats may already be functionally extirpated in portions of the central Appalachians.

Key words: Myotis septentrionalis, roost, colony, recruitment, failure

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Prior to the advent and spread of white-nose syndrome (WNS; *Pseudogymnoascus destructans*), northern long-eared bats (*Myotis septentrionalis*) were common throughout much of eastern North America (Silvis et al. 2016). In the central Appalachians, they were often the most commonly captured bat in upland forest habitats (Menzel et al. 2002, Owen et al. 2002, Ford et al. 2006). Northern long-eared bats have since experienced precipitous population declines from WNS and estimates of regional mortality exceed 90% (Turner et al. 2011, Francl et al. 2012, Moosman et al. 2013, Reynolds et al. 2016). Moreover, Francl et al. (2012) and Reynolds et al. (2016) observed what appears to be altered reproductive phenology and a reduction in recruitment rates among surviving bats. In response to effects from WNS, the U.S. Fish and Wildlife Service (USFWS) listed the northern long-eared bat as a federally-threatened species in 2015 (USFWS 2016), and the species is currently under review for endangered status (USFWS 2020).

Northern long-eared bats form non-random assorting maternity colonies in the summer, with reproductive females cohabiting a social network of day-roosts in trees and snags (Johnson et al. 2012). Roost use and switching occurs in a fission-fusion dynamic with individuals in smaller groups periodically fusing in to and diffusing out of larger groups (Silvis et al. 2014). Maternity colony use by temperate biome bats provides reproductive benefits that include social thermoregulation, which hastens gestation and juvenile development (Racey and Swift 1981, Willis and Bringham 2007, Altringham 2011), allonursing (Watkins and Shump, 1981, Wilkinson 1992), and communal juvenile care (Beck and Rudd 1960, Kerth 2008). Prior to WNS, northern long-eared bat maternity colonies, depending on reproductive state (i.e., pregnancy, parturition, lactation, or weaning) typically included 20–30 adults (Sasse and Perkins 1996, Owen et al. 2002, Perry and Thill 2007, Patriquin et al. 2012, Silvis et al. 2014), but colonies of 60–100 indi-

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viduals were not uncommon (Owen et al. 2002, Rojas et al. 2017). Long-term philopatry to summer habitat (>5 years) has been observed and appears related to colony social structure (Perry 2011, Silvis et al. 2014).

The summer day-roosting habits of northern long-eared bats prior to WNS have been reasonably well-documented with the species observed using >50 tree species throughout its distribution (Silvis et al. 2016). At local and regional scales, consistent preference for several species have been observed, notably: black locust (*Robinia pseudoacacia*), red maple (*Acer rubrum*), sugar maple (*A. saccharum*), sassafras (*Sassafras albidium*), and oaks (*Quercus* spp). Northern long-eared bats generally roost in tree cavities and under sloughing bark, and day-roost selection often has been linked to successional processes or past disturbance events (i.e., fire, forest pests, and forest management; Lacki and Schwierjohann 2001, Menzel et al. 2002, Ford et al. 2006, Perry and Thill 2007, Johnson et al. 2009, O'Keefe 2009, Silvis et al. 2016, Rojas et al. 2017). Anthropogenic structures such as buildings or bat houses also are utilized but less frequently (Krynak 2010, De La Cruz et al. 2018).

Roost microclimate is regarded as one of the most important factors of energy expenditure in bats (McNab 1982), and reproductive females have the greatest demand for maintaining normothermic body temperature (Foster and Kurta 1999). Depending on study site and extant forest condition, reproductive females have been observed selecting larger overstory trees in some settings (Sasse and Pekins 1996, Foster and Kurta 1999, Lacki and Schwierjohann 2001, Garroway and Broders 2008, O'Keefe 2009, Rojas et al. 2017, Hyzy et al. 2020), and smaller, midstory or suppressed trees in others (Owen et al. 2002, Ford et al. 2006, Johnson et al. 2009, Silvis et al. 2012). Selection for maternity day-roosts under reduced canopy cover (Sasse and Perkins 1996, Perry and Thill 2007, Garroway and Broders 2008, O'Keefe 2009) and positions on ridgetops and sideslopes with southeast- to west-facing aspects in areas with topographic relief is commonly associated with increased solar exposure and consequent thermal benefits (Lacki and Schwierjohann 2001, Johnson et al. 2009, Silvis et al. 2012). Overall, maternity roost selection appears to be driven by opportunistic use of a variety of roost characteristics over different climate and landscape conditions to achieve optimal thermal conditions that enable reproductive success (Sedgeley 2001).

Northern long-eared bats face plausible risk of widespread extirpation from WNS across much of their distribution in eastern North America (Frick et al. 2015). Furthermore, changes in reproductive timing and output observed by Francl et al. (2012) and Reynolds et al. (2016) suggest maternity colony size, structure, viability, and day-roosting habits also have been affected, yet potential secondary WNS impacts remain unknown due to recent scarcity of northern long-eared bats where WNS mortality has been extreme (Turner et al. 2011). Recent observations have documented several populations of northern long-eared bats with successful recruitment in coastal areas of North Carolina, Washington, D.C., New York, and Massachusetts (Dowling et al. 2017; Jordan 2020; K.M. Gorman, Virginia Tech University, personal communication). Their persistence has been attributed to either avoidance of karst hibernacula where the fungal pathogen that causes WNS is present or shorter dormancy periods that prevent deleterious infection (Dowling et al. 2017, Jordan 2020). Aside from these anomalous coastal populations, there have been few observations of northern long-eared bat maternity colonies post-WNS. Thalken and Lacki (2018) documented maternity colonies at Mammoth Cave National Park, Kentucky, in 2015 and 2016 following the local arrival of WNS in 2013, but few comparisons were made with pre-WNS observations beyond finding lower roost exit counts.

To evaluate potential changes to northern long-eared bat maternity roosting and recruitment from the effects of WNS in the central Appalachians, we assessed colonies in 2015 and 2016 following the regional onset of WNS in 2009 and compared roost selection with Johnson et al. (2009) from 2007 and 2008, before WNS had reached the region. We expected colonies to be comprised of fewer individuals than pre-WNS, and that reduced colony numbers may alter social structure and delay, or even eliminate, successful recruitment as posited by Francl et al. (2012).

Study Area

In 2015 and 2016, we observed northern long-eared bat day-roosting post-WNS at two locations within the central Appalachians of western Virginia, Back Creek Mountain (BCM) in the Ridge and Valley physiographic province and Rapidan Camp (RC) in the Blue Ridge physiographic province. Pre-WNS roosts were characterized in 2007 and 2008 by Johnson et al. (2009) at the Fernow Experimental Forest (FEF) in the Allegheny Mountain portion of the Appalachian Plateau physiographic province in northeastern West Virginia. Back Creek Mountain is approximately 130 km west-southwest of RC, and FEF is approximately 120 km west-northwest of RC and 110 km north of BCM (Figure 1).

Back Creek Mountain lies within George Washington National Forest and includes adjacent private lands in Bath County, Virginia. It is part of a linear ridge system with relatively broad intermontane valleys, and elevations range from approximately 700 m to 1000 m. Forests of this area are mesic oak and montane pine associations dominated by chestnut oak (*Q. montana*), white oak (*Q. alba*), Virginia pine (*Pinus virginiana*), and pitch pine (*P. rigida*). In lower elevations and along riparian corridors, more me-

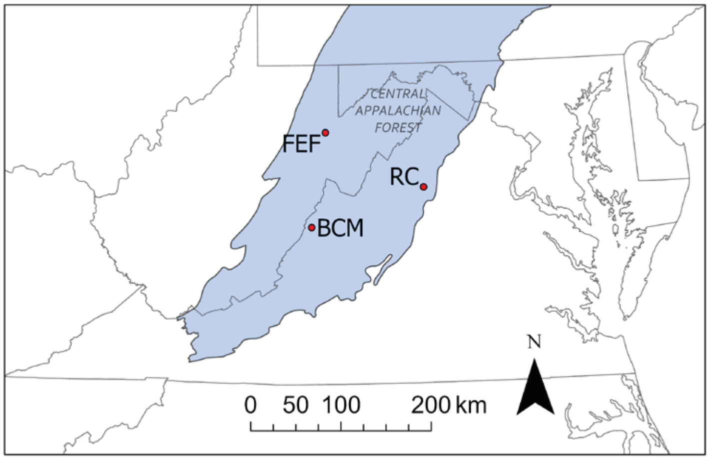

Figure 1. Locations of northern long-eared bat (*Myotis septentrionalis*) maternity roost sites at Fernow Experimental Forest, West Virginia (FEF), Back Creek Mountain (BCM), and Rapidan Camp, Virginia (RC). Roosts at FEF were observed in 2007 and 2008, prior to the regional introduction of white-nose syndrome (WNS) in 2009. Post-WNS roosts were observed in 2015 and 2016 at BCM and in 2016 at RC.

sic species such as white pine (*P. strobus*), eastern hemlock (*Tsuga canadensis*), and red maple predominate. Fire suppression has increased abundance of fire-intolerant species such as red maple, American beech (*Fagus grandifolia*), blackgum (*Nyssa sylvatica*), and tulip poplar (*Liriodendron tulipifera*). Rapidan Camp is in the Madison County, Virginia, portion of Shenandoah National Park. Topography consists of steep slopes with narrow, sheltered side-slope ravines and valley floors. Shenandoah National Park is 95% forested with most forest cover comprised by mature oak-hickory (*Carya* spp.) and cove hardwood (Young et al. 2009, Costanzo et al. 2016). Located in the basin of the Rapidan River headwaters, elevations at RC are approximately 750–800 m. Forest cover is a cove hardwood-riparian type, previously dominated by eastern hemlock recently killed by the hemlock woolly adelgid (*Adelges tsugae;* HWA) with a dense, early successional understory of yellow birch (*Betula alleghaniensis*), black birch (*B. lenta*), and striped maple (*A. pensylvanicum*).

Our pre-WNS central Appalachian comparison site, FEF, is a 1900-ha forest research site managed by the US Forest Service in Tucker County, West Virginia. Topography is similar to BCM and elevations range from 530 to 1100 m (Johnson et al. 2009). Forests are a mosaic of mixed mesophytic and northern hardwoods managed with a variety of silvicultural practices (Johnson et al. 2009). Dominant tree species include northern red oak (*Q. rubra*), sugar maple, red maple, black cherry (*Prunus serotina*), tulip poplar, American beech, black birch, and black locust. Like BCM, it has undergone a shift toward greater presence of shade-tolerant species following a lack of fire and harvest disturbance since the mid-20th century, though some prescribed fire research occurred prior to Johnson et al. (2009)'s study.

Methods

We captured bats using mist nets (38 mm, low-bag, Avinet Research Supplies, Portland, Maine) set across closed forest corridors, ephemeral pools, and first-order streams or adjacent known roost trees from May through July in 2015 and 2016 at BCM and in 2016 at RC. For all northern long-eared bats captured, we recorded sex, age, reproductive condition, mass, forearm length, and wing damage index and placed 2.4-mm uniquely numbered aluminum bands on their forearms (Porzana Ltd., Icklesham, East Sussex, U.K.; Menzel et al. 2002). We determined age by examining epiphyseal-diaphyseal fusion and reproductive condition in females by palpating the abdomen and examining mammaries (Menzel et al. 2002). We attached radio transmitters (0.27 g; Holohil Systems, Ltd., Woodlawn, Ontario, Canada) between the scapulae using Perma-Type surgical cement (Perma-Type Company, Inc., Plainville, Connecticut; Silvis et al. 2012). We released bats near the site of capture within 15 minutes. Our capture and handling of bats adhered to the guidelines of the Virginia Polytechnic Institute and State University Institutional Animal Care and Use Committee permit #16-049 and Virginia Department of Game and Inland Fisheries threatened and endangered species permit #055802.

We located radio-tagged female northern long-eared bats using TRX1000S receivers and three-element Yagi antennas (Wildlife Materials, Inc., Carbondale, Illinois). At each day-roost, we recorded roost tree species, diameter at breast height (dbh), height, percent bark, canopy cover, decay stage (Cline et al. 1980), and crown class (Nyland 1996). When possible, we identified roosts as in a cavity or under exfoliating bark. We georeferenced roosts with a Garmin GPSmap 64st global positioning system (Garmin International, Inc., Olathe, Kansas). We measured tree height using a clinometer (model PM-5/1520, Suunto, Finland) and used a Model-A spherical concave densiometer (Forestry Suppliers, Inc., Jackson, Mississippi) to measure canopy cover by averaging measurements at four cardinal directions from the base of the tree. When we identified roosts located in canopy-dominant snags bearing no foliage and receiving no shade, we recorded canopy cover as zero, rather than estimating canopy cover from the ground where solar exposure was not representative of roost conditions (Drake et al. 2020). We estimated basal area (m^2 ha^{-1}) of the surrounding stand with a 20-factor prism (Forestry Suppliers, Inc., Jackson, Mississippi). To compare roosts with surrounding trees, we used ArcMap 10.3 (ESRI, Redlands, California) to generate two random points per roost, one within the minimum convex polygon (MCP) of roost trees identified, and one within a larger 65-ha buffer surrounding roosts, which is reflective of the mean home range size of female northern long-eared bats during the maternity season in the central Appalachians pre-WNS (Owen et al. 2003). For all random points, we selected the nearest potential roost tree that featured cavities or sloughing bark and measured the same characteristics as for roost trees (Owen et al. 2002, Johnson et al. 2009).

To compare roosts at BCM and RC with their respective random trees and similarly measured pre-WNS northern long-eared bat maternity roosts at FEF, we used two-sample Wilcoxon tests for the following variables: dbh, tree height, canopy cover, decay class, slope, aspect, and basal area (Johnson et al. 2009, Silvis et al. 2012). We used Fisher's exact test to evaluate the equitability of proportion of roost use among available tree species, crown class, and live or dead status (Johnson et al. 2009). We held $\alpha = 0.05$ for significance in all pairwise comparisons. Lastly, we used a principal component analysis (PCA) to evaluate roost structure characteristics more broadly among all three sites using centered and scaled independent variables: dbh, tree height, crown class, canopy cover, basal area, and decay stage (McConville et al. 2013). We performed all analyses with the *stats* package in R version 4.0.3 (R Core Team 2020). We generated a PCA biplot using package *FactoMineR* version 2.4 (Lê et al. 2008).

Results

At BCM, we mist-netted on 16 nights between 28 May to 25 July in 2015 (including two nights at occupied roosts) and 14 nights between 23 May and 23 July in 2016 (including eight nights at occupied roosts), capturing 10 female northern long-eared bats: three pregnant in 2015, and one pregnant and six lactating in 2016. At RC, we mist-netted for 15 nights between 16 May and 14 July in 2016 and captured six female northern long-eared bats that exhibited no identifiable signs of pregnancy or lactation and one male northern long-eared bat. Capture frequency was 0.088 captures per net-night over 2015 and 2016 at BCM, excluding effort at occupied roosts, and 0.058 at RC in 2016. Capture frequencies were very low compared with pre-WNS results in West Virginia prior to 2008 (1.438) and even post-WNS results (0.330) in 2010 (Francl et al. 2012). We tracked these 16 female northern long-eared bats to 26 unique day-roosts, 15 at BCM and 11 at RC. Colony groups at BCM appeared distinct as there was no overlap of roosts or bats from 2015 to 2016. The highest exit counts we observed from individual roosts were five bats in 2015 and seven in 2016 at BCM and three bats at RC in 2016. Bats at these locations roosted within an area close to the capture site (<1 km), shared roosts, and occupied roosts in close proximity (<10 m) through early June. However, the presumed maternity colony groups disassociated and were absent by mid-June in all three cases. We last documented female northern long-eared bats on 7 June 2015 and 10 June 2016 at BCM and 13 June 2016 at RC.

Back Creek Mountain

Roosts at BCM included: red maple ($n = 5$), chestnut oak ($n = 4$), black gum ($n = 2$), sassafras ($n = 2$), black locust ($n = 1$), and an unidentified hickory ($n = 1$; Table 1). Four of five red maple roosts were live trees and all other roosts were snags in variable stages of decay. Red maple was used significantly more than were available ($P = 0.002$), whereas chestnut oak, black gum, sassafras, and black locust were used in proportion to occurrence. Roosts occurred in snags more than were available ($P = 0.003$), and roost decay stage was greater than random ($P = 0.008$; Table 2). Roost dbh, tree height, crown class, canopy cover, aspect, slope position, and surrounding basal area were not significantly different from that of random trees. For comparison of BCM roosts to pre-WNS roosts at FEF, we found BCM roosts to be smaller in dbh ($P = 0.001$), shorter ($P = 0.005$), under greater canopy cover ($P = 0.043$), of more westerly aspect ($P < 0.001$), and of differing crown class distribution ($P = 0.005$), but similar in basal area, slope, decay stage, and proportion of snag use (Table 3). Species use was similar between BCM and FEF with roosts occurring in maples, oaks, and sassafras at both sites, but preference for black locust exhibited at FEF was not observed at BCM. Thirteen of 15 BCM roosts were in tree cavities and two were under sloughing bark, similar to FEF. Mean aspect of BCM roosts was 257.8 ± 65.2°, with the majority falling between west and north, whereas mean aspect of FEF roosts was 153.3 ± 38.5°. Spatially, most roosts at BCM were situated on

Table 1. Tree species day-roost use by northern long-eared bats (*Myotis septentrionalis*) and availability as indicated by random sampling at Back Creek Mountain (BCM) and Rapidan Camp (RC), Virginia, in 2015 and 2016 relative to pre-white-nose syndrome (WNS) at the Fernow Experimental Forest (FEF) in West Virginia, 2007–2008. Values are counts (percent) of trees.

	BCM		**RC**		**FEF**
Tree species	**Roosts**	**Random**	**Roosts**	**Random**	**Roosts**
Acer spp.	5 (33*)	0 (0)	0 (0)	2 (9.1)	11 (15.9)
Quercus spp.	4 (27)	11 (36.7)	0 (0)	6 (27.3)	10 (14.5)
Nyssa sylvatica	2 (13)	7 (23.3)	0 (0)	0 (0)	0 (0)
Sassafras albidum	2 (13)	1 (3.3)	0 (0)	0 (0)	4 (5.8)
Carya spp.	1 (7)	1 (3.3)	0 (0)	0 (0)	1 (1.4)
Robinia pseudoacacia	1 (7)	2 (6.7)	1 (9.1)	0 (0)	33 (47.8)
Tsuga canadensis	0 (0)	0 (0)	10 (90.1*)	2 (9.1)	0 (0)
Betula spp.	0 (0)	2 (6.7)	0 (0)	8 (36.4)	0 (0)
Liriodendron tulipifera	0 (0)	1 (3.3)	0 (0)	4 (18.2)	1 (1.4)
Prunus serotina	0 (0)	0 (0)	0 (0)	0 (0)	3 (4.3)
Oxydendrum arboreum	0 (0)	0 (0)	0 (0)	0 (0)	3 (4.3)
Magnolia acuminata	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.4)
Fraxinus spp.	0 (0)	2 (6.7)	0 (0)	0 (0)	0 (0)
Pinus spp.	0 (0)	2 (6.7)	0 (0)	0 (0)	0 (0)
Unknown	0 (0)	0 (0)	0 (0)	0 (0)	2 (2.9)

*denotes the species was used more than was available by Fisher's exact test

Table 2. Characteristics (mean ± 1 SD; observed % for snags and crown classes) of northern long-eared bat (*Myotis septentrionalis*) roosts compared with available random trees at Back Creek Mountain (BCM) and Rapidan Camp (RC), Virginia. Comparisons of diameter at breast height (dbh), tree height, canopy cover, decay class, and basal area were made using two-sample Wilcoxon tests (*W*), and Fisher's exact test was used to compare distribution of crown class and live or dead status for percent snags between roosts and random trees.

	BCM				RC			
Characteristics	**Roost trees**	**Random trees**	***P***	***W***	**Roost trees**	**Random trees**	***P***	***W***
n	15	30	–	–	11	22	–	–
Dbh (cm)	16.33 ± 7.11	18.32 ± 12.95	0.828	215.5	49.03 ± 22.19	28.40 ± 20.59	0.015	57.0
Tree height (m)	10.00 ± 6.44	11.11 ± 6.13	0.462	256.0	19.12 ± 7.37	15.77 ± 8.02	0.214	88.0
Decay[a]	3.3 ± 1.8	2.1 ± 2.0	0.008	123.5	3.8 ± 0.6	1.4 ± 1.1	0.000	19.5
Snags (%)	69	23	0.003	–	100	13.6	0.000	–
Basal area (m^2 ha^{-1})	32.73 ± 16.07	28.60 ± 8.99	0.451	193.5	27.53 ± 10.31	29.41 ± 12.07	0.490	139.5
Canopy cover (%)	88.0 ± 14.1	92.0 ± 17.6	0.205	278.0	32.5 ± 43.0	89.7 ± 3.4	0.003	199.0
Dominant (%)[b]	40	26.7	0.531	–	63.6	22.7	0.029	–
Codominant (%)	20	16.7	–	–	9.1	40.9	–	–
Intermediate (%)	40	43.3	–	–	9.1	31.8	–	–
Suppressed (%)	0	13.3	–	–	18.2	4.5	–	–

a. Decay class based on Cline et al. (1980)
b. Crown class based on Nyland (1996)

Table 3. Characteristics (mean ± 1 SD; observed % for snags and crown classes) of northern long-eared bat (*Myotis septentrionalis*) roosts recorded prior to the regional onset of white-nose syndrome (WNS) at the Fernow Experimental Forest (FEF), West Virginia, and roosts at Back Creek Mountain (BCM) and Rapidan Camp (RC), Virginia. Comparisons of diameter at breast height (dbh), tree height, roost height, basal area, canopy cover, and decay class were made using two-sample Wilcoxon tests (*W*), and Fisher's exact test was used to compare distribution of crown class and live or dead status for percent snags between FEF roosts and BCM and RC roosts, respectively.

			BCM vs. FEF			RC vs. FEF	
Characteristics	**FEF (pre-WNS)**	**BCM**	**P**	**W**	**RC**	**P**	**W**
n	69	15	–	–	11	–	–
Dbh (cm)	28.53 ± 17.22	16.33 ± 7.11	0.001	795.0	49.03 ± 22.19	0.004	157.0
Tree height (m)	15.45 ± 7.46	10.00 ± 6.44	0.005	757.0	19.12 ± 7.37	0.096	260.0
Decay[a]	4.0 ± 1.6	3.3 ± 1.8	0.181	630.5	3.8 ± 0.6	0.559	421.0
Snags (%)	81	69	0.492	–	100	0.196	–
Basal area (m^2 ha^{-1})	34.18 ± 10.40	32.73 ±16.07	0.706	550.0	27.53 ± 10.31	0.027	537.0
Canopy cover (%)	81.3 ± 18.1	88.0 ± 14.1	0.043	344.0	32.5 ± 43.0	0.001	578.5
Dominant (%)[b]	4.3	40	0.005	–	63.6	0.000	–
Codominant (%)	39.1	20		–	9.1	–	–
Intermediate (%)	33.3	40		–	9.1	–	–
Suppressed (%)	23.2	0		–	18.2	–	–

a. Decay class based on Cline et al. (1980)
b. Crown class based on Nyland (1996)

mid- and low sideslope positions. The MCP of the six BCM roosts in 2015 was 20.8 ha and nine roosts in 2016 was 39.8 ha.

Rapidan Camp

At RC, bats roosted almost exclusively in eastern hemlock snags ($n = 10$) except for one black locust roost. Selection for eastern hemlock was strong ($P < 0.001$) whereas oak, birch, and tulip poplar were avoided despite prevalence. Notably, seven eastern hemlock roosts were in positions above the canopy and received no shade. Compared to random trees, roost trees were larger in dbh ($P = 0.015$), more often snags ($P < 0.001$), of greater decay stage ($P = 0.008$) and had lower canopy cover ($P = 0.003$). Six of 11 roosts were in cavities; we were unable to identify specific locations of the other five. Compared to FEF, RC roost trees were larger in dbh ($P = 0.008$), within lower basal area ($P = 0.027$) and under lower canopy cover ($P = 0.001$). Rapidan Camp roosts were oriented linearly along a basin, with most occurring on flat terrain. The MCP of the 11 roosts was 6.5 ha.

Principal Components Analysis

Our PCA identified significant overlap in roost characteristics among all three sites (Figure 2). The first two components account-

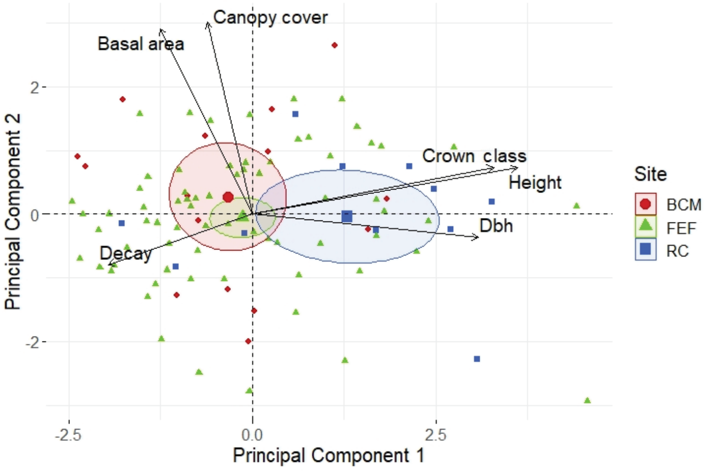

Figure 2. Northern long-eared bat (*Myotis septentrionalis*) day-roost principal components (see text) at Back Creek Mountain (BCM) and Rapidan Camp (RC), Virginia, in 2015 and 2016 compared to pre-white nose syndrome roosts at Fernow Experimental Forest (FEF), West Virginia, in 2007 and 2008. Large shapes denote mean coordinates of roost principal components by site and corresponding ellipses represent mean 95% confidence intervals.

ed for 60.1% of roost characteristic variance with the first component explaining 40.2% and second component explaining 19.9%. The first component was most representative of roost size with high loadings of tree height (0.577), crown class (0.525), and dbh (0.493). The second component was most representative of stand characteristics with high loadings of canopy cover (0.684) and basal area (0.661). Decay class had negative loadings in both first and second components (–0.313, –0.181) and was most positively represented in the third component (0.802), which explained 15.9% of data variance and also included high loadings of dbh (0.490) and canopy cover (0.332).

Discussion

Overall, female northern long-eared bat roosting at post-WNS sites in the central Appalachians was consistent with maternity colony roosting habits reported prior to the onset of WNS. Our observations generally conformed to two previously documented strategies: selecting smaller, suppressed midstory trees at BCM (Owen et al. 2002, Ford et al. 2006, Johnson et al. 2009, Silvis et al. 2012) and selecting large, dominant exposed roosts at RC (Garroway and Broders 2008, O'Keefe 2009, Rojas et al. 2017). Roosting patterns at BCM were similar to pre-WNS patterns at FEF in terms of condition, landscape position, and species composition, whereas roost selection at RC was different but did correspond with studies in other regions (Sasse and Perkins 1996, Garroway and Broders 2008, O'Keefe 2009, Rojas et al. 2017). Roost MCPs at BCM (20.8 ha and 39.8 ha) and RC (6.5 ha) were comparable with the range of MCPs at FEF (18.3 ± 11.5 ha; Johnson et al. 2012).

Our PCA results showed that BCM mean coordinates were similar to FEF but were slightly lower in the first component and slightly higher in the second, reflecting trends of smaller tree use among denser stands with more continuous canopy cover. Whereas the RC mean coordinates represented greater tree size compared to FEF and unexpectedly similar stand structure components of basal area and canopy cover despite differences in forest composition. Notably, overlap among several large FEF roosts with low canopy cover and RC roosts demonstrated that similar conditions were used at FEF though they were outliers to overall trends observed there.

Despite low sample sizes in both locations and only one season of data from RC that limits our inference (Silvis et al. 2015), our ability to examine post-WNS day-roosts from BCM and RC with a more robust pre-WNS dataset from FEF provides a novel opportunity to explore potential secondary effects of WNS on maternity roosting among similar habitats within the central Appalachians. Of course, direct comparisons among sites should be interpreted with caution as differences in selection could be attributable to differing forest stand characteristics, cavity availability, or other unmeasured factors that reflect the generalist, highly adaptive nature of northern long-eared bat day-roost use. The BCM and FEF sites were very similar in forest composition and topography, though FEF had a greater range of stand ages. Although Shenandoah National Park overall was also similar, the RC portion differed as it was a sheltered riparian cove forest largely in a gap-phase, understory re-initiation stage in response to eastern hemlock mortality (Oliver and Larson 1996). Both BCM and FEF had similar management regimes that include regular forest harvest and use of prescribed fire (Johnson et al. 2009, Lorber et al. 2018), whereas RC has been largely left to natural disturbance regimes (i.e., wind, insect attack, and infrequent wildfire; Young et al. 2009, Austin et al. 2019).

Back Creek Mountain

At BCM, bats used smaller snags that did not significantly differ from random trees in any attribute other than live or dead status. This was unsurprising given the relative uniformity and continuous canopy of mid-successional to early mature stands present. Functionally, roosts at BCM closely resembled the midstory and suppressed pre-WNS roosts of FEF and other pre-WNS studies from more heavily-managed forests or that regenerated from similar disturbance events in the past (Menzel et al. 2002, Silvis et al. 2012). Canopy cover was slightly higher at BCM than FEF, which we attribute to greater presence of canopy gaps at FEF, especially in fire treatment locations (Johnson et al. 2009). The BCM roosts were considerably smaller in dbh and height than pre-WNS roosts at FEF. We hypothesize that use of smaller roosts could be the result of reduced colony numbers. At BCM, cavities in small diameter roosts might provide better physical structure for thermoregulation by solitary and small groups of northern long-eared bat females compared to larger roosts at FEF where mean exit count was 18.7 bats (Johnson et al. 2012).

Red maple was strongly selected for at BCM, comprising a third of total roosts despite low prevalence. Red maple was used pre-WNS at FEF, although in lower proportion to availability, and larger trees were chosen. Red maple was the most commonly-used species of post-WNS roosts in Mammoth Cave National Park (Thalken and Lacki 2018) and in coastal North Carolina (Jordan 2020). Given the suitability of red maple, its use by remaining northern long-eared bats could increase as this tree species is becoming increasingly abundant in the eastern United States (Nowacki and Abrams 2008). All other roost tree species at BCM have been used prior to WNS at FEF and elsewhere (Lacki and Schwierjohann 2001, Johnson et al. 2009, O'Keefe 2009, Silvis et al. 2016). Unexpectedly, black locust at BCM was used only once and in proportion to occurrence. This is contrary to findings of selection for black locust in a variety of stand conditions from FEF (Ford et al. 2006, Johnson et al. 2009), the nearby Westvaco Wildlife and Ecosystem Research Forest, West Virginia (Menzel et al. 2002, Owen et al. 2002), and Governor Dodge State Park, Wisconsin (Hyzy et al. 2020). Measurements of random trees indicate characteristics and availability of black locust were similar among BCM and FEF (Johnson et al. 2009). Fewer bats in post-WNS colonies may also explain the lack of preference for black locust snags at BCM. Sap in live tree stems causes slower heat conductance and provides a greater capacity to buffer internal temperatures compared to dead trees (McComb and Noble 1981, Coombs et al. 2010). Greater thermal inertia may have contributed to better microhabitat in small, live red maple trees for individuals and small groups with reduced capacity for social thermoregulation over moderate-size black locust snags.

Rapidan Camp

At RC, bats took advantage of high, exposed roosts that received full sun in large, dominant eastern hemlock snags. Preference for large snags with high solar exposure to presumably aid thermoregulation has been observed in many pre-WNS studies (Kunz and Lumsden 2003, Perry and Thill 2007, Garroway and Broders 2008, O'Keefe 2009, Rojas et al. 2017). Exaggeration of this trend at RC compared to pre-WNS studies may indicate increased reliance on solar exposure as a means of compensating for decreased social thermoregulatory potential in small post-WNS colony groups. Thalken and Lacki (2018) also observed small colony groups roosting in large trees with relatively low canopy cover at Mammoth Cave National Park post-WNS.

Results from RC highlight the adaptability of northern long-eared bats to exploit stochastic disturbances that create pulse recruitment of snags (Carter and Feldhamer 2005, Perry and Thill 2007, Ford et al. 2016). Similar use of insect-killed trees by northern long-eared bat females has been documented by Perry and Thill (2007) who observed preference for dominant shortleaf pine (*Pinus echinata*) snags killed by engraver beetles (*Ips* spp.) in the Ouachita Mountains and by Rojas et al. (2017) in Great Smoky Mountain National Park where most roosts were large white pine snags associated with southern pine beetle (*Dendroctonus frontalis*) infestation.

Northern long-eared bat colony selection for riparian cove forest at RC was contrary to a widely observed affinity for upland habitats (Silvis et al. 2016). However, it was consistent with pre-WNS findings at Nantahala National Forest, North Carolina, that O'Keefe (2009) attributed to selection for roosts near productive foraging habitat to minimize energetic costs of commuting. We agree with this reasoning, especially in locations with high topographic relief where energetic expenditure for commuting could be great. Cove roosts observed by O'Keefe (2009) were not specifically linked to disturbance events but were correlated with high snag densities and were similarly in large, dead and dying trees that received high solar exposure. Accordingly, solar warming is likely desirable for bats day-roosting in riparian cove locations generally associated with cold air drainage (Dobrowski 2010).

Colony Dispersal

Colony groups inexplicably vacated BCM and RC sites in early June prior to when potential juveniles would have become volant. In the region, juvenile volancy is typically observed beginning in early July (Francl et al. 2012). Fates of colonies could have been collective relocation, movement to combine with other groups, or disassociation and dispersal, especially if recruitment failed (Francl et al. 2012, Reynolds et al. 2016). Scenarios of failed recruitment after evidence of pregnancy and lactation are supported by Reynolds et al. (2016) who observed similar proportions of pregnant and lactating northern long-eared bats throughout western Virginia post-WNS but found a 76.7% decrease in proportion of juveniles and an eventual cessation of recruitment. Failed recruitment by northern long-eared bats at BCM and RC would be consistent with predicted secondary physiological and allee effects from WNS-induced population collapses and suggestive that northern long-eared bats may already be functionally extirpated in parts of the central Appalachians (Cryan et al. 2010, Ford et al. 2016, Reynolds et al. 2016).

Our findings indicate that small colonies of 10 or fewer northern long-eared bats may constitute a threshold whereby successful recruitment is no longer viable. Roosting behavior at BCM and RC was highly typical in reliance on snags created by forest successional processes and disturbance regimes, yet could be indicative of shifts towards greater dependence on smaller cavities and roosts with high solar exposure where colony groups are reduced following WNS. Also, red maples and HWA-killed eastern hemlocks may constitute preferred roosting conditions for remaining northern long-eared bats in changing central Appalachian forests.

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