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Cover Photograph: Photograph of a *Myotis lucifugus* (Little Brown Bat) colony in the rafters of a barn located in Wythe Co., VA. Photograph © Karen Powers.

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Reproductive Trends in Little Brown Bats Before and After the Onset of White-nose Syndrome in Virginia

Karen E. Powers[1,*], W. Mark Ford[2], Richard J. Reynolds[3], William D. Orndorff[4], David E. Yates[5], and Thomas E. Malabad[4]

Abstract - *Myotis lucifugus* (Little Brown Bat) declines in Virginia following white-nose syndrome (WNS) prompted an investigation into reproductive behaviors of surviving individuals. To examine reproductive change, we examined female bats prior to, during and after endemism establishment. We also examined capture trends of juveniles at maternity colonies. Timing and proportion of reproductive conditions did not differ except for reductions in lactating females during WNS invasion relative to the pre-WNS period. There was no significant difference in the proportion of juveniles between WNS-endemic years, indicating population recovery is slow. Of 78 recaptures, 2 individuals survived >8 years, suggesting individual longevity after WNS endemism. Our findings emphasize the value of long-term datasets to assess reproductive status of Little Brown Bats.

Introduction

White-nose syndrome (WNS), first documented in New York in 2006, is caused by an ascomycete fungus, *Pseudogymnoascus destructans* (Blehert and Gargas) Minnis and D. L. Lindner (*Pd*), that grows on hibernating bats. The fungus inhabits exposed (hairless) epidermal tissue of hibernating bats, whose body temperatures can drop to ambient levels. Hibernating bats are largely immune-incompetent (Bouma et al. 2010, Prendergast et al. 2002), allowing *Pd* to grow unabated (Hoyt et al. 2021) and destroy tissue (Meteyer et al. 2022). This drives a cascade of physiological changes that ultimately causes bats to arouse too often and expend fat stores before spring insects are reliably available (Cryan et al. 2010, Warnecke et al. 2013). To date, WNS has negatively impacted hibernating bats in 40 states in the continental United States and 8 Canadian provinces (USFWS 2023). Studies conducted shortly after the invasion of WNS suggested that surviving *Myotis* females in West Virginia shifted reproductive timing (pregnancy, lactation) into narrower windows (Francl et al. 2012), but longer-term impacts are less studied. In Virginia, long-term trends in summer reproductive activities of *Myotis* remain understudied, despite WNS population declines.

The first documented WNS cases in Virginia were in February and March 2009 at hibernacula in Giles and Highland counties (VDGIF 2009). Because WNS detection was extremely limited in 2009, we considered 2010 the advent of disease invasion (i.e., rapid expansion) in Virginia. By winter 2011, WNS was documented south and west of Virginia, and was endemic in Virginia caves. Although early estimates remain imprecise, WNS may have killed up to 6.7 million bats in eastern North America by 2012 (USFWS 2012). Mortality rates of *Myotis lucifugus* (Le Conte) (Little Brown Bat) exceeded 95%, as determined by counts at hibernacula and summer surveys in the central Appalachians,

[1]Radford University, Radford, VA 24142. [2]U.S. Geological Survey, Virginia Cooperative Fish and Wildlife Research Unit, Blacksburg, VA 24061. [3]Virginia Dept. of Wildlife Resources, Verona, VA 24482. [4]Virginia DCR-Natural Heritage, Richmond, VA 23219. [5]Biodiversity Research Institute, Portland, ME 04103. *Corresponding author – kpowers4@radford.edu.

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including Virginia (Francl et al. 2012, Johnson et al. 2021, Powers et al. 2015). By 2011, only 4 known summer maternity colonies for the Little Brown Bat remained in Virginia; today, Little Brown Bats remain markedly absent from most of the state. Current documentation of Little Brown Bats in Virginia is limited to these known maternity colonies and 1 summer bachelor cave. Counts at hibernating bats at 24 caves in western Virginia that each historically housed >100 Little Brown Bats remain low. By our counts, 75% of these caves house <10 hibernating individuals in biennial surveys after the onset of WNS. To date, we have located just 4 hibernacula where counts are increasing (R.J. Reynolds, unpubl. data). Though lacking in pre-WNS counts to which we can compare, maternity colonies remain among the locations in Virginia where more than a few individual Little Brown Bats can be captured in a single night. These colonies also allow for season-wide trends in reproduction to be studied.

To understand the impacts WNS has on the reproductive status of Little Brown Bats in Virginia, we examined several reproductive trends in adult females. In West Virginia, Francl et al. (2012) identified 3 measures of reproductive condition that would directly relate to colony population performance: percent of females pregnant in early summer, percent of females lactating mid-summer, and percent of post-lactating females in mid-to-late summer. We asked whether these metrics differed in our datasets pre-WNS (prior to 2010), during WNS invasion (2010–2012), and once WNS was endemic (after 2012). After the onset of WNS, when the surveys focused on maternity colonies, we examined the percent of captures that were juveniles in mid-summer. We postulated a decline in the proportion of juveniles to adults on the landscape after the onset of WNS, similar to the observations of Francl et al. (2012). We also predicted that reproductive performance (i.e., proportion of adult females pregnant, lactating, and showing evidence of post-lactation) would be lower during the WNS-invasion period relative to the pre-WNS period, and that performance would improve in the years following invasion. Furthermore, we predicted that the proportion of juvenile captures at maternity sites after the onset of WNS would show a modest increase consistent with persisting Little Brown Bat colonies elsewhere in the Northeast and mid-Atlantic. To further investigate natural history patterns in surviving Little Brown Bats after onset of WNS, we examined longevity trends of recaptured individuals. We predicted low recapture rates but high site fidelity, similar to Dobony and Johnson (2018) in New York, as remaining individuals seem to coalesce into a few to single maternity colonies on the local landscape.

Field-site description

We obtained our pre-WNS (1992–2009) and early WNS invasion (2010) data from opportunistic surveys conducted by the Virginia Department of Wildlife Resources (VDWR) and private contractors at summer locales. Projects consisted of VDWR mist-net and harp-trap efforts plus surveys by consulting firms that targeted *Myotis sodalis* Miller and G. M. Allen (Indiana Bat), Little Brown Bats, and *Myotis grisescens* A.H. Howell (Gray Bats) (Fig. 1). Habitats at netting sites varied based on project goals, but typically conformed to suitable habitat for Indiana Bats and *Myotis septentrionalis* (Trouessart) (Northern Long-eared Bats) as defined by USFWS (2022). These surveys were not intentionally associated with known Little Brown Bat maternity colonies, though many surveys likely occurred near colonies, as the species was abundant and widespread in western Virginia pre-WNS.

We focused our surveys on 4 maternity colonies and made the assumption that they could serve as performance surrogates for unknown colonies in Virginia and the surrounding mid-Atlantic to track reproductive trends since the onset of WNS. All 4 maternity colonies that we monitored were associated with barns on rural landscapes in Augusta (*n* = 1), Fauquier (*n* = 1), Rockingham (*n* = 1), and Wythe (*n* = 1) counties. Three barns were privately-owned, while the fourth was on state-managed land (Fauquier). We estimated straight-line distance between barns as follows: Rockingham Co. to Fauquier Co., 106 km; Rockingham Co. to Wythe Co., 240 km; Wythe Co. to Fauquier Co., 345 km; Augusta Co. to Fauquier Co. was 117 km, while Augusta Co. to Wythe Co. was 229 km. Distance between Augusta Co. and Rockingham Co. was 11 km (Fig. 1).

Methods

For pre-WNS efforts, we surveyed sites between Julian date 135 (15 May) and 227 (15 August), in line with the standardized netting protocol for the Indiana Bat (USFWS 2022). After the onset of WNS, we utilized data from the same range of Julian dates, with the focus on maternity colonies. We visited barns during 9 years from 2011 to 2021, typically once in late May during presumed gestation, and once mid-July when offspring are volant. Depending on the barn's structure and the location of the colony, we used 1 or more capture techniques: harp traps, mist-netting systems up to 9 m in height, and in some instances, hand captures. We opened nets and traps at sunset or shortly after. Because of variance in our timing of net opening and in trapping techniques, we did not examine capture-per-unit effort. Further, Little Bown Bats no longer utilized the Augusta Co. barn after summer 2012 and we did not trap there after failed netting efforts in May 2013. The remaining 3 sites continued to house maternity colonies.

We determined reproductive status of adult females, and we applied a uniquely numbered band (2.9-mm, Porzana Ltd., East Sussex, UK) to the forearms of both juveniles and adults. We confirmed reproductive status (pregnant, lactating, post-lactating, or non-

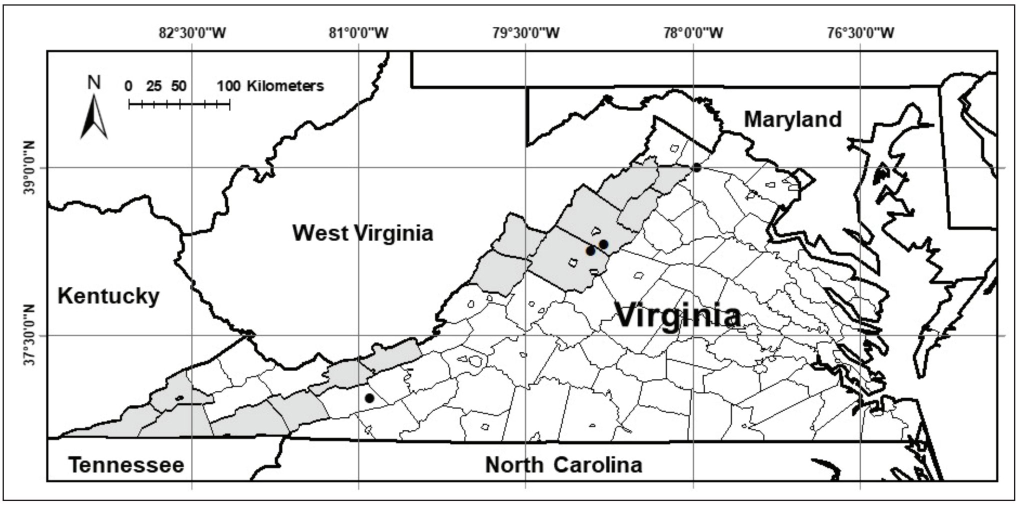

Figure 1. Location of 14 Virginia counties (shaded) in which surveys for Little Brown Bats were completed, 1992–2010. Black circles indicate locations of Little Brown Bat maternity colonies surveyed during the invasion (2010–2012) and WNS-endemic advent (2013–2021). Colonies from north to south: Fauquier, Rockingham, Augusta, and Wythe Counties. The Augusta County colony was extirpated after summer 2012.

reproductive) following standard techniques (Haarsma 2008). We identified juveniles versus adults by the degree of epiphyseal-diaphyseal fusion (visible in backlit examination of wings; Anthony 1988). Recapture of banded bats allowed us, in some instances, to document age relative to initial capture and banding. All handling of bats followed protocols approved by the Institutional Animal Care and Use Committee of Radford University and the Virginia Polytechnic Institute and State University, and field work was conducted under the authority of permits issued by the Virginia Department of Wildlife Resources.

Analyses

We quantified recapture rate of bats from maternity colonies after onset of WNS. Site revisits by bats were confirmed if the same individual was recaptured at the same maternity colony as its initial capture, regardless of starting "age." Recaptures were not analyzed statistically, but simply reported as the proportion of total captures. We noted anecdotal finds in the recapture dataset.

To assess if reproductive condition or juvenile capture proportions changed from pre-WNS to the WNS invasion period or the WNS-endemic phase, we used a generalized linear mixed model with a beta distribution and logit function in SAS 9.4 (PROC GLIMMIX; SAS Institute 2020). Collection site was included as a random variable. We entered numerical day of year as a continuous covariate because reproductive condition changes over the course of the maternity season. We also included the interaction of day of year with WNS period to examine if the timing of the proportional peak of any of the 3 reproductive conditions changed across disease status (Francl et al. 2012). For lactation, day of year was entered as a quadratic term because we expected a rise and fall in proportions across the summer season, per the reproductive phenology of Little Brown Bats (Silvis et al. 2016). When analyzing data on the reproductive condition of adult females, we included all data from mid-April until the last date of sampling in August. To determine the proportion of juveniles, we only used captures beginning on Julian day 180 (29 June) of the summer, reflecting initiation of volancy. Because of the inherent bias (known concentration) towards juvenile captures at maternity sites, we limited juvenile analyses to 2012 and beyond and examined trends at colonies over time. We checked each model for goodness-of-fit and over- and under-dispersion by examining residual plots.

Results

We captured 1590 Little Brown Bats from 1992–2021: 1183 adults (964 female, 219 male) and 407 juveniles (1471 unique individuals). We documented 250 pregnant, 232 lactating, 280 post-lactating, and 202 nonreproductive adult females.

Recapture data

One hundred nineteen recaptures (10.6% of maternity captures) of 101 individuals occurred at maternity sites sampled during the WNS-endemic period. Of these, 18 were recaptured more than once; 89 were first captured as adults (4 male, 85 female) and 12 as juveniles (1 male, 11 female). Only 26 of the 119 recaptures occurred within the same sampling year; the remaining 93 recapture events originated from 78 individuals recaptured across multiple years. Of 93 recaptures across years, 47 (50.5%) were ≤2 years of age, 15 (16.1%) were 2.1–4.0 years old, 20 (21.5%) were 4.1–6.0 years old, 9 (9.7%) were 6.1–8.0 years old, and 2 (2.2%) were >8 years of age. The oldest individual recaptured was an adult female first captured on 11 May 2012 and last captured on 7 July 2021, which indicates a minimum age of 9 years, 1 month, and 25 days. This individual was captured in 4 years (2012, 2015, 2019, and 2021) and was reproductively active for 3 captures. Of the 12 juveniles later recaptured, 7 (58.3%) were ≤2 years of age, 2 (16.7%) were 2.1–4.0 years old, and 3 (25.0%) were 4.1–6.0 years of age. All recaptures were at the site of first capture (99.2% of recaptures), except a single adult female first captured at a hibernaculum in Highland County on 1 November 2011 (Powers et al. 2015), and later recaptured on 10 May 2012 after migrating to the Rockingham County maternity site, 64 km east.

Reproductive trends

As expected, the predicted proportion of adult females that were pregnant, lactating, and post-lactating varied significantly over the summer by day (Tables 1–3, Fig. 2–4). However, the phenology did not substantially differ before invasion, during invasion, and when WNS was endemic (Fig. 2–4). The proportion of pregnant and post-lactating females was similar among the 3 WNS periods (Tables 1, 3; Fig. 2, 4). As expected, the proportion of lactating females was higher earlier in the summer than later in the summer across all WNS period, however the seasonal rise and peak was lower in the WNS-endemic relative to the pre-WNS period (Table 2, Fig. 3). The proportion of juveniles captured differed among years (Table 4) and was significantly lower in 2012 and 2013 during the WNS invasion and first WNS-endemic year than in subsequent years (Fig. 5).

Discussion

The effects of WNS on reproductive trends of Little Brown Bats in Virginia did not change as much as expected during the WNS invasion (2010–2012) and endemic (2012–present) phases. Our findings of a drop in the proportion of lactating females in the WNS-invasion years was similar to the findings from Francl et al. (2012) during the

Table 1. Generalized linear mixed model parameter estimates to predict proportion of pregnant *Myotis lucifugus* (Little Brown Bats, n = 250) in western Virginia across day of the year and among 3 white-nose syndrome (WNS) periods: pre-WNS (1992–2010), WNS-invasion (2010–2012) and WNS-endemic (2013–2021).

Parameter	Estimate	*SE*	*t*	*P*
Intercept	12.34	4.32	2.86	0.0042
Day of year	-0.9	0.03	-2.93	0.0034
WNS period				
WNS-endemic[a]	.	.	.	.
WNS-invasion	-5.19	5.81	-0.89	0.371
Pre-WNS	4.92	5.80	0.85	0.396
WNS*Day				
WNS-endemic[a]	.	.	.	.
WNS-invasion	0.03	0.04	0.89	0.373
Pre-WNS	-0.02	0.04	-0.62	0.373

[a]Reference condition in analysis

Table 2. Generalized linear mixed model parameter estimates for predicted proportion of lactating adult female *Myotis lucifugus* (Little Brown Bats, $n = 232$) in western Virginia across day of year and among three white-nose syndrome (WNS) periods: pre-WNS (1992–2010), WNS-invasion (2010–2012), and WNS-endemic (2013–2021).

Parameter	Estimate	*SE*	*t*	*P*
Intercept	-89.55	28.31	-3.16	0.0016
Day of year	1.09	0.34	3.21	0.0013
Day of year^2	-0.003	0.01	-3.28	0.0013
WNS period				
WNS-endemic[a]	.	.	.	.
WNS-invasion	6.34	8.35	0.76	0.448
Pre-WNS	-14.10	7.24	-1.95	0.051
WNS Day				
WNS-endemic[a]	.	.	.	.
WNS-invasion	-0.04	0.05	-0.73	0.463
Pre-WNS	0.08	0.04	2.04	0.041

[a]Reference condition in analysis

Table 3. Generalized linear mixed model parameter estimates for predicted proportion of post-lactating adult female Little Brown Bats ($n = 280$) in western Virginia across day of year and among 3 white-nose syndrome (WNS) periods: pre-WNS (1992–2010), WNS-invasion (2010–2012), and WNS-endemic (2013–2021).

Parameter	Estimate	*SE*	*t*	*P*
Intercept	-89.55	28.31	-3.16	0.0016
Day of year	1.09	0.34	3.21	0.0013
Day of year^2	-0.003	0.01	-3.28	0.0013
WNS period				
WNS-endemic[a]	.	.	.	.
WNS-invasion	6.34	8.35	0.76	0.448
Pre-WNS	-14.10	7.24	-1.95	0.0514
WNS*Day				
WNS-endemic[a]	.	.	.	.
WNS-invasion	-0.04	0.05	-0.73	0.463
Pre-WNS	0.08	0.04	2.04	0.041

[a]Reference condition in analysis

Table 4. Generalized linear mixed model parameter estimates for predicted proportion of juvenile Little Brown Bats ($n = 289$) at maternity colony areas ($n = 25$ site-nights) in western Virginia, 2013–2021.

Parameter	Estimate	*SE*	*t*	*P*
Intercept	-55.36	127.46	0.43	0.664
Year	0.03	0.06	0.42	0.673

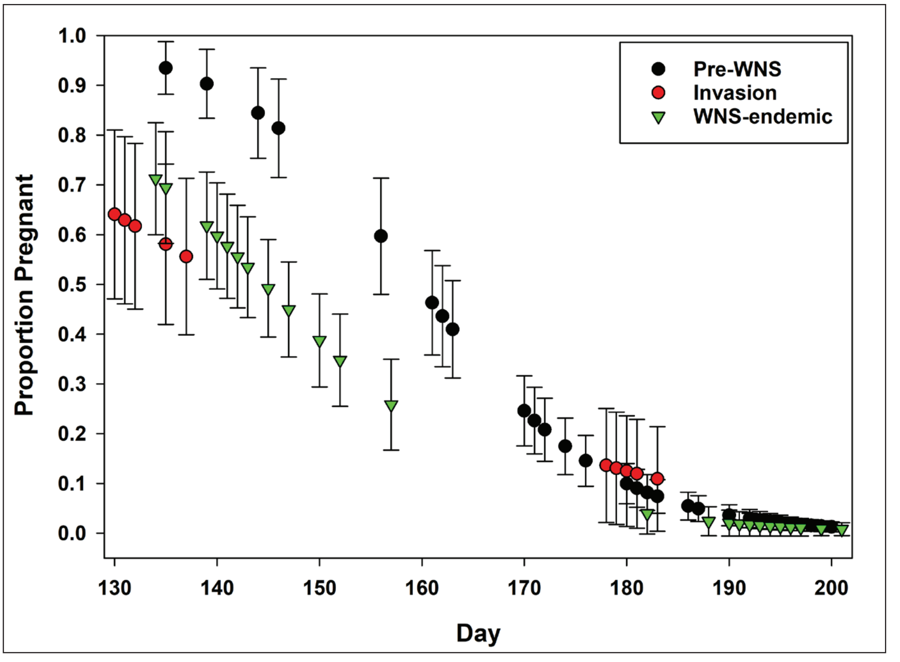

Figure 2. Generalized linear mixed model fit plot with 95% confidence intervals for predicted proportion of pregnant adult female Little Brown Bats (n = 250) in western Virginia across Julian day of reproductive season and among 3 white-nose syndrome (WNS) periods: pre-WNS (1992–2010), WNS-invasion (2010–2012), and WNS-endemic advent (2013–2021).

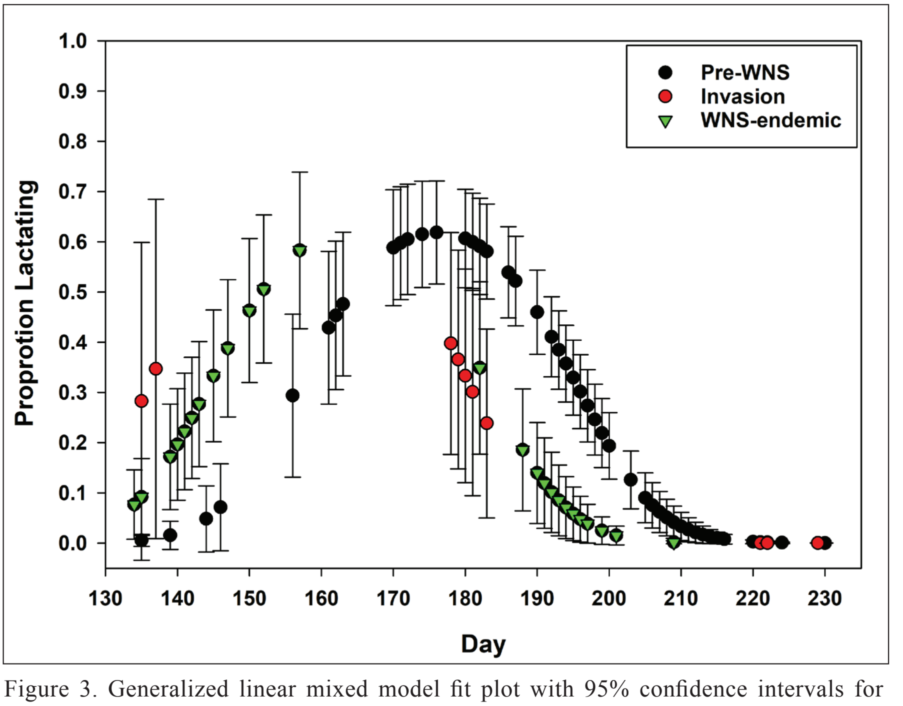

Figure 3. Generalized linear mixed model fit plot with 95% confidence intervals for predicted proportion of lactating adult female Little Brown Bats (n = 232) in western Virginia across day of year and among 3 white-nose syndrome (WNS) periods: pre-WNS (1992–2010), WNS-invasion (2010–2012), and WNS-endemic advent (2013–2021).

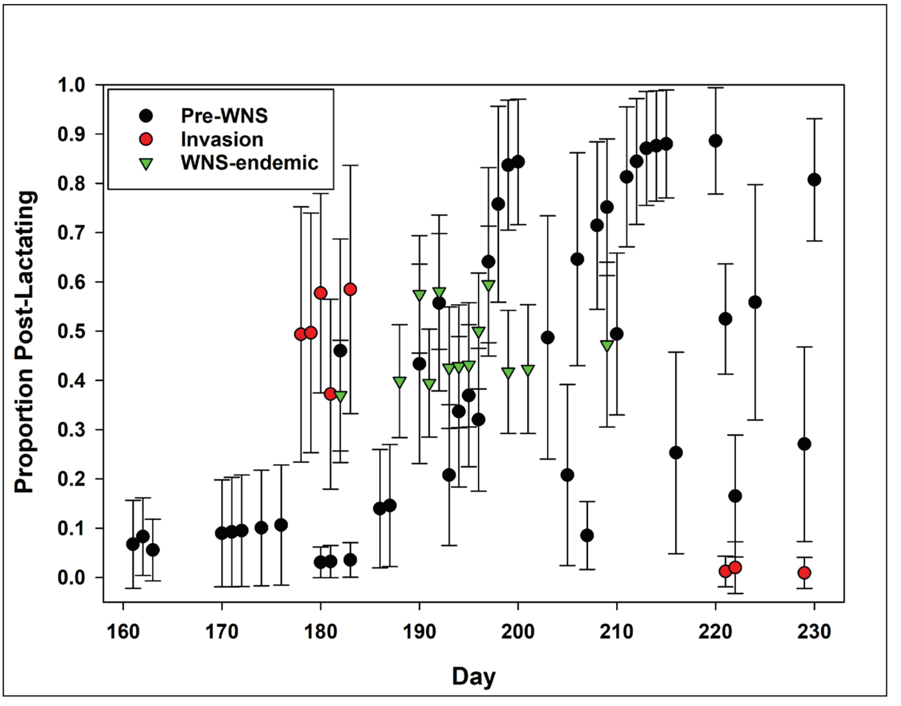

Figure 4. Generalized linear mixed model fit plot with 95% confidence intervals for predicted proportion of post-lactating adult female Little Brown Bats (n = 280) in western Virginia across day of year and among 3 white-nose syndrome (WNS) periods: pre-WNS (1992–2010), WNS-invasion (2010–2012), and WNS-endemic advent (2013–2021).

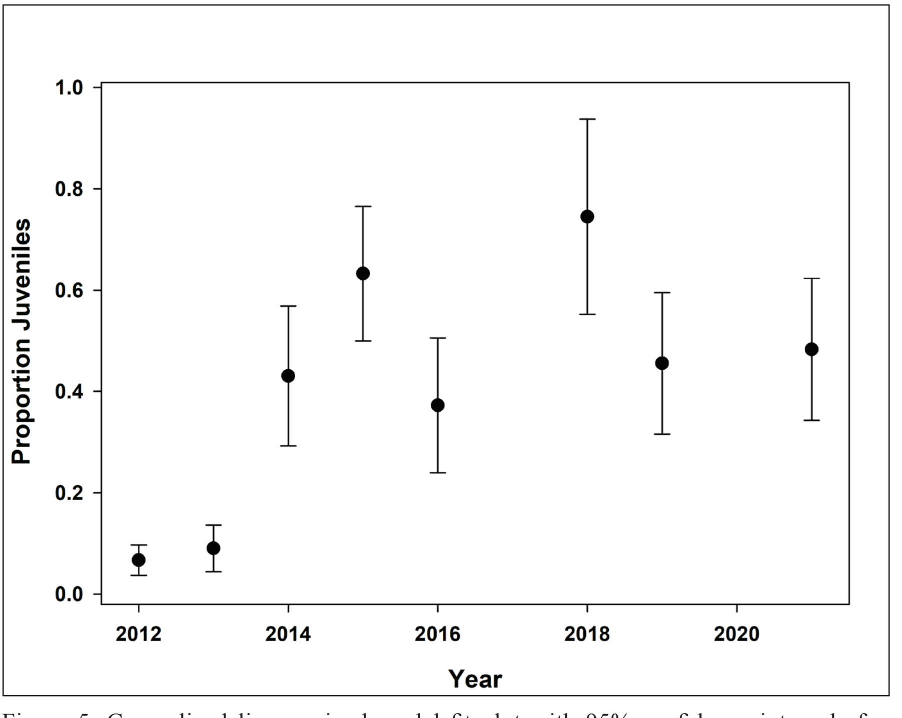

Figure 5. Generalized linear mixed model fit plot with 95% confidence intervals for predicted proportion of juvenile Little Brown Bats (n = 289) at maternity colony areas in western Virginia, 2013–2021.

same WNS period in West Virginia. However, unlike that study, we observed no shift in the timing of peak pregnancy between pre-WNS and WNS-invasion periods. Over the single WNS-invasion year surveyed by Francl et al. (2012), a decrease in the proportion of juveniles among Little Brown Bats was also observed. Although we found no trend in the proportion of juveniles captured in Virginia from the WNS-invasion period through the contemporary WNS-endemic period, we note that 2 of the sampled maternity colonies had low proportions of juveniles in 2012 and 2013. After 2013, the proportion of juveniles appeared to rebound. Although numbers of Little Brown Bats on the landscape have markedly declined, survivors continue to reproduce. However, our weak shift to earlier timing of lactating females in the WNS-endemic period may result in 1 of 2 outcomes: (1) surviving females are lactating earlier in the summer with no negative impact on juvenile birth rate, or (2) failed reproductive efforts led females to cease lactating earlier in the summer. This second scenario is supported by conclusions of Kalen et al. (2022) who tracked reproductive failures of Northern Long-eared Bats during WNS-endemic years in central Appalachia. A limitation of our dataset is that we cannot differentiate between these 2 outcomes.

Over WNS-endemic years at maternity colonies, ~70% of adult females were reproductive. This is lower than 85–100% of reproductive females in the WNS-endemic era reported by Dobony and Johnson (2018) in upstate New York. Whether or not our findings for the WNS-endemic era are reflective of any undiscovered Little Brown Bat colonies in Virginia is unknown. The difference in reproductive females in Virginia versus New York (Dobony and Johnson 2018) may suggest that WNS-endemic era population increases as seen in the Northeast (Ford et al. 2020) are not as apparent in Virginia, at present.

Despite a low recapture rate, we anecdotally noted that banded bats appeared to be trap-averse, or at least avoid human activity on trap nights (Robbins et al. 2008, Winhold and Kurta 2008). Due to visibility of bats between ceiling joists, we noted that banded bats would not exit eaves until 2–3 hours after dusk (thus, avoiding nets and subsequent capture), whereas juveniles and unbanded adults comprised most early-evening (1–2 h after sunset) captures. This bias affected our analyses of recaptures and juvenile proportions. This perhaps is a driver for the non-significant increase in juveniles (as a proportion of captures) across years and makes conclusive comparisons to the pre-WNS mist-net capture data on the wider landscape less certain. We did not disturb non- or late-emerging banded bats out of concern for causing undue stress, but do acknowledge this bias. Dobony and Johnson (2018), who had a 26.4% recapture rate for Little Brown Bats at bat houses on Fort Drum, New York across 8 years of surveys, also noted that banded bats remained in their bat boxes, avoiding capture.

The trend in decreases in numbers of Little Brown Bats on the landscape in Virginia matched that of Northern Long-eared Bats, as did the negligible declines in reproductive trends (Reynolds et al. 2016). However, Reynolds et al. (2016) documented a significant (>70%) decline in juvenile Northern Long-eared Bat captures during mist-netting efforts. In West Virginia, Francl et al. (2012) found that the proportion of pregnant Little Brown Bats peaked earlier in summer in the WNS-endemic era and that the proportion of juveniles among all captures declined in the WNS-endemic era.

We acknowledge that the differences in collection of our data before and after the onset of WNS may limit our ability to implicate directly WNS as the cause for reproductive changes. Indeed, the WNS quandary remains: the substantial declines in Little Brown Bats on the landscape directly limit availability of Virginia sampling sites in the WNS-endemic era. Netting at locations other than known maternity roosts could not have produced a sufficient sample to examine reproductive trends for this species. The reduction of Little Brown Bats on the landscape (away from known maternity colonies) was shown in Virginia in the WNS-endemic period by Brack et al. (2022). Brack and others noted a significant reduction in Little Brown Bat abundance at utility rights-of-way mitigation features; reduced Little Brown Bat recruitment could have contributed to that finding. Given our WNS-endemic focus on maternity colonies, we were unable to compare our juvenile trends in the same manner as Reynolds et al. (2016) in Virginia or with Francl et al. (2012) in West Virginia. Minor shifts in reproductive peaks may also be a feature of more limited survey dates in the WNS-endemic era. This limitation in comparing long-term datasets in the pre-WNS and WNS-endemic periods will continue to be a concern for wildlife managers.

Longevity of some of our recaptures (1 bat > 9 years old) is remarkable in the WNS-endemic era (e.g., Kurta et al. 2020). This suggests that some individuals persist for many years after WNS exposure and continue to reproduce. In Virginia, it remains unanswered if long-term, survivors in the WNS-endemic era have a genetic (Lilley et al. 2020) or behavioral advantage (e.g., hibernating in cool sites or isolated locations that are less suitable for *Pd*; Frank et al. 2019, Johnson et al. 2014) or some combination thereof.

Given the rarity of maternity colonies on the landscape, we suggest continued monitoring of remaining sites. Although yearly surveys may cause undue disturbance, occasional visits every 2–3 years may ensure that population increases or collapses do not go unnoticed or uninvestigated. To monitor thoroughly the success or failures of colonies, we recommend trapping or hand-capturing bats to determine their reproductive status, rather than using passive observation methods that would not produce these data.

Further, we suggest a renewed effort to locate additional Little Brown Bat maternity colonies in Virginia. We detected 2 of the colonies in our study by radio-tracking bats during netting projects. Given that more than 15 years have elapsed since this effort, we suggest renewed efforts by VDWR to locate additional barns or bat boxes that house summer colonies of Little Brown Bats. New maternity colonies may be located by tracking radio-tagged individuals at spring emergence (Copperhead Consulting 2023). One of our maternity colonies was discovered around the time of WNS onset in Virginia when a landowner responded to a state agency press release requesting summer sites that could be investigated. With the proliferation of social media use, wide-ranging campaigns on social media platforms could produce additional maternity sites. Communication with local and statewide citizen-scientist groups also may garner new colony locations. Finally, we emphasize the importance of initiating and maintaining good relationships with private landowners. Individual outreach to private landowners can emphasize the value of housing and maintaining summer colonies for the continuation of this now-rare species.

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Literature Cited

Anthony, E.L.P. 1988. Age determination. Pp. 47–57, *In* T.H. Kunz (Ed.). Ecological and Behavioral Methods for the Study of Bats. Smithsonian Institution Press, Washington, DC, USA. 533 pp.

Bouma, H.R., H.V. Carey, and F.G. Kroese. 2010. Hibernation: The immune system at rest? Journal of Leukocyte Biology 88:619–624.

Brack, V., D.W. Sparks, and S. Kennedy. 2022. Case study: Upland ponds provide on-site mitigation for bat habitat along American Electric Power's 765-kV powerline ROW in the Appalachian Mountains, USA. *In* L. Hufnagel (Ed.). New Insights into Protected Area Management and Conservation Biology. Available online at https://www.intechopen.com/chapters/85169. Accessed 14 August 2024.

Copperhead Consulting. 2023. Little Brown Bat (*Myotis lucifugus*) spring migration to maternity colonies. Pennsylvania Game Commission, Harrisburg, PA, USA. 118 pp.

Cryan, P.M., C.U. Meteyer, J.G. Boyles, and D.S. Blehert. 2010. Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. BMC Biology 8:135. DOI: 10.1186/1741-7007-8-135

Dobony, C.A., and J.B. Johnson. 2018. Observed resiliency of Little Brown Myotis to long-term white-nose syndrome exposure. Journal of Fish and Wildlife Management 9:168–179.

Ford, W.M., C.A. Dobony, D.S. Jachowski, L.S. Coleman, T. Nocera, and E.R. Britzke. 2020. Case study one: Acoustic surveys at Fort Drum Military Installation – The value of long-term monitoring. Pp. 78–81, *In* E.E. Fraser, A. Silvis, R.M. Brigham and Z. Czenze (Eds.). Bat Echolocation Research: A Handbook for Planning Acoustics Studies. Bat Conservation International, Austin, TX, USA. 116 pp.

Francl, K.E., W.M. Ford, D.W. Sparks, and V. Brack, Jr. 2012. Capture and reproductive trends in summer bat communities in West Virginia: Assessing the impact of white-nose syndrome. Journal of Fish and Wildlife Management 3:33–42.

Frank, C.L., A.D. Davis, and C. Herzog. 2019. The evolution of a bat population with white-nose syndrome (WNS) reveals a shift from an epizootic to an enzootic phase. Frontiers in Zoology 16:40. DOI: 0.1186/s12983-019-0340-y

Haarsma, A.-J. 2008. Manual for assessment of reproductive status, age, and health in European vespertilionid bats. Available online at https://www.researchgate.net/publication/228874636. Accessed 14 August 2024.

Hoyt, J.R., A.M. Kilpatrick, and K.E. Langwig. 2021. Ecology and impacts of white-nose syndrome on bats. Nature Reviews Microbiology 19:196–210. DOI: 10.1038/s41579-020-00493-5

Johnson, C., D.J. Brown, C. Sanders, and C.W. Stihler. 2021. Long-term changes in occurrence, relative abundance, and reproductive fitness of bat species in relation to arrival of white-nose syndrome in West Virginia, USA. Ecology and Evolution 11:12453–12467.

Johnson, J.S., D.M. Reeder, J.W. McMichael III, M.B. Meierhofer, D.W.F. Stern, S.S. Lumadue, L.E. Sigler, H.D. Winters, M.E. Vodzak, A. Kurta, J.A. Kath, and K.A. Field. 2014. Host, pathogen, and environmental characteristics predict white-nose syndrome mortality in captive Little Brown Myotis (*Myotis lucifugus*). PLoS ONE 9(11):e112502. DOI: 10.1371/journal.pone.0112502

Kalen, N.J., M.S. Muthersbaugh, J.B. Johnson, A. Silvis, and W.M. Ford. 2022. Northern Long-eared Bats in the Central Appalachians following white-nose syndrome: Failed maternity colonies? Journal of the Southeastern Association of Fish and Wildlife Agencies 9:159–167.

Kurta, A., R.W. Foster, B.A. Daly, A.K. Wilson, R.M. Slider, C.D. Rockey, J.M. Rockey, B.L. Long, G.G. Auteri, J.D. Collins, J.P. White, H.M. Kaarakka, J.A. Redell, and D.M. Reeder. 2020. Exceptional longevity in Little Brown Bats (*Myotis lucifugus*) still occurs, despite presence of white-nose syndrome. Journal of Fish and Wildlife Management 11:583–587.

Lilley, T.M., I.W. Wilson, K.A. Field, D.M. Reeder, M.E. Vodzak, G.G. Turner, A. Kurta, A.S. Blomberg, S. Hoff, C.J. Herzog, B.J. Sewall, and S. Paterson. 2020. Genome-wide changes in genetic diversity in a population of *Myotis lucifugus* affected by white-nose syndrome. G3 Genes|Genomes|Genetics 10:2007–2020.

Meteyer, C.U., J.Y. Dutheil, M.K. Keel, J.G. Boyles, and E.H. Stukenbrock. 2022. Plant pathogens provide clues to the potential origin of bat white-nose syndrome *Pseudogymnoascus destructans*. Virulence 13:1020–1031.

Powers, K.E., R.J. Reynolds, W. Orndorff, W.M. Ford, and C.S. Hobson. 2015. Post-white-nose syndrome trends in Virginia's cave bats, 2008-2013. Journal of Ecology and the Natural Environment 7:113–123.

Prendergast, B.J., D.A. Freeman, I. Zucker, and R.J. Nelson. 2002. Periodic arousal from hibernation is necessary for initiation of immune responses in ground squirrels. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 282:R1054–R1062.

Reynolds, R., K.E. Powers, W. Orndorff, W.M. Ford, and C. Hobson. 2016. Changes in rates of capture and demographics of *Myotis septentrionalis* (Northern Long-eared Bat) in western Virginia before and after onset of white-nose syndrome. Northeastern Naturalist 23:195–204.

Robbins, L.W., K.L. Murray, and P.M. McKenzie. 2008. Evaluating the effectiveness of the standard mist-netting protocol for the endangered Indiana Bat (*Myotis sodalis*). Northeastern Naturalist 15:275–282.

SAS Institute, Inc. 2020. SAS user's guide: Statistics, version 9.4 edition. SAS Institute, Inc. Cary, NC, USA.

Silvis, A., R.W. Perry, and W.M. Ford. 2016. Relationships of three species of white-nose syndrome-impacted bats to forest condition and management. US Forest Service Southern Research Station General Technical Report. SRS–214, Ashville, NC, USA. 57p.

US Fish and Wildlife Service (USFWS). 2012. Press release: North American bat death toll exceeds 5.5 million from white-nose syndrome. Available online at https://www.whitenosesyndrome.org/press-release/north-american-bat-death-tool-exceeds-5-5-million-from-white-nose-syndrome. Accessed 9 August 2023.

U.S. Fish and Wildlife Service (USFWS). 2022. Range-wide Indiana Bat and Northern Long-eared Bat survey guidelines. Available online at https://www.fws.gov/sites/default/files/documents/USFWS_Range-wide_IBat_%26_NLEB_Survey_Guidelines_2022.03.29.pdf. Accessed 26 October 2023.

U.S. Fish and Wildlife Service (USFWS). 2023. Where is WNS now? Available online at https://www.whitenosesyndrome.org/where-is-wns. Accessed 9 August 2023.

Virginia Department of Game and Inland Fisheries (VDGIF). 2009. Progress report for Federal Assistance in Wildlife Restoration Conservation Project WE99R18 – July 1, 2008–June 30, 2009. VDGIF, Richmond, VA, USA. 207 pp.

Warnecke, L., J.M. Turner, T.K. Bollinger, V. Misra, P.M. Cryan, D.S. Blehert, G. Wibbelt, and C.K.R. Willis. 2013. Pathophysiology of white-nose syndrome in bats: A mechanistic model linking wing damage to mortality. Biology Letters 9(4):20130177. DOI: 10.1098/rsbl.2013.0177

Winhold, L., and A. Kurta. 2008. Netting surveys for bats in the Northeast: Differences associated with habitat, duration of netting, and use of consecutive nights. Northeastern Naturalist 15:263–274.