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Source: Acta Chiropterologica, 11(2):457-464. 2009.

Published By: Museum and Institute of Zoology, Polish Academy of Sciences

DOI: <http://dx.doi.org/10.3161/150811009X485684>

URL: <http://www.bioone.org/doi/full/10.3161/150811009X485684>

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White-nose syndrome inflicts lasting injuries to the wings of little brown myotis (*Myotis lucifugus*)

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White-nose syndrome (WNS) is an emerging disease causing massive mortality of hibernating bats in the northeastern United States. At hibernacula, bats affected with WNS typically exhibit growth of a white psychrophilic fungus (*Geomyces destructans*) on the nose, wings and ears; many individuals seem to prematurely die of starvation owing to depleted fat reserves. Conspicuous scarring and necrosis of the wings on WNS-affected bats that survive hibernation may have lasting consequences for survival and reproductive success during the active season. We monitored two maternity colonies of little brown myotis, *Myotis lucifugus*, in Massachusetts and New Hampshire from 14 May to 8 August 2008 to assess body conditions after expected exposure to WNS over the previous winter. We developed a 4-point wing damage index (WDI = 0 to 3) to assess the incidence and severity of wing damage in the months following emergence from hibernation. Severe wing damage was observed up to 4 June and moderate damage was observed through 9 July. Light wing damage was observed on both adult and juvenile bats throughout the study period, but was not exclusively attributed to WNS. The most severe wing damage was associated with a lower body mass index which may reflect reduced foraging success. Overall, reproductive rate was 85.1% in 2008; slightly lower than reported in previous studies. The incidence, timing, and geographic range of wing damage observed on little brown myotis in 2008 correspond to the occurrence of WNS at hibernacula. Monitoring wing conditions of affected and healthy bats will be important tool for assessing the spread of this disease and for establishing baseline data for unaffected bats. The simple scale we propose should be useful for monitoring wing conditions in any bat species.

Key words: disease monitoring, flight performance, white-nose syndrome, wing damage index, WNS

INTRODUCTION

White-nose syndrome (WNS) is an unprecedented, recently described condition that affects hibernating bats in the northeastern United States (Blehert *et al.*, 2009). First reported from Howe Cavern near Albany, New York in February 2006 and in a handful of nearby hibernacula in the winter of 2006–2007, WNS had spread to 37 counties in New Hampshire, Vermont, New York, Massachusetts, Connecticut, New Jersey, Pennsylvania, West Virginia, and Virginia by the end of the winter of 2008–2009. WNS is linked to massive mortality of four hibernating species in the region — *Myotis lucifugus*, *M. septentrionalis*, *M. leibii*, and *M. sodalis*, and expected mortality in two other species — *Perimyotis* (formerly *Pipistrellus*) *subflavus* and *Eptesicus fuscus* (Blehert *et al.*, 2009). Local declines at several hibernacula reach 90% in New England (J. Reichard, personal observation; S. Darling, personal communication; T. French, personal

communication) and 100% in New York State (A. Hicks, personal communication). WNS is associated with a psychrophilic, or cold-adapted fungus (*Geomyces destructans*) growing on the nose, ears and membranes of hibernating bats (Gargas *et al.*, 2009); individuals that succumb to WNS presumably die of starvation owing to prematurely depleted fat reserves during winter. At present, the cause and consequences of this syndrome are not fully understood.

Premature depletion of fat reserves during hibernation has implications that threaten the survival and sustainability of affected bat populations. Upon approaching depletion of critical fat reserves, some bats may emerge and attempt to forage (Turbill and Geiser, 2008) or relocate to warmer microclimates within the hibernaculum, presumably to conserve energy (Boyles and Willis, 2009). Bats may also vacate affected hibernacula prematurely to seek alternate roosts for the remainder of the winter and early spring. In cold climates, these behaviors exact high

energetic costs and risk injuries such as frostbite (Thomas *et al.*, 1991). At the end of hibernation, bats rely on their remaining fat reserves to complete migration to summer roosts (Kunz *et al.*, 1998). Moreover, females rely on fat reserves for the production of leptin to induce the cascade of other hormones that lead to ovulation and subsequent gestation (Zhao *et al.*, 2003). Thus, the adverse impacts of WNS likely extend beyond the hibernation period by limiting spring migration and potentially reducing reproductive success during the summer.

A large proportion of bats leaving WNS-affected hibernacula exhibit varying degrees of scarring, necrosis, and atrophy of flight membranes. Insectivorous bats rely on the unique mechanical properties of their wings to capture prey, evade predators, and to access roosts (Swartz *et al.*, 2003). Wings are also important for circulatory regulation (Wiegman *et al.*, 1975; Davis, 1988a, 1988b), thermoregulation (Thomas and Suthers, 1972), gas exchange (Herreid *et al.*, 1968; Makanya and Mortola, 2007), and water balance (Kluger and Heath, 1970; Thomson and Speakman, 1999; Bassett *et al.*, 2009). Wounds or infections on the wing membranes of bats can adversely affect these properties or functions, and ultimately may affect foraging success. In this way, WNS poses another threat to affected bat populations during the active season.

Our study was designed to characterize the physical damage to wing membranes and to document phenological changes in wing conditions in little brown myotis (*Myotis lucifugus*) at maternity roosts in the spring and summer months following emergence from hibernation. We postulated that bats affected by WNS during winter, but that survived and arrived at maternity roosts with damaged wing membranes, would have poorer body condition than bats with healthier flight membranes. We predicted that bats with the most severely damaged wings may succumb to starvation or predation during the summer. We also predicted that bats affected by WNS would be at increased risk of failed reproduction.

MATERIALS AND METHODS

Study Sites

The study was conducted from 14 May and 8 August 2008 at two maternity colonies of *M. lucifugus* within 60 km of each other in the northeastern US (Framingham, Massachusetts and Milford, New Hampshire). Both sites are within 160 km of Aeolus Cave, East Dorset, Vermont and Chester Emery Mine, Chester, Massachusetts, where hibernating bats experienced high prevalence of WNS in the winter of 2007–2008 and 2008–2009. Thus, the distances between the summer colonies

and two highly affected hibernacula are within the putative seasonal migratory range of this species in eastern North America (Davis and Hitchcock, 1965; Griffin, 1970; Fenton, 1970; Humphrey and Cope, 1976). The maternity colonies are located in barns used for hay and household storage and for housing assorted livestock (e.g., chickens, geese, and sheep). The landscape surrounding these sites is composed of mixed hardwood forest, agricultural grassland, and residential communities. These roosts are also inhabited by smaller numbers of the northern long-eared myotis (*M. septentrionalis*), tri-colored bat (*P. subflavus*), and big brown bat (*E. fuscus*). Because *M. lucifugus* is the most common of the species affected by WNS and has a rich history of scientific study in this region, it is an ideal species for the current study. The study period we report spans the early active season of *M. lucifugus* in the northeastern US, extending from arrival at maternity roosts following spring migration to departure for swarming sites and hibernacula in late summer.

Field Methods

Except for two weeks in late June, colonies were visited at biweekly intervals and bats were trapped with double-frame harp traps (0.9 m wide by 1.0 m high or 1.5 m wide by 1.9 m high) placed in a doorway of the barn at dusk (Kunz *et al.*, 2009). Other large openings were partially obstructed with coarse nylon nets to increase trapping success. Captured *M. lucifugus* were transferred to and temporarily held in individual cotton bags until trapping was complete at the end of the evening emergence period. Other species, when captured, were transported several meters away from the barn and released without further processing. Traps and nets used for blocking alternate exit routes were removed once 60 *M. lucifugus* were trapped or after one hour, to allow bats to return and emerge freely from the barn.

Sex, age, reproductive condition, body mass (M_b), and length of forearm were recorded. Bats were banded with 2.9 mm numbered and lipped alloy bat bands (Porzana Ltd. Icklesham, UK). The wings and uropatagium were inspected by transillumination, using a 3-LED light source (Dot-It, OSRAM, Billerica, MA, US). Alternatively, portable light boxes from arts and crafts suppliers provide excellent transillumination of wings (D. Reeder, personal communication). Each bat was assigned a single wing damage index (WDI) to describe scarring and necrosis on the flight membranes (see below). For each bat that was scored with a $WDI \geq 1$, we recorded digital photographs of the transilluminated wings (Fig. 1). Wings were photographed on the camera's automatic setting with the flash turned off, by extending the wing on the translucent surface that was positioned above the diffuse LED light source (or portable light box). The identification number (band number) of each individual, the date of capture, and a metric ruler were included in each digital photograph. All methods were conducted in accordance with American Society of Mammalogists Guidelines for the Capture, Handling, and Care of Mammals, Boston University's Institutional Animal Care and Use Committee, and the US Fish and Wildlife Service's Disinfection Protocol for Bat Field Studies.

Wing Damage Index

Five types of wing damage were identified: splotching, flaking, necrosis, holes, and membrane loss (Table 1 and Figs. 1–5).

The wing damage index, described below, is a four-point scale ranging from 0 (no / minimal damage) to 3 (severe damage) for recording the occurrence of these symptoms. After examining both wings and the uropatagium, each bat was assigned a single WDI corresponding to the highest score for which it exhibited one or more types of damage for that level (Table 2). Thus, the WDI is a composite assessment for the wing membranes and uropatagium. Because the severity of forearm flaking, when present, was fairly consistent, other categories of damage characteristic of WDI = 2 and WDI = 3 were considered for assigning these scores.

WDI scores were determined based on the physical conditions of the wings, without consideration of the causes of observed damage. When a cause could be hypothesized (e.g., bites from ectoparasites or tears from assorted environmental hazards) these notes were recorded in addition to WDI.

Analytical Methods

Separate contingency tables were created for adult females and juveniles to test for changes in the relative abundance of

TABLE 1. Wing conditions observed in *M. lucifugus* used for developing the wing damage index (WDI) for assessing the physical condition of flight membranes

| Symptom | Description | Example |
|-----------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| Spotting, splotching and depigmented membrane | Light spots appear on the darker wing and tail membranes. These spots are often more visible when the membrane is backlit | Fig. 1 |
| Flaking and depigmented forearm | Dry skin appears along the forearm. Some spots appear lighter brown or pink where skin appears to have flaked off | Fig. 2 |
| Necrotic tissue | Membranes may have visible scabs, open wounds, or infections. In more severe cases, large sections of membrane are sloughing from the wing | Fig. 3 |
| Holes | Some very small pin-holes appear to be associated with ectoparasite wounds. Other holes are larger and often surrounded by depigmented or necrotic tissue. The appearance of the edges of holes may be likened to singed nylon | Fig. 4 |
| Membrane loss | Wing areas are notably reduced along edges. Most commonly, the trailing edge of the plagiopatagium is receded in an arc from the leg to the fifth digit. Such damage may be severe, greatly reducing the overall surface area of the wings | Fig. 5 |

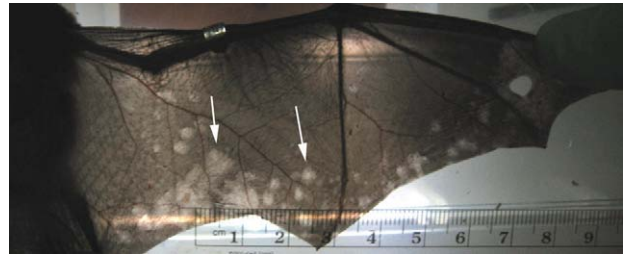


FIG. 1. Spotting, splotching, and depigmented tissue associated with scarring on wings of *M. lucifugus*



FIG. 2. Depigmentation and flaking skin along the forearm of *M. lucifugus*

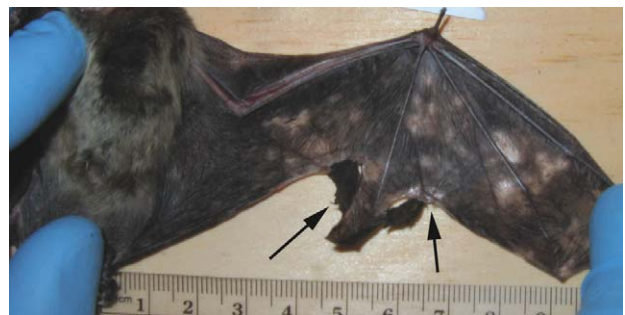


FIG. 3. Necrotic tissue and sloughed membrane on *M. lucifugus*



FIG. 4. Small holes surrounded by necrotic tissue and spots on *M. lucifugus*



FIG. 5. Loss of flight membrane on *M. lucifugus*

TABLE 2. Criteria used for the wing damage index (WDI) to assess bat flight membrane conditions. Each bat received the highest WDI for which it exhibits one or more of the indicated conditions for that level. The WDI score is recorded as a single composite score for both wings and the uropatagium, as a whole

| Wing condition | Condition | | | | |
|---------------------------------------|-------------------------------------------------|-----------------------------------------------|-----------------------|-----------------------------------------------------------------------|---------------------------------------------------------------------------------------|
| | Spots / splotches | Discolored / flaking forearm | Necrotic tissue | Holes | Membrane loss |
| WDI = 0 No damage / Minimal damage | ≤ 5 small spots visible with trans-illumination | Not present | Not present | No holes, or possibly very small pin-sized holes | Fully intact |
| WDI = 1 Light damage | Present on < 50% of flight membranes | Present | Not present | No holes, or possibly very small pin-sized holes | Fully intact |
| WDI = 2 Moderate damage | Present on > 50% of flight membranes | Present (this condition alone scores WDI = 1) | Few areas of necrosis | Small holes < 0.5 cm diameter – often associated with necrotic tissue | Necrosis on edges of patagium, but no loss of membrane area Tears < 1 cm |
| WDI = 3 Severe damage | Present on > 90 % of flight membranes | Present (this condition alone scores WDI = 1) | Abundant necrosis | Large holes > 0.5 cm diameter – often associated with necrotic tissue | Noticeable loss of membrane, often along trailing edge of plagiopatagium Tears > 1 cm |

bats with different WDI over time. Body mass index (BMI = M_b (g) / length of forearm (mm)) was calculated for adult females and for juveniles captured up to 9 July (when WDI ≥ 2 was last observed) to compare relative body conditions among WDI scores with a Kruskal-Wallis test. Reproductive rate of each colony was estimated by maximum percentage of adult females that were pregnant on a given sample night.

RESULTS

A total of 603 *M. lucifugus* were captured between 14 May and 8 August 2008. Pregnant females were captured in the greatest proportions on 28 May in Framingham (89.2%) and 4 June in Milford (81.1%). Mean M_b was 8.6 ± 1.0 g for pregnant females ($n = 91$), 7.6 ± 0.9 g for nonpregnant adult females (including undetectable pregnant females in early summer; $n = 338$), 6.8 ± 1.0 g for adult males ($n = 8$), and 6.6 ± 0.6 g for juveniles ($n = 166$). Volant juveniles were first captured on 2 July in Milford.

Bats with WDI ≥ 1 were captured on each sampling night. For adult females, the incidence of different WDI scores was not independent of date ($G = 107.96$, $d.f. = 27$, $P < 0.001$ — Fig. 6). Relative abundance of bats with obvious wing damage peaked in June when more than 60% of bats in the colonies had WDI ≥ 1 . Bats with WDI = 3 were most prevalent in May and were not observed after 4 June. Bats with WDI = 2 were not observed after 9 July. The incidence of different WDI scores for juveniles was not independent of date ($G = 12.05$, $d.f. = 5$, $P < 0.05$ — Fig. 7). Juveniles exhibited WDI ≤ 1 throughout the study period; wing damage on juveniles was most abundant from late July to early August when about 20% of juveniles had WDI = 1.

Body mass index (BMI) differed among WDI scores for adult females ($\chi^2 = 15.04$, $d.f. = 3$, $P < 0.01$, Kruskal-Wallis test) (Fig. 8). Median BMI (range) was greatest for bats with WDI = 0 ($n = 173$) and WDI = 1 ($n = 108$), being 0.22 g/mm (0.17–0.29 g/mm) and 0.22 g/mm (0.16–0.31 g/mm), respectively. Median BMI was 0.20 g/mm (0.16–0.28 g/mm) for adult female bats with WDI = 2 ($n = 29$) and 0.19 g/mm (0.15–0.20 g/mm) for WDI = 3 ($n = 6$). BMI did not differ among juveniles with different WDI ($\chi^2 = 0.01$, $d.f. = 1$, $P = 0.92$, Kruskal-Wallis test); median BMI was 0.17 g/mm (0.14–0.23 g/mm) and 0.17 g/mm (0.17–0.20 g/mm) for juveniles with WDI = 0 ($n = 152$) and WDI = 1 ($n = 16$), respectively.

Of the 603 bats captured, 549 bats (380 adults, 166 juveniles) were banded. However, all adult bats that were recaptured were initially banded on or

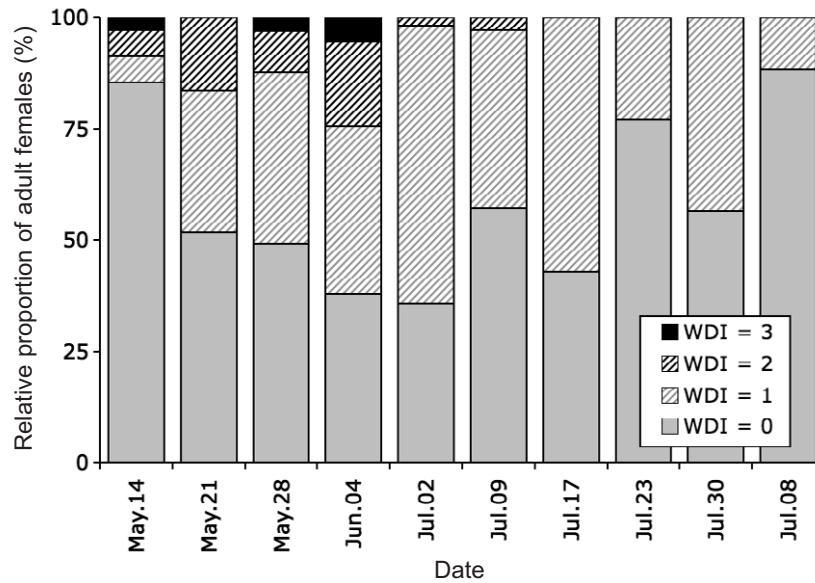


FIG. 6. Relative proportion of adult female *M. lucifugus* exhibiting various degrees of wing damage (WDI) at summer maternity colonies in the northeastern US

before 9 July. Thus, of 362 adult bats banded up to that date, 34 (9.4%) were recaptured. Recapture rates differed among wing damage scores with borderline significance ($G = 6.89, d.f. = 3, P = 0.08$ — Table 3). Wing conditions of only three recaptured bats improved over the study period; one from WDI = 2 to WDI = 1 and two from WDI = 1 to WDI = 0. All other recaptured bats had the same WDI as recorded at the time of initial capture.

DISCUSSION

Damaged wings may lose surface area, elasticity and dexterity, thus compromising maneuverability and foraging success (Arita and Fenton, 1997). If their flight abilities were compromised during the active season, bats would be less likely to achieve sufficient energy and nutrient intake to sustain gestation and lactation. Increasing severity of wing

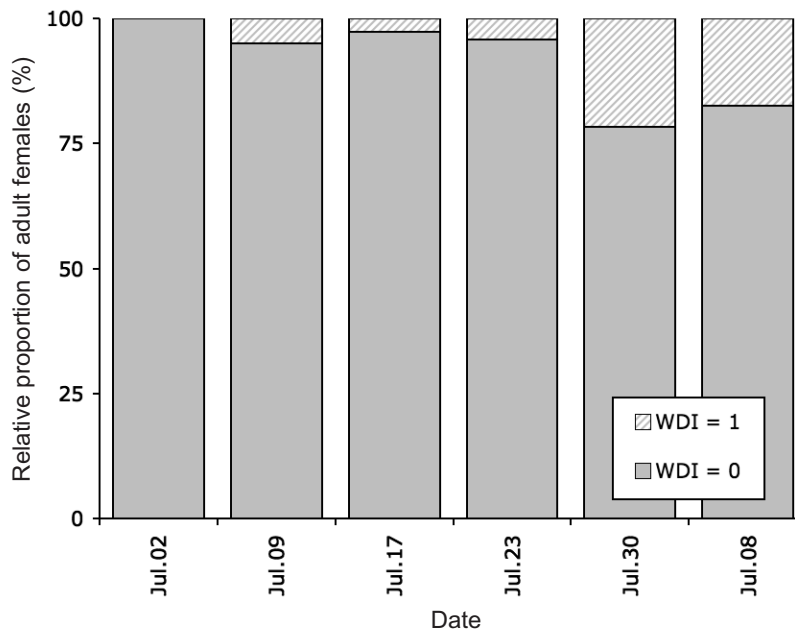


FIG. 7. Relative proportion of juvenile *M. lucifugus* exhibiting various degrees of wing damage (WDI) at summer maternity colonies in the northeastern US

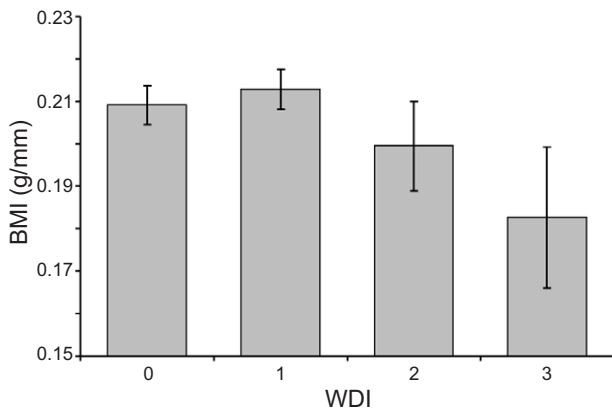


FIG. 8. Mean body mass index [$BMI = M_b$ (g) / forearm length (mm)] of adult female *M. lucifugus* with different wing damage indices (WDI) at summer maternity colonies in the northeastern US from 14 May to 9 July 2008. Error bars are 95% confidence intervals

damage was associated with poorer body condition, suggesting foraging success may have been compromised. Moreover, reproductive rate in the current study (~85%) was slightly lower than previously reported (> 93%) for *M. lucifugus* (Humphrey and Cope, 1976; Reynolds, 1998). Although wing damage, low body mass, and a decline in reproductive success may result from many possible factors, including, but not limited to WNS, this study reveals an unexpectedly high prevalence of wing damage on little brown myotis in the affected range of the recent syndrome. Further research is needed to clarify the connection between WNS and wing damage and to fully quantify the impact that wing damage during spring and early summer has on subsequent reproductive success and survival.

Numerous dead bats were found on floors of barns and surrounding landscapes during this study period (J. Reichard, personal observation). Unfortunately, these were in various stages of decay that prevented accurate assessment of WDI or BMI. However, we expect that wing damage led to poorer survival of affected bats during the active season. Reduced flight performance of bats would compromise foraging success and make them more vulnerable to predators and other environmental hazards (Norberg and Rayner, 1987; Norberg, 1998). We suggest that the decrease in proportion of captured bats with $WDI \geq 2$ into early July likely reflects either fatalities or emigration rather than recovery from damage. Mean M_b of pregnant females in 2008 was lower than for pregnant females in 1995 (9.69 g), before WNS had been reported (Reynolds and Kunz, 2000). While it is possible that poorer

body condition in the summer of 2008 is associated with reduced insect abundance or other factors not measured in this study, we predict that it is more likely associated with WNS exposure in winter and wing conditions or foraging success in spring and summer. Bats that survive hibernation at affected sites may be unable to fully recover from emaciated conditions. Moreover, poor body condition may continue through the swarming and prehibernation fattening period. If the wing damage experienced by little brown myotis compromises their ability to recover lost energy and nutrient reserves incurred during pregnancy and lactation, then we can expect that these compounding factors directly and indirectly associated with WNS will lower their survival.

Wing Damage and WNS

In most cases, light wing damage ($WDI = 1$) on adult bats occurred in similar locations on the wings to more severe damage ($WDI > 1$). However, since BMI for these bats was not significantly different from bats with $WDI = 0$, we do not expect that light wing damage affects foraging success. It is important to note that some wing damage is likely to occur independently of WNS-related infections, and light damage may reflect 'normal' wing conditions. Documenting wing conditions at control sites not affected with WNS will elucidate the incidence and impact of wing damage in affected populations.

Bats occasionally sustain injuries from agonistic encounters with conspecifics, would be predators, and environmental obstacles in roosts and in foraging areas. Although such injuries may be acknowledged (Sachanowicz *et al.*, 2006), they are probably underrepresented in the published literature (but, see Davis, 1968). Exceptions include investigations of injuries caused by wing bands (e.g., Kunz and Weise, 2009). Rapid regeneration time of damaged wings may be triggered by naturally occurring injuries to membranes or from taking wing biopsies

TABLE 3. Banding and recapture rates for adult *M. lucifugus* banded up to 9 July grouped by wing damage index (WDI) during the first capture. The bats banded up to 9 July included all adult bats recaptured through the entirety of the study

| WDI | Bats banded before 9 July | Recaptured bats (%) |
|-------|---------------------------|---------------------|
| 0 | 213 | 15 (7.0) |
| 1 | 111 | 17 (15.3) |
| 2 | 33 | 2 (6.1) |
| 3 | 5 | 0 (0) |
| Total | 362 | 34 (9.4) |

that may heal in less than four weeks (Worthington Wilmer and Barratt, 1998), but may be delayed by bacterial or fungal infections of wounded tissue. Although damaged membranes are capable of healing, greater than 80% of recaptured bats that initially scored $WDI \geq 1$ showed no obvious change in wing conditions. Thus, we expect that reduced abundance of bats with severe and moderate damage ($WDI \geq 2$) as the summer progressed may be due to death from starvation or predation. Alternatively, bats with severe wing damage could have emigrated from maternity roosts if their conditions prevented successful pregnancies. The rate and extent to which wings of free-ranging bats recover following injury are not well understood and deserve further study.

Most of the scarring observed in the present study was markedly different from wounds inflicted by environmental obstacles and far more abundant than has been previously reported. The location of scars and necrotic tissue on active bats captured in spring and early summer is consistent with areas of fungal growth observed in hibernating *M. lucifugus* in the winter of 2007–2008. Histopathologic investigation of wing injuries on bats captured outside of WNS-affected hibernacula has linked fungal infection to severe inflammatory responses and sloughing of serocellular crusts containing hyphae of *Geomyces* sp. (Meteyer *et al.*, 2009). Moreover, the timing and geographic distribution of wing damage is consistent with the known geographic range of WNS. Thus, it is likely that the scars and necrotic tissue observed in *M. lucifugus* in the summer of 2008 are consequences associated with WNS. We suggest that most of the wounds and scars observed on bats at summer colonies are a direct consequence of exposure to *G. destructans* causing fungal infection, associated bacterial infections, or necrosis resulting from frostbite incurred at times when bats flew outside hibernacula during subfreezing conditions. Bats observed flying during extreme cold periods near WNS-affected hibernacula may also be prone to collisions with trees, rocks, and buildings, and freezing, thus risking further injury to flight membranes.

Wing damage is not limited to bats exposed to WNS. For example, Davis (1968) reported 28 of 63 pallid bats (*Antrozous pallidus*) exhibited varying degrees of wing damage. The gleaning behavior of this species makes it more likely to encounter thorns and cactus spines, or suffer bone fractures than aerial insectivores. Juveniles of *M. lucifugus* in the current study also showed varying degrees of light

scarring on the wings, but they had not previously hibernated at sites affected by WNS. We expect that many of these spots were caused by bites from ectoparasites (e.g., mites), a condition that, in another study, did not seem to effect flight performance (Fenton, 1970).

The recent emergence and spread of WNS has drawn special attention to wing conditions, both within and outside of the affected geographic range. Bat researchers and wildlife managers studying and monitoring WNS should record wing conditions to determine the impact wing damage has on bats during the active season. Researchers and managers not directly involved in WNS research will also benefit from recording WDI to establish a baseline for wing damage in healthy populations. Early detection of changes in wing conditions in these populations will be critical for assessing the future spread of WNS. Although the vector or mode of transmission of *G. destructans* has not been determined, hypotheses suggest that movements of bats among roosts and differential degrees of sociality may lead to transmission at summer roosts. Thus, dispersal of bats from the WNS-affected hibernacula may explain the continued spread of the syndrome beyond its current range. This protocol for monitoring wing damage provides a standard for quantifying wing damage quickly and consistently among different researchers.

ACKNOWLEDGEMENTS

We thank R. and V. Putnam and A. and A. Howe for access to maternity colonies on their properties. E. Buckles, M. Moore, D. Reeder, S. Reynolds, and G. Turner provided early feedback on the wing damage index. Additional field-testing of the index was facilitated by J. Coleman, S. Darling, and S. von Oettingen. We also thank C. Kang, T. Murtha, and L. Vanhkhon who assisted in the field and laboratory. We are also grateful to P. Cryan and two anonymous reviewers for their suggestions and comments on an earlier version of this manuscript. This study was funded, in part, by the U.S. Fish and Wildlife Service, Boston University's Undergraduate Research Opportunities Program (UROP), Boston University's Center for Ecology and Conservation Biology, and the National Science Foundation (IIS-0326483).

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Received 15 April 2009, accepted 29 July 2009