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The Effects of White-nose Syndrome on Cave-dwelling Bats During Their Pre-hibernation Season in Eastern Nebraska

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In Conjunction with the Biology Department of the University of Nebraska at Omaha, Supervised by Dr. Jeremy White

Keywords: White-nose syndrome (WNS), Nebraska, bat, population(s), Pseudogymnoascus destructans (Pd), decline, Myotis septentrionalis (MYSE)

Subject Categories: Biology, mammalogy, ecology

Abstract

White-nose syndrome (WNS) is currently the leading cause of death to North American bats and was first documented in the United States in 2006 on the East Coast. Infection with the fungus Pseudogymnoascus destructans (Pd) causes bats to arise from hibernation with unnecessary frequency, leading to depletion of fat reserves that are imperative for extended periods of torpor. In October 2014, biologists surveyed the activity levels of bats at Fontenelle Forest in eastern Nebraska using acoustic detectors and determined what species of bats were present and their relative abundance before being exposed to WNS. By returning to the same region in October of 2020 and collecting a new sample of acoustic data, we were able to determine how bat populations in their pre-hibernation period have been affected since WNS arrived in eastern Nebraska. Our study shows that fall populations of northern long-eared bats have experienced drastic declines in this area since the arrival of WNS. This corroborates the findings of a study that observed reduced activity at the same site during the summer and echoes the results of surveys conducted throughout the eastern portion of the United States documenting population declines of northern long-eared bats due to WNS.

Introduction

Bats are widely considered to be one of the most diverse and ecologically important mammalian taxa on Earth (Puechmaillle et al. 2011). They are vital to local economies as pollinators and controllers of insect populations; one colony of bats, numbering about 150 individuals, has the capacity to consume over 1 million insects a year that may otherwise pose a serious threat to domestic agriculture (Boyles et al. 2011). Unfortunately, bats across North America are currently facing a serious threat in the form of a deadly pathogen. White-nose syndrome (WNS) is caused by a psychrophilic fungus called Pseudogymnoascus destructans (Pd) (Puechmaillle et al. 2011, Cryan et al. 2010). Since its discovery in Howes Cave of eastern New York in 2006 (Blehert et al. 2009) the pathogen has caused rampant destruction to bat colonies throughout the United States; the most recent estimate published suggests that white-nose syndrome has killed close to six million bats in North America (USFWS, 2013) and has spread westward to 35 US states and seven Canadian provinces according to the White-nose
Syndrome Response Team (2019) (Figure 1). Due to the highly mobile nature of bats, complete containment of the disease is unrealistic and the continued infection of virgin colonies must be considered unavoidable. While there is still much unknown regarding the exact nature of this fungus, scientists consider WNS to be an invasive pathogen (Nocera et al. 2019) as it is presenting like an emergent disease on the North American continent (Puechmaillle et al. 2011). Biologists hypothesize that $Pd$ has long been present in Europe and was recently introduced to North America by anthropogenic means (Leopardi et al. 2015). This hypothesis is supported by the fact that this same fungus has been observed on bats in Europe with no record of mass mortality amongst these populations (Puechmaillle et al. 2011). Warnecke et al. (2012) found that North American bats were highly susceptible to both the European and North American isolates of WNS; the data from this study provides strong support for the novel pathogen hypothesis and implies that European bats have coevolved a level of resistance to and/or tolerance of infection with $Pd$.

Bats that have contracted WNS are easily identifiable owing to the presence of white filaments on their muzzle caused by fungal spores replacing healthy hair follicles (Blehert et al. 2009, Cryan et al. 2010, Meteyer et al. 2009). The wings of hibernating bats are at great risk of destruction by the disease as $Pd$ grows on exposed epithelial tissue (Cryan et al. 2010, Puechmaillle et al. 2011). Clinical signs of infection with WNS is necrosis of the wing membrane (Pannkuk et al. 2015) as well as loss of wing membrane elasticity, irregular pigmentation, and stickiness of wing tissue (Warnecke et al. 2012, Meteyer et al. 2009). WNS causes infected bats to awaken more frequently than is necessary during their period of hibernation (Frank et al. 2014, Reeder et al. 2012); this is perhaps the fungus’ most deadly effect. These periods of alertness cause a rapid depletion of fat reserves during a time of the year when resources are scarce. It is estimated that up to 90% of a bat’s fat reserves are used during their arousals from torpor despite these periods accounting for a mere 1% of their time spent overwintering (Reeder et. al 2012). Thus, infection with $Pd$ results in bats dying of starvation, dehydration, and/or exposure to natural elements (Nocera et al. 2019). The spread of this pathogen is affected by a number of factors including ambient cave temperature where the bats are roosting, the proximity of individual bats to one another while hibernating, and the population density of a given hibernaculum (O’Keefe et al. 2019). Because this fungus displays saprotrophic growth behavior, it is likely that it is able to grow throughout the year in certain cave systems; this increases the risk of infection in new bats as well as infection of previously untouched caves via contaminated sediment samples (Reynolds & Barton 2014). Experimental inoculation shows that WNS is spread through direct physical contact between bats (Warnecke et al. 2012) although it can be spread anthropomorphically as $Pd$ is able to survive on shoes, clothing, and gear that is moved from one hibernaculum to another, infecting previously healthy colonies (What is White-nose Syndrome, n.d.).
Figure 1: This map displays the steady migration of Pd from east to west across the United States and Canada.
Bats experience WNS differently based on several factors including species, life history, and geographic distribution. Species that are most severely impacted are those that undergo a period of hibernation throughout the winter months (Mayberry 2020). Our survey focused on the population declines of two cave-dwelling species—the tricolored bat (*Perimyotis subflavus*) and the northern long-eared bat (*Myotis septentrionalis*)—as these two species were once plentiful across the Midwest but have proven to be highly susceptible to WNS in other parts of the country (Langwig et al. 2012, O’Keefe et al. 2019). According to White et al. (in preparation), *Pd* was first documented in Nebraska in 2015 and the first bats found dead due to WNS were discovered in 2017. Although surveys have been completed measuring the decline of summer populations of at-risk species (White et al. in preparation, Nocera et al. 2019, O’Keefe et al. 2019) and a number of winter counts have been done in an attempt to establish population sizes of hibernating communities (Frank et al. 2014, Langwig et al. 2012, Langwig et al. 2015, Reeder et al. 2012) few studies have examined the pre-hibernation population patterns of the northern long-eared bat or the tricolored bat (see Powers et al. 2015). Furthermore, coastal states lacked the opportunity to establish an understanding of native bat populations pre-WNS due to rapid exposure with the pathogen. In preparation for its eventual arrival, acoustic detectors were deployed in 2014 in eastern Nebraska to establish the relative activity of bats in this region before the arrival of WNS. By comparing the 2014 population survey with data collected post-WNS, we can form a clearer idea of how different species of Midwestern bats have been affected by WNS since it entered the state.

**Hypothesis**

Preliminary echolocation recordings taken in 2014 showed that there were several different species of bats present along the eastern edge of Nebraska including the big brown bat (*Eptesicus fuscus*, EPFU), the silver-haired bat (*Lasionycteris noctivagans*, LANO), the northern long-eared bat (*Myotis septentrionalis*, MYSE), and the tricolored bat (*Perimyotis subflavus*, PESU). We expect to observe a decrease in activity of the northern long-eared and tricolored bat when compared to the 2014 survey as these bats are more susceptible to *Pd* than other species; they are communal roosters and WNS relies on density-dependent transmission (Langwig et al. 2012). In contrast, silver-haired bats are not known to be impacted by WNS (Nocera et al. 2019) and there has yet to be a documented case of *Pd* infection among bats that do not overwinter in a shared hibernaculum. Therefore, tree-roosting, migratory species of bats like the silver-haired bat are not considered susceptible to death by WNS (Jachowski et al. 2014). For this reason, we hypothesize that activity levels of silver-haired bats will exhibit little to no change in the region. Meanwhile, the big brown bat has exhibited lower mortality rates after becoming infected with *Pd* (Frank et al. 2014, Mayberry et al. 2020) and there are several instances where the populations of big brown bats increase in areas that have come into contact with *Pd* (Frank et al. 2014). This could be because they are not regular communal hibernators and have a larger body size (Mayberry et al. 2020, Frank et al. 2014, Nocera et al. 2019). Thus, we expect the big brown bat to also maintain a fairly constant level of activity post-exposure to WNS.
Figure 2: A picture of the location of one detector, illustrating the ideal placement of an acoustic detector in an uncluttered portion of the lowland Nebraska floodplain. The lowland in the foreground is bordered by the wooded highlands and highlights the differences in the two neighboring habitats.
**Methods**

To evaluate the activity levels of Nebraska bats during their pre-hibernation period after the arrival of WNS, we deployed acoustic detectors within the same geographic area that was utilized in the 2014 study at Fontenelle Forest. Fontenelle Forest is a conservation organization based in eastern Nebraska that works to preserve habitats such as Loess Hill land formations and the Missouri River floodplain (About Fontenelle Forest, n.d.). Due to its relative proximity to active hibernacula in southeastern Nebraska—a series of mines located approximately 50 km south of Omaha—Fontenelle Forest is frequently visited by bats during their pre-hibernation foraging period. On 7 and 8 October 2020, we placed four acoustic detectors in the floodplain forest (Figure 2). Each detector was affixed to a sturdy tree approximately two meters off of the ground in an uncluttered portion of the floodplain in order to increase the likelihood of recording long, search-phase calls of any passing bats (Figure 3). A call emitted by a bat as it passes by the microphone is referred to as a bat pass and was recorded for five-second intervals by the detectors. The detectors were placed an average of 233 meters from one another and were hiked off of the primary trail to avoid attracting the attention of the general public (Figures 4 & 5). The tools initially used for this project were the SM2Bat+ acoustic detector and the associated SMX-US ultrasonic microphones (White et al. 2016) whereas we employed the SM4BAT FS detectors with SMM-U2 microphones in 2020. In both surveys the calls were recorded as soundbites at the original frequencies generated by the bat; these ultrasonic frequencies were then converted to WAV format and automatically identified to species using Kaleidoscope Pro by Wildlife Acoustics. One limitation of automated programs is that while they are largely accurate, their identifications are not infallible. Thus, in order to establish the smallest margin of error, each pass recorded in the 2020 survey was processed with a secondary identification program called AnalookW and then manually vetted. While some discretions existed, the two programs (Kaleidoscope and AnalookW) typically agreed and were usually supported by the manual identification. The most common error exhibited by the automated programs was the habit of confusing *E. fuscus* and *L. noctivagans* passes for one another. For the purposes of this study this error can be considered largely inconsequential due to the fact that both *E. fuscus* and *L. noctivagans* populations have remained largely unaffected by the spread of WNS. In this analysis we will be using big brown bat and silver-haired bat calls as a baseline in our experiment to give context to the population fluctuations displayed by the two other species—the tricolored and northern long-eared bat—monitored in this survey.
Figure 3: Detector 46 secured to a tree. Each detector was placed approximately four feet off of the ground to ensure the attached microphones reached an adequate height. The detectors contained a battery and memory card to record and save the passes collected by the ultrasonic microphone.
Figure 4: A map showing the locations of each detector when placed in the field in October 2020. The letter “A” refers to the detector’s location on 7 October while the letter “B” identifies where the detector was placed on 8 October.

Table 1: This graph shows the exact latitude and longitude of each detector.

<table>
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<th>Date</th>
<th>Detector Name</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
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<tr>
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<td>62A</td>
<td>41.1746°</td>
<td>-95.8921°</td>
</tr>
<tr>
<td>7 October 2020</td>
<td>65A</td>
<td>41.1768°</td>
<td>-95.8937°</td>
</tr>
<tr>
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<td>89A</td>
<td>41.1737°</td>
<td>-95.8904°</td>
</tr>
<tr>
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<td>46B</td>
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<td>-95.8906°</td>
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<tr>
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<td>8 October 2020</td>
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<td>41.1725°</td>
<td>-95.8898°</td>
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Results

On 7 and 8 October 2020, we recorded a total of 1,832 bat passes and 1,346 of these passes were generated by species focused on within this study (Table 2). The number of bat passes recorded per night varied wildly among detectors, ranging from a mere 31 passes to over 700. Throughout the two nights of recording, Kaledoscope and AnalookW failed to identify a single northern long-eared bat call, although the process of manual identification yielded a single pass that could be attributed to *M. septentrionalis* (Figure 6). This exemplifies a dramatic decrease in activity when compared to the 2014 survey (Figure 5). While there are likely still northern long-eared bats in the Fontenelle Forest area, they remain largely absent from the 2020 survey and act as the best source of evidence supporting the hypothesis of local population decline due to WNS. The automated programs identified a total of six tricolored bat calls that were supported by manual vetting (Table 2 and Figure 7), constituting a decrease in acoustic activity compared to the 2014 data (Figure 5). Meanwhile, 1,339 EPFU/LANO calls were recorded (Table 2); this means that big brown and silver-haired bat passes compromised nearly 75% of total passes recorded in 2020.

Discussion

Our results show a drastic decline in northern long-eared bats in eastern Nebraska since the arrival of WNS and are consistent with the findings reported by Nocera et al. (2019), Blehert et al. (2009) and other surveys examining the effect of *Pd* on North American bat species. Furthermore, our findings support other fall, pre-hibernation population studies that have been completed, such as that by Powers et al. (2015). The 2014 survey was completed with the understanding that white-nose syndrome would eventually reach Nebraska and wreak havoc on local bat communities. This period preceding the arrival of *P. destructans* allowed biologists to gain a more thorough understanding of the bat population demographics within the state and therefore allowed us to more fully understand the toll the disease has taken on bats in eastern Nebraska. Because our survey shows that northern long-eared bats have declined during their pre-hibernation season, we can now state with confidence that it is not merely their summer populations at Fontenelle Forest that are suffering from infection with WNS (White et al. in preparation), but also individuals moving through the area in the fall before arriving at their winter roost site.

The northern long-eared bat experienced a definitive population decline once WNS entered the Midwest (Langwig et al. 2015). However, we did not observe a large decline in activity of tricolored bats at Fontenelle Forest in our study. The low activity of tricolored bats at Fontenelle Forest in early October both before and after the arrival of *Pd* in Nebraska may be attributed to their extended hibernation period; that is, most individuals had likely already left the area for their wintering grounds. While still present in the region, they have also suffered since coming into contact with *Pd*. Their more moderate decrease in activity may be due to the fact that they are typically solitary roosters within hibernacula (O’Keefe et al. 2019).
Figure 5: These three bat graphs show the pre-WNS findings from the survey conducted in October 2014 in red compared to the data collected in October 2020, after WNS arrived in the state of Nebraska.

<table>
<thead>
<tr>
<th>Date</th>
<th>Detector</th>
<th>MYSE</th>
<th>PESU</th>
<th>EPFU/LANO</th>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>140</td>
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<td>89A</td>
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<td>2</td>
<td></td>
<td>126</td>
</tr>
<tr>
<td>8 Oct 2020</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>46B</td>
<td>1</td>
<td>0</td>
<td></td>
<td>111</td>
</tr>
<tr>
<td>62B</td>
<td>0</td>
<td>2</td>
<td></td>
<td>73</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td></td>
<td>706</td>
</tr>
<tr>
<td>89B</td>
<td>0</td>
<td>0</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td><strong>Total Number of calls for 2020</strong></td>
<td><strong>1</strong></td>
<td><strong>6</strong></td>
<td></td>
<td><strong>1,339</strong></td>
</tr>
</tbody>
</table>

Table 2: A chart outlining the individual calls recorded per detector and the total number of passes recorded throughout the 2020 survey. These calls were confirmed by manual vetting to ensure accurate identification.
Figure 6: The single northern long-eared bat call recorded by the acoustic detectors in 2020, as displayed by the program AnalookW. The vertical axis is measured in kilohertz while the horizontal axis is measured in milliseconds.

Figure 7: One of six tricolored bat calls recorded by the acoustic detectors in 2020, as displayed by the program AnalookW. The vertical axis is measured in kilohertz while the horizontal axis is measured in milliseconds.
The population decline of both of these species has had indirect effects on other bat communities in the area. The rampant destruction of the intraspecific competition for resources has likely allowed the big brown bat to increase their foraging activity, displaying an alteration in niche partitioning (Nocera et al. 2019). In addition, the large number of big brown and silver-haired bat passes recorded when conducting the 2020 population survey is also likely due to the fact that silver-haired bats complete an annual migration through this region during October as they move south to their wintering grounds. Over half of the big brown/silver-haired bat calls from 2020 were recorded at a single site on 8 October (Table 2) and might represent a migratory wave of silver-haired bats moving through Fontenelle Forest. Furthermore, any population fluctuations displayed by the silver-haired bat cannot be wholly attributed to the effects of WNS. As a migratory species and tree-roosting bat, silver-haired bats are susceptible to death by wind turbines along with the eastern red bat (Lasiurus borealis) and the hoary bat (Lasiurus cinereus) (Cryan & Barclay 2009). Collision with wind turbines impacts migratory bat species (Johnson et al. 2004) and the Great Plains is considered to have high wind turbine fatality rates on average (Arnett & Baerwald 2013). The majority of wind turbine related fatalities among these species occur during their autumnal migration (Cryan & Barclay 2009) and thus have the capacity to affect any pre-hibernation population surveys conducted. This information must be kept in mind when analyzing data collected during the migratory season. The general pattern detected in this study, however, is consistent with other population surveys completed which show minimal changes in silver-haired and big brown bat activity following the introduction of Pd to a given community.

The data collected in these acoustic surveys is not infallible; there is the potential for automated programs to misidentify species emitting passes and for the manual examination of the data to fail to catch mistakes made. However, our findings contribute to the overall story of northern long-eared bats experiencing shocking declines in many regions due to infection with WNS. Nebraska bats have proven to be equally susceptible to death by WNS as those on the east coast and have experienced an astonishing number of fatalities. Although this outcome was expected, it is particularly troubling when considered alongside the economic value associated with these creatures within this part of the country. Eating tons of insects every night, their role within the agricultural community can be valued at over three billion dollars annually (USFWS, 2017). This research is currently of the utmost importance as US Fish and Wildlife Service is in the process of evaluating whether northern long-eared bats should be considered an endangered species. As of April 2, 2015, *M. septentrionalis* was listed as threatened under the Endangered Species Act (USFWS, 2020). By elevating its status to endangered, the northern long-eared bat would be awarded increased federal protection, an increased amount of funding directed towards their conservation, and would be identified as a critical species at risk of immediate extinction. It is clear that northern long-eared bats are in immediate and extreme danger. We must all work towards preserving the wonderful biodiversity that has long since been established in the United States amongst our varied bat species.
Acknowledgments

We would like to thank Brett Andersen of the University of Nebraska at Omaha for collecting the initial data in 2014. Furthermore, we wish to thank Cliff Lemen and Trish Freeman of the University of Nebraska at Lincoln for analyzing the data from 2014 which allowed us to complete the 2020 post-WNS survey. Finally, we thank the staff at Fontenelle Forest for allowing us to conduct research there.
References


