A Population of Little Brown Myotis (*Myotis lucifugus*) in Southern Ontario Exhibits Apparent Adaptation to White-nose Syndrome

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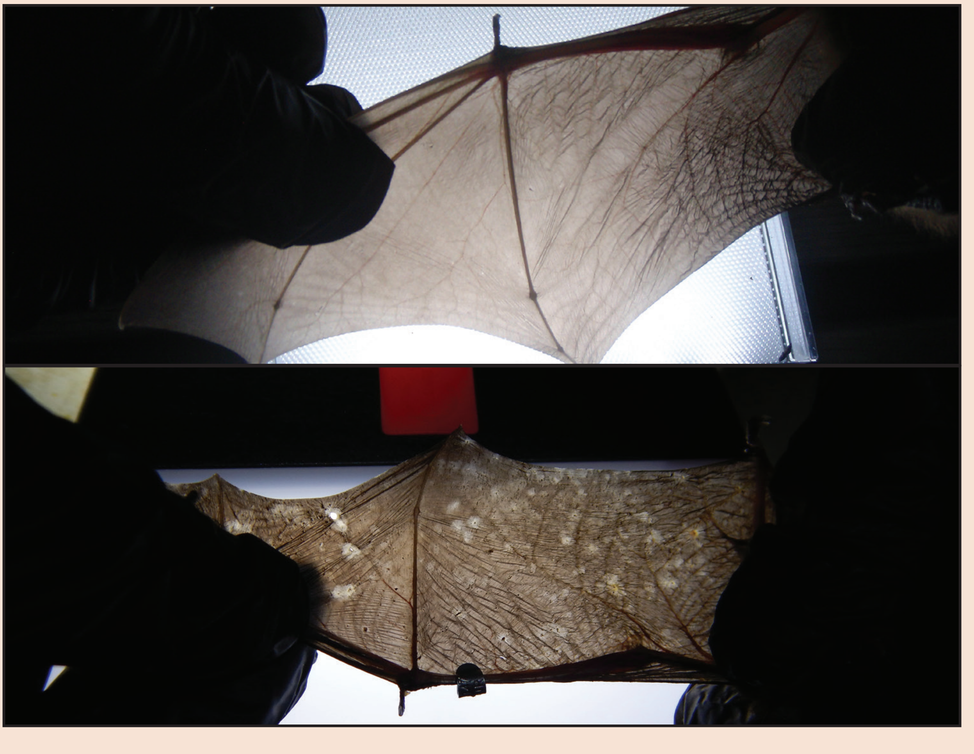

Volume 2, 2024 Journal of North American Bat Research No. 5

Journal of North American Bat Research

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Cover Photograph: Top: *Myotis lucifugus* wing with no damage from white-nose syndrome. Bottom: Damage from white-nose syndrome (score of 2 on the Reichard's Wing Damage Index) on the wing of a *Myotis lucifugus*. This was the highest wing damage score observed during the study. Photographs © Derek E. Morningstar.

The ***Journal of North American Bat Research*** (ISSN 2994-1075) is published by the Eagle Hill Institute, PO Box 9, 59 Eagle Hill Road, Steuben, ME 04680-0009. Phone: 207-546-2821 Ext. 4. E-mail: office@eaglehill.us. Webpage: https://www.eaglehill.us/nabr.

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A Population of Little Brown Myotis (*Myotis lucifugus*) in Southern Ontario Exhibits Apparent Adaptation to White-nose Syndrome

Al Sandilands[1,*] and Derek E. Morningstar[2]

Abstract - Novel wildlife diseases are an increasing concern, as they can cause population declines in species unable to adapt, or result in crossover events, sickening humans. *Myotis lucifugus* (Little Brown Myotis), exhibited precipitous declines after exposure to *Pseudogymnoascus destructans* (*Pd*), the fungus that causes white-nose syndrome (WNS). Our objectives were to determine if Little Brown Myotis at a roost in southern Ontario had been exposed to WNS and, if so, what effects this had on the bats. All tested positive for *Pd* in spring, but had minimal wing-tissue damage (mean = 0.3 on the Reichard's Wing Damage Index in May). Annual survival rate of adult females was approximately 0.83 ± 0.03, comparable to pre-WNS populations. The percentage of adult females that were lactating was 83.1%. All 3 subadult females that returned as yearlings lactated in their first year. Despite having been exposed to WNS, females had normal survival rates and reproductive success. This population appears to have developed resistance or tolerance to WNS. The prognosis for the Little Brown Myotis in southern Ontario may have shifted from imminent extinction to slow recovery.

Introduction

Emerging infectious diseases are increasing and they threaten both biodiversity and human health (Daszak et al. 2000). More than 60% of human infectious diseases are shared with domestic or wild animals (Karesh et al. 2012). Wildlife species exposed to a novel disease are at risk of population decline (Blehert et al. 2009, De Castro and Bolker 2005), but the evolution of traits that minimize the costs of infection can reduce this risk (Vander Wal et al. 2014). The host may reduce the effects of the pathogen through avoidance, resistance, or tolerance (Medzhitov et al. 2012, Vander Wal et al. 2014). Resistance involves limiting the pathogen burden, leading to antagonistic coevolution between the host and pathogen (Woolhouse et al. 2002). Resistance is detrimental to the pathogen, reducing its prevalence in the host population (Råberg et al. 2009). Tolerance decreases host susceptibility to tissue damage and other fitness costs caused by the pathogen (Medzhitov et al. 2012), without having adverse effects on the pathogen (Råberg et al. 2009).

North American bats have recently been exposed to a disease called white-nose syndrome (WNS), which is caused by an invasive fungal pathogen, *Pseudogymnoascus destructans* (Blehert and Gargas) Minnis and D.L. Lindner (*Pd*). *Pd* was introduced in New York State in 2006 (Blehert et al. 2009) and has since spread to at least 38 states and 9 provinces (White-Nose Syndrome Response Team 2023). As of 2021, 12 bat species had been confirmed with WNS and an additional 6 species had been confirmed with *Pd* (Cheng et al. 2021). The most obvious sign of WNS is a white, filamentous growth on the muzzle and powdery growth on the surface of ears, wings, and tail membranes of hibernating bats (Blehert et al. 2009). The most serious effect of WNS is that it disrupts the normal torpor

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Associate Editor: Craig Willis, University of Winnipeg.

patterns during hibernation, resulting in more frequent arousals (Reeder et al. 2012, Warnecke et al. 2012), depletion of fat reserves, and death of most bats (Frank et al. 2019). Dehydration caused by excess evaporative water loss is a key contributor to mortality (reviewed by Hoyt et al. 2021). Early indicators of WNS infection include acidosis, an increase in metabolic rate, and elevated blood potassium levels (Hoyt et al. 2021). Infected *Myotis lucifugus* (Le Conte) (Little Brown Myotis) that survive hibernation may be subject to increased physiological stress due to skin damage and decreased fat reserves (Davy et al. 2016). Wing damage may decrease flight efficiency, compromise bats' ability to forage and escape predators (Reichard and Kunz 2009), and cause dehydration (Cryan et al. 2010). High levels of cortisol associated with chronic stress have the potential to reduce reproductive success (Davy et al. 2016).

WNS has caused populations of Little Brown Myotis to decline precipitously in the northeastern United States (Frick et al. 2010a) and eastern Canada (Forbes 2012). WNS has spread rapidly throughout the northeastern United States, killing a high proportion of bats within hibernacula. Populations at these hibernacula decreased by >90%, with some local populations becoming extirpated. Summer captures declined at similar rates (Frank et al. 2014; Frick et al. 2010a, 2015; Langwig et al. 2012; Moosman et al. 2013; O'Keefe et al. 2019; Pettit and O'Keefe 2017). Little Brown Myotis were predicted to be extirpated in the northeastern United States as early as 2026 (Frick et al. 2010a, Langwig et al. 2012). Cheng et al. (2021) reported that WNS was prevalent in 36% of the range of the Little Brown Myotis and its population had declined by 98%. In eastern Ontario, from 2009 to 2015, Little Brown Myotis experienced declines of 91–100% at 11 hibernacula (Hooton et al. 2023). Counts declined to 0 or near 0 at small hibernacula with <1000 bats (Hooton et al. 2023). Similarly, summer activity of Little Brown Myotis, determined by acoustic techniques, declined by 81.7% on average, across 13 locations in southern Ontario, after WNS arrival (Morningstar et al. 2019).

We banded and implanted passive integrated transponder (PIT) tags in Little Brown Myotis at a roost in southwestern Ontario, Canada. Our objectives were to determine if this population of Little Brown Myotis had been exposed to WNS and what effects the infection had. This bat is listed as endangered in Canada and Ontario because of WNS, so a better understanding of how populations are responding to WNS may assist in determining future status designations. We tested for *Pd* on bats and examined them for wing damage. We used annual survival rates of adult females, percentage of females that were lactating, and percentage of returning yearling females that were lactating as metrics of population health.

Field-site Description

We studied bats at a maternity roost near Cambridge, in the Regional Municipality of Waterloo, Ontario, Canada. The study roost was on the Paris-Galt Moraine. The moraine is characterized by gently to steeply rolling topography with numerous wetlands, ponds, small lakes, and large deciduous and mixed forests embedded in an agricultural matrix. The roost was in a stone shed attached to a stone house on a rural residential lot. The shed has 2 main entries: an open window on the west side and an opening above a wooden door on the east side. WNS was not previously documented in the Regional Municipality of Waterloo but was confirmed in an adjoining county as early as 2010–2011 (White-Nose Syndrome Response Team 2023). There are no known hibernacula in the Regional Municipality of Waterloo.

Methods

We captured and banded bats on 10 occasions over 4 years (2014–2017) on 3 July 2014; 23 May, 10 July, and 28 August 2015; 13 May, 9 July, and 26, 27, and 29 August 2016; and 8 July 2017. To capture bats, we covered both entrances to the roost with harp traps and erected a 12-m wide by 8-m high mist net in the yard outside the roost. We placed each captured bat in a bag (1 bat per bag) until processing. We banded bats with 2.9-mm bat bands (Porzana Limited, Icklesham, East Sussex, United Kingdom), branded with "OMNR" and a unique identification number. For each bat, we recorded time of capture, the net it was captured in, species, sex, age class (subadult or adult; Adams 1992), reproductive condition when possible (scrotal, lactating, non-reproductive), mass, forearm length, and Reichard's Wing Damage Index (RWDI; Reichard and Kunz 2009).

Beginning in 2015, we implanted most captured bats with PIT tags. On 23 May 2015, we implanted bats with HPT12 (12-mm size tags, 12.5 x 2.12 mm, 0.12 g, BioMark, Boise, ID). The Ontario Ministry of Natural Resources and Forestry (OMNRF) subsequently required use of 9-mm tags (HPT9, 9 x 2.12 mm, 0.08 g, BioMark). We used the 9-mm tags for the next 2 sampling periods in 2015. Due to apparent high losses of the smaller tags in 2015 (Sandilands and Morningstar 2021), we used 12-mm tags in subsequent years, with permission from OMNRF. We implanted the tags subdermally between the scapulas of the bats using individual sterilized hypodermic needles and a gun applicator for the HPT12 tags or a syringe applicator for the HPT9 tags. We used a small amount of skin cement (Osto-Bond, Montreal Ostomy, Vaudreuil-Dorion, QC, Canada) to accelerate the healing process and help retain the tag.

We qualitatively evaluated bats for evidence of damage from WNS. We used a white light to assess wing damage of all bats. We shone a hand-held ultraviolet (UV) light on 34 bats in May 2015 to look for evidence of orange-yellow fluorescence. This fluorescence is correlated with the presence of fungal cupping erosions used to diagnose WNS by histopathology (Turner et al. 2014). We categorized damage on the bats using the RWDI (Reichard and Kunz 2009). We swabbed the wings of 1 bat and took a 3.5-mm skin punch from another in May 2015 to test for the presence of *Pd*. In 2016, we swabbed 6 bats in May, 7 in July, and 4 in August. We sent the swab and punch samples to Canadian Wildlife Health Cooperative at the University of Guelph Laboratory for testing. During all procedures, we followed the WNS decontamination protocols of the Canadian Wildlife Health Cooperative (2014) and the United States Fish and Wildlife Service (2012) in place at the time.

On 26 June 2015, we installed 2 PIT-tag readers (BioMark IS1001) in the shed. The readers monitored both openings without restricting access by bats. The readers collected data on detections of individuals entering and leaving the roost until the end of 2020.

We used the PIT-tag data to calculate annual adult female survival rates and probability of recapture between years. Ellison et al. (2007) demonstrated that annual capture rates averaged >2 times higher when calculated from PIT-tag data than from conventional capture techniques. We used only females tagged with 12-mm PIT tags in these analyses. The return rate and number of dates recorded per year were significantly less for 9-mm than 12-mm tags. The 9-mm tags had greater tag losses and lower detectability by the PIT-tag readers than the 12-mm tags, resulting in a lower estimated survival rate of females tagged with 9-mm tags (Sandilands and Morningstar 2021).

We used a Cormack-Jolly-Seber (CJS) model to test whether survival and recapture probabilities were constant or time-dependent. We captured bats in the nets in 2014–2017. We used PIT-tag data from 2015–2020 and counted each individual only once per calendar year. We used an open population model that assumed individuals could immigrate and emigrate because the PIT-tag data indicated that this was occurring. We tested 4 models: whether annual survival changed with time or not and whether recapture probability changed with time or not. We used the RMark package (Laake 2013) version 2.2.7 for program R (version 4.0.4; R Core Team 2021) to conduct the capture-recapture analysis in program MARK (White and Burnham 1999). Akaike Information Criterion (AIC; Burnham et al. 2011) was used to identify the most parsimonious model structure. We used the goodness-of-fit test for the CJS model implemented in R2ucare (Gimenez et al. 2017).

Results

We captured 208 Little Brown Myotis: 107 adult females, 44 subadult females, 5 adult males, and 49 subadult males. We tagged 65 adult females with 12-mm PIT tags. The PIT-tag readers detected these females 9516 times during the study (Table 1).

All 8 bats tested in May 2015 and 2016 were positive for *Pd*. All 11 bats we tested in July and August were negative for *Pd*, including 1 individual that had first tested positive in May. Fourteen of 34 (41.2%) bats examined under UV light showed small flecks of orange-yellow fluorescence in the wing membranes.

The overall mean RWDI score was 0.1 (n = 140; on a scoring system of 0–3); the mean in May was 0.3 (n = 34) (Table 2). The highest score recorded was 2; only 4 of 140 bats attained this score (Table 2). We recaptured 2 bats within the same year. These bats had venation and healing marks in May that were absent in July, indicating that healing had occurred. Healing marks may consist of thinner and paler patches around the wound, new blood vessels in the tissue surrounding the wound, and scabs (Ceballos-Vasquez et al. 2015).

The analysis of annual survival of adult females and the probability of recapture determined that the CJS model was a good fit to the data when only adult females tagged with 12-mm tags were considered ($X^2 = 18.2$, $P = 0.052$, df = 11). Forty of the 65 adult females (61.5%) tagged with 12-mm tags returned in a subsequent year.

The model comparison (Table 3) indicated that the best model included adult female annual survival that was constant over time and probability of recapture that changed with time (2016–2020). The best model indicated that annual survival was 0.83 and that recapture probability increased each year from 0.68 in 2016 to 0.98 in 2020 (Fig. 1). The second-best model included both annual survival and probability of recapture that varied over time ($\Delta AIC_c = 2.05$). In this model, annual survival ranged from 0.81 in 2015 to 0.84 in 2019 and probability of recapture ranged from 0.68 in 2016 to 0.98 in 2020 (Fig. 1). Estimates resulting from the 2 models were virtually identical.

Forty-nine of 59 adult females (83.1%) captured in July were lactating, suggesting that they had birthed a pup that year. The percentage of females lactating was 92.3–95.8% in 2014, 2015, and 2017, but only 50% in 2016. All 3 subadult females that returned as yearlings were lactating or had extended nipples, suggesting that they had given birth in their first year.

Discussion

The swabbing and punch sampling indicated that all bats that were tested in May 2015 and 2016 had been exposed to *Pd*. Forty-one percent of bats exhibited small flecks of orange-yellow fluorescence under UV light. Bats showed minimal signs of WNS, with a small proportion exhibiting minor damage to their wings (mean RWDI = 0.1, Table 2). Some

Table 1. Number of adult female Little Brown Myotis captured and recaptured during each sampling period. The number of PIT tags implanted and the reproductive status of females is provided. Reproductive status could only be determined during the July sampling events.

Year	Month	Captures	Recaptures	New Females	9-mm PIT tags	12-mm PIT tags	Reproductive	Non-reproductive	% Reproductive
2014	July	14	0	14	0	0	12	1	92.3
2015	May	34	2	32	0	33			
2015	July	20	8	12	13	0	18	1	94.7
2015	August	29	9	20	17	0			
2016	May	16	4	12	0	10			
2016	July	19	13	6	0	9	7	7	50.0
2016	August	8	4	4	0	2			
2017	July	19	12	7	0	11	12	1	92.3

Table 2. Summary of Reichard's Wing Damage Index (RWDI) scores for adult Little Brown Myotis for the 8 sampling periods. Mean scores are presented for each sampling period and for each month. Score definitions are: 0, no or minimal damage with ≤5 small spots visible; 1, light damage with spots present on <50% of flight membranes, and discoloration or flaking on the forearm; 2, moderate damage with spots on >50% of flight membranes, discoloration or flaking on the forearm, necrosis, and small holes <0.5 mm diameter (Reichard and Kunz 2009). The RWDI scale goes up to 3, but we did not observe any bats with a score higher than 2.

RWDI score	Sampling Period							
	May 2015	May 2016	July 2014	July 2015	July 2016	July 2017	Aug. 2015	Aug. 2016
0	18	9	14	19	12	17	28	9
1	0	4	2	1	0	2	1	0
2	0	3	0	1	0	0	0	0
Means	0.0	1.2	0.1	0.1	0.0	0.1	0.0	0.0
Monthly means		May = 0.3				July = 0.1		August = 0.0

healing probably had occurred before we sampled the bats. Those that tested positive for *Pd* in spring tested negative when captured later in the season, and all wing damage was healed. Our study provides more evidence that bats that survive WNS recover from the infection in the summer months. This fungus is heat intolerant and can only grow at temperatures <20°C (Verant et al. 2012). Bats that emerge from hibernation begin to clear the infection within 10 days following emergence, and by midsummer, fungal levels are almost undetectable. Over this period, damaged tissue is regenerated and most lesions are healed 25–40 days from the start of recovery (Hoyt et al. 2021). Other studies have documented healing of wing tissues later in the season (Dobony and Johnson 2018, Fuller et al. 2011, Greville et al. 2018, Langwig et al. 2015).

The annual survival rate of adult females was approximately 0.83 in our study, within the range reported for Little Brown Myotis pre-WNS. In eastern Ontario, Keen and Hitchcock (1980) reported survival rates of 0.82 for males and 0.72 for females; in Indiana, the estimated survival rates were 0.77 for males and 0.86 for females (Humphrey and Cope

Table 3. Akaike Information Criterion (AIC) selection of best models used to explain whether survival (φ) and recapture probabilities (*p*) varied with time (~Time) or not (~1). AICc is AIC corrected for small sample sizes. ΔAICc is the difference for models relative to the smallest AICc in the model set. AIC weight is the approximate probability in favor of the given model from the set of models considered.

Model	Parameters	AICc	ΔAICc	AIC weight
φ(~1)*p*(~Time)	3	213.8	0.000	0.6602
φ(~Time)*p*(~Time)	4	215.8	2.045	0.2374
φ(~1)p(~1)	2	218.6	4.864	0.0580
φ(~Time)*p*(~1)	3	219.2	5.400	0.0444

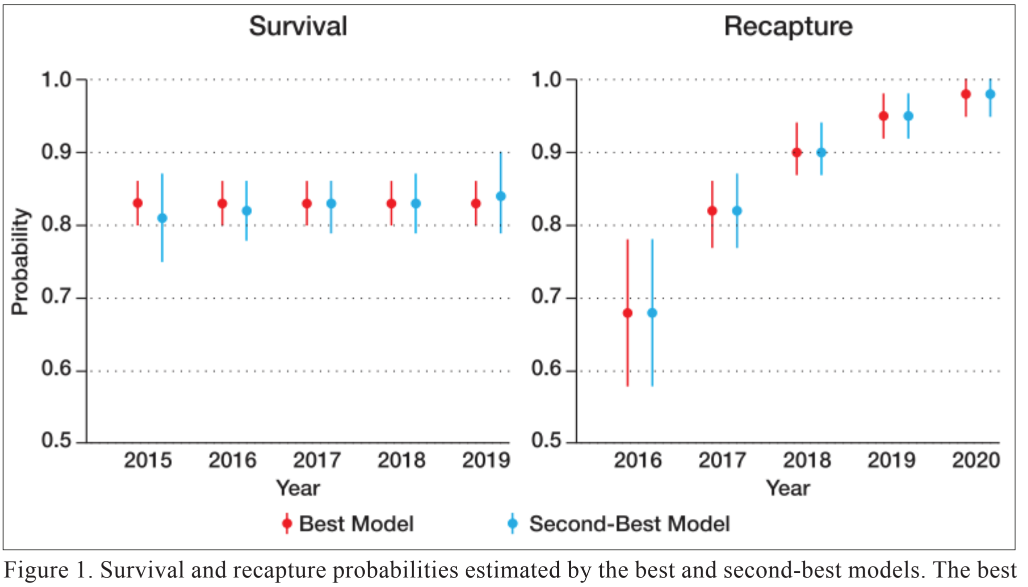

Figure 1. Survival and recapture probabilities estimated by the best and second-best models. The best model included adult female annual survival that was constant over time and probability of recapture that changed with time. The second-best model included both female annual survival and probability of recapture that changed with time. Estimates resulting from the two models were virtually identical.

1975). Annual survival rates for temperate insectivorous species ranged from 0.63 to 0.90 (Frick et al. 2010b).

In our study, the survival rate of Little Brown Myotis remained constant or increased only slightly over time. Other studies found an increase in survival rate over time after the initial decline from WNS: from 0.68 to 0.75 for males and from 0.65 to 0.70 for females in New Jersey over 4 years (Maslo et al. 2015), and from 0.41 to 0.87 for the sexes combined over 4 years in New York (Dobony and Johnson 2018).

Approximately 83% of adult females were lactating over the 4 years of netting (Table 1), suggesting that they may have birthed a pup. Before *Pd* exposure in New Hampshire, approximately 95% of adult females reproduced annually, but this declined to 87% after exposure (Frick et al. 2010b). Other studies determined that adult females are capable of reproduction after WNS exposure (Dobony and Johnson 2018, Fuller et al. 2011, Reichard et al. 2014). Adult fecundity at the study roost was comparable to pre-WNS populations in 2014, 2015, and 2017, but was considerably depressed in 2016. One possible explanation for this is that some females may not breed in consecutive years. We captured 4 adult females in consecutive Julys. Three were lactating in both years; 1 was lactating in 2015, but non-reproductive in 2016. It is possible that in 2016 the non-reproductive years of many females coincided. Of the 7 non-reproductive females documented in 2016, 5 were not detected at the roost again that year. The other 2 returned for 3–4 days in mid- to late July and 1 reappeared again for 2 days in mid-September. Of the 7 females that were non-reproductive in 2016, 6 were present in subsequent years and may have raised a pup in those years. There were no major differences in temperatures or precipitation in 2016 from other sampling years (Government of Canada 2023).

All 3 subadult females that returned as yearling adults lactated in their first year. Yearling females frequently reproduce, but those born early in the previous year are more likely to do so (Frick et al. 2010b); those born at higher latitudes are less likely to do so (van Zyll de Jong 1985). Reichard et al. (2014) reported that only 60% of females returning to their natal area bred in their first year. Although our sample size is small, results suggest that yearlings are reproducing normally in this population.

Bats at our roost had been exposed to *Pd,* but appeared to be resistant or tolerant to WNS. Reproductive rates of adult females were normal, with the exception of 1 anomalous year. The survival rate was within the range of pre-WNS populations and was constant or slightly increasing with time, indicating a stable population. Although the entire population was probably exposed to *Pd*, 61.5% of adult females returned in subsequent years. All returning yearling females apparently birthed a pup, whereas lower reproductive rates of yearlings have been reported in other populations exposed to WNS (Reichard et al. 2014).

The results of this and other studies suggest that the Little Brown Myotis population is beginning to recover in Ontario, after being decimated by WNS. In eastern Ontario after sharp declines during initial exposure to WNS infection, there was an increase in numbers at the 2 largest hibernacula in the province, consistent with the hypothesis that surviving Little Brown Myotis exhibit immunological, physiological, or behavioral traits that confer resistance or tolerance to the disease (Hooton et al. 2023). Populations of this species should continue to be monitored throughout Canada to ensure that their official conservation status reflects the best available data.

Similar results have been reported in the northeastern United States, where some populations have stabilized approximately 4 years after detection of WNS (Dobony and Johnson 2018, Frick et al. 2017, Langwig et al. 2012, Maslo et al. 2015, Reichard et al. 2014). These studies examined only those individuals that survived hibernation. Frank et al.

(2019) conducted a 9-year study in a hibernaculum where WNS was first detected in winter 2007–2008. By 6–7 years post-exposure, signs of *Pd* infection of bats under UV illumination were absent, mean body fat content of bats increased over time, the proportion of bats with normal torpor bout lengths increased, and the proportion that developed only moderate or no *Pd* infections increased. After initial exposure, populations in 2 hibernacula were reduced to 9–12% of their original size but, by 9 years after exposure, had increased to 30–41% of their original size. Our study adds to the growing body of evidence that suggests Little Brown Myotis exhibit some resistance or tolerance to *Pd* (Frank et al. 2019) and that some populations recover after initial exposure to WNS.

Our study did not determine how this population is coping with exposure to WNS or whether its apparent adaptation to the disease is a result of tolerance or resistance. Cheng et al. (2019) found that body fat levels in early winter were significantly higher in persisting populations than those that declined due to WNS. These higher fat stores could reduce mortality by 58–70%. Three studies (Auteri and Knowles 2020, Donaldson et al. 2017, Gignoux-Wolfsohn et al. 2021) found significant differences in allele frequencies between survivor and non-survivor populations. Allele frequencies did not follow the same path in different locations, suggesting that selection is operating differently in different populations. One gene under apparent selection by WNS is linked to obesity in mammals, and it may be beneficial for bats facing premature depletion of winter fat reserves (Auteri and Knowles 2020). There was no consensus among these studies as to whether the genetic adaptations were indicative of resistance or tolerance to WNS.

Little Brown Myotis in the population we studied had been exposed to WNS, but exhibited minor symptoms. Wing damage levels were minimal and yearling and older females apparently reproduced at normal rates, with the exception of 1 study year. Our results suggest that this population is developing resistance or tolerance to WNS. Further research is required in Ontario and elsewhere in Canada to determine the effects of WNS on Little Brown Myotis.

Acknowledgments

Funding for the 2015 and 2016 studies was provided in part by the Canadian Wildlife Service. We are grateful to J. Girard for awarding and overseeing these contracts. Funding for the PIT tags was provided by the National Speleological Society under its WNS Rapid Response Fund. We thank those who helped with fieldwork and decontamination of the equipment: A. Alic, G. Buck, A. Ceballos-Vasquez, R. Del Giudice, S. Fraser, L. Greville, L. Haines, K. Hoo, S. Labrie, O. Morningstar, L. Owens, R. Perdiao, C. Risley, P. Ronald, M. Russel, B. Talbot, N. Taylor, and R. Valdizon. G. Betini conducted the statistical analyses and provided helpful comments on the relevant text. A. McLennan of Cartographics Mapping & Design prepared the figure. Two anonymous reviewers made helpful constructive comments that improved the manuscript. The OMNRF issued the necessary permits to conduct this research, and we captured and handled bats under animal care protocols of the University of Guelph and the Wildlife Animal Care Committee of Ontario.

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