June 2017

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Articles

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Abstract

White-nose syndrome (WNS) is an emerging fungal disease suspected to have infected Indiana caves in the winter of 2010–2011. This disease places energetic strains on cave-hibernating bats by forcing them to wake and use energy reserves. It has caused >5.5 million bat deaths across eastern North America, and may be the driving force for extinction of certain bat species. White-nose syndrome infection can be identified in hibernacula, but it may be difficult to determine whether bats in a particular area are affected if no known hibernacula exist. Thus, our aim was to use long-term monitoring data to examine changes in a summer population away from hibernacula that may be attributable to WNS effects during winter. We used capture data from a long-term bat-monitoring project in central Indiana with data from 10 repeatedly netted sites consistent across all reproductive periods. We modeled capture data by WNS exposure probability to assess changes in relative abundance of common species and reproductive classes as WNS exposure probability increases. We base exposure probability on a cokriging spatial model that interpolated WNS infection from hibernaculum survey data. The little brown bat *Myotis lucifugus,* the Indiana bat *M. sodalis,* and the tri-colored bat *Perimyotis subflavus* suffered 12.5–79.6% declines; whereas, the big brown bat *Eptesicus fuscus,* the eastern red bat *Lasiurus borealis,* and the evening bat *Nycticeius humeralis* showed 11.5–50.5% increases. We caught more nonreproductive adult females and postlactating females when WNS exposure probabilities were high, suggesting that WNS is influencing reproductive success of affected species. We conclude that, in Indiana, WNS is causing species-specific declines and may have caused the local extinction of *M. lucifugus*. Furthermore, WNS-affected species appear to be losing pups or forgoing pregnancy. Ongoing long-term monitoring studies, especially those focusing on reproductive success, are needed to measure the ultimate impacts of WNS.

Keywords: bats; capture rates; disease; endangered species; *Eptesicus fuscus;* mist-netting surveys; species declines

Received: October 7, 2016; Accepted: January 11, 2017; Published Online Early: January 2017; Published: June 2017

Citation: Pettit JL, O'Keefe JM, 2017. Impact of white-nose syndrome observed during long-term monitoring of a midwestern bat community. *Journal of Fish and Wildlife Management* 8(1):69–78; e1944-687X. doi:10.3996/102016-JFWM-077

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Introduction

White-nose syndrome (WNS), a fungal disease caused by *Pseudogymnoascus destructans,* has resulted in significant declines of hibernating bats in eastern North America. White-nose syndrome has spread steadily from its origin in New York, where it was discovered in 2006 (Blehert et al. 2009), south through the Appalachian

Mountains, and into southern and Midwestern states (see maps in Turner et al. 2011 and Cryan et al. 2013). The fungus infects the exposed skin of cave-hibernating bats; infection is linked to the depletion of fat stores and often leads to death of the bat host (Cryan et al. 2013). Bats' immune responses are suppressed during hibernation (Bouma et al. 2010) and, thus, recovery from WNS typically does not begin until spring emergence from hibernation when the immune response returns to normal levels (Meteyer et al. 2011) and insect prey are readily available to resupply lost fat stores. However, because WNS causes wing damage in addition to fat store depletion, spring-emerging bats may face energy depletions over an extended period (Reichard and Kunz 2009). Bats that survive the winter may still succumb to the effects of WNS in spring or may have lower fecundity when compared with pre-WNS.

Jonasson and Willis (2011) make the case that because female bats enter hibernation with larger fat stores and consume them more slowly than do male bats, they may be more likely to survive hibernation in a WNS-infected hibernaculum (cave or mine where bats hibernate during the winter months). However, survival does not ensure successful reproduction. In fact, any harm to body condition is likely to have implications for reproductive success. Even indirect factors such as excess precipitation (Grindal et al. 1992) or latitude (Kunz et al. 1998) have been shown to influence the proportion of reproductive bats. White-nose syndrome has been shown to directly influence body condition and, thus, is likely to have a significant impact on reproduction; Frick et al. (2010) suggested this connection when they found decreased reproductive rates for little brown bats *Myotis lucifugus* within 4 y of WNS being detected in hibernacula in the northeastern United States. Francl et al. (2012) found reductions in encounter rates for reproductive females and juveniles during capture surveys in West Virginia immediately after the onset of WNS. Declines in recruitment paint an even grimmer picture for WNS-affected bat species and, thus, it may be prudent to study the effects of WNS on reproduction.

The effects of WNS are most visible in hibernacula, which are generally restricted to karst areas, specifically caves or mines, but it is not always feasible to use hibernacula surveys to determine WNS status. Annual or biannual cave surveys have allowed detection of the disease in hibernating bat populations; these surveys showed that WNS arrived in Indiana caves as early as the winter of 2010–2011. However, in areas with no known hibernation sites, it is difficult to determine whether bat populations have been affected by WNS. This is the case for many summer maternity sites. Currently, there is no simple and cost-effective way to detect the fungus on bats during summer. Surveys of maternity areas offer a less direct assessment of WNS influence in a particular area, but are desirable in that information on abundance and reproduction can be gained. Previous research during the summer has shown that WNS is detectable through changes in capture rates during mist-net surveys (Francl et al. 2012; Moosman et al. 2013) and in bat activity via acoustic monitoring (Brooks 2011; Dzal et al. 2011). In this study, we aimed to record the arrival and impact of WNS to a part of central Indiana where there are no known winter hibernacula. We used data from long-term monitoring of 10 repeatedly netted sites, with >8 y of pre-WNS data taken consistently across all reproductive periods (pregnancy, lactation, and post-lactation), to 1) discern which species were declining coincident with the arrival of WNS to Indiana, and 2) determine whether reproductive frequencies or timing were affected by WNS presence.

Study Area

Our study area was located along a riparian corridor southwest of the Indianapolis International Airport in Hendricks County, Indiana. The Indianapolis Airport Authority owns 1,045 ha of natural areas adjacent to the East Fork of White Lick Creek, where 10 permanent netting sites were established along the creek in 1997. One net site consists of a fixed location for which similar net arrangements were used on successive visits. Two major site changes happened in 2003: one site was moved 460 m upstream because of a tree fall, and highway construction forced the removal of another site; subsequently, a replacement net site was established near other sites ~2.5 km upstream.

The seven most common species of bats caught in this study area were *Myotis lucifugus* (little brown bat), *M. septentrionalis* (northern long-eared bat), *M. sodalis* (Indiana bat), *Perimyotis subflavus* (tri-colored bat), *Eptesicus fuscus* (big brown bat), *Lasiurus borealis* (eastern red bat), and *Nycticeius humeralis* (evening bat; Whitaker et al. 2004). The first four species have been heavily affected by WNS in northeastern North America (Blehert et al. 2009; Cryan et al. 2010; Langwig et al. 2012). Less common, but also captured in this area, were *Lasiurus cinereus* (hoary bat) and *Lasionycteris noctivagans* (silver-haired bat).

Methods

We mist-netted each of the 10 sites for bats 1 night/30-d period, 2002–2014, (3 nights/y) for an average of 4.5 h/night, starting at dusk, from 15 May to 15 August. We defined one night of netting at a particular site as one site visit. We timed site visits to evenly represent all reproductive periods. During each visit we deployed a combination of 1–3 single-, double-, or triple-high nets 6–18 m wide. Nets were designed for capturing bats, with a fine weight (75/2 denier), 38-mm polyester mesh, and reduced bags (Avinet, Dryden, NY). For a more detailed description of the site and methods, see Whitaker et al. (2004). We followed American Society of Mammalogists' guidelines for use of wild mammals in research (Sikes et al. 2011; Indiana State University Institutional Animal Care and Use Committee protocols for J. O. Whitaker and J. M. O'Keefe) and, beginning in 2011, WNS protocols as specified by the U.S. Fish and Wildlife Service (2012). We recorded species, reproductive condition, sex, age, and body condition for each bat, using degree of ossification of finger joints to assess age

(juvenile or adult). We attached uniquely numbered aluminum forearm bands (Porzana, Ltd., East Sussex, England, UK) before releasing bats at the capture site. We conducted field work under federal permits held by J. M. O'Keefe (TE206872), J. O. Whitaker (TE839763), and B. L. Walters (TE106220), as well as State of Indiana permits by these individuals and lead technicians.

Analysis

We examined effects of WNS presence in Indiana on 1) frequency of capture by species and 2) frequency and timing of reproductive states or "condition" (i.e., pregnancy, lactation, postlactation, and nonreproductive). Only 20 bats banded at this study area have been observed at Midwest hibernacula; all band recoveries were within Indiana (Table S1); but, with so few recoveries, we lack robust information on when all bats in this area were first exposed to WNS. Therefore, we approximated exposure probabilities using cokriging, an extrapolation tool in ArcGIS Geostatistical Analyst (v.10.1, Redlands, CA). Cokriging uses $\geq$2 spatial data sources to estimate values of exposure for pixels where exposure data are lacking or unmeasurable. Cokriging models were based on Indiana bat hibernacula population data curated by Andrew King (U.S. Fish and Wildlife Service, data accessed 3 January 2014; see also Thogmartin et al. 2012). These data included current and historical surveys of Indiana bat population size and the infection year for 506 hibernacula in 136 counties across 25 U.S. states (189 hibernacula were infected in the United States at the time of data access). We used yearly hibernaculum infection status (WNS confirmed or not observed) and the 10-y maximum Indiana bat population of a monitored county to predict probability of WNS exposure for all species of cave-hibernating bats. We used population data for the Indiana bat as a proxy for total population size of hibernacula because total population data of all species were not available for all hibernacula. Cokriging models output a continuous prediction surface giving probability of infection of hypothetical hibernacula. This method is subject to higher error rates where data representation is low (e.g., low cave density or areas where Indiana bat hibernacula are not present; see Figure S1). From the output map, we inferred a high probability of WNS presence where cokriging probabilities were $>$50%. While this cokriging model attempts to predict hibernaculum infection, it was a simple method for approximating the probability of exposure on the summer landscape.

Using a mixed modeling approach to accommodate for zero-inflation, we analyzed captures of adult female bats per site visit with the pscl package in R (Zeileis et al. 2008; Jackman 2015). To test for changes in species abundance, we assessed the effects of WNS exposure probability (output from the cokriging analysis), species, and the interaction between WNS exposure and species on capture rates. To test for changes in reproduction, we assessed capture rates by WNS exposure, reproductive condition, and a reproductive condition by WNS exposure interaction. Other factors of interest were month of capture to control for any seasonal differences in species abundance, net effort (net area $\times$ hour netting) to account for differences in net setup between site visits, and net site to control for species abundance differences between sites. We log-transformed net effort to normalize the data. We tested each variable for normality, homogeneity, and independence. Although we did recapture some individuals in this dataset, we treated all captures as new bats for this analysis.

We tested 14 zero-inflated models, of which 12 used a negative binomial distribution and two used a Poisson distribution (Table S2). We used zero-inflated models because there were many site visits where we caught no bats or not all bat species. The zero-inflated model was a two-part hierarchical model; part one was a logistic model that estimated the probability of obtaining a structural zero (bats are not present at the time of sampling and thus, are not detected) and part two was a generalized linear "count" model determining the impact of factors on capture frequencies. We built a global zero-inflated mixed model that included all factors mentioned above in both the logistic and count models. To choose the appropriate model distribution (Poisson versus negative binomial), we compared models using likelihood ratio tests. We then refined the global model by plotting each factor by the model residuals, and checking for overdispersion to ensure all important factors were present. We further refined models to eliminate factors that we deemed nonsignificant, as determined by likelihood ratio tests (Zuur et al. 2009). To create the final, refined model, we sequentially dropped nonsignificant factors from the global model, until all remaining factors significantly contributed to model performance.

Similar to the methods of Francl et al. (2012), we used locally weighted regression (loess.sd in the msir package Scrucca 2011) in R (R Core Team 2014) to assess changes in the timing of reproductive condition across WNS time periods. Locally weighted regression offers a flexible method of fitting a line to the data, but requires large sample sizes; thus, we assessed changes in timing of reproduction for the most commonly captured species, the big brown bat. We were also interested in the timing of reproduction in *Myotis* species that are highly affected by WNS and have similar life-history timing (Fenton and Barclay 1980; Thomson 1982; Caceres and Barclay 2000). To account for small sample sizes for each *Myotis* species, we pooled these samples and performed locally weighted regression analyses on the pooled dataset. We considered differences between the lines meaningful where 95% confidence intervals did not overlap. We used a conservative Loess span of 0.75 for all reproduction timing models. For interpretation, we defined a reproductive period as times when $\geq$50% of adult female captures exhibited a particular reproductive condition (e.g., pregnant, lactating, postlactation, or nonreproductive). Finally, to discern shifts in capture frequencies of juvenile bats and recaptured bats, we chose to analyze two additional datasets, one of juvenile captures and one of recaptured bats. The zero-inflated models and the

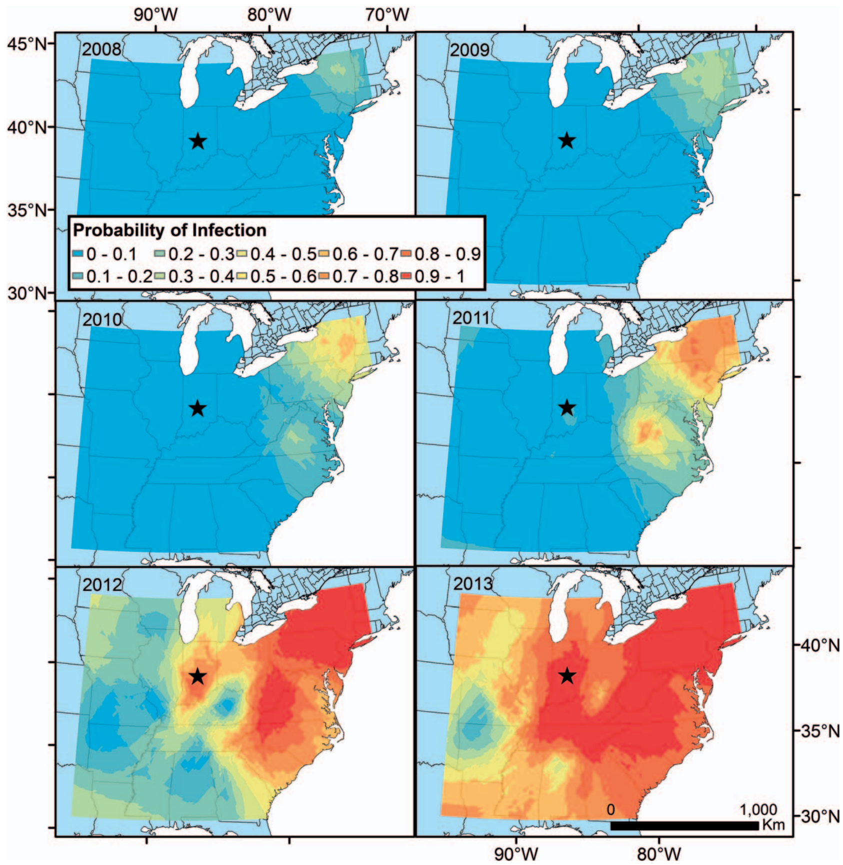

Figure 1. Probability of white-nose syndrome (WNS) infection for the eastern United States from 2008 to 2013; surface was created by interpolating infection data for Indiana bat *Myotis sodalis* hibernacula with cokriging models. The black star represents the central Indiana long-term monitoring site where we measured WNS impacts in summer. White-nose syndrome data from Canada were not included in this analysis.

sample size of the full capture dataset did not allow for models complex enough to test for significant shifts in in captures of these demographics and, thus, we analyzed them separately using descriptive statistics.

Results

We surveyed the East Fork of White Lick Creek on 380 site visits from 2002 to 2014, capturing 2,511 bats (Table S3). Silver-haired and hoary bats were captured irregularly ($n = 5$ and $n = 7$, respectively) and, thus, we excluded them from further analyses. We refined the dataset for our analysis to use only capture records of adult female bats for the seven most common species caught at our study area (1,376 bats).

Cokriging models based on hibernacula infection data showed the probability of WNS exposure in Hendricks County began to increase in summer 2011, and bats summering there were probably exposed to WNS by 2012 (Tables S1 and S4). This model showed WNS spreading from the original infection site in New York, south through the Appalachians, and then west (Figure 1). By 2013, probability of WNS exposure was 0.5–1.0 for bat populations in all states east of Indiana, except Florida. Based on these distinct probabilities, we delineated two exposure time periods at our study site: the pre-WNS period as all years with probabilities approaching zero (1998–2010); and the WNS period (2011–2014), which can be separated into initial-WNS (the year with a probability >0 and <0.5 [2011]), and late-WNS period (years with >0.5 probability of WNS presence [2012–2014]).

Our mixed-modeling analysis showed that impact from WNS was detectable in long-term summer capture data. The final model from the mixed-model analysis contained a logistic zero-inflation component (Table S35) and a negative binomial count component (Table S6), both containing the same factors. Model refinement did not produce a significantly better model by removing nonsignificant or near-significant factors. Therefore, our models contained the following factors: WNS exposure probability, species, reproductive status, month, log net effort, net site, and interactions between WNS exposure and species and between WNS exposure and reproductive status. Species, reproductive status, month, and site variables were significant within the logistic model, providing support that these variables were influential in determining the probability of structural zeros in the data (bats were not present and, thus, not observed). Structural zeros were most common for evening bats, nonreproductive females, early spring captures, and at

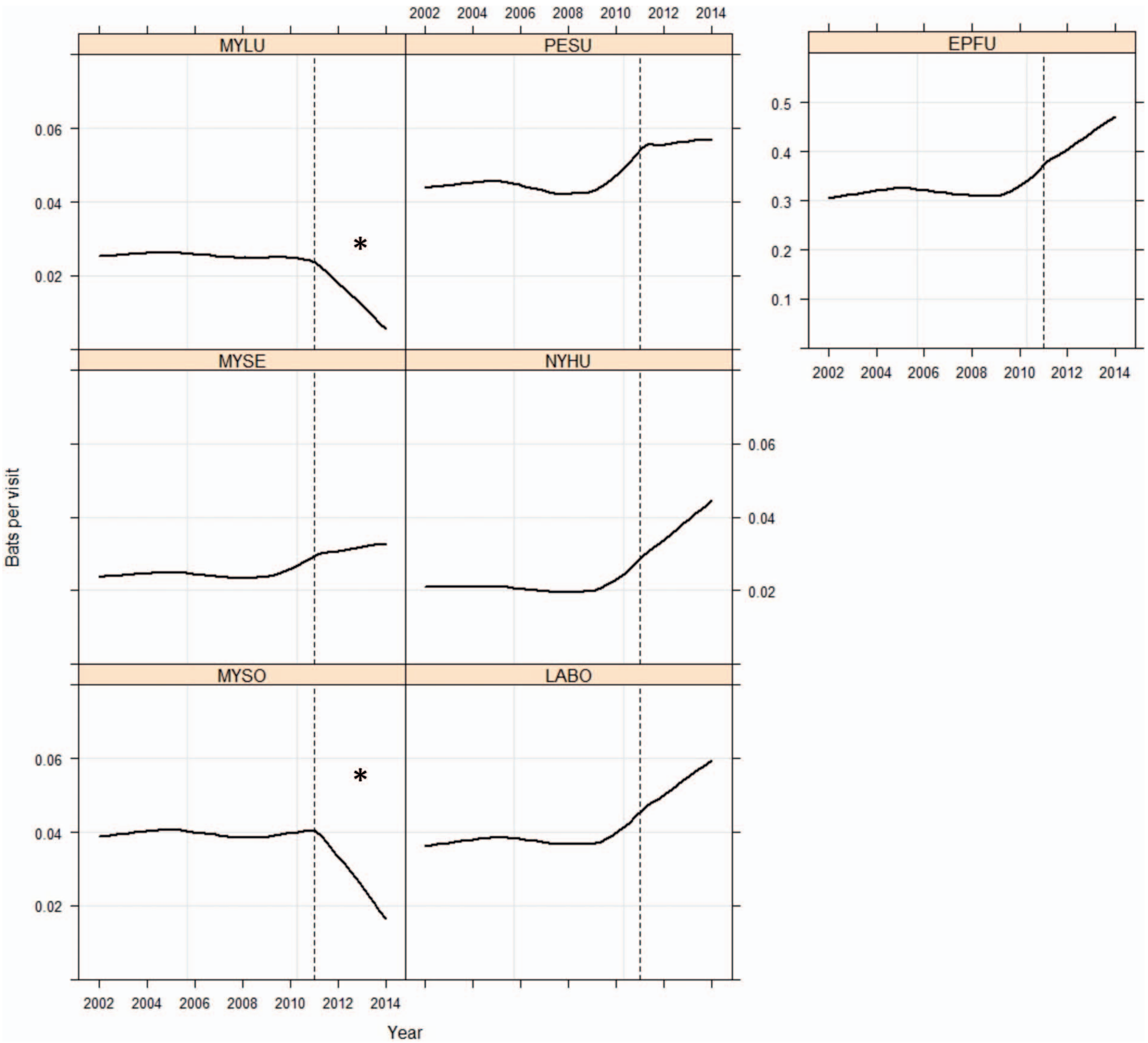

Figure 2. Loess-smoothed line of fitted values organized by species from a zero-inflated mixed-model analysis of bat captures at a long-term monitoring site in central Indiana (2002–2014). An asterisk denotes a significant change in the count portion of the model during the white-nose syndrome (WNS) period. Loess span = 0.5. Vertical dashed line represents the first year of WNS observation in Indiana. Big brown bat *Eptesicus fuscus* (EPFU), eastern red bat *Lasiurus borealis* (LABO), little brown bat *Myotis lucifugus* (MYLU), northern long-eared bat *M. septentrionalis* (MYSE), Indiana bat *M. sodalis* (MYSO), evening bat *Nycticeius humeralis* (NYHU), tri-colored bat *Perimyotis subflavus* (PESU).

one particular net site. Month was a significant factor in the count model because capture rates varied over time within a particular year. We captured bats at higher rates in July and August and lower rates in May. In the count model, WNS exposure probability alone was not a statistically significant predictor variable, but the WNS exposure $\times$ species interaction was significant for Indiana bats and little brown bats (Figure 2); thus, WNS exposure probability was maintained in the final fitted model. We captured significantly fewer little brown bats and Indiana bats as the WNS exposure probability increased. Empirical data summaries show captures per night dropped for little brown bats from 0.19 ($\pm$0.5 SD, $n = 271$) prior to 2011 to 0.09 ($\pm$0.3 SD, $n = 119$) from 2011 to 2014; there were no little brown captures during the summer of 2014. Indiana bat captures per night dropped from 0.32 ($\pm$0.8 SD, $n = 271$) to 0.15 ($\pm$0.5 SD, $n = 119$) over the same interval.

Capture rates varied with reproductive condition in the count model; lactating females were most common (1.39 $\pm$ 2.5 SD bats/night 2002–2014, $n = 380$) and nonreproductive adults were least common (0.14 $\pm$ 0.5 bats/night 2002–2014, $n = 380$). In the count model, the WNS exposure $\times$ reproductive condition interaction showed a significant increase in the number of postlactating adult females as WNS exposure probability increased (Figure 3 and Table S46). Captures of nonreproductive females also increased notably with WNS exposure but the WNS exposure $\times$ reproductive condition interaction parameter was not significant in

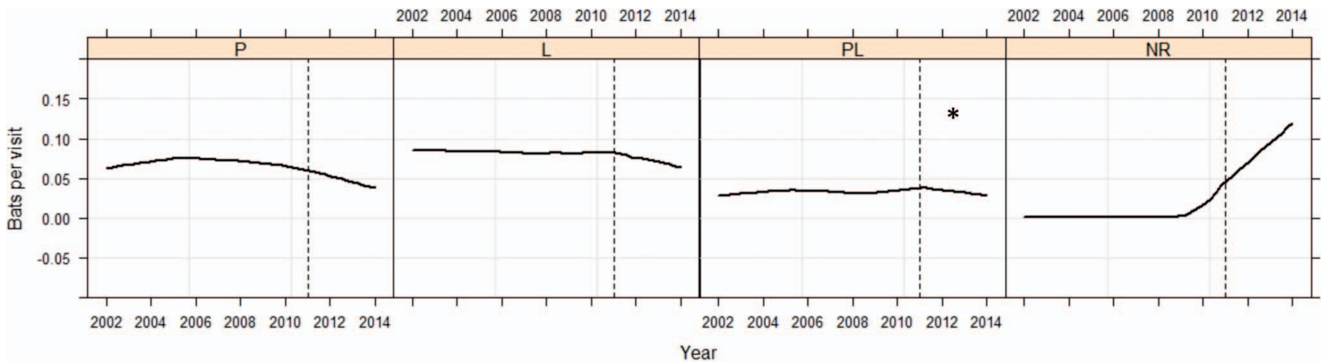

Figure 3. Loess-smoothed line of fitted values for seven pooled bat species organized by reproductive condition from a zero-inflated mixed-model analysis of bat captures at a long-term monitoring site in central Indiana (2002–2014). Loess span = 0.5. Vertical dashed line represents the first year of white-nose syndrome observation in Indiana. Lactating (L), nonreproductive (NR), pregnant (P), and postlactating (PL).

the count portion of the zero-inflation model. It was significant in the logistic portion, indicating that structural zeros were more common at low exposure probabilities (i.e., nonreproductive bats were not present at low infection probabilities and could not be detected). Prior to 2011, only three nonreproductive adult females were observed, compared with the 51 observed from 2011 to 2014.

White-nose syndrome increased the incidence of nonreproductive adult females, but WNS did not significantly influence timing for reproductively active bats. Most nonreproductive big brown bats were caught after the parturition date during the time we would expect to catch lactating females (Figure 4), but nonreproductive *Myotis* species were caught during the entire summer. The big brown bat lactation period (when >50% of adult female captures were lactating) was 15 d shorter during the WNS period than it was pre-WNS (Figure 4), and the *Myotis* lactation period was 12 d shorter (Figure 5). However, because the confidence intervals overlapped across WNS periods, we determined that this shortening of the lactation period was not significant for either big brown bats or *Myotis* bats.

Juvenile captures and recaptures of all bats increased after WNS affected Indiana caves. We caught 407 juvenile bats of all species during the 261 pre-WNS period site visits and 271 juveniles during the 119 WNS period site visits. However, when only *Myotis* species juveniles were considered, we caught 42 juveniles pre-WNS versus 11 during WNS. We recaptured 98 bats of all species during the 261 pre-WNS period site visits and 100 during the 119 WNS period site visits. Of the pre-WNS recaptures, 62% were big brown bats, 12% were Indiana bats, 8% were tri-colored bats, and 7% were northern long-eared bats. Little brown bats, evening bats, and eastern red bats made up the remaining 11% in order of abundance. During the WNS period, 70% of recaptures were big brown bats, 18% were evening bats, and the remaining 12% were eastern red bats, Indiana bats, northern long-eared bats, tri-colored bats, and little brown bats.

Discussion

By examining capture data from a long-term study in a WNS-affected area and extrapolating data from WNS-affected hibernacula to the eastern United States, we have demonstrated that WNS effects can be detected in

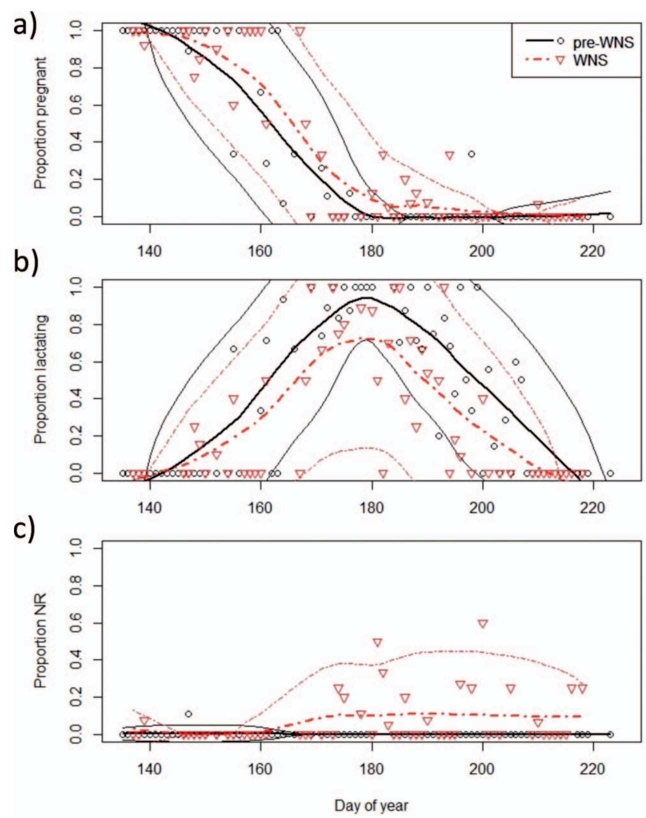

Figure 4. Loess-smoothed lines showing the proportion of big brown bat *Eptesicus fuscus* captures that are **(a)** pregnant, **(b)** lactating, or **(c)** nonreproductive by day of year; data are from a long-term study in central Indiana (2002–2014). Solid lines represent the pre-white-nose syndrome (WNS) fitted values and dashed lines represent the WNS period fitted values; thin lines are 95% confidence intervals. Loess span = 0.75.

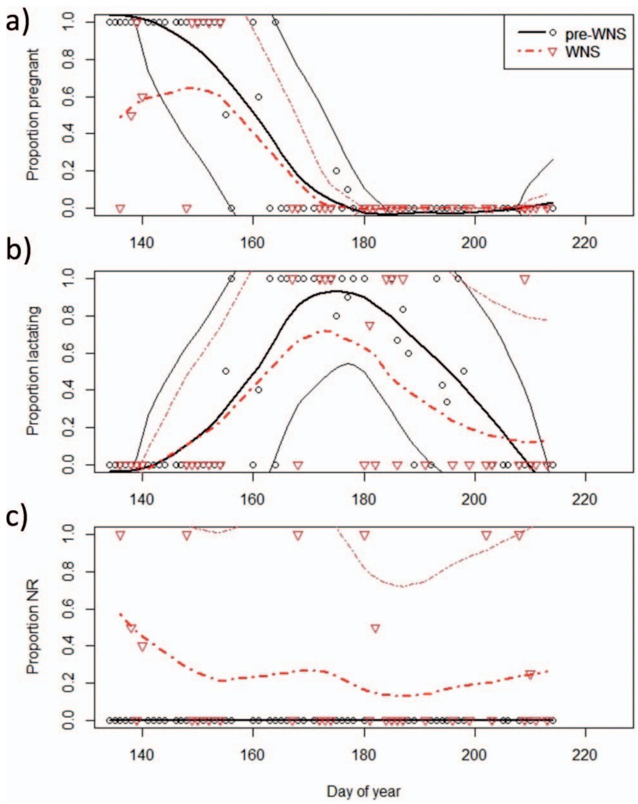

Figure 5. Loess-smoothed lines showing the proportion of captures that were **(a)** pregnant, **(b)** lactating, or **(c)** nonreproductive *Myotis* species (little brown bats, northern long-eared bats, and Indiana bats) by day of year; data are from a long-term study in central Indiana (2002–2014). Solid lines represent the pre-white-nose syndrome (WNS) fitted values and dashed lines represent the WNS period fitted values; thin lines are 95% confidence intervals. Loess span = 0.75.

summer populations, possibly predictably, in areas away from hibernacula. The cokriging model made predictions matching changes in capture rates and changes in species-specific reproductive condition that we observed at our central Indiana site. Changes were more apparent for *Myotis* species, with little brown bats suffering the greatest impact. Big brown bats did not show WNS-induced declines, nor did eastern red bats or evening bats, two species not known to be affected by WNS. At high WNS exposure probabilities, we observed more nonreproductive and postlactating adult females than expected based on pre-WNS captures and noticed shifts in timing and proportion of certain reproductive classes for WNS-affected species. However, our small sample size during the WNS period did not allow for detection of statistically significant changes in reproductive timing.

The cokriging model suggests a timeline of WNS impacts in the study area that mirrors the spread of the infection to Indiana caves in January 2011. The probability of WNS infection was low during 2011 and then increased dramatically in 2012. Based on our data showing bats that summer in Hendricks County are hibernating in WNS-infected caves (Table S1) and the close proximity of infected caves, we know the probability of WNS exposure of Hendricks County bats was 0.95 in 2013 and presumably is rising. These data also support the idea that bats could be a vector for moving *Pseudogymnoascus destructans* to our summer study area, adding validity to the application of the cokriging model on the summer landscape (Miller-Butterworth et al. 2014; Meyer et al. 2016).

Data from this study and previous studies using capture surveys (Francl et al. 2012), population modeling (Frick et al. 2010; Moosman et al. 2013), and acoustic surveys (Brooks 2011; Dzal et al. 2011) have demonstrated declines in *Myotis* species during the maternity period after regional detection of WNS. This epidemic disease has had the greatest impact on populations of little brown bats (e.g., 73% declines in the northeast, Frick et al. 2010; 78% declines in New York, Dzal et al. 2011). We observed a 79.6% decline in little brown captures from pre-WNS to the WNS period (Table 1), and 2014 was the only year that we did not capture little brown bats during this 13-y study. Declines in Indiana bat captures were greater in this study (59.6% decline) than the 10.8% declines from a study in West Virginia (Francl et al. 2012). It should be noted, however, that Indiana bats were caught in greater proportion in this study compared with the Francl et al. (2012) study (6.8% of captures versus 0.3% respectively). The fact that Indiana bats were more common in our study area could explain some of the difference in declines observed between studies. We observed a slight increase in captures of northern long-eared bats that contrasted with findings in other regions (e.g., 77% decline in West Virginia, Francl et al. 2012; or 95% declines in the same area found by Reynolds et al.

Table 1. Mean number of bats captured at a long-term monitoring site in central Indiana during three time periods centered around the year white-nose syndrome (WNS) was first detected in Indiana (January 2011). Captures are standardized (std.) by net effort and percent rate change is reported for each species. Negative changes are bolded.

	Pre-WNS mean std. captures/year 2002–2010	Initial-WNS mean std. captures/year 2011	WNS mean std. captures/year 2012–2014	Percent rate change (WNS–Pre)/Pre × 100
Eptesicus fuscus, big brown bat	90.4	81.5	134.4	11.5
Lasiurus borealis, eastern red bat	15.7	17.9	31.5	50.5
Myotis lucifugus, little brown bat	10.5	8.6	2.9	**−79.6**
Myotis septentrionalis, northern long-eared bat	6.9	7.0	9.4	2.1
Myotis sodalis, Indiana bat	16.7	8.1	9.0	**−59.6**
Nycticeius humeralis, evening bat	8.6	7.8	16.7	46.5
Perimyotis subflavus, tri-colored bat	16.1	20.0	18.8	**−12.5**

2016), though the increase we observed was driven mostly by an increase in captures during 2012.

In Indiana hibernacula, the Indiana Department of Natural Resources (2015) has observed declines for little brown bats, Indiana bats, tri-colored bats, and big brown bats (90%, 27%, 71%, and 51%, respectively). As we observed in this study, two of Indiana's most common cave dwellers have declined significantly since *Pseudogymnoascus destructans* was first detected in Indiana caves; the little brown bat declined from 7,603 bats in 2009 to 794 bats in 2015 and the Indiana bat declined from 166,891 bats in 2009 to 121,582 bats in 2015 (Indiana Department of Natural Resources 2015). Although the numbers of tri-colored bats and big brown bats have dropped significantly in Indiana caves, we did not detect significant declines for these species in this study. This discrepancy could be due to methodological differences and the wintering ecology of these bats. Big brown bats can hibernate in man-made structures in addition to caves and mines (Whitaker and Gummer 2000) and, thus, may have a natural refuge from WNS. Tri-colored bats are typically not observed in large numbers during cave surveys because they usually roost singly in deep portions of the cave (Fujita and Kunz 1984).

We did not detect a negative effect of WNS on capture rates for evening bats, eastern red bats, or big brown bats. Other studies have also shown the big brown bat and eastern red bat capture rates and acoustic activity increasing after WNS infection (Brooks 2011; Francl et al. 2012), despite the fact that *Pseudogymnoascus destructans* had been found present in skin swabs of these species (Bernard et al. 2015). Ours is the first study to show changes in capture rates for evening bats after WNS infection. Big brown bats amass greater amounts of fat prior to hibernation and, thus, may be able to better survive WNS-induced resource depletion during hibernation (Frank et al. 2014). Both Francl et al. (2012) and Ford et al. (2011) hypothesized that capture rates might be increasing for some species because of a decrease in competition as populations of other bat species decline. We surmise that during the WNS period we may have been more likely to capture big brown bats in mist nets because of fewer WNS-affected bats using the airspace around mist nets. We detected an 11.5% increase in capture rates for big brown bats, but the observed reduction in reproductive females suggests WNS could still affect population sizes by altering fecundity for affected bats.

Our data show that WNS is affecting the successful completion of the reproductive cycle for little brown bats, northern long-eared bats, Indiana bats, and big brown bats. We observed an increase in captures of nonreproductive and postlactating adult females at high WNS exposure probabilities. The increased encounter rate for nonreproductive adult females of these species indicates that even bats that survive hibernation in a WNS-infected cave may lose too much energy to successfully produce viable offspring. Adult female little brown bats enter hibernation with greater fat reserves and consume them more slowly than their male counterparts (Jonasson and Willis 2011). This saves more energy for spring reproduction, which should increase the reproductive success of female bats. However, females conserve said fat stores through greater use of torpor, a behavior that is interrupted by WNS-induced arousals (Reeder et al. 2012). Therefore, females may emerge from hibernation with insufficient energy to support fetal development. An alternative hypothesis is that some bats are losing pups rather than foregoing pregnancy; it follows that WNS-induced resource depletion could be decreasing reproductive success in a number of ways. The fact that 19 of the 20 nonreproductive adult female big brown bats were caught after the parturition date suggests that big brown bats in our study area were losing pups after becoming pregnant in spring. Also, our data suggest that recruitment of *Myotis* species juveniles is declining and similar results were found by Francl et al. (2012). We recommend that future studies monitor the relative proportion of nonreproductive adults and juvenile recruitment in a study population as indicators of WNS impacts and encourage studies that observe females during pregnancy to reveal mechanisms responsible for pup loss.

Management Implications

In our long-term study area, WNS-affected species represent a broad ecological niche that may be severely affected as populations decline (Jachowski et al. 2014). Though we demonstrated an impact of WNS 4 y after it reached Indiana caves, there is no guarantee that the full effect of WNS to the bats of Indiana has been realized. Declines in capture rates for Indiana bats and little brown bats were significant, and we expect that as WNS progresses the impact on populations of tri-colored bats and northern long-eared bats will be more apparent. Also, because WNS is impacting the reproductive cycle of affected bats, further research is needed to detect changes in recruitment, especially for highly affected species. This WNS study, unparalleled in the length of the study term, provides an example of how we may use summer capture numbers to analyze the impact of a disease that infects bats during the hibernation period.

Supplementary Material

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Table S1. Records of Indiana bats observed in Indiana caves during hibernation surveys in 2011 and 2013. Bats were originally banded at a long-term monitoring site in central Indiana between 2002 and 2012. The white-nose infection year, distance, and direction is listed for each cave. Caves are grouped by proximity to our study area and represent two major hibernacula concentrations in Indiana.

Found at DOI: http://dx.doi.org/10.3996/102016-JFWM-077.S1 (DOCX 21.0 KB).

Table S2. List of factors and distributions for zero-inflated mixed models used to predict bat captures at a long-term monitoring site in central Indiana (2002–2014). The most supported model, as shown by likelihood ratio tests, is the full model with a negative binomial distribution, Model 4. Found at DOI: http://dx.doi.org/10.3996/102016-JFWM-077.S2 (DOCX 27.4 KB).

Table S3. Data containing information on bat captures from 2002 to 2014 organized by site visit for a long-term monitoring study in central Indiana. Found at DOI: http://dx.doi.org/10.5061/ 10.3996/102016-JFWM-077.S3 (CSV 1.2 MB).

Table S4. Predicted probability of white-nose syndrome (WNS) exposure for a long-term bat monitoring site in central Indiana and number of WNS-infected caves in Indiana by year (2008–2013). Annual values for the central Indiana site were extracted from cokriging spatial models developed using population data from Indiana bat *Myotis sodalis* hibernacula. Found at DOI: http://dx.doi.org/10.3996/102016-JFWM-077.S4 (DOCX 18.8 KB).

Table S5. Parameter estimates, standard errors, *z*-values, and *P*-values for model parameters in the logistic portion of the final zero-inflated model predicting bat captures at a long-term monitoring site in central Indiana (2002–2014). Found at DOI: http://dx.doi.org/10.3996/102016-JFWM-077.S5 (DOCX 34.5 KB).

Table S6. Parameter estimates, standard errors, *z*-values, and *P*-values for model parameters in the count portion of the final zero-inflated model predicting bat captures at a long-term monitoring site in central Indiana (2002–2014). Found at DOI: http://dx.doi.org/10.3996/102016-JFWM-077.S6 (DOCX 35.2 KB).

Figure S1. Standard error estimate for a 2013 cokriging spatial model predicting white-nose syndrome (WNS) exposure probability across a continuous landscape in eastern North America. The standard error estimate for our long-term monitoring site in central Indiana was 0.06, suggesting that predicted values for WNS presence (see Figure 1) were accurate. Found at DOI: http://dx.doi.org/10.3996/102016-JFWM-077.S7 (PPT 462 KB).

Acknowledgments

Data collection was funded by the Indianapolis Airport Authority. J. Pettit was supported by a grant from the Indiana Department of Natural Resources and assistantships from Indiana State University. We thank many field technicians and graduate students for data collection and B. Walters, D. Sparks, and N. Gikas for data management to 2012. We greatly appreciate helpful comments provided by the Associate Editor and reviewers.

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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